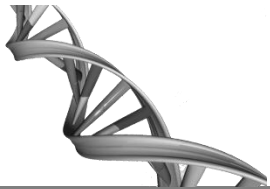


**HUNGARIAN MOLECULAR
LIFE SCIENCES
2019**

**PROGRAMME
&
BOOK OF ABSTRACTS**

**HOTEL EGER-PARK
EGER, HUNGARY
29-31 MARCH 2019**

**PUBLISHED BY:**

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Róbert Hohol

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Dear Colleagues,

The Hungarian Biochemical Society (MBKE) representing the disciplines of biochemistry and molecular biology, and the Hungarian Genetics Society (MAGE) acting for genetics, cell- and developmental biology, organise their fourth joint conference, entitled

„Hungarian Molecular Life Sciences 2019”,

in Eger, Hungary, between 29-31 March 2019. By organising this event, our scientific societies are committed to maintaining the traditions which started with the first “Hungarian Molecular Life Sciences” meeting in 2013. Scientists from institutions of higher education and research institutes of Hungary, as well as of its neighbouring countries, are anticipated to attend the meeting. The goal of the conference is to establish a common forum for colleagues working in the fields of biochemistry, cell and structural biology, developmental biology, classic and molecular genetics, molecular biology of human diseases, systems biology, synthetic biology, genomics and bioinformatics. We aim to organise a conference with a creative and friendly atmosphere, where former acquaintances are renewed and new collaborative partnerships are formed; a symposium of class and uniqueness.

The meeting is held in the conference centre of Hotel Eger-Park in Eger, the same venue where we already had a very successful meeting in two previous years. It has been selected so that the lecture halls, the poster exhibition area and that of the company exhibitors will all be hosted in one building, together with the restaurants and social rooms, providing ample possibilities for professional discussions and recreational activities alike.

Hereby we kindly invite you to participate in the Hungarian Molecular Life Sciences 2019 conference. We count on your contribution to a scientific symposium with great atmosphere that will be memorable and beneficial for the whole Community of the Hungarian molecular life sciences. The official language of the conference is English.

The congress is organised in collaboration with Diamond Congress Ltd. Their staff is happy to respond to questions you might have on organisational details.

On behalf of the organisers:

László Virág

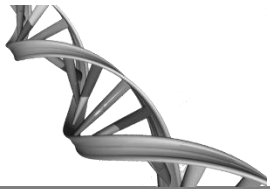
Miklós Erdélyi

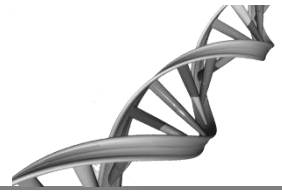
László Buday

Mihály Kovács

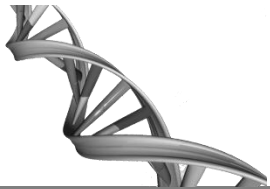
József Mihály

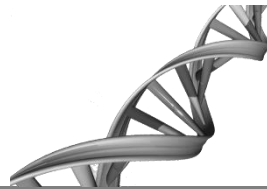
Máté Varga





GENERAL INFORMATION





GENERAL INFORMATION

CONGRESS ORGANISERS

- Magyar Biokémiai Egyesület (Hungarian Biochemical Society)
- Magyar Genetikusok Egyesülete (Hungarian Genetics Society)

ORGANISING COMMITTEES

**László Buday¹, Miklós Erdélyi², Mihály Kovács³, József Mihály², Máté Varga⁴,
László Virág⁵**

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TECHNICAL ORGANISERS

Diamond Congress Ltd.

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Tel: +36-1-225-0209

<http://www.diamond-congress.hu/>

WEBSITE OF THE CONGRESS

<http://www.hunlifesci.hu>

WEBSITE OF THE HUNGARIAN BIOCHEMICAL SOCIETY

<http://www.mbkegy.hu>

WEBSITE OF THE HUNGARIAN GENETICS SOCIETY

<http://www.magenegy.hu/hu/>

VENUE

Hotel Eger-Park

3300 Eger

Szálloda utca 1-3.

Tel.: +36-36/522-200

OPENING HOURS OF THE REGISTRATION

Friday, 29 March, 2019	-	12.00 – 18.00
Saturday, 30 March, 2019	-	08.30 – 19.00
Sunday, 31 March, 2019	-	08.30 – 14.00
(Daylight Saving Time – Óráátállítás!)		

ONSITE CONTACT NUMBERS

Éles-Etele Nóra / Varga Attila

+36/70-9438-543, +36/20-936-2969

Diamond Congress Ltd.

OFFICIAL LANGUAGE

Official language of the Conference is English (no translation is available).



Registration fee (incl. VAT)	Payment till 15 February	Payment after 15 February
Registration fee for industrial participants	70.000 Ft	75.000 Ft
Registration fee for senior researchers*	65.000 Ft	70.000 Ft
Registration fee for junior researchers**	49.000 Ft	55.000 Ft
Registration fee for exhibitors	45.000 Ft	45.000 Ft
Registration fee for accompanying persons	38.000 Ft	38.000 Ft

*Junior researcher: Ph.D. and university student, or researcher under 30;

**Only for participants with academic background

Registration fees include admission to the scientific sessions, admission to the exhibition, welcome reception, banquet dinner, coffee breaks, lunches.

Accompanying persons' registration fee and exhibitor's registration fee is not valid for admission to the scientific part of the Conference, and these fees do not include conference materials, only meals and social events.

ORAL PRESENTATIONS (PL-1 – PL-4, TA-1 – TA-2, O-1 – O-66)

The schedule of the oral presentations can be seen in the detailed programme of this booklet. Speakers and session chairs are kindly requested to keep the time of the presentations. Make sure to bring your presentation file written on a USB flash drive. Presenters are kindly requested to give their presentation file to the technicians in the lecture rooms preferably half day before beginning of the corresponding session.

Oral presentations: Congress Hall, Session Room Liget I.

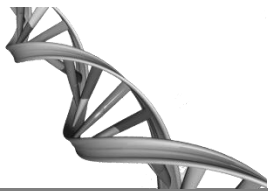
POSTER PRESENTATIONS (P-1 – P-225)

Poster presenters of poster numbers P-1 – P-114 are kindly requested to mount their posters **before** the opening of the conference (or in the coffee break) and remove them on Saturday till 11:00 the latest (during the coffee break), to enable the authors of the next poster session to mount their posters in time.

Poster presenters of poster numbers P-115 – P-225 are kindly requested to mount their posters on Saturday from 13:30 **before** the afternoon session starts and remove them latest at lunch time on Sunday.

Posters will be identified by poster numbers, which are indicated in the abstract booklet, in the authors' index.

For mounting and removing the posters, please take it into consideration, that there will be oral presentations in the room! Please use the breaks for these actions.



Poster sessions: Room Liget I.

Pins are to be provided to fix the posters by the technical organisers.

Authors of posters from P-1 to P-114 will present their work between 20.30 –22:00 on Friday, 29 March. Authors of posters from P-115 to P-225 will present their work between 17.00 – 19.00 on Saturday, 30 March.

AWARDS OF BEST POSTER PRESENTATIONS

A panel of experts will evaluate the posters and select the best three poster presentations.

EXHIBITON

In accordance with the conventions of the conference, parallel to the scientific sessions a professional exhibition is to be organised. Please have a look at the exhibition floor plan of the booklet.

ACCOMMODATION

Hotel rooms are booked under the name of the participants. Conference participants may occupy the rooms from 14:00 on the day of arrival and should arrange the check out until 10:00. The hotel ensures a luggage room. The guarded parking lot of the hotel is available for our participants free of charge. Guests are kindly requested to settle their extra room bills (such as phone calls, drinks and minibar) prior to departure.

The room prices include buffet breakfast, VAT and city tax. In the Hotel Eger-Park the price also contains the usage of wellness facilities (pools, jacuzzi, sauna park and steam bath).

SOCIAL PROGRAMMES (*incl. in the registration fees*)

Friday, 29 March, 2019

Welcome dinner

(Restaurant of Hotel Park)

Saturday, 30 March, 2019

Banquet (Congress Hall of Hotel Eger)

Saturday & Sunday, 30-31 March, 2019

Lunches (Restaurant of Hotel Park)

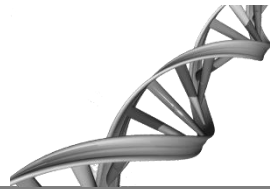
Friday - Sunday, 29 March - 31 March, 2019

Coffee breaks

(indicated in the programme)

All participants and accompanying persons will receive a personal badge upon registration. You are kindly requested to wear your name badge when attending the meetings or social events.

Extra consumption, which is not included in the menus are kindly requested to settle prior to departure.



CANCELLATION POLICY

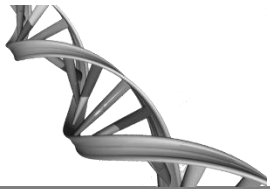
Cancellations on registration and hotel reservation can be made only in writing. The refund for cancellations made on and prior to 28 February, 2019 is 100%. After this date the conference secretariat has to pay the advanced payments to the hotel, and there is no way to refund in case of latter cancellation.

PAYMENT, INVOICES

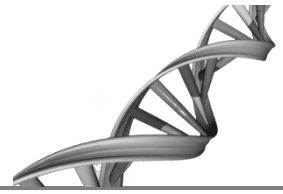
The price of the ordered services will be indicated on the final invoice according to the Hungarian official financial rules. Official final invoices and receipts for fees paid by the participants will be handed over on site at the registration desk. Please forward them to the financial department of your institution.

LIABILITY AND INSURANCE

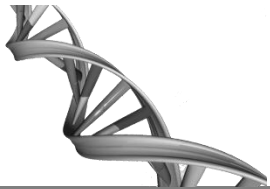
The organisers cannot accept liability for any personal accidents, loss of belongings or damage to private property of participants and accompanying persons that may occur during the conference.



Eger, 29-31 March 2019



SCIENTIFIC PROGRAMME





Day 1 – Friday, 29 March 2019

14:00 **Opening**

Plenary session / Congress Hall

- 14:10 PL-1 Engines of carcinogenesis; what determines the speed of mutagenesis?**
Lajos Haracska, Lili Hegedűs, Lajos Pintér, Szilvia Juhász, Kata Dudás, Ernő Kiss, Li Qiuzhen, Péter Burkovics and Mónika Mórocz
- 14:45 PL-2 Unexpected functions of the actin cytoskeleton in meiotic divisions of oocytes**
Péter Lénárt, Natalia Wesolowska, Pedro Machado and Yannick Schwab

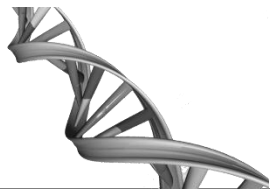
Tankó awards / Congress Hall

- 15:20 TA-1 From thermosensor membranes to membrane lipid therapy**
 László Vigh
- 15:45 TA-2 Forty years with protein phosphatases: past, present and future**
 Ferenc Erdődi
- 16:10 Coffee break**

Cell differentiation & Signaling / Congress Hall

Chair: László Buday

- 16:50 O-01 Ca²⁺ mobilization dependent reduction of the ER lumen is due to influx of cytosolic glutathione**
 Beáta Lizák, Julia Birk, Melinda Zana, Gergely Kosztyi, Alex Odermatt, Miklós Geiszt, Christian Appenzeller-Herzog and Gábor Bánhegyi
- 17:10 O-02 Identification of novel substrates of the evolutionarily conserved PP4 phosphatase to better understand its role in mitosis**
Zoltán Kármán, Zsófia Kormányos, Edit Ábrahám, Lilla Fábri-Ördögh, Katalin Kesserű-Lukács, Margit Pál and Zoltán Lipinszki
- 17:25 O-03 Role of RYBP in balancing between early and late neural processes**
Gergő Kovács, Enikő Sutus, Bertalan Vilmos Takács and Melinda Katalin Pirty
- 17:40 O-04 Functional characterization and gene expression patterns of human adipocytes from the neck support high thermogenic potential in the deep region**
Endre Kristóf, Abhirup Shaw, Rini Arianti, Attila Vámos, Klára Varga, Ferenc Győry, Szilárd Póliska, Beáta Bartáné Tóth, Péter Bai and László Fésüs
- 17:55 Technical break**



Hungarian Molecular Life Sciences 2019

Stem cells / Session Room Liget I.

Chair: András Nagy

16:50	O-05	Treating disease with organoids requires solutions for two major hurdles András Nagy
17:10	O-06	Talpid³ regulates multiple aspects of neuromuscular patterning during gastrointestinal development in animal models and human <u>Nándor Nagy</u> , Jean Marie Delalande and Alan J. Burns
17:25	O-07	Extracellular vesicles transmit epithelial growth factor activity in the intestinal epithelial stem cell niche <u>Ádám Oszvald</u> , Orsolya Gyöngyvér Sándor and Zoltán Wiener
17:40	O-08	Generating new human vascular smooth muscle cells from pluripotent stem cells <u>Annamária Nemes</u> , Mária Husvéth-Tóth, Nikolett Pahor, Béla Merkely and Gábor Földes
17:55		<i>Technical break</i>

Sponsored talks / Congress Hall

18:00	ST-1	Roche Molecular Solutions in life science <u>Ádám Józsa</u> , Anikó Stágel and Zoltán Várhelyi
18:15	ST-2	Fast and gentle 3D superresolution Gert Sonntag
18:30	ST-3	Ion GeneStudio S5 systems: New tools for further NGS pipelines by ThermoFisher Scientific <u>Salvatore Conti</u> , Eszter Tihanyi and Gyula Csanádi
19:00		Welcome dinner (Restaurant of Hotel Park)
20:30		Poster discussion with drinks – Poster session I. (P001 – P114)



Day 2 – Saturday, 30 March 2019

Developmental Genetics / Congress Hall

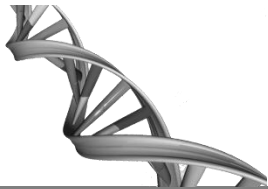
Chair: Rita Sinka

9:00	O-09	Deciphering the nodal role of p38 family of mitogen-activated protein kinases (MAPKs) towards specifying primitive endoderm (PrE) fate in the inner cell mass (ICM) of late-blastocyst stage mouse preimplantation embryo Pablo Bora, Vasanth Thamodaran, Lenka Gahurova (nee Veselovska), Ravishankar Ram Mani, Zbynek Zdrahal, David Potesil and Alexander W. Bruce
9:20	O-10	Analysis of sessile tissue integrity and function in <i>Drosophila melanogaster</i> Viktor Honti , Gergely István Varga, Gábor Csordás, Róbert Márkus, Gyöngyi Cinege, Szilárd Szikora, Ede Migh, Péter Horváth, Éva Kurucz and István Andó
9:35	O-11	Zebrafish as a model for Bloom's syndrome Tamás Annus , Dalma Müller, Bálint Jezsó, Gábor Harami, Máté Varga and Mihály Kovács
9:50	O-12	Changes of mitochondria in <i>Drosophila melanogaster</i> spermatids Viktor Vedelek , Barbara Laurinyecz, Kinga Szilasi, Zoltán Lipinszki, Zsuzsanna Darula, Attila L. Kovács, Gábor Juhász and Rita Sinka
10:05	O-13	Nanoscale reconstruction of flight muscle sarcomeres Szilárd Szikora , Tamás Gajdos, Tibor Novák, Dávid Farkas, Péter Lénárt, Miklós Erdélyi and József Mihály
10:20		Coffee break

Plant biology / Session Room Liget I.

Chair: László Szabados

9:00	O-14	Light control of salt-induced proline accumulation is mediated by ELONGATED HYPOCOTYL 5 in Arabidopsis Dávid Aleksza, Hajnalka Kovács, Abu Imran Baba, Anita Hajdu, Laura Zsigmond, Szilvia Zita Tóth, Anna Mária Király, László Kozma-Bognár and László Szabados
9:20	O-15	SMALL PARAQUAT RESISTANT protein controlling stress responses in higher plants Dóra Faragó , Gábor Rigó, Laura Zsigmond and László Szabados
9:35	O-16	Involvement of RNA binding proteins in plant antiviral responses Károly Fátyol



Day 2 – Saturday, 30 March 2019

Plant biology / Session Room Liget I. (continued)

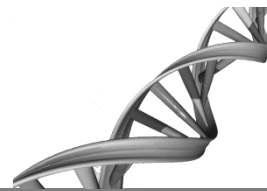
Chair: László Szabados

- | | | |
|-------|------|--|
| 9:50 | O-17 | Ubiquitin protease 12 affects the function of the circadian clock in <i>Arabidopsis thaliana</i>
Anita Hajdu, Ferenc Nagy and <u>László Kozma-Bognár</u> |
| 10:05 | O-18 | The protective effect of ERD14 - the versatility of an intrinsically disordered chaperon
<u>Nikoletta Murvai</u> , Bianka Szalaine Ágoston, Ágnes Tantos, Beáta Szabó, András Láng, András Perczel, Lajos Kalmár, Dénes Kovács and Péter Tompa |
| 10:20 | | Coffee break |

Epigenetics & Regulation of Gene expression / Congress Hall

Chair: Imre Boros

- | | | |
|-------|------|--|
| 11:00 | O-19 | Transposase and host factor determinants of target site selection by DNA transposons
<u>Zoltán Ivics</u> , Lisa Kesselring, Csaba Miskey, Irma Querques, Andreas Gogol-Döring, György Abrusán, Orsolya Barabás and Zsuzsanna Izsvák |
| 11:20 | O-20 | Small ovary (sov): a novel heterochromatin regulator in <i>Drosophila</i>
<u>Ferenc Jankovics</u> , Karam Ibrahim, Melinda Bence, Rita Sinka, László Bodai, Aladár Pettkő-Szandtner and Miklós Erdélyi |
| 11:35 | O-21 | R-loop binding proteins: from chromatin to therapeutic targeting
<u>Lóránt Székvölgyi</u> , Zsolt Karányi, Orsolya Feró, Dénes Nagy, Tibor Csorba and Ágnes Mosolygó-Lukács |
| 11:50 | O-22 | Regulation via PTMs, histone variants and extrachromosomal interactions are all mediated by large changes of nucleosome stability
<u>László Imre</u> , Gergő Kalló, Péter Nánási, Éva Csősz, Kristian Helin, Kerstin Bystricky, Giovanna Lattanzi, Hiroshi Kimura, Juan Ausio, Masahiko Harata and Gábor Szabó |
| 12:05 | O-23 | Ccr4-Not complex is at the core of the gene expression circuitry
<u>Zoltán Villányi</u> , Ishaan Gupta, Sari Kassem, Imre Boros and Martine Collart |



Epigenetics & Regulation of Gene expression / Congress Hall (continued)

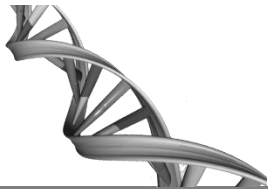
Chair: Imre Boros

12:20	O-24	Germ cells unleashed: role of Rybp in regulating germ cell determinants <u>Izabella Bajusz</u> , Enikő Sutus, Viktória Szabó, Surya Henry, Gergő Kovács and Melinda Katalin Pirity
12:35	O-25	Chromatin level determinants of cellular identity <u>Bálint L. Bálint</u> , Dóra Bojcsuk, Edina Erdős, Noura Faraj, Péter Gargya and Lilla Ozgyin
12:50		Lunch

Protein structure & function / Session Room Liget I.

Chair: László Nyitray

11:00	O-26	Two tales of protein tails: regulation by S100 protein binding and phosphorylation Péter Ecsédi, Bence Kiss, Gergő Gógl and <u>László Nyitray</u>
11:20	O-27	Insights into the structural background of dUTPase inhibition by a proteinaceous inhibitor <u>András Benedek</u> , Fanni Temesváry-Kis, Tamjidmaa Khatanbaatar, Ibolya Leveles, Éva Surányi, Judit Eszter Szabó, Lívius Wunderlich and Beáta G. Vértessy
11:35	O-28	Disordered regions of Mixed Lineage Leukemia 4 (MLL4) protein are capable of RNA binding <u>Ágnes Tantos</u> , Beáta Szabó, Nikoletta Murvai, Rawan Abukhairan, Éva Schád, József Kardos, Bálint Szeder and László Buday
11:50	O-29	Studying the catalytic cycle of Pgp and ABCG2 transporters <u>Katalin Goda</u> , Szabolcs Tarapcsák, Zsuzsanna Gyöngy, Dóra Türk and Gergely Szakács
12:05	O-30	Characterization of a cis-acting protein stabilization motif that functions as a universal STABILON in higher Eukaryotes Margit Pál, Katalin Udvardy, Éva Klement, Róbert L. Katona, Vilmos Tubak, Andor Udvardy and <u>Zoltán Lipinszki</u>
12:20	O-31	Functional redundancy within the S100 protein family <u>Márton András Simon</u> , Gergő Gógl, Péter Ecsédi and László Nyitray
12:35	O-32	Multiscale quantum modeling in biocatalysis: a tool for predicting enzymatic reactivity and selectivity <u>Jitrayut Jitonnorn</u> , Wijitra Meelua
12:50		Lunch



Day 2 – Saturday, 30 March 2019

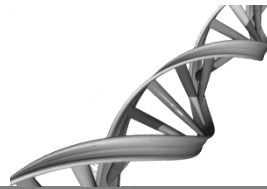
Plenary session / Congress Hall

14:10	PL-3	Nucleoside-modified mRNA therapeutics-developing a new class of drugs Norbert Pardi
14:45	PL-4	Asymmetric movements reveal distinct roles of the two ATP binding sites in the CFTR anion channel Ben Sorum, Beáta Törőcsik and <u>László Csanády</u>
15:20		<i>Technical break</i>

Genome manipulation & archeogenetics / Congress Hall

Chair: Anna Szécsényi-Nagy

15:30	O-33	Downregulating insertional mutagenesis in bacterial genomes using CRISPR-interference Ákos Nyerges, Balázs Bálint, Judit Cseklye, István Nagy, Csaba Pál and <u>Tamás Fehér</u>
15:45	O-34	Haploinsufficiency: mechanisms and evolution <u>Andreea Daraba</u> , Zoltán Farkas, Gábor Boross, Dorottya Kalapis, Karola Almási, Zoltán Bódi, Éva Boros, Gergely Fekete, Ákos Nyerges, Balázs Bálint, László Nagy, István Nagy, Balázs Papp and Csaba Pál
16:00	O-35	Blackjack mutations improve the on-target activities of all increased fidelity variants of SpCas9 <u>Péter István Kulcsár</u> , András Tálas, Eszter Tóth, Zoltán Ligeti and Ervin Welker
16:15	O-36	Genetic investigation of archeological sites in Russia that can be associated with early Hungarians <u>Bea Szeifert</u> , Veronika Csáky, Balázs Stégmár, Dániel Gerber, Balázs Gusztáv Mende, Attila Türk, Balázs Egyed and Anna Szécsényi-Nagy
16:30	O-37	Y-chromosome haplogroups from Hun, Avar and conquering Hungarian nomadic people of the Carpathian Basin <u>Endre Neparáczki</u> , Zoltán Maróti, Tibor Kalmár, Kitti Maár, István Nagy, Dóra Latinovics, Ágnes Kustár, György Pálfi, Erika Molnár, Csilla Balogh, István Raskó and Tibor Török
16:45	O-38	Archaeogenetic evidence on the origin and social organization of the Avar period elite (7-8th centuries AD) <u>Anna Szécsényi-Nagy</u> , Veronika Csáky, Dániel Gerber, István Koncz, Gergely Csiky, Bea Szeifert, Balázs Egyed, Horolma Pamjav, Balázs G. Mende, Choongwon Jeong, Johannes Krause and Tivadar Vida



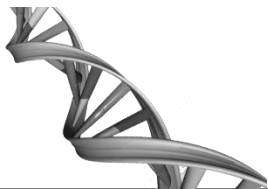
Pathobiochemistry / Session Room Liget I.

Chair: Ferenc Gallyas

- | | | |
|--------------|-------------|--|
| 15:30 | O-39 | <p><i>CARD14</i> gene variants and nuclear factor κB (NFκB) activation in pityriasis rubra pilaris</p> <p><u>Anikó Göblös</u>, Judit Danis, Brigitta Gál, Katalin Farkas, Nikolett Nagy, Lajos Kemény, Zsuzsanna Bata-Csörgő and Márta Széll</p> |
| 15:45 | O-40 | <p>Apc mutation induces extracellular vesicle release from intestinal organoids</p> <p><u>Zsuzsanna Szvicsek</u>, Anikó Zeöld, Andrea Kelemen, Edit I. Buzás and Zoltán Wiener</p> |
| 16:00 | O-41 | <p>Disruption of primary cilia function initiates region-specific craniofacial defects</p> <p>Marek Hampl, Petra Cela Koliskova, Natalia A. Shylo, Petra Nevorankova, Scott D. Weatherbee and <u>Marcela Buchtova</u></p> |
| 16:15 | O-42 | <p>Epigenetic role of ascorbate compartmentalization in human diseases</p> <p><u>Éva Margittai</u>, Zsófia Nemoda, Péter Löw, Pál Szabó, Erzsébet Z. Horváth, Paul J. Coucke, Marina Colombi and Gábor Bánhegyi</p> |
| 16:30 | O-43 | <p>Loss of transglutaminase 2 sensitizes for diet-induced obesity-related inflammation, insulin resistance and hepatic steatosis</p> <p><u>Tibor Sághy</u>, Krisztina Köröskényi, Miklós Antal, Krisztina Hegedűs, Csaba Bankó, Zsolt Bacsó, Attila Papp, Rinke Stienstra and Zsuzsa Szondy</p> |
| 16:45 | O-44 | <p>Pattern recognition receptors and inflammasome activation in cerebral endothelial cells and pericytes</p> <p>Ádám Nyúl-Tóth, Mihály Kozma, Csilla Fazakas, János Haskó, Kinga Molnár, Attila E. Farkas, Attila G. Végh, Imola Wilhelm and <u>István A. Krizbai</u></p> |

17:00	Poster discussion with coffee break – Poster session II. (P115 – P225)
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20:00	Banquet (Congress Hall of Hotel Eger)
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Day 3 – Sunday, 31 March 2019

Systems biology & Bioinformatics / Congress Hall

Chair: Zsuzsanna Dosztányi

9:30	O-45	Cell cycle arrest due to proteostasis decline in aging yeast cells <u>Attila Csikász-Nagy</u> , David F. Moreno, Kirsten Jenkins, Sandrine Morlot, Gilles Charvin and Martí Aldea
9:45	O-46	ChIPSummitDB: a comprehensive database of human transcription factor binding sites and the topological arrangements of transcription factor complexes on the DNA <u>Erik Czipa</u> , Mátyás Schiller, Levente Kontra, Júlia Koller, Orsolya Pálné Szén, Tibor Nagy and Endre Barta
10:00	O-47	Identifying driver regions in hypermutated cancer genomes <u>Borbála Hajdu-Soltész</u> , Bálint Mészáros and Zsuzsanna Dosztányi
10:15	O-48	Linking the resistome to the microbiome in complex microbial communities <u>Lajos Kalmár</u> , Srishti Gupta, Iain R.L. Kean, Xiaoliang Ba, Liz Lay, Ruby Coates, Andrew J. Grant and Mark A. Holmes
10:30	O-49	Fast and sensitive detection of pathogens from high throughput sequencing data <u>Balázs Ligeti</u> , Éva M. Kistóth, Regina Kalcsevszki, Bálint. S. Steller, János Juhász and Sándor Pongor
10:45	O-50	Defining transmembrane regions using cryo-EM maps: the MemBlob database and server <u>Georgina Csizmadia</u> , Bianka Farkas, Eszter Katona, Gábor E. Tusnády and Tamás Hegedűs
11:00		Coffee break

DNA repair & Carcinogenesis / Session Room Liget I.

Chair: Lajos Haracska

9:30	O-51	A new modulator of the synthetic activity of Polymerase eta <u>Éva Bálint</u> , Ildikó Unk
9:45	O-52	Saccharomyces cerevisiae Mgs1 protein contributes to G-quadruplex replication Ágnes Tóth, Gábor Harami, Karola Almási, Enikő Sajben-Nagy, Theresa Schmitz, Qianlu Yang, Katharina Wanzek, Mihály Kovács, Katrin Paeschke and <u>Péter Burkovics</u>



DNA repair & Carcinogenesis / Session Room Liget I. (continued)

Chair: Lajos Haracska

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|--------------|-------------|--|
| 10:00 | O-53 | Capturing the transient profile of uracil accumulation in human genomic DNA by super-resolution microscopy and genome-wide uracil-DNA sequencing analysis
<u>Hajnalka Laura Pálincás</u> , Gergely Róna, Gergely Tihanyi, Lőrinc Pongor, Angéla Békési, Carolina Gemma, Simak Ali, Michael Morten, Eli Rothenberg, Balázs Györfy and Beáta G. Vértessy |
| 10:15 | O-54 | Gene targeting of dUTPase in mice leads to early embryonic lethality
<u>László Hiripi</u> , Hajnalka Laura Pálincás, Gergely Attila Rácz, Zoltán Gál, Orsolya Ivett Hoffmann, Gergely Tihanyi, Gergely Róna, Elen Gócza and Beáta G. Vértessy |
| 10:30 | O-55 | Molecular mechanism of legitimate DNA homology sensing by RecQ helicase
<u>Gábor M. Harami</u> , Yeonee Seol, Keir C. Neuman and Mihály Kovács |
| 10:45 | O-56 | Analysis of the mutagenic effect of platinum-based chemotherapeutic agents in cell line based model system
<u>Bernadett Szikriszt</u> , Ádám Póti and Dávid Szüts |
| 11:00 | | <i>Coffee break</i> |

Proteomics / Congress Hall

Chair: Katalin Medzihradszky

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|--------------|-------------|--|
| 11:30 | O-57 | A neglected post-translational modification: extracellular O-glycosylation
Ádám Pap, Éva Klement, Éva Hunyadi-Gulyás, <u>Katalin F. Medzihradszky</u> and Zsuzsa Darula |
| 11:50 | O-58 | Complex proteomics and metabolomics examination of wound healing markers in glaucoma patients following trabeculectomy
<u>Éva Csősz</u> , Gergő Kalló, Noémi Tóth, Eszter Deák, József Tőzsér and Adrienne Csutak |
| 12:05 | O-59 | Proteomics and genomics studies on pathological and physiological functioning of the nervous system
Katalin Adrienna Kékesi, Botond Penke, Balázs Györfy, Katalin Völgyi, Éva Szegő, József Kardos, András Micsonai, Tamás Janáky, László Drahos, Katalin F. Medzihradszky, Éva Hunyadi-Gulyás, Zsuzsa Darula, James Eberwine, Tamás Bártfai and <u>Gábor Juhász</u> |



Day 3 – Sunday, 31 March 2019

Proteomics / Congress Hall (continued)

Chair: Katalin Medzihradsky

- 12:15 O-60 Analysis of the N-glycosylation of the HeLa cell lysate**
Simon Sugár, András Ács, Ágnes Gömöry, Gábor Tóth, Károly Vékey, László Drahos and Lilla Turiák
- 12:25 O-61 Quantitative proteomics at peak: evaluation of novel approaches in mass spectrometry based proteomics**
Zoltán Szabó, Bella Bruszel, Gábor Kecskeméti and Tamás Janáky

Autophagy and intracellular trafficking / Session Room Liget I.

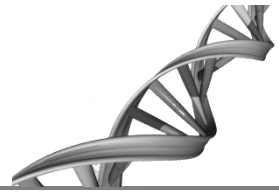
Chair: Tibor Vellai

- 11:30 O-62 The role of the small GTPases Arl8 and Rab7 in crinophagic degradation**
Attila Boda, Tamás Csizmadia, Luca Varga, Péter Lőrincz and Gábor Juhász
- 11:50 O-63 Macrophages engulf apoptotic and primary necrotic thymocytes through similar phosphatidylserine-dependent mechanisms**
Zsófia Budai, László Ujlaky-Nagy, Gréta Nikoletta Kis, Miklós Antal, Csaba Bankó, Zsolt Bacsó, Zsuzsa Szondy and Zsolt Sarang
- 12:05 O-64 Drosophila Sec20 regulates autophagy and endocytosis independently of the Golgi-to-ER retrograde transport**
Zsolt Lakatos, Zoltán Szabó, Péter Lőrincz, Péter Benkő, Lili Kenéz and Gábor Juhász
- 12:15 O-65 Hemocytes regulate selective autophagy during regeneration of germline stem cells in Drosophila**
Tibor Kovács, Virginia B. Varga, Fanni Szikszai, Anna Manzéger, Viktor A. Billes, Janka Szinyákovics, Bernadette Hotzi, Gina Puska and Tibor Vellai
- 12:25 O-66 SNARE protein Ykt6 as swiss army knife in vesicular fusion events**
Gábor Glatz, Zsófia Gyetvai, Tamás Csizmadia and Gábor Juhász

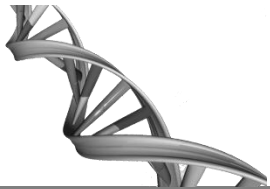
12:40 Closing and award ceremony

13:00 Lunch

Eger, 29-31 March 2019



PLENARY TALKS





PL-1

Engines of carcinogenesis; what determines the speed of mutagenesis?

Lajos Haracska, Lili Hegedűs, Lajos Pintér, Szilvia Juhász, Kata Dudás, Ernő Kiss, Li Qiuzhen, Péter Burkovics and Mónika Mórocz

Institute of Genetics, Biological Research Centre, Szeged, Hungary

Cancer is a genetic disease caused by mutations in the DNA. Why mutations develop with a faster speed during carcinogenesis is an exciting and still open question.

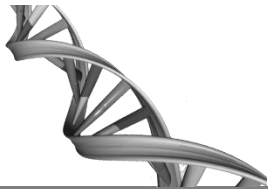
It is known that the genome is constantly exposed to different exogenous and endogenous DNA-damaging factors which, by generating DNA lesions, block the movement of the replication machinery, since the replicative DNA polymerase is unable to insert nucleotides opposite certain DNA lesions. Stalling of the replication fork can lead to strand breaks, chromosomal rearrangements, genome instability, and eventually cancer. To rescue the stalled replication fork, DNA damage tolerance pathways have evolved facilitating mechanisms that help to achieve replication across the lesion. Such DNA damage tolerance pathways are translesion synthesis and template switching. During translesion synthesis, specialized DNA polymerases can insert nucleotides opposite the damaged bases on the template, while in template switching the blocked primer is switched from the damaged template to the newly replicated nascent strand of the sister duplex, which then serves as a template for DNA synthesis.

Our research now focuses on the regulatory mechanisms of the above DNA damage tolerance pathways, by which we aim to reveal the molecular origins of mutagenesis. In particular, we investigate the following questions: What are the molecular mechanisms of chromosomal rearrangements and the formation of point mutations? Why do we observe increased genome instability during carcinogenesis? What is the role of the DNA damage tolerance genes in cancer suppression?

Our recent studies have described new players and pathways of DNA damage tolerance that can bring us closer to answering the above questions. To further characterise these players, we reconstitute DNA lesion bypass using purified proteins and apply single-cell-based methods such as DNA-fiber and comet assays.

In addition to shedding more light on DNA damage tolerance, our research has the potential to discover new tumour suppressors and provide novel cancer therapeutic targets.

Keywords: *mutations, DNA replication, DNA repair, carcinogenesis*



PL-2

Unexpected functions of the actin cytoskeleton in meiotic divisions of oocytes

Péter Lénárt¹, Natalia Wesolowska², Pedro Machado² and Yannick Schwab²

¹ Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

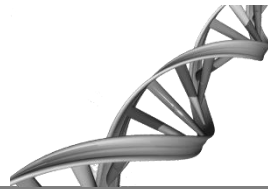
² European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Our general aim is to understand how the cell division machinery adapted to divide the very large oocyte in a very asymmetric manner to produce the fertilizable egg. Intriguingly, while chromosome capture and segregation is largely independent of the actin cytoskeleton in somatic cells, actin apparently has taken on diverse essential functions in mediating these processes in oocyte meiosis, observed in species ranging from *Drosophila* to mammals.

We use starfish oocytes, which are particularly suited for live-cell imaging to systematically characterize the functions of the actin cytoskeleton in early steps of meiosis. We have identified at least three distinct functions of actin each mediated by a specific actin structure: actin is required for nuclear envelope breakdown (NEBD), it is required to transport chromosomes to the forming microtubule spindle, and actin is also responsible for coordinating chromosome capture.

In this presentation, I will focus on the actin-driven mechanism required to break the exceptionally large oocyte nucleus. This involves the assembly of a massive but very transient actin 'shell' under the nuclear envelope mediated by the Arp2/3 nucleator complex. Specifically, by live-cell and super-resolution light microscopy we showed that at NEBD a transient cortex-like F-actin structure assembles within the lamina. This F-actin shell sprouts filopodia-like protrusions forcing apart the lamina and nuclear membranes. By correlated electron microscopy, we visualized that these F-actin spikes protrude pore-free nuclear membranes, while adjoining stretches accumulate packed NPCs. These NPC conglomerates sort into distinct membrane tubules and vesicles, while breaks appear in pore-free regions. We thus propose that the F-actin shell destabilizes the NE by forcing apart the lamina and nuclear membranes.

Keywords: *cell division, actin, nuclear envelope*



PL-3

Nucleoside-modified mRNA therapeutics-developing a new class of drugs

Norbert Pardi

Department of Medicine, University of Pennsylvania, Philadelphia, PA, United States

In vitro transcribed messenger RNA (mRNA) emerged as a potential new drug class to deliver genetic information. Recent technological advances overcame the inherent challenges of mRNA (stability, immune-stimulatory activity, translatability, potency) and mRNA-based therapeutic approaches became highly effective options for cancer and infectious disease vaccine development, protein replacement therapy and genome editing. Dr. Pardi explored the development of a novel, versatile vaccine delivery platform using nucleoside-modified mRNA encapsulated in lipid nanoparticles (LNPs). His talk will highlight some of his recent findings in the fields of infectious diseases mRNA vaccines and delivery of mRNA-encoded therapeutic proteins.



PL-4

Asymmetric movements reveal distinct roles of the two ATP binding sites in the CFTR anion channel

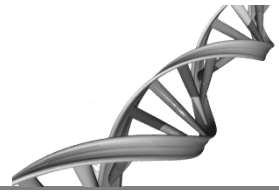
Ben Sorum^{1,2}, Beáta Törőcsik^{1,2} and László Csanády^{1,2}

¹ Semmelweis University, Budapest, Hungary

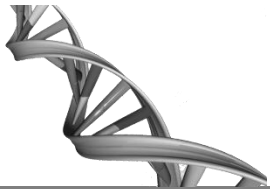
² MTA-SE Momentum Ion Channel Research Group, Budapest, Hungary

Gating of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) anion channel is driven by ATP binding/hydrolysis at two cytosolic nucleotide binding domains (NBDs). The pore opens upon formation of a head-to-tail dimer of ATP-bound NBDs, and closes upon disruption of this dimer following ATP hydrolysis. The tight NBD dimer occludes two ATP molecules in non-equivalent composite binding sites. One site hydrolyzes ATP in each gating cycle, whereas the other is inactive and keeps ATP bound unhydrolyzed. Relative timing of opening-associated motions in a protein position is reported by the slope Φ of its Brønsted plot. Here we report Φ values of multiple positions stretching from the two cytoplasmic ATP sites to the extracellular surface. We find markedly asynchronous motions in the two ATP sites: the active site is already dimerized in the opening transition state, but the degenerate site tightens only on reaching the open state. Thus, in asymmetric ABC proteins, ATP bound within the active site speeds the inward- to outward-facing transition, whereas ATP in the degenerate site prevents reversal to the inward-facing state without ATP hydrolysis, ensuring unidirectional catalytic cycling. Phenylalanine 508 maps to a region that experiences strain, is just on the move in the transition state for opening, and is involved in stabilizing interactions that become stronger in the transition state and stronger still in the open state: loss of those interactions readily explains the gating defect caused by its deletion ($\Delta F508$), the most common cystic fibrosis (CF) mutation. Despite its extracellular location, CF mutation R117H causes a severe gating defect. We identify formation of a strong stabilizing interaction of the R117 side chain as one of the final events during pore opening. That interaction occurs neither in the closed, nor in the opening transition state, and its loss fully explains the striking phenotype of R117H.

Eger, 29-31 March 2019



TANKÓ AWARDS





TA-1

From thermosensor membranes to membrane lipid therapy

László Vigh

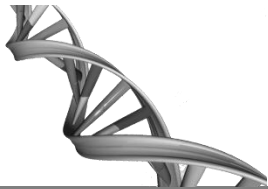
Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Common to all organisms is a highly conserved and exquisitely regulated cellular response to suboptimal physiological stress conditions such as the extreme environmental temperatures. Cells that have been pre-exposed to low or high “priming” temperature can acquire a transient resistance against the killing effect of a subsequent temperature stress.

By introducing the method of catalytic membrane hydrogenation (in collaboration with Ferenc Joó) to biological systems, we have firstly shown that isothermal, *in vivo* lipid saturation-mediated surface membrane rigidification of algal cells is able to stimulate the transcription of specific desaturase genes in the same way, as cooling. In other words, the change in plasma membrane physical state acts as a primary low temperature sensor which regulates some desaturases being key components of membrane remodeling at cooling. In order to extend the validity of the emerging “membrane sensor model” based on cyanobacteria later we have also evidenced, that under heat shock conditions disturbing of the preexisting membrane physical state/microdomain organization can cause the formation and transduction of primary signal(s) that subsequently upregulates the stress protein genes in bacteria, yeast, plants and mammalian cells.

Due to their multiply and vital functions, stress protein molecular chaperones (also called heat shock proteins, HSPs) play a fundamental role in the pathology of several human diseases. Aberrantly high level of certain HSPs is characteristic in cancer cells and the opposite situation applies for type-2 diabetes, neurodegenerative and cardiovascular disorders, etc. In accordance, understanding the mechanism whereby cells can elicit a stress protein response is of key importance. As a “central dogma” earlier it was suggested (also for mammalian cells), that stress-induced protein denaturation serves as a primary stress-sensing machinery which triggers *hsp*-gene expression. The introduction of our new but not exclusive “membrane thermosensor” model firstly pointed to the existence and operation of the membrane-associated stress sensing- and signaling mechanisms. As we explored, changes in the physical state (fluidity) and/or composition of specific lipid molecular species can serve as a “molecular switch” for the operation of “cellular thermometers”.

Correcting the defects of membrane domains, engaged in the generation and transmission of stress signals (“membrane lipid therapy”) may be of paramount importance for the design of new drugs with the ability to induce or attenuate the level of a particular class of heat shock protein molecular chaperones. In fact, some stress protein co-inducer hydroxylamine derivatives (Bimoclomol, BGP-15, or Arimoclomol, which is under “Phase 3 Clinical Trials”), whose function is based on membrane lipid therapy, have enormous potential therapeutic implications.



TA-2

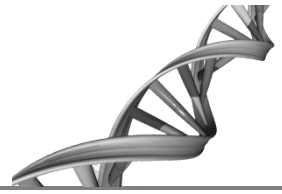
Forty years with protein phosphatases: past, present and future

Ferenc Erdődi

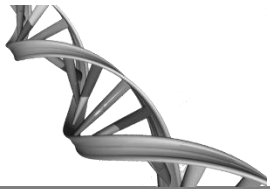
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The discovery that protein phosphorylation on serine (Ser), threonine (Thr) and tyrosine (Tyr) residues mediates numerous cellular processes directed attention toward the regulation of the interconverting enzymes, the phosphorylating protein kinases and the dephosphorylating protein phosphatases. For long, kinases appeared to be the major regulatory enzymes in signal transduction, and phosphatases were almost an afterthought, needed only to make modification by phosphorylation reversible. From the beginning of the 21st century, protein phosphatases have been recognized as the „winners” of the competition between kinases and phosphatases as major signaling proteins. It is believed that phosphatases may control both the time it takes a signal to travel from the cell surface to its intracellular target and the duration of the signal. In addition, debates about whether protein phosphatases are “drugable” or not, have taken positive turns attracting further attention to these enzyme families as possible drug targets. This presentation is designed to summarize the major events in protein phosphatase research in the past forty years via showing also the author’s and his research group’s achievements on this interesting field. The focus is on the roles of the phospho-Ser/Thr specific protein phosphatase-1, -2A and -2B types in the regulation of distinct cellular processes. Attempts will also be made to predict future research directions on this field.

Keywords: *protein phosphorylation, protein kinases, protein phosphatases, cellular regulation*



SPONSORED TALKS (COMPANIES)





ST-1

Roche Molecular Solutions in life science

Ádám Józsa, Anikó Stágel and Zoltán Várhelyi

Roche Diagnostics, Budaörs, Hungary

Roche is as pioneer in molecular diagnostics as in life science. Since 1997, when Roche acquired Boehringer Mannheim become one of the market leader on the field of molecular biology. Thanks to our wide range of products, services and solutions we are able to cover the needs of different types of applications worldwide. Roche provides solutions for areas such as oncology, genomics and microbiology. Roche also provides state-of-the-art PCR and real-time PCR-based nucleic acid tests. Our systems are designed to improve workflow efficiencies, providing modular and automated solutions for labs of all types and sizes.

Our product portfolio covers the area from nucleic acid isolation to sample detection with different throughput capabilities to serve the customer needs. We also provide solutions for target region enrichment for sequencing. We offer fully automated systems and also manual solutions for nucleic acid purification to achieve the best results.

The MagNa Pure systems are designed to reduce the hands-on time of nucleic acid isolation at a high level of quality due to the magnetic isolation technique. The same quality can be achieved by manual isolation using our wide range of nucleic acid purification kits.

Our qPCR instruments are designed with great solidity to receive the most accurate results for diagnostic as well as for research purposes. The Roche LightCycler® Systems are the hallmark of real-time PCR Systems in terms of accuracy, flexibility and performance. Performance in temperature homogeneity, fast ramping rates, and reproducibility allows labs to generate consistent quality results they can trust. The throughput of the product portfolio is designed for labs of all sizes. This ideal combination of accuracy, temperature homogeneity, and reproducibility is matched with powerful software so intuitive that it is accessible to any user.

Keywords: *qPCR, nucleic acid isolation, life science*



ST-2

Fast and gentle 3D superresolution

Gert Sonntag

Carl Zeiss Microscopy GmbH, Jena, Germany

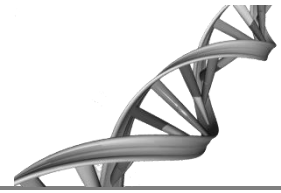
Life sciences research often requires you to measure, quantify and understand the finest details and sub-cellular structures of your sample. You may be working with tissue, bacteria, organoids, neurons, living or fixed cells and many different labels. Elyra 7 takes you beyond the diffraction limit of conventional microscopy to image your samples with superresolution. You can examine the fastest processes in living samples – in large fields of view, in 3D, over long time periods, and with multiple colors.

The new Lattice SIM technology of your Elyra 7 brings structured illumination microscopy (SIM) to a new level. Groundbreaking light efficiency gives you gentle superresolution imaging with incredibly high speed – at 255 fps you will get your data faster than ever before.

Elyra 7 lets you combine Lattice SIM with single molecule localization microscopy (SMLM) for techniques such as PALM, dSTORM and PAINT. You can now choose freely among your labels when imaging with resolution down to 20 nm laterally and 50 nm axially. High power laser lines allow you to image your sample with ease, from green to far red.

Elyra 7 is also very flexible: you can employ a wealth of contrasting techniques and combine them with optical sectioning. The new Apotome mode gives you superfast optical sectioning of your 3D samples. All that, plus Elyra 7 works seamlessly with your ZEISS SEMs in a correlative workflow.

This lecture will focus on all the benefits coming together with Lattice SIM technology for all kinds of very sensitive living samples combining superfast and superresolution imaging capabilities the first time win a single instrument.



ST-3

Ion GeneStudio S5 systems: New tools for further NGS pipelines by ThermoFisher Scientific

Salvatore Conti¹, Eszter Tihanyi² and Gyula Csanádi³

¹ NGS Business Developer, South Europe and V3, Thermo Fisher Scientific

² Genetic Analysis systems sales representative, Hungary, Thermo Fisher Scientific

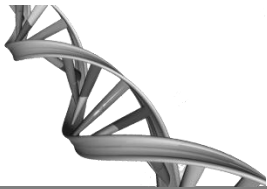
³ QPCR technical sales representative, V4, Thermo Fisher Scientific

The next-generation sequencing (NGS) technologies are revolutionary tools which have made possible achieving remarkable advances in genetics thanks to even the parallel increase of the large applications of NGS protocols allowing the genetic decoding of biological systems through studies of genome anatomy and gene mapping, coupled to the transcriptome pictures. Today Thermo Fisher Scientific provides the new Ion Torrent GeneStudio S5 System (with Ion Chef) in order to speed up the NGS applications for Human Genetics, Oncology, Metagenomics and Animal breeding (Figure 1).



Figure 1: Ion GeneStudio S5

The GeneStudio S5 allows users to apply a lot of NGS pipelines such as (1) genomic sequencing by whole-genome shotgun strategies; (2) Targeted gene sequencing for human and other genomes; (3) metagenomics of large bacterial communities (4) and RNA-seq methods including whole transcriptome and targeted sequencing. Recently, the availability of advanced protocols such as the AmpliSeq™ (Thermo Fisher Scientific) targeted technology has allowed to overcome the usual sequencing technical issues related to low coverage uniformity and specificity over the standard library preparation methods. The new Ion AmpliSeq HD technology is a new library amplification technology for Ion Torrent that gives user the power to design their own gene panels and find variants with very low limits of detection—down to 0.1% for cell-free DNA samples. This can be used to find low-frequency alleles in circulating tumor DNA, within DNA or RNA samples extracted from

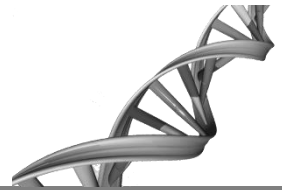


formalin-fixed paraffin-embedded (FFPE) tissue, or to find rare microbial species or resistant strains.

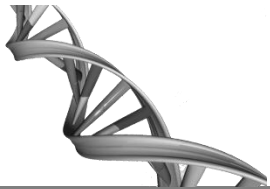
For any hypothesis free experiment can be validated with our Real-Time PCR instruments, the QuantStudio family from 1 to 12KF. The whole needed reagent portfolio for QPCR runs are also provided by Thermo Fisher Scientific.

Keywords: *NGS¹, GeneStudio S5², Applications³*

Eger, 29-31 March 2019



ORAL LECTURES





O-01

Ca²⁺ mobilization dependent reduction of the ER lumen is due to influx of cytosolic glutathione

Beáta Lizák¹, Julia Birk², Melinda Zana^{3,4}, Gergely Kosztyi¹, Alex Odermatt², Miklós Geiszt^{3,4}, Christian Appenzeller-Herzog^{2,5} and **Gábor Bánhegyi**^{1,6}

¹ Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary

² Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland

³ Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest

⁴ "Momentum" Peroxidase Enzyme Research Group of the Semmelweis University and the Hungarian Academy of Sciences, Budapest, Hungary

⁵ University Medical Library, Basel, Switzerland

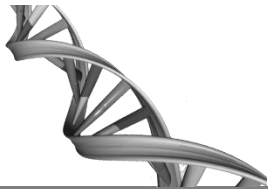
⁶ Pathobiochemistry Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

Depletion of luminal Ca²⁺ from the endoplasmic reticulum (ER) provokes a rapid and reversible shift towards a more reducing poise, but the molecular basis for this shift is currently unclear.

We found that the Ca²⁺ mobilization-dependent reduction was sensitive to the inhibition of glutathione synthesis or the dilution of cytosolic glutathione by selective permeabilization of the plasma membrane. A glutathione-centered mechanism was further indicated by a sharp increase in ER-luminal glutathione levels that occurred concomitant to Ca²⁺ efflux. We also show that inducible reduction of the ER lumen by glutathione is independent of the Ca²⁺-binding chaperone calreticulin, which has previously been implicated in this process. However, opening the translocon channel by puromycin or the addition of cyclosporine A mimicked the glutathione-related effect of Ca²⁺ mobilization. While the action of puromycin was ascribable to a Ca²⁺ mobilizing mechanism, the mechanism of cyclosporine A-induced influx of glutathione was independent of calcineurin and cyclophilin A and B and remained unclear.

We conclude that the influx of cytosolic glutathione rather than the inhibition of local oxidoreductases is responsible for the reductive shift upon Ca²⁺ mobilization and postulate the existence of a Ca²⁺- and cyclosporine A-sensitive glutathione transporter in the ER membrane.

Keywords: endoplasmic reticulum, redox, glutathione, translocon, cyclosporine A, cyclophilin, calreticulin



O-02

Identification of novel substrates of the evolutionarily conserved PP4 phosphatase to better understand its role in mitosis

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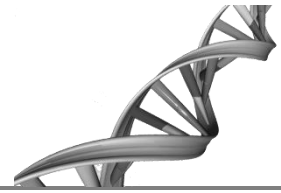
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Reversible protein phosphorylation governed by the antagonistic activity of protein kinases and phosphatases (PPases) is an elegant way of regulating the function of different protein complexes to ensure proper cell division in Eukaryotes. While the role of kinases in this process is well established, the molecular details of how PPases reverse this activity is not fully understood, mainly because most of the substrates are not known. Our group aims to identify and characterise novel mitotic substrates and regulators of the essential, but poorly-known Ser/Thrprotein phosphatases and explore how dephosphorylation of substrates contributes to cell division. We focus on the fruit fly Protein Phosphatase 4 (PP4), a member of the PP2A-type Ser/Thr PPases, which is an evolutionarily conserved and essential heterotrimeric enzyme with an important, but relatively unexplored role in mitosis.

Applying proteomics and biochemical approaches, we have identified more than 60 putative mitotic targets of PP4. Using the N-terminal EVH1 domain of the regulatory R3 subunit of PP4 as bait that has been shown to be responsible for substrate recognition and binding, we identified and validated 12 direct interacting partners of the enzyme. This includes centrosomal, centromeric, kinetochore and spindle assembly checkpoint-related proteins. Interestingly, we also found interacting partners bound exclusively to the SMK1domain of Falafel that lies downstream to the EVH1 and so far its role has been unknown. Further investigation of the newly identified interacting partners and the examination of how the SMK1 domain takes part in substrate recognition would help us to better understand the role of the PP4holoenzyme in mitosis.

This work was supported by grants from The National Research, Development and Innovation Office (OTKA-PD115404), Ministry for National Economy of Hungary (GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00032) and Hungarian Academy of Sciences (Bolyai Fellowship (bo_329_15)) and Lendület Program Grant (LP2017-7/2017).

**O-03****Role of RYBP in balancing between early and late neural processes****Gergő Kovács**, Enikő Sutus, Bertalan Vilmos Takács and Melinda Katalin Pirty*Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary*

RING1 and YY1 Binding Protein (RYBP) is a core member of the non-canonical type-1 polycomb repressive complexes (ncPRC1s) and several other multimeric protein complexes (MPCs) with essential role in embryonic development. Mice lacking functional RYBP die at the time of implantation and a portion of heterozygotes harboring only one functional *Rybp* allele exhibit serious developmental defects of the central nervous system (CNS). Besides that, the complete absence of *Rybp* in mouse embryonic stem (ES) cells causes accelerated neural stem cell generation and dramatic decrease in the formation of mature neurons.

We used ES cell based *in vitro* neural differentiation model system to uncover underlying molecular events of the RYBP action during neural lineage specification. We performed label-free pulldown of endogenous RYBP from nuclear extracts of pluripotent ES cells, early stage and terminal stage of neural cultures, and analyzed the hits with mass spectrometry (MS).

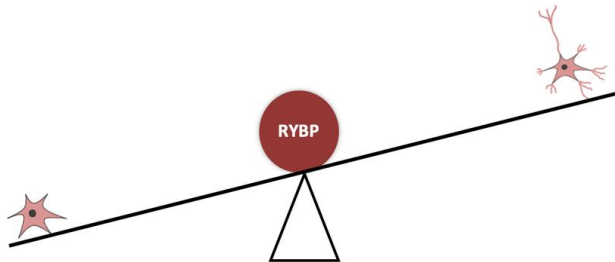
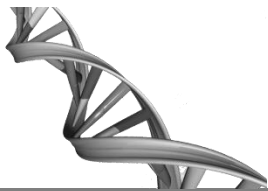


Figure 1: RYBP has important role in balancing between early and late neural processes

We found that RYBP is present more abundantly at the beginning of *in vitro* neurogenesis and its level decreasing afterwards. Our MS results indicate that in ES cells RYBP interacts with the ncPRC1.1, ncPRC1.2 and ncPRC1.6 complexes. Our other finding was that extra copy of RYBP causes de-repression of pluripotency genes, resulting impaired neural differentiation. These results shed light on that high level of RYBP plays important role in maintaining pluripotency. Later, during differentiation processes RYBP interacts with the members of the retinoic acid signalling pathway, the members of the PRC2 and npBAF/nBAF complexes. The composition change between npBAF and nBAF complexes plays important role in commitment of neural progenitors to mature neurons. These results show that the altered assembly of MPCs can shift the finely tuned balance of transcription



factors governing neural progenitor pool generation and maturation, which can obscure the process of neural differentiation in the lack of RYBP.

These findings broaden our knowledge about how RYBP balances between early and terminally differentiated stages of neurogenesis and have implications for understanding neural lineage commitment, learning, memory and neurological diseases.

This work was supported by GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00039.

Keywords: *Rybp, polycomb, neurogenesis, pluripotency, BAF complex*

O-04

Functional characterization and gene expression patterns of human adipocytes from the neck support high thermogenic potential in the deep region

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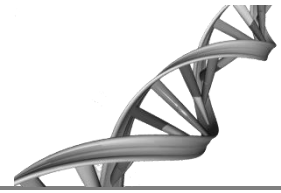
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Thermogenic brown adipose tissue depots amounted to 1.5% of total body mass and are mostly enriched in the supraclavicular, deep neck (DN) and paravertebral regions. There are at least two types of thermogenic adipocytes, classical brown and beige, which have different origins and distribution. Their thermogenic capacity is mainly dependent on the uncoupling protein 1 (UCP1) activated in response to a β -adrenergic stimulus.

We intended to characterize differentiated adipocytes from DN and cervical subcutaneous (SCN) human adipose tissue depots. Therefore, preadipocytes of the same donor obtained from the two sites were differentiated into white and beige adipocytes with or without irisin or BMP7. Global gene expression analysis was performed by RNA-sequencing. Oxygen consumption and extracellular acidification were measured using an XF96 oxymeter.

Majority of the beige markers, including UCP1, were upregulated in DN as compared to SCN adipocytes, indicating the large potential of thermogenesis in the deep depots. Adipocytes differentiated from progenitors that were isolated from DN fat showed elevated mitochondrial respiration and extracellular acidification compared to SCN adipocytes. However, oxygen consumption and acidification did not increase in response to irisin and BMP7 in both types of adipocytes. In contrast to abdominal subcutaneous adipocytes, irisin and BMP7 induced distinct expression programs, unrelated to thermogenesis. More than 20 genes, expressed at a higher extent in DN compared to SCN adipocytes and clustered closely



to UCP1, were identified with unknown role in thermogenesis. To find out if they directly regulate browning, specific experiments have been designed for the near future.

The work is supported by the GINOP-2.3.2-15-2016-00006 and ÚNKP-18-4 projects.

Keywords: *obesity, adipocyte browning, mitochondria*

O-05

Treating disease with organoids requires solutions for two major hurdles

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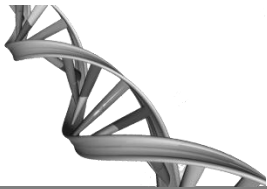
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Organoids have proven to be powerful tools for studying organ development and disease processes, evaluating disease treatments and treating illness by transplantation into patients. The most obvious example of such a promise is the pancreatic islet organoids faithfully responding to blood glucose levels with insulin secretion and liver organoids that rescue liver failure. Here I report solutions for the two major hurdles faced for the safe and long-term-tolerated allogeneic organoid products. We provide a definition and quantitation of safety as calculating the odds of accidentally generating potentially dangerous therapeutic cells and ameliorate these odds through gene-editing. Furthermore, the results of a proof-of-principle experiment will be shown to cure diabetes in an animal model.

Keywords: *organoids, safe cells, diabetes mellitus*



O-06

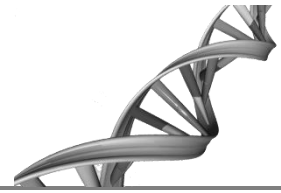
Talpid³ regulates multiple aspects of neuromuscular patterning during gastrointestinal development in animal models and human

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Talpid³ is an evolutionary conserved protein localized at the centrosome, which plays a critical role for the coordination of protein trafficking, in particular ciliary proteins. Although Talpid³ has been recognized to have essential functions during embryonic development, its role during gastrointestinal (GI) and enteric nervous system (ENS) development has not been well studied. To address this, we analysed chicken and mouse embryonic GI tracts bearing Talpid³ mutation, as well as human GI tissues obtained from a fetus with Joubert syndrome. Results showed that the GI tract of Talpid³ chicken embryos was reduced in length and severely malformed, with tracheoesophageal fistula or atresia and open hindgut. Gut smooth muscle was mispatterned and was not arranged in concentric layers. Likewise, enteric neural crest cells (ENCC) were scattered throughout the gut wall, rather than arranged in the presumptive plexuses of the ENS. Analysis of the Hedgehog (Hh) signalling pathway showed altered expression of the Hedgehog ligand patched (*Ptc1*) underlying the smooth muscle defects. Analysis of the gut extracellular matrix suggested that deficient ENCC repellent cues contributes to the ENS defects. To specifically assess the cell autonomous requirement of Talpid³ during ENS development, we used intra-species neural tube grafting between Talpid³ and GFP expressing chick embryos. Analysis of the chimeric embryos demonstrated that patterning of the ENS does not require Talpid³, but is dependent on correct environmental cues. Interestingly, although the lack of Talpid³ in vagal ENCC did not affect the gross morphology of the ENS, it affected smooth muscle and epithelial morphology in a non cell-autonomous manner. In accordance with these results, a mouse model where Talpid³ was mutated conditionally in NCC also showed grossly normal ENS but smooth muscle and epithelium defects. Lastly, gut defects observed in animal models were strikingly similar to defects observed in human gut fetal tissues with Joubert syndrome, bearing a homozygous null mutation in KIAA0586, the human ortholog of chicken Talpid³. Overall, our results reveal an evolutionary conserved Talpid³-dependent mechanism essential for neuromuscular patterning and neuronal-mesenchymal-epithelial interactions in the developing GI tract.



O-07

Extracellular vesicles transmit epithelial growth factor activity in the intestinal epithelial stem cell niche

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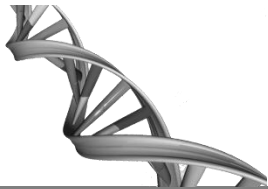
The intestinal epithelium is continuously renewing by a proliferating stem cell population, residing in the bottom of the intestinal crypts. The intestinal stem cells (ISC) critically depend on niche factors, secreted into the surrounding microenvironment by intestinal fibroblasts or Paneth cells. Recently, the three-dimensional organoid culture technology has emerged as a novel, cutting edge tool. Stem cell containing murine small intestinal crypts form self-organizing organoids called „miniguts” after embedded into 3D matrix and administered with the critical stem cell niche factors epidermal growth factor (EGF), the Wnt agonist R-spondin and the Bmp pathway inhibitor noggin. In case of colon organoids, Wnt3a must be added as a niche factor as well.

Extracellular vesicles (EV) are membrane-enclosed vesicles secreted by virtually all cell types. The exosomes are the smallest and so far best characterized EV subtype, released from multi-vesicular bodies of the endosomal compartment. Although understanding factors that critically contribute to the ISC niche is central for regenerative medicine, the effect of EVs on this process is unknown.

Here we provide evidence that both mouse and human intestinal fibroblasts secrete EVs. By using small intestinal and colon organoids and the Lgr5-EGFP-IRES-CreER mouse model that had been developed to visualize the EGFP+ ISCs, we found that fibroblast-derived EVs do not increase the number of ISCs when all niche factors are present. Furthermore, EVs do not modify the effect of cytokines acting in the ISC niche. As expected, we observed a massive organoid death when R-Spondin1, noggin or EGF were removed from the culture medium one-by-one. Strikingly, fibroblast-derived EVs could rescue organoid death in the absence of EGF, but they had no effect when either R-Spondin1 or noggin lacked. Importantly, fibroblasts express a wide range of EGF family members and we found that at least one of these molecules, amphiregulin is transported via EVs. Collectively, we provide evidence that fibroblast-derived EVs are key players in forming the ISC niche by transmitting EGF activity.

This work was funded by the International Centre for Genetic Engineering and Biotechnology (Italy, CRP/HUN16-04_EC) and by the National Excellence Program in Higher Education (Hungary).

Keywords: extracellular vesicles, EGF, organoids, stem cell



O-08

Generating new human vascular smooth muscle cells from pluripotent stem cells

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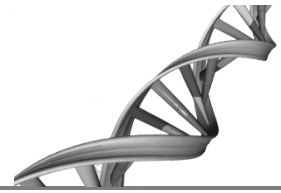
Smooth muscle cells (SMCs) have an important role in physiological regulation of the vascular system and cardiovascular diseases such as atherosclerosis and hypertension. The aim of this project was to develop a feasible and robust SMC differentiation protocol for *in vitro* disease modelling as well as for tissue engineering and further developing cell therapy applications.

In our laboratory we used human induced pluripotent stem cells (hiPSC, IMR90-4 line) for the differentiation. Cells were cultured in 2D cell culture system in 24 well-plates and under standard culture conditions (37  C, 5% CO₂). The starting cell number was 10⁵ cells/cm²; we expanded cells for two days in stem cell medium. For the differentiation stage, we used glucose-based RPMI 1640 medium was used, in the presence of supplemental growth factors (VEGF-A, FGF2) and B27-supplement. Upon the start of the differentiation contractile subgroup of SMC, human PDGF-BB and TGF   proteins were added to the basal medium.

We measured changes in the expressions of SMC markers (CNN1, ACTA2) during differentiation period and in hiPSC-derived SMC. As assessed by real time PCR, mRNA levels of SMC markers were markedly upregulated by the end of the differentiation (ACTA2: day 14 vs. day 0: 970x, $p < 0.0001$, $n = 5$; CNN1: 6.2x, $p < 0.01$, $n = 5$), reaching a similar expression to those in mature human aortic SMC. We performed immunocytochemistry for   -SMA antibody and analysed the fluorescent images by ImageJ software. We found that 32% of the cell culture was stained positive for SMC specific   -SMA. In our pilot experiment also suggested that differentiated contractile SMCs were able to contract in response to carbachol.

Human iPSCs can be utilised as unlimited source for new SMCs differentiation. Our further goal is therefore to generate immature SMC cultures which are much more proliferative than those of mature contractile SMCs. These cells can be used for future scale-up for cell therapy.

Keywords: stem cell, smooth muscle cell, differentiation



O-09

Deciphering the nodal role of p38 family of mitogen-activated protein kinases (MAPKs) towards specifying primitive endoderm (PrE) fate in the inner cell mass (ICM) of late-blastocyst stage mouse preimplantation embryo

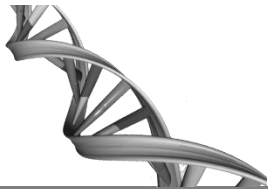
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p38 MAPKs are classically defined as the effectors of stress-induced signalling pathways but are also associated with cell cycle regulation, survival, differentiation, senescence, development, tumorigenesis and immune responses, among others. In the preimplantation mouse embryo, inhibition of p38 MAPKs from the 2-cell stage has been reported to cause reversible loss of filamentous actin and arrested development at the 8- and 16-cell stages. Inhibition at later stages (post-8-cell stage) has been associated with cavitation and hatching defects, partly associated with impaired fibroblast growth factor (Fgf) signalling and the abnormal expression and localisation of TJ Protein 1, Na/K-ATPase and Aquaporins within the trophectoderm (TE). Recently, our lab has identified p38 α/β as occupying a nodal position in the differentiation of inner-cell-mass (ICM) cells, under the control of Fgf-signalling, towards primitive endoderm (PrE) fate between E3.5 to E4.0 in the mouse blastocyst. Here we report the resolution of a short temporal window of p38 function in this process, having identified a minimal ~3 hour window of required p38 activity to initiate PrE differentiation. Furthermore, to identify which one, or combination of the four, expressed p38 paralogue(s) (i.e. p38 α , p38 β , p38 γ and p38 δ) are required, we are utilising the PiggyBAC cloning strategy to express both dominant negative and constitutively-active mutant constructs of each p38 paralogue, in a doxycycline inducible manner. Additionally we report preliminary data/ findings aimed at identifying downstream protein substrate effectors of p38 function, using a combination of phospho-proteomic mass spectrometry and transcriptomic mRNA-Seq screens of p38 α/β inhibited blastocysts. Collectively, these approaches are beginning to elucidate the mechanistic action of p38 function during the specification of PrE in the mouse blastocyst ICM.



O-10

Analysis of sessile tissue integrity and function in *Drosophila melanogaster*

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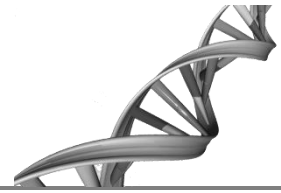
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In the *Drosophila* larva, three hemocyte compartments were described: the lymph gland, the sessile tissue and the circulation. Although regulatory networks of hematopoiesis are well characterized in the lymph gland, less information is available on the factors that maintain the integrity and ensure the function of the sessile tissue. Wounding of the larva leads to the differentiation of a specific cell type, the lamellocyte, which encapsulates the potential invader. The sessile tissue was shown to serve as a pool of phagocytic plasmatocytes, precursors for lamellocyte development, and is assumed to be disintegrating upon immune induction, thus contributing to the effector hemocyte pool.

To understand the connection between sessile tissue integrity and function, we analyzed hemocyte motility with *in vivo* confocal video microscopy. We found that wounding does not lead to the disintegration of the sessile tissue. Sessile tissue integrity was also kept in tumorous larva, in which lamellocytes differentiate vigorously. However, lamellocytes did not differentiate in *eater* mutant larvae, in which the sessile tissue is completely disintegrated. These data suggest that the integrity and function of the sessile tissue are regulated by genetically distinct elements, and that the sessile tissue is not a *bona fide* blood cell niche.

We used a combination of CoinFLP and UAS-transgenes for labelling hemocyte clones and to identify signal transduction pathways that regulate sessile tissue integrity either in a cell-autonomous or a non-cell-autonomous manner. We established a novel method which enables culturing hemocytes *ex vivo*. Our preliminary data suggest that lamellocyte differentiation requires active signalization after the wounding of the larva. We used the expression of hemocyte type specific *in vivo* transgenes in combination with machine learning to identify intermediate cell types, and found that the intermediate stages of transdifferentiation can be pinned down. This approach can serve as a basis for the identification of key factors instrumental in plasmatocyte-lamellocyte transition and lead to a better understanding of macrophage plasticity, in general.

Our work is supported by OTKA NK-101730 (LA), PD-115534 (VH) and GINOP-2.3.2-15-2016-00001 grants. The research of V. Honti and G. I. Varga was supported by the European



Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

Keywords: blood cell, differentiation, hematopoiesis, *Drosophila*

O-11

Zebrafish as a model for Bloom's syndrome

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Also known as the “guardians of the genome”, RecQ helicases play crucial roles in genome integrity maintenance through their involvement in various DNA metabolic pathways. Aside from being conserved from bacteria to vertebrates, their importance is also reflected in the fact that in humans impaired function of certain RecQ helicase orthologs are known to cause severe sets of symptoms such as Bloom's, Werner's and Rothmund-Thomson syndromes. Zebrafish is a promising model organism for the study of RecQ helicases, as all five human paralogs have single zebrafish orthologs.

In order to gain a better insight into the specific roles of RecQ helicases in the genome maintenance of vertebrates, we create and characterize zebrafish models for mutations in the five RecQ helicase genes (*recql*, *blm*, *wrn*, *recql4* and *recql5*). The first such gene we have examined more closely is *blm*. As mentioned above, humans lacking BLM exhibit Bloom's syndrome, an autosomal disorder characterized by short stature, skin rashes, reduced fertility, increased risk of carcinogenesis and shortened life expectancy caused by genomic instability. To determine whether zebrafish is indeed a viable model for Bloom's syndrome we analyzed lifespan, fertility, histology and DNA repair efficiency in our mutant strain.

Our results show that our model recapitulates major hallmarks of the human disease. Moreover, some functions of zebrafish Blm bear additional importance in germ line development. Therefore, our model will be a valuable tool for further understanding the developmental and molecular attributes of this rare disease, along with providing novel insights into the role of genome maintenance proteins in somatic DNA repair and fertility.

Keywords: RecQ, Bloom's syndrome, BLM, genome integrity, aging, cancer



O-12

Changes of mitochondria in *Drosophila melanogaster* spermatids

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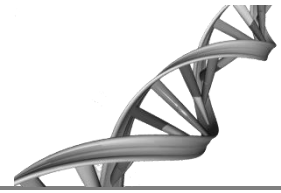
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Drosophila melanogaster sperm reach an extraordinary long size, 1.8 mm, at the end of spermatogenesis. *Drosophila* sperm tail contains two mitochondrial derivatives and both derivatives run along the entire length of the sperm. The mitochondrial derivatives have an important role in mediating the elongation of developing cysts during spermatogenesis. These mitochondria are unique because their structure hardly resembles the classical mitochondrion structure. The two mitochondrial derivatives differentiate and by the end of spermatogenesis the minor one reduces its size and the major one accumulates paracrystalline material inside it. The extremely large mitochondrion is thought to provide structural rigidity for flagellar movement, but its precise function and organization is incompletely understood. Also the molecular constituents and precise function of the paracrystalline material have not yet been revealed. We purified paracrystalline material from mature sperm and identified S-Lap proteins as major protein components of it. We characterized several male sterile mutant alleles of S-Lap genes and provide evidence that the function of S-Lap genes is not redundant and they are necessary for the formation of normal paracrystalline material. Our results can provide a better understanding, how the mitochondrial derivatives develop and perform their function in the late stages of spermatogenesis and in the fully developed sperm.

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O-13

Nanoscale reconstruction of flight muscle sarcomeres

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During muscle development, actin and myosin containing filaments assemble into highly organized, contractile units, known as sarcomeres. The actin and myosin filaments are assembled into parallel bundles, organized by two transverse structures, the Z-discs and M-bands. Despite their fundamental significance, understanding the molecular mechanisms underlying their assembly and maturation remains one of the major unresolved issues of the field. Elucidating the precise molecular architecture of sarcomeric proteins is indispensable for understanding these processes. However, determining their accurate localization is not possible with conventional fluorescence microscopy, since the resolution of optical microscopes is limited by the physical properties of light. To circumvent this obstacle, we used a nanoscopic approach to visualize the protein distribution in sub-diffraction sized compartments of *Drosophila* indirect flight muscle sarcomeres, i.e. in their H-zone and I-band. We collected dSTORM (direct stochastic optical reconstruction microscopy) images of about 9000 sarcomeres and developed a new software tool to determine the precise position of 15 epitopes in the H-zone and 20 epitopes in the I-band. We combined this comprehensive localization information with the results of former structural, biochemical and genetics studies to compile a sarcomere model with a previously unprecedented scope and resolution.

Keywords: *super-resolution microscopy, sarcomere, cytoskeleton*



O-14

Light control of salt-induced proline accumulation is mediated by ELONGATED HYPOCOTYL 5 in Arabidopsis

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Plants have to adapt their metabolism to constantly changing environmental conditions, among which the availability of light and water is crucial in determining growth and development. Proline accumulation is one of the sensitive metabolic responses to extreme conditions; it is accumulated during drought, upon exposure to salt and is regulated by light. The Δ^1 -pyrroline-5-carboxylate synthase (P5CS) and the proline dehydrogenase (PDH) enzymes regulate the biosynthetic and catabolic pathways, respectively. In Arabidopsis both enzymes are encoded duplicated genes. The *P5CS1* gene is induced by drought and salt stress and upregulated by ABA and light, while the *PDH1* gene is repressed by osmotic stress and light.

Here we show that red and blue but not far-red light is essential for salt-induced proline accumulation, upregulation of *P5CS1* and downregulation of *PDH1* genes, which control proline biosynthetic and catabolic pathways, respectively. We demonstrated that the transcription factor ELONGATED HYPOCOTYL 5 (HY5) binds to conserved G-box and C-box elements of both *P5CS1* and *PDH1* genes. Salt-induced proline accumulation and *P5CS1* expression are reduced in the *hy5hyb* double mutant, suggesting that HY5 connects light and stress signals in promoting proline biosynthesis. Our results shed light to previously unknown interactions between stress, ABA and light signals, with HY5 as key regulator in proline metabolism.

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Keywords: ELONGATED HYPOCOTYL 5, proline accumulation, Arabidopsis, light signalling, gene expression regulation



O-15

SMALL PARAQUAT RESISTANT protein controlling stress responses in higher plants

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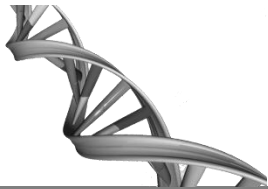
Small proteins in plants can regulate many physiological activities, including cell-to-cell communications, and plant defenses. Cell-to-cell communication is essential for the plant growth and cell differentiation as well as in controlling responses to biotic and abiotic stimuli. Extremophile plants are valuable sources of genes conferring tolerance traits, which can be explored to improve stress tolerance of crops.

Previously we have isolated several cDNA clones from the halophytic plant *Lepidium crassifolium* a relative of the model plant *Arabidopsis thaliana*, which could enhance tolerance to salt, osmotic or oxidative stresses (Rigó et al., 2016). Overexpression of one of these cDNAs rendered remarkable tolerance to paraquat and was therefore named SMALL PARAQUAT RESISTANT (SPQ). The full length cDNA encoded a small protein (69 amino acid), with closest similarity to the predicted gene product of *AT3G52105* (92% identity) of *Arabidopsis*. Similar small proteins are present in many plant species, information about their function is however missing. Structure predictions suggest presence of an N-terminal signal peptide and a SCOP:d1e4mm domain. Sequence similarities were found with plant defensins implicated in material transport, pathogen and hormone response and development.

Overexpression of the *Lepidium* or *Arabidopsis* SPQ (LcSPQ, AtSPQ, respectively) in *Arabidopsis* conferred similar degree of paraquat tolerance. Transgenic plants exhibited increased tolerance to drought stress and displayed higher electron transport rate (ETR) than wild type plants during dehydration. The knockout *spq1* T-DNA insertion mutant had elongated petiole and longer hypocotyl when compared to the wild-type plants. Moreover, the *spq1* mutant was insensitive to ABA *in vitro* growth assays, while LcSPQ and AtSPQ overexpressing plants had slightly enhanced sensitivity to ABA. Our results suggest, that the previously unknown SPQ genes have multiple functions in higher plants, regulate plant development, modulate responses to herbicides and ABA. Evaluation of biotechnological potential of the *Lepidium* and *Arabidopsis* SPQ genes to improve drought tolerance is in progress.

This research was supported by Bayer CropScience and NKFI project KH 129510. Dóra Faragó is supported by Young Scientist Fellowship of the Hungarian Academy of Sciences.

Keywords: paraquat resistance, drought tolerance, ABA



O-16

Involvement of RNA binding proteins in plant antiviral responses

Károly Fátol

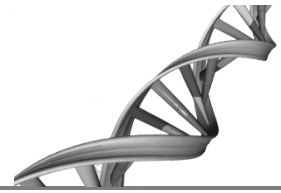
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Plants employ multiple defense mechanisms to restrict viral infections, among which RNA silencing is the best understood. In addition, plants also resort to both local and systemic resistance responses, which limit the virus to the infected cells and can also provide resistance to distal, non-infected parts of the organism. Unlike RNA silencing, the mechanistic details and regulation of the latter processes are just beginning to emerge.

Systemic necrosis (SN), which is regarded as a special form of the local hypersensitive response (HR), results in the necrosis of the apical stem region, usually causing the death of the plant. It is generally assumed that HR and SN are related processes, however the exact mechanisms leading to SN are still largely unexplored. Recently, we have demonstrated that Argonaute 2 (AGO2) is one of the main components of antiviral RNA silencing in *Nicotiana benthamiana*. Using the CRISPR/Cas9 genome editing system *ago2* mutant plants were also generated. Infection of these plants with various viruses results in apical necrosis, closely resembling SN. The main objective of our work is to uncover the mechanistic details of the above process.

Here we report on the involvement of the RNA binding protein, DRB2 (double stranded RNA binding protein 2) in virus induced SN. We show that DRB2 inhibits vsiRNA biogenesis by blocking DCL (dicer-like protein) activity. Upon engagement with viral RNA, DRB2 also elicits a necrotic response. Importantly, downregulation of DRB2 expression can rescue *ago2* mutant plants from virus induced SN. Based on our results we present a model, which describe the complex interplay between RNA silencing and virus induced hypersensitive responses in the context of plant antiviral defenses.

Keywords: *RNA binding protein, RNA silencing, virus*



O-17

Ubiquitin protease 12 affects the function of the circadian clock in *Arabidopsis thaliana*

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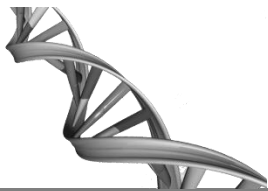
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In order to identify novel components of the circadian system in *Arabidopsis thaliana*, we carried out a large-scale forward genetic screen. Several mutants, displaying altered rhythmic expression pattern of a clock regulated luminescent reporter gene in continuous light conditions, were isolated. The mutant *rs24* (*red screen 24*) showed about 2 h period shortening under the screening conditions and was selected for further analysis. The mutation affected the expression of several clock-controlled genes in the same manner and the short period phenotype was independent of the light conditions. These findings indicated that the function of the core oscillator was altered in the mutant. In fact, expression of the core clock genes showed the expected short period phenotype, but the level of their expression was not affected significantly. This suggests that RS24 does not affect transcription of clock components directly. Consistent with the basic circadian dysfunction, *rs24* showed early flowering phenotype in short day conditions. *rs24* mutants produce long hypocotyls in red light, suggesting a positive role for RS24 in phytochrome-mediated light responses.

Genetic mapping followed by transgenic complementation showed that the RS24 gene encodes an **ubiquitin protease**, known as UBP12. This enzyme has been shown to influence the ubiquitination state of histone H2A and via the modulation of the activity of the Polycomb group protein complex, it affects heterochromatic gene repression. Although we have not discovered the direct molecular link yet, our results underline the important function of chromatin remodeling in the regulation of the plant circadian oscillator.

Keywords: *plant, circadian clock, ubiquitin*



O-18

The protective effect of ERD14 - the versatility of an intrinsically disordered chaperon

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ERD14 (Early Response to Dehydration 14) is an intrinsically disordered chaperon found in *Arabidopsis thaliana*, which is expressed during abiotic stress conditions in large quantities [1]. The activity of ERD14 chaperone was demonstrated *in vitro* and *in vivo* studies and the purpose of our research was to explore the molecular mechanism and the connections between structure and function.

We conducted a systematic examination of sequentially randomized and deletion mutants in an *E. coli* bacterial test system. The protein is largely disordered both *in vitro* and *in vivo*, with its short, conserved regions transiently sampling helical conformations *in vitro* that are involved in recognizing partner proteins in the cell [2]. Overexpressed ERD14 raises viability of cells from 40% to 75% following heat stress (50°C x 15 min). Deleting Kc- and S-segments decreases chaperone activity, whereas scrambling the entire protein impairs activity.

In-cell NMR data were measured and mapped to the sequence of ERD14 revealing that disappearing resonances are located mainly at the regions of K-segments, which have a significant effect on survival rate in the applied stress assay. It is a strong presumption that these conserved regions of ERD14 (Ka through Kc) participate in partner binding.

Preceding measurements revealed that ERD14 is capable to bind RNAs. This was confirmed by Biolayer Interferometry and Electromobility Shift Assay measurements. Furthermore, wild-type ERD14 protein forms liquid droplets in the presence of multiple RNAs *in vitro* and the liquid-liquid phase separation can be observed in ERD14-CFP overexpressing *A. thaliana* plants *in vivo*.

In all, our data provide evidence that the structural disorder and protein function could be connected in live cells, leading to a testable molecular hypothesis about molecular mechanism of the intrinsically disordered chaperones.

Keywords: intrinsically disordered chaperon protein, structure-function, phase separation

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O-19

Transposase and host factor determinants of target site selection by DNA transposons

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DNA transposons, including *Sleeping Beauty* (SB) and *piggyBac* (PB) are useful tools for genetic engineering. There are currently 11 clinical trials listed in Gene Therapy Clinical Trials Worldwide (<http://www.abedia.com/wiley/vectors.php>) employing SB-engineered primary human cells. One factor that largely contributes to the mutagenic potential of these transposons (desired in mutagenesis screens and undesired in gene therapy applications) is their target site selection properties.

Comparative analyses of genome-wide distributions of transposon and retrovirus insertions in human CD4⁺ T cells revealed unexpected parallels between PB transposon and MLV retrovirus insertions. Both PB and MLV insertions are enriched at transcriptional start sites (TSSs). We demonstrate physical interaction between the PB transposase and chromatin-reader BET-domain proteins (including BRD4), suggesting a tethering mechanism that directs integration into TSSs. We provide evidence for a tethering mechanism in SB transposition that involves interaction between the transpositional nucleoprotein complex and chromatin-bound excess transposase molecules.

We have begun to dissect the molecular determinants of target site selection by the SB transposase. Selected amino acid positions, implicated to be involved in making contacts with the target DNA during transposition, were subjected to saturation mutagenesis. We identified novel SB transposase mutants that display efficient and precise excision but practically unmeasurable genomic re-integration. We further show that the transposons excised by these transposase mutants form extrachromosomal circles that cannot undergo a further round of transposition, thereby representing dead-end products of the excision reaction. We demonstrate the utility of the exc⁺/int⁻ transposase in cassette removal for the generation of reprogramming factor-free induced pluripotent stem cells. Lack of genomic integration and formation of transposon circles following excision is reminiscent of signal sequence removal during V(D)J recombination and implies that cut-and-paste DNA transposition can be converted to a unidirectional process by a single amino acid change. Our screen also revealed mutants with altered target site selection properties, which may be useful for the development of molecular strategies for target-selected transposon integration for therapeutic applications.

Keywords: *transposon, gene therapy, mutagenesis*



O-20

Small ovary (sov): a novel heterochromatin regulator in *Drosophila*

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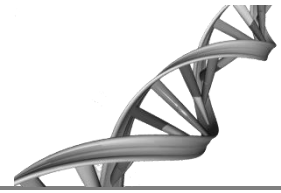
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The reliable transmission of the genetic information between generations requires the preservation of genome integrity of the germ cells. Mobilisation of transposons in germ cells can induce double strand breaks leading to deleterious mutations and genome damage. Thus, a small non-coding RNA-based defence system (piRNA-pathway) has been evolved against transposon-induced mutagenesis. Multiple aspects of piRNA-mediated transposon silencing depend on heterochromatin. Long precursors of the piRNAs are transcribed from piRNA clusters located at the heterochromatic regions of the genome. Following their biogenesis, piRNAs suppresses transposon transcription through promoting heterochromatin formation at the active transposon loci. The *Drosophila* oogenesis provides an excellent model for understanding the role of heterochromatin mediated gene expression regulation in transposon silencing.

In a large scale RNAi screen, the *small ovary (sov)* gene was identified to be essential for germ cell development. *Sov* mutant flies showed abnormal germline stem cell development accompanied by high transposon mobility. Position effect variegation tests showed that *sov* is a repressor of variegation suggesting that *sov* promotes heterochromatin formation. Protein interactome study of Sov revealed a physical interaction between Sov and Heterochromatin protein 1a (HP1a), a central component of the heterochromatin. In addition, Sov co-localises with HP1a in the nuclei suggesting that Sov affects chromatin function through HP1a regulation. FRAP studies revealed an increased HP1a mobility in *sov* mutants indicating that *sov* promotes heterochromatin formation by supporting the association between HP1 and the chromatin. We propose a model of how Sov functions in transposon silencing as a regulator of the heterochromatin. Since Sov shows a remarkable structural similarity to the proteins driving the formation of phase-separated nuclear domains, we hypothesise that Sov functions in the establishment of the unique physical properties of heterochromatin.

Keywords: stem cell, heterochromatin, piRNA



O-21

R-loop binding proteins: from chromatin to therapeutic targeting

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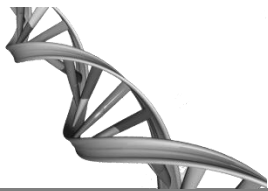
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Unraveling the origin of genetic alterations from point mutations to chromosomal rearrangements was greatly enhanced by the discovery of RNA-DNA hybrids (R-loops) that behave as hotspots of genomic instability in a variety of organisms. Current models posit that unscheduled R-loops are a hazard to genome integrity as their single-stranded portion is susceptible to breakage. Therefore, identifying proteins that are involved in sensing, forming, stabilizing, and eliminating R-loop structures are of key importance.

Herein we studied potential R-loop binding proteins from various species that were identified recently¹⁻³. We show that Nodulin homeobox (Ndx) is a new chromatin architectural protein in *Arabidopsis thaliana* that binds to heterochromatin and transposable elements and promote RNA-DNA hybrid formation. Inactivation of Ndx led to the disappearance of R-loop structures and *de novo* formation of chromatin loops (detected by DRIP-seq⁴ and chromosome conformation capture (Hi-C) techniques) that were accompanied by massive transcriptional changes in homeotic genes governing crucial developmental functions.

Besides *Arabidopsis*, we also analyzed recent mass spectrometric screening data in humans¹⁻² identifying >900 RNA-DNA hybrid binding proteins. Taking advantage of large-scale gene expression, survival rate, and drug-sensitivity data from various cancer databases, we show that high (or low) mRNA expression of different RNA-DNA hybrid binding proteins in different cancer types can have contrasting outcomes in responding to different chemotherapeutic treatments. Based on the revealed pharmacogenomic landscape of human RNA-DNA hybrid binding proteins, we propose that R-loops and R-loop binding proteins are potentially relevant new diagnostic markers and therapeutic targets in multiple cancer types that are associated with drug effectiveness and resistance.

Keywords: genome structure, chromosome conformation capture (Hi-C), RNA-DNA hybrid, Nodulin homeobox, survival association, drug sensitivity, cancer



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O-22

Regulation via PTMs, histone variants and extrachromosomal interactions are all mediated by large changes of nucleosome stability

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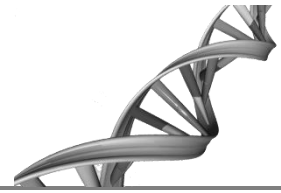
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Nucleosomal structure is repressive, therefore, the mechanisms involved in the regulation of nucleosome stability are of great importance in the control of gene expression. It is well established that nucleosome free regions (NFRs) are generated at the transcriptional start sites, flanked by nucleosomes with high turnover rate and of special histone composition. K4 trimethylated H3 histones and H2A.Z histone variants are also characteristic for the +1 and -1 nucleosomes neighbouring these NFRs. H2A.Z has other preferential locations in more compact heterochromatic regions as well. The effects of the presence of the different PTMs or histone variants on the stability features of such nucleosomes are still unknown.

We have developed an *in situ* assay for the measurement of the intrinsic and superhelicity dependent nucleosome stability (1). Using H2A.Z specific antibodies we observed unusually stable H2A.Z containing nucleosomes that could be detected in an antibody clone/brand-dependent manner. We have shown that increased stability of H2A.Z was independent of the acetylation of the N-terminal histone tail, or the H2A.Z isotype. Through the spectacles



of superhelicity dependent stability, the unusually stable nucleosomes appear to constitute a subpopulation of all H2A.Z containing nucleosomes that appears to be of perinucleolar localization. Using cells expressing mutant H2A.Z histones we have shown that the deletion of the C-terminus containing the binding site of the H2A.Z reader protein PWWP2A has a destabilizing effect, suggesting that interactions involving these entities are major determinants of the increased stability of H2A.Z containing histone dimers.

Stability of H3K4me3 containing nucleosomes was analyzed in an experimental system where the H3K4me3 reader protein genes Jmjd1, Jmjd2 and Jmjd3 were knocked out in mouse embryonic stem cells. Jmjd proteins are responsible for the removal of H3K9me3 from the H3K4me3 containing or the neighbouring nucleosomes. We show that knocking out Jmjd genes leads to nucleosome stabilization. These data, together with our recent observations on a nucleosome stabilizing effect of certain other histone variants and on the histone dimer destabilizing effect of a lamin mutation, allow us to conclude that nucleosome stability is a common point of convergence playing a central role in gene regulation.

This work has been supported by OTKA K128770 and GINOP-2.3.2-15-2016-00044.

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O-23

Ccr4-Not complex is at the core of the gene expression circuitry

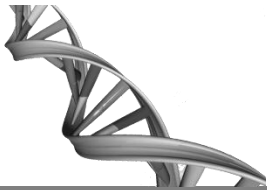
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The Ccr4-Not complex is a conserved multi-protein complex that regulates gene expression at all stages, from production of the mRNA in the nucleus to its degradation in the cytoplasm. Challenging the general understanding of gene expression that considers transcription and translation to be independent processes in eukaryotes, we recently demonstrated that translation capacity of the cell is determined during transcription elongation through imprinting of ribosomal protein encoding mRNAs with Not1, the central scaffold of the Ccr4-Not complex. We have confirmed that this mRNA imprinting in the nucleus is important for polysomal presence of the imprinted mRNAs and in turn for normal translation rate of ribosomal proteins. We also determined that Not5-dependent Not1 association with specific complex subunit encoding mRNAs is important during translation for assembly of protein complexes involved in transcription, such as RNA polymerase II and the SAGA histone acetyltransferase. This effect of the Ccr4-Not complex on



transcription by translation and translation by transcription identifies Ccr4-Not as a regulator acting at the core of the gene expression circuitry. I will summarize published and ongoing work about the functions of the Ccr4-Not complex that we identified in yeast and I will discuss how I think these might be relevant to understand why Ccr4-Not and ribosome protein mutations accumulate in numerous cancers.

O-24

Germ cells unleashed: role of Rybp in regulating germ cell determinants

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Repression of germ lineage program in the soma is crucial for proper development. Non-canonical forms of Polycomb Repressive Complex1s (ncPRC1s), which contain RING1 and YY1 Binding Protein (RYBP), can specifically ubiquitinate histone 2A and block nucleosome remodeling *in vitro*. The role of several ncPRC1 subunits in meiosis and in the regulation of germ cell development both in the testes and in the ovary has been confirmed recently, but the function of RYBP in these processes has not been studied so far. We and others have shown earlier, that germ cell specific genes and meiotic regulators are de-repressed in *Rybp null* mutant embryonic stem (ES) cells. RYBP protein is detectable in primordial germ cells (PCGs) and spermatogonia, and its level decrease as germ cells differentiate.

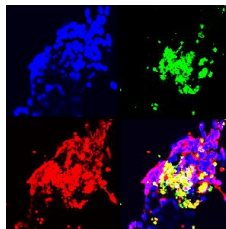
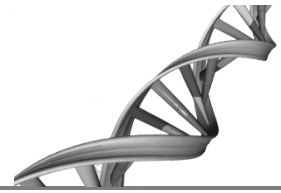


Figure 1: During neural differentiation (day 10) germ cell marker proteins SSEA1 and DDX4 are detectable in a subset of *Rybp* mutant cells, which are prone to apoptosis

In order to study the role of Rybp in germ cell development we applied several *in vitro* ES cell-based differentiation systems. Our results show that RYBP is required for differentiation of PCGs and for the progression of meiosis during *in vitro* PCG differentiation. We demonstrated that the level of several key germ cell proteins (DDX4, SSEA1, SYCP3) are significantly elevated in the *Rybp null* mutant ES cells. We suppose that the increased transcription of germ cell specific genes might not be a mere consequence of de-repression



but can be a result of direct NANOG or retinoic-acid (RA) dependent activation. NANOG is a well-known pluripotency factor, which alone is able to initiate germ cell specific differentiation, when overexpressed. The level of Nanog is elevated both at mRNA and at protein level in the *Rybp null* mutant ES cells. We could demonstrate that RA metabolism is also disturbed in the mutant cells. We proved that the early meiotic regulator, Stimulated by retinoic acid8 (STRA8), is found only in *Rybp null* mutant ES cells. The level of germ cell specific proteins decreases during *in vitro* cardiac and neural development but remain high in the *Rybp null* mutant cells. We propose that RYBP has a specific function in the repression of germ cell specific genes in the soma. The involvement of RYBP in the development of germ cells will be discussed in current talk.

Keywords: *Rybp, germ cells differentiation, Polycomb repression*

O-25

Chromatin level determinants of cellular identity

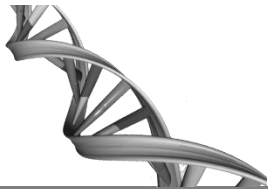
Bálint L. Bálint, Dóra Bojcsuk, Edina Erdős, Noura Faraj, Péter Gargya and Lilla Ozgyin

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In cancer, the genome is transformed in such a manner that the uncontrolled cell division and growth of the cancer is destroying the organism itself. The activity of the genome is controlled by distinct mechanisms on the chromatin level by determining the boundaries of active and inactive regions. Components of these regulatory processes are the super-enhancers, or clustered enhancers with their transcription factors and the protein complexes that determine the boundaries of chromatin domains. The changes in cell identity can be identified both on genotype and phenotype levels and their initial phase is initiated by changes on the chromatin level.

We have investigated the impact of personal genome variability on chromatin level determinants in LCL cells, and the formation of clustered enhancers in breast cancer cells. Interestingly the chromatin level changes regulated by ERalpha and COUP-TFII highlight some important features. Based on our results COUP-TFII has cell independent roles related with angiogenesis, mediated via VEGF. On the other hand COUP-TFII is involved in chromatin loop formation via CTCF. This later effect is influencing the copy number variability observed in cancer cells and correlates with changes in breast cancer prognosis

Keywords: *chromatin, epigenetics, enhancers, personal genome*



O-26

Two tales of protein tails: regulation by S100 protein binding and phosphorylation

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Protein termini are often unstructured and involved in the regulation of protein function. These intrinsically disordered regions (IDRs) contain many phosphorylation and other PTM sites and also linear motifs responsible for protein-protein interactions (PPIs). PTMs and PPI can regulate protein function synergistically or competitively, and they could even provide isoform-specific regulations. Here we present two stories comparing the regulatory effect of phosphorylation and S100 protein binding at terminal IDRs on the function of annexin A2 (ANXA2) and non-muscle myosin 2s (NM2s).

ANXA2 has a versatile role in membrane-associated functions including membrane aggregation, and it is regulated by PTMs and PPIs through an N-tail IDR. Structural studies indicated that the flexibility of the N-tail and the core domain are interrelated and oppositely regulated by Tyr24 and Ser26 phosphorylation, the former being inhibitory to the membrane-bridging function of ANXA2. This negative effect is relieved by S100 binding. Our results provide a structural model for regulation of ANXA2-mediated membrane aggregation by phosphorylation and S100 binding.

NM2 motor protein isoforms (NM2A, -B, -C in mammalian cells) assemble into functional homo- and heterotypic “minifilaments” and are involved in controlling cell adhesion and cell migration. We present evidence that the equilibrium between these filaments and single molecules can be regulated via S100 protein interactions and phosphorylation at the C-tail IDR. Importantly, S100 proteins can selectively remove NM2A from heterotypic filaments. On the other hand, tail phosphorylation by various kinases down-regulates NM2B filament assembly in an additive fashion and has a comparatively minor effect on NM2A filament stability. These two mutually exclusive mechanisms are likely to contribute to the temporal and spatial sorting of the two NM2 paralogs within heterotypic filaments. (Supported by NKFI grant K119359)

Keywords: *regulation, phosphorylation, protein-protein interaction, structural dynamics*



O-27

Insights into the structural background of dUTPase inhibition by a proteinaceous inhibitor

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The dUTPase enzyme family plays a key role in maintaining genomic integrity throughout preventing uracil incorporation into DNA. Their importance is underlined by their presence in almost all type of organisms from bacteria to primates. Their dysfunction poses severe consequences to most cells or organisms, as increased uracil incorporation leads to DNA double strand breaks and eventually cell death. Therefore dUTPases are regarded as chemotherapeutic targets in certain diseases like tuberculosis and malaria, and also in some aspects of cancer treatment.

A recently discovered staphylococcal protein termed Stl is able to inhibit a couple of dUTPase homologues (1,2). However, its inhibitory potential may be different according to species-specific structural differences among dUTPases. We have pointed out that protein Stl which was originally identified as a bacterial repressor is also able to form a stable protein complex with the eukaryotic *Drosophila melanogaster* dUTPase and inhibit remarkably its enzymatic activity (3).

Our results indicated that the amino acid side-chains being involved in this interaction are partially evolutionarily conserved among bacterial and eukaryotic dUTPase homologues. Interestingly, we have also found that while Stl forms a strong complex with *Escherichia coli* dUTPase, still, no inhibition was observed under steady-state experimental conditions – this is exceptional among the numerous investigated complexes (1,2,3). Consequently we are committed to uncover the structural bases for the lack of steady-state enzymatic inhibition upon complex formation in the *E. coli* dUTPase.

Relying on our recent results on the interaction surface of the human dUTPase and protein Stl, we designed a rational mutation screen. We identified specific amino acid side-chains in the *E. coli* dUTPase sequence as potentially relevant factors for the lack of inhibition, and found that among the generated mutants, two became sensitized to Stl-induced inhibition. We successfully grew crystals from one these mutants and obtained a complete X-ray diffraction dataset at 1.8 Å resolution. We solved its structure which is currently under deposition at Protein Data Bank under the ID 6HDE. Together with our experimental data it provides novel insights into the mechanism of Stl induced dUTPase inhibition.

Funding: EMMI FIKP-BME Biotechnology grant



Keywords: dUTPase, protein complex, enzyme, inhibition

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O-28

Disordered regions of Mixed Lineage Leukemia 4 (MLL4) protein are capable of RNA binding

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Long non-coding RNAs (lncRNAs) are emerging as important regulators of cellular processes and are extensively involved in the development of different cancers; including leukemias. As one of the accepted methods of lncRNA function is affecting chromatin structure lncRNA binding has been shown for different chromatin modifiers¹. Histone lysine methyltransferases (HKMTs) are also subject of lncRNA regulation as demonstrated for example in the case of Polycomb Repressive Complex 2². Mixed Lineage Leukemia (MLL) proteins that catalyze the methylation of H3K4 have been implicated in several different cancers³; yet many details of their regulation and targeting remain elusive. In this work we explored the RNA binding capability of two, so far uncharacterized regions of MLL4 with the aim of shedding light to the existence of possible regulatory lncRNA interactions of the protein. We demonstrated that both regions, one that contains a predicted RNA binding sequence and one that does not, are capable of binding to different RNA constructs *in vitro*. This recognition highlights a yet unrecognized possibility for the regulation of MLL4 functions through RNA binding.

Keywords: histone methyltransferase, lncRNA, RNA binding

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O-29

Studying the catalytic cycle of Pgp and ABCG2 transporters

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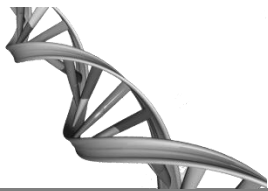
ABC transporters form a large evolutionary conserved family of membrane proteins. Certain human ABC proteins such as P-glycoprotein and ABCG2 are drug exporters expressed in various tissues having barrier functions. Thanks to their broad substrate spectrum they can affect the distribution of various drugs in our body and often cause chemotherapy resistance in tumours. Pgp consists of two transmembrane domains (TMDs) responsible for substrate binding and translocation and two nucleotide binding domains (NBDs) responsible for nucleotide binding and hydrolysis, while ABCG2 reaches this structure by homodimer formation.

Although these transporters are subjects of intense research for a long time the experimental data and models regarding the details of their catalytic mechanism are still controversial. Coupling between the ATP hydrolysis cycle and the steps of substrate translocation is the key unresolved question of the catalytic mechanism of both transporters. To try to solve the above problem we have developed several assays for studying them in their natural plasma membrane environment applying the UIC2 and 5D3 conformation sensitive antibodies reacting with the ATP-free inward facing conformation of Pgp and ABCG2, respectively.

We have shown that nucleotide binding drives both Pgp and ABCG2 from the inward- to the outward-facing conformation and in case of Pgp we have proved that this conformation switch coincides with the flipping of the transporter from the high to a low substrate-affinity state leading to export of substrates.

We have also proved that several previously characterized NBD point mutations completely inactivate the ATPase and transport function of Pgp when present in both NBDs simultaneously in accordance with literature data. However, when these mutations are induced in only one of the catalytic sites the mutant Pgp variants can pass through the catalytic cycle repeatedly, although their turnover rate was found to be dependent on the site of the point mutation. These data support that opposed to previous concepts a single intact catalytic site is sufficient for the activity of Pgp.

ABC transporters can be trapped in a state following the hydrolysis of ATP using phosphate mimicking anions such as vanadate (V_i) or beryllium-fluoride (BeF_x). We have shown that the progressive accumulation of Pgp and ABCG2 in the V_i or BeF_x trapped state is facilitated not only by substrates that increase the rate of ATP hydrolysis, but also by



competitive inhibitors that inhibit ATP hydrolysis. A likely explanation of the above observations is that binding of both kinds of agents can increase the duration of the state sensitive to the phosphate mimicking anions.

Support: OTKA grant K124815 and GINOP-2.2.1-15-2017-00079

Keywords: *P-glycoprotein, ABCG2, catalytic cycle*

O-30

Characterization of a cis-acting protein stabilization motif that functions as a universal STABILON in higher Eukaryotes

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In vivo protein stability that is regulated by specific degron sequences, is a key factor in maintaining a healthy proteome in Eukaryotes. While a large variety of degrons had been characterized, our knowledge on cis-acting protein motifs that can *in vivo* stabilize otherwise short-lived proteins is very limited. We have identified and characterized a short, evolutionarily conserved protein segment derived from the extreme C-terminus of the p54/Rpn10 polyubiquitin receptor subunit of the Drosophila 26S proteasome, which fulfils all the characteristics of a protein stabilization motif. Attachment of this 15-mer stabilon sequence to the carboxyl-terminus of short-lived model proteins or polypeptides differing in structure, cellular localization, *in vivo* processing and C-terminal amino acid sequences, have significantly extended their half-life by preventing their proteasomal degradation. The consequence of its stabilization activity was most conspicuous on the production of a secreted protein. The quantity of the secreted human erythropoietin fused to stabilon in the tissue culture medium was orders of magnitude higher than that of the nascent erythropoietin. Finally, we found that this motif functions as a universal stabilon in higher Eukaryotes.

This work was supported by grants from the Ministry for National Economy of Hungary (GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00032) and Hungarian Academy of Sciences (Bohyai Fellowship (bo_329_15) and Lendület Program Grant (LP2017-7/2017)).



O-31

Functional redundancy within the S100 protein family

Márton András Simon, Gergő Gógl, Péter Ecsédi and László Nyitray

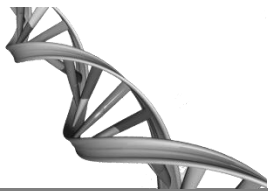
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The vertebrate-specific S100 proteins are members of an EF-hand containing, Ca^{2+} -binding superfamily, consisting of 20 paralogs. They are small homodimeric proteins with two Ca^{2+} -binding motifs per monomer. They regulate Ca^{2+} homeostasis and interact in a Ca^{2+} -dependent manner with proteins localized both intra- and extracellularly.

Mainly upregulation and occasionally downregulation of S100 genes, through crucial protein-protein interactions, is associated with various cancers and inflammatory disease. It is assumed that functional redundancy can occur within the family, thus changes in their expression level in a pathological state can lead to dysfunction via competition in their interactions. Therefore, our research goals were to characterize the affinity of individual S100 proteins with various partners and functional classification according to the deduced specificity-map, which can be important for future inhibitor-based therapeutic application.

In the first part of the project, we produced a full panel of recombinant S100 proteins, and studied their interactions, using various biophysical methods with binding partners, including RSK1, MK2, MNK1, LATS1/NDR1, ANXA2, p53, ezrin, FOR20, FOP, CapZ, NCX1, SIP, TRPM4 and NMIIA. It has been found that S100 proteins can be divided into two groups and further into subgroups according to their binding specificity and affinity pattern. The first group consists of low-specificity, promiscuous S100 proteins, while the other one contains either high-specificity, or non-interacting members. Compared to strictly sequence-based classification, considerable differences are observed, probably due to structural reasons. Therefore, it is now possible not only to classify but also to predict interactions more accurately between different paralogs of S100 proteins and their binding partners.

Keywords: *EF-hand, Ca^{2+} -binding, biophysical measurements, fluorescence polarization, protein-protein interactions, systems biology*



O-32

Multiscale quantum modeling in biocatalysis: a tool for predicting enzymatic reactivity and selectivity

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Nature has engineered enzymes to catalyze nature itself. Enzymes are used to produce drugs as an alternative to the chemical synthesis to reduce cost, to improve stereoselectivity, overcome practical concerns like high temperature and pressure and to make the process eco- friendly. Such advantages of enzymes lead to the emerge of biocatalysis field where scientists all around the world are now searching for novel biocatalysts or finding ways to manipulate them for desired properties. Designer enzymes mimicking evolution on a laboratory timescale is the trend in the global enzyme market. However, conventional methods such as directed evolution and protein engineering for enzyme modification depend on re-sampling and *in vitro* confirmation studies, which make them time-consuming and costly exercises. The advent of multi-scale modeling methods such as QM-cluster, QM/MM (or ONIOM), and QM/MM molecular dynamics (MD) simulations has unveiled the complexity of enzymatic reactions and provides accurate atomistic picture and reasonable information on the kinetics and thermodynamics of the reactions. These methods have been proved in recent years to reproduce reactivity and selectivity of natural and laboratory-engineered enzymes, providing a rationale for suitable modifications to achieve the maximum benefit of biotransformation. In this talk, we will give a brief on current state-of-the-art multiscale modeling to elucidate reactivity and selectivity for a number of enzymes with different biocatalytic processes, such as dihydropyrimidine-metabolizing enzymes, glycosidases, and isomerase. These particular enzymes are potential biocatalysts with specifically relevant to the industrial production of valuable chemicals and biofuels. Our findings will provide useful guidance for further enzyme engineering and the design of improved biocatalysts for industrial applications.

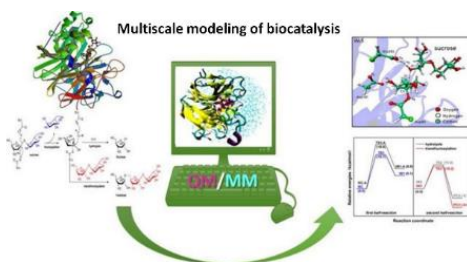
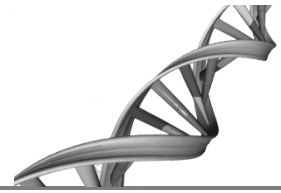


Figure 1: Multiscale modeling in enzyme biocatalysis

Keywords: biocatalysis, multiscale modeling, enzyme mechanism, QM-cluster, QM/MM, MD



O-33

Downregulating insertional mutagenesis in bacterial genomes using CRISPR-interference

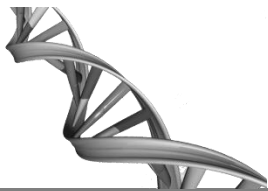
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Insertion sequences are one of the simplest autonomous mobile genetic elements, predominating in prokaryotic genomes. Their spontaneous and stress-induced transpositions make measurable contributions to the mutation rate, and therefore the genetic diversity of bacterial cells. The resulting phenotypic changes may be advantageous for the cell and facilitate population survival by relieving environmental stresses. This process however, also takes place during bacterial fermentation, where the overexpressed genes pose a significant burden on the cell. In this case, mutations inactivating the genes of interest increase cellular fitness, and non-producer cells quickly become dominant in the culture. Several strategies have been developed to deal with this issue, including efforts to reduce the rates of genetic mutation within the production strain. For example, we have participated in multiple projects removing mobile genetic elements from certain bacterial genomes. These projects however were time and effort-consuming, and potentially had to be repeated for the many different production strains used in the industry. To circumvent these difficulties, we introduce here a plasmid-based system capable of silencing IS-element transposition relying on the tunable nature of the CRISPR/dCas machinery. We demonstrate the portability and specificity of the system, along with its effect on mutation rates measured at chromosomal and episomal loci. Our results suggest that CRISPR-based IS-knockdown is a useful strategy for the rapid elimination of insertional mutagenesis.



O-34

Haploinsufficiency: mechanisms and evolution

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Heterozygous loss-of-function mutations are occasionally dominant over the wild-type allele and thereby result in abnormal phenotype, a phenomenon termed haploinsufficiency. Previous studies indicate that haploinsufficiency is highly plastic across environments and genetic context. However, it remains unclear, why certain genes are haploinsufficient in one but not in the other related species. To investigate this issue systematically, we performed laboratory evolution with 184 diploid heterozygotes knock-out baker's yeast strains all of which displayed reduced fitness in the laboratory (i.e. haploinsufficient). 44.4% of the parallel evolving populations displayed increased fitness in stress-free conditions, suggesting that haploinsufficient strain can readily mitigate their fitness defects during evolution. Genomic analysis revealed two main molecular mechanisms behind this pattern. However, these genetic changes have associated fitness costs in specific environmental settings. In sum, mutations that shape haploinsufficiency are ubiquitous and have a wide-range of pleiotropic side effects.

O-35

Blackjack mutations improve the on-target activities of all increased fidelity variants of SpCas9

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Cas9 proteins are RNA-guided endonucleases that can be directed to cleave chosen DNA sequences. The utility of *Streptococcus pyogenes* Cas9 (SpCas9) nuclease for genome engineering, although it has been proved, is limited by its off-target activity – i.e., the nuclease also cleaves targets that show only limited, imperfect complementarities with the associated



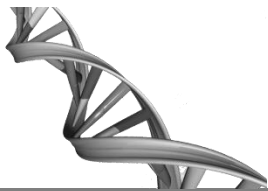
guide RNA (sgRNA) – that interferes with several research applications and the therapeutic uses of the technology.

The propensity for off-target activity of SpCas9 has been considerably decreased by rationally engineered variants with increased fidelity (eSpCas9¹; SpCas9-HF1²; HypaCas9³, evoCas9⁴, HeFSpCas9⁵), however we and other former studies also revealed that these variants work poorly with 5' G-extended sgRNAs⁵. It is a critical issue, because the U6 and T7 promoters that are most frequently employed for the expression of the sgRNA in mammalian cells or by *in vitro* transcription, respectively, require a G nucleotide at the 5'-end of the sgRNA. In cases of target sites which start with a non-G nucleotide there are different practices to fulfil this requirement of the U6 and T7 promoters, from which the most commonly used one is the extension of the sgRNA with an extra 5' G. This way 5' non-G sequences could be targeted with wild type SpCas9. Thus, the incompatibility of increased fidelity nucleases with 5' G-extended sgRNAs limits their applicability due to the limited number of available target sites.

By structure-based rational design, we identified “Blackjack” mutations that rescue the activity of increased fidelity nucleases with 5' G-extended sgRNAs. Exploring these mutations, we developed two variants, *eSpCas9-plus* and *SpCas9-HF1-plus* that possess up to 24-fold increased on-target activities with 5' G-extended sgRNAs yet matching fidelities and on-target activities with non-extended sgRNAs to those of eSpCas9 and SpCas9-HF1, respectively. Furthermore, it is reported that increased-fidelity variants do not work effectively when transfected to the cells in pre-assembled ribonucleoprotein form.^{6,7} Here we generated a series of increased fidelity variants containing the Blackjack mutations that works effectively in pre-assembled ribonucleoprotein form and provides high fidelity editing to the full target spectrum available to wild type SpCas9.

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O-36

Genetic investigation of archeological sites in Russia that can be associated with early Hungarians

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Although linguists, historians, archaeologists have been researching it for a long time, the exact origin, route and chronology of the early Hungarians' migration is still unclear. According to our current knowledge the first relics from archaeological cultures that are most probably connected with Hungarians' ancestors were found in the Central and Southern Urals. The Hungarians migrated westwards from the Ural region through the Middle-Volga region and the East-European steppe, until they arrived in the Carpathian Basin around 895 AD.

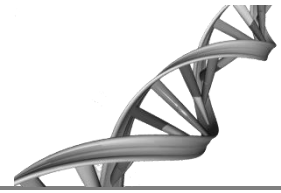
Aiming to better understand the early Hungarians' origin and migration, our research group analyse medieval populations from the principal sites of the supposed migration. These populations are from diverse geographic locations (Ural region, Volga-Kama region) and represent different periods (6-12th AD) and cultures, but they have archaeological connection with each other and with the early Hungarians in the Carpathian Basin.

At the center of our ongoing investigations is the mitochondrial DNA. Our work ranges from sampling from archaic bones to the determination of the whole mitochondrial genomes by next generation sequencing and to the statistically and phylogenetically analysis of the data.

We observed in all tested populations (>70 individuals) both European and Asian mitochondrial haplogroups, but in different proportions. We compared statistically these medieval populations with other archaic and recent populations available in international databases. We detected genetic connections among the examined populations, but their level and extent is different. Some observed lineages have shown close or direct relations between and within the studied populations and even connected them to the Hungarian Conquerors in the Carpathian Basin.

Our preliminary results show that the investigated populations have important role in the Hungarian prehistory, whereas their analysis has revealed Central-and East-Asian relations that have not been genetically tested.

Keywords: *archaeogenetics, mitochondrial DNA, Hungarian prehistory*



O-37

Y-chromosome haplogroups from Hun, Avar and conquering Hungarian nomadic people of the Carpathian Basin

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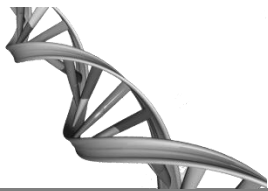
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Population history of the Carpathian Basin was profoundly determined by the invasion of various nomadic groups from the Eurasian Steppes during the Middle Ages. Between 400-453 AD the Huns held possession of the region and brought about a major population reshuffling all over Europe. From 568 AD the Avars established an empire in the region lasting nearly for 250 years. Presence of the Hungarians in the Carpathian Basin was documented from 862 AD and between 895-905 they took full command of the region. Our recent analysis of conquering Hungarian (hence shortened as Conqueror) mitogenomes revealed that the origin of their maternal lineages can be traced back to distant parts of the Eurasian steppe. One third of the maternal lineages were derived from Central-Inner Asia and their most probable ultimate sources were the Asian Scythians and Asian Huns, while the majority of the lineages most likely originated from the Bronze Age Potapovka-Poltavka-Srubnaya cultures of the Pontic-Caspian steppe. Population genetic analysis indicated that Conquerors have closest connection to the Onogur-Bulgar ancestors of Volga Tatars.

In order to generate sufficient data for statistical evaluation and compare paternal and maternal lineages of the same population, we have determined the Y-Haplogroups (Hg) from the same cemeteries whose mtDNA Hg-s were described. As the studied Conqueror cemeteries represent mainly the Conqueror elite, we supplemented our Y-Hg studies with samples from the preceding Avar elite and available samples from the Hun period to test possible genetic relations.

We selected 191 phylogenetically informative Y chromosome SNP-s defining all major Hg-s and the most frequent Eurasian sub-Hg-s, as well as 87 autosomal SNP. DNA segments containing above SNP-s were enriched from the next generation sequencing (NGS) library of 49 samples with hybridization capture.



We compared the Hg distribution of the Avar and Conqueror elite Y-Hg-s to that of 79 modern Eurasian populations. The Avars were obviously mapped among Siberian populations, while the Conquerors between Central Asians and eastern Europeans.

The origin and composition of the Conqueror paternal lineages fairly mirrors that of their maternal ones; 1/3 of the Hg-s originated from East Eurasia, there are Hg-s characteristic both for eastern and north-western Europeans and lineages of Caucasus-Middle origin are also present, affirming that both males and females of same origin migrated together.

The Avar group carried predominantly East Eurasian lineages in accordance with their known Inner Asian origin inferred from archaeological/anthropological parallels and historical sources. The unanticipated prevalence of the Siberian Y-Hg N1a among the Avars sheds new light on their prehistory. The genetic profile of the Avar and Conqueror elite seems considerably different, as latter group is distinguished by the significant presence of Hgs I2a1a2b-L621, R1b1a1b1a1a1-U106 and the Finno-Permic N1a1a1a2-Z1936 branch. On the other hand Y-Hg R1a1a1b2a2-Z2124, also found in Hungarian King Béla III, seems to link the Hun, Avar and Conqueror elite.

Keywords: *ancient DNA, population genetics, NGS sequencing*

O-38

Archaeogenetic evidence on the origin and social organization of the Avar period elite (7-8th centuries AD)

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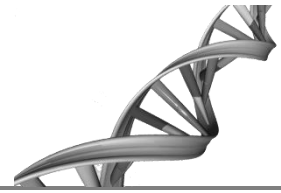
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After 568 AD the nomadic Avars settled in the Carpathian Basin and founded the Avar Quaganate that was an important empire in Central Europe until the 9th century. At least a part of the Avar population was probably of Central or East Asian origin, however location of their homeland is hampered by the scarcity of historical and archaeological data.

Here, we present whole mitochondrial genomes and Y chromosomal variability of twenty-five individuals, most of them assigned to a well-characterized Avar elite group buried in the center of the Carpathian Basin more than a century after the Avar conquest.



The majority (69.5% of n=23) of the mitochondrial DNA variability belongs to Asian haplogroups. The Y-STR variability of the 16 analyzed males is assigned only to five lineages, three N-Tat with mostly Asian parallels and two Q lineages. The Avar elite group has maternal and paternal genetic affinities to several modern Inner and some North Asian populations and the comparative analyses of available ancient mitochondrial DNA datasets also point toward Asia.

The homogeneity of the Y chromosomes reveals paternal kinship as an important factor in the organization of the Avar elite strata. The Y chromosomal data along with the mitochondrial genepool indicate that the Avar elite arrived in the Carpathian Basin as a group of families, and remained mostly endogamous for several generations after the conquest.

Keywords: *archaeogenetics, mitochondrial DNA, Y chromosome, nomadic societies*

O-39

CARD14 gene variants and nuclear factor κ B (NF κ B) activation in pityriasis rubra pilaris

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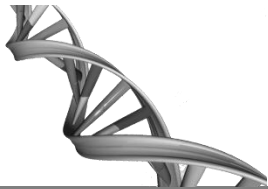
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Pityriasis rubra pilaris (PRP) is a rare, chronic inflammatory papulosquamous skin disease with unknown etiology. PRP is phenotypically related to psoriasis and other skin diseases making the diagnosis often difficult. Based on phenotypical features we distinguish certain subtypes (I-V) of the disease. The rare familial cases show associations with particular *CARD14* gene mutations, while in the genetic background of the sporadic form of the disease *CARD14* polymorphisms have been implicated. Data in the literature suggest that particular *CARD14* variants alter the activation of NF κ B signal transduction thus contributes to disease pathogenesis.

The genetic screening of *CARD14* gene was performed in 19 PRP patients. Half of the patients carried different *CARD14* variants alone or in combination, revealing two mutations (rs114688446, rs117918077) and six polymorphisms (rs28674001, rs2066964, rs34367357, rs11653893, rs11652075, rs2289541).

The atypical juvenile (type V) PRP patient carries four *CARD14* variants, thus to examine the consequences of the identified variants further *in vitro* and *in situ* functional studies were carried out. Immunofluorescent staining revealed nuclear localization of the NF κ B p65 subunit in PRP skin specimen, indicating the activation of NF κ B, in contrast in healthy skin sections only cytoplasmic staining of inactivated NF κ B was detected. Moreover, p65 staining



showed higher p65 positivity in PBMCs derived from the PRP patient compared to healthy samples. NF κ B–luciferase reporter assay demonstrated significantly increased NF κ B activity in keratinocytes from the PRP patient compared to healthy keratinocytes. Our results also show that the higher NF κ B activation in PRP cells had functional consequences leading to significantly higher inflammatory cytokine expression and secretion in both keratinocytes and PBMCs; moreover, PRP samples demonstrated elevated cytokine responses to inflammatory stimuli compared to healthy cells.

The higher inflammatory state established in the biological samples of a type V PRP patient cannot be explained solely by any of the carried *CARD14* variants alone but the cumulative effects of the four variants may contribute to the increased NF κ B activity. The multifactorial nature of the disease indicates the involvement of additional genetic factors; therefore, a large scale genetic investigations would be helpful for the better understanding of the molecular mechanisms of the disease.

O-40

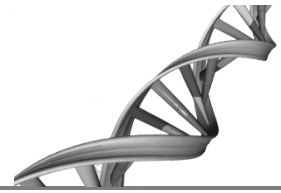
Apc mutation induces extracellular vesicle release from intestinal organoids

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Colorectal cancer (CRC) is one of the most frequent causes of cancer-related death in the Western countries. In the majority of CRC patients, the tumorigenesis starts with a mutation in the Apc gene, leading to the uncontrolled activation of the Wnt pathway and thus to adenoma formation. Some of these adenomas may then further progress to CRC with the accumulation of other mutations. Extracellular vesicles (EVs) are membrane-surrounded structures that represent a novel way of intercellular communication by delivering biologically important molecules from the releasing to the target cells. Since EVs carry their cargo in a protected form and their secretion is generally increased in tumorigenesis, EVs hold a great potential for early cancer diagnosis. The recently developed 3D organoid technology maintains the cellular and genetic heterogeneity of *in vivo* tissues and has proved to be so far the best *ex vivo* model of human cancers. Here we characterize the EV production of CRC cell lines and patient-derived organoids and we identify major factors influencing EV production in CRC.



We used CRC cell lines and patient-derived organoids. Since CRC cells represent a late stage of tumorigenesis and carry a wide variety of mutations, we used a mouse model with well-defined genetic background as well, by introducing Apc mutation into wild type small intestinal organoids by CRISPR-Cas9.

We provide evidence that EVs can be detected in CRC patient-derived organoids by a simple flow cytometry-based method. When testing the effects of major factors promoting CRC progression, we observed that Apc mutation critically induces EV release by activating the Wnt pathway in intestinal organoids. As another important progression factor, the extracellular matrix component collagen enhances EV secretion as well. Furthermore, we show that fibroblast-derived EVs induce the colony formation of CRC organoid cells under unfavourable conditions. Collectively, our results with the organoid system identify Apc mutation and collagen deposition as key factors for increasing EV release from tumors and they highlight the importance of fibroblast-derived EVs in CRC.

This work was funded by OTKA-NN 118018 and by the National Competitiveness and Excellence program NVKP_16-0007 (National Research, Development and Innovation Office, Hungary).

O-41

Disruption of primary cilia function initiates region-specific craniofacial defects

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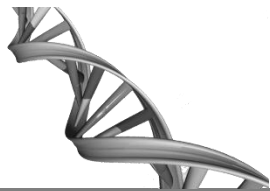
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Primary cilia play an important role during craniofacial development and several ciliopathies exhibit defects in oral structures. Interestingly, there are common differences in the level and appearance of phenotype in the rostral and caudal area of the jaw.

Here, we analyzed mouse deficient in Tmem107, which is an essential factor for primary cilia formation and SHH signaling during embryonic development. Craniofacial mesenchyme exhibits decreased cilia number and their abnormal morphology. Detailed analysis of primary cilia revealed region-specific changes in ciliary morphology. Also teeth phenotype exhibited region-specific features. Most prominent phenotype was observed in the rostral jaw area, where absence of upper incisors was accompanied with normal morphology in molars. Lower incisors display smaller size and in some cases there were completely missing. However, what determines the different response to alteration in cilia



signaling in the molar and incisor area is not known and we decided to uncover this feature in the recent study.

First, we analyzed differences in gene expression profiling by microarray analysis of early mandible. This microarray screen revealed significant differences in WNT signaling with its most prominent upregulation in the rostral part of the jaw. Based on this results, we proposed the WNT gradient in the jaw determines the primary cilia sensitivity.

To test this hypothesis, we established primary mesenchymal cultures separately from the rostral and caudal areas of the jaw and analyzed possible differences in the primary cilia size and morphology between these cells. Then, we treated cells from both areas with WNT3a or WNT5a for 24h to reveal cell response to canonical or non-canonical signaling.

In conclusion, based on our preliminary results, we propose the specific role of non-canonical WNT signaling in primary cilia function, which can provide insight into the causations of region-specific response of individual craniofacial structures in ciliopathic models.

This study was supported by the Czech Science Foundation (17-14886S) and by the Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/15_003/0000460 to MB and CZ.02.2.69/0.0/0.0/16_027/0008502 to MH).

O-42

Epigenetic role of ascorbate compartmentalization in human diseases

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Ascorbate requiring Fe²⁺/2-oxoglutarate dependent dioxygenases located in the nucleoplasm have been shown to participate in epigenetic regulation of gene expression *via* histone and DNA demethylation. Transport of dehydroascorbic acid is impaired in the endomembranes of fibroblasts from arterial tortuosity syndrome (ATS) patients, due to the mutation in the gene coding for glucose transporter GLUT10. We hypothesized that altered nuclear ascorbate concentration might be present in ATS fibroblasts, affecting dioxygenase activity and DNA demethylation. Therefore, our aim was to characterize the subcellular



distribution of vitamin C, the global and site-specific changes in 5-methylcytosine and 5-hydroxymethylcytosine levels and the effect of ascorbate supplementation in control and ATS fibroblast cultures. Diminished nuclear accumulation of ascorbate was found in ATS fibroblasts upon ascorbate or dehydroascorbic acid addition. Analyzing DNA samples of cultured fibroblasts from controls and ATS patients, lower global 5-hydroxymethylcytosine level was found in ATS fibroblasts, which could not be significantly modified by ascorbate addition. Investigation of the (hydroxy)methylation status of specific regions in six candidate genes related to ascorbate metabolism and function showed that ascorbate addition could stimulate hydroxymethylation and active DNA demethylation at PPAR- γ gene region in control fibroblasts only. Altered DNA hydroxymethylation pattern in patient cells both at global level and at specific gene regions accompanied with decreased nuclear accumulation of ascorbate suggests the epigenetic role of vitamin C in the pathomechanism of ATS. The present findings represent the first example for the role of vitamin C transport in epigenetic regulation suggesting that ATS is a compartmentalization disease.

Keywords: *ascorbate/dehydroascorbate, DNA demethylation, glucose transporter GLUT10, arterial tortuosity syndrome, compartmentalization*

O-43

Loss of transglutaminase 2 sensitizes for diet-induced obesity-related inflammation, insulin resistance and hepatic steatosis

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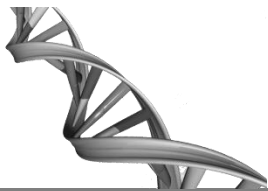
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Transglutaminase 2 (TG2) is a multifunctional protein. Among many others, it contributes to the clearance of apoptotic cells acting as integrin $\beta 3$ coreceptor. Increasing amount of evidence indicates that defective clearance of dying cells can contribute to the development of chronic inflammatory diseases. Obesity is characterized by accumulation of dead adipocytes and inflammatory macrophages in the adipose tissue leading to obesity-related metabolic syndrome. Here we report that loss of TG2 from bone marrow derived cells sensitizes for high fat diet induced obesity-related pathologies. Surprisingly, TG2 null macrophages clear dying adipocytes more efficiently than the wild type ones, but they



express more integrin $\beta 3$ and consequently are more inflammatory. Anti-inflammatory treatment with an LXR agonist reverts the HFD-induced phenotype in mice lacking TG2 in bone marrow derived cells with less hepatic steatosis than in wild type mice. Interfering with inflammation and enhancing dead adipocyte clearance might be beneficial in obesity.

O-44

Pattern recognition receptors and inflammasome activation in cerebral endothelial cells and pericytes

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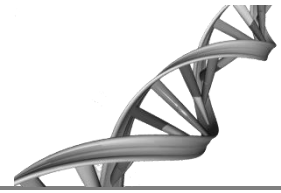
Neuroinflammation is a common characteristic of the majority of central nervous system (CNS) diseases and ageing as well. Most prominent cells of the neurovascular unit (NVU) involved in inflammatory responses are microglia, astrocytes and neurons, but cerebral endothelial cells (CECs) and pericytes can also participate in this reaction.

Chronic inflammation can be mediated by increased levels of damage-associated molecular patterns, which activate pattern recognition receptors (PRRs). PRRs, such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs) may be expressed not only in cells of the innate immune system but also in other cells, including cells of the neurovascular unit (NVU).

Therefore, we systematically tested pattern recognition receptor expression of CECs and brain pericytes. In our studies we detected expression of several TLRs and NLRs in both CECs and pericytes and these receptors were highly regulated by different factors including inflammatory stimuli or oxidative stress. Moreover, CECs and pericytes are able to assemble functional inflammasomes and thus produce active IL-1 β as well. We could show that activation of inflammasomes in CECs occurs by the canonical pathway, whereas in pericytes the activation occurs by the non-canonical pathway. Inflammasome activation has serious consequences on the functionality of the BBB.

Our findings demonstrate that pattern recognition receptors and inflammasomes can be activated in endothelial cells and pericytes of the brain. Cells of the NVU might have an important and well-controlled regulatory role in neuroinflammation.

Keywords: *pericytes, cerebral endothelial cells, inflammasomes*



O-45

Cell cycle arrest due to proteostasis decline in aging yeast cells

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Loss of proteostasis and cellular senescence are two key hallmarks of cell aging, but their cause-effect relationships are still unknown. Here we show that most yeast cells arrest in G1 before death with low nuclear levels of cyclin Cln3, a key activator of Start extremely dependent on chaperone status. The G1 arrest correlates with aggregation of a chaperone reporter and chaperone availability is seriously compromised in aged cells. A mathematical model integrating autocatalytic protein aggregation and a minimal Start network recapitulates empirical observations. Notably, Cln3 overexpression increases lifespan in a chaperone-dependent manner and, as also predicted, inducing protein aggregation in young cells decreases chaperone mobility and nuclear levels of Cln3 causing a G1 delay. Moreover, lifespan shortening by enforced protein aggregation is suppressed by increased expression of specific chaperones or cyclin Cln3. Overall, our data configure a molecular pathway whereby proteostasis collapse acts as a direct effector of cell senescence.

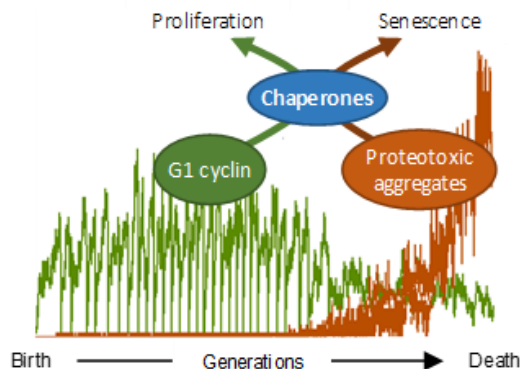
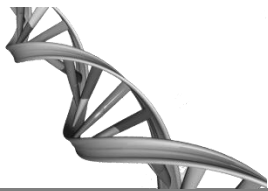


Figure 1: By compromising chaperone availability, proteostasis deterioration would exclude cyclin Cln3 from the nucleus and, as a direct consequence, drive the cell into senescence

Keywords: ageing, cell cycle, systems biology, mathematical modelling, chaperons, microfluidics



O-46

ChIPSummitDB: a comprehensive database of human transcription factor binding sites and the topological arrangements of transcription factor complexes on the DNA

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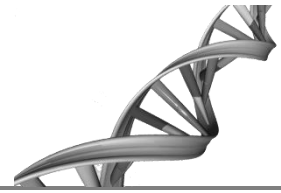
Transcription factors (TF) are proteins, which recognize specific regions on the genome and influence the expression of corresponding genes. The role of these factors can be investigated by analysing ChIP-seq experiments. ChIP-seq is powerful technique for genome wide measurement of transcription factor binding sites. In our previous studies, we showed (Nagy et al. BMC Genomics, 2016) that the exact positions of DNA binding proteins on the DNA can be extracted from the ChIP-seq data by identifying the peak summit positions. These summits correlate with the accurate contact positions of the proteins on the DNA and can be used to determine the topological arrangements of the binding proteins relative to the strand specific transcription factor binding sites.

Our goal was to create a genome wide transcription factor binding site database with combination of the JASPAR non-redundant TF binding profile set and data from bioinformatical analysis of more than 3700 ChIP-seq sequencing data downloaded from SRA database. The data are uniquely processed, which makes the result comprehensive. The result of the data processing is not only a transcription factor binding site (TFBS) database, but complete pipelines for deep ChIP-seq analysis. The pipelines automatize the data collection from SRA database, proper naming of experiments, basic ChIP-seq analysis (peak, summit prediction), binding site prediction with motif optimization (identification of real transcription factor binding sites) and distance measurement for topological data extraction.

	Processed ChIP-seq data	Transcription factor ChIP-seq	Cofactor ChIP-seq data	Number of all peaks	Genome coverage of peaks (bp)	Identified motif number	Coverage of motifs (bp)
Human	3782	2921	861	93 445 309	1,04 Gbp	5 532 773	40,8 Mbp

Table 1: Quantity information of processed data on the ChIPSummitDB

The database contains position information (distance information measured in base pair) about the surrounding ChIP-seq summits (targeted different factors from several cell lines)



around each identified transcription binding sites. Our data is freely downloadable and viewable.

The ChIPSummit database has an intuitive web interface with six different views (Motif, PairShift, VennDiagram, Experiment, Genome and SNP) and is freely available for browsing at the following address: <http://summit.med.unideb.hu/summitdb/>.

Keywords: *chip-seq, transcription factor, database*

O-47

Identifying driver regions in hypermutated cancer genomes

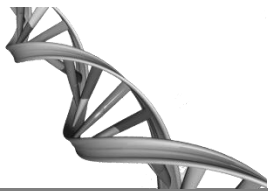
Borbála Hajdu-Soltész, Bálint Mészáros and Zsuzsanna Dosztányi

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Cancer is a genetic disease arising largely from the accumulation of somatic mutations. Cancerous genomes can contain thousands of mutations, although only a few key mutations drive tumour development directly by conferring selective advantage to cancerous cells. The identification of the driver genes that contain these key mutations has been enhanced by the improvement of whole genome (or exome) sequencing technologies. Nevertheless, discriminating driver mutations from randomly occurring passenger mutations remains challenging, especially in the case of hypermutated samples (Watson et al., 2013).

In a recent analysis, we identified driver genes by combining cancer samples with low mutation rate collected from the COSMIC and the TCGA databases (Mészáros et al., 2016). The identification of driver genes was based on the iSiMPRe method. This method takes advantage of the observation that driver mutations are not evenly distributed throughout the genome, instead they accumulate at specific sites within genes. This approach therefore not only identifies drivers but also points out the functionally important sites in the encoded protein that are important for cancer. By restricting our analysis to samples with low mutation rate helped to increase the signal/noise ratio by filtering out samples with very high rate of neutral passenger mutations.

In this work now, we seek to extend our analysis to hypermutated samples. In order to address the higher rate of neutral passenger mutations we utilize mutational signatures. Signatures are the imprints of distinct mutational processes, which prefer different channels of mutation (Alexandrov et al., 2013). For many signatures, the underlying mutagen factor or process is already known, e.g. exposure to UV radiation or DNA mismatch repair deficiency. In this work we group the samples by signature types, and identify significantly mutated regions within these clusters of samples. Here, we extend the list of driver genes and regions to hypermutated samples and show interesting examples for mutational mechanisms by which driver genes can contribute to cancer development. We also address the connection of mutational processes to the generation of neoantigens which can trigger immune response against cancerous cells.



Keywords: *bioinformatics, cancer drivers, mutated regions, signatures*

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O-48

Linking the resistome to the microbiome in complex microbial communities

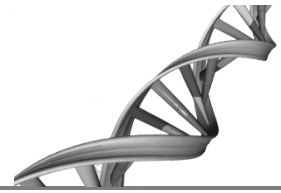
Lajos Kalmár, Srishti Gupta, Iain R.L. Kean, Xiaoliang Ba, Liz Lay, Ruby Coates, Andrew J. Grant and Mark A. Holmes

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Bacterial infectious diseases contribute to more than 54% of global infectious disease and the ever-increasing multitude of antimicrobial resistant (AMR) pathogens is of global concern for animal and public health. Acquired resistance traits are often located on mobile genetic elements (MGEs) thereby increasing the chance of their horizontal transfer. Next generation sequencing (NGS) has substantially increased our understanding of the microbiome, but current NGS analysis fails to accurately identify the location of specific genes of interest within any single host genome of a mixed community. Chromosome conformation capture and its derivative Hi-C are powerful techniques that allow the physical crosslinking of prokaryotic genomes and the associated MGEs. This study aims to demonstrate AMR gene identification within complex microbial communities, and link these elements to their host bacteria, without culturing and the usage of known reference sequences.

In this study we processed 100 faecal samples from 5-5 British conventional and organic pig farms (10 samples/farm) and performed shotgun metagenomics and Hi-C sequencing on them in parallel. De novo metagenomic assemblies were constructed from the processed NGS data, followed by contig binning based on the pairwise linkage information from the Hi-C read pairs. The end products of the bioinformatics pipeline are the core-communities (CCs), collection of contigs that were physically close to each other, potentially in the same cell. We calculated pairwise genetic distance between all final big CCs (>500kbase sequence/community, n=3590), defined clades from closely related CCs, identify AMR genes using ResFinder database, and try to speciate the clades by using Metaxa, Mash and CheckM software.

We showed, that combination of traditional metagenomics sequencing with Hi-C chromosome capture linked AMR genes (on mobile genetic elements) successfully, and with



high reliability to the host genome. The developed laboratory and bioinformatics frameworks represent the first published, sustainable, culture independent and high-throughput solution to reliably associate antibiotics resistance to host species in complex microbial communities.

In the 100 pig faecal samples, we found 139 different AMR genes, categorised in 14 main- and 41 sub-categories based on the antibiotics family they provide resistance against and the molecular mechanism respectively. Several AMR genes were represented on both the conventional (high antibiotics usage) and organic (low antibiotics usage) farms, but we found significantly more AMR genes and AMR gene categories on the conventional farms, highly correlating with the amount of used antibiotics.

Keywords: *metagenomics, antibiotics resistance, Hi-C, bioinformatics*

O-49

Fast and sensitive detection of pathogens from high throughput sequencing data

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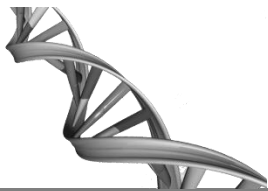
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Whole Genome Shotgun Sequencing is a more and more standard approach for characterizing the bacterial communities in environmental samples. These methods do not only help to gain insight into the microbiome in a culture-independent way and help us to explore the species composition of the community but even makes it possible to detect novel, unknown pathogens or resistance genes. However, accurately and sensitively detecting such pathogens, especially the novel clones in WGS data is a challenging task.

We have developed a taxonomic binning algorithm, partly based on fast, short read aligner programs i.e. Bowtie2/BWA, and a dedicated, non-redundant nucleotide microbial database. The program takes advantage of the lowest common ancestor principle, where each read is mapped to a taxon in the taxonomic tree, covering the most significantly hit taxa.

This approach proved to be an efficient strategy to balance between sensitivity and running time. The performance of the pipeline was evaluated on Mock community sequencing data as well as on synthetic and experimental data.

Our method in some cases outperformed the state-of-the-art BLAST-based methods or performed as good as those, however it runs two magnitudes faster. Furthermore, it was able to detect accurately a taxon being present only in small abundance, even in cases where the genome is unknown and not present in the database. To investigate such scenarios we analyzed a well-characterized *B. anthracis* strain with known genome and an unknown



Japanese isolate (*B. anthracis* strain BA104). The majority of the reads (96.50%) was assigned to *Bacillus anthracis* and only 1.2% of the reads were classified as other species from the *Bacillus* genus.

Here we present that it is feasible to evaluate high-throughput sequencing data in a reasonable time with good classification accuracy.

Acknowledgments: *Funded in part by Hungarian government grants OTKA 120650, EFOP-3.6.2-16-2017-00013 (supported by the European Union and co-financed by the European Social Fund) and ED_17-1-2017-0009 (within the National Bionics Program).*

Keywords: *metagenomics, taxonomy binning, pathogens, microbiome, b. anthracis*

O-50

Defining transmembrane regions using cryo-EM maps: the MemBlob database and server

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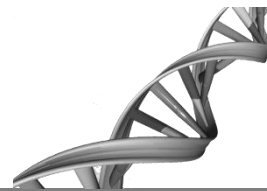
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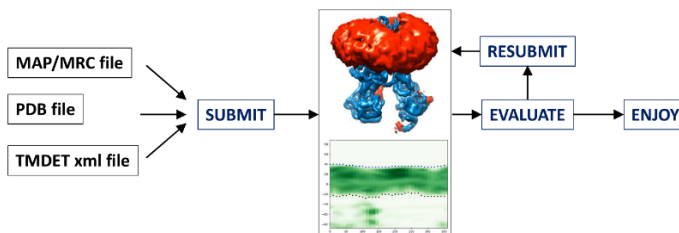
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* equally contributed

Transmembrane (TM) proteins play an important role in many cellular processes and are highly significant drug targets. In order to understand TM protein function and develop novel therapies targeting TM protein associated diseases, it is crucial to define the localization of transmembrane regions. While experimental data on the boundaries of membrane embedded regions are sparse, this information is present in cryo-electron microscopy (cryo-EM) density maps and it has not been utilized yet for determining membrane regions. We developed a computational pipeline, where the inputs of a cryo-EM map, the corresponding atomistic structure, and the potential bilayer orientation determined by TMDET algorithm of a given protein result in an output defining the residues assigned to the bulk water phase, lipid interface, and the lipid hydrophobic core. Based on this method, we built a database involving published cryo-EM maps with a resolution better than 4 Å and also a web application to allow the analysis of unpublished densities. Unlike previous approaches, our method presents the membrane region as a volume with boundaries that follows the shape of the lipid environment, not as a slab with parallel edges. Furthermore, the information we extracted is the first data set at atomic resolution that allows the comparison of *in silico* predictions with experiments.



The database and server is available at <http://memblob.hegelab.org>.



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Keywords: membrane protein structure, transmembrane region detection, cryo-electron microscopy

O-51

A new modulator of the synthetic activity of Polymerase eta

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DNA Polymerase eta (Pol η) is a low fidelity translesion synthesis polymerase that rescues damage-stalled replication by inserting deoxyribonucleotides opposite DNA damage sites resulting in error-free or mutagenic damage bypass.

Previously we found that Pol η of *Saccharomyces cerevisiae* is required for the efficient transcription of several genes *in vivo*, and it is enriched over actively transcribed regions. We could also show that strains deficient in Pol η exhibited defects in the elongation step of transcription. Moreover, we found evidence that the polymerase activity of Pol η is required for its transcription-associated function. We demonstrated that Pol η is able to extend RNA primers by incorporating ribonucleotides (rNTPs) *in vitro*. These reactions are an order of magnitude more efficient than the misinsertion of rNTPs into DNA. Furthermore, during RNA extension, Pol η bypasses the 8-oxoguanine and thymine dimer DNA lesions in an error-free manner. However, we also found that besides rNTPs, Pol η could insert dNTPs into RNA as well, with similar efficiencies. Therefore, we aimed to find cofactors that would enhance the RNA synthetic activity of Pol η . Here we show that we have identified a cofactor that increases the affinity of Pol η towards ribonucleotides, and augments the RNA synthetic activity, while preserving its fidelity. Even the DNA damage bypass activity of Pol η is increased $\sim 10^3$ - fold during RNA extension.

Our results demonstrate that certain conditions could change the preference of Pol η from using dNTP during extension reactions to rNTP insertions.



O-52

***Saccharomyces cerevisiae* Mgs1 protein contributes to G-quadruplex replication**

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The basic criterion for staying alive for any living organism is the preservation of the genomic integrity, therefore the greatest challenge in this process is the correct replication of the genetic information during cell division. In order to create an accessible template for the replication process, single-stranded DNA is formed by the unwinding of the parental double-stranded DNA. Since single-stranded DNA sequences often form stable secondary structures, which represent replication blockades, for complete replication, cell division, and for the prevention of apoptosis and cell death these structures have to be resolved.

One of the most studied blocking DNA structure is the G quadruplex (also called G4), which in the recent years has emerged as a key regulatory *in vivo* element in essential cellular processes, like transcription, translation, replication and recombination.

The replicative polymerases are primarily blocked by the stable secondary structure-forming DNA sequences, therefore, the cooperation of DNA helicases and DNA polymerases is needed for replication. Some key players of this process are already known, recently it has been described that the mutation rate of G4 sequences increases in the absence of the yeast Pif1 or human FANCD1 protein. In order to gain more information about this complex process we searched for uncharacterized players of G4 replication. Here we report the genetic and biochemical characterization of a novel *Saccharomyces cerevisiae* G quadruplex binding protein, the Mgs1.



O-53

Capturing the transient profile of uracil accumulation in human genomic DNA by super-resolution microscopy and genome-wide uracil-DNA sequencing analysis

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Non-canonical bases escape detection by usual sequencing methods, prohibiting the true understanding of the accurate chemical composition of genomic DNA. Importantly, several such bases play substantial roles in epigenetic processes. Base-specific chemical- or antibody-based detection methods have been reported for several of those non-canonical bases. However, no straightforward method has yet been published for description of endogenous uracil distribution patterns in human DNA. Using a catalytically inactive uracil-DNA glycosylase (UNG) [1] enzyme equipped with different tags, we developed a versatile approach that allows for the first time *in situ* visualization of uracil in cells with elevated uracil-DNA level by both conventional light microscopy and by super-resolution microscopy. Importantly, the approach also allows efficient pull down of uracil enriched genomic segments, providing genome-wide mapping of uracil-DNA distribution patterns. Comparison of U-DNA-Seq data with other ChIP-seq databases show preferential localization of uracil within heterochromatic regions. Co-staining of genomic uracil and different epigenetic markers via dSTORM microscopy reinforced our findings based on whole-genome sequencing data. The presented approach can be readily applied to study the dynamic spatio-temporal nature of uracil content of the genome.

Funding: EMMI FIKP-BME Biotechnology grant

Keywords: uracil-DNA detection, super-resolution microscopy, whole-genome sequencing

Reference:

[1] Róna, G. et al., *Nucleic Acids Res.* 2016 Feb 18;44(3):e28



O-54

Gene targeting of dUTPase in mice leads to early embryonic lethality

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dUTPase is a key enzyme for maintaining DNA integrity by preventing misincorporation of uracil into DNA, which results in DNA toxicity and cell death. dUTPase has an important function in dTTP formation also. It is encoded by the *dut* gene.

Despite its physiological significance, dUTPase gene targeting experiments have only been performed in unicellular organisms, such as bacteria and yeast.

To explore exact functional roles of the enzyme during early mammalian development we have generated a CRISPR/Cas9-mediated dUTPase knock-out in mice. Heterozygous *dut* +/- animals are viable and fertile with decreased dUTPase level.

Homozygous *dut* -/- embryos can develop till blastocyst stage but die *in vivo* shortly after implantation. Blastocyst stage embryos show decreased cell number both in the inner cell mass and trophoctoderm. It seems that dUTPase enzyme has an indispensable role in embryonic development.

Additional combined knock-out experiments that may potentially rescue the *dut* -/- phenotype are in progress to address and analyze the *dut* -/- phenotype.

Keywords: CRISPR/CAS9, dUTPase, embryonic lethality

**O-55****Molecular mechanism of legitimate DNA homology sensing by RecQ helicase****Gábor M. Harami¹, Yeonee Seol², Keir C. Neuman² and Mihály Kovács^{1,3}**

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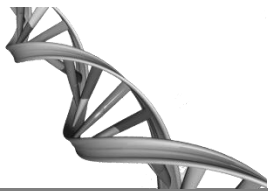
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Homologous recombination (HR) is an essential molecular process for all cells as it allows error-free repair of DNA double strand (ds)-breaks and stalled replication forks. Also, in meiotically dividing cells, HR is required for faithful chromosome segregation and generation of genetic diversity. During HR, single stranded (ss) DNA regions are generated by resection of dsDNA and covered by recombinase enzymes. The recombinase nucleoprotein filament can search for homologous dsDNA segments and form a displacement loop (D-loop) DNA structure through a strand invasion reaction. Progression of HR occur through enzymatic processing of the D-loop. Strand invasions mostly occur between allelic, "legitimate" DNA regions; however, D-loops can also be formed between non-allelic DNA segments through partial homologies, leading to illegitimate HR with detrimental effects for genome stability. Ultimately, illegitimate recombination must be avoided by the cell, but the underlying mechanisms are unknown.

Previously we showed that *E. coli* RecQ helicase (member of the RecQ family, present in both bacteria and eukaryotes) is a key player in the quality control of bacterial HR as the enzyme is able inhibit illegitimate recombination via DNA-geometry dependent unwinding activities. RecQ binds to D-loops in an orientation that leads to unwinding of the strand invasions. This orientational binding bias, along with the complex unwinding activity pattern of RecQ, could allow selective disruption of illegitimate D-loops over legitimate ones.

Here we propose a mechanistic model of how RecQ can discriminate between illegitimate and legitimate strand invasions based on a combination of single molecule magnetic tweezers and ensemble single turnover rapid kinetic experiments. We show that the so-called HRDC, auxiliary ssDNA binding domain of RecQ stabilizes the intrinsic DNA sequence-dependent pauses of the helicase core during unwinding. Ultimately, this leads to a non-linear amplification of transient pauses by the controlled ssDNA binding of the HRDC domain. In other words, the helicase will stop unwinding for exponentially longer periods in relation of the increasing stability of a small dsDNA region ahead of the helicase.

Importantly, this mechanism can serve as a sequence homology sensor that allows rapid disruption of partially homologous (illegitimate) and stabilization of extensively homologous (legitimate) DNA strand invasions. We also observed a similar HRDC domain-dependent



unwinding activity for the human Bloom's syndrome helicase (RecQ family member), suggesting that these activities are conserved in eukaryotes to safeguard genome stability.

Keywords: *DNA-repair, single molecule biophysics, DNA unwinding*

O-56

Analysis of the mutagenic effect of platinum-based chemotherapeutic agents in cell line based model system

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Cytotoxic drugs have been in use for cancer therapy since the 1950s, and remain the first line treatment for most cancers today. These drugs inhibit cell proliferation through a range of different mechanisms, including directly damaging DNA. Successful treatments kill tumour cells, but also exert side effects attributable to a number of factors including the inhibition of cell proliferation in healthy tissues. Genomic mutations caused by cytotoxic agents used in cancer chemotherapy may cause secondary malignancies as well as contribute to the evolution of treatment resistant tumour cells. The most widely used cancer chemotherapeutics are the platinum-base agents, namely cisplatin and its derivatives, carboplatin and oxaliplatin.

Our main goal was to measure the mutagenicity of selected commonly used chemotherapeutic agents that cause DNA damage or inhibit DNA repair. In the presented experiments we treated human (TK6) and chicken (DT40) lymphoblast cells using the IC75 and IC50 concentration of the platinum-based antineoplastic drugs cisplatin, carboplatin and oxaliplatin, as measured by cytotoxicity- and colony survival assays. These agents cause crosslinking of DNA as monoadduct, interstrand, intrastrand or DNA-protein crosslinks. We analyzed γ H2AX DNA damage marker in these cells by immunoblotting and immunofluorescent assay. The mutation rates were determined by next-generation sequencing (Illumina HiSeq X Ten platform) of the whole genomes of treated cell clones. The mutagenic spectra of the tested agents were analysed using the newly designed IsoMut mutation detection tool. We found that cisplatin was considerably mutagenic and induced both single nucleotide variations and short insertions / deletions. The detailed analysis of the sequence context of the mutations showed that most occur at cisplatin-induced intrastrand DNA crosslinks, typically at GG or AG dinucleotides. At equitoxic concentration, carboplatin and oxaliplatin treatments caused a lower number of mutations than cisplatin, although induced similar mutational spectra.

Our data show that cisplatin induces a far greater number of mutations in chicken DT40 BRCA1 or BRCA2 deficient cells than in a matched wild type cell line. Furthermore, we found two of the newly defined cisplatin-specific mutation types as causes of published cases



of the reversion of BRCA2 mutations in emerging cisplatin-resistant tumours or cell clones. Our results suggest genetic reversion due to treatment-induced mutations as a distinct mechanism for developing resistance.

This study is supported by National Research, Development and Innovation Office (NKFIH) 121381 OTKA-PD grant.

Keywords: *cisplatin, carboplatin, oxaliplatin, DNA damage*

O-57

A neglected post-translational modification: extracellular O-glycosylation

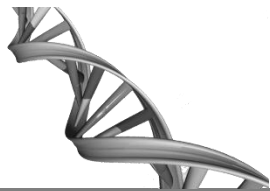
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Genomic and transcriptomic information might not be not sufficient for understanding cellular processes. Sooner or later researchers have to cast a wider net, and turn to other ‘omics’. Proteomic studies in general aim to describe the ‘normal’ composition of protein complexes, cell organelles, tissues or body fluids, and then to detect the changes occurring as the result of certain stimuli, or diseases. Such research produced impressive results, but we still have limited knowledge about the occurrence and changes of post-translational modifications (PTMs) that most certainly are significant players in the regulation of complex-formation, protein-protein interactions, and biological activity. In addition, PTMs that modify proteins located in the extracellular space have been neglected for a long time despite the ever growing evidence that i) they are really important for our wellbeing ii) they may influence/control/regulate intracellular processes.

Our research focuses on the Golgi-derived glycosylation of Ser, Thr and Tyr residues. These modifications are exceptionally hard to tackle, but their biological role(s) – at least what we know about them – are really exciting. We are developing protocols for the enrichment of modified peptides from readily available body fluids and then use reversed phase nanochromatography linked to high sensitivity tandem mass spectrometry to analyze the resulting mixtures. We are gathering data on the proteins and the sites modified. Presently we work with urine samples of healthy volunteers and bladder cancer patients. We have mined the urinary O-glycoproteome at an unprecedented level: we identified unexpected structures on secreted glycoproteins, such as disialic units, mono- and di O-acetyl sialic acids, as well as different blood-type antigens that have never been linked to an unambiguously identified site in a particular protein before [1]. We do hope that our basic research project will lead to translational results.



Keywords: PTM, glycosylation, mass spectrometry

References:

[1] Extended Sialylated O-Glycan Repertoire of Human Urinary Glycoproteins Discovered and Characterized Using Electron-Transfer/Higher-Energy Collision Dissociation. Darula Z, Pap Á, Medzibradský KF. *J Proteome Res.* 2018 Nov 19. doi: 10.1021/acs.jproteome.8b00587.

O-58

Complex proteomics and metabolomics examination of wound healing markers in glaucoma patients following trabeculectomy

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The most important form of glaucoma treatment is the reduction of high intraocular pressure. Patients are first treated with eye drops but when the desired effect cannot be reached surgery is required. The gold standard surgery is trabeculectomy with relatively high complication and failure rate. Here I will present the results of a complex proteomics and metabolomics strategy applied to study the protein profile changes in the tear and aqueous humour (AH) collected from patients who underwent trabeculectomy in order to better understand the phenomena leading to the appearance of complications.

First we compared tear to AH and observed that they are not identical from the inflammatory point of view, however, tear can be used instead of AH for the follow-ups. At the same time we could identify tear IL5, IFN gamma and GMCSF as potential predictive biomarkers for the appearance of complications in the preoperative tear samples.

The time-course of the tear protein profiles was examined using proximity extension assay (PEA). PEA allows for a sensitive and scalable analysis of multiple proteins in a single run from a 1 µL sample, so we applied this technique and examined the amount of 184 proteins in tears collected at different time points after trabeculectomy. Patients with or without complications were tested, and proteins that have roles in the immune response and wound healing could be observed with altered frequency and amounts in the cases of patients with complications. Our results highlight the importance of inflammation in wound-healing complications, and at the same time, indicate the utility of PEA in tear analysis.

Based on our results, the most important events in the appearance of late complications are related to the early phases of ocular wound healing when the proinflammatory processes dominate. Most probably a shifted pro - antiinflammatory balance along with the change in the amount and abundance of proteins having role in wound healing leads to an altered wound healing starting in the first few postoperative days and finalizing with the appearance of late (1 year) complications.



Further verification and the extension of the study with a higher number of recruited patients is required in order to validate our data and possibly identify new diagnostic and therapeutic target proteins.

The work was funded by Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences, OTKA PD116817, PD121075 and GINOP-2.3.3-15-2016-00020.

Keywords: *proximity extension assay, glaucoma, tear, wound healing, inflammation*

O-59

Proteomics and genomics studies on pathological and physiological functioning of the nervous system

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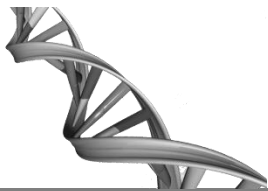
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There is no causal therapy of brain diseases but the number of patients exceeds several Millions which generates enormous social pressure to science. Drugs used in the medical practice bind the same 6-7 receptors in each disease inducing numerous side effects and the number of non-responding patients is high. We need new targets and novel ideas but literature search points to the same 6-7 receptors. It was the reason for us to initiate proteomics studies of neuronal processes and animal models of brain diseases. In 2006 and recently we started single cells sequencing based transcriptomics. The explorative work run in the laboratories of ELTE Proteomics Laboratory, the MS studies were done in the laboratories of SZTE, and MTA. On the way of our studies we revealed proteomics changes in the brain induced by oestrogen dependent cytoprotection, we studied changes in the suicide brains of human subjects and under sleep waking cycle. We obtained proteomics data on differences in synaptic and non-synaptic mitochondria in synaptosomes and in MAM region in Alzheimer disease animal model (APP/PS1 mice). Analyzing the proteome changes in C1q tagged synapses prepared for selective pruning and revealed a local apoptotic process in them. Using single cell transcriptomics we disclosed transcriptome level differences between pyramidal and fast spiking cells of the prefrontal cortex and suggested several novel targetable molecules in ion-channels and receptor subunits. We introduced the DIGE



proteomics in Hungary and we added protein network analysis based interpretative methods in proteomics analysis of hereditary diseases. We trained several PhD students for making proteomics and genomics methods. The lecture is a review of 13 years work in proteomics.

O-60

Analysis of the N-glycosylation of the HeLa cell lysate

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Glycosylation is one of the most abundant post-translational modifications present in humans. It contributes greatly to the dynamic changes and the fine tuning of protein functions, thus it is potentially prognostic of pathophysiological conditions.

The analysis of glycoproteins in biological samples is a challenging task. Routine glycoproteomics approaches involve an enrichment step, then proteolytic digestion, followed by de-glycosylation, the analysis of components with HPLC-MS/MS, and the data assessment via database search. However, during de-glycosylation the site-specific information is lost, and during MS/MS analysis many minor components are overlooked.

HeLa cell lines are commonly used for studying cancer, and are also among the most common reference standards for proteomic research. Although they have been extensively studied, there is very little available on protein glycosylation in HeLa cells. Our aim was the determination of the glycosylation pattern of the proteins in a commercially available HeLa cell lysate. This will be useful both for studying cancer, and will likely be used as a standard for future research.

Here we introduce a novel workflow, which combines a glycopeptide enrichment and LC-MS analysis; the latter involving structural analysis using data dependent and energy resolved MS/MS, and quantitative analysis using MS1. This provides both excellent sensitivity, and reliable structure assignments.

Using this method, we identified over 50 glycoproteins, more than 70 glycosylation sites, and 200 distinct glycoforms. Among these we have identified both core- and antenna-fucosylated glycoproteins, complex, high-mannose and hybrid N-glycosylated structures using special low-energy MS/MS experiments. This presents the first detailed, protein- and site-specific glycosylation analysis of HeLa cell lines.



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Keywords: glycoproteomics, HPLC-MS/MS, HeLa cell lysate

O-61

Quantitative proteomics at peak: evaluation of novel approaches in mass spectrometry based proteomics

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Proteomics is an emerging field, and its advancement is highly dependent on technical developments. Achievements in instrumentation, new ways of data collection, novel software algorithms help to increase the depth of proteome analysis or shorten analysis time, improve sensitivity, reproducibility or let us use information earlier hidden in our data.

Here we evaluate novel developments in mass spectrometry based proteomics on different sample types and tasks presenting different challenges. Solutions for both targeted and untargeted approaches are discussed. Some of these approaches are generally applicable (eg. specific data independent acquisition modes, retention time standards), some of them are limited to hybrid orbitrap mass spectrometers exploiting instrument specific benefits (eg. Boxcar scanning, spectral multiplexing).

Acknowledgement: This work was supported by the GINOP-2.3.2-15-2016-00060 and 20391-3/2018/FEKUSTRAT projects.

Keywords: proteomics, mass spectrometry

O-62

The role of the small GTPases Arl8 and Rab7 in crinophagic degradation

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In the beginning of metamorphosis, *Drosophila* salivary gland cells undergo massive glue granule secretion. The exocytosed material helps to attach the forming pupa to a solid surface. A subset of the granules is degraded by direct fusion with lysosomes instead of



exocytosis. This type of secretory granule-specific autophagy is known as crinophagy. Our group has previously described a molecular model for secretory granule-lysosome fusion, including the tethering complex HOPS (Homotypic fusion and protein sorting), the small GTPases Rab2 and Rab7 and a SNARE (Soluble N-ethylmaleimide-sensitive factor attachment protein receptors) complex consisting of Syntaxin13, Snap29, and Vamp7.

Arl8 (Arf-like protein 8) is a lysosomal small GTPase crucial for the anterograde transport of lysosomes in human cells. We have recently found that Arl8 is required for autophagosome-lysosome fusion in the *Drosophila* fat body and it is also needed for the proper function of endolysosomal system in Garland nephrocytes. Here we report that Arl8 is indispensable for degradation of glue granules by mediating secretory granule-lysosome fusion. Similarly to the fat body, we found that the three shared subunits (Blos1, Blos2 and Snapin) of two similar protein complexes, the BLOC-1 (Biogenesis of lysosome-related organelles complex 1) and BORC (BLOC-1-related complex), are also important for this process, most likely by recruiting Arl8 to lysosomes. However, the specific subunits of these complexes did not seem to be necessary for crinophagy. Interestingly, a study on mammalian cells showed that all subunits of the BORC complex is needed for Arl8 binding. Thus, it seems that Arl8-mediated lysosomal fusions are regulated differently in *Drosophila* than in mammals.

Arl8 and Rab7, a small GTPase required for various lysosomal fusions, are known to bind the HOPS complex and we have previously showed that glue granules are positive for Rab7. Here we demonstrate that Mon1/Ccz1, a complex previously described as a guanine nucleotide exchange factor for Rab7, is necessary for Rab7 recruitment to secretory granules, proper crinosome formation and crinophagic degradation in salivary gland. In conclusion, we hypothesize that Rab7 positive secretory granules dock to Arl8 positive mature lysosomes by the HOPS complex and Arl8 is recruited to lysosomes by the act of the shared subunits of BLOC-1 and BORC complexes. Thus, we provide an extended molecular model for secretory granule-lysosome fusion.

Keywords: *crinophagy, Arl8, lysosome, fusion*



O-63

Macrophages engulf apoptotic and primary necrotic thymocytes through similar phosphatidylserine-dependent mechanisms

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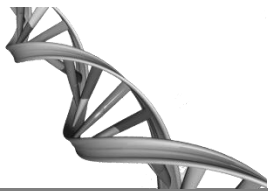
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One of the major roles of professional phagocytes is the removal of dead cells in the body. We know less about the clearance of necrotic cells than apoptotic cell phagocytosis, despite the fact that both types of dead cells need to be cleared together, and necrotic cells appear often in pathological settings. In the present study, we examined phagocytosis of heat- or H₂O₂-killed necrotic and apoptotic thymocytes by mouse bone marrow-derived macrophages (BMDMs) *in vitro*, and found that the two cell types are engulfed at equal efficiency and compete with each other when added together to BMDMs. Phagocytosis of both apoptotic and necrotic thymocytes was decreased by (i) blocking phosphatidylserine on the surface of dying cells; (ii) inhibition of Mer tyrosine kinase, Tim-4, integrin $\beta 3$ receptor signaling, or Rac1 activity; or (iii) using BMDMs deficient for transglutaminase 2. Stimulation of liver X, retinoid X, retinoic acid or glucocorticoid nuclear receptors in BMDMs enhanced not only apoptotic, but also necrotic cell uptake. Electron microscopic analysis of the engulfment process revealed that the morphology of phagosomes and the phagocytic cup formed during the uptake of dying thymocytes is similar for apoptotic and necrotic cells. Our data indicate that apoptotic and necrotic cells are cleared via the same mechanisms, and removal of necrotic cells *in vivo* can be facilitated by molecules known to enhance the uptake of apoptotic cells.

This study was supported by the National Research, Development and Innovation Office (124244), by the GINOP-2.3.2-15-2016-00006 project and EFOP-3.6.3-VEKOP-16-2017-00009 (co-financed by the European Union and the European Regional Development Fund), and University of Debrecen (RH/751/2015). Zsolt Sarang was a recipient of Lajos Szodoray fellowship given by the University of Debrecen.



O-64

Drosophila Sec20 regulates autophagy and endocytosis independently of the Golgi-to-ER retrograde transport

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Sec20/BNIP1 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 1) is a SNARE protein first described in yeast as an endoplasmic reticular SNARE. It mediates the fusion between the ER and retrograde vesicles from the Golgi. Other studies have also suggested that it may have a role in many other cellular functions including autophagy, ER homeostasis and apoptosis. As the role of Sec20 in autophagy (and other lysosomal pathways) has yet to be elucidated we used *Drosophila melanogaster* as an *in vivo* model organism. Starved larval fat cells and garland nephrocytes were used to detect any defects in autophagy or endocytosis, respectively. We found that Sec20 not only regulates autophagy, but it surprisingly has a role in endocytic transport as well. Ultrastructural analyses revealed abnormal lysosomal compartment and dilated ER in both fat cells and nephrocytes. Moreover, the latter contained enlarged late endosomes and was nearly devoid of endolysosomes. As we found that depletion of other SNAREs or tethering proteins (previously shown to regulate Golgi-to-ER retrograde traffic) did not cause any defect in either autophagy or endocytosis, we suggest that Sec20 has a novel role in lysosomal degradation that seems to be independent of Golgi-to-ER transport.

Keywords: *Sec20, BNIP1, autophagy, endocytosis, SNARE*

O-65

Hemocytes regulate selective autophagy during regeneration of germline stem cells in Drosophila

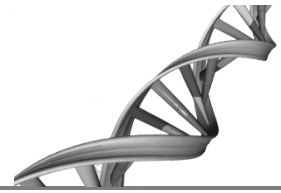
Tibor Kovács¹, Virginia B. Varga¹, Fanni Szikszai¹, Anna Manzéger¹, Viktor A. Billes^{1,2}, Janka Szinyákovics¹, Bernadette Hotzi¹, Gina Puska³ and Tibor Vellai^{1,2}

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Autophagy is a highly conserved lysosome-dependent catabolic process of eukaryotic cells which has an essential role in the degradation of damaged proteins and organelles. It also plays important roles in cellular differentiation and development, by mediating the remodelling of cytoplasmic constituents. In early spermatogenesis of *Drosophila*, there are two different populations of stem cells, the somatic stem cells and the germline stem cells



(GSCs). GSCs divide by asymmetric division to give rise to two distinct daughter cells. One of them will leave the stem cells' niche and start differentiation into spermatogonial cells. Both aging and accumulation of cellular damage can cause the loss of GSCs. Lost stem cells can be replenished by dedifferentiation of spermatogonial cells into functional GSCs.

Autophagy is implicated in tissue regeneration. Furthermore, macrophages provide special conditions for cellular transformation. Here we examined the potential role of autophagy during dedifferentiation, a type of tissue regeneration, and its regulation by immune surveillance cells called hemocytes in *Drosophila*. During GSC regeneration, we detected elevated levels of lysosomal breakdown. In addition, silencing of autophagy-related genes led to the inhibition of this regeneration process. We also observed two different mechanisms of mitochondrial degradation (amphisome- and autophagosome-mediated) in somatic and germline cells during regeneration. Interestingly, we found elevated numbers of hemocytes during dedifferentiation. The immune depletion of hemocytes decreased the regeneration capacity of the germline. We found that autophagic activity in spermatogonial cells is regulated by JAK-STAT signalling emitted by hemocytes. Together, we suggest that lysosomal degradation has an important function in spermatogonial dedifferentiation, and that mitophagy may be critical for GSCs' regeneration.

Keywords: *autophagy, regeneration, dedifferentiation, stem cell, hemocyte, JAK-STAT signalling, Drosophila*

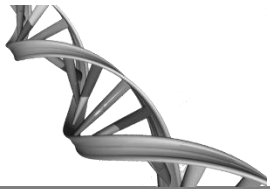
O-66

SNARE protein Ykt6 as swiss army knife in vesicular fusion events

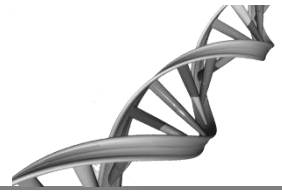
Gábor Glatz, Zsófia Gyetvai, Tamás Csizmadia and Gábor Juhász

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Ykt6 is a highly conserved SNARE protein, which has a critical role in several vesicular fusion events from yeast to human. It has been shown that Ykt6 promotes fusions in ER-Golgi and trans-Golgi pathways as a canonical R-SNARE. Normally R-SNAREs contain a transmembrane domain but Ykt6 is unique as it lacks TM domain and its 'open' and 'closed' state is mediated by palmitoylation. Furthermore, the presence of this lipid anchor enables that Ykt6 is not constitutively localized to the vesicular membranes, resulting in a higher mobility of the protein. We show here that Ykt6 promotes autolysosome formation and crinophagy as well. Knock-down and immunohistochemical methods show that loss of Ykt6 results in fusion defects between lysosomes and the autophagosomes and secretory vesicles in *Drosophila*. We provide evidence that similar to the Vamp7 (the cognate R-SNARE) Ykt6 is also localized to lysosomes. Furthermore, both *in vitro* and *in vivo* binding assays show that it interacts with HOPS and forms functional complexes with Q-SNAREs involved in both autophagy (Syntayin17 and SNAP29) and crinophagy (Syntaxin13 and SNAP29). Thus, our results identify novel fusion capable SNARE complexes in the lysosomal pathway.

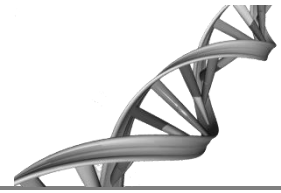


Eger, 29-31 March 2019



POSTER LECTURES





P-001

Ablation of cranial neural crest affects development of the thymus

Krisztina H.-Minkó¹, Emese Huszár¹, Nándor Nagy¹, Imre Oláh¹, Sophie Creuzet² and Ildikó Bódi¹

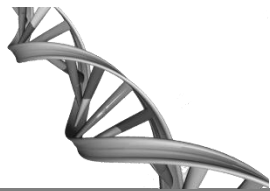
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Thymus is the central organ of the lymphoid system, where the maturation of T cells take place. The epithelial anlage of the thymus develops from the 3rd and 4th pharyngeal pouches which grows into the neural crest derived mesenchyme. The capsule, septae and myoid cells of the medulla have neural crest origin as it was shown by quail-chick chimera experiments. Haemopoietic cells colonise the organ later. According to classic histological description the thymus is separated to cortex and medullary region but anti-cytokeratin immunostaining reveals sub-compartments in the medulla such as keratin positive network (KPN) and keratin negative area (KNA). We revealed the presence of extracellular matrix molecules (fibronectin, tenascin, collagen) in the KNA by immunohistochemistry and concluded that this area contains reticular connective tissue which is in continuation with the connective tissue of the septae (Bódi et al. 2015). These data together with previous results suggest that the KNA of the medulla has neural crest origin.

The aim of our present study is to prove that the keratin negative areas of the thymic medulla have neural crest origin. In the first experiments we ablated unilaterally the part of the neural crest thought to be responsible for the development of the thymus (from the 5th somite level to the 4th rhombomers) in chicken embryos having 10 pairs of somites (HH10, 33-38 hours of development). Operated and control embryos were examined by immunohistochemistry and thymus morphology was compared. In ablated embryos we observed the following phenotype of the thymus: absence of medullary region, enlarged keratin free areas in the cortex with lack of capillarisation. We conclude from these experiments that the morphological abnormalities are due to the lack of neural crest cells which cause abnormal thymus epithelium differentiation.

Keywords: *thymus, neural crest, chicken embryo*



P-002

Beige cells of tissue transglutaminase knock-out mice possess attenuated response to adrenergic agonists

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It has been discovered that cold exposure or adrenergic stimuli can provoke the appearance of clusters of uncoupling protein1-(UCP1)-positive heat producing cells in white adipose tissue (WAT) through the process of browning. These inducible cells have been named 'beige' adipocytes and have an overlapping, but distinct gene expression pattern compared to classical brown adipocytes. Interestingly, TG2 in some cells can act as a G (Gh α) protein (Nakaoka, 1994). This TG2 function can transmit the α 1B-adrenoceptor (α 1B-AR) signal to phospholipase C (PLC) through its GTPase activity (Feng, 1996). In order to investigate the possible role of TG2 in browning mechanism dependent on mitochondrial functions, first we cold exposed mice (Madi, 2017). Then, we isolated preadipocytes from epididymal fat and differentiated them to beige direction. We treated the beige cells with activators for adrenergic pathways: arterenol (general AR agonist), CL-316,243 (specific β -AR agonist), phenylephrine (specific α 1-AR agonist) and forskolin (adenylate cyclase activator). The differentiation process was successful as adipocytes were obtained with significantly increased expression of beige marker genes UCP1, TBX1, TNFRSF9 and TMEM26 compared to preadipocytes similarly in both TG2^{-/-} and TG2^{+/+} cells. However, the expression of UCP1 and TBX1 markers was significantly lower in TG2^{-/-} beige cells activated by 10 μ M of adrenergic agonists for 4 h compared to TG2^{+/+}, except in case of forskolin. Preliminary results obtained from MitoTracker staining and Seahorse measurements combined with Western-blot on UCP1 and Mitochondrial Complexes suggest that in the absence of TG2 mitochondrial functions might be affected leading to lower heat generation capacity of TG2^{-/-} beige cells compared to TG2^{+/+} cells.

The work is supported by the ÚNKP-18-3 New National Excellence Program of the Ministry of Human Capacities, by the GINOP-2.3.2-15-2016-00006 and by the Hungarian Research Fund [OTKA-NK129139] projects. The project is co-financed by the European Union and the European Regional Development Fund.

**P-003****Circadian rhythms during early chondrogenesis****Csaba Matta, Abdulhadi M. Alagha, Judit Vágó and Róza Zákány***Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Hungary*

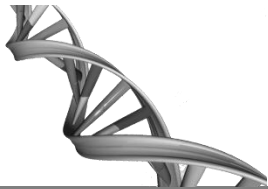
Osteoarthritis (OA) is a debilitating musculoskeletal disorder, and is currently untreatable. The severe disability caused by OA stems from the fact that articular cartilage has a very limited intrinsic capacity for self-repair. Recent research indicates that a dysregulated molecular clock in articular chondrocytes may contribute to OA pathogenesis. The circadian rhythm is not only determined by the central clock located in the hypothalamus but it is also adjusted by tissue- and cell-specific molecular clocks. The molecular basis of the circadian clock is the rhythmic expression and activity of conserved clock genes (*Clock*, *Bmal1*, *Per2/3* and *Cry1/2*, *cRev*), which also regulate a wide range of downstream genes. It has recently been shown that the disruption of *Bmal1* induces an OA-like phenotype. However, the putative links between the molecular clock and the regulation of chondrogenesis have not been studied. Therefore, our aim was to assess the expression pattern of the circadian clock genes and key chondrogenic/osteogenic marker genes (*Sox9*, *Runx2*, *Col2a1*, *Acan*) during cartilage formation and identify the time points for clockwork stimulation.

As an experimental model to study *in vitro* chondrogenesis, we used an embryonic limb-bud derived micromass culture system. Chicken eggs were incubated for 4 days, distal limb buds were collected, dissociated with trypsin, and high density cultures of chondrogenic cells were established. The micromass cultures were cultured for 1 day, and then serum shocked (using 50% FBS for 1 hour) to synchronise the molecular clock. Samples were collected at various time points post-synchronisation. The components of the molecular clock were assessed at an early (3-day) and a late (6-day) time point during chondrogenesis. RNA was isolated and reverse transcribed to cDNA. Primers were designed and tested for specificity. Following serial dilutions, gene expressions were analysed using RT-qPCR (absolute quantification using SYBR Green method). Gene expression values of target genes were normalised to the most stably expressed reference genes.

We detected statistically significant changes at various time-points in the expression of the clock genes *Bmal1/2*, *Per2/3*, *Cry1/2* and *cRev*, revealing an oscillatory expressional pattern. Interestingly, transcripts for *Clock* could not be detected in chondrogenic cultures, despite the well-established oscillatory expressional pattern of the other clock genes. The key chondrogenic marker genes also showed oscillatory patterns, revealing a circadian regulation of chondrogenesis. Based on our results, differentiating chondrocytes in chicken limb bud-derived micromass cultures contain self-sustained circadian clocks. The molecular clockwork may have a strong influence on the regulation of chondrogenesis. However, the molecular link between the clock and the chondrogenic pathways needs to be elucidated, along with the physiological cues that adjust and fine-tune the clock during chondrogenesis.

This work was supported by the Premium Postdoctoral Research Fellowship from the Hungarian Academy of Sciences.

Keywords: *molecular clock, circadian rhythm, chondrogenesis*



P-004

Connecting RING1 and YY1 binding protein to the retinoic acid signalling pathway during neural differentiation

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Retinoic acid (RA) is a natural morphogen, which is essential for embryonic development and a common inducer of neural differentiation of embryonic stem cells (ES) *in vitro*. Signalling through RA regulates the proliferative capacity of certain cell types and can induce apoptosis during development.

RING1 and YY1 Binding Protein [(RYBP), also known as Death Domain Associated Factor (DEDAF)] is essential for neural development both *in vitro* and *in vivo*. Insufficient dosage of RYBP led to neural tube defects in mice, and accelerated neuroprogenitor formation of ES cells *in vitro*. Moreover, incomplete *in vitro* neural differentiation was coupled with elevated mRNA level of several RA inducible genes, such as Pax6 in the *Rybp* null mutant neural cultures. This suggested a possible link between RYBP and the RA signalling. Here we show that in the lack of *Rybp*: (1) key members of the apoptotic (*Bcl2*, *Apaf1*, *Diablo*, *Caspase 9*, *Fas*, *Fadd*, *Tnf-R1*, *Caspase8*) and RA signalling pathways (*Stra6*, *Rdb10*, *Raldb1*, *Cyp26a1*) are upregulated; (2) amongst these, the normal mRNA levels of the retinoid converting enzymes *Rdb10* and *Raldb2* can be restored by lentiviral rescue of the *Rybp* null mutant ES cells; (3) and most importantly, that the increased level of apoptosis is RA dependent. Based on these results we hypothesize that in the lack of *Rybp*, elevated RA signalling can lead to increased apoptosis resulting impaired terminal differentiation from progenitors.

These results enhance our ability to understand the role of RA and *Rybp* in stem cell differentiation, progenitor formation and apoptosis. Since RA and *Rybp* have also a profound effect on complement cancer chemotherapy treatments, our results may improve therapeutics strategies for the treatment of cancer or neurodegenerative diseases.

Keywords: *Rybp*, retinoic acid signalling, *in vitro* neural differentiation, apoptosis



P-005

Distinct gene expression patterns, unrelated to thermogenesis, are induced in human adipocytes from the neck in response to Irisin and BMP7

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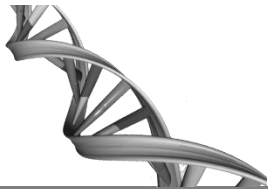
PET/CT verified the presence of uncoupling protein (UCP-1) dependent, thermogenic brown adipose tissue (BAT) in healthy adult humans and highlighted the negative correlation between obesity and BAT amount. These thermogenic fat depots are enriched in “deep neck” (DN) regions but it has not been clarified how they respond to different natural browning-inducers.

We intended to characterize differentiated adipocytes from DN and cervical subcutaneous (SC) human adipose tissue depots. Preadipocytes obtained from the two sites were differentiated into adipocytes with or without Irisin or BMP7 treatments. These endogenous browning-inducers can have distinct effects on different adipose depots; they have been shown to induce functional browning in human abdominal subcutaneous adipocytes. Global gene expression analysis was performed by RNA-sequencing.

More than 200 genes were upregulated and 50 were down-regulated in response to Irisin in differentiated adipocytes from DN or SCN depots; out of those approximately 100 were regulated similarly in adipocytes derived from both sites. Genes involved in cytokine and interferon signaling (e.g. OAS2, IRF1) and those of chemokines (e.g. IL8, CXCL1, CXCL6) but not the thermogenic genes were altered as a result of Irisin treatment. When BMP7 was added the expression of 250 or 100 genes changed in DN or SCN depots, respectively, with less overlap between adipocytes from the two sites. BMP7 upregulated class I/A rhodopsin-like receptors (e.g. CELSR1, CMKLR1) in SCN adipocytes; however, thermogenic genes were not induced at either site. Our data clearly shows that in contrast to abdominal subcutaneous adipocytes, Irisin and BMP7 induce distinct gene expression programs, unrelated to thermogenesis, in differentiating human adipocytes from the neck.

The work is supported by the GINOP-2.3.2-15-2016-00006 project.

Keywords: Human neck adipocytes, Irisin, Bmp7



P-006

Examining the role of Wnt/PCP proteins in axon growth and neuronal cytoskeleton regulation

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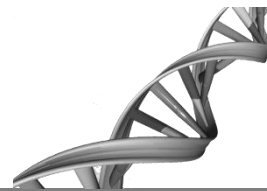
In *Drosophila melanogaster* there are 6, evolutionary highly conserved, core Planar Cell Polarity (PCP) proteins that are essential in the formation of planar polarity in epithelial tissues. Besides this function, these proteins are also known to play multiple roles in neuronal development. Neurons are highly polarised cells with extended neuronal protrusions, during the growth and maintenance of which the neuronal cytoskeleton plays a crucial role. The aim of this project is to study how core PCP proteins regulate the neuronal cytoskeleton, and to understand how they contribute to axon growth in *Drosophila melanogaster*.

To this end two strategies were applied: we used primary embryonic cell cultures which provide powerful subcellular readouts to determine the state of the neuronal cytoskeleton; in addition, we studied the embryonic and the adult nervous system in order to assess the *in vivo* relevance of our findings.

Our results showed that most PCP proteins are present in the embryonic nervous system and the loss of some of these components causes motor axon stalls in embryos. In addition, we found that the PCP proteins control axon growth and guidance in the ventral lateral neurons (LNv) in the adult brain as the LNv axons project ectopically in some of the PCP mutants. We are currently examining whether this phenomenon affects the circadian clock or the sleeping behaviour of the adult flies.

In accordance with these, we found that PCP proteins affect axon growth in primary neuronal cell cultures as well. The lack of PCP components alters the state of the microtubule (MT) and the actin cytoskeleton in cultured neurons. In order to investigate the mechanism by which PCP proteins can control the neuronal cytoskeleton we also studied Daam, a formin type of protein which has previously been linked to PCP signalling. Besides its known actin-regulatory function, we found that Daam strongly affects the MT cytoskeleton as well. Together these findings suggest that PCP proteins are necessary to properly regulate the neuronal cytoskeleton, presumably by controlling Daam, and contribute to axon growth *in vivo*. We are currently investigating the relationship between PCP proteins and Daam to better understand their role in neuronal cytoskeleton regulation.

Keywords: planar cell polarity, neuron, cytoskeleton



P-007

Gene expression signature of BRAF inhibitor resistant melanoma spheroids

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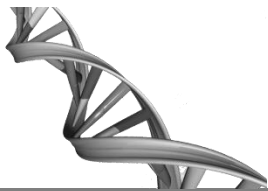
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Approximately 50% of melanomas harbour oncogenic BRAF mutations, which became the basis for one of the main treatment strategies for melanoma. Majority of currently available data have been obtained from two-dimensional (2D) melanoma cell cultures. However, tumour cells grow in 3D *in vivo*, therefore the reconstruct of 3D melanoma spheroid models can provide an advanced screening possibility to study molecular mechanisms not only the development of tumours but to investigate the therapy resistance in the future.

The major aims of this study were 1.) to develop 3D melanoma spheroid models from BRAFV600E mutant melanoma cell lines that are sensitive and resistant to BRAF inhibitors (BRAFi), 2.) to compare the gene expression signature of the 2D and 3D melanoma cell lines in both (sensitive and resistant) model systems.

Spheroids were prepared from sensitive (WM983A and WM983B) and BRAFi (PLX4720) resistant (WM983ARES and WM983BRES) melanoma cell lines using hanging drop method. Affymetrix Human Gene 1.0 ST arrays were used to determine the gene expression pattern of cell lines growing a monolayer (2D) and spheroid (3D).

Univariate t- tests were performed on log-transformed expression values of the probes to determine differentially expressed genes ($P \leq 0.05$). In case of sensitive cells, the analysis resulted in 1,038 genes that expressed differentially between cells growing in 2D and 3D. Approximately half of the differentially expressed genes were upregulated (559 genes), whereas 479 genes were significantly downregulated in the spheroid model. The overexpressed genes in the spheroids included many genes with reported functional roles in cell cycle regulation, p53 signalling- and DNA damage pathways, while downregulated genes were associated with oxidative stress, apoptosis- and TGF- beta signalling pathways. Comparison of the gene expression signature of the resistant cell lines (2D and 3D) resulted in 279 differentially expressed genes in the spheroid. The number of upregulated genes were a lot lower in the resistant than in the sensitive 3D cells (71 genes), and associated with cell adhesion molecules and IGF1R signalling. The down-regulated genes ($n=212$) in the BRAFi resistant spheroid are involved in VEGF signalling, mTOR signalling, axon guidance pathway and p53 pathway. By comparative analysis of the sensitive- and resistant spheroids, we found that completely different pathways involved in 3D formation of sensitive and resistant cells. Only 1% of upregulated- and 5.7% of downregulated genes were commonly altered. Common down-regulated gene included MMP16, CEP19, IGF1R and FLOT1. Interestingly, we recognized some genes which were up-regulated in the sensitive and down-



regulated in the resistant spheroids such as CENPF, LOXL2 and BNIP3 which are actively involved in cell cycle regulation. On the other hand, part of the down-regulated genes in the sensitive spheroids were upregulated in resistant ones including SCN8A, RING1, and ABHD4 that have crucial role in rapid membrane depolarization, transcriptional repression and anoikis resistance, respectively.

In summary our data shows that BRAFi resistant cells are using different molecular pathways in 3D structure formation than the sensitive cells. These findings could be useful for the better understanding of the resistance profile in melanoma cells.

Keywords: 2D and 3D cell culture, spheroids, gene expression, BRAF inhibitor resistance

P-008

Headcase is a regulator of hemocyte differentiation in *Drosophila melanogaster*

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One of the main criteria of the effective immune system is the precise regulation of blood cell development. In the fruit fly, *Drosophila melanogaster* the differentiation of the immune cells, the hemocytes, is controlled by a complex network of signaling pathways. Recently we isolated *headcase* (*hdc*), the ortholog of the human tumor suppressor HECA, which is a regulator of hemocyte differentiation. *hdc* is expressed in one of the immune compartments of the larva, the lymph gland, and it is downregulated in the differentiating hemocytes that leave the organ upon immune induction. The aim of our studies was to identify interacting partners of Hdc in order to place it into the regulatory network of hemocyte development.

We generated a *hdc-Gal4* driver by P-element conversion to analyze the changes of the expression pattern of *hdc* during the larval development. In the lymph gland of the second instar larva, *hdc* expression was detected in the blood cell progenitors (MZ), in the differentiated hemocytes (CZ) and in the hematopoietic niche (PSC) as well, however the proportion of the cells expressing *hdc* dramatically reduced by the end of the larval development. In the course of the investigation of our newly generated amorphic *hdc* allele, we observed spontaneous lamellocyte differentiation. By silencing *hdc* with various hemocyte specific drivers, we mapped the focus of the mutation to the hematopoietic niche. In a PSC-specific misexpression- and silencing screen, we identified the JAK/STAT, the Decapentaplegic (Dpp) and the Hedgehog (Hh) pathways as potential interacting partners of Hdc.



Our work is supported by OTKA NK-101730 (LA), PD-115534 (VH) and GINOP-2.3.2-15-2016-00001 grants. The research of V. Honti and G. I. B. Varga was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TAMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program'.

Keywords: *Drosophila*, hematopoiesis, headcase, differentiation

P-009

Individual immunity of a social insect the honey bee (*Apis mellifera*)

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In insects the individual defense involves the immune system, which consists of humoral and cell-mediated responses. The hemocytes play a key role in the cell-mediated immunity, by phagocytosing the microorganisms, produce matrix proteins, antimicrobial peptides and form capsules around the intruders which are too large to be taken up by phagocytosis. The individual immune responses of eusocial insects are complemented with communal defense mechanisms as hygienic behavior or hive fever therefore their immune system may have unique features, different from other non-eusocial insects.

Apis mellifera is an ideal model organism to investigate the immune defense mechanisms triggered by different stress factors in a eusocial caste system. Until now the hemocytes of the honey bee were described only by their morphological and lectin binding characteristics, which does not allow proper definition of hemocyte subsets and lineages. We developed monoclonal antibodies against honey bee hemocytes which distinguished the melanizing oenocytes, the phagocytic granulocytes and the plasmatocytes which are involved in aggregation of the hemolymph.

We have identified key effector molecules of the plasmatocytes and the oenocytes - components of two main hemocyte classes - : the hemolectin which is expressed by the plasmatocytes and the prophenoloxidase expressed by the oenocytes. These and other cell type specific molecules allowed us to set up a model for the differentiation of the hemocytes in this species.

Support comes from NKFI K 120140, GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00035.

Keywords: honey bee, hemocyte differentiation, immunity



P-010

Inflammatory cytokines transdifferentiate mesenteric mesothelial cells into macrophages

Sándor Katz, Viktória Zsiros and Anna L. Kiss

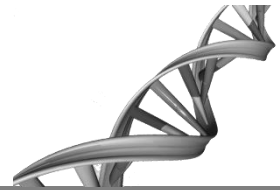
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In our previous work we showed, that during inflammation induced epithelial-to-mesenchymal transition (EMT) mesenteric mesothelial cells express ED1 (pan-macrophage marker), indicating that they are transformed into macrophage-like cells. In our recent work we provide additional evidences about this transition by following the phagocytic activity, IL6 and IL10 expression as well as the TNF α production in mesenteric mesothelial cells during inflammation and GM-CSF treatment.

When we injected India ink particles or fluorescent labelled bioparticles (pHrodo) into the peritoneal cavity of rats pretreated with Freund's adjuvant, we found that mesothelial cells efficiently engulfed these particles. A similar increase of internalization could be observed by mesothelial cells in GM-CSF pretreated primary mesenteric culture. By the time of inflammation the pro-inflammatory IL-6 expression significantly increased in mesenteric mesothelial cells both *in vivo* and *in vitro* circumstances. Since macrophages are the major producers of tumor necrosis factor, TNF α , we investigated expression level of TNF α in mesothelial cells during inflammation induced EMT as well as in GM-CSF treated primary mesenteric culture. We found that as the inflammation proceeded TNF α was indeed expressed in these cells, reaching the highest level at the day 5 of inflammation. GM-CSF treatment resulted in similar result. TNF α is one of the target genes of early growth response (EGR1) transcription factor, playing important role in monocyte-macrophage differentiation. We followed the expression of EGR1 in mesothelial cells by Western blot and immunocytochemistry. We found that control mesothelial cells did not express EGR1, a marked increase was observed in mesothelial cells by the time of inflammation. Parallel to this, nuclear translocation of EGR1 was shown by immunocytochemistry at the day 5 of inflammation.

Our recent data strongly support the idea that during inflammation induced EMT mesenteric mesothelial cells can really transdifferentiate into macrophages.

Keywords: *inflammation, inflammatory cytokines, mesenteric mesothelial cells, macrophages*



P-011

Inflammatory signal-induced IL-10 production of marginal zone B cells depends on CREB

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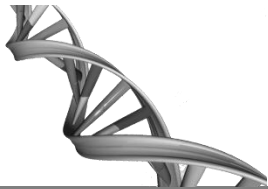
IL-10 is a potent anti-inflammatory cytokine which can be produced by different cell types such as regulatory B cells. We showed previously that marginal zone (MZ) B cells are able to differentiate into IL-10 producing cells under inflammatory conditions and contribute to the downregulation of collagen-induced arthritis. Based on these observations, we aimed to characterize the signalling cascades lead to the IL-10 production of MZ B cells particularly focused on the involvement of the CREB transcription factor.

Throughout our experiments we used sorted MZ B cells from the spleens of DBA/1 mice and mimicked inflammatory conditions *in vitro* by stimulated MZ B cells through their BCR, TLR-9 and IFN- γ receptors. The activation and IL-10 production of MZ B cells were analysed by IL-10-specific ELISA, Western blot, flow cytometry, real-time RT-PCR and confocal microscopy.

Our results show that IL-10 production mediated through the BCR, TLR-9 and IFN- γ receptors is clearly depends on the CREB transcription factor. We demonstrated that the phosphorylation of CREB is prolonged in MZ B cells but transient in IL-10 non-producing follicular (FO) B cells. Likewise, the association of activated CREB and its partner in IL-10 transcription, the CREB-binding protein (CBP) is sustained in MZ B cells while transient in IL-10 negative FO B cells. Moreover, inhibition of the binding between CREB and CBP negatively regulates the IL-10 production by MZ B cells.

Based on these data, we can conclude that CREB is an essential transcription factor involved in the IL-10 production of MZ B cells and its prolonged activation is needed for the regulatory differentiation of MZ B cells during inflammation.

This project was supported by the MTA Premium Post Doctorate Research Program.



P-012

Internalization of GM-CSFR β is essential to initiate mesothelial-to-macrophage transition

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During Freund's adjuvant induced inflammation rat mesenteric mesothelial cells lose their epithelial phenotype and differentiate into mesenchymal, macrophage-like cells by epithelial-mesenchymal transition type II (EMT type II), meanwhile they express macrophage markers (like ED1), produce inflammatory cytokines (TGF- β , TNF- α , etc.) and express their special receptors. Our *in vitro* experiments, when primary mesenteric cultures were treated with GM-CSF, TGF- β and their mixture, showed that both cytokines induced similar phenotypic changes as Freund adjuvant *in vivo*; GM-CSF treatment increased ED1 expression; mesothelial cells produced GM-CSF and expressed its receptors (GM-CSF receptor α and β). Our experiments with GM-CSF, dynasore and their combination suggested that internalization of GM-CSF receptor-ligand complex is indispensable for GM-CSF signalling to initiate mesothelial-macrophage transition (EMT). The intracellular route of the internalized receptor however is not known. In our present work we used immunocytochemical, statistical and biochemical analysis to follow the intracellular fate of the internalized GM-CSF receptor β , that initiate the signalling. We carried out co-localization experiments with various endocytotic markers (Cav-1, EEA1, Rab7, Rab11a). Since STAT5 is the downstream element of GM-CSF signalling, we also studied the STAT5 transcription factor expression and activation.

Our results showed that in mesothelial cells GM-CSFR β was internalized by caveolae, delivered into early endosomes where the signalling events could occur. In early endosomes STAT5 was phosphorylated, then migrated to the nucleus, to initiate mesothel/macrophage transition. When the internalization of GM-CSFR β was blocked, STAT5 was not phosphorylated, supporting the idea that internalization of the receptor was the crucial event in signalling. As the inflammation proceeded the receptor β recycled back to the plasma membrane, but after the peak time of inflammation (day 5, 8), when the recovery process started, the internalized receptor was detected in multivesicular bodies indicating that the degradation has already started. As the receptor degraded, the EMT stopped and the regeneration of mesothelial cells started by the mesenchymal-epithelial transition (MET).

Keywords: mesothel/ macrophage transition, internalization, GM-CSFR β , STAT5 signalling



P-013

Investigating the role of septins in chondrogenic high density cultures

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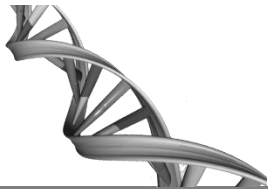
Septins in eukaryotes belong to a strongly conserved group of proteins, the members of which are increasingly noted as a new component of the cytoskeleton. All septin types have a GTP binding domain, form heterohexamer complexes that can arrange into higher order structures, such as filaments and rings. Septins are also known to form cell membrane domains in the proximity of endoplasmic reticulum punctae, which are believed to play a key role during STIM1–Orai1 coupling during store-operated calcium entry (SOCE). Knock down of certain septin types, or the inhibition of their remodelling impedes SOCE and relevant downstream signalling processes. The role of septins has not been described in chondrogenesis and literature is generally scarce regarding the role of these proteins in cell differentiation.

As previous results of our laboratory suggest SOCE as a key signalling process in chick chondrogenic high density cultures, we set out to study septins as regulators of these mechanisms via SOCE.

Our experiments were carried out during the critical period of chondrogenesis of chick high density cultures established from limb buds of 4-day-old chicken embryos. Treatments with the inhibitor of septin remodelling (forchlorfenuron; FCF) took place on the first or the second day of culturing. mRNA and in certain cases protein expression of SOCE components and relevant septin types has been examined. Chondrogenic marker gene mRNA expression in treated and control cultures has been quantified. Production of metachromatic extracellular matrix has been analysed by visual and semiquantitative histological methods. Furthermore, we have examined the effect FCF exerted on collagen synthesis, proliferation rate and mitochondrial activity. Hypothesised changes in the cytosolic calcium concentration of chondrogenic cells is currently under investigation.

Our results demonstrate a clear reduction in the rate of proliferation and cartilage specific matrix production under the inhibition of septin rearrangement without a negative effect on the viability of our chondrogenic cells. This set of data together with previous findings clearly demonstrate the tight connection between septins, SOCE and chondrogenesis in our experimental model.

Keywords: *septins, chondrogenesis, primary cell culture*



P-014

Investigation of molecular clock elements in WM35 melanoma cells

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Many of the cell functions show circadian rhythm which is controlled by an endogenous rhythm- generator system. The clock machinery is composed of a central neural oscillator located in the brain and peripheral clock systems which are found in the different cells and tissues. The core regulators CLOCK (Circadian Locomotor Output Cycles Kaput) and Bmal1 (also called as ARNTL1, Aryl hydrocarbon receptor nuclear translocator-like protein 1) activate the expression of period (PER2/3) and cryptochrome (Cry1/2) genes. These latter two proteins form a complex in the cytoplasm, that translocates to the nucleus and inhibits the CLOCK/Bmal1 transcription. The secondary regulators of this system are the ROR and Rev-Erb proteins by activating and inhibiting Bmal1, respectively.

Recently, more and more data accumulated suggesting that cancer development and progression can be linked to the disruption of the circadian homeostasis of the cells. In case of melanoma, little is known about the role of the biological clock, although recent studies suggest that loss of circadian rhythm may correlate with increased proliferation of tumour cells.

Based on the above data, the aim of our research is to investigate the expression profile of the molecular clock genes in WM35 melanoma cells. In next steps, we synchronize the clock genes with serum shock, a well-established intervention applied in circadian clock investigations. We also aim to find more physiological possibilities to modify disrupted clock gene expressions. To this end, we hypothesize that fasting can re-organize the rhythmicity of the molecular clock genes of melanoma cells. Following clock-gene profiling, we study the changes the cell proliferation, viability and migration capability of melanoma cells upon clock synchronization. The experiments and analysis of the data are in progress, the explored details obtained by the time of the congress will be presented.



P-015

p38 MAP kinase mediates degradation of the putative metastasis suppressor PMCA4b in BRAF mutant melanoma cells

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Intracellular Ca²⁺ homeostasis is altered during tumorigenesis and plasma membrane Ca²⁺ ATP-ases (PMCA) play a key role in this process. Previously, we identified PMCA4b as a putative metastasis suppressor in BRAF mutant melanoma cells. The aim of the present study is to identify regulators to prevent the loss of PMCA4b during tumorigenesis.

Previously, we showed that PMCA4b is downregulated in BRAF mutant melanoma cells and inhibition of mutant BRAF with vemurafenib increases PMCA4b at both the mRNA and protein levels. The present study identified p38 MAPK as another possible regulator of PMCA4b in the BRAF mutant A375 cell line, while NF- κ B and Jun kinase did not show any effect. Inhibition of p38 MAPK enhanced PMCA4b abundance at the protein level without changing its expression at the mRNA level, suggesting post-translational regulation. When p38 inhibitor and vemurafenib were combined, the results did not show additivity between the inhibitors suggesting some common downstream regulatory pathways.

A critical issue in PMCA4b protein function is its proper localization in the plasma membrane that is tightly regulated at the post-translational level. To study PMCA4b turnover we used markers of the endo/lysosomal system (EEA1, Rab7, LAMP1) and found that PMCA4b is targeted to the lysosomes for degradation. Our study showed that p38 inhibition rescued PMCA4b from degradation by inhibiting its internalization from the plasma membrane and consequently increased PMCA4b stability. We showed that inhibition of the proteasome pathway, however, did not change PMCA4b level. We demonstrated that inhibition of p38 MAPK markedly inhibited migration of the BRAF mutant melanoma cells that might be partly mediated by stabilization of PMCA4b.

In summary, we showed that the p38 MAPK pathway plays a key role in the PMCA4b degradation. Increasing the stability of the metastasis suppressor PMCA4b might provide a novel therapeutic potential for targeting BRAF mutant melanoma and prevent metastasis.

Keywords: PMCA4b, BRAF mutant melanoma, calcium signaling, metastasis, protein degradation, mitogen-activated protein kinase (MAPK), p38



P-016

Proteoglycan-mediated glucose uptake and mitochondrial respiration in skeletal muscle cells

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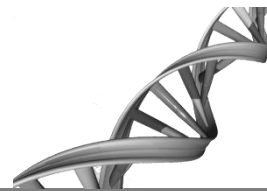
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The insulin-regulated GLUT4 (glucose transporter type 4) is responsible for glucose uptake in adipose tissue and muscle. Following the stimulation, GLUT4 storage vesicles are transported and redistributed to the plasma membrane from the cytosol. The impaired GLUT4 translocation is involved in the development of insulin resistance and diabetes mellitus. On the one hand the classical insulin-stimulated translocation of GLUT4 can be mediated by PI3K, Akt, AS160 (Akt substrate of 160 kDa) pathway. The AS160 Rab GAP (GTPase activating protein) is a key molecule in GLUT4 translocation by controlling the activity of Rab GTPases. On the other hand, the AMPK (5' AMP-activated protein kinase) can also control the AS160 phosphorylation. AMPK can be activated by LKB1, AMP/ATP ratio, or Ca²⁺-triggered CaMKK, and the phosphorylation of AMPK is required to activate the PGC-1 α transcription factor, which is a master regulator of mitochondrial biogenesis. Syndecan-4 (SDC4) is a transmembrane, heparan sulfate proteoglycan linked to the actin cytoskeleton, and the rearrangement of actin filaments is essential for exocytosis. We were used shRNA to reduce SDC4 expression in C2C12 mouse myoblast cells. The silencing increased the phosphorylation of AS160, and the 2-deoxy-2-(18F)fluoro-D-glucose radiotracer uptake of the cells was also significantly higher. Furthermore, it increased the phosphorylation of AMPK and the mitochondrial reserve capacity, but other mitochondrial oxygen consumption parameters (Routine, Leak, ROX) have not changed. Our results suggest, that beside the previously known options (such as metformin), a new mechanism can also increase glucose uptake by means of insulin-independent signal transduction.

This research was supported by EFOP-3.6.2-16-2017-00006 grant.

Keywords: AMPK, GLUT4, proteoglycan

**P-017****Role of protein phosphatase 2A B55 α in angiogenesis****Zsófia Thalwieser, Csilla Csontos and Anita Boratkó***Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

Protein phosphatase 2A (PP2A) is one of the major phospho-Ser/Thr specific phosphatase in mammalian cells. A typical PP2A holoenzyme consists of a catalytic C subunit, a structural A subunit, and a regulatory B subunit which are assigned into B, B', B'' and B''' families. The variable B subunit defines the substrate specificity and/or subcellular localization of a given PP2A holoenzyme. PP2A has a critical role to play in the regulation of cell cycle, signal transduction, cell differentiation, cytoskeletal remodeling and even cellular dysfunction. Previously our workgroup has shown the role of B55 α regulatory subunit in endothelial cell junction regulation.

Our recent aim was to explore the role of the PP2A B55 α holoenzyme in the angiogenesis. B55 α subunit was depleted in endothelial cells by siRNA mediated transfection. Cell lysates and supernates of nonsiRNA and B55 α specific siRNA treated cells were studied by Proteom Profiler Angiogenesis Array. The array detects 55 angiogenesis related protein level simultaneously. We found that due to B55 α silencing, thrombospondin-1 (TSP-1) level of the cells decreased greatly. TSP-1 is known as an angiogenesis inhibitory protein by regulating cell migration. To compare the effect of B55 α silencing on RNA level, first mRNA was isolated from treated samples and cDNA synthesis was done. PCR was carried out using specific primers. Interestingly, not only the protein level, but also the mRNA level of TSP-1 decreased due to the depletion.

In endothelial cells the interaction between B55 α and TSP-1 was confirmed by immunoprecipitation and also by pull down. To test the B subunit isoform specificity of the interaction, endothelial cells were transfected with recombinant plasmids coding B55 α and B56g. Protein were purified and samples were tested by Western blot. TSP-1 interacted only with B55 α . The localization of TSP-1 was examined by immunofluorescence staining or cell fractionation followed by Western blot. Next we would like to test how TSP-1 degradation is regulated by B55 α and how the tube formation of endothelial cells is affected by depletion of B55 α .

This work was supported by grants ÚNKP-18-4-New National Excellence Program of the Ministry of Human Capacities and Bolyai Fellowship (A.B.).



P-018

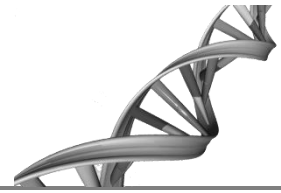
Role of TIMAP-PP1c complex in neuronal cell differentiation

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TIMAP, TGF- β inhibited membrane associated protein, has been considered as a member of the myosin phosphatase targeting protein (MYPT) family. Our workgroup previously demonstrated specific protein-protein interaction between TIMAP and the δ isoform of protein phosphatase 1 catalytic (PP1c) subunit and proved the regulatory role of TIMAP on PP1 activity. TIMAP is highly expressed in endothelial cells and our workgroup identified ERM (ezrin-radixin-moesin) proteins, merlin (neurofibromin 2), endothelin-converting enzyme 1 and eukaryotic elongation factor 1A1 as TIMAP-PP1c substrates in endothelial cells. ERM proteins and merlin have a prominent role in neuronal cell functions and their altered expression or posttranslational regulation are associated with serious diseases, like neurofibromatosis. Several studies reported that TIMAP is also expressed in neuronal cells, but its exact function was never studied in details. Our recent aim was to study the role of TIMAP in neuronal cells, especially during differentiation. First the expression of TIMAP was proved on mRNA and protein level in B50 rat neuronal cells and SH-SY5Y human neuroblastoma cells. The interaction with PP1c was checked by immunoprecipitation and pull down assay using specific antibodies and GST-tagged recombinant proteins, respectively. In both cell type TIMAP forms a complex with PP1c. Next, the previously identified TIMAP-PP1c substrates and their interactions with TIMAP were verified. ERM proteins and merlin, the most interesting from our aspect, showed strong interaction with TIMAP. Differentiation of B50 and SH-SY5Y cells was optimized using all-trans retinoic acid (ATRA) and dibutyryl-cAMP. Neuronal differentiation markers, like β 3-tubulin, nestin and ID1 protein expression were checked by Western blot. To study the role of TIMAP during the differentiation of the cells, we have already started experiments using shRNA mediated silencing of TIMAP. Selection of positive clones is still in progress. In the future we would like to compare the mock and TIMAP depleted cells differentiation ability. Also during the progress phosphorylation level of the target proteins, ERM and merlin will be checked.

This work was supported by grants ÚNKP-18-4 New National Excellence Program of the Ministry of Human Capacities and Bolyai Fellowship (A.B.).



P-019

RYBP is essential for the maturation of cardiomyocytes *in vitro*Viktória Szabó^{1,2}, Surya Henry^{1,2} and Melinda Katalin Pirity¹¹ Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary² Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Mutations and irregular changes of molecular pathways that govern embryonic heart development often lead to congenital heart defects (CHD). Our laboratory focuses on the early onset of cardiac development by utilizing embryonic stem (ES) cell based *in vitro* cardiac differentiation systems. We have previously showed that lack of the Polycomb Group (PcG) member RYBP (RING1- and YY1-Binding Protein; UniGene Mm.321633; MGI:1929059) impairs cardiac differentiation resulting cardiomyocytes (CMCs) without contractile activity (Ujhelly *et al.*, 2015). To reveal the underlying molecular mechanisms, we have differentiated wild-type (*Rybp*^{+/+}), *Rybp* null mutant (*Rybp*^{-/-}) and *Rybp* overexpressing (*Tg^{Rybp}*) mouse ES cells to CMCs and (I) characterized the presence and localization of key cardiac marker, cardiac Troponin T in the *Rybp* null mutant and RYBP overexpressing cell lines (II) and analyzed the gene expression of several Hedgehog pathway members that are essential for cardiac development (Fair *et al.*, 2016). Our results indicate that (1) the maturation of cardiomyocytes is impaired in the *Rybp* null mutant cells (Fig. 1, *Rybp*^{-/-}), (2) RYBP overexpressing cells can form cardiomyocytes with high efficiency (Fig. 1, *Tg^{Rybp}*), (3) and the gene expression of several key Hedgehog pathway members are altered in the *Rybp* null mutant cells. Moreover, analyzed ChIP-seq data we found that RYBP can regulate key cardiac transcription factors such as *Shh*, *Nkx2-5*, *Gata4*, *Mef2c* and *Isl1* and these transcription factors have the potential to target *Tnnt2*. In summary, we present evidence the first time that RYBP is a key factor of the proper cardiac differentiation and RYBP may be a new player of facilitating CHD development.

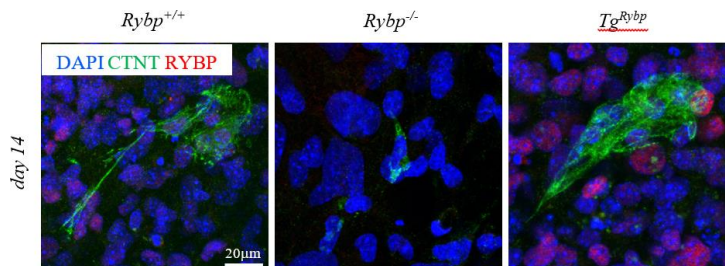
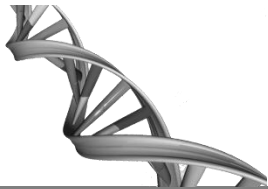


Figure 1: Immunostaining of cardiomyocytes for cardiac Troponin T (CTNT, green) and RYBP (RYBP, red) at day 14.

Keywords: RYBP, cardiac Troponin T, Hedgehog pathway



P-020

Specific detection of the cell polarity regulator LKB1

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The immunological methods play unambiguous role in the progression of cell biology research. Western blot technique and ELISA make quantification of protein levels possible, whereas immunohistochemistry and immunocytochemistry provide spatial information on distribution of the protein of interest. However, the reliability of these methods is fundamentally based on the specificity of the applied antibodies.

The liver kinase B1 (LKB1), the human paralogue of Par4, is a conserved serine/threonine protein kinase that plays pivotal role in the regulation of epithelial and neuronal cell polarity. The subcellular localization of LKB1 has been controversial in the literature. It has also been reported that translocation of LKB from the nucleus to the cytosol or its accumulation at the plasma membrane can profoundly contribute to its function. It should, however, be noted that most of these experiments are based on immunological detection of LKB1 in an overexpression system or in a knockdown animal model. The physiological regulation of the localization and activity of the human LKB1 (hLKB1) therefore remains unclear.

A sensitive and specific detection method is an essential prerequisite to reveal the exact cellular localization of hLKB1 and its translocation upon various cellular stimuli. In the present work, therefore, we screened commercially available antibodies against hLKB1 for their specificity. For this purpose, we have developed an assay system, which is based on HeLa cells, having no endogenous LKB1 expression, and a plasmid containing the FLAG-tagged human LKB1 gene. The cells previously transfected with this construct were simultaneously immunostained with an anti-FLAG antibody and the tested antibody, then visualized by confocal microscopy. We found that only one antibody out of the six yielded bright, specific immunofluorescence staining despite the fact that all of them were claimed by the manufacturers to recognize hLKB1 and five of them were recommended for immunofluorescence experiments. Ironically, the single one that specifically stained hLKB1 was recommended by the manufacturer only for Western blot application. In addition, another antibody was proven to be suitable for immunofluorescence labelling; however, it also produced a dim but evenly distributed non-specific background.

These results calls our attention to that the specificities claimed and applications listed by the manufacturers should be taken with reservation. More importantly, the published results obtained with these non-validated antibodies should be reconfirmed. Thus, our results may resolve the controversy found in the literature on the subcellular localization of LKB1.

This work has been supported by the National Research, Development and Innovation Office (OTKA_K 128123).

Keywords: LKB1, antibody recognition, specificity, immunofluorescence



P-021

The effect of the formin DAAM on cytoskeleton rearrangements in *Drosophila melanogaster*

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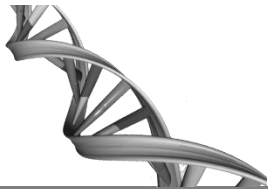
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The cytoskeleton has an essential role in normal cell functions, such as cell shape changes, motility, division and intracellular transport. Microtubules (MT) and actin-filaments (F-actin) are the two main building blocks of cytoskeleton. The coordinated changes of these filamentous systems are the main principle of proper cytoskeleton functions. The regulation of cytoskeleton is especially important in neuronal axonal growth. Axons have to navigate over long distances to find their appropriate synaptic partners. During axonal growth splayed MTs emanating from the central region of axonal growth cone and dynamically invade and scan the F-actin rich periphery. Filopodial F-actin bundles can act as guides for these dynamic MTs, and it is thought that this actin-MT crosstalk followed by MT and filopodia stabilization is a key step of growth cone advance. However, despite the relatively large number of proteins implicated in actin-MT crosstalk, the mechanisms whereby actin polymerization is coupled to MT stabilization and advancement in the peripheral growth cone, remained largely unclear.

Our group recently found that the *Drosophila* formin DAAM plays a key role in regulating both the actin and the MT cytoskeleton in neurons. We proved that, beyond the well-known actin barbed end binding, DAAM is able to directly bind to and stabilize MTs *in vitro*, and it has an actin-MT co-aligning activity. In *DAAM* null mutant *Drosophila* primary neurons MT dynamics increases significantly which suggests that MTs are less stable in the absence of DAAM. In this study, constitutively active forms of DAAM were used to examine their effect on cytoskeleton regulation. We used two types of constitutively active forms, one of them lacks the N-terminal regulatory domains (cDAAM), while the other is lacking the C-terminal regulatory domain (Δ DAD-DAAM). Point mutant versions affecting the actin-processing activity of DAAM were also created. The effect of cDAAM and Δ DAD-DAAM constructs were investigated in the nervous system of whole embryos, primary neuronal cell cultures and in S2 cells. Our results revealed that cDAAM and Δ DAD-DAAM behaved differently in all experiments. The expression of cDAAM had a negative effect on axonal growth and it completely disrupted the organization of fasciclinII (FasII)-positive motoraxons in whole embryos. Conversely, the expression of Δ DAD-DAAM promoted axonal growth and it had a weak effect on FasII-positive axons. I will present data that might explain this marked difference in the cellular behavior of these two different activated versions.

Keywords: cytoskeleton, axon growth, formin, *Drosophila*



P-022

The effects of a hyperosmotic environment on cartilage formation

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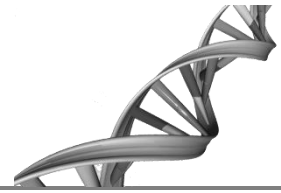
Therapeutic attempts aimed at the restoration of damaged articular cartilage are still challenging. This is mostly caused by the unique biological properties of articular hyaline cartilage. The extracellular matrix of articular cartilage has an extremely complex structure: the matrix is rich in highly negatively charged glycosaminoglycans which, on one hand, bind a significant amount of water, improving the biomechanical characteristics of the tissue, and, on the other hand, it also attracts many positively charged ions, which act as electrolytes. Due to the high concentration of these electrolytes, the osmolality of the immediate micro-environment of chondrocytes residing in cartilage matrix is higher than in other tissues of the body. However, the osmolality of commercially available cell culture media is conventionally lower than the physiological needs of cartilage cells. Therefore, we hypothesised whether a hyperosmotic environment promotes the development of cartilage tissue.

In our experiments, we used chondrifying micromass cultures isolated from the developing limb buds of 4-day-old chicken embryos. We investigated the effects a hyperosmotic environment by applying culture media supplemented with differing concentrations of saline solutions. Media with altered osmotic characteristics were applied on various days of culturing during the 6-day-long differentiation process. We measured mitochondrial activity by MTT assay, cell division by monitoring the incorporation of tritiated thymidine, and detected the amount of matrix produced by a metachromatic staining using dimethyl methylene blue and toluidine blue. We also studied the expression of cartilage specific genes (*Sox9*, *col2a1* and *acan*) with RT-qPCR. We also analysed the short-term effects of altered osmolality on intracellular calcium signalling on cells loaded with the calcium-sensitive fluorescent dye Fluo-8 with or without the TRPV4 inhibitor HC067047. Calcium measurements were carried out on a DSD spinning disk microscope.

When cultures were exposed to the hyperosmotic environment during the entire culturing period, it inhibited the process of chondrogenesis. However, exposure to hyperosmotic conditions later in the process (from day 4 onwards or for 24 hours on day 6) enhanced cartilage formation, which was confirmed by metachromatic staining and qPCR. Although hyperosmotic media evoked Ca²⁺ transients in chondrogenic cells, this was apparently not mediated through the osmosensitive TRPV4 ion channels. In conclusion, a hyperosmotic environment may promote the development of mature cartilage and could contribute to the effectiveness of cartilage regeneration procedures.

This work was supported by the New National Excellence Program of the Ministry of Human Capacities.

Keywords: *chondroprogenitor cell, osmolality, extracellular matrix*



P-023

The formation of Multinucleated Giant Hemocytes, the effectors of cell-mediated immune response in *Drosophila ananassae*

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Insects developed a powerful immune response, consisting of humoral and cellular elements, to combat infections. In *Drosophila* species, the cellular elements of immunity, the hemocytes participate in killing of the invaders. In the *melanogaster* subgroup, the phagocytic plasmatocytes, the melanizing crystal cells and the encapsulating lamellocytes take part in these processes. In *Drosophila melanogaster*, three hematopoietic compartments were identified: the lymph gland, the sessile hematopoietic tissue and the circulation. All three compartments are known to contribute to the differentiation of the effector hemocyte pool following immune induction. Besides plasmatocytes and crystal cells, we identified a novel blood cell type in the species of the *ananassae* subgroup, which we dubbed as Multinucleated Giant Hemocyte (MGH). In this study, we followed the differentiation of these cells upon immune induction, and defined their compartmental origin.

With the aid of a *Drosophila ananassae* cell-specific immunological marker system, we identified different hemocyte subsets. We also developed a transgenic reporter system, which allowed *in vivo* detection of hemocytes and hematopoietic compartments in this species. We observed that the MGHs which appear in the circulation of the larva after infestation with parasitoid wasps are syncytia of fused hemocytes and develop from phagocytic plasmatocytes. MGHs originate both from the sessile hematopoietic tissue and the circulation. Electron microscopic analysis showed that the MGHs contain a special ultrastructure with unique and so far unrecognized compartmentalization. As we investigated the role of the nitric-oxide (NO), a precursor of reactive oxygen- and nitrogen species, in the cell-mediated immune response, we found that NO may serve as a signal for MGH differentiation.

The MGHs are important effector cells of the cell-mediated immune response of *Drosophila ananassae*. Similarly to the lamellocytes of *Drosophila melanogaster*, MGHs also differentiate from phagocytic cells, however their formation and compartmental origin show substantial differences. The organization of the subcellular components suggest active metabolism, secretion and vesicular transport and this unique structure could provide a basis for the effective immune response against parasites. As MGHs are similar to mammalian multinuclear giant cells, they may serve as a powerful model to understand the basic events of granuloma formation in vertebrates.

Keywords: Multinucleated Giant Hemocyte, cell-mediated immunity, hemocyte differentiation



P-024

The loss of scaffold protein Tks4 induces EMT like changes in HCT116 human colorectal carcinoma cells

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Epithelial to mesenchymal transition (EMT) and the reversing mesenchymal to epithelial transition (MET) are multipurpose processes involved in wound healing, development, and certain pathological processes. Previous studies had characterized EMT in detail and identified hallmarks such as the transcriptional factors Snail1, Snail2 and TWIST, mesenchymal markers such as fibronectin, also described a certain change in phenotype, moving from a dense „cobblestone” like monolayer to more mobile, less connected „spearhead” like cells. During our research, we found that the loss of scaffold protein Tks4, the product of gene SH3PXD2B, initiates EMT like changes in human colorectal carcinoma cell line HCT116. We generated Tks4 knock-out stable cell line using the CRISPR/Cas9 system. In our investigation RT-PCR analysis we found that absence of Tks4 results in elevated Snail2, TWIST and fibronectin mRNA levels. Concurrently the stable KO cell lines showed reduced adherence to surfaces, and a change in morphology moving towards a more mesenchymal phenotype, increased motility, and invasion into collagen matrices. Our findings suggest that the Tks4^{-/-} genotype might initiate EMT-like alterations.

P-025

The potential role of transglutaminase 2 in skeletal muscle regeneration

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The broadly expressed, protein cross-linking enzyme transglutaminase 2 (TG2) was shown to play a role both in apoptotic cell death and in the clearance of apoptotic cells. TG2 was also implicated as a regulator of wound healing, and tissue fibrosis via participating in the



inflammatory cytokine TGF β production and activation. Moreover, efferocytosis plays a major mechanistic role in the resolution of inflammation by at least two ways. First, proper clearance of dead cells prevents their secondary necrosis. Second, efferocytosis itself triggers several different anti-inflammatory and pro-resolving signaling pathways. In addition, *in vitro* and *in vivo* TG2^{-/-} macrophages show decreased efferocytosis. Muscle injury also involves cell death during the regeneration phases, such as degeneration, clearance of dead cells, inflammation, regeneration, and remodeling-repair. Short-term inflammation is required for muscle stem cell activation in regenerating muscle and phagocytosis of apoptotic cells is known to regulate inflammation. Thus we decided to determine the role of TG2 in the skeletal muscle regeneration of mice.

In our experiments we induced muscle injury by cardiotoxin injection into the tibialis anterior muscle of TG2^{+/+} and ^{-/-} mice. The cross-sectional area of muscle fibers was determined by microscopy and digital image analysis. Immunohistochemistry and flow cytometry were used to quantify the number of intramuscular inflammatory cells. In addition, the *in vitro* phagocytic ability of muscle-derived macrophages was also measured by flow cytometry. Finally, CD45⁺ leukocytes were isolated from muscles 2, 3 and 4 days post-injury by magnetic cell separation and the gene expression of these cells was analyzed by RT-qPCR.

Based on cross-sectional area measurements we found that the frequency of smaller fibers was higher in regenerating TG2^{-/-} muscles compared to wild type ones. Quantifying the number of infiltrating neutrophils and macrophages we have found slightly, but not significantly, increased neutrophil number in TG2^{-/-} muscles at day 1 and 2 after injury. The expression of TNF α , IL1 β and IL6 proinflammatory cytokines at day 2, and the expression of Mrc1 and TGF β 1 M2 macrophage markers at day 4 is lower in leukocytes isolated from TG2^{-/-} muscles. Compared to wild type leukocytes the GDF3 expression was also lower in the absence of TG2. These results indicate that TG2 affects cytokine and growth factor expression in regenerating skeletal muscle which results in disturbed muscle regeneration.

This work was supported by the National Research, Development and Innovation Office (124244), European Union project titled: Institutional Developments for Intelligent Specialization program (grant number: EFOP-3.6.1-16-2016-00022 „Debrecen Venture Catapult Program”), and by the GINOP-2.3.2-15-2016-00006 project and EFOP-3.6.3-VEKOP-16-2017-00009 (co-financed by the European Union and the European Regional Development Fund).



P-026

The role of Mesr4 in germline stem cell differentiation

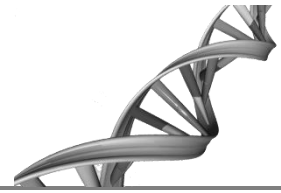
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Adult stem cells are unspecialized cells, which divide continuously to maintain tissue homeostasis. These cells usually reside in a special microenvironment called stem cell niche. One of the best known stem cell niches is the *Drosophila* ovarian germ line stem cell (GSC) niche. In the *Drosophila* ovary the balance between stem cell maintenance and differentiation is regulated by the Tgf β signalling pathway which maintains one of the GSC daughter cells in the undifferentiated stem cell state. In the other GSC daughter cell, however, the Tgf β signalling pathway is inactive, which permits the expression of *bag of marble* (*bam*), the key regulator of the differentiation, in the generation of a committed progenitor cell called cystoblast.

Here we show, that Misexpression suppressor of Ras4 (*Mesr4*) is required for the differentiation of the GSCs. Cell type- specific silencing of *Mesr4* revealed that it controls the differentiation process in the germ line in a cell-autonomous manner. By a series of genetic epistasis and transgenic rescue experiments we placed *Mesr4* into the hierarchy of developmental events of the GSC-to-cystoblast differentiation program. We demonstrate that *Mesr4* is required for the upregulation of the expression of the differentiation factor *bam*. The mutant phenotype of *Mesr4* can be rescued by restoring *Bam* expression identifying *bam* as the major target of *Mesr4*. *Mesr4* contains one AAA-ATPase domain, nine zinc-finger domains, a nuclear localisation signal and a plant homeodomain finger suggesting a role for *Mesr4* in transcriptional regulation. Interactome analysis of *Mesr4* revealed an association between *Mesr4* and *host cell factor* a component of the ATAC histone acetyltransferase complex. We hypothesise that *Mesr4* contributes to stem cell differentiation as an epigenetic regulator by promoting transcription of differentiation genes.



P-027

The role of syndecan-4/Rac1 signalling in myoblast differentiation and fusion

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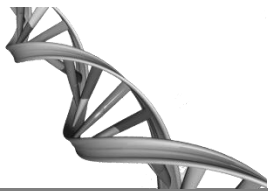
Skeletal muscle is constantly renewed in response to injury, exercise, or muscle diseases. The muscle progenitor satellite cells are quiescent in healthy muscle, they are stimulated by local damage to proliferate extensively and form myoblasts that will subsequently migrate, differentiate and fuse to form muscle fibers. The ubiquitously expressed transmembrane, heparan sulfate proteoglycan syndecan-4 (SDC4) has an important role in outside-in and inside-out signalling events by influencing cell-matrix adhesion, endocytosis, exosome biogenesis, cytokinesis, regulating the activity of Rac1 GTPase and the level of intracellular calcium. The Rac1-mediated actin remodelling is required for myoblast fusion.

SDC4 KO mice are unable to regenerate damaged muscle; however, the precise mechanism has not been characterized. Here we show that shRNA-mediated silencing of SDC4 in myoblasts increased the expression of the myogenic regulatory factor MyoD, increased the differentiation index (myotube number / total number of cells) and fusion index (nuclei in myotubes / total number of nuclei), number of nuclei per myotube, myotube size, and length. The inhibition of Rac1 activity (NSC23766; 50 μ M) blocked the fusion of SDC4 silenced myoblasts, indicating the role of Rac1-GTP in SDC4-mediated cell-cell fusion. According to our model the gradually decreasing level of SDC4 during the differentiation allows the activation of Rac1 leading to myoblast fusion. On day 4, 5 and 7 of the differentiation, the basal $[Ca^{2+}]_i$ and depolarization-induced $[Ca^{2+}]_i$ levels showed significant differences following SDC4 silencing.

Our results may contribute to the understanding of the essential role of SDC4 in the development and regeneration of skeletal muscle.

This research was supported by GINOP-2.3.2-15-2016-00040 grant of the National Research, Development and Innovation Office, Hungary.

Keywords: syndecan-4, myoblast, differentiation



P-028

Basal body composition during spermatogenesis of *Drosophila melanogaster*

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Drosophila melanogaster have a highly regulated spermatogenesis, which results in an extremely long, 1.8mm, sperm. *Drosophila* sperm has two mitochondrial derivatives and a very long axoneme, which is nucleated from the basal body. Late stages of spermatogenesis is controlled mainly by testes-specific proteins. We started to investigate the testis specific basal body components. We have identified several testis-specific basal body components by testis-specific RNA sequencing of different region of wild type *Drosophila* testis. We present the characterization of the identified genes by phenotypic description of the classical mutants. We generated transgenes and present the localization pattern of them in cultured cells and in transgenic animals. Our results help to understand the molecular composition of basal body, which could provide information of nucleation and growth of the extreme long axoneme of the sperm of *Drosophila melanogaster*.

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P-029

Examination of the redundancy between DAAM and FRL formins during pigment cell development in the *Drosophila* eye

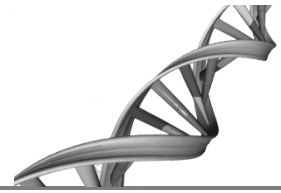
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The major aim of my studies is to examine the potentially redundant role of two formins, DAAM (Dishevelled associated activator of morphogenesis) and FRL (Formin related in Leukocytes/ Formin-like), during eye development in *Drosophila melanogaster*.

Formins are highly conserved cytoskeleton regulatory proteins that act in dimers and support the assembly and elongation of new actin filaments. The dynamic assembly and disassembly of the actin filaments is controlled by a large set of regulatory proteins some of which also contribute to the formation of higher-order actin structures, such as bundles and networks of filaments. Among the three major types of actin nucleation factor that have so



far been identified, we focus our studies on the formin protein family. Beyond actin regulation, some formins were shown to be able to interact with microtubules as well, and they play a role in coordination of the actin and microtubule cytoskeleton.

We have previously shown that these two actin assembly factors play redundant roles during axonal growth in the mushroom bodies of the adult brain. We also noticed that although DAAM is strongly expressed in the developing eye and in axons of the photoreceptor cells, *DAAM* mutant animals do not exhibit any developmental abnormalities in the eye. On the contrary, *dDAAM; frl* double mutant adult flies exhibit rough eyes. These results suggested that these formins may act redundantly during eye development. In accordance with this, we found developmental aberrations of the pigment cells in the double mutants, and shape of the ommatidia (unit eyes) were also abnormal.

To determine the cellular role of these two formins, we follow eye development in the pupal stages in the double mutants, whereas the single mutants are used as controls. Moreover, recently we generated a verified *frl* null allele with the CRISPR/Cas9 system (*frl^{ko}*), and we raised a specific antibody against the FRL protein. Remarkably, in the *DAAM; frl^{ko}* double mutants we observed a unique and novel eye phenotype: the pigment cells (also known as interommatidial cells) can not reach the lower layers of the retina and fail to undergo the typical cell shape changes required to properly seal the bottom of the unit eyes.

My poster will summarize our novel findings regarding the role of these two apparently redundantly acting formins required for eye development.

Keywords: *formin, DAAM, FRL, eye development, pigment cell, Drosophila*

P-030

Role of *Su(var)2-10* gene in germ cell development of *Drosophila melanogaster*

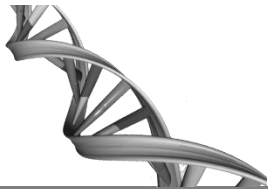
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Sumoylation is a post-translational modification which attaches a small ubiquitin-like modifier (SUMO) to the target proteins, thereby modulating their functions. Similar to ubiquitination, sumoylation is catalyzed by E3 ligases. In the course of a large scale RNAi screen, we have identified the E3 SUMO-ligase *Su(var)2-10* gene as a key regulator of gonadal development in *Drosophila melanogaster*. Our purpose is to reveal the biological functions of *Su(var)2-10* in germ cell development.

To determine which cell types and which stages of oogenesis are affected by the *Su(var)2-10*, we depleted its mRNA in the ovary using the GAL4>UASshRNA system. According to our results, depletion of *Su(var)2-10* mRNA in the germ line during the early developmental



stages resulted in a highly penetrant rudimentary adult ovary. Detailed analysis of the germarium revealed that it does not contain stem cells and differentiating germ line cells. Contrarily, RNA silencing in the germ cells of later egg chambers did not influence the oogenesis. Furthermore, we demonstrated, that depletion of Su(var)2-10 in the somatic niche cells does not affect the proper niche formation and stem cell maintenance. However, *Su(var)2-10* silencing in the follicular cells of the ovary resulted in abnormal germaria, as the developing germline cysts failed to be encapsulated by follicle cells.

For further analysis, we generated homozygous Su(var)2-10 mutant clones in the adult ovary using FLP/FRT-based mitotic recombination. The ovary of the flies was analyzed one and two weeks after clone induction. The percentage of the Su(var)2-10 mutant germline stem cell and cytoblast clones was significantly lower compared to the percentage of wild type clones. Contrarily, percentage of the Su(var)2-10 mutant cystocysts was comparable to the wild type. The percentage of the mutant follicle clones was much lower compared to the wild type. The size of the mutant follicle clones was also smaller than the size of the wild type clones.

These results indicate that the Su(var)2-10 is required for the initial stage of germline development and it is essential for the stem cell/cystoblast maintenance but it is dispensable for the later stages of oogenesis and differentiation of cystocytes. We also demonstrated that the Su(var)2-10 is essential for the proper development and viability of follicle cells.

This study is supported by grant from the National Research, Development and Innovation Office (K-117010; PD 124446; GINOP-2.3.2-15-2016-00001; GINOP-2.3.2-15-2016-00032). M.B. is supported by the János Bolyai Research Fellowship of the Hungarian Academy of Sciences.

Keywords: *Su(var)2-10, sumoylation, germ cell, Drosophila*

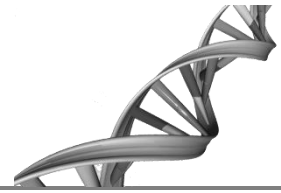
P-031

Small ovary (sov) regulates transposon silencing by prompting heterochromatin formation in Drosophila

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Germ cells are specialised to transmit the genetic information of species to the next generation. Thus, protection of the germ cells genome against detrimental mutagenic effects is indispensable for the maintainance of the species. In the germ cells, a small RNA-based defence system, the so called piRNA pathway protects the genome against transposone induced mutagenesis, the pathway includes post-transcriptional gene silencing and transcriptional silencing through heterochromatin formation at the transposon loci. *Drosophila small ovary* gene (*sov*) was identified through large scale RNAi screening to be



important in germ line development. *sov* mutant flies showed abnormal germline stem cell survival and differentiation accompanied by higher transposon mobility.

Position effect variegation test showed *sov* as variegation repressor and protein interaction studies revealed *sov* to colocalize with HP1 in the nucleus, a key component of the heterochromatin, suggesting *sov* to play a role in chromatin regulation. FRAP studies shows a higher HP1 mobility in *sov* mutants indicating that *sov* supports the association between HP1 and the chromatin. Using qPCR analysis, we found that transposon derepression in *sov* mutants was not accompanied with a decreased production of piRNA precursors. All together, *sov* is suggested to help transposon repression in the germline cells through transcriptional gene silencing mechanism as a structural component of the heterochromatin.

Keywords: *Drosophila*, piRNA, chromatin, heterochromatin, HP1a

P-032

Testing the biological significance of the nuclear localization of actin

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In recent years it has become clear that the main cytoskeletal component of eukaryotic cells, Actin is present also in the nucleus. However, there was no attempt so far to investigate the biological significance of this localization therefore, we don't know today how essential is nuclear Actin for the organism. The aim of our work is to understand the importance of nuclear Actin in *Drosophila melanogaster*.

Out of the six Actin coding genes of *Drosophila melanogaster*, two (*Act5C* and *Act42A*) encode ubiquitously expressed, essential proteins which are also present in the nucleus. We chose *Act5C* in our experiments because its lack is lethal, and the gene has a simple structure, small size and well characterized regulation. To investigate the biological significance of Actin's nuclear function at the organism level, we created a two-component genetic system of *Act5C*. One element of this system is an *Act5C* null-mutant line which lacks the whole protein coding region, and the other element is represented by animals carrying various *Act5C* transgenes. The transgenes express different forms of the Act5C protein including one which is tagged with a Nuclear Export Signal (NES). The NES tag dramatically decreases the amount of nuclear Act5C, without damaging the protein's cytoplasmic functions. Our subsequent experiments revealed that the *NES-Act5C* transgene is able to rescue the lethal phenotype of the null-mutants suggesting that the nuclear localization of Actin is not essential. However, the efficiency of the rescue was apparently lower than in the control experiments. Based on this finding and other results, we hypothesize that Actin has essential



role in the nucleus but its nuclear functions are most likely redundantly secured. The ways through which the nuclear functions of Actin are assured are under investigation and will be discussed.

Keywords: *actin, nucleus, Nuclear Export Signal, Drosophila melanogaster*

P-033

Testis specific proteasome subunits in *Drosophila melanogaster*

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Proteasomes have an important function in selective protein degradation. Malfunction in the ubiquitin proteasome system may cause severe neurodegenerative diseases, tumors or infertility. Recent studies have revealed that the proteasome has dynamic and heterogeneous structure in different cell types, such as immune cells or spermatids. In *Drosophila melanogaster*, about one third of the proteasome genes are found to have testis-specific paralogs, including both the regulatory and catalytic subunits. We examined the role of these testis-specific proteasome subunits in spermatogenesis. We studied the phenotypes of classical mutants and RNSi lines and we found male sterility and spermatogenic defects in 6 cases. We found that both the testis-specific core and the regulatory subunits have important role during spermatogenesis. We determined the localization of testis-specific proteasome subunits in wild type testis by tagged proteins. The phenotypical characterization of the mutants revealed that testis-specific proteasomes play crucial role in the post-meiotic stages of spermatogenesis.

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Keywords: *proteasome, spermatogenesis*



P-034

The role of linker Histone H1 variant in early *Drosophila* embryogenesis

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Metazoan genomes usually encode several linker Histone (H1) variants which are often expressed in a developmental stage or tissue specific manner. In many vertebrate species a specific H1 variant is expressed during the earliest developmental steps. In *Drosophila*, H1 complexity is much lower than in vertebrates since only dH1 is expressed in somatic cells and only the germ line and early embryonic cells express an alternate Histone H1 variant, dBigH1. It has been shown that dBigH1 is essential during early embryogenesis where it replaces somatic dH1. Interestingly, homology between these proteins is rather low: 30% overall. Our questions are: why does *Drosophila* need an alternate linker Histone during early embryogenesis, and what are the specific features of this protein. To answer these questions, we modified the endogenous dBigH1 using CRISP/Cas9 gene editing. We created chimeric H1-dBigH1 genes, in which dBigH1 domains were changed to dH1 domains. We also replaced the entire dBigH1 coding sequence to dH1. The expression patterns of chimeric proteins are similar to the wild type dBigH1. We found that these modifications do not strongly influence fly fertility or viability. However, mutants where the C-terminal domain was changed, showed increased nuclear fallout phenotype in precellular embryos, indicating that the C-terminal domain of dBigH1 plays a role in determining its specific feature. We also show that nuclear volume is higher in precellular embryos when dBigH1 is replaced to dH1. To reveal the biological significance of these observations we measured cell cycle lengths of this mutant in precellular embryos, and we tested our mutants under stress conditions (cold and heat).

This work was supported by grants from The National Research, Development and Innovation Office (OTKA-116372) and Ministry for National Economy of Hungary (GINOP-2.3.2-15-2016-00032).



P-035

Uracil in the DNA during the early development of zebrafish

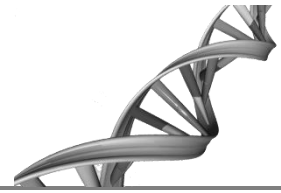
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The presence of uracil in DNA is usually the sign of erroneous polymerase activity (dUTP being incorporated instead of dTTP) or cytosine deamination. As the latter results in mutagenic U:G mismatches in the double helix, cells have evolved error repair mechanisms to ensure that such mismatches are repaired promptly, and the freely available dUTP pool is kept at relatively low steady-state levels, to prevent uracil incorporation into the DNA. Recent data suggests that the impairment or absence of the two enzymes that guard the cell from permanent uracil accumulation, uracil–DNA glycosylase (UNG) and dUTPase, can have profound morphological consequences on the development of animals. Using zebrafish (*Danio rerio*) as a model we explore the dynamics of uracil incorporation into the DNA during development, and show that prior to the activation of the zygotic genome (MZT) uracil levels are significantly increased and only later reduced. This suggests the presence active regulatory mechanism that is only active post-MZT. We apply a combination of loss-of-function techniques to explore the developmental and genomic consequences of UNG and dUTPase impairments.



P-036

Chromatin fractionation sequencing for the detection of early heterochromatin alterations in Hutchinson-Gilford progeria syndrome

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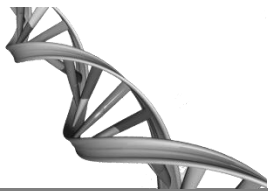
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[§] Equal contribution

^{*} Equal contribution

Here we present a new high-throughput sequencing-based technique, named Sequential Analysis of MacroMolecules accessibilitY (SAMMY-seq), for the genome-wide mapping of chromatin regions separated by differential accessibility. The method is based on the sequential extraction of multiple chromatin fractions, corresponding to increasingly compacted and less accessible chromatin regions, which are mapped along the genome using high-throughput sequencing. Using SAMMY-seq we analyzed Hutchinson-Gilford progeria syndrome (HGPS) skin fibroblasts and normal control fibroblasts. HGPS is characterized by the progressive accumulation of progerin, an aberrant form of lamin A leading to chromatin structure disruption, transcriptional changes, and accelerated aging, in particular by interfering with Lamina Associated Domains (LADs). In normal fibroblasts SAMMY-seq allows the mapping of LADs in heterochromatin regions. In HGPS primary fibroblasts we were able to detect early-stage changes of chromatin structure, where the most compact heterochromatic regions are not overlapping with normal fibroblast LADs anymore. Of note, these structural changes do not alter the distribution of H3K9me3, a mark of constitutive heterochromatin, but are associated with transcriptional deregulation of Polycomb target genes. These results suggest that progerin could affect Polycomb group dependent maintenance of heterochromatin structure. Taken together, our results show that SAMMY-seq is a versatile and sensitive tool to characterize heterochromatin alterations in primary cells.

Keywords: *Genome structure, lamin A, Chromatin conformation*



P-037

Constrained and extranucleosomal superhelicity impose a barrier to intercalation in native chromatin

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DNA intercalating agents cause structural changes to DNA upon binding and have thus been utilized as probes for chromatin structure and as DNA-targeting chemotherapeutic drugs. Despite their relative success in therapy, the mechanisms by which these drugs cause cell death are not completely understood and the mechanisms controlling their intercalation into DNA in native chromatin have not been elucidated either. In the wake of recent reports on intercalator-induced nucleosome eviction^{1,2} the area has become the focus of intense research.

Ethidium bromide (EBr) is a classical intercalators, whose interaction with isolated chromatin and nucleosomes is well characterized. In live HeLa cells however, we observed that the chromatin exhibited a conspicuous resistance to EBr intercalation even when the nuclear interior was already saturated by the dye (patch clamp set-up). Using confocal microscopy and laser scanning cytometry, we have examined EBr binding in HeLa cells expressing GFP-tagged histones and in human peripheral blood lymphocytes, to elucidate the mechanisms regulating intercalation *in vivo*. We have also extended our study to other intercalators including psoralen and YOYO-1.

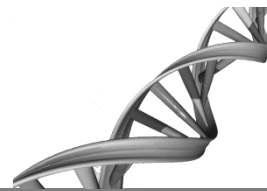
Our results demonstrate that the nucleosome structure is a highly effective barrier to intercalation into nucleosomal DNA. This barrier is overcome when the nucleosomes are disassembled by salt treatment. Further, introducing a single nick per 50 kb chromatin loop by x-ray radiation, we observed an increase in EBr binding at salt concentrations where even the linker histones stay chromatin-bound. This observation is taken as evidence of net torsional strain in the linker and nucleosome free regions within the chromatin loops *in vivo*. Elucidation of the mechanism of resistance of native chromatin to intercalators can be exploited in medical applications of anthracyclin derivatives.

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Keywords: Intercalators, native chromatin, superhelicity

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P-038

Developing novel gene integration and amplification techniques for enhanced transgene expression in *Escherichia coli*

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Escherichia coli has been the organism of choice for hosting the production of numerous different high value compounds. In the majority of such enterprises, the heterologous genes and pathways required for production were expressed from plasmids. Such systems however, display genetic instability, a need of constant selection, and a high cell to cell variability in transgene copy number and expression level. There is therefore a strong need to find a method to efficiently integrate entire metabolic pathways in a rapid and marker-less manner into the *E. coli* genome to facilitate testing and engineering of novel production strains. Larger size integrations have been reported using the lambda Red machinery and the CRISPR/Cas system, but the requirement of landing pads is usually a limiting factor for general and robust applicability. We aim to develop a system that allows multiple integration of transgenes and their further amplification to obtain stable, multi-copy chromosomal expression.

P-039

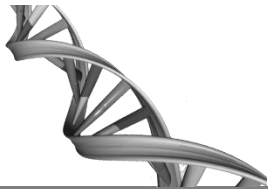
Early and long term behavioral and molecular patterns evoked by toxic stresses in *Caenorhabditis elegans*

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Early life memories are particularly stable and form enduring behavioral patterns. Imprinting is characterized by a sensory cue at the critical perinatal period that evokes a life-long response critical for survival. Adult preference for odors associated perinatally with food have widely been documented both in vertebrates and nematodes, but little is known about responses to cues representing danger. We study the early and persistent (adult) molecular and behavioral responses of the nematode *C. elegans* evoked by odors that have been associated with toxic insults in newly hatched animals.

We observed that exposure to either antimycin (terminal oxidation inhibitor, AM) or paraquat (mitochondrial superoxide inducer, PQ) in *E. coli* OP50 food source employed for



24 hours from hatching resulted in a dose-dependent developmental retardation and aversion of the bacterial lawn. At the molecular level, AM treatment induced the expression of *hsp-6::GFP* mitochondrial stress marker and PQ treatment induced the expression of *gst-4::GFP* phase II detoxification enzyme reporter in early life. Intriguingly, worms that did not avoid the food lawn exhibited a significantly higher expression of the abovementioned molecular surveillance markers.

Early learned behavioral responses have been assayed in L1/L2 larvae by a food leaving-food choice test that had been set up in our lab. AM and PQ treated, compared to control, worms both exhibited (i) an aversive associative memory to *E. coli* OP50 sensory cues (i) an increased preference for previously not encountered *B. subtilis* bacteria. Animals expressing higher *hsp-6::GFP* or *gst-4::GFP* fluorescence respectively, preferentially remained on OP50, suggesting a connection between inducible somatic cytoprotective responses and learned behavior.

We did not find, yet, that AM and PQ induced aversive behavior and food preference would persist in adulthood. However, in response to early life AM or PQ exposure a re-encounter of young adult with OP50 sensory cues alone significantly increased the expression of *hsp-6::GFP* or *gst-4::GFP* reporters, respectively.

In summary, perinatal toxic insults associated sensory cues trigger a complementary pattern of cytoprotective and early learned aversive responses as well as a re-activation of toxin-induced molecular defenses in adulthood. To our knowledge, this is the first demonstration of an imprinted „cellular memory” as well as an imprinted response to perceived danger. Our findings suggest that such connection between cellular and behavioral responses exist in mammals and may shed light on persistent aberrant somatic symptoms after adverse early life experiences.

P-040

Effect of DNA targeting drugs on DNA topology

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DNA- and epigenetic targeted drugs have become of pivotal importance in cancer chemotherapy. In addition to the complexity of their effects, the lack of information on their interactions at the chromatin level imposes a significant limitation on their combinative application. Cisplatin, a widely used agent in cancer chemotherapy, is able to crosslink the two strands of the DNA. These interstrand crosslinks (ICLs) are considered to be important factors in the cytotoxicity of this compound. The well-known relationship between DNA superhelicity and ICL formation implies the possibility that crosslinking efficiency could be



modulated via changing superhelicity using intercalating agents like Daunomycin, which is frequently used in the clinic in combination with Cisplatin. In addition, reactivity of both drugs with DNA could be sensitive to possible shielding effect of the nucleosomes, the stability of which may be altered by intercalators (1). We set out to experimentally analyze these interconnected chromatin features that might influence the individual reactivities of these agents with the DNA so that we can better understand how they might interact at the chromatin level, *in vivo*. Our experimental model involves agarose embedded, permeabilized nuclei of parental and histone-GFP expressing HeLa cells, treated with different concentrations of salt to evict the dimers or also the tetrasomes. To measure Cisplatin adduct levels, including those of the ICLs, we have developed a laser scanning cytometric (LSC) method based on the alkaline nuclear comet assay and also performed mass spectrometric imaging. We measured DNA-bound Daunomycin via its quenching Hoechst 33342 fluorescence. We attempt to interpret the cell type- and concentration-dependent synergy and antagonism of the two drugs, what we observed via the isobologram analyses of Alamar Blue cytotoxicity assays, based on our observations in the above model system.

Acknowledgements: EFN is sponsored by the Richter Talentum Fund. This work has been supported by OTKA K128770 and GINOP-2.3.2-15-2016-00044.

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P-041

Epigenetic regulation of chondrogenesis in chicken micromass cell cultures

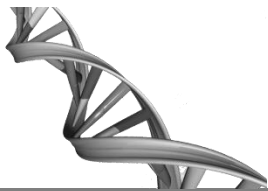
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During ontogenesis, epigenetic mechanisms create a suitable nuclear milieu for cell type- and differentiation stage-specific gene expression. Differentiating chondrocytes first proliferate rapidly along with intense cartilage matrix synthesis, but divide rarely and maintain low-metabolic activity as they mature. Epigenetic factors are supposed to play a crucial role in regulating such cellular changes during cartilage formation, therefore our aim was to investigate this less elucidated field of tissue specific epigenetics.

Chicken embryos of Hamburger-Hamilton 22-24 stages were used to create primary micromass cell cultures of chondrifying mesenchymal cells. Some cultures were treated with GSK-126 (2 μ M) altering histone methylation, while others got 5-azacytidine (10 μ M)



inhibiting DNA methylation. Treatments were applied from the first or third day of culturing for 72 hours. Changes in the quantity of cartilage-specific extracellular matrix and bone-specific calcium deposits were examined by specific histological stainings (DMMB: dimethyl-methylene blue, TB: toluidine blue, AR: alizarin red staining). qPCR reactions were carried out to study the expression of cartilage-specific and bone-specific genes (Sox9: SRY-box 9, Col2a1: collagen type II alpha 1 chain, Acan: aggrecan, Col10a1: collagen type X alpha 1, Runx2: runt related transcription factor 2). Protein expression of Sox9, aggrecan, and the phosphorylation status of Sox9 was analyzed by Western blot. Measurements of cell proliferation with ³H-thymidine labelling and mitochondrial activity with MTT-assay were also carried out.

According to our first results, the inhibitor treatments applied during early stages of differentiation affected chondrogenesis in a negative way. The addition of chemicals in later chondrification stages, however, stimulated chondrogenesis. The experiments and analysis of data are in progress, the obtained results will also be presented by the time of the congress.

Keywords: *chicken chondrogenic cell culture, inhibition of DNA and histone methylation, 5-azacytidine, GSK-126*

P-042

Functional studies of the domesticated human PGBD1 transposon

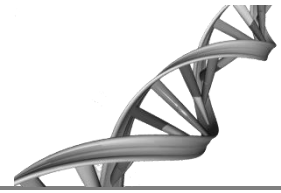
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Mobile genetic elements (transposons) make up approximately 45% of the human genome and although some of them were known to be still active, they were mostly considered as junk DNA. However, recent studies revealed that several transposons have been domesticated by the host genome: they have lost the ability of transposition during evolution, but gained a new function that is advantageous for the host.

DNA transposons make up only a small fraction of the human transposons but they still represent 3% of the whole genome, representing approximately 300.000 sequences. There are several piggyBac-like element derived sequences among them, and our research group focuses on the five potentially domesticated transposase genes (PGBD1-5). We could show that one of these genes, namely PGBD1 is expressed in several tissues in various isoforms, for example in the brain and in certain blood cell types. However, the exact function of PGBD1 is currently unknown.

We tested the transposition activity of the fusion PGBD1 variant and the transposase domain in excision and in colony assays and could provide evidence for the lack of its transposition ability. We have investigated the potential domesticated functions of PGBD1: by genotyping Hungarian haemochromatosis patients with control groups, we showed that



one variant of the gene shows a linkage with the disease, indicating that the protein(s) might have leukocyte specific functions. In addition, based on previous bioinformatics predictions, we tested if PGBD1 is involved in RNA regulation, such as in splicing pathways but could exclude that possibility. We have established cell lines stably expressing PGBD1 variants and we are currently investigating if the expressed proteins influence cell proliferation, and attempting to decipher what cellular functions the PGBD1 protein(s) potentially have.

Our research was supported by the OTKA K-112112 and the VEKOP-2.1.1-15-2016-00156 grants.

Keywords: *DNA transposon, PGBD1*

P-043

Investigation of meiotic DNA Double-Strand Break (DSB) proteins with chromosome conformation capture techniques

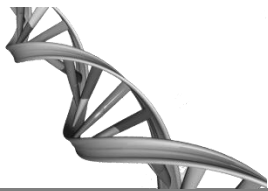
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Meiotic recombination is initiated by the formation of double-stranded DNA breaks (DSBs) catalyzed by the Spo11 enzyme. DSBs occur during the first reductional division of meiosis at particular genomic sites called hotspots. The proteins involved in DNA cleavage and repair are highly conserved among organisms including mammals. Spp1, a member of the Set1C/COMPASS histone methylase complex, contains a H3K4me3-reader PHD (plant homeodomain) finger domain and a Mer2-binder CxxC zinc finger motif, and plays a key role in the DNA cleavage process. In the absence of functional Spp1, H3K4me3- and DSB levels are greatly reduced because DSB protein Mer2 is not tethered to the chromosome axis where DSB formation occurs (1, 3, 5). However, molecular details of the Spp1-mediated chromatin tethering process remain largely unknown.

In order to investigate the spatial interaction between Spp1 and the DSB protein Mer2, we applied a modified chromosome conformation capture approach called Hi-ChIP (or PLAC-seq) (2, 4). We used wild-type and separation-of-function mutant yeast cells in which Spp1 and Mer2 were tagged with 9xmyc epitopes that were subsequently immunoprecipitated by anti-myc antibodies. With this enrichment step, we mapped the Spp1-mediated and Mer2-anchored 3D interactome with high specificity and superior spatial (1-3 kb) resolution. Our data are expected to clarify the roles of Spp1 and Mer2 in loop-axis tethering leading to a mechanistic understanding of DSB formation in the context of 3D genomic structure.

Keywords: *Hi-C, Hi-ChIP, meiosis, recombination, Set1C, Spp1, Mer2, H3K4me3*



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P-044

Investigation the role in mRNA export of the actin binding protein, moesin

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Accurate and precise control of gene expression is critical for cell survival in order to respond to cellular stress and environmental stimuli. Gene activity is tightly regulated at the level of transcription and translation but mRNA export which links the two processes also plays key role in gene regulation. During RNA export, several specific proteins are recruited to the transcribed RNA molecule where they form an RNA-protein complex, called messenger Ribonucleoprotein Particle (mRNP). In our laboratory we are studying the nuclear function of moesin, the single cytoskeletal actin-binding ERM protein in *Drosophila melanogaster*. ERMs (Ezrin, Radixin and moesin) compose a highly conserved group of proteins and carry out many crucial cytoplasmic functions including reorganization of the actin cytoskeleton, cell survival, membrane dynamics or cell migration. Previously we demonstrated that the moesin protein is present also in the nucleus where it shows clear co-localization with mRNA export factors. In a functional assay we observed the accumulation of total mRNA in the nucleus upon RNAi against *moesin* in cultured cells and *in vivo* as well, demonstrating that the inhibition of moesin's function impairs mRNA export. As the detailed molecular mechanism underlying the nuclear activity of moesin is still not known, we aim to identify the nuclear protein interaction partners of moesin. Mass spectrometry analysis verified by protein co-immunoprecipitation suggests that moesin's function is



related to the NXF2-mediated mRNA export pathway as a possible new binding partner of the Mediator complex. Moesin also shows colocalization with Mediator proteins on *Drosophila* larval giant chromosomes. *In vitro* assays will further confirm these protein interactions in order to get a deeper insight into the role and significance played by the actin-binding ERM proteins in nuclear mRNA export.

Keywords: *moesin, nucleus, mRNA export, Mediator complex, Drosophila*

P-045

Prior IL-4 exposure induces chromatin remodeling and the rearrangement of LPS-activated p65 cistrome, leading to synergistic transcriptional activation of a specific gene set in macrophages

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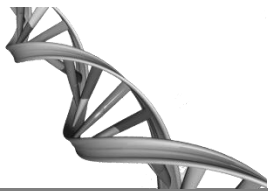
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In response to various environmental signals or pathogen-derived molecules, macrophages undergo extensive phenotypic shift. The two extreme types are called classical or M(INF γ) and alternative or M(IL-4) phenotype, but in complex tissue environments these cells often display a broad spectrum of macrophage polarization states. Using genome-wide technologies such as ChIP-seq, ATAC-seq and RNA-seq, we investigated the epigenetic and transcriptomic outcomes of inflammatory response in alternatively polarized mouse macrophages. Unexpectedly, we found that IL-4 pretreatment enhances the LPS-responsiveness of 216 genes including cytokines, chemokines, and several immune response-related genes in a STAT6 dependent manner. This phenomenon is associated with the IL-4-mediated remodeling of chromatin structure and the elevated LPS-activated NF κ B-p65 binding at the regulatory regions of synergistically activated genes in alternatively polarized macrophages. Finally, both the enhanced LPS-responsiveness of this gene set and also elevated NF κ B-p65 binding at the enhancers proved to be at least partially dependent on the



presence of the IL-4-induced EGR2 transcription factor. Taken together, these findings suggest that the complex interaction between alternative polarization and inflammatory signals in macrophages is orchestrated at the epigenomic level and can lead to synergistic gene regulatory events.

P-046

STAT6-mediated direct repression of inflammatory enhancers limits inflammasome activation in alternatively polarized macrophages

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Cellular response to environmental cues requires reorganization of the transcriptional program by activating novel pathways and turning off unneeded ones. The molecular basis of signal-dependent transcriptional activation has been extensively studied in macrophage polarization, however our understanding remains quite limited regarding the relevance and molecular determinants of repression.

Here we show that IL-4-activated STAT6 is required for the direct transcriptional repression of a large number of genes during *in vitro* and *in vivo* alternative macrophage polarization. At the molecular level, repression results in decreased lineage-determining transcription factor, p300 and RNA polymerase II binding followed by reduced enhancer RNA expression, H3K27 acetylation and chromatin accessibility. In addition, STAT6-repressed enhancers show extensive overlap with the NF- κ B/p65 cistrome and exhibit decreased responsiveness to LPS following IL-4 stimulus on a subset of genes. As a



consequence, macrophages exhibit dampened inflammatory response, including diminished inflammasome activation, decreased IL-1 β production and pyroptosis.

Our findings reveal a novel molecular circuit and biological activity of IL-4/STAT6 signaling in establishing and maintaining the alternative polarization-specific epigenetic signature and rendering macrophages less responsive to inflammatory stimuli.

P-047

The effect of the CaPpz1 protein phosphatase on gene expression in *Candida albicans* under oxidative stress conditions

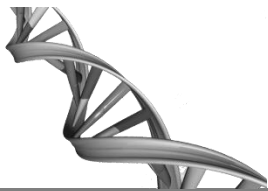
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Candida albicans is an opportunistic pathogen which is the most common cause of nosocomial fungal infections worldwide. It has one CaPPZ1 gene that codes for the CaPpz1 protein. This protein is a “novel” type, fungus specific serine/threonine protein phosphatase, which has several important physiological functions. In this study our aim was to investigate the role of CaPpz1 in oxidative stress response by a global transcriptomic method. To this end we compared the *cappz1* phosphatase deletion mutant with its genetically matching wild type strain under normal conditions and in oxidative stress induced by tert-butyl hydroperoxide (tBOOH). Total RNA was isolated from the fungal cells and the efficiency of the tBOOH treatment was verified. Three replicates of each sample were analyzed by Next Generation RNA sequencing. Transcript data were interpreted by bioinformatic tools. Biological triplicates showed only slight differences according to cluster and principal component analyses indicating that our experiments were reproducible. Differentially expressed genes with more than two-fold transcriptional changes were selected for further analysis. These were categorized based on gene ontology enrichment. We found that the deletion of the phosphatase and/or the oxidative treatment affected among others membrane transport, cell wall/surface components, translation, and metabolism. In general, we noted that either the tBOOH treatment or the lack of CaPpz1 caused only moderate transcriptional changes, but the combination of these resulted in robust alterations of gene expression. The amount of mRNAs associated with transport processes, cytosolic ribosomal proteins, oxidoreductases activity, and cell signaling were reduced, while the expression of genes coding for mitochondrial ribosomal proteins, enzymes of RNA metabolism, and cell surface proteins was downregulated by oxidative stress in the mutant



C. albicans. These preliminary results still have to be validated by RT-qPCR, but even in their present form they suggest that CaPpz1 protects the wild type fungus from oxidative damage.

Our work was supported by the NKFIH K108989 grant, and by the ÚNKP-18-3 New National Excellence Program of the Ministry of Human Capacities.

Keywords: *protein phosphatase, Candida albicans, gene expression*

P-048

An improved system for *in vitro* analysis of protein ubiquitination

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A complex network of proteins is responsible for the early recognition and repair of DNA double strand breaks of eukaryotic cells. Key components of this network are histone 2A and histone 2B and their respective post-translational modifications. It was previously demonstrated in mammalian cells that the phosphorylation of H2AX Ser139 (γ -H2AX) is required for the monoubiquitination of H2AX Lys119/120, and this ubiquitination is catalysed by a RING finger ubiquitin ligase [1]. We study whether a similar mechanism is involved in *Arabidopsis thaliana* DNA damage pathway by *in planta* studies and *in vitro* protein interaction and functional analysis.

The wheat-germ based protein expression system has been proven to be appropriate for production of eukaryotic proteins of native conformations by a cost- and time effective-manner. One of the most generally applied methods for protein interaction analysis is the pull-down assay. First, we set out to increase the sensitivity of this approach by creating a double-hexahistidine (12XHis) tag encoding *in vitro* translation vector and demonstrated that detection level of 12XHis labelled proteins is at least a magnitude larger than those of traditional hexahistidine tagged proteins. Next, we developed a protocol for *in vitro* investigation of ubiquitin conjugating and ubiquitin ligase activity of proteins of interest. One of the main bottlenecks of straightforward analysis of these enzymes is the lack of selective detection of ubiquitination, which is implemented by the protein of interest. Application of anti-ubiquitin antibodies is limited by the high level of endogenous ubiquitination of protein extracts. On the other hand, the use of commercially available, tagged ubiquitin is hindered by their high price [2]. In order to tackle these restraints, we optimized a protocol for the bacterial expression and purification of FLAG-tagged ubiquitin and tested the applicability of the purified protein in the wheat germ-based *in vitro* translation system. The obtained results demonstrated that the FLAG-tag provides the needed



selectivity and sensitivity for detection of *de novo* ubiquitination of proteins. We propose that the above described developments could aid analysis of ubiquitination cascades.

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Keywords: DNA damage, ubiquitination, *in vitro*

P-049

Cellular trafficking of ABCG2 variants assessed by a novel dynamic approach

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ABCG2, an integral membrane protein belonging to the ATP-Binding Cassette transporter superfamily, is expressed in stem cells and in polarized cells of several physiological interfaces. Using the energy of ATP binding and hydrolysis, ABCG2 mediates the transport of numerous molecules across the plasma membrane of these cells, providing protection against endo- and xenobiotics. Urate is also a physiologically relevant substrate of ABCG2, thus, the dysfunction of the transporter may lead to hyperuricemia or gout. Proper targeting of ABCG2 to the correct membrane compartment is essential for its normal function. Frequently, mutations or polymorphisms, such as the M71V and Q141K substitutions, affect the cellular routing of the protein rather than its transport function. Better understanding of the trafficking of these ABCG2 variants may therefore help to develop effective and personalized treatment for gout patients. Localization of these transporters is most frequently studied by antibody staining in fixed samples, which can only reflect the steady state distribution of the protein. In our study, the cellular routing of the ABCG2 transporter was assessed by a dynamic method, the Retention Using Selective Hooks (RUSH) system, which is capable of tracking the transporters in various subcellular compartments. Wild type and polymorphic variants of ABCG2 were expressed in HeLa cells. Using the RUSH system, the ABCG2 variants were initially retained in the endoplasmic reticulum, from which they were released by the addition of biotin. The glycosylation properties and the cell surface appearance of transporter variants were assessed by Western blotting and confocal microscopy, respectively. We found that Q141K and M71V polymorphisms in ABCG2 cause a trafficking defect, where the rate limiting step is the transfer from the endoplasmic reticulum to the Golgi apparatus. Our results and the developed assay method can assist to



identify pharmacological chaperones that promote plasma membrane delivery of ABCG2 variants with trafficking defect, opening new perspectives to the treatment of gout patients.

This work has been supported by the National Research, Development and Innovation Office (OTKA_K 128123).

Keywords: ABCG2, cellular trafficking, RUSH, gout, polymorphism

P-050

Characterization of Trehalose-6-phosphate synthase and Na⁺/H⁺ antiporter genes in Turkish endemic plant species *V. turcica*

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V. turcica is an endemic to Turkey and endangered plant species. Although this species was discovered in 1983, since genomic knowledge is not known, molecular studies have been few till now. As in the rest of the world, abiotic stress factors in our country seriously affect production. Salt stress, which is one of the abiotic stress factors, affects the photosynthesis and physiological functions of plants negatively and causes crop loss. Trehalose-6-phosphate synthase and vacuolar Na⁺/H⁺ antiporter genes are known to be useful in salt tolerance. It has been found in our previous studies that the *V. turcica* plant is also salt tolerant. In this study, the *TPS* and *NHX* – like genes in *V. turcica* were partially sequenced and submitted to the NCBI database (accession numbers MK120983 and MH757417, respectively), and gene profiles of identified *TPS* and *NHX* were investigated. According to literature, this is the first attempt to identify the genes such as *TPS* and *NHX* in *V. turcica*.

Acknowledgments: *This study was funded by Scientific Research Projects Coordination Unit of Mersin University (Project Code: 2018-2-AP3-2961)*

Keywords: *Characterization, NHX, Salt tolerance, TPS, Vuralia turcica*



P-051

CRK5 protein kinase exhibits an essential role in embryogenesis of *Arabidopsis thaliana*

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Plant embryogenesis requires fine tuning of hormonal signals including ethylene, gibberellin, brassinosteroid and auxin. The directional movement of auxin is ensured by coordinated action of specific transporters such as the PIN efflux, the AUX1/LAX1 influx and the ABCB proteins. Both efflux and influx auxin transporters were found to be responsible for embryo polarity determination. Earlier we reported that the *Arabidopsis thaliana* CRK5 protein kinase can phosphorylate PIN2 protein and modulate root gravitropic response (Rigó et al., 2013 Plant Cell, 25:1592-1608). Here we present evidence that AtCRK5 is implicated in the maintenance of the local auxin concentration during embryo formation and mediate the auxinGA crosstalk in embryo development. Embryogenesis is delayed in the *Atcrk5-1* mutant, which is accompanied with reduced gibberellic acid (GA) content. The abundance of the polar auxin transport (PAT) proteins PIN1, PIN4 and PIN7 in the *Atcrk5-1* mutant embryos decreased (Figure 1). Moreover, AtCRK5 can phosphorylate *in vitro* the hydrophilic loops of additional PIN proteins including PIN1 and PIN3 which are involved in auxin transport during embryogenesis. Our results suggest, that in addition to its regulatory role in root gravitropic responses, AtCRK5 can also govern the embryo development in *Arabidopsis* through fine tuning auxin-GA levels by phosphorylation of polar auxin transport (PAT) proteins.

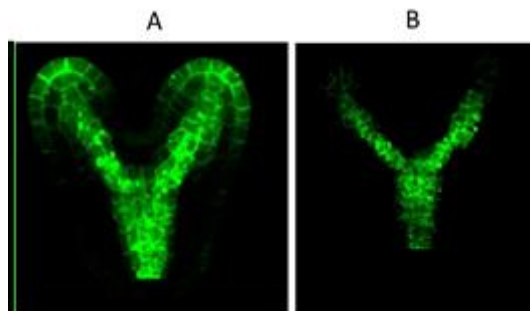
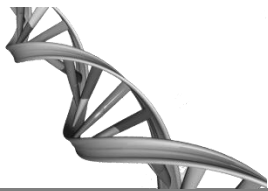


Figure 1: PIN1-GFP abundance decrease in *Atcrk5-1* mutant in torpedo stage embryo. (A) PIN1GFP pattern in wild type (*Col-0*) and (B) in mutant (*Atcrk5-1*) backgrounds.



This research was supported by OTKA project No. PD115502 and No. PD128055, NKFI project No. NN118089, OTKA grant No. 124828 and GINOP-2.3.2-15-2016-00001. AIB was supported by the Tempus Public Foundation, and the Doctoral School in Biology, University of Szeged, Hungary.

Keywords: *Ca²⁺-calmodulin-dependent kinase-related kinases (CRKs), polar auxin transport (PAT) proteins, embryogenesis, Arabidopsis thaliana*

P-052

Determining the optimal cell conservation method with highest proportion of cells alive after culture re-initiation

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Introduction: There is no uniformly accepted standard for cell culture protocols. Various protocols for freezing and thawing are available in the literature and on the websites of different companies producing lab instruments. Our goal was to compare previously published protocols and to provide a comprehensive evaluation of their usefulness.

Methods: Five different of freezing and two melting protocols were compared. One million cells were used for each freezing, the difference was in the composition of the freezing medium: 0/90/10, 20/70/10, 50/50/5, 60/20/20, 95/0/5 for the percentages of medium/FBS/DMSO, respectively. The two thawing methods include a “slow” multi-step approach a “rapid” direct de-freezing by directly adding the media to the cells.

The experiments were performed on two cell lines, A549 WT and MCF-7 LUC in triplicates. Before freezing and after melting, we counted cells and measured viability with Bürker chamber and MUSE Count & Viability Assay Kit. Apoptosis measurement were performed after 2 days with MUSE Annexin V and Dead Cell Assay Kit. The results were evaluated by a Kruskal-Wallis test, statistical significance was set at $p < 0.05$.

Results: The best results were achieved using the 20/70/10 (medium/FBS/DMS) method with rapid direct de-freezing in the MCF-7 LUC cells. In this setting, 71.06% of the cells were alive ($p = 0.01$). The worst result was delivered by the 60/20/20 combination with slow thawing on both cell lines.

In the A549 WT, the most efficient method was the 95/0/5 with fast thawing, when 77.33% of the cells were alive ($p = 0.0069$), and the second best was the same freezing as in the MCF-7 LUC but using slow de-freezing technique. In almost all cases, fast thawing proved to be better.

Discussion: Across all measurements, the best performing combination was the 20/70/10 (percentage of medium/FBS/DMSO, respectively) method with rapid direct de-freezing. However, there may be minor differences in the efficiency on different cell lines and the appropriate freezing and de-freezing method depends on the used cell line.



P-053

Discreet charm of the smallest APC/C subunit in *Drosophila*

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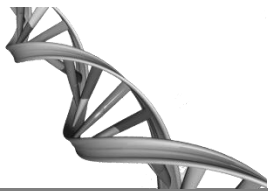
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The ubiquitin-proteasome system is responsible for the selective degradation of most cellular proteins, therefore it plays important regulatory role in many cellular processes. This includes the cell cycle, in which progression from one phase to the next requires the degradation of regulatory proteins. The Anaphase Promoting Complex or APC/C, a large ubiquitin-protein ligase complex, is one of the main players in cell cycle regulation, its activity is essential for metaphase-anaphase transition, mitotic exit and also for the maintenance of the G1 phase.

The APC/C contains at least fifteen evolutionarily conserved subunits, from which many appear as homodimers. The smallest, but still essential subunit is Cdc26, which plays a role in the structural stability of the complex. Data suggest that this small protein is present in all eukaryotes, from yeast to human, but only a short section of its N-terminal sequence is conserved. Using this motif we have identified not one, but two putative Cdc26 proteins in *Drosophila melanogaster*, which is unique among eukaryotes examined so far. The two proteins are – apart from the first 20 amino acids – different in size and amino acid sequence. They could be the product of gene duplication at the beginning of the Drosophilidae evolution, since a database search revealed that this arrangement is specific only to this family. One of the two proteins are essential, so we named it DmCdc26, while the nonessential one was named DmCdc26-like. Both of them are connected to the APC/C, but only DmCdc26 shows the expression pattern characteristic to other subunits, while Cdc26-like seems to be ovary specific. Our aim is to characterize these unique APC/C subunits and clarify their function.

This study is supported by the Hungarian Research Fund K116372, and the National Research Development and Innovation Office grant GINOP-2.3.2-15-2016-00032.

Keywords: cell cycle, mitosis, APC/C, ubiquitin-proteasome system, Cdc26, Cdc26-like



P-054

Dissection of *Plasmodium falciparum* developmental stages with multiple imaging methods

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Every year, more than 200 million people are infected with malaria. Five species of the *Plasmodium* genus cause human malaria infection. The protozoon is transmitted into the human body by a mosquito bite, leading to fever, anaemia, splenomegaly and finally death. In the blood stream the malaria parasites invade red blood cells, multiply, mature and then burst out of the cells, ready to invade further ones. This cycle has been the subject of intense research because it is the cause of clinical symptoms and therefore the major target of antimalarial treatment. The digestion of hemoglobin by all *Plasmodium* species results in the accumulation of a metabolic byproduct, the malaria pigment, and in morphological changes of the red blood cell which are typically characterized with bright-field microscopy. These changes are likely associated with alteration of red-blood-cell topology and mechanics, which are little understood.

To explore correlations of the *Plasmodium*-induced molecular, topological and mechanical changes, we investigated infected red blood cells with atomic force microscopy (AFM), phase contrast and total internal reflection fluorescence (TIRF) microscopy. The last is a sensitive probe of the malaria pigment, due to the autofluorescence of these crystals. By combining these imaging methods, we could correlate the morphological changes of an red blood cell with the amount of malaria pigment crystals produced therein. Furthermore, the comparative analysis of the optical and AFM images facilitated a more detailed identification of parasite development stages, compared to bright-field microscopy, without the need of contrast materials.

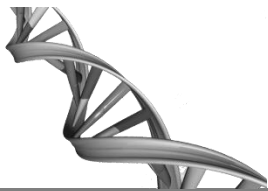
We are going to extend these studies, carried out on fixed cells, by investigating infected red blood cells by AFM in cold aqueous solutions, slowing down parasite maturation. This protocol may provide conditions close to those realised in the human body to trace morphological and mechanical properties of the infected cells during the maturation of the parasites.

Keywords: *Plasmodium falciparum*, atomic force microscopy, total internal reflectance fluorescence microscopy

**P-055****Double sterility barrier and its breakdown in reproductively isolated yeast species****Mátyás Sipiczki, Zsuzsa Antunovics and Adrienn Szabó***Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary*

The yeast genus *Saccharomyces* includes eight natural species that are biologically isolated by post-zygotic sterility. Their hybrids are viable but do not produce viable ascospores (gametes). The failure of gamete production is assumed to be due to the failure of the homeologous chromosomes of the parental subgenomes to pair in meiosis (no allosyndetic pairing). We noticed that genome duplication upon hybridisation makes the hybrid able to form viable spores. Segregation analysis and genome sequencing revealed that the meiosis of the allotetraploid hybrid cells is autodiploidised (the subgenomes perform autodiploid meioses) and each spore receives a complete haploid set of chromosomes from both subgenomes. These spores are viable but allodiploid and thus sterile as the original allodiploid hybrid was before the duplication of its genome. The sterility of the allodiploid hybrid and the sterility of the allodiploid spores of the allotetraploid hybrid are the two sterility barriers that ensure the reproductive isolation of the *Saccharomyces* species. This mechanism is different from the sterility barrier operating in the higher plants whose allopolyploid hybrids become fertile upon genome duplication. The difference is due to the different modes of genetic determination of the sexual processes in yeasts and plants. In yeasts, the *MAT* locus located on Chr. III (or its equivalent) determines both the meiotic programme and the mating (fertilisation) programme. *MATa/MAT α* cells cannot fertilise but can perform meiosis, provided the chromosomes can pair. In allodiploids, no chromosome pairing can take place (low synteny between the sequences of certain homeologous pairs), so the allodiploid hybrids and the allodiploid spores of the allotetraploid hybrids are sterile. Then we found that the malsegregation of the *MAT*-carrying chromosome pair of one of the subgenomes during allotetraploid meiosis eliminates the sterility barrier: the alloaneuploid spores monosomic for this chromosome are fertile because they are either *MATa* or *MAT α* and thus capable of fertilisation. The loss of this chromosome destabilises the genome and subsequent malsegregation of additional chromosomes and allosyndetic recombination between the chromosomes of the subgenomes reduce the size of the hybrid genome and chimerise it (mosaic genomes). Electrophoretic karyotyping and sequencing of hybrid and segregant genomes revealed high diversity of genomic changes during chimerisation.

Keywords: *interspecies hybridisation, hybrid sterility, chimeric genome*



P-056

Establishment of an MRP4 (ABCC4) knock-out cell line using CRISPR/Cas9 system

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MRP4 (ABCC4) is an ATP-dependent efflux transporter that belongs to the ABC protein family. This protein mediates the transport of cAMP/cGMP, bile acids, steroid hormones, and also clinical drug substrates, such as antivirals, antibiotics and cytotoxic agents. MRP4 is highly polymorphic, and some of the polymorphisms may have clinical relevance, however current FDA and EMA guidances make no specific recommendations for MRP4.

In this work we aimed to knock-out the MRP4 gene from the HEK293FT, an embryonic kidney cell line of human origin with hypotriploid chromosome set, to establish a parental cell line for a cell-based test system in which the effects of physiological or pathological polymorphisms on the protein's transporter activity can be examined.

CRISPR/SpCas9 is an RNA guided endonuclease that became widely used due to its simplicity and modularity that can be exploited in multiple ways in genome engineering tasks such as tagging genes or establishing knock-in or knock-out cell lines.

A conventional way to knock-out a gene is the disruption of the gene function by causing frameshifts in its coding region utilizing the non-homologous end joining (NHEJ) DNA repair machinery. This can be done by causing a double stranded break in the coding region by SpCas9 or other RNA guided endonucleases. As the DNA ends are processed and ligated during the repair inaccuracies may occur, leading to insertions or deletions that, as the double strand breaks were caused in the coding region, may cause frameshifts and disrupted gene expression.

One hardship of establishing knock-out cell lines using this approach is the amount of invested time, resources and work that is required for the identification and confirmation of the gene knock-out cell clones. Without a marker that flags every knock-out event, a large number of clones has to be maintained and tested whether any intact gene locus remained in them or not.

To simplify the search for the right clones we disrupted the genes by exploring the capability of the NHEJ repair to inserting extrachromosomal DNA fragments into the double strand breaks. The inserted DNA fragments contain expression cassettes of different (blue, green or red) fluorescent proteins, and thus, acted as labels for each cleavage sites where disrupted the gene expression. Using FACS we gathered those cells that expressed all three fluorescent proteins, cloned a few cells to find clones that contained no intact MRP4 genes without having to work with hundreds of candidate clones of the transgenic cells.



After we confirmed the successful disruption of every MRP4 gene locus in the cells by flow cytometry, Sanger-sequencing of the amplicons and western blotting, we knocked out the fluorescent markers from the MRP4 knockout cells to prevent their interference with the signals of the fluorescent markers used in the subsequent drug transport assays.

Keywords: CRISPR/ Cas9, genome editing, gene knock-out, NHEJ, MRP4

P-057

Expression patterns of motilin, gastrin, ghrelin, and somatostatin in association with the Left Displaced Abomasum in cattle

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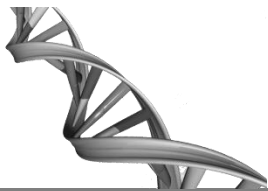
Cattle is one of the most frequent livestock of agriculture. Breeding of cattle provide not just food and milk, but also gives leather and other important products. The management requires the exact knowledge of the physiology and pathology of this species including detection and prevention of the most common diseases.

Left-sided displacement of the abomasum (LDA) is a quite common disease amongst dairy cows which can decrease the milk production and even can lead to mortality. It seems to be a close correlation between the presence of LDA and the a gastrointestinal hormone called motilin (MLN). Based on genome association studies a single nucleotide polymorphism in the promoter region of MLN affect the expression level of the hormone. Despite the fact that in other species this hormone is well characterised we have quite a few information about the expression levels of motilin and other gastrointestinal (GI) hormones like gastrin (GAST), ghrelin (GHRL) and somatostatin (SST) in bovine.

The aim of our study was to investigate and characterize the expression level of the above mentioned hormones amongst the different parts of the GI tract of cattle. Beside this our goal was to perform a High Resolution Melting (HRM) procedure to easily observe the mentioned SNP in the bovine motilin gene.

We collected our samples from the farmland of the University of Veterinary Medicine with the help of a skilled veterinary surgeon. Total RNA was extracted and cDNA was syntetised from all the samples (rumen, reticulum, omasum I-II., abomasum, pylorus, duodenum, jejunum, appendix, colon I-II., rectum). The samples were investigated with MLN, GAST, GHRL, SST specific and endogenous primers in RT-PCR and QPCR assays. HRM analysis to the bovine MLN gene was elaborated and routinely performed.

QPCRs were resulted with the same pattern of the hormones in cattle. Briefly, the expression of the hormones can be detected in every part of the gastrointestinal track and



there were outstanding expression values in some cases. With the HRM assay we could easily distinguish the different genotypes.

In the future we are going to involve more individuals to our study and we are also going to compare the expression levels of healthy and LDA affected animals.

This study was supported by OTKA 111964 and OTKA 124708.

Keywords: *Bos taurus*, GI hormones, Left Displaced Abomasum

P-058

Frequency of a single nucleotide substitution of ABCB1 gene in herding dog breeds in Hungary

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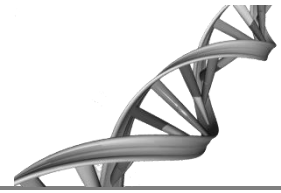
P-glycoprotein is a multidrug resistance (MDR) transporter a member of the ATP binding cassette (ABC) family and is highly expressed in capillary endothelial cells in the brain. Its physiological function is to protect the body from potentially toxic compounds. In certain dog breeds a 4-bp deletion mutation of P-glycoprotein encoding ABCB1 (MDR1) gene is frequently occurring, and consequently veterinary drugs accumulate in the central nervous system resulting in lethal toxicosis. In addition to this some SNPs are present in the ABCB1 gene and one of them is situated at intron 1 near the 5'-end of the gene (c.-6-180T>G), where the promoter elements are located

The aim of our study is to determine the prevalence of the substitution of thymine for guanine; c.-6-180T>G in canine ABCB1 gene in Hungarian population of two herding dog breeds; Shetland sheepdog and border collie.

Blood samples were collected from forty-four client-owned dogs; the owners gave signed, informed consent for study enrolment. Genomic DNA was purified via spin column-based method from all samples. ABCB1 mutation was determined by modified allele specific detection method via real-time PCR analysis. In order to distinguish the polymorphism in ABCB1 PCR-restriction fragment length polymorphism (RFLP) assay were carried out. The PCR product was digested with *MboI* restriction endonuclease.

Eight of twenty-two Shetland sheepdogs were homozygous and eleven were heterozygous for the mutant ABCB1 allele. None of the investigated border collies were carrying the 4-bp deletion mutation of MDR1. The homozygous T/T wild type genotype was present in ten dogs, the heterozygous T/G genotype was found in eleven and the G/G homozygous mutant genotype in three dogs. The frequency of the mutant G allele was 29% in our study.

Our future plan is to involve more dogs and other dog breeds to further screening. It is advantageous to possess as much information as possible about SNPs that are determinative



in drug delivery processes. It is presumed according to the location of the c.-6-180T>G SNP that it results in increased P-glycoprotein expression. Determining the prevalence of allele frequency at a population level would allow more conscious veterinary therapy of the dogs affected.

Keywords: *ABCB1, dog, ivermectin-sensitivity*

P-059

Genetic risk variants in the major histocompatibility complex region in a Hungarian schizophrenia sample

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Schizophrenia is a severe, chronic debilitating psychiatric disorder, resulting in decreased functionality and considerable personal suffering. According to genetic investigations, this disorder is highly heritable. Common single nucleotide polymorphisms, rare copy number variants and de novo mutations have all been implicated in the genetic background of schizophrenia. Recent data from genome-wide association studies (GWAS) has demonstrated the association of SNP in the 6p21-23 locus, the extended major histocompatibility (MHC) region. Moreover, genotype-phenotype studies revealed the effect of variants in this region on symptom severity. The MHC region is located on chromosome 6, spanning 3.6 megabase pairs and containing 224 genes, many of them still of unknown function. In this region, some loci have a quite high variability (5-17%), which has been by far the highest level found in the human genome so.

We conducted a genome-wide association study on a sample which consist of 246 patients' samples from Hungarian Schizophrenia Biobank, and 494 controls. In our work we analyzed more than 5000 SNPs spanning the MHC region. The PLINK software was used, for data analyses. We explored the association of these SNPs with disease risk and symptom clusters. Results are presented in light of recent schizophrenia genetics findings.

Keywords: *genome-wide association study, schizophrenia, major histocompatibility complex region*



P-060

High-throughput targeted microsatellite marker development in *Vuralia turcica* (Piyan)

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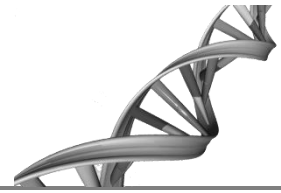
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Vuralia turcica (Piyan) is a member of the Fabaceae family and is a critically endangered endemic plant species in Turkey. The species' most striking characteristic is its multi-carpellate ovaries. Worldwide, the majority of human nutrition is derived from plant products. Turkey is a significant exporter of legume products. Cultivating highly productive plants using breeding techniques offers an important opportunity to increase production and exports. Therefore, *V. turcica* is a valuable genetic resource for breeding programs aiming for the development of high-yield legume species. Besides, *V. turcica* is the only species of the Papilionoideae subfamily that grows naturally in Turkey. Since *V. turcica* is an endemic endangered plant species with little known about its genetic diversity, the development of a protection strategy is essential. For this reason, The identification of simple sequence repeats (SSRs) was carried out, and the SSR marker system was improved using Illumina sequence data from *V. turcica*. The literature on *V. turcica* to date includes no study regarding the determination of SSR loci in *V. turcica* and the design of SSR markers to identify these loci. This research is expected to enable the population genetics of this important species to be assessed for the first time.

Acknowledgments: The authors express appreciation to the Ali Nihat Gökyiğit Foundation, Neẓabat Gökyiğit Botanical Garden for providing research materials for the study. The work was fully supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) (Project No. 117Z797)

Keywords: Fabaceae, Illumina sequencing, Neẓabat Gökyiğit Botanical Garden, SSR, *Vuralia turcica*



P-061

***In vivo* diffusion tensor imaging of the brains of stressed rats**

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Aim: Stress is the most important triggering factor for the development of various psychiatric disorders, but the underlying neurobiological events are not completely understood. Stress exposure can affect neuroplasticity and structural integrity of limbic brain areas. Here, we used diffusion tensor imaging (DTI) to study the temporal dynamics of stress induced structural changes in the brains of laboratory rats.

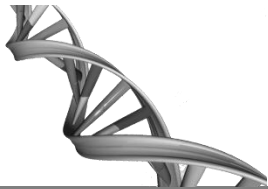
Method: Young adult male Sprague-Dawley rats (control group: 16 animals, stress group: 16 animals) were subjected to restrain stress (6 hours/day) for 21 days. DTI and T2-weighted images were acquired with a 4.7T Bruker PharmaScan pre-clinical MR scanner. Baseline measurements were performed before stress and the protocol was repeated three times: one week (acute stress), three weeks (chronic stress) after stress initiation and two weeks after the end of the stress (recovery). A pre- and post-processing pipeline was built up by using FMRIB Software Library for both DTI and T2-weighted measurements.

Results: Diffusion data were corrected for eddy currents and subject movements by the detection and the replacement of positive and negative outliers and then fractional anisotropy (FA), mean diffusivity (MD), eigenvalues ($L_{1,2,3}$) and eigenvectors ($V_{1,2,3}$) were calculated. After manual corrections, different brain areas were used for diffusivity and volumetric analysis.

Conclusion: We have developed an image processing pipeline for volumetric and diffusion analysis in order to identify rat brain areas affected by stress.

This work was financially supported by Hungarian Brain Research Program (KTLA_NAP_13-2-2014-0019 and 2017-1.2.1-NKP-2017-00002) and EFOP-3.6.2-16-2017-00008

Keywords: stress, rat MRI, diffusion tensor imaging, data processing



P-062

Investigation of cholinergic enzymes in heroin and polydrug addiction

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Addiction of drug is a chronic relapsing disorder. The development of addiction effect various neurobiological processes which include both drug-use and drug use-dependence. According to the United Nation Office of Drug and Crime (UNODC) report 2015, worldwide incidence of drug dependence has increased more than 29 million in 2014 from 27 million in previous years and heroin is found to be leading addictive drug. Cholinergic enzymes i.e. acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) has been reported to play role in addiction physiology but scarcely investigated. The present study was designed to investigate the status of cholinergic enzymes in heroin and polydrugs addicted individuals in comparison to non-addicted healthy subjects. Ethical approval was taken from departmental ethical committee. Prior consent was obtained from participants of the study. Spectrophotometric measurement of enzymes was based on Ellman's method. The results revealed an increase level of AChE and BChE in heroin addicted individuals than healthy non-addicted subjects. The increase was statistically significant. It is concluded that cholinergic enzymes have physiological roles in heroin and polydrug addiction. Further study is recommended to find a cholinergic enzyme based therapy for heroin addiction.

P-063

Isolation and culturing of human cutaneous melanocytes

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Melanocytes of the integument are pigment producing cells located in hair follicles and in the basal layer of the epidermis. Cutaneous melanocyte related pathological phenomena include wide range of abnormalities from pigmentation disorders to the infamous melanoma. Primary melanocyte cultures serve as a useful *in vitro* model for the better understanding of pigment cell disease pathogenesis and behaviour. For this purpose, we aimed to isolate



melanocytes from human skin and to establish primary cell cultures which maintain proliferative capacity for several passages.

To this end, we used human juvenile prepuce samples for our experiments. Samples were obtained from the Paediatric Surgery Division, Department of Paediatrics, University of Debrecen, under the licence of the appropriate authorities. After several steps of sterilization and overnight incubation in dispase II the epidermis can be carefully removed from the tissue. Followed by trypsinization and centrifugation, the resuspended pellet can be plated in a desired density. From a single piece of skin with the size of 1 cm² approximately 2-3×10⁶ cells can be isolated. The addition of special mitogens and factors to the culture medium enhances melanocyte selection and proliferation from the initial cell population. A vital part of our work is to verify the melanocyte content of the primary cell cultures. Morphological analysis of haematoxylin-eosin stained cultures showed bi-, tri- and multipolar, gracile, pigment containing cells. Light microscopy using full bright field optics also proved the presence of dendritic, pigmented cells in the culture. Fluorescent microscopic evaluation of melanocyte specific protein expression (MITF, TYRP1) proved positive immune signals in every cell in each examined visual field. Presence of contaminating cells (keratinocytes, fibroblasts) was not detected. Based on the thorough verification processes we can state that the isolation and initiation procedure of our primary melanocyte cultures is successful (n=4).

In our poster, we present the steps of isolation and culturing of human cutaneous melanocytes and the data proving the purity of the cell cultures. Optimization of our protocol resulted in four different primary melanocyte cultures derived from skin samples of different donors. The cost-efficient method, the flexible protocol and the sustainable source of samples provides an ideal approach for isolation and culturing of human cutaneous melanocytes.

Supported by the ÚNKP-18-2 New National Excellence Program of the Ministry of Human Capacities

Keywords: *primary cell culture, human, melanocyte*

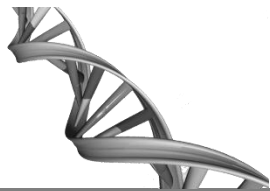
P-064

Metagenomic analysis of acquired antibiotic resistance genes in the gut microbiome of wild boars (*Sus scrofa*)

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Antibiotics are widely used in human medicine, for disease prevention and treatment of animals and had also been applied for promoting growth in animal farming, leading to the emergence of antibiotic-resistant strains in the gut microbiota. The land application of animal manure that contains resistant bacteria and antibiotics facilitates the establishment of an



environmental reservoir and the selection of antibiotic-resistant microbes (1, 2), promoting their dissemination into natural habitats and wild animal populations.

In this study shotgun metagenomic sequencing data of gastrointestinal samples from a free-living wild boar in the Zemplén Mountains in Hungary (3), as well as from free-living and domesticated wild boars in Japan (4) were analysed to assess the occurrence of acquired antibiotic resistance genes. The shotgun metagenomic sequencing data were analysed by a variety of bioinformatic tools for metagenomic contig assembly and for the identification of acquired antibiotic resistance genes. Furthermore, DNA samples purified from the faeces of four additional free-living wild boars sampled in the Zemplén Mountains in Hungary were screened for the presence of the identified resistance genes by PCR. The genetic environments of the antibiotic resistance genes, detected in the Zemplén Mountains, were investigated by functional analysis of the metagenomic contigs harbouring them.

Three different tetracycline resistance genes and a macrolide resistance determinant were identified in the gastrointestinal samples of all five free-living wild boars sampled in Hungary. Their genetic environments included conjugative elements with variable levels of similarity to those of earlier isolated and characterised bacterial strains available in public databases. An acquired tetracycline resistance gene and an acquired β -lactam resistance gene were detected in the faecal microbiome of a free-living wild boar hunted in the mountains in Shiga prefecture in Japan. On the other hand, as opposed to the analysed free-living animals the domesticated wild boar in Japan carried diverse acquired resistance genes against eight different classes of antibiotics. Our results suggest that the assumable level of exposure to antibiotics is an indicator for the diversity and prevalence of acquired antibiotic resistance determinants in the gut microbiome of wild boars from different habitats.

Keywords: *antibiotic resistance, gut microbiome, natural habitats*

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**P-065****Osteopontin splice variants expression in malignant melanoma cell lines**

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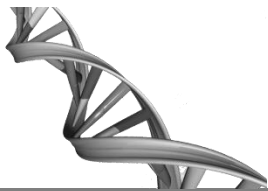
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Based on our gene expression data on primary and metastatic melanomas using Affymetrix U133 Plus2.0 array, we have demonstrated that osteopontin (OPN) is significantly overexpressed in melanomas with less favorable prognosis. OPN is an extracellular matrix glycoprophosphoprotein. Besides playing a role in bone remodeling, maturity and activation of osteoclasts, OPN also involved in inflammatory processes and cell adhesion, furthermore, it can function as an anti-apoptotic factor promoting cancer cell survival by preventing programmed cell death. It is encoded by a highly conserved, single copy gene (SPP1). Unmature mRNA of OPN is subject to alternative splicing resulting in 5 distinct isoforms and among these types, three isoforms (OPNa, OPNb and OPNc) have been already described in human cancer. The importance of OPN splicing in cancer has been increasingly recognized, however there are insufficient data about the role of each isoform in specific cancer types, including melanoma.

In the present study, our aim was to determine what type of OPN splice variants exist in primary and metastatic melanomas using gene expression analysis (qRT-PCR). We aimed to compare the relative gene expression pattern of melanoma cell lines developed from primary (n=12) and metastatic (n=9) melanoma tissues. On the other hand, we also aimed to investigate the effect of BRAF inhibitor on the expression of the three splice variants on BRAFV600E mutated melanoma cell line (WM278).

We found that the relative expression of OPN isoforms are different in cell lines originated from different subtypes of primary tumors. Out of the 12 primary melanoma originated cell line, 6 were SSM subtype and the relative expression of the OPN variants were very low (e.g. average expression of OPNa in SSM subtype = 0.07 ± 0.1 ; in NM subtype = 3.6 ± 2.1), the OPNb and OPNc expressions were similar. The metastases originated cell lines showed a lot lower expression of all OPN isoforms. The relative gene expression of the OPN variants after BRAF inhibitor treatment (0-96hr) using the concentration of 50 nM, 500 nM and 5000 nM showed interesting pattern. After 96 hr of incubation time the highest expression was seen for OPNa at 50 nM BRAFi concentration. Similarly, high expression of OPNa was detected at 500 nM BRAFi treatment after 96 hr incubation and the tendency was similar at 5000 nM.

In summary, melanoma cell lines originated from primary tumors show different patterns of the OPN variants. Higher expression is associated with more aggressive phenotype. Based on our preliminary data BRAF inhibitor treatment is probably associated with elevated OPNa expression, but this statement requires more investigation.



This study was supported by OTKA K112327). The project was co-financed by the European Regional Development Fund (GINOP-2.3.2-15-2016-00005).

Keywords: *osteopontin, alternative splicing, melanoma cell lines, BRAF inhibitor treatment*

P-066

PlantSize: an affordable non-destructive method to measure plant size and color *in vitro*

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Plant phenotype is determined by the genetic background and environmental conditions. Interaction of the genotype and environmental factors influences plant growth and development, physiological and molecular traits. Characterization of phenotypes is crucial to understand the regulation of stress in responses to climate changes. Non-destructive analysis of plants through color imaging is an increasingly popular method to define growth parameters, characterize plant development in time.

We have developed a non-invasive method, which simultaneously measures basic morphological and physiological parameters of *in vitro* cultured plants such as *Arabidopsis thaliana*. Changes of plant size, shape and color is monitored by repeated photography with a commercial digital camera. Images are analyzed with the Matlab-based computer application PlantSize, which simultaneously calculates several parameters including projected rosette area (pixel area, fresh weight, convex area and ratio), and color (chlorophyll and anthocyanin contents). Numerical data are exported in MS Excel format. Subsequent data processing provides information on growth rates, chlorophyll and anthocyanin contents. Utility of the system is demonstrated by revealing small but significant differences between wild type and transgenic *Arabidopsis* plants overexpressing the HSFA4A transcription factor or the *hsfa4a* knockout mutant, subjected to different stress conditions. While HSFA4A overexpression was associated with better growth, higher chlorophyll and lower anthocyanin content in saline conditions, the mutant showed hypersensitivity to various stresses. Morphological differences were revealed by comparing rosette size, shape and color of wild type plants with phytochrome B (phyB-9) mutant.

The developed technology offers a simple, affordable and fast way to measure several morphological and physiological parameters of *Arabidopsis* plants. The methods are based on non-destructive imaging allowing repeated measurements and monitoring changes of various growth parameters in time. PlantSize is publicly available (<http://www.brc.hu/pub/psize>).

This research was supported by Bayer CropScience and NKFI project KH 129510. Dóra Faragó is supported by Young Scientist Fellowship of the Hungarian Academy of Sciences.

Keywords: *imaging, rosette area, chlorophyll and anthocyanin contents*



P-067

Role of the *Arabidopsis thaliana* CRK5 protein kinase in the gravitropic response

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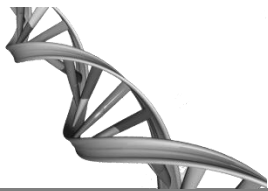
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The gravitropic responses, in roots and shoots, are controlled by unequal distribution of the plant phytohormone, auxin. Both positive and negative gravitropic responses, directing downward and upward bending of horizontally placed roots and shoots, respectively, are controlled by asymmetric distribution of auxin. The PIN2 protein plays crucial role in regulation of root gravitropic response; the PIN3 protein is a key regulator of shoot gravitropic and phototropic responses in stem endodermis. Basal-to-apical switching of the PIN proteins in cell membranes is stimulated by different kinases, which can phosphorylate various PIN's hydrophilic loop in different manner. We have identified and partially characterized the CRK5 protein kinase from *Arabidopsis thaliana*. We showed that inactivation of AtCRK5 causes a root and shoot gravitropic defect, reduced root growth and enhanced lateral root formation. Our preliminary studies suggest that relocation of PIN3 in stem cells might be influenced by AtCRK5 phosphorylation as well. In order to study this phenomenon we cloned the hydrophilic loop of PIN1, PIN2 and PIN3 proteins, and subsequently overexpressed and purified them from *E. coli* cells. Performing *in vitro* radioactive kinase assay we found that the hydrophilic loop of all three PIN proteins was phosphorylated by AtCRK5 protein kinase. Cooperation with the Proteomics Research Group (BRC, Szeged, Hungary), we identified various possible phosphorylation sites. For mutant complementation and protein interaction studies, we generated the gCRK5:GFP construct. After confirmation of functionality we used the plant lines for immunoprecipitation studies to find the interaction partners of the AtCRK5 protein kinase. In order to verify the possible interactions we used the BiFC method.

This work was supported by OTKA PD115502, PD128055, FK128920 and GINOP-2.3.2-15-2016-00001

Keywords: *Gravitropism, CRK5 protein kinase, phosphorylation, polar auxin transport, BiFC, Arabidopsis thaliana*



P-068

Selection of aptamers of therapeutic potential for respiratory syncytial virus infection

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The respiratory syncytial virus (RSV) is the most common cause of acute lower respiratory tract infections during infancy and late elderhood, yet it still remains one of the unmet vaccine need. The current treatment for RSV is based on a monoclonal antibody vaccination, which is accessible for only a small portion of the patients. Severe RSV infection correlates with higher probability of developing asthma during childhood. In 2017, approximately 60 000 children under the age of 5 died due to RSV infection and 99% of these cases occurred in developing countries. Therefore, an affordable and safe RSV treatment is desperately needed.

Aptamers are short, single stranded oligonucleotides that bind their target molecules with high affinity and specificity. In contrast to the generally applied monoclonal antibodies, aptamers are produced by an *in vitro* process within weeks and can be synthesized chemically with on-demand labelling. Consequently, aptamers outperform antibodies in terms of cost, thermal stability, shelf-life, low toxicity and batch-to-batch variation.

Our aim was to select aptamers with therapeutic potential of RSV infection. The target molecule in our experiment was one of RSV's main surface proteins, the so-called protein F, that is responsible for the host cell membrane fusion and therefore the viral infection itself. During fusion, protein F goes under a conformational change thus two forms of the protein F can be distinguished i.e. prefusion and postfusion form. We hypothesize that RSV infection of host cells can be prevented by binding of an aptamer to the prefusion form. An iterative procedure, referred to as SELEX (Systematic Evolution of Ligands by EXponential enrichment) was used for aptamer selection. The process consists of aptamer-target molecule binding, partitioning, recovery and amplification of the oligonucleotides, which steps are repeated with increasing partition stringency. The SELEX target molecule, prefusion F, was immobilized onto paramagnetic beads. Five cycles of selection were performed with increasing selection pressure, using the postfusion F protein in the counter-selection steps to eliminate the aptamers that bind the postfusion form of the virus protein. After the *in silico* analysis of the aptamer candidates, 2 different approaches were applied to further characterize the candidates: the traditional filter-binding assay, and the simple mix-and-measure Amplified Luminescent Proximity Homogenous Assay (ALPHA).

We demonstrated that our SELEX protocol yielded aptamers that selectively bind to the prefusion form of RSV's surface protein F. Our collaborating partner laboratory is analysing the virus infection inhibitory effect of selected aptamers by using a cell-based assay to identify aptamers of therapeutic potential.

Keywords: *aptamer, therapeutic aptamer, respiratory syncytial virus*



P-069

Sequence specificity of Cas9 variants on one million target sequences

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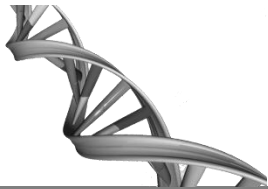
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In the last five years the CRISPR/Cas9 system has revolutionized genome engineering from molecular biology research to human clinical applications. The Cas9 protein associated with the guide RNA (gRNA) can recognize a specific sequence in the DNA and introduce a sitespecific double stranded DNA break (DSB). The 20 base pair long so-called spacer sequence of the guide RNA determines the target sequence. Modifying the spacer sequence on the gRNA the ribonucleoprotein complex can be targeted seemingly to any sequence in the genome. With this technology genes can be efficiently knocked in or out exploiting the DNA repair of the DSB.

Although, this system is far more powerful than former genome editing techniques, it has its own limitations. Different targeted sequences are not cut equally well, and the rules underlying this selectivity are not yet fully understood. Experiment-based target-cleavage efficacy prediction tools are available only for the *Streptococcus pyogenes* Cas9 (SpCas9) and *Staphylococcus aureus* Cas9, but not for their mutant variants or orthologs. One of the major obstacles in developing such tools is the rarity of accurate cleavage-activity data.

Here, we report the development of a method termed “self-targeting gRNA library screen” for assaying the activity of Cas9 nucleases by random variant gRNA/target libraries of selftargeting gRNAs. We demonstrated the approach on SpCas9 exploiting more than a million different sequences and developed an *in silico* on-target prediction algorithm that outperforms former prediction tools on several target datasets. We also analyzed the activity of the High Fidelity1 variant of SpCas9 and developed a scoring algorithm for predicting its on-target activity with an accuracy matching that of the one for SpCas9. Our approach facilitates the easy generation of a sufficient amount of accurate cleavage-activity data for increased fidelity and altered specificity variants and orthologs of SpCas9 to facilitate their target-cleavage efficacy prediction.



P-070

The Heat Shock Factor A4A regulates responses to combined salt and heat stresses

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Heat shock factors regulate responses to high temperatures, salinity, water deprivation or heavy metals through activation of stress-induced genes, by binding to heat shock elements on promoters of these target genes. Their function in different stress combinations is however not well known. The *Arabidopsis* heat shock factor A4A (HSFA4A) was previously shown to regulate responses to salt and oxidative stresses, and is phosphorylated by MPK3/6 MAP kinases. The heat shock factor A5 (HSFA5) is a repressor of HSFA4A by forming heterooligomers. To describe more precisely the interaction between these heat shock factors we started the characterization of single (*hsfa4a* and *hsfa5*) and double (*hsfa4ahsfa5*) mutant lines in different stress conditions.

We showed that the *HSFA4A* gene is induced not only by salt, but elevated temperature and by combination of these conditions. We observed fast translocation of HSFA4A-YFP protein from cytosol to nuclei in salt-treated root cells. HSFA4A can be phosphorylated not only by MAP kinases MPK3/6 but also by MPK4 and Ser309 is the dominant MAPK phosphorylation site. Changing Ser309 to Asp or Ala has altered intramolecular multimerisation. Using chromatin immunoprecipitation assays we were able to confirm binding of HSFA4A to promoters of target genes encoding the small heat shock protein HSP17.6A and transcription factors WRKY30 and ZAT12. HSFA4A overexpression enhanced tolerance to individually and simultaneously applied heat and salt stresses through reduction of oxidative damage. Our results suggest that heat shock factor A4A is a component of a complex stress regulatory pathway, connecting upstream signals mediated by MAP kinases MPK3/6 and MPK4 with transcription regulation of a set of stress-induced target genes.

This research was supported by OTKA NN-110962 and NKFI KH-129510 grants.

Keywords: *Arabidopsis, combined stress, heat shock factor A4A, heat, MAP kinases, phosphorylation, promoter binding, salinity, transcription regulation*



P-071

The role of *Arabidopsis thaliana* mitochondrial proteins in stress responses

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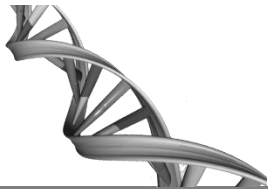
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Plant responses to unfavorable environmental conditions broadly encompassed the various changes in photosynthesis, respiration, metabolite assimilation and catabolism. High soil salinity or drought result in accumulation of reactive oxygen species (ROS), leading to oxidative stress. Although chloroplasts are main source of ROS, mitochondria are also important in the maintenance of the cellular redox homeostasis, where Complex I and III of mitochondrial electron transport chain are major sites for ROS production.

To reveal the importance of genes encoding the mitochondrial proteins in stress responses, we are characterizing mutants of 13 *Arabidopsis thaliana* genes, encoding the subunits of Complex I and III of the mitochondrial electron transport. When compared to wild type several mutants showed morphological and physiological changes under salt and osmotic stress conditions. Phenotypic alterations and differences in tolerance to drought and salinity were revealed through *in vitro* germination and growth tests, as well as by complex phenotyping in collaboration with European Plant Phenotyping Network. Several mutants were identified, which showed altered tolerance to osmotic or salt stress. One mutant was characterized in detail in which the mutations disrupted the *NDUSF8.2* gene. The *ndusf8.2-1* mutant was hypersensitive to oxidative stress, although less hydrogen peroxide formed in them and lipid peroxidation level was lower in high osmotic stress. Changes in chlorophyll fluorescence under stress treatments suggested that these Complex I mutations influence photosynthesis as well. Our data shows that the *NDUSF8.2* gene has important affect on plants stress responses, and we found a strong correlation between the mutations and the photosynthetic activity and energy production.

This research was supported by NKFI FK128920 and NKFI NN110962 (G.R., D.A., L.Sz., L.Zs.), Hungarian Ministry for National Economy GINOP-2.3.2-15-2016-00001 (I.V., G. R., D.A., L.Sz., L.Zs.), and EPPN program (N.K., F.F).

Keywords: *abiotic stress, ROS, mitochondrial proteins, Arabidopsis thaliana*



P-072

Triacylglycerol-deficient mutants exhibit altered surface membrane properties in fission yeast

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Heat shock (HS) is one of the most common and important stressor for organisms. HS facilitates the formation of several thermoprotectant molecules - such as chaperones or trehalose - as well as the remodelling of the cellular membranes in the fission yeast, *Shizosaccharomyces pombe* (*S. pombe*). Interestingly, the intracellular amount and the desaturation level of the triacylglycerol (TG) is highly increased during HS. We assume that the TG - and/or its precursors - have an important role in the heat stress management. Therefore we have inactivated the two genes (*plb1* and *dga1*) involved in TG biosynthesis. Although all three mutants (both single- and the double mutant (DKO)) displayed elevated heat sensitivity, they were able to acquire thermotolerance. Besides these observations, the wt and DKO cells have significantly differed in their lipid composition under either normal or HS conditions. We assumed, that these alterations might also modulate the physical/structural properties of the membranes. In order to check our hypothesis, a fluorescent lipophilic dye (di-4-ANEPPDHQ) have been used to follow the “fluidity” changes of the plasma membranes of the wt and DKO cells under different conditions.

Since the heat induction of the molecular chaperones might also be affected by the physical state of surface membranes, we have analyzed the expression levels of different Hsps in wt and TG mutants. Besides Hsps, trehalose is also a well known membrane protectant, we have investigated whether the elimination of TG-synthesizing enzymes influences the accumulation of trehalose during heat stress.

Our experiments have been shown firstly that the regulation of the plasma membrane physical state during HS management is controlled by the TG synthesis. Furthermore these findings suggest a strong connection between the lipid homeostasis and the other major players of the heat-stress management system indicating their crucial role in thermal stress management.

This work was supported by GINOP 2.2.1-15-2016-00007

Keywords: *fission yeast, triacylglycerol, heat stress, membrane physical state*



P-073

Ubiquitin pool dynamics revealed by quantifying ubiquitin forms in *Drosophila* UPS mutants

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Due to the reversible nature of ubiquitination, the ubiquitin (Ub) pool of cells is divided into distinct fractions that include free monoubiquitins as well as covalently linked mono- and polyubiquitin-protein conjugates. These ubiquitin forms reach a dynamic intracellular equilibrium in which the availability of free monoubiquitins appears to be essential for normal cell physiology. The deubiquitinating enzymes or DUBs play an essential role in maintaining physiologically required ubiquitin levels by liberating free ubiquitin from their conjugated forms.

In order to study the effect of different DUB mutants on Ub pool dynamics, a simple, fast and reliable assay was required to monitor Ub changes in these mutants. For this purpose, we modified an immunoblot-based densitometric assay and used it to simultaneously quantitate free monoubiquitin and total ubiquitin contents from whole protein extracts of three ubiquitin pathway mutants. Our data show that disrupting either proteasome function in the Rpn10/p54 mutants, or Ub recycling in the Usp5 and Usp14 DUB mutants led to quantifiable changes in the ubiquitin pool, although with visible differences. These phenotypic differences might represent unique responses to different perturbations of the ubiquitin equilibrium. These data indicate that the immunoblot-based ubiquitin quantification assay can be used to detect dynamic changes in the ubiquitin pool.

This study is supported by the Hungarian Research Fund K116372, and the National Research Development and Innovation Office grant GINOP-2.3.2-15-2016-00032.

Keywords: *ubiquitin quantification, ubiquitylation, deubiquitylation, protein degradation, *Drosophila melanogaster**



P-074

A new approach to the experimental analysis of the human transmembrane proteome

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The main aim of the novel experiments designed by our Research Group is to label the extracytosolic lysine side chains of plasmamembrane proteins with a special agent containing biotin. The exact place of the label is determined by mass spectrometry after cell lysis, membrane preparation and the purification of the labelled peptides.

In my work, I settled the goal for a new labelling strategy on the side chains of aspartic and glutamic acid. Carboxyl groups can be modified only in a two-step protocol based on scientific literature. We need to apply an activation step before the labeling.

First, I optimized the procedure on a unique and pure modelprotein then continued with experiments on living cells. The targeted transmembrane proteins were labelled via more methods. In order to enrich the labelled peptides, the affinity chromatography was utilised based on the generally known high affinity interaction between avidin and biotin.

During the purification steps, the peptides with biotin side chains bind to the surface of high capacity agarose beads. While eluating, the disulfide bonds are cleaved in a one-step reaction. This way, the eliminated peptides will contain a known covalent modification that becomes an input parameter in the HPLC-MS/MS analysis.

By labelling the extracellular side of transmembrane proteins, their structure could be better examined because the resulted positions of the labelled amino acids are utilized in the CCTOP prediction algorithm as extracellular constraints which leads to a more accurate structure prediction. I proved that the carboxyl chains are also available targets of the original protocol.

Keywords: *transmembrane protein, topology, biotinylation*



P-075

A simple *E. coli* system for studying sequence-specific DNA binding of proteins

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Sequence-specific interactions between DNA and proteins are at the heart of fundamental mechanisms underlying the maintenance and regulated expression of genetic information. Analysis of these interactions (*e.g.* detection of binding to a specific nucleotide sequence, determination of the binding strength, investigation of the effect of mutations in the binding site and/or in the protein) requires suitable *in vitro* and *in vivo* methods. In contrast to the powerful and widely used *in vitro* techniques such as the electrophoretic mobility shift assay or the SELEX technique, there are very few published methods for studying DNA-protein binding *in vivo*. The available *in vivo* methods are laborious because they require construction of protein fusions. Our goal was to develop a simple assay, which can detect sequence-specific binding of proteins to DNA in *E. coli*, but does not involve construction of protein fusions.

The assay reported here utilizes a LacI repressor deficient *E. coli* host strain and two compatible plasmids. One of the plasmids (generic name pLacI) carries the gene of the Lac repressor (*lacI*) with the recognition sequence of the tested protein inserted into the *lacI* promoter in a position so that it does not impair *lacI* transcription. The tested protein is expressed from a compatible plasmid (pDBP). Constitutive β -galactosidase synthesis characterizing the *E. coli* $\Delta lacI$ host is repressed upon introduction of the pLacI plasmid. If the protein of interest expressed from pDBP binds to the target site on pLacI, it interferes with *lacI* transcription and leads to induction of β -galactosidase synthesis, which can be easily measured.

The method was tested with two zinc finger proteins recognizing 18 bp target sites and with CRISPR-dCas9 targeted to different sequences of the *lacI* promoter. The assay can be performed using samples of standard cultures as well as with cultures grown in microplate wells. Moreover, it can be adapted to screen for binding-positive colonies on the surface of X-gal indicator plates.

Keywords: DNA-protein interaction, *lacI* gene, β -galactosidase



P-076

Activities of Flightless-I (Fli-I) in the organisation of actin cytoskeleton

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Flightless-I (Fli-I) is a relatively newly identified protein, which alloys leucine-rich repeats (LRR) and gelsolin-homology domains (GH1-6). GH family proteins are involved in actin cytoskeletal rearrangements. Flightless-I is expressed widespread in tissues, mostly in skeletal, myocardial and nerve cells. Fli-I presents in the nucleus where it acts as a hormone-regulated nuclear receptor coactivator. In the presence of serum, Fli-I can translocate from the nucleus to the cytoplasm, where it plays a key role in cell migration, which is thought to be linked to its negative influences on wound healing and tissue regeneration. However, the actin activities of Flightless-I underlying its cytoplasmic functions are not completely resolved. To address this issue the interactions of different, recombinantly produced Fli-I constructs with actin were studied *in vitro* by using fluorescence spectroscopy and TIRFM approaches.

According to our results, Fli-I interacts with both actin monomers and polymers, which endows it with multifunctional activities including *de novo* nucleation and capping. Unlike gelsolin, the actin activities of Fli-I are not *calcium* dependent, which can be explained by the lack of the conservation of type-2 *calcium-binding sites between the two proteins*. *In addition, the small actin-binding protein profilin directs the activities of Fli-I to filament end capping.*

In conclusion, our results suggest that in the cytoplasmic environment Flightless-I interferes with actin dynamics by preventing monomer addition to polymer ends, which may explain its negative effects on cell migration, and thus wound healing and tissue regeneration.

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P-077

Analysis of Heterodimeric “Mutual Synergistic Folding”-complexes

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* Both authors contributed equally to this work.

Several intrinsically disordered proteins (IDPs) are able to adopt stable structures during interactions without a folded partner. The folding of all participating protein partners happens at the same time: coupled with the interaction in a synergistic manner through a process called Mutual Synergistic Folding (MSF). In an MSF complex, all interacting partners are IDPs without stable tertiary structures outside of the complex. These complexes represent a new subset of IDPs.

Lately, we collected information on their complexes and created the Mutual Folding Induced by Binding (MFIB, available at <http://mfib.enzim.ttk.mta.hu>) database. In a recent study, we compared the residue composition of homodimer MSF-complexes with homodimeric and monomeric globular proteins with similar amino acid sequence lengths. We conclude that MSF homodimers have a greater solvent-accessible main-chain surface area on the contact surface of the subunits, compared to globular homodimeric proteins. The main driving force of the dimerization is the mutual shielding of the water-accessible backbones and the formation of additional intermolecular interactions.

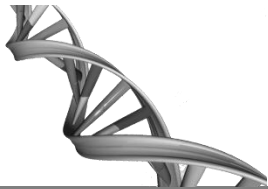
In this work, we investigated heterodimeric MSF complexes. We found that MSF-heterodimers have a different amino acid composition from the MSF-homodimers; therefore, another mechanism of their macromolecular interactions is expected.

Funding: This work was financially supported by the National Research, Development and Innovation Office (grant no. K115698). IS was supported by project no. FIEK_16-1-2016-0005 financed under the FIEK_16 funding scheme (National Research, Development and Innovation Fund of Hungary). The work of AM was supported by the ÚNKP-18-3-III-ELTE-184 New National Excellence Program of the Ministry of Human Capacities (Hungary).

Keywords: protein interaction, intrinsically disordered protein, mutual synergistic folding, heterodimer complex

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P-078

Annexin A2 is a new interacting partner of TIMAP in endothelial cells

Nikolett Király, Csilla Csontos and Anita Boratkó

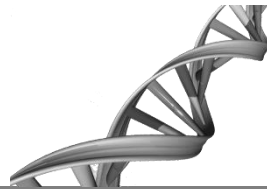
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TIMAP, TGF-beta-inhibited membrane-associated protein is highly expressed in endothelial cells, where it acts as a regulatory subunit of protein phosphatase 1 (PP1). TIMAP contains within its structure a potential nuclear localization signal and a prenylation CAAX-box motif, accordingly it was found in the nucleus and in the plasma membrane of pulmonary artery endothelial cells. It plays an important role in the regulation of the endothelial barrier maintenance, through the dephosphorylation of its substrate proteins.

Our recent aim was to identify new interacting partners or substrates of TIMAP-PP1c in endothelial cells. Bacterially expressed GST-tagged recombinant full length and truncated TIMAP proteins were utilized in pull down assay made with bovine pulmonary artery endothelial cells (BPAEC). Annexin A2 protein was identified as an interacting partner of TIMAP by LC-MS/MS analysis and it was confirmed by Western blot. We found that Annexin A2 binds to the N-terminal region of TIMAP. To further verify the interaction, Annexin A2 was cloned from endothelial cDNA and recombinant pGEX-4T-2 Annexin A2 plasmid was created. GST-Annexin A2 was successfully purified and it was able to bind TIMAP-PP1c and the presence of Ca^{2+} enhanced their interaction. By immunofluorescence staining and by subcellular fractionations we showed that endogenous Annexin A2 localized mainly in the nucleus. To check the interaction of the endogenous proteins, immunoprecipitation experiments were made. To further study the interaction in endothelial cells, coding region of Annexin A2 was subcloned into pCMV plasmid, which is suitable for mammalian expression. The recombinant plasmid was transfected into endothelial cells and the expression and localization of Annexin A2 was checked by Western blot and immunofluorescent staining.

In the future, we would like to study the physiological significance of TIMAP-Annexin A2 interaction with special emphasis on their nuclear functions.

This work was supported by grants ÚNKP-18-4-New National Excellence Program of the Ministry of Human Capacities and Bolyai Fellowship (A.B.).



P-079

Atomistic modelling of gas molecule diffusion in H-NOX proteins**Ahmed Rozza¹, Dóra K. Menyhárd² and Julianna Oláh¹**¹ Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Hungary² MTA-ELTE Protein Modelling Research Group, Budapest, Hungary

Over the past decades there has been an ever-growing recognition of the role of nitric oxide (NO) in living organisms. In higher organisms it is a messenger molecules: among others in the nervous and immune systems. Dysfunctioning of the signalling network has been linked to cardiovascular, neurodegenerative and inflammatory diseases. In man soluble guanylyl cyclase (sGC) is the only known receptor of nitric oxide: sGC can bind NO at its H-NOX (Heme-nitric oxide/oxygen binding) domain which leads to signal transduction and a 200-fold increase in the cyclase activity of the enzyme to produce cGMP, which turns on a signalling cascade.

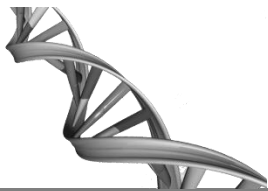
As sGC is an attractive target for the pharmaceutical industry (with only a single drug presently on market), therefore a detailed understanding of its functioning is highly desirable. In the present contribution we address the first step of sGC functioning: the mechanism of NO-binding. As there is no X-ray structure available of sGC that could be used for our modelling studies, we focus on two homologues proteins: on the H-NOX domains of the facultative anaerob *Nostoc* genus, and on the H-NOX domain of the obligate anaerob *Thermoanaerobacter tengcongensis*. Following the protocol set out in our previous work¹ we perform extended molecular dynamics simulations on these two proteins and analyze the trajectories with a simple three-state Markov model and obtain rate constants for ligand diffusion. The calculated data will be compared to the values published for myoglobin.¹ The results of the cluster analysis of the obtained trajectories will also be presented which help in the identification of diffusion routes and of the gas storing pockets in these proteins.

Acknowledgements: The financial support of a *Stipendium Hungaricum Fellowship*, of *Bolyai János Research Fellowship*, of *NKFIH Grant 115503* and of the *Protein Science and its Applications National Programme (HumProtEx, 2018-1.2.1-NKP-2018-00005)* is thankfully acknowledged. MD simulations were carried out at the facilities of the Hungarian NITF Institute.

Keywords: modelling, diffusion, molecular dynamics simulation

References:

[1] A. Lábás, D. K. Menyhárd, J. N. Harvey, J. Oláh *Chem. Eur. J.* **(2018)**, 24, 5350



P-080

Characterization of the direct and indirect interactions between formins and microtubules

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It is widely known that the coordinated changes of actin and microtubule (MT) cytoskeleton are regulated by several cytoskeleton-associated proteins. Formins are best known for having role in the processive assembly of unbranched actin filaments. It has been proved that some formins are able to bind to MTs via direct and indirect interactions. Recent studies revealed that the actin- and MT-binding regions of formins partially overlap. However, the fact that some formins are able to crosslink actin and MT filaments directly, strongly suggests that some of the cytoskeletal element-binding surfaces of formins are specific for either actin or MT filaments. Besides the direct formin-MT interactions, some formins can bind to MT plus-end tracking proteins (+TIPs) which suggests that formins may be part of the +TIP complex, which plays important roles in cytoskeleton coordination. Mapping the direct and indirect MT lattice-binding surfaces of formins is important to understand the cytoskeleton regulatory role of this family of proteins.

The aim of this study is to characterize the interaction between the *Drosophila* formin, DAAM (Dishevelled-associated activator of morphogenesis) and MTs and a +TIP, EB1. Our previous results revealed that DAAM interacts with MTs via its formin-homology (FH) and C-terminal domains (CT). In this study several truncated forms of DAAM were generated to identify MT interaction surfaces within the FH1-FH2 domains. Our results showed that unlike the FH1 domain, the FH2 domain binds to MTs and also to α/β -tubulin dimers. More detailed analysis of the FH2 domain revealed that this region of DAAM has multiple MT interaction surfaces. Protein binding assays were also used to examine the direct interaction between DAAM and EB1. Our results showed that the C-terminal region of DAAM containing the FH1-FH2-DAD-CT domains can interact with EB1 directly. DAAM contains an EB1-binding motif (SxIP) in its FH2 domain, although our results suggest that this motif is not functional or DAAM has multiple EB1 interacting surfaces. Co-immunoprecipitation assays showed that both the inactive and constitutively active form of DAAM co-purified with EB1 which suggests that the DAAM-EB1 interaction does not require the activation of DAAM. GST-pull down assays revealed that not only DAAM, but Dia and FRL formins also co-purifies with EB1. This suggests that formins can be a part of the +TIP complex in general and this may have an important role in the coordination of actin and MT cytoskeleton.

Keywords: *cytoskeleton, formin, microtubules, microtubule-binding proteins*



P-081

Comprehensive analysis of intrinsically disordered regions in postsynaptic scaffold proteins

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The chemical synapse is the most important structure in interneuronal communication. The postsynaptic density (PSD) is a characteristic part of excitatory chemical synapses. It forms a disk-shaped electrodense entity about 200-800 nm wide and 30-50 nm thick beneath the postsynaptic membrane. This dense, protein-based network provides a complex connection between the intracellular portions of membrane receptors and adhesion molecules and the cytoskeleton. The PSD is substantially more than a passive scaffold for receptors and signal transducers, as many observations show that it is undergoing dynamic reorganization during the course of various neural processes.

We investigated whether intrinsic disorder tendency plays a role in the organizing, variability of postsynaptic density proteins and the dynamic rearrangement of postsynaptic density. While there are domains such as PDZ domains that have been shown to be abundant in postsynaptic proteins, to our knowledge, a comprehensive analysis of intrinsic disorder in SD proteins is lacking. In the present work we considered the consensus of two different prediction methods, IUPred and VSL2B, to minimize the number of false positives. In addition to this, we define a so-called 'genuine disorder' instead of ordinary disorder. This means that we exclude fibrillar motifs such as coiled-coil structures from our analysis. In order to put our results into perspective, we have chosen a number of external reference sets: the entire human proteome, the immunome, nuclear proteins, histone methylases, proteins involved in signaling pathways as well as cytoskeletal proteins. Binding regions within disordered segments were identified with ANCHOR and the number of linear motifs participating in actual binding events was estimated using the regular expressions listed in the ELM database. To assess the potential of the proteins to be engaged in multivalent interactions, we introduced a descriptor called 'diversity of potential interactions'.

Our results suggest that intrinsic disorder is indeed an important feature of postsynaptic proteins and it might comprise one of the main factors contributing to the versatility and dynamics of the postsynaptic network. We show that postsynaptic proteins are particularly enriched in disordered segments. When considering largely disordered proteins, the PSD is enriched in long proteins with a high number of IDRs. Although the number of interacting partner proteins is not exceptionally large, the estimated diversity of the combinations of putative complexes is high in postsynaptic proteins. We propose that the number of disordered regions, rather than overall disorder content, is key in determining the versatility of interactions in most proteins investigated.

Keywords: postsynaptic density, intrinsic disorder tendency, binding sites



P-082

Developing a novel protein-tagging, immunodetection and purification system

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Over the past years our laboratory has produced several monoclonal antibodies (mAb) that specifically recognize the p54 protein, the polyubiquitin receptor subunit of the 26S proteasome. We found that anti-p54 mAb1 recognizes the C-terminal region of the protein with unusually high specificity. Preliminary results suggested that the epitope of the antibody is relatively short, hence, it can be used to generate an epitope-tagging system for protein labelling, immunodetection or purification. Therefore, we aimed to compare the anti-p54 mAb1 with a commercially available immunoglobulin (anti-Flag M2) that is widely used (however very expensive) in immunological applications due to its high specificity towards an artificial epitope (tag) fused to the protein of interest. We did the comparison on whole cell lysates made from *Drosophila*, Chinese Hamster Ovary or human cells, representing different proteome complexities, using the same concentration of the two antibodies in Western-blot experiments. We found that anti-p54 mAb1 performed significantly better under the same conditions and did not produce any background at exposure when anti-Flag M2 already generated non-specific bands. Therefore, we started to map the epitope of anti-p54 mAb1 and found that an 8-mer sequence localized in the carboxyl-terminal half of the p54 protein serves as the epitope of anti-p54 mAb1. When this 8-mer epitope was fused to a model protein it behaved as an immuno-tag and the chimeric protein could be detected with the anti-p54 mAb1 in Western-blot.

The main goal of our research is to establish a protein labelling plasmid vector system. This would allow us to tag any proteins of interest with this 8-mer epitope for immune-detection, staining or purification from bacteria or tissue culture cells.

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P-083

Effect of protein aggregation on protein complex assemblies

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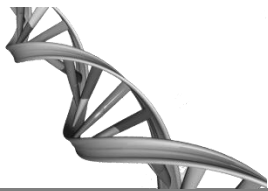
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Although protein complexes and structures are widely researched, most of them are still undiscovered. To understand cellular regulatory mechanisms and support new drug development, we need to get detailed information about the abundance and composition of these complexes. A new approach is being proposed with SiComPre (Simulation based Complex Prediction software), which makes stochastic simulations of protein complex formation, while combining multi-source data, for instance protein abundances and domain-domain interactions. It generates a computational model of all proteins and their dynamic interactions, and the results provide an approximation of protein complexes and their abundances.

The method can be used to investigate how protein complexes are perturbed when certain proteins are aggregating. Such protein aggregation can cause intracellular perturbations that lead to toxicity and cell death. In humans, protein aggregation can cause neurodegenerative diseases, including Parkinson's and Alzheimer's disease, and the expression of prion-like proteins is toxic, also in yeast cells. Proteomics data series are available for a yeast protein aggregation experiment, in which several amyloidogenic proteins, for instance amyloid-beta and alpha-synuclein were expressed in *S. cerevisiae* for 24 hours. During that experiment, in order to characterize cellular events triggered by amyloidogenic activity, large-scale proteomics analysis was applied in every 6 hours. By using this proteomics data series as an input for SiComPre it is possible to predict and compare structural and quantitative changes of protein complexes. For instance, we have identified loss of proteins from essential protein complexes and found situations where abundance of a complex is perturbed. Further research about the functionality of the changed protein complexes can help us to understand the mechanisms how aggregation-prone proteins cause cell death.

Acknowledgement: The research has been supported by the European Union, co-financed by the European Social Fund (EFOP-3.6.2-16-2017-00013).

Keywords: protein complex, protein aggregation, neurodegeneration



P-084

Elucidation of the conformational changes of ABCG2 in permeabilized cells

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ABCG2 (Breast Cancer Resistance Protein, BCRP) is an ABC transporter that is able to extrude a large variety of chemotherapeutic drugs from cells and it is often involved in the multidrug resistance of cancer cells. Based on crystal structures of several ABC transporters ABCG2 is believed to alternate between nucleotide-free inward and nucleotide-bound outward facing conformations. Previous studies revealed that the reactivity of ABCG2 with the 5D3 anti-ABCG2 mAb is dependent on the conformational changes of the transporter.

In the present study we show that after washing out the ATP from cells permeabilized by bacterial toxins the ABCG2 molecules become trapped in a 5D3-reactive conformation. Addition of ATP at conditions either allowing (presence of ATP/Mg²⁺ at 37 °C) or preventing ATP hydrolysis (absence of Mg²⁺ or low temperature) switches ABCG2 into a 5D3-dim state in a nucleotide concentration dependent manner. Interestingly, ADP also brings about a switch of ABCG2 to the 5D3-dim conformation, although it binds to the transporter with lower affinity compared to ATP. Taken together, these results suggest that the conformational changes between the 5D3-reactive and non-reactive conformations are caused by nucleotide binding and dissociation. Addition of vanadate (V_i) increased the apparent affinity of nucleotide binding under hydrolysis conditions (presence of ATP/Mg²⁺ at 37 °C) consistent with the formation of the stable ABCG2-ADP-V_i complex. However, when ATP hydrolysis was prevented, the apparent affinity was not influenced at all by V_i, confirming that ATP hydrolysis and the subsequent release of the γ -phosphate is a prerequisite for the formation of the ABCG2-ADP-V_i complex. In our kinetic studies the ABCG2 substrate quercetin increased the rate of the formation of the vanadate trapped post-hydrolytic complex in accordance with the substrate stimulation of the ATPase activity of the protein. We also found that the binding of 5D3 antibody to ABCG2 is reversible; the dissociation rate of 5D3 is enhanced by ATP/Mg²⁺ and substrates due to the function dependent conformational changes of the transporter.

Based on the above data our novel 5D3 antibody based assays are applicable for studying the details of the catalytic cycle of ABCG2.

Support: TÁMOP 4.2.4.A/2-11-1-2012-0001; OTKA grants PD75994 and K72762, Szodoray fellowship (Katalin Goda) and TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" project.



P-085

Examination of BFSP1 (Beaded Filament structural Protein 1 or Filensin) tumor specific splice variants in ex vivo human tumorous tissue and human serum samples

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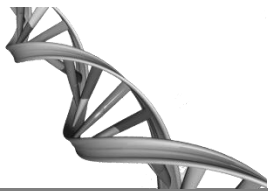
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BFSP1 (beaded filament structural protein one, or Filensin) is an eye lens specific cytoskeletal protein forms intermediate filaments (IFs) with its assembly partner (BFSP2) in the fiber cells of the eye lens. Preliminary experiments have proved the evidence that BFSP1 is expressed in various cancer cell lines as well.

Here, we show the presence of BFSP1 in *ex vivo* human tumor samples, compared to non-tumor tissue samples by Western Blotting, MS and immunohistochemistry. Furthermore, we quantitatively examined BFSP1 concentration in human sera *pre-op*, as well as *post-op* after 1 and 3 weeks. We performed qPCR in order to detect different splice-variants of the protein form human cancer cell lines.

Since there are few antibodies against BFSP1 and there are no antibodies recognizing only the splice variants of BFSP1 respectively, we immunized rabbits with a BFSP1 antigen presenting self-assembling nanoparticle (SANP). The BFSP1-antigen linked SANP was expressed in *E. coli* strain then purified via His-tag affinity chromatography. This new antibody will be essential for further research like detection of the different splice variants in human tumorous tissue and serum.

According to the literature, BFSP1 has been known as a cytoskeletal protein expressing particularly in eye lens so far. The presence of BFSP1 in cancer cells seems unlikely and it indicates a new exciting approach in the field of tumor biology. To establish the possible role of a new cytoskeletal protein as a tumor marker might have extraordinary significances in cancer diagnosis.



P-086

***In vitro* characterisation of human transglutaminase 4 activity and biological stability**

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The prostate transglutaminase (TG4) is one of the less studied member of the transglutaminase family. Originally it was described in rodents responsible for the viscous copulatory plug formation. In humans it is present in prostate cancer cells as negative prognostic marker contributing to higher invasiveness of cancer cells (Jiang et al, 2013), could be involved in male fertility (Landegren et al, 2015) and masking of sperm immunogenicity in the female genital tract (Mukherjee et al, 1983). Perez et al. (2013) have published that TG4 is present in the saliva in the form of an approximately 50-55 kDa fragment. This raises the possibility of a still unknown gastrointestinal role of TG4.

In this study our goal was the *in vitro* characterisation of recombinant human TG4.

TG4 was expressed in His₆-tagged form and purified by affinity and ion exchange chromatography yielding more than 90% purity. Its crosslinking activity was analysed using microtiter plate and kinetic fluorescent assays under various conditions because based on the available data this transglutaminase has a relatively low catalytic activity. Similarly to the rodent orthologues, transamidase activity of human TG4 was increased either at lower pH or in the presence of 1.5 mM SDS. Based on sequence alignment with other transglutaminases, TG4 has neither GTP nor fibronectin binding property which was confirmed experimentally. TG4 does not bind BODIPY-FL-GTPγS and TG4 activity does not influenced by GTPγS. Immobilized fibronectin is not able to anchor TG4 in ELISA experiment.

Potential proteolytic cleavage and stability of the enzyme have been checked in the presence of intestinal digestive enzymes, duodenal juices, thrombin and dispases. It was found that activated pancreatic juice generated similar TG4 fragments compared to the human recombinant trypsin. Dispace I quickly degraded recombinant human TG4.

Eukaryotic expression, natural substrates and posttranslational modifications of human TG4 are targets of our future studies to reveal so far unknown functions of human TG4.

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P-087

Intrinsically disordered linkers impart processivity on enzymes by spatial confinement of binding domains

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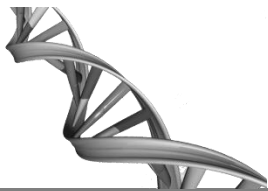
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Processivity is common among enzymes and mechanochemical motors that synthesize, degrade, modify or move along polymeric substrates, such as DNA, RNA, polysaccharides or proteins. Processive enzymes, once committed, can make multiple rounds of modification without releasing the substrate/partner, making their operation extremely effective and economical. The molecular mechanism of processivity is rather well understood in cases when the enzyme structurally confines the substrate, such as the DNA replication factor PCNA, and also when ATP energy is used to cause conformational changes that determine the succession of molecular events, such as with mechanochemical motors. It is much less appreciated that processivity may also result from the kinetic bias of binding imposed by spatial confinement of two binding elements connected by an intrinsically disordered (ID) linker. By statistical physical modeling, we show that this arrangement results in processive systems, in which the linker ensures an optimized effective concentration around novel binding site(s), favoring rebinding over full release of the polymeric partner. By collecting and analyzing 12 such proteins, such as cellulase, RNase-H and matrix metalloproteinase 9, we illustrate that in these proteins linker length and flexibility, and the kinetic parameters of binding elements, are fine-tuned for optimizing processivity. Conservation of structural disorder and special amino acid composition of linkers, and the correlation of their length with the step size on the substrate in the given enzyme suggest a unique type of entropic chain function of ID proteins, that is widespread and imparts functional advantages on diverse enzymes in a variety of biological contexts.



P-088

Investigating the regulatory function of IBAR and IRSp53 proteins in the formation of membrane nanotubes

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Cell protrusions, such as lamellipodia, filopodia, membrane ruffles appearing on the surface of the cells are associated with different cellular functions, they play role e.g. in cell migration, exocytosis, sensing, wound healing, cell-cell interaction, as well as viral and bacterial infection of cells. Although these structures differ in their morphology, similar molecular mechanisms are responsible for their formation. Membrane nanotubes – as filopodium-like intercellular bridges – were identified in 2004 as a new form of intercellular communication and matter transport. Membrane nanotubes are thin, actin-based, membrane covered cell protrusions that physically connect two – even distant – cells. These tubes were described *in vitro* in several cell lines and *in vivo* in mouse, chicken and zebrafish embryos. These membrane protrusions may be involved in the transfer of calcium ions, different cell organelles (e.g. mitochondria), lipids, various proteins, prions, vesicles, DNA and RNA molecules and presumably they have role in the intercellular spreading of bacterial and viral infections. Membrane nanotubes show similarity in several properties with the previously mentioned membrane protrusions, but possess characteristic differences from them (uniquely they provide direct contact between cells and they differ for instance in their length and diameter). A proposed way of membrane nanotube formation is that they develop as actin-dependent membrane protrusions from directed, filopodium-like structures. It is well-known that IBAR domain proteins, e.g. insulin receptor substrate protein 53 (IRSp53) induce negative membrane curvature, consequently promote filopodia formation, and they interact with several actin regulator proteins.

In this work laser-scanning confocal (LSCM) and total internal reflection fluorescence (TIRF) microscopy were applied to investigate the effect of IBAR and IRSp53 proteins on the formation and morphology of filopodia and membrane nanotubes as well as on individual actin filaments.

Based on our studies on cell cultures and individual actin filaments the examined proteins result in a significant change in the morphology of membrane nanotubes and affect the polymerization of microfilaments. Our results suggest that albeit membrane nanotubes show similarity in several aspects with filopodia also show differences from them in the basic mechanism of formation.



This work was supported by the GINOP-2.3.2-15-2016-00036, 20765-3/2018/FEKUTSTRAT and the EFOP-3.6.1-16-2016-00004 grants.

Keywords: *membrane nanotube, actin, IRSp53, IBAR, confocal microscopy*

P-089

Mislocalized phase separation by oncogenic fusion proteins: a frequent cause of cancers

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Membraneless organelles (MOs), like P-bodies, stress granules and signalosomes, form through liquid-liquid phase separation (LLPS). MOs have unique material properties, ensure reversibility and rapid response to triggers, and represent functional and structural units of cellular organization, conferring a range of benefits on cells. The assembly of MOs is mostly dictated by multivalent weak interactions between disordered, compositionally biased LLPS driver regions (DRs) of only one or a few associated proteins. Importantly, mutations within LLPS DRs can remarkably influence the formation, dynamics or maturation of MOs, and are thus implicated in a number of neurodegenerative disorders, muscular atrophies and cancers. It has only been sparsely investigated, however, what happens if such LLPS DRs are not mutated but placed to an altered molecular context due to chromosome translocation-derived gene fusions.

To address this question, we have collected all the hitherto described human LLPS DRs that are capable of driving phase separation on their own. Then we have integrated diverse information on all the in-frame oncogenic fusion proteins from the literature and dedicated databases that incorporate those self-sufficient LLPS DRs (as a whole or at least partly). We first focused on the fusions published in low-throughput, clinical studies where they were suggested to be essential drivers of the respective cancerous transformation. Then we obtained those fusion proteins for reference that have been only detected in high-throughput sequencing analyses of multiple cancer samples and thus are less likely to be the major drivers of oncogenic transformation. We compared the fusion partners between these datasets, analysed their enrichments for GO terms and evaluated the associated cancer types.

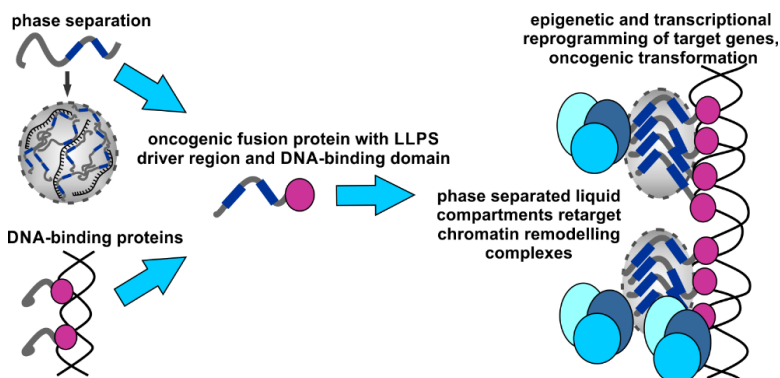
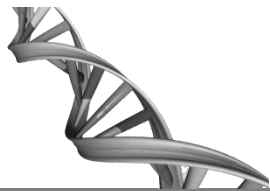


Figure 1: *Oncogenic transformation by fusion proteins capable of phase separation*

Based on our results, we propose that 1) LLPS DRs are frequently incorporated into oncogenic fusion proteins with high transformation potential in specific, rare cancer types, 2) fusions with different DNA-binding domains are likely to drive oncogenic transformation and 3) that such uncontrolled phase transitions along the DNA are prone to retarget chromatin modifying complexes, thereby leading to the epigenetic and transcriptional reprogramming of target genes. Thus, such fusion proteins drive cancer through a yet unappreciated molecular mechanism.

Keywords: *Liquid-liquid phase separation, oncogenic fusion proteins, gene fusion, chromosome translocation, transcription reprogramming*

P-090

Multiscale modelling of protein-ligand interactions in heme enzymes

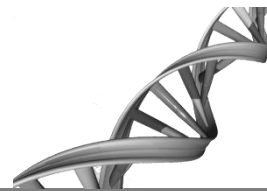
Julianna Oláh¹, Anikó Lábas², Dóra K. Menyhárd² and Jeremy N. Harvey³

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Understanding the nature of protein-ligand interactions is vital for getting insight into the functioning of living organisms. Complementing versatile experimental methods, computational tools have become increasingly important in elucidating the factors that are responsible for the functioning of biomolecules. By their ability to give atomistic information on local interactions between ligand and host they have also become indispensable for the discovery of new drugs in the pharmaceutical industry. We have recently used various modelling methods such as molecular dynamics simulations, docking, quantum chemical and



and hybrid quantum chemical molecular mechanics calculations to address various aspects of heme chemistry: the binding of small ligands to myoglobin,¹ the biosynthesis of estrone by human aromatase,² the bioactivation of estrone by human cytochrome P450 enzymes.³

In the present contribution, through the example of small ligand (NO and CO) binding by myoglobin, we would like to demonstrate the ability of composite methodologies to tackle the complex nature of the ligand binding process. We address the problem of ligand diffusion to the active site of myoglobin with MD simulations and using a simple Markov-state model we obtained rate-constants for diffusion in close agreement with experiment. The chemical step of the binding of the ligand by the heme group was studied using quantum chemical calculations which provided accurate activation energies and rate constants for the spin-forbidden binding of NO and CO by the heme group. Combination of the rate constants obtained for the two steps of the reaction (diffusion and chemical binding) allowed us to reveal the differences in the mechanisms of the binding of the two ligands and to obtain absolute rate constants for binding to better than two orders of magnitude. The protocol opens the way for the study of other protein-ligand reactions such as enzymatic reactions.

Acknowledgements: *The financial support of COST Action CM1305 (ECOSTBio), of Richter Gedeon Talentum Foundation, of OTKA Grant No. K116305 and PD11503 and of a Bolyai János Research Fellowship is acknowledged. MD simulations were carried out at the facilities of the Hungarian NIIF Institute.*

Keywords: *modelling, diffusion, heme protein*

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P-091

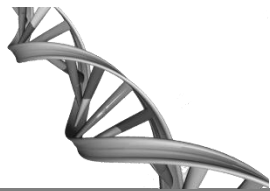
Mutational analysis of a deubiquitylating enzyme-coding gene in *Drosophila melanogaster*

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Ubiquitylation is a reversible post-translational protein modification that regulates many biological processes. It is reversed by the activity of a large family of deubiquitylating enzymes or DUBs; many of them is still without known physiological function. Genetic analysis of mutant phenotypes in *Drosophila* could reveal novel new roles for the still uncharacterized DUBs.



In this study, we have used the powerful new gene editing approach, the CRISPR/Cas9 technique to generate site-directed mutations in one of the uncharacterized *Drosophila* DUB genes, *Usp34*. Forty independent lines were established and investigated to detect any mutation within the gene. Among these, seven lines carried a frameshift mutation, and eight lines carried nucleotide insertions and/or deletions that resulted in alteration in the amino acid sequence of the gene product. The different mutations could be correlated with different phenotypes, and we hope that a detailed functional analysis of these mutants will help us elucidating the function of this DUB.

This study is supported by the Hungarian Research Fund K116372, and the National Research Development and Innovation Office grant GINOP-2.3.2-15-2016-00032.

Keywords: Ubiquitin, deubiquitylating enzyme, CRISPR/Cas9, *Drosophila*

P-092

Nanobodies as potent tools in structural and functional studies of large multidomain partially disordered proteins

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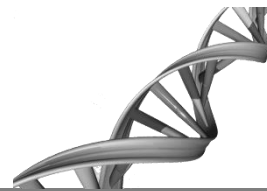
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Structural-functional studies of proteins possessing significant intrinsic disorder means great challenge per se. The CREB-binding protein (CBP) and its paralogue histone acetyl transferase p300 (p300) are pivotal transcription co-regulators with large size, hundreds of post-translational modifications and interacting partners, nine structural domains, and ~60% intrinsic disorder that makes understanding their structure function relationships extremely difficult [1]. On the other hand, nanobodies are small ~15 kDa recombinant proteins derived from variable regions of cameloid heavy chain antibodies [2]. Specific nanobodies are state-of-the-art tools in many aspects [3]: might serve as protein crystallization aids, specific labels in low resolution structural studies, markers in live-cell imaging and tracking, and *in vivo* diagnostic or even therapeutic tools.

Here we produced both recombinant full-length CBP and p300, immunized llamas with them and selected nanobody libraries using wild type and inactive forms of CBP and p300 [4]. These libraries were characterized by next generation sequencing (NGS). From NGS data, 559 988 individual amino acid sequences were deduced, and 355 023 clonotypes were



defined based on their complementarity-determining region 3 (CDR3). Within these clonotypes, 162 strong candidates were identified expected to be strong and specific binders to CBP and/or p300. Parallely, specific nanobodies were isolated also by low scale screening, and their binding abilities were characterized in depth. Three non-related nanobodies from the 'CBP'-library bind to linear epitopes on the KIX domain of CBP with K_d in the nM range. These nanobodies could be used as specific labels and/or molecular switches in future studies. A fourth nanobody (E12) was isolated from the 'p300'-library that is specific for the p300/CBP acetyltransferase (HAT) domain. Detailed structural analysis by HDX-MS suggested the existence of a new conformation of the HAT domain that escaped from crystallization till now. Nanobody E12 could definitely be used as crystallization aid that might provide missing pieces of the catalytic mechanism.

Keywords: CBP/p300, nanobody, supertertiary structure

References:

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P-093

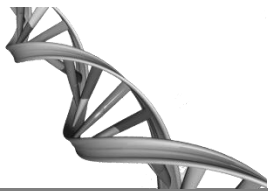
Relationship between interfaces, binding strength and specificity of disordered protein complexes

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The concept of structural disorder is now widely accepted, bioinformatic predictions suggest that about 50% of human proteins have at least one long disordered region. Most of intrinsically disordered protein (IDP) functions are carried out by molecular recognition, i.e. via transient or permanent binding to a structured partner. To understand the role and functioning of disordered proteins, it is very important to map the mechanism(s) of recognition and binding. The common view is that disorder separates binding strength from specificity. This feature would enable IDPs to engage in highly specific, yet rather weak and reversible interactions. Scattered observations, however, show that this may not be true



without exceptions. Our hypothesis is that the binding strength of IDP complexes depends on the mode of recognition and the type of interaction, which enables weak and strong, specific and non-specific interactions alike.

The aim of the project is to find out what influences the binding strength and specificity of protein complexes of which at least one of the partners is disordered. We have 260 disordered protein complexes for which both the structure and the dissociation constant (K_d) are known, from the publicly available Database of Disordered Binding Sites.

First we analyze the collected data from several aspects: distribution of K_d values, physical and chemical properties of the interface, eg. size, proportion of hydrophobic interface, amino acid composition, distribution of contact types etc. We examine and compare these properties in the case of strong (low K_d values) and weak (high K_d values) complexes. We use a reference database built from complexes of completely ordered proteins.

We also want to analyze the functional binding specificity of protein-protein interactions so we showed that the local patterning of the physicochemical properties of amino acids within surface patches is uniquely characteristic of interfaces and defined a statistical potential, iPat, as a measure of surface patterning.

We also would like to examine and compare the evolutionary conservation of interface and surface amino acids in disordered and globular protein complexes.

Keywords: *disordered protein complexes, specificity, conservation*

P-094

Revealing the Catalytic Mechanism and Stereoselectivity of the Dinuclear Zinc Enzyme Dihydropyrimidinase with Quantum Chemical Approach

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Dihydropyrimidinase (DHPase) catalyzes the reversible conversion of 5,6-dihydrouracil (DHU) or 5,6-dihydrothymine (DHT) to the corresponding N-carbamoyl- β -amino acids in pyrimidine metabolism. This enzyme has been shown to become a promising target for the stereoselective production of α -amino acids, alternative to the currently industrial used enzyme hydantoidase. Here, the reaction mechanism and origin of stereoselectivity of DHPase from *Saccharomyces kluyveri* is investigated using density functional theory. Two different models of the active site are designed on the basis of the X-ray structure: a small one to study the mechanism and characterize the stationary points and a large one to study the stereoselectivity and the role of stereo gate loop (SGL) residues. It is shown that the proton abstraction from hydroxide by Asp358 occurs concertedly with a protonation of ring nitrogen. For DHT substrate, the enzyme is preference to the L-configuration, in good



agreement with experimental observation. Comparison of the reaction energetics of the two models reveals the importance of SGL residues in stereoselectivity of the catalysis. The role of conserved Tyr172 in transition state stabilization is also investigated and its mutant (Y172F) reduce the activation barrier of the reaction compared to the wild-type enzyme. Detailed understanding of the catalytic mechanism of the enzyme may allow it to be engineered in order to enhance its activity and stereospecificity.

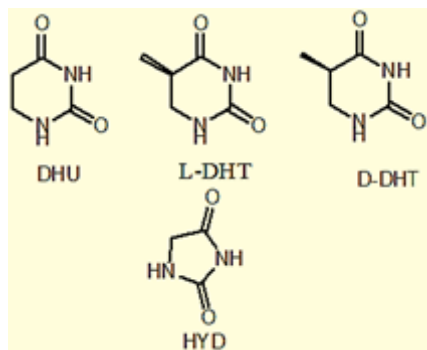


Figure 1: Substrates of dihydropyrimidinase

Keywords: dihydropyrimidinase, enzyme mechanism, stereoselectivity, substrate specificity, DFT

P-095

Structural and functional characterization of the GKAP post-synaptic density scaffold protein

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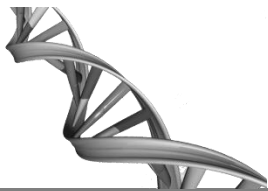
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* These authors contributed equally to this work

Scaffold proteins participating in the formation and maintaining the spatial organization of the post-synaptic density (PSD) actively take part in the regulation and modulation of signalling through glutamate receptors as well. GKAP (guanilate kinase-associated protein), an essential member of the PSD, dynamically connects several proteins through its highly flexible disordered regions, which are not yet fully characterized. On the N terminal the GK-binding region consisting of five 14 amino acid long repeats associates with the GK region of the well-known PSD-95 protein, while the dynein binding region (GKAP-DLC) binds



the light chain of the dynein motor protein. A known globular, helical region called GH1 domain is located on the C terminal.

Several *in silico* techniques have been applied to the GKAP protein to predict its structure. Multiple alignment of the different GKAP isoforms was performed to compare the isoforms and to identify consensus residues in the binding sites. Repeat sequences were also detected to identify recurring motifs. Besides secondary structure and disorder prediction, aggregation prediction and coiled coil prediction were performed to accurately localize the disordered regions. Protein-protein interactions were also extracted from the STRING database. With the ITasser web service, a 3D structural model was built, and has been further refined with Pulchra.

Using *E. coli* bacteria, a recombinant protein was produced including 6x His tag, TEV protease cleavage site and the two dynein binding sequences of GKAP (GKAP-DLC domain). Probably due to the disorder in the structure, this construct was mainly expressed in inclusion bodies, therefore denaturing-renaturing affinity chromatography protocol was applied to purify it. Size-exclusion chromatography and TEV protease digestion were performed. A preliminary NMR measurement was carried out validating the disorder in this region.

Similarly dynein light chain production and purification was performed. The dynein-binding activity of GKAP-DLC was shown in a pull-down experiment, an analysis with isothermal titration calorimetry and also through detecting the conformational change of the proteins when participating in the interaction via measuring their tryptophan fluorescence spectra.

¹⁵N labeled production of this construct is on the way for more informative NMR measurements. We plan to focus on the detailed structural and functional characterization of the disordered regions of GKAP. So far our experimental results are consistent with our *in silico* predictions and also with the data described in the literature.

Keywords: GKAP, protein structure, post-synaptic density

P-096

Structural determinants of the long-distance interaction between human UPF1 and UPF2

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Intrinsically disordered proteins/regions (IDPs/IDRs) function as ensembles of unfolded conformations. Scaffold proteins and those that bridge large distances to bind partners often harbour long IDRs, however, current models are insufficient to describe their contribution.



According to the classical fly-casting model, freely diffusing IDPs recognize their partners faster than globular proteins due to their increased capture radius, but molecular dynamics simulations disprove this model. Based on the architectures of known assembly proteins we propose a modified model termed „real fly-casting” (RFC), wherein complex assembly and long-distance binding require modular proteins that are anchored to large, slowly-diffusing entities of the cell, like membranes, DNA or RNA, by dedicated domains, but also harbour tentacle-like long IDRs rich in binding motifs. This arrangement could grant the efficient reduction of search space dimensions and thus the productive sequestering of distant partners.

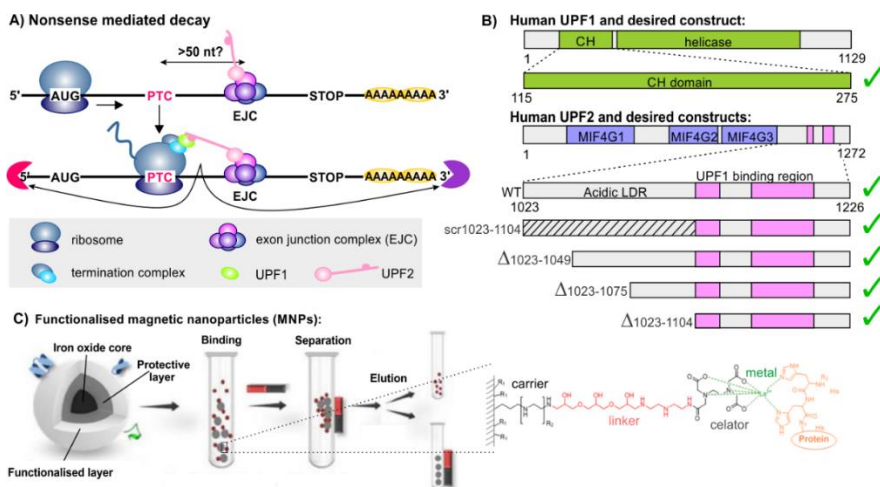


Figure 1: As a model for RFC we study the interaction between two proteins of the nonsense mediated decay (NMD) pathway, human UPF1 and UPF2. The protein constructs and the functionalised magnetic nanoparticles developed to efficiently purify them are presented.

NMD is a system for degrading mRNAs with premature stop codons (Figure 1A). Here, the critical long-range interaction between UPF2 and UPF1 likely relies on RFC and is enabled by a long, acidic IDR in UPF2 (Figure 1B). We plan to check how varying the length or scrambling the sequence of this acidic IDR will influence the kinetics and thermodynamics of UPF2-UPF1 interaction. The efficient production of the required protein constructs is finally optimized by functionalised MNPs (Figure 1C). Since the RFC mechanism can only be validated by studying immobilized proteins, we plan interaction experiments exploiting this feature: the results of surface plasmon resonance, biolayer interferometry and atomic-force microscopy measurements will be compared to those of microscale thermophoresis used for the direct characterization of the interaction thermodynamics in solution.

Keywords: structural disorder, complex assembly, nonsense mediated decay



P-097

Structural determination of p53 – S100A4 complex using annexin A2 as a novel crystallization chaperone

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X-ray crystallography is a powerful tool to precisely understand protein function and mechanism. As structure solving methods developed over time, the production of high-quality diffracting crystals has become the most challenging part of protein crystallography. The process of crystallization is affected by numerous factors including chemical characteristics of the protein, environmental factors, conformational heterogeneity and the amount of available purified protein. An often used method for purifying proteins is affinity tagging. Before crystallization these affinity tags are usually removed, but in some cases these tagged chimeric proteins are utilized to help crystal formation. For this purpose mannose binding protein (MBP), glutathion-S transferase (GST) and thioredoxin (TRX) were previously used beside other well-crystallizing proteins like lysozyme, however, so far only MBP appears to be generally effective. Here we show that annexin A2 (ANXA2) with a cleavable His₆-tag could be a similar or even better crystallization chaperon expanding the repertoire of these helper molecules.

ANXA2 is a non-EF-hand Ca²⁺-binding protein with 12 paralogs in humans. Its ability to aggregate (“annex”) phospholipid membranes in a Ca²⁺-dependent manner is the basis of its biological functions such as vesicular transport, exo- and endocytosis. ANXA2 has many useful properties; it is highly soluble and stable, and its bound calcium ions allow anomalous data collection. Its ability to form crystals very easily was revealed in our previous studies. In this work we fused ANXA2 to the small EF-hand Ca²⁺ binding protein called S100A4. Using this chimeric construct we were able to solve the structure of the p53 – S100A4 complex, which previously resisted to crystallize of its own or with fused MBP or GST (Supported by NKFI grant K119359)

Keywords: *protein crystallography, fluorescence polarization, protein-protein interaction*

**P-098****Structural differences between Gelsolin and Flightless-I proteins and their behaviour in the presence and absence of Ca^{2+} -ion****Péter Gaszler**, Péter Bukovics, Rauan Sakenov, Réka Pintér, Tamás Huber and Beáta Bugyi*University of Pécs, Department of Biophysics, Pécs, Hungary*

Gelsolin homology (GH) domain family proteins are central to the control of the actin cytoskeleton. Its eponymous member, the six-GH-domain gelsolin (GSN) is a calcium-activated multifunctional actin regulator. Recently, a unique member; Flightless I (Fli-I) was identified characterized by the six-GH-segment structure, but preceded with a leucine rich repeat. Both GSN and Fli-I are implicated in pathologies, including sepsis and tissue regeneration, respectively. Albeit, the structural-functional relation in GSN upon Ca^{2+} -dependent activation is well described the role of Ca^{2+} in the actin activities of Fli-I is unravelled. Our functional analysis indicates that the GH16 domains of GSN and Fli-I respond to Ca^{2+} differently implying different conformational characteristics of the GH domains in the two proteins. To assess this question we aimed to investigate the structural behaviour of GSN and Fli-I in the presence and absence of calcium-ion by using fluorescence spectroscopy and biochemical approaches.

The change in the fluorescence parameters (emission and maximum wavelength) of Trp in GSN upon quenching by acrylamide or guanidine-hydrochloride induced chemical denaturation indicates marked conformational changes in response to Ca^{2+} . This is in agreement with the high-resolution structural model of GSN activation. In contrast, our data suggest that the microenvironment of Trp residues in Fli-I GH16 is different from that of GSN even in Ca^{2+} -free conditions and not markedly affected by the presence of the divalent cation. Spectroscopic data is supported by the different kinetics of limited proteolysis observed for GSN and Fli-I GH16. Our experimental findings are supported by bioinformatics analysis, which reveals that the type-2 Ca^{2+} binding sites and the C-terminal tail mediating structural changes in GSN upon Ca^{2+} -activation are not conserved in Fli-I GH16. Altogether, our work reveals different Ca^{2+} -response and predicts distinct mode of activation of GSN and Fli-I.

Acknowledgements: *The project has been supported by the ÚNKP-18-2-1 New National Excellence Program of the Ministry of Human Capacities (to PG) and by grant from the Faculty of Medicine, University of Pécs (PTE ÁOK-KA No: 2018-01, to PB). We thank József Mihály (Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary) for providing us with the plasmid of D. melanogaster Flightless I.*



P-099

The effect of phosphorylation on the RNA-binding capacity of the Ezh2 disordered loop region

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The protein Enhancer of zeste homolog 2 (Ezh2) is responsible for the maintenance of the trimethylated lysine 27 on histone H3 (H3K27me3) as the enzymatic component of Polycomb Repressive Complex 2 (PRC2). Ezh2 is overexpressed in various cancers and promotes metastasis formation primarily through the epigenetic silencing of E-cadherin¹. It has been recently proposed that the targeting of PRC2 is mediated by different long non-coding RNAs (lncRNAs) capable of binding to Ezh2². The RNA binding region of Ezh2 is suggested to reside between residues 342-370, in a disordered loop that also contains a threonine phosphorylation site (T345) important for RNA binding³.

In order to understand the molecular background of RNA binding and the effect of phosphorylation, we expressed the wild-type and a phosphomimetic mutant version of Ezh2 loop region and studied their binding affinities to different RNA constructs. We also measured the CD spectra of the different constructs either in free form or bound to RNA. For detailed structural analysis NMR spectra of the protein constructs were also recorded.

Keywords: *histone methyltransferase, RNA binding, phosphorylation, intrinsically disordered protein, protein structure*

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P-100

The effect of the Myo16 Ankyrin domain on the actomyosin system

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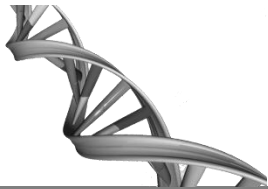
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The unconventional myosin 16 (Myo16) is a lesser-known member of the myosin superfamily. First it was described in the developing nervous system and it might play a role in neuronal development, in cell cycle regulation and in signal transduction. It could be involved in the development of human neural diseases, such as schizophrenia and autism. Myo16 has a unique N-terminal extension consisting of eight ankyrin repeats (Myo16Ank). It has been already showed, that the ankyrin domain of Myo16 might play a role in the regulation of the motor function: it enhanced the motor activity of skeletal and non-muscle myosins in NADH-coupled ATPase assay.

The regulation of motor function is essential for the proper behaviour of myosin proteins. In our research, we were studying how Myo16Ank interacts with skeletal muscle heavy meromyosin (skHMM), and which step of the ATPase cycle is influenced by this domain. In our studies we performed steady-state anisotropy and ATPase activity measurements, *in vitro* motility assays (IVMA) using total internal reflection fluorescence (TIRF) microscopy and rapid kinetic measurements with a stopped-flow device as well. Our experiments were performed using skeletal muscle actomyosin model system.

The results showed that Myo16Ank binds directly to the S1 fragment of the skHMM ($K_D \sim 7,01 \mu M$) and increases the ATPase activity of the S1 fragment. During IVMA we observed that Myo16Ank significantly increased the actin-activated ATPase activity of the skHMM in a concentration dependent manner. Performing IVMA at different ATP concentrations we observed that the motor domain in the presence of Myo16Ank reached its maximal reaction-rate at a lower ATP concentration. Since Myo16Ank does not bind actin filaments directly, the effect is presumably achieved by increasing the ADP dissociation from the motor domain. Using stopped-flow measurements we were able to observe the enhancement of ADP release-rate of actoS1 complex in the presence of Myo16Ank.

Based on our results, one possible intramolecular role of Myo16Ank is the regulation of myosin 16 motor function, primarily through the direct enhancement of the motor activity.



P-101

The role of adenosine 2A receptor activation in the vesicular trafficking of macrophages

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Macrophages have important functions in the innate immune system, such as antigen presentation, pathogen killing and communication with the environment.

These immune cells identify and eliminate pathogens. During these processes the membrane's structure changes dynamically. The membrane repair mechanism is responsible for maintaining the homeostasis of the cells. If this mechanism is incomplete, it causes apoptosis, necrosis, lysosomal permeabilization or disturbances. Moreover, it leads to neurodegenerative disorders, metabolic or cardiovascular diseases and muscular dystrophy. Lysosomes also play a major role in the development of these diseases. Besides this evidence, our workgroup previously identified twenty-eight proteins which interact with the C-terminal domain of the Adenosine 2A receptor (A2AR). Eight of these have a role in vesicular transport processes.

Based on the preliminary results and facts, the aims of the study were to examine the expression of genes identified previously as A2AR interactors and investigate the role of A2AR in the vesicular trafficking of macrophages.

Firstly, we investigated the expression of genes by q-RT-PCR method. We treated the cells with A2AR agonist and antagonist on lipopolysaccharide (LPS) activated primary macrophage cells. Considering the eight genes together we can distinguish two groups based on the gene expression pattern. In the first group LPS alone and in combination with the A2AR agonist and antagonist significantly reduced the level of the selected gene expression. In the other group considerable gene expression growth was observed with the effect of A2A receptor antagonist combined with LPS compared to the LPS treated cells. We also started to scan the protein levels of Niemann-Pick disease, type C1 (NPC-1) and Early endosome A1 (EEA1) genes by western blot and immunostaining methods. Based on the results there was no significant change at the protein level on western blot for EEA-1. During the immunostaining we found that the EEA-1 gene showed significant growth in fluorescence intensity due to A2A receptor antagonist treatment but NPC-1 has not shown any change so far.

In order to examine the vesicular trafficking we chose the Lysosome-associated membrane protein-2 (LAMP-2) as a lysosome marker which can translocate to the plasma membrane in case of possible injury. We treated the LPS activated primary macrophage cells with A2AR agonist and measured the translocation (membrane over cytoplasm region) of the LAMP-2 protein by Opera Phenix confocal microscope and the photos were analyzed with Harmony 4.8 software. We found that the fluorescence intensity decreased due to the A2A agonist



treatment compared to the untreated cells. Consequently A2AR influences the appearance of LAMP-2 protein in the lysosomal membrane.

In summary, the A2AR create a part in regulating the expression of genes involved in vesicular trafficking. We confirmed the A2AR regulatory role in the cell surface appearance of the LAMP-2 protein.

P-102

The role of polyQ region in the RNA binding of Mixed Lineage Leukemia 4 (MLL4) Protein

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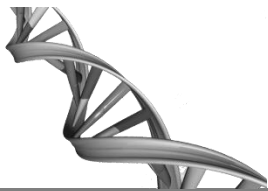
Long non-coding RNAs (lncRNAs) are transcribed RNA molecules longer than 200 nucleotides that do not code for translated proteins. These lncRNAs are more and more recognized as central players in a plethora of biological processes. They can act as flexible scaffolds, they can interfere with other endogenous RNAs and they can modify chromatin state¹. LncRNAs have also been shown to play a role in several layers of epigenetic regulation: they are involved in DNA methylation and demethylation, they can modify chromatin conformation through binding to remodelers and many of them interact with histone modifier enzyme complexes such as PRC2, coREST or SMCX².

In a recent publication we have provided evidence for a so far unrecognized interaction between MLL4 and different lncRNAs³. One of the RNA-binding regions contains a 14-residue long polyQ stretch and exerts phase separation-like behavior upon binding to certain RNAs. As it was suggested earlier that polyQ regions are be involved in phase separation of RNA binding proteins⁴, we hypothesized that this region might be responsible for the anomalous behavior in our studies. In order to understand the importance of the polyQ region in the RNA binding and the structural properties of the protein, we deleted the polyQ region and measured the structural and RNA binding properties of the mutant region.

Keywords: *histone methyltransferase, RNA binding, phase separation, lncRNA*

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P-103

Using ubiquitinated positions to gather membrane protein topology data

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Ubiquitination is a cytosolic post-translational modification where ubiquitin ligases covalently attach one or more ubiquitin proteins to lysines of other proteins. Depending on the number and place of the attached ubiquitins, the modification can have several different results. For example, poly-ubiquitination leads to the degradation of proteins by the proteasomes. Monoubiquitination of certain proteins can lead to DNA repair or chromatin structure remodeling. In case of membrane proteins, multi-monoubiquitination could initiate the internalisation of membrane proteins from the cell membrane, hence playing important part in several signaling pathways. As ubiquitination exclusively happens in the cytosol and only marks the inner side of intact membrane proteins, it could provide a great opportunity for gathering topology data without the need of artificial chemical modifications of the membrane proteins. Previously, our research group has developed a method, called MSTOP, for the extracellular modification of membrane proteins by a covalent modification of extracellular lysines. One drawback of the method is that according to the ‘positive-inside rule’ more positively charged lysines can be found in the cytosolic loops of membrane proteins. However, these lysines are accessible for natural ubiquitination. In our work, we modified the MSTOP method by applying deubiquitinase and protein synthesis inhibitors on intact cells. The latter was important, as badly folded membrane proteins are retrotranslocated to the cytosol and got polyubiquitinated. Then, the cells were subjected to subsequent steps of the protocol: creation of membrane preparation and digestion of membrane proteins, followed by the purification of labeled peptides by GG motif (remnant of ubiquitin) specific antibodies and mass spectrometry analysis.

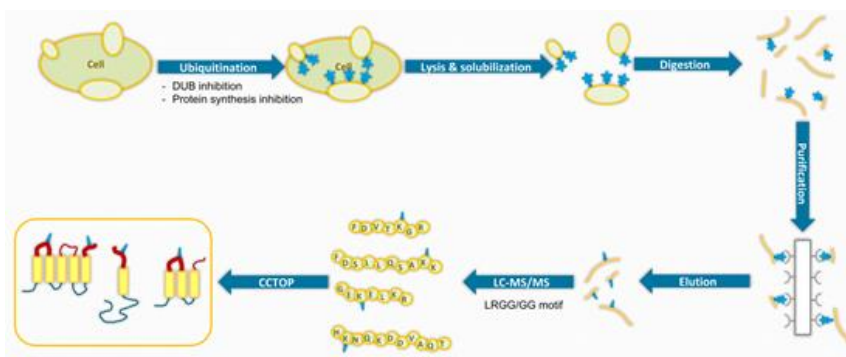


Figure 1. Workflow of developed protocol.

Keywords: ubiquitination, membrane protein, topology

P-104

Acute and chronic health effects of extracellular vesicles from irradiated mice

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Introduction: Radiation-induced biological effects in cells that are not directly exposed to ionizing radiation are defined as bystander effects. Due to radiation-induced bystander effects the number of cells affected by radiation is larger than the actual number of irradiated cells. This has important consequences in radiation protection and in radiation induced health effect.

Our aim was to investigate extracellular vesicles (EVs) mediated ionizing radiation effects in the bone marrow. In this study, we focused on the bone marrow stem cells and progenitors.

Methods: Male 9-12-week old C57Bl/6 mice were exposed to total body irradiation using different X-ray doses (0 Gy, 0.1 Gy, 0.25 Gy, 2 Gy). The mice were sacrificed 4 or 24 hours or 3 month after the irradiation and the bone marrow and spleen as well as the EVs from bone marrow were isolated. The EVs were injected into the tail vein of non-irradiated mice (bystanders) and the bystander bone marrow and spleen were isolated 4 or 24 hours or 3 month after injection. The effects of EVs on the bone marrow cells of these bystander mice were compared to radiation effects in the directly irradiated animals. Apoptosis induction was analyzed by TUNEL assay and the level of DNA double strand breaks was measured



by flow cytometry using an anti- γ -H2AX antibody. The following cell types were measured in the bone marrow by flow cytometry: long-term-hematopoietic stem cells (LT-HSC), short-term-hematopoietic stem cells (ST-HSC), multipotent progenitors (MPP), mesenchymal stem cells (MSC), lymphoid progenitors (LP), granulocyte and monocyte precursors (GMP), megakaryocytes and megakaryocyte progenitors (MK) and erythroid precursors (EP).

Results: The role of EVs in transmitting radiation effects was confirmed in the bystander mice. As an acute effect we detected increased rate of apoptosis in the HSC and L.PROG populations 4 hours after injection of EVs isolated from mice irradiated with 2 Gy. Migratory HSC were detected in the spleen and the number of HSC in the bone marrow significantly decreased 24 hours after injection of EVs from mice irradiated with 0,25 ; 2 Gy. The composition of HSC has changed significantly due to the decrease in MPP population 24 hours after injection of EVs as well as moderately 3 months after. Bone marrow MSC significantly decreased 24 hours after injection of EVs from mice irradiated with 0,1; 0,25 ; 2 Gy, but this effect was not detected 3 months later. Exclusively chronic effect was the decrease in cell number of LP and GMP populations 3 months after injection of EVs.

Conclusion: We show here that the injection of EVs from irradiated mice is able to mediate acute and chronic changes in the bone marrow of non-irradiated mice. This study sheds light on possible mechanisms of radiation-induced direct and bystander damage in the bone marrow.

The work described in this abstract has been supported by the European Commission, within the CONCERT project. This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287.

Keywords: *extracellular vesicles, bone marrow, ionizing radiation, bystander effects*



P-105

CRISPR genome editing based isogenic induced pluripotent stem cell system for schizophrenia disease modeling

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Schizophrenia (SCZ) is a devastating neuropsychiatric disorder of complex, poorly understood etiology. Genetic and environmental factors both play a role in the development of SCZ. While several mutations have been associated with SCZ, in most cases their biological significance remains unclear. De novo mutations (DNMs) represent a recently described new source of genetic variation in the background of SCZ. The aim of this study was to investigate the biological significance of DNMs in SCZ by combining induced pluripotent stem cell (IPSC) based disease modeling and CRISPR based genome editing.

To this end we selected a SCZ case-parent trio, where the affected patient carries a potentially disease causing DNM. Based on exome sequencing studies we chose a patient harboring a zinc finger MYND domain-containing protein 11 (ZMYND11) 1495C>T nonsense DNM resulting in a R399X stop codon. ZMYND11 encodes a chromatin reader protein that specifically binds H3.3K36me3, co-localizes with highly expressed genes and promotes intron retention. The heterozygous mutation observed in our patient could potentially lead to haploinsufficiency of ZMYND11.

First we introduced monoallelic or biallelic frameshift mutations into a control wild type IPSC line using CRISPR non-homologous end joining (NHEJ). Next IPSC lines were generated from each member of the case-parent trio using Sendai virus based reprogramming. The investigated ZMYND11 mutation was corrected using CRISPR homology-directed repair (HDR) in the affected IPSC line. These isogenic IPSC pairs were taken forward to neuronal differentiation experiments and neuronal progenitor cells were differentiated (NPC).

In ongoing experiments we are investigating morphological, functional and molecular differences in our isogenic system to establish the biological significance of the investigated ZMYND11 DNM.

This study is funded by the Richter Gedeon Pharmaceuticals Téma Pályázat Grant to DS and the National Brain Research Program (Grant NAP-B KTLA_NAP_13-2014-0011 to JR).

Keywords: schizophrenia, disease modeling, induced pluripotent stem cells, genome editing, CRISPR



P-106

Discovery the role of miR-302 cluster in chicken and rabbit stem cells

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Chicken primordial germ cells (cPGCs) are future pioneers of stem cell and developmental biology. The cPGCs can be easily cultured in the laboratory using a well-defined condition. Rabbit induced pluripotent stem cells (rabiPSCs) have also important role in stem cell biology. Many studies have identified miRNAs as factors controlling the pluripotency of the stem cells. To explore the relevant miRNAs expressed in cPGCs, a complex microarray analysis was done using LC microRNA microarray. The miRNA expression pattern showed different expression in slowly and highly proliferating cPGCs lines. We found high gga-miR-302b-3p and low expression of gga-miR-302b-5p in slow proliferating lines. The 5p/3p arm ratio was high in highly proliferating cPGCs cell lines (FEK/M2/ZZ, FS/101/ZZ, FS111/ZW). To functionally validate the role of these miRNAs, we performed miRNA inhibition studies in these PGC lines (*Figure 1*).

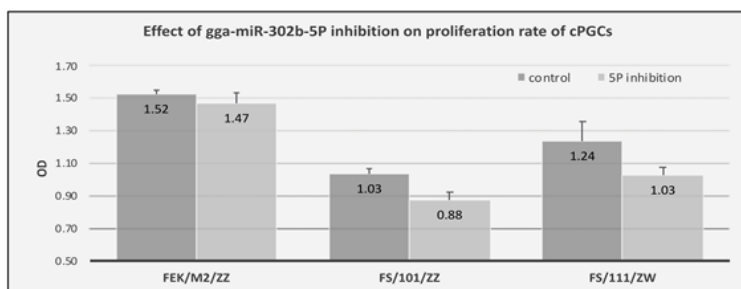


Figure 1: Inhibition of gga-miR-302b-5p decreased the proliferation rate in cPGCs

We found that ocu-miR-302a-3p is highly expressing in rabbit stem cells. We analyzed the role of this miRNA in rabbit iPSC. Our result revealed that both ocu-miR-302a overexpression and inhibition of ocu-miR-302a-3p increased the proliferation rate of rabbit iPSC cells.

From the above studies it can be concluded that there is concordant dysregulation between the gga-miR-302b-5p and 3p arms. In the future, we would like to characterize the role of ocu-miR-302b-5p and 3p in controlling the proliferation and apoptosis rate of rabiPSCs.

Keywords: PGC, iPSC, miR-302 cluster



P-108

Effect of microglial cells on the neurite outgrowth of human stem cell-derived neural progenitor cells

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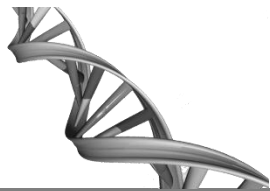
Neural progenitor cells (NPCs) are multipotent self-renewing stem cells, which provide a good model system for studying neuronal polarization. During early development, progenitor cells, which have initially a round shape, protrude more or less equally long projections, the so-called neurites. Later during differentiation, one of the neurites differentiates into an extended axon, whereas the others become shorter dendrites. The formation and maintenance of such cellular structures are fundamental for the proper neural development and regeneration. It has become more and more accepted that external stimuli, such as injury and inflammation, can stimulate NPC proliferation and differentiation.

Microglia, the main immune modulating cells in the neurogenic niche, have also an important role in neuronal regeneration after injury. When damage occurs, basal state microglia rapidly become reactive, i.e., undergo morphological changes and acquire pro-(M1) or anti-inflammatory (M2) properties depending on the incoming signal.

In this study, we aimed at elucidating the interaction between microglia and neural progenitor cells at the initial stage of neuronal differentiation. BV2 microglial cells were first activated either into M1 or M2 direction. Then human induced pluripotent stem cell-derived NPCs expressing GFP were subjected to the supernatants collected from the activated BV2 cultures, or co-cultured with these microglial cells. Cell proliferation and neurite outgrowth of the NPCs were assessed by a high-content, automated microscope system. We found that basal state microglial cells reduce the neurite outgrowth of human NPCs, whereas activation of microglial cells toward M1 direction results in reversal of this effect. In addition, we observed that lipopolysaccharide, as well as certain cytokines, such as IF- γ , IL-4, and IL-13, promote the NPCs proliferation without affecting neurite outgrowth. Our results provide new additions to our better understanding of human neuronal regeneration.

This work has been supported by the National Research, Development and Innovation Office (OTKA_K 128123)

Keywords: NPC, BV2, microglia activation, co-culture



P-109

Establishment and characterization of DGCR8 knock-out human embryonic stem cells for modelling DiGeorge syndrome

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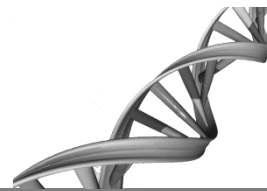
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Human pluripotent stem cells (hPSCs) provide an excellent and indefinite cell source to study early stages of embryonic development, thus can be used for toxicity testing and disease modelling. Our aim is to investigate *in vitro* cellular phenotypes of a complex multi-organ disease, the DiGeorge (DG) syndrome. DG syndrome is the most common micro-deletion syndrome resulting in a broad range of developmental defects including congenital cardiac anomalies, parathyroid and craniofacial manifestations, immune deficiency, as well as neurological disorders. These abnormalities are caused by hemizygous deletions in the 22q11.2 chromosomal region. The affected region contains about 40-50 protein-coding genes. Primary candidate gene in the deleted regions is DGCR8, which is a key player in the canonical microRNA biogenesis. MicroRNAs are small non-coding RNAs, which participate in various cellular functions such as cell cycle regulation, apoptosis, aging, cell fate decisions, and different signaling pathways. Our first goal was to establish a DGCR8 knock-out human embryonic stem cell line (hESC) by the insertion of reporter and resistance genes into the 3rd exon of the gene to create a frameshift-mutation and thereby the formation of downstream premature stop codons. We performed this targeted genetic modification by the CrispR/Cas9 system, and created two hemizygous gene-edited clonal hESC lines. We plan to differentiate these DGCR8 deficient cells towards cardiovascular and neuronal lineages and to examine the formed mature cell types. This genetic modification may cause several changes in cell growth, survival, and differentiation potential of the stem cells, or may result in phenotypic or functional alterations in their progenies (neurons, cardiomyocytes, and endothelial cells). We plan to examine these „*in vitro* anomalies” by immunocytochemistry, Ca-imaging, and patch-clamp technique. Our investigations could contribute to the understanding of the molecular mechanisms underlying the complex symptoms of the DG syndrome. Furthermore, this *in vitro* model system can also be used for drug screening and pharmacological testing purposes.

This study has been funded by the National Brain Research Program of Hungary (NAP 2017-1.2.1-NKP-2017-00002 and KTLA_NAP_13-2014-0011), and by the National Research, Development and Innovation Office (NVKP_16-1-2016-0017 and OTKA- K128369).

Keywords: stem cells, disease modelling, CRISPR/Cas9



P-110

How expression pattern of ABCG2 does change during neural differentiation?

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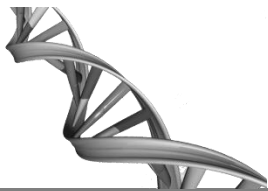
ABCG2 is a member of the G family of the ATP-binding cassette transporters (ABC). It is a multidrug transporter, which protects the cells against endo and xenobiotics, helps to withstand the different kind of cellular stresses and plays an important role to emergence of the multidrug resistance during tumorigenesis [1]. Moreover ABCG2 expression and the related side-population characteristics were shown earlier as a marker for tissue-derived stem cells [2]. In our previous work, we showed that, ABCG2 has a heterogeneous expression pattern in human pluripotent stem cells (hPSC), but during the process of differentiation from hESC to isogenic mesenchymal stem cells (MSC) we observed the significant decrease in the level of ABCG2 [3]. Now we are interested whether ABCG2 is detectable in the hippocampal *Gyrus Dentatus* (GD) neural progenitor cells (NPC). NPCs have fundamental role in regenerative processes in the central nervous system. These cells can differentiate towards different cell types, such as neurons or glia cells, contributing to the repair of the damaged neuronal functions. In our laboratory we are able to generate neuronal progenitor cell types matching GD progenitors using *in vitro* differentiated hPSC cells [4].

Here we present our findings about the expression pattern of the ABCG2 in our hPSC derived NPC lines. It is important to know the ABC transporters expressed by the NPC's in order to understand how drugs and stress conditions affect NPC's and neural regeneration. Answering the questions conceived in this project, we receive new information on human NPC's ABC protein pattern, which can provide important information for setting up disease models or designing drug testing systems.

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P-111

Role of CXCR4-CXCL12 signaling in colonization of developing bursa of Fabricius

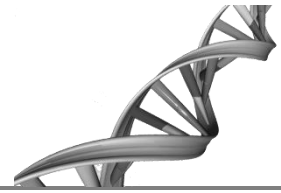
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The bursa of Fabricius is a central lymphoid organ of the birds responsible for the B cell maturation and the antigen specific IgM-IgG switch. In the developing embryo B cell precursors migrate to the epithelio-mesenchymal rudiment of the bursa where they proliferate and diversify their B cell receptor repertoire using gene conversion. Around hatching, these diversified B cells start to emigrate from the cortical region of the bursal lymphoid follicles in order to populate the peripheral lymphoid organs.

Chemokines are the central regulators of directed cell migration. In mammals the presence of CXCR4, a receptor for the chemokine stromal cell-derived factor-1 (SDF1, also named CXCL12) mediates migration of leukocytes and hematopoietic progenitors in response to CXCL12. Recent microarray work reveals different expression levels of CXCR4/CXCL12 signaling in developing avian B cells but very little is known about the regulation of the B cell migratory processes.

The first aim of this work was to analyze the normal expression pattern of CXCR4 and CXCL12 during development of the bursa of Fabricius and for this purpose we have used *in situ* hybridization technique. CXCR4 receptor is first expressed by B cell progenitors in the bursa at embryonic day 10 (E10) and show an increasing expression of the receptor from the B cell immigration until hatch. After hatching CXCR4 expression is slowly downregulating from chB6+ medullary B cells. CXCL12 mRNA expression is detected in the bursa mesenchyme during all stages of B cell colonization: earliest CXCL12 expression was observed at E10 in the subepithelial mesenchyme. As lymphoid cells colonize the surface epithelium and induce follicle bud formation CXCL12 expression decrease from the mesenchyme and becomes more pronounced inside the developing follicles. To determine whether the CXCR4/CXCL12 signaling mediates migration of avian B cell precursors we have investigated the effect of inhibiting endogenous CXCR4 signaling using choriallantoic membrane (CAM) assays. During this experiment E9 old precolonized embryonic bursa of Fabricius was injected with AMD3100 (CXCR4 antagonist) and transplanted to the surface



of age matched GFP-chicken CAM. The effect of AMD3100 treatment was assessed in tissue sections immunostained with B cell, dendritic cell and macrophages markers. Analysis of CAM grafts showed considerable reduction of B cells in follicle buds. In conclusion, our results demonstrate that the CXCR4/CXCL12 pathway represent a significant signal for the migration of the B cell precursors into the bursa primordium and the colonization of the bursal follicles.

Grant NFKI: 124740

P-112

Stem cell-based modelling of cardiac and neuronal phenotypes in DiGeorge syndrome

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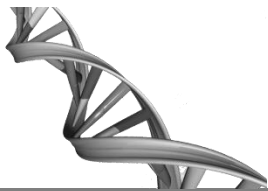
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Human pluripotent stem cells (hPSCs) can be differentiated towards all the three germ layers and can develop into all the cell types of the human body. hPSCs and their differentiated derivatives are new, promising models for studying disease-related phenotypes *in vitro* regarding cell types which cannot be investigated directly or in long-term cultures, or when appropriate animal models are not available. hPSCs can be derived from the inner cell mass of the blastocyst (human embryonic stem cells - hESC), or generated from somatic cells via reprogramming process by forced expression of certain transcription factors (human induced pluripotent stem cells - hiPSC). In our disease model system we use hiPSCs derived from patients and healthy controls to investigate the pathomechanism at cellular level.

Our aim is to study DiGeorge syndrome, a genetically well-determined, multiorgan disease caused by the hemizygous deletion of the 22q11.2 chromosome region. The phenotypic manifestation of the disease is highly variable in terms of affected organs and severity of the symptoms. In our lab we focus on modelling cardiovascular and neuronal symptoms.

We generated iPSCs from peripheral blood mononuclear cells (PBMCs) of members of a family where the disease is present in three generations. The manifestation of the disease differs between family members as grandfather and mother have milder symptoms such as minimal facial dysmorphism and hypocalcaemia, while progeny had severe symptoms



including pulmonary atresia, ventricular and atrial septal defect, hypoparathyroidism and asymmetrical brain ventricles. For control cell line we generated iPSCs from PBMCs of grandmother (not affected by the disease) and we also use a commercially available healthy hiPSC line (XCL1).

Reprogramming of peripheral blood mononuclear cells to pluripotent state was performed by Sendai virus (SeV) transduction. The established hiPSC lines were characterized by mRNA and protein expressions of pluripotency markers and were genetically analysed by comparative genomic hybridization. iPSCs were then differentiated towards cardiac and neuronal lineages to carry out comparative analyses of cellular morphology, viability, transcriptomics, proteomics and functionality.

Supported by the National Brain Research Program of Hungary (NAP 2017-1.2.1-NKP-2017-00002 and KTLA_NAP_13-2014-0011), and by the National Research, Development and Innovation Office (NVKP_16-1-2016-0017 and OTKA- K128369).

P-113

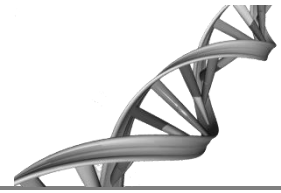
The role of BMP signaling and the embryonic ceca in the enteric nervous system development

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The enteric nervous system (ENS) is principally derived from vagal-level neural crest cells that migrate rostrocaudally along the entire length of the gastrointestinal tract, giving rise to neurons and glial cells in two ganglionated plexuses. Incomplete migration of vagal-derived enteric neural crest cells (ENCCs) leads to Hirschsprung's disease (HD), a congenital disorder characterized by the absence of enteric ganglia along variable lengths of the distal intestine. Preliminary data suggests that the ceca, a paired structure present at the junction of midgut and hindgut in the avian intestine, plays an important role in ENS development, since removal of the ceca leads to incomplete ENCC colonization of the hindgut. Bone morphogenetic protein-4 (BMP4) is highly expressed in the ceca, leading us to hypothesize that cecal BMP4 is critically important for normal ENCC colonization of the developing hindgut.

To study the role of BMP4, we have developed an embryonic intestinal organ culture technique, where we can study the effect of Noggin (BMP specific inhibitor protein) on the developing enteric nervous system. Hindgut dissected from 5 days old chicken embryo, when ENCCs have not yet reached the ceca, was cultured in the presence of Noggin. After 72 hours culture the distal hindgut remained aganglionic, while in the proximal end ectopic ganglia developed. BMP4 inhibitor treatment modified the expression pattern of



extracellular matrix proteins of the intestinal wall. Abnormalities of neural crest cell (NCC) migration and extracellular pattern formation are characteristic of two human intestinal disorders, Hirschprung's disease and intestinal neuronal dysplasia. Our results support an essential role for BMP4 signaling in the ceca in promoting normal ENCC migration and complete formation of a hindgut ENS.

Grant NFKI: 124740

Keywords: *stem cell, neural crest, enteric nervous system, Hirschprung's disease, developmental biology*

P-114

***Zbtb46* mediated suppression of the embryonic stem cell derived myeloid lineage development**

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Myeloid lineage commitment is precisely coordinated by key transcription factors (TFs) during developmental procedures. Understanding the regulatory mechanisms governed by these factors are critical and highly focused topic of today's immunology. Recently we have examined the gene expression profiles of several DC specific transcription factors in embryonic stem cell derived dendritic cells (ES-DCs). The newly identified *Zbtb46* was barely expressed in ES-DCs and progenitors. Based on this result we were encouraged to further examine the role of this factor during DC differentiation. In our chemically inducible ES model system we overexpressed the *Zbtb46* in various stages of ES-DC development to assess the modulatory effect of this factor on DC generation. Unexpectedly, introduction of this factor negatively affected the development of CD45⁺ myeloid precursors. Consistent with this the myeloid specific PU.1 and Irf8 TFs showed an impaired expression profile in *Zbtb46* instructed cells. In addition to this finding it is also revealed that overexpression of *Zbtb46* has a strong suppressive effect on the early stage development of Flk1⁺ lateral plate mesoderm compartment. In contrast to these negative effects introduction of *Zbtb46* resulted an enhanced generation of erythroid progenitor colonies. Our results suggest that overexpression of this factor has a general regulatory effect on hematopoietic cell differentiation, represses the myeloid development and steers the cell fate specification towards to the erythroid commitment.



P-115

A new relationship between miniCORVET and HOPS tethering complexes

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Lysosomal degradation plays an important role in maintaining cellular homeostasis by sequestering and degrading extracellular (endocytosis) and intracellular (autophagy) cargo in eukaryotic cells. Cargo containing vesicles mature into lysosomes which requires several fusion steps. During vesicle fusions tethering factors bind GTP bound Rab proteins and tether target membranes.

Two related multisubunit tethering complexes have been identified in the endocytotic and autophagic pathways in yeast. In general HOPS promotes the fusion of Rab7 positive late endosomes and autophagosomes with the vacuole, whereas CORVET tethers Rab5 positive early endosomes. Both are composed of six subunits, sharing 4 class C subunits. Two Rab-binding complex specific proteins are located on the opposing ends (Vps41 and Vps39 in HOPS, Vps8 and Vps3 in CORVET, respectively). We have recently identified miniCORVET which contains only one Rab5-binding complex specific Vps (Vps8) and three class C proteins in *Drosophila*.

Although they share similar structure and experiments made on yeast suggest that CORVET and HOPS can be transformed into each other via intermediate complexes, their interplay is incompletely understood.

The goal of our research was to examine the relationship of these complexes by overexpressing the Rab binding subunits in different fruit fly tissues. Surprisingly, the overexpression miniCORVET specific Vps8 could block all HOPS dependent processes but the overexpression of the HOPS specific Vps41 did not prevent the function of miniCORVET. Mechanistically, Vps8 overexpression abolishes the late endosomal localization of HOPS-specific Vps41 and decreases the amount of assembled HOPS. Based on these results we suggest that Vps8 can displace Vps41 from the class C core, pointing to a competition between the specific subunits. Our data – challenging the current model of CORVET/HOPS formation – give novel insights into the interplay and functions of these tethering complexes.



P-116

A systems biological analysis of AMPK-mTOR-ULK1 module-controlled autophagy

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Cellular homeostasis is controlled by an evolutionary conserved cellular digestive process, called autophagy. This mechanism is tightly regulated by the two sensor molecules of nutrient conditions, called mTOR and AMPK kinases. The mTOR complex is the master regulator of proteostasis by integrating different external and internal signals, while AMPK also senses cellular energy status and maintains energy homeostasis. AMPK is able to promote the self-eating mechanism by phosphorylating ULK1, the key inducer of autophagosome formation. Besides, AMPK directly inhibits mTOR complex via phosphorylation upon nutrient depletion. mTOR down-regulates the self-eating process under nutrient rich condition by phosphorylating ULK1. We claim that the feedback loops of AMPK-mTOR-ULK1 regulatory triangle guarantee the appropriate response mechanism when nutrient and/or energy supply changes.

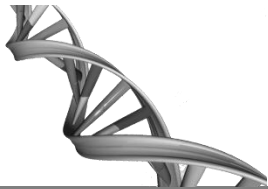
In our opinion, there is an essential double negative feedback loop between mTOR and AMPK kinases. Namely, not only AMPK down-regulates mTOR, but mTOR also inhibits AMPK. We claim that this inhibition is required for keeping AMPK inactive at physiological conditions.

The aim of the present study is to explore the regulatory connection between mTOR and AMPK. We approach our scientific analysis from a systems biological perspective by using both theoretical and molecular biological techniques. For molecular biological experiments HEK293T cell line is used, meanwhile the dynamical characteristic of the regulatory network is described by mathematical modelling.

We claim that, the ULK1-regulated autophagy is always preceded by AMPK activation. AMPK is essential to induce autophagy, but not sufficient to maintain it. AMPK activation is followed by ULK1 induction, and that protein has a key role in keeping autophagy active. Either mTOR hyper-activation (i.e. via TSC1/2 down-regulation) or ULK1 depletion verify that AMPK-mTOR double negative feedback loop is crucial for the proper dynamical features of the control network. Our computer simulations further confirm the dynamical characteristic of AMPK-mTOR-ULK1 regulated cellular nutrient sensing.

Supported by the ÚNKP-18-3-I-SE-19 New National Excellence Program of the Ministry of Human Capacities

Keywords: mTOR, AMPK, ULK1, autophagy, mathematical modelling



P-117

C11.1 protein is a new regulator of actin network rearrangement involved in exocytosis of secretory granules

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Crinophagy is a degradative mechanism of secretory granules in gland tissues. In this process, secretory granules which avoid exocytosis fuse with lysosomes via a HOPS tethering complex dependent manner and their content is degraded. The *Drosophila* salivary gland produces glue protein containing secretory granules at high levels and then crinophagy gets activated before puparium formation. Glue is required for fastening of the pupa to a solid surface and ensuring the stable position of it during metamorphosis. Such glue granules, which do not undergo exocytosis, are degraded by crinophagy. Actin cytoskeleton has a diverse role in the regulation of exocytosis such as cortical actin rearrangement, fusion pore expansion and compression of the vesicle in order to empty the cargo.

We found a new factor, C11.1 protein, in the regulation of secretory granules exocytosed in *Drosophila* salivary gland. In the absence of C11.1, actin positive and Vps26 as well as Rab7 positive vesicles accumulate in the cytoplasm. Later, when puparium formation has happened, glue protein is lacking from the lumen of the gland while in control animals we can detect luminal glue. Moreover, upon loss of C11.1, we found early activation of crinophagy and furthermore, we observed autophagic activity, however, autophagy related (ATG) genes are dispensable for crinophagy and (macro)autophagic activity is low in the salivary gland.

Our results suggest that C11.1 is a new regulator of the secretion of glue granules in *Drosophila* salivary gland and it is required for actin cytoskeleton network rearrangement during secretory granule exocytosis. We found abnormal autophagic activity in the C11.1 deficient salivary gland cells, which has been unknown yet.



P-118

Cardiomyocytes are protected by kynurenic acid against simulated ischemia/reperfusion injury

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Introduction: Kynurenic acid (KYNA) is an endogen tryptophan metabolite, which acts as neuroprotective and immunosuppressor agent, however, its effect on cardiac muscle is barely investigated. In this study, we aimed 1) to investigate the effect of KYNA in simulated ischemia/reperfusion (SI/R) injury on cardiomyocytes and 2) to explore underlying mechanisms of action focusing on the involvement of apoptosis and autophagy.

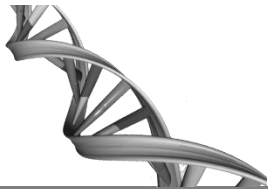
Materials and methods: Primary cardiomyocytes were isolated from neonatal Wistar rats and were treated with KYNA in different concentrations (8-512 μ M) or its vehicle 20 hours before and during the SI/R protocol. Cardiac myocytes were subjected to 4 h simulated ischemia and 2 h reperfusion followed by viability measurement. To explore the effect of KYNA on SI/R-induced cellular processes such as apoptosis and autophagy or well-known cardioprotective signaling pathways including reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) pathways, cardiomyocytes were treated with 128 μ M KYNA and were subjected to SI/R followed by immunostaining of cleaved caspase-3 and determination of protein levels of pro- and cleaved caspase-7, LC3 I/II (microtubules-associated protein 1A/1B light chain 3), mTOR (mammalian target of rapamycin), AMPK (AMP-activated protein kinase), Akt (Protein Kinase B), ERK1/2 (extracellular signal-regulated kinase 1/2) and STAT3 (signal transducer and activator of transcription-3) via Western blot.

Results: We have shown that KYNA treatment dose-dependently protected primary cardiomyocytes from SI/R-induced cell death. KYNA administration significantly attenuated the SI/R-induced increase in caspase-3 and -7 activation. SI/R resulted in increased autophagy, which was diminished by KYNA treatment through reduction of LC3II/I ratio, AMPK phosphorylation and increase in mTOR phosphorylation. In case of RISK or SAFE pathways, KYNA had no effect on phosphorylation level of Akt, ERK1/2 or STAT3.

Conclusion: We conclude that KYNA protects myocardial cells from SI/R injury via antiapoptotic mechanisms and inhibition of autophagy overactivation. The well-known cardioprotective pathways are not involved in KYNA-induced cytoprotection.

Support: GINOP-2.3.2-15-2016-00034; NKFIH K115990; 20391-3/2018/FEKUSTRAT

Keywords: cardiocytoprotection, apoptosis, autophagy, cardiac myocytes



P-119

Connections between genes of PERK pathway upon endoplasmic reticulum stress

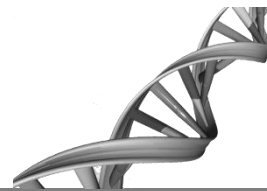
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One of the most important tasks of living organisms is to maintain its intrinsic homeostasis against external stimuli. Keeping the right balance of both secreted and membrane proteins is strictly controlled in the endoplasmic reticulum (ER). Accumulation of incorrectly folded proteins due to different ER stress events leads to the activation of unfolded protein response (UPR). The signalling pathway of UPR has three branches that are regulated by IRE1, PERK and ATF6 transmembrane proteins. The main role of UPR is to avoid cell damage in respond to ER stress and to restore the homeostatic state by self-eating dependent autophagy. Besides, excessive level of ER stress results in apoptotic cell death. PERK induces ATF4 upon ER stress, which transcription factor has two essential targets, called CHOP and Gadd34. While CHOP mainly controls gene transcription involved in apoptosis, the role of Gadd34 seems to be unclear. Our hypothesis is that Gadd34 has a key role in autophagy induction upon ER stress. This immediately arises a question, namely how the two different stress response mechanisms (i.e. autophagy and apoptosis) can be regulated by the same PERK pathway. Using molecular biological techniques our goal is to give a qualitative description about the dynamical behaviour of the control system by exploring the key regulatory motifs.

Using human embryonic kidney (HEK293T) cell line as a model system, we confirmed a strict order between autophagy and apoptosis, controlled by PERK pathway. We studied both on the protein and mRNA level the dynamical characteristic of the crucial elements of PERK pathway (such as ATF4, Gadd34 and CHOP) upon ER stress. We claim that Gadd34 activity correlates to autophagy induction and always precedes the appearance of CHOP. Gadd34 can be activated even at tolerable level of ER stress, while it has only a transient activity peak at excessive level of ER stress. In contrast the protein level of CHOP shows a high increase only when ER stress is non-tolerable for the cell. We also suggest some structures of the regulatory network to describe Gadd34-CHOP-ATF4 controlled ER stress response mechanism of the cell.

Keywords: *endoplasmic reticulum stress, UPR, PERK*



P-120

Deciphering the role of the small GTPase Rab27 in *Drosophila* autophagy

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Rab GTPases are members of Ras superfamily proteins. They are GTP/GDP binding proteins linked to their activation/inactivation cycle, respectively. GTPases have a major role in vesicular trafficking pathways, which are responsible for endocytosis, exocytosis as well as autophagosome-lysosome fusion. Former studies have demonstrated that Rab27 GTPase has a role in breast cancer, melanoma, and other human cancers. Other researchers showed that Rab27b loss-of-function hinders axonal regeneration in different organisms. A previous study performed an RNAi screen for Rab GTPases for their role in autophagosomal processes in human cells, where hRAB27A & hRAB27B were identified as major candidates that altered the number of WIPI2 (human homolog of *Drosophila* Atg18a) punctate structures (phagophores). However, little is known about the role of Rab27 in autophagy. Here, we show that Rab27 RNAi in *Drosophila* alters autophagosome size (Atg8a-positive vesicles), and Rab27 structures colocalize with anti-GABARAP(Atg8a)-positive autophagosomes and to a lesser extent with Atg16-positive structures in starved larval fat cells. Overexpression of a GTP-locked, constitutively-active variant of Rab27 reversed these phenotypes. These data indicate that Rab27 has a putative role in autophagy, and further experiments are being conducted to detail its molecular mechanism of action.

Keywords: *GTPase, autophagy, autophagosome*

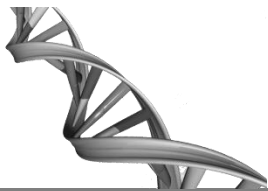
P-121

Developing genetic tools to study the role of autophagy in zebrafish development and tissue homeostasis

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Autophagy is an intracellular self-degradation process of Eukaryotes by which cytoplasmic components are delivered for degradation to lysosomes. A low autophagic flux can be detected in almost every cell and this ensures the clearance of damaged organelles and turnover of long half-life proteins. However, autophagy is not only important to maintain cellular homeostasis, it also has important roles in development, elimination of pathogens



and certain stress conditions where increased autophagic activity helps the cells to adapt to environmental changes.

While during the past few decades autophagy has become a highly popular research topic, there is very little known about this process in zebrafish. The absence of high profile research projects in this field can be explained by the lack of reliable genetic tools that could be used to study the role of autophagy in zebrafish biology. To address this problem, we decided to develop novel transgenic lines that allow us to monitor and interfere with the autophagic process. With the help of these lines we would like to gain better understanding of the role of autophagy in zebrafish development and in certain stress conditions.

To monitor the autophagic process we are generating a transgenic line (Tg(*ubi:mCherry-miniSOG-lc3b*)) carrying two fluorescent proteins translationally fused to the autophagosome marker Lc3b. This reporter line will be unique as it allows for correlative (fluorescent and EM) microscopic observation of the autophagic process. This is achieved combining the activities of the mCherry and miniSOG reporters, respectively.

Most of the research data examining autophagy's role in zebrafish comes from either using small molecular inhibitors or synthetic oligonucleotides. Although these chemicals can serve as valuable tools in deciphering a process, they are also prone to produce aspecific results. To interfere specifically with autophagy and minimize the chances of observing an artefact, we have decided to modulate the expression of the endogenous *atg5* gene. The well-conserved Atg5 acts in a complex of other proteins to elongate the phagophore, which is a crucial step before the autophagosome closure. We believe that by comparing the effects of knocking-down and knocking-out this gene we could gain more insights into the functions of autophagy in zebrafish. Taking advantage of the CRISPR/Cas9 system we have developed a line ubiquitously expressing a guide RNA specific to *atg5*, while the Cas9 protein is under the control of a heat-shock promoter. These fish can be used in knock-down experiments and their offspring will increase our knowledge about the gene's role in early development.

P-122

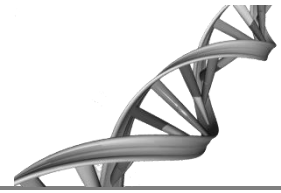
Doxorubicin induces large-scale and distinct H2A and H2B redistribution in live cells

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Anthracyclines are widely used anti-cancer drugs exhibiting pleiotropic effects. At the chromatin level these include topoisomerase inhibition, DNA intercalation, aggregation of chromatin, histone eviction as well as direct binding to histones.

Using Confocal Microscopy (CLSM), we observed marked differences between the effect of doxorubicin (Dox), applied in a concentration range between 0.6-36 μ M, on the intracellular distribution of H2A vs. H2B in Jurkat cells. Aggregation was assessed by Laser



Scanning Cytometry (LSC), via the retention and consequential accumulation of histones in permeabilized nuclei of cells showing no signs of apoptosis.

Aggregation was observed only in the case of H2A, while the dominant effect of the anthracyclin on H2B was the massive accumulation of the histone in the cytoplasm concomitant with its disappearance from the nuclei. The latter phenomenon was not affected by inhibitors of protein and RNA synthesis, by Tanespimycin, an hsp70 inhibitor, and by leptomycin B, a nuclear export inhibitor. On the other hand, cytoplasmic accumulation was completely diminished by PYR41, an inhibitor of ubiquitylation, when H2B showed a H2A-like aggregated pattern in the nuclei. The cytoplasmic accumulation of H2B was confirmed also by mass spectrometric identification of elevated levels of H2B following Dox treatment in the dechromatinized samples.

The above large-scale effects were detected already at Dox concentrations that overlap serum peak levels reached in the typical clinical setting and therefore can be a factor both in the anticarcinogenic mechanism and in the side-effects of this anthracyclin.

Support: *This work has been supported by OTKA K128770 and GINOP-2.3.2-15-2016-00044.*

P-123

EDTP and MTMR14 lipid phosphatases promote brain aging by progressively downregulating autophagy throughout adulthood

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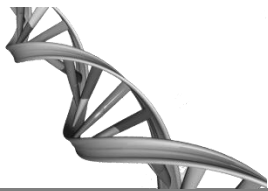
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Understanding why neurons become increasingly sensitive to demise at advanced ages remains a fundamental problem in neurobiology, with significant medical implications. Aging is driven by the progressive accumulation of molecular damage interfering with cellular homeostasis. Such damages mainly include misfolded, oxidized and aggregated proteins, and can predominantly be degraded by autophagy, a major catabolic process of eukaryotic cells. Many neurodegenerative diseases are characterized by the formation of protein aggregates in neurons, which can lead to different neurological dysfunction. Therefore, studying autophagy is of great importance from a biomedical point of view as well.



In *Drosophila*, the myotubularin-related lipid phosphatase EDTP, the sole fly ortholog of human MTMR14/Jumpy, is known to downregulate the autophagic process by antagonizing phosphatidylinositol-3 kinase, which catalyzes the formation of autophagic membranes. This inhibitory step helps the cell to maintain autophagic activity at an optimal level. However, it is well known that the capacity of autophagy declines during aging, resulting in the progressive accumulation of toxic protein aggregates. Using a Western blot analysis, semi-qPCR and a trojan gene trap system, we show that EDTP gradually accumulates in brain neurons during the adult life span, thereby establishing an age-associated gradual decline in the capacity of autophagy in these cells. Consistently, genetic inhibition of EDTP delays the development of age-related neuropathological symptoms. Inhibition of EDTP did not just improve climbing ability but also resulted in lifespan extension in treated animals.

The orthologous human protein MTMR14 displays a similar tendency to accumulate during aging. These results reveal that after a certain life period EDTP and MTMR14 turn into endogenously toxic proteins promoting brain aging. Thus, age-related brain deterioration is a genetically determined process.

Keywords: *brain aging, autophagy, lipid phosphatase, MTMR14/Jumpy, EDTP, Drosophila, dog, protein accumulation*

P-124

FRAP analysis of the nuclear import of the cytoskeletal moesin protein

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The moesin protein of *Drosophila melanogaster* belongs to the family of evolutionary conserved actin-binding ERM (Ezrin-Radixin-Moesin) proteins, and it is the only family member in the fruit fly. Similarly to vertebrate ERM proteins, moesin's well-known function in the cytoplasm is to anchor the actin cytoskeleton to plasma membrane proteins. It was shown recently in our laboratory that moesin localizes also to the interphase nucleus where the amount of the protein greatly increases under certain conditions. The molecular mechanisms through which moesin enters and exits the nucleus are not known therefore, it is a reasonable assumption that the nuclear transport of moesin is tightly linked to the dynamic nuclear shuttling of its primary binding partner, actin.

To gain deeper insight into the mechanism of nuclear moesin's transport we conducted Fluorescent Recovery After Photobleaching (FRAP) assays on *Drosophila* S2R+ cultured cells expressing fluorescently labeled moesin protein. In these experiments confocal microscopy was used to bleach the entire nucleus and then to monitor the fluorescence recovery from the cytoplasm, which process reflects the nuclear import activity of moesin.



The import mechanism of actin was analyzed by the same method earlier, therefore we used GFP-Actin as positive control.

After the initial FRAP assays, we examined the nuclear import of moesin under different conditions, such as inhibiting the nuclear mRNA export which causes the accumulation of the protein in the nucleus, or applying Jasplakinolide drug treatment which reduces the pool of free monomeric actin. We also investigated the nuclear import of moesin using the inactive and the constitutively active mutant forms of the protein.

Our experiments revealed that in contrast to actin, the actin-binding moesin protein does not constantly shuttle between the cytoplasm and the nucleus, consequently the cytoplasmic and nuclear moesin pools are not connected dynamically. Our results surprisingly also suggest that the nuclear import of moesin is independent from actin.

Keywords: *moesin, nuclear import, FRAP, confocal microscopy, Drosophila*

P-125

Investigation of sorting nexin functions in *Drosophila* tissues

Dalma Feil-Börcsök¹, Tamás Maruzs¹, Enikő Lakatos¹ and Gábor Juhász^{1,2}

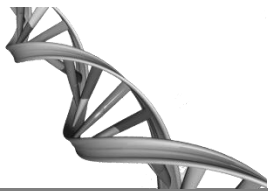
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Transmembrane proteins play essential roles in various functions of the eukaryotic cells including signaling, secretion, vesicle trafficking and fusion. Beside their de novo synthesis, recycling of these molecules contribute to the maintenance of a pool of functional proteins in the interconnected network of the endolysosomal system.

Several protein complexes have been identified that are responsible for trafficking of transmembrane proteins either towards the lysosome for degradation or back to their original localization via recycling. In the recycling pathway, the endosomal retromer complex have a well-established role in directing a wide variety of transmembrane proteins away from the degradation route and recently, the structurally and functionally similar retriever complex has been identified in mammalian cells. Both complexes interact with members of the sorting nexin (Snx) protein family that are characterized by the presence of a PX-domain that mainly binds to phosphatidylinositol-3-phosphate, a lipid enriched in the membrane of early endosomes and autophagosomes.

The mammalian sorting nexin family contains more than 30 proteins and subgroups of the family display different domain structures that enable the different Snx proteins to interact with a variety of binding partners. However, many Snx proteins are poorly characterized, presumably because of the potentially overlapping functions of the individual proteins belonging to the same sorting nexin subgroup. Showing a lower level of genetic redundancy, the fruitfly *Drosophila melanogaster* is an ideal model to study the functions of such protein families and indeed, only 10 snx genes have been identified in flies.



Using fluorescent microscopy and various fly tissues, we aim to explore the functions of sorting nexins in flies and their relations to retromer and retriever complexes. Our results show that Snx3 and Snx6 have retromer-dependent functions in autophagy through the retrograde trafficking of lysosomal enzyme receptors in the hepatocyte-like larval fat body cells while Snx21 and Snx25 are required to maintain a fully functional endolysosomal system in the highly endocytic garland nephrocytes. Ongoing investigation of the tissue-specificity and subcellular localization of the fly sorting nexins will enable us to understand their relations to retromer and retriever complexes and their functions in the trafficking of particular transmembrane proteins.

Keywords: *Drosophila*, *Sorting nexin*, *vesicular transport*

P-126

Investigation of the interaction of two endosomal tethering factors, Rabenosyn-5 and Vps18

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* These authors contributed equally to this work

Rabenosyn-5 (Rbsn-5) is an effector protein of Rab5, which plays an important role in the early endosomal system. Earlier our research group indentified Vps18 as Rbsn-5 binding protein in *Drosophila*. Vps18 is a subunit of two major tethering complexes CORVET and HOPS. CORVET complex plays a key role in the early endosomal fusion mechanism.

Our goal to investigate the interaction between human Rbsn-5 and Vps18.

During our research we successfully verified the interaction between human Rbsn-5 and Vps18 in a yeast two-hybrid system. Then we created truncated proteins to identify the regions, that are responsible for the actual binding: we found that the N-terminal region (1-421 aa) in the case of Rbsn-5 and the region between 482-854 amino acid in the case of Vps18 are responsible for the binding. We used co-immunoprecipitation to verify the interaction between the whole proteins: we transiently transfect HEK293 cells with hemagglutinin (HA) and FLAG tagged proteins. Then we precipitated the proteins with anti-HA and anti-FLAG agarose and the samples were examined with western blot. The interaction between our proteins was shown successfully in both cases. We verified the co-localization of the proteins with immunocytochemistry as well. After this we compared the expression level and the localization of Rbsn-5 in the case of the wild type and the Vps18 null-mutant HEK293 cells using western blot and epifluorescens microscopy. Based on



these we can determine that the absence of Vps18 do not disturb the level and distribution of Rbsn-5.

During our work we verified the interaction between human Rbsn-5 and Vps18 in yeast two-hybrid system and in human cell culture as well. Moreover we proved that Vps18 does not influence Rbsn-5 expression and localization.

In our further studies we would like to specify the exact binding locations with site directed mutagenesis, to establish new mutant cell lines with CRISPR/Cas9 (regarding Rbsn-5) and to investigate the viability and the adhesion activity of the mutant cells.

This work was supported by BO/00652/17, PD124594 and ÚNKP-18-4-ELTE-409 for ZS-V and LP-2014/2 and K119842 for GJ.

Keywords: *endosomes, tethering factor, protein-protein interaction*

P-127

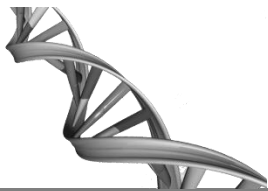
Links between DNA repair proteins and autophagy regulation

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Numerous endogenous and exogenous agents can cause DNA damage, which may lead to deleterious consequences. The response to DNA damage is the activation of DNA damage sensing proteins and DNA repair mechanisms. DNA repair mechanisms and DNA damage tolerance are required for the correction of DNA damage occurring during normal cellular life or due to physical or chemical agents. Autophagy is responsible for the breakdown and recycling of damaged cellular components. There are evidences that the two processes are connected, and they can regulate each other. In the scope of our work we attempted to evaluate the role of selected DNA repair genes in the regulation of autophagy.

We examined changes in DNA Double Stranded Breaks (DSB) and general autophagic markers (changes in hp62 and hLC3B protein levels) during different treatments (autophagy induction or block) in HEK293 human cell lines. We used stable transformant lines silenced for each of 11 different DNA repair genes (BRCA1, BRCA2, SPARTAN, FAN1, ZBTB1, RAD18, PAR1, XPA, RAD52, FANCA, FANCD2). We performed 1h starvation (induction of autophagy), Bafilomycin A1 (100 nM) and Chloroquine (100 μ M) (both blocking autophagy) treatments. We used anti-phospho-H2AX (Mouse monoclonal 1:2000), anti-hp62 (Rabbit polyclonal 1:1000), and anti-hLC3B (Rabbit polyclonal 1:1000) antibodies to detect DSBs and autophagic activity, respectively, for fluorescent western blot analysis followed by quantification with Li-Cor ODYSSEY Blot Imager and Image Studio 5.2 analysis software.



Although BRCA2 and BRCA1 are in a common pathway we detected opposite changes in the case of LC3-II and gamma-phospho-H2AX levels during autophagy induction or block. Interestingly, p62 levels strongly decreased in SPARTAN shRNA lines.

The age of the cell cultures strongly influenced the autophagic flux and the efficiency of the DNA repair mechanisms. There were several silenced lines (BRCA1, BRCA2, RAD18 and SPARTAN) which exhibited unique changes in the examined parameters during different treatments.

We plan the examination of DSBs and autophagic markers in selected single and double silenced cell lines after the above mentioned treatments + 2h starvation. Also we are going to check TOR kinase (the main inhibitor of autophagy) activity of single and double silenced cell lines after Bafilomycin A1, Chloroquine treatments, 1h and 2h starvation.

Keywords: *autophagy regulation, DNA repair proteins, HEK 293*

P-128

Lipids of autophagic membranes and their functional roles

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Autophagy is a „self-eating” process of the eukaryotic cells, during which damaged or obsolete cytoplasmic components are removed and degraded in lysosomes. The breakdown products are then recycled for use in biosynthesis or energy production, which ensures the homeostasis of the cells. Misregulation of autophagy leads to neurodegenerative diseases, cancer, accelerated aging, etc. This interesting catabolic process relies on the biogenesis of unique membranes and membrane-bound autophagic vesicles. Lipids and proteins are the main constituents of these special membranes: they maintain membrane structure and function. Autophagy research has mainly focused on autophagy related proteins and very little is known about the lipids composing autophagic membranes and about their roles in autophagy, especially lipid-protein interactions during autophagic processes. Therefore the main aim of this project is to study the regulatory roles of lipids during autophagy in animals. We used *Drosophila* as model organism to study the lipid composition of autophagic membranes. For lipid-protein interaction studies we applied a combination of biochemical, biophysical, molecular and cell biology methods, focusing on how lipids influence Syntaxin 17 protein loading to autophagosomes, which is necessary to mediate fusion with lysosomes.



The results obtained by this multidisciplinary approach will help understanding the lipid constituents of autophagic membranes and their functional relevance.

Keywords: *autophagic membranes, lipids, Syntaxin 17*

P-129

Loss of Vps18 affect autophagy and cell adhesion of mammalian cells

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Eukaryotic cells have to maintain constant flux of proteins and lipids between different organelles, which is sustained by transport vesicles formed as a donor membrane and fuse with the acceptor membrane. This latter process is supported by conserved fusion machinery that consists of Rab-GTPases, their interacting effectors -such as tethering factors- and SNARE proteins. Vps18 is a central subunit of CORVET and HOPS tethering complexes, which mediate the fusion of early and late endosomes, respectively; moreover, HOPS mediates autophagosome-lysosome fusion as well. These hexamers are composed of shared class-C subunits such as Vps11, Vps16, Vps18 and Vps33 and additional subunits: Vps3 and Vps8, which are CORVET-, and Vps39 and Vps41 that are HOPS-specific subunits.

We aimed to establish a Vps18 KO HEK293 cell line using CRISPR/Cas9 system and to investigate the role of this protein regarding autophagy, cell adhesion and structure.

We generated nineteen potential KO cell line with the method published by Tálas et al.¹. We verify the targeted cleavage of Cas9 in the coding region of Vps18 by PCR and sequencing, and the absence of the Vps18 protein by Western blot and immunocytochemistry. We found six cell lines that match the criteria for Vps18 deficiency and we used two for further analysis.

First we investigated the effect of the loss of Vps18 on autophagic markers, such as lipidation of LC3 and the alteration of p62 level. We found that the levels of lipidated form of LC3 (LC3 II) are elevated in the mutants, in the chloroquine-treated and in the starved HEK293 cells as well, compared to the control, non-starved HEK cells. We could observe a slight accumulation of p62 in the Vps18 KO cells, such as in chloroquine treated cells due to the improper fusion of autophagosomes and lysosomes.

Next we investigated the adhesion capacity of the mutants: we counted the number of attached cells on coverslip coated with poly-L-lysine in given time points -6, 12, 24 and 48 hour after passage². We found that significantly less mutant cells could attach in all



investigated time point, than the wild types. Based on earlier findings³ Vps18 can interact with actin, which can affect cell motility and attachment. To reveal if Vps18 has an effect on actin organization, we examined the cytoskeleton with phalloidin staining. We observed that the actin network of Vps18 deficient cells is altered compared to the wild type.

In our work we established new Vps18 KO cell lines, and we showed that the loss of Vps18 disturbed autophagosome-lysosome fusion, and affect the adhesion capacity and the actin network organization of HEK293 cells. We plan to investigate the expression level and distribution of cell adhesion molecules -such as integrins- in Vps18 deficient cells and to examine the motility of these cells.

This work was supported by BO/00652/17, PD124594 and ÚNKP-18-4-ELTE-409 for ZS-V and LP-2014/2 and K119842 for GJ.

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P-130

Rab3GAP-Rab18 module promotes autolysosome maturation through Atg14-PI3K complex

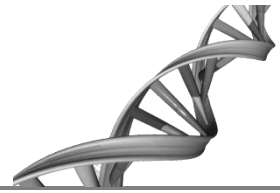
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Autosomal recessive mutations of the RAB18, RAB3GAP1 and RAB3GAP2 genes are associated with Warburg Micro Syndrome, a hereditary neuromuscular disorder. The genetic background of the disease is well known, however our current knowledge of the exact pathomechanism is still limited. The Rab3GAP complex, consisting of Rab3GAP1 and 2, serves as a guanosine exchange factor, therefore as activator for Rab18. In our study we found that loss of the Rab3GAP-Rab18 module leads to perturbed autolysosome maturation and morphology and impaired autophagic degradation. Despite the absence of the Rab3GAP-Rab18 module, cells were able to maintain the early but not late endosomal compartments and exhibited partial failure in the delivery of the lysosomal hydrolase Cathepsin L. We also demonstrate that Rab18 interacts with the Atg6 subunit of the Atg14 containing PI3K complex and loss of Atg14 results in similar phenotype to Rab3GAP-Rab18 module lacking cells. Finally, we show that Rab3GAP mutant *Drosophila* is useful as a model for Warburg Micro Syndrome, as aged flies' climbing abilities were compromised



and this defect is likely due to impaired autophagy in the thoracic muscles. Our study suggests that defect in autolysosomal maturation and autophagic degradation in muscle cells may contribute to the onset of Warburg Micro Syndrome.

Keywords: *Rab18, Rab3GAP, Warburg Micro Syndrome, autophagy*

P-131

The role of the autophagy machinery in glial engulfment of axon debris

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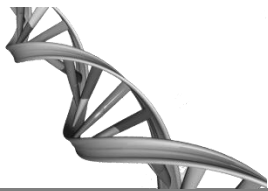
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Glia are non-neuronal cells in the nervous system. They take part in the fate determination, differentiation and survival of neurons, form myelin sheaths and protect neurons from injuries. If neurons get injured, it triggers reactive gliosis whereby morphological and gene expression change happens at the injured site and it results in clearance of the dying neuronal debris by the phagocytic activity of glia and macrophages both in *Drosophila* and in mammals. *Drosophila* ensheathing glia have been known to participate in the clearance of axon debris generated after nerve injury. In neurodegenerative disease such as Alzheimer's disease, microglia and astrocytes play important roles having both beneficial and detrimental effects on affected neurons by plaque and apoptotic cell removal and excessive synaptic pruning, respectively. Autophagy (more specifically macroautophagy) is a cellular process that protects the cell from the intracellular accumulation of non-essential materials and regulatory proteins by involving a serial membrane-limited degradation pathway regulated by conserved Atg proteins.

In order to shine a light on new regulators of the glial engulfment pathway, we have systematically tested the functional importance of different autophagy genes (*Atg*-s) in an engulfment assay using the injured *Drosophila* wing nerve in diverse *Atg* mutant backgrounds. Our data suggest that canonical autophagy may be essential for glial clearance of axon debris. Glial knockdown of the corresponding *Atg* transcripts with RNA interference (RNAi) lead to similar engulfment defects hinting to a role for glial autophagy in this process. Next, we have investigated the role of Ref(2)P, the *Drosophila* orthologue of mammalian p62, an autophagy adaptor participating in selective autophagy using a domain-specific mutant and glially delivered RNAi-s. Our results reveal selective autophagy of ubiquitylated proteins or organelles at the heart of the engulfment pathway. In follow-up studies, we aim to pin down the responsible glial subtype and potential autophagy cargoes that mediate this effect on glial phagocytosis.



These findings are likely to open a new window on the autophagic modulation of glial engulfment functions and may be beneficial in addressing the mechanistic details in several pathologies such as traumatic injury-related neuronal toxicity and neurodegeneration caused by protein aggregates as well as ageing-related decline in neuronal and glial function.

Keywords: *glia, autophagy, neurodegeneration, Drosophila*

P-132

The role of the SNARE protein Ykt6 in crinophagy

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The Ykt6 is a common SNARE protein in eukaryotic cells which has an important role in several intracellular vesicle fusion events. Its contribution is proven in ER-Golgi trafficking among others, and based on the earlier results of our laboratory, also in autophagy. The goal of the research was to determine if this protein plays a role in crinophagy, a special type of autophagy.

Crinophagy is a lysosomal self-degrading pathway, characteristic of cells carrying out secretion. During crinophagy the old or redundant secretory granules degrade through fusing with lysosomes, thereby it takes part in the maintenance of the homeostasis of the glandular cells. Its abnormal operation can contribute to the development of type 2 diabetes and acute pancreatitis.

According to a model set up for macroautophagy, Ykt6 is necessary for the fusion of lysosomes and autophagosomes. It is not part of the final SNARE complex, but it takes part in the recruitment of the factors included in the process. During my work I tested if this model can be applied to crinophagy.

The *in vivo* experiments were performed on the salivary gland of *Drosophila* larvae with fluorescent microscopy. I determined, with the application of special transgenic fly stocks, that the loss of Ykt6 causes fusion defect in crinophagy. Preliminary immunocytochemical studies suggest that Ykt6 localises to lysosomes. Also, I cleared up the interactions between the participator SNARE proteins in crinophagy with the help of *in vitro* interaction essay. My study suggests that Ykt6 plays a crucial role in the fusion of secretory granules and lysosomes in *Drosophila* salivary gland cells during crinophagy.



P-133

Two myotubularin phosphatase, EDTP and Mtmr6, differentially regulate autophagy in *Drosophila melanogaster*

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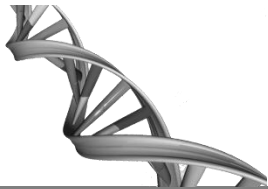
* These authors contributed equally to this work

Autophagy is a highly-conserved lysosomal dependent degradation pathway which is required to the maintenance of cellular homeostasis. During this process, superfluous and harmful cytoplasmic constituents are sequestered by a double membrane structure called autophagic isolation membrane. After the closure of the isolation membrane the generated structure termed autophagosome fuses with lysosomes and the inner content of the formed autolysosome is degraded by lysosomal enzymes. Although many components and the process of autophagy are well characterised, its regulation remains still poorly unresolved.

Different stages of autophagy require various lipid-signalisation molecules like phosphatidylinositol 3-phosphate (PtdIns3P) and phosphatidylinositol 3,5-bisphosphate (PtdIns3,5P₂) which act at the initial step and the late stage of the process, respectively. Myotubularin (MTM) and myotubularin-related (MTMR) phosphatases remove the D3-positioned phosphate group from these phosphatidylinositol derivatives thereby producing phosphatidylinositol (PtdIns) and phosphatidylinositol 5-phosphate (PtdIns5P), respectively. These enzymatic activities implicate that MTMRs are involved in autophagy control.

Here we examined two active *Drosophila* MTMRs, EDTP/MTMR14 and CG3530/MTMR6, -7, -8, and found that these proteins affect autophagy differently under normal and amino acid-depleted conditions. EDTP inhibited basal autophagy while it did not influence starvation-induced autophagy. In contrast, CG3530, which we termed Mtmr6, promoted the autophagic breakdown under normal (physiological) conditions but markedly inhibited the process under starvation-induced stress conditions. These findings may contribute to understanding better the regulation of autophagy under physiological and pathological circumstances.

Keywords: *autophagy, EDTP, Mtmr6, myotubularins, phosphoinositides*



P-134

Ykt6 acts as a non-canonical SNARE in autophagosome-lysosome fusion

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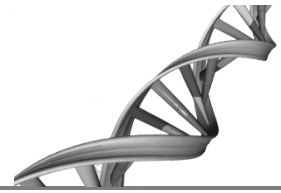
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Autophagy is a highly conserved eukaryotic lysosomal degradation pathway, which is responsible for the removal of the damaged or superfluous cellular constituents. During this process, the phagophores envelop the cargo, seal to double-membrane autophagosomes, which ultimately fuse with acidic lysosomes. In the formed autolysosomes the uptaken materials get degraded and the released monomers can be recycled.

For eukaryotic membrane fusion events, the consecutive and coordinated action of Rab small GTPases, tethering factors and SNARE proteins are required. During the fusion reaction the SNARE proteins in opposing membranes form a trans-SNARE complex, which promotes the distortion of membranes and drive them towards the formation of a fusion pore, the most critical step of the fusion reaction. The trans-SNARE complex — responsible for autophagosome-lysosome fusion — is composed of Syntaxin 17, Snap29 and VAMP7. We found that the presence of Ykt6, an other SNARE protein, is also necessary for the proper autophagosome clearance in *Drosophila*. We revealed, that Ykt6 localizes to lysosomes and autolysosomes and forms a classical SNARE complex with Syntaxin 17 and Snap29. Interestingly, Vamp7 can outcompete Ykt6 from this forming complex and the overexpression of Vamp7 can fully restore the fusion defect of ykt6 loss of function cells. We also unveiled that both Vamp7 and Ykt6 N-terminal domains are able to interact with HOPS tethering complex.

These data suggest that Vamp7 is directly involved in autophagosome-lysosome fusion, while Ykt6 acts rather as a non-canonical, regulatory SNARE in this process through the early recruitment of the fusion factors to the lysosomal fusion sites.



P-135

Analyzing the effect of splicing factor mutations with information theoretic methods

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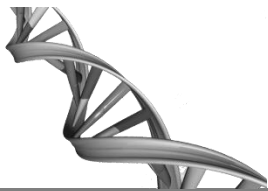
RNA splicing factor mutations are frequent in different cancer types, including myelodysplastic syndrome (MDS) patients. However, it is unclear how these mutations contribute to the molecular pathomechanism of MDS and how they lead to cancer. The alternative splicing events correlated with splicing factor mutations seem to be minor and highly variable between patients. It is hard to detect specific splicing changes, which occur in known oncogenes and tumor suppressors.

We hypothesize that splicing factor mutations break down the coordinated post-transcriptional regulation in a cell. Therefore, the relative ratio of the expressed transcript isoforms becomes random, leading to accidental changes in splicing. Finally, these changes cause large random fluctuations in gene regulatory networks, leading to aberrant cellular phenotypes and cancer. In order to characterize the randomness of transcript isoform expression, we are developing a novel statistical method, based on Shannon entropy. This method will quantify transcriptome noise in different conditions. Transcriptome noise might be driven by splicing factor mutations, characteristic of MDS and other hematological conditions, like chronic myelomonocytic leukemia (CMML) or acute lymphoid leukemia (AML). Frequently mutated splicing factors in MDS, CMML and AML include SF3B1, U2AF1, SRSF2 and ZRSR2, components of the core spliceosome complex.

We are developing our tool based on the general form of Shannon entropy, regularly used in information technology. Using this measurement, we are planning to do a genome-wide analysis of splicing randomness in different conditions, using publicly available data. Currently, we are analyzing transcriptome data from hematological cancer patients with a read mapping-free technique and state-of-the-art bioinformatics software. Our tool will be available as an R package in Bioconductor.

In the future, we will extend the number and type of available methods to characterize splicing randomness, or calculate the significance of detected differences between conditions. Our results will lead to a more accurate understanding of the molecular mechanisms of MDS and cancer in general, besides the development of novel targeted therapies.

Keywords: cancer, splicing noise, transcriptomics, Shannon entropy



P-136

Analyzing the prognostic value of immunomodulatory genes in multiple types of solid tumors

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Introduction: Over the past decade, cell surface signaling molecules have emerged as crucial targets for the treatment of cancer and immune disorders. Several therapeutic agonistic monoclonal antibodies targeting tumors expressing death domain-containing TNFRs (e.g. GITR, HVEM, DR3) induce cancer cell destruction and are currently in clinical trials. Here, our aim was to examine the prognostic effect of the established immunomodulatory genes in a large cohort of distinct cancer types.

Methods: The raw htseq counts of RNA-seq and survival data of 26 different tumor types were acquired from the TCGA (The Cancer Genom Atlas) repository. The “DESeq” package was used to normalize the read counts data. The “AnnotationDbi” package was applied to annotate Ensembl transcript IDs with gene symbols. Correlation between gene expression and overall survival (OS) was computed using Cox proportional hazards regression and by plotting Kaplan-Meier survival plots. We applied the “survival” R package for Cox regression analysis and “survplot” R package to generate Kaplan-Meier plots. Finally, corrected the p-values for multiply hypothesis testing. Data processing and statistical computations were performed in the R v3.2.3 statistical environment.

Results: We performed survival analysis using the established immune modulatory genes (n=17) across the 26 cancer types. Among these genes, GITR (TNFRSF18) was the best performing gene. We obtained significant association between the GITR expression and OS in glioblastoma multiforme (HR=0.52; p=5.7E-04), head and neck squamous cell carcinoma (HR=1.6; p=6.9E-04), kidney renal clear cell carcinoma (HR=0.41; p=1.3E-07), skin cutaneous melanoma (HR=2.0; p=3.2E-06), stomach adenocarcinoma (HR=1.9; p=8.3E-04) and uterine corpus endometrial carcinoma (HR=2.4; p=1.8E-05). The second and third best performing genes were HVEM (n=5 tumors at p<0.01) and CD27 (n=4 tumors at p<0.01). Of the 13 immunomodulatory genes in clinical investigation, ten was significant in at least one tumor type.

Discussion: We evaluated immunomodulatory genes in 9,742 cancer patient samples. Expression change of GITR, HVEM and CD27 genes was associated with poor survival outcome in multiple solid tumors. Our results help to prioritize targeted immunomodulatory therapies in different types of solid tumors.



P-137

COMPARE1K+, a bioinformatic pipeline and an R package to analyse the evolution of thousands of genomes

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Ongoing large-scale genome sequencing projects, such as the 1000 Fungal Genomes Project or 10k Plant Genomes Project generate a big demand for analytical tools capable of processing hundreds to thousands of genomes. COMPARE1K+ pipeline uses a genome-wide collection of protein families and a species phylogeny to resolve gene duplication and loss events in each of the nodes of the species phylogeny, resulting in a gene family evolution database which can be further processed with a newly created R package, CompareTools. This allows testing hypotheses on the tempo and mode of genome evolution through time, the composition of ancestral genomes as well as the genomic background of a phenotypic trait of interest.

To achieve a massive scale-up, we set out to improve both the sensitivity and specificity of the pipeline adapting it to a supercomputer environment. We tested alternative measures of protein similarity, (BLASTP, DELTA-BLAST), protein family reconstruction techniques (e.g. Markov clustering) and several phylogenetic tools (RAxML, FastTree, etc.). In addition, a set of tree reconciliation software (e.g. Treefix, Notung) was tested to resolve disagreements between gene trees and the species tree. Manually curated protein families in the Carbohydrate Active Enzymes Database (CAZy) and the Hierarchical Catalog of Orthologs (OrthoDB) were used as reference sets for pipeline benchmarking and optimization.

To facilitate the interpretation of the evolutionary inferences we constructed an R package called CompareTools. This package contains functions which can efficiently handle gene duplication and loss data. The package can be used to visually interpret the data by plotting a species tree annotated with gene duplication/loss events. Furthermore, to get a global view of the evolutionary history of thousands of species a function is being developed that plots pairwise similarity in the evolutionary histories of species as a heatmap. With the help of CompareTools users can perform statistical tests, detect gene families with similar evolutionary histories and construct an output which can be easily handled by downstream functional annotation pipelines.

We anticipate that the COMPARE1K+ pipeline together with the CompareTools R package will greatly facilitate the analysis of the vast genomic data generated by ongoing large-scale genome sequencing projects and will allow the research community to test broad evolutionary hypotheses in a rigorous phylogenetic framework across the tree of life.



P-138

Comprehensive analysis of signal peptide prediction algorithms

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The majority of secreted and membrane proteins enter the secretory pathway due to the presence of hydrophobic signal sequences that translocate nascent peptides into the ER.

Secreted and membrane proteins are one of the most important instruments of communication between tissues and cells. Albeit, the availability of open access tools for signal peptide prediction and the complete human genome sequence, annotation with respect to protein compartmentalization is still ambiguous or non-existent for a large portion of the proteins.

The precise determination of protein localization (i.e. secreted, membrane) is important for the selection of candidate proteins from global proteome and transcriptome profiling experiments because secreted protein systems are often drug targetable and may serve as biomarkers. The *in silico* tools for signal peptide prediction algorithms were developed during pre-genomic times, and today sufficient biological evidence has evolved for unambiguous determination of protein localization for a large number of proteins.

Here, we present a comprehensive performance analysis of multiple signal peptide prediction tools (i.e.: SignalP, SecretomeP) using the peptide sequence of 200 secreted and 200 non secreted proteins for which sufficient biological data was available to accept their designation as “correct”. We used the selected peptide sequences as queries and recorded various parameters of signal peptide predictions; the dataset was then used to develop a weighted table for improved signal peptide prediction. The approach is being validated via biological experiments.

Keywords: *secreted proteins, prediction analysis, bioinformatics*



P-139

Effect of dietary fructooligosaccharide (FOS), carotenoids, anthocyanins and probiotics supplementation on the intestinal microbiome in broiler chickens

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Introduction: The poultry industry during the past two decades have been one of the most efficient animal productive system, and form the basis of global protein production in the world. Modern chickens require increasingly less feed to achieve their desired weight, for example broiler chickens that were selected for meat production gained a higher body weight (~ 3 kg) within 42 days. However, such intensive, extreme growth rate may have inadvertently resulted in changes in gastro-intestinal development during growth of the animal. Some other negative effects may also eventuate at times, like metabolic disorders, poor immunocompetence and increased susceptibility to pathogens. We started to develop feed additives, which are highly rich in different bioactive components, like FOS, carotenoids, anthocyanins, and probiotics. These bioactive components could reduce the stress and may have an immunomodulatory effect on broiler chickens. Furthermore, they are produced from food industries waste, so they are cheap and available in a large amount.

Objective: The following study was conducted to investigate the effect of dietary supplementation of FOS, carotenoids, anthocyanins, and probiotics on the intestinal microbial composition of broiler chickens.

Methods: The experiment lasted for 40 days. One-day-old Ross 308 broiler chicks, were divided into six equal groups, four experimental groups, one internal group (β -glucan supplementations) and one control group. Stool samples were collected on 7, 19, 31, 40 day of age to determine bacterial population. DNA was isolated using phenol/chloroform/isoamyl alcohol protocol. 96 DNA samples were utilized to construct the 16S rDNA metagenome sequencing on Illumina Miseq platform based on V4 and V3 regions for microbiome analyses. Paired end reads were demultiplexed by the software of the Illumina sequencing machine. The reads were imported into the Qiime2 pipeline according to the „Atacama Soil microbiome” tutorial. Alpha diversity differences were calculated using the Kruskal-Wallis test. Beta diversity differences were calculated by using the PERMANOVA pseudo-F test. Taxonomic identification was performed and we determined the Gram positive and negative ratio of our samples.

Results and conclusion: Faith's phylogenetic diversity significantly increased with chicken development in both the treated and control groups ($P < 0.005$). No significant difference



was observed in Chao-1 index. Shannon diversity index was significantly higher in the treated groups ($P < 0.005$) compare with the control group, indicating that dietary supplementation had effect on bacterial diversity. Unweighted and weighted unifrac also showed some but not total separation of the sample of each treated groups. In addition, there were significant differences of *Faecalibacterium prausnitzii* (which is commonly associated with gut health) in treated groups ($P < 0.005$). The result demonstrated that the microbial composition in the gastrointestinal tract was considerably affected by the diet. Gut microbiome can be affected by diet, and different dietary interventions are used by poultry producers to enhance bird growth and reduce risk of enteric infection by pathogens. From this perspective, the study of the gastrointestinal microbiota has nowadays an enormous potential and importance.

Keywords: *microbiome, probiotics, prebiotics, 16S rDNA metagenome sequencing*

P-140

Epigenetic determinants of gene expression noise in hematopoietic stem cells

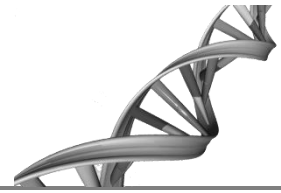
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Eukaryotic cells show significant non-genetic heterogeneity, the result of noisy molecular processes. One of the most investigated of these processes is gene expression. Gene expression noise is usually attributed to differences in the genetic background or the environment of organisms. However, even isogenic cell lines show a significant variation in their expression. Various genomic features influence expression noise, ranging from DNA base composition at the promoter to histone modifications along the gene body or large-scale chromatin structure. Even though gene expression noise is investigated in various model systems, there is a lack of information on its role during the development of various diseases, including cancer.

We hypothesize that histone modifications do not simply silence or activate gene expression, as generally assumed, but also influence expression variance. This variance might have important roles at the early stages of cancer development. During early cancer development, expression variance might lead to outlier oncogene or tumor suppressor expression patterns in otherwise healthy cells or tissues. Additionally, histone modifications themselves are variable across genomic regions, when compared between biological samples or cells, introducing even more randomness into gene expression.

Myelodysplastic syndrome (MDS) is a hematological cancer originating from the clonal expansion of mutated CD34⁺ hematopoietic stem cells. MDS mutations frequently affect genes with a role in DNA methylation or chromatin modification. Mutated genes include TET2, ASXL1, DNMT3A, EZH2 or BCOR among others. Considering these mutation patterns, we decided to analyze histone modification patterns in CD34⁺ hematopoietic stem



cells, using sequencing datasets available from the literature. The ENCODE project contains data on the H3K4me1, H3K4me3, H3K27me3, H3K36me3, H3K9me3 and H3K27ac histone marks for this cell type, including ChIP-seq signal distribution and called peaks.

Using the general form of Shannon entropy as a measure of variance in different genomic regions and across different samples, we characterized the “randomness” or consistency of the above-mentioned histone marks. In the future, we will aim to correlate our histone mark entropy with gene expression variance, and check if known oncogenes or tumor suppressors have a biased location or expression variance. Hopefully, our results will lead to a more comprehensive understanding of early cancer development.

Keywords: *expression noise, histone modifications, epigenetics, hematopoiesis*

P-141

Features of exceptional survivors – defining advanced stage cancer patients with prolonged overall survival

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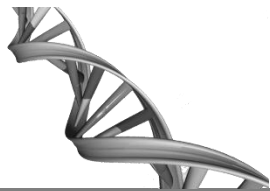
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Background: Between different tumor types, the overall survival rates show notable differences. Patients presenting with any kind of metastases at the time of diagnosis have significantly worse prognosis compared to non-metastatic patients. However, even some of these patients experience exceptional long survival rates.

Aim: Our aim was to characterize those cohorts of patients where survival rates surpass majority in a positive manner.

Methods: We examined seven tumor types: colorectal carcinoma (mCRC), breast cancer (mBC), melanoma (mML), lung cancer (mLC), gastric cancer (mGC), pancreas carcinoma (mPC) and ovarian cancer (mOC). Only metastatic or high grade patients were selected. Using the National Comprehensive Cancer Network (NCCN) database, we obtained those publications which were phase II or III clinical trials with available survival plots (Kaplan-Meier plots). The webplotdigitizer web-tool (<https://apps.automeris.io/wpd/>) was employed to re-digitalize the survival plots. We calculated the median survival time of the 10th percentile of the proportion of longest living patients with simultaneously including censoring events in the analysis. Trials for the same tumor type were processed separately and the results were averaged.

Results: We analyzed 53 eligible clinical trials from NCCN data, including mLC: n= 9, mBC: n= 8, mCRC: n=8, mML: n=10, mGC: n= 5, mOC: n=5, and mPC: n= 8. The median survival at the 10th percentile of the longest living patients for the selected tumors: mlc: 23.21 months, (14.83-35.4 months), mbc: 39.81 months (30.08-49 months), mcr: 54.5



months(31.5-70.8 months), mml: 76.1 months(51.96-165.56 months), mgc: 22.82 months(17.39-37.52months), moc: 38.67 months(26.65-151.9 months), mpc: 17.37 months(11.72-30.09 months).

Conclusion:In our study, using previous clinical trials we re-computed the survival percentiles and defined the time necessary to achieve an exceptional survivor status in different kinds of solid tumors.

Keywords: *exceptional survivors, advanced cancer, metastatic tumor, long overall survival*

P-142

Impact of different alterations of sample preparation, lysis protocols and DNA separation on shifts in composition of broiler chicken microbiota

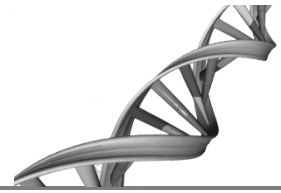
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Background: Poultry industry accounts for the majority of meat production worldwide, and according to OECD/FAO surveys the poultry meat production will reach 130 million tons by 2020. With this dramatic increase maintaining livestock became much more difficult. In order to preserve the health of the animals antibiotic treatment was used to prevent infections until the European Union restricted its application in 2006. There is a dire need for other strategies to manage the immune status of the animals and to provide high quality meat. Several studies report a mutualistic relationship between the gut microbiome and the host. The gut microbiota helps digestion of food, produce metabolites that are beneficial, and can influence the host immune system to prevent infections. On the other hand, disruption of the diversity of the gut microbes can lead to disbiosis and several health problems. Next generation sequencing technics provide a good opportunity to investigate the microbiome, especially when using the 16S metagenomics approach. However, there is no consensus in sample preparation and DNA isolation process of chicken feces, which factors can highly affect the result of sequencing. The aim of this study was to assess the impact of different protocol modifications on the sequencing outcomes (total reads vs. quality read count, No. of OTUs) and on biodiversity (Shannon index, FaithPD, Simpson evenness, Chao1 index) and to identify consistent batch-effects facilitating reproducibility.

Methods: Fecal samples were collected from mature broiler chickens, pooled and distributed to 96 aliquots. A comprehensive analysis was carried out to assess 96 different metagenomic DNA purification platforms by comparing two preprocessing protocols (bacterial cell suspension, direct lysis of the feces), three lysis protocols (bead beating,



chemical lysis, mixed lysis) and three type of DNA purification methods (robotic magnetic bead based, silica membrane based, alcoholic precipitation of DNA). Library preparation was carried out according to the Illumina 16S library preparation protocol. Paired end sequencing was done on the Illumina MiSeq platform, targeting the V3-V4 regions of the bacterial 16S rDNA. Data analysis was performed using the QIIME2 (ver. 2018.8) pipeline. Consistency factor of the protocol variables was assessed by PCoA analyses.

Results: Along with the yield, DNA purity (A260/280) proved to be the best indicator for good library preparation, but the DNA integrity had a low effect. According to biodiversity and consistency we found significant differences between the two preprocessing protocols. The bacterial suspension based preprocessing gave the highest consistency and showed the best biodiversity in combination with robotic (Roche MagNa Pure 24) conventional DNA purification. Altogether the different lysis protocols had low impact on sequencing outcomes. Samples with extraordinarily high read counts provided the lowest biodiversity values.

Summary: We analyzed numerous bacterial DNA purification approaches which can be suited to investigate different biological questions when studying chicken microbiome.

Keywords: *animal microbiome, 16S rDNA metagenome analysis, next generation sequencing*

P-143

Potential new therapeutic targets in colorectal carcinoma linked to driver mutations

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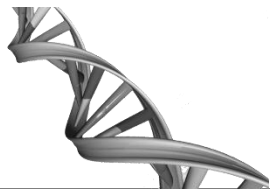
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Despite well described driver genetic alterations in colorectal carcinoma (CRC), the availability of targeted therapies for this malignancy are limited, and development of novel treatments has been slow. Mutations may impact downstream signaling pathways and alter expression of potentially actionable genes. We aimed to identify upregulated, potentially druggable genes, linked to disruptive mutations as potential therapeutic targets in CRC.

Somatic mutations and gene expression data were available for 582 CRC patients, incorporating the mutational status of 39,916 genes and the expression of 20,500 genes. We uncovered the most frequently disruptive mutations and identified differentially expressed genes between wild type and mutant samples with Mann-Whitney U test. Gene expression and survival data were available for 2,100 patients. Up-regulated genes associated with worse



survival were filtered for potential druggability. We validated the impact of the identified mutations/genes on survival in an independent set of 171 patients.

Altogether 426 upregulated genes associated with disruptive mutations were subjected to survival analysis, of which 95 overexpressed genes were linked to worse Recurrence Free Survival (RFS; $p < 0.05$). Based on expression and potential druggability, we filtered out 7 genes and validated their expression on an independent clinical sample. The high expression of four genes, *TRIB2* ($p = 0.0003$), *VSIG4* ($p = 0.0003$), *BMP4* ($p = 0.023$) and *DUSP4* ($p = 0.043$) linked to *ACVR2A*, *ANK3*, *SOX9* and *AMER1* mutations was associated with worse RFS, providing potentially actionable targets in CRC. The identified driver mutations may help future patient stratification for therapeutic relevance.

Keywords: colon cancer, disruptive mutations, oncogenes, molecular targeted therapy, survival

P-144

Prediction of single α -helices in large sequence sets

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Single alpha helices (SAHs), stable in aqueous solution in monomeric form, are formed by sequences containing repeating blocks of positively and negatively charged residues. There are still very few proteins in which the presence of SAHs is experimentally demonstrated. We have previously developed two algorithms, SCAN4CSAH and FT_CHARGE, which were used in synergy for a consensus-based prediction. The slower of these methods is FT_CHARGE, a program that uses Fourier transformation to identify the charge pattern characteristic of SAHs. The original implementation of this method provides a major bottleneck in large-scale sequence analysis of SAHs. In this work, we have implemented this algorithm on FPGA, a hardware platform allowing massive parallelization. In addition, after a review of SAHs recently characterized by experimental methods, we have adjusted the parameters of FT_CHARGE used for SAH identification. Using these improvements we present the latest version of our SAH detection web service and the SAH database we can now build on a monthly basis, following UniProt releases. Our comprehensive analysis of SAH-containing sequences highlights the presence of SAHs in multiple proteins participating in RNA binding and some neuronal processes. Our methods and data are available at <http://csahserver.itk.ppke.hu>.

Keywords: single alpha helix (SAH), structure prediction, hardware acceleration



P-145

An old player in a new role: HLTF tumour suppressor fighting shoulder to shoulder with FANCF against replication stress

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Lesions in DNA block the synthesis of nascent DNA by the replicative polymerases leading to replication stress, and unless replication is rescued by lesion bypass processes, stalled replication forks can collapse, resulting in genomic instability. In the eukaryotic cells, the Rad6–Rad18 pathway governs at least three alternative pathways to promote replication through DNA lesions. Two pathways are activated by PCNA monoubiquitination and are carried out by specialized translesion synthesis (TLS) polymerases which are able to copy DNA directly from the damaged template in an error-free or error-prone way. The third pathway is activated by PCNA polyubiquitination and operates via template switching using the information of the newly synthesized strand on the sister duplex for DNA synthesis across damaged DNA.

In yeast, this error-free template-switching pathway is mediated by Rad5. It was revealed by our group, that the evolutionarily highly conserved PCNA polyubiquitylation process in human cells is governed by two Rad5 homologs: HLTF and SHPRH (Unk I et al., 2008). HLTF, as a dominant factor of postreplication repair, has a ubiquitin ligase activity, which promotes the polyubiquitination of PCNA in collaboration with Rad6–Rad18 and Mms2–Ubc13. HLTF expression is altered in various cancers via two mechanisms: gene silencing through promoter hypermethylation or alternative mRNA splicing, and this alteration indicates poor prognosis.

The BRCA-associated FANCF DNA helicase is mutated in hereditary breast and ovarian cancer as well as in Fanconi Anemia (Cantor S, et al., 2004). FANCF is needed in case of endogenous replication stress and in response to genotoxic agents as well; however, it was not known that depletion of FANCF causes an imbalance of HLTF level at the chromatin. We found that FANCF-knockout (FANCF-KO) cells suffer from enhanced replication stress resulting in an aberrant accumulation of the fork remodelling factor HLTF. HLTF contributes to fork degradation in FANCF-KO cells. Loss of HLTF in FANCF-KO cell leads to unrestrained DNA synthesis and enhances fork degradation. These results suggest that FANCF and HLTF promote replication fork integrity by keeping fork remodelling and elongation in check. FANCF and HLTF fight against replication stress shoulder to shoulder: one compensates for the loss of the other; the loss of both HLTF and FANCF causes a more severe replication stress response (Peng M, et al. 2018).

Keywords: HLTF, FANCF, replication fork



P-146

Analysis of the function of *Saccharomyces cerevisiae* Mgs1 protein in Gquadruplex replication

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Preservation of genome stability is crucial for normal cell growth, and it is maintained by the complex repair and surveillance mechanisms of the cell. Our field of interest is connected to the replication of DNA with stable secondary structures, namely G-quadruplexes (G4). G4 regions represent blockades for complete replication and cell division. Recently, several publications described the contribution of intrachromosomal G4 sequences in transcriptional and translational regulation processes, therefore the inaccurate replication of them could result far-reaching consequences (cell cycle arrest, apoptosis, mutations, cancer and genetic diseases).

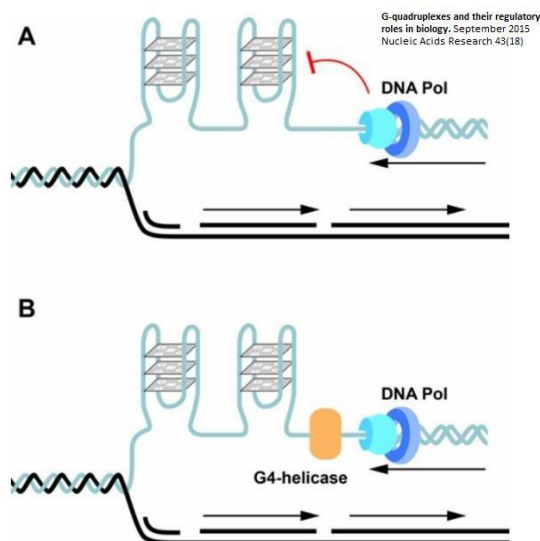


Figure 1: *G-quadruplexes is formed during replication when the DNA is transiently single stranded. This can impede replication and has to be resolved to permit the replication machinery, including DNA polymerase to proceed for both leading and lagging strand DNA synthesis.*

We discovered a potential candidate which might contribute in the process of G4 replication. The *yeast* Mgs1 protein has strong G4 binding ability and it is an important contributor of genome maintenance, but its exact role is not clarified yet. Based on our



current observations it is possible, that Mgs1 can act as a regulatory protein between DNA replication machinery and G4 unwinding helicases during intrachromosomal G4 replication. Our major goal is the detailed analyses of Mgs1 in this process, therefore it is important to follow the replication machinery through G4-rich intrachromosomal segments.

Keywords: *G-quadruplex, genome maintenance, yMgs1*

P-147

Correlation of homologous recombination deficiency induced mutational signatures with sensitivity to PARP inhibitors and cytotoxic agents

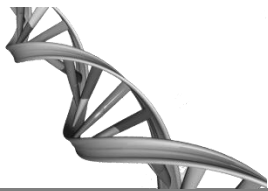
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Carriers of heterozygous germline BRCA1 or BRCA2 mutations have an elevated risk of developing breast, ovarian or pancreatic cancer during their lifetime. Several cytostatic agents, including the most recently discovered family of the polyADP-ribose polymerase (PARP) inhibitor compounds, show an elevated cytotoxic effect on cells having defects in the homologous recombination (HR) pathway, due to harboring deleterious mutations in these genes. However, the use of PARP inhibitors was shown to be effective not only against tumors with germline BRCA1 or BRCA2 variants, but often it was also beneficial for patients having no such mutations, only showing a disturbed HR phenotype. It raises the possibility that the lack of other HR components or double strand break (DSB) signaling proteins may also sensitize these tumors to PARP inhibitors or other selective drugs.

Recent advances in sequencing technology have reduced sequencing costs, enabling a detailed examination of all genomic alterations in cancerous samples. Tumors harboring inactivating BRCA mutations were shown to have a distinct single nucleotide variation spectrum, termed COSMIC signature 3, in addition to short indels and long range structural variations, often showing signs of microhomology. Previously we have shown that these patterns are indeed mechanistically caused by the loss of BRCA1 or BRCA2. These observations may permit to detect defective HR repair even if the causative mutation is unsure, thus this mutational pattern may be a satisfactory indication of PARP inhibitor use. Our aim was to verify that the genomic scars found in a panel of HR defective isogenic cells lines correspond to the BRCA-specific mutational patterns, and if these genomic imprints can predict the sensitivity of these cells to a range of chemotherapeutic agents.

In the present work we showed that in our cell line panel the patterns of genomic alterations in a set of strictly taken HR mutants are highly similar to BRCA1 or BRCA2 cells and real HR defective tumors, while the DSB signaling mutants did not show elevated mutagenesis. By measuring the half-lethal doses of 10 different chemotherapeutic drugs



including PARP inhibitors and platinum agents to this cell line panel, we found that the overall mutagenic tendencies are well correlated with PARP inhibitor sensitivities, but are less efficient predictors in case of other compounds. By monitoring the mutagenic outcomes of the repair of a DSB in a CRISPR-Cas9 based assay, we found similar patterns compared to wild type cells, so we propose that the elevated mutagenic burden is rather a consequence of hindered replication fork rescue.

P-148

Development of new reporter systems to monitor the accuracy of G4 replication in *Saccharomyces cerevisiae*

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DNA replication is a basic, highly regulated process in the cell. During replication and transcription, the genomic DNA is transiently in single-stranded form, and can adopt different secondary structures. Nowadays the guanine-rich regions are in the focus of the scientific interest, which can spontaneously fold a very specific and stable secondary structure, called G-quadruplex (G4). The building blocks of G4s are planar guanine tetrads stabilized with Hoogsteen hydrogen bonds. Two or more G-tetrads can stack on top of each other to form the complex. Computational analyses showed that the human genome contains more than 350 000 potential G4 forming sequences. Moreover, the distribution is not random, they are accumulated e.g. at promoter regions and clustered at telomeres. At physiological salt concentration, this quadruplex is thermodynamically more stable than the common form of the DNA, and might block both replication and transcription. So, these structures pose challenges to replicative processes, and G4-interacting proteins, like helicases and polymerases, must recognize and unwind G4 DNA to maintain the genetic stability. Their exact replication is essential, mutations in the G4 sequence can lead to the loss of genome integrity and activation of oncogenic pathways. Deficiency of G4-recognizing helicases and polymerases can cause serious problems, like early ageing or immunodeficiency.

Without a high-throughput system investigation of the failures in G4 replication is very difficult. *Saccharomyces cerevisiae* could be a suitable model organism, because of the similarities in G4 processing in yeast and human. Until now there was no simple and sensitive method published to detect the mutations in the G4 sequence as a result of the replication.

In this study we provide a plasmid based reporter system developed in *S. cerevisiae* which is suitable for detection of G4 replication failures.

Keywords: *G-quadruplex, replication*



P-149

Dissecting molecular pathways to understand breast cancer chemoprevention

Patricia Kálmán, Sham Jdeed, Júlia Pap and Iván Uray

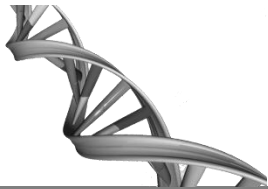
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Cancer chemoprevention refers to the administration of natural or synthetic agents to delay or block the development of malignant disease. Previously, by high throughput screens of drug libraries, we determined that the RXR selective retinoid bexarotene, and carvedilol, a non-selective beta blocker synergistically suppress cell proliferation and inhibit the formation of Her2-induced breast cancer, even at low doses. However, given the pleiotropic nature of both of these agents, the way they exert their chemopreventive effect is unclear.

We hypothesized that the molecular changes leading to the observed effects will be reflected in the patterns of gene expression, and at the proteomic landscape. We used low dose bexarotene and carvedilol treatment in normal and *in situ* breast carcinoma cells. Microarray, next generation sequencing, reverse phase protein array (RPPA) experiments were performed to identify a set of targets related to cell proliferation and genomic stability. All were validated by western blot and q-PCR assays.

In genomic studies, we found significant change in SPTLC3, IGFBP3, DUSP5, and DEPP gene expressions upon bexarotene and carvedilol treatment in normal breast cells. Our proteomic analyses revealed that the Thr202/Tyr204 phosphorylation of Erk 1/2 and Tyr416 phosphorylation of Src cell growth proteins are decreased. Furthermore, we found increased levels and phosphorylation of the DNA damage sensor ATM, along with elevated levels of ARID1A in normal breast epithelial cells upon combined bexarotene and carvedilol treatment, but not in *in situ* breast carcinoma cells. ARID1A, a tumour suppressor and an integral subunit of the SWI/SNF chromatin remodelling complex, controls nucleosomal organization and modulates DNA accessibility. Chromatin Immunoprecipitation followed by deep sequencing (ChIP-Seq) for H3K27 acetyl complemented with histones H3K4 mono and dimethyl forms will identify enhancers in ARID1A overexpressing and deficient states. Another target we are also studying in more detail is DEPP. DEPP is frequently underexpressed in breast cancer patients and its low expression correlates with poor prognosis. However its function is not well characterized, so we are working on overexpressing DEPP in normal and malignant breast cell lines, to determine its effect on cell proliferation and viability.

Keywords: cancer prevention, retinoids, breast cancer, genomic stability



P-150

Identification of differentially expressed miRNAs in plasma samples of ovarian cancer patients

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Introduction: Ovarian cancer (OC) is the 6th most common tumor in women. Its high mortality rate is partially due to lack of effective screening methods for early diagnosis. Given their ability to regulate gene expression and presence in bio-fluids miRNAs could be non-invasive biomarkers for cancer diagnosis.

Materials and Methods: Total RNA was isolated from plasma samples of 18 OC (6-6 FIGO stage I, III and IV) patients and 6 healthy controls. MiRNA copy number was determined using the Nanostring System with nCounter Human v3 miRNA Panel. Background and technical variations were corrected for the mean $\pm$ 2SD of negative controls and the positive code-set, respectively. Data was normalized by the geometric mean of 10 housekeeping miRNA counts. Significant differentially expressed (DE) miRNAs were identified by Kruskal $\pm$ Wallis test with *post hoc* Dunn's test. Target genes, candidate pathways and lncRNA associations for significant miRNAs were identified by a network-based analysis using miRNet, mirTargetLink and Networkanalyst tools. Functional annotation clustering of target genes and pathway analysis was done with the DAVID tool.

Results: 26 miRNAs were upregulated, the tumor suppressor miR-584-5p was down-regulated. In our DE miRNA set miR-25-3p/26b-5p/301a-3p/19b-3p/144-39 had the highest degree and betweenness centrality values, their common targets are PTEN, BCL2L11, KAT2B, SMAD4, TP53, MALAT1, XIST, HOTAIR.

Conclusion: Functional annotation and gene-GO term enrichment analysis of targets identified the involvement of cell cycle regulation, FOXO/PI3-AKT/TP53/TGFB/SMAD4 signaling pathways, negative regulation of apoptosis, positive regulation of proliferation and epithelial-mesenchymal transition in OC development. Specific plasma miRNA profiles could represent potential diagnostic biomarkers for OC.



P-151

Investigation of the mutator effects of various genotoxic stresses provides insight into the mechanism of drug resistance development in *Mycobacteria*

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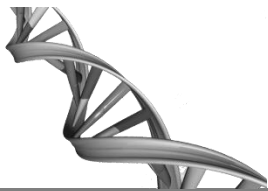
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Tuberculosis is still one of the top 10 causes of death worldwide in spite of century-long efforts to combat it. In addition, multidrug-resistant tuberculosis also denotes a public health crisis. WHO estimates that there were over a half million new cases in 2017 with resistance to rifampicin – the most effective first-line drug - of which 82% had multidrug resistance. Horizontal gene transfer is a major molecular mechanism responsible for generating genetic diversity and plays a particularly important role for driving evolution of drug resistance in most bacteria. However, mycobacterium strains acquire antibiotic resistance merely by single point mutations. Interestingly, in contrast to the remarkable genomic diversity displayed in isolates from tuberculosis patients, the basal mutation rate in mycobacteria is very low *in vitro*, suggesting that genotoxic stress and harsh conditions within the host cell may elicit transient or constitutive mutator phenotypes. We promote that the detailed understanding of the mutator effects of various genotoxic stresses would provide insight into the mechanism of drug resistance development and allow better management of current therapeutics.

To investigate the mutator effect of different environmental stress factors (oxidative stress, starvation, hypoxia, alkylating agents, UV, etc.) or currently used tuberculosis drugs (first and second line antibiotics), we analyzed the mutation rates and the spectrum of mutations by whole-genome sequencing. We also correlated the changes in the expression pattern of the DNA repair enzymes of *Mycobacterium smegmatis* upon genotoxic stress to these data. Correlating dNTP pool imbalances with a defined mutator effect and with the activated DNA repair responses yields an unprecedented insight into the mechanisms underlying mutagenesis by genotoxic stress.

Funding: EMMI FIKP-BME Biotechnology grant



P-152

Investigation of uracil-DNA repair in *Staphylococcus aureus*

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The core genome of the biomedically relevant *Staphylococcus aureus* (*S. aureus*) bacterium lacks the usually essential deoxyuracil triphosphate pyrophosphatase (dUTPase) enzyme, which is responsible for preventing uracil incorporation into the genome. In the lack of dUTPase the elevated uracil content of the DNA may overload uracil-DNA repair, resulting in the increase of mutational rate and double strand breaks. Interestingly, the mobile genetic elements of the bacterium that encode antibiotic resistance and/or pathogenicity factors carry dUTPases, or the SaUGI (*S. aureus* Uracil-DNA Glycosylase Inhibitor) protein which protects from overloading uracil-DNA repair.

Our aim is to investigate I) how does *S. aureus* survive without the usually essential dUTPase enzyme II) whether the presence of mobile genetic elements integrated into the genome of the bacterium has any role in counterbalancing the lack of genomic dUTPase III) whether the mobile genetic elements themselves take any advantage of possessing the dUTPase and/or the SaUGI protein.

For this purpose, we measured the uracil level and the deoxynucleoside-triphosphate (dNTP) pools of *S. aureus* strains with different genetic background (+/- mobile genetics). Besides, we also introduced the UNG inhibitor SaUGI to an *E. coli* – *S. aureus* shuttle vector, pRMC2 which allows for the directed and controlled expression of the protein in *S. aureus*.

Although we found that all investigated *S. aureus* strains have an elevated uracil-DNA level, we neither could see a remarkable difference in the uracil content of the genomic DNA nor in the dNTP pools of the different strains. However, the directed expression of the SaUGI protein from the pRMC2 plasmid resulted in an even higher uracil-DNA level. These results together indicate that i) the SaUGI protein acts as a uracil DNA repair inhibitor *in vivo*; ii) the SaUGI protein is not expressed in the investigated strains from its integrated, resting mobile genetic element. Based on the present result we assume that *S. aureus* cannot take advantage directly from the dUTPase and the SaUGI proteins carried by integrated mobile genetic elements. To answer our further research questions, further investigations are necessary.



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Keywords: uracil-DNA repair, *Staphylococcus aureus*, mobile genetic elements, dUTPase, dNTP pool

P-153

PARylation and DNA accessibility during the DNA damage response

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The tight packaging of DNA in chromatin hinders the access of DNA-templated processes including DNA damage repair. The rapid activation of PARP1 upon DNA damage response results in the addition of poly(ADP-ribose) chains (PAR) along the chromatin fiber at the sites of DNA lesions. The PAR signal initiates fast relaxation of the chromatin and regulates the recruitment of several factors to the DNA lesions. We have developed a new method to study the correlation among PARylation, DNA accessibility and DNA repair. Our results indicate that the DNA damage induced PAR-dependent chromatin relaxation underlies changes to DNase hypersensitivity normally associated with open chromatin regions in genomic studies.

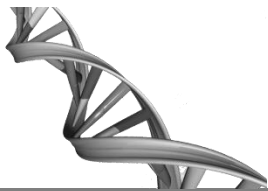
P-154

Regulatory function of ZBTB1 in the DNA damage tolerance pathways

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Our cells are constantly exposed to different exogenous and endogenous factors that can affect our genome. During S phase, when the genome is especially vulnerable, lesions can block replication, because the replicative polymerase cannot bypass the damaged site. Rescuing the stalled replication fork is crucial to preserve the integrity of the genome and to prevent carcinogenesis.



The Rad18-dependent DNA damage tolerance (DDT) pathways are specialized to help to complete replication in case of DNA damage. Post-transcriptional modifications of PCNA play a central role in the regulation of these mechanisms. The Rad6/Rad18-mediated monoubiquitylation of PCNA initiates the translesion synthesis (TLS), where the replicative polymerase is replaced by a TLS polymerase, which can insert nucleotides opposite to the lesions, mostly in an error-prone manner. In contrast to that, polyubiquitylation of PCNA by Mms2-Ubc13 and HLTf/SHPRH proteins activates the template switching (TS), where the newly synthesized, error-free strand is used as a template for the replication, avoiding the damaged region.

ZBTB1 protein was firstly identified as a transcription factor and an important regulator of chromatin remodeling. Recently, it was also described as an upstream regulator of the DNA damage tolerance pathways. It can assist in the phosphorylation of KAP-1, which leads to local chromatin relaxation. This way, Rad18 can access the damaged sites and can ubiquitilate PCNA. Mutations of the ZBTB1 gene occur mostly in gastrointestinal, ovarian and breast tumors.

Although we have an extensive knowledge about the Rad18-dependent pathways, there are still many unknown factors among its upstream regulators. In order to gain further understanding of the regulation of DNA damage tolerance, we examined the relationship between already known regulators and ZBTB1, with cell biological methods. Thus, we can understand the molecular mechanisms, which protect the stability of our genome.

Keywords: DNA damage tolerance, UV damage

P-155

Single-molecule techniques reveal how proteins can access DNA coated by SSB proteins

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Single-stranded (ss) DNA binding (SSB) proteins are essential for genome maintenance in all kingdoms of life, as they cover ssDNA generated during DNA metabolism to prevent formation of secondary DNA structures and protect ssDNA from nucleolytic degradation. In addition, SSB also acts as a hub protein by coordinating and altering the activity of a variety of DNA processing enzymes. *Escherichia coli* SSB is the best-characterized SSB. It



functions as a homotetramer, and each monomer consists of an N-terminal DNA-binding OB fold and a C-terminal intrinsically disordered linker followed by an acidic tail (TIP). The TIP region is responsible for the interaction with 18 known partner proteins.

One essential partner of SSB is *E. coli* RecQ helicase, which can unwind various double- or multi-stranded DNA structures in an ATP-dependent manner during genome maintenance processes. Members of the RecQ family are often referred to as the “guardians of the genome” as mutations of several human RecQ helicases (WRN, BLM, and RECQ4) lead to genetic disorders with symptoms of premature aging and increased cancer predisposition.

Despite the fact that SSBs from all investigated bacteria and eukaryotes form specific physical interactions with RecQ helicases, the cellular and mechanistic role of the interaction is unknown.

Previously we showed in ensemble assays that RecQ can induce a DNA-binding mode change in the SSB, allowing RecQ to gain access to ssDNA and begin DNA unwinding. In addition, SSB has a modest stimulatory effect on the unwinding rate of RecQ via its TIP region. Consequently, a bidirectional communication occurs between the two proteins for efficient DNA processing.

Now, by utilizing a combination of single-molecule magnetic tweezers and total internal reflection fluorescence (TIRF, single-molecule detection) microscopic experiments, we investigated further the role of interaction on the activities of *E. coli* RecQ and SSB on the single molecule level. Our results show that RecQ shortens SSB-ssDNA filaments only if a physical interaction occurs between the two proteins. SSB-ssDNA complexes lacking the TIP region do not undergo structural changes in the presence of RecQ helicase. We propose a mechanistic model in which RecQ gains access to SSB-covered DNA by inducing a DNA-binding mode change in SSB and local SSB dissociation via interaction with the SSB TIP region.

Keywords: *single molecule, helicase, protein-protein interaction*



P-156

The effects of genotoxic stress factors on DNA repair system in Mycobacteria

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Tuberculosis is still among the most challenging global health problems. One of the main reasons for that is the quick and efficient adaptation of the pathogen *Mycobacterium tuberculosis* to the variety of harsh conditions in the host. Mycobacteria uncommonly develop resistance only by chromosomal mutations, in particular, single-nucleotide polymorphisms [1]. Based on the remarkable genetic diversity of mycobacteria in isolates from tuberculosis patients [2], a great mutation rate could be expected in the bacteria. Although, *in vitro* studies show a very low basal mutation rate in mycobacteria ($\sim 10^{-10}$) [3]. These contradictory results suggest that genomic stress factors like the immune pressure and antituberculous drugs within the patients may provoke increased mutational rates in the bacteria. Cells provide the genome stability and low mutation rates by presenting countless DNA surveillance and correction processes. Mycobacteria has a unique, yet not quite understood DNA repair system with several redundant enzymes, absent canonical mismatch repair pathway, and proteins with special functionality [4].

Our aim is to study the activation pattern of mycobacterial DNA repair system upon *in vitro* genotoxic stress with the help of qPCR. In our experiments, we use the non-pathogen *Mycobacterium smegmatis*, which shares the same metabolic and repair routes as the medicinally relevant strains.

Our results will hopefully contribute to a better understanding of the adaptive events resulting in the resistance of mycobacteria.

Funding: EMMI FIKP-BME Biotechnology grant.

Keywords: Mycobacteria, DNA repair, gene expression

References:

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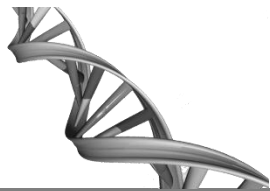
**P-157****A novel fluorescence-based assay to characterize transporters involved in hepatic detoxification****Virág Székely¹, Éva Bakos¹, Izabel Patik², Nóra Kucsma¹ and Csilla Özvegy-Laczka¹**¹ Membrane Protein Research Group, Institute of Enzymology, RCNS, HAS, Budapest, Hungary² Department of Pathology, Brigham and Women's Hospital, Boston, United States

Liver has central role in the defense of the body against harmful compounds. Detoxification in hepatocytes is a strictly controlled process, in which governed action of membrane transporters involved in the uptake and efflux of potentially dangerous molecules has crucial role. Major drug transporters of the liver belong to the ABC (ATP Binding Cassette) and Organic Anion Transporting Polypeptides (OATPs) protein families. OATP1B1, one of the major drug uptake transporters of the liver, is exclusively expressed in hepatocytes. It transports bile acids, bilirubin, sex hormones and also interacts with many clinically used drugs (e.g. antiviral and chemotherapeutic drugs, statins). The removal of toxic molecules from hepatocytes is accomplished by ABC-MDR multidrug transporters of the ABC (ATP-binding Cassette) family. Multipsecific OATP and ABC-MDR transporters have overlapping substrate specificities. Their mutations, polymorphism, and the co-administration of their substrates causes altered pharmacokinetics. Hence, monitoring these proteins during drug development is obligatory. Therefore, reliable and cost effective *in vitro* assays for ABC-MDR and OATP proteins are of high relevance.

Fluorescent dyes, recognized by these multispecific transporters are good candidates to develop safe and sensitive functional transporter assays. Our group has already identified such fluorescent OATP substrates. In the frame of the current project, we investigated the interaction of these novel OATP substrates with ABC-MDR transporters. For this aim, inside-out membrane vesicles were applied. Additionally, we established stable cell lines co-expressing OATP and ABC-MDR proteins to characterize fluorescent dye interactions. Common OATP ABC-MDR fluorescent substrates were identified. These novel dye substrates represent a novel tool for studying the activity and drug interactions of OATP and ABC-MDR proteins.

Funding: This project was supported by research grant of NKFIH (OTKA FK 128751). Csilla Özvegy-Laczka is a recipient of the Bolyai János Scholarship of the Hungarian Academy of Sciences.

Keywords: OATP, ABC-MDR, fluorescent dye, drug interaction



P-158

Design of novel heterocyclic molecules for apoptosis induction

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Pancreatic adenocarcinoma is one of the most aggressive tumor with poor prognosis, the five-year survival rate is 5% [1]. The resistance of pancreatic carcinoma to treatment regimens represents a major challenge, whereas pancreatic carcinoma cell resistance to apoptosis is well known and may contribute to treatment failure [2]. A potential drug selectively restoring the regulation of apoptosis or inducing apoptosis of the tumor cells may reduce the therapeutic failure.

Objectives of our study were: (i) to design a new library of heterocyclic molecules for apoptosis induction; (ii) to screen the novel molecules on different tumor cell lines by HTS impedimetry; (iii) to identify the molecular target of the hit molecules; (iv) to check how the hit molecules act on the apoptotic pathway.

Methods: the drug-like library were developed upon non-flat 3-dimensional templates and libraries (Smart Diversity Approach™). Compounds with small molecular weight, more favorable physicochemical properties, higher sp³/sp² atom ratio, and novel 3-dimensional shapes with various functionalities were selected and synthesized. Biological tests were performed on different tumor cell lines, PANC1 (pancreatic adenocarcinoma), COLO 205 (colon cancer), A2058 (melanoma), EBC1 (lung cancer). Cytotoxicity was measured by impedance based technique in xCELLigence SP (ACEA) system. Flow cytometry were applied to detect the apoptotic effect of the molecules, caspase 3/7 activity were measured.

Results: In total 193 novel heterocyclic molecules containing linker connection functional groups with a relatively low molecular weight, below 600 Daltons were synthesized in high purity (> 95 %). 18 of the tested compounds were significantly cytotoxic on pancreatic tumor cells (PANC-1). These molecules were able to reduce the cell viability of other tumor cell lines (COLO 205, A2058 and EBC1) as well. Screening in databases based on structural similarities revealed that the potential target of several components is the XIAP (X-linked inhibitor of apoptosis). However, the apoptotic pathway analysis proved, that some of the hit molecules were able to induce caspase 3/7 activation in the model cells, further examination is required to test the interaction of the XIAP and the hit molecules.

Recently XIAP is proved to be overexpressed in several tumor - as pancreatic carcinoma-, associate with invasiveness and shortens the survival of pancreatic cancer patients [2].



Therefore, our novel heterocyclic molecules selectively inhibiting XIAP may open up new therapeutic option.

The project was supported by the National Research, Development and Innovation Office (NVKP_16-1-2016-0036)

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P-159

Endoplasmic reticulum stress: major player in size-dependent inhibition of P-glycoprotein by silver nanoparticles in multidrug-resistant breast cancer cells

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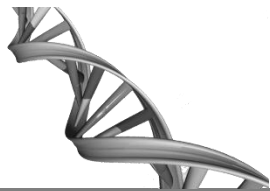
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Background: Development of multidrug resistance (MDR) is a major burden of successful chemotherapy, therefore, novel approaches to defeat MDR are imperative. Although the remarkable anti-cancer propensity of silver nanoparticles (AgNP) has been demonstrated and their potential application in MDR cancer has been proposed, the nanoparticle size-dependent cellular events directing P-glycoprotein (Pgp) expression and activity in MDR cancer have never been addressed. Hence, in the present study we examined AgNP size-dependent cellular features in multidrug resistant breast cancer cells.

Results: In this study we report that 75 nm AgNPs inhibited significantly Pgp efflux activity in drug-resistant breast cancer cells and potentiated the apoptotic effect of doxorubicin, which features were not observed upon 5 nm AgNP treatment. Although both sized AgNPs induced significant ROS production and mitochondrial damage, 5 nm AgNPs were more potent than 75 nm AgNPs in this respect, therefore, these effects can not to be accounted for the reduced transport activity of ATP-driven pumps observed after 75 nm AgNP treatments. Instead we found that 75 nm AgNPs depleted endoplasmic reticulum



(ER) calcium stores, caused notable ER stress and decreased plasma membrane positioning of Pgp.

Conclusion: Our study suggests that AgNPs are potent inhibitors of Pgp function and are promising agents for sensitizing multidrug resistant breast cancers to anticancer drugs. This potency is determined by their size, since 75 nm AgNPs are more efficient than smaller counterparts. This is a highly relevant finding as it renders AgNPs attractive candidates in rational design of therapeutically useful agents for tumor targeting. In the present study we provide evidence that exploitation of ER stress can be a propitious target in defeating multidrug resistance in cancers.

P-160

Impact of *Stellaria media* tea lyophilizate on hypercholesterolemia in rats

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Introduction: Common chickweed (*Stellaria media*, SM) tea is nowadays popular due to its potential cholesterol-lowering effect, although this is not scientifically proven. Therefore we aimed to examine the effect of SM tea lyophilizate on blood cholesterol levels.

Methods: Adult male Wistar rats were fed a special cholesterol-enriched diet, i.e. a standard laboratory rat chow supplemented with 2% (w/w) cholesterol and 0.25% (w/w) sodium-cholate-hydrate (HC, n=16) or standard laboratory rat chow (C, n=8) for 8 weeks. The diet of half of the cholesterol-fed rats (HC+SM group, n=8) was further supplemented with 100 mg/kg SM tea lyophilizate for 8 weeks once a day, mixed into cookie balls. Blood samples were collected at the end of the study from the abdominal aorta, and total cholesterol and triacylglycerol, as well as parameters of liver and kidney function were measured from serum. Before termination, echocardiography was performed in order to evaluate the effects of experimental hypercholesterolemia and SM on cardiac morphology and function.

Results: In the HC group serum total cholesterol level was significantly higher (HC: 3.55 ± 0.52 vs. C: 1.46 ± 0.11 mmol/l, $p < 0.05$), which was not affected by SM tea lyophilizate (HC+SM: 3.50 ± 0.47 mmol/l). Compared to the C group, triacylglycerol level showed no significant difference in the HC group, and SM tea lyophilizate did not influence these results. The SM tea lyophilizate did not worsen the parameters of liver and kidney



function in comparison to the HC group, furthermore no significant alterations were detected in cardiac morphology and function by echocardiography.

Conclusion: Our results did not confirm the cholesterol-lowering effect of SM tea, since the tea lyophilizate in the examined dose showed no beneficial effect in our diet-induced hypercholesterolemia model. Regarding to safety, the SM treatment seems to have no harmful effects on liver and kidney function.

P-161

Impedance-based analysis - A dedicated technique to characterize efficacy of novel antitumour compounds

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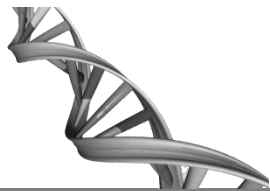
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Evaluation of candidate molecules as dedicated antitumour compounds is a complex, multistep process, which prefers high throughput and real-time cell physiological assays. Application of impedimetry-based systems (ECIS, xCELLigence etc.) in combination with other cell physiology/molecular biology high content screening platforms (e.g. CellDiscoverer7) provides the possibility to perform large number of accurate measurements required by pharmaceutical research.

In the last decade, hundreds of drug candidates (free active drugs, targeting moiety and their conjugates) have been screened in our laboratory.

- i. In these investigations the most frequently tested drug-carrier constructs were belonging to peptide- or protein-based conjugates: daunomycin- (Dau), doxorubicin- (Dox) or methotrexate- (Mtx) peptid hormone (e.g. GnRH, neurotensin) conjugates; while there were other targeted constructs selected by phage display technique as well as another list of novel chemotherapeutic agents (e.g. cinchona alkaloids, tamoxifens, TIC-10 and their ferrocene containing hybrids).
- ii. The most often used target cells were: human pancreas ductal carcinoma (PANC-1), human acute monocytic leukemia (Mono Mac-6); human melanoma (A2058 and HT168-M1); human colorectal carcinoma (COLO-205) and choriocarcinoma (BeWo) cell lines.
- iii. While the monitored cell physiological characteristics (proliferation, cell adhesion, migration) as well as apoptosis were analysed by impedimetry and colorimetric assays (e.g. AlamarBlue). The registered antitumour effects were confirmed by computer assisted analysis of morphometry and migratory behaviour in holographic microscopy.



In impedimetry-based cytotoxicity/viability assays (e.g. in PANC-1) showed that in the group of peptide-based compounds (i) some conjugates containing Dau (e.g. KK031), selected by phage display technique, expressed the highest cytotoxic effect (viability: 8.6 % at 10^{-5} M); (ii) the neurotensin-based conjugates were also effective (viability 4.6 %) in tumour cells, nevertheless in these type of constructs the dual targeting or formation of bifunctional conjugates could also enhance the cytotoxicity of the monofunctional compound. In the group of non-peptide-type ingredients (iii) cinchona alkaloids and TIC-10 derivatives proved to be the most effective ones ($IC_{50} = 3E-6 - 6E-6$ and $IC_{50} = 7E-7 - 3E-6$ respectively); (iv) formation of ferrocene hybrids of cinchona alkaloids resulted also enhanced cytotoxic effects both in PANC-1 and COLO-205 cells; while (v) the increased cytotoxicity of the halogenated TIC-10 ($IC_{50} = 3.4E-7$) vs. the parent molecule was also detected ($IC_{50} = 1.7E-6$).

Results presented above as well as series of data gained by other probes of cell physiology (to be presented in the oral presentation) support our opinion that impedimetry is a strong arm of experimental screening and complex evaluation of a wide range of antitumour compounds.

The project was supported by the National Research, Development and Innovation Office (NVKP_16-1-2016-0036)

Keywords: impedimetry, tumour cell lines, drug-delivery, GnRH, tamoxifen

P-162

***In vitro* modelling the protective effect of rosuvastatin against doxorubicin-induced endothelial injury**

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Chemotherapeutic drugs, such as doxorubicin (DOX), have a well-known off-target effect in the cardiovascular system. However, it is less known about the molecular mechanism of DOX-induced toxicity in endothelial cells (EC) and potential prevention of this side effect. Our aims were to model the DOX-induced toxicity in arterial and venous ECs *in vitro* as well as to identify putative drugs to prevent the vascular injury.

DOX (0.1 μ M, 24h)-induced apoptosis was quantified in human coronary artery (HCAEC) and umbilical vein ECs (HUVEC) cultures. The cell survival was measured in the presence and absence of rosuvastatin (RO, 5 μ M, 6h) or human recombinant neuregulin-1 (rNRG1, 2000 pg/ml, 30h). The endogenous NRG1 secretion was assessed by ELISA; Nrg1 and NRG1-receptor domain ErbB2/3/4 gene expressions were measured by real-time PCR.



DOX-treatment induced apoptosis in arterial (10.8 % vs DMSO 5.13 %, $p < 0.01$, $n = 3$) and venous (14.5 % vs DMSO 5.04 %, $p < 0.05$, $n = 3$) EC cultures. After DOX treatment, the Nrg1 mRNA level was increased significantly (HCAEC: 1.8x vs DMSO, HUVEC: 1.77x vs DMSO, $p < 0.001$, $n = 3$). RO pretreatment reduced DOX-induced endothelial injury ($p < 0.05$, $n = 3$) and increased the NRG1 secretion in HUVEC (2237.3 pg/ml, $p < 0.01$ vs control). The rNRG1 pretreatment had not effect on cell survival, but RO-induced protective effect was inhibited after short interfering RNA treatment to silence Nrg1 (NRG1 siRNA) in HUVEC but not in HCAEC. The ErbB2 domain expressed during the treatments constitutively, but ErbB3 and ErbB4 expressed in other myocardial cells were not observed.

We found that RO pretreatment can prevent the DOX-induced proapoptotic effect in ECs *in vitro*, and induces the secretion of NRG1 in HUVEC. The RO-induced protective effect does not act via ErbB2/3 or ErbB2/4 receptors, but endogenous NRG1 signalling can play role in the survivor of venous endothelial cells.

P-163

New perspective in melanoma treatment: Targeting endoplasmatic reticulum stress

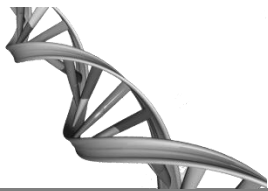
István Szász^{1,2}, Viktória Koroknai^{1,2}, Vikas Patel¹, Krisztina Jámor¹, Róza Ádány^{1,2} and Margit Balázs^{1,2}

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Melanoma is one of the most aggressive cancer, with an incidence that doubles every ten years. Encouraging results have been obtained with BRAF and MEK inhibitors, however, after a short period (~ 6 months) most tumours develop drug resistance and the tumour regrows. The mechanism of drug resistance is highly heterogeneous and mostly leads to restoration of MAPK signalling. Other therapies, like anti-CTLA4 and anti-PD1, have been developed to reactivate the anti-tumour immune response of patients, however, despite these improvements, there is an urgent need to discover more effective treatments. A new drug candidate, named HA15 recently was described as a compound which triggers endoplasmatic reticulum (ER) stress and exert deleterious effect on melanoma cell viability due to autophagy and apoptosis, regardless driver mutations or drug resistance.

In the present study our aim was to define the HA15 effect on melanoma cell lines including BRAF inhibitor (BRAFi) resistant ones. We investigated the effect of HA15 on viability/proliferation on 5 BRAFi sensitive and 4 resistant cell lines using WST-1 assay. Apoptosis was measured by Annexin V/Dead Cell Apoptosis Kit. To generate HA15 resistant lines we used high dose (30 μ M) HA15 treatment. To investigate ER stress we used qRT-PCR, to detect of spliced XBP1, CHOP and BIP mRNA.



In contrary to the literature where the average viability of cells were $\sim 17\%$ at $10\ \mu\text{M}$ HA15, we did not detect significant cell death compared to the control (viability $\sim 90\%$) using the published concentration. We also performed flow cytometric experiment (Annexin V/PI) to distinguish between apoptotic, necrotic and alive cells as a result no significant cell death were seen at $10\ \mu\text{M}$ HA15. After a discussion with the authors, it turned out that they used cell starvation during the whole experiment. During our experiment we found significant decrease in cell viability due to starvation solely. Not surprisingly, because it is well known, that starvation induce cell death by autophagy and apoptosis. The viability further decreased if we combined the starvation of cells with HA15 treatment. Determining the gene expression of ER stress indicators also showed that the HA15 compound does not trigger ER stress alone, but could synergistically increases the ER stress under starving conditions. Last but not least, we could generate HA15 resistant cell lines, in which the authors were failed.

In summary, we have found that the $10\ \mu\text{M}$ HA15 (treatment) effects the viability of melanoma cells only in case of starvation, therefore the use of HA15 in the clinic is questionable. On the other hand our data clearly shows that melanoma cells can acquire HA15 resistance, therefore revision of the effect of HA15 is highly recommended.

The work was supported by the Hungarian National Research Fund (OTKA K112327), EFOP-3.6.1-16-2016-00022 project, GINOP-2.3.2-15-2016-00005 project, UNKP-18-3 new national excellence program of the ministry of human capacities.

Keywords: melanoma, ER stress, chemotherapy, HA15

P-164

Structural dissection of 13-epiestrones based on the interaction with the steroid transporting human Organic Anion-Transporting Polypeptide, OATP2B1

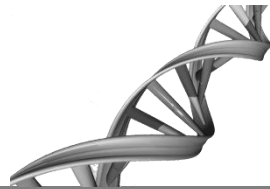
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Human Organic Anion-Transporting Polypeptides (OATPs) encoded by the SLCO genes are membrane proteins that mediate the cellular uptake of large organic molecules. The endogenous substrates of OATPs are hormones, bilirubin, prostaglandins and bile acids. Additionally, several OATPs also recognize clinically applied drugs, including statins, antivirals, antihistamines and chemotherapeutic agents. Some OATPs are overexpressed in tumors of the breast, colon, lung or pancreas. Therefore, although their exact role in tumor



development and therapy response is still unclear, OATPs are potential targets of anti-cancer therapy.

One of the main strategies to fight hormone dependent tumors is the inhibition of their hormone uptake, synthesis or metabolism. To prevent estrogen formation and proliferation of hormone dependent cancer cells, inhibition the 17β -HSD1 (17β -hydroxysteroid dehydrogenase 1) enzyme, e.g. by 13α -estrone is a promising strategy. Since OATP2B1, a multispecific steroid hormone transporter, is overexpressed in various hormone dependent tumors, this protein could provide a new strategy to promote cellular entry of steroid-based 17β -HSD1 inhibitors.

The purpose of our investigation was to unfold the interaction between 13α -estrone derivatives and OATP2B1. For this aim, we characterized steroid interactions of OATP2B1 in several ways. We analysed the inhibitory potential of the steroids in a fluorescence-based transport assay. Additionally, we measured direct transport of the steroid derivatives by OATP2B1, and finally, we determined the cytotoxic effect in parental and OATP2B1 overexpressing cells.

We identified potent inhibitors and also potential substrates of OATP2B1. Moreover, some 13α -estrone derivatives proved to be more toxic in A431 cells overexpressing OATP2B1, suggesting that OATP2B1 can be exploited to kill tumor cells. Based on the interaction profile of 13 -epiestrones we could define structural elements important for OATP2B1 interaction and selective cytotoxicity.

Funding: This project was supported by research grants of NKFIH SNN 124329 and FK 128751. Erzsébet Mernyák and Csilla Özegey-Laczka are recipients of the János Bolyai Scholarship of the Hungarian Academy of Sciences.

Keywords: OATP, steroid dependent tumor, HSD1 inhibitor

P-165

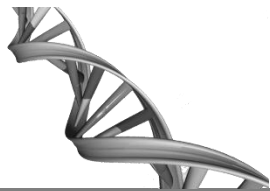
The effect of sour cherry flavonoids and Anthocyanins on Pgp activity

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P-glycoprotein (Pgp) is a member of one of the largest family of active transporter proteins called ABC transporters. They play a multi-task role in the protection of our body from toxic compounds including xenobiotics and toxic metabolic side products. Pgp is present in the capillary endothelial cells of the blood-brain, blood-placenta and blood-testis barrier systems, in epithelium of alimentary canal, liver and in the kidney. Thanks to its expression pattern



and broad substrate spectrum it is an important determinant of the absorption, metabolism and excretion of many drugs. Pgp and/or some other drug transporting ABC proteins (e.g., ABCG2, MRP1) are overexpressed in nearly all cancers and cancer stem cells by which cancer cells become resistant against many drugs. Thus, Pgp inhibition might be a strategy to fight against drug-resistant cancer cells.

Previous studies have shown that certain flavonoids and anthocyanins interact with human Pgp and ABCG2. Since many flavonoids are often used as food supplements or as components of cosmetics their interaction with drug transporting ATPases also might be of particular interest. We are testing the effect of many sour cherry origin compounds on Pgp activity using ATPase assay applying membrane samples isolated from NIH 3T3 mouse fibroblast cells expressing human Pgp at high level as well as Pgp substrate accumulation assays using intact Pgp-positive and Pgp-negative cells.

We have found that quercetin, catechin and ellagic acid inhibited the ATPase activity of Pgp and increased the calcein accumulation of Pgp-positive cells similarly to a known Pgp inhibitor cyclosporinA. Pgp-negative cells exhibited high calcein accumulation which was not affected by flavonoids. Transferulic-acid and narcissoside did not affect the ATPase of Pgp and the intracellular accumulation of calcein suggesting that these compounds do not interact with Pgp. Although, quercetin-glucoside had inhibitory effect in ATPase assay it was ineffective in calcein assay probably because of its lower membrane permeability. Interestingly catechin and epicatechin behave differently, although they are stereoisomers. Taken together our preliminary data suggest complicated interactions between the tested compounds and the complex drug binding pocket of Pgp.

Support: OTKA grant K124815 and GINOP-2.2.1-15-2017-00079

Keywords: *P-glycoprotein, flavonoids, ATPase activity*

P-166

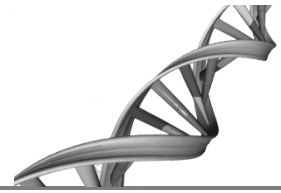
Advances of bioinformatic methodology in archaeogenetics

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Although archaeogenetics is not a new research topic itself, its advances and successes only became substantial and well recognized in the last few years. This can be acknowledged by the introduction of Next Generation Sequencing (NGS) in the field, which led to large-scale genome wide studies of past populations. However, the highly increased amount of data demands extensive bioinformatic background. Continuous development of bioinformatic methodology is obligatory for the competent analysis of growing datasets,



where our laboratory aimed to take a prominent role. Here we present the general methodology of NGS analysis of archaeogenetic datasets, including the classical approaches and the latest trends as well. Moreover, a customized bioinformatic pipeline is presented along with the comparison of information obtainable from both NGS and classical molecular genetic methods. Also, as a case study, we present a dataset comprising 22 individuals from the Bronze Age (2000-1500 BCE) Hungary, where we utilized both techniques, and we show how these methods could complement each other.

Keywords: *ancient DNA, bioinformatics, NGS, palaeogenomics*

P-167

Chromosome 22q11 copy number variations in pediatric and adult patients with congenital heart defects

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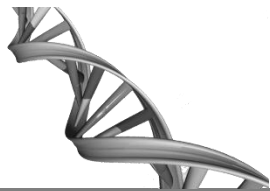
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Congenital heart defect (CHD) is the most common form of birth defects and approximately 1% of newborns are affected. Copy number variants (CNVs) are important genetic contributors to CHDs, usually in the presence of additional birth defects and developmental delay. Chromosome 22q11 microdeletion syndrome is often associated with congenital heart defects. It is the most common human microdeletion syndrome and occurs in approximately one in 4000 live births in the general population. 22q11 CNVs are caused by non-allelic meiotic recombination events in the flanking low copy repeat regions (LCRs), labeled A-H.

Our aim was to evaluate the prevalence of 22q11 CNVs in Hungarian CHD patients. 175 blood samples were obtained from children and adults with CHDs (tetralogy of Fallot, septal defects, stenosis or coarctation of the aorta, transposition of the great arteries and complex defects). Samples were analyzed using multiplex ligation-dependent probe amplification (MLPA) to detect microdeletion or microduplication in the given chromosomal region.

CNVs were detected in overall ten cases (5,7%): typical microdeletions (between LCRs A and D) - also known as DiGeorge syndrome - in six cases, microduplications (in LCR A-D and LCR F-H) in two cases and combined CNVs (microdeletion in LCR A-D with microduplication in LCR E-H and microdeletion in LCR C-D with microduplication in LCR D-E) in two cases. Positive MLPA results were confirmed with conventional cytogenetic



methods. Genotype-phenotype comparison and family screening was also performed. In three families the CNVs were also detected in other family members.

The diagnosis of 22q11 microdeletion/microduplication syndrome may be challenging due to its variable clinical manifestation. Thus, systematic genetic testing of CHD patients may be beneficial in setting up the diagnosis and contribute to the proper management of these patients.

Keywords: 22q11 microdeletion, copy number variation, congenital heart defect, MLPA

P-168

Computational and functional analyses of angiogenin mutations identified in Hungarian patients with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of motor neurons leading to paralysis and death 3–5 years after disease onset. Mutations in the *angiogenin* (*ANG*) gene are known to be associated with both familial and sporadic amyotrophic lateral sclerosis (ALS). The angiogenin protein belongs to the pancreatic ribonuclease superfamily, and it plays an important role in rRNA biogenesis and cellular proliferation in addition to its crucial role in inhibiting protein translation by cleaving tRNA. The majority of disease-causing mutations of *ANG* result in loss of either ribonucleolytic activity, nuclear translocation activity or both. We sequenced *ANG* gene from a total of 136 sporadic ALS patients and 112 healthy controls of Hungarian origin. Mutation screening revealed a mutation located in the signal peptide (M-24I) and two mutations that affect the mature protein (R33W, V103I). The R33W mutation, which has not been previously detected in ALS patients, affects the key amino acid of the nuclear translocation signal of the angiogenin protein. To elucidate the role of the R33W mutation in the disease mechanism, computational and functional analyses were performed. Molecular dynamics simulation of the R33W protein was carried out using AMBER 14 software to model structural and dynamics changes in the functional sites compared to the wild-type protein. Ribonucleolytic assay and nuclear translocation assay of the R33W angiogenin protein were performed to investigate the enzymatic and nuclear translocational activity of



the mutant protein. Molecular dynamics simulations suggested that the R33W mutation results in partial loss of ribonucleolytic activity and reduced nuclear translocation activity. The functional assays of the R33W angiogenin protein confirmed the molecular dynamics results. In the Hungarian ALS population, the observed frequency of *ANG* mutations was 2.9%, which is higher than previously reported for sporadic cohorts. Our findings confirm the relevance of *ANG* mutations in ALS and points out the variability among populations of different ethnic origin.

Keywords: *amyotrophic lateral sclerosis, angiogenin, mutation screening, molecular dynamics, nuclear translocation, ribonucleolytic activity*

P-169

Maternal lineages from 10th century commoner cemeteries of the Carpathian Basin

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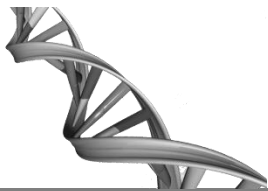
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The examination of ancient DNA (aDNA), extracted from archeological remains, can reveal the origin and relationship of individuals and populations. We set out to clarify the genetic origin of the conquering Hungarians (Conquerors) with aDNA methods. Recently we described the maternal origin of samples from the Karos-Eperjesszög 1-2-3 and Kenézlő graveyards, representing the early Conqueror elite. The highest resolution whole mitogenome analysis from these remains indicated that the origin of their maternal lineages can be traced back to distant parts of the Eurasian steppe: One third of the them were derived from Central-Inner Asia and their most probable ultimate sources were the Asian Scythians and Asian Huns, while the majority of the lineages most likely originated from the Bronze Age Potapovka-Poltavka-Srubnaya cultures of the Pontic-Caspian steppe. These results raise the question as to what extent can our findings be generalized to the entire Conqueror population? To answer this question we embarked on completing our Conqueror mitogenome database with sequences from other contemporary cemeteries, which predominantly belonged to 10th century commoners.

We chose the large commoner cemeteries of Sárrétudvari-Hízóföld, Püspökladány-Eperjesvölgy, Vörs-Papkert-B, Homokmégy-Székes and Magyarhomorog all of which were



used for a long time, starting from the 10th century characterized by pagan ritual burials continuing into the 11th- early 12th centuries with Christian tombs of the Árpadian-Age. We selected a representative number of samples, usually 20-30/cemetery, based on archaeological and anthropological evaluation both from the early pagan and late Christian parts of these graveyards. The aDNA was extracted from pars-petrosa then Illumina-specific sequencing libraries were prepared, provided with a dual index combination. Shotgun sequencing was used to determine the endogenous aDNA content of the samples, and samples with similar endogenous DNA-content were enriched in groups using hybridization techniques with our own mitogenome bait kit. After NGS sequencing the mitochondrial haplogroup of individuals was determined, followed by phylogenetic and population genetic evaluation.

Our sample selection makes it possible to find out what is the genetic connection between the smaller elite and larger commoner cemeteries of the Conqueror era, as well as to determine the relation between different commoner cemeteries. We may also be able to answer if the older Christian parts represent descendants of the original 10th century population or belong to newcomers relocated from other parts of the kingdom.

Keywords: ancient DNA, mitogenome, NGS sequencing

P-170

A comprehensive analysis of the contribution of genotoxic stress conditions to the development of antibiotic resistance genes in *Mycobacteria*

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The fidelity of replication is essential for every organism to maintain their genome integrity. *Mycobacterium tuberculosis* (*M. tuberculosis*), the causative agent of tuberculosis has an especially low *in vitro* mutation rate even though it lacks the mismatch repair enzymes¹. However, *in vivo* samples from patients often exhibit resistance to more than one drugs², caused exclusively by various single-nucleotide mutations (SNP). Based on these contradictory observations, our aim is to investigate several key factors contributing to the fast adaptability when genotoxic stress conditions occur. Such key factors are i) the level of the deoxyribonucleoside 5'-triphosphate (deoxynucleotide, or dNTP) pool ii) the activation of the DNA repair enzymes and iii) the mutation rate and spectra of the genome (Fig 1).

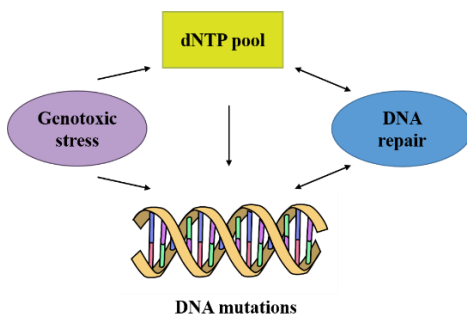


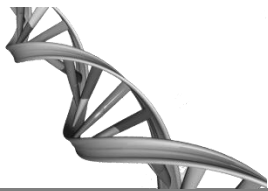
Figure 1: The context of the fast adaptation to genotoxic stress: besides direct mutational effects on the DNA molecule, deoxynucleotide pool and DNA repair activation should also be investigated to understand changes in the mutation rate

As a valid model of *M. tuberculosis*, we used the non-pathogenic *Mycobacterium smegmatis*, since they share the same DNA metabolic and repair routes. Several stresses are important in the lifecycle of *M. tuberculosis*: these are oxidative stress, ionizing radiation, nutrient starvation, alkylating agents, hypoxia and currently used antimycobacterial drugs. The dNTP concentration following genotoxic treatments were measured applying a fluorescent-based DNA-polymerization method. The level of the DNA repair enzymes was approximated with the corresponding mRNA level via a qPCR based method and last, the mutation rate and spectra was evaluated via whole-genome sequencing. Our results showed, that the abovementioned processes do change upon genotoxic stress treatments, which highlight the importance of all of these investigations when an adequate model of the antibiotic resistance genes development is constructed.

Funding: EMMI FIKP-BME Biotechnology grant

References:

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- [2] Sun, G. et al. *J. Infect. Dis.* 206, 1724–1733 (2012)



P-171

A new phenotype in the thymus of children operated with atrial septal defect

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The epithelial network of thymus, responsible for the development of T cells has endodermal origin which requires the presence of mesenchyme developing from the neural crest as it is demonstrated on animal models. Hematoxylin-eosin staining shows the histological units of the human thymic tissue as cortex and medulla but the anti-pancytokeratin immunostaining reveals two sub-compartments in the organ described as keratin negative area (KNA) and keratin positive network (KPN) of epithelial cells (Bódi et al., 2018). According to these results we conclude that the structure of the human thymus is much more complex than previously described. The Foxn1 transcription factor known to be specific to the thymic epithelial cells is expressed during embryogenesis by several other cell types in the liver, lung, kidney and urinary tract [Itoi et al., 2007], and during the migration and maturation of neural crest cells participating in thymus development [Nowell et al., 2007]. According to our present knowledge, Foxn-1 also plays a role in the development of thymus vascularization [Mori et al., 2010].

Human thymuses were obtained from children under 6 years of age from cardiac surgical intervention and from children diagnosed with sudden infant disease syndrome (SIDS) as a control group. The thymic lobes were analysed on frozen sections by numerous monoclonal antibodies and histological methods (light, fluorescence, confocal and transmission electron microscopy).

Based on our observations, we found evidence that Foxn1 is expressed in the nucleus of cytokeratin positive cells and in the cytokeratin negative cells of the KNA. In children where congenital heart disease was classified as septal defect we could not observe cytokeratin and Foxn1 expression in the cortex of the thymuses. Additionally, our electron microscopical studies show that the blood vessels are obliterated in the cortical area of these thymuses.

We observed recently an interesting new phenotype in the thymus of children operated with atrial septal defect. It is assumed that the abnormally developed thymus is a parallel symptom to the septal defect and the real cause of this phenomenon could be the defective regulation of signaling processes in the neural crest cells.

Keywords: *thymus and heart development, atrial septal defect, cortical thymic epithelial cell abnormality*



P-172

A new zebrafish model for Pseudoxanthoma elasticum (PXE)

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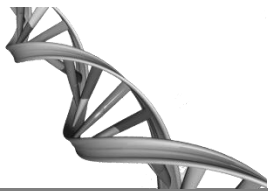
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Calcification of various tissues is a serious global issue, which has been associated with ageing, cancer and autoimmune diseases. There are both environmental and genetic factors behind this phenomenon and exploring and understanding them is essential for the development of efficient therapeutic techniques.

Pseudoxanthoma elasticum (PXE) is a rare genetic disease, resulting from the dysfunction of *ABCC6*, a transport protein found in the membranes of cells. It is characterised by early onset calcification in various tissues (e.g. eyes, skin, arteries) and currently it has no cure, treatments target symptoms only. Preclinical studies of PXE have been successful in mice, proving the usefulness of animal models for the study of the disease.

Zebrafish (*Danio rerio*) has recently become one of the most popular models of genetic diseases, because of its short generation time and the relative ease to disrupt gene function with novel genome engineering techniques. Additionally, it is ideally suited for the cheap and efficient testing of drug-precursors in preclinical trials.

Here we present a new zebrafish model for PXE. By resolving some ambiguous assemblies in the zebrafish genome, we show that there are two functional and one non-functional paralogs for *ABCC6* in fish (*abcc6a*, *abcc6b.1* and *abcc6b.2*, respectively). We created single and double mutants for the functional paralogs and characterized their calcification defects with a combination of techniques.



P-173

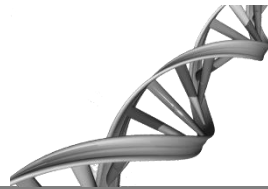
ABCG2 mutations found in gout patients have various effects on the expression of the protein

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ABCG2 is a member of the ABC-type multidrug transporter protein family and plays an important role in the protection of our cells by exporting toxic substances. In our research we focused on the role of ABCG2 in gout. Increased serum uric acid level (hyperuricaemia) is the precondition of the development of gout and ABCG2, among many other molecules, actively transports urate. The ABCG2 protein is expressed in the proximal tubules of the kidney, in the intestine, and in several other tissues. ABCG2 maintains the balance of uric acid secretion and absorption, which, in the case of a dysfunctioning ABCG2 protein, may shift towards absorption, and lead to hyperuricaemia and gout. According to our previous study, there are ABCG2 polymorphisms and mutations that are more common among gout patients. By further examining these mutant variants we suggest that in these cases the dysfunction or lower expression level of ABCG2 may play a role in the development of the disease. It is important to characterize these clinically relevant mutations, as a directed genetic analysis can help to find the best personal treatment for the patients. ABCG2 is also present in the blood-brain barrier and has many substrates that are commonly used drugs in medicine. By knowing that a patient has a dysfunctional variant of the ABCG2 protein, a personalized treatment with an ABCG2-substrate drug should be used. A recent study showed the presence of as yet unknown missense ABCG2 mutations in gout patients, while no information on the cellular effects of these mutations were presented. Therefore, we have decided to examine these mutant variants. First, we have transfected HEK cells with a vector containing the mutant variants and examined the protein expression levels, transport activity and localization of ABCG2. To better understand the effect of these mutations, we have generated stable HeLa cell lines that express the mutant ABCG2 variants and performed further experiments on these cell lines. Our data show greatly variable functional effects of these previously uncharacterized mutant variants.

Keywords: *ABCG2, transporter, mutations*



P-174

Acquired cellular stress resistance in the absence of heat shock protein induction

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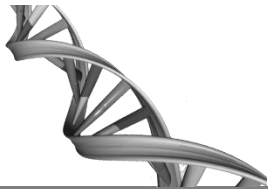
To understand how different organisms react and adapt to environmental changes, especially during pathological conditions, it is crucial to investigate the cellular stress response. The main focus of research aims at the classical theory claiming protein denaturation as the sole cause for heat shock (stress) response. However, protective heat shock proteins (HSPs) can also be induced under conditions in which no protein denaturation is observed. Considerable evidence has been accumulated in favor of the "Membrane Sensor Hypothesis" which predicts that the level and ratio of HSPs change as a result of alterations to the plasma membrane. Our goal was to delineate this phenomenon under mild fever-type conditions and to characterize the acute effect of heat on membrane organization and lipid composition for the better understanding of how cells are sensing and adapting to heat.

We analyzed the effect of short (1h) and long (12h) 40°C heat treatments on membrane organization, HSP synthesis and localization in mammalian cells (Chinese Hamster Ovary). We have also studied how cells acquire cross-tolerance, that is their ability to respond to one stressor (heat) and at the same time acquire tolerance to a second heterologous stressor (benzyl alcohol). Our experiments have revealed two distinct protection windows depending on the dose of stress. We have identified a novel mild type of cellular eustress domain, where cells adapt to fever-type mild heat by maintaining membrane homeostasis, activating lipid remodeling, membrane domain reorganization and redistributing preexisting chaperone proteins. Membrane structural changes also occurred at two levels. Image FCS data revealed that under mild stress conditions cells can cope with the stress by preserving the fluidity (lateral diffusion of lipid and protein probes) of their plasma membranes by membrane domain reorganization.

Surprisingly, this mild stress response led to acquired stress resistance without the induction of HSPs. At higher stress levels additional defensive mechanisms are activated, including classical HSP expression, which contribute to an extended stress memory and acquired stress tolerance. The general validity of the above findings will be discussed.

Acknowledgements: *This research was supported by the Hungarian Basic Research Fund (OTKA ANN112372) and the Ministry for National Economy (GINOP-2.3.2-15-2016-00001, GINOP-2.3.2-15-2016-00060).*

Keywords: *stress management, membrane, lipid, HSP, cross-tolerance*



P-175

Alterations of PACAP signalling in testis of Alzheimer's disease modelling mice

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a 38 amino acid neuropeptide which is detectable in the CNS and various peripheral organs. PACAP is ligand of G protein coupled PAC1, VPAC1 and VPAC2 receptors and induces the activity of adenylate cyclase. Therefore, expression of various genes happens by activation of PKA and transcription factors. In peripheral organs, the highest concentration of PACAP was detected in testis. The peptide plays important role in differentiation and maturation of male gonad and it is essential in male reproduction by supporting spermatogenesis. Fertility can be harmed by lack of PACAP.

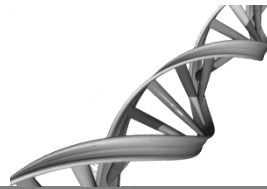
Alzheimer's disease is the most common cause of dementia in elderly humans. It has been demonstrated that expression of PACAP decreases in Alzheimer's disease. On the other hand, the neuropeptide has important protective effect against the progression of dementia. Similarly to the brain, pathologic amyloid protein can be formed and accumulated in the testis in Alzheimer's disease.

The main goal of our research was to examine PACAP signalling in testis of Alzheimer's disease modelling mice. We compared the testicles of wild type and PDAPP transgenic mice by performing immunohistochemistry staining, RT-PCR and Western blot analysis.

Decreased expression of PAC1 receptor, phosphorylated PKA, PP2A and phosphorylated Sox9 was detected in testes reflecting on reduced activity of PACAP signalling. Another notable observation was the abnormal collagen type IV content of basal membrane in seminiferous tubules of PDAPP mice compared with that of the testis of wild type mice.

Based on our results and previous investigations it may be hypothesized that examination of signalling pathways in testis can be appropriate for modelling pathologic conditions of CNS in Alzheimer's disease.

Keywords: PACAP, PKA (*protein kinase A*), Sox9, A β (*amyloid β*), Alzheimer's disease



P-176

Atypical *in vitro* chondrogenesis upon Transient Receptor Potential Vanilloid 4 modulation

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The gain of function mutations of the calcium permeable TRPV4 ion channel causes skeletal dysplasia. Abnormal calcium homeostasis, protein-protein interactions and irregular gene expression of chondrocytes may induce these skeletal defects, but the background molecular mechanisms which generate the phenotypic changes have not been revealed yet. Therefore, we aimed to monitor the effect of TRPV4 activity in chondrogenic micromass cultures to unveil the reasons behind these defects.

Multinodular chondrogenesis spontaneously takes place *in vitro* in high density mesenchymal micromass cultures. Elongated, mainly less differentiated cells occupy internodular (IN) regions, while nodular (N) regions are populated by spherical and young chondrocytes. The ratio of these two regions changes during the chondrogenic differentiation process. We monitored the spatio-temporal presence of TRPV4 in these cultures, the morphology of cultures and the proteoglycan content of the matrix together with the proliferation rate of the cells. The intracellular calcium changes of differentiating cells was analysed during short- and long-term application of TRPV4 ligands.

Gradually elevating TRPV4 protein level characterizes these cultures up to the 10th day of culturing, then its expression seems to decrease. The distribution of TRPV4 protein is different in the N and IN regions. The long-term treatments of TRPV4 pharmacons result in distinct and significant morphological differences between nodular and internodular regions. Application of the TRPV4 ligands generated detectable calcium-concentration changes in differentiating cells. On the basis of the characteristics of the Ca-signals, differentiating cells can be classified into groups and it seems that various responses to the TRPV4 ligands are in correlation with the ratio of the N /IN regions, thereby the process of chondrogenesis.

Supported by the ÚNKP18-3-III New National Excellence Program of the Ministry of Human Capacities, DETEP and Hatvani István Szakkollégium.

Keywords: *chondrogenesis, intracellular calcium-measurements, micromass*



P-177

Base excision repair in rheumatoid arthritis - a correlation of DNA repair efficiency with the BER genes polymorphisms

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Rheumatoid arthritis (RA) is a systemic, inflammatory disease of the joints and surrounding tissues. RA manifests itself with severe joint pain, articular inflammation and oxidative stress. RA is associated with certain types of cancer. We have assumed that increased susceptibility to cancer of RA patients may be linked with genomic instability induced by disturbed DNA repair of oxidative DNA lesions by BER.

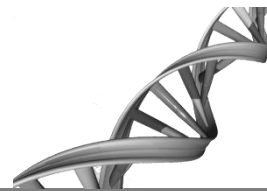
In the present work we determined the level of basal and oxidative DNA damage and the kinetics of removal of DNA damage induced by tert-butyl hydroperoxide in peripheral blood mononuclear cells (PBMC) of 20 RA patients and 20 healthy individuals. The data from DNA damage and repair study were correlated with the genotypes of functional polymorphisms of the key BER genes including XRCC1, hOGG1, UNG, SMUG1, TDG, MBD4, MUTYH.

DNA damage and repair were evaluated by alkaline single cell gel electrophoresis (comet assay). The genotypes of the polymorphism were determined by TaqMan SNP Genotyping Assays.

We observed an association between RA occurrence, impaired DNA repair in PBMC and the polymorphisms of XRCC1, and hOGG1 genes. Therefore, our result suggest that polymorphism of the XRCC1, and hOGG1 genes may be linked with RA by the modulation of the cellular response to oxidative stress and these polymorphisms may be a useful additional marker in this disease along with the genetic or/and environmental indicators of oxidative stress.

Acknowledgement: This work was supported by the National Science Center (Poland) - UMO-2017/25/B/NZ6/01358.

Keywords: rheumatoid arthritis, BER, polymorphisms



P-178

Clinically used PARP inhibitor olaparib protects against oxidative stress-induced epithelial barrier dysfunction *in vitro*

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Background: Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract. Although the exact pathogenesis of IBD is not clear yet, number of factors (genetic, environmental, microbial, and immunological) have been associated with the development of IBD. Overactive immune response can distract the intestinal epithelium which results in increased permeability, amplification of inflammatory response and tissue damage. Oxidative stress, which plays a key role in the development of inflammation can cause the excessive activation of poly (ADP-ribose) polymerase (PARP), which leads to cell death and stimulates inflammation. Olaparib is a clinically approved and available potent PARP inhibitor for cancer therapy. In this project we examined the effect of olaparib on oxidative stress-induced epithelial barrier disruption and mitochondrial dysfunction.

Methods: Caco-2 colon epithelial cell monolayer was used as an *in vitro* model of experimental intestinal epithelial barrier. For monitoring epithelial barrier integrity we used a real-time cell electric impedance sensing system (RTCA-DP, xCELLigence) and a fluorescein isothiocyanate (FITC)-conjugated dextran (4 kDa) flux assay in a transwell plate. In Caco-2 cells H₂O₂-induced reactive oxygen species (ROS) production was determined using dihydrorhodamine 123 fluorescent dye. Cell viability was detected by MTT assay and Muse Annexin V & Dead Cell Assay. To assess mitochondrial dysfunction, oxygen consumption and extracellular acidification rates were measured by Seahorse XFp Analyzer.

Results: Olaparib significantly increased the normalized Cell Index of H₂O₂-treated cells and reduced the flux of FITC-dextran across the monolayer, so olaparib protected the integrity of Caco-2 monolayer from H₂O₂-induced damage. Morphological changes were also detected by microscopic imaging. Olaparib improved mitochondrial function, reduced H₂O₂-induced ROS production and apoptosis in Caco-2 cells.

Conclusions: We have demonstrated that clinically approved PARP inhibitor olaparib enhances intestinal epithelial barrier integrity via the reduction of mitochondrial dysfunction. Based on these result our future aim is to test the efficacy of olaparib in experimental colitis.

Acknowledgment: GINOP-2.3.2-15-2016-00049, GINOP-2.3.3-15-2016-00025

Supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Supported by the ÚNKP-18-4. New National Excellence Program of the Ministry of Human Capacities.



Keywords: olaparib, intestinal epithelial barrier, mitochondrial dysfunction, inflammatory bowel disease



P-179

Decorin protects cardiomyocytes against doxorubicin-induced cytotoxicity

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Doxorubicin is one of the most effective antitumor agents, however, its widespread use is limited by its well-known cardiotoxic effect. Due to improvement in the survival of cancer patients, the long-term side effects and complications of various cancer therapies are becoming more relevant. It has a great importance to investigate and find substances capable of reducing the cardiotoxic effects of doxorubicin. In our study, we aimed to investigate the effect of decorin, a small leucine-rich proteoglycan on doxorubicin-induced cardiocytotoxicity.

Primary cardiomyocyte cultures were isolated from 1-3 days old newborn Wistar rats. The potential effect of various concentrations of decorin (0.25-30 nM) in the doxorubicin-induced cytotoxicity model was examined in two experimental arrangements. To investigate the short-term effect of decorin 30 min pretreatment, while for looking at long-term effects 20 hr decorin pretreatment was applied prior to a 24 hr treatment with 300 ng/ml doxorubicin. Decorin treatments were maintained during the doxorubicin challenge. Cell viability was measured by calcein assay.

We found that doxorubicin treatment significantly decreased the viability of cardiomyocytes by $26 \pm 7\%$ (one-way ANOVA, $p=0.0122$). We have shown that doxorubicin-induced cell death was significantly attenuated by 0.5 nM decorin in case of both 30 min and 20 hr pretreatments (one-way ANOVA, $p=0.032$ or $p=0.042$). Decorin treatment did not affect cell viability in stress-free conditions.

Our results indicate that decorin treatment exerts a dose-dependent cardiocytoprotective effect in our doxorubicin-induced cytotoxicity model. These data may be a good starting point for our research into the attenuation of long-term cardiac side effects of chemotherapeutic agents.

Keywords: *decorin, doxorubicin, cardiotoxicity*



P-180

Diet-induced hypercholesterolemia is associated with alterations in cardiac gene expression profile

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Hypercholesterolemia is one of the major risk factors of ischemic heart diseases due to its proatherogenic effect. Beyond its impact on the vasculature, direct cardiac consequences are also responsible for the adverse effects of elevated blood cholesterol level such as increased oxidative/nitrosative stress, slight systolic and diastolic dysfunction and disturbed ischemic adaptation.

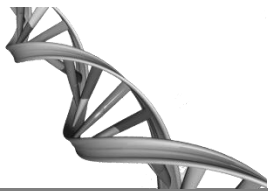
The aim of the present study is to identify mRNAs whose altered expressions are correlated with the direct cardiac consequences of hypercholesterolemia. Furthermore, expression pattern of the well-known posttranscriptional modulator miRNAs is also investigated.

Male Wistar rats were fed with a laboratory chow supplemented with 2% cholesterol and 0.25% sodium-cholate hydrate for 8 weeks, while the control group was fed with standard rat chow. At the end of the diet period serum LDL, HDL, total cholesterol and triglyceride levels were measured. Total RNA were isolated from left ventricles and next generation sequencing was performed in order to investigate cardiac gene expression.

Elevation of serum cholesterol level was observed due to cholesterol-enriched diet (4.01 ± 0.28 vs. 1.56 ± 0.07 mmol). In the hearts of hypercholesterolemic rats 54 mRNA and 13 miRNA transcripts showed significantly altered expression compared to the control group. Among these transcripts, mRNAs related to intracellular cholesterol transport and prooxidant functions showed upregulated expression, while transcripts of genes important in cardiac contractility and stress adaptation showed both down- and upregulated expression. Several mRNA transcripts are putative targets of certain miRNAs showing altered expression due to hypercholesterolemia, however, many of the differentially expressed miRNAs have not been implicated in relation with the adverse cardiac consequences of hypercholesterolemia so far.

Based on our results we may conclude that diet-induced hypercholesterolemia markedly influences cardiac gene expression pattern.

Keywords: *hypercholesterolemia, mRNA, miRNA*



P-181

***dkc1* mutations are associated with changes in *tp53* expression and growth impairment**

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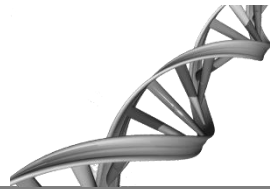
Several important studies during the past few years have provided unequivocal evidence about the important role that posttranscriptional epigenetic modifications play in the regulation of gene expression. Paradoxically, despite of the growing interest in such modifications, we still know very little about the most abundant posttranscriptional modification of the eukaryotic cells, pseudouridylation. Previous studies from different organisms suggest that several RNA types can be targeted by pseudouridylation and the deficient pseudouridylation of rRNA will result in malfunctioning ribosomes, causing classic ribosomopathies.

Pseudouridylation is catalyzed by pseudouridine-synthase enzymes (PUS) that can have snoRNA-dependent or -independent activities. The *DKC1* gene encodes the major snoRNA-dependent PUS enzyme, dyskerin. This protein is highly conserved, widely expressed and available data suggests that it functions in two distinct complexes. It has an active role in telomerase stabilization and maintenance, but more importantly, associates with hundreds of H/ACA small RNAs to generate a multitude of functionally distinct ribonucleoproteins (RNPs) which will catalyze the pseudouridylation of specific RNA residues.

Recently we created an allelic series in zebrafish for the *dkc1* gene, with multiple null and hypomorphic alleles. Using these mutants we can reveal the biological consequences of dyskerin impairment and explain how translational dysfunction through ribosome shortage or deficiency leads to disease. Combining experimental biological assays with bioinformatics we aim to provide a functional explanation for the emergence of phenotypes resulting from dyskerin loss-of-function.

Here we provide evidence that our hypomorphic *dkc1* mutants have abnormal pigmentation and show significant growth impairments. Importantly, similar growth defects are often associated with erroneous translation in other species and a recent studies suggest that many ribosomal protein mutations in zebrafish also lead to growth defects.

The whole transcriptome analysis of null mutants shows that the differentially expressed genes have role in the modification of RNAs and the biogenesis of ribosomes. These results suggest that dyskerin-deficient cells try to compensate for the lack of functional ribosomes.



In addition, we detect the upregulation of the short, anti-apoptotic isoforms of *tp53*, which can explain, at least in part, the developmental abnormalities of these mutants. We hypothesize that the mutant phenotype is caused by the impaired control of IRES-mediated translation, based on the faulty rRNA biogenesis and defective ribosomes.

P-182

Evaluation of *BDNF* and *HSP70* gene expression in the pathogenesis of multiple sclerosis in a Polish population

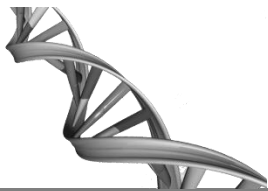
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Multiple sclerosis (MS) is a chronic disease of the central nervous system commonly diagnosed in people aged 20-40. It is estimated that the incidence of multiple sclerosis in Poland ranges about 2,000 new cases reported each year. Despite many studies, the etiology of MS is not fully understood. The aim of this study was an evaluation of BDNF (brain-derived neurotrophic factor) and *HSP70* (heat shock protein-70) gene expression levels as a molecular mechanism potentially involved in multiple sclerosis (MS) development. We postulate that disturbances in mRNA signaling pathway may conduct to changes in expression of genes encoding proteins involved in neuroprotection in MS. The study group consisted of 44 patients with multiple sclerosis and matched 30 subjects from control group. We evaluated the expression level of BDNF and *HSP70* genes using real-time qPCR method. The expression level of both genes was lower in patients with multiple sclerosis. The results showed significantly lower level of mRNA of BDNF as well as *HSP70* in whole blood of MS patients in comparison to control group ($P=0.001$, $P=0.026$, respectively). In conclusion, the decreased expression level of BDNF and *HSP70* genes may suggest the impairments in the regulation of neuroprotective factors expression in the pathogenesis of multiple sclerosis patients.

This work was supported by grant OPUS no. 2017/27/B/NZ7/02527 from National Science Center (NCN) Poland.

Keywords: *multiple sclerosis, gene expression, BDNF, HSP70*



P-183

Gene expressional changes in the dorsomedial prefrontal cortex of suicide victims – an RNA sequencing study

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Recent studies revealed that the resting state (RSN) network is a critical element in psychiatric disorders, especially in mood disorders. Alterations of the synaptic plasticity in the RSN may contribute to the development of mood disorders. The dorsomedial prefrontal cortex (DMPFC) is a major component of the RSN network. Still, gene expressional alterations related to depression has not been performed in this cortical area. Therefore, in the present study, we addressed RNA level changes in the DMPFC in relation to depression in suicide victims by RNA sequencing. Subjects were assigned into three groups: control subjects without psychiatric disorders, depressed suicide victims, and suicides without any known signs of chronic depression. Brain tissue collections were performed within 2-10 hours after death. Cluster analysis suggested that the suicide and depressed suicide groups were quite similar, while both of them differed markedly from the control subjects. We found significant changes in the expression of over 200 genes between the control and suicide / depressed suicide groups. One of the significantly expressed genes between the control and suicide / depressed suicide groups was glial fibrillary acidic protein. Since this gene may have great importance as an indicator of astrocyte cell number, we validated its change by qRT-PCR and confirmed its reduction in suicide victims. The altered pathways were also investigated using gene set enrichment analysis. Genes belonging to oxidative phosphorylation, endocrine cannabinoid signalling and cytokine receptor pathways were over-represented in suicide victims compared to controls suggesting that these processes are involved in the suicidal behaviour. Altogether, the data imply extensive gene expressional alterations in the DMPFC related to depression and suicidal behaviour, which indicates an important role of this cortical area during such pathological conditions.

Support: NKFIH NAP program 2017-1.2.1-NKP-2017-00002 for ER and MP, and NKFIH-4300-1/2017-NKP_17 for AD.



P-184

Investigating the effects of a PARP inhibitor, HO-3089, on signaling pathways in monocrotaline-induced pulmonary hypertension in rat lung tissue

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Pulmonary hypertension (PH) is a rare and progressive disease with unclarified pathogenesis. Some characteristics for PH are abnormal proliferation of smooth muscle cells and endothelial cells in the lung arteries which results in pulmonar vascular hypertrophy and structural remodeling. Chronic inflammation is also present in patients suffering from PH.

Up to this day there is no effective cure for PH, however different treatment options are available (endothelin receptor antagonists, vasodilators, anticoagulants).

The monocrotaline (MCT)-induced model of PH is one of the most frequently used rodent model. MCT is a prodrug, which transformation to its active form is catalyzed by cytochrome P450 in the liver. Based on previous results MCT has harmful effects on pulmonary endothelial cells, but the specific mechanism leading to PH and involved in the progression is still not known.

Poly(ADP-ribose) polymerases (PARP) are a family of enzymes that catalyze the transfer of ADP-ribose to target proteins. They have many functions in different cellular processes, their role in DNA damage repair being one of the most researched topic of interest.

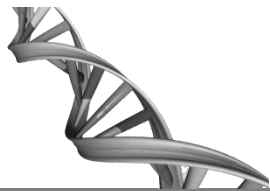
Increased ROS formation, and elevated oxidative stress are proved to be characteristics for PH. Olaparib which is the most widely used PARP inhibitor on the market has shown protective effects against oxidative stress in rat cardiomyocytes and LPS-induced acute lung injury.

In our research the effects of another PARP inhibitor, HO-3089 were investigated on MCT-PH rat model. As the PI-3K-Akt pathway and MAP kinase pathway has significant roles in vascular remodeling investigating the changes of these pathways were our primary goal. Protein nitrosylation, heart/body weight ratio and survival were also measured.

Results showed that HO-3089 had positive effects on survival, heart/body weight ratio, and its effect on MAP kinase and PI-3K-Akt pathways implicate that HO-3089 might be a potential drug candidate in pulmonary hypertension therapy.

Acknowledgement: GINOP-2.3.2-15-2016-00049, GINOP-2.3.3-15-2016-00025

Keywords: *pulmonary hypertension, monocrotaline, cell signaling, PARP inhibitor*



P-185

Investigation of the activation of certain DNA repair enzymes in *Mycobacterium smegmatis* under stress conditions

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The cure of tuberculosis is often difficult due to the occurrence of antibiotics resistance. Interestingly, the causative agent of this disease, *Mycobacterium tuberculosis* develops antibiotic resistance genes exclusively by single nucleotide polymorphisms. Although these bacteria have very low *in vitro* mutation rate, mutations arise much more frequently *in vivo*¹. The reason behind it can be the genotoxic stress conditions that affect the pathogen during infection. To have a better understanding of the development of resistance genes under genotoxic stress conditions, we aimed to investigate the expression levels of DNA repair enzymes upon stress treatments. We hypothesize that the emergence of drug resistance is in connection with specific changes in the DNA repair pathways upon genotoxic stress (Figure 1).

As *Mycobacterium tuberculosis* is able to spread in aerosol droplets keeping its infectivity to humans, we identified UV radiation in the atmosphere as a natural environmental stress factor affecting this pathogen. We expect that a specific pattern of DNA repair enzymes are activated to overcome the eventual genetic damage.

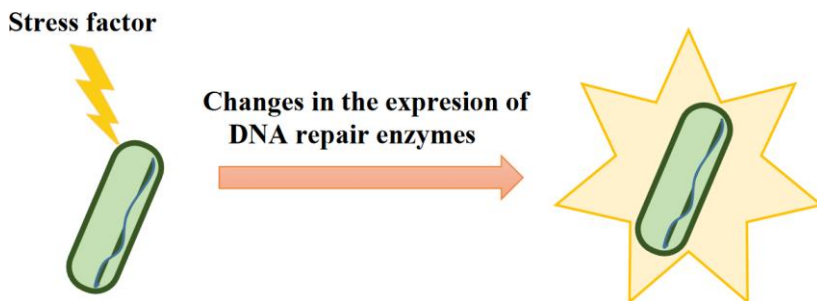


Figure 1: According to our hypothesis, under genotoxic stress conditions, the expression of certain DNA repair enzymes is altered and is characteristic to antibiotics resistance

The stress treatments were executed on the non-pathogenic *Mycobacterium smegmatis* model organism of the pathogenic agent of tuberculosis. The two bacteria possess almost the same DNA repair system including the UV damage repair enzymes². Therefore, our results can be projected to *M. tuberculosis*, as well. Our results showed that there are several changes in the



expression pattern of the enzymes involved in DNA repair upon UV treatment. We are still in the process of data acquisition and analysis and will be able to synthesize our results in the near future.

Funding: EMMI FIKP-BME Biotechnology grant

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P-186

Lysophosphatidylcholine depletion in head and neck squamous cell carcinoma identifies MAGL and LYPLA1 as potential biomarkers

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Pathological characterization of head and neck squamous cell carcinoma (HNSCC) relies on morphological analysis. Apart from occasionally used HPV and EGFR testing molecular markers are not available that could help define resection margins and patient prognosis. Here we used MALDI mass spectrometry imaging to carry out an in-depth screening for localization and expression of potential protein and lipid markers in human tumor samples. Comparing expressions of low molecular weight proteins in healthy vs tumor tissue areas revealed limited differences such as for S100A8 and S100A9. More strikingly, lipid imaging unraveled numerous dramatic differences in healthy vs tumor tissue areas. Remarkably, a specific lipid-ion adduct of 519 Da was absent in tumorous areas of the tissue, which was identified by lipidomics as (palmitoyl)-lysophosphatidylcholine. Level of other lysophospholipid species (LPC, LPE, LPI, LPS, LPG) was also reduced in tumor regions, showing a strong pattern of potential biomarker value and pointing to a recently raised lysolipid and fatty acid dependence of tumor cells. Finally, based on immunohistochemical staining of a set of tumor samples, lysolipid degrading enzymes monoacylglycerol lipase (MAGL) and lysophospholipase A1 (LYPLA1) may be further considered clinically as tumor biomarkers.

Keywords: biomarker, lipid tumor marker, MS image screening



P-187

Maternal and fetal folate metabolism gene polymorphisms as risk factors for congenital heart disease

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Background: The folate metabolism pathway plays an important role in cardiac development. The related gene polymorphisms have been associated with congenital heart disease (CHD), however, the data are inconclusive. Our aim was to evaluate the MTHFR C677T/A1298C, MTR A2756G, and MTRR A66G polymorphisms as risk factors for CHD.

Methods: Genotypes were assessed in maternal and fetal sample groups collected from 20 vs. 20, and 19 vs. 19 case-control pregnancies, respectively. FRET-based RT-PCR followed by melting curve analysis was performed using polymorphism specific probes. Allele frequencies were determined and odds ratios (OR) were calculated for CHD in each case.

Results: There was no significant difference in the allele frequencies of MTHFR polymorphisms in the maternal case-control group. The MTR 2756G allele meant a minor risk for CHD: 0.22 vs. 0.15, OR= 1.65. Interestingly, the MTRR 66G allele appeared as a protective factor: 0.45 vs. 0.72; OR= 0.31. In the prenatal case-control group, the MTHFR 1298C allele turned out as a possible risk factor: 0.34 vs. 0.18, OR= 2.30. From the fetal side, carrying the MTRR 66G allele also reduced the risk of CHD: 0.50 vs. 0.63 OR= 0.58.

Conclusion: The investigated folate metabolism gene polymorphisms meant a risk in different degrees in case of maternal or fetal involvement. We have not found significant associations between the maternal MTHFR polymorphisms and CHD, which may be due to the protective effect of periconceptional folic acid supplements. Prenatally, the MTHFR 1298C allele turned out as a possible risk factor for developing CHD. The MTRR 66G allele appeared as a protective factor in both maternal and fetal scenario.

Keywords: *congenital heart disease, folate metabolism, MTHFR C677T/A1298C, MTR A2756G, MTRR A66G*



P-188

Mitochondrial exchange between myeloma cells and bone marrow stromal cells

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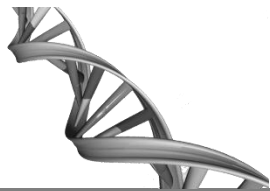
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Multiple myeloma (MM) is a malignant growth of clonal, long lived, immunoglobulin-producing plasma cells primarily located in the bone marrow (BM) and is the second most common hematologic malignancy. Neoplastic and stromal cells continuously interact with each other in the marrow and have the capacity to connect with both contiguous and distant cells through a wide range of communication networks, such as: paracrine signaling via cytokines and chemokines, transport through gap junctions, extracellular vesicle secretion (EV) and tunneling nanotubes (TNT). The different types of cell-to-cell interactions include the transfer of mitochondria between cells which can occur via direct cellular fusion, TNTs, or EVs. Because the acquisition of cancer drug resistance was associated with mitochondrial transfer in several solid tumors and in acute myeloid and lymphoid leukemias, a finding that relates to the role of mitochondria as a junction for many metabolic pathways, we investigated the possibility of a similar phenomenon between BM mesenchymal stromal cells and myeloma cells *in vitro*, using a stromal cell–malignant plasma cell co-culture system.

BM aspirates from MM patients were collected after written informed consent and ethical approval from the Institutional Review Board. Primary MM cells were isolated from BM aspirates via subsequent isolation of mononuclear cells from the samples of 24 newly diagnosed and 36 relapsed patients. In stromal cell–myeloma cell co-cultures the exchange of MitoTracker-labeled mitochondria was investigated by flow cytometry and confocal microscopy. We found a fast, bidirectional mitochondrial transfer between BM mesenchymal stromal cells and malignant plasma cells in MM. The exact mechanism is still not fully understood, however, TNTs are definitely involved in this process. Furthermore other cell-to-cell interactions must be involved as the transfer of mitochondria comes to pass very quickly. According to our findings, cytotoxic drugs are able to increase the velocity and intensity of the transfer process in a dose dependent manner, which may contribute to the chemical resistance of myeloma cells. Cell adhesion mediated drug resistance during myeloma therapy has been a phenomenon recognized two decades ago. Our results provide additional explanation for better understanding of the underlying biological processes.

This work was supported by grant NVKP_16-1-2016-0005 from the NKFLA, Hungary

Keywords: *mesenchymal stromal cells, mitochondrial transfer, myeloma multiplex, tunneling nanotubes*



P-189

Pancreatic cancer patient-derived 3D organoids to analyze extracellular vesicles

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* contributed equally to this work

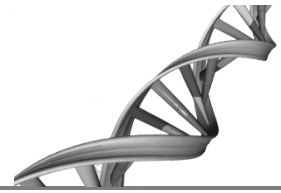
Pancreatic ductal adenocarcinoma (PDAC) is one of the most dangerous cancers with a 5-year survival rate of less than 8%. Despite the intensive research efforts, there is only a slow progress in the early diagnosis and treatment efficiency of this disease. PDAC consists of not only tumor cells, but also of a high amount of stroma, containing mostly cancer-associated fibroblasts (CAF). The intensive communication between the heterogeneous cell populations evolves the tumor niche and contributes to tumor progression.

Extracellular vesicles (EV) are membrane-surrounded particles that transfer biologically active molecules (e.g. lipids, proteins, RNAs) from the releasing to target cells in a pre-packaged form, thus representing a novel way of intercellular communication. Importantly, since tumor-derived EVs transport their tumor-specific cargo in a protected way in the body fluids, they hold a great promise for early tumor detection. However, the potential use of EVs as biomarkers is largely hindered by the lack of proper methods to study their release and cargo composition.

Most studies have so far used 2D cell cultures in EV research which do not properly model the *in vivo* situation. Here we prove that 3D pancreas-derived organoid technology is a proper method to study EVs. Interestingly, EV release from PDAC cell lines and pancreas organoids depends on the mutational status of tumor-driver genes. Surprisingly, we observed a massive EV secretion not only from PDAC cells and organoids, but also from stromal cells, suggesting that a substantial proportion of tumor-derived EVs in the body fluids of PDAC patients is of stromal origin. Furthermore, we show that EVs released from 3D and 2D cultures differ in their molecular cargo, highlighting the importance of model systems when analysing EV molecular composition.

Collectively, our results show that the 3D organoid technology is a promising model system to study EV functions in PDAC for diagnostic or therapeutic purposes.

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Fellowship (Hungarian Academy of Sciences; BO/01012/16/8). A.Z. and A.Á.S. are supported by the ÚNKP Program (ÚNKP-18-4-SE-98 and ÚNKP-18-2-I-ÁTE-15).

Keywords: PDAC, pancreas, adenocarcinoma, extracellular vesicles, organoid technology

P-190

The role of endogenous retroelements in neural aging in the canine model of human senescence

Sára Sándor¹, Kitti Tátrai², Kálmán Czeibert¹, Enikő Kubinyi¹ and Balázs Egyed²

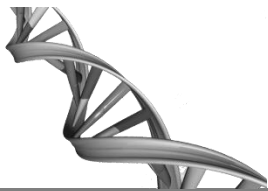
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An increasing body of experimental evidence suggests that age-related mobilization of endogenous transposable elements may be at least partly responsible for the genomic instability which will eventually lead to cellular senescence and aging of model organisms, from yeast to mice. The same mechanism might be present in aging human tissues, as general hypomethylation of Alu and LINE-1 (long interspersed nuclear element 1) retrotransposon sites were indicated in aging cohort studies. Although direct evidence about retrotransposon mobilization in human tissues have been limited so far, recent findings suggested that retrotransposon mobilization may be induced by tau pathology in patients with neurodegeneration. Dogs have been proposed as natural models of human age-related dementia, as they develop Canine Cognitive Dysfunction Syndrome (CCD), which shares many hallmarks with early stage Alzheimer's disease. Thus, the identification of retrotransposon mobilization in canine brain samples could help scientists to clarify the contribution of retroelements to dementia. So far, somatic activation of LINE-1 retrotransposons has not been investigated in dog brains. A main limitation is the accessibility of post mortem brain tissue from aged or demented dogs.

In our study, we used brain cortical samples from our new initiative, the Canine Brain and Tissue Bank (CBTB), to measure expression of the canine LINE-1 open reading frame 2 (ORF2), which is highly conserved between dogs and humans. The first step was the collection of suitable sample material. Samples were provided from the bodies of pet dogs, which had been previously donated by their owners with consent of the veterinarian who euthanized the animal for justified medical reasons. The tissue pieces intended for RNA and protein isolation were stabilized in appropriate buffers immediately after removal from the body, within 4 hours post mortem.

Our preliminary results showed that ORF2 was present as translated protein in the prefrontal cortex of both young and old dogs. A tendency of age-related increase in ORF2 levels was observed in our first western blot runs, which were based on 8 dogs. We will include additional samples in further western blots to specifically address the question whether aging or CCD causes an increase in ORF2 levels. We have also designed a SYBR



Green based RT-qPCR test to detect LINE-1 ORF2 mRNA, as we plan to assess possible differences in the transcriptional and translational regulation of LINE-1 (ORF2) expression. As a third step, we will quantify novel, somatic insertion events in genomic DNA. Thus, our research aims to provide a three-way approach to investigate the activity and mobilization of LINE-1 elements in dogs. Our long term goal is to provide a comprehensive description of LINE-1 activity in canine cortical samples of aged and demented dogs.

P-191

The role of HCN2 in the development of ileus

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Introduction: Approximately 30% of trauma patients develop ileus, a decrease or cessation of intestinal motility resulting in pathological distension of the intestinal wall. Mechanosensitive ion channels play a role in both the physiological and pathological response to mechanical stimuli.

Objective: The objective of this study was to determine if mechanosensitive ion channels play a role in the development of ileus and the response of the intestine to pathological stretch.

Methods: In a rodent model, we induced ileus by a combination of hemodilution and mesenteric venous hypertension. We measured intestinal function using an *ex vivo* organ bath system. We also measured smooth muscle myosin light chain phosphorylation and intestinal wall fluid content. We collected tissue for microarray analysis.

Results: Intestinal wall fluid content increased significantly, indicating the development of intestinal edema. Edema development resulted in distension of the intestinal wall, decreased intestinal smooth muscle contractile activity, and decreased smooth muscle myosin light chain phosphorylation. We used microarray analysis to compare normal (unstretched) intestinal smooth muscle to edematous (stretched) intestinal smooth muscle to characterize changes in ion channel expression. We identified hyperpolarization-activated cyclic nucleotide-gated 2 (HCN2), a cyclic nucleotide gated ion channel, which is also stretch-activated. HCN2 gene expression decreased in edematous intestinal tissue. In the *ex vivo* organ bath system, we tested the effects of HCN2 inhibition on intestinal function. HCN2 inhibition with ZD7288 resulted in a significant decrease in response to carbachol, while spontaneous contractile activity increased slightly. Treatment with ZD7288 also decreased calcium sensitivity in the small intestine significantly.

Conclusions: Our data suggest that HCN2 downregulation/inhibition can suppress intestinal contractile activity. Thus, HCN2 is a potential therapeutic target for improving intestinal motility. In future experiments, we will determine the role of HCN2 in the intestinal response to stretch.

Keywords: *smooth muscle, gastrointestinal, ion channel*



P-192

Toxic stress-specific molecular cytoprotective responses determine behavioural adaptation in *C. elegans*

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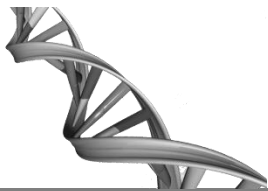
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Dangerous insults, such as toxins and pathogens trigger an evolutionarily conserved survival response in mammals as well as in the soil nematode *Caenorhabditis elegans*. This response includes aversive behaviour and various molecular defenses exemplified by stress, immune and detoxification processes. Such cytoprotective responses induced by a preconditioning stress play a role in stress tolerance, i.e. increased organismal survival in a subsequent lethal challenge. It is also known that behavioural avoidance of some sensory cues may diminish with time, a phenomenon called habituation. However, whether there is a relationship between cellular defenses and behavioural adaptation and tolerance remains unclear.

To address this, we employed two odours, benzaldehyde (BA) and diacetyl (DA) that has been shown to be aversive at high doses. We found that exposure to aversive doses of concentrated BA and DA induced toxicity and impaired survival. Interestingly, preconditioning exposure to BA diminished BA-avoidance, while that to DA further increased avoidance of DA odour source. As a potential underlying mechanism, BA exposure induced specific and widespread set of molecular surveillance responses including nuclear translocation of the stress regulator DAF-16/FOXO, *glo-1*-dependent Lysosome-Related Organelle (LRO) accumulation and *sen-1*/NRF-dependent expression of detoxification enzymes, while we did not observe any of these responses upon DA exposure. Genetic downregulation of these cytoprotective effectors and the chaperone *hsp-90* augmented BA-induced toxicity and decreased behavioural tolerance. We detected the same molecular responses upon exposure to methyl-salicylate (MS), an odorant structurally related to BA. Finally, behavioural cross-tolerance to aversive MS exposure developed after preconditioning BA exposure.

Our results indicate that extended exposure to some toxic agents induce a specific set of cytoprotective molecular surveillance responses characteristic to the nature of the compound and the damage, which protects from toxicity and results in behavioural tolerance. In the absence of efficient cytoprotection, behavioural avoidance and further sensitization remains the only adaptive response. Thus, efficacy of toxic stress-specific molecular responses determine behavioural adaptation.



P-193

A neglected post-translational modification: extracellular O-glycosylation

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Genomic and transcriptomic information might not be sufficient for understanding cellular processes. Sooner or later researchers have to cast a wider net, and turn to other ‘omics’. Proteomic studies in general aim to describe the ‘normal’ composition of protein complexes, cell organelles, tissues or body fluids, and then to detect the changes occurring as the result of certain stimuli, or diseases. Such research produced impressive results, but we still have limited knowledge about the occurrence and changes of post-translational modifications (PTMs) that most certainly are significant players in the regulation of complex-formation, protein-protein interactions, and biological activity. In addition, PTMs that modify proteins located in the extracellular space have been neglected for a long time despite the ever growing evidence that i) they are really important for our wellbeing ii) they may influence/control/regulate intracellular processes.

Our research focuses on the Golgi-derived glycosylation of Ser, Thr and Tyr residues. These modifications are exceptionally hard to tackle, but their biological role(s) – at least what we know about them – are really exciting. We are developing protocols for the enrichment of modified peptides from readily available body fluids and then use reversed phase nanochromatography linked to high sensitivity tandem mass spectrometry to analyze the resulting mixtures. We are gathering data on the proteins and the sites modified. Presently we work with urine samples of healthy volunteers and bladder cancer patients. We have mined the urinary O-glycoproteome at an unprecedented level: we identified unexpected structures on secreted glycoproteins, such as disialic units, mono- and di O-acetyl sialic acids, as well as different blood-type antigens that have never been linked to an unambiguously identified site in a particular protein before [1]. We do hope that our basic research project will lead to translational results.

Keywords: PTM, glycosylation, mass spectrometry

References:

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P-194

An attempt to identify interacting partners and substrates of transglutaminase 4

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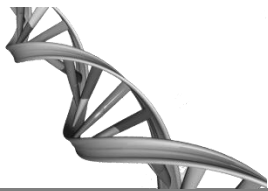
The transglutaminase 4 (TG4, prostate transglutaminase) is one of the less studied member of the transglutaminase family. In humans, it is expressed in the prostate gland regulating the viscosity of the semen and masking the autogenicity of sperm cells in the female genital tract (Mukherjee et al, 1983). Recently, an approximately 50-55 kDa molecular weight protein fragment of TG4 was detected in the insoluble fraction of human saliva but its function here is not known (Perez et al, 2013).

Our goal is to explore the biological role of TG4 in the intestinal tract by identifying its substrates and interaction partners.

First, we confirmed the presence of TG4 fragment in the human saliva. Then, the cDNA of human TG4 was cloned into pTriEx-4 eukaryotic expression vector after an N-terminal His₆-tag using ligase independent cloning. The created vector was transfected into endogenously TG4 negative AD-293 and PC-3 cells with Lipofectamine 2000 reagent. 48 hours after transfection on Western blot a strong 72 kDa and a faint 50-55 kDa size TG4 fragment were detected by either anti-HIS/HRP or monoclonal anti-TG4 antibodies. This suggests that in these cells TG4 is mostly expressed in the full length form and it undergoes limited proteolysis.

In order to produce large amounts of TG4 containing cell extract Ca precipitation transfection protocol was set up but it worked only with AD-293 cells. After large scale expression of TG4 in AD-293 cells 34 potential TG4 interacting proteins were identified using Ni-NTA affinity chromatography and LC-MS/MS analysis. For the identification of TG4 substrate proteins biotin-pentylamine was incorporated into AD-293 cell extract using recombinant human TG4 because the exogenously expressed protein did not show enzymatic activity in the cell extract. The analysis of the modified peptide fragments are in progress.

The first two authors contributed equally to this study. The authors are grateful to the Proteomics Core Facility at University of Debrecen, Faculty of Medicine for the MS analysis. This work was supported by NKFI K120392 and the ÚNKP-18-4 New National Excellence Program of the Ministry of Human Capacities. R.K. was supported by Janos Bolyai Research Fellowship of the Hungarian Academy of Science.



P-195

Analysis of salivary protein profiles in oral leukoplakia, oral lichen planus and oral squamous cell carcinoma using proteomics techniques

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Oral squamous cell carcinoma (OSCC) accounts for about 90% of malignant oral lesions worldwide. Hungarian males and females exhibit the highest age-standardized rates for both the incidence and the mortality of oral cavity and pharyngeal cancers in Europe, without substantial improvements in the last decades. It is crucial to find suitable biomarkers for the early detection of the disease because diagnostic delay may be one of the reason for the relatively small 5-year survival rate.

The development of the OSCC takes years and the precancerous conditions often precede the appearance of OSCC. In the literature, two precancerous conditions, oral lichen planus (OLP) and oral leukoplakia (OLK) have been studied intensively, but the exact mechanism of tumor development is still not understood.

The goal of our research is to analyze the salivary protein profiles of oral leukoplakia, oral lichen planus and oral squamous cell carcinoma in order to get more information on the precancerous-cancerous transitions and to find potential biomarkers suitable for early OSCC detection.

Saliva samples originating from four groups: controls, patients with OLK, patients with OLP and patients with OSCC were examined. Proximity extension assay and gel electrophoresis coupled to mass spectrometry were applied to analyze the protein changes characteristic to each group. Functional and network analysis was carried out to unravel the pathways that play important role in the disease development.

We could identify proteins characteristic to each group and hopefully some of them will be further validated as potential biomarkers for the early detection of OSCC. According to our results, the inflammatory pathways play an important role in the pathomechanism and development of OSCC.

The work was funded by Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences, Makovecz Mobility Programme, OTKA K105034 and GINOP-2.3.3-15-2016-00020.

Keywords: oral squamous cell carcinoma, salivary biomarkers, proximity extension assay, oral lichen planus, oral leukoplakia



P-196

Chronic cerebral hypoperfusion induced synaptic proteomic changes in rat central nervous system

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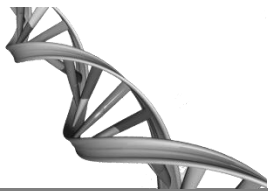
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Chronic cerebral hypoperfusion (CCH) is an ischemic state where cerebral blood flow is gradually and permanently reduced. CCH leads to cognitive impairment and neurodegenerative diseases, such as vascular dementia and Alzheimer's disease. It can induce neuroplasticity damage, inflammatory response and hypoperfusion-related metabolic changes in different brain regions, such as various cortical areas and hippocampus. Experimental animal models are effective tools to study the mechanism of neurodegeneration to achieve potential therapeutic targets of CCH. The most widely used model of CCH is the permanent bilateral common carotid artery occlusion in rats. In our study, we have performed an unbiased survey of synaptosome proteome changes of hippocampus and two cortical areas (frontal and occipital cortex) by a high-resolution quantitative proteomic approach. Stepwise bilateral common carotid artery occlusion or sham operation were performed on rats. The occlusion was monitored by 3D TOF angiography. Eight weeks after the first occlusion, rats were sacrificed and synaptosome samples were prepared from the 3 brain areas of 12 animals. Then, the proteins were separated by two-dimensional differential gel electrophoresis and analyzed with DeCyder software. Finally, 94, 32 and 15 proteins were identified from the altered spots by LC-MS from the occipital, frontal cortex and hippocampus, respectively. The interaction network of the proteins with significantly changed levels was analyzed, common regulator and common target analyses were performed.

This work was supported by the National Research, Development, and Innovation Office of Hungary Grants 2017-1.2.1-NKP-2017-00002 and FIEK_16-1-2016-0005.

Keywords: Chronic cerebral hypoperfusion, proteomics



P-197

Dissecting the uterotrophic effect of estradiol in rodents by mass spectrometry-based proteomics

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The mammalian uterus is a well known organ target for estrogens functioning as primary female sex hormones. Their uterotrophic effects also have been the foundation of bioassays for known or suspected endocrine disrupting chemicals. However, classical rodent uterotrophic assays merely use uterine weights as measurement endpoints. „Omics”-based methods promises to reveal gene- and protein-level details of estrogens’ *in vivo* pleiotropic action. This presentation will summarize progress in proteomics in this field due to the introduction of newer instrumentation surpassing earlier generations in performance, followed by a compilation of current knowledge derived through advanced bioinformatics (Ingenuity Pathway Analysis®, Qiagen) to process results of protein identifications and quantifications.

Tissues were harvested from ovariectomized female CD1 mice and Sprague-Dawley rats injected subcutaneously with 17 β -estradiol (E₂) and vehicle (control), respectively. Excised uteri were processed first by incubating in aqueous urea solution followed by a conventional workflow involving tryptic digestions for bottom-up shotgun proteomics. Digested samples were analyzed using nanoflow liquid chromatography coupled with data-dependent mass spectrometry and tandem mass spectrometry on a linear ion trap-Fourier transform ion cyclotron resonance (LTQFT), a linear ion trap-Orbitrap (LTQ Velos Orbitrap Pro), or a linear ion trap-quadrupole-Orbitrap (Orbitrap Fusion Tribrid) hybrid instrument—all from Thermo Fisher Scientific (San Jose, CA, USA). UniProt mouse and rat protein sequences, respectively, and Mascot (Matrix Science, Boston, MA, USA) search engine were used to identify peptides and proteins. Validation of the results and label-free quantifications were performed through Scaffold (Proteome Software, Portland, OR, USA).

Although newer hybrid instruments clearly outperformed the predecessor Fourier transform mass spectrometer in acquisition speed and, thus, in the number of identified proteins, increase in false discovery has become an issue to control. Nevertheless, pathway analyses afforded a meaningful dissection of E₂’s uterotrophic effect to several protein interaction networks and associated biological processes.

Acknowledgments: US National Institutes on Aging (AG031535); US National Eye Institute and Office of Research on Women’s Health (EY027005); The Welch Foundation (BK-0031); Hungarian Government Funds (GINOP-2.3.3-15-2016-00020 and 20428-3/2018/FEKUTSTRAT).

Keywords: *estrogen, uterus, proteomics*



P-198

Effect of repeated measurements and choice of database search engines in bottom-up proteomics

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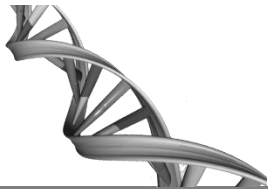
Shotgun proteomics, via tandem mass spectrometry (MS/MS), is widely used to identify proteins in complex biological samples. In the data-dependent acquisition approach, an MS spectrum is recorded first, followed by MS/MS spectra for the most intense peaks in the preceding MS spectrum. The identification of peptides is then performed by searching against a database of known sequences. For complex samples, there may be significant run-to-run variability in the peaks selected for MS/MS analysis, and the results of the database search themselves are sensitive to the search engine chosen and to the specific search parameters. We therefore set out to investigate the performance of two commonly used software packages, Byonic and Mascot in the analysis of repeated measurements of the same sample.

We carried out 12 repeated nano-LC-MS/MS runs on a HeLa tryptic digest standard sample on a Bruker Maxis II Q-TOF instrument. The data were searched against the SwissProt Human 2017 database. Further analysis and comparison of the output of the search engines files were done using the Scaffold 4 program.

Combined analysis of the repeated LC-MS/MS runs showed that further peptides and proteins could be identified from *every* additional LC-MS/MS run included. Nevertheless we found that 3 to 4 repeats are sufficient to find about 90% of proteins and peptides in a sample with this complexity. A direct comparison of search engines using the LFDR-based (re)scoring system in Scaffold showed that a higher number of proteins and peptides could be identified by Byonic, most probably due to the more efficient handling of modifications. Additionally, we assessed the effect of the size of the database on the outcome of the searches via a shortlisted and an all-organisms database.

Acknowledgements: *Ágnes Révész is grateful for support from the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.*

Keywords: *tandem mass spectrometry, bottom-up proteomics, database search*



P-199

Identification and validation of H1 interacting partners

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Histones are essential proteins in eukaryotes responsible for packaging the genetic material. By this they affect every DNA related processes including but not limited to gene regulation, replication, DNA damage repair mechanisms, centromere and telomere function. To fulfil these functions properly histones have a large number of interacting partners and post-translational modifications. However, classical genetic approaches to study histones and their posttranslational modifications are extremely difficult since histone genes are present in the genome in many copies organized in clusters, containing sets of core and linker histone coding sequences. Nonetheless, post translational modifications and interacting partners of the four core histones are fairly well characterized. In case of linker histone H1 post translational modifications are described, but the enzymes and interacting partners that deposit and read those are mostly unknown. The presence of numerous H1 variants in mammals with redundant roles makes the exploration of H1 functions even more difficult. Previous proteomic studies reported among H1 interacting partners many ribosomal proteins and spliceosome factors casting doubts on the validity of those data, and leaving open the possibility that H1 interactions with abundant proteins (like proteins in the nucleolus) could mask its less frequent interacting partners.

Our aim was to identify and validate H1 histone interacting partners. To do so we chose *Drosophila melanogaster* since in this species only two H1 variants exist and only one is expressed in somatic cells. We expressed histone H1 and immobilized it in affinity matrix to fraction S2 cell nuclear extracts on it. Proteins which bound to the immobilized histone were analysed with mass spectrometry. The potential interacting partners were filtered and analysed as a network. Proteins that have nuclear function and were identified as hits with lower abundance were selected for further validation by micro-scale thermophoresis.

Support was provided by OTKA (OTKA-116372) and GINOP (GINOP-2.3.2-15-2016-00032, GINOP 2.3.2-15-2016-00001)

Keywords: linker histone, H1 histone, *Drosophila*, interaction, mass spectrometry, MST



P-200

Interaction of the postsynaptic scaffold proteins GKAP and Shank-1

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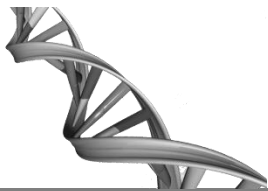
The postsynaptic density (PSD) is an elaborate network of proteins capable of dynamic rearrangement upon stimuli. Although most of pairwise interactions have been described, their full three-dimensional arrangement and dynamic is not known. Guanylate-kinase-associated-protein (GKAP) can interact with several scaffold proteins of the PSD, including the Shank family. Shank proteins are prominent organizers of PSD and are associated with several neuronal disorders, such as mental retardation and autism.

The PDZ domain of Shank can bind the C-terminus of GKAP, providing a link between PSD95 and Homer proteins, known to bind GKAP and Shank, respectively. Thus, the Shank-GKAP interaction can be a key element in the regulation of PSD structure and function. The C-terminal part of GKAP contains a globular domain, GH1, followed by the Shank PDZ binding segment. At present, only X-ray structures of the isolated Shank PDZ and GKAP GH1 domains are known, but no structure of a larger complex including the full GH1 domain has been characterized.

In this study, we aimed to design and produce these interacting protein domains suitable to investigate the structure and dynamics of their complex by NMR spectroscopy and other methods. Recombinant, 6xHis tagged proteins were expressed in BL21(DE3) cells, purified by immobilized metal affinity chromatography (IMAC), followed by ion exchange chromatography (IEC) and size exclusion chromatography (SEC). Purified protein samples were tested by PAGE and MS prior to performing pilot NMR experiments. Interaction of the constructs was investigated by native PAGE, SEC and pull-down assays.

Good quality NOESY and HSQC spectra indicate compact, well-folded globular structure of both constructs. Preliminary results of pull-down assays and native PAGE suggest that our recombinant constructs are able to interact with each other *in vitro*.

Keywords: postsynaptic density, Shank-1, GKAP, protein interaction



P-201

Investigation of serum protein biomarkers for human glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most common malignant disease of the central nervous system. Its prognosis is unfavourable, and the median survival of patients is 14 months from diagnosis. Diagnosis is currently based on CT scans and/or MRI and molecular characterization is possible only with specimens obtained by open surgery or biopsy. One of the important goals of oncology is to develop biomarkers that can be identified through less invasive methods. Like other neoplasms, GBM releases molecular information into the circulation. Tumour-derived biomarkers include proteins, nucleic acids and extracellular vesicles (EVs) that accumulate in plasma, urine, cerebrospinal fluid and serum. EVs high number in body fluids, their stability and the presence of many tumour-associated molecules in their cargo make them potentially optimal biomarkers. The aim of this study was to identify protein biomarkers in serum and serum EVs that may be useful as easily accessible diagnostic, prognostic and/or predictive biomarkers to guide patient management.

Based on literature data and previous studies, a list of feasible GBM and invasion related protein biomarkers was created. In this list 20 proteins (proteolytic enzymes, extracellular matrix proteins, cell adhesion molecules, cytoskeletal components and glia specific elements etc.) were selected for targeted liquid chromatography-mass spectrometry (LC-MS) analysis. In order to determine the LC-MS detectability of these targets and to build a library of additional possible candidates, serum, serum EV and glioblastoma tissue samples were analysed both in targeted and comprehensive untargeted LC-MS measurement modes. Level of detectable proteins were compared in serum and serum EV samples of GBM patients and healthy controls. In order to enhance the dynamic range of analysis the top 12 most abundant proteins were depleted from serum samples.

Biological relevance of proteins showing altered expression levels in healthy and GBM individuals is evaluated, and most promising candidates are validated by different methods (eg. Western blot).

Acknowledgement: *This work was supported by the 20391-3/2018/FEKUSTRAT project.*

Keywords: *biomarker, glioblastoma, mass spectrometry*



P-202

Investigation of the effect of overexpression of different ABC transporters on the cell membrane proteome

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ATP-binding cassette (ABC) transporters are very common in both prokaryotes and eukaryotes, generally consisting of transmembrane domains and membrane-associated ATPase domains. These transporters transfer different substrates across the cell membrane using the energy of ATP hydrolysis¹. In human these are expressed in brain, small intestine, liver and kidney under physiological conditions. Quantification of ABC transporters is really important in drug kinetics studies as these determine the drug concentrations in the cells. Overexpression of these transporter proteins in cancer cells may cause drug resistance during chemotherapy².

Cell lines overexpressing specific ABC transporters are widely used in study of membrane transport of drugs. Our aim was to observe the effect of an ABC transporter protein overexpression on concentration of other transporters and composition of cell membrane proteome. Membrane fractions of transfected human embryonic kidney (HEK-293) cells were analysed using liquid chromatograph coupled with mass spectrometer (LC-MS).

Absolute concentrations of MDR1, BCRP, MRP4, OATP1B1, OATP1B3, OCT2, OAT1, OAT2 and OAT3 were determined in HEK-293 cells overexpressing one of these transporters and compared to cells with no overexpression. In addition to these targeted measurements relative differences in concentration of more than 2000 proteins were evaluated in the various cells.

Acknowledgement: *This work was supported by the GINOP-2.2.1-15-2016-00009 and 20391-3/2018/FEKUSTRAT projects.*

Keywords: *ABC transporter, membrane proteome, mass spectrometry*

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P-203

On-tissue digestion of prostate cancer biopsies for proteomics

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Biopsies, in the form of tissue microarrays (TMA) were studied to identify changes in the proteomic profile throughout prostate cancer progression. TMAs offer a valuable source of well-characterized biological material. Due to the small size of TMA samples, method development was essential to provide the necessary sensitivity and reliability of analysis.

Surface digestion of TMA cores was followed by peptide extraction and shotgun proteomics analysis. Conventional in-solution digestion and surface digestion methods were thoroughly evaluated. The methodology provides sufficient sensitivity to identify over 500 proteins from individual prostate TMA cores (<1µg total protein content) using strict identification criteria. The number of identified proteins strongly increased with the progression of prostate cancer. Reliability of quantitation was tested using at least 3 replicate analysis of each sample analyzed.

We have identified 189 proteins which showed statistically significant abundance changes (t-test p-value <0.05) between healthy and cancerous tissue samples. The proteomic profile showed significant changes according to cancer grade (Principal Component Analysis, XLStat). Results of this study were evaluated using bioinformatics tools (STRING and CytoScape ClueGO plugin), identifying various protein pathways affected by prostate cancer progression indicating the usefulness of studying TMA cores to identify quantitative changes in tissue proteomics.

Acknowledgements: Lilla Turiák and Károly Vékey are grateful for funding from the National Research Development and Innovation Office (NKFIH PD-121187 and NKFIH K-119459). LT and ÁR are grateful for support from the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Keywords: tissue microarray, prostate cancer, mass spectrometry



P-204

Phosphorylation-dependent diversification of RETINOBLASTOMA RELATED protein complexes in *Arabidopsis thaliana*

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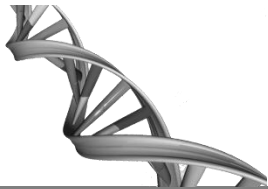
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To address phosphorylation-dependent changes in certain protein complexes we selected a well studied, biologically significant, evolutionary conserved pathway, which is a key component of tuning meristem activity and cell proliferation to environmental conditions. This pathway is fundamental for plant growth and crop yield [1], yet only poorly understood. The plant orthologue of the animal Retinoblastoma (Rb), the RETINOBLASTOMA RELATED (RBR) protein is of central importance in this process. RBR is an ancient signal-dependent scaffold for protein interactions, regulated by post-translational modifications, largely by multisite phosphorylation [2,3]. According to the textbook model, based on animal studies, Rb represses cell proliferation through binding to cell cycle regulatory E2F transcription factors when hypo-phosphorylated, and this association is dissolved by sequential phosphorylation, initially by G1-, followed by G1/S- and mitosis-specific cyclin-dependent kinases (CDKs) [2]. The initial phosphorylations, e.g. on the conserved Serine-807 (S807) site of Rb (S911 on RBR), might prime further CDK sites for phosphorylation [4]. During the transition through G1, Rb was shown to become phosphorylated on alternative individual sites, leading to mono-phosphorylated Rb forms with distinct properties; such as the association with activator E2F1 to regulate checkpoint arrest and apoptosis during DNA damage, or the dissociation from repressor E2F4 to enable the entry into G1/S suggesting a diversification of the Rb functions through phosphorylation during early G1 phase [5]. Approximately 20 phosphorylation sites were predicted on RBR. In our work, we unambiguously identified 14 of these sites by mass spectrometry (MS) using immunoprecipitation (IP) of RBR-GFP. In pull down experiments with the available, well known RB interactors: E2FA-, E2FB-, E2FC-, DPA-, DPB- genomic complementing constructs 8 of the 14 phosphosites were found suggesting that the effect of RBR phosphorylation is more complex than the textbook model.

Keywords: phosphorylation, protein complexes, mass spectrometry

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P-206

Quantitative analysis of tear proteins from patients with diabetic retinopathy with different mass spectrometry-based methods

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Analysis of body fluids has high impact in medical sciences. It has been proved that the qualitative and quantitative changes in the proteome of several body fluids such as tears, saliva or sweat can provide information about the presence of local and systemic pathological changes. The basal tear fluid is a protein rich body fluid with high clinical relevance. Studies show that changes in the tear proteome can indicate local pathological changes like diabetic retinopathy and dry eye disease and also have relevance in case of systemic pathological changes like Alzheimer's disease. As far as the volume of the collectable non-stimulated tears is very low the careful selection of the appropriate analytical method for the examination of tear proteins is very important.

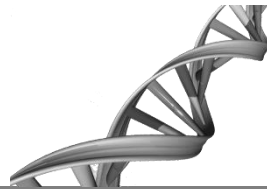
In this study our aim was to compare different quantitative mass spectrometry strategies in order to find the appropriate methods for the analysis of the tear proteome. We have analyzed tear pools from healthy volunteers, from patients with type II diabetes and from patients with non-proliferative and proliferative stages of diabetic retinopathy.

iTRAQ chemical labeling-based quantification was applied on a 4000QTRAP (ABSciex) and on an Orbitrap Fusion (Thermo Scientific) hybrid tandem mass spectrometer. By using the Orbitrap Fusion mass spectrometer label-free quantification experiments were done as well. Besides the applied shotgun techniques targeted mass spectrometry methods were also applied. SRM-based targeted proteomics analyses were carried out on the 4000QTRAP mass spectrometer and PRM-based targeted mass spectrometry experiments were carried out on the Orbitrap Fusion mass spectrometer.

The results indicate that the combination of label-free quantification and PRM in further studies can be a good strategy to analyze the quantitative changes of the tear proteome. According to our data the higher sensitivity provided by the Orbitrap Fusion mass spectrometer is required for the proper examination of the tear samples.

Funding: This research was funded by János Bolyai Research Scholarship of the Hungarian Academy of Sciences, PD121075 and PD 116817 funds.

Keywords: tears, mass spectrometry, quantitative proteomics

**P-207****Statistical investigation of citrulline effect on a citrullinome database**Arnold Steckel^{1,2}, Gitta Schlosser^{2,3}¹ Hevesy György PhD School of Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary² MTA-ELTE Research Group of Peptide Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary³ Department of Analytical Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary

Protein citrullination or deimination is a post-translational modification converting certain arginines to citrullines¹. The modification results in a 0.9840 gain in molecular mass per occurrence. Citrullination could be associated with several pathological conditions including neurodegenerative and autoimmune diseases². Full characterization of modified peptides is still far from straightforward despite some progress in the field recently³. Protein sequencing by mass spectrometry usually relies on the generation of *b* and *y* type ions from peptides via collision-induced dissociation (CID). It is well-described that bond scission *N*-terminal to proline is strongly preferred in CID resulting in very intense *y* ions for tryptic peptides⁴. Previously we described a similar phenomenon occurring *C*-terminal to citrulline (citrulline effect)⁵. In this study we statistically analyzed ~300 peptides containing citrulline based on the human deep proteome database mining work of Lee et al⁶ to investigate the significance of citrulline effect. Our results indicate that the overall occurrence of citrulline effect was slightly inferior to proline effect. On the other hand, cleavage preference was remarkably enhanced at the *C*-terminus of Cit for most evaluable cases.

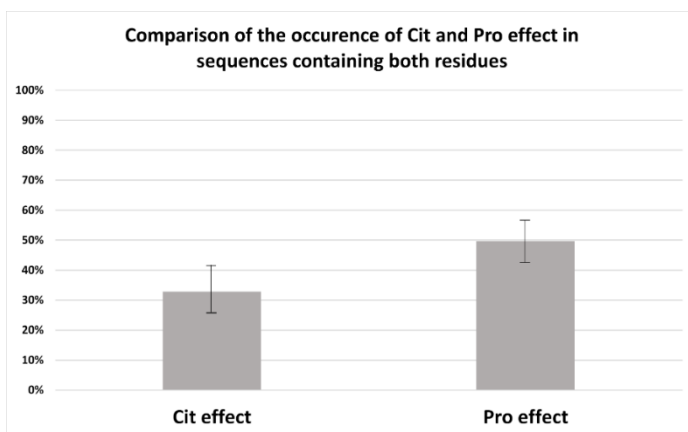
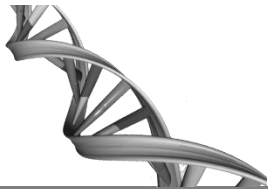


Figure 1: Comparison of the overall relative sequential base peak occurrences for deiminated peptides containing both citrulline and proline residues



Acknowledgement: *The research was supported by the MTA Premium Post-Doctorate Research Program of the Hungarian Academy of Sciences (HAS, MTA).*

Keywords: *citrullination, citrulline effect, MS/MS*

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P-208

Synaptic functional disturbances leading to C1Q deposition in health and disease

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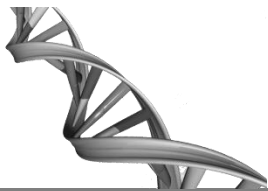
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Mounting evidence supports the role of the microglia-derived local complement system in selective elimination of synapses. This mechanism of synaptic pruning relies on the deposition of C1q to particular synapses that triggers their selective microglial engulfment via a yet not fully deciphered mechanism. Complement-dependent synaptic pruning is fundamental during early brain ontogenesis, and possibly in adult synaptic plasticity. Overactivation of the microglia-complement axis has been repeatedly implicated in neurodegenerative and neuropsychiatric disorders ranging from Alzheimer's disease to schizophrenia. To get a better insight into the functional impairments underlying synaptic C1q-tagging, we employed an unbiased proteomic strategy. Fluorescence-activated sorting of C1q-tagged and untagged synaptosomes from healthy mice and a mouse model of Alzheimer's disease were conducted followed by proteomic comparisons. Our results pointed out the role of synaptically activated, local apoptotic-like processes in C1q-tagged synapses that was verified on human brain specimens as well. Triggering of the apoptotic-like machinery and the concomitant synaptic C1q deposition could be elicited by lower synaptic strength as we demonstrated in a model of sensory deprivation. We unveiled excessive mitochondrial oxidative stress in C1q-tagged synapses in the mouse model of Alzheimer's disease. Moreover, we observed a striking enrichment of septin proteins, which are known to isolate individual dendritic spines via creating a molecular barrier. Overall, our results revealed the importance of apoptotic-like processes in synaptic C1q deposition and



gave insights into the functional impairments that characterize the tagged synapses in neurodegeneration.

This work was supported by grants KTLA_NAP_13-2-2014-0017, 2017-1.2.1-NKP-2017-00002, and FIEK_16-1-2016-0005.

Keywords: *complement C1q, synaptic pruning, local apoptosis*

P-209

Effect of dietary fructooligosaccharide (FOS), carotenoids, anthocyanins and probiotics supplementation on the intestinal microbiome in broiler chickens

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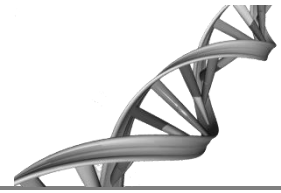
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Introduction: The poultry industry during the past two decades have been one of the most efficient animal productive system, and form the basis of global protein production in the world. Modern chickens require increasingly less feed to achieve their desired weight, for example broiler chickens that were selected for meat production gained a higher body weight (~ 3 kg) within 42 days. However, such intensive, extreme growth rate may have inadvertently resulted in changes in gastro-intestinal development during growth of the animal. Some other negative effects may also eventuate at times, like metabolic disorders, poor immunocompetence and increased susceptibility to pathogens. We started to develop feed additives, which are highly rich in different bioactive components, like FOS, carotenoids, anthocyanins, and probiotics. These bioactive components could reduce the stress and may have an immunomodulatory effect on broiler chickens. Furthermore, they are produced from food industries waste, so they are cheap and available in a large amount.

Objective: The following study was conducted to investigate the effect of dietary supplementation of FOS, carotenoids, anthocyanins, and probiotics on the intestinal microbial composition of broiler chickens.

Methods: The experiment lasted for 40 days. One-day-old Ross 308 broiler chicks, were divided into six equal groups, four experimental groups, one internal group (β -glucan supplementations) and one control group. Stool samples were collected on 7, 19, 31, 40 day of age to determine bacterial population. DNA was isolated using phenol/chloroform/isoamyl alcohol protocol. 96 DNA samples were utilized to construct the 16S rDNA metagenome sequencing on Illumina Miseq platform based on V4 and V3 regions for microbiome analyses. Paired end reads were demultiplexed by the software of



the Illumina sequencing machine. The reads were imported into the Qiime2 pipeline according to the „Atacama Soil microbiome” tutorial. Alpha diversity differences were calculated using the Kruskal-Wallis test. Beta diversity differences were calculated by using the PERMANOVA pseudo-F test. Taxonomic identification was performed and we determined the Gram positive and negative ratio of our samples.

Results and conclusion: Fatt’s phylogenetic diversity significantly increased with chicken development in both the treated and control groups ($P < 0.005$). No significant difference was observed in Chao-1 index. Shannon diversity index was significantly higher in the treated groups ($P < 0.005$) compare with the control group, indicating that dietary supplementation had effect on bacterial diversity. Unweighted and weighted unifracs also showed some but not total separation of the sample of each treated groups. In addition, there were significant differences of *Faecalibacterium prausnitzii* (which is commonly associated with gut health) in treated groups ($P < 0.005$). The result demonstrated that the microbial composition in the gastrointestinal tract was considerably affected by the diet. Gut microbiome can be affected by diet, and different dietary interventions are used by poultry producers to enhance bird growth and reduce risk of enteric infection by pathogens. From this perspective, the study of the gastrointestinal microbiota has nowadays an enormous potential and importance.

Keywords: *microbiome, probiotics, prebiotics, 16S rDNA metagenome sequencing*

P-210

Examination of R-loops and chromatin loops with chromatin conformation capture techniques

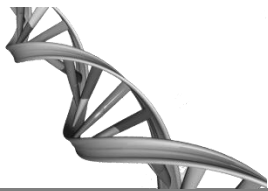
Beáta Boros-Oláh¹, Orsolya Feró¹, Adrienn Horváth¹, Csaba Fillér¹, Zsolt Karányi^{1,2} and Lóránt Székvölgyi¹

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Formation of spatial chromatin interactions (e.g. promoter-enhancer contacts) is essential for normal gene functions. Errors in the 3D chromatin interactome can result in misregulation and misexpression of genes that lead to the formation of diseases [1]. A crucial unresolved question in the field is the specificity of long-range DNA interactions: what are the molecular components that mediate 3D chromosome contacts and how do these factors ensure the formation of correct interactions at the right time and place in the genome? Non-coding RNAs, including RNA-DNA hybrids (R-loops), could represent an important regulatory layer governing these processes [2].

In the current project we sought to identify 3D chromosome interactions that are mediated by R-loop structures. We identified the chromosomal location of RNA-DNA hybrids by DNA-RNA hybrid immunoprecipitation sequencing (DRIP-seq) [3]. Subsequently we



performed HiChIP (a genome conformation capture method) with an RNA-DNA hybrid-specific antibody that enriched all 3D chromatin interactions that involve RNA-DNA hybrids.

Sequencing R-loop HiChIP samples resulted in 60 million paired-end reads that ensured sufficient coverage to reliably identify intra-chromosomal interactions, however, it was less efficient in detecting inter-chromosomal R-loop contacts. Comparing DRIP seq and R-loop HiChIP data we assessed RNA-DNA hybrids that participate in chromatin looping interactions.

Based on the above experiences with normal (healthy) human cell lines, our goal is to map the pattern and frequency of R-loop-facilitated chromatin interactions in non-physiological settings as neurodegenerative- and autoimmune disorders. Thus, we can expect a more comprehensive understanding of these severe diseases in the context of 3D genome structure and R-loop formation.

Keywords: *chromatin conformation capture, R-loops, chromatin loops*

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P-211

Identification of RNA interference-related genes in barley (*Hordeum vulgare* L.)

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RNA interference (RNAi) is a genetic regulation mechanism which is evolutionally conserved among all eukaryotic species. Its main components in plants are Dicer-like (DCL), Argonaute (AGO) and RNA-dependent RNA polymerase (RDR) proteins. DCLs are endonuclease type proteins, responsible for small RNA (sRNA) biogenesis from double-stranded precursors. AGOs are the executors of RNA induced silencing complex (RISC). For biological activity of sRNAs it is crucial to associate with adequate AGO proteins. sRNA-loaded RISC can act transcriptionally or post transcriptionally via mRNA degradation or translational inhibition. RDRs could produce double-stranded RNA from RNA template giving new precursors to the RNAi machinery.



In model plant, *Arabidopsis thaliana* 10 AGOs, 4 DCLs and 6 RDRs were identified. During the past decade several crop plants RNAi-associated proteins were investigated, but there is no available study on barleys respective gene families. To identify and characterise *AGO*, *DCL* and *RDR* genes in barley, *Arabidopsis*' maize's and rice's corresponding genes were used as queries.

The predicted RNAi components phylogenetic relationship, conserved motifs and expression in different tissues and under abiotic stress treatments were studied with bioinformatical methods and semiquantitative PCR analyses. To further investigate single RNAi-associated components function, CRISPR/Cas9 constructions were designed against AGO4, DCL3a, DCL3b and AGO6.

According to our preliminary results, the presence of truncated DCL3a in homozygous mutants does not have any strong effect on barley's phenotype. AGO4 mutants showed delayed flowering and altered miRNA expression pattern in embryonic tissues compared to wild type in T₂ generation. As both proteins involved in RNAi's DNA methylation pathway their effect may cumulate during generations.

Our research was founded by NKFI 125300, 130384. Éva Hamar is a student of University of Pannonia Festetics Doctoral School.

Keywords: RNAi, CRISPR/Cas9, barley

P-212

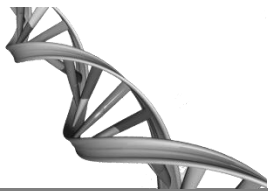
The role of lncRNAs and histone methyltransferases in epithelial-mesenchymal transition

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Histone lysine methyltransferases (HKMTs) catalyse the mono-, di- and trimethylation of lysine residues in histone tails, controlling complete genetic programs. In recent years it has become commonly accepted knowledge that some HKMT complexes are capable of binding to long non-coding RNAs (lncRNAs) with a functional outcome. One of the many processes HKMT-lncRNA binding has been implicated in is epithelial-mesenchymal transition (EMT), with polycomb repressive complex (PRC2) as HKMT and HOTAIR as lncRNA being major players [1]. Apart from PRC2, other HKMTs, like MLL proteins have also been suggested to participate in EMT regulation. Our recent finding that MLL4 (KMT2D) has regions capable of binding to HOTAIR (and other lncRNAs) *in vitro* [2] raises the possibility lncRNAs being directly involved in targeting KMT2D *in vivo*. Our aim in the work presented here was to investigate the involvement of HOTAIR and different HKMTs in the EMT-like behavior of different cancer cell lines.

Keywords: histone methyltransferase, lncRNA, epithelial-mesenchymal transition



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P-213

Tissue-specific promoter usage regulates primary microRNA processing efficiency of the human Chromosome 19 MicroRNA Cluster

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We investigated the maturation of microRNAs (miRNAs) from Chromosome 19 MicroRNA Cluster (C19MC), which is the biggest human miRNA cluster containing 59 miRNAs. These miRNAs are currently believed to be expressed simultaneously and in terms of expression pattern, exclusively in human embryonic stem cells (hESC) and placenta. MiRNA maturation is a complex pathway, which is regulated both transcriptionally and post-transcriptionally. In case of a miRNA cluster, multiple miRNAs encoded by a single primary transcript (pri-miRNA) and regulated together on the transcriptional level. On the other hand, post-transcriptional processing alters each miRNA separately through sequential nucleolytic cleavages first by the Drosha/DGCR8 complex and then by Dicer to form mature miRNA molecules.

Comparing the expression of C19MC miRNAs in hESC and placenta samples revealed different level of expressional changes within the cluster. MiRNAs from the 5' region of the cluster have smaller disparity than downstream miRNAs. The correlation between expressional differences and miRNA positions is significant. This suggests a transcription-related mechanism, which is strengthened by the identification of two tissue-specific promoters, each with a diverse set of enhancers. However, the pri-miRNA level measurements do not correlate with the mature miRNA results. For further investigation, we used JAR, a choriocarcinoma cell line and characterized the connection between transcription and the Drosha/DGCR8 complex with Drosha knock-down by siRNA and blocking co-transcriptional pri-miRNA processing by flavopiridol.

Our data from multiple steps of the miRNA maturation pathway suggest that C19MC is regulated by a previously unknown tissue-specific co-transcriptional pri-miRNA processing mechanism, which is determined by its promoter usage.

This work was supported by the OTKA K112112 and the VEEKOP-2.1.1-15-2016-00156 grants.

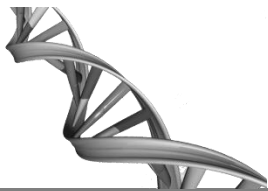
Keywords: *Drosha, DGCR8, siRNA treatment, JAR*

**P-214****Translation termination factor is regulated by readthrough and NMD through 3'UTR intron in *N. crassa*****Anita Kurilla, Dániel Silhavy***Agricultural Biotechnology Centre, Gödöllő, Hungary*

Translation termination factor (eRF1) has a crucial role in protein synthesis through stimulating both peptide release and ribosome recycling. The level of such factor should be strictly controlled, both over and under expression of eRF1 lead to strong phenotypes. Previously our group has shown that eRF1 protein level is controlled by a complex autoregulatory circuit in plants. We hypothesize that a similar regulatory system functions in filamentous fungi. Thus I study the regulation of eRF1 in *Neurospora crassa*. The results has an evolutionary relevance, because the eRF1 autoregulation circuit in plant and fungi may supports our understanding about the origin of this mechanism.

Aberrant transcripts containing intronic 3'UTR can induce NMD (Nonsense-mediated RNA decay), an eukaryotic surveillance system which degrades faulty transcripts to avoid the accumulation of harmful peptides or to balance the optimal protein level. Plant *eRF1* mRNA has a unique structure, this transcript has an intronic 3'UTR and its stop codon is present in a readthrough stimulating context. *eRF1* transcript level is decreased by NMD through its intronic 3'UTR, while the readthrough stop context can partially protect the mRNA from NMD. Increased eRF1 protein level decreases the frequency of readthrough, so the *eRF1* mRNA will be degraded by NMD more efficiently. These attributions make the eRF1 autoregulated by readthrough and NMD. The very special structure of *eRF1* transcript also appears in *Neurospora*. We demonstrated that the readthrough stop context of *eRF1* can also protect the *eRF1* mRNA from NMD in *Neurospora*. The results suggest that autoregulatory circuit of eRF1 also exists in filamentous fungi, so it has evolved in the common ancestor. Our further plan is to study the effect of the overexpression of eRF1 and make an allelspecific gene replacement of *eRF1* by CRISPR system to clarify the physiological relevance of the specific structure of *eRF1* mRNA.

Keywords: NMD, 3'UTR, translation termination factor, *Neurospora crassa*



P-215

Utilization of CRISPR/Cas9 system for investigation of a potential new wheat miRNA targeting RNA polymerase V (Pol V) subunit

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RNA polymerase V (Pol V) is one of the key proteins of the RNA-directed DNA methylation (RdDM) pathway, which represents the small RNA-mediated epigenetic pathways in plants. The RdDM induced transcriptional gene silencing (TGS) mechanism responsible for repression transposons and genes and also implicated in pathogen defense, stress responses and reproduction. The main pathway of RdDM is mediated by siRNAs derived from Pol IV transcripts which are loaded onto ARGONAUTE4 and then linked to the nascent Pol V transcripts in the nucleus by sequence complementarity recruiting enzymes responsible for methylation of cytosine.

We produced miRNA libraries from developing wheat (*Triticum aestivum* L. cv. 'Bánkúti') grain. RNA samples were isolated from 10-20-30 day-old seeds with two replicates. We identified an extremely abundant pool of previously unknown 21 nt long small RNA. Its presence was validated by small RNA northern hybridization and *in silico* analysis revealed that predicted microRNA like RNA precursors can be found in two genomes of wheat (B and D). This predicted miRNA is seed-specific since it was not found in other small RNA libraries prepared from wheat leaves. We identified PolV mRNA as potential target of this predicted miRNA. To reveal the biological function we designed three CRISPR/Cas9 single guide (sg) RNAs to the conservative segment of its predicted precursor RNAs to produce loss of function mutants wheat lines. We targeted one sgRNA to the miRNA mature strand on the 3'-arm of the precursor (sg1), a second one to the miRNA* region on complementary strand (sg2) and a third one downstream from the miRNA strand (sg3). To reduce the OFF-target effects, we used the TECCDNA (Transient expression of CRISPR/Cas9 DNA) method. Constructs containing sg1 or sg2-sg3 RNAs in pBUEdsRED vector were delivered to immature wheat (cv. 'Fieldier') embryos by biolistic gene transfer (Gene Gun). Embryos showing the highest dsRED expression were selected and growing on antibiotic-free callus induction medium.

In the future, we would like to select the regenerating plants for the presence of mutations in the miRNA precursors using PCR/restriction digestion (PCR/RE) assays. Moreover, we will investigate the expression pattern the miRNA in grain tissue by *in situ* hybridization and its regulatory activity on its putative target, Pol V by 5' RACE-PCR and transient expression methods.

This work was supported by NKFI grants, 125300 and 130384.

Keywords: miRNA, RdDM, wheat, CRISPR/Cas9



P-216

Viral diagnostics of rootstock plantations and the complex pathogen elimination methods of grapevine by small RNA NGS

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Several pathogens infect grapevine, including viruses and viroids. Considering that there are no effective plant protection treatments against these pathogens and vineyards are cultivated through decades usage of high quality and pathogen-free propagation material (rootstocks and scions) is essential. Virus elimination from grapevine based on meristem and shoot tip cultures, although another micropropagation method, somatic embryogenesis seems to be a more effective tool. Although presence of regulated pests is routinely checked using ELISA or rarely RT-PCR, these diagnostics methods can detect only particular pathogens moreover can fail to detect variant strains. Next generation sequencing of small RNAs can be an effective, alternative method to avoid these disadvantages.

The aim of our study was to evaluate the viral infection both of the grapevine rootstock plantations in Hungary and verify the effectiveness of different pathogen elimination methods using highly sensitive small RNA NGS as a diagnostics method.

For rootstock survey, rootstock plantations (17 plantations and 2 variety collections) throughout the country were sampled. For comparing virus elimination methods field grown mother plants: two 'Muscat Ottonel' clones, and 'Szirén' and 'Trilla' new varieties, infected with different viruses and viroids were selected for sanitation via somatic embryogenesis and meristem culture. Following RNA isolation, RNA extracts from individuals from the same plantation, different organs of the same mother plant, or different lines resulted from the same method were combined, generating a pool. From these pooled RNAs, small RNA fraction was isolated and we prepared small RNA sequencing libraries. After sequencing at Illumina platform, the sequenced reads were analysed by CLC Genomic Workbench 10.1.1. and the results were validated by RT-PCR.

However, rootstock plantations, seemed almost clear from regulated viruses, viruses that do not require testing (GRSPaV) or have been identified in recent years (GPGV, GSyV-1) and the viroids (HSVs, GYSVd-1) have shown high prevalence. Somatic embryogenesis proved to be more effective than meristem cultures, eliminating all viruses originally present in the mother plants; however, none of the methods were suitable for totally removing viroids.

This work was supported by National Research, Development and Innovation Office K-119783. Emese Demian is a PhD student of Doctoral School of Biology Sciences, SZIU.

Keywords: small RNA NGS 1, *Vitis vinifera*, grapevine viruses, diagnostics, plant regeneration



P-217

Virome of Hungarian peach plantations identified by small RNA NGS

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Virus elimination is indispensable for the maintenance of stone fruit plantations, because these pathogens can cause serious crop damage and crop losses. Currently we do not possess efficient plant protection methods against viruses therefore prevention has a prominent role. In order to prevent infections, pathogen-free propagation material production and application of effective diagnostic methods have essential role. Our examined peach samples derived from isolator houses and stock nurseries of Fruitculture Research Institute of NARIC.

With the help of highly sensitive metagenomic diagnostic methods: such as next generation sequencing of small RNAs, we are able to detect all of the presenting pathogens within our samples. During preparation steps, total RNA was isolated from leaf samples, RNA pools were made from the varieties, and then small RNA libraries were prepared. Sequencing was performed on Illumina platform and we used CLC Genomics Workbench for the bioinformatics evaluation. Results were verified by RT-PCR and Northern-blot. PCR products were cloned into pJET vector and Sanger sequenced.

As a result, we detected nectarine stem pitting-associated virus (NSPaV), peach associated luteovirus (PaLV) and also peach latent mosaic viroid (PLMVd) which presence has to be checked regularly. Moreover we proved the incidence of PLMVd and PaLV first time in Hungary. We suspect, that the source of the viral infection might be the propagation material, which was used as a base for the variety collection in this isolator house.

During the past few years significant tree death occurred in the peach isolator house, why we carried out a PLMVd survey and found that 65% of the individuals were PLMVd infected. The viroid possess high genetic variability, we found several point mutations, but the structure of the viroid was not affected by this SNPs. Furthermore, we identified a 12 nt long insertion in the case of Öb 166/1 variety, which located at the same position, where the peach calico phenotype causing mutation can be found.

Our work was supported by the National Research, Development and Innovation Office K127951 and the Hungarian Ministry of Agriculture. Dániel Baráth and Nikoletta Jaksa-Czotter participate in the Program for Reinforcement of Scientists. Dániel Baráth is a PhD student of Doctoral School of Biological Sciences, SZIU.

Keywords: virus diagnostics, small RNA sequencing, NGS, stone fruit



P-218

Cellular interactions in yeast colonies

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After the more thorough understanding of the intracellular processes, nowadays more and more researchers are interested in understanding the interactions occurring between cells. During our experiments, we focus on examining the interactions that arise, in our case, between yeast cells. This is important because in their natural environment yeast strains grow in mixed colonies, while in most laboratory experiments of microbial colony growth researchers are focusing on single strains. The most common interaction that occurs in every microbial colony is the competition for nutrients, but besides this microbes can develop many strategies to survive, like cooperating with each other or producing toxins against each other. In order to get a bigger picture about these interactions and their molecular background, we examine these interactions between different natural strains of the budding yeast *Saccharomyces cerevisiae*. In order to differentiate the strains in a mixed colony we use two fluorescent proteins EGFP and mCherry to label our strains. Our main goal is to determine how these interactions between the different strains influence the future of the mixed colonies.

Along with the laboratory experiments an agent-based model is being developed to simulate the growth of single and mixed colonies. Because of this, our laboratory experiments are also used to provide better parameters for the model.

Preliminary results of these studies will be present on this poster.

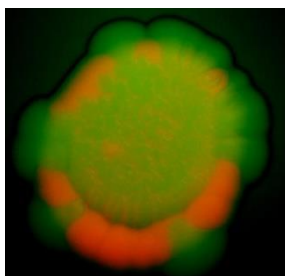


Figure 1. Co-culture of *Sgu165* (GFP, 20%) and *Y55* (mCherry, 80%) picture taken with Olympus MVX10 Macro Zoom microscope

Keywords: yeast, *Saccharomyces cerevisiae*, colony growth, cellular communication



P-219

Generation of successive random deletions in *E. coli* reveals the evolutionary gains and limits of genome reduction

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Artificial genome reduction can reveal evolutionary processes shaping the genome, and reducing complexity and improving metabolic efficiency might aid biotech processes. Genome reduction efforts are primarily based on targeted approaches. However, rational design assumes *a priori* knowledge of gene functions, and successive targeted deletions allow limited choice of targets and elimination pathways.

In contrast to the targeted approaches, we sought to develop a random, iterative deletion scheme. Coupled with selection for reduced-genome variants most fit for survival, this approach could provide a straightforward path to streamlined, robust cells.

Here we present an untargeted streamlining method. It is based on random transposon insertion, followed by introduction of a DSB (double strand breaks) at the transposon, and repair by alternative non-homologous end joining. The process creates random deletions of various sizes in nearly all cells, and can be applied cyclically to a large population of cells. The growth phases in the multistep procedure ensure selection of the fittest variants in the population.

Application of the method to *E. coli* MG1655 revealed a hierarchy of preferred deletions, as well as structural features promoting deletions. Importantly, it was demonstrated that loss of certain genomic regions can be beneficial (increasing growth rate or yield) for the cell under the conditions applied throughout the process. On the other hand, cells bearing these beneficial changes showed rapid decline of fitness, when measured in another environment. Interestingly, emergence of point-mutations compensating for fitness loss was observed under selective conditions.

Keywords: genome reduction, untargeted streamlining, random transposon insertion

**P-220*****In-silico* modelling of yeast colony growth**

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Saccharomyces cerevisiae (baker's yeast) is an important eukaryotic model organism of molecular life sciences. Yeast cells are able to form colonies on solid media with diverse morphologies depending on their genetics and the properties of their environment (e.g.: the quantity and quality of nutrients or the density of the agar plate). Interactions between different strains in a colony also affect the shape and composition of microbial communities. These connections can play an important role in nature and can be crucial for agriculture and industry (e.g.: winemaking and brewing).

We are developing an agent-based computational model for studying interactions between various yeast strains by simulating the growth and morphology dynamics of single and mixed colonies. This simple and minimal model can reproduce qualitatively the shapes and patterns of natural yeast colonies on solid media. The parameters can be set to represent different fitness levels (e.g. different nutrient uptake or metabolism) that result in communities with different size and cell number (Figure 1A). Competition between two strains for available nutrients can be also simulated with our tool (Figure 1B). The parameters of the computational model are being estimated based on laboratory experiments. We hope that our *in silico* modelling tool will help to understand details about intercellular interactions and colony formation of different yeast strains.

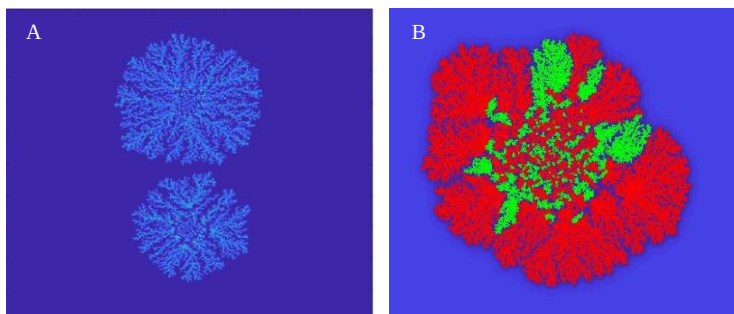
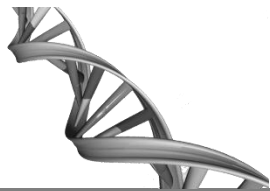


Figure 1: A: Simulated yeast colonies, the upper strain has higher fitness. B: Simulated mixed yeast colonies, the red strain has higher fitness.



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Keywords: *Saccharomyces cerevisiae*, computer modelling, colony growth, systems biology

P-221

Integrating molecular databases in a proteome-wide simulation environment

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In the last few decades due to new high-throughput techniques, such as mass spectrometry, we gained a large amount of data about Protein-Protein Interactions (PPI) and Domain-Domain Interactions (DDI).

This opened the opportunity for computational methods to predict protein complexes.

Here we propose a new tool, based on the SiComPre software¹. We developed a simulation based protein complex prediction tool, which uses Gillespie's Multiparticle algorithm. This combines the classic Gillespie's algorithm² with diffusion by splitting the simulation space into sub-volumes (SVs) which are small enough that we can assume that the reactant are able to find each others. In given times the molecules diffuse between the SVs¹. The SiComPre program had some limitations; the model of the cell was only two dimensional and due to the performance limitations it could not consider real protein abundances. In our simulation tool, we implemented a three dimensional cell model, thus the diffusion is be less limited. Our implementation resulted in magnitudes of higher-performance and the code more flexible for the future features, such as more specified internal bounds in complexes, protein level conformation changes, complex specific diffusion properties.

Protein complex predictions can be used to investigate the effects of drugs in protein complex level behaviour in the cells. Depending on the drug we can change the protein interactions, reaction rates and protein abundances for the simulation. After that it is possible to predict the behaviour of the cell by analysing the most varied protein complexes. Examples of the use of this framework will be presented.

Acknowledgments: This research has been carried out partly in the project Thematic Research Cooperation Establishing Innovative Informatic and Info-communication Solutions, which has been



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P-222

Morphological characteristics of yeast colonies

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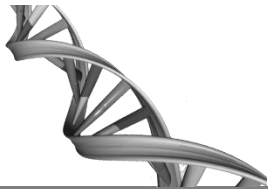
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Yeasts are eukaryotic unicellular microorganisms and they are one of the most often used model microorganisms in cell biology research. Colonies built by natural isolate strains of the budding yeast, *Saccharomyces cerevisiae* show various morphologies. To understand the differences in the growth behaviour of these strains we study the quantitative features of yeast colonies. Our major focus is on two laboratory strains, the BY4743 and Sigma1278b strains. The later one can show true budding morphotype, but can also grow in a filamentous form, while BY4743 can only grow in yeast form. We quantify the number of the cells, the density, the size and the shape of the colonies initiated from various inoculation scenarios to reveal how the density of the colony affects the proliferative capacity of the cells, both in the yeast and the filamentous growth modes.

We develop an agent-based mathematical model and parameterize it, based on the experimental results to create a simulation environment that can predict how yeast colonies will form from a custom initial layout of cells. This will help us in the future to design synthetic yeast colonies with desired spatial layouts.

Keywords: yeast, *Saccharomyces cerevisiae*, colony growth, agent-based model, cell morphology



P-223

Structural ensembles of PDZ tandem generated by externally restrained MD simulation

Bertalan Kovács, Nóra Epresi and Zoltán Gáspári

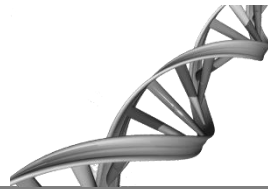
3in Research Group, Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Esztergom, Hungary

In tandem arrangement, PDZ domains usually fold and function in an interdependent way. The PDZ1-2 tandem of the PSD-95 protein was shown to possess a rigid structure in the apo-state, however ligand binding induces considerable interdomain mobility. We presume that regulation of the relative orientation and dynamics of the two PDZ domains might be a key feature in organizing the distribution of the binding partners of the post-synaptic intracellular protein PSD-95.

In order to elucidate the atomic-level mechanism of the inner motions in the PDZ tandem, we performed all-atom molecular dynamics (MD) simulations, including NMR-derived experimental dynamic parameters (NOEs and S2 order parameters) as external restraints. As a result, we obtain a number of dynamic structural ensembles of the modeled structure that properly describe its dynamics on the fast (ps-ns) timescale while maintaining good correspondence with the observable dynamic parameters.

Analysis of the structural ensembles proved an interdependence between inter- and intra-domain motions in the PDZ1-2 tandem. The intradomain motions reveal the β 2- β 3 loop, near the ligand binding groove, as a region that changes dynamic behavior upon ligand binding. This region was earlier reported to have a role in ligand binding specificity of PDZ domains. Furthermore, the displacement of the two domains in the PDZ1-2 tandem relative to each other is exhaustively sampled in our simulations, which allows for clustering the resulting ensembles according to the mode of interdomain interactions.

Keywords: *dynamic structural ensembles, NMR spectroscopy, PDZ tandem*



P-224

The effect of local rifampicin treatment on the composition and resistance of the intestinal microbiota: the first steps taken

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The gut microbiome of an infant was screened for resistance to antibiotics that it had not been previously exposed to. Strains resistant to ampicillin, chloramphenicol, kanamycin, spectinomycin, rifampicin and tetracycline were isolated, but no resistance to ciprofloxacin was observed. The effect of local (intranasal) rifampicin and oral probiotic treatment on the microbiota was thereafter examined. We performed 16S rRNA deep sequencing of the fecal DNA obtained at different time points, and observed that even the local rifampicin treatment led to intestinal dysbiosis. The altered microbiome went through further changes after termination of the therapy, and successive probiotic treatment could achieve only a partial restoration of the original microbial composition. We also found that the rifampicin treatment caused a dramatic expansion of rifampicin-resistance within the gut microbial community. After completion of the therapy the fraction of the resistant subpopulation displayed a slow spontaneous reduction, which was accelerated after probiotic administration. Our case study provides insight on the antibiotic resistance present in the gut microbiome prior to exposure to antibiotics, and underlines the need to study more extensively the effects of local antibiotic treatment on the composition and resistance of enteric bacterial communities.



P-225

The genomic imprint of cancer therapies helps timing the formation of metastases

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A retrospective determination of the time of metastasis formation is essential for a better understanding of the evolution of oligometastatic cancer. This study was based on the hypothesis that genomic alterations induced by cancer therapies could be used to determine the temporal order of the treatment and the formation of metastases. We analysed the whole genome sequence of a primary tumour sample and three metastatic sites derived from autopsy samples from a young never-smoker lung adenocarcinoma patient with an activating EGFR mutation. Mutation detection methods were refined to accurately detect and distinguish clonal and subclonal mutations. In comparison to a panel of samples from untreated smoker or never-smoker patients, we showed that the mutagenic effect of cisplatin treatment could be specifically detected from the base substitution mutations. Metastases that arose before or after chemotherapeutic treatment could be distinguished based on the allele frequency of cisplatin-induced dinucleotide mutations. In addition, genomic rearrangements and late amplification of the EGFR gene likely induced by afatinib treatment following the acquisition of a T790M gefitinib resistance mutation provided further evidence to tie the time of metastasis formation to treatment history. The established analysis pipeline for the detection of treatment-derived mutations allows the drawing of tumour evolutionary paths based on genomic data, showing that metastases may be seeded well before they become detectable by clinical imaging.

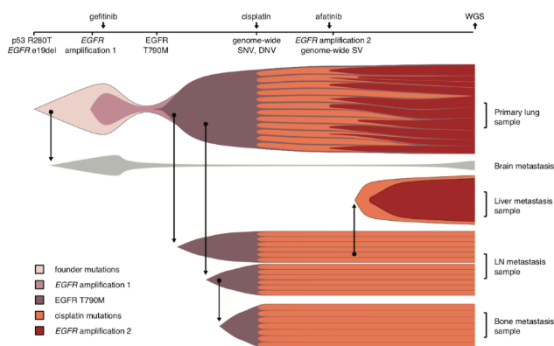
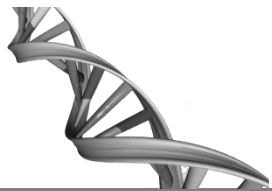


Figure 1: The final model of metastatic spread was built based on the clonality of therapy caused mutations in the genome.

Keywords: cisplatin, chemotherapy, whole genome sequencing

AUTHORS' INDEX



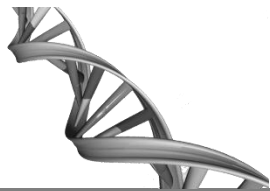


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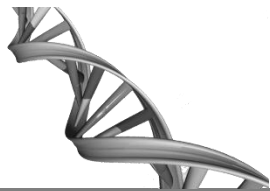
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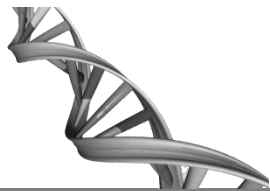
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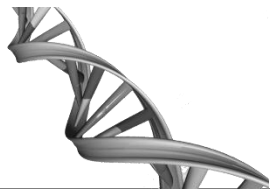


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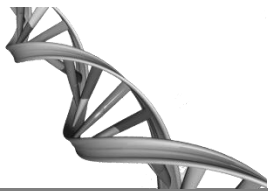
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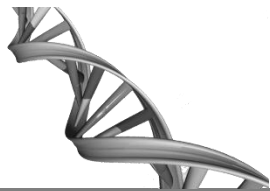


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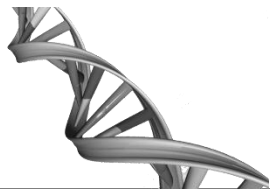
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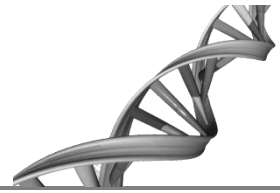
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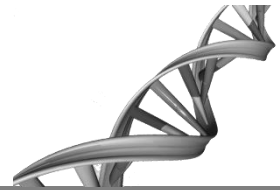
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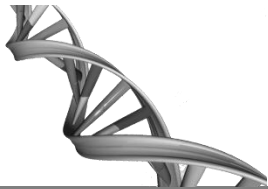
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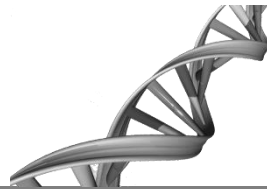
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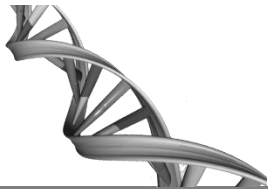
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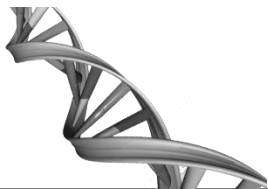
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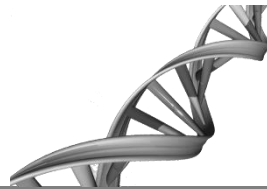
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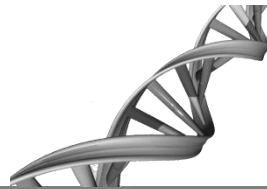
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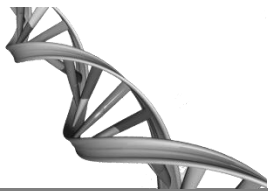
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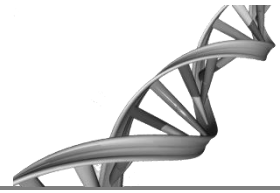
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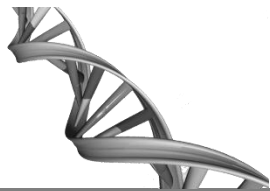
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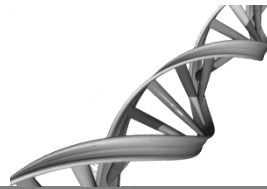
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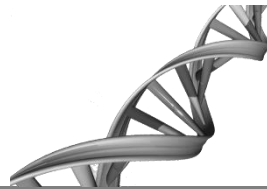
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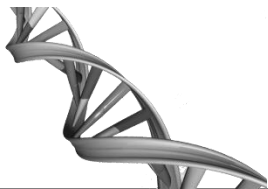
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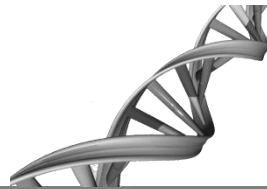
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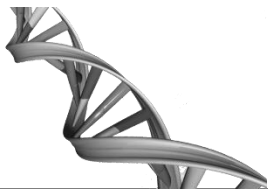
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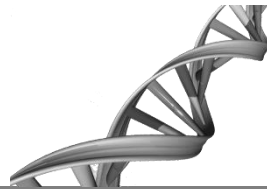
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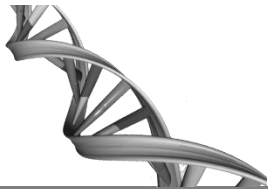
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