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ABSTRACTS

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tested chimaeras showed 100 % germline transmission. We have used these cells to generate mouse models by random transgene integration and by both homologous recombination-based and CRISPR-induced gene-targeting strategies with the same good results. The simplicity and efficiency of this derivation protocol, the feeder-free culture conditions and the high-quality of the mESC lines obtained make this powerful tool accessible and suitable to any transgenic mouse laboratory.

50. 100 % Coat-coloured chimaera generation by single es cell injection

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Murine embryonic stem cells (mESC) are defined as 'pluripotent' i.e. they can generate all cell types of the adult mouse as well as the germ cells. The gold standard assessment of pluripotency is the generation of chimaeras by reintroducing mESCs into pre-implantation embryos. Traditionally, multiple cells have been injected into blastocysts or, more recently, into morulae. The extent of chimaerism is routinely scored as the percentage of coat pigmentation, and this can vary dramatically between individual mice and experiments, reflecting the heterogeneity of mESC cultures. Here, we investigate the pluripotency of mESC derived and maintained in different conditions, using a very stringent test where single cells are injected into early embryonic stages. We have found that a single cell can give rise to 100 % coat-coloured chimaeras when injected into 2-cell embryos. In these animals, the contribution of the injected ES cell to all the main organs is comparable to that found in 100 % coat-coloured chimaeras generated by multiple cell injection. Remarkably, only serum-free culture conditions (KOSR + LIF and 2i + LIF), but not serum-containing medium, support this ability of the cells when injected individually. This work reveals that serum-free conditions enhance the clonogenicity of mESCs and describes a rigorous technical procedure to assess this ability of the cells.

51. Novel tools to discern the pluripotency gene network establishment

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Not only does the reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) by exogenous transcription factors remain highly elusive, but there is also characteristic discrepancy among the discoveries of different labs.

In order to address this, first we developed a system for a robust and reproducible systematic mechanistic analysis of reprogramming. Briefly, we generated a single targeting vector carrying both the reverse tetracycline-controlled transactivator (rtTA) and the doxycycline (Dox)-inducible reprogramming factors (2A-peptide-linked c-Myc-Klf4-Oct4-Sox2 = MKOS) for a novel genomic locus that assured optimal transgene expression. One round of targeting with this vector makes any ES cell (ESC) line a source of Dox-inducible reprogrammable cells/mouse.

Intriguingly, there is a variety of reprogramming cassettes used by different labs to perform mechanistic analysis; thus, we compared the existing cassettes, and to our surprise, they generated different number of both fully reprogrammed and partially reprogrammed colonies. Keeping that in mind, we used our novel reprogramming system to generate Nanog-GFP ESC lines with the reprogramming factors in a different order (TNG MKOS/OKMS ESCs). TNG OKMS reprogramming gave rise to ~10 times more colonies upon the addition of Dox, but with poor activation of the Nanog-GFP reporter, compared to the TNG MKOS one. CD44 and ICAM1 expression, which we recently utilized to dissect the reprogramming process, along with E-cadherin staining, enabled us to identify a clear roadblock in OKMS reprogramming. Finally, RNA sequencing of these reprogramming intermediate subpopulations provided us with many candidate genes which potentially affect the pluripotency acquisition.

The tools described here enable the precise analysis of the reprogramming intermediates and will make a large contribution to reveal the molecular mechanisms for the establishment of the pluripotency gene network and to shed light to the existing conflict.

52. Characterization the developmental potential of cultured chicken primordial germ cells

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The avian embryos are widely used for studying early embryogenesis. The chicken embryos are also important model system for studying primordial germ cell (PGC) development. Researchers used the avian model to reveal the cellular origins and migratory pathways of PGCs. The most recent work focuses on the molecular mechanisms regulating early germ cell development. Avian PGCs have significant potential as a cell-based system for the study and preservation of avian germ cells. Long-term maintenance of avian PGCs in vitro has tremendous potential because it can be used to deepen our understanding of the biology of PGCs and also a promising option for gene banks and genome preservation.

Avian embryos have also proven to be useful organisms for transgenic research. PGCs can be cloned and maintain the germline competency, so they are a good source for modifying the chicken genome and producing chimeras. This provides enormous benefits in advancing animal biotechnology and aids

in the development of unique technologies for bioreactor production and experimental model development.

In our laboratory, we adapted the method of feeder-free *in vitro* cultivation of PGCs. We isolated the PG cells from the blood of HH16 chicken embryos and investigated the expression profile of stem cell and PGC-specific genes. cPouV, cNanog, cSox2, cGATA6, cGATA4, cCDX2, cKlf4, cDAZL, cBrachyury, cDDX4, cStra8 expression were checked in chicken embryos and cultivated PGCs. We also analysed the expression profile of stem cell-specific miR-302 cluster. miRNAs regulate the lineage-specific differentiation of cells and maintain the pluripotency in stem cells. The post-transcriptional regulation through miRNAs are also important role for the control of differentiation in the chicken PGCs. Using SSEA-1 and EMA1 immunostaining, we annotated that our cultivated cells are PGC cells.

In order to check the viability and functionality of the cultured PG cells *in vivo*, cultivated GFP expressing PGCs were injected back to the HH16 embryos and followed their way to the developing gonads.

53. Rapid generation and screening of high-percentage mouse chimeras utilizing CRISPR modified ESCs

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Our current project combines new advances in CRISPR technology, ES cell chimera technology and targeted phenotyping to generate mouse mutants within 2 months, for a fraction of the current cost of mouse knockouts. This technology enables large numbers of disease candidate genes to be inexpensively and quickly assessed by individual labs for their relevance to human disease. The screen is not intended to define the definitive phenotype associated with candidate genes rather the screen is designed to quickly determine whether the candidate gene has disease-related function that can be further pursued in follow-up experiments with traditional knockout approaches. RFP + host embryos were used to determine the chimeric contribution of modified ESC to early developmental disorders. We determined that using RFP + host embryos facilitated rapid detection of RFP + host-derived tissue still present in high percentage of chimeric pre- and postnatal animals.

54. Identification of rabbit microRNA expression profile in rabbit pluripotent stem cells

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In the context of biomedical research, precise genetic engineering in rabbits (*Oryctolagus cuniculus*) would be precious model to generate genetically defined rabbit models of human diseases. Rabbit embryonic stem cells (ESCs) would be a valuable tool for both creating second-generation transgenic models of human diseases and testing stem cell therapies for human applications.

We identified new miRNA sequences in several rabbit samples sequenced by solidTM System and validated them using real-time quantitative PCR and Custom TaqMan[®] Array Plates. Then, we aimed to compare the miRNA expression patterns of rabbit samples with the read numbers of solidTM sequencing. Our results underlined that solidTM sequencing is really good at identifying new miRNA sequences, but it could be established that the read numbers of solidTM sequencing cannot be directly compared to the relative expression levels of real-time PCR analysis.

Using our new rabbit specific miRNA sequences, we analysed the expression profile of pluripotency-associated miRNAs in rabbit embryos and embryonic stem-like (ES-like) cells. The rabbit specific ocu-miR-302, ocu-miR-290 clusters and three homologs of human C19MC cluster (ocu-miR-512, ocu-miR-520e and ocu-miR-498) were identified in rabbit preimplantation embryos and pluripotent stem cells. The ocu-miR-302 cluster was highly similar to its human homolog, while ocu-miR-290 revealed a low level of evolutionary conservation with its mouse homologous cluster. The expression of ocu-miR-302 cluster members began at 3.5 dpc early blastocyst stage and they stayed highly expressed in rabbit pluripotent stem cells. In contrast, high expression level of ocu-miR-290 cluster members was detected during preimplantation embryonic development, but low level of expression was found in rabbit ES-like cells.

55. Czech centre for phenogenomics: new research infrastructure for transgenic mouse model production and phenotyping

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The Czech Centre for Phenogenomics (CCP), a newly set up infrastructure, is combining genetic engineering capabilities, advanced phenotyping and imaging modalities, specific pathogen-free (SPF) animal housing and husbandry, as well as cryopreservation and archiving, all in one central location—located within the new BIOCEV complex (Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec) and the hosting institution, the Institute of Molecular Genetics (IMG) in Prague.

The Czech Centre for Phenogenomics (CCP), through its memberships in INFRAFRONTIER and the International Mouse Phenotyping Consortium (IMPC), is a partner in a collective global network that aims to comprehensively and systematically analyse the effect of loss-of-function gene mutations in mice. The goal is to produce a comprehensive 'encyclopedia' of gene function that will help identify causative factors of human diseases as well as novel targets for therapeutic intervention.

Current services in model production include custom-targeted mutagenesis and transgenesis, blastocyst injection, pronuclear microinjection, sperm and embryo cryopreservation, *in vitro* fertilization and breeding on demand. Over 80