

Integrating the molecular, mechanistic and
physiological diversity of autophagy

(w21-51)

Eger, Hungary, 27 June – 1 July 2022

Programme

Day 1 – 27 June 2022, Monday

13:00-16:00

Registration

16:00-16:15

Opening remarks

Gabor Juhasz, HU

16:15-17:00

Remote keynote: The regulation of autophagy in yeast

Dan Klionsky, US

Session 1: Biochemistry of autophagy

Chair: Gabor Juhasz

17:00-17:30

Mechanisms of Cargo Selection and Autophagosome Biogenesis in Selective Autophagy

Sascha Martens, AT

17:30-18:00

Structural basis of Atg18 oligomer binding to lipid membranes

Carsten Sachse, DE

18:00-18:30

Coffee break

18:30-19:00

Ultrastructural characterization of autophagy by cryo electron tomography

Brenda Schulman, DE

19:00-19:15

Short talk: Membrane wetting of phase-separated compartments organizes autophagosomal cargo sequestration

Roland Knorr, DE

19:15-19:30

Short talk: Lipid composition determines Syntaxin17 recruitment to autophagosomes

Hajnalka Laczko-Dobos, HU

19:30-19:55

Flash talks 1-5

Uxue Ballesteros, Elias Adriaenssens, Lena Berning, Carla Ribeiro, Valgerdur Hjaltalin

20:00-23:00

Networking

Dinner

Day 2 – 28 June 2022, Tuesday

Session 2: Cell biology of autophagy

Chair: **Christian Behrends**

9:00-9:30

ATG9A and ATG2A form a heteromeric complex essential for autophagosome formation

Sharon Tooze, UK

9:30-10:00

Neuroprotective properties of the autophagy inducer spermidine

Frank Madeo, AT

10:00-10:30

Spatial confinement and avidity drive autophagosome formation

Claudine Kraft, DE

10:30-11:00

Coffee break

11:00-11:30

ER-remodelling: lessons from SARS-CoV-2

Antonella De Matteis, IT

11:30-11:45

Short talk: Lipid transfer via Vps13 ensures robust autophagosome biogenesis

Rahel Dabrowski, DE

11:45-12:00

Short talk: Autophagy-mediated plasma membrane removal promotes the formation of epithelial syncytia

Parisa Kakanj, DE

12:00-12:25

Flash talks 6-10

Angelina Gross, Ikuko Koyama-Honda, Hyun Jin Kim, Arindam Bhattacharjee, Kathryn Jacobs

12:25-15:00

Poster session I – odd numbers (1, 3, ...)

Buffet-style lunch at the poster area

Session 3: Stem cells and (de)differentiation

Chair: Katja Simon

15:00-15:30

Autophagy, a key player in inflammation and tumor driven lymph/angiogenesis

Patrizia Agostinis, BE

15:30-16:00

Selective autophagy in cancer

Simon Wilkinson, UK

16:00-16:30

Autophagy facilitates reprogramming in somatic cells

Morten Petersen, DK

16:30-17:00

Coffee break

17:00-17:30

EMBO Young Investigator Lecture: How to engineer human pluripotent stem cells to understand human development and disease

Nuria Montserrat, ES

17:30-17:45

Short talk: Tumor progression requires coordination of systemic lipid catabolism and local fatty acid synthesis

Szabolcs Takats, HU

17:45-18:00

Short talk: Polycomb-mediated regulation of Dram1 governs autophagy levels during mouse embryonic stem cell differentiation

Deepika Puri, IN

18:00-18:25

Flash talks 11-15

Saska Ivanova, Silvia Vega-Rubin-de-Celis, Tibor Kovacs, Margarita Papandreou, Helene Knaevelsrud

19:00-24:00

Traditional Hungarian folk dance and meet the speakers dinner I.

Dinner

Day 3 – 29 June 2022, Wednesday

Session 4: Selective autophagy

Chair: **Sascha Martens**

9:00-9:30

Regulation of PRKN-independent mitophagy

Anne Simonsen, NO

9:30-10:00

Investigating the interplay between mitophagy and pexophagy

Ian Ganley, UK

10:00-10:30

Initiating PINK1/Parkin mitophagy

Michael Lazarou, AU

10:30-11:00

Coffee break

11:00-11:30

Proteomic approach of purified p62-body in selective autophagy-defective mice reveals a novel p62-mediated selective autophagy

Masaaki Komatsu, JP

11:30-12:00

EMBO Young Investigator Lecture: Mitophagy induction by misfolded mitochondrial proteins

Christian Münch, DE

12:00-12:15

Short talk: Leveraging evolutionary diversity to discover new autophagy mechanisms in plants and humans

Yasin Dagdas, AT

12:15-12:30

Short talk: Regulation of ER-phagy during starvation response

Giorgia Di Lorenzo/Carmine Settembre, IT

12:30-15:00

Poster session II – even numbers (2, 4, ...)

Buffet-style lunch with vegetarian food only at the poster area

Session 5: Cellular defense mechanisms

Chair: Sharon Tooze

15:00-15:30

Membrane atg8ylation and autophagy in immunity, quality control, and metabolism

Vojo Deretic, US

15:30-16:00

Autophagy's role in function and differentiation of immune cells

Katja Simon, DE

16:00-16:30

DRAM1 confers resistance to intracellular infections through autophagy and
LAP

Annemarie Meijer, NL

16:30-17:00

Regulation of selective autophagy via phosphoribosyl-linked serine
ubiquitination

Ivan Dikic, DE

17:00-17:15

Short talk: TBK1 phosphorylation activates LIR-dependent degradation of the
inflammation repressor TNIP1

Joern Dengjel, CH

17:15-17:45

Coffee break

Session 6: Focus on early career scientists

Chair: **Claudine Kraft**

17:45-18:00

Short talk: FAM134-dependent ER-phagy is regulated by mTOR and CK2 via phosphorylation-dependent ubiquitination

Alexandra Stolz, DE

15:00-18:25

Flash talks 16-20

Laurene Andre, Alexis Gonzalez, Ivana Novak, Vasilis Promponas, Florian Wilfling

18:25-18:40

Short talk: NDP52 restricts KSHV entry through recruitment of selective autophagy to virus-containing endosomes

Katarina Wendy Schmidt, CH

18:40-19:05

Flash talks 16-25

Anusca Rader, Peter Lorincz, Baptiste Pradel, Beren Aylan, Shweta Santra

Session 7: Accessibility of careers

Chair: **Sharon Tooze, Christian Behrends**

19:05-19:15

Women in science: in memory of Beth Levine (1960-2020)

Vojo Deretic, US

19:15-20:00

Publishing

Petra Gross (JCS), William Teale (EMBO), Gabor Juhasz (Autophagy)

20:00-21:30

Meet the speakers dinner II.

Dinner

21:30-24:00

Poster session III – odd numbers again (1, 3, ...)

Day 4 – 30 June 2022. Thursday

Session 8: Aging

Chair: **Vojo Deretic**

9:30-10:00

Mitophagy contributes to long term neuron survival and function during ageing

Nektarios Tavernarakis, GR

10:00-10:30

Rewiring of the mTORC1-autophagy pathway in senescence: role of the lysosome

Bernadette Carroll, UK

10:30-11:00

Coffee break

11:00-11:30

Remote: Physiology of Chaperone-mediated Autophagy

Ana Maria Cuervo, US

11:30-11:45

Short talk: Selective autophagy at an organismal scale: balancing starvation survival and recovery dynamics

Joel Tuomaala, CH

11:45-12:00

Short talk: BAG3 is capable of directly targeting its cargo clients to the autophagic degradation via its LC3-interacting regions

Christian Behl, DE

12:00-12:25

Flash talks 26-30

Karolina Pircs, Tobias Eisenberg, George Kelly, Adriana Covarrubias, Samuel Rodgers

12:25-15:00

Poster session IV – even numbers again (2, 4, ...)

Buffet-style lunch at the poster area

Session 9: Autophagy-related pathways

Chair: Anne Simonsen

15:00-15:30

Roles of ubiquitination in lysophagy

Christian Behrends, DE

15:30-16:00

Crinophagy: A mechanism for insulin loss and compromised autophagy in diabetic pancreatic beta cells

Romeo Ricci, FR

16:00-16:30

LC3-associated phagocytosis in Drosophila

Gabor Juhasz, HU

16:30-17:00

Coffee break

17:00-17:30

Survival-independent functions of neuronal autophagy

Natalia Kononenko, DE

17:30-17:45

Short talk: Beyond autophagy: Noncanonical functions of the autophagy machinery

Bradlee Heckman, US

17:45-18:00

Short talk: Physiological role of endosomal microautophagy

Andreas Jenny, US

18:00-18:25

Flash talks 31-35

Karsten Nalbach, Elif Begum Gokerkucuk, Quentin Frenger, Tereza Humhalova, Ollie Haworth

18:25-19:15

Keynote: Recent advances in autophagy and autophagy-related pathways

Noboru Mizushima, JP

19:15-19:30

Concluding remarks

Gabor Juhasz, HU

19:30-21:00

Meet the speakers dinner III.

Dinner

21:00-24:00

Poster session V – all posters again

Brainstorming and speed networking

Day 5 – 1 July 2022, Friday

9:00-12:00

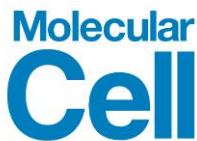
Excursions

Visiting the castle or the city or the vine valley

12:00

Departure

Sponsors



Integrating the molecular, mechanistic and
physiological diversity of autophagy

(w21-51)

Eger, Hungary, 27 June – 1 July 2022

Abstracts

Invited talks

Autophagy, a key player in inflammation and tumor driven lymph/angiogenesis

Patrizia Agostinis 1, 2, Odetta Mece 1, 2, Diederik Houbaert 1, 2, Jelle Verhoeven 1, 2, Madhur Agrawal 1, 2

1 Laboratory of Cell Death Research and Therapy, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium

2 Center for Cancer Biology, VIB-KU Leuven, Leuven Belgium

Autophagy is a major lysosomal pathway for the degradation and recycling of cytoplasmic components, with vital homeostatic functions. In cancer, autophagy has been shown to shape tumor growth and dissemination by regulating vital cell autonomous and non-autonomous processes affecting cancer cell-stromal cells interface. Our previous work highlighted that melanoma growth is delayed in mice harboring genetic deletion of ATG5 specifically in blood and lymphatic vessels (i.e. ATG5^{ECKO}) suggesting that EC-associated autophagy may provide a conducive microenvironment favoring melanoma progression (Maes et al, Cancer Cell, 2014). Emerging evidence indeed indicates that the aberrant tumor vasculature supports cancer outgrowth, not only by promoting the hypoxic and pro-angiogenic status of the tumor, but also by actively regulating tumoral immune responses. To clarify the specific contribution of blood EC (BECs) or lymphatic EC (LECs)-associated autophagy in melanoma dissemination, we generated transgenic mouse models whereby ATG5 is specifically deleted either in BECs or LECs. Here I will present unpublished data underscoring the crucial role of EC-associated autophagy in inflammation- and tumor-driven (lymph)angiogenesis.

Roles of ubiquitination in lysophagy

Christian Behrends

Ludwig-Maximilians-Universität (LMU) München

Lysosomes represent the terminal subcellular compartment for a number of degradative and recycling pathways, which makes their integrity crucial for cell homeostasis. However, under certain stress and pathophysiological conditions (e.g. neurodegeneration) the lysosomal membrane can be damaged resulting in functional impairment, leakage of lysosomal content (e.g. protons, reactive oxygen species and degradative enzymes) into the cytosol and eventually lysosomal cell death. To cope with lysosomal damage the cell therefore developed several defense strategies which are summarized under the term endo-lysosomal damage response. Selective autophagy of the ruptured lysosome, termed lysophagy, presents the ultimate step for incurable damage and is accompanied by a fast and robust ubiquitylation of the lysosome. We are particularly interested in identifying the ubiquitylation machinery and their substrates upon lysosomal damage.

Rewiring of the mTORC1-autophagy pathway in senescence: role of the lysosome

Bernadette Carroll

University of Bristol

Cellular senescence is a well-established driver of tissue and organismal ageing. While senescence is beneficial as a tumour suppressor mechanism, age-related accumulation of senescent cells in lung, muscle, liver and bone leads to a decline in tissue function, integrity and regeneration capacity.

Senescence is defined as an irreversible exit from the cell cycle and occurs in response to potentially transformative cell stress such as oncogene activation, DNA damage or excessive stress. Despite not being able to divide, growth pathways are still active in senescence leading to the characteristic increase in cell size and increased organelle content. These senescence phenotypes can be dampened or reversed by rapamycin, demonstrating an important role for the mTORC1-autophagy pathway.

We are working to explore the mechanisms responsible for dysregulation of the mTORC1-autophagy pathway in senescence. We have previously described how growth factor signalling and amino acid sensing are perturbed, and this metabolic rewiring is important for senescent cell survival. Lysosome health underlies proper function of the mTORC1-autophagy pathway, and changes in lysosome biochemistry and morphology are one of the defining features of senescence. I will focus here on our recent, unpublished work investigating the mechanisms controlling upregulation of lysosomes and autophagy in senescence. Our overall aim is to establish a more complete understanding of the metabolic rewiring of senescent cells, and I will explore our ideas on whether targeting these pathways has senolytic (senescence killing) potential.

Physiology of Chaperone-mediated Autophagy

Ana Maria Cuervo

Albert Einstein College of Medicine

Chaperone-mediated autophagy (CMA) degrades a subset of the cellular proteome in lysosomes. The activity of this pathway decreases with age and in different human disorders.

Cytosolic proteins substrate for CMA are delivered to the lysosomal lumen for degradation by directly crossing the lysosomal membrane. This process is mediated by a set of cytosolic and lysosomal chaperones and by a receptor protein at the lysosomal membrane, the lysosome-associated membrane protein type 2A (LAMP-2A). The limiting step of this type of autophagy is the binding of substrates to LAMP-2A and the organization of this protein as a translocation complex at the lysosomal membrane.

In recent years, to gain a better understanding of the physiology of CMA, we have generated conditional mouse models with blocked or enhanced CMA, systemically or in specific organs. Using these models we have learned about the importance of CMA in selectively and timely remodeling of the cell proteome. In this talk I will summarize some of the recent connections of CMA with organismal metabolism, neuronal function and circadian rhythms. I will also comment on our recent efforts to genetically and pharmacologically upregulate CMA in age-related conditions.

ER-remodelling: lessons from SARS-CoV-2

Antonella De Matteis

TIGEM

Membrane atg8ylation and autophagy in immunity, quality control, and metabolism

Vojo Deretic

University of New Mexico School of Medicine

The process of membrane atg8ylation, defined herein as the conjugation of the ATG8 family of ubiquitin-like proteins to membrane lipids, is beginning to be appreciated in its broader manifestations, mechanisms and functions. Classically, membrane atg8ylation with LC3B, one of six mammalian ATG8 family proteins, has been viewed as the hallmark of canonical autophagy entailing the formation of characteristic double membranes in the cytoplasm. However, ATG8s are now well-established as being conjugated to single membranes as well as having other functions. Collectively, atg8ylation is a modification akin to ubiquitylation and is utilized by different systems participating in membrane stress responses and membrane remodeling activities. The process of atg8ylation is co-opted by multiple downstream processes, one of which is canonical autophagy. Collectively, these biological outputs impact metabolism, quality control, and immunity. We will cover the overall concept and provide examples including those of relevance for inflammation and immunity.

Regulation of selective autophagy via phosphoribosyl-linked serine ubiquitination

Ivan Dikic

Institute of Biochemistry II, Faculty of Medicine, Goethe University

Max Planck Institute of Biophysics

Legionella pneumophila is a Gram-negative pathogenic bacteria which causes a severe form of pneumonia termed Legionnaires disease. SidE effector proteins of *Legionella* poisons the host ubiquitylation system by non-canonical phosphoribosyl-linked serine ubiquitination (PR-Ub). Attachment of PR-Ub to serine residues of host proteins regulates protein function and signalling pathways. Selective autophagy is one of the chief targets of serine ubiquitination during the first few hours of bacterial infection. Serine ubiquitinated substrates include the autophagosomal SNARE proteins syntaxin17 (STX17), SNAP29 and the autophagy adaptor SQSTM1 (p62). Modification of these proteins manoeuvre autophagy to promote intracellular bacterial proliferation. Using a combination of cell biology, bacterial infection and proteomic studies we deciphered the molecular mechanism of how serine ubiquitination of the autophagosomal SNARES help to initiate autophagosome formation but block their fusion with the lysosome. These autophagosome-like vesicles serve as a replicative niche to ensure intracellular bacterial proliferation.

Investigating the interplay between mitophagy and pexophagy

Ian Ganley

University of Dundee

Over the past decade, a great body of work has elaborated mitophagy mechanisms but relatively little is known about that of mammalian pexophagy. Likewise, whether different organelles are turned over under the same conditions and how this might be coordinated remains unclear. Here, I will present data demonstrating that mitophagy and pexophagy can happen under hypoxic stimuli and that they both require the protein NIX. NIX expression thus provides a means whereby autophagy of the two organelles can be coordinated. We find that NIX can also localise to peroxisomes, independently of mitochondria, where it interacts with the autophagy machinery to drive pexophagy.

LC3-associated phagocytosis in *Drosophila*

Gabor Juhasz, Aron Szabo

Biological Research Centre, Szeged, Hungary

Autophagy is especially important in long-lived cells such as neurons, as others and we showed many years ago that core Atg gene knockout mice and flies exhibit neurodegeneration, ataxia and short lifespan. These papers also established that autophagy is required for the selective turnover of polyubiquitinated proteins, but how much the large-scale accumulation of protein aggregates containing p62 and ubiquitinated proteins contribute to these pathologies was unclear. We have recently reported that p62 knockin flies, in which we disrupted the LC3/Atg8a interacting motif of p62 via CRISPR gene editing, are viable and fertile without any obvious defects despite massive accumulation of ubiquitinated aggregates in all cells including neurons. Thus, surprisingly, the loss of selective ubiquitinated protein autophagy is not detrimental to cells and animals.

In search for other functions of *Drosophila* autophagy genes, we find that the breakdown of axon debris during Wallerian degeneration is disrupted in Atg8a and Atg16 mutant flies. Our further experiments reveal that Atg1 kinase complex subunits are not required for the timely clearance of axon fragments after traumatic nerve injury, pointing to a possible non-autophagic process. Indeed, glial (but not neuronal) disruption of Atg8 lipidation or of the Rubicon-containing Vps34 lipid kinase complex prevents the breakdown of axon debris, indicating that glial cells use LC3-associated phagocytosis (LAP) to promote debris-containing phagosome maturation and fusion to lysosomes. In line with the genetic evidence, glial phagosomes containing axon debris become positive for Atg8a and Rubicon, and Rubicon overexpression accelerates debris clearance. Taken together, our *in vivo* data raise the possibility that induction of LAP in phagocytic glia could aid the recovery from nervous system injury.

The regulation of autophagy in yeast

Daniel Klionsky

University of Michigan, Life Sciences Institute, Ann Arbor, MI, USA

Autophagy is a fundamental pathway of cellular homeostasis, and it is involved in a wide array of physiological processes. Along these lines, defects in autophagy are associated with numerous diseases in humans. Accordingly, there is tremendous interest in manipulating autophagy therapeutically. However, this process is currently difficult to modulate pharmacologically, and there is an added problem that either too little or too much autophagy can be deleterious. Therefore, we are interested in learning more about the mechanisms involved in autophagy regulation within the cell, as the regulatory components may provide additional targets for clinical application. Autophagy is conserved from yeast to human, so the information we gain from studying the more genetically tractable yeast system is generally applicable to other organisms. We find that autophagy is controlled through multiple mechanisms from epigenetic, through transcriptional, translational and post-translational modifications. Interestingly, the regulation of autophagy often happens in a manner that is opposite to that of processes that occur during normal growth conditions. This presentation will highlight some of our recent work on the transcriptional and translational regulation of autophagy.

Proteomic approach of purified p62-body and selective autophagy-defective mice reveals a novel p62-mediated selective autophagy

Masaaki Komatsu, Reo Kurusu, Yuki Fujimoto, Hideaki Morishita

Department of Physiology, Juntendo University School of Medicine

Autophagy contributes to the selective degradation of large cytoplasmic p62-bodies that are formed by liquid-liquid phase separation, but the constituents, which changes in response to cellular conditions, largely remain unclear. Here, we establish a fluorescence-activated particle sorting-based purification for p62-bodies and determine their constituent elements by mass spectrometry. By combining it with the quantitative mass spectrometry using selective autophagy-defective mouse tissues, we identify several proteins that localize in p62-bodies and are degraded by autophagy such as known autophagy substrates such as ubiquitinated proteins and KEAP1 and selective autophagy receptors including NBR1 and TAX1BP1. In addition, we found vault, a large cytoplasmic ribonucleoprotein particle as a component of p62-bodies and as a selective substrate. Mechanistically, major vault protein, a predominant subunit of vault, directly interacts with the Ubiquitin-associated domain of NBR1 independently of its ubiquitination, which is required to recruit vault to p62-bodies and subsequent degradation. Therefore, suppression of selective autophagy in mouse livers increases the p62-structures containing vault. Meanwhile, liver-specific depletion of Nbr1 in mice results in diffuse accumulation of vault in the cytosol. Collectively, this study provides a unique proteomic approach to identify substrates for p62-mediated autophagy and reveals the molecular mechanism for the degradation of the non-membranous organelle, vault.

Survival-independent functions of neuronal autophagy

Natalia Kononenko

Center for Physiology and Pathophysiology & CECAD, Faculty of Medicine and University Hospital
Cologne, University of Cologne,

Autophagy provides nutrients during starvation and eliminates detrimental cellular components. However, accumulating evidence indicates that autophagy is not merely a housekeeping process. Here, we show that the protein AuTophagy 5 (ATG5) functions in neurons to regulate the cAMP-dependent protein kinase A (PKA)-mediated phosphorylation of a synapse-confined proteome. This function of ATG5 is independent of bulk turnover of synaptic proteins and requires the targeting of PKA inhibitory R1 subunits to autophagosomes. Neuronal loss of ATG5 causes synaptic accumulation of PKA R1, which sequesters the PKA catalytic subunit and diminishes the cAMP/PKA-dependent phosphorylation of postsynaptic cytoskeletal proteins mediating AMPAR trafficking. Glutamatergic neurons-confined ATG5 deletion augments AMPAR-dependent excitatory neurotransmission and causes the appearance of spontaneous recurrent seizures in mice. Our findings identify a novel role of autophagy in regulating PKA signaling at glutamatergic synapses and suggest the PKA as a target for restoration of synaptic function in neurodegenerative conditions with autophagy dysfunction.

Spatial confinement and avidity drive autophagosome formation

Claudine Kraft

University of Freiburg, Germany

Autophagosomes form at the endoplasmic reticulum in mammals, and between the vacuole and the endoplasmic reticulum in yeast. However, the roles of these sites and the mechanisms regulating autophagosome formation are incompletely understood. In budding yeast, Vac8 is required for autophagy initiation and recruits several autophagy factors to the site of autophagosome formation at the vacuole. It acts as an assembly hub at the vacuole. However, ectopic Vac8 can drive autophagosome formation also at other locations in the cell, indicating that the vacuolar membrane is not specifically required. We propose that multiple avidity-driven interactions drive the initiation and progression of selective autophagy.

Initiating PINK1/Parkin mitophagy

Michael Lazarou

Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Monash Biomedicine Discovery Institute, Monash University, Clayton, Australia

Cargo sequestration is a crucial step of selective autophagy in which cells generate autophagosomes de novo on the surface of cargo. During PINK1/Parkin mitophagy, the cargo adaptor NDP52 binds to FIP200 which recruits the ULK1/2 kinase complex to initiate autophagosome biogenesis. Optineurin is another key mitophagy adaptor, but how it initiates autophagosome formation during mitophagy remains largely unclear. Here, we uncover the mitophagy initiation mechanisms of Optineurin, revealing which kinases it requires and how it engages the autophagy machinery. We find that Optineurin follows an unconventional path of mitophagy activation that involves initial engagement with the lipid kinase machinery (PI3KC3-C1) through its interaction partner TBK1. How the lipid kinase machinery subsequently recruits the ULK1/2 kinase complex is also explored. Overall, our work reveals that NDP52 and Optineurin mediated mitophagy initiation is mechanistically distinct, demonstrating that there is more than one path of autophagosome formation for PINK1/Parkin mitophagy.

Neuroprotective properties of the autophagy inducer spermidine

Frank Madeo

University of Graz

Mechanisms of Cargo Selection and Autophagosome Biogenesis in Selective Autophagy

Sascha Martens

Max Perutz Labs, University of Vienna

Autophagy is an intracellular lysosomal bulk degradation pathway that ensures cellular homeostasis by the removal of damaged and dangerous material from the cytoplasm. This is achieved by the sequestration of the cytoplasmic cargo material within double membraned organelles called autophagosomes. The selective sequestration of only specific cargo material is mediated by cargo receptors that link the cargo to the nascent autophagosomal membrane. How cargo selection, membrane nucleation and growth are coupled is unclear. I will present our recent work on the human cargo receptors and the autophagy machinery derived from in vitro reconstitution systems and cell biology. In particular, I will discuss how cargo receptors and the autophagy machinery act sequentially during cargo condensation, membrane nucleation and elongation to mediate the specific sequestration and subsequent degradation of cellular material.

DRAM1 confers resistance to intracellular infections through autophagy and LAP

Annemarie Meijer

Institute of Biology Leiden, Leiden University

The membrane protein DRAM1, for Damage-Regulated Autophagy Modulator 1, is the founding member of a family of five stress-inducible autophagy modulators. DRAM1 functions as a target of p53 signaling in the UV-damage response and as a target of NF κ B signaling during infection. Using mammalian macrophages together with in vivo zebrafish models for tuberculosis and salmonellosis, we have shown that DRAM1 confers resistance against different intracellular pathogens, including *Mycobacterium marinum* and *Salmonella typhimurium*. Interestingly, the mechanisms through which these pathogens interact with the autophagy machinery are different. *M. marinum* is targeted by xenophagy, following invasion of the cytosol, whereas *S. typhimurium* is subject to LC3-associated phagocytosis (LAP). These results show that DRAM1 functions as a central hub in pathogen degradation, increasing the intrinsic capacity of immune cells to kill intracellular pathogens through both xenophagy and LAP. We have evidence that DRAM1 augments LC3 recruitment in these pathways, as well as promotes the fusion of bacteria-containing vesicles with lysosomes. This DRAM1-mediated vesicle fusion depends on the activity of the PIKfyve lipid kinase. As a consequence of reduced vesicle maturation, DRAM1-deficient macrophages fail to contain pathogens intracellularly and succumb to disease-exacerbating pyroptotic cell death. Due to its central role in host resistance to intracellular infection, DRAM1 is a promising target for therapeutic modulation of the microbicidal capacity of macrophages.

Recent advances in autophagy and autophagy-related pathways

Noboru Mizushima

Department of Biochemistry and Molecular Biology, Graduate School of Medicine, The University of Tokyo

Autophagy research has grown exponentially in various organisms, and the basic mechanisms and physiological functions of autophagy have been extensively investigated. However, there remain many fundamental questions and technical problems. One is that measuring autophagic flux is still challenging, particularly in mammalian cells. This limitation is a bottleneck in both basic and applied research. To overcome this problem, we recently established a pulse-chasable HaloTag (Halo)-based reporter processing assay. This new assay is highly quantitative and objective, and can detect even bulk nive autophagy.

Another hot topic in this field is organelle degradation. It is well established that autophagy can selectively degrade organelles such as depolarized mitochondria, damaged lysosomes, and fragments of the endoplasmic reticulum. Besides these membranous organelles, membrane-less organelles produced by liquid-liquid phase separation are also targeted by autophagy. We recently found that NCOA4, which has been considered a receptor of ferritin autophagy (ferritinophagy), drives phase separation of ferritin, and ferritin-NCOA4 condensates are subjected to both macroautophagy and microautophagy. In addition to these autophagy-dependent pathways, we discovered a new organelle degradation system in the lens, which involves the cytosolic phospholipase PLAAT, but not autophagy. Thus, various mechanisms exist for organelle degradation. I would also like to talk about our recent progress on a novel modification of phospholipids in organellar membranes.

How to engineer human pluripotent stem cells to understand human development and disease

Núria Montserrat

Barcelona Institute of Science and Technology

In recent years considerable progress has been made in the development of faithful procedures for the differentiation of human pluripotent stem cells (hPSCs). An important step in this direction has also been the derivation of three-dimensional cell cultures that represent micrometer to centimeter size versions of human organs, the so-called organoids. The convergence of stem cell biology and bioengineering now offers the possibility to provide physiologically relevant stimuli in a controlled fashion, resulting in the development of naturally inspired approaches to overcome major limitations of the organoid field.

Here we will discuss current developments in the kidney organoid field and emphasize the achievements and ongoing challenges of bringing together hPSC organoid differentiation, bioengineering and disease modelling with a particular focus on genetic and systemic disorders as well as COVID19 research.

Mitophagy induction by misfolded mitochondrial proteins

Jonas Michaelis 1, Süleyman Bozkurt 1, Jasmin Schäfer 1, Christian Münch 1, 2, 3

1 Institute of Biochemistry II, Goethe University Frankfurt, Faculty of Medicine, Frankfurt, Germany

2 Frankfurt Cancer Institute

3 Cardio-Pulmonary Institute Frankfurt

Mitophagy is essential to maintain mitochondrial function and prevent diseases. It activates upon mitochondria depolarization, which causes PINK1 stabilization on the mitochondrial outer membrane. Strikingly, a number of conditions, including mitochondrial protein misfolding, can induce mitophagy without a loss in membrane potential. The underlying molecular details remain unclear. Here, we report that a loss of mitochondrial protein import, mediated by the pre-sequence translocase-associated motor complex PAM, is sufficient to induce mitophagy in polarized mitochondria. A genome-wide CRISPR/Cas9 screen for mitophagy inducers identifies components of the PAM complex. Protein import defects are able to induce mitophagy without a need for depolarization. Upon mitochondrial protein misfolding, PAM dissociates from the import machinery resulting in decreased protein import and mitophagy induction. Our findings extend the current mitophagy model to explain mitophagy induction upon conditions that do not affect membrane polarization, such as mitochondrial protein misfolding.

Autophagy facilitates reprogramming in somatic cells

Jakob Kanne 1, Jeppe Ansboel 1, Masaki Ishikawa 2, Mitsuyasu Hasebe 2, Eleazar Rodriguez 1, Yasin Dagdas 3, Morten Petersen 1

1 Department of Biology, Copenhagen University

2 Department of Basic Biology, The Graduate University for Advanced Studies, SOKENDAI, Okazaki, Japan

3 Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna BioCenter (VBC), Vienna, Austria.

Autophagy has been reported to regulate many different cellular processes. Autophagy has also been implicated in induced pluripotent stem cell formation, cellular regeneration, and stem cell survival in animals. Using the plant models *Arabidopsis thaliana* and *Physcomitrium patens*, we provided comprehensive evidence that autophagy functions in many types of cellular reprogramming. Stimuli as diverse as phytohormones, danger signals and microbial elicitors triggered robust activation of autophagy. Using quantitative proteomics, we showed that autophagy mediates the switch between several somatic cell programs by removing cellular components that are no longer required. Moreover, pluripotent cells of autophagy mutants exhibit de-regulated cell growth and organogenesis, while somatic cells of autophagy deficient plants struggle to dedifferentiate and produce pluripotent cells (A). In addition, overexpressing ATG8 has a positive effect on cellular reprogramming in wild type plants but not in an autophagy-deficient background (B). Interestingly, autophagic activity and dedifferentiation provoked by wounding depends upon the activation of a wound activated MAP kinase. These observations indicate that autophagy promotes phenotypic plasticity in plants and functions in wound induced regeneration.

Crinophagy: A mechanism for insulin loss and compromised autophagy in diabetic pancreatic beta cells

Romeo Ricci

IGBMC

A very fundamental conundrum in the beta cell field was the basic observation that β cells showed very moderate or no induction of macro-autophagy in response to nutrient deprivation or fasting. This somehow contradicted the classical concept in which mammalian cells engage in cellular autodigestion via macro-autophagy to overcome shortage of nutrients. Our laboratory put forward a fundamentally new concept explaining these unresolved biological problems. Several cellular metabolites and nutrients are capable of stimulating insulin secretion and it was reasonable to assume that non-selective macro-autophagy produces potent insulin secretagogues. In our study, we discovered that β cells specifically induce lysosomal degradation of insulin granules while keeping macro-autophagy inhibited through lysosomal induction of mTORC1 activity. Forcing macro-autophagy under nutrient-poor conditions stimulated insulin secretion from β cells. Hence, conceptually, generation of nutrients by lysosomal degradation of insulin granules is an ideal strategy to replace macro-autophagy and to counteract fasting as at the same time, it removes insulin that otherwise would be secreted in response to autodigestion. In fact, it would be detrimental to secrete insulin during fasting, as it could lower glucose levels even further. For these reasons, we believe that β cells employ this evolutionary adapted and fundamentally distinct response to fasting. Intriguingly, we have very recently demonstrated that lysosomal insulin granule degradation is dysregulated in diabetic β cells potentially representing a major mechanism underlying insulin loss. Together with our previous studies, this stimulated an extensive collaboration with Sanofi France, where my laboratory with a team of chemists, biologists and structural biologists identified a promising compound that acts to inhibit lysosomal insulin granule degradation. Strikingly, this compound is capable of attenuating β cell failure and diabetes in mice, thus opening future avenues for further pre-clinical therapeutic exploitation.

Structural basis of Atg18 oligomer binding to lipid membranes

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Autophagy-related protein 18 (Atg18) is a critical membrane adapter in yeast macroautophagy, a conserved catabolic pathway involved in cellular maintenance and homeostasis in eukaryotes. The seven WD40-repeat PROPPIN family protein Atg18 participates in the elongation of early phagophores when complexed with Atg2 and Atg9. How Atg18 contributes to the structural and mechanistic coordination of Atg2 and Atg9 assemblies remains to be understood. Here, we determined the near-atomic resolution cryo-EM structures of Atg18 organized in helical tubes as well as soluble oligomers. Moreover, we show by biochemical binding studies that smaller Atg18 oligomers are capable to associate with lipid membranes through its PI(3)P and PI(3,5)P₂ FRRG-motif while giving up large helical assemblies. Subtomogram averaging identified a twisted Atg18 dimer at 26 Å resolution bridging two juxtaposed membranes at constant distances of 80 Å. The observed structural properties of Atg18 membrane binding and oligomeric organization provide a molecular framework for the positioning of downstream components of the autophagy machinery.

Ultrastructural characterization of autophagy by cryo electron tomography

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Autophagosome biogenesis involves a series of membrane transformations with distinctly shaped intermediates whose ultrastructure is poorly understood. As a step toward obtaining a high-resolution structural understanding of autophagy, we have combined cell biology, correlative cryo-electron tomography (ET) and novel data analysis to reveal the step-by-step structural progression of autophagosome biogenesis at high resolution directly within yeast cells. By mapping individual structures onto a timeline based on geometric features, we uncover dynamic changes in membrane shape and curvature. Moreover, we reveal the organelle interactome of growing autophagosomes, highlighting a polar organization of contact sites between the phagophore and organelles such as the vacuole and the ER. Collectively, these findings have important implications for the contribution of different membrane sources during autophagy and for the forces shaping and driving phagophores towards closure without a templating cargo.

Autophagy's role in function and differentiation of immune cells

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Across all arms of the immune system, the process of autophagy is key in development, function, homeostasis and prevention of senescence . However, the mechanisms involved are much less clear, and encompass control of metabolism, cell survival, and selective degradation of substrates and organelles. However, a technique is lacking that determines the autophagic degradome systematically in immune cells and other primary tissues in vivo. Here I present a novel proximity labelling mouse model which we use to identify autophagic cargo in T cells. So far the autophagy field has focused on degradation rather than provision. How important is the provision of amino acids by autophagy and how is that linked to metabolism and differentiation is a topic we investigated in hematopoietic stem cells.

Regulation of PRKN-independent mitophagy

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The mechanisms involved in programmed or damage induced removal of mitochondria by mitophagy in response to different stimuli remains elusive. We have recently screened for regulators of Parkin-independent mitophagy by treating cells expressing a mitochondrial matrix reporter with inducers of HIF1A-mediated mitophagy. I will present data showing mechanistic insight into the function of novel regulators of mitophagy and their role in disease development, using zebrafish as a model organism.

Mitophagy contributes to long term neuron survival and function during ageing.

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Ageing is driven by the inexorable and stochastic accumulation of damage in biomolecules vital for proper cellular function. Although this process is fundamentally haphazard and uncontrollable, senescent decline and ageing is broadly influenced by genetic and extrinsic factors. It is becoming increasingly apparent that most such interventions ultimately interface with cellular stress response mechanisms, suggesting that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Key determinants of this capacity are the molecular mechanisms that link ageing to main stress response pathways, and mediate age-related changes in the effectiveness of the response to stress. Mitochondria, the indispensable and highly dynamic, energy-generating organelles in all eukaryotic cells, play essential roles in fundamental cellular processes. Neuronal cells depend, perhaps more than any other cell type, on proper mitochondrial function. Mitochondrial impairment is a major hallmark of several age-related neurodegenerative pathologies, including Alzheimer's disease. Interestingly, accumulation of damaged mitochondria has been observed in post-mortem brain of Alzheimer's disease patients. Although disease-associated tau and amyloid β are known to deregulate mitochondrial function, it remains elusive whether they also directly influence the efficiency of mitophagy. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to their metabolic state. We developed an in vivo imaging system to monitor mitophagy in neurons. We demonstrated that neuronal mitophagy is impaired in *C. elegans* models of Alzheimer's disease. Urolithin A- and nicotinamide mononucleotide-induced mitophagy ameliorates several pathological features of Alzheimer's disease, including cognitive defects. Mitophagy stimulation restores memory impairment through PINK-1-, PDR-1 or DCT-1-dependent pathways. Our findings suggest that impaired removal of damaged mitochondria is a pivotal event in Alzheimer's disease pathogenesis highlighting mitophagy as a potential therapeutic intervention.

ATG9A and ATG2A form a heteromeric complex essential for autophagosome formation

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ATG9A and ATG2A are essential core members of the autophagy machinery. ATG9A is a lipid scramblase that allows equilibration of lipids across a membrane bilayer, while ATG2A facilitates lipid flow between tethered membranes. Although both have been functionally linked during the formation of autophagosomes, the molecular details and consequences of their interaction remain unclear. By combining data from peptide arrays, crosslinking and hydrogen deuterium exchange mass spectrometry, together with cryo-electron microscopy, we propose a molecular model of the ATG9A-2A complex. Using this integrative structure modelling approach, we identify several interfaces mediating ATG9A-2A interaction which would allow a directional transfer of lipids from ATG2A into ATG9A. Mutational analyses combined with functional activity assays demonstrate their importance for autophagy, thereby shedding light on this protein complex at the heart of autophagy.

Selective autophagy in cancer

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Genetic studies have revealed numerous roles for bulk autophagy in cancer. However, selective autophagy has been less frequently investigated. We propose that investigating selective autophagy across different cancers and stages will illuminate tumourigenesis and treatment, and provide fundamental discovery tools. To illustrate, I will present new data on ER-phagy and lysophagy.

Our data show that ER-phagy inhibits pancreatic cancer onset. The mutant Kras oncogene transcriptionally compromises ER-phagy in mouse pancreatic acinar cells. Kras mutant cells are initially indistinguishable from healthy cells. However, we identify a transient cell state characterised by ER-phagy dysfunction, which represents a novel intermediate between healthy cells and a known pre-malignant cell state (ductal metaplasia). Genetic experiments modifying autophagy by targeting Ccpg1 (ER-phagy receptor) and Atg5 in mouse pancreas confirm the role of ER-phagy in preventing progression to this intermediate cell state. This work also involved development of new SRAI-based ER-phagy reporters for use both in vitro and in vivo. These and their application in parallel screening projects for novel ER-phagy regulators will be outlined.

To contrast, I will finish by briefly outlining lysophagy involvement in cancer therapeutic responses; our data identify clinical drugs that cause lysophagy by damaging lysosomes and triggering TBK1 activation. If cellular lysophagy capacity is exceeded, these associate with mitochondria at novel contact sites, whereupon TBK1 drives leakage of mitochondrial DNA, triggering interferon responses.

Short talks

BAG3 is capable of directly targeting its cargo clients to the autophagic degradation via its LC3-interacting regions

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The co-chaperone BAG3 is a hub for various cellular pathways via its multiple domains and its interaction with HSP70 and HSPB8. Besides its function in maintaining myofibrils or promoting survival, growth and invasiveness in cancer, it is also associated with the cellular protein quality control driving a BAG3-mediated selective autophagy pathway. Under cellular stress conditions in particular, together with different molecular chaperones, BAG3 ensures the sequestration of aggregated or aggregation-prone ubiquitinated proteins to the autophagic-lysosomal system via ubiquitin receptors. Presently there are emerging indications that the BAG3-mediated macroautophagy also copes with non-ubiquitinated cargo. Phylogenetically, BAG3 comprises several highly conserved putative LIRs, LC3-interacting regions, which might directly target its cargo to ATG8 proteins and drive their autophagic degradation. Based on the interaction of BAG3 with LC3 proteins peptide array and pull-down data indicate the functionality of these LIRs. In addition, we found that mutations within the LIRs impair colocalization with ATG8 proteins via immunofluorescence. A BAG3 variant mutated in all LIRs demonstrates a substantial decrease of BAG3 levels within purified native autophagosomes compared to the wildtype. These results strongly suggest LC3-mediated sequestration of BAG3, including its cargo. Therefore, we conclude that in addition to being a pivotal co-chaperone to HSP70, BAG3 may also act as a cargo co-/receptor for non-ubiquitinated client proteins and significantly extend BAG3's role in selective autophagy. (Funded by the CRC1177)

Lipid transfer via Vps13 ensures robust autophagosome biogenesis

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During autophagosome biogenesis, small single-membrane phagophores nucleate from vesicular precursors. Phagophores then rapidly expand as cup-shaped intermediates to form large double-membrane autophagosomes. The core autophagy protein Atg2 organizes phagophore-ER contact sites and channels phospholipids between membranes in vitro. However, whether Atg2-mediated lipid transfer (LT) in vivo is sufficient to provide the millions of phospholipids required for phagophore expansion within a few minutes is still unclear.

Using quantitative live-cell imaging, we find that autophagosome biogenesis required the same time independently of autophagosome size. These findings indicate a coupling between the LT rate driving phagophore expansion and the size of the resulting autophagosome. However, we show that the number of Atg2 molecules at a phagophore poorly correlated with the size of the resulting autophagosome, suggesting that additional LT mechanisms contribute to phagophore expansion during autophagosome biogenesis. Indeed, we find that the Atg2-related LT protein Vps13 quantitatively associated with forming autophagosomes and critically contributed to autophagosome biogenesis. In the absence of Vps13 or its LT activity, the duration of autophagosome biogenesis correlates with the size of the resulting autophagosome. Thus, Vps13 is required to allow for an efficient formation of differently sized autophagosomes in the same time. Furthermore, our data show that upon loss of Vps13, the number of Atg2 molecules positively correlates with autophagosome size. Therefore, in the absence of the additional LT protein Vps13, LT via Atg2 becomes rate limiting for autophagosome biogenesis. In summary, our work reveals that Vps13 and Atg2 cooperate in channeling phospholipids into growing phagophores and the presence and LT activity of Vps13 ensures robust autophagosome biogenesis.

Leveraging evolutionary diversity to discover new autophagy mechanisms in plants and humans

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Quality control pathways mediate phenotypic plasticity which is crucial for adaptation to adverse conditions. Selective autophagy is a highly conserved quality control mechanism that remodels cytoplasmic contents during cellular reprogramming. The selective nature of the process is encoded by selective autophagy receptors (SARs) that bridge the cargo with the core autophagy machinery and ensure selective cargo degradation. SARs recruit the autophagy machinery by interacting with ATG8, a ubiquitin-like protein conjugated to the phagophore, via a conserved ATG8-interacting motif (AIM). Despite recent advances in metazoans, the catalog of SARs is incomplete in plants. Here, we leverage a state-of-the-art proteomics approach to identify novel autophagy players conserved across land plants. Unlike conventional proteomics approaches, we devised a simple, high-throughput approach to specifically identify direct ATG8 interactors. We biochemically validated candidate interactors using in vivo and in vitro approaches. I will present our latest findings on the mode of action of CESAR, a novel selective autophagy receptor that we have identified through our pipeline that is conserved over more than 500 million years of plant evolution.

TBK1 phosphorylation activates LIR-dependent degradation of the inflammation repressor TNIP1

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Limitation of excessive inflammation due to selective degradation of pro-inflammatory proteins is one of the cytoprotective functions attributed to autophagy. In the current study, we highlight that selective autophagy also plays a vital role in promoting the establishment of a robust inflammatory response. Under inflammatory conditions, here TLR3-activation by poly(I:C) treatment, the inflammation repressor TNIP1 (TNFAIP3 interacting protein 1) is phosphorylated by TBK1 (Tank-binding kinase 1) activating a LIR motif that leads to the selective autophagy-dependent degradation of TNIP1, supporting expression of pro-inflammatory genes and proteins. This selective autophagy efficiently reduces TNIP1 protein levels early (0-4 h) upon poly(I:C) treatment to allow efficient initiation of the inflammatory response. At 6 h TNIP1 levels are restored due to increased transcription avoiding sustained inflammation. Thus, similarly as in cancer, autophagy may play a dual role in controlling inflammation depending on the exact state and timing of the inflammatory response.

Regulation of ER-phagy during starvation response

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Selective degradation of the endoplasmic reticulum (ER) via autophagy (ER-phagy) is initiated by ER-phagy receptors, which facilitate the incorporation of ER-fragments into autophagosomes. FAM134 reticulon family proteins (FAM134-A, -B and -C) are ER-phagy receptors with structural similarities and non-redundant functions. Whether they respond differentially to the stimulation of ER-phagy is unknown. Here we describe an activation mechanism unique to FAM134C during starvation. In fed conditions, FAM134C is phosphorylated by Casein kinase 2 (CK2) at critical residues flanking the LIR domain. Phosphorylation of these residues negatively affects binding-affinity to the autophagy proteins LC3. During starvation, mTORC1 inhibition prevents FAM134C phosphorylation by CK2, hence promoting receptor activation and ER-phagy. Using a novel tool to study ER-phagy in vivo, we demonstrated the physiological relevance of FAM134C phosphorylation during starvation induced ER-phagy in mouse liver. These data provide a new mechanistic insight into ER-phagy regulation and an example of autophagy selectivity during starvation.

Beyond autophagy: Noncanonical functions of the autophagy machinery

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Over the past decade the diverse roles of the autophagy machinery in unique cellular mechanisms have come to light. We, along with our collaborators including Douglas Green, have characterized new functions for the autophagy machinery in the lipidation of LC3 to unique membrane depots and the physiological implications of these pathways including LC3-associated phagocytosis (LAP) and endocytosis (LANDO). We have extended these earlier studies and will present new data demonstrating the use of the autophagy machinery and in particular the key noncanonical regulator Rubicon, in the trafficking of intracellular effector proteins. We detail two instances where Rubicon and the autophagy machinery are of paramount importance, 1) in the regulation of necroptosis through mediating MLKL plasma membrane translocation and 2) in the regulation of mitochondrial dynamics. In both instances and as will be presented, activation of this unique intracellular LC3-associated pathway that is distinct from canonical autophagy, LAP, and LANDO, is protective against cell death and loss of mitochondrial function. We found that AD patient brain has reduced expression of autophagy proteins including Rubicon with commensurate increases in necroptotic activation and plasma membrane localization of active MLKL. Using murine models of Alzheimer's Disease (AD) we have identified a novel key role for Rubicon in preventing neurodegeneration by limiting necroptotic activation and mitigating neuroinflammation as well as memory loss. Furthermore, we have characterized that Rubicon and the autophagy machinery function during ischemic stroke to prevent mitochondrial dysfunction and apoptotic death of neurons, improving stroke outcome and reducing neurological consequences. These findings further add to the expanding roles for the autophagy machinery in health and disease. Moreover, they provide a new understanding for putative therapeutic intervention in difficult to treat diseases such as AD and beyond.

NDP52 restricts KSHV entry through recruitment of selective autophagy to virus-containing endosomes

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Kaposi sarcoma-associated herpesvirus (KSHV) is a human oncogenic γ -herpesvirus associated with Kaposi sarcoma and with two B cell malignancies. Kaposi sarcoma is one of the most common cancers in sub-Saharan Africa and in immunocompromised individuals, such as patients suffering from HIV/AIDS. Autophagy is an evolutionarily conserved cellular degradation process that is involved in defending the cytosol against intracellular pathogens, such as viruses, in a selective form of autophagy called xenophagy. In xenophagy, selective autophagy receptors bind to cytosol-invading pathogens and direct them to autophagy which leads to subsequent degradation of the pathogen within the lysosome. Studies have shown that KSHV lytic reactivation can be restricted by xenophagy, however the relationship between xenophagy and KSHV at the start of infection during viral entry is less understood. Using an in vitro infection model with human epithelial cell lines, we show that the lipidation of LC3 is induced shortly after KSHV entry, and when autophagy is inhibited, it seems to facilitate infection, suggesting that autophagy has an antiviral role on KSHV. We then investigated more in detail the molecular mechanism taking place at the site of newly endocytosed viral particles. We observe that several autophagy-related proteins (ATG) are recruited to endocytosed viral particles, which also seems to be decorated by autophagy receptors p62 and nuclear dot protein 52 kDa (NDP52/CALCOCO2). Interestingly, upon KSHV entry, two virus structural proteins, capsid and tegument were observed co-localizing with the autophagic eat me signal galectin-8 and NDP52. Thus, we hypothesize that galectin-8 may be recruited to glycans exposed on virus-damaged endosomes, and that NDP52 then binds to galectin-8 to which it then recruits the autophagy machinery to clear the damaged endosome and the pathogenic contents therein. This cell-autonomous mechanism for KSHV restriction could provide new insights for future medical treatments of patients suffering from KSHV-associated malignancies.

Physiological role of endosomal Microautophagy

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Autophagy is a catabolic process degrading damaged organelles and aggregate prone proteins and thus is essential for development and cellular homeostasis. As part of stress responses, it counteracts various human diseases, and its reduction leads to aging like phenotypes. Of the three major forms of autophagy, Macroautophagy can degrade organelles or aggregated proteins, and Chaperone-mediated autophagy (CMA) is specific for proteins containing KFERQ-related targeting motifs. The physiological role of endosomal Microautophagy (eMI), a form of autophagy during which substrates are taken up into multivesicular bodies for degradation in a KFERQ-specific manner or in bulk, is the least understood.

Using a KFERQ-tagged biosensor, we have developed an *in vivo* model of eMI and found that eMI in flies is partially Hsc70-4 and fully ESCRT machinery dependent. Our data further suggest that starvation, oxidative stress, and DNA damage, but not ER stress can elicit an eMI response, implying a stress-selectivity of the process.

In contrast to CMA, for which the lysosomal translocation channel LAMP2A is the key limiting factor, eMI should be independent of a LAMP protein. We therefore made mutants in *Lamp1*, the *Drosophila* ortholog of the mammalian LAMP1/2. Surprisingly and in contrast to *lamp1 lamp2* double-mutant mice, *Drosophila Lamp1* is not required for viability or autophagy, suggesting that fly and vertebrate LAMP proteins acquired distinct functions, or that autophagy defects in *lamp1 lamp2* mutants have indirect causes. However, *Lamp1* deficiency results in an increase in the number of acidic organelles in flies. Furthermore, we find that *Lamp1* mutant larvae show elevated levels of sterols and diacylglycerols (DAGs). Because DAGs are the main lipid species used for transport through the blood in insects, our results indicate broader functions of *Lamp1* in lipid transport which thus can be studied in *Drosophila* without the confounding effects of lipid storage or autophagy.

Autophagy-mediated plasma membrane removal promotes the formation of epithelial syncytia

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Epithelial wound healing in *Drosophila* involves the formation of multinucleate cells surrounding the wound. We show that autophagy, a cellular degradation process often deployed in stress responses, is required for the formation of a multinucleated syncytium during wound healing, and that autophagosomes that appear near the wound edge acquire plasma membrane markers. In addition, uncontrolled autophagy in the unwounded epidermis leads to the degradation of endo-membranes and the lateral plasma membrane, while the apical and basal membranes and the epithelial barrier function remain intact. Proper functioning of TORC1 is needed to prevent autophagy from destroying the larval epidermis, which depends on membrane isolation and phagophore expansion, but does not require the fusion of autophagosomes to lysosomes. Our findings reveal a function for TORC1-mediated regulation of autophagy in maintaining membrane integrity and homeostasis in the epidermis and during wound healing. Finally, autophagy can counteract experimentally induced nuclear defects resembling laminopathies.

Membrane wetting of phase-separated compartments organizes autophagosomal cargo sequestration

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Compartmentalisation of cellular material in condensed, droplet-like structures is a hallmark of liquid-liquid phase separation. Evidence suggests that such proteinaceous condensed phases can be degraded by autophagy, a highly-conserved degradation system in which membrane sheets bend to isolate portions of the cytoplasm within double-membrane autophagosomes. However, the mechanisms of how such biomolecular condensates mediate cargo sequestration are poorly understood.

We recently identified membrane-wetting condensates as a novel key autophagy structure [Nature 2020]. Further, we observed that closing autophagosomes divide condensate droplets for piecemeal degradation and uncovered a switching mechanism that allows condensed phases to act either as liquid assembly platforms for cytosol-degrading autophagosomes or as specific autophagy substrates [Nature 2021]. In plant embryos, we identified that the formation of protein storage vacuoles underlies similar physical principles [PNAS, 2021], revealing the importance of membrane wetting in cytosolic organization.

Lipid composition determines Syntaxin17 recruitment to autophagosomes

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Membrane bound organelles play key roles in autophagy, but we know very little about their lipid composition and its relevance, e.g. by lipid-protein interactions. In this project, we study these in the context of autophagosomal recruitment of the SNARE Syntaxin17, which is an important factor for lysosomal fusion. This is an unusual protein, as it has been shown to be present both in the cytosol and in the autophagosomal (and other intracellular) membrane(s). To be able to perform lipid-protein interaction studies first we had to decipher the lipid composition of autophagic membranes. We successfully established an easy biochemical method for the isolation of these autophagic structures from wild type and lipid transport mutant (Atg2-) *Drosophila*. Our recently published lipidomics data showed that autophagic membranes contain highly unsaturated lipids. To explore the role of specific lipids in the recruitment of Syntaxin17 to the autophagic membranes, we applied a multidisciplinary approach by combining in vitro and in vivo methods: biochemistry, biophysics, molecular biology and genetics. We managed to optimize the expression and purification of soluble forms of both full-length *Drosophila* and human Syntaxin 17. By adding these recombinant Syntaxin 17 proteins to liposomes of various lipid composition, we show that this unusual SNARE protein is able to associate with vesicles resembling autophagosomes even in the absence of any other co-factors. In line with this, manipulating the level of a key autophagosomal lipid in vivo impairs the autophagosomal recruitment of Syntaxin17 and thus lysosomal fusion. Taken together, the lipid composition of autophagosomes has a crucial role during autophagy, by providing the necessary physico-chemical environment for the recruitment of autophagic proteins such as Syntaxin 17.

Polycomb-mediated regulation of Dram1 governs autophagy levels during mouse embryonic stem cell differentiation.

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Autophagy is a constitutive, cytoprotective, catabolic process, aberrations in which lead to many degenerative disorders (Bento et al, 2016). While the field has primarily focused on the cytosolic constituents and processes of autophagy, recent studies increasingly appreciate the role of chromatin modifications and epigenetic regulation in autophagy maintenance (Füllgrabe et al, 2014; Puri & Subramanyam, 2019). Reports have also implicated autophagy genes in regulating the embryonic stem cell state and differentiation (Xu et al, 2020). However, how autophagy genes themselves are regulated in stem cells is poorly understood. Our study identifies the Polycomb protein, EZH2, as a novel epigenetic regulator of autophagy in mouse embryonic stem cells (mESCs) and describes the importance of a balance between autophagy and cell death during mESC differentiation. We observed an induction of autophagy, accompanied by an upregulation of autophagy gene expression when mESCs were differentiated along the neural lineage. Bioinformatics analysis revealed distinct epigenetic profiles over autophagy gene promoters in stem cells vs differentiated cells. Using a small molecule epigenetic inhibitor screen, we identified chromatin modulators such as G9A/GLP, BET proteins and EZH2 as novel regulators of autophagy in mESCs. We further identified Dram1, a regulator of autophagic cell death (Guan et al, 2015, 1), as a target for EZH2-mediated autophagy repression. Increased Dram1 expression upon EZH2 inhibition led to context-specific induction of autophagy and autophagic cell death in mESCs and differentiating ESCs respectively. Using siRNA based knockdown and epigenetic characterisation of the Dram1 promoter, we elucidate a previously unknown function of Polycomb proteins in regulating autophagy gene expression in mESCs and implicate precise levels of Dram1, regulated by EZH2, in maintaining the autophagy-cell death balance during ES cell differentiation.

FAM134-dependent ER-phagy is regulated by mTOR and CK2 via phosphorylation-dependent ubiquitination

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The dynamic regulation of ER shape is important to maintain cellular homeostasis upon environmental changes. Selective degradation of ER fragments via autophagy (ER-phagy) are also driven by the FAM134 protein family acting as ER-phagy receptors. We have recently shown, that FAM134C seems to work in concert and as an enhancer of FAM134B driven ER-phagy. Activity of both receptors could be significantly increased upon inhibition of mTORC1 with Torin1, indicating active regulation by the master kinase of autophagy in response to stresses. In this study we analyzed dynamic phosphorylation of FAM134 proteins upon mTOR inhibition. We identified several phosphorylation sites clustering within the reticulon homology domain (RHD) of the proteins to be positively regulated by Torin1 treatment. FAM134B was shown to be oligomerize in order to facilitate bending and fragmentation of ER membrane. We predict, that phosphorylation of FAM134B and FAM134C is a prerequisite of and signal for ligase recruitment and subsequent ubiquitination. In a kinase screen, we identified CK2 as the downstream kinase for Torin1-induced, FAM134-dependent ER-phagy. Consistently, the sequences of identified phosphorylation sites were predicted CK2 targeting sites and treatment with the CK2 inhibitor SGC-CK2-1 prevented Torin1-induced FAM134 phosphorylation and ubiquitination. Inhibition of ATM and ATR, respectively, as upstream kinases of CK2 also partially prevented Torin-induced, FAM134-dependent ER-phagy. Point mutation of identified phospho-sites prevent FAM134 ubiquitination and oligomerization of the receptors, indicating the importance of CK2 in FAM134B and C mediated ER-phagy.

Tumor progression requires coordination of systemic lipid catabolism and local fatty acid synthesis

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Increased mobilization of nutrients is critical for tumor progression and cancer cachexia. By using a *Drosophila* tumor model, we recently demonstrated that malignant tumors activate autophagy to mobilize sugar and amino acids into the circulation. The elevated levels of nutrients in the hemolymph eventually supports the growth of tumor biomass and results in the wasting of peripheral host tissues. Here we aim to understand how lipid metabolism and mobilization is affected. We performed a comprehensive lipidomic analysis on tumors and various host tissues from fly larvae carrying malignant tumors in control, and autophagy or lipase mutant background. We observed that loss of autophagy or the major cytoplasmic lipase, Bmm (Brummer) differentially affect lipid storage and metabolism in the adipose tissue, but neither of these affected the levels of mobilized lipids in the hemolymph upon early stages of tumor progression. In contrast, in later stages, intensive Bmm-dependent lipid redistribution from adipose tissue into the hemolymph was apparent. Moreover, we also found that lipid composition of tumors undergoes extensive Bmm-dependent remodeling already in early stages of progression. As loss of Bmm only slightly decreased tumor growth and tissue wasting, we assayed for a compensatory mechanism, that can maintain tumor progression in these circumstances. We found that systemic loss of Bmm and tumor specific knock down of fatty acid synthesis genes almost completely abolish tumor growth and tissue wasting. Taken together, we found evidence that coordination of systemic lipid mobilization with cancer specific lipid synthesis is essential for malignant tumors to achieve their complete growth potential and induce cachexia.

Selective autophagy at an organismal scale: balancing starvation survival and recovery dynamics

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Under starvation, organisms upregulate autophagy to provide energy for vital processes. Although the signalling pathways that control autophagy are well-known, it is not understood how these pathways tune the selectivity and rate of autophagy to maximize starvation survival. We addressed this question by a combination of quantitative mass-spectrometry and live imaging using *C. elegans*.

We observe that protein turnover during starvation is strongly biased towards selected targets: biosynthesis-related proteins, such as ribosomes, are preferentially degraded, whereas the nuclear envelope and collagens are protected from degradation. The decline in ribosomes is fully dependent on autophagy and leads to a 50% reduction of the ribosomal concentration by day 10 of starvation. Although autophagy is indeed crucial for survival under starvation, we found that the associated loss of ribosomes has a negative impact on recovery: When food returned, previously starved animals had a lag in growth onset that scaled proportionally to the depletion of ribosomes.

We propose that selective autophagy towards ribosomes maximizes survival by preferentially degrading proteins dispensable under starvation. However, the depletion of ribosomes creates a metabolic bottle neck and limits protein synthesis and growth when food returns. The selectivity of autophagy may thus quantitatively balance a trade-off between starvation survival and recovery.

Poster presentations

Studying the effect of Cariprazine in induced neurons directly reprogrammed from Huntington's disease patient's fibroblasts

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Huntington disease (HD) is an incurable autosomal dominant progressive neurodegenerative disorder. HD is caused by PolyQ repeat expansions in the N-terminus of the HTT gene, which mainly causes motor, cognitive and psychiatric symptoms, such as depression, apathy and behavioral disinhibition. The role of the dopaminergic system in the development of HD symptoms is crucial, as the central dopaminergic pathways are overactivated in HD. The dopaminergic overactivity can be reduced by several drugs. However, their effectivity on psychiatric symptoms is limited. Moreover, the treatment of apathy and cognitive symptoms still remains challenging in HD. Cariprazine, a third-generation antipsychotic is acting as a dopamine D3 and D2 receptor agonist. Previous results shown positive effect in HD patients after cariprazine treatment. Clinical studies indicated positive effects in early stage of HD patients after cariprazine treatment in some psychiatric symptoms such as depressed mood, apathy and cognitive function in patients. Moreover, cariprazine also improved dopamine imbalance in the prefrontal cortex.

In this project we aim to study the effect of cariprazine in a novel in vitro model system of HD using donor-derived aged induced neurons. Using reverse translational strategy, we use cariprazine treatment in induced neurons directly reprogrammed from 5 ctrl, 5 HD drug naive and 5 cariprazine treated HD patients' fibroblasts. Our goal is to understand the putative beneficial effects of Cariprazine in HD patients and to better understand its mechanism of action by focusing on autophagy.

Mitophagy initiation is tightly regulated by its own factors

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Mitophagy is a tightly controlled process in which dysfunctional mitochondria are isolated and selectively degraded by autophagy. Many cell types critically rely on this pathway and the importance of mitophagy is underscored by its link to human diseases, most evident for Parkinson's disease. While most factors required for mitophagy are known, it remains incompletely understood how these factors come together to build a mitophagosome. Here, we combined biochemical reconstitution with cell biological approaches to investigate the role of TBK1-adaptors NAP1 and SINTBAD during mitophagy. We find that both NAP1 and SINTBAD are recruited to the mitochondrial surface upon induction of mitophagy, where they subsequently colocalize with autophagic machinery. However, in contrast to their role in xenophagy, NAP1 and SINTBAD are found to antagonize mitophagosome formation as their genetic deletion accelerates mitophagy. Biochemical reconstitution reveals that NAP1/SINTBAD compete with OPTN for TBK1-binding. Our data reveal that one of the primary roles of OPTN is to recruit TBK1 to the mitochondrial surface, where TBK1 fulfils a more extensive role than previously thought in OPTN-driven mitophagy. NDP52-driven mitophagy, on the other hand, benefits from the presence of NAP1/SINTBAD as in vitro experiments reveal that both factors stabilize the NDP52-FIP200 interaction. Based on our data, we speculate that NAP1/SINTBAD put a break on mitophagy activation initially, before switching to a more supportive role. This would suggest a model in which mitophagy initiation is tightly regulated by its own factors, to prevent it from initiating too early.

PAK6 activates TFEB to enhance autophagy in neurons

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Autophagy has been shown to be deregulated in several neurodegenerative diseases and its activation is frequently considered a promising therapeutic approach. Autophagy is mainly controlled by the activity of the transcription factor EB (TFEB), which modulates the expression of several genes involved in the autophagic-lysosomal pathway. The subcellular localization of TFEB is regulated by the binding of 14-3-3 proteins, which prevent its nuclear translocation. Considering that the neuronal-enriched kinase PAK6 has been demonstrated to modulate the interaction of 14-3-3 proteins with their binding partners, we were interested to assess whether PAK6 may induce TFEB activation, by phosphorylating 14-3-3 and promoting their dissociation from TFEB. Using both cellular and in vivo *C. elegans* models we demonstrated that the activity of PAK6 promotes TFEB nuclear translocation. Accordingly, the overexpression of PAK6 lead to the enhancement of the autophagic-lysosomal pathway, as demonstrated by alterations in the level of some crucial autophagic markers, while the neuronal-specific silencing of the *D. melanogaster* ortholog of PAK6 modulated the autophagic process, causing variations of lysosomal amount and defects in the activity of the lysosomal enzyme glucocerebrosidase (GBA).

Interestingly, our data suggest that PAK6 may regulate TFEB both indirectly, via 14-3-3 phosphorylation, and through direct interaction with the transcription factor. Moreover, we demonstrated that PAK6 acts downstream of the TFEB negative modulator mTOR.

Taken together, these results show a novel and conserved mechanism by which the kinase activity of PAK6 enhances the autophagic-lysosomal pathway through the activation of TFEB, pointing to PAK6 as a potential key target to regulate autophagy specifically in neurons.

CELLULAR: A story of a cell in autophagy

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Autophagy is a catabolic process that is activated upon starvation to ensure the availability of energy and building blocks that sustain cellular functions. Basal cellular quality control is ensured by autophagy, which if defective can accelerate aging, disturb development, imbalance immunity and promote cancer. Modulation of autophagy in diseases constitutes a potential target for therapy and hence places an ever-increasing demand for a better understanding of the autophagy landscape in a cell. Monitoring autophagy activity in cultured cells has proven to be instrumental in widening our understanding of the autophagic response to nutritional stress such as starvation. On the other hand, in recent years, machine learning (ML) has been applied to nearly all fields of research and found much success especially in automated image analysis. The main aim of this study is to establish a machine learning framework for monitoring autophagy from high-content imaging data and characterize the different states of autophagy in a cell under both nutrient-rich and nutrient-deprived conditions. For this, we employ ML algorithms to process time-lapse live-cell imaging data of *Drosophila* S2 cells expressing RFP-GFP-Atg8a in either basal or activated autophagy levels. Here we present CELLULAR, a segmented autophagy dataset aiming to train ML models to automatically segment and classify cells into different autophagic states. This improved toolkit for automated analysis of a cell's autophagy states will contribute to a more detailed grasping of the phenotypes at hand and potential future therapeutic discovery.

Homeostatic mitophagy scales mitochondrial networks

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Maintaining organelle size to scale is vital for physiological function in all cells, but how this is regulated is poorly understood. Here, we uncover a dual role for autophagy in regulating mitochondrial network size, topology, and activity by promoting mitochondrial biogenesis and homeostatic mitophagy.

Specifically, non-selective autophagy sustained mitochondrial biogenesis in starving non-dividing yeast cells, driving initial expansion of mitochondrial networks. Strikingly, mitochondria are subsequently scaled by Atg32-mediated mitophagy to pre-starvation size. Without mitophagy, mitochondria size increased two-fold while retaining wildtype-like key structural, compositional, and metabolic features. These data indicate that mitophagy functions predominantly in determining mitochondrial network size rather than quality during starvation. Based on single-cell analyses, we identified two metabolically tunable setpoints for mitochondrial network scaling for autophagy-driven biogenesis (mitochondria-to-cell) and degradation (mitochondria-to-cytosol). Therefore, our work shows how cells use autophagy to shape mitochondria networks according to metabolic and cellular size parameters.

Remarkably, knockdown of mammalian Atg32 homologue Bcl2l13 increased mitochondria network size in MEFs, consistent with an evolutionary conserved function of Atg32/Bcl2l13-mediated mitophagy in scaling. Intriguingly, despite increased network size, cells with a Bcl2l13 knockdown displayed increased mitophagy levels, suggesting induction of compensatory mitophagy possibly to counteract mitochondrial expansion. These data point towards a more complex interplay of mitophagy receptor systems for quantity and quality control in mammalian cells that we are currently exploring.

The role of autophagy protein LC3C N-terminus on LC3C binding to phospholipid and interaction with lipid membranes

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Autophagosome generation in macroautophagy is a complex event, in which many proteins are involved. Among the latter, yeast Atg8 or its mammalian orthologues are essential in autophagosome membrane elongation, shaping and closure. The LC3 subfamily of the human Atg8 orthologues includes the proteins LC3A, LC3B, and LC3C. The N-terminus of LC3C is quite different from that of LC3A and LC3B. The present study was devoted to exploring the effect of mutating the LC3C N-terminus on its covalent binding to lipid vesicles, and on the ensuing protein-dependent perturbing effects. The mutants were incubated with liposomes containing a maleimide-derivatized phosphatidyl ethanolamine, in order to ensure their lipidation, an essential step for the Atg8 involvement in autophagosome elongation. Ultracentrifugation, surface pressure measurements, and spectroscopic techniques revealed that the N-terminus was involved in vesicle aggregation and inter-vesicular lipid mixing induced by LC3C, including inner-monolayer lipid mixing, consistent with in vitro vesicle-vesicle fusion. The data support the idea that the LC3C N-terminus would be important for the protein role in autophagosome elongation/fusion in autophagy phenomena.

Elevating autophagy-dependence of hypoxic glioblastoma cells for increased therapeutic benefit

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The majority of solid human tumors, including glioblastoma, contain regions that are poorly oxygenated. Tumor hypoxia is a very heterogeneous and dynamic feature and is associated with a more malignant phenotype due to higher resistance to chemo- and radiotherapy. From a clinical point of view, reducing tumor hypoxia is highly desired. Glioblastoma treatment response and survival is associated with the level of hypoxia and expression of hypoxia-associated genes with a median overall survival of only 14.6 months.

One important pathway identified for the survival of hypoxic cells, is the activation of autophagy. We showed that inhibiting autophagy (chloroquine) sensitizes cells to hypoxia, reduced the viable hypoxic fraction in tumors and subsequently sensitizes glioblastoma tumors to irradiation.

Alterations in intracellular signaling change the cells' dependence on autophagy for their survival during hypoxia, e.g. through ectopic expression of the mutated kinase EGFRvIII. Targeting autophagy results in an increased reduction the hypoxic fraction in glioblastoma, leads to sensitization of tumors to therapy and showed improved prognosis compared to EGFRvIII- tumors. Hence, changing kinase signaling may result in inhibition of autophagy redundancy pathways and sensitize hypoxic cells to chloroquine-treatment.

Therefore, we aim to find druggable targets that, when inhibited, increase the dependence of hypoxic cells on autophagy and synergize with chloroquine and radiotherapy in hypoxic cell killing.

Using two independent CRISPR-Cas9 drop-out screens (kinome and whole-genome), we have identified promising hits in a library of primary and established glioblastoma cell lines. Currently, we are assessing the potential therapeutic gain of the selected targets using chemical inhibition and genetic targeting.

Ultimately, understanding the mechanism of hypoxia-induced autophagy will provide us with additional therapeutic possibilities for treatment of tumor hypoxia in glioblastoma.

Role of bulk and specific autophagy during nutritional starvation, organelle turnover and plasma membrane homeostasis in fission yeast

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To study the molecular basis of autophagy, the cellular model *Schizosaccharomyces pombe* has proved to be a useful genetic tool to highlight the evolutionary similarity and at the same time diversity of Atg components in autophagy. Our study aims to take a global approach to investigate the signals, the pathways and the consequences of autophagy activation.

Using general reporters of bulk autophagy, such as GFP-Atg8 and Pgk1-GFP cleavage, we have described a synergistic role of two well-known signalling pathways, the MAP kinase and TOR, in regulating the onset of macroautophagy under different nutrient-deprivation conditions. Furthermore, we have studied the role of mitophagy in lifespan extension during chronological aging using Sdh2-GFP cleavage as a reporter and the deletion of *atg43* as a specific regulator of organelle processing.

In parallel, we are investigating the role of autophagy in plasma membrane proteostasis. When cells are facing adverse conditions, such as environmental stress or nutritional starvation, certain membrane components need to be relocated or even internalized for degradation into the vacuoles. Although the remodelling of the polarized area of the plasma membrane is a tightly regulated process, little is known about the signals and the molecular pathways controlling the internalization of integral or lipid-bound plasma membrane proteins. Several components of the polarity module in fission yeast are temporarily internalized during stress conditions from the cells tips. In particular, the master regulator of cell polarity Cdc42, which is bound to the membrane through a lipid moiety, and the glucan synthase Bgs4, which present a transmembrane domain, have shown different depolarization from active growth areas during stress conditions. Using GFP-tagged Cdc42 and Bgs4, we have found a differential participation of autophagy in their internalization. We have performed unbiased and hypothesis-driven screenings of genes required for this specific autophagy process, and the updated results will be shown.

TRIM27- a potential regulator of autophagy through lysosome biogenesis

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In the process of autophagy, the lysosomes are important, as they serve as endpoints to which the content of the autophagosome is degraded. The normal function of lysosomes is important as dysregulation for example seen in lysosomal storage diseases, can have a negative effect on autophagy (Seranova et al., 2017). TRIM27 is part of the TRIM family of ubiquitin E3 ligases characterized by the N-terminal RING, B-Box and Coiled-coil domains, and several members of this family is known to have a role in autophagy (Di Rienzo et al., 2020). TRIM27 is involved cellular processes such as innate immunity and endosomal recycling, and its expression is upregulated in various cancer cells (Hao et al., 2015; Cai et al., 2018; Xiao et al., 2021). The aim of this study is to identify whether TRIM27 has a role in autophagy, as a previous study (Overå et al., 2019) identified TRIM27 as an autophagic substrate.

We have performed a mass spectrometry study to identify potential interaction partners of TRIM27. Applying the list of putative interaction partners to the Bioinformatic tool DAVID (Huang et al., 2007), predicted that TRIM27 may be implicated in lysosome and autophagosome biogenesis. One of the potential interaction partners is the lysosomal protein LAMP2, which is important for the fusion between the lysosome and autophagosome (Zhai et al., 2021). We observed that depletion of TRIM27 expression in HEK293 cells led to enlarged LAMP2 structures and enhanced LAMP2 expression. We are currently performing immunoprecipitations and confocal analyses to confirm the interactions between TRIM27, and a set of lysosomal proteins identified in the MS screen, including LAMP2. As TRIM27 associates with the retromer complex and is implicated in tubulation of sorting endosomes (Hao et al., 2015), an interesting question we will address is whether TRIM27 may be implicated in the autolysosomal reformation process.

Identification of GRASP55 as a novel target of the natural compound prodigiosin

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The natural compound prodigiosin has been shown to exert various biological activities, including antimicrobial, antimalarial, and anticancer properties. The anticancer activity has been linked to the autophagy-modulating and lysosome-alkalizing properties of prodigiosin. However, the existence of target proteins of this natural compound remains unclear. GRASP55 is a peripheral membrane protein, which is located at the trans cisternae of the Golgi apparatus, where GRASP proteins are crucial to establish the structure of the Golgi apparatus. In addition, GRASP55 has been shown to play a role in the fusion of autophagosomes and lysosomes. In this project, we investigate the effects of prodigiosin on autophagy with particular emphasis on the role of GRASP55. Consistent with previous findings, we found that prodigiosin is cytotoxic in nanomolar concentrations, increases LC3 levels and inhibits the degradation of SQSTM1 in cancer cells. In combination with the inhibition of cathepsin activity, we postulate that prodigiosin blocks late stages of the autophagic process. In a thermal proteome profiling approach, we discovered that prodigiosin binds to GRASP55 and dose-dependently stabilizes it against thermal degradation. Hence, we propose GRASP55 as a direct target protein of prodigiosin. These findings are supported by a prodigiosin-induced impairment of Golgi apparatus structure in wild type, but not in GRASP55 KO HeLa cells. Interestingly, treatment with prodigiosin leads to co-localization of GRASP55 and LC3 in the perinuclear region. Based on these observations, we hypothesize that binding of prodigiosin to GRASP55 inhibits the autophagosome-lysosome fusion and thereby supports cancer cell death.

TFEB-independent promotion of autophagic flux by TRPML-mediated lysosomal Ca²⁺ release

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Autophagy depends on lysosomal acidic hydrolase activity. Proper acidification of active lysosomes (pH 4.5-5.0) is necessary for this, which is achieved by inward proton pumping by the lysosomal V-ATPase. Recent studies implicated the role of TRPML/MCOLN1, an endolysosome-localized ion channel in autophagy. Amino acid starvation was found to activate TRPML currents, and TRPML-released Ca²⁺ appears to promote autophagosome biogenesis, retrograde lysosomal migration and TFEB activation to induce autophagy/lysosomal pathway genes. The immediate consequences following TRPML activation are however unclear.

Here we find that starvation-induced lysosome acidification is abrogated by ML-SI1 (TRPML antagonist) treatment and in Trpml- larval Drosophila fat cells. Using our novel GCaMP6m-TRPML1 reporter, we show that Ca²⁺ efflux inversely correlates with lysosomal pH. Moreover, acidification is blocked by Ca²⁺ chelation or by expression of the dominant negative TRPML pore-forming mutant D471K/D472K (DDKK). Thus, TRPML-mediated Ca²⁺ efflux is important for lysosome acidification.

We then used the synthetic, specific TRPML agonist ML-SA1 to characterize early (10-15 min treatment) autophagic and lysosomal effects that take place well before TFEB activation and autophagosome biogenesis (hereafter SA1early). SA1early led to elevated cathepsin activity and a decrease in lipidated LC3-II and p62 levels. ML-SA1 feeding in flies similarly triggered lysosome acidification, and Trpml overexpression decreased the number of Atg8a-II and Ref(2)P/p62 puncta in larval fat cell clones.

Interestingly, SA1early increased autophagosome-lysosome fusion independent of lysosomal migration. In support, treatment with vinblastine, a microtubular transport inhibitor also increased fusion of stalled lysosomes with autophagosomes in the periphery of cultured cells in a TRPML- and PIKfyve-dependent manner. Further supporting increased fusion upon SA1early, this treatment increased Rab7 recruitment to autophagosomes. Taken together, we show that early responses to TRPML-mediated Ca²⁺ release promote autophagic flux via increased lysosomal degradation activity and fusion with autophagosomes.

Identification of a novel translocation mechanism of TFEB in neurodegeneration.

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Autophagy is a highly conserved catabolic process that degrades unnecessary cellular materials by the fusion of autophagosomes and lysosomes. Autophagy is essential in the maintenance of cellular homeostasis and in the development of multicellular organism. Dysregulated autophagy has been found in various neurodegenerative diseases including Alzheimer disease and Parkinson disease. Recent studies have reported that TFEB (transcription factor EB), a transcriptional regulator of the autophagy, plays a critical role in regulating several autophagic genes related autophagosome formation and lysosome biogenesis. In this process, it has been demonstrated that phosphorylation status of TFEB and subsequent cytosol/nucleus shuttling of TFEB are comprised an important step. As such, phosphorylated TFEB (pS211-TFEB) can be sequestered within the cytoplasm by binding 14-3-3 proteins. Upon various autophagic stimuli, dephosphorylated TFEB is translocated from the cytosol to the nucleus. This event leads to induce transcription of several autophagic gene. In this study, we hypothesized that other post-translation modifications can affect the cytosol/nucleus shuttling of TFEB in experimental models of neurodegeneration. We tested several post-translational modifications of TFEB and found the potentially intriguing modification using both in vitro and cell-based assays. We are attempting to identify the specific site of modification of TFEB by LC-MS/MS assay. We believe this novel posttranslational modification plays an important role in regulation of autophagy and the resulting neurodegeneration (supported by NRF-2017M37A1025369).

Sigma-1 Receptor binds to GABARAP and its activation induces autophagy

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Dysfunction of autophagy and a disturbed protein homeostasis are linked to pathogenesis of human neurodegenerative diseases and the modulation of autophagy has become one key pharmacological target. Due to the pleiotropic neuroprotective effects of sigma-1 receptors (Sig-1R) in various experimental paradigms, Sig-1R activation is recognized as a potential target for prevention and therapy of neurodegeneration. Interestingly, mutations in the Sig-1R gene are associated with different neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) and ALS-associated mutations cause a disturbed autophagy. Previously, we analyzed the effects of Sig-1R activation by PRE-084, a selective Sig-1R agonist, and by ANAVEX2-73, a muscarinic receptor ligand and Sig-1R agonist, on autophagy and proteostasis in vitro and in vivo. We found that pharmacological Sig-1R activation a) induces ULK1 phosphorylation at Serin555, b) enhances autophagic flux, and c) increases proteostasis capacity, ameliorating paralysis caused by overexpression of aggregation-prone proteins in *C. elegans*. Interestingly, we now found that this effect may be mediated by Sig-1R interaction with the ATG8 protein GABARAP through a highly conserved canonical LIR sequence, which is only accessible in the activated state of the receptor (unpublished data). Employing Sig-1R KO and mutant cells, as well as additional Sig-1R activating or inhibiting compounds, we further analyze the interplay between Sig-1R and GABARAP and its role in autophagy induction to ultimately understand the link between Sig-1R, autophagy, proteostasis, and neurodegeneration. Taken together, our findings show a direct impact of Sig-1R activation on proteostasis maintenance underlining Sig-1R as a potential target for different neurodegenerative disorders linked to protein aggregation. Moreover, they may fuel detailed knowledge on the molecular mechanisms of autophagy modulation by Sig-1R activation.

Inside out: Novel autophagy mechanisms in non-canonical IL-1 β secretion and release of extracellular autophagy vesicles by human dendritic cells

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Secretory autophagy drives non-canonical secretion of inflammatory mediators (including IL-1 β , lysozyme) and release of LC3+ autophagy vesicles into the extracellular milieu, with implications in inflammatory output and viral dissemination. Although roles of degradative autophagy in dendritic cell (DC)-driven immunity are well described, cues and molecular machinery that route cargo to secretory autophagy in DCs remain elusive. Here, we aimed to (i) identify autophagy receptors and trafficking pathways that govern IL-1 β secretion by stimulated human DCs, and (ii) quantify and characterize the LC3+ extracellular vesicles (EVs) subset.

We demonstrate that RNA interference (RNAi)-mediated knockdown of selected autophagy molecules (e.g. ATG5, ATG13, ATG16L1) reduced IL-1 β secretion by DCs exposed to curdlan, without impacting IL-1 β transcription or processing. Large-scale RNAi screening uncovered additional regulatory molecules and networks directing autophagy-mediated IL-1 β secretion, including membrane trafficking proteins (Galectins, Rab proteins) and autophagy receptors (Sorting nexins, p62). Strikingly, upon treatment of DCs with a panel of stimuli, discrete cargo receptors appeared to direct IL-1 β to secretory autophagy pathways in a stimulus-dependent manner.

To confirm the presence of LC3+ autophagy vesicles within the extracellular milieu, DC-derived EVs were purified by ultracentrifugation or immunomagnetic positive selection for canonical EV markers. Phenotypic characterization and quantification of highly purified EVs by imaging flow cytometry confirmed that approximately 30% of CD9+CD81+CD63+ EVs co-expressed LC3, underscoring the impact of secretory autophagy pathways on extracellular release of both inflammatory output and specialized EVs.

Our data describe new autophagy-mediated regulatory mechanisms of IL-1 β secretion by human cells, confirm the presence of secreted LC3+ autophagy extracellular vesicles, and illustrate that secretion of IL-1 β and autophagy vesicles is regulated by discrete mechanisms in a stimulus-dependent manner. As activation of secretory autophagy mechanisms by DCs has implications in inflammatory disease pathogenesis, intercellular communication, and viral dissemination, these data have potential to inform future autophagy-targeted therapeutic interventions.

ER-phagy driven by heteromeric clusters of ubiquitinated ER shaping proteins prevents neurodegeneration

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ER shaping proteins characterized by reticulon homology domains (RHDs) play an important role for the dynamic remodeling of the endoplasmic reticulum (ER). One example is FAM134B, which can bind to LC3 and thus mediates the degradation of ER sheets via selective autophagy (ER-phagy) {Khaminets, 2015 #10}. FAM134B mutations result in a neurodegenerative disorder mainly affecting sensory and autonomic neurons {Kurth, 2009 #21}. Here, we report that ARL6IP1, another RHD-containing ER-shaping protein associated with sensory loss {Novarino, 2014 #18}, interacts with FAM134B and thus promotes the formation of multi-protein clusters required for ER-phagy. We further reveal that the binding of such clusters to LC3 depends on the ubiquitination of the RHDs of ARL6IP1. Consequently, the disruption of ARL6IP1 in mice causes an expansion of ER sheets in sensory neurons, which degenerate over time. Because primary cells obtained from ARL6IP1-deficient mice or patients display an incomplete budding of ER membranes and a severe impairment of ER-phagy flux, we propose that the clustering of ubiquitinated ER-shaping proteins drives the dynamic remodeling of the ER during ER-phagy and thus contributes to neuronal maintenance.

ARN2: An autophagy-focused network resource to aid omics analysis and experiment planning

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Autophagy is a highly-conserved catabolic pathway eliminating unnecessary, dysfunctional components and invading pathogens. Autophagy plays a role in disorders such as neurodegenerative, inflammatory diseases and cancers. Although mutations of core autophagy genes are well-researched in pathological conditions, the perturbation of the regulation of the core machinery also plays a role in these diseases. Hence, understanding the regulation of autophagy is necessary, and its precise analysis is only possible on a systems-level. We previously developed the Autophagy Regulatory Network (ARN) web resource to provide an integrated database for autophagy research. For the last seven years, ARN has been used by thousands of users from around the world. Here, we introduce ARN2 - developed based on user feedback and developments in the field - containing updated, experimentally verified connections between core autophagy proteins and their regulators. In ARN2, we separated five regulatory levels: direct regulators - direct protein interactors of autophagy proteins; transcriptional regulators; post-translationally modifying enzymes; miRNAs and lncRNAs. With integrating the large-scale signaling pathway database Signalink3, we defined connections between pathway members and core autophagy proteins. This new release is also upgraded with: annotations of core proteins in different types of autophagy (microautophagy, xenophagy, etc.); grouping autophagy proteins into distinct stages of autophagy (initiation, elongation, termination); subcellular localization and tissue-specific protein localization. These can be used to filter the dataset, providing customizable download options tailored to the user's needs. The database can be downloaded in various file formats (CSV, BioPAX, PSI-MI, etc.), and visualized directly in Cytoscape. With combining the ARN2 network with transcriptomics and other omics datasets, the resource provides the possibility for (multi-)omics analysis of autophagy regulation. Hence, regulation of autophagy can be examined more in depth and on the systems-level, leading to understanding its functions in tissue specific diseases and identifying potential drug targets in autophagy-related diseases.

AUTag – a novel fluorescent reporter for autophagy

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Autophagy (“self-eating”) is an evolutionarily conserved survival mechanism in living organisms during which intracellular components (‘cargo’) are identified and delivered to lysosomes for degradation. Basal autophagy plays a vital role in maintaining cellular homeostasis; dysregulation of autophagy have been found to associate with diseases ranging from neurodegeneration, early stage of cancers, cardiovascular disease to infectious disease, with different stages of autophagy being impaired. Research efforts have identified multiple molecular targets to rectify autophagy with the promise for therapeutic intervention. Modulators that enhance or inhibit autophagic activities have largely contributed to mechanistic understanding of autophagy and their implications in diseases. As autophagy is a multi-step process, it is important to identify and assess which stage of autophagy is affected by a modulator, to quantify the extent of the regulation and to draw a clear mechanistic picture. However, tools that allow real-time monitoring of the dynamics of autophagy, especially with quantitative readout, are still scarce and highly desirable. Conventional methods involving the expression of fluorescent protein tagged autophagy markers require tedious and complexed transfection procedures and fail to be applicable in clinical samples. Here, we design and synthesize the first fluorescent chemical probe that is highly specific to autophagy. Also, the introduction of a pH-sensitive fluorophore into the molecular design enables the discrimination between autophagosomes and acidic autolysosomes. This tailor-made probe, named AUTag, has been proven to be applicable in live cells and in vivo under conditions that induce/inhibit autophagy, or with ATG deletion, which was validated with standard autophagy methods. Also, by virtue of AUTag, protocols for quantitative analysis of cellular autophagic flux have been established. Compared to the conventional methods only evaluate the average autophagy flux within certain period, AUTag provides a robust tool to quantify the transient autophagy flux in situ, highlighting its great potential in autophagy studies.

Fatty acid and acyl chain requirements of autophagy in aging yeast

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Autophagy is a catabolic process that ensures survival and stress resistance of aging cells. Although lipids are central molecules in the autophagy process as part of autophagic membranes, signaling molecules and cargo, the complex lipid requirements of autophagy are only partly understood. Using lipogenesis-impaired autophagy-compromised yeast (Gross et al. 2019, JBC), we screened for fatty acid-dependent restoration of autophagy. Our results reveal specific fatty acids that are capable of restoring autophagy upon their supplementation to de novo lipogenesis-impaired yeast, while others fail to do so. The capacity to restore autophagy depends on the fatty acyl chain length and degree of saturation. Using lipidomics, we identify potential lipid species that drive the autophagy phenotypes and correlate them to autophagy flux perturbations induced by mutation of cytosolic acetyl-coenzyme A carboxylase 1 (Acc1), the rate-limiting enzyme in de novo fatty acid biosynthesis, or of the transcriptional repressor Opi1 involved in the regulation of phospholipid biosynthetic genes. Our results suggest specific lipid requirements to maintain autophagy in cellular aging.

Learning from reconstitution: metamorphosis of HORMA domain proteins ATG13 and ATG101 as rate-limiting step in assembling autophagy initiation complexes

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In response to starvation, bulk cytosol is taken up by autophagosomes within minutes, transported to the lysosome for degradation, and recycled into small-molecule biosynthetic precursors. After autophagy induction, a membrane contact site is assembled on the ER. This contact site is required to initiate the process, arguing for a membrane interconnection between the ER membrane and newly formed autophagic membrane. These contact sites have a largely uncharacterized architecture. Genetic studies have proposed that autophagy in mammalian cells is initiated by the recruitment of three initiation complexes to the ER contact site, which includes HORMA-domain proteins ATG13-101 as upstream factors. Lipid flow is likely mediated by ATG2, which is also proposed to be part of an initial tether between the ER and the autophagosome.

A crucial unresolved question is how the initial contact sites are assembled on-demand, and thereby build the autophagy initiation machinery at the right time and place. Do the autophagy initiation complexes and lipid transfer machinery coalesce in a stable contact site super-complex? What triggers the assembly of these complexes at the right place and time? Building on our previous biochemical reconstitutions of HORMA-domain based signaling networks, we hypothesized that the interaction of HORMA-domain proteins ATG13-101 with other initiation complexes might represent a rate-limiting and obligatory step in the assembly of a larger effector complex. With the aim of a full biochemical reconstitution with full-length human recombinant proteins, we have purified the majority of the initiation and lipid transfer machinery. We explore the complex binding behavior of ATG13-101 with their client proteins, as well as their coordinating role of the initiation complexes and lipid transfer.

Involvement of non-canonical autophagy in B-cell receptor trafficking

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Antigen presentation by B cells is central in the humoral immune response. B cells present antigens to helper T cells which stimulate their differentiation into plasma cells or memory cells. Autophagy-related (ATG) proteins participate in antigen presentation, but their precise contribution is still unclear. We recently showed that ATG proteins are involved in immobilized antigen processing after engagement by the B cell receptor (BCR) through polarized endocytosis. We aim now at precising how ATG proteins participate in BCR trafficking.

We generated human B cell lines expressing tagged ATG16L1, ATG5 or MAP1LC3B. Using mass spectrometry approaches, we identified novel ATG partners in the context of BCR crosslinking. We focused on SNAP23 (synaptosome-associated protein 23), involved in lysosome exocytosis and LC3-associated phagocytosis (LAP). SNAP23 colocalization with BCR after crosslinking is impaired upon ATG5 or ATG16L1 depletion. We are now generating SNAP23-deficient B cell lines to validate the involvement of this protein in BCR trafficking. To define if LC3-conjugation to single membranes is involved in BCR trafficking, we studied the impact of RUBCN depletion, essential for LAP/LC3-associated endocytosis. We stimulated primary B cells isolated from a Rubcn^{-/-} mice, with microbeads-tethered antigens. Upon RUBCN depletion, super-resolution imaging show polarization defects of internalized BCR-containing vesicles. This suggests the involvement of RUBCN-dependent processes in BCR endocytosis that we will confirm using correlative-light and electron microscopy. We are now investigating whether RUBCN participates in antigen presentation by B cells after BCR engagement.

Altogether we showed that ATG proteins polarize BCR trafficking and identify new partners in this RUBCN-dependent process. We are now validating involvement of identified partners and generating mouse models to address the question of the in vivo role of this pathway.

Targeting the ATG13-ATG101 protein-protein-interaction within the autophagy inducing ULK1 complex for cancer therapy

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The dysregulation of autophagy promotes the development of several diseases like neurodegeneration, infection, or cancer. To keep up with their metabolic demand under low nutrient and/or oxygen conditions, which are typical for the tumor microenvironment, cancer cells upregulate autophagy. Therefore, the inhibition of autophagy is desired in clinical settings. However, to date drugs targeting autophagy selectively remain rare. The autophagy-inducing ULK1 complex comprises ULK1/2, FIP200 and a heterodimer consisting of ATG13 and ATG101. Our group previously described that the assembly of the ULK1 complex is disrupted and autophagic activity is significantly reduced upon the exchange of amino acids of ATG13 that mediate binding to ATG101. Thus, in this project we are aiming to selectively inhibit autophagy by targeting the ATG13-ATG101 protein-protein interaction. For this purpose, we utilized homogeneous time-resolved fluorescence (HTRF) as an in vitro tool to measure the interaction of recombinant GST-ATG13 and His-ATG101 in a pilot screening with a library of 15488 compounds. The resulting hit list was reviewed concerning PAINS (pan-assay interference compounds), redox potential and signal specificity and was reprioritized depending on molecular weight and in vitro IC50 values. Eventually, 29 compounds were prioritized for cell-based assays. Biomolecular fluorescence complementation (BiFC) and NanoBiT technology® were used for the detection of compound-induced interference with the cellular ATG13-ATG101 interaction. Thus far, five compounds produced promising results in BiFC experiments and are currently tested for their impact on cellular autophagic activity.

Ubiquitination regulates ER-phagy and remodeling of the endoplasmic reticulum

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The endoplasmic reticulum (ER) is the largest endomembrane system in the cell that constantly adapts to various cellular requirements^{1 2}. Among critical pathways driving ER remodelling is ER-phagy, a selective autophagy pathway that targets ER components for lysosomal degradation³. ER-phagy receptors play a central role in this process⁴, but how they are regulated *in vivo* remains largely unknown. Here, we report that ubiquitination of the ER-phagy receptor FAM134B within its reticulon-homology domain (RHD) promotes its clustering and increases the binding to lipidated LC3B, thereby stimulating ER-phagy. Molecular dynamics (MD) simulations revealed how ubiquitination broadens the characteristic wedge-shape of the FAM134B-RHDs and accelerates the induction of membrane curvature. Ubiquitin mediates trans-interactions with neighbouring RHDs to form dense receptor clusters that facilitate the large-scale remodelling of lipid bilayers. The ubiquitin-mediated receptor clustering within ER membranes was monitored and quantified using super-resolution imaging. Furthermore, we identified the E3 ligase AMFR/gp78 within multimeric ER-phagy receptor clusters that catalyses FAM134B ubiquitination and facilitates ER-phagy. Taken together, ubiquitination triggers changes in the conformation and composition of ER-phagy receptors thus controlling the ER remodelling in response to cellular demands.

A metabolite sensor interacts with Atg11 to regulate selective autophagy

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Cells have to sense and convert complex metabolic information into adaptive autophagy responses to maintain cellular homeostasis. Canonically, protein kinase networks convey metabolic information by converging on the Atg1/Ulk1 kinase complex (AKC) to regulate autophagy. How the AKC integrates these metabolic signals to regulate selective and non-selective forms of autophagy is poorly understood.

Here, we show that components of the yeast AKC complex, essential during nitrogen starvation, are dispensable for autophagy induction upon phosphate starvation, indicating compositional plasticity of the AKC depending on metabolic conditions. To identify phosphate starvation-dependent components of the AKC complex, we used unbiased immuno-purification and found the metabolite-sensor Pho81. Pho81 binds Atg11, the selective autophagy adaptor subunit of the AKC, via a conserved Atg11/FIP200 consensus sequence. We find that the Pho81-Atg11 interaction is critical for the selective degradation of peroxisomes. Strikingly, our data suggest that changes in inositol phosphate metabolites are sensed by Pho81 and promote its physical interaction with both, Atg11 and peroxisomal proteins to induce pexophagy.

In summary, we propose that Pho81 regulates receptor-mediated selective autophagy by directly conveying metabolic information to the AKC.

Loss of Aryl Hydrocarbon Receptor Interacting Protein (AIP) results in a Novel Paediatric Metabolic Disorder Characterised by Defective Autophagy and Lysosome Function

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Aryl Hydrocarbon Receptor Interacting Protein (AIP) is a ubiquitously expressed co-chaperone molecule, but the molecular functions of AIP are poorly understood. We have identified children born with homozygous loss of AIP had a novel metabolic disorder that shares features of a lysosomal storage disease that failed to thrive, put on weight and died at an early age.

Aip KO mouse embryonic fibroblasts (MEFs) had a shortage of amino acids indicating a defect in proteostasis within cells. Aip KO MEFs had decreased proteasome function and an increase in ubiquitinated proteins. Aip KO MEFs underwent autophagy under fed conditions but couldn't support autophagy upon starvation. To acquire more nutrients, Aip KO MEFs had increased macropinocytosis but could not digest the engulfed material due to defective lysosomes. To compensate for the shortage of amino acids, Aip KO MEFs experienced metabolic reprogramming, including increased glycolysis, TCA cycle activity and glutaminolysis.

Together, our results provide evidence that the co-chaperone molecule AIP supports protein recycling - proteasome function, autophagy, lysosomes and trafficking within cells and provide a rationale for the AIP deficient children's failure to thrive.

DEPLETION OF MXA EXPRESSION IN OESOPHAGEAL CANCER CELL LINES ELEVATES ENDOGENOUS AND DRUG-INDUCED AUTOPHAGY

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Oesophageal cancer is the eighth most common cancer worldwide and the sixth leading cause of cancer death. Five-year survival remains below 20% across the EU and novel therapeutic options are required to combat resistance to chemotherapy. The ISGylation network is frequently dysregulated in oesophageal cancer cells. This pathway leads to the conjugation of ISG15 to target proteins using specific E1, E2, & E3 enzymes, in a similar manner to ubiquitination. This group has already demonstrated that ISG15 and the E2 enzyme involved - UBE2L6, are negative regulators of autophagy.

We have investigated the role of Myxovirus resistance A (MxA) protein, a known target of ISGylation, in the response of oesophageal cancer cells to chemotherapeutics. MxA is an interferon-induced antiviral protein with homology to the dynamin superfamily of large GTPases. Using siRNA or CRISPR/Cas9 technology we have depleted expression of MxA in three oesophageal cancer cell lines (OE21, KYSE270 and FLO-1) and examined the impact on autophagy induced by the chemotherapeutic agents 5-fluorouracil and oxaliplatin. Autophagy was assessed by western blot analysis of LC3B-II, P62 and quantification of autophagosomes using the fluorescent dye Cyto-ID and flow cytometry. Cells were co-treated with the lysosomal inhibitor chloroquine, to distinguish new synthesis of autophagosomes from a block in turnover. Loss of MxA expression significantly increased the level of endogenous and drug-induced autophagosomes in all cell lines. Autophagosome levels exceeded those induced by lysosomal inhibition alone, indicating upstream regulation of autophagosome synthesis.

The impact of MxA depletion on cell survival was assessed using clonogenic assays following drug treatment. In 2/3 cell lines, loss of MxA impeded the recovery of drug-treated cells.

This study indicates, for the first time, that MxA plays a role in the negative regulation of autophagy. Further mechanistic work should indicate if MxA represents a useful target for future therapeutic intervention.

Lipid and protein content profiling of isolated native autophagic vesicles

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Autophagy is responsible for clearance of an extensive portfolio of cargoes, which are sequestered into autophagic vesicles, called autophagosomes, and are delivered to lysosomes for degradation. The pathway is highly dynamic and responsive to several stress conditions. However, the exact phospholipid composition and protein contents of autophagosomes under changing autophagy rates are elusive so far. Here, we introduce a FACS-based approach for isolation of native autophagic vesicles and ensured the quality of the preparations. Employing quantitative lipidomics, we analyzed phospholipids present within autophagosomal structures purified upon basal autophagy conditions, starvation, and proteasome inhibition. Importantly, besides phosphoglycerides, we identified sphingomyelin within autophagosomal membranes and showed that the phospholipid pattern was maintained under different autophagy conditions. Employing quantitative proteomics, we obtained cargo profiles of autophagic vesicles isolated upon the different treatment paradigms. Interestingly, starvation showed only subtle effects, while proteasome inhibition resulted in the enhanced localization of proteins of the ubiquitin proteasome pathway within autophagic vesicles. Thus, here we present a powerful method for isolation of autophagic vesicles, which enabled profound phospholipid and cargo analyses.

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A novel region within a conserved domain of ATG7 could harbor a vertebrate specific function

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Autophagy is a highly conserved degradation pathway important for maintaining homeostasis in eukaryotic cells. The E1-like enzyme ATG7 belongs to a group of ATG proteins that mediate the autophagy process. Several autophagy independent functions of ATG7 have been described and studies have suggested a more important function of the protein in mammalian models. We set out to investigate the evolutionary history of ATG7. We wanted to know whether a new functional region had emerged in the history of eukaryotes that could harbor a novel function. The study revealed a region that emerged early in vertebrates. Interestingly, this vertebrate specific region (VSR) is situated within the active domain of the protein. As this is the most highly conserved domain of all E1-enzymes, we conducted a comparative analysis of this protein family. The analysis revealed that this is a unique event in ATG7, but the domain has not changed in this respect in other E1-enzymes. Structural models of ATG7 in various eukaryotic species predict that the VSR forms an intrinsically disordered loop that extends out from the protein, leaving it open to interactions with other macromolecules. The function of the VSR remains to be elucidated, but two residues within the region have been linked to cancer in humans. To address the putative differences in the function of ATG7 between vertebrates and invertebrates we are utilizing CRISPR/Cas9 modified *Drosophila melanogaster* strains. Studies on novel functions of ATG7 have exclusively been performed in mammalian models and, therefore, it has not been explored whether other taxa share these functions – nor have any been assigned to a specific region of the protein. Our data suggests that the VSR could harbor a novel vertebrate specific function of ATG7.

Linking Autophagy to PARPi-Resistance in Triple Negative Breast Cancer

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Electrophilic fatty acids, or nitrated fatty acids (NFA), are endogenously synthesized lipid signaling molecules with well-documented anti-inflammatory properties. Their substantial therapeutic efficacy and safety have been demonstrated in healthy obese patients and in phase II clinical trials for pulmonary arterial hypertension. Through reversible alkylation of hyperactive protein cysteines, NFAs demonstrate anti-tumor efficacy in diseases like colon cancer. Our group has recently shown that NFAs selectively target triple negative breast cancer (TNBC) over benign breast epithelium. TNBC has a critical unmet need for targeted therapy, as it has the poorest prognosis among breast cancer subtypes and lacks effective response to targeted therapy. Current targeted therapy only exists for TNBC patients carrying germline loss of function mutations in BRCA1/BRCA2 genes that are essential for DNA double strand break (DSB) repair by homologous recombination. Loss of BRCA1/2 function critically sensitizes these patients to poly-ADP ribose polymerase inhibitor (PARPi) treatment which causes single and double strand DNA breaks. Unfortunately, resistance to PARPi is a pervasive obstacle caused by various mechanisms, of which macroautophagy, hereafter autophagy, is understudied.

Autophagy is a necessary homeostatic mechanism to degrade dysfunctional cellular components and replenish energy. Autophagy is induced under various stimuli including DNA damage. In cancer treatment, autophagy promotes drug resistance and tumor survival. TNBC patients with elevated autophagy have poor prognosis. In ovarian cancer, PARPi treatment enhances autophagy and autophagy inhibition causes re-sensitization to PARPi. However, the role of autophagic PARPi resistance is lacking in TNBC. Our novel preliminary data show that PARPi induces autophagy in TNBC cells and that NFAs inhibit autophagy and promote cell death. Through bioconjugation pulldown we show that NFAs interact with cysteines of essential autophagy proteins. Therefore, our hypothesis is that PARPi-induced DNA damage initiates autophagy-mediated drug resistance that can be overcome by NFA-targeting of critical cysteine residues of essential autophagy proteins.

Transgenic reporter zebrafish models for whole-organism autophagy monitoring

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Activation of adaptive autophagy is a promising strategy to promote cellular function and prevent the onset of age-related diseases. Transparency of zebrafish larvae facilitates the image-based measurement of autophagy in vivo, a strategy that can be used to identify novel inducers of autophagy. To this end, we developed and characterized muscle-specific transgenic zebrafish reporters; Map1lc3 protein, a protein found on autophagy cargo vesicles, and transcription factor EB (Tfeb), a transcription factor inducing the expression of autophagy and lysosome-related genes, were tagged to the fluorescent protein ZsGreen through generation of reporter constructs. These constructs were injected to derive the stable reporter zebrafish lines Tg(actc1b:map1lc3-ZsGreen;cryaa;tdTomato) and Tg(actc1b:Tfeb-ZsGreen). Here, I show results from a high-content image-based acquisition and analysis setup that I used to screen natural bioactives on these lines. The images of treated zebrafish larvae are classified into responders and non-responders using an A.I.-based image analysis script based on the presence and absence of Tfeb nuclei, respectively. This new platform represents a rapid technology for the identification of new autophagy inducers for potential development of human interventions.

WASH complex: membrane attachment revised

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The endosomal protein sorting influences a broad range of cellular processes, such as nutrient uptake, signaling, secretion, protein degradation, and autophagy. Actin and its regulator WASH complex are one of the key elements of the endosomal protein sorting machinery. The pentameric WASH complex is recruited to the endosomal membrane, where it promotes the nucleation of actin filaments by activating the Arp2/3 complex. The actin network then isolates sorted proteins in distinct microdomains and facilitates the fission of transport vesicles from endosomes.

The recruitment to the endosomal membrane is a fundamental step for the WASH complex function. Early studies postulated that WASH complex membrane recruitment requires interaction between WASH complex subunit FAM21 and retromer – master regulator of endosomal protein sorting.

We show WASH complex does not require the FAM21-retromer interaction to localize to the endosomal surface and carry out its function. Moreover, using a set of knock-out cell lines for individual subunits of the WASH complex, we determine that the subunit SWIP associates with the membrane independently of FAM21 and helps recruit the rest of the WASH complex to the endosomal membrane. Thus, our data reveal that there is another, retromer-independent mechanism of the WASH complex recruitment to the endosomal membrane, mediated by its subunit SWIP.

The UFM1 system regulates ER-phagy through the ufmylation of CYB5R3

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Protein modification by ubiquitin-like proteins (UBLs) amplifies limited genome information and regulates diverse cellular processes, including translation, autophagy and antiviral pathways. A UBL, ubiquitin-fold modifier 1 (UFM1), is covalently conjugated with intracellular proteins through ufmylation, a reaction analogous to ubiquitylation. Ufmylation was recently shown to play unexpected roles in processes such as endoplasmic reticulum (ER)-associated protein degradation, ribosome-associated protein quality control at the ER and ER-phagy. However, it remains unclear how ufmylation regulates such distinct ER-related functions. Here we identify a reductase, NADH-cytochrome b5 reductase 3 (CYB5R3) localizing on ER-membrane, as a novel UFM1 substrate. Ufmylation of CYB5R3 occurs dependent on the E3 components UFL1 and UFBP1 on ER, and it converts CYB5R3 into its inactive form. Ufmylated CYB5R3 is recognized by UFBP1 through the UFM1-interacting motif, which increases the E3-ligating activity of UFL1–UFBP1 against CYB5R3. Ufmylated CYB5R3 is degraded in lysosomes in an autophagy-related protein Atg7- and an autophagy-adaptor protein CDK5RAP3-dependent fashion. Mutations of genes involved in the Ufm1-system as well as CYB5R3 causes hereditary developmental disorders, and the ufmylation-defective Cyb5r3 knock-in mice exhibited microcephaly. Our results indicate that CYB5R3 ufmylation is a signal that induces ER-phagy, which is indispensable for brain development.

TP53INP2 regulates autophagy cargo composition

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TP53INP2 is a versatile protein involved in different processes such as transactivation of a number of nuclear hormone receptors, myogenic and adipogenic differentiation, autophagy and apoptosis among others (1-4). In addition, TP53INP2 mRNA and protein levels are down-regulated in muscle and adipose tissue in diabetic and obese mice and humans (1, 2). TP53INP2 positively regulates autophagy by binding to all Atg8 proteins through LIR motif, and expression of TP53INP2 increases the number of autophagosomes in basal and starvation conditions (3). On the contrary with the increased autophagy, TP53INP2 up-regulates protein levels of some autophagy receptors as for example p62 and NBR1 (1), and decreases their degradation through autophagy. Moreover, in TP53INP2 expressing cells p62 phase separated (in 30% of the cells), which as well as increased protein levels of p62 was abolished by mutations in LIR sequence of TP53INP2. Surface Plasmon Resonance studies showed that the LIR sequence of TP53INP2 has the highest affinity for the Atg8 proteins that any other LIR described so far, which explains why TP53INP2 displaces p62 and some other autophagy receptors from the autophagosomes. Mass spectrometry of the purified autophagosomes revealed that TP53INP2 expressing cells have increased levels of mitochondria proteins in the autophagosomes, suggesting an increase in mitophagy. We are currently investigating if TP53INP2 is an autophagy receptor.

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exploration into the role of autophagy in lymph node high endothelial venules

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Lymph nodes (LNs) sequester antigens to prime immune cells and mount immunological responses. Under homeostasis, lymphatic vessels (LVs) deliver immune cells and antigens from peripheral tissues and are the major route of lymphocyte efflux, meanwhile high endothelial venules (HEVs) extract lymphocytes from the blood stream to be primed by antigens, thus both are essential to lymphocyte priming. Under homeostasis, HEVs express adhesion molecules to attract naïve lymphocytes, however upon inflammation, they upregulate genes associated with an inflamed endothelium (eg. Selectin-P, 4-1BB etc) to allow activated lymphocytes through as well. Autophagy is a major degradative pathway implicated in cellular homeostasis and stress responses. Recently, much attention has been placed on targeting it across a variety of diseases, including cancer, however, potential off-target effects on immune responses must be elucidated. Our data reveal an important role of autophagy in the functioning of HEVs within LNs during inflammation and cancer. Using mouse models with endothelial cell deletion of autophagy (ATG12 /ATG7/ ATG5) we observe loss of HEV “plump” morphology as well as reduced expression of inflamed endothelial genes in the LNs of knock out mice during inflammation, and B16F10 melanoma growth. Functionally, this was accompanied by reduced recruitment of both Naïve and activated T-cells to the LN during inflammation. Therefore, HEV autophagy may play an important role in HEVs ability to respond to stress.

Discovery and characterisation of ER-phagy protein networks

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The endoplasmic reticulum (ER) is a ubiquitous and dynamic organelle involved in many cellular processes and it is subject to turnover and remodeling – this being particularly relevant under ER stress conditions – to ensure an optimal function. To maintain ER homeostasis, ER is removed by a selective form of autophagy, known as ER-phagy, which is emerging as a fundamental pathway in the maintenance of cellular health. ER-phagy is mediated by ER-phagy receptors; these are membrane bound or soluble cargo receptors that will target the ER to the autophagy machinery. Recently, a few ER-phagy key players including cargo receptors have been identified but still, the molecular composition and regulation of this pathway is incomplete, particularly between different cell types. To address this, we performed proximity labelling proteomics in cultured pancreatic cells and organoids to identify proteins showing association with different ER-phagy receptors coupled with the biotin ligase TurboID (CCPG1, FAM134B, FAM134C, TEX264, SEC62). Next, to identify which of these might be potential ER-phagy candidates, we carried out a targeted secondary screen using CRISPR/Cas9 technology against high-confidence candidates identified by the proteomics, coupled with automated imaging of ER-phagy flux as read out by fusions of the ER-phagy receptor with the new signal-retaining autophagy indicator (SRAI) FRET-enhanced probe. A number of clear novel regulators of ER-phagy were thus identified. These are undergoing mechanistic characterization. Currently, these fall into two categories, those that appear to regulate ER stress responses and those independent of ER stress. This presentation will reveal the identity of these and discuss latest mechanistic data. Overall, this information will identify new proteins involved in this process and will provide a better understanding of the ER-phagy pathway.

PINK1/Parkin/p62-dependent basal mitophagy is suppressed in cellular senescence and mediates senescence phenotypes

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Selective degradation of damaged mitochondria by autophagy (mitophagy) is proposed to play an important role in cellular homeostasis. However, the molecular mechanisms and the requirement of mitochondrial quality control by mitophagy for cellular physiology are poorly understood. We demonstrated that primary human skin fibroblasts in cell culture maintain highly active basal mitophagy driven by mitochondrial ROS signalling. Mitophagy was found to be partially mediated by PINK1/Parkin-dependent pathway involving p62 as a selective autophagy receptor. Importantly, this pathway was disturbed upon the induction of cellular senescence leading to a robust shutdown of mitophagy. Conversely, re-activation of mitophagy was necessary for anti-senescence effects of NAD precursors or rapamycin. Furthermore, activation of mitophagy by p62-targeting small molecule suppressed markers of senescence establishing mitochondrial quality control as a promising target for the development of novel anti-ageing interventions.

AICAR and NAM synergistically attenuate senescence-associated changes in mesenchymal stem cells: The interplay of autophagy and mTORC1

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Tissue engineering has yet to reach its ideal goal, i.e. creating profitable off-the-shelf tissues and organs. One of the major challenges of this field that affects the outcome of the cell transplantation is the in-vitro aging and replicative senescence of the cell source. In the present work, Passage 5 (P5) adipose-derived mesenchymal stem cells (MSCs) were treated with control media, nicotinamide (NAM), AICAR, or the combination of AICAR+NAM till P10. Proliferative capacity, senescence-associated changes, multi-lineage differentiation potential, CDK-inhibitors -P16 and P21-, ROS, and apoptotic markers were compared between cells of P5 and P10. Furthermore, we studied the effects of AICAR and NAM on mTORC1 activity, and autophagy.

Our results showed that MSCs treated with NAM, AICAR, or both demonstrated an increase in proliferation and osteogenic differentiation potential, which was amplified in the group receiving both. AICAR or NAM treatment resulted in reduced expression of β -galactosidase and decreased accumulation of dysfunctional lysosomes. Also, NAM or AICAR+NAM significantly reduced the cellular ROS in aged MSCs. Moreover, AICAR and NAM administration attenuated mTORC1 activity and boosted autophagy.

In general, inhibition of mTORC1 by AICAR and NAM upregulates autophagy, retains MSCs' self-renewal capacity, and delays senescence of MSCs after prolonged in-vitro culture. Furthermore, our findings showed that concomitant use of AICAR and NAM shows a synergistic effect on the attenuation of cellular senescence. Our findings provide a viable option to slow down the in-vitro aging of the stem cells and conquer aging as a limiting factor in tissue engineering.

The leukemic oncogene MLL-AF4 induces autophagy and cell death in *Drosophila*

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Cells are constantly confronted with mutagenic challenges that could lead to activation of oncogenes. Traditional cancer therapies are mostly aimed at unselectively killing all rapidly dividing cells, resulting in undesirable side-effects and failing to exterminate slowly dividing cancer stem cells that induce relapse of the disease. Therefore, there exists a need for more specific therapies that can also rewire signaling to eradicate cancer stem cells.

Here we present that expression of the human oncogene MLL-AF4 in the hematopoietic system of *Drosophila* leads to development of a leukemia-like phenotype, whereas expression of the very same oncogene in a non-hematopoietic tissue results in cell death through autophagy and caspase activity. We find that fat body cells expressing the leukemic oncogene initially develop as the surrounding wild-type cells, but thereafter their growth is restricted and these cells are eventually eliminated. Autophagy plays an essential role in this context, whereas it is not required for the similar phenotype of cells with general aberrant transcriptional activity. By understanding these contrasting effects induced by the leukemic oncogene in different tissues, our aim is to exploit this knowledge to rewire pro-tumorigenic cellular signals into anti-tumor defense signaling.

A targeted screen of the human ubiquitome identifies novel regulators of autophagic flux

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During nutrient deprivation, cytoplasmic components are sequestered into double-membrane vesicles called autophagosomes and delivered to the lysosome/vacuole for degradation and recycling. However, it has been observed that organelles, pathogens, or specific protein complexes are selectively targeted for degradation by autophagy. Ribosomes are highly abundant long-lived protein-RNA complexes, that undergo efficient removal by autophagy during conditions of nutrient limitation. To gain molecular insight toward this process, we performed a targeted high-content screen against 1200 genes of the human ubiquitome, including E2- and E3-ligases, ubiquitin-binding proteins, and deubiquitinases. Using 3 different siRNAs per gene in a live-cell Keima-based imaging readout in human fibroblasts, we identified a group of high-confidence hits and validated their efficiency in a secondary screen using different Keima-tagged autophagy reporters (Keima-LC3B, Keima-RPL10A, RPL22-Keima) during nutrient starvation. In mechanistic follow-up studies, we focused on the E3 ligase PELI3, which we found to co-immunoprecipitates with ATG8 and ATG16L1. Currently, we are working to understand the possible role of ubiquitin as a signaling molecule for this process and to understand whether PELI3 has a role in mediating ATG8's recruitment to the marked ribosomes for their degradation by autophagy.

Nesprin-2/ANC-1 regulates nuclear autophagy

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Preservation of nuclear ultrastructure and function is crucial for the maintenance of cellular homeostasis. Phenotypic alterations in nuclear morphology, as well as, aberrant nuclear activity, dynamics and signaling are universal hallmarks of ageing and age-related pathologies. Nucleophagy, a process of selective targeting and degradation of damaged nuclear components by the autophagic machinery, serves as a nuclear quality control mechanism. Nucleophagic events have been implicated in a broad range of pathological contexts such as neurodegeneration, cancer and DNA damage. We find that the nuclear envelope anchor protein Nesprin-2 and its *Caenorhabditis elegans* orthologue ANC-1 are key nucleophagy regulators. Nesprin-2/ANC-1 functions to maintain small nucleolar size, which is a common denominator of diverse lifespan extension regimes. In addition, Nesprin-2/ANC-1 prevents nuclear shape abnormalities and accumulation of Lamin, the major structural component of the nuclear lamina. Under DNA damaging conditions, Nesprin-2-positive and Fibrillarin-positive nuclear protrusions are formed. Nesprin-2/ANC-1 deficiency causes enlargement of nucleoli and accumulation of Fibrillarin, a protein component of nucleolar ribonucleoproteins, in an autophagy-dependent manner. Genetic or pharmacological inhibition of autophagy leads to accumulation of Nesprin-2/ANC-1. Moreover, *anc-1* or Nesprin-2 deficiency causes accumulation of autophagosomes and autophagosome precursor membranes *in vivo* and in primary mouse embryonic fibroblasts respectively. Finally, we show that ANC-1 confers organismal stress resistance against nutrient deprivation, heat stress and DNA damage in *C. elegans*. Our findings indicate that Nesprin family members preserve nuclear and nucleolar architecture, by serving as evolutionarily conserved regulators of autophagic nuclear and nucleolar material degradation.

Upregulation of Mitophagy by JAK-STAT Signaling Enables Stem Cell Regeneration

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Regeneration of undifferentiated stem cells retaining proliferation capacity from differentiated, non-dividing cells underlies carcinogenesis and tissue restoration following injury and disease. Despite its medical and physiological significance, the molecular and cellular basis of stem cell renewal remains largely uncharacterized. We demonstrate that in the *Drosophila* testis in which germline stem cells were eliminated, JAK-STAT signaling induced by specialized immune cells, invading hemocytes (macrophages), upregulates mitophagy in gonialblast and spermatogonial cells, which then dedifferentiate into germline stem cells. The selective degradation of mitochondria is accomplished by an amphisome-mediated autophagic process that may promote constitutive glycolysis, a metabolic hallmark of stem cells. Thus, mitophagy is likely to have a role in metabolic reprogramming (a transition from oxidative phosphorylation to aerobic glycolysis) that influences cell fate decisions relevant to both tissue repair and tumorigenesis.

Characterization of the phagophore rim during autophagosome formation

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The autophagosome is a double-membrane vesicle that sequesters cytoplasmic components for autophagy. During autophagosome formation, a single-membrane flat phagophore elongates and bends into a cup-shaped structure. Finally, it becomes a spherical structure by closing the rim. Throughout this process, the volume of the phagophore is maintained small, keeping the rim highly curved and energetically unstable. We proposed a mathematical model in which the elastic bending energy and the partitioning entropy of the curvature generator determine the shape of the phagophore (Sakai et al., iScience, 2020). The mathematical model predicts that, under conditions of excessive membrane curvature generators, tube-like structures would be formed from the rim of flat vesicles. In fact, such tube-like structures were observed by live-cell imaging. These structures often appeared immediately before expanded flat phagophores started bending. At that time, the amount of the curvature generator should reach its maximum. These observations well support our mathematical model and suggest that the amount of the curvature generator is regulated independently of the diameter of flat phagophores. The remained question is what the curvature generator is. Fluorescence microscopy revealed that autophagy-related (ATG) proteins are classified into those localized at the rim of phagophores and those distributed uniformly on phagophores. Among the former group, ATG2 has an amphipathic helix motif that is required for the localization to the rim. Thus, we hypothesize that ATG2 has a curvature-generating or stabilizing activity, in addition to the well-established lipid transfer activity.

RUFY3 links Arl8b and JIP4-Dynein complex to regulate lysosome size and positioning

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The bidirectional movement of lysosomes on microtubule tracks regulates their whole-cell spatial arrangement. Arl8b, a small GTP-binding (G) protein, promotes lysosome anterograde trafficking mediated by kinesin-1. Herein, we report an Arl8b effector, RUFY3, which regulates the retrograde transport of lysosomes. We show that RUFY3 interacts with the JIP4-dynein-dynactin complex and facilitates Arl8b association with the retrograde motor complex. Accordingly, RUFY3 knockdown disrupts the positioning of Arl8b-positive endosomes and reduces Arl8b colocalization with Rab7-marked late endosomal compartments. Moreover, we find that RUFY3 regulates nutrient-dependent lysosome distribution, although autophagosome-lysosome fusion and autophagic cargo degradation are not impaired upon RUFY3 depletion. Interestingly, lysosome size is significantly reduced in RUFY3 depleted cells, which could be rescued by inhibition of the lysosome reformation regulatory factor PIKFYVE. These findings suggest a model in which the perinuclear cloud arrangement of lysosomes regulates both the positioning and size of these proteolytic compartments.

Domain-specific interactome of BAG3 identifies the precise binding region of proteins within the BAG3-HSP70-HSPB8 module

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The co-chaperone BAG3 is a hub for various cellular pathways via its multiple domains and its interaction with HSP70 and HSPB8. Besides its function in maintaining myofibrils in muscle cells or promoting survival, growth and invasiveness in cancer, it is also associated with the cellular protein quality control driving a BAG3-mediated selective autophagy pathway. Under cellular stress conditions in particular, together with different molecular chaperones, BAG3 ensures the sequestration of aggregated or aggregation-prone ubiquitinated proteins to the autophagic-lysosomal system via ubiquitin receptors. We previously defined the proteostasis stress-specific BAG3 interactome revealing numerous BAG3 interactors (>250) influencing many cellular pathways. To define the exact binding domain of key BAG3 interacting proteins we now introduced a BAG3 domain-dependent interactome. Our results confirm previously postulated binding regions of known binding partners and identify a plethora of hitherto unknown binding partners of the BAG3-HSP70-HSPB8 complex. We uncovered the binding of highly interesting target proteins, such as 42 kinases or kinase-regulating proteins and nine E3 ubiquitin ligases, to certain domains. Further experiments support the assumption that some E3 ligases do not interact in a ternary complex via HSP70, but rather bind directly to the BAG domain. Our results contribute to a clearer understanding of the composition and dynamics of this key player complex and may introduce a novel function of BAG3 in the protein degradation process.

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Battle between bacterial effector and plant autophagy

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Plant pathogenic effectors perturb the proteolytic pathways autophagy and ubiquitin-proteasome system (UPS), disrupting host cellular processes to enhance virulence. We study the bacterial pathogen *Xanthomonas campestris* pv. *vesicatoria* (Xcv) which is a significant agricultural pest. Results from our lab show that Xcv subverts host autophagy during infection. We identified XopL as an effector with a role in this subversion of host autophagy. We showed that XopL interacts with and ubiquitinates a component of the host autophagic machinery, SH3P2. This results in SH3P2 degradation via the proteasome, which leads to higher Xcv bacterial growth. In turn, the plant defends itself using defence-related selective autophagy receptor NBR1. We showed that NBR1 is upregulated and accumulates during coexpression with XopL, colocalizes with XopL in aggregates, is present in a complex with XopL, and causes the autophagic degradation of XopL. Knockdown of NBR1 also resulted in higher Xcv bacterial growth. To provide further mechanistic insight, NBR1 mutants lacking ubiquitin-binding domains were tested for interaction with XopL. We found that ubiquitin-dependent and -independent mechanisms drive this interaction. Our study is a novel example in plants of “effectorphagy”, where selective autophagy targets bacterial effectors, and further gives mechanistic insight behind this process.

The KFERQminer project – development of novel bioinformatics tools for finding KFERQ motifs

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Autophagy plays a key role in the maintenance of cellular homeostasis and its disturbances have been extensively described in a plethora of human diseases. Chaperone-mediated autophagy (CMA) can be characterized by a high substrate specificity. It only selects proteins with a degradative KFERQ motif tag in their sequence. The structure of this motif has been proposed based on empirically tested substrates in the 1980s.

To summarize past decades' accomplishments in KFERQ motif research we develop the KFERQminer project which includes several motif collection and analysis steps. The collection step involves a thorough manual review of papers published in PubMed in search of CMA-related keywords. We extract described KFERQ motifs and combine them into the KFERQminer database. The collected set is used for sequence analysis and the development of new *in silico* KFERQ motif searching tools. In the final step proteins with the most promising motif targets are validated as potential CMA substrates in a cell line model.

Based on our database we develop a novel web-based KFERQminer bioinformatics tool that is intended to gather all the necessary data about KFERQ-related motifs in one place. The motif review we made suggests the presence of functional motifs that fall outside the canonical KFERQ definition. We find these noncanonical motifs in proteins described as interacting with the CMA machinery but regarded as not containing any KFERQ motif. Moreover, because intracellular pathogens undergo cellular processes of their hosts, we have analyzed proteomes of human viruses. Interestingly, we observed an abundance of KFERQs in several virus families, which may have significant medical implications. Our observations suggest the legitimacy to extend the search for KFERQ motifs with some additional rules and to intracellular pathogens of CMA-competent organisms, which by their nature are subjected to the cellular processes of their hosts.

Autophagy keeps the most common intracellular bacterium under control

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Wolbachia pipientis is considered one of the most common parasites on Earth: it infects approximately 40% of terrestrial arthropods including fruit flies, but it also parasitizes other invertebrates, such as human pathogenic filarial nematodes. The intracellular way of living led *Wolbachia* to lose its ability to live outside of a host, so *Wolbachia* spares the host's life and utilizes it to spread the infection to the next generation. For its own survival and reproduction, the bacterium gives the host resistance against viruses and other pathogens in return. Thus, *Wolbachia* is widely used to control mosquito-borne diseases. However, it is still not clear whether this prokaryote has evolved into a symbiont or remained a pathogen. Autophagy is a conserved process of eukaryotic cells in which cells isolate a portion of cytoplasm and degrade it by the lysosomal system. Hence, autophagy is also used to eliminate intracellular pathogens. Therefore, we can expect *Wolbachia* infection to be also controlled by this process. However, our knowledge is still very limited about the relationship of *Wolbachia* and autophagy. Most studies focused mainly on the reproductive tissues of *Drosophila* to explore how it is passed onto new generations and how it affects its host immune system. In this work, we demonstrate that fruit fly somatic cells use autophagy to selectively degrade and eliminate damaged *Wolbachia*. Without autophagy, the amount of this bacterium greatly increases therefore significantly decreasing the lifespan of the host. This suggests that autophagy is used to control both the quality and quantity of this intracellular prokaryote.

Novel role of the E3 RING ligase Ubr2 as autophagy modulator in *Saccharomyces cerevisiae*

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Maintenance of protein homeostasis is pivotal for the preservation of functional cell metabolism. It is safeguarded by chaperones and the two proteolytic systems, the ubiquitin proteasome system and autophagy. In the present study, we investigated the role of Ubr2, a RING E3 ligase negatively regulating expression of proteasome-related genes in *Saccharomyces cerevisiae*, in chronological lifespan (CLS) and autophagy. We found that the deletion of UBR2 prolonged CLS in an autophagy-dependent manner. Likewise, mRNA levels of several autophagy-related genes were elevated upon UBR2 deletion. Rim15, a kinase positively regulating transcription of autophagy-related genes, was required for CLS extension. However, Rim15 was not stabilized upon UBR2 deletion and therefore is not directly targeted by Ubr2 for degradation. These data demonstrate the novel role of Ubr2 as an autophagy repressor in *S. cerevisiae*. However, the target of Ubr2 which mediates CLS extension and autophagy induction remains elusive.

Spatial proteomics reveals secretory pathway disturbances upon loss of neuropathy-associated TECPR2

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Hereditary sensory and autonomic neuropathy 9 (HSAN9) is a rare neurological disease caused by mutations in the gene encoding for Tectonin β -propeller repeat containing protein 2 (TECPR2). TECPR2 consists of a N-terminal WD40 domain, a series of C-terminal TECPR2 motifs and a LC3-interacting region (LIR). Several pathogenic mutations lead to the expression of truncated TECPR2 variants missing some of its C-terminal features and are thought to be rapidly degraded. TECPR2 was identified as an interactor of hATG8 proteins and to support autophagy and autophagosome-to-lysosome targeting. Additionally, TECPR2 regulates ER export through stabilization of SEC24D, a component of the COPII-coat that plays a key role in the secretory pathway. While both processes are connected, it remains elusive how loss of TECPR2 affects them, especially regarding the consequences for trafficking cargo or downstream compartments.

To address this, we employed a series of spatial proteomic approaches to systematically examine defects along the secretory pathway upon TECPR2 deficiency in an HSAN9-mimicking cell model. This global analysis unveiled profound alterations at distinct trafficking steps including ERES formation, COPII-mediated transport to the Golgi, sorting to the lysosome or PM, and secretion into the extracellular space. Importantly, numerous proteins with neuronal function were affected, providing a potential link to HSAN9 pathogenesis. In parallel, we mapped TECPR2's extensive association with components of the ER-Golgi interface. Overall, our systematic profiling of an HSAN9 cell model points to specific but detrimental protein sorting and ER export defects which might precede autophagy dysfunction upon TECPR2 deficiency. Given TECPR2's effects on autophagy and ERES formation, further studies integrating both processes might be beneficial in elucidating HSAN9 pathogenesis.

Interplay between phosphorylation and dimerization of the BNIP3L/NIX as a new mechanism of receptor-mediated mitophagy regulation

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A form of autophagy specialized for selective removal of mitochondria, mitophagy, is essential for the elimination of dysfunctional mitochondria whose accumulation can lead to the development of neurodegenerative diseases and tumors. Programmed mitophagy of healthy mitochondria is crucial for the differentiation of specialized cell types, such as erythrocytes. Autophagy receptor BNIP3L/NIX is shown to be important for the removal of healthy mitochondria during terminal erythropoiesis but molecular mechanisms of selectivity are still unclear. Here, we have investigated BNIP3L/NIX dimerization and phosphorylation as a novel molecular mechanism underlying receptor-mediated mitophagy. Stable BNIP3L/NIX homodimers provide the formation of new and strong interactions between the receptor and autophagosomal proteins, more robust recruitment of autophagosomes, and efficient removal of mitochondria. This dimerization is achieved by specific Ser212 dephosphorylation in the C-terminal part of the receptor located in the intermembrane mitochondrial space and has the same effect on mitophagy initiation and progression as LIR phosphorylation. Thus, the interplay between BNIP3L/NIX phosphorylation and dimerization indicates that the combined mechanism of LIR phosphorylation and receptor dimerization is needed for proper BNIP3L/NIX-dependent mitophagy initiation and progression. Currently, the focus of our research is on a detailed analysis of interactions between BNIP3L/NIX and identified kinases and phosphatases to unveil upstream signaling pathways that trigger and regulate mitophagy, especially in erythroid cell lines. Lastly, this knowledge of the molecular basis of BNIP3L/NIX-dependent mitophagy regulation is crucial for better understanding the mechanisms of individual cell differentiation and the development of pathological conditions that underlie the disturbed mitophagy process.

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Autophagy is a conserved catabolic pathway in eukaryotic cells, where a newly formed organelle, the autophagosome, transports cytoplasmic material, organelles, pathogens or aggregates to the vacuole/lysosome for degradation and reuse. During autophagy, a membrane disc forms, expands by lipid import, thereby surrounds its cargo, and finally forms a double membrane structure, the so-called autophagosome. After maturation, the autophagosome fuses with the vacuole/lysosome, where the engulfed material gets degraded into its monomers for reuse by the cell. In yeast, fusion of autophagosomes with vacuoles requires SNARE proteins, the homotypic vacuole fusion and protein sorting (HOPS) tethering complex, the Rab7-like Ypt7 GTPase, and its guanine nucleotide exchange factor (GEF) Mon1-Ccz1. Recently, we showed that Rab5 on endosomes can directly promote Mon1-Ccz1 dependent Rab7 activation. Mon1-Ccz1 also targets to pre-autophagosomal structures by binding to Atg8 and phosphatidylinositol-3-phosphate (PI3P). Previous data show that Mon1-Ccz1 and Ypt7 are recruited early to the autophagosomes, but the activity of Mon1-Ccz1 on Ypt7 is only observed on mature autophagosome. We hypothesize that the additional regulation of Mon1-Ccz1 requires the autophagy related kinase Atg1 in complex with Atg13. For Atg1-Atg13 it is known that it regulates through phosphorylation different proteins involved in autophagy, for example Atg9 or the SNARE protein Ykt6. Here, we could purify the Atg1-Atg13 kinase complex and Mon1-Ccz1 from yeast and established an in vitro kinase assay to show that Mon1-Ccz1 is a substrate of Atg1-Atg13. We identified phosphorylation sites by mass spectrometry, and test their effect on autophagy in vivo. We suggest that phosphorylation of Mon1-Ccz1 by Atg1-Atg13 controls a metabolic switch of the cell from normal growth conditions to autophagy. We postulate that Mon1-Ccz1 is differentially regulated in its activity on endosomes and autophagosomes.

Nuclear autophagy delays ageing while maintaining germline immortality

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Recycling of nuclear material is essential for cellular and organismal homeostasis. Here, we show that selective autophagy of nuclear material is an important determinant of germline immortality and somatic ageing under conditions of stress. Impairment of autophagic recycling of nuclear components diminishes stress resistance, germline immortality and longevity. We find that the *Caenorhabditis elegans* nuclear envelope anchor protein, ANC-1 and its mammalian orthologue Nesprin 2 are key autophagy regulators that restrict nucleolar size, a common denominator of diverse lifespan extension regimes. We identify and characterize a novel germline immortality assurance mechanism, which involves nucleolar degradation at the most proximal oocyte by ANC-1. Clearance of aberrant germ cells during their differentiation by autophagic cell death requires ANC-1 and LGG-1. Notably, perturbation of this clearance pathway causes tumour-like structures in the *C. elegans* germline. Similarly, genetic ablation of Nesprin 2 in female mice causes ovarian carcinomas, indicating that the relevant molecular pathways are evolutionarily conserved, across distant phyla. Thus, autophagic recycling of nuclear envelope-associated and nucleolar components is an essential soma longevity and germline immortality mechanism that promotes youthfulness and delays ageing under conditions of stress, by preserving nuclear architecture and preventing nucleolar expansion.

Exploiting lipophagy for Atlantic salmon farming.

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Scotland's salmon farming sector is worth over £500 million to the country's economy. In Atlantic salmon aquaculture, the commercial feed used in the final grow-out production phase contains high levels of lipids. These lipids provide the main source of energy, promoting rapid growth and high feed efficiency. However, this strategy comes at the expense of a marked increase in visceral fat deposition (through lipid droplets in fat cells), reversal of PUFA ratio (n3:n6), atherosclerosis, susceptibility to viral infections and mortality (up to 20%) in winter months, bringing significant economic loss. It is therefore crucial to reduce lipid deposition in these animals.

We hypothesized that lipophagy is functional in Atlantic salmon. We further hypothesized that high lipid content in salmon feed blocks lipophagy and this block can be restored by mTOR inhibition.

Using CRISPR/Cas9, we knocked out ATG7 in salmon head kidney cell line (SHK). Our pilot data reveals that knocking out ATG7 leads to accumulation of lipid droplets in SHK cells ($p < 0.001$). Furthermore, we see a significant reduction in number and size of lipid droplet ($p < 0.001$) in SHK cells cultured in a lipid rich diet and treated with 200nM Rapamycin for 48hrs. Quantitative proteomics analysis of rapamycin treated cells show an upregulation of lipid catabolism by lipophagy and downregulation of inflammatory response.

Currently, we are investigating lipophagy in primary adipocytes from final grow-out production phase salmon. Concurrently, we are performing lipidome profiling to analyse free fatty acid release on rapamycin treatment along with beta-oxidation measurements.

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*This is my first work as an independent principle investigator

Adaptive role of ER-phagy in response to chronic endoplasmic reticulum stress in neuronal cells

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Calcium dysregulation induces the accumulation of misfolded and aggregated proteins and may play a crucial role in the pathogenesis of neurodegenerative diseases. The hippocampal cell line HT22 has recently been used to illustrate the adaptive ability in response to oxidative stress by enhancing autophagic activity via especially BAG3-mediated selective macroautophagy. ER-phagy, a selective turnover of endoplasmic reticulum (ER) fragments via the autophagy-lysosome system, has been described to function in ER homeostasis and ER quality control under normal as well as stress conditions. Here, we generated a novel HT22 cell line that is resistant to up to 1.5 μ M thapsigargin, an inducer of Ca^{2+} release from intracellular stores and a potent ER stressor. We observed a significantly increased LC3B-II and p62 flux in thapsigargin-resistant (TgR) cells compared to HT22-WT cells at both, basal and starved conditions. However, phosphorylation of mTOR remains unchanged in TgR cells compared to HT22-WT. Intriguingly, when looking at ER-phagy receptors, we found the long isoform of RTN3, which facilitates tubular ER degradation, increased and ATL3 slightly reduced in TgR cells. Activation of ER-phagy at nutrient-sufficient conditions in TgR cells is further confirmed using the ER-phagy tandem reporter ssRFP-GFP-KDEL, as indicated by RFP-positive but GFP-negative puncta. Proteomics analysis unveiled a highly significant differential protein expression pattern in TgR cells, with many changes in lipid metabolism-related proteins. Moreover, the number of lipid droplets in TgR cells is reduced compared to HT22-WT cells. Hence, we suggest that ER-phagy could be a mechanism promoting resistance to the ER stressor, which is likely to be linked to lipid droplet turnover.

UFMylation regulates C53-mediated autophagy at the endoplasmic reticulum

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The endoplasmic reticulum (ER) processes around 40% of the proteins inside the cell. Cellular stress stalls the ER bound ribosomes and impedes proteins synthesis, which leads to the accumulation of toxic products that need to be removed to maintain homeostasis. One of the main pathways involved in resolving ER-stress is autophagy, a selective quality control process that recognizes stress signals and removes faulty components. How selective autophagy of the ER (termed as ER-phagy) is regulated remains largely unknown. Here, we identified a highly conserved receptor protein C53 that is activated upon stalling of membrane bound ribosomes. We show that C53 has evolved non-canonical shuffled ATG8 interacting motifs (sAIMs) that are essential for ATG8 interaction and autophagy activation. C53 senses proteotoxic stress through the components of the UFMylation machinery. UFMylation is an elusive posttranslational modification system where substrate proteins are modified with the ubiquitin-like protein UFM1. We demonstrate that C53 forms a complex at the ER with UFL1, the E3 ligase that mediates UFMylation and its membrane adaptor DDRGK1. Under normal conditions, C53 is kept inactive by UFM1 bound to the sAIMs. Upon ribosome stalling, UFM1 is covalently transferred to the ribosomal subunit RPL26. This leads to ATG8 binding and recruitment of the core autophagy machinery, activating ER-phagy. We finally observe that mutants of both C53 and UFMylation pathway are highly susceptible to ER-stress. Thus, C53 forms an ancient quality control mechanism that bridges UFMylation and selective autophagy with ribosome quality control at the endoplasmic reticulum.

Distinct subcellular autophagy impairments in induced neurons from Huntington's disease patients

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Many neurodegenerative disorders of the CNS are characterized by the presence of intracellular protein aggregates. These disorders, often termed proteinopathies, include Huntington's Disease (HD). The age-dependent neuronal loss that occurs in HD correlates well with the accumulation of these protein aggregates. However, why these protein aggregates fail to be cleared as they develop at a subcellular level in HD remains largely unknown.

Modelling HD in the lab has proven challenging as rodent models poorly reproduce the disease process and cellular models fail to include age-dependent processes crucial to the disease. In this project, we established a new model of HD based on induced neurons (generated by direct reprogramming). This HD-iN model allows for a systematic study of a wide range of differentially affected human neurons derived from patients with HD that retained age-dependent characteristics. Proteomics analysis demonstrated an alteration in autophagy in HD-iNs. In line with this, HD-iNs displayed a distinct alteration in autophagy activity, specifically in the neurites, characterized by a reduction in the transport of late autophagic structures from neurites to the cell-body and subsequent cargo degradation. In addition, we found another neurite specific HD-iN phenotype, which consistently displayed shorter and fewer neurites compared to ctrl-iNs. Lastly, silencing of HTT using CRISPRi could not rescue autophagy or the less elaborate neuronal morphology in the HD-iNs.

These results identify a post-transcriptionally regulated autophagy coupled with neurite impairments in HD-iNs. This newly gained information will also allow us to design novel therapeutic strategies.

The autophagic machinery favours HIV-1 entry in CD4+T lymphocytes

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HIV-1 is the etiological agent of AIDS. CD4+ T lymphocytes (CD4+TL), in which it replicates efficiently, are the main targets of this virus. Like others pathogens, HIV-1 has evolved strategies to block or use the autophagic machinery to its own benefit. Indeed, we previously shown that autophagy is transiently induced in CD4+TL in the first steps of HIV-1 replication cycle before being controlled and completely inhibited by the action of viral proteins. The aim of the present work was to determine the interplay between the autophagic machinery and HIV-1 at the very early step of HIV-1 infection of CD4+TL.

Our results showed that pre-treating CD4+T cells with PI3C3 inhibitors leads to a decrease in HIV-1 infection, suggesting that PI3P production favours HIV-1 replication. As we observed a lipidation of LC3 early after HIV-1 cells exposure, we used a sensitive and specific enzyme-based assay to detect HIV-1 virion fusion in CD4+TL and shown that inhibiting PI3KC3, ATG5 or ATG7, by pharmacological and genetic approaches, dampen the HIV-1 entry step in TCD4+ cells. Consistently, we found that overexpressing LC3B in CD4+T cells leads to an increase of HIV-1 entry. In parallel, we started to visualize the LC3B dynamics upon CD4+ T cells HIV-1 exposure. For that, we generated a “GFP-LC3B CRISPR-Knock-IN” HEK.293T cell line, allowing the expression of GFP-LC3B at endogenous levels, expressing the HIV-1 receptors. Experiments of live-cell imaging showed that, upon viral exposure, dots of GFP-LC3B transiently reach the cell surface and polarize near viruses. In addition, by biochemical experiments, we observed a specific interaction between HIV-1 capsid and LC3B in the early steps of infection of CD4+T cells.

In conclusion, our results show that HIV-1 uses the autophagy machinery to facilitate its entry in CD4+TL lymphocytes and therefore to complete the first step of a successful infection.

Role of PLAC8 in regulating autophagy in Chinese Hamster Ovary (CHO) cells

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We have previously shown that Placenta-specific 8 (PLAC8), a cysteine-rich lysosomal protein is overexpressed in Hs578Ts(i)8 cells, an invasive subclone derived from the parental breast cancer cell line, Hs578T. In comparison to the parental cells, the Hs578Ts(i)8 cells showed a significant increase in growth rate and culture duration. Recent publications have shown that PLAC8 may play a role in autophagy in colon cancer. Over the last 30 years CHO cells have become the workhorse for the biopharmaceutical industry and advances in cell line development have increased CHO productivity and product quality. However, process termination due to cell death is still an issue and extending the lifetime of cells and processes is crucial.

The objective of our research was to examine the role PLAC8 in autophagy in CHO-K1 cells. We have transfected the cDNA for PLAC8 into CHO-K1 cells and generated stable clones, referred to as CHO-PLAC8. Analysis of growth kinetics in CHO-PLAC8 cells over 9 days showed significant differences in growth rate, maximal cell density and culture duration in comparison to control CHO-K1 cells. Using flow cytometry, we simultaneously analyzed apoptosis and autophagy over 9 days culture and performed western blot analysis for PLAC8, LC3 and p62 on cell extracts. Preliminary results showed an increase in PLAC8 protein from Day 3-9 which is accompanied by activation of LC3-I to LC3-II suggesting a potential role for PLAC8 in autophagy. Preliminary analysis of autophagy using CYTO-ID® showed an equivalent increase in autophagy corresponding with the extended culture lifetime of CHO-PLAC8 cells. We have transfected CHO-K1 cells with a GFP-PLAC8 fusion protein so that we can directly investigate the involvement of PLAC8 in the autophagic process. In the long-term results from this research may lead to the development of cell lines with an extended lifetime in culture which will have applications for biomanufacturing.

LIRcentral - towards a centralized bioinformatics portal for data and tools to study selective macroautophagy

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Members of the Atg8 protein family (1) are small proteins with a ubiquitin-like fold and are key components of the macroautophagy machinery, participating essentially in all steps of macroautophagy from phagophore nucleation, to autolysosome formation (2). They are involved in an increasing number of biological processes, including LC3-associated phagocytosis (3,4), endosomal microautophagy (4,5), and unconventional secretion (6).

In selective macroautophagy, selectivity is mainly achieved through receptor proteins that bind to Atg8 by anchoring a small linear peptide (AIM in plants and fungi, LIR-motif in mammals) (7). After the discovery of the first few LIR-containing proteins (LIRCPs), several definitions of the LIR-motif have appeared in the literature, highlighting a short core consensus sequence described by the regular expression pattern [WFY]xx[VLI] (8-12). Accumulating experimental evidence pinpoints the importance of regions flanking the core LIR-motif, while several instances of atypical LIR-motifs and LIR-independent interactions are being uncovered.

We have developed LIRcentral as a publicly available, comprehensive collection of experimentally confirmed LIR-motifs, based on extensive biomedical literature search, assisted by text mining techniques and careful manual curation. Interested researchers are able to retrieve information about literature-validated LIR-motifs and display them along with sequence features annotated in UniProt.

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Autophagy on demand: Using host-directed therapies to outsmart emerging SARS-CoV-2 variants

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The current dominant SARS-CoV-2 variant of concern (VOC) classified as Omicron has accumulated a greater number of mutations as compared to earlier VOCs situated within the Spike protein receptor-binding domain. Although antibodies generated post-infection or upon vaccination do not completely lose their ability to neutralize Omicron, there is a significant drop in antibody neutralization efficacy. Furthermore, the trend towards clustering of mutations in key areas targeted by existent direct-acting antivirals suggests that post-infection therapeutics may show reduced efficacy to novel VOCs. Thus, different antiviral approaches are needed to overcome new challenges in addressing the COVID-19 pandemic.

Host-directed therapies have the potential to both interfere with host cell mechanisms hijacked by viruses for infection, and boost cell-mediated resistance mechanisms. Host-directed antivirals are therefore unlikely to lose efficacy upon emergence of novel variants that escape antibodies or direct-acting therapeutics. A known target of host-directed therapies is autophagy. Degradative autophagy is considered a key player in cellular antiviral defense mechanisms. However, several viruses manipulate autophagy to facilitate infection. It is well supported in literature, and by our own data, that SARS-CoV-2 and other respiratory coronaviruses, including SARS-CoV-1 and MERS, rely on host autophagy mechanisms for entry and replication. Here, we investigated the impact of autophagy-modulating therapeutics on SARS-CoV-2 acquisition of primary human epithelial cells.

Our findings demonstrate that selected autophagy-inducing and -blocking therapeutics robustly inhibit SARS-CoV-2 entry into primary human epithelial cells. The inhibition of virus entry was exerted via autophagy, and not by other mechanisms of action, because knockdown of key autophagy molecules abrogated the inhibiting properties of the compounds. Thus, our findings underscore the therapeutic potential of host-directed therapeutics targeting autophagy to restrict SARS-CoV-2 spread. Our data prove that repurposed host-directed therapeutics could be a realistic alternative to combat rapidly emerging SARS-CoV-2 VOCs.

The basal autophagic turnover of the anti-inflammatory protein TNIP1 is mediated through both ATG7-dependent and -independent pathways.

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In a recent study, we showed that the anti-inflammatory protein TNIP1 is an autophagy substrate with the ability to interact with ATG8 proteins through LC3-interacting regions (LIRs). Upon stimuli with poly(I:C), which mimics viral RNA, TBK1 phosphorylates and activates one of the TNIP1 LIRs, leading to LIR- and ATG7-dependent degradation of TNIP1. However, we also observe that ATG7 knockout only partially affects the basal lysosomal degradation of TNIP1 in unstimulated cells. Here, we show that TNIP1 is still degraded in lysosomes independently of ATG7 and ATG8 proteins under basal conditions. We propose that TNIP1 can be delivered to the lysosome in an ATG7-independent, but ATG9- and FIP200-dependent manner in unstimulated cells. We are currently working to unravel the mechanistic details involved. By employing this ATG7-independent autophagy pathway, the cell can maintain a steady state level of TNIP1 in unstimulated cells, yet efficiently activate the selective ATG7-dependent degradation of TNIP1 during the early phase of immune activation.

Endosome maturation links nutrient signaling to lysosome repopulation during basal autophagy

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Autophagy is dependent on the repopulation of lysosomes to degrade intracellular components and recycle nutrients. There has been significant progress in understanding how lysosomes are repopulated during starvation-induced autophagy via TFEB/TFE3-dependent lysosome biogenesis and autophagic lysosome reformation. However, these pathways do not operate during basal autophagy, which occurs constitutively under nutrient-rich conditions and is associated with longevity, neurodegeneration and cancer. Thus, it remains unclear how cells co-ordinate lysosome repopulation during basal autophagy. Here, we identify an endosome-dependent phosphoinositide pathway that links nutrient signaling to lysosome repopulation during basal autophagy. We show that a Vps34-independent pool of PI(3)P on endosomes was required for basal but not starvation-induced autophagic degradation. PI(3)P signals were maintained as endosomes matured into endolysosomes to promote lysosome reformation. Inhibition of this phosphoinositide pathway resulted in lysosome depletion and autophagosome accumulation, as well as protein aggregate accumulation and cytotoxicity under conditions of proteotoxic stress. Therefore under nutrient-rich conditions, Vps34-independent generation of PI(3)P contributes to basal autophagic degradation and protection from proteotoxic stress via regulation of lysosome reformation from endolysosomes. These findings reveal an origin for lysosomes during basal autophagy whereby endosome maturation couples nutrient signaling to lysosome reformation.

Exploitation of Non-Canonical Autophagy by *Streptococcus pneumoniae* for prolonging intracellular persistence

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Autophagy is primarily a cytoprotective mechanism, activated to maintain cellular homeostasis. Apart from clearing damaged organelles and misfolded proteins, a selective form of autophagy called xenophagy, is pivotal for clearance of pathogens. Xenophagy is induced due to damage to the pathogen containing vacuolar membrane and the extent of damage depends on the expression of specific bacterial factors inducing such damage. For a vacuolar membrane disrupting toxin expressing pathogen, such as *Streptococcus pneumoniae*, the extent of toxin pneumolysin (Ply) secretion triggers variety of fates. Our findings suggest that while high Ply producers are easily eliminated by the host, low Ply expressors experience prolonged intracellular persistence. Surprisingly, the low Ply expressing SPN-containing vacuoles though are devoid of all damage sensors but are decorated with LC3, the classical autophagy marker. We observed that osmotic/pH imbalance created due to Ply created small pores halts maturation of the vacuole and disallows fusion with lysosomes. FIP200 knock-out and simultaneous inhibition of PI3K further revealed that LC3 decoration onto pneumococcal containing vacuole happens through a novel non-canonical pathway and is distinct from LC3 associated phagocytosis (LAP). This unique subset of SPN confined within a LC3 marked endosome is protected from lysosomal degradation and acquires Rab11, the characteristic marker of recycling endosomes. Thus such pneumococci may possibly recycle to the airway lumen for subsequent transmission. In conclusion, our study suggests that by regulating the expression of its key virulence factor Ply, SPN hijacks the host cell signaling pathway to prolong its survival, promoting plausible onward transmission.

FIP200 controls the TBK1 activation threshold at SQSTM1/p62-positive condensates

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The protein kinase TBK1 is a central regulator of innate immune responses and autophagy, and ablation of either function has been linked to neuroinflammatory or degenerative diseases. Autophagy is an intracellular process that recycles old or damaged proteins and organelles. In recent years, the TBK1-dependent regulation of autophagy pathways has been characterized. However, the autophagy-dependent regulation of TBK1 activity awaits further clarification. Here, we observed that TBK1 is recruited to SQSTM1/p62-containing aggregates via the selective autophagy receptor TAX1BP1. In these aggregates, TBK1 phosphorylates SQSTM1/p62 at serine 403 and thus presumably regulates the efficient engulfment and clearance of these structures. We found that TBK1 activation is strongly increased if FIP200, a component of the autophagy-inducing ULK1 complex, is not present or cannot bind to TAX1BP1. Given our collective findings, we hypothesize that FIP200 ensures the inducible activation of TBK1 at SQSTM1/p62 condensates.

Genetic variation in the autophagy machinery is associated with enhanced antiviral T-cell immunity and reduced T-cell exhaustion signatures in HIV-1-infected individuals

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Although antiretroviral therapy is effective, treated HIV-1 patients suffer from chronic inflammation and immune dysfunction. Autophagy functions as an antiviral defense mechanism and regulator of T-cell-mediated immunity. We have identified a genetic polymorphism within the Tripartite motif-containing protein 5 (TRIM5 α)-mediated autophagy pathway, which is associated with delayed disease progression and improved survival of HIV-1-infected individuals from the Amsterdam Cohort Studies. These findings underscore the *in vivo* relevance of autophagy activity in chronic HIV-1 infection. Here, we present the functional impact of this autophagy polymorphism on T-cell proliferation and immunophenotype, including signatures associated with antiviral and inflammatory T cell responses in HIV-1-infected individuals.

Cryopreserved blood lymphocytes from healthy and HIV-1-infected individuals were used per genotype: either homozygous for the major allele (wild-type) or homozygous for minor allele (carrying autophagy polymorphism). Advanced flow-cytometry in combination with next-generation RNA-sequencing was employed to determine autophagy levels, T-cell immunophenotype, polyfunctionality, and transcriptional signatures.

T cells from homozygous minor individuals displayed enhanced basal levels of autophagy and upregulated transcription of core autophagy genes. Heightened autophagy activity in the minor genotype was associated with increased CD4 and CD8 T cell proliferative capacity, increased frequencies of CD127-CD25⁺FoxP3⁺ regulatory T cells, and a reduction in IL-17A/IL-17F-producing ROR γ ⁺ T-helper (Th)17 cells. Minor genotyped T cells displayed increased expression of receptors associated with immune responsiveness (CCR7/CD28/CD127) and downregulation of exhaustion markers (CTLA-4/PD-1). Gene set enrichment analyses identified unique transcriptome signatures in minor genotyped T cells with enhanced regulation of antiviral responses as well as suppressed inflammasome assembly and IL17-production.

Our findings on this newly described autophagy polymorphism underscore the protective role of autophagy activity in the clinical outcome of HIV-1 infected individuals. We demonstrate the functional impact of heightened autophagy on adaptive T-cell immunity and skewing of Th subsets toward enhanced immune control and suppressed T-cell exhaustion in chronic HIV-1 infection.

Combined targeting of autophagy and the MNK1/2-eIF4E protein synthesis axis for breast cancer cells.

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One of the central events in the adaptation to cellular stress is reprogramming of protein synthesis. The one of critical nexus, connecting stress response signaling and selective protein translation are the MNK1/2 kinases and its downstream target eIF4E, the protein that regulates the rate-limiting step of translation initiation. Considering the indispensable crosstalk between protein synthesis, proteostasis and autophagy, the impact of targeting MNK1/2 signaling together with autophagy in resistant breast cancer cells is the main goal of our project. Recently, we have developed EB1, the first in-class non-ATP competitive inhibitor of the kinases MNK1/2, and found that inhibition MNK1/2 signaling leads to the induction of autophagy. Moreover, combining EB1 with the autophagy inhibitor Chloroquine increases cell death in breast cancer cells, but not normal epithelial cells. This requires the concurrent effects in the sequential induction of macroautophagy and later chaperone-mediated autophagy. Supporting these results, we present evidence that the MNK1/2 kinases negatively regulate the autophagic machinery. Our results identify a new potent combination for the treatment breast cancer cells targeting both autophagy and MNK1/2 signaling.

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Ubiquilin-2 (UBQLN2) is part of the ubiquilin family which is involved in the degradation of proteins through the ubiquitin-proteasome system (UPS) and autophagy. UBQLN2 is linked to neurodegenerative diseases, as UBQLN2 mutations were identified in familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) patients, and UBQLN2-positive inclusions were identified in degenerating motor neurons of ALS patients. UBQLN2 has the ability to undergo liquid liquid phase separation and to regulate the formation of stress granules, membrane-less organelles believed to underlie the formation of RNA-binding protein (RBP) aggregates in ALS and FTD, such as TDP-43 or FUS aggregates. Recent data suggest that UBQLN2 interacts with FUS and other stress granule proteins, and that FUS phase separation behavior and RNA binding dynamics are altered by UBQLN2. However, the exact function of UBQLN2 in stress granules, its role in protein degradation via the UPS or autophagy, and its connection to TDP-43/FUS pathology are very poorly understood.

Here we show that purified nucleic acid free UBQLN2 is able to form droplets under physiological conditions. By conducting in vitro separation assays, we found that UBQLN2 suppresses the formation of FUS or TDP-43 condensates in the presence of RNA, however it does not have an effect on pure FUS or TDP-43 droplets. Furthermore, electrophoretic mobility shift assays show that RNA binding of TDP-43 and FUS is enhanced by UBQLN2. Further studies will reveal which regions of UBQLN2 are required for the regulation of TDP-43/FUS phase separation and RNA-binding and for the regulation of stress granule dynamics. Moreover, we will examine if ALS-linked mutations in UBQLN2 disrupt these regulatory functions of UBQLN2.

Understanding the role of SHP-1 in transcription regulation

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Histone post-translational modifications are essential during eukaryotic transcription. However, the role of histone dephosphorylation and the enzymatic machinery that are engaged in this modification during transcriptional events are unexplored. Here, we show that tyrosine phosphatase SHP-1 dephosphorylates histone H2B and plays a critical role during the transition of initiation to elongation stage of transcription. Nuclear localized SHP-1 associates with the Paf1 complex at chromatin and dephosphorylates H2B. Mechanistically, we demonstrate that H2B phosphorylation precludes its interaction with the E2 enzyme and thus SHP1 mediated dephosphorylation is a prerequisite for efficient H2B ubiquitination. Functionally, we show that SHP-1 dependent H2B dephosphorylation maintains basal autophagic flux in cells through efficient transcription of autophagy and lysosomal genes. Collectively, our study reveals an important modification of histone H2B regulated by SHP-1 that has an essential role during eukaryotic transcription.

Neurotropic flaviviruses modulate autophagy in human astrocytes

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Astrocytes, a heterogeneous subtype of neuroglial cells, are key homeostasis providing cells in the brain. By executing various homeostatic and metabolic functions they support neurons. Since they are structurally a constituent of the blood-brain barrier, they are considered as one of the first targets of neurotropic viruses, which invade the brain from the blood by breaching the blood-brain barrier. Recent discoveries revealed that astrocytes can produce and release high amounts of certain flaviviruses, and are often resistant to virus-induced cell death. Therefore, astrocytes are now considered as one of the key cell types for viral replication and dissemination in the central nervous system. Viral infections can affect various cellular processes, including autophagy. The interplay between autophagy and the life cycle of viruses is context-dependent, as it varies between different viruses, cell types and host species. The mechanisms and the role of autophagy in neuroinfections of astrocytes are still largely unexplored. We have investigated whether neurotropic flaviviruses affect autophagy dynamics in human astrocytes. Cultured human astrocytes were first transfected with plasmid encoding reporter protein mRFP-EGFP-LC3, which enables identification of autophagosomes and autolysosomes, and then infected with selected flaviviruses. Single cells expressing mRFP-EGFP-LC3 were imaged by fluorescence microscopy and flavivirus-induced alterations in autophagy dynamics were assessed based on the following parameters: the number of LC3-positive compartments normalized to the cell volume, the ratio of autolysosomes to autophagosomes (an indicator of autophagosome maturation efficiency) and the size of autophagic compartments. The exposure of astrocytes to neurotropic flaviviruses induced an increase in the number of LC3-positive compartments. In contrast, the ratio of autolysosomes to autophagosomes was not affected by flaviviruses, nor was the diameter of autophagic compartments. These results demonstrate that neurotropic flaviviruses increase the autophagy rate without affecting the autophagosome maturation process in human astrocytes.

Analyses of Ubiquitin - Proteasome and Autophagy systems reveal the components of common and tissue selective subsets of genes in human.

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The autophagy-lysosome and the ubiquitin proteome (UPS) systems are complementary protein degradation pathways that maintain cellular proteostasis under various physiological conditions. Both systems have been extensively studied, yet their tissue and cell-type-specific properties are just beginning to emerge. Here, we elucidate the architecture of these systems by analyzing bulk and single-cell transcriptomes and proteomics data from over 30 human tissues. We focused on 419 autophagy-related proteins from initiation to lysosome degradation and 558 UPS-related proteins, including ubiquitin ligases and proteasome subunits. We first analyzed the global characteristics of these proteins. Relative to other protein-coding genes, they were ubiquitously expressed and more highly expressed across tissues and cell types. Additionally, they were more essential than other protein-coding genes, supporting the fundamental roles of both systems. Next, we asked whether these systems show tissue-selective behavior. For this, we compared the expression patterns of autophagy and UPS machinery across tissues. Whereas most autophagy and UPS genes showed 2-fold changes in their expression across tissues, a small core subset of 36 and 42 genes, respectively, was uniformly expressed. This core subset was enriched for the components of early stage complexes in autophagy and E2s and proteasome subunits. Relative to other autophagy and UPS genes, the core subset was more highly expressed in most tissues and cell types and was significantly more essential. This organization was established in development and was also apparent at the protein level. Interestingly, highly variable components of those systems, such as lysosomal genes and E3s, were enriched for disease-related genes. In contrast, core components, such as ESCRT and E2s, were depleted for disease-related genes. Taken together, we propose that while the degradation and clearance machinery are fundamental, they are also tailored to the different demands posed by tissue and cell-type-specific proteomes.

De novo phospholipid metabolism in the regulation of autophagy in aging yeast

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Autophagy is a highly conserved catabolic process and plays a role in healthy aging. Recent work has highlighted the need of efficient fatty acid (FA) supply into de novo phospholipid synthesis to ensure autophagic competence of yeast and mammalian cells (Andrejeva et al. 2020, In: Autophagy 16; Schütter et al. 2020, In: Cell 180). Acetyl-Coenzyme A carboxylase 1 (Acc1) is the rate limiting enzyme of de novo fatty acid synthesis and has been implicated in autophagy regulation in aging yeast, but the underlying mechanisms remain unclear (Gross et al. 2019, In: The Journal of biological chemistry 294). Using Soraphen A to inhibit Acc1 we here present a model to study the phospholipid metabolism requirements downstream of FA utilization for maintaining autophagy.

We found that supplementation of specific FAs restores autophagy upon Acc1 inhibition. In order to identify the crucial lipid pathways required for FA-dependent autophagy, we conducted a genetic screen using the knockouts of several enzymes involved in yeast lipid metabolism. Our screen revealed several enzymes involved in the de novo phosphatidylcholine (PC) biosynthesis pathway, including the activation of free FAs and their incorporation into phosphatidylethanolamine (PE) and PC lipid species. In subsequent genetic analysis, we identify a crucial function of mitochondria to maintain FA-dependent autophagy by the PE-PC biosynthesis pathway in aging yeast.

Cupric chloride-Induced Autophagic Flux Impairment Is Linked to Dopaminergic Neuronal Cell Death:
Focus on role of cytosolic calcium

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Heavy metals such as copper, iron and zinc are essential trace elements in many cellular mechanisms. However, high levels of them are neurotoxic and promote neurodegeneration. Previously, it has been demonstrated that metals imbalance can induce mitochondrial dysfunction and thereby deplete ATP, produce reactive oxygen species (ROS), and ultimately lead to cell death. Metal disruption has been reported in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS). Here, we specifically attempted to examine whether cupric chloride (CuCl₂) leads to impaired autophagic flux and to induce neuronal cell death. As determined by ultrastructural and biochemical analyses, we found that CuCl₂ induces an autophagy in MN9D dopaminergic neuronal cells. Study using co-treatment with the lysosomal acidification inhibitor, bafilomycin A1 indicated that CuCl₂-induced autophagy is ascribed to impairment of autophagy flux. Interestingly, we showed that the buffering of Ca²⁺ by BAPTA-AM co-treatment or overexpression of calbindin-D28K, reverses impaired autophagic flux and consequently rescued cells from death. Similarly, we demonstrated that CuCl₂-induced overload of cytosolic Ca²⁺ is linked to lysosomal pH change that eventually promotes abnormal autophagic flux and cell death. Taken together, our results suggest that abnormal accumulation of redox-active heavy metals is directly linked to neuronal death by perturbing normal range of autophagic flux at the post-lysosomal fusion step.

GABARAP meets EGFR – Structural clues to the GABARAP-specific phenotype during EGFR degradation

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The autophagy-related protein 8 (Atg8) family member γ -aminobutyric acid type A receptor-associated protein (GABARAP) and its closest paralogs GABARAPL1 and GABARAPL2 have been described to be involved in a wide variety of autophagy-related and -unrelated processes. Owing to their unique C-terminal lipid conjugation, these proteins carry the potential to functionalize membranes by either serving as docking sites for other proteins or by deforming membranes on their own. We recently found that the deficiency of GABARAP, but not its paralogs, enhances the degradation of epidermal growth factor receptor (EGFR) upon ligand stimulation, affecting both signal transduction and target gene expression of EGFR. Here, we demonstrate that the EGFR C-terminal regulatory tail is sufficient for affinity enrichment by GABARAP. In vitro interaction studies targeting putative LC3-interacting regions (LIRs) in this EGFR segment revealed binding of a peptide spanning positions 1052-1075 of mature EGFR to GABARAP and its closest relative GABARAPL1, but not to GABARAPL2 and the LC3 proteins LC3A and LC3B. Using a chimeric EGFR(1052-1075)-GABARAP protein we investigated the mode of interaction by X-ray crystallography. Our data indicate participation of both hydrophobic pockets of GABARAP and the EGFR(1062-1065) core-LIR residues, FLPV, in the interaction, suggesting a canonical binding mode. Interestingly, although EGFR(1052-1075) does occupy the LIR docking site (LDS), residues Y49 and L50 do not contribute significantly in this case, as no reduction in binding was observed with the commonly used LDS-mutant GABARAP (Y49A/L50A) when compared with the wild-type GABARAP. Taken together, GABARAP seems to influence the fate of the EGF receptor after EGF stimulation, at least in part, in a direct manner. Pinpointing the endosomal compartment(s) in which this interaction takes place will be the next challenge.