Visegrád Group Society for Developmental Biology: Inaugural Meeting

PROGRAM & ABSTRACTS

7th - 9th September 2018, Brno, Czech Republic


Visegrád Group Society for Developmental Biology
est. 2018

V4 Society for Developmental Biology

Program and Abstract Book

September 6-9, 2018

Brno, Czech Republic

Organizers

M. Buchtová, A.W. Bruce, V. Bryja, D. Fabián,

A. Piliszek, M. Varga, P. Vilmos

Editors:

M. Buchtová, A.W. Bruce, V. Bryja, D. Fabián, A. Piliszek, M. Varga, P. Vilmos

V4 Society for Developmental Biology – Program and Abstract Book

1st edition, September 2018

Published by: Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Rumburská, 277 21 Liběchov, Czech Republic

Printed by: Tribun EU s.r.o., Cejl 892/32, 602 00 Brno, Czech Republic

SPONZORS AND EXHIBITORS

supported by

Visegrad Fund

eppendorf

pragolab

ThermoFisher SCIENTIFIC

Roche

OLYMPUS

Your Vision, Our Future

MUNI
MENDEL
MUSEUM

The Story of a Humble Genius

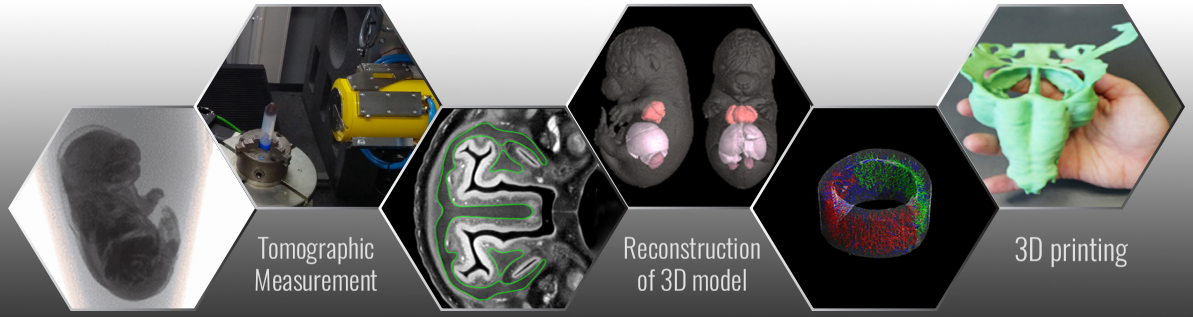
Mendlovo náměstí 1a
603 00 Brno
+ 420 549 496 669
info@mendelmuseum.muni.cz
www.mendelmuseum.muni.cz

Opening hours:

April — October
Tuesday — Sunday
10 a.m. — 6 p.m.

November — March
Tuesday — Sunday
10 a.m. — 5 p.m.

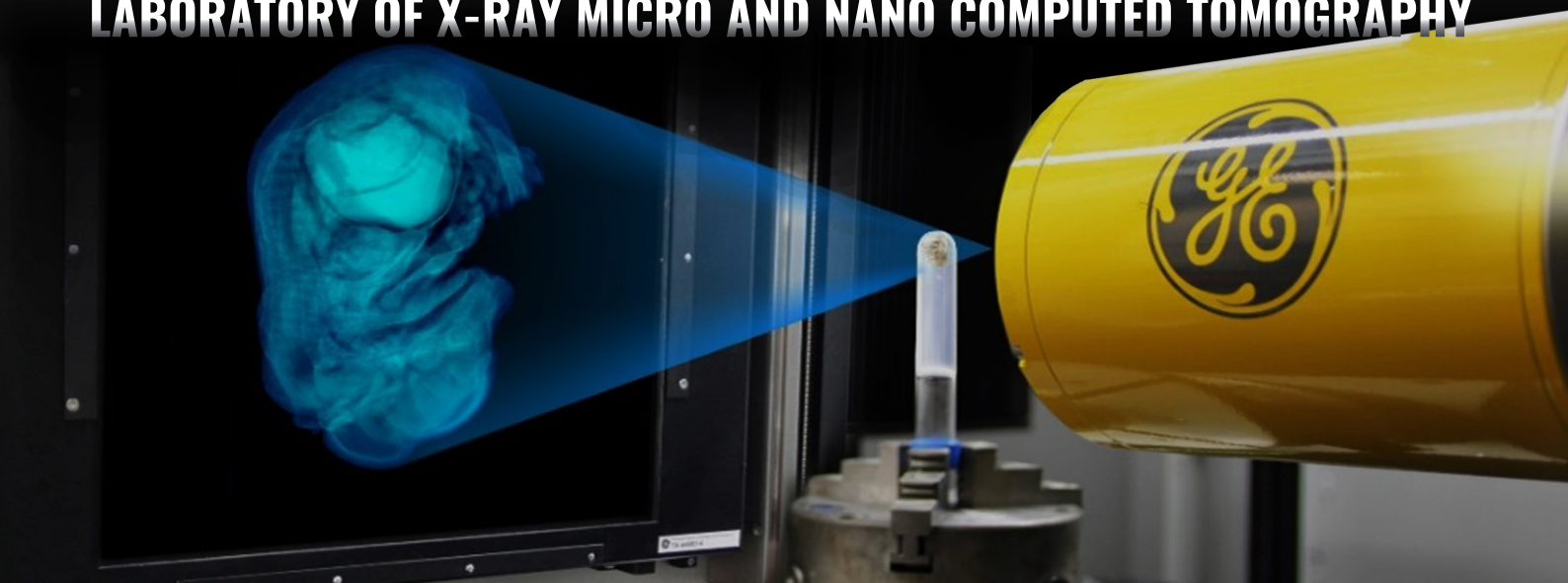
MASARYKOVA
UNIVERZITA



CEITEC CTLAB
BRNO | CZECH REPUBLIC

ctlab.cz

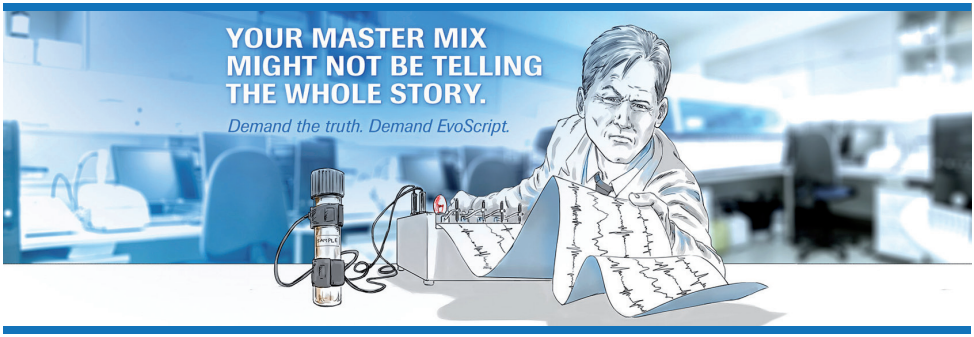
LABORATORY OF X-RAY MICRO AND NANO COMPUTED TOMOGRAPHY



NOVÁ GENERACE TECHNOLOGIE REVERZNÍ TRANSKRIPCE

EvoScript Master Mixes

Produkty EvoScript jsou vhodné pro vysoce specifickou a přesnou jednokrokovou RT-qPCR, zejména pro genovou expresi



Proč použít EvoScript Master Mixy

- **Vynikající citlivost RT-qPCR, specifita, provedení genové exprese a detekce patogenu**
- **Robustní výsledky pro cílovou RNA izolovanou z různých vstupních materiálů**
- **Vynikající účinnost**
- **Časnější hodnoty Cq**
- **Vyšší citlivost**
- **Široký dynamický rozsah**
- **Práce s vysoce variabilním obsahem GC párů**
- **Variabilní délka cílové sekvence**
- **Vysoce termostabilní RT**
- **Kompatibilní UNG**
- **Vyšší fluorescence**

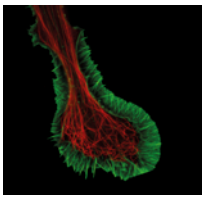
Více informací najdete na stránkách:
www.lifescience.roche.com

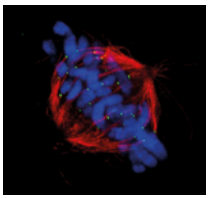
Objednávky prosím posílejte:
E-mailem: prague.objednavkydia@roche.com
Tel.: +420 220 382 565

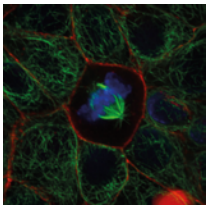
Roche, s.r.o.,
Diagnostická divize
Na Valentince 3336/4
150 00 Praha 5

OLYMPUS®

Your Vision, Our Future







CONFOCAL SUPER RESOLUTION

for all live cell samples

- Super resolution down to 120nm XY resolution
- Prolonged cell viability in confocal time-lapse imaging due to less phototoxicity and bleaching
- Switch between wide-field, confocal and super-resolution observations in the IXplore SpinSR system in one step
- Accurate 3D reconstruction with Olympus silicone oil immersion objectives

www.olympus-lifescience.com/ixplore-spinHIGH-SPEED CONFOCAL IMAGING

Scientific Solutions Division

OLYMPUS CZECH GROUP, S.R.O., ČLEN KONCERNU

Evropská 176/16, 160 41 Praha 6 | Tel.: +420 221 985 211 | mikroskopy@olympus.cz | www.olympus.cz

pragolab

LEICA S9

GREENOUGH STEREOMIKROSKOP
ZOOM **9:1**

TECHNOLOGIE **FUSIONOPTICS™**

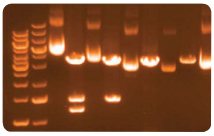
VYVINUTO PRO POTŘEBY
VÝVOJOVÉ BIOLOGIE

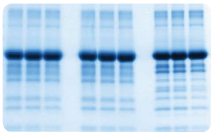
VYZKOUŠEJTE SI NA VISEGRAD GROUP SOCIETY FOR DEVELOPMENTAL BIOLOGY: **INAUGURAL MEETING 2018**

invitrogen

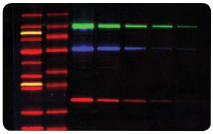
Invitrogen™ iBright™ Imaging Systems simplify western blot imaging with features like:

- Push-button, optimized exposure
- User-focused, intuitive interface and workflows
- Brilliant 4-color fluorescence multiplexing


Nucleic acid gels


Protein gels


Chemiluminescent blots


Fluorescent blots

For more information or to schedule a live demo, contact:

Ing. Jan Pokorný

00420 725 349 380

jan.pokorny2@thermofisher.com

Learn more about iBright Imaging Systems at
thermofisher.com/ibright

ThermoFisher SCIENTIFIC

Enantis

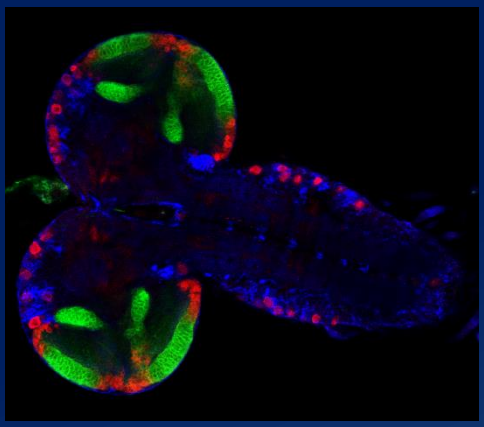
We are the Protein pro team and we can do wonders in the field of protein engineering.

Let us rebuild your unstable protein or optimize your biocatalytic process.

Find out more at **www.enantis.com**

We are looking for new talent. Are you the one we are looking for? Send your CV to polednakova@enantis.com

Visegrád Group Society for Developmental Biology



Our aims:

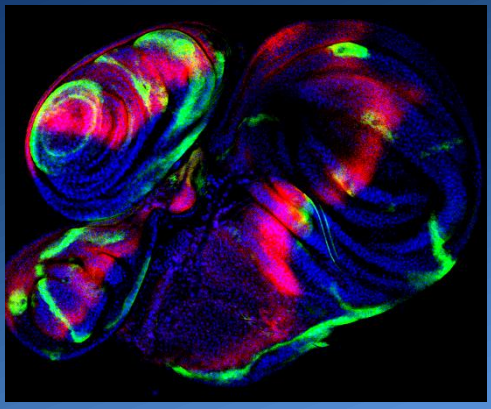
Support & popularise developmental biology research in Czech Republic, Hungary, Poland & Slovakia (i.e. the' V4 group')

Organise rotating biannual V4SDB flagship meeting & focussed (member driven) events in developmental biology.

Cooperate with sister societies in Europe (e.g. joint meetings & collaborations).

Promote cooperation & research collaboration within V4 group its (e.g. 'Jobs notice board').

Dedicated support for student developmental biologists (e.g. student specific sessions, workshops, careers advice & travel awards).



MEMBERSHIP:
PI/post-docs: 10 EUR
Students: 5 EUR

www.v4sdb.com

TABLE OF CONTENTS

Welcome Message

Dear colleagues,
it is a great pleasure to welcome you to Inaugural Meeting of the Visegrad Society for Developmental Biology (V4SDB; http://www.v4sdb.org/). We sincerely hope that you will enjoy the four days of excellent science being performed in our area of Central Europe and in a location just a few hundred meters from the monastery where the genetic fundamentals of our field were formulated by Johann Gregor Mendel more than 150 years ago. We are especially happy to welcome you to place with such a rich history of genetics, where science is now taking a new deep breath after the rough interruption of scientific continuity during the cold war. You are very welcome to explore both the history and future of science in Brno by visiting the Mendel Museum as well as the Masaryk University Campus, both located very close to the conference venue.

This meeting aims to establish "Visegrád Four Society for Developmental Biology" (V4SDB). Societies for Developmental Biology are non-profit scientific associations that are active globally as well as locally in many non-European and western European countries, plus pan-international. These societies exist with the aim to promote functional integration of researchers working in the field of Developmental Biology (*i.e.* the study of process by which animals & plants grow and develop); specifically, they help to promote exchange of relevant knowledge and experimental methodologies, to establish cooperation in the active research projects, to establish professional networks (especially relevant to junior researcher career development), to improve education and finally, to publicize this fundamental branch of biological research with the impact on the human health and animal production. Despite these advantages, there was not, until now, any such societies in any of the V4 countries (Czech Republic, Hungary, Poland, Slovak Republic). Therefore, the launch of V4SDB is expected to provide the missing forum to foster more efficient and integrated research in Developmental Biology across Central Europe. Specifically, the V4SDB meeting itself will act to assimilate the most recent and fundamental insights into the cellular/molecular mechanisms of development, stem cell biology and possible application of developmental biology principles for understanding and treating of human diseases.

This meeting was made possible by a financial support grant from the "Visegrád Fund" and all our commercial sponsors. Great thanks are also paid to Masaryk University, Institute of Animal Physiology and Genetics and University of South Bohemia for their valued support.

We wish all participants an exciting and fruitful meeting, complete with many interesting discussions and new scientific interactions. Have a great time in Brno!

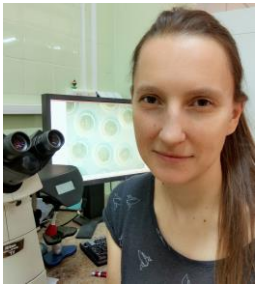
Marcela Buchtová, Alexander W. Bruce, Vítězslav Bryja,
Anna Piliszek, Dušan Fabián, Máté Varga, Peter Vilmos

Organizing Committee

Marcela Buchtová
Institute of Animal Physiology and Genetics/
Masaryk University
Brno, Czech Republic

Vítězslav Bryja
Masaryk University
Brno, Czech Republic

Alexander W. Bruce
University of South Bohemia
České Budějovice
Czech Republic



Anna Piliszek
Institute of Genetics
and Animal Breeding
Jastrzebiec, Poland

Dušan Fabián
Institute of animal Physiology
Kosice, Slovak Republic

Máté Varga
ELTE Eötovös Loránd University
Budapest, Hungary

Peter Vilmos
Biological Research Centre
Szeged, Hungary

Conference Information

Venue:

Main meeting:

Faculty of Economics and Administration (The large hall)
Lipová 41a, Brno - Pisárky

Student sessions:

Masaryk University campus
Kamenice 753/5, Brno - Bohunice

Contacts

Conference organizers:

Marcela Buchtová	+420 776 461 222
Vítězslav Bryja	+420 601 500 857

City taxi (+420 542 321 321, +420 777 014 004)

Wi-fi

Connectivity to the Eduroam network will be available at all times.
If you do not have an account at Eduroam, please use login received at the registration.

Public Transportation

The easiest possibility for travelling around Brno is to purchase Travel Ticket through your mobile phone application and send SMS on number **90206.**

Time	Text SMS	Price
20 min	BRNO20	20 CZK
75 min	BRNO	29 CZK
24 h	BRNOD	99 CZK

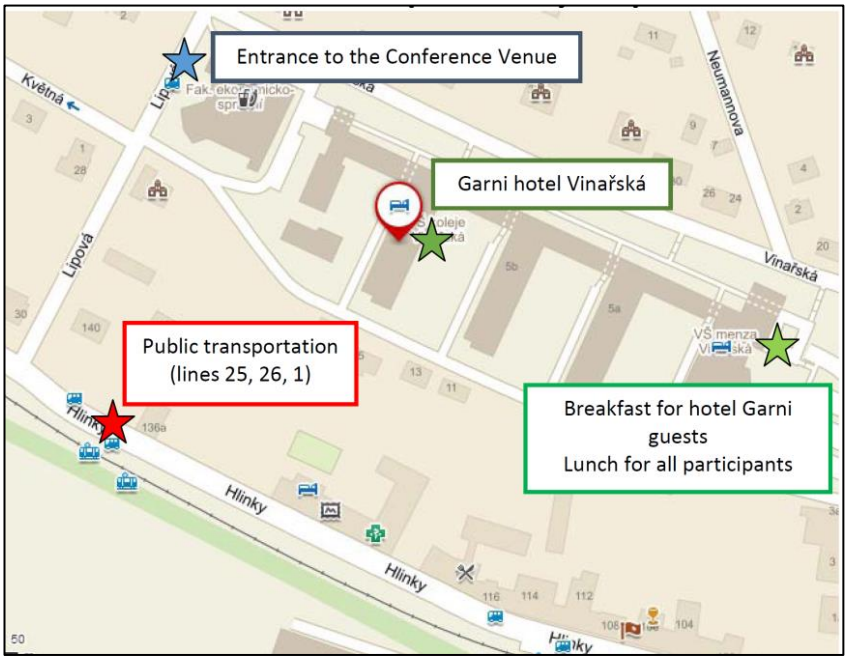
Or ring to automatic machine and SMS will come to your phone about 2 min later.

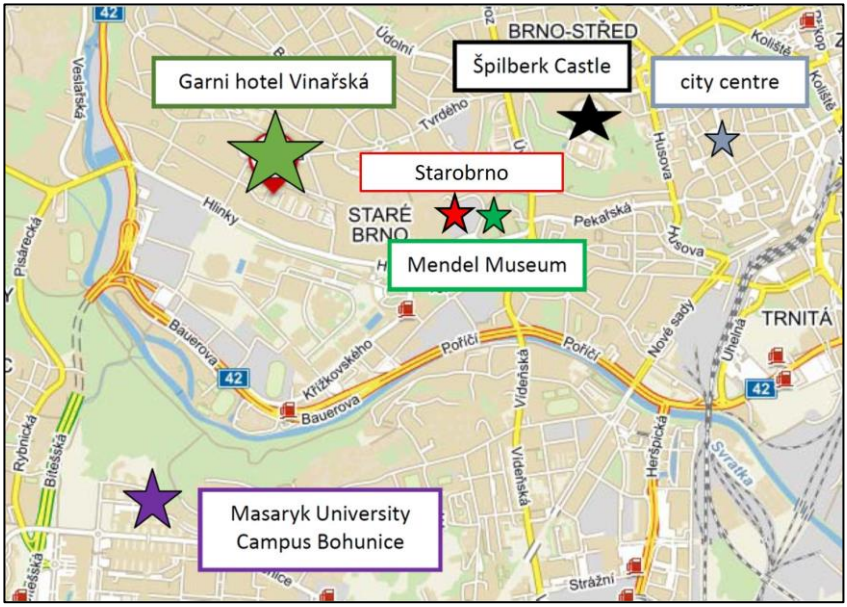
Time	Number to ring	Price
20 min	**+420 910 902 020**	20 CZK
75 min	**+420 910 902 029**	29 CZK
24 h	**+420 910 902 099**	99 CZK

You can travel to city from "Lipová" stop (tram no. 1 or trolleybus no. 25/26). Tram no. 1 take you to the main train station "Hlavní nádraží" and trolleybuses will take you to Mendel square. Here you can visit the Mendel Museum or enjoy a beer at Starobrno brewery. Connecting tram 5/6/11 will take you to stop "Česká", which is close to the main old city area.

Between the hours of 23:00 to 5:00, public transports operate according to the night regime (there are special night bus lines calling every hour).

Conference venue and simplified city map

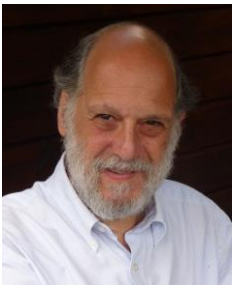




Map of Masaryk University campus

- student sessions (Thursday 6.9. – A11, Friday 7.9. – A36)

KEYNOTE SPEAKERS



Claudio D. Stern
University College London
London, UK

Andrei Chagin
Karolinska Institutet
Stockholm, Sweden

Marie-Hélène Verhlac
College de France
Paris, France

Petra Hájková
Imperial College London
London, UK

Jeremy B. Green
King´s College London
London, UK

Pavel Tomančák
Max Planck Institute of MCBG
Dresden, Germany

Olov Andersson
Karolinska Institutet
Stockholm, Sweden

Abigail S. Tucker
King´s College London
London, UK

PROGRAM

STUDENT SESSION

DAY 1 Thursday 6th of September - student flash talks

WHERE? Masaryk University Campus – Bohunice (building A11, room 306)

from 11:00	Registration open
13:00-13:30	Opening by organizers

SESSION 1	***Chair: V. Bryja***	
13:00-13:05	Ibrahim Karam	Small ovary (sov) regulates transposon silencing by promoting heterochromatin formation in Drosophila
13:05-13:10	Borkuti Peter	Testing the biological significance of the nuclear localization of actin
13:10-13:15	Kovacs Zoltan	Analysis of the nuclear transport of the cytoskeletal Moesin protein
13:15-13:20	Kovarikova Petra	Analysis of Cep164 interactome identifies KIF14 as novel regulator of ciliogenesis
13:20-13:25	Annus Tamas	The role of RecQ-family helicases in the genome maintenance of zebrafish
13:25-13:30	Fedorova Veronika	Expression and Regulations of p16/INK4A in Human Embryonic and Neural Stem Cells
13:30-13:35	Lavicky Josef	Expansion and response of human pluripotent stem cells in synthetic cell culture environment
13:35-13:40	Svoradova Andrea	Ultrastructure of chicken embryonic stem cells
13:40-13:45	Rabata Anas	3D cell culture models demonstrate a role for FGFs and WNT signalling in regulation of adult lung epithelial stem cell fate

13:45-14:15 *Coffee break*

SESSION 2 ***Chair: A. Pilliszek***

14:15-14:20	Khan Anzer	Deciphering effects of *Adar* on *Drosophila* metamorphosis
14:20-14:25	Anand Mahek	Comparison the miRNA expression profile in chicken male and female primordial germ cells
14:25-14:30	Baldovska Simona	Phytoestrogenic Effect of Pomegranate on Human Ovarian Cells
14:30-14:35	del Llano Edgar	Age related differences in the translational landscape of mammalian oocytes
14:35-14:40	Trebichalska Zuzana	Analysis of human oocyte ultrastructure by advanced electron microscopy technologies
14:40-14:45	Bora Pablo	Deciphering the nodal role of p38 family of mitogen-activated protein kinases (MAPKs) towards specifying primitive endoderm (PrE) fate in the inner cell mass (ICM) of late-blastocyst stage mouse pre-implantation embryo
14:45-14:50	Duricek Tomas	DNA damage checkpoints in early mammalian embryos
14:50-14:55	Florek Wiesława	Blastomere removal from 8-cell stage embryos programs post-natal body weight, food intake and the level of leptin in mice
14:55-15:00	Knoblochova Lucie	The Role of CDC25A Phosphatase in Meiosis and First Embryonic Division in Mouse

15:00-15:30 ***COFFEE BREAK***

SESSION 3	***Chair: A.W. Bruce***	
15:30-15:35	Tetkova Anna	FSH influences amino acid incorporation in mammalian oocyte and early embryo
15:35-15:40	Machacova Simona	Evolution of gene network regulating gastrulation: Insights via *Chordin*
15:40-15:45	Stachowiak Anna	Differentiation potential of rabbit inner cell mass
15:45-15:50	Winiarczyk Dawid	*SIRT1* may control apoptosis resistance in preimplantation mouse development
15:50-15:55	Knop Filip	*Caenorhabditis elegans* SEL-5 kinase role in anterio-posterior cell outgrowth and migration
15:55-16:00	Henry Surya	Characterization of cardiac transcription factor PLAGL1, a newly identified putative downstream target of RYBP
16:00-16:05	Kovacs Tamas	Intraganglionic macrophages: a new population of cells in the enteric ganglia
16:05-16:10	Drozdova Angelika	Development of melatonin rhythm in the pineal gland of chick embryos incubated under different wavelength of light
16:10-16:15	Pejtsik Diana	Early-life social isolation-induced serotonergic deficits and their behavioural consequences in zebrafish (*Danio rerio*)
16:15-16:20	Zietek Marta	Effect of advanced maternal age on reproductive outcomes in a mouse model of autism

16:20-16:50 COFFEE BREAK

SESSION 4	***Chair: M. Buchtova***	
16:50-16:55	Nickl Petr	The role of transcription factors of Meis family during development of neural crest cells in *Danio rerio*
16:55-17:00	Fabik Jaroslav	Inactivation of *Meis2* in neural crest cells results in craniofacial developmental defects
17:00-17:05	Hampl Marek	Ciliary protein TMEM107 regulates rostro-caudal patterning in craniofacial development
17:05-17:10	Dalecka Linda	Incisor development in *Eda* mutants
17:10-17:15	Steklikova Klara	Early tooth development and segmentation of oral epithelium in mice
17:15-17:20	Kavkova Michaela	Digital dissection of 3D model of mouse skeleton: x-ray micro computed tomography analysis
17:20-17:25	Sumbal Jakub	Deciphering Roles of FGF Signaling in Mammary Gland Development
17:25-17:30	Radaszkiewicz Tomasz	The role of RNF43/ZNRF3 in non canonical Wnt signaling and its impact on human melanoma

17:30-22:00 ***ROUND TABLE WITH BEER***

DAY 2 Friday 7th of September - student practical worshop

WHERE? Masaryk University Campus – Bohunice (building A36)

Name	Group	Time	Room
Jipa Andras	Invertebrate	8:00-9:00	A36, 212
Olbertova Kristyna	Invertebrate	8:00-9:00	A36, 212
Szarka-Kovács Alexandra B.	Invertebrate	8:00-9:00	A36, 212
Killinger Michael	Invertebrate	8:00-9:00	A36, 212
Ibrahim Karam	Invertebrate	8:00-9:00	A36, 212
Landová Marie	Invertebrate	8:00-9:00	A36, 212
Kovacs Zoltan	Invertebrate	8:00-9:00	A36, 212
Smutna Tereza	Invertebrate	8:00-9:00	A36, 212
Nkubito Claudia	Invertebrate	9:00-10:00	A36, 212
Vincze Katalin	Invertebrate	9:00-10:00	A36, 212
Fedorova Veronika	Invertebrate	9:00-10:00	A36, 212
Knop Filip	Invertebrate	9:00-10:00	A36, 212
Annus Tamas	Invertebrate	9:00-10:00	A36, 212
Bora Pablo	Invertebrate	9:00-10:00	A36, 212
Fabik Jaroslav	Invertebrate	9:00-10:00	A36, 212
Khan Anzer	Invertebrate	9:00-10:00	A36, 212
Knoblochova Lucie	Invertebrate	10:00-11:00	A36, 212
Magnin Valentina	Invertebrate	10:00-11:00	A36, 212
Machacova Simona	Invertebrate	10:00-11:00	A36, 212
Maronek Martin	Invertebrate	10:00-11:00	A36, 212
Stachowiak Anna	Invertebrate	10:00-11:00	A36, 212
Steffal Pavel	Invertebrate	10:00-11:00	A36, 212
Virnicchi Giorgio	Invertebrate	10:00-11:00	A36, 212
Ziętek Marta	Invertebrate	10:00-11:00	A36, 212

Borkuti Peter	Invertebrate	11:00-12:00	A36, 212
Cada Stepan	Invertebrate	11:00-12:00	A36, 212
Kompanikova Petra	Invertebrate	11:00-12:00	A36, 212
Luza Bernadette	Invertebrate	11:00-12:00	A36, 212
Muller Dalma	Invertebrate	11:00-12:00	A36, 212
Novakova Barbora	Invertebrate	11:00-12:00	A36, 212
Winiarczyk Dawid	Invertebrate	11:00-12:00	A36, 212

Baldovska Simona	Vertebrate	8:00-9:00	A36, 215
Benc Michal	Vertebrate	8:00-9:00	A36, 215
Dave Zankruti	Vertebrate	8:00-9:00	A36, 215
del Llano Edgar	Vertebrate	8:00-9:00	A36, 215
Drozdova Angelika	Vertebrate	8:00-9:00	A36, 215
Duricek Tomas	Vertebrate	8:00-9:00	A36, 215
Fenclova Tereza	Vertebrate	8:00-9:00	A36, 215
Florek Wiesława	Vertebrate	8:00-9:00	A36, 215
Gomoryova Kristina	Vertebrate	8:00-9:00	A36, 215
Hencz Alexandra	Vertebrate	8:00-9:00	A36, 215

Henry Surya	Vertebrate	9:00-10:00	A36, 215
Kavkova Michaela	Vertebrate	9:00-10:00	A36, 215
Kolkova Miroslava	Vertebrate	9:00-10:00	A36, 215
Kovarikova Petra	Vertebrate	9:00-10:00	A36, 215
Kravec Marek	Vertebrate	9:00-10:00	A36, 215
Novakova Monika	Vertebrate	9:00-10:00	A36, 215
Paclikova Petra	Vertebrate	9:00-10:00	A36, 215
Pisko Jozef	Vertebrate	9:00-10:00	A36, 215
Pospisilova Anna	Vertebrate	9:00-10:00	A36, 215
Rabata Anas	Vertebrate	9:00-10:00	A36, 215

Steklikova Klara	Vertebrate	10:00-11:00	A36, 215
Sutus Eniko	Vertebrate	10:00-11:00	A36, 215
Szabo Viktoria	Vertebrate	10:00-11:00	A36, 215
Turmer Katalin	Vertebrate	10:00-11:00	A36, 215
Beckerova Deborah	Vertebrate	10:00-11:00	A36, 215
Borbely Noemi	Vertebrate	10:00-11:00	A36, 215
Dalecka Linda	Vertebrate	10:00-11:00	A36, 215
Dudas Krisztina	Vertebrate	10:00-11:00	A36, 215
Gregorova Michaela	Vertebrate	10:00-11:00	A36, 215
Kovacs Tamas	Vertebrate	10:00-11:00	A36, 215

Nickl Petr	Vertebrate	11:00-12:00	A36, 215
Pasovska Martina	Vertebrate	11:00-12:00	A36, 215
Pejtsik Diana	Vertebrate	11:00-12:00	A36, 215
Psutkova Viktorie	Vertebrate	11:00-12:00	A36, 215
Anand Mahek	Vertebrate	11:00-12:00	A36, 215
Sumbal Jakub	Vertebrate	11:00-12:00	A36, 215
Takács Bertalan	Vertebrate	11:00-12:00	A36, 215
Varga Zsombor	Vertebrate	11:00-12:00	A36, 215
Knapova Livia	Vertebrate	11:00-12:00	A36, 215
Sumberova Veronika	Vertebrate	11:00-12:00	A36, 215

PROGRAM

MAIN SESSION

DAY 1 Friday 7th of September

	from 11:00	Registration open	
	13:00-13:30	Opening by organizers	
	SESSION 1	*Chair: V. Bryja*	
T1	**13:30-14:10**	**KEYNOTE TALK 1 Claudio Stern**	**Two for the price of one: twinning - the ultimate regeneration**
T2	14:10-14:25	Ondrej Machon	Transcription factors of Meis family control differentiation of neural crest cells in vertebrates
T3	14:25-14:40	Cecilia Winata	Genomics dissection of the zebrafish heart
T4	14:40-14:55	Ildiko Kristo	Investigation the role in mRNA export of the actin binding protein, Moesin
T5	14:55-15:10	Adriana Kolesarova	A prominent and promising impact of grape skin extract on human ovarian cells
T6	15:10-15:15	Krisztina Herberth-Minko	A new theory of the thimic structure
T7	15:15-15:20	Karol Kaiser	WNT5A governs branching morphogenesis of choroid plexus in the developing brain
	15:20-15:50	*Coffee break*	
	SESSION 2	*Chair: A. Hampl*	
T8	**15:50-16:30**	**KEYNOTE TALK 2 Andrei Chagin**	**A novel stem cell niche discovered in the epiphyseal growth plate**
T9	16:30-16:45	Ales Hampl	Early lung epithelial progenitors originating from human pluripotent stem cells
T10	16:45-17:00	Agnieszka Bernat	The model of intragenic homologous recombination for studying genetic stability in ESC cells
T11	17:00-17:15	Vladimir Krylov	Identification and characterization of immature Sertoli cell progenitors derived from Xenopus tropicalis testis
T12	17:15-17:30	Lukas Cajanek	Acentrosomal divisions prevent self-renewal and trigger p53-dependent differentiation in human pluripotent stem cells
T13	17:30-17:35	Eniko Sutus	Connecting RING1 and YY1 Binding Protein to the retinoic acid signaling pathway during neural differentiation
T14	17:35-17:40	Josef Vecera	HIF1α role in early mammalian neural development
	17:40-23:00	**POSTERS 1**	*(with welcome reception)*

DAY 2 Saturday 8th of September - morning

	SESSION 3	*Chair: A. Bruce*	
T15	**9:00-9:40**	**KEYNOTE TALK 3 Marie-Helene Verlhac**	**Active fluctuations modulate gene expression in mouse embryos**
T16	9:40-9:55	Martin Anger	Functional correlation between Spindle Assembly Checkpoint and Anaphase Promoting Complex activity during mammalian meiosis I
T17	9:55-10:10	Petr Solc	Roles of three Aurora kinases (A, B, C) in spindle formation and chromosome segregation during mammalian oocyte meiosis
T18	10:10-10:25	Rita Sinka	Changes of mitochondria in *Drosophila melanogaster* spermatids
T19	10:25-10:40	Ferenc Jankovics	Small ovary (sov): a novel heterochromatin regulator in Drosophila
T20	10:40-10:45	Dmitry Dedukh	Cytological insights about hybrid male gametogenesis from *Cobitis taenia* complex
T21	10:45-10:50	Viktor Vedelek	Stage-specific transcriptom analysis in Drosophila testis
	10:50-11:20	*Coffee break*	
	SESSION 4	*Chair: A. Piliszek*	
T22	**11:20-12:00**	**KEYNOTE TALK 4 Petra Hajkova**	**Epigenetic reprogramming in vivo: what have we learnt?**
T23	12:00-12:15	Anna Ajduk	Optical coherence microscopy as a novel, non-invasive method for the 4D live imaging of early mammalian embryos
T24	12:15-12:30	Marta Czernik	Mitochondrial fusion in pre-implantation mouse embryos
T25	12:30-12:45	Lenka Gahurova	Stage specific mTOR-regulated translation affects the relative spatial positioning of cells during mouse preimplantation embryo development
T26	12:45-13:00	Aneta Suwinska	The role of paracrine interactions involving FgfR1 and FgfR2 receptors in regulation of development of the preimplantation mouse embryo
T27	13:00-13:05	Zofia E. Madeja	Pluripotency and lineage specific signalling affects the quality of bovine preimplantation embryos obtained *in vitro*
T28	13:05-13:10	Jan Nevoral	SIRT1 in oocyte: epigenetic and non-epigenetic mode of action
	13:10- 14:30	*Lunch*	

DAY 2 Saturday 8th of September - afternoon

	SESSION 5	*Chair: M. Varga*	
T29	**14:30-15:10**	**KEYNOTE TALK 5 Jeremy B.A. Green**	**Epithelial bending for organ formation: forces, molecular signals, and novel ensemble cell behaviours**
T30	15:10-15:25	Zuzana Koledova	Regulation of epithelial branching morphogenesis by fibroblasts: lessons from mammary gland
T31	15:25-15:40	Alena Krejci	NADH metabolism as regulator of *Drosophila* development
T32	15:40-15:55	Stefan Cikos	Maternal stress during early pregnancy: short-term and long-term consequences
T33	15:55-16:10	Jozsef Mihaly	A STORM analysis of sarcomere structure and assembly in Drosophila flight muscles
T34	16:10-16:15	Ahmed Gad	Expression profiling of miRNAs in porcine oocytes with different developmental competence
T35	16:15-16:20	Ondra Bernatik	Tau tubulin kinase 2 substrates phosphorylation in cilia initiation
	16:20-16:45	*Coffee break*	
	SESSION 6	*Chair: P. Vilmos*	
T36	**16:45-17:25**	**KEYNOTE TALK 6 Pavel Tomancak**	**A new force awakens: comparative approach to tissue morphogenesis in insects**
T37	17:25-17:40	Marek Jindra	Tissue-restricted juvenile hormone signaling simultaneously permits larval growth and adult development during advanced *Drosophila* metamorphosis
T38	17:40-17:55	Jiří Pergner	Evolution of photoreception in chordates – insights from development of amphioxus photoreceptors
T39	17:55-18:10	Vladimir Soukup	Hyoid breathing: Heterochrony underlies development of larval adaptive structures
T40	18:10-18:20	Viktor Demko	The mechanism and evolution of positional signalling in plants – understanding the DEK1 pathway
	18:20-19:00	**POSTERS 2**	
	19:00-24:00	*Banquet*	*(Restaurant Pivovarska Starobrno)*

DAY 3 Sunday 9th of September

	SESSION 7	*Chair: D. Fabian*	
T41	**9:00-9:40**	**KEYNOTE TALK 7 Olov Andersson**	**Pancreatic beta-cell regeneration in zebrafish**
T42	9:40-9:55	Jan Krivanek	How do the continuously growing teeth regenerate?
T43	9:55-10:10	Radek Sindelka	Role of nitric oxide during wound healing
T44	10:10-10:25	Zdenek Trachtulec	An ancient pathway of the initiation of meiotic DNA repair present in *Mus musculus*
T45	10:25-10:30	Josef Jaros	3D bioprinting of stem cells and their environment
	10:30-11:00	*Coffee break*	
	SESSION 8	*Chair: M. Buchtova*	
T46	**11:00-11:40**	**KEYNOTE TALK 8 Abigail S. Tucker**	**Development of the human ear canal sheds light on external ear defects in patients**
T47	11:40-11:55	Dusan Fabian	Transgenerational mouse obesity model – an ideal choice for reproductive and developmental studies
T48	11:55-12:10	Mate Varga	Defective pseudouridylation results in impaired differentiation during zebrafish development
T49	12:10-12:25	Tomas Barta	miR-183 cluster: a microRNA family regulated by light in human retinal organoids
T50	12:25-12:40	Michaela Kunova Bosakova	Regulation of ciliary function by fibroblast growth factor signaling identifies FGFR3-related disorders achondroplasia and thanatophoric dysplasia as ciliopathies
T51	12:40-12:45	Eva Hruba	Pathophysiology of *Sprouty2* and its association with ciliopathies
T52	12:45-12:50	Katarzyna Filimonow	Rabbit as a model for inner cell mass and trophectoderm differentiation
	12:50-13:00	Closure of the meeting	
	13:00-14:30	*Lunch*	
	14:30-15:00	Round table of organizers about future meeting and society running	

List of Posters

Odd posters will be presented: Friday 7.9. 18:00-19:30

Even posters will be presented: Friday 7.9. 19:30-21:00

More discussion in front of posters: during coffee breaks or on Saturday (8.9. 17:45-19:00)

	Presenting author	**Title of poster**
P1	Mahek Anand	Comparison the miRNA expression profile in chicken male and female primordial germ cells
P2	Tamas Annus	The role of RecQ-family helicases in the genome maintenance of zebrafish
P3	Janka Babelova	Can maternal obesity influence the effect of Fipronil on *in vitro* development of mouse preimplantation embryos?
P4	Csaba Bajusz	The biological significance of the nuclear localization of an actin-binding cytoskeletal protein
P5	Simona Baldovska	Phytoestrogenic Effect of Pomegranate on Human Ovarian Cells
P6	Tomas Duricek	DNA damage checkpoints in early mammalian embryos
P7	Melinda Bence	Role of *Su(var)2-10* gene in germ cell development of *Drosophila melanogaster*
P8	Pablo Bora	Deciphering the nodal role of p38 family of mitogen-activated protein kinases (MAPKs) towards specifying primitive endoderm (PrE) fate in the inner cell mass (ICM) of late-blastocyst stage mouse pre-implantation embryo
P9	Peter Borkuti	Testing the biological significance of the nuclear localization of actin
P10	Linda Dalecka	Incisor development in *Eda* mutants
P11	Angelika Drozdova	Development of melatonin rhythm in the pineal gland of chick embryos incubated under different wavelength of light
P12	Jana Dumkova	Development of tooth-bone interface in reptiles
P13	Jaroslav Fabik	Inactivation of Meis2 in neural crest cells results in craniofacial developmental defects
P14	Veronika Fedorova	Expression and Regulations of p16/INK4A in Human Embryonic and Neural Stem Cells
P15	Katarzyna Filimonow	EMT (epithelial-mesenchymal transition) in mouse blastocyst

P16	Wiesława Florek	Blastomere removal from 8-cell stage embryos programs post-natal body weight, food intake and the level of leptin in mice
P17	Marek Hampl	Ciliary protein TMEM107 regulates rostro-caudal patterning in craniofacial development
P18	Katalin Turmer	*In vivo* examination of membrane nanotubes in developing zebrafish embryos
P19	Laszlo Henn	A novel approach to investigate the effects of Histone post-translational modifications in *Drosophila*
P20	Surya Henry	Characterization of cardiac transcription factor PLAGL1, a newly identified putative downstream target of RYBP
P21	Zuzana Holubcova	From totipotency to pluripotency: non-invasive time-lapse imaging provides insight into initial stages of human life
P22	Karam Ibrahim	Small ovary (sov) regulates transposon silencing by promoting heterochromatin formation in Drosophila
P23	Rajan Iyyappan	Reprograming of translation during oogenesis and embryogenesis
P24	Andras Jipa	Characterization of mutant alleles of Atg8 genes in *Drosophila melanogaster*
P25	Aneta Suwinska	The role of Fgf4/MAPK signaling pathway in regulation of development of mouse chimeric embryo
P26	Michaela Kavkova	Digital dissection of 3D model of mouse skeleton: x-ray micro computed tomography analysis
P27	Anzer Khan	Deciphering effects of *Adar* on *Drosophila* metamorphosis
P28	Michael Killinger	Gene expression profiling of anterior-posterior differences in early limb bud
P29	Veronika Kinterova	Polyspermy in bovine zygotes after SCF complex inhibition
P30	Katarzyna Klimczewska	The role of paracrine interactions involving FgfR1 and FgfR2 receptors in regulation of development of the preimplantation mouse embryo
P31	Lucie Knoblochova	The Role of CDC25A Phosphatase in Meiosis and First Embryonic Division in Mouse
P32	Filip Knop	*Caenorhabditis elegans* SEL-5 kinase role in anterio-posterior cell outgrowth and migration
P33	Marketa Koncicka	Detection of Lamin C2 in the mouse oocyte
P34	Tamas Kovacs	Intraganglionic macrophages: a new population of cells in the enteric ganglia

P35	Zoltan Kovacs	Analysis of the nuclear transport of the cytoskeletal Moesin protein
P36	Petra Kovarikova	Analysis of Cep164 interactome identifies KIF14 as novel regulator of ciliogenesis
P37	Veronika Kovarikova	Glutamic acid can influence development of preimplantation embryo in vitro
P38	Michaela Kunova Bosakova	Loss of GRK2 produces asphyxiating thoracic dystrophy via impaired Hedgehog and canonical Wnt signaling
P39	Marie Landova	Regulation of complex tooth shape during reptile odontogenesis
P40	Josef Lavicky	Expansion and response of human pluripotent stem cells in synthetic cell culture environment
P41	Petra Lesakova	Metabolic restriction influences phenotype of embryonic stem cell-derived cardiomyocytes?
P42	Edgar del Llano	Age related differences in the translational landscape of mammalian oocytes
P43	Bernadette Luza	New cell dynamics during the enamel patterning using live cell imaging
P44	Simona Machacova	Evolution of gene network regulating gastrulation: Insights via *Chordin*
P45	Matej Murin	Developmental potential of porcine oocytes from large and small follicles
P46	Petr Nickl	The role of transcription factors of Meis family during development of neural crest cells in *Danio rerio*
P47	Monika Novakova	Deficiency of Cdk13 leads to improper organogenesis and late embryonic lethality in mice
P48	Kristyna Olbertova	The expression of stem cell markers during odontogenesis
P49	Piotr Pawlak	The influence of stearic and oleic fatty acids supplementation on early embryo development, gene expression and phenotypic divergence of lipid droplets in cumulus cells
P50	Diana Pejtsik	Early-life social isolation-induced serotonergic deficits and their behavioural consequences in zebrafish (*Danio rerio*)
P51	Jozef Pisko	Morphological assessment phagocytized apoptotic cells in mouse blastocysts
P52	Anna Pospisilova	Comparative cranial skeletogenesis in non-teleost fishes: towards understanding of developmental strategies of fish craniofacial diversity

P53	Vladimir Soukup	Patterning of teeth at the dichotomy of ray- and lobe-finned fishes
P54	Leszek P. Pryszcz	Deciphering the role of RNA editing in vertebrate development
P55	Viktorie Psutkova	The unique development of the primary mouth in basal ray-finned fishes
P56	Anas Rabata	3D cell culture models demonstrate a role for FGFs and WNT signalling in regulation of adult lung epithelial stem cell fate
P57	Deborah Beckerová	Phorbol ester increases cardiomyogenesis in mES cells through ERK signalling
P58	Katarzyna Anna Radaszkiewicz	Transient hypoxia drives a ventricular-like cardiomyocyte phenotype in *in vitro* cardiomyogenesis of mouse embryonic stem cells
P59	Tomasz W. Radaszkiewicz	The role of RNF43/ZNRF3 in non-canonical Wnt signaling and its impact on human melanoma
P60	Iva Sovadinova	Advanced *in vitro* model of Leydig cell development for male reproductive toxicity assessment
P61	Anna Stachowiak	Differentiation potential of rabbit inner cell mass
P62	Klara Steklikova	Early tooth development and segmentation of oral epithelium in mice
P63	Jakub Sumbal	Deciphering Roles of FGF Signaling in Mammary Gland Development
P64	Marek Supak	Function of Cdk12 during mammary gland development in mice
P65	Gergo Kovacs	Proteomic approaches to identify new interactors of Rybp during neuronal specification
P66	Andrea Svoradova	Ultrastructure of chicken embryonic stem cells
P67	Viktoria Szabo	Lack of RYBP impairs sarcomere formation and the development of multiple cell types during *in vitro* cardiac differentiation
P68	Alexandra Brigitta Szarka-Kovacs	The role of Mesr4 in stem cell differentiation
P69	Veronika Sumberova	The role of HIF-1α in early neurogenesis
P70	Bertalan Takacs	Investigating the role of polycomb protein Rybp in neural progenitor formation
P71	Anna Tetkova	FSH influences amino acid incorporation in mammalian oocyte and early embryo
P72	Tereza Toralova	Protein degradation during bovine preimplantation development

P73	Zuzana Trebichalska	Analysis of human oocyte ultrastructure by advanced electron microscopy technologies
P74	Jaromir Vasicek	Ultrastructural characterization of rabbit adipose tissue derived mesenchymal stem cells
P75	Giorgio Virnicchi	The Role of WWC2 during preimplantation mouse embryo
P76	Dawid Winiarczyk	*SIRT1* may control apoptosis resistance in preimplantation mouse development
P77	Oldrich Zahradnicek	Continuously replacing dentition in anoline lizards: the way to change teeth shapes
P78	Marta Zietek	Effect of advanced maternal age on reproductive outcomes in a mouse model of autism
P79	Krystyna Zyzynska-Galenska	The influence of pluripotency state of mouse ESCs on their ability to colonize blastocysts
P80	Barbora Putnova	WNT signaling in pathogenesis of odontogenic tumors

Abstracts

Oral presentations

T1. KEYNOTE TALK 1

Two for the price of one: twinning – the ultimate regeneration

Claudio Stern[1], Kaustubh Adhikari[1], Federica Bertocchini[1], Irene de Almeida[1], Macarena Fuentes Guajardo[1], Mohsin Khan[1], Hyung Chul Lee[1], Grace Lu[1], Isaac Skromne[1] and Angela Torlopp[1]

[1] *Department of Cell & Developmental Biology, University College London, U.K.*

Amniote (birds and most mammals) embryos have the remarkable ability to undergo "embryonic regulation": this is a property by which a fragment of the embryo can reconstitute the entire embryo. This can occur right up to the appearance of the primitive streak (14th day in humans), and gives rise to identical (monozygotic) twins. Understanding the mechanisms that position the primitive streak, the site of gastrulation, within the embryo is essential to understand how identical twins form. Experimental studies on chick embryos are now starting to uncover these mechanisms. A cascade of genes (including transcription factor Pitx2 and several signalling molecules like GDF1/Vg1, Nodal, BMP and Wnt) is important for primitive streak formation, but until now we have not understood the mechanisms upstream of this, which position the expression of these genes in the right place. I will review some very recent work implicating some unexpected gene regulatory interactions and some non-genetic mechanisms. In parallel, we have been conducting genetic studies on human populations with a high rate of spontaneous twinning, especially in Brazil and India. The results are just starting to come out, and seem to reinforce the findings from chick experiments.

T2.

Transcription factors of Meis family control differentiation of neural crest cells in vertebrates

Ondrej Machon[1]

[1] *Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic*

Neural crest cells (NCC) represent a multi-potent embryonic cell population that generates a very diverse range of cell types including cranial nerves, neurons and glia of the peripheral nervous system, enteric neurons, melanocytes, cranial bones and cartilages. Our previous work indicated that transcription factor Meis2 plays an important role in differentiation of neural crest cells during mouse embryogenesis. We employ conditional knock-out mutagenesis in mouse to specifically inactivate Meis1, Meis2, or both, in neural crest cells. We show that Meis2 absence leads to multiple defects in craniofacial morphogenesis, namely in the palate, hyoid bone, middle ear bones and parietal bone. Also other structures originating from the first and second pharyngeal arch (PA) are severed, such as the tongue and submandibular gland. Using in situ hybridization and immunohistochemistry we attempt to decipher the mechanism of Meis function in NCC by analyzing cell specification, proliferation and migration. Further, we study NCC-dependent craniofacial defects in zebrafish after inactivation of Meis genes by CRISPR-Cas9 technology.

Acknowledgement: This study is supported by Czech Science Foundation, grant 18-00514S.

T3.

Genomics dissection of the zebrafish heart

Michal Pawlak[1], Maciej Migdał[1], Katarzyna Kedzierska[1], Hashimoto Kosuke[2], Jordan Ramilowski[2], Lukasz Bugajski[3], Karim Abu Nahia[1], Katarzyna Piwocka[3], Piero Carninci[2] and Cecilia Winata[1,4]

[1] *International Institute of Molecular and Cell Biology in Warsaw, Poland*
[2] *RIKEN Center for Life Science Technologies, RIKEN Yokohama Institute, Japan*
[3] *Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*
[4] *Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany*

The development of vertebrate organs is a complex process which involves interactions between multiple signaling pathways at the molecular level, as well as interactions at the cell and tissue level. Heart development is an example of such complex process which, when disrupted, results in congenital heart defect. So far, molecular pathways regulating heart development have been identified. However, despite these advances, there is still a lack of understanding of their downstream regulatory networks and how they interact. To better understand the mechanism regulating heart development in zebrafish, we employ RNA-seq and ATAC-seq to profile the transcriptome and chromatin state in cardiomyocytes of zebrafish wild-type and mutant lines for *gata5*, *tbx5*, and *hand2*. Taking advantage of transgenic lines Tg(*nkx2.5*:EGFP) and Tg(*myl7*:EGFP) with cardiomyocyte-specific EGFP expression, we FACS-isolated cardiomyocytes at three developmental stages corresponding to heart tube formation, looping, and maturation. Our analyses revealed major gene expression changes and chromatin rearrangements between 24 hpf and 48 hpf and identified genetic regulatory hubs with distinct expression and chromatin dynamics during key stages of heart development. I will present our ongoing study on this topic and discuss the crosstalk between genetic and epigenetic regulation of heart development and potential insights it will contribute to understanding congenital heart diseases.

T4.

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

Ildiko Kristo[1], Csaba Bajusz[1], Peter Borkuti[1], Zoltan Kovacs[1] and Peter Vilmos[1]

[1]HAS BRC, Institute of Genetics, Szeged, Hungary

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

T5.

A prominent and promising impact of grape skin extract on human ovarian cells

Adriana Kolesarova[1], Simona Baldovska[1], Katarina Michalcova[1], Branislav Galik[2]

[1]*Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*
[2]*Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*

One of the popular and widely cultivated fruits in the world, grape is a prominent source of bioactive compounds with antioxidant activity. In particular, resveratrol, well known for its various medicinal properties, is a major biocomponent in grapes. Current progress in the field of pharmacology and physiology have enhanced the significance of grapes as a nutritional food against various ailments. Phytochemicals present in grapes may have important role in inhibiting of cancer cell proliferation. Human ovarian granulosa cell line (HGL-5) presents model system for understanding the molecular mechanisms of action of phytocompounds. The aim of our study was to evaluate the effect of grape skin extract (*Vitis vinifera* L., cultivar *Pinot gris*) at the doses 0; 6.25; 12,5; 25; 50; 100 μg/ml on the viability of HGL-5 cells, secretion of steroid hormones and on the viability of human ovarian carcinoma cell line (OVCAR-3). The metabolic activity was evaluated by *alamarBlue™ cell viability* assay and the release of hormones was assayed by ELISA methods. The level of viable OVCAR-3 cells significantly ($P \leq 0.05$) decreased after addition of extract at the concentrations 12.5; 25; 50; 100 μg/ml compared to the control. On the other hand, the number of vital HGL-5 cells was not significantly influenced ($P \geq 0.05$) at all concentrations. The secretion of 17ß-estradiol and progesterone was significantly ($P \leq 0.05$) decreased at the highest concentration 100 μg/ml. The mechanisms of action are not yet fully discovered, however, grape skin as a byproduct of winemaking contains phytocompounds whose are suggestive of health benefits and anticancer potential.

Acknowledgment: This work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects VEGA 1/0039/16, VEGA 1/0411/17, KEGA 011SPU-4/2016, APVV-16-0170 and EU project no. 26220220180: Building Research Centre „AgroBioTech".

T6.

A new theory of the thimic structure

Krisztina Herberth-Minko[1], Ildiko Bodi[1] , Nandor Nagy[1], Sophie Creuzet[2] and Imre Olah[1]

[1] Semmelweis University, Faculty of Medicine, Department of Anatomy, Histology and Embryology, Budapest, Hungary
[2] Paris-Saclay Institute of Neuroscience, Paris, France

The thymus gland develops from three different sources. The thymic epithelial anlage is a derivative of the foregut endoderm. The branching epithelial cords of thymic primordium grows into the surrounding mesenchyme of neural crest (NC) origin. This epithelial-mesenchyme primordium is colonized by hemopoietic cells. The blood vessels reach the thymic tissue from the capsule and interlobular trabecules, the latter developing from the NC cells. The epithelial framework of thymus consists of reticular epithelial cells (RECs). The anti-cytokeratin immunostaining identifies two sub-compartments in the medulla. One is continuous with the cortical epithelial cells, which expresses keratin (keratin positive network-KPN) and the other does not show keratin staining (keratin negative area-KNA) and is devoid of RECs. The connective tissue space of interlobular septae is continuous with KNA, that is “a dilation” of the septae and the supporting tissue of the KNA is identical with the septae, which may indicate the common NC origin of KNA and septae. The immunologically competent T cells from the KPN have to enter to the KNA, where the blood vessels and thymic dendritic cells are localized. The hemopoietic cells (T-cells, B-cells, dendritic cells) show a topographical arrangement in medulla, which indicates some cell-sorting mechanism at the KNA and KPN border. According to our hypothesis, NC cells invade not only the capsule and septae of the thymus, but make up the KNA in the medulla. The precise origin of the KNA is unknown, so our aim is to show its NC origin and characterize the changes in thymic development of cranial NC ablated and transplanted chicken embryos and to obtain deeper knowledge about the exact structure of the functional cortico-medullary border, which selects the T-cells.

T7.

WNT5A governs branching morphogenesis of choroid plexus in the developing brain

Karol Kaiser[1], Petra Kompanikova[1], Jan Prochazka[2], Daniel Gyllborg[3] Michaela Prochazkova[2], Renee van Amerongen[4], Rocio Laguna-Goya[5], Roger Barker[5], Radek Sedlacek[2], Carlos Villaescusa[6], Ernest Arenas[3] and Vitezslav Bryja[1]

[1] Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2] Czech Centre for Phenogenomics and Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics, CAS, Vestec, Czech Republic
[3] Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden
[4] Swammerdam Institute for Life Sciences, Faculty of Science, University of Amsterdam, Netherlands
[5] John van Geest Centre for Brain Repair, University of Cambridge, Cambridge, UK
[6] Psychiatric Stem Cell Group, Neurogenetics Unit, Center for Molecular Medicine, Department of Molecular Medicine and Surgery, Karolinska University Hospital, Stockholm, Sweden

Morphogenesis is a complex process arising from coordinated action of large number of signaling pathways during embryonic development. Wnt signaling pathway is one of the crucial cascades involved in proper regulation of the most fundamental developmental processes required for generation of cell and tissue diversity. Wnt5a represents one of the most well studied Wnt ligands, which was shown to play major role in execution of developmental functions associated with Wnt pathway activity, including cell polarity as well as establishment and outgrowth of multiple structures in vertebrate animals. Choroid plexus (ChP), located within the lumen of brain ventricles, plays the role of the major site for the production of cerebrospinal fluid (CSF). Developing ChP exhibits high levels of Wnt5a expression and given its growth pattern and complex branching architecture, we decided to investigate potential involvement of Wnt5a-mediated signaling in the process of its development. Here we report identification of Wnt5a as a key morphogen involved in the embryogenesis of all choroid plexuses in the developing brain. We demonstrate that Wnt5a is expressed in a specific spatial pattern shared by all the choroid plexus, playing particularly prominent role in the morphogenesis of hindbrain choroid plexus. Furthermore, we show deleterious effects of Wnt5a ablation and overexpression on the hindbrain plexus formation and highlight distinct differences in non-canonical Wnt pathway activity between embryonic choroid plexuses.

T8. KEYNOTE TALK 2

A novel stem cell niche discovered in the epiphyseal growth plate

Andrei Chagin[1]

[1] *Karolinska Institutet, Stockholm, Sweeden*

Longitudinal growth of children occurs in thin cartilage discs, called growth or epiphyseal plates, which are located near the ends of all growing long bones. Growth plates provide a continuous supply of the cells crucial for the maintenance of normal bone growth but it is not yet known how these discs maintain themselves. Employing clonal genetic tracing, we show here that in mice longitudinal growth during the fetal and neonatal periods occurs via small clones arranged into multi-clonal columns, a pattern of clonality that strongly supports direct depletion of the progenitor cells. In contrast, later in life this pattern changes dramatically, with the formation of mono-clonal chondrocyte columns and a 2-5 fold increase in clone size. Since no other drastic changes in cell kinetics could be observed this radical switch in clonality suggests that chondro-progenitors acquire the capacity for self-renewal. This acquisition occurs simultaneously with the formation of the secondary ossification center, the expression of stem cell markers and initiation of symmetric cell divisions. Furthermore, the pool of self-renewing progenitors could be expanded by specifically activating the mTORC1-signaling pathway in the growth plate, suggesting a novel target for the treatment of growth disorders.
Overall our present findings show that a stem cell niche develops postnatally in the epiphyseal growth plate and that this niche is essential for the maintenance of postnatal bone growth.

T9.

Early lung epithelial progenitors originating from human pluripotent stem cells

Ales Hampl[1,2]

[1] *Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[2] *International Clinical Research Center, St. Anne´s University Hospital Brno, Czech Republic*

Currently, it is a challenge to obtain sufficient numbers of primary lung epithelial progenitor cells that could possibly be used for therapy and/or tissue engineering applications. Here we describe the cells differentiated *in vitro* from human embryonic stem cells (hESC) that can be propagated for long-term in culture and most likely represent equivalent of early lung progenitors (ELEP) occurring in development. We have shown that these cells can be maintained in culture for a minimum of 65 passages without losing their key characteristics. ELEP maintain their population doubling time at an average of 26.5 hours and the activity of their telomerase holds at about 50% of that typical for undifferentiated hESC. ELEP express high levels of anterior foregut marker SOX2 (also typical for self-renewing cells), marker of definitive endoderm SOX17, and marker of early lung epithelial lineage, thyroid transcription factor-1. As found by transmission EM, ELEP also possess morphological features of cells differentiating towards airway epithelia, multivesicular and lamellar bodies. When induced to terminally differentiate, ELEP increase levels of FOXJ1 (ciliated cells), pro-surfactant protein B (alveolar epithelial cells), Club cell specific protein (Club cells), aquaporin A (type I pneumocytes), and surfactant proteins A and C (type I pneumocytes and Club cells). Under 3D conditions, differentiating ELEP then develop morphologies of alveolar- and airway-like structures.

Acknowledgement: Grant no. 16-31501A (Ministry of Health of the Czech Republic) and project LQ1605 from the National Program of Sustainability II (Ministry of education, Youth, and Sports of the Czech Republic).

T10.

The model of intragenic homologous recombination for studying genetic stability in ESC cells

Paulina Santus[2], Nathalie Doerflinger[3], Pierre Savatier[3] and Agnieszka Bernat[1,2]

[1] Laboratory of Molecular Diagnostics, Laboratory of Genetic Analysis, Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Gdansk, Poland
[2] Laboratory of Experimental Embryology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland
[3] INSERM U1208, Stem Cell and Brain Research Institute, Laboratory of Pluripotent Stem Cells in Mammals, Bron, France

As any signs of genetic instabilities that appear during pluripotent stem cells culture, hamper their use in medical applications, there is a need to monitor the population welfare. In response to genotoxic stress, if repair is possible and apoptosis is not triggered, embryonic stem cells (ESC) predominantly use homologous recombination (HR) to repair the most treacherous damage - DNA double-strand breaks (DSBs). Here, we present the generation of the model for monitoring the intragenic HR events, that reflect the rate of DSBs in mouse ESCs. For this, we constructed eukaryotic pCAG-LaacZ vector containing *LacZ* reporter gene with inactivating sequence duplication. Emerging intragenic HR events remove duplication and restore *LacZ* function, as detected by simple X-gal staining. Next, mouse ES cell line carrying pCAG-LaacZ vector was created and the frequency of DSBs repair was assessed after exposure to various gentotoxic stressors and measured as number of X-Gal (+) cells appearing in the population. We have observed the increased frequency of intragenic HR in response to common DSBs inducers like doxorubicin. Uncommon agents, like blue light (411nm) and photosensitizers, that in contrast to doxorubicin treatment, do not impair cell proliferation, also caused higher rate of DSBs rapair by HR, in comparison to untreated cells. This shows that the described model enables the detection of DNA damage in form of DSBs and it can be used for monitoring population well-being and testing possible cytotoxic and genotoxic agents.

T11.

Identification and characterization of immature Sertoli cell progenitors derived from Xenopus tropicalis testis

Thi Minh Xuan Nguyen[1], Marketa Vegrichtova[1], Tereza Tlapakova[1], Magdalena Krulova[1] and Vladimir Krylov[1]

[1] *Charles University, Faculty of Science, Prague, Czech Republic*

Sertoli cells in testis are indispensable for germ cell protection, nourishment and differentiation. Their immunosuppressive potential and biological similarity with well-studied mesenchymal stem cells make them interesting for the transplantation and regenerative medicine. However, origin of Sertoli cells in ontogenesis, migration and differentiation potential and functioning during germ cell maturation on the subcellular and molecular level are still poorly understood. We have established a cell culture derived from the testis of juvenile Xenopus tropicalis male. Gene expression analysis revealed the upregulation of pluripotency genes (klf4, myc and tert) and Sertoli cell and peritubular myoid cell markers (sox9 and acta2). Germ cell markers (dazl, ddx4 and ddx25) were absent. Additional observation of simultaneous presence of cytokeratin and vimentin provided the evidence that we described as a first, an immature precursor of Sertoli and peritubular myoid cells (XtiSCs). To determine their migration and differentiation potential we employed three inducers of epithelo-mesenchymal transition (EMT) - GSK-3 inhibitor (CHIR99021), FGF2 and TGF-β1 ligands. XtiSCs underwent full EMT with CHIR99021 accompanied by the morphological changes and upregulation of mesenchymal and downregulation of epithelial markers. In contrast to the wt cells CHIR99021 treated XtiSCs were able to differentiate in chondrocytes, osteocytes and adipocytes in vitro likewise mesenchymal stem cells and into functional cardiomyocytes after their microinjection to the peritoneal cavity of X. tropicalis tadpoles. Moreover, XtiSCs migrated towards injury site in tadpole´s tail after intraperitoneal transplantation indicating their relatively broad differentiation potential and wound healing activity.

Acknowledgement: The research was funded by the Charles University programs SVV 260435 and 20604315 PROGRES Q43.

T12.

Acentrosomal divisions prevent self-renewal and trigger p53-dependent differentiation in human pluripotent stem cells

Tereza Renzova[1], Dasa Bohaciakova[1], Milan Esner[1,2], Veronika Pospisilova[1], Tomas Barta[1], Ales Hampl[1,3] and Lukas Cajanek[1]*

[1] Department of Histology and Embryology, Masaryk University, Faculty of Medicine, Brno, Czech Republic
[2] Cellular Imaging Core Facility, Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic
[3] International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Centrioles account for centrosomes and cilia formation. Recently, a link between components of centrosome and human developmental disorders has been established. However, the exact mechanisms how centrosome abnormalities affect embryogenesis and contribute to cell fate are not fully understood. We have analyzed consequences of centrosome removal for early events of embryogenesis using human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). We will demonstrate that the loss of centrosome leads to both p53-dependent and independent defects, including prolonged cell divisions, upregulation of p53 levels, chromosome instability, and, importantly, reduction of pluripotency markers and induction of differentiation. We show that the loss of key stem cells properties after centrosome depletion is connected to alterations in mitotic timing and protein turnover. In sum, our data define a novel link between the centrosome and the regulation of pluripotency and differentiation in pluripotent stem cells.

T13.

Connecting RING1 and YY1 Binding Protein to the retinoic acid signaling pathway during neural differentiation

Eniko Sutus[1,2], Gergo Kovacs[1] and Melinda Katalin Pirity[1]

[1] *Laboratory of Embryonic and Induced Pluripotent Stem Cells, Institute of Genetics, BRC, HAS, Szeged, Hungary*

[2] *Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary*

Retinoic acid (RA) is a natural morphogen, which is essential for normal regulation of development, differentiation, proliferation, apoptosis and has profound effect on complement cancer chemotherapy treatments. RA is also a common inducer of neural differentiation of embryonic stem cells (ES) *in vitro* and signaling through RA regulates the proliferative and neurogenic capacity of certain cell types. We have previously showed that the insufficient dosage of RING1 and YY1 Binding Protein (RYBP) led to neural tube defects (exencephaly) in mice, and incomplete terminal differentiation of ES cells *in vitro*. Moreover, accelerated progenitor formation was coupled with elevated *Pax6* mRNA expression in the Rybp null mutant cells, which suggested a possible link between RYBP and RA signaling. Here we show that in the lack of *Rybp* key members of the RA signaling pathway (*Stra6, Rdh10*, *Raldh1, Cyp26a1*) are upregulated during the time-course of *in vitro* neural differentiation. Furthermore, we also demonstrate that increased mRNA level of the retinoid converting enzymes *Raldh1*, *Rhd10* was restored to the level of wild-type cells by lentiviral rescue of the Rybp null mutant ES cells. These results suggest that Rybp is critical for setting the right level of retinoid converting enzymes and facilitate to understand how accelerated progenitor formation and impaired consequent terminal differentiation is taking place in the lack of *Rybp*.
These results also enhance our ability to understand the role of RA and *Rybp* in stem cell differentiation and may improve therapeutics strategies for the treatment of cancer or neurodegenerative diseases.

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

T14.

HIF1α role in early mammalian neural development

Josef Vecera[1], Vojtech Konir[1], Jana Kudova[2], Veronika Sumberova[1], Lukas Kubala[2], Emma R. Andersson[3] and Jiri Pachernik[1]

[1] Faculty of Science, Masaryk University, Brno, Czech Republic
[2] Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[3] Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

Most embryonic and adult stem cell populations are naturally present in hypoxic conditions and oxygen level plays a crucial role in the determination of stem cell fate. Cellular responses to hypoxia are mainly mediated via hypoxia inducible factors (HIF). Besides their role in the regulation of metabolic and angiogenic responses, HIFs have been found to be involved in the control of stem cell proliferation and differentiation. In our research, we have focused on role of HIF1α in early neural development. For tracking and analysis of early embryonic fate, we use several approaches – *in vitro* organoid cultures derived from mouse embryonic stem cells (embryoid bodies, neurospheres), cell culture derived from embryonic neuroepithellium and whole embryo explants. HIF1α loss-of-function has been introduced either through gene total knockout or through the delivery of lentivirus carrying shRNA interfering with HIF1α mRNA. Our results reveal that absence/knockdown of HIF1α leads to loss of pluripotency markers and to increase of neural stem/progenitor cell markers together with increased neuronal maturation during long-term differentiation. Importantly, there seem to be strict temporal and spatial requirements for HIF1α endogenous function, depending on type of morphogen gradient and signalling pathways activated during neurogenesis. We have found that interaction of HIF1α and β-catenin inhibit neural differentiation during spontaneous differentiation. On the other hand, increased neural phenotype after destabilization of HIF1α in neuroepithelium is rather associated with disrupted HES1/Notch signaling. By detailed research, including gene silencing experiments *in vivo,* we would like to further uncover fundamental mechanisms underlying hypoxic regulation of early neurogenesis.

T15. KEYNOTE TALK 3

Active fluctuations modulate gene expression in mouse oocytes

Maria Almonacid[1], Stephany El-Hayek[1], Alice Othmani[2], Isabelle Queguiner[1], Fanny Coulpier[2], Sophie Lemoine[2], Leila Bastianelli[2], Christophe Klein[3], Tristan Piolot[1], Philippe Mailly[1], Raphaël Voituriez[4], Auguste Genovesio[2] and Marie-Hélène Verlhac[1]

[1] CIRB, Collège de France, and CNRS-UMR7241 and INSERM-U1050, Equipe Labellisée FRM, Paris, France
[2] Ecole Normale Supérieure, Institut de Biologie de l'ENS, IBENS, and CNRS-UMR8197 and Inserm, U1024, Paris, France
[3] Centre de Recherche des Cordeliers UMRS 1138, INSERM, UPMC, 15 rue de L'Ecole de Médecine, Paris, France
[4] CNRS/UPMC, UMR7600 and UMR 8237, Paris, France

In mammals, the nucleus is central in oocytes, not defining the future embryo axis. Nucleus centring depends on an F-actin mediated pressure gradient. In *Fmn2*$^{-/-}$ oocytes, lacking the F-actin nucleator Formin 2, the nucleus is off-centre and can be centred by re-expressing Formin 2. Here, we addressed the biological significance of nucleus positioning in mammalian oocytes. Using a dedicated computational 3D imaging approach, we observed nuclear architecture alterations in mouse *Fmn2*$^{-/-}$ oocytes. RNA sequencing of control versus *Fmn2*$^{-/-}$ oocytes detected 2285 mis-regulated genes. Rescue experiments showed that the process of nuclear positioning impacts nuclear architecture and gene expression. Using signal processing methods coupled to biophysical modelling allowing the extraction of *in vivo* mechanical properties of the nuclear envelope, we showed that F-actin-mediated activity promotes nuclear envelope shape fluctuations and chromatin motion. We thus propose a mechano-transduction model whereby nucleus positioning via microfilaments modulates oocyte transcriptome, essential for further embryo development.

T16.

Functional correlation between Spindle Assembly Checkpoint and Anaphase Promoting Complex activity during mammalian meiosis I

Martin Anger[1], Kristina Kovacovicova[1], Lenka Radonova[1], Jaroslava Sebestova[1], Thuraya Awadova[1], Michal Skultety[1], Michal Kubelka [2], Michael Hopkins[3] and Bela Novak[3]

[1] Central European Institute of Technology – CEITEC, Veterinary Research Institute, Brno, Czech Republic
[2] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libechov, Czech Republic
[3] Oxford Centre for Integrative Systems Biology, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, UK

Mammalian oocytes and embryos are frequently affected by aneuploidy arising during chromosome segregation. The reason why chromosome segregation errors are more frequent in female germ cells and embryos in comparison to somatic cells is still not completely understood. We believe that the problem is partially caused by differences in function of chromosome segregation and anaphase control mechanisms operating in these cells. In our study we focused on relationship between activities of surveillance checkpoint mechanism called Spindle Assembly Checkpoint (SAC) and ubiquitin ligase controlling anaphase entry called Anaphase Promoting Complex (APC) in individual oocytes. Using mainly micromanipulation and whole cell volume confocal imaging we monitored the activity of SAC on individual kinetochores and global APC activity and correlated these with chromosome positions, spindle formation and polar body extrusion simultaneously in individual cells progressing through the first meiotic division and approaching anaphase I. Our results show similarities between oocytes and somatic cells in relationship between SAC and APC activity as well as important and unexpected differences. Apart from other valuable insights into function of these two mechanisms in oocyte meiosis this approach allowed us to analyze quantitatively the dependence of APC activation on the number of kinetochores producing SAC signal or consequences of SAC reactivation in time of APC activity. Our results revealed that the checkpoint mechanisms involved in monitoring chromosome segregation and the pathways controlling anaphase entry in oocytes show remarkable differences compared to somatic cells, which might contribute to the high incidence of aneuploidy in these cells.

Acknowledgement: This work was supported by Czech Science Foundation Projects 17-20405S and by the Ministry of Education, Youth, and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601). We acknowledge the core facility Cellular Imaging sup- ported by the MEYS CR (LM2015062 Czech-BioImaging).

T17.

Roles of three Aurora kinases (A, B, C) in spindle formation and chromosome segregation during mammalian oocyte meiosis

David Drutovic[1], Alexandra L. Nguyen[2], Ahmed Z. Balboula[2], Amanda S. Gentilello[2], Berta N. Vazquez[2], Warif El Yakoubi[2], Suzanne M. Quartuccio[2], Lucie Knoblochova[1], Michaela Vaskovicova[1], Karen Schindler[2] and Petr Solc[1]

[1] Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic
[2] Department of Genetics, 145 Bevier Road, Rutgers, The State University of New Jersey, Piscataway, NJ, USA

Oocytes lack centrosomes and, therefore, bipolar spindle assembly depends on clustering of acentriolar microtubule-organizing centers (MTOCs) into two poles. Unlike somatic cells that use two Aurora kinase homologs to support mitosis (AURK; AURKA, AURKB), mammalian germ cells use a third (AURKC). Here we combined mammalian genetics and small molecule inhibitors with advanced live confocal imaging to uncover roles of three different AURKs during oocyte meiosis. In mitosis, AURKB is the catalytic subunit of the chromosomal passenger complex (CPC). Loss of AURKB results in abnormal mitosis and cell death. In oocytes AURKC is main AURK associated with CPC. The kinase haspin (GSG2) is required to regulate AURKC localization at chromosomes during meiosis I. We show that inhibition of haspin or genetic disruption of Aurkc perturbed MTOC clustering into two poles and the stability of the clustered MTOCs. Furthermore, we show that AURKC localizes to MTOCs in mouse oocytes. Inhibition of haspin perturbed the localization of AURKC at MTOCs, Surprisingly, females with oocytes deficient for both AURKB and AURKC are fertile because AURKA localizes to chromosomes in a CPC-dependent manner and compensates. The data suggests that AURKC is required to keep AURKA at MTOCs by competing for CPC binding, a mechanism that does not occur in mitotic cells. Although inhibition of AURKA in wild-type oocytes has only partial effect on spindle formation, inhibition of AURKA in AURKC or AURKB/C deficient oocytes results in destruction of spindle structure suggesting that AURKA compensate loss of AURKC. Taken together, we uncover a role for haspin as a regulator of bipolar spindle assembly by regulating AURKC function at MTOCs and cooperation of all AURKs in mammalian oocytes.

Acknowledgement: Czech part of this work was supported by Inter-Excellence program by project LTAUSA17097 and this work was done also in the frame of the project LO1609 from National Sustainability Programme of the Czech Ministry of Education, Youth and Sports. U.S. part of this work was supported by grants from NIH: R00 HD061657 and R01 GM112801 to K.S and F31 HD089591 to A.L.N.

T18.

Changes of mitochondria in *Drosophila melanogaster* spermatids

Viktor Vedelek[1], Barbara Laurinyecz[1], Kinga Szilasi[1], Zsuzsanna Darula[3], Attila L. Kovacs[2], Gabor Juhasz[2] and Rita Sinka[1]

[1] *Department of Genetics, University of Szeged, Szeged, Hungary*
[2] *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary*
[3] *Laboratory of Proteomics Research, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary*

Mitochondria are crucial elements of the sperm in most organism by providing energy to the movement of the sperm. Drosophila sperm tail contains two mitochondrial derivatives. The mitochondrial derivatives have an important role in mediating the elongation of developing cysts during spermatogenesis. These derivatives are unique because their structure hardly resembles the classical mitochondrion structure. Both derivatives run along the entire 1.8 mm length of the sperm. The extremely large mitochondrion is thought to provide structural rigidity for flagellar movement, but its precise function and organization is incompletely understood. The two mitochondrial derivatives differentiate and by the end of spermatogenesis the minor one reduces its size and the major one accumulates paracrystalline material inside it. The molecular constituents and precise function of the paracrystalline have not yet been revealed. We purified paracrystalline material from mature sperm and identified the major protein components of it. We characterized several male sterile mutant alleles of genes that function is necessary for the formation of paracrystalline and showed that their phenotype is caused by defects in paracrystalline accumulation and abnormal structure of the elongated major mitochondrial derivatives. Our results can provide a better understanding, how the mitochondrial derivatives develop and perform their function in the late stages of spermatogenesis and in the fully developed sperm.

Acknowledgement: GINOP 2.3.2-15-2016-00032 and GINOP-2.3.2-15-2016-00035, EFOP-3.6.1-16-2016-00008

T19.

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

Ferenc Jankovics[1], Karam Ibrahim[1], Melinda Bence[1] and Miklos Erdelyi[1]

[1] *Institute of Genetics, Hungarian Academy of Sciences, Szeged, Hungary*

The reliable transmission of the genetic information between generations requires the preservation of genome integrity of the germ cells. Mobilisation of transposons in germ cells can induce double strand breaks leading to deleterious mutations and genome damage. Thus, a small non-coding RNA-based defence system (piRNA-pathway) has been evolved against transposon-induced mutagenesis. Multiple aspects of piRNA-mediated transposon silencing depend on heterochromatin. Long precursors of the piRNAs are transcribed from piRNA clusters located at the heterochromatic regions of the genome. Following their biogenesis, piRNAs suppresses transposon activity by silencing transcription through promoting heterochromatin formation at the active transposon loci. The Drosophila oogenesis provides an excellent model for understanding the role of heterochromatin mediated gene expression regulation in transposon silencing. In a large scale RNAi screen, the *small ovary* (sov) gene was identified to be essential for germ cell development. S*ov* mutant flies showed abnormal germline stem cell development accompanied by high transposon mobility. Position effect variegation tests showed that sov is a repressor of variegation suggesting that sov promotes heterochromatin formation. Protein interactome study of Sov revealed a physical interaction between Sov and Heterochromatin protein 1a (HP1a), a central component of heterochromatin. In addition, Sov co-localises with HP1a in the nuclei suggesting that Sov affects chromatin function through HP1a regulation. FRAP studies revealed an increased HP1a mobility in *sov* mutants indicating that sov promotes heterochromatin formation by supporting the association between HP1 and the chromatin. We propose a model of how Sov functions in transposon silencing as a regulator of the heterochromatin.

T20.

Cytological insights about hybrid male gametogenesis from *Cobitis taenia* complex

Dmitry Dedukh[1,3], Zuzana Majtanova[1], Lukas Choleva[1,2], Anatol Marta[1] and Karel Janko[1,2]

[1] Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, CAS, v.v.i, Liběchov, Czech Republic

[2] Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic

[3] Department of Cytology and Histology, Saint Petersburg State University, Saint Petersburg, Russia

Despite numerous prezygotic barriers, closely related animals species can interbreed and produce hybrids. However, postzygotic isolation can prevent hybrids development and/or reproduction usually through affecting meiosis. Alterations of gametogenesis can rescue hybrids reproduction ability and allow forming of gametes. Cellular pathways leading to hybrid sterility remain mostly unknown for the majority of hybrid species. In current work, we aim to describe cytological mechanisms of hybrid sterility in the European spined loaches fish from *Cobitis taenia* hybrid complex. This complex includes sexually reproducing species *C. taenia* (TT) and *C. elongatoides* (EE), as well as their diploid (ET) and triploid (ETT, EET) hybrids. Remarkably, hybrid males and females drastically differ in their ability to reproduce: diploid and triploid males are sterile while females produce progeny through gynogenesis. To find out why diploid and triploid hybrid males are sterile we analyzed chromosomal pairing during meiosis. In contrast to parental species, hybrids demonstrate the high number of cells during prophase and first metaphase of meiosis with bivalents and improperly paired chromosomes. Using FISH with PNA probes for telomeric sequences and CGH we found that some bivalents consist of chromosomes from separate species while individual chromosomes cannot properly pair and exist as either unsynapsed chromosomes or univalents. To confirm abnormal bivalent formation we performed analysis of synaptonemal complexes and recombination points during pachytene in diploid and triploid hybrid males. We suggest that abnormal pairing of orthologous chromosomes prevents successful propagation of meiosis and leads to the sterility of hybrid males.

T21.

Stage-specific transcriptome analysis in Drosophila testis

Viktor Vedelek[1], Gabor Grezal[2], Barbara Laurinyecz[1], Bence Kovacs[1], Imre Boros[2], Laszlo Bodai[2] and Rita Sinka[1]

[1] Department of Genetics, University of Szeged, Szeged, Hungary
[2] Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary

To gain a better understanding of cellular differentiation of spermatogenesis, we applied RNA-Seq to analyse the testis-specific transcriptome, including coding and non-coding genes. We isolated three different parts of the wild-type testis by dissecting and cutting the different regions: 1.) the apical region, which contains stem cells and developing spermatocytes 2.) the middle region, with enrichment of meiotic cysts 3.) the basal region, which contains elongated post-meiotic cysts with spermatids. Total RNA was isolated from each region and analysed by next-generation sequencing. We collected data from the annotated 17412 Drosophila genes and identified 5381 genes with significant transcript accumulation differences between the regions, representing the main stages of spermatogenesis. We demonstrated for the first time the presence and region specific distribution of 2061 lncRNAs in testis, with 203 significant differences. Using the available modENCODE RNA-Seq data, we determined the tissue specificity indices of Drosophila genes. Combining the indices with our results, we identified genes with region-specific enrichment in testis. By multiple analyses of our results and integrating existing knowledge about *Drosophila melanogaster* spermatogenesis to our dataset, we were able to describe transcript composition of different regions of Drosophila testis, including several stage-specific transcripts. Our visualization could help us to identify genes responsible for the early stages of spermatogenesis and the organisation of post-meiotic elongation, the individualisation of spermatids, and give more detailed information about the composition of the mature sperm.

Acknowledgement: GINOP 2.3.2-15-2016-00034, GINOP-2.3.2-15-2016-00035 and EFOP-3.6.1-16-2016-00008.

T22. KEYNOTE TALK 4

Epigenetic reprogramming in vivo: what have we learnt?

Petra Hajkova[1]

[1] *MRC London Institute of Medical Sciences, London, U.K.*

During mouse embryonic development early postfertilisation zygotes and the developing primordial germ cells (PGCs, the precursors of gametes) undergo global epigenetic reprogramming. This process involves genome-wide erasure of DNA methylation as well as global changes in chromatin structure and histone modifications. Despite the efforts of numerous research teams the molecular mechanisms underlying these developmental reprogramming processes remain elusive.

I will present our recent results regarding the dynamics of DNA modifications and chromatin during the epigenetic reprogramming in mouse gonadal primordial germ cells (PGCs). I will also discuss our current understanding of mechanistic links between the global DNA demethylation and the execution of the germline developmental programme.

T23.

Optical coherence microscopy as a novel, non-invasive method for the 4D live imaging of early mammalian embryos

Anna Ajduk[1], Karol Karnowski[2], Bartosz Wieloch3, Szymon Tamborski[2], Krzysztof Krawiec[3], Maciej Wojtkowski[2,4] and Maciej Szkulmowski[2]

[1] Department of Embryology, Faculty of Biology, University of Warsaw, Miecznikowa 1, Warsaw, Poland
[2] Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Torun, Poland
[3] Institute of Computing Science, Poznan University of Technology, Poznan, Poland
[4] Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

Recent advancements of imaging technology based on traditional fluorescence and confocal laser scanning microscopy has significantly increased our knowledge of oogenesis and early embryonic divisions, allowing us to pinpoint the properties of oocytes and embryos that are crucial to ensure their high developmental potential and may serve as biomarkers of oocyte/embryo quality. However, until now, we typically were not able to visualize many of those properties non-invasively, without fluorescent dyes or tags and sample pre-processing, thus, their usability in oocyte/embryo selection procedures at in vitro fertilization clinics or animal breeding facilities has been limited. Optical coherence microscopy (OCM) is a promising alternative that circumvents the technical limitations of fluorescence imaging techniques and provides unique access to fundamental aspects of early embryonic development, without the requirement for sample pre-processing or labelling. We utilized the internal motion of cytoplasm, as well as custom scanning and signal processing protocols, to effectively reduce the speckle noise typical for standard OCM and enable high-resolution time-lapse imaging. We visualised intracellular structure (nuclei with chromatin conformation and nucleoli, spindle structure, networks of endoplasmic reticulum and mitochondria) of mouse and pig oocytes and embryos and through fertilization and the first embryonic division (4D imaging), as well as at selected stages of oogenesis and preimplantation development (3D imaging). Because all morphological and morphokinetic properties recorded by OCM are believed to be biomarkers of oocyte/embryo quality, OCM may represent not only a novel tool for basic research, but also a new chapter in imaging-based preimplantation embryo diagnostics.

Acknowledgement: The research was founded by the Applied Research Programme grant (PBS1/A9/20/2013) from the National Centre for Research and Development (Poland).

T24.

Mitochondrial fusion in pre-implantation mouse embryos

Marta Czernik[1,2], Dawid Winiarczyk[1], P. Greda[1,3], P. Loi[2] and Jacek A. Modlinski[1]

[1] *Department of Experimental Embryology, Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzebiec, Poland.*
[2] *Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy*
[3] *Faculty of Veterinary Medicine Department of Morphological Sciences, Warsaw University of Life Science, Warsaw, Poland*

Mitochondria are found in high numbers in oocytes and embryos. They provide the energy for oocyte maturation, fertilization, and embryo formation *via* oxidative phosphorylation. Consequently, any adverse influence on mitochondrial function may negatively impact the development of the pre-implantation embryos. To maintenance high quality of function, mitochondria have numerous periods of fusion and fission. The effect of mitochondrial fusion in pre-implantation embryos remains unknown. Here, we wanted to check whether *in vitro* culture (IVC) effect on mitochondrial fusion, distribution, expression of mRNA and major mitochondrial proteins. IVC as well as natural mated (NM) mouse embryos (2-4 cell stage and blastocysts) were subjected to mitochondrial analysis (fusion, organisation, activity, expression of genes involved in regulation of mitochondria functions (mRNA and proteins level)). We have observed that mitochondria in IVC embryos were less numerous, distributed more peripherally while NM embryos mitochondria were mainly localised close to the nucleus. Basing on time-lapse analysis reduced fusion process in IVC early embryos was observed. Additionally, fusion process has been observed only in blastocyst stage embryos (regardless of the experimental group). Moreover, expression of major mitochondrial proteins as well as mRNA have different expression pattern *in* IVC embryos. Preliminary results clearly shown that experimental conditions affect on proper mitochondrial functionality.

Acknowledgement: This project was financed by National Science Centre grant no. 2016/21/D/NZ3/02610 and has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 734434.

T25.

Stage specific mTOR-regulated translation affects the relative spatial positioning of cells during mouse preimplantation embryo development.

Lenka Gahurova[1,2], M. Kubickova[1], Andrej Susor[2], Kristina Kovacovicova[3,4], Martin Anger[3,4] and Alexander Bruce[1]

[1] Laboratory of Early Mammalian Dvelopment, Department of Molecular Biology and Genetics, University of South Bohemia, Czech Republic
[2] Laboratory of biochemistry and molecular biology of germ cells, Institute of Animal Physiology and Genetics CAS, v.v.i, Czech Republic
[3] Central European Institute of Technology, Veterinary Research Institute, Brno, Czech Republic
[4] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

The earliest appearing inner cells (inner cell mass precursors) of mouse preimplantation development are formed following the 8-cell (8C) to 16C division and have been reported to be biased towards eventually contributing to the pluripotent blastocyst stage epiblast (by avoiding differentiating cues imposed on outer cells). Alternatively, cells internalised after the 16C to 32C cleavage division are more likely to contribute to the differentiating primitive endoderm. The generation of inner cells after the 8C stage cell divisions has been shown to be affected by intra-cellular nuclear positioning and relative orientation of the resulting mitotic spindle, plus other constrictive actomyosin driven processes following cytokinesis. However, the underlying mechanisms regulating these processes remain largely unknown.
mTOR-regulated translation of mRNAs containing 5' terminal oligopyrimidine (TOP) motifs was previously shown to affect chromosomal segregation and meiotic spindle positioning in mouse oocytes. Here we demonstrate that mTOR has a previously unrecognised role during the 8C to 16C stage division, as short chemical inhibition immediately prior to division, negatively influences the formation of the first inner cells. Whilst actomyosin constriction appears unaffected, time-lapse confocal microscopy reveals spindle orientations and nuclear positions ordinarily associated with the generation of inner cells often give rise to two outer cells under mTOR inhibition - presumably via translational regulation of relevant mRNAs. Indeed, RNAi-mediated downregulation of candidate TOP-containing cytoskeletal gene mRNAs, Ank2 and Dctn2, also impairs inner cell generation by the 16C stage. We are currently investigating detailed spatial and temporal patterns of translational regulation imparted by mTOR during this period.

T26.

The role of paracrine interactions involving FgfR1 and FgfR2 receptors in regulation of development of the preimplantation mouse embryo

Katarzyna Klimczewska[1], Aneta Suwinska[1] and Marek Maleszewski[1]

[1] *Department of Embryology, Faculty of Biology, University of Warsaw, Poland*

The aim of our study is to investigate whether paracrine interactions involving FgfR1 and FgfR2 receptors, which are the components of the Fgf4/MAPK signaling pathway, play an important role in regulation the development of the mouse embryo. To this end, we constructed a chimeric embryo composed of the 8-cell mouse embryo and inner cell mass (ICM) cells of E3.5 mouse blastocyst. To investigate whether both components communicate via Fgf4/MAPK transduction pathway, we blocked the possibility of receiving a Fgf4 signal, secreted by the ICM cells, through the suppression of *FgfR1* and/or *FgfR2* expression in the 8-cell embryo. We revealed that chimeric embryos, in which intercellular communication is not disturbed, can reconstruct normal blastocyst and undergo full development. However, the disturbance of intercellular interactions between the 8-cell embryo and ICM cells by the suppression of *FgfR2* results in impaired formation of cell lineages of the chimeric blastocyst. Moreover, we observed that the absence of *Fgfr2* does not reduce the number of primitive endoderm (PE) cells in blastocysts, whereas the suppression of *Fgfr1 at least* partially disrupts PE development. Our preliminary results indicate that FgfR1 and FgfR2 receptors cooperate with each other in PE lineage establishment within the ICM of the mouse blastocyst. We plan to verify how the suppression of *FgfR1* and both receptors at the same time will have impact on the normal embryogenesis of the chimeric embryo.

Acknowledgement: The project is financed by the grant PRELUDIUM 14 2017/27/N/NZ3/00625 from the National Science Centre (Poland).

T27.

Pluripotency and lineage specific signalling affects the quality of bovine preimplantation embryos obtained *in vitro*

Zofia E. Madeja[1], Ewelina Warzych[1], Piotr Pawlak[1] and Dorota Lechniak[1]

[1] Department of Genetics and Animal Breeding, Faculty of Veterinary Medicine and Animal Sciences, Poznan University of Life Sciences, Poznań, Poland

The success rate of embryo related technologies strictly depends on embryo quality. Culture conditions determine embryo quality, which may be affected on many levels (including: timing of development, blastomere count, transcripts and metabolites content, apoptosis). Furthermore, molecular interactions of signalling pathways like MAPK and WNT/β-catenin are critical for cell-to-cell communication and induction of cellular differentiation. Both pathways are also important regulators of apoptosis. Therefore, we have aimed to verify the prolonged effect of MAPK silencing and WNT activation by chemical inhibitors (2i/3i systems) on bovine IVP embryos. Apoptotic index, total cell count and transcription of embryo quality markers were evaluated. A higher rate of apoptosis was observed in 2i/3i blastocysts, but was not accompanied by changes in transcript content of genes controlling apoptosis (*BAX, BCL2, BAK*). Therefore, alternative pathways of apoptotic activation cannot be ruled out. The expression of genes related to embryo quality determination (*HSPA1A, SLC2A1*) was not affected. *GJA1* gene expression was significantly higher in 3i blastocysts (compared to controls), what indicates a stimulatory effect of the applied inhibitors on cell-to-cell interactions. The lowest transcript level of the *IFNT2* gene was found in 2i embryos. We have also observed a variation in the *SDHA* gene transcript with the highest content in the 3i embryos, what may suggest their reduced quality. It may be concluded that the modifications of culture environment with inhibitors, which alter signalling pathways crucial for embryo development are not necessarily detrimental to the embryo, as alternative signalling might support proper development.

Acknowledgement: National Science Centre, Poland (NCN) Grant no.: OPUS3 2012/05/B/NZ9/03349

T28.

SIRT1 in oocyte: epigenetic and non-epigenetic mode of action

Jan Nevoral[1], Lukas Landsmann[1,2], Miriam Stiavnicka[1], Petr Hosek[1], Jiri Moravec[1], Sarka Prokesova[1,3], Tereza Zalmanova[4], Hedvika Rimnacova[1], Tereza Fenclová[1] and Milena Kralickova[1]

[1] Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2] Faculty of Science, Charles University, Prague, Czech Republic
[3] Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague 6-Suchdol, Czech Republic
[4] Institute of Animal Sciences, Prague-Uhrineves, Czech Republic

Meiotic maturation of oocytes is ingeniously orchestrated by a machinery of enzymes responsible for post-translational modifications of proteins that are necessary for meiotic progress. In addition to the well-known protein phosphorylation driven by phosphatases/kinases, NAD^+-dependent histone deacetylase SIRT1 represents an impactful protein modulating acetylation status of many epigenetic targets, such as core histones and up-stream factors, and/or cytoskeletal proteins. In accordance with that, we hypothesized that SIRT1 plays a role of a signal molecule in a mature oocyte through epigenetic and non-epigenetic regulation. Based on in vitro maturation of mouse oocytes and immunostaining of target proteins, we observed SIRT1 to be exclusively localized in germinal vesicle of immature oocytes. Subsequently, re-localization of SIRT1 occurred forming a spindle-like pattern in matured metaphase II oocyte. After treating mature oocytes with BML-278, a SIRT1 selective activator, we observed several changes in their histone code, i. e. increased trimethylation of lysine K9 on H3 (H3K9me2/3) and ubiquitination of H2A (H2AK199ub), while H3K4me2 decreased. These chromatin changes were accompanied by a suppression of TUNEL signal, revealing the protective effect of BML-278 on DNA integrity of matured oocyte. Considering non-histone targets, SIRT1 decreased α-tubulin acetylation. Taken together, our observations point out dual SIRT1 action through epigenetic and non-epigenetic regulation, leading to protective changes in matured oocytes.

Acknowledgement: This study was supported by the Charles University Research Fund (Progres Q39), the National Sustainability Programme I (NPU I) Nr. LO1503 provided by the Ministry of Education, Youth and Sports of the Czech Republic, European Human Biomonitoring Initiative HBM4EU provided by H2020, the National Agency of Agriculture Sciences (NAZV QJ1510138) and the Czech Ministry of Agriculture (MZeRO 0714).

T29. KEYNOTE TALK 5

Epithelial bending for organ formation: forces, molecular signals, and novel ensemble cell behaviours

Jeremy B. A. Green[1]

[1] *Centre for Craniofacial Biology & Regeneration, King's College London, London, UK*

Epithelial bending is a fundamental process of developmental morphogenesis from the earliest stages of gastrulation to the final stages of organogenesis. Classically, epithelia bend by cell-autonomous shape changes such as apical constriction or wedging. In principle, this is not the only way a sheet of cells can bend itself. We have investigated invagination of epithelia to form mouse tooth buds, hair follicles, mammary ducts and salivary glands. A novel family of cell-on-cell migration-driven epithelial bending mechanisms will be presented, including some of the signals and forces involved. These findings reveal cell-behavioral mechanisms underpinning both similarities and differences in these evolutionarily diversified epithelial organs and highlight a global framework for analysis of morphogenesis that lends itself to quantification.

T30.

Regulation of epithelial branching morphogenesis by fibroblasts: lessons from mammary gland

Zuzana Koledova[1] and Jakub Sumbal[1,2]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Development of mammary gland takes place from the most part postnatally. During puberty a rudimentary mammary epithelium that was established during embryogenesis is activated and undergoes branching morphogenesis to form a complex ductal network. This process is regulated by multiple signalling mechanisms, including epithelial-stromal interactions. In this study, we investigated the role of stromal fibroblasts in mammary epithelial branching morphogenesis using advanced 3D co-culture models combined with time-lapse imaging. We found that mammary fibroblasts induce epithelial branching by paracrine signalling, extracellular matrix (ECM) remodelling, and using mechanical force. Our data suggest that direct fibroblast-epithelium contact is necessary for fibroblast-mediated epithelial branching. Investigation of candidate signalling pathways in fibroblasts revealed that fibroblast growth factor (FGF) signalling regulates several important fibroblast functions for mammary tissue morphogenesis, including ECM production and remodelling, and production of paracrine signals. Taken together, our studies reveal a novel role for fibroblasts in epithelial branching morphogenesis and bring new insights into the mechanisms by which fibroblasts and FGF signalling regulate mammary gland development.

Acknowledgement: This project has been supported by grant number GJ16-20031Y form Czech Science Foundation (GACR) and by funds from the Faculty of Medicine MU to junior researcher Zuzana Koledova.

T31.

NADH metabolism as regulator of *Drosophila* development

Raquel Perez-Gomez[1,2], Valentina Magnin[1,2], Vladimira Tuckova[2], Nora Hagleitner[2], Zorana Mihajlovic[1,2], Vera Slaninova[1,2], Sarah Bray [3] and Alena Krejci [1,2]

[1] *Czech Academy of Sciences, Biology Centre, Insitute of Entomology, Ceske Budejovice, Czech Republic*
[2] *University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic*
[3] *University of Cambridge, Physiology, Development and Neuroscience, Cambridge, UK*

NADH is a vital cofactor for cellular metabolic pathways but it also influences important cellular events by the employment of NAD(H)-sensitive metabolic sensors. Through an RNAi based screen in Drosophila wings we identified NADH-binding subunits of respiratory complex I as mediators of major signalling changes during wing disc development. Downregulation of respiratory complex I, III or IV in the wing disc leads to robust stimulation of TOR activity, which in turn orchestrates a downstream signalling network. Specifically, after downregulation of the complex I subunit ND-49 (mammalian NDUFS2) TOR activates JNK to induce cell death and ROS production essential for the stimulation of compensatory apoptosis-induced proliferation within the tissue. Additionally, TOR robustly upregulates Notch and JAK/STAT signalling and it also directs a metabolic switch towards increased glycolysis by the stimulation of glucose uptake and massive upregulation of lactate dehydrogenase expression. Our results highlight the central role of TOR signalling in mediating the complex response to mitochondrial respiratory dysfunction. As TOR overactivation balances between stimulation of apoptosis and proliferation, the model we present suggests a possible mechanism for the observations when complex I inhibition supports cell death and/or proliferation in different contexts. The model also provides a rationale why the disease symptoms associated with respiratory dysfunctions are often alleviated by TOR inhibitors.

T32.

Maternal stress during early pregnancy: short-term and long-term consequences.

Stefan Cikos[1], Jan Burkus, Zofia Janstova, Janka Kubandova, Alexandra Spirkova, Veronika Kovarikova, Zuzana Sefcikova, Dusan Fabian and Juraj Koppel

[1] Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, Košice, Slovak Republic

Negative effects of prenatal stress on embryo development and offspring health are well documented. In our experiments, we investigated whether maternal stress can affect embryo at the very early stages of development. We demonstrated previously that cell receptors capable to bind stress mediators are expressed in preimplantation embryos of several mammalian species. We used mouse restraint stress model, and females in the stressed group were subjected to restraint stress exclusively during the preimplantation developmental period. Embryos isolated from stressed mothers showed lower developmental capacity than control embryos. Analysis of embryos that reached blastocyst stage showed reduced cell amount in blastocysts isolated from stressed mothers, and the two blastocysts cell lineages (TE and ICM) were affected differently. To analyze possible impact of maternal physiological status, embryos were isolated from stressed and unstressed females that differed in body fat amount and endocrine profile. Our results showed that effect of stress on early embryo can depend on the actual physiological status of the maternal organism exposed to stress. We also examined whether offspring of mothers exposed to stress during preimplantation developmental period will be influenced by the early intervention, and we found significant differences in body weight, fat deposits as well as in behavior of adult offspring. These results indicate that exposure to stress during preimplantation developmental period can have long-term consequences reaching even into late postnatal life.

Acknowledgement: This work was supported by the Slovak Academy of Sciences project VEGA 2/0039/15.

T33.

A STORM analysis of sarcomere structure and assembly in Drosophila flight muscles

Jozsef Mihaly[1], Szilard Szikora[1], David Farkas[1], Tibor Novak[2], Tamas Gajdos[2] and Miklos Erdelyi[2]

[1] *Biological Research Centre, Hungarian Academy of Sciences, Szeged, Institute of Genetics, Hungary*
[2] *University of Szeged, Department of Optics and Quantum Electronics, Hungary*

Myofibrillogenesis requires the coordinated assembly of the sarcomeric filament systems and their integration into the highly organized contractile structures. Genetic analysis combined with biochemistry and cell biology revealed many players involved in sarcomere assembly. However, visualization of these proteins is limited by the diffraction limit, and for this reason, molecular reconstruction of these assemblies remained speculative. Application of the recently developed nanoscopic techniques allowed us to visualize the molecular organization of individual sarcomeres at a previously unprecedented resolution level. We determined the localization of about 25 epitopes with a roughly 10 nm precision in developing Drosophila indirect flight muscles. This pioneering approach led us to gain novel insights into the mechanisms of thin filament elongation. In addition, by integrating our comprehensive localization information with previously generated protein structure and interaction data, we provide a refined model of sarcomere formation and sarcomere structure. Detailed presentation of the results of these single molecule localization microscopy studies is planned to be provided at the meeting.

T34.

Expression profiling of miRNAs in porcine oocytes with different developmental competence

Ahmed Gad[1], Lucie Nemcova[1], Matej Murin[1], Jiri Kanka[1], Jozef Laurincik[1,2] and Radek Prochazka[1]

[1] Laboratory of Developmental Biology, Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Libechov, Czech Republic
[2] Constantine the Philosopher University in Nitra, Nitra, Slovak Republic

Oocyte developmental competence is acquired during folliculogenesis, closely linked with follicle size and regulated by complex molecular mechanisms. Several molecules are involved in these regulation mechanisms including microRNAs (miRNAs) that are essential for oocyte-specific processes during development. The objective of this study was to identify the expression profile of miRNAs in porcine oocytes aspirated from follicles of different sizes using RNA high throughput sequencing technology. Two small RNA libraries were constructed from oocytes aspirated from large (3-6 mm) and small (<2 mm) follicles and then sequenced on an Illumina NextSeq500. In total, 203 and 192 known miRNAs were detected in large and small oocyte groups, respectively with 178 miRNAs were commonly expressed in both groups. Further analysis showed that 8 miRNAs were differentially expressed (DE) between both groups (>2 fold change) with 4 up- and 4 down-regulated miRNAs in large compared to small oocyte groups. Target gene prediction followed by KEGG pathway analysis revealed 61 pathways that were enriched with miRNA-target genes. Oocyte meiosis and signaling pathways including PI3K-AKT, TGF-beta, FoxO, and MAPK related genes were targeted by the up-regulated miRNAs in large compared to small oocytes. However, genes related to endocytosis and cAMP signaling pathways were the targets for down-regulated miRNA. These results can help us to further understand how oocyte development is regulated by miRNAs.

Acknowledgement: This work was supported by projects CZ.02.1.01/0.0/0.0/15_003/0000460 from OP RDE, VEGA 1/0022/15, VEGA 1/0327/16 and APVV-14-0001.

T35.

Tau tubulin kinase 2 substrates phosphorylation in cilia initiation

Ondrej Bernatik[1], Petra Kovarikova[1], David Vyslouzil[1], Katerina Hanakova[2], Zbynek Zdrahal[2], Vitezslav Bryja[3] and Lukas Cajanek[1]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] Research Group Proteomics, Central European Institute of Technology, Brno, Czech Republic
[3] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Primary cilium is one of the cellular organelles whose proper function is indispensable for proper execution of developmental processes like neural tube formation, limb patterning or kidney development. Primary cilium formation is initiated by series of events that enable docking of mother centriole to the plasma membrane. Proteins of mother centriole distal appendages (DA) and proteins associated with DA are necessary for this docking step, while one of the crucial factors is Tau tubulin kinase 2 (TTBK2), its kinase activity indispensable for PC initiation. Despite TTBK2 importance for the process of PC formation, only very few substrates have been identified so far and the identity and functions of TTBK2 induced phosphorylations are basically unknown. We are therefore trying to identify new TTBK2 substrates, analyze their TTBK2 induced phosphorylation sites and study consequences of such events functionally. So far, by combining proteomics and biochemical approaches we have identified several new TTBK2 substrates and mapped TTBK2 induced phosphorylations *in vivo* and *in vitro.* Using these datasets, we have been able to delineate kinase motif for TTBK2 by comparing sequences of phosphorylation sites, which surprisingly does not correspond to one identified by previous study (Bouskila *et al.,* 2011). To study the function of TTBK2 in transfection unperturbed way we established TTBK2$^{-/-}$ RPE1 and HEK293 cell lines by CRISPR and reintroduced doxycycline inducible TTBK2 variants and we plan to analyze their influence on the identified substrates. We will present data on this ongoing project.

T36. KEYNOTE TALK 6

A new force awakens: comparative approach to tissue morphogenesis in insects

Stefan Munster[1,2,3], Akanksha Jain[1], Alexander Mietke[2], Stephan W. Grill[2,3] and Pavel Tomancak[1]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[2] Max Planck Institute for the Physics of Complex Systems, Dresden, Germany
[3] Technische Universität Dresden, Dresden, Germany

The early embryo of the red flour beetle, Tribolium castaneum, initially consists of a single-layered blastoderm covering the yolk uniformly that differentiates into an embryonic rudiment as well as extraembryonic amnion and serosa. The germband anlage forms inside the egg during gastrulation when the embryonic rudiment condenses and folds along the ventral midline; this process is accompanied by large-scale flow and expansion of the extraembryonic serosa which ultimately covers the entire surface of the egg, thus engulfing the growing embryo. The mechanical properties of these tissues and the forces governing these processes in Tribolium, as well as in other species, are poorly understood. Here, we present our findings on the dynamics of myosin in the early blastoderm of Tribolium using multiview lightsheet live imaging of transiently labeled wild type embryos. We quantitatively measure the global distribution of myosin throughout the flow phase and present a physical description that couples the contractile forces generated by myosin to the mechanical properties of the blastoderm. In particular, we describe the overall tissue as a thin, actively contractile, viscous bulk material. This description accurately captures the large-scale deformation the tissue undergoes during the initial stages of gastrulation and it points to the existence of an attachment of the blastoderm tissue to the surrounding vitelline envelope. We provide several lines of evidence that such an attachment indeed exists and provides a fundamentally new external force required for tissue morphogenesis during insect gastrulation. Our findings lay a foundation for the physical description of gastrulation in Tribolium and will allow, in combination with the well-studied Drosophila paradigm, for the first time the comparative analysis of blastoderm tissue morphogenesis.

T37.

Tissue-restricted juvenile hormone signaling simultaneously permits larval growth and adult development during advanced *Drosophila* metamorphosis

Marek Jindra[1] and Mykola Yatsenko[1]

[1] Biology Center CAS, Institute of Entomology and Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

Internalized development of wings is a key innovation in insect metamorphosis, the most successful mode of animal development. In the highly advanced *Drosophila*, the entire head and thorax with the eyes, wings and legs of the adult arise from primordial cells called imaginal discs, deposited within the larva; the abdomen comes from histoblasts. Imaginal disc cells proliferate throughout larval life, then differentiate during metamorphosis while most larval tissues die. Juvenile hormone (JH), promotes larval growth while preventing adult development until larvae attain an appropriate size. However, imaginal discs develop in the presence of JH, and ectopic JH application at the onset of metamorphosis cannot block formation of the adult structures; it only blocks differentiation of the abdominal histoblasts. We hypothesized that imaginal discs may develop independently of JH because they lack JH receptors. Having identified the transcription factors Met and Gce as JH receptors in *Drosophila* (*PLoS Genet* 2015, 11:e1005394), we could test this hypothesis. We show that imaginal discs indeed lack the Gce protein, which at the same time abounds in larval tissues and in the JH-responsive histoblasts. Transgenic expression of either Gce or its paralog Met in the eye or wing discs caused malformation or complete loss of the affected adult structures. No anomalies resulted from misexpression of mutated receptors that cannot bind JH. We conclude that a natural suppression of JH receptor genes is part of the evolutionary adaptation that permits JH to promote larval growth without interfering with formation of the prospective adult structures.

T38.

Evolution of photoreception in chordates – insights from development of amphioxus photoreceptors

Jiri Pergner[1], Pavel Vopalensky[2], Iryna Kozmikova[1] and Zbynek Kozmik[1]

[1] Department of Transcriptional Regulation, Institute of Molecular Genetics of the Academy of Sciences, Prague, Czech Republic
[2] Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Amphioxus is a representative of basally branching chordate clade cephalochordates. Thanks to their phylogenetic position, cephalochordates serve as a reasonable proxy for studying the evolution of chordate-specific characters. Our aim was to get some insights into the evolution of light detection in chordates. Amphioxus possesses four morphologically distinct photoreceptor organs - frontal eye, lamellar body, Joseph cells and Hesse organs. From the point of the evolution of vertebrate vision, amphioxus frontal eye is the most interesting, since it is widely accepted as homolog of vertebrate eyes. Recently, it was shown that despite huge differences in their morphology, amphioxus frontal eye and vertebrate eyes share similar set of genes necessary for their development and physiological function. In our study we aimed on shedding new light on the development of frontal eye and its comparison with known facts about the development of vertebrate eyes. By chemical manipulation of BMP, FGF, Nodal, Notch, Shh and Wnt cascades we studied contribution of these signalling cascades to the development of frontal eye. Since all of the previously mentioned signalling cascades were shown to be involved in the development of vertebrate eyes, we would like to understand what the conserved chordate features are and what is specific only for the development of frontal eye or vertebrate eyes, respectively.

T39.

Hyoid breathing: Heterochrony underlies development of larval adaptive structures

Jan Stundl[1,2], Anna Pospisilova[1], David Jandzik[1], Peter Fabian[1], Barbora Dobiasova[1], Martin Minarik[1], Brian D. Metscher[3], Vladimir Soukup[1] and Robert Cerny[1]

[1] *Department of Zoology, Charles University in Prague, Czech Republic*
[2] *Department of Zoology, National Museum, Prague, Czech Republic*
[3] *Department of Theoretical Biology, University of Vienna, Vienna, Austria*

The vertebrate head is an extremely evolvable part of the body, and this evolvability is, to a large extent, driven by the plasticity of its constituent parts producing features that, over time, may become adaptive to the environment. The developmental cues standing behind the origin of these features are however largely unexplored. We set out to analyze the morphogenesis of adaptive structures of the vertebrate head, the external gills of the ray-finned fish, bichir. We show that these structures are associated, uniquely among extant vertebrates, with the hyoid arch and are one of the first functional organs of early larvae. The accelerated appearance of these organs results from the heterochronic development of tissues of the embryonic hyoid segment. More specifically, the conserved anteroposterior sequence in pharyngeal development, typical for other vertebrates, is disrupted by premature morphogenesis and differentiation of hyoid neural crest, mesoderm, and endoderm. The orchestrated heterochronic differentiation of tissues of the hyoid segment accounts for the premature development and early establishment of this larval adaptive organ. Our findings thus show a substantial alteration to the serially arranged ground plan of vertebrate head development and highlight the role of heterochrony in the evolution of adaptive structures.

T40.

The mechanism and evolution of positional signalling in plants – understanding the DEK1 pathway

Viktor Demko[1]

[1] *Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovak Republic*

Positional signalling plays instrumental role during plant growth and development. Positional cues control what type of cell will form (cell fate determination) and how big the cell will grow (cell expansion). We focus on the membrane-anchored calpain protease DEFECTIVE KERNEL 1 (DEK1), an essential regulator of plant growth and development. Our hypothesis is that DEK1 evolved both sensory and effector functions to control robust morphogenetic outputs such as cell expansion, asymmetric cell division and cell fate determination in response to positional cues. The exact mechanism of DEK1 activation and signal transmission is unknown. In Arabidopsis thaliana, DEK1 is essential for embryonic protoderm determination and maintenance of epidermal layers throughout the development. In the moss Physcomitrella patens we showed that DEK1 is essential for developmental transition from filamentous growth to 3D growth via asymmetric cell division control. In this presentation, I shall discuss our current efforts to understand the DEK1 pathway. We combine genetics, cell biology, proteomics, transcriptomics and structural biology in order to determine the mechanism of DEK1 activation and to identify potential downstream-acting effectors in the pathway.

Acknowledgement: This project is supported by the Slovak Research and Development Agency grant APVV-17-0570.

T41. KEYNOTE TALK 7

Pancreatic β-cell regeneration in zebrafish

Olov Andersson[1]

[1] *Karolinska Institutet, Stockholm, Sweden*

Processes such as β-cell regeneration are hard to replicate *in vitro* since the endogenous micro-environment most likely plays an important role in regeneration *in vivo*. We previously generated a zebrafish model in which insulin-expressing cells are conditionally targeted for ablation by using tissue specific expression of nitroreductase. This model provides a unique opportunity to take a 'high-throughput chemical' approach to identify small molecules that can enhance β-cell regeneration. In the mouse, a screen for small-molecule modulators of β-cell regeneration would be prohibitively time consuming, whereas in the zebrafish the analysis is simplified by the fact that zebrafish embryos have only one endocrine islet and can fit into 96-well plates. We are currently performing targeted screens for different cellular mechanisms of β-cell regeneration, as well as validating our putative hits. Several small molecules affect both regeneration of β-cells and subsequent recovery of normoglycemia in zebrafish. The increased regenerative response is mainly mediated through either neogenesis of β-cells or proliferation of newly formed β-cells. In support of this notion, several of the identified compounds enhance differentiation of mouse ES cells to β-cells or proliferation of mouse β-cells. In sum, this project aims to identify drugs that enhance regeneration of β-cells and could provide new avenues for the development of therapeutics for diabetes.

T42.

How do the continuously growing teeth regenerate?

Jan Krivanek[1,2], Ruslan A. Soldatov[3], Marie Landova[4], Bernadette Luza[5], Maria-Eleni Kastriti[6], Anna Nele Herdina[6], Brian D. Metscher[7], Marc Bajenoff[8], Peter V. Kharchenko[3], Kaj Fried[9] and Igor Adameyko[1,6]

[1] *Department of Molecular Neurosciences, Center for Brain Research, Medical University Vienna, Vienna, Austria*
[2] *Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[3] *Department of Biomedical Informatics, Harvard Medical School, Boston, MA, USA*
[4] *Institute of Animal Physiology and Genetics, v.v.i., Academy of Science of the Czech Republic, Brno, Czech Republic*
[5] *Center for Molecular Biology, University of Vienna, Vienna, Austria*
[6] *Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*
[7] *Department of Theoretical Biology, University of Vienna, Vienna, Austria*
[8] *Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, Inserm, CNRS, Marseille, France*
[9] *Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden*

Mouse incisor a continuously growing tooth serves for many decades as an excellent model system for studying stem cell niche, developmental and differentiation pathways and importantly to study mutually interdependent epithelial-mesenchymal interactions. In our study we used single cell transcriptomics method and revealing gene expression patterns in each single selected cell building such a teeth. We analysed vast transcriptomics data by elegant algorithms, produce user friendly graphical database which enabled us to distinguish different cell (sub)types, cluster them into different (sub)families and run multiple analyses using these data. Our results show expression of various genes in different cell types which can be find in tooth. Based on these findings we can explain complicated interactomic pathways between distinct cell types, suggest developmental pathways responsible for differentiation of stem cells through transit-amplifying cells into pulp cells, odontoblasts or ameloblasts and moreover to follow their aging from maturation stage until apoptosis using knowledge of their transcriptome (including of expression changes of multiple transcription factors, receptors or ligands) in different intermediate stages and finally to find new cell types in silico and subsequently validate the results in vivo. Our findings provides deeper understanding of cell heterogeneity and homeostasis in teeth and suggest a new direction of how to decipher physiology of continuously growing incisors in unprecedented details.

T43.

Role of nitric oxide during wound healing

Radek Sindelka[1], Pavel Abaffy[1], Silvie Tomankova[1], Ravindra Naraine[1] and Mikael Kubista[1]

[1] *Institute of Biotechnology, Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic*

Wound healing and regeneration are complicated processes regulated by various signaling factors including small gaseous molecules. Reactive oxygen species, hydrogen peroxide and nitric oxide (NO) are crucial components of the inflammation phase in the early stages of adult wound healing. *Xenopus laevis* embryo is a prime model for embryonic healing studies in our laboratory. We have found NO production in epidermal tissue during embryonic development and its level is rapidly increased in healing and regenerating tissues at the early developmental stages. In addition, wound healing and tail regeneration is blocked/slowed down in embryos with inhibited NO production. Our goal is to determine NO function and its downstream targets during wound healing. Here we present several genes, which expression depend on NO production and mechanism of NO function during healing and regeneration.

T44.

An ancient pathway of the initiation of meiotic DNA repair present in *Mus musculus*

Zdenek Trachtulec[1], Ondrej Mihola[1], Florencia Pratto[2], Kevin Brick[2], Petr Flachs[1] and R. Daniel Camerini-Otero[2]

[1] Division BIOCEV, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
[2] National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA

In order to be fertile and reshuffle their alleles, eukaryotes produce germ-line-specific programmed double-strand DNA breaks during meiotic recombination. Histone-methyltransferase PRDM9 (PR/SET-domain 9) determines the positions (hotspots) of these DNA breaks initiating meiotic recombination in the mouse, human, and cattle. Due to high variability in the DNA-binding domain of PRDM9, the hotspots positions differ even among individuals in these species. Deletion of the *Prdm9* gene from the laboratory mouse induces a shift of the recombination initiation hotspots to other sites including promoters and a complete arrest of meiosis. However, dog, finch, swordfish, and *Saccharomyces* yeast that lack PRDM9 have recombination hotspots often located near promoters. Moreover, a human individual that produced offspring without PRDM9 function has been identified. We therefore investigated if PRDM9 is required for meiosis in wild-derived mouse of the subspecies *Mus m. musculus*. Despite decreased fertility in comparison to littermate controls and relocation of recombination hotspots to other sites including promoters, *Prdm9*-deficient *Mus m. musculus* males produced sperm. Some mice can thus still use the ancient pathway of meiotic DNA repair employed until today by some other eukaryotes. These *Mus m. musculus* males can be utilized to resolve the key differences between the ancient and PRDM9-directed modes of meiotic recombination initiation. Moreover, they are a great model of human germ cell development due to their capability to accomplish meiosis using both PRDM9-determined and other recombination sites.

T45.

3D bioprinting of stem cells and their microenvironment

Josef Jaros[1,2], Jakub Pospisil[1], Karolina Spustova[1], Josef Lavicky[1], Vaclav Chochola[1], Mario Kandra[2], Veronika Valouchova[1], Richard Mackovic[1], Vladimír Proks[3] and Ales Hampl[1,2]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] Cell and Tissue Regeneration, International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic
[3] Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic

Human pluripotent stem cells plays crucial role for studies of embryonic development, morphogenesis, and regenerative medicine and their microenvironment is vital for their expansion and differentiation. Stem cell niche is composed by proteins of the extracellular matrix, cell junctions and soluble factors. All these molecules influence wide spectrum of cellular responses.
To understand these stimuli, we developed unique synthetic environment, where we immobilize specific ligand(s) of protein(s) of our interest. We analyzed and successfully defined several of peptides, their concentration and distribution for hPSC adhesion, expansion and long-term cultivation. However standard planar cultivation represents biologically artificial conditions, as the cells adhere just from one side, nutrients from the other side, cell-cell contact is performed in thin region, etc.
To bring cells into three-dimensional organization, we utilized 3D extrusion for printing of stem cells and hydrogels. We control spatial distance and organization of printed cells as well as cellular aggregates. We build simple structures as well as complex 3D objects with tens of micrometers resolution. Moreover we modify physio-chemical properties of hydrogels (natural and synthetic) and with these parameters we are prepared to induce mechanical and biochemical response of cells. We observe and analyze cell migration, expansion, growth, interaction and coupling into printed shapes.
Finally we started to print simple tissue structures to address cell organization and behavior in natural tissues.

Acknowledgement: This project was supported by grants no. LQ1605 from the National Program of Sustainability II (MEYS CR), by Czech grant foundation GACR16-02702S and the project from Masaryk University (MUNI/A/1369/2016, MUNI/A/1298/2017).

T46. KEYNOTE TALK 8

Development of the human ear canal sheds light on external ear defects in patients

Juan M. Fons Romero[1], Mona Mozzafari[1], Dean Malik[1] and Abigail S. Tucker[1]

[1] Centre for Craniofacial & Regenerative Biology, Department of Craniofacial Development and Stem Cell Biology, King's College London, London, UK

The ear is a complex structure formed from an integration of external, middle and inner ears. Defects in one part of the ear influence neighbouring structures and lead to hearing loss and deafness. Here we have investigated development of the external ear canal, following its development in both mouse and human embryos. The ear canal undergoes a complex system of closure and reopening during its development. The more superficial part of the canal was shown to form from an originally open primary canal, which later collapsed and then reopened. In contrast, the deeper part of the canal formed from a solid meatal plate that extended from the primary canal and later opened. This dual mechanism of canal formation was conserved across mammals, but was distinctly different from ear canal development in non-mammalian vertebrates. As the ear canal developed, the different parts showed distinct patterns of gene expression and proliferation. Interestingly, the forming human ear canal epithelium expressed HOXA2, suggesting a direct role for this gene in human ear canal extension, and explaining the ear canal defects observed in patients with Hoxa2 mutations. Understanding canal development can therefore shed light on the underlying causes of canal defects.

T47.

Transgenerational mouse obesity model – an ideal choice for reproductive and developmental studies

Dusan Fabian[1], Janka Babelova[1], Zuzana Sefcikova[1], Martina Ksinanova[1] and Stefan Cikos[1]

[1] *Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Slovak Republic*

A transgenerational dietetic model producing adult female mice with obesity-like phenotype was used for the study of the effect of altered maternal body condition on the development of in vivo–derived oocytes, zygotes and preimplantation embryos. Model is based on over-nutrition of experimental animals during intrauterine and early postnatal development and possesses several advantages: it produces obese animals with stable and long-lasting body condition (in standard diet-induced obesity models, after removal of the high-fat diet, the body weight of experimental animals frequently decreases); it allows to study the effect body condition minimalizing the impact of actual nutrition (during experiments, both control and obese animals are fed the same type diet, the only difference is in their caloric intake); and it allows to study the effect body condition in juvenile animals and animals at early adulthood age. In standard diet-induced obesity model, it usually takes several weeks (up to 16) to breed obese animals, while the mice continue aging. Since aging is accompanied with significant decrease in reproductive abilities of animals as well as decrease in oocyte and embryo quality, the last listed advantage makes transgenerational model an ideal for reproductive and developmental studies. However, it does not lead to production of mice with the amount of body fat overcoming 15%. Results of the study showed that maternal overweight might adversely disturb the physiology of several events occurring during oocyte maturation and early embryo development.

Acknowledgement: Work was supported by national grants VEGA 2/0039/15 and VEGA 2/0001/14.

T48.

Defective pseudouridylation results in impaired differentiation during zebrafish development

Mate Varga[1,2], Eszter Balogh[2,3], Renata Hamar[1], Regina Legradi[2,3], Dora K. Menyhard[4] and Kalman Tory[2,3]

[1] Department of Genetics, Eötvös Loránd University, Budapest, Hungary
[2] MTA-SE "Lendület" Nephrogenetic Research Laboratory, Budapest, Hungary
[3] 1st Department of Paediatrics, Semmelweis University, Budapest, Hungary
[4] MTA-ELTE Protein Model. Res. Group and Laboratory of Structural Chemistry and Biology, Eötvös Loránd University, Budapest, Hungary

One of the most important post-transcriptional modification of RNA molecules in eukaryotic cells is pseudouridylation catalyzed by the multifunctional Dyskerin enzyme. Interestingly, despite its abundance and importance, we still know very little about the role of this modification during cell function. Previous results suggest that the pseudouridylation of ribosomal RNA will result in faulty ribosome function that would manifest as a classic ribosomopathy. As the pathogenesis of this group of diseases is still mostly unexplored, dissecting the function of Dyskerin will be an important step towards their possible treatment. To get a holistic view on the function of this enzyme, in a recently initiated collaborative work we started to probe the function of the molecule from the organismal to the quantum level. On the organismal level we created an allelic series in zebrafish for the *dkc1* gene (the fish gene encoding Dyskerin), and we started the molecular and phenotypic characterization of these mutant lines. We were able to show that the absence of *dkc1* function results in very peculiar developmental abnormalities, related to impaired cell differentiation. Whole transcriptome analysis suggests that the upregulation of specific *tp53* isoforms could explain the observed cell-cycle blockade. We speculate that the mutant phenotype is probably due to errors in rRNA biogenesis, which results in defective ribosomes and the ribosomopathic phenotype is related to erroneous IRES-recognition.

T49.

miR-183 cluster: a microRNA family regulated by light in human retinal organoids

Tomas Barta[1], Denisa Jurcikova[1], Lucie Peskova[1], Livia Knapova[1] and Ales Hampl[1,2]

[1] Deparment of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] International Clinical Research Center, St. Anne´s University Hospital Brno, Brno, Czech Republic

Recent progress in human pluripotent stem (hPS) cell research has enabled *in vitro* differentiation of hPS cells into retinal tissues providing a valuable tool for retinal therapies, human retinal developmental studies, and drug testing. During differentiation of hPS cells numerous distinct microRNAs are dynamically expressed in tissue-specific manner and control diverse biological processes including cell fate specification, yet their contribution to the regulation of development and function of human retina is not fully elucidated. In this project, we study the role of light-regulated miR-183 cluster on differentiation and function of hPS cells-derived retinal organoids. We use a novel approach of optical stimulation in combination with genetic manipulation to study miR-183 cluster role in differentiating human retina. The proposed strategy combines the innovative optogenetic approach with stem cell biology research and opens up the possibility to understand molecular mechanisms underlying development and function of human retina with potential impact on regenerative medicine.

Acknowledgement: This study was supported by GJI6-24004Y (Czech Science Foundation), Brno Ph.D. Talent and supported by funds from the Faculty of Medicine MU to junior researcher TB.

T50.

Regulation of ciliary function by fibroblast growth factor signaling identifies FGFR3-related disorders achondroplasia and thanatophoric dysplasia as ciliopathies

Michaela Kunova Bosakova[1,2], Miroslav Varecha[1,2], Bohumil Fafilek[1,2], Alexandru Nita[1], Tomas Gregor[3], Iva Gudernova[1], Marketa Tomanova[1], Tomas Barta[4], Lukas Balek[1], Marcela Buchtova[5,6], Marek Hampl[5,6], Hana Dosedelova[6], David Potesil[3], Ivan Duran[7], Jennifer Zieba[7], Jieun Song[10], Yangli Xie[11], Zhenhong Ni[11], Peter Konik[12], Lukas Trantirek[3], Zbynek Zdrahal[3], Gert Jansen[13], Zheng Fu[14], Lin Chen[11], Hyuk Wan Ko[10], Ales Hampl[2,4], Deborah Krakow[7,8,9], Pavel Krejci[1,2]

[1]Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic
[3]Central European Institute of Technology, Masaryk University, Brno, Czech Republic
[4]Department of Histology and Embryology, Masaryk University, Brno, Czech Republic
[5]Institute of Experimental Biology, Faculty of Sciences, Masaryk University, Brno, Czech Republic
[6]Institute of Animal Physiology and Genetics AS CR, 60200 Brno, Czech Republic
[7]Department of Orthopaedic Surgery, David Geffen School of Medicine at UCLA, Los Angeles, USA
[8]Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, USA
[9]Department of Obstetrics and Gynecology, David Geffen School of Medicine, Los Angeles, USA
[10]Department of Biochemistry, CLSB, Yonsei University, Seoul, Korea
[11]Department of Rehabilitation Medicine, Third Military Medical University, Chongqing, China
[12]Institute of Chemistry and Biochemistry, University of South Bohemia, Ceske Budejovice, CR
[13]Department of Cell Biology, Erasmus MC, Rotterdam, the Netherlands
[14]Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, USA

Cilia project from almost every cell integrating extracellular cues with signaling pathways. Constitutive activation of FGFR3 signaling produces the skeletal disorders achondroplasia (ACH) and thanatophoric dysplasia (TD), but many of the molecular mechanisms underlying these phenotypes remain unresolved. Here, we report *in vivo* evidence for significantly shortened primary cilia in ACH and TD cartilage growth plates. Our data demonstrate that transient versus constitutive FGFR activation produced differing cilia consequences. Transient FGF pathway activation elongated cilia, while constitutive activity shortened cilia. Employing a GFP-tagged IFT20 construct to measure intraflagellar (IFT) speed in cilia, we showed that FGF signaling affected IFT velocities, as well as modulating cilia-based Hedgehog signaling. Transient FGF signaling extended primary cilia via ERK MAP kinase and mTORC2 signaling, but not through mTORC1. To gain a further insight into molecular mechanisms by which FGFR regulates cilia length, we employed proteomic analysis of the complexes associated with the FGF receptor, FGFR3, where we identified the serine/threonine kinase Intestinal Cell Kinase (ICK), a conserved cilia regulator, as a FGFR interactor. Detailed mapping revealed protein segments responsible for FGFR-ICK interaction, and the ICK tyrosines phosphorylated by FGFR which we correlated with changes of the ICK activity and subcellular localization. Activation of FGFR affected both primary cilia length and function in a manner consistent with cilia effects caused by inhibition of ICK activity. Moreover, knockdown and knockout of ICK rescued the FGF-mediated effect on cilia. We provide conclusive evidence that FGF signaling controls cilia via ICK.

T51.

Pathophysiology of *Sprouty2* and its association with ciliopathies

Eva Hruba[1], Michaela Kavkova[2], Tomas Zikmund[2], Maria Hovorakova[4], Linda Dalecka[4], Miroslav Varecha[5], Pavel Krejci[5] and Marcela Buchtova[1,3]

[1] Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic
[2] CEITEC BUT, Brno University of Technology, Brno, Czech Republic
[3]Department of Experimental Biology, Masaryk University, Brno, Czech Republic
[4]Department of Developmental Biology, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic
[5] Department of Biology, Masaryk University, Brno, Czech Republic

Sprouty proteins are evolutionarily conserved intracellular modulators of receptor tyrosine kinases (RTK) signaling, including fibroblast growth factor receptor (FGFR) pathway. During embryonic development and postnatal bone remodeling, FGFRs play essential roles in regulating chondrogenesis, osteogenesis, and bone and mineral homeostasis. Sprouty2 has been shown to be implicated in endochondral bone formation in Sprouty2$^{-/-}$ mice with reduced postnatal skeletal size and trabecular bone mass. However, the underlying mechanism of Sprouty proteins´ action in skeletogenesis at the molecular level remains to be elucidated. Number of our skeletal and other phenotypic findings in Sprouty2 deficient mice strikingly resemble phenotype of mouse models bearing mutations in genes associated with primary cilia structure and function. Moreover, *in vivo* evidence for disruption of primary cilia length in achondroplasia and thanatophoric dysplasia cartilage growth plates with sustained activation of FGF signaling has been reported and new links between ciliopathies and FGF-related syndromes have been discovered recently. Our analysis of embryonic tissues revealed alterations in length of ciliary axonemes in Sprouty2$^{-/-}$ compared to corresponding wt tissues and expression of several genes associated with ciliogenesis were downregulated in tissues with missing Sprouty2. Therefore, we propose the hypothesis, that Sprouty2$^{-/-}$ mice may be actually ciliopathic in nature and Sprouty2 deficient mice represent valuable model for studying association of FGF signaling overactivation and primary cilia dysfunction.

Acknowledgement: This research was supported by the Czech Science Foundation (14-37368G) and by the Ministry of Education, Youth and Sports of the Czech Republic from the Operational Programme Research, Development and Education (CZ.02.1.01/0.0/0.0/15_003/0000460).

T52.

Rabbit as a model for inner cell mass and trophectoderm differentiation

Katarzyna Filimonow[1,&], Anna Stachowiak[1,&], Katarzyna Barlowska[1], Dawid Winiarczyk[1], Berenika Plusa[2] and Anna Piliszek[1]

[1] Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland
[2] Division of Developmental Biology and Medicine, University of Manchester, Manchester, UK
[&]these authors contributed equally

Early stages of embryonic development of placental mammals are the time of differentiation of the first cell lineages - pluripotent epiblast (Epi), and extra-embryonic lineages of primitive endoderm (PrE also called hypoblast) and trophectoderm (TE). The proper differentiation and segregation of these lineages is a prerequisite for further development, and abnormalities in this process may result in stunted development already at implantation. Here we present analysis of the first cell lineages differentiation in the rabbit embryos. We demonstrate that such factors as Cdx2 and Gata3, which are crucial for TE specification in the mouse, are absent at the initial stages of TE differentiation in the rabbit, and their expression is only detected after TE can be morphologically distinguished. To determine timing of lineage commitment we analyse differentiation of immunosurgically isolated inner cell masses, showing that they lose ability to regenerate TE soon after cavitation. We further demonstrate that the differentiation of inner cell mass into PrE and Epi strongly depends on the activity of the MEK / ERK signalling pathway, in a manner that partially differs from that previously described for mouse embryos. Namely, the activity of the MEK / ERK signal pathway is necessary and sufficient to differentiate the embryonic cells towards the PrE lineage, but inhibition of this pathway is not sufficient for their differentiation into the Epi lineage. Our research suggests that the rabbit embryos show a greater resemblance to placental mammals other than rodents.

Acknowledgement: This work was supported by National Science Centre (NCN) grant 2011/03/D/NZ3/03992.

Abstracts

Posters

P1.

Comparison the miRNA expression profile in chicken male and female primordial germ cells

Mahek Anand[1,2], Bence Lazar[2,3], Roland Toth[1,2], Eszter Patakine Varkonyi[3], Krisztina Liptoi[3] and Elen Gocza[2]

[1] SZIU, Doctoral School of Animal Husbandry Science, Gödöllő, Hungary
[2] NARIC, ABC, Animal Biotechnology Department, Gödöllő, Hungary
[3] Research Centre for Farm Animal Gene Conservation, Gödöllő, Hungary

Primordial germ cells (PGCs) are precursors of functional gametes in chickens. They are also potent pluripotent stem cells. PGCs can be easily obtained from the blood of chicken embryos and cultured *in vitro* for long time. MiRNAs are a class of non-coding RNAs. They are cited to play important role in regulating the stem cell renewal and pluripotency. They also regulate the stemness of PGCs. However, the exact molecular pathways along with the precise miRNA network regulating the pluripotency not fully elucidated. We established and characterized male and female chicken PGC lines. In order to identify this complex miRNA cascade that are responsible for the pluripotency network in male and female PGCs; a complex miRNA analysis was performed. The analysis identified novel miRNA signatures as well as class of miRNA differentially expressing in PGCs with reference to be specific to the genotype as well as depending upon the proliferation rate of PGCs in the cell culture. In this study, we identified the members of the miR-302 cluster, showing different expression in the fast and slowly proliferating PGCs cell lines. These results were followed up by qPCR analysis. In future, we would like to perform functional validation studies on the gga-miR-302 cluster via miRNA inhibition experiments and proliferation and apoptosis test to characterize the exact role of this gga-miR-302 cluster in PGCs.

P2.

The role of RecQ-family helicases in the genome maintenance of zebrafish

Tamas Annus[1], Dalma Muller[1], Mate Varga[1,2] and Mihaly Kovacs[3]

[1] Department of Genetics, Eötvös Loránd University, Budapest, Hungary
[2] MTA-SE Nephrogenetic Laboratory, 1st Department of Paediatrics, Semmelweis University, Budapest, Hungary
[3] Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary

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

In order to gain a better insight into the specific roles of RecQ helicases in the genome maintenance of vertebrates, we aim to create and characterize zebrafish models for mutations in the five RecQ helicase genes (*recql, blm, wrn, recql4* and *recql5*). We are also working on the development of a reporter construct that would allow us to quantify the incidence of homology directed repair (HDR) versus non-homologous end joining (NHEJ) events after induction of DNA lesions in the presence or absence of RecQ activity.

P3.

Can maternal obesity influence the effect of Fipronil on *in vitro* development of mouse preimplantation embryos?

Janka Babelova[1], Zuzana Sefcikova[1], Stefan Cikos[1], Juraj Koppel[1] and Dusan Fabian[1]

[1] *Centre of Biosciences, Slovak Academy of Sciences, Institute of Animal Physiology, Slovak Republic*

Our previous results suggest that Fipronil-induced changes during the preimplantation period of embryonic development could be considered as a potential risk factor in mammalian reproduction. The aim of this study was to evaluate the potential toxicity of Fipronil on development and quality of mouse preimplantation embryos in the context of actual physiological and metabolic status of embryo donors. During *in vitro* test, 2-cell stage embryos were isolated from spontaneously ovulated mice dams obtained from two groups of mice with different physiological and metabolic status: CN - "Normal" controls with physiologically normal body weight and body fat (7-8%) and EXF - Fat mice with significantly elevated body weight and fat (>11%). Embryos from CN and EXF groups were cultured in media with or without addition of Fipronil at 1 µM concentration until blastocysts formation. Stereomicroscopic examination of collected embryos showed that Fipronil supplementation negatively affected embryonic development of 2-cell embryos isolated from mice females and cultured for 96 h *in vitro* in both groups (CN, EXF). As shown by fluorescence staining, the sensitivity of mouse embryonic cells to the tested Fipronil concentration was confirmed by decreased cell proliferation in blastocysts derived from EXF group. However, the negative effect of 1 µM Fipronil on the qualitative parameters of blastocysts derived in CN group did not reach statistical significance. The obtained preliminary results indicate that embryotoxic potential of Fipronil could be influenced with respect to body condition and metabolic state of maternal organism.

Acknowledgement: Study was supported by the Slovak Research and Development Agency under contract APVV 14-0763.

P4.

The biological significance of the nuclear localization of an actin-binding cytoskeletal protein

Csaba Bajusz[1], Ildiko Kristo[1], Zoltan Kovacs[1], Peter Borkuti[1] and Peter Vilmos[1]

[1] Institute of Genetics, Biological Research Centre Hungarian Academy of Sciences, Szeged, Hungary

The members of Ezrin-Radixin-Moesin family of proteins play important role in cytoskeletal rearrangements. The majority of ERM proteins localize in the cytoplasm and to the cell cortex, however the Moesin protein of *Drosophila melanogaster*, the only representative of the ERM protein family in the fly, has been detected by our laboratory also in the cell nucleus. The cytosolic role of Moesin is well characterized but since its nuclear transport mechanism is unknown, the direct study of its nuclear function is not possible. We decided to overcome this problem with the CRISPR-Cas9 system. Our aim was to tag the *moesin* gene *in situ* with a nuclear export signal (NES). As a result, the Moesin-NES protein would be constantly cleared out from the nucleus while it can still perform its cytoplasmic functions. To achieve our goal, we co-injected Cas9 producing embryos with the gRNAs and the donor construct, then screened for succesful recombination. Four *Moe*[NES] mutant lines have been recovered. Their analysis revealed that the lack of nuclear Moesin causes various developmental defects and maternally inhibits germ cell development. Current results suggest that the loss of germ cells in the developing early embryo is most likely due to increased maternal mRNA level(s). Our experiments provide direct evidence to the importance of the actin binding and organizing activity of Moesin in the nucleus.

P5.

Phytoestrogenic Effect of Pomegranate on Human Ovarian Cells

Simona Baldovska[1], Katarina Michalcova[1], Marek Halenar[1], Angel A. Carbonell-Barrachina[2] and Adriana Kolesarova[1]

[1] *Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*
[2] *Department of Agro-Food Technology, Faculty of Experimental Sciences, University of Miguel Hernández, Orihuela (Alicante), Spain*

Pomegranate fruit is a reliable and rich source of bioactive compounds which has the health-promoting potential with strong antioxidant activity. Recent studies have demonstrated that the phytochemicals present in pomegranate may play an important role in decreasing the risk of chronic disease. Pomegranates have been shown to inhibit the growth of several types of cancer and contain phytoestrogens, which are structurally similar to 17ß-estradiol. This could be of interest for study ovarian functions. The aim of study was to determine the effect of dry pomegranate extract (Spain) at concentrations 0; 12,5; 25; 50; 100; 200 µg/ml on the viability of human ovarian carcinoma cell line (OVCAR-3), the viability of human ovarian granulosa cell line (HGL-5) and secretion of steroid hormones. The metabolic activity was evaluated by *alamarBlue™ cell viability* assay, the release of hormones was assayed by ELISA methods. The number of vital HGL-5 cells was significantly increased ($P \geq 0.05$) at the concentrations 12.5 µg/ml, but significantly decreased ($P \geq 0.001$) at concentrations 100 and 200 µg/ml compared to the control. In addition, all used concentrations of the extract led to significant ($P \leq 0.001$) inhibition of growth of OVCAR-3 cells. Our results indicated a significant ($P \leq 0.001$; $P \geq 0.05$) affected of the 17ß-estradiol secretion by extract at concentrations 12.5; 25; 50 µg/ml (increase) and 100; 200 µg/ml (decrease). On the other hand, level of progesterone was not significantly ($P \geq 0.05$) influenced. The understanding the mechanisms of action of phytocompounds is essential due to its effectiveness in regulation of steroidogenesis and reproduction functions, however, it warrants further studies.

Acknowledgement: This work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects VEGA 1/0039/16, KEGA 011SPU-4/2016, APVV-15-0543 and EU project no. 26220220180: Building Research Centre „AgroBioTech"

P6.

DNA damage checkpoints in early mammalian embryos

Vladimír Baran[1], Tomas Duricek[2], Michaela Vaskovicova[2], David Drutovic[2], Lucie Knoblochova[2], and Petr Solc[2]

[1] Centre of Biosciences - Inst. Animal Physiology SAS, Košice, Slovak Republic
[2] Institute of Animal Physiology and Genetics AS CR, Liběchov, Czech Republic

In somatic cells, DNA damage checkpoints are activated after increased level of double-strand DNA breaks (DSBs) following the delay cell cycle progression to provide time for DNA repair. Ineffective checkpoint signalling compromises the genome integrity and it may leads to uncontrolled cell divisions. Two pronuclei in 1-cell stage embryo (zygote), unobvious lengths of cell cycle stages and reprogramming of genome during the first two cell divisions make the cell cycles of the early mammalian embryos significantly different from somatic cells. It was shown that G1/S checkpoint is not active in zygotes and the zygotes arrest only in G2-phase after increased level of DSBs. Using confocal imaging of live and also fixed embryos we demonstrate that low level of DSBs, induced by short treatment with the radiomimetic drug Neocarzinostatin (NCS) in the first G1-phase, increases the incidence of chromosome fragments but do not activate checkpoints in zygotes. Although NCS-treated embryos form micronuclei in 2-cell stage very often, the onset and progress of the second S-phase is not affected suggesting the absence of the G1/S and intra S-phase checkpoints also in 2-cell stage embryos. Finally, NCS-treated embryos arrest in G2-phase of the 2-cell stage. In summary, our data suggest that G2-checkpoint in 2-cell embryo is the first active and robust DNA damage checkpoint during early mammalian development.

Acknowledgement: This work was supported by the project LO1609 from National Sustainability Programme of the Czech Ministry of Education, Youth and Sports and also by Strategy AV21, programme Qualitas. Sci project VEGA 2/0039/15 from Ministry of Education of Slovakia.

P7.

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

Melinda Bence[1], Ferenc Jankovics[1], Claudia Karina Nkubito[1], Laszlo Henn[2] and Miklos Erdelyi[1]

[1]*Institute of Genetics, Biological Research Centre, HAS, Szeged, Hungary*
[2]*Institute of Biochemistry, Biological Research Centre, HAS, Szeged, Hungary*

Sumoylation is a post-translational modification which attaches a small ubiquitin-like modifier (SUMO) to the target proteins, thereby modulating their functions. Similar to ubiquitination, sumoylation is catalyzed by E3 ligases. In the course of a large scale RNAi screen, we have identified the E3 SUMO-ligase *Su(var)2-10* gene as a key regulator of gonadal development in *Drosophila melanogaster*. Our purpose is to reveal the biological functions of *Su(var)2-10* in germ cell development. To determine which cell types and which stages of oogenesis are affected by the *Su(var)2-10*, we depleted its mRNA in the ovary using the GAL4>UASshRNA system. According to our results, depletion of *Su(var)2-10* mRNA in the germ line during the early developmental stages resulted in a highly penetrant rudimentary adult ovary. Detailed analysis of the germarium revealed that it does not contain stem cells and differentiating germ line cells. Contrarily, RNA silencing in the germ cells of later egg chambers did not influence the oogenesis. Furthermore, we demonstrated, that depletion of Su(var)2-10 in the somatic niche cells does not affect the proper niche formation and stem cell maintenance. However, *Su(var)2-10* silencing in the follicular cells of the ovary resulted in abnormal germaria, as the developing germline cysts failed to be encapsulated by follicle cells. Our study suggests that sumoylation exerts its function during the early stages of oogenesis and regulates key events of germ cell as well as follicular cell development in Drosophila ovary. Subsequent studies are aimed to reveal mechanisms by which sumoylation regulates oogenesis.

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

P8.

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

Pablo Bora[1], Lenka Gahurova (nee Veselovska)[1], Vasanth Thamodaran[1,2], Ravishankar Ram Mani[1] and Alexander W. Bruce[1]

[1] Department of Molecular Biology and Genetics, University of South Bohemia in Ceske Budejovice, Czech Republic
[2] Current affiliation: Centre for Stem Cell Research, Christian Medical College, Vellore, India

p38 MAPKs are classically defined as the effectors of stress-induced signalling pathways but are also associated with cell cycle regulation, survival, differentiation, senescence, development, tumorigenesis and immune responses, among others. In the pre-implantation mouse embryo, inhibition of p38 MAPKs from the 2-cell stage has been reported to cause reversible loss of filamentous actin and arrested development at the 8- and 16-cell stages. Inhibition at later stages (post-8-cell stage) has been associated with cavitation and hatching defects, partly associated with impaired fibroblast growth factor (Fgf) signalling and the abnormal expression and localisation of TJ Protein 1, Na/K-ATPase and Aquaporins within the trophectoderm (TE). Recently, our lab has identified p38α/β as occupying a nodal position in the differentiation of ICM cells, under the control of Fgf-signalling, towards PrE fate between E3.5 to E4.0 in the mouse blastocyst. Currently, we are resolving the temporal window of p38 function in this process and have identified a minimal ~3 hour window of required p38 activity. Furthermore, to identify which one, or combination of the four, expressed p38 paralogue(s) (i.e. p38α, p38β, p38γ and p38δ) are required, we are utilising the PiggyBAC cloning strategy to express both dominant-negative and constitutively-active mutant constructs of each p38 paralogue, in a doxycycline-inducible manner. Simultaneously, we are identifying downstream effectors of p38 function, using a combination of phosphoproteomic mass spectrometry and transcriptomic mRNA-Seq screens of p38α/β inhibited blastocysts. Collectively, these approaches aim to elucidate the mechanistic action of p38 function during the specification of PrE in the mouse blastocyst embryo.

P9.

Testing the biological significance of the nuclear localization of actin

Peter Borkuti[1], Csaba Bajusz[1], Ildiko Kristo[1], Zoltan Kovacs[1] and Peter Vilmos[1]

[1] *Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary*

In recent years it has become clear that actin is present also in the nucleus where it participates in fundamental nuclear events. The manifold and essential activities of actin in the cytoplasm hinder the direct analysis of its nuclear functions, therefore the biological significance of the nuclear localization is still unclear. To resolve this problem and to separate the cytoplasmic functions from the nuclear activities, we planned to equip the actin protein with a Nuclear Export Signal (NES), which ensures its constant clearing out from the nucleus without disturbing cytoplasmic functions. For this aim, first we generated a null mutant for *Act5C*. Sequencing, phenotyping and rescue experiments confirmed that we have successfully deleted the protein coding region of *Act5C*. Next, we created transgenic stocks carrying the entire *Act5C* gene with different modifications. In the present phase of our work we are performing rescue experiments of the null mutant. Mutants rescued with the NES-Act5c transgene help us to determine the physiological consequences of nuclear actin deficiency.

P10.

Incisor development in *Eda* mutants

Linda Dalecka[1,2], Lucie Horakova[1,3], Oldrich Zahradnicek[1] and Maria Hovorakova[1]

[1] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague 4, Czech Republic
[2] Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, Czech Republic
[3] Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

Ectodysplasin A (Eda) is a transmembrane protein of the TNF family which plays an important role in the development of ectodermal derivates, such as teeth. *Sonic hedgehog* (*Shh*) as a downstream of *Eda* is an important signalling molecule involved in the initiation of tooth development. We aim to evaluate the involvement of *Eda* gene during the development of mouse teeth and its relation to *Shh*. To elucidate this relationship in the early tooth development, we focused on *Shh* expression in *Eda* mutants with spontaneous mutation in *Eda* gene. Using epithelial dissociation and fluorescent microscopy, we performed comparative study of tooth development in *Eda* mutant and WT. According to our results, the development of teeth in *Eda* mutants seems to be approximately one day delayed. Using *Shh* whole-mount in situ hybridization in *Eda* mutant mice, we showed that there are two antero-posterior subsequently appearing *Shh* signalling areas during the lower incisor development similarly to what was published in WT mice. The early superficial anterior *Shh* signalling centre being responsible for the initiation of the posterior one appeared normally in *Eda* deficient mandible, but the successional later appearing posterior domain working as the signalling centre of the functional tooth was noticeably reduced. Based on this, we evaluated the level of SHH protein using western blot. According to our results, *Eda* gene with its protein seems to have a limiting function in the posterior *Shh* incisor centre formation. Substantial reduction of EDA protein level causes the reduction of the successional *Shh* expression.

Acknowledgement: This study was supported by the Czech Science Foundation (14-37368G).

P11.

Development of melatonin rhythm in the pineal gland of chick embryos incubated under different wavelength of light

Angelika Drozdova[1], Monika Okuliarova[1] and Michal Zeman[1]

[1] *Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*

Our previous data demonstrated that chick embryos incubated under polychromatic white light developed the circadian rhythm of melatonin production during the final stages of embryonic development, with high melatonin levels during the dark- and low levels during the light-time. It is not known if the embryonic pineal gland is selectively sensitive to different wavelengths of light, which can entrain circadian rhythms of melatonin production and influence embryonic development. In this study, broiler breeder eggs (Ross 308; n = 450) were incubated under the light-dark cycle 12L:12D using white, red, green or blue light. Pineal glands were excised, immediately frozen and stored at -20°C until melatonin measurement. Melatonin was measured in extracts of pineal glands by radioimmunoassay. Daily rhythm of pineal melatonin concentration in 20-day-old chick embryos was confirmed under all four wavelengths of light with expected higher pineal melatonin concentrations during the dark- than the light-time under all lighting conditions. The highest levels were measured in chick embryos incubated under red and white light and the lowest levels under blue light. Incubation under green light induced intermediate results. Melatonin synthesis increased significantly after hatching. We did not find differences in melatonin levels between 2-day-old chicks incubated and subsequently kept under white and green light. Our results showed a selective sensitivity of the chick embryo pineal gland to different wavelengths of light and possible implication on the development and welfare of broiler chicken will be further studied.

Acknowledgement: This study was supported by grants APVV-17-0371 and UK/135/2018.

P12.

Development of tooth-bone interface in reptiles

Jana Dumkova[1,2], Marie Landova[3], Oldrich Zahradnicek[4], Michaela Kavkova[5], Tomas Zikmund[5], Ales Hampl[2], Jozef Kaiser[5], Abigail S. Tucker[6] and Marcela Buchtova[1,3]

[1] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno,, Czech Republic
[2] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[3] Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic
[4] Department of Developmental Biology, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic
[5] Laboratory of Computed Tomography, CEITEC BUT, Brno, Czech Republic
[6] Department of Craniofacial Development and Stem Cell Research, King's College London, London, United Kingdom

Teeth are firmly anchored to the jaw bones during development of lepidosaurian reptiles. Several modes of ankylotic attachment evolved in this reptilian lineage. Teeth of the most of lizards and snakes are ankylosed to the inner side of the high labial wall (pleurodont ankylosis). However, lizards from Acrodonta clade (agamas and chameleons) have teeth fused to the crest of the tooth-bearing bone (acrodont ankylosis). The aim of our study was to reveal the developmental processes of formation tooth-bone interface in acrodont dentition. Chameleon (*Chamaeleo calyptratus*) was selected as a model species, which exhibits monophyodont (one generation of teeth) and acrodont type of dentition. During in ovo development, enamel organs of tooth germs grow asymmetrically with higher progress within the labial part of the cervical loop. Later in development, cervical loops were re-oriented towards to the bone ridge. Extracellular matrix (ECM) of dentin appeared analogical to ECM of bone, however, cells producing this matrix were clearly distinct. On the outer side of tooth germ, ameloblasts with discontinuous basal lamina sharply ended and ECM was more mineralized. On the inner side of tooth germ, the border between odontoblasts and osteoblasts was distinct only in electronograms. Outstanding mineralization of ECM in tooth-bone interface was observed similar to the outer side. In conclusion, acrodont dentition in its early development resembles pleurodont dentition, and tooth-bone interface displays exceptional mineralization in chameleon with acrodontly ankylosed teeth.

Acknowledgement: The project was supported by the Czech Science Foundation (project No. 17-14886S) and by the Mobility grant of the Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.2.69/0.0/0.0/16_027/0008502) to ML.

P13.

Inactivation of *Meis2* in neural crest cells results in craniofacial developmental defects

Jaroslav Fabik[1,2] and Ondrej Machon[1]

[1] *Department of Developmental Biology, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic*
[2] *Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Neural crest cells (NCC) represent a multipotent stem cell population that originates from lateral borders of the neural plate. After the formation of neural tube, NCC delaminate and migrate to distant parts of the embryonic body. Subsequently, these cells differentiate into various different types, including the connective tissues of head and neck, the peripheral nervous system and pigment cells. Inactivation of the transcription factor Meis2 in the mouse leads to a defective development of NCC derivatives. We aim to elucidate the cellular and molecular mechanisms leading to the abnormal development of the head and neck structures in Meis1 and Meis2 deficient embryos after tissue-specific conditional knock-out. We have investigated the expression of major determinants of NCC differentiation – Barx1, Dlx5, Gsc, Hand1, Hand2, Msx1, Pax3, Runx2, Sox9, Tbx1, Tbx2 and Twist1. Early in development, we detected an abnormal apoptosis in the pharyngeal arches along with a decreased expression of Gsc in the pharyngeal arch (PA) 1. Later, Meis2 mutants displayed an absence of submandibular glands and hypoplasia of palatal shelves and tongue. Immunohistochemical analysis revealed a decreased expression of Runx2 in palatal shelves and disorganised muscle fibers in the tongue. In addition, Meis2 mutants exhibited malformations of structures originating from the PA2, such as anomalous cartilaginous rod connecting lesser horns of the hyoid with styloid process of petrous bones. Meis2 knock-out also disrupted the cardiac outlow tract septation – the mutant embryos showed a persistent truncus arteriosus.

P14.

Expression and Regulations of p16/INK4A in Human Embryonic and Neural Stem Cells

Veronika Fedorova[1], Martin Barak[1], Lina Elrefae[1], Martina Petrasova[1] and Dasa Bohaciakova[1]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Human embryonic stem cells (hESCs) have the ability to differentiate and to unlimitedly self-renew without undergoing senescence. This is partially ensured by rapid cell division and specific cell cycle regulatory mechanisms. Importantly, length of G1 phase, and activity of specific cell cycle regulators determine the cell fate decision and differentiation. Here we aimed to study the cycle inhibitor p16/INK4A (p16), an important regulator of G1 phase transition that has been asocciated with cellular senescence and is frequently deregulated in human glioblastoma. Initially, we noticed that expression of p16 in hESCs is low in early passages (<40) and increases with high number of passages (>60). Surprisingly, the elevated level of p16 in cells of high passage does not affect their proliferation. Further analysis of this phenomenon, including methylation of p16 promoter, are currently being performed. Next we studied possible regulations of p16 expression in hESCs and in hESC-derived neural stem cells (NSCs). Our functional experiments suggest that regulations of p16 change upon differentiation of hESCs into NSCs. While p16 protein level increased in hESCs after inhibition of miRNA biogenesis, suggesting regulation by miRNA, in NSCs p16 seems to be modulated by proteasomal degradation. Finally, we also studied relationship between p16, p53, and Bmi1, oncogene necessary for self-renewal where we are uncovering interesting regulatory loops. Altogether, our results reveal novel expression patterns of protein p16 during early human development *in vitro* and point to several molecular pathways by which its expression is being regulated.

Acknowledgement: This study was supported by GAMU-Rector´s programme to V.F. (MUNI/C/1436/2017)), funds from the Faculty of Medicine MU to junior researcher D.B. (ROZV/25/LF/2017), MUNI/A/1369/2016, GACR GJ15-18316Y, GJ18-25429Y, and by the National Program of Sustainability II of the Ministry of education, Youth, and Sports of the Czech Republic (LQ1605).

P15.

EMT (epithelial-mesenchymal transition) in mouse blastocyst

Katarzyna Filimonow[1,2], Aneta Suwińska[2], Marek Maleszewski[2] and Berenika Płusa[3]

[1] Institute of Genetics and Animal Breeding, Polish Academy of Scienciesm Jastrzębiec, Poland
[2] Department of Embryology, Faculty of Biology, University of Warsaw, Warsaw, Poland
[3] Faculty of Biology, Medicine and Health, University of Manchester, Manchester, Poland

In the moment of implantation of mouse embryo in uterus primitive endoderm cells (PrE) are migrating away from ICM and form parietal endoderm. For last decades there was discussion in the field if EMT occurs at that moment in PrE cells or not. Here we show evidences that PrE cells are going through EMT process in the preimplantation stage of the embryo. We show presence of EMT markers like vimentin, Twist and downregulation of E-cadherin. In the near future we plan firther investigation of this process to characterise details of EMT in PrE lineage.

P16.

Blastomere removal from 8-cell stage embryos programs post-natal body weight, food intake and the level of leptin in mice

Wiesława Florek [1], Magdalena Kotlarska [1], Jacek A. Modlinski [1] and Silvestre Sampino [1]

[1] *Department of Experimental Embryology, Institute of Genetics and Animal Breeding, PAN, Jastrzębiec, Poland*

Blastomere Biopsy (BB) from 8-cell human embryos is commonly used for preimplantation genetic diagnosis (PGD) to select genetically healthy embryos before their transfer *in utero*. Little is known about the potential side effects induced by BB on the future health and wellbeing of the offspring obtained after PGD. Here, we used a mouse model to explore the long-term effects of BB on offspring's growth rates and food intake, as well as on the level of leptin - hormone which is known to regulate energy balance in mammals. C57Bl10 x CBA hybrid mice were obtained after embryo transfer from biopsed (BB), sham-biopsed (SB, blastomere removal followed by its re-introduction into the embryo) and only *in vitro* cultured (IVC) embryos. Body weight was measured in pups and adult offspring; food intake was measured in adults; leptin protein expression was measured in blood and fat tissues in adults by immunoassays. BB mice displayed increased body weight as well as increased food intake compared to SB and IVC controls. The expression of leptin in fat was increased in BB animals compared to controls. Conversely, systemic leptin levels were decreased in IVC mice compared to BB and SB. Obtained results demonstrate that single blastomere removal from preimplantation embryos induces obesity and hyperphagia, as well as altered hormonal regulation of energy homeostasis in mice. This suggests that PGD procedures could be a risk factor for late-onset metabolic disorders in offspring.

Acknowledgement: This research was supported by KNOW Leading National Centre Scientific Consortium "Healthy Animal-Safe Food".

P17.

Ciliary protein TMEM107 regulates rostro-caudal patterning in craniofacial development

Marek Hampl[1,2], Petra Cela Koliskova[1,3], Michaela Kavkova[4], Natalia A. Shylo[5] , Tomas Barta[6], Tomas Zikmund[4], Jozef Kaiser[4], Scott D. Weatherbee[5] and Marcela Buchtova[1,2]

[1] Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic
[2] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3] Department of Physiology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.
[4] Laboratory of Computed Tomography, CEITEC BUT, Brno, Czech Republic
[5] Department of Genetics, Yale University, School of Medicine, New Haven, CT, USA
[6] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Primary cilium is an immotile microtubule-based solitary organelle that emanates from cell surface. It is localized in one copy probably on each cell during the resting period and is considered to be important signaling center of the cell. The cilium consists of microtubular axoneme covered by cellular membrane, which is anchored to the cell through the basal body at the ciliary base. TMEM107 protein is expressed in the transition zone, which is located at the base of the cilium. This zone selectively transmits proteins and thus regulates protein composition, morphology and function of the cilium. Mutation in TMEM107 causes developmental defects in mice such as preaxial polydactyly, exencephaly, disrupted ventral neural tube patterning, cleft palate, and malformation of incisor tooth germs. Based on morphological observation, that the mutation causes defects only in incisors and not in molars, we decided to uncover differences in cellular response to Tmem107 deficiency in the rostral and caudal structures of the head. Primary mesenchymal embryonic fibroblast cell cultures were established separately from the rostral and caudal parts of palatal shelves and the mandible. Then, we transfected the cells either with Tmem107 overexpressing vector, or with anti Tmem107 shRNA. Next day, cells were treated with either FGF2, WNT3a, or WNT5a. Using immunocytochemistry and confocal microscopy, we analyzed the primary cilia length following the treatment. This comparison revealed differences in primary cilia response depending on presence or absence of TMEM107 protein and this reaction was region-specific.

Acknowledgement: This project was supported by the Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/15_003/0000460) to MB and by the Mobility grant of MEYS (CZ.02.2.69/0.0/0.0/16_027/0008502) to MH.

P18.

In vivo examination of membrane nanotubes in developing zebrafish embryos

Alexandra Hencz[1,*], Katalin Turmer[1,*], Miklos Nyitrai[1,2] and Edina Szabo-Meleg[1,2]

[1] *Department of Biophysics, Medical School, University of Pécs, Pécs, Hungary*
[2] *Szentágothai Research Centre, University of Pécs, Pécs, Hungary*
[*] *Equal contributions.*

Membrane nanotubes (NT) were first reported in 2004 between rat pheochromocytoma (PC12) cells. These actin-based cell protrusions seem to be channels for intercellular communication [1]. Membrane nanotubes were described both *in vitro* (in cell cultures) and *in vivo* (eg. between dendritic cells in mouse cornea [2] or between zebrafish embryos [3]). The mechanism of NT formation is not completely understood yet, nevertheless based on *in vitro* studies conducted on cell cultures, these structures can be developed as actin-driven membrane protrusions from directed, filopodium-like structures or they can be formed when migratory cells separate after close contact. Although convincing results are available about the presence of NTs *in vivo*, the important aspects of their growth and function need to be elucidated. Based on zebrafish studies NTs *in vivo* may play role not only cell migration, but also cell differentiation processes. Therefore, *in vivo* study of membrane nanotubes is an exciting and important research field nowadays. In our work, we focused on the *in vivo* characterization of membrane nanotubes in developing zebrafish embryos. For visualization unstained and DiI labelled pre-gastrulation phase embryos were used. Laser-scanning confocal microscope was applied to examine the NTs between the epiblast cells of the embryos. The characterization of membrane nanotubes shows that their length is approximately identical with the individual cell diameters, and their occurrence is pronounced in the animal pole of the embryo. Our data clearly support the *in vivo* existence of NTs, but further experiments are needed to reveal their possible function in zebrafish embryo.

References:

[1] Gerdes, H.-H. (2008) Intercellular transfer mediated by tunneling nanotubes. Curr Opin Cell Biol 20(4):470–475.
[2] Davis D.M., Sowinski S. (2008) Membrane nanotubes: dynamic long-distance connections between animal cells. Nature Reviews Molecular Cell Biology 9. 431–436.
[3] Caneparo L., Pantazis P., Dempsey W., Fraser S. E. (2011) Intercellular Bridges in Vertebrate Gastrulation. PLoS One 6(5):e20230.

P19.

A novel approach to investigate the effects of Histone post-translational modifications in *Drosophila*

Laszlo Henn[1], Andrea Abraham[1,2] and Imre Boros[1,2]

[1] Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
[2] Department of Biochemistry and Molecular Biology, University of Szeged, Hungary

The effects of histone post-translational modifications (PTMs) have been mostly revealed by indirect evidence so far. Since each canonical Histone protein is encoded by around one hundred gene copies, it is not possible to investigate the direct effects of histone mutations inhibiting or mimicking PTMs by classical alleles. *Drosophila melanogaster* is a unique model organism, where each Histone protein is encoded by multiple genes having the same nucleotide sequence. Therefore, it is possible to silence a given histone by RNAi. Here we present a novel approach where canonical histones can be replaced by the overexpression of their modified or mutated version on RNAi background. This method allows us to detect the direct phenotypic effect of histone point mutations in living animals.

Acknowledgement: OTKA K116372, GINOP-2.3.2-15-2016-00032

P20.

Characterization of cardiac transcription factor PLAGL1, a newly identified putative downstream target of RYBP

Surya Henry[1], Gergo Kovacs[1], Viktoria Szabo[1] and Melinda Katalin Pirity[1]

[1] Laboratory of Embryonic and Induced Pluripotent Stem Cells, Institute of Genetics, Biological Research Centre, Hungarian Academy of Science, Szeged, Hungary

RYBP (RING1 and YY1 Binding Protein) is a member of the non-canonical Polycomb Repressive Complex (ncPRC) with essential role in early embryonic development. We have previously reported that mouse embryonic stem (ES) cells lacking RYBP do not form beating cardiomyocytes *in vitro*. Transcriptome analyses revealed that the expression of several key cardiac transcription factors have altered in the RYBP null mutants including Pleiomorphic adenoma gene like 1 (PLAGL1), a key cardiac transcription factor affiliated to the formation of ventricular septum defects which leads to congenital heart disorders (CHD). To analyse the nature of the relationship between RYBP and its putative target *Plagl1*, and to gain more insight into the role of PLAGL1 in cardiac development; we (1) characterised PLAGL1 and its expression during the time course of *in vitro* cardiac differentiation and (2) defined its possible relationship with RYBP. Our findings showed that *Plagl1* has a complex genomic structure containing three promoter regions (P1,P2 and P3) and two non-coding RNAs (ncRNAs): *Hymai* & *Plagl1 I.T*. qRT-PCR analysis of *Hymai* and *Plagl1 I.T* revealed that the expression of these two ncRNAs were impaired in the *rybp*$^{-/-}$ cells. By using *in vitro* luciferase promoter assays, we have also demonstrated that RYBP was able to activate the P3 *Plagl1* promoter. Current work broadens our limited knowledge on how repressor complex members can also exert their functions as activators during embryonic development or early developmental disorders, like CHD.

Acknowledgement: This work is supported by GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00039.

P21.

From totipotency to pluripotency: non-invasive time-lapse imaging provides insight into initial stages of human life

Zuzana Holubcova[1,2], Martina Martonova[2], Darja Paralova[2], Drahomira Kyjovska[2], Tereza Klenkova[2] and Pavel Otevrel[2]

[1] *Masaryk University, Faculty of Medicine, Department of Histology and Embryology, Brno, Czech Republic*
[2] *Reprofit International, s.r.o., R&D Department, Brno, Czech Republic*

Despite all the achievements of assisted reproduction techniques we still know very little about early embryonic development in humans. Unravelling the complexity of human development poses a particular challenge due to technical, ethical and legal constraints. Introduction of non-invasive time-lapse imaging into IVF practice has shed a new light on early stages human embryogenesis. Due to technical advancements we can now observe how totipotent zygote develops into blastocyst in high resolution without compromising its developmental potential. Here, we seek to leverage the power of continuous monitoring and systematically analyse three-dimensional time lapse image data to provide comprehensive spatio-temporal map of earliest stages of human embryogenesis. Unlike other studies, we focus on embryos conceived using gametes donated by young and healthy donors with no history of infertility. Initial stages of development such as formation of a zygote and early cleavage can be tracked in unprecedented detail as well as dynamics of first cell fate decision events including morula compaction, blastulation and recruitment of cell population forming pluripotent inner cell mass. Previously described features as well as newly identified morpho-kinetic variables are correlated with embryo developmental competence, results of preimplantation genetic testing and clinical outcome. Improving our understanding of developmental program is orchestrated in human embryos is crucial not only for evidence-based (de)selection of embryos for fertility treatment but also for development of more efficient cloning techniques and refined protocols for derivation of human embryonic stem cells.

P22.

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

Karam Ibrahim[1], Ferenc Jankovics[1], Melinda Bence[1] and Miklos Erdelyi[1]

[1] *Institute of Genetics, Hungarian Academy of Sciences, Szeged, Hungary*

Germ cell are specialised to transmit the genetic information of the species to the next generation. Thus, protection of the germ cells genome against detrimental mutagenic effects is indispensable for the maintenance of the species. In the germ cells, a small RNA-based defence system, the so called piRNA pathway protects the genome against transposon induced mutagenesis. The piRNA pathway inhibits transposon activity by post-transcriptional gene silencing and by transcriptional silencing through heterochromatin formation at the transposon loci.The Drosophila *small ovary* (sov) gene was identified through a large scale RNAi screen to be important in germ line development. *Sov* mutant flies showed abnormal germ cell development accompanied by derepression of transposon mobility. Position effect variegation tests showed that *sov* is a variegation repressor, which suggests a positive regulatory function for *sov* in heterochromatin formation. In *sov* mutants, we detected by FRAP analysis an increased mobility of HP1, a key component of the heterochromatin, indicating that Sov supports the association between HP1 and the chromatin. Using RT-qPCR, we found that transposon derepression in *sov* mutants was not accompanied with a decreased production of piRNA precursors. Altogether, we propose that *sov* promotes transposon repression in the germline cells through the transcriptional gene silencing mechanism as a structural component of the heterochromatin.

P23.

Reprograming of translation during oogenesis and embryogenesis

Rajan Iyyappan[1], Anna Tetkova[1], Michal Kubelka[1] and Andrej Susor[1]

[1] *Institute of Animal Physiology and Genetics, CAS, Libechov, Czech Republic*

A subset of maternal transcripts is stored in a dormant state in the oocyte, and the timely driven translation of specific mRNAs guides meiotic progression, the oocyte-embryo transition, and early embryo development. In the absence of transcription, the regulation of gene expression in oocytes is controlled almost exclusively at the level of transcriptome and proteome stabilization and at the level of protein synthesis. Gene expression and cell cycle control are tightly controlled process in every cell. Recently, studies shows that, tightly controlled translational program occurring during the cell cycle, thus understanding the molecular mechanism of translational control through cell cycle assume very important. We analysed activity of positive translation initiation, elongation factors, and their repressors during interphase and M-phase of meiosis or mitosis of germ cells and early embryos. We found that number of key players in the translational machinery change their actions based on the cell cycle stage. Here we found that after resumption of oocyte meiosis or entering to the first and second mitosis of early mouse embryos, the translational program significantly reprograms to specifically regulate the expression of the subset of transcripts which are necessary for mammalian oogenesis and early embryo development.

P24.

Characterization of mutant alleles of Atg8 genes in *Drosophila melanogaster*

Andras Jipa[1,4], Szabolcs Takats[3], Agnes Varga[3], Viktor Vedelek[2], Adel Urmosi[1,4], Margaret Mukami[1], Rita Sinka[2], Gabor V. Horvath[1] and Gabor Juhasz[1,3]

[1] *Institute of Genetics, BRC HAS, Szeged, Hungary*
[2] *Department of Genetics, University of Szeged, Szeged, Hungary*
[3] *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary*
[4] *Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary*

Autophagy is a conserved intracellular degradation process in eukaryotic organisms. This catabolic pathway plays a very important role in cells: the continuous turnover of macromolecules and cell organelles is important for longevity, and it is essential in adaptation to nutrient-poor conditions during starvation. As the autophagy-related (Atg) genes were first described only about 26 years ago in yeast, we still have only limited information about their functions. Via molecular genetic, cell and developmental biology investigations of the *Drosophila* autophagy-related genes (*Atg*), we can clarify their roles. The main focus of my work is the investigation of *Atg8* genes in *Drosophila*. Atg8 is an ubiquitin-like protein that is conjugated to the lipid phosphatidylethanolamine (PE) on the phagophore membrane during autophagy. Only two Atg8 homologs are found in *Drosophila*: the *Atg8a* and *Atg8b* genes. To generate a null mutant allele of *Atg8a* we generated a gene trap construct. During the characterization of this new allele, we proved that the created allele functions as an *Atg8a* null mutant, and all homozygotes die during the pharate adult stage. Additionally, generate a nonsense mutant *Atg8a* allele encoding a truncated (G116stop) protein defective in PE conjugation. Animals expressing this non-lipidatable Atg8a are autophagy defective, but viable and fertile. As for *Atg8b*, it is not required for autophagy, and animals homozygous for this allele are adult viable and female fertile but nearly completely male sterile. *Atg8b* mutant sperm cells can develop until the latest stages of spermatogenesis but they are immobile, which explain the male sterility.

Acknowledgements: Lendület LP-2014/2, GINOP-2.3.2-15-2016-00006, GINOP 2.3.2.-15-2016-00032, OTKA 119842.

P25.

The role of Fgf4/MAPK signaling pathway in regulation of development of mouse chimeric embryo

Anna W. Kasperczuk[1], K. Wolukanis[1] and Aneta Suwinska[1]

[1] *Department of Embryology, Faculty of Biology, University of Warsaw, Warsaw, Poland*

Molecular mechanisms underlying developmental plasticity of the mouse embryo are not fully unraveled. Chimeric embryo may serve as a tool to study the regulative abilities of the embryo. Our previous research showed that in chimeric embryos constructed by microinjection of embryonic stem cells (ES cells) into the cleaving 8-cell mouse embryos the interactions between blastomeres of the host embryo and the introduced ES cells are responsible for the pattern of their cleavage and specification of the first cell lineages. Our results suggest the existence of a controlling mechanism, based on the communication between the cells of both components, which regulates the plasticity of chimeric mouse embryos. We aimed to determine whether Fgf4/MAPK signaling pathway is involved in these interactions. Using *Fgf4*$^{-/-}$ ES cells we showed that development of primitive endoderm (PE) of chimeric embryos, generated by injection of these cells into the 8-cell embryos, is heavily disrupted, leaving the inner cell mass comprised mainly of epiblast cells. Both the proportion of blastocysts containing PE cells and the number of these cells in *Fgf4*$^{-/-}$ ES cells-injected embryos were significantly reduced when compared with the embryos containing wild type ES cells. Moreover, we revealed that modulating the Fgf4/MAPK signaling by changing the levels of its components in the culture medium biases the direction of differentiation of cells contributing to the chimeric embryo. Ability to steer the fate of cells in chimeric embryo could contribute to optimization of the procedure of obtaining transgenic animals.

Acknowledgements: This work was supported by the grant from National Science Centre, Poland (2014/15/D/NZ3/02435).

P26.

Digital dissection of 3D model of mouse skeleton: x-ray micro computed tomography analysis

Michaela Kavkova[1], Eva Hruba[2], Tomas Zikmund[1], Marcela Buchtova[2], Marketa Novotna[1] and Jozef Kaiser[1]

[1] Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic
[2] Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic

The X-ray computed microtomography (micro CT) plays an important role in the 3D imaging of a large variety of biological samples of both soft and hard tissues. Micro CT imaging of fully mineralized adult bones has prior advantage over imaging of soft tissues – it is abscence of staining step, which is needed in soft tissues in order to make them properly radiopaque. Skipping of the staining step makes the whole process of imaging faster and avoids possible shrinking of the tissue. Micro CT is also nondestructive imaging method so the sample can be further analyzed by other techniques. Several bones and structures have been segmented for demonstration of possible outputs from micro CT data. Tissue segmentation and construction of accurate 3D models was possible due to the high-resolution tomographic sections in all coronal, sagittal and horizontal planes. Complex anatomical changes caused by mutations in cell signaling pathways involved in formation of bones or the neoplastic transformations of the bone is hard to display and analyze using only 2D methods. Applying fast and efficient methods such as micro CT imaging and subsequent 3D visualization can provide alternative approach to conventionally used methods and bring new perspective into developmental biology research.

Acknowledgement: This research was carried out under the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II and CEITEC Nano Research Infrastructure (MEYS CR, 2016–2019).

P27.

Deciphering effects of *Adar* on *Drosophila* metamorphosis

Anzer Khan[1], Nagraj Sambrani[1], Barbora Novakova[1], Mary A. O'Connell[1] and Liam P. Keegan[1]

[1] CEITEC , Masaryk University, Brno, Czech Republic

One of the most prevalent type of RNA editing is the conversion of adenosine to inosine in double-stranded RNAs that is mediated by adenosine deaminases acting on RNA (ADAR) enzymes. A→I RNA editing can lead to a codon change as the nucleoside inosine (I) is interpreted as guanosine (G) by ribosomes, resulting in a diversification of protein function. The ADAR family of proteins is present in all metazoans. In *Drosophila*, a single *Adar* is present on the distal X chromosome and is an orthologue of vertebrate *ADAR2*. In spite of major progress in the identification of editing sites, little is known about the regulatory mechanism of ADAR proteins in normal development and in disease. In this present study, we performed a genetic screen that has uncovered a novel effect of *Adar* on ecdysone signaling which is a crucial regulator of *Drosophila* development. Ubiquitous expression of *Adar* with the *act*5c-Gal4 driver results in pupal lethality with defects in ecdysis and head eversion. The lethality caused by ubiquitous expression of *Adar* can be rescued by blocking ecdysone synthesis and signaling. Tissue specific over-expression of *Adar* in the Prothoracic Gland (PG) with *phm*-Gal4 causes extended larval life with a long delay in pupation, with major reductions in prothoracic gland transcripts encoding the enzymes of ecdysone synthesis and signaling. These defects may be due to either aberrant RNA editing or RNA binding by ADAR protein. We hypothesize that *Adar* expression in *Drosophila* is a prerequisite to regulate ecdysone signaling during metamorphosis.

P28.

Gene expression profiling of anterior-posterior differences in early limb bud

Michael Killinger[1,2] and Marcela Buchtova[1,2]

[1] Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Brno, Czech Republic
[2] Department of Experimental Biology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

Limb development is characterized by strict regulation of patterning in all three axis. Anterior-posterior (thumb-pinkie)axis is known to be specify by the zone of polarizing activity, which establishes finger identity. However, detailed information about gene expression pattern during very early limb development is not well known and it is crucial for understanding of anterior-posterior patterning defects occurring in zeugopodial area. Here, we performed gene expression analysis of early stage of chicken forelimb bud to uncover regional differences before Shh expression onset and the establishment to the zone of polarizing activity.Using Chicken Genome Array (Affymetrix),we obtained gene expression data for anterior and posterior areas separately. Distinct pattern in gene expression of several signaling pathway was observed. Molecules associated with FGF pathway (Fgf5, Fgf13, Fgf16, Dusp5) were mostly expressed in the posterior area while only few of them were expressed in the anterior zone (Fgf23, Dusp7, Dusp8, Fgfr2). WNT members were also found to be expressed in specific areas of early limb bud with Apc, Wnt2, Wnt8b, Axin2 and Vangl2 to be located in the anterior area while Wnt9b, Fzd3, Dvl1 or Sfrp4 expressed in the posterior tissue. Interestingly, higher expression of genes associated with exocytosis and vesicular transport was detected in the posterior area (Syt9, Stx6, Stx19 and Gosr2). Increase expression of several genes associated with cytoskeletal activity was observed in the anterior tissue, including Rock2, Itga4, Itgb8, Mylk3, Dock1 or Apc. Only few cytoskeletal genes were highly expressed in the posterior area (Itga2, Bdkrb2, Mos, Diaph1).Moreover, genes associated with primary cilia function (Evc, Evc2, Gas1, Gli2, Cdon) were found to be expressed in the anterior limb bud. As our previous experiments discovered differences in primary cilia response in the anterior and posterior areas of early limb bud, our new data could provide answer how differential primary cilia regulation is controlled during early limb bud patterning.

Acknowledgement: This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic from the Operational Programme Research, Development and Education (CZ.02.1.01/0.0/0.0/15_003/0000460).

P29.

Polyspermy in bovine zygotes after SCF complex inhibition

Veronika Kinterova[1,2], Jiri Kanka[1], Veronika Petruskova[1,3] and Tereza Toralova[1]

[1] Laboratory of Developmental Biology, Institute of Animal Physiology and Genetics Academy of Science of Czech Republic, v.v.i., Libechov, Czech Republic
[2] Department of Veterinary Sciences, Czech University of Life Sciences in Prague, Prague, Czech Republic
[3] Faculty of Science, Charles University in Prague, Prague, Czech Republic

SCF complex, as one of the most abundant family of E3 ubiquitin-ligases, seems to play important role during the early embryogenesis. This complex consists of three invariant members: Cullin, Rbx1, Skp1 and one of the F-box protein family, which determines the substrate specificity. SCF complex is activated by neddylation, process in which is NEDD8 protein conjugated to Cul1. According to our previous results, SCF complex activity is necessary throughout the whole preimplantation development and its activity during oogenesis is also high. That's why we decided to inhibit this complex and see impact on in vitro fertilization. We inhibit this complex by cultivation of GV oocytes for 24 hours with MLN4924, an inhibitor of SCF ligases controlled by neddylation. Despite, we found no expansion of cumulus cells after this treatment, oocytes were able to be fertilized. After in vitro fertilization, the average rate of normally fertilized embryos in MLN4924 matured oocytes was significantly reduced to 22.00 ± 16.28 % (mean ± S.D.) compared to 71.57 ± 14.39 % in untreated group ($p=0.014$). An amount of unfertilized MLN4924 treated oocytes was significantly higher (26.2% ± 14.70) in comparison to untreated control (11.91% ±14.88) ($p<0.05$). Herewith, we observed significantly higher rate of polysperm embryos in MLN4924 treated oocytes (51.798 ± 20.721 %) in comparison to control group (16.52 ± 5.13 %; $p=0.016$).

Acknowledgement: This work was supported by CIGA 20172013.

P30.

The role of paracrine interactions involving FgfR1 and FgfR2 receptors in regulation of development of the preimplantation mouse embryo

Katarzyna Klimczewska[1], Aneta Suwinska[1] and Marek Maleszewski[1]

[1] Department of Embryology, Faculty of Biology, University of Warsaw, Warsaw, Poland

The aim of our study is to investigate whether paracrine interactions involving FgfR1 and FgfR2 receptors, which are the components of the Fgf4/MAPK signaling pathway, play an important role in regulation the development of the mouse embryo. To this end, we constructed a chimeric embryo composed of the 8-cell mouse embryo and inner cell mass (ICM) cells of E3.5 mouse blastocyst. To investigate whether both components communicate via Fgf4/MAPK transduction pathway, we blocked the possibility of receiving a Fgf4 signal, secreted by the ICM cells, through the suppression of *FgfR1* and/or *FgfR2* expression in the 8-cell embryo. We revealed that chimeric embryos, in which intercellular communication is not disturbed, can reconstruct normal blastocyst and undergo full development. However, the disturbance of intercellular interactions between the 8-cell embryo and ICM cells by the suppression of *FgfR2* results in impaired formation of cell lineages of the chimeric blastocyst. Moreover, we observed that the absence of *Fgfr2* does not reduce the number of primitive endoderm (PE) cells in blastocysts, whereas the suppression of *Fgfr1 at least* partially disrupts PE development. Our preliminary results indicate that FgfR1 and FgfR2 receptors cooperate with each other in PE lineage establishment within the ICM of the mouse blastocyst. We plan to verify how the suppression of *FgfR1* and both receptors at the same time will have impact on the normal embryogenesis of the chimeric embryo.

Acknowledgement: The project is financed by the grant PRELUDIUM 14 2017/27/N/NZ3/00625 from the National Science Centre (Poland).

P31.

The Role of CDC25A Phosphatasein Meiosis and First Embryonic Division in Mouse

Lucie Knoblochova[1], Ivana Ferencova[1] and Petr Solc[1]

[1] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libechov, Czech Republic

Cyclin-dependent kinases (CDKs) are important regulators of cell cycle progression. CDKs are modulated by many factors. Among these factors is family of CDC25 phosphatases, its members being CDC25A, CDC25B and CDC25C. We are interested in the role of CDC25A phosphatase in cell cycle progression in oocytes and early embryos in mouse. Using Cre-lox system, we have depleted Cdc25A gene specifically in oocytes (Cdc25A ooKO). We investigated the impact of Cdc25A depletion on meiosis and early embryonic development by long-term time-lapse confocal imaging, microinjection of fluorescently marked sensors, and immunofluorescence staining. Results from our model show that Cdc25A ooKO oocytes reach both meiosis I and meiosis II in the same rate as wild-type oocytes. Thereafter we focused on investigating cell cycle progression in Cdc25A ooKO early embryos. Cdc25A ooKO zygotes (with a Cdc25A wild-type paternal allele) divide into two-cell stage embryos after 5 hours delay, in comparison with wild-type embryos. These results indicate that CDC25A phosphatase does not have a major function in meiosis progression in mouse. On the other hand, the results show that Cdc25A phosphatase plays an important role in the first embryonic division in mouse. CDC25A phosphatase regulates both replication and mitosis progression through activation of CDK2 and CDK1, respectively. Presently, we are inspecting which CDC25A function is significant for the first mitosis in zygote.

Acknowledgement: This study has been supported by National Sustainability Programme, project No. LO1609 (Czech Ministry of Education, Youth and Sports) and by Charles University in Prague, project GA UK No. 1402217.

P32.

***Caenorhabditis elegans* SEL-5 kinase role in anterio-posterior cell outgrowth and migration**

Filip Knop[1] and Marie Macurkova[1]

[1] Laboratory of Molecular Genetics of Development, Department of Cell Biology, Charles University, Prague, Czech Republic

During nematode *Caenorhabditis elegans* development several cells migrate stereotypically and/or extend processes in a defined direction. Several signaling pathways regulate these events, among them the Wnt pathway. Q neuroblast descendants migration is one example of a Wnt-regulated migration. In search for novel regulators of this pathway we looked for enhancers of a weak retromer mutant *vps-29*. We uncovered a Ser/Thr kinase SEL-5 as a new regulator of the Wnt signalling pathway. *sel-5* mammalian homolog AP2-associated kinase (AAK1) was previously described as a regulator of clathrin-mediated endocytosis. GFP tagged SEL-5 kinase shows localization proximal to the plasma membrane. This suggests SEL-5 role in clathrine-mediated endocytosis regulation similarly to its mammalian counterpart AAK1. We have found that *sel-5* mutation strongly enhances the QL migration defect seen in *vps-29* mutants, suggesting a role for *sel-5* in Wnt signaling. Tissue-specific rescue experiments suggest a role of SEL-5 in the Wnt producing cells. We also found that *vps-29 sel-5* double mutant animals tend to have shortened excretory cell posterior canals. Moreover, we uncovered that Wnt signaling pathway components *lin-17/Fz*, *lin-44/Wnt* and *mig-14/Wls* show the opposite phenotype – an overgrowth of the excretory cell posterior canals. While Q neuroblast migration depends on the canonical Wnt signaling via EGL-20/Wnt, the excretory cell canals extension seems to be regulated by a non-canonical Wnt signaling via LIN-44/Wnt. This suggests a general role of SEL-5 kinase in multiple Wnt signaling pathways. In summary, we identified SEL-5 kinase as a new Wnt signaling regulator, that affects two distinct migrational and outgrowth events during *C. elegans* development.

Acknowledgement: This work was supported by Czech Science Foundation grant (16-17966Y) and Charles University Grant Agency (243-250951).

P33.

Detection of Lamin C2 in the mouse oocyte

Marketa Koncicka, Jakub Cervenka, Rita Sucha, Petr Vodicka, Michal Kubelka and Andrej Susor

[1] *Institute of Animal Physiology and Genetics, CAS, Libechov, Czech Republic*

Lamin C2 (LMN C2) is a shortest product of alternative splicing of the Lamin A/C gene. LMN C2 is germ cell specific lamin and previously has been extensively studied in the male germ cells. We found that despite of absence of mRNA coding for Lmn c2 in the mouse oocyte, the protein persist in the fully grown germinal vesicle stage oocyte, however this protein is not expressed in the cumulus cells. Expression of LMN C2 tagged with GFP showed localization on the nuclear membrane of the mouse oocyte. Our results obtained by western blotting and mass spectrometry clearly showed that LMN C2 is expressed in the fully grown mouse oocyte.

Acknowledgement: This project was supported by GACR (15–22765S), EXCELLENCE (CZ 02.1.01/0.0/0.0/15 _003/0000460), OP RDE and Institutional Research Concept RVO67985904.

P34.

Intraganglionic macrophages: a new population of cells in the enteric ganglia

Tamas Kovacs[1], David Dora[1], Csilla Barad[1] and Nandor Nagy[1]

[1] Semmelweis University; Department of Anatomy, Histology and Embryology, Hungary

The enteric nervous system (ENS) shares embryological, morphological, neurochemical, and functional features with the CNS. In addition to neurons and glia, the CNS includes a third component, microglia, which are functionally and immunophenotypically similar to macrophages, but a similar cell type has not previously been identified in enteric ganglia. In this study we identify a population of macrophages in the enteric ganglia, intermingling with the neurons and glia. These intraganglionic macrophages (IMs) are highly ramified and express the hematopoietic marker CD45, MHC class II antigen, and chB6, a marker specific for B cells and microglia in avians. These IMs do not express antigens typically associated with T cells or dendritic cells. The CD45+/ChB6+/MHCII+ signature supports a hematopoietic origin and this was confirmed using intestinal chimeras in GFP-transgenic chick embryos. The presence of GFP+/CD45+ cells in the intestinal graft's ENS confirms that IMs residing within enteric ganglia have a hematopoietic origin. IMs are also found in the ganglia of $CSF1R^{GFP}$ chicken and $CX3CR1^{GFP}$ mice. Based on the expression pattern and location of IMs in avians and rodents, we conclude that they represent a novel non-neural crest-derived microglia-like cell population within the enteric ganglia.

P35.

Analysis of the nuclear transport of the cytoskeletal Moesin protein

Zoltan Kovacs[1], Ildiko Kristo[1] and Peter Vilmos[1]

[1] Biological Research Center, HAS, Szeged, Hungary

The Moesin protein of *Drosophila* belongs to the family of evolutionary conserved actin-binding ERM (Ezrin-Radixin-Moesin) proteins, and it is the only family member in the fruit fly. Moesin's well-known function in the cytoplasm is to anchor the actin cytoskeleton to plasma membrane proteins. It was shown recently in our laboratory that Moesin localizes also to the interphase nucleus where the amount of the protein greatly increases under certain conditions. The molecular mechanisms through which Moesin enters and exits the nucleus are not known therefore, it is a reasonable assumption that the nuclear transport of Moesin is tightly linked to the dynamic nuclear shuttling of its primary binding partner, Actin. To gain deeper insight into the mechanism of nuclear Moesin transport we conducted Fluorescent Recovery After Photobleaching (FRAP) assays on Drosophila S2R+ cultured cells expressing fluorescently labeled Moesin protein. In these experiments confocal microscopy was used to bleach the entire nucleus and then to monitor the fluorescence recovery from the cytoplasm, which process reflects the nuclear import activity of Moesin. The import mechanism of Actin was analyzed by the same method earlier, therefore we used GFP-Actin as positive control. After the initial FRAP assays, we examined the nuclear import of Moesin under different conditions, such as inhibitng the nuclear mRNA export which causes the accumulation of the protein in the nucleus, or applying Jasplakinolide drug treatment which reduces the pool of free monomeric Actin. Our results suggest the the nuclear import of Moesin is an active process and it is independent from Actin.

Acknowledgements: This work was supported by the National Research, Development and Innovation Office – NKFIH (GINOP-2.3.2-15-2016-00001, GINOP-2.3.2-15-2016-00032 and PD127968). The research was supported also by the European Union, State of Hungary, and the European Social Fund in the framework of TÁMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program'.

P36.

Analysis of Cep164 interactome identifies KIF14 as novel regulator of ciliogenesis

Petra Kovarikova[1], Zbynek Zdrahal[2] and Lukas Cajanek[1]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] Research Group Proteomics, Central European Institute of Technology, Brno, Czech Republic

Primary cilium (PC) is an organelle, build on a surface of mammalian cells in G0 phase of the cell cycle. Defects in formation or maintenance of the PC can lead to a broad range of human diseases known as ciliopathies. In this work we follow up on study of Cep164 (Centrosomal Protein of 164kDa) key role in initial steps of ciliogenesis (1). First, we focused on screen for novel potential interacting partners of Cep164, structural protein of basal body distal appendages. Our goal was to validate obtained candidates and verify their role in process of PC formation. Candidate interactors of Cep164 were obtained by results intersection of two independent methods: proximity-dependent biotinylation (BioID) and coimmunoprecipitation (coIP) of endogenous bait in HEK293 (Human Embryonic Kidney) cell line, respectivelly. For validations and verifications were used RPE-1 (Retinal Pigment Epithelium) cells transfected with siRNA. Following proteomics analyses we shortlisted 5 potential interactors of Cep164. Their further analysis has uncovered, that depletion of Kif14 (Kinesin-like Protein KIF14) in RPE-1 cells causes ciliogenesis defects. Our subsequent follow up analyses based on combination of IF microscopy and live imaging has proposed a role of KIF14 in regulation of cilia initiation and elongation. Although protein Kif14 has so far been almost exclusively associated with process of cytokinesis (2), our study has revealed its novel role in ciliogenesis. Precise mechanism of how does Kif14 regulate ciliogenesis is currently being investigated.

References:

1. Čajánek L, Nigg EA. Cep164 triggers ciliogenesis by recruiting Tau tubulin kinase 2 to the mother centriole. Proc Natl Acad Sci U S A. 2014;111(28):E2841-2850.
2. Gruneberg U, Neef R, Li X, Chan EHY, Chalamalasetty RB, Nigg EA, et al. KIF14 and citron kinase act together to promote efficient cytokinesis. J Cell Biol. 2006;172(3):363–72.

P37.

Glutamic acid can influence development of preimplantation embryo in vitro

Veronika Kovarikova[1], Janka Babelova[1], Zuzana Sefcikova[1], Dusan Fabian[1], Juraj Koppel[1] and Stefan Cikos[1]

[1] *Centre of Biosciences, Slovak Academy of Sciences, Institute of Animal Physiology, Kosice, Slovak Republic*

Sodium glutamate is used in the food industry as a flavor enhancer with an umami taste that intensifies the meaty, savory flavor of food, as naturally occurring glutamate does in foods. Sodium glutamate is the sodium salt of glutamic acid (one of the most abundant naturally occurring non-essential amino acids), and orally received sodium glutamate is found in blood circulation as a glutamate anion. Beneficial effect of low concentrations of amino acids added into preimplantation embryo culture medium is well known, and we examined the effect of increasing concentration of glutamic acid on development and quality of mouse preimplantation embryos *in vitro*. Embryos were cultured in KSOM medium (except of glutamic acid and glutamine, no other amino acid was added, not to induce osmotic stress or increase ammonium level) from 2-cell stage for 72 hours. Embryos were divided into two groups: i) without addition of glutamic acid (control group), ii) with addition of glutamic acid at final concentrations 1mM, 2 mM, 5 mM and 10 mM. No significant influence of glutamic acid on development ability was found. Analysis of embryos at the blastocyst stage showed that glutamic acid at 2 mM concentration significantly decreased cell number in blastocysts Analysis of dead cell per blastocyst showed increased numbers of dead cells in 5 mM concentration of glutamic acid. These results showed that higher concentration of glutamic acid can negatively influence development of preimplantation embryo, and this effect is probably not connected with increased ammonium formation.

Acknowledgement: This work was supported by the Slovak Research an Development Agency project APVV-14-0763.

P38.

Loss of GRK2 produces asphyxiating thoracic dystrophy via impaired Hedgehog and canonical Wnt signaling

Michaela Kunova Bosakova[1,2], S. Paige Taylor[3], Lukas Balek[1], Ivan Duran[3], Jorge Martin[3], Miroslav Varecha[1], Eva Hruba[6], Jitka Krenova[1], Marcela Buchtova[6], Jiri Kohoutek[7], Tomasz Radaszkiewicz[8], Vitezslav Bryja[8], Deborah Nickerson[9,10], Michael Bamshad[9,10,11,12], Rajad Rohatgi[13], Daniel H. Cohn[3,14], Pavel Krejci[1, 2, 6], Deborah Krakow[3,4,5*]

[1]Department of Biology, Faculty of Medicine, Masaryk University, 62500 Brno, Czech Republic
[2]International Clinical Research Center, St. Anne's University Hospital, 65691 Brno, Czech Republic
[3]Departments of Orthopaedic Surgery, [4]Human Genetics, and [5]Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
[6]Institute of Animal Physiology and Genetics of the CAS, 60200 Brno, Czech Republic
[7]Veterinary Research Institute of the CAS, 62100 Brno, Czech Republic
[8]Institute of Experimental Biology, Masaryk University, 62500 Brno, Czech Republic
[9]Department of Genome Sciences, [10]University of Washington Center for Mendelian Genomics,
[11]Department of Pediatrics, University of Washington, Seattle, WA 98195, USA
[12]Division of Genetic Medicine, Seattle Children's Hospital, Seattle, WA 98105, USA
[13]Departments of Medicine and Biochemistry, Stanford University School of Medicine, Stanford, CA 94305, USA
[14]Department of Molecular Cell and Developmental Biology, University of California at Los Angeles, Los Angeles, CA 90095, USA

Mutations in genes affecting primary cilia cause ciliopathies, a diverse group of disorders often affecting skeletal development. This includes asphyxiating thoracic dystrophy (ATD) or Jeune syndrome, an autosomal recessive ciliopathy characterized by short and horizontal ribs, shortened long bones and occasional polydactyly. Unraveling the molecular pathology underlying ATD helps illuminate mechanisms responsible for functional primary cilia. We identified ATD caused by homozygosity for a nonsense mutation (p.R158*) in the gene encoding adrenergic receptor kinase 1 (*ADRBK1* or *GRK2*). The mutation resulted in loss of GRK2 transcript and protein, and markedly disrupted chondrocyte growth and differentiation in the cartilage growth plate of endochondral bones. *GRK2*$^{-/-}$ cells displayed normal cilia morphology and distribution of cilia component proteins, yet loss of GRK2 compromised response to Hedgehog (Hh) pathway activation due to defective translocation of the Hh receptor Smoothened (Smo) to the cilia. Canonical Wnt signaling was also impaired, manifested as a failure to respond to Wnt ligand due to impaired activating phosphorylation and total levels of the Wnt co-receptor LRP6. We identify GRK2 as an essential regulator of skeletogenesis, and demonstrate that Hh and now Wnt signaling mechanistically contribute to skeletal ciliopathies.

P39.

Regulation of complex tooth shape during reptile odontogenesis

Marie Landova[1,2], Oldrich Zahradnicek[3], Hana Dosedelova[1], Jana Dumkova[4], Michaela Kavkova[5], Tomas Zikmund[5], Jozef Kaiser[5], Abigail S. Tucker[6] and Marcela Buchtova[1,2]

[1] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, Czech Republic
[2] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3] Department of Developmental Biology, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic
[4] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[5] CEITEC, Brno, University of Technology, Brno, Czech Republic
[6] Department of Craniofacial Development and Stem Cell Biology, Dental Institute, King's College London, UK.

In mammals, tooth development and their shaping are regulated by signaling centres called enamel knots. They play important role in differential proliferation within the developing tooth germs. The number of enamel knots determines the number of future cusps and final shape of the tooth crown. However, the mechanisms of formation of reptilian dental structures such as crests and cusps is still not known. The aim of our study is to elucidate whether signalling centres appear also during reptilian tooth development and whether they are responsible for tooth shaping similar to mammals. We selected two squamate species to resolve this question: chameleon with simple shaped rostral teeth at the most rostral part of jaws and multicuspid teeth within the caudal part of the jaws, and gecko with unicuspid teeth and labial and lingual enamel ridges at the tip of each tooth. In both analyzed species, we determined the distinct morphological structure within the inner enamel epithelium resembling mammalian enamel knot. No proliferating cells and only few apoptotic cells were found in this area. At the cap stage, only one cluster of cells resembling the enamel knot of mice was observed within the inner enamel epithelium of enamel organ in both chameleon and gecko. The lateral cusps, which form later in chameleon, did not exhibit similar morphologically distinguishable structures. Strong expression of Shh and β-catenin was observed within the EK-like structure of both species. In contrast to cusps, ridges formation was associated with rearrangement of cells and cellular shape changes at later stages of odontogenesis. In squamate reptiles, the development of central tooth cusp seems to be driven by cluster of cells resembling the mammalian enamel knot. Tooth ridge formation is driven by dissimilar cellular mechanisms, which has to be further investigated.

Acknowledgement: This study was supported by the Czech Science Foundation (17-14886S).

P40

Expansion and response of human pluripotent stem cells in synthetic cell culture environment

Josef Lavicky[1], Jakub Pospisil[1], Ilya Kotelnikov[2], Vladimir Proks[2], Ales Hampl[1] and Josef Jaros[1]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic

The interaction between the human pluripotent stem cells (hPSC) and their microenvironment influence cell fate and behavior including proliferation, differentiation, migration, etc. Stem cell niche is represented by numerous extracellular matrix components, cell junctions and soluble factors.
In vitro hPSC cultivation is highly dependent on modification of culture surfaces, which are usually treated i) by supportive cell feeder layer or ii) by surrogate extracellular matrix (e.g. Matrigel). Both methods are expensive and batch-to-batch variable. Another way is to modify surfaces with synthetically prepared peptide motifs (ligands derived from proteins of ECM or to specific cell receptors), which provides advantage in low-cost preparation and precise surface concentration. Moreover these can give us biological answers to “how does a single peptide affect cell behavior” and “which signaling pathways are influenced”.
We developed a unique system combining covalently bound short amino acid sequencesonto a protein-repulsive brush made of PEG chains. This brush prevents the non-specific adhesion of cells and the adsorption of soluble proteins from the cell culture medium, so that adhesion of cells is provided just by offered peptide ligands.
We analyzed several ligands and concentrations. We found peptide motif RGDT successful for hPSC adhesion and long-term cultivation, as well as differentiation. We observed specific expression profiles of adhesion receptors by qPCR, western blot and immunofluorescent staining. In comparison with cultivation on Matrigel, hPSCs preserved similar proliferation rate and level of pluripotency, which confirms its possible application for hPSC xeno-free cultivation.

Acknowledgement: This work was supported by grants from: Grant agency of Czech Republic GA16-02702S, Grant Agency of Masaryk University (MUNI/A/1369/2016, MUNI/A/1298/2017).

P41.

Metabolic restriction influences phenotype of embryonic stem cell-derived cardiomyocytes?

Petra Lesakova[1], Katarzyna Anna Radaszkiewicz[1] and Jiri Pachernik[1]

[1] *Institute of Experimental Biology, Faculty of Sciences, Masaryk University, Brno, Czech Republic*

Pluripotent cells have the unique ability to form all cells of adult body including cardiomyocytes. Nowadays in the stem cell field, there is a number of effective protocols for cardiomyocyte differentiation from pluripotent stem cells. However, only a few of them focus on phenotype regulation in produced cardiomyocytes. Here, we show possible regulation of cardiomyocyte phenotype in vitro by metabolic restriction. Metabolic restriction, such as high concentration of lactate and absence of glucose, leads to purification of pluripotent stem cell-derived cardiomyocytes (ESdC) from mixed population of differentiating cells. High lactate concentration and glucose withdrawal reduce atrial-like and support ventricular-like phenotype of ESdC. In conclusion, our results document that modulation of cellular metabolism through metabolic restriction by eliminating of glucose and increasing lactate concentration enables us to regulate ESdC phenotype.

P42.

Age related differences in the translational landscape of mammalian oocytes

Edgar del Llano[1], Tomas Masek[2], Lenka Gahurova[1], Anna Tetkova[1], Denisa Jansova[1], Marketa Koncicka[1], Martin Pospisek[2], Andrej Susor[1] and Michal Kubelka[1]

[1] Institute of Animal Physiology and Genetics, Libechov, Czech Republic
[2] Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Czech Republic

Oocyte aneuploidy is the result of abnormal chromosome segregation during meiosis giving rise to a ready to be fertilized oocyte which, however, possesses an aberrant number of chromosomes. These anomalies are inherited by the future embryo drastically reducing its developmental potential. Furthermore, aneuploidy is not an infrequent event in mammalian oocytes but rather a common feature, the frequency of which increases in correlation with female age. Importantly, maturing oocytes are transcriptionally silent and rely on the utilization of a pool of mRNAs synthesized and stored during the growth period. We have applied a polysome fractionation method to isolate RNA population involved in the active translation during maturation from as little as 200 oocytes from both young and aged mouse females. After sequencing through Illumina NextGen, we have identified several genes with potential different expression levels between oocytes from young and aged females and therefore might intervene in age-related aneuploidy. Moreover, gene ontology enrichment analysis of polysome-bound RNAs reveal biological function categories related with translation initiation and regulation with higher incidence on oocytes from aged females. This results shed some light upon the reasons behind the genomic instability or loss of quality in oocytes from women of advanced age.

P43.

New cell dynamics during the enamel patterning using live cell imaging

Bernadette Luza[1], Jan Krivanek[2], Marie Landova[3] and Igor Adameyko[4,5]

[1] Center for Molecular Biology, University of Vienna, Vienna, Austria
[2] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[3] Institute of Animal Physiology and Genetics, v.v.i., Academy of Science of the Czech Republic, Brno, Czech Republic
[4] Department of Molecular Neurosciences, Center for Brain Research, Medical University Vienna, Vienna, Austria
[5] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

The continuously growing mouse incisor retains an epithelial stem cell niche. This so called labial cervical loop gives rise to the progenitors of enamel-producing ameloblasts. In contrast to the mouse molars as well as human dentition, mouse incisors do not lose the ability of enamel layer regeneration. Here, we investigated the dynamics of epithelial stem cells during tooth renewal using inducible lineage tracing combined with live cell imaging microscopy. Our aim was to visualize proliferation and migration of stem cells out of the labial cervical loop by taking advantage of this clonal color-coding technique using time lapse function over several hours. Furthermore, we are interested in pattern formation of the enamel layer by imaging movements of transit amplifying cells and ameloblasts. This study gives insights to cell dynamics of amelogenesis and provides solid ground for any further investigations in enamel patterning.

P44.

Evolution of gene network regulating gastrulation: Insights via *Chordin*

Simona Machacova[1], Zbynek Kozmik[1] and Iryna Kozmikova[1]

[1] *Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic*

To understand evolution of vertebrate program regulation gastrulation, it is necessary also to obtain knowledge about the development from animal lineages proxy to vertebrates. Cephalochordates (e.g., amphioxus) have simple body plan and belong to the clade chordates together with urochordates and vertebrates. For these reasons, the study of amphioxus is important for understanding of evolution of vertebrates. In all animals, gastrulation is controlled by a complex program, in which protein chordin plays an important coordinating role to form the dorso-ventral body axis in vertebrates. Nevertheless, the regulation of gene *Chordin* is not clear and the evolution of vertebrate genetic program regulating gastrulation remains vague. Concurrently, expression pattern of Chordin reliably marks the organizer of gastrulation, what allows the tracking changes after system disruption. We use combination of chemical manipulation of signaling pathways and transgenesis (knock-in of reporter gene driven by amphioxus regulatory sequence into vertebrate model, zebrafish) in order to study similarities and differences of *Chordin* regulation between the vertebrate and the invertebrate model. By taking advantage of comparisons regulatory systems in distinct animals, we can discuss possible evolutionary scenario of changes in gene network regulating dorso-ventral body axis in vertebrates.

P45.

Developmental potential of porcine oocytes from large and small follicles

Matej Murin[1], Ahmed Gad[1], Radek Prochazka[1] and Jozef Laurincik[1, 2,]

[1] The Czech Academy of Sciences, Institute of Animal Physiology and Genetics, Liběchov, Czech Republic
[2] Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, Nitra, Slovak Republic

It is very well known that developmental potential of porcine oocytes from small follicles (S group; 1-2mm) is worse than developmental potential of those from large follicles (L group; 3-6mm). In our experiments we have evaluated developmental potential of oocytes originated from these groups. We found small statistically significant difference between the diameter of these oocytes (L group=121.49±1.84µm vs. S group=117.72±0.98µm; P<0.05). Using Hoechst 33342 and Orcein staining we have analyzed the ability of the oocytes to reach metaphase II (MII) stage *in vitro*. According our results, first oocytes from L group reached MII stage after 32 hours of culture (16.82±3.18%). First oocytes from S group reached MII stage after 36 hours of culture (22.39±7.22%). Also, our results showed statistically significant difference in ability to reach MII stage after 44 hours of culture (L group 82.83±4.11% vs. S group 51.73±2.74%; P<0.001). Regarding the ability to reach 2-cell stage after parthenogenetic activation, we have not observed statistically significant difference (L group=59.84±6.58% vs. S group=52.84±8.10%; P>0.05). However, in ability to reach the blastocyst stage we have found statistically significant difference (L group 27.41±7.66% vs. S group 14.30±2.07%; P<0.05). In conclusion, our preliminary results indicate that the oocytes originated from L follicles have better maturation and developmental competence as compared to the S follicles.

Acknowledgement: This work was supported by Slovak Research and Development Agency under the contract No. APVV-14-0001, and also by the project "EXCELLENCE in molecular aspects of the early development of vertebrates", CZ.02.1.01/0.0/0.0/15_003/0000460 from the Operational Programme Research, Development and Education and was co-funded by the projects VEGA 1/0022/15 and VEGA 1/0327/16 and the European Community under the project No. 26220220180: Building Research Centre "Agrobiotech".

P46.

The role of transcription factors of Meis family during development of neural crest cells in *Danio rerio*

Petr Nickl[1] and Ondrej Machon[1]

[1] Department of Developmental Biology, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic

Neural crest cells (NCC) are a population of multi-potent embryonic cells that plays crucial role in formation of structures, such as cranial nerves, bones and cartilages, neurons and glia of the peripheral nervous system, melanocytes and enteric neurons. Our previous work on mouse model indicates that Meis transcription factors might be important for differentiation of NCC during embryogenesis. Apart from a mouse model, our experiment is conducted on a zebrafish model also. In previous morpholino experiments, we proved zebrafish to be convenient model organism for studying the function of Meis transcription factors within NCC in early embryogenesis, mainly because of its short generation time. The aim is to establish Meis1a and Meis1b double knock-out fish lineage using CRISPR/Cas9 technology. The double knock-out lineage would allow us to breed mutants and define the phenotype throughout generations. Main developmental abnormalities are expected in heart, craniofacial bones and cartilages.

P47.

Deficiency of Cdk13 leads to improper organogenesis and late embryonic lethality in mice

Monika Novakova[1], Marek Hampl[2,3], Jan Prochazka[4,r], S. Petrezselyova[4,5], M. Prochazkova[4,5], Radek Sedlacek[4,5], Michaela Kavkova[6], Tomas Zikmund[6], Jozef Kaiser[6], H.-C. Juan[7], M.-J. Fann[7], Marcela Buchtova[2,3] and Jiri Kohoutek[1]

[1] Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, Czech Republic
[2] Institute of Animal Physiology and Genetics, Brno, Czech Republic
[3] Institute of Experimental Biology, Faculty of Sciences, Masaryk University, Czech Republic
[4] Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics, Prague, Czech Republic
[5] Czech Centre for Phenogenomics, Institute of Molecular Genetics, Prague, Czech Republic
[6] CEITEC-Central European Institute of Technology, University of Technology, Brno, CR
[7] Department of Life Sciences, Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan, Republic of China

Cyclin dependent kinase 13 (Cdk13) is the serine/threonine kinase that in complex with cyclin K (CycK) phosphorylates RNA polymerase II in the carboxyl-terminal domain. Besides that, Cdk13 participates in the alternative splicing of mRNA, maintenance of stemness and axonal elongation suggesting its important role in gene regulation. Nevertheless, its precise function is still unknown. Recently, various CDK13 mutations were identified in patients with different congenital disorders, including heart defects, developmental delay and facial dysmorphism. Thus, our goal is to elucidate function of Cdk13 during mouse development and reveal its potential role in molecular mechanisms governing adverse phenotype observed in patients. We employed gene trap knock-out allele in *Cdk13* gene and observed embryonic lethality of homozygous Cdk13-deficient mice by the embryonic day E15.5, most likely due to heart failure followed by insufficient blood circulation. Moreover, developmental delay, growth retardation, kidney malformation and facial features were observed in Cdk13-deficient mice demonstrating similar phenotype as in patients with CDK13 mutations. Based on our results, Cdk13 is most likely important for transcription control of developmental regulatory genes in tissue specific manner in later stage of organogenesis. Using Western blot analysis, we identified higher expression of Cdk13 in E14.5 forelimbs and hindlimbs; using Alizarin staining, we found differences in ossification of limbs between wild-type and Cdk13-deficient embryos. At the moment we are identifying Cdk13-dependent genes employing the high-throughput RNA sequencing analyses to pinpoint signaling circuits or developmental pathways affected by the deficiency of Cdk13.

Acknowledgement: This research was supported by grants n. 16-34215A of the Czech Science Foundation and the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0518.

P48.

The expression of stem cell markers during odontogenesis

Kristyna Olbertova[1,2], Dusan Hrculak[3], Marek Hampl[1,2], Hana Dosedelova[1], Lucie Vrlikova[1], Vladimir Korinek[3] and Marcela Buchtova[1,2]

[1] Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Brno, Czech Republic
[2] Department of Experimental Biology, Masaryk University, Brno, Czech Republic
[3] Laboratory of Cell and Developmental Biology, Institute of Molecular Genetics, Prague, Czech Republic

Functional tooth germs in vertebrates are initiated from a dental lamina. The longevity of the lamina plays a role in governing the number of tooth generations. Monophyodont species (with one generation of teeth, mouse) have only rudimental replacement dental lamina, while polyphyodont species (multiple generations of teeth, snake) have a permanent continuous dental lamina. Here, we propose the existence of stem cell niche in the dental lamina similar to that described for other ectodermal structures and we aim to analyze the localization, gene expression and behavior of these progenitor cells in the dental lamina. To detect progenitor cells in the dental lamina, we selected two progenitor markers. LGR5 (Leucine-rich repeat containing G-protein-couple receptor 5) represents a transmembrane receptor of WNT pathway. The second marker, transcription factor SOX2 (SRY-Box 2) regulates division and maintenance of stem and progenitor cells. Progenitor cells were analyzed in pre- and postnatal stages in transgenic mice LGR5-EGFP-IRES-CreERT2. LGR5-positive cells were observed in the epithelial thickening, dental stalk and the base of the rudimentary successional dental lamina or surrounding mesenchyme. The expression of LGR5-positive cells was dissimilar in the labial and lingual side of the dental stalk. In the lingual side, the signal was located in the epithelium of dental stalk, but in the labial side, LGR5-positive cells were found in the surrounding mesenchyme. SOX2-possitive cells were detected in the epithelium of the lingual side of the dental stalk similar to LGR5 but they were not found in the mesenchyme. In conclusion, we observed distinct area of LGR5- and SOX2-positive cells co-expression in the lingual side of dental stalk epithelium of developing molar. This overlap of both progenitor markers indicates the existence of a new stem cell niche which significance for molar development has to be functionally proven.

Acknowledgement: This work was supported by the Czech Science Foundation (18-04859S) and Masaryk University (1403/2017).

P49.

The influence of stearic and oleic fatty acids supplementation on early embryo development, gene expression and phenotypic divergence of lipid droplets in cumulus cells

Piotr Pawlak[1], Natalia Malyszka[1], Izabela Szczerbal[1], Ewelina Warzych[1], Zofia E Madeja[1] and Dorota Lechniak[1]

[1] Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Poznan, Poland

The pig oocyte maturation procedure differs from other mammalian species due to the need of follicular fluid supplementation. It is known from the bovine and the human model that saturated fatty acids can negatively affect the developmental competence of oocytes. The influence of fatty acid supplementation on porcine oocyte maturation in vitro has not been analyzed broadly so far. Therefore, we aimed to investigate its effect on the lipid content and the expression of genes related to fatty acid metabolism in both oocytes and the surrounding cumulus cells, which have been shown to store significant amounts of lipids acting as a protecting agent facilitating oocyte's homeostasis. We found significantly increased expression of *ACACA, FASN, SCD, PLIN2, FADS1, FADS2* genes ($P<0.01$) in cumulus cells upon maturation with 100 µM supplementation with stearic and oleic acid compared to control, while the expression in oocytes did not change. The increase in gene expression was more pronounced in response to oleic acid addition (in comparison to stearic acid), showing up to 30 fold increase in *PLIN2* transcript level compared to control. The area occupied by the lipid droplets and their number did not vary in oocytes while increased significantly in the cumulus cells. Stearic acid did not inhibit embryo development to the blastocyst stage (27% vs 32% in control) while oleic acid supplementation improved the blastocyst yield (48%). Additionally, we have discovered a phenotypic divergence in cumulus cells lipid droplets in response to stearic and oleic fatty acids. Such differences were not noted in oocytes and adipocytes cultured as control cells to observe the process of lipogenesis.

Acknowledgement: Ministry of Science and Higher Education, Iuventus Plus (0051/IP1/2013/72).

P50.

Early-life social isolation-induced serotonergic deficits and their behavioural consequences in zebrafish (*Danio rerio*)

Diana Pejtsik[1], Zoltan Kristof Varga[1,2], Aron Zsigmond[3], Laszlo Biro[1], Kornel Demeter[1], Eva Mikics[1], Blanka Toth[4], Vilmos Salamon[1], Mate Varga[3] and Mano Aliczki[1]

[1] Laboratory of Translational Behavioural Neuroscience, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2] János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary
[3] Department of Genetics, Eötvös Loránd University, Budapest, Hungary
[4] Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Hungary

Disturbed social environments, e.g. parental neglect in childhood, serve as risk factors for developing psychiatric disorders. Social neglect can be modelled in rodents, employing social isolation in a critical developmental period, when the brain is most amenable to changes. However, despite its numerous advantages, similar models have not been developed in zebrafish. Here we aimed to develop a model of social neglect based on the isolation of zebrafish in a critical developmental period we previously characterised. We found that isolated fish displayed decreased reactivity in the Light-Dark Test compared to socially-housed control. Interestingly, these changes were specific to the Light-Dark Test, since in other tests measuring different domains of avoidance behaviour, isolation resulted in increased responsiveness. Furthermore, whole-brain serotonin content analysis revealed lower basal levels in isolated animals, which swiftly increased to acute stress, as opposed to the long-lasting decrease in socially-reared animals. Immunohystochemical analysis of serotonin showed that this difference appeared in brain areas homologous to the mammalian hippocampus and amygdala, involved in the regulation of avoidance behaviour of zebrafish. To assess possible causal relationships between elevated serotonergic activity and behavioural changes of isolated animals, we pharmacologically dampened 5-HT signaling using 5-HT1A partial agonist buspirone. Buspirone treatment abolished isolation-induced increase in forebrain serotonergic activity along with behavioural changes in Light-Dark Test. Taken together, social isolation in the critical period changes the way zebrafish respond to challenges by disrupting the same neuromodulatory system described in higher vertebrates, allowing for further use of this model in developing treatment for psychiatric disorders.

Acknowledgement: This project was supported by the Hungarian Genetics Society in the form of a travel, and accommodation grant, which enabled me to attend this conference.

P51.

Morphological assessment phagocytized apoptotic cells in mouse blastocysts

Jozef Pisko[1], Veronika Kovarikova[1] and Dusan Fabian[1]

[1] *Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice, Slovak Republic*

Aim of our study was to standardize methodology for evaluation of physiological capacity of blastomeres to eliminate apoptotic cells by phagocytosis in *in vivo* obtained blastocysts. Expanded blastocysts were obtained from ICR mice stimulated by hormonal treatment. *In vivo* developed blastocysts were freshly isolated from fertilized females on Day 4 of pregnancy (*120* h post hCG). The incidence of phagocytosis was evaluated by two types of fluorescence morphological staining followed by confocal laser scanning microscopy: 1) F-actin staining combined with Hoechst 33342 DNA staining and TUNEL labeling, 2) Lysotracker assay combined with Hoechst 33342 DNA staining. Fragmented nuclei (Hoechst 33342 DNA staining) showing specific DNA fragmentation (TUNEL labeling) localized within the cytoplasm of neighboring cells (F-actin staining), were classified as phagocytized apoptotic cells. Apoptotic nuclei co-localized with the presence of acidic organelles (Lysotracker labeling) were classified as phagocytized apoptotic cells. *In vivo* derived blastocysts showed 5.18 of apoptotic cells *per* blastocyst at average, and 8.97% of them were extruded to blastocoele cavity, i.e. they escaped phagocytosis. In blastocysts assessed by F-actin staining combined with DNA staining and TUNEL labeling, average number of non-extruded phagocytized apoptotic cells reached 1.36 and average number of dubious cases reached 1.57 per blastocyst. In blastocysts assessed by Lysotracker assay combined with DNA staining, average number of non-extruded phagocytized apoptotic cells reached 2.26 per blastocyst. The results indicate, that Lysotracker staining appears more suitable for the assessment phagocytized apoptotic cells in mouse blastocysts.

Acknowledgement: Study was supported by the Slovak Research and Development Agency under contract APVV 14-0763.

P52.

Comparative cranial skeletogenesis in non-teleost fishes: towards understanding of developmental strategies of fish craniofacial diversity

Anna Pospisilova[1], Jan Stundl[1,2], Martin Minarik[1], Brian Metscher[3] and Robert Cerny[1]

[1] *Department of Zoology, Charles University, Prague, Czech Republic*
[2] *Department of Zoology, National Museum, Prague, Czech Republic*
[3] *Department of Theoretical Biology, University of Vienna, Vienna, Austria*

Skeletal tissues signify unique and key novelties of vertebrates. Among all recent vertebrates, the most numerous and successful lineage is represented by the Ray-finned fishes that consequently show amazing variety of skeletal architectures and phenotypic adaptations. In order to depict fundamental principles of fish cranial skeletogenesis, we analysed members of early branching lineages, that also exemplify very different strategies of skeletogenesis: bichirs, sturgeons, and gars. Whereas bichirs and gars are heavily armored forms with massive exoskeleton and hyperossified dental structures, sturgeons are, on the contrary, mostly cartilaginous fishes with secondarily reduced skeleton and teeth that get lost during larval development. Our comparative analyses detected differences in the initiation, number, and sequences of formation of corresponding developmental modules, as well as in their growth dynamics. Moreover, we also determined disproportions in patterns of temporal and topographical organization of cartilaginous and bony condensations. Our data thus revealed several crucial factors responsible for dissimilar patterns of fish craniofacial architectures and we further seek to identify developmental dynamics of craniofacial mesenchyme during the processes of condensation and skeletogenesis. This will allow to define developmental strategies of craniofacial mesenchyme which provide the developmental basis generating disparate patterns of craniofacial architectures.

Acknowledgement: This study was supported by: Aktion grant no. ICM-2016-03562, Charles University Grant Agency GAUK 640016 (Anna Pospisilova) and GAUK 1448514 (Jan Stundl), GAČR grant no. 16-23836S.

P53.

Patterning of teeth at the dichotomy of ray- and lobe-finned fishes

Anna Pospisilova[1], Jan Stundl[1,2], Robert Cerny[1] and Vladimir Soukup[1]

[1] Faculty of Science, Charles University, Prague, Czech Republic
[2] National Museum, Prague, Czech Republic

Dentition composed of teeth is a key innovation that underlines the evolutionary success of jawed vertebrates. To be functional, each dentition has to be precisely patterned through the molecular machinery that regulates sequential addition of new teeth. How this machinery looks like and how did early vertebrates pattern their teeth is largely unexplored due to inaccessibility of embryos of species that reflect the ancestral state of vertebrate dentitions. Here, we aim to study molecular toolbox for tooth patterning and the sequential addition of new tooth germs in representatives of three basal fish lineages (bichir, sturgeon, and gar) and one tetrapod (axolotl). Specifically, we study the expression of tooth patterning genes in relation to the actual dentate field composition visualized by the alizarin red epifluorescence. We show that each dentate field is prefigured by a region of odontogenic competence, in which focal expression of tooth-specific genes demarks positions of individual tooth germ foci in all three species. Notably, the shape of the odontogenic competence indicates the final shape of the dentate field and the tooth germ-specific gene expression prefigures individual positions and spatial relations of teeth within the dentate field. Thanks to the access to the rare embryonic material of species that reside at the dichotomy of ray- and lobe-finned fishes and that supposedly reflect the ancestral state of dentitions, we propose that patterned dentitions share a common underlying molecular signature from the very beginning of the vertebrate tooth evolution.

P54.

Deciphering the role of RNA editing in vertebrate development

Leszek P. Pryszcz[1], Katarzyna Nieścierowicz[1], Cristina Navarrete Hernández[1], Eugeniusz Tralle[1], Katarzyna Misztal[1], Matthias Bochtler[1,2] and Cecilia L. Winata[1,3]

[1] IIMCB, Warsaw, Poland
[2] IBB, Warsaw, Poland
[3] Max Planck Institute, Germany

RNA editing refers to the post-transcriptional modification of RNA sequence. It has been reported to function in an array of biological processes, affecting physiology and behaviour of animals. Our aim is to elucidate the role of RNA editing in early embryo development. We have sequenced two parental genomes of zebrafish and transcriptomes of their offspring at multiple developmental stages. We found RNA editing to be present throughout embryo development, with more than 20,000 edited sites per stage. RNA editing occurs predominantly in 3'-UTRs (close to 40%), with less than 2% in coding sequences (no amino acid changes). Interestingly, the pattern of RNA editing changes with time and it peaks around maternal-to-zygotic transition and at later stages in development. This is conserved across several model organisms: fruitfly, worm, zebrafish and Xenopus. We're currently characterising ADAR knock-out and knock-down both, phenotypically and biochemically. Adar KD affects gastrulation, the midline structures, and posterior body axis (severe reduction in the posterior embryonic structures). KD phenotype can be reverted completely by injection of adar mRNA along morpholino construct. Interestingly, adar mRNA overexpression has no effect on posterior embryonic structures development, but results in cyklopy and head deformation. All of these, suggests that RNA editing may play crucial role in the development.

P55.

The unique development of the primary mouth in basal ray-finned fishes

Viktorie Psutkova[1], Vladimir Soukup[1] and Robert Cerny[1]

[1] *Faculty of Science, Department of Zoology, Charles University, Prague, Czech Republic*

The primary mouth is the first connection of the digestive system with the outer environment. It develops during early embryogenesis at the anterior end of the head, where the ectoderm and the endoderm are directly juxtaposed. In the majority of vertebrates, the mouth develops through stages of deep stomodeal invagination and establishment of the oral membrane, whose rupture causes opening of the mouth. However, this general scheme does not hold true for actinopterygian fishes. We have discovered that the primary mouth development of the basal actinopterygian fishes, bichir (*Polypterus senegalus*), sturgeon (*Acipenser ruthenus*), gar (*Atractosteus tropicus*), is special and different. In these fishes, the primary mouth formation is influenced by the large endodermal domain, the so called pre-oral gut. This endodermal domain forms the cement gland of bichir and gar, and the oral epithelium, the oral teeth and the barbels of sturgeon. Presence of the endoderm on the surface of the head is unique for vertebrates, and my work will describe contribution and morphodynamics of ectoderm and endoderm germ layers during the primary mouth formation in basal fishes.

P56.

3D cell culture models demonstrate a role for FGFs and WNT signalling in regulation of adult lung epithelial stem cell fate

Anas Rabata[1], Ales Hampl[1] and Zuzana Koledova[1]

[1] *Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

The ability of adult lung tissue to maintain itself, remodel, regenerate and repair after injury depends on the activity of resident adult lung stem/progenitor cells (LSPCs). However, our understanding of the cellular hierarchy in adult lung and microenvironmental signals that regulate LSPC self-renewal and differentiation remains incomplete. In this study, a special protocol has been developed to isolate LSPC that takes advantage of the unique abilities of stem cells to survive in non-adherent conditions and maintain self-renew. In this assay, it has been approved that LSPCs had the ability to form spheroids (lungospheres) of several distinct phenotypes. Furthermore, lungospheres may be serially passaged and their proliferation, self-renewal and differentiation are regulated by FGF signalling. In addition, lungospheres could be embedded in 3D ECM, such as Matrigel, and cultured for a long time, after that lungospheres proliferate and form large cystic or branched structures in response to FGF and Wnt signalling. Our lungosphere and 3D cell culture assays provide useful tools to assess stem/progenitor properties of distinct lung epithelial cell populations and to study lung epithelial-stromal interactions in vitro.

P57.

Phorbol ester increases cardiomyogenesis in mES cells through ERK signalling

Katarzyna Anna Radaszkiewicz[1], Deborah Beckerova[1], Lucie Woloszczukova[1], Tomasz Witold Radaszkiewicz[1], Lukas Kubala[1,2], Petr Humpolicek[3] and Jiri Pachernik[1]

[1] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2] Department of Free Radical Pathophysiology, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[3] Centre of Polymer Systems and Faculty of Technology, Tomas Bata University in Zlin, Zlin, Czech Republic

12-O-Tetradecanoylphorbol-13-acetate (TPA), a diacylglycerol mimetic agent, is one of the most widely used protein kinase C (PKC) inductors. TPA has been proposed as a factor for differentiation of pluripotent stem cells, although the obtained results were inconsistent. Here we show that TPA can be used to promote cardiomyogenesis in a culture of mouse embryonic stem cells *in vitro*. The effect of TPA was at least partially mediated by induction of extracellular signal regulated kinase (ERK) activity, although the use of other mitogenic growth factors and known inducers of ERK pathway did not simulate the TPA-mediated effect. We also demonstrated that the increase in cardiomyocyte yield is not mediated by cross reaction of TPA-induced response with canonical Wnt signalling pathway. Although the precise mechanism and mode of action of TPA remains to be clarified, proposed treatment can be used to increase cardiomyogenesis in ES cells.

P58.

Transient hypoxia drives a ventricular-like cardiomyocyte phenotype in *in vitro* cardiomyogenesis of mouse embryonic stem cells

Katarzyna Anna Radaszkiewicz[1], Marketa Bebarova[2], Lucia Bino[3], Petr Humpolicek[4], Jarmila Navratilova[5], Lukas Kubala[1,3,5] and Jiri Pachernik[1]

[1] *Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2] *Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic,*
[3] *Department of Free Radical Pathophysiology, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic*
[4] *Centre of Polymer Systems and Faculty of Technology, Tomas Bata University in Zlín, 760 01 Zlín, Czech Republic*
[5] *International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic*

Hypoxia and hypoxia inducible factor 1 (HIF1) play an important role in cardiomyogenesis. *In vivo* studies indicate that heart development in a HIF-1α deficient embryo is associated with various abnormalities leading to insufficient heart function. However, HIF1-mediated response to low oxygen level is required at a specific time of heart growth. Inactivation of HIF-1α at the later stages of heart morphogenesis, when maternal hypoxia decreases, does not influence its development. *In vitro* hypoxia is suggested to promote the cardiomyogenesis of differentiating pluripotent stem cells. The depletion or silencing of HIF-1α lead to a reduction in cardiomyocyte formation and impaired cardiomyocyte maturation. Here we elucidated how different hypoxic time windows affect the cardiomyogenesis of differentiating mouse embryonic stem cells. We demonstrated that exposure of cells to transient hypoxia, at a time when mesoderm and early cardiomyocyte progenitors are formed, promotes the formation of ventricular-like cardiomyocytes. Contrary, prolonged culture of these cells under hypoxia has the most pronounced effect on cardiomyocyte formation. Importantly, the described effects of low oxygen level on cardiomyogenesis were dependent on the presence of functional HIF-1α. Thus our results provide a new insight into the role for hypoxia and HIF1α in the regulation of cardiomyogenesis. Differentiation of pluripotent cells under hypoxic condition can be an alternative approach for formation of specific cardiomyocyte subtypes and cardiomyocytes propagation.

P59.

The role of RNF43/ZNRF3 in non canonical Wnt signaling and its impact on human melanoma

Tomasz W. Radaszkiewicz[1], Michaela Noskova[1], Tomas Gybeľ[1], Karol Kaiser[1], Anna Kotrbova[1], Lucia Demkova[2], Lucia Kucerova[2], Zbynek Zdrahal[3], David Potesil[3] and Vitezslav Bryja[1]

[1] Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2] Laboratory of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovak Republic
[3] Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Proteins RNF43 and related ZNRF3 are transmembrane E3 ubiquitin ligases with an ability to inhibit canonical Wnt signaling. Their activity leads to internalization and degradation of Frizzleds, Wnt ligand receptors. Loss of function mutations of RNF43 are frequent in various cancers and they are correlated with hyper-activation of beta-catenin dependent transcription, which supports proliferation and survival. Thus, RNF43 and ZNRF3 are tumor suppressors.
In our work we have focused on RNF43 in Wnt/Planar Cell Polarity (PCP) pathway by exploring RNF43 interactome and impact on key signaling events. Unbiased proteomic method for the detection of transient protein-protein interactions called BioID allowed us to identify that RNF43 interacts with the components of PCP receptor complex - namely Ror1, Ror2, Vangl1, Vangl2, Dvl1, Dvl2, Dvl3, Casein kinase 1ε and Casein kinase 1δ. Overexpression of RNF43 in an enzymatic dependent manner lead to desensitization of cells to Wnt5a, accompanied the by decreased level of surface and total Ror1, Vangl2 and Dvl2 and Dvl3 dephosphorylation and proteasomal degradation.
We further show that RNF43 regulates Wnt/PCP pathway in melanoma cells. Melanoma is the deadliest skin cancer that is driven by Wnt5a. Although, RNF43 is not frequently mutated in this cancer, its expression dramatically decreases with cancer progression. When we overexpressed RNF43 in A375 metastatic melanoma cell line, it suppressed Wnt5a signaling and blocked migration, invasion and invadopodia formation. Therefore, we propose that RNF43 downregulation allows efficient Wnt5a signaling and is beneficial for melanoma progression.

P60.

Advanced *in vitro* model of Leydig cell development for male reproductive toxicity assessment

Iva Sovadinova[1], Eliska Sychrova[1], Petra Laboha[1], Amrita Basu[1], Affiefa Yawer[1], Aneta Dydowiczova[1] and Pavel Babica[1]

[1] *RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*

In the last decades, there is an increasing concern about male reproductive health. The adverse male reproductive health outcomes are associated with exposures to drugs, cosmetics and personal care products as well as environmentally relevant contaminants, especially during critical periods of human development. Leydig cells in adult testes play pivotal roles in the maintenance of male reproductive functions by producing testosterone. Any disturbance of Leydig cell development during both prenatal and postnatal periods can lead to male reproductive system dysfunctions later on. Therefore, there is a pressing need for a better *in vitro* model addressing different developmental stages of Leydig cells that may be used for male reproductive toxicity assessment and t replace the traditional animal-based models. The talk will be about recent progress in understanding the mechanisms of Leydig cell development and differentiation with a focus on current available *in vitro* models with their advantages and disadvantages. In addition, we will introduce our advanced *in vitro* model of pre-pubertal Leydig cells based on spheroids formed by testicular Leydig TM3 cells which we have used for male reproductive toxicity assessment of potential reproductive toxicants. We will demonstrate that a 3D spheroid model of pre-pubertal Leydig cells in combination with an automatic image acquisition and evaluation represents a valuable tool for prescreening of male reproductive toxicity and for the assessment of cellular and molecular mechanisms.

Acknowledgment: This research is supported by Czech Science Foundation Project No. GA16-10775Y.

P61.

Differentiation potential of rabbit inner cell mass

Anna Stachowiak[1,*], K. Filimonow[1,*], K. Barłowska[1], D. Winiarczyk[1] and Anna Piliszek[1]

[1]*Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland*
[*] *these authors contributed equally*

Proper differentiation and segregation of the first three cell lineages of the mammalian embryo: pluripotent epiblast (EPI), and extraembryonic primitive endoderm (PrE) and trophectoderm (TE) play a key role in early mammalian development. However, timing of lineage differentation differs among mammalian species. Here we examine the ability and timing of the rabbit preimplantation embryos to specify these first lineages. Using time-lapse microscopy we follow rabbit development *in vitro* and assess timing of developmental time points (cleavage, compaction, cavitation). To determine timing of lineage commitment, embryos obtained from natural matings at 3-4 *dpc* were assigned to groups based on total cell number (stage V, 32-63 cells (morula); stage VI, 64-127 cells (compact morula/ early blastocyst); stage VII, 128-255 cells; stage VIII, 256-511 cells). The ICMs from every group were isolated by immunosurgery and cultured for 24 and 48 h. Cell number of particular lineage and their proper differentiation were based on localization of lineage-specific transcription factors: CDX2 for TE, SOX2 for EPI, and SOX17 for PrE. We observed that stage VII and VIII ICMs are not able to regenerate CDX2-positive trophectoderm layer. However, they are able to specify EPI and PrE cells, and in some cases sort and outer SOX17-positive (PrE) layer, and inner SOX2-positive (EPI) layer. Interestingly, some of the cultured ICMs contained inner layer of triple negative cells, suggesting that some cells are not properly differentated.

Acknowledgement: This work was supported by National Science Centre (NCN) grant 2011/03/D/NZ3/03992 and KNOW – The Leading National Research Centre grant KNOW2015/CB/PRO1/48.

P62.

Early tooth development and segmentation of oral epithelium in mice

Klara Steklikova[1,2], Sophie Pantalacci[3], Alexa Sadier[3], Martina Pasovska[1] and Maria Hovorakova[1]

[1] Institute of Experimental Medicine, The Czech Academy of Sciences, Prague, Czech Republic
[2] Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic
[3] Laboratoire de Biologie et de Modélisation de la Cellule, UMR5239, ENS Lyon, France

Patterning, the process whereby patterns arise during development, is a general process forming different organs and organ systems. Here, we aim to use the mouse molar development with special focus on segmentation of oral epithelium during sequential development of the tooth germ to solve the question of tooth pattern formation. The tooth development represents an efficient model to bring knowledge on sequential organ formation in general. Using *in vitro* culturing we have documented that rudimentary (R2) signaling center is actively competed with the first molar (M1) early signalling center during formation of primary enamel knot (pEK) of a nascent fuctional tooth. After the separation of the anterior and posterior segments of M1 germ at 14.3ED a signalling center in the anterior part was recovered the following day and the epithelium formed a well developped tooth germ after 6 days of culturing. In the separatelly cultured posterior part, a signalling center formed one day sooner than pEK of second molar (M2) in the intact controls and formed a similarly sized tooth germ as in the anterior part. In controls M2 doesn´t occur before the third day of cultivation. The size of the second tooth in cultured segments was obviously larger compared to the control M2. Our study confirmed the developmental potential of R2 rudiment, normally incorporated in the M1 germ. Separated from M1 germ, R2 is able to revive its *Shh* signalling center and give rise to a full-size tooth germ because of loss of active inhibition of posteriorly located developing early M1 *Shh* signalling center.

Acknowledgement: This study was supported by the Czech Science Foundation (14-37368G); Boursier du gouvernement Francais – Stage 2017 and Nadation of Josef Hlávka.

P63.

Deciphering Roles of FGF Signaling in Mammary Gland Development

Jakub Sumbal[1,2], Tereza Vranova[1,2,3], Anas Rabata[1] and Zuzana Koledova[1]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] International Clinical Research Centre, St. Anne's University Hospital, Brno, Czech Republic
[3] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Mammary gland is a unique organ undergoing massive postnatal morphogenesis and repeated cycles of growth and involution during each pregnancy. During puberty, tiny epithelial rudimental tree is activated by female sex hormones and the epithelium starts to fill surrounding fat pad by a process called branching morphogenesis. This process is strictly regulated by interaction with stromal cells including paracrine signaling, ECM remodeling and mechanosignaling. Mechanisms regulating the growth of tissue are often deregulated in pathogenesis of breast cancer and thus attracting a huge research interest.In this study we analyzed the effect of hyperactivation of FGF signaling on mammary epithelial morphogenesis. For this purpose we employed hyperstable FGF2 with increased thermostability and 3D culture of primary mammary organoids. We found that FGF2 hypersignaling leads to enrichment of highly proliferative luminal progenitors, altered mechanosensing, polarization and distribution of myoepithelial cells, all leading to epithelial hyperplasia. We identified global hyperactivation of FGF receptor downstream pathways (ERK, AKT, STAT3, PLCγ) and their target genes (*Etv4, Etv5, Spry2, Dusp6*) as a result of FGF2 hypersignalling. Our findings will bring new insights into the roles of FGF signaling in mammary gland development and tumourigenesis.

Acknowledgement: This work has been supported by grant GJ16-20031Y from Czech Science Foundation (GACR), by grant TH02010219 from Technology Agency of the Czech Republic (TACR), and by funds from the Faculty of Medicine MU to junior researcher Zuzana Koledová.

P64.

Function of Cdk12 during mammary gland development in mice

Marek Supak[1], Monika Novakova[1] and Jiri Kohoutek[1]

[1] Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, Czech Republic

Cyclin dependent kinase 12 (Cdk12) belongs to the group of transcription-associated kinases including Cdk7, Cdk8, Cdk9, Cdk11 and Cdk13. By associating with cyclin K (CycK), Cdk12 phosphorylates C-terminal domain of RNA polymerase II and facilitates transcription elongation. Disruption of this process results in the decreased expression of DNA damage response genes, such as Brca1, followed by genomic instability and tumorigenesis. Cdk12 is also required for self-renewal of murine embryonic stem cells and its deficiency leads to increased expression of the various differentiation markers and reduced axonal outgrowth controlled by lowered Cdk5 expression in neurogenesis. Recently was found that the loss of Cdk12 results in the embryonic lethality after implantation in mice. However, we also observed significant postnatal mortality of newborn pups of heterozygous mice, where more than 75% of Cdk12 heterozygous female failed to lactate after first parturition. The main development of mammary gland takes place postnatally, when gland begins to branch and forms terminal end buds (TEB) in puberty and alveolar buds in pregnancy. Among others, prolactin induces mammopoiesis and lactogenesis by the phosphorylation of Stat5a gene that promotes the expression of specific differentiation genes. In contrast, Brca1 binds Stat5a and suppresses its transcriptional activity, thereby damages terminal differentiation of alveolar buds and followed milk production. We compared mammary gland architecture of wild-type and Cdk12 heterozygous virgin mice in 14 weeks. We found significantly longer penetration of ducts to the fat pad in Cdk12-deficient mice, the branching was not affected. Moreover, smaller amount of TEBs was presented in heterozygous female signifying cessation of the normal development. Investigation of the Cdk12-deficient mammary gland after parturition revealed deregulated expression of milk proteins confirming affected terminal differentiation of alveolar buds. We are going to confirm our hypothesis, that Cdk12 deficiency in mice impairs differentiation and enhance proliferation of mammary gland, observing MCF10A cell alveogenesis in Matrigel after Cdk12 depletion. The elucidation, if this assumption is caused by the Brca1 deficiency and disrupted polarization of alveolar cells, is our next point of interest.

P65.

Proteomic approaches to identify new interactors of Rybp during neuronal specification

Gergo Kovacs[1], Eniko Sutus[1] and Melinda Katalin Pirity[1]

[1] *Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary*

We have previously reported that polycomb protein RYBP (RING1 and YY1 Binding Protein) plays imporant role in mammalian central nervous system (CNS) development and neuroprogenitor differentiation. However, the precise molecular meachanism of how RYBP excerts its diverse biological functions is still unknown. Since several studies described RYBP as a member of multimeric protein complexes (MPCs) we applied mass spectrometry (MS) to identify interactors of RYBP during *in vitro* neural differentiation. To achieve our goal, we have utilized wild type (*Rybp*$^{+/+}$) and Rybp null mutant (*Rybp*$^{-/-}$) embryonic stem (ES) cells, differentiated them towards neural lineages *in vitro* and collected samples from both early and late stage of neural differentiation for MS analyses. We performed label-free pulldown of endogenous RYBP from nuclear extracts from each designated timeponts of *in vitro* neural differentiation and analyzed the hits with MS. Our results indicated possible interactions between RYBP and several members of the Polycomb repressive complex 2 (PRC2) and with several members of the npBAF/nBAF (SWI/SNF-like) complex, which composition change play essential role in commitment of neuronal progenitors to terminally differentiated stage. These findings broaden our knowledge about how RYBP balances between early, progenitor and differentiated stages of neural differentiation and have implications for understanding neural lineage commitment, learning, memory and neurological diseases.

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

P66.

Ultrastructure of chicken embryonic stem cells

Andrea Svoradova[1*], Jaromir Vasicek[2,3], Lucia Olexikova[2] and Peter Chrenek[2,3]

[1] Constantine the Philosopher University, Faculty of Natural Sciences, Nitra, Slovakia
[2] Research Institute for Animal Production in Nitra, National Agricultural and Food Centre, Lužianky, Slovakia
[3] Slovak University of Agriculture, Faculty of Biotechnology and Food Science, Nitra, Slovakia

Ultrastructural characterisation of chicken blastodermal cells (BCs) and primordial germ cells (PGCs) were carried out to investigate their detailed morfology. BCs used in this study were isolated from X stage of developing embryo and PGCs were obtained from gonads of six-dayold embryo. After isolation, BCs and PGCs were immediately fixed, dehydrated, embedded in epoxy resin, cut into ultrathin sections, contrasted and examined on a transmission electron microscope. Our study showed differences between these cell types in the case of yolk distribution. The abundant presence of yolk inclusions of different size in the intercellular space of fresh BCs were observed. Moreover, the cell membrane was relatively compact without any pseudopodia and cell size varied from 40 to 60 µm. In contrast, PGCs contained a smaller amount of yolk inclusions that is the result of embryo development, however glycogen substances were presented in the PGCs cytoplasm. The cell membrane was compact with pseudopodia and cell size varied from 15 to 20 µm. Our findings indicate that different sources of chicken stem cells have different structure, size and that care should be taken choosing the appropriate sources of chicken stem cells for aplication in different field of biological research. The knowledge about detailed ultrastructure of embryonic stem cells are essential for their using in biomedicine or preservation programs.

Acknowledgement: The study was financially supported by the Slovak Research and Development Agency (No. APVV-17-0124), VEGA 1/0611/15, VEGA 1/0160/18, KEGA 026 SPU -4/2018 and „Špičkový tím - CeRA.

P67.

Lack of RYBP impairs sarcomere formation and the development of multiple cell types during *in vitro* cardiac differentiation

Viktoria Szabo[1,2] and Melinda Katalin Pirity[1]

[1] Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
[2] Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Congenital heart defects (CHD) are caused by mutations and irregular changes of molecular pathways that govern embryonic heart development. Our laboratory focuses on the early onset of cardiac development by utilizing embryonic stem (ES) cell based *in vitro* cardiac differentiation systems. We have previously showed that lack of the Polycomb Group (PcG) member, RYBP (RING1- and YY1-Binding Protein; UniGene Mm.321633; MGI:1929059) in ES cells impairs cardiac differentiation resulting cardiomyocytes (CMCs) without contractile activity. To reveal the molecular background of this defect we have differentiated wild-type ($Rybp^{+/+}$) and *Rybp null* mutant ($Rybp^{-/-}$) mouse ES cells to CMCs and (I) characterized the gene expression changes of thick- and thin-filament sarcomeric components and their protein products, (II) investigated the presence of cardiomyogenic and non-cardiomyogenic cell types in the cardiac cultures, (III) and analyzed the gene expression of some Hedgehog pathway member that are essential in the cardiac development and some other genes, which are closely linked to this pathway. We used quantitative real-time PCR (qRT-PCR) and immunocytochemistry (ICC) analyses in our experiments. Our results indicate that (1) the cardiac sarcomere structure was not properly formed in the $Rybp^{-/-}$ cells, (2) the differentiation of CMCs and other cell types was improper in the lack of *Rybp*, (3) and the gene expression of several key Hedgehog pathway members altered in these mutants. In summary, we present evidence the first time that RYBP is a key factor of the proper cardiac differentiation and RYBP may be a new player of facilitating CHD development.

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

P68.

The role of Mesr4 in stem cell differentiation

Alexandra Brigitta Szarka-Kovacs[1,2], Ferenc Jankovics[1] and Miklos Erdelyi[1]

[1] Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
[2] Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Adult stem cells are unspecialized cells, which divide constitutively reproducing themselves and generating a differentiating daughter cell. The balance between stem cell-renewal and differentiation is regulated by intrinsic mechanisms of the stem cells and by extrinsic factors originating from the specific microenvironment of the stem cells, called stem cell-niches. One of the best known stem cell-niches is the ovarian germ line stem cell niche of Drosophila melanogaster. At the tip of the niche, the germline stem cells (GSCs) divide constitutively reproducing themselves and generating the cystoblasts, which differentiate into oocytes. The balance between stem cell-renewal and differentiation is regulated by the TGF-β pathway, which blocks the expression of the differentiation gene bag of marbles (bam) in the GSCs and permits bam expression in the cystoblasts. Here, we show a cell biological analysis of the Misexpression Suppressor of Ras4 (Mesr4) gene function in the niche. Mesr4 contains nine zinc-finger motifs, a plant homeodomain-finger and localises to the nuclei in all cells of the niche. In the absence of Mesr4, GSCs do not differentiate resulting in the formation of germ cell tumours. Cell-type specific RNAi revealed that Mesr4 promotes germ cell differentiation intrinsically in the cystoblasts. In the Mesr4 RNAi germ cells, the TGF-β pathway is functional, nevertheless the bam expression is reduced in the cystoblasts. The mutant phenotype of Mesr4 can be rescued by forced bam expression suggesting that Mesr4 is required upstream of bam. Our results indicate that Mesr4 promotes germ cell differentiation as a positive regulator of bam transcription.

P69.

The role of HIF-1α in early neurogenesis

Veronika Sumberova[1], Josef Vecera[1] and Jiri Pachernik[1]

[1] *Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*

The majority of stem cell populations naturally occurs under hypoxic conditions. The key molecule in terms of low O_2 concentration is hypoxia-inducible factor 1, i.e. HIF-1. It triggers gene transcription related to hypoxia adaptation and is also of significance to other signalling pathways. HIF1 itself, and most importantly its alpha subunit, plays a crucial role in neurogenesis as it affects proliferation and differentiation processes of both embryonic and adult stem cells. The present research establishes the *in vitro* cultivations that are instrumental in studying the role of HIF-1α in early development of a nervous system. The experiments made use of a culture of adherent neuroectodermal cells derived from embryonic neuroepithelium. The loss of HIF-1 function during the early stages of development was induced by introducing a lentivirus carrying shRNA interfering with HIF-1α mRNA. In order to create hypoxic conditions, the cells were cultivated at low-oxygen tension. Phenotype changes during stem cells differentiation were observed both visually and with the aid of western blot analysis and fluorescent immunohistochemistry. The results confirm that the action of retinoic acid causes the cells to differentiate into neurons and glial cells. Under non-serum conditions, differentiation into neurons and oligodendrocytes is accelerated while glial markers are suppressed. Neural differentiation is also enhanced by a knockdown of HIF-1α and accompanied by a low level of neuronal repressor HES1. Concurrently with *in vitro* experiments, this research looks into the optimization of *ex vivo* cultivations of embryos so as to better understand the role of HIF-1α during organogenesis.

P70.

Investigating the role of polycomb protein Rybp in neural progenitor formation

Bertalan Takacs[1], Gergo Kovacs[1] and Melinda Katalin Pirity[1]

[1] Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Our group have previously shown that lack of polycomb protein RYBP (RING1 and YY1 Binding Protein) leads to impaired neural differentiation *in vitro* including drastic decrease in the number of terminally differentiated neurons and glial cells, and abundance of neural precursors. Our aim was to reveal: (1) is there any specific lineage (either glial or neuronal) with altered kinetics in the lack of RYBP, (2) whether the lack of RYBP alters the kinetics of any neural progenitor gene expression and (3) whether the accelerated progenitor formation is sustained during the entire time course of differentiation? In order to answer these questions we performed *in vitro* neural differentiation of embryonic stem (ES) cells and analyzed the kinetics of neural progenitor formation by quantitative real-time PCR (qRT-PCR) and immunocytochemistry (ICC) using neural lineage specific markers. We found that the lack of RYBP alters the kinetics of progenitor formation in every examined cell lineage and that the progenitor pool formation was accelerated during the entire time course of *in vitro* neural differentiation. These results suggest that RYBP is important for the proper balance between progenitor formation and terminal differentiation and that *Rybp* may function as a cell fate decision making gene during neural development.

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

P71.

FSH influences amino acid incorporation in mammalian oocyte and early embryo

Anna Tetkova*[1,2], Jaroslav Kalous*[1] and Andrej Susor[1]

[1] *Laboratory of Biochemistry and Molecular Biology of Germ Cells, Institute of Animal Physiology and Genetics, CAS, Libechov, Czech Republic*
[2] *Department of Developmental Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic*
** shared co-authorship*

The culture media used in the programs of assisted reproduction are supplemented with gonadotropic hormones in order to support the development of *in vitro* cultured oocytes. However, the effect of gonadotropins on protein synthesis in oocytes remains to be elucidated. Fully grown mammalian oocytes are transcriptionally inactive and completion of meiosis relies on stabilization and translation of stored maternal RNA, thus any changes in translational regulation might affect proper oocyte development. Our results reveal that presence of the follicle stimulating hormone (FSH) in the cultivation media significantly decrease the incorporation of methionine and homopropargylglycine (amino acid analogue) into de novo synthetized proteins in mouse oocytes and early embryos. Moreover we confirm that FSH-receptor is expressed at protein level in cumulus-free oocytes from various mammalian species. In conclusion, decrease of amino acid incorporation is not the cause of activation of translational repressor eIF2a, but the result of impaired amino acid transport from cultivation media to the cytoplasm of oocyte or early embryo.

P72.

Protein degradation during bovine preimplantation development

Tereza Toralova[1], Veronika Kinterova[1, 2], Veronika Petruskova[1,3] and Jiri Kanka[1]

[1] Laboratory of Developmental Biology, Institute of Animal Physiology and Genetics, Libechov, Czech Republic
[2] Department of Veterinary Sciences, Czech University of Life Sciences in Prague, Prague, Czech Republic
[3]Faculty of Science, Charles University in Prague, Prague, Czech Republic

The early embryogenesis is initially driven by maternally inherited mRNAs and proteins. As the development proceeds, the stocks are degraded and replaced by embryonic molecules. Even though there is only limited number of data concerning maternal protein degradation, it is clear that it is necessary for normal course of embryonic genome activation (EGA) in both mice and bovines and the development of preimplantation embryos with inhibited ubiquitin-proteasome pathway (UPS) is deteriorated. We have found that for normal development of preimplantation embryos, normal protein degradation is more important during oocyte maturation than during period just before and during EGA. The inhibition of SCF complexes, one of the most important parts of UPS, leads not only to deteriorated fertilization but also to delayed development of the embryos. This deterioration is most evident at 8 cell stage (8c) but persists until the blastocyst stage. When bovine embryos were treated from 4c to late 8c (i.e. before and during EGA in bovines), the onset of EGA was delayed and the developmental deterioration was found. However, some of the treated embryos were able to overcome this handicap and there was no statistically significant difference between treated group and controls at late blastocysts. In conclusion, it seems that degradation of proteins by UPS is necessary during early embryogenesis, but it is likely driven by strict rules with degradation of concrete protein at very specific time and/or place.

Acknowledgement: This work was supported by CIGA 20172013 and GACR 13-24730P

.

P73.

Analysis of human oocyte ultrastructure by advanced electron microscopy technologies

Zuzana Trebichalska[1] and Zuzana Holubcova[1]

[1] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

The human oocyte is a highly specialized cell uniquely equipped for a single function in propagation of life. The oocyte quality is crucial for proper embryo development. Although the influence of the oocyte quality on the developmental potential of the embryo has been recognized, there is still a lack of a clear understanding of the essential features of a good quality egg. In this project, we aim to systematically study ultrastructural morphology of high quality human oocytes to determine structural bases underlying acquisition of egg´s developmental competence. With the use of advanced electron microscopy technologies, the qualitative and quantitative analysis of three-dimensional organelle organisation and dynamics of structural rearrangements associated with normal oocyte maturation can be addressed. The transmission electron microscopy (TEM) of chemically fixed samples is used as a default approach to examine intracellular morphology at the nanoscale level; moreover, to analyse spatial relationship between subcellular components within the context of large oocyte we take advantage in advances of high resolution 3D imaging. Namely, we harvest the power of Focused Ion Beam Scanning Electron Microscopy (FIB-SEM). The study will provide important morphological dataset for further comparative analyses and evidence-based selection of good quality oocytes in clinical practice.

Acknowledgement: We would like to thank our colleagues at TESCAN for their technical support.

P74.

Ultrastructural characterization of rabbit adipose tissue derived mesenchymal stem cells

Jaromir Vasicek[1,2], Maria Tomkova[1], Lucia Olexikova[2] and Peter Chrenek[1,2]

[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Nitra, Slovak Republic
[2] National Agricultural and Food Centre, Research Institute for Animal Production Nitra, Lužianky, Slovak Republic

At the present, rabbit mesenchymal stem cells (MSCs) are of a great interest for regenerative medicine. Beside rabbit bone marrow and amniotic fluid, adipose tissue seems to be an accessible source of MSCs that can be obtained with relative ease from any individual and can be stored in stem cell bank. As far we know, the ultrastructure of rabbit adipose MSCs was not yet studied. The aim of this preliminary study was to observe the basic ultrastructural morphology and organelle composition of rabbit adipose MSCs. Samples for transmission electron microscopy were prepared as described in our previous studies and analysed using TEM microscope JEM 100CX-II (JEOL company, Japan). The observation of rabbit adipose MSCs confirmed their typical stem cell ultrastructure comparable to rabbit MSCs derived from bone marrow or amniotic fluid. These cells exhibit eccentrically located nucleus with nucleolus and numerous pseudopodia and vacuoles. Moreover, the ultrastructural analysis revealed an abundant presence of granulated endoplasmic reticulum, mitochondria and vesicles that are typical for active cells with extensive protein synthesis. On the other hand, the lack of lipid droplets and Golgi apparatus was observed. In conclusion, rabbit MSCs derived from adipose tissue possess typical ultrastructural composition of adult stem cells obtained from other biological sources.

Acknowledgement: This work was supported by the grant of Slovak Research and Development Agency: APVV-17-0124 and the grants of Scientific Grant Agency of the Ministry of Education, science, research and sport of the Slovak Republic and the Slovak Academy of Sciences: VEGA 1/0611/15 and VEGA 1/0160/18 and KEGA 026SPU-4/2018.

P75.

The Role of WWC2 during preimplantation mouse embryo

Giorgio Virnicchi[1] and Alexander W. Bruce[1]

[1] *Laboratory of Development Biology and Genetic, Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic*

Mouse preimplantation embryo development lasts 4.5 days generating a blastocyst structure, comprising three distinct cell lineages; the epiblast/ pluripotent inner cells, that will form the embryo proper, and two differentiating extraembryonic epithelial lineages trophectoderm and primitive endoderm encapsulating the epiblast and demarking a boundary versus blastocyst cavity. The three cells lineages are derived by cleavage divisions typified by atypically long cell cycles (12h) when compared to other somatic mitotic divisions. However, insight into such unique and atypical cell cycles is markedly lacking. Additionally, published embryonic stem cell studies have highlighted the importance of cell cycle length, particularly the G1 phase, in the reception of differentiative cues. Here, we show that the WW and C2 domain containing protein family member, Wwc2 (related to the Hippo-signalling pathway component Kibra/ Wwc1) has a role in regulating cell cycle. Through a combination of global and clonal RNAi-mediated knockdown approaches, we observe that ablation of *Wwc2* expression causes prolonged cell cycles, often exhibiting unusually long mitotic phases, that are often associated with abnormal chromosomal segregation and cytokinesis defects (*e.g.* micronuclei formation, bi-nucleated cells, mid-body chromosome association; *n.b.* we also report meiotic defects during oocyte maturation); therefore producing embryos with statistically significant reductions in cell number. Moreover, the clonal dysregulation of *Wwc2* expression favours the production of cells preferentially contributing fewer inner cells and more outer trophectoderm cells (Cdx2 +ve), by the late blastocyst/ E4.5 stage. We suggest Wwc2 is a novel and key regulator of early embryonic cell cycle progression and cell fate.

P76.

SIRT1 may control apoptosis resistance in preimplantation mouse development

Dawid Winiarczyk[1], Adrian Stankiewicz[1], Jacek Modliński[1] and Anna Piliszek[1]

[1]*Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzębiec, Republic of Poland*

Apoptosis is a process of physiological cell suicide which is genetically programmed. During apoptosis chromatin undergoes condensation and aggregation near nuclear membrane, followed by cytoplasmic shrinkage and fragmentation of the nucleus. These events are usually preceded by DNA degradation to segments corresponding to the length of the nucleosome.
It is assumed that every cell has the potential ability to enter the path of apoptosis. This is a naturally occurring phenomenon also in preimplantation embryos. However our observations, supported by literature data, suggest that apoptosis in the mouse does not occur until blastocyst stage. To confirm this, we investigated regulation of apoptosis in mouse preimplantation development on transcription level by qPCR. We performed analysis of various pro- and antyapoptotic genes in 5 developmental stages of preimplantation mouse development (oocytes, zygotes, 2-cells, 8-cells and blastocysts) after 24h *in vitro* culture with proapoptotic factor (staurosporine or TNFα). We observed features of apoptosis in oocytes and blastocysts only. No apoptotic cells were found between zygote and late morula stages. Our results indicate that *SIRT1* may be one of key regulators of apoptosis in early mammalian development. We observed highest peak of *SIRT1* expression in zygote group and then expression of that gene gradually decreased. Despite high expression levels of proapoptotic genes (*p53, Casp3, Bax*) observed at zygote and 2-cell stage, apoptosis did not occur at these stages. This coincides with high expression levels of *SIRT1,* that may block ability to induce apoptosis in cells of early mouse embryos.

Acknowledgement: Supported by KRP/KNOW01/2015/01/DAWWIN grant

P77.

Continuously replacing dentition in anoline lizards: the way to change teeth shapes

Veronika Zahradnickova[1,2], Maria Hovorakova[3], Milan Bartos[4,5] and Oldrich Zahradnicek[3]

[1] *Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic*
[2] *The Prague Zoological Garden, Prague, Czech Republic*
[3]*Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic*
[4] *Department of Stomatology, First Faculty of Medicine, Charles University, Prague, Czech Republic*
[5] *Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic*

The ability to form multiple tooth generations gives the oportunity to change morphology and function of dentition during the life of individuals. Here we focus on four species of Cuban false chameleons (*Anolis* of *Chamaeleolis* group) and show postnatal changes in morphology of their snail shells crushing dentitions. Such specialized tool evolved from anole dentition adapted to insectivory and saurivory. Dentition morphologies were evaluated from SEM photographs and internal structure of teeth was examined from mCT scans. The morphology of the first functional tooth generation of all studied species showed ancestral state as in anoles (tricuspid teeth at the back of jaws). The typical molluscivorous dental characters as miniaturized or missing accessory cusps, robust teeth, rounded tooth tops and vertical ridges on crow are missing. These characters are fully developed only in caudal teeth of adults and are species specific. These characters were the most expressed in *A. barbatus* and the least in *A. guamuhaya*. Except this only one (*A. guamuhaya*) in all other studied species significantly increased the number of tooth positions during their life and resembles dentition of anoles. Other species added smaller numbers of tooth positions or did not add tooth positions at all. Adults of all molluscivorous species significantly increased the thickness of dentine at the tooth bases. This is the most apparent in *A. guamuhaya* and *A. porcus*. This points to the fact that *A. guamuhaya* adapted to molluscivory through increasing of dentine width while other species rather through the teeth shape and size changes.

Acknowledgement: We thank for the financial support to GACR 14-37368G.

P78.

Effect of advanced maternal age on reproductive outcomes in a mouse model of autism

Marta Zietek[1,2], Artur H. Swiergiel[2], Jacek A. Modlinski[1] and Silvestre Sampino[1]

[1] Department of Experimental Embryology, Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Poland
[2] Department of Animal and Human Physiology, Faculty of Biology, University of Gdansk, Poland

Advanced Maternal Age (AMA) negatively affects reproductive function and pregnancy outcomes, as well as is associated with an increased risk of neurodevelopmental disorders in offspring. The aim of this study was to investigate the effects of AMA on reproductive/pregnancy outcomes in the BTBR mouse model of autism, as well as in the C57BL/6J control strain. Young (YMA, 11-23 weeks old) and old (AMA, 27-40 weeks old) females were mated with young strain-matched males. Fetuses and placentas were collected at embryonic day 12.5 (E12.5) and fetus/placenta weight ratio was calculated; moreover, litter size and the percentage of retarded/resorbed fetuses were calculated. AMA females showed decreased pregnancy rate after mating, decreased litter size at E12.5 and at delivery and increased percentage of retarded/resorbed fetuses, compared to YMA controls. Furthermore, conceptuses of BTBR females showed increased fetus/placenta weight ratio compared to C57BL/6J. Aged BTBR females also showed decreased fetus/placenta weight ratio compared to young BTBR females. Overall, these results demonstrate that AMA negatively influence reproduction and pregnancy outcomes and that some of these effects are strain-specific and more evident in BTBR mice.

Acknowledgement: This work was supported by the Polish National Science Center (grant no. 2014/15/D/NZ4/04274 to SS) and by the KNOW Leading National Centre Scientific Consortium "Healthy Animal-Safe Food".

P79.

The influence of pluripotency state of mouse ESCs on their ability to colonize blastocysts

Krystyna Zyzynska-Galenska[1], Paulina Santus[1], Pierre Savatier[3] and Agnieszka Bernat[1,2]

[1] Laboratory of Experimental Embryology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland
[2] Laboratory of Molecular Diagnostics, Laboratory of Genetic Analysis, Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Gdansk, Poland
[3] INSERM U1208, Stem-Cell and Brain Research Institute, Pluripotent Stem Cells in Mammals Group, Bron/Lyon, France

Mouse embryonic stem cells (mESCs) can assume distinct states of pluripotency that reflect upon their key features like self-renewal capacity, proliferation kinetics, metabolic features and competence to produce chimeras. When mouse ESCs are maintained in cell culture, distinct pluripotency states can be achieved by manipulating and changing cell culture conditions. Our experiments were designed to test the possibility that pluripotency state of ESCs may affect the ability of mESC to colonise the blastocyst. First, mouse ESCs that were tagged with different genetic barcodes and GFP, were cultured in 3 different conditions: (a) LIF/serum, (b) STAT3 overexpression /serum, (c) LIF/ inhibitors of MAPK and GSK3 pathways (2i). Next, cells in various combinations were injected into cleaving 8-16 cell embryos, under zona pellucida and cultured until blastocyst stage. Blastocysts were analysed by confocal microscopy for cell visualization, by immunocytochemistry for expression of markers specific for ICM and TE and by MALDI-TOF mass spectrometry for detection of cell-identifying genetic barcodes. Our results show that all three types of cells have high affinity to ICM of the developing blastocysts. However, cells grown in STAT3 overexpression condition had lower ability to integrate with ICM. Analysis of barcodes show that at least two types of cells are able participate in ICM colonization.

Acknowledgement: This project was funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

P80.

WNT signaling in pathogenesis of odontogenic tumors

Barbora Putnova[1,2], Eva Hruba[1], Iveta Putnova[1,3], Jan Stembirek[1,4], Pavel Hurnik[4], Zdenek Danek[5] and Marcela Buchtova[1,6]

[1] Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Academy of Sciences, Brno, Czech Republic
[2] Department of Pathological Morphology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[3] Department of Anatomy, Histology and Embryology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[4] Department of Maxillofacial Surgery, University Hospital Ostrava, Ostrava, Czech Republic
[5] Clinic of Oral and Maxillofacial Surgery of Faculty Hospital, Masaryk University, Brno, Czech Republic
[6] Department of Experimental Biology, Masaryk University, Brno, Czech Republic

Ameloblastoma is the most common odontogenic tumor in humans. This tumor is mostly benign, but can invade local structures including bones. The reoccurrence after surgical treatment is very common. Here, we investigated the role of Frizzled6 (FZD6) in tumorigenesis of ameloblastoma. FZD6 is one of the receptors of WNT signaling pathway, which plays role in determination of planar cell polarity. We also investigated expression pattern of beta-catenin – major activator in canonical WNT signaling pathway and a cell-cell adhesion regulator.
Samples of benign and malign ameloblastoma were embedded into paraffin and further processed for immunofluorescence labelling (FZD6, beta-catenin, E-cadherin). To visualize expression pattern on the cellular level, confocal microscope Leica SP8 (Leica Microsystems, Germany) was used. Moreover, we analyzed differences in expression of key molecules of WNT signaling pathway in both types of ameloblastoma by PCR microarray. Oral epithelium of health patients was used as a control tissue.
As a receptor participating on the cell polarity establishment, we expected FZD6 to be expressed asymmetrically in ameloblastoma cells and preferentially in epithelial protrusions growing into the mesenchyme. Nevertheless, FZD6-positive cells were located mostly in the central area of epithelial islets. Expression of beta-catenin exhibited strong dotted pattern on membranes, which was in contrast to smooth expression in healthy gingival tissues. PCR Array uncovered upregulation of non-canonical WNT molecules in ameloblastoma samples. In conclusion, our findings revealed significant changes in WNT signaling during ameloblastoma pathogenesis.

Acknowledgement: This research was supported by the Czech Science Foundation (14-37368G).

Addresses

KEYNOTE SPEAKERS

Andersson Olov
Department of Cell and Molecular Biology, Karolinska Institutet
Berzelius väg 35, 171 65 Solna, Stockholm, Sweden
olov.andersson@ki.se

Chagin Andrei
Department of Physiology and Pharmacology, Karolinska Institutet
Solna Vägen 9, D6 171 65 Solna, Sweden
andrei.chagin@ki.se

Green B. Jeremy
Craniofacial Development & Stem Cell Biology King's College London
Guy's Tower, Floor 27, London SE1 9RT, United Kingdom
jeremy.green@kcl.ac.uk

Hájková Petra
Institute of Clinical Sciences, Faculty of Medicine, Imperial College London
South Kensington Campus
London SW7 2AZ, United Kingdom
petra.hajkova@imperial.ac.uk

Stern D. Claudio
UCL Research Department of Cell & Developmental Biology, University College London
Gower Street Anatomy Building, London
WC1E 6BT, United Kingdom
c.stern@ucl.ac.uk

Tomančák Pavel
Max Planck Institute of Molecular Cell Biology and Genetics
Pfotenhauerstrasse 108, D-01307
Dresden, Federal Republic of Germany
tomancak@mpi-cbg.de

Tucker S. Abigail
Craniofacial Development & Stem Cell Biology King's College London
Guy's Hospital London Bridge, SE1 9RT
London, United Kingdom
abigail.tucker@kcl.ac.uk

Verlhac Marie-Hélène
Centre Interdisciplinaire de Recherche en Biologie (CIRB), Collège de France
Cnrs/ Umr 7241 - Inserm U1050
11, Place Marcelin Berthelot
75231 Paris Cedex 05, France
marie-helene.verlhac@college-de-france.fr

ORGANIZERS

Buchtová Marcela
Institute of Animal Physiology and Genetics CAS, v.v.i.
Veveří 967/97, 602 00 Brno, Czech Republic
buchtova@iach.cz

Bruce Alexander W.
University of South Bohemia in České Budějovice
Branišovská 1760, 370 05 České Budějovice, Czech Republic
awbruce@prf.jcu.cz

Bryja Vítězslav
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
bryja@sci.muni.cz

Fabián Dušan
Institute of Animal Physiology - Centre of Biosciences, Slovak Academy of Sciences
Šoltésovej 4-6, 040 01 Košice, Slovak Republic
fabian@saske.sk

Piliszek Anna
Institute of Genetics and Animal Breeding, Polish Academy of Sciences
Postepu 36A, 05-552 Jastrzebiec, Republic of Poland
a.piliszek@ighz.pl

Varga Máté
Eötvös Loránd University, Department of Genetics
Pázmány Péter stny. 1/C, 1117 Budapest, Republic of Hungary
mvarga@ttk.elte.hu

Vilmos Peter
Biological Research Centre of the Hungarian Academy of Sciences
62. Temesvari krt., 6725 Szeged, Republic of Hungary
vilmosp@brc.hu

CONFERENCE SUPPORT

Hampl Marek
Institute of Animal Physiology and Genetics CAS, v. v. i.
Veveří 967/97, 602 00 Brno, Czech Republic
marekhampl12@gmail.com

Hyršl Pavel
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
hyrsl@sci.muni.cz

Nesvadbová Lucie
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
lus@sci.muni.cz

Zemanová Irena
Institute of Animal Physiology and Genetics CAS, v. v. i.
Veveří 967/97, 602 00 Brno, Czech Republic
i.zemanova@iach.cz

LIST OF PARTICIPANTS

Ajduk Anna
Department of Embryology, University of Warsaw
Krakowskie Przedmiescie 26/28, 00-927, Warsaw, Republic of Poland
aajduk@biol.uw.edu.pl

Aleshkina Daria
Czech Academy of Sciences Institute of Animal Physiology and Genetics, v. v. i.
Rumburská 89, 277 21 Liběchov, Czech Republic
alyoshkina_darya@list.ru

Anand Mahek
Szent Istvan University
Pater Karoly u.1, H-2100 Godollo, Republic of Hungary
sweet_anand2@yahoo.com

Anger Martin
CEITEC / Veterinary Research Institute
Hudcova 70/36, 621 00 Brno, Czech Republic
anger@vri.cz

Annus Tamás
Eötvös Loránd University
Pázmány Péter stny. 1/C, 1117 Budapest, Republic of Hungary
tamasannus@gmail.com

Babeľová Janka
Centre of Biosciences, Slovak Academy of Sciences, Institute of Animal Physiology
Šoltésovej 4-6, 04001 Košice, Slovak Republic
kubandova@saske.sk

Bajusz Csaba
Biological Research Centre - Hungarian Academy of Sciences
Temesvári krt. 62, 6726 Szeged, Republic of Hungary
csaba.bajusz@gmail.com

Bajusz Izabella
Biological Research Center Szeged Hungarian Academy of Sciences
Temesvári krt. 62., H-6726 Szeged, Republic of Hungary
iza@brc.hu

Baldovská Simona
Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
sbaldovska@gmail.com

Baran Vladimir
Centre of Biosciences - Inst. Animal Physiology SAS
Soltesovej 4, 04011 Kosice, Slovak Republic
baran@saske.sk

Bárta Tomáš
Department of Histology and Embryology, Masaryk University
Kamenice 126/3, 625 00 Brno, Czech Republic
tom.barta@gmail.com

Beckerová Deborah
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
417832@mail.muni.cz

Benc Michal
Institute of Animal Science
Přátelství 815/107, Uhříněves, 104 00 Praha, Czech Republic
benc.michal@gmail.com

Bence Melinda
Institute of Genetics, Biological Research Centre, HAS
Temesvari krt 62., 6726 Szeged, Republic of Hungary
bence.melinda@brc.mta.hu

Bernat Agnieszka
Institute of Genetics and Animal Breeding
Postepu 36 Jastrzebiec, 05-552 Magdalenka, Republic of Poland
a.bernat@ighz.pl

Bernatík Ondřej
Department of Histology and Embryology, Masaryk university
Kamenice 753/5, 625 00 Brno, Czech Republic,
ondrej.bernatik@gmail.com

Bílková Barbora
Institute of Animal Physiology and Genetics CAS, v. v. i.
Veveří 967/97, 602 00 Brno, Czech Republic
460989@mail.muni.cz

Bohačiaková Dáša
Department of Histology and Embryology, Faculty of Medicine, Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic,
dasa.bohaciakova@gmail.com

Bora Pablo
University of South Bohemia in České Budějovice
Branišovská 1760, 370 05 České Budějovice, Czech Republic
pablo.bora@hotmail.com

Borbély Noémi
ELTE Eötvös Loránd University Department of Genetics
Pázmány Péter sétány 1/c, 1117 Budapest, Republic of Hungary
borbelynoemi9702@gmail.com

Borkúti Péter
Biological Research Centre - Hungarian Academy of Sciences
Temesvári krt. 62, 6726 Szeged, Republic of Hungary
borkutip@brc.mta.hu

Bubelínyová Lucia
Institute of Animal Physiology and Genetics
Veveří 967/97, Veveří, 602 00 Brno, Czech Republic
460412@mail.muni.cz

Čajánek Lukáš
Department of Histology and Embryology, Masaryk university
Kamenice 753/5, 625 00 Brno, Czech Republic
cajanek@med.muni.cz

Chrenek Peter
Slovak University of Agriculture Nitra
Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic
peter.chrenek@uniag.sk

Czernik Marta
University of Teramo and The Institute of Genetics and Animal Breeding of the Polish Academy of Sciences
ul Postepu 36A, Jastrzebiec 55-052, Republic of Poland
mczernik@unite.it

Čada Štěpán
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk Unviersity
Kamenice 5, 625 00 Brno, Czech Republic
451280@mail.muni.cz

Čikoš Štefan
Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology
Šoltésovej 4-6, 04001 Košice, Slovak Republic
cikos@saske.sk

Dalecká Linda
Institute of Experimental Medicine CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
linda.dalecka@iem.cas.cz

Dave Zankruti
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
432167@mail.muni.cz

Dedukh Dmitrii
Institute of Animal Physiology and Genetics CAS
Rumburská 89, Liběchov, 277 21 Liběchov, Czech Republic
dmitrov89@yandex.ru

del Llano Edgar
Institute of Animal Physiology and Genetics
Rumburská 89, Liběchov, 277 21 Liběchov, Czech Republic
llano@iapg.cas.cz

Demko Viktor
Comenius University in Bratislava
Ilkovicova 6, 84215 Bratislava, Slovak Republic
demko1@uniba.sk

Drozdová Angelika
Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University
Iľkovičová 6, 84215Bratislava, Slovak Republic
angelikadrozdova@gmail.com

Drutovic David
Institute of Animal Physiology and Genetics CAS, v.v.i.
Rumburská 89, 277 21 Liběchov, Czech Republic
drutovic@iapg.cas.cz

Dudas Krisztina
Biological Research Centre, Szeged, Hungary
Temesvari krt. 62, H-6726 Szeged, Republic of Hungary
dudas.kriszti93@gmail.com

Dumková Jana
Department of Histology and Embryology, Masaryk University
Kamenice 126/3, 625 00 Brno, Czech Republic
jdumkova@med.muni.cz

Ďuríček Tomáš
Institute of Animal Physiology and Genetics AS ČR, v.v.i.
Rumburská 89, 277 21 Liběchov, Czech Republic
tom.duricek@gmail.com

Erdelyi Miklós
Institute of Genetics, BRC, Hungarian Academy of Sciences
Temesvari krt., H-6726 Szeged, Republic of Hungary
erdelyi.miklos@brc.mta.hu

Fabik Jaroslav
Institute of Experimental Medicine, the Czech Academy of Sciences
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
jaroslav.fabik@iem.cas.cz

Fedorová Veronika
Masaryk University, Department of Histology and Embryology
Kamenice 126/3, 625 00 Brno, Czech Republic
veronika.fed@volny.cz

Fenclová Tereza
Biomedical Center
Alej Svobody 1655/76, Severní Předměstí, 323 00 Plzeň, Czech Republic
fenclova.tereza@gmail.com

Filimonow Katarzyna
Institute of Genetic and Animal Breeding, Polish Academy of Sciences
Postepu 36A, 05-552Jastrzebiec, Republic of Poland
k.filimonow@ighz.pl

Florek Wiesława
Institute of Genetics and Animal Breeding, Polish Academy of Sciences
Postepu 36A, 05-552 Jastrzebiec, Republic of Poland
w.florek@ighz.pl

Fulková Helena
Institute of Animal Science
Přátelství 815/107, Uhříněves, 104 00 Praha, Czech Republic
fulkova.helena@vuzv.cz

Gad Ahmed
Institute of Animal Physiology and Genetics, Czech Academy of Science
Rumburská 89, 277 21 Liběchov, Czech Republic,
gad@iapg.cas.cz

Gahurová Lenka
University of South Bohemia in České Budějovice
Branišovská 1760, 370 05 České Budějovice, Czech Republic
lveselovska@prf.jcu.cz

Gocza Elen
National Agricultural Research and Innovation Centre
Szent-Gyorgyi A. Str. 4., 2100 Godollo, Republic of Hungary
goczaelen@me.com

Gömöryová Kristína
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
423436@mail.muni.cz

Gregorová Michaela
Masaryk University
Mánesova 892/24, Hradec Králové, 500 02 Hradec Králové, Czech Republic
michaela.gregorova13@gmail.com

Hampl Aleš
Department of Histology and Embryology, Masaryk university
Kamenice 753/5, 625 00 Brno, Czech Republic
ahampl@med.muni.cz

Hampl Marek
Institute of Animal Physiology and Genetics
Rumburská 89, 277 21 Liběchov, Czech Republic
marekhampl12@gmail.com

Hencz Alexandra
University of Pecs, Medical School, Department of Biophysics
Szigeti Street 12., 7624 Pécs, Republic of Hungary
alexandra.hencz@aok.pte.hu

Henn László
Institute of Biochemistry, Hungarian Academy of Sciences
Temesvari krt., 62., 6726 Szeged, Republic of Hungary
henn@brc.hu

Henry Surya
Biological Research Institute, Hungarian Academy of Science, Szeged, Hungary
Temesvári krt. 62., H-6726 Szeged, Republic of Hungary
regissurya10@gmail.com

Herberth Minkó Krisztina H.
Semmelweis University
Tűzoltó, 1094 Budapest, Republic of Hungary
hminkokriszta@gmail.com

Hlubcová Zuzana
Department of Histology and Embryology, Masaryk University
Kamenice 126/3, 625 00 Brno, Czech Republic
zholub@med.muni.cz

Hovořáková Mária
Institute of Experimental medicine, CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
marhor@biomed.cas.cz

Hrubá Eva
Institute of Animal Physiology and Genetics CAS, v. v. i.
Veveří 967/97, Veveří, 602 00 Brno, Czech Republic
uzievama@gmail.com

Huszár Emese
Semmelweis University
Tűzoltó, 1094 Budapest, Republic of Hungary
michaemeril@gmail.com

Ibrahim Karam
Institute of Genetics, Hungarian Academy of Sciences
Temesvari krt. 62, 6726 Szeged, Republic of Hungary
jankovics.ferenc@brc.mta.hu

Iyyappan Rajan
Institute of Animal Physiology and Genetics
Rumburská 89, 277 21 Liběchov, Czech Republic
iyyappan@iapg.cas.cz

Jakešová Veronika
Institute of Animal Physiology and Genetics
Rumburská 89, 277 21 Liběchov, Czech Republic
veronikam@volny.cz

Jankovics Ferenc
Institute of Genetics, Hungarian Academy of Sciences
Temesvari krt. 62, 6726 Szeged, Republic of Hungary
jankovic@brc.hu

Janovská Pavlína
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
pavlina.janovska@gmail.com

Jaroš Josef
Department of Histology and Embryology, Masaryk university
Kamenice 753/5, 625 00 Brno, Czech Republic
jaros@med.muni.cz

Jindra Marek
Department of Genetics,
Biology Center ASCR
Branisovska 31, 37005 Ceske Budejovice, Czech Republic
jindra@entu.cas.cz

Jipa András
Biological Research Centre Szeged
Temesvári krt. 62., 6726 Szeged, Republic of Hungary
jipaandras@gmail.com

Kaiser Karol
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
karol.kaiser1@gmail.com

Kalous Jaroslav
Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic
Rumburská 74, 277 21 Liběchov, Czech Republic
kalous@iapg.cas.cz

Kasperczuk Anna
Department of Embryology, Faculty of Biology, University of Warsaw
Ilji Miecznikowa 1, 02-096 Warsaw, Republic of Poland
anna.kasperczuk@uw.biol.edu.pl

Kavková Michaela
CEITEC Brno University of Technology
Purkyňova 656/123, 612 00 Brno, Czech Republic
michaela.kavkova@ceitec.vutbr.cz

Khan Anzer
CEITEC Masaryk university
Kamenice 753/5, 625 00 Brno, Czech Republic
anzer.khan@ceitec.muni.cz

Killinger Michael
Institute of Animal Physiology and Genetics CAS, v. v. i.
Veveří 967/97, Veveří, 602 00 Brno, Czech Republic
423455@mail.muni.cz

Kinterová Veronika
Institute of Animal Physiology and Genetics
Rumburská 89, 277 21 Liběchov, Czech Republic
kinterova@iapg.cas.cz

Klimczewska Katarzyna
Department of Embryology, Faculty of Biology, University of Warsaw
Krakowskie Przedmieście 26/28, 00-927 Warsaw, Republic of Poland
k.klimczewska@biol.uw.edu.pl

Knapová Lívia
Masaryk university
Kamenice 126/3, 625 00 Brno, Czech Republic
liviaknapova@gmail.com

Knoblochová Lucie
Institute of Animal Physiology and Genetics AS CZ v.v.i.
Rumburská 89, 277 21 Liběchov, Czech Republic
knoblochova@iapg.cas.cz

Knop Filip
Charles University
Albertov 2038/6, Nové Město, 128 00 Praha, Czech Republic
knopf@natur.cuni.cz

Kohoutek Jiří
Veterinary Research Institute
Hudcova 296/70, Medlánky, 621 00 Brno, Czech Republic
kohoutek@vri.cz

Koledová Zuzana
Masaryk University, Faculty of Medicine
Kamenice 126/3, Bohunice, 625 00 Brno, Czech Republic
koledova@med.muni.cz

Kolesárová Adriana
Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
adriana.kolesarova@uniag.sk

Kolková Miroslava
Institute of Molecular Genetics, CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
miroslava.kolkova@img.cas.cz

Kompaníková Petra
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
450952@sci.muni.cz

Končická Markéta
Institute of Animal Physiology and Genetics, CAS
Rumburská 89, 277 21 Liběchov, Czech Republic
makykonc@centrum.cz

Kotasová Hana
Department of Histology and Embryology, Masaryk University
Kamenice 126/3, 625 00 Brno, Czech Republic
151025@mail.muni.cz

Kovács Gergo
Biological Research Centre - Hungarian Academy of Sciences
Temesvári krt. 62., H-6726 Szeged, Republic of Hungary
kovacs.gergo@brc.mta.hu

Kovács Tamás
Semmelweis University
Viola street, 1094 Budapest, Republic of Hungary
kovitomi.sote@gmail.com

Kovács Zoltán
Biological Research Centre of the HAS
Temesvári krt. 62., 6726 Szeged, Republic of Hungary
kovacs96zoltan@gmail.com

Kovaříková Petra
Department of Histology and Embryology, Masaryk university
Kamenice 753/5, 625 00 Brno, Czech Republic
kovarikova@med.muni.cz

Kovaříková Veronika
Centre of Biosciences, Slovak Academy of Sciences, Institute of Animal Physiology
Šoltésovej 4-6, 04001 Košice, Slovak Republic
kovarikova@saske.sk

Kravec Marek
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
mar.kravec@gmail.com

Krejčí Alena
University of South Bohemia, Faculty of Science
Branišovská 1160/31, 370 05 České Budějovice, Czech Republic
akrejci@prf.jcu.cz

Kristó Ildikó
Biological Research Centre, Hungarian Academy of Sciences
Temesvári krt. 62., 6726 Szeged, Republic of Hungary
kristoi@brc.hu

Křivánek Jan
Faculty of Medicine, Masaryk University
Kamenice 753/5, Bohunice, 625 00 Brno, Czech Republic
kriv.jan@gmail.com

Krylov Vladimír
Charles University, Faculty of Science
Viničná 1594/7, Nové Město, 128 00 Praha, Czech Republic
vkrylov@natur.cuni.cz

Kubelka Michal
Institute of Animal Physiology and Genetics CAS, v.v.i.
Rumburská 89, 277 21 Liběchov, Czech Republic
kubelka@iapg.cas.cz

Kunová Bosáková Michaela
Faculty of Medicine, Masaryk University
Kamenice 753/5, Bohunice, 625 00 Brno, Czech Republic
bosakovam@med.muni.cz

Landová Marie
Institute of Animal Physiology and Genetics CAS, v.v.i.
Veveří 979/5, 602 00 Brno, Czech Republic
maruska.landova@seznam.cz

Lavický Josef
Department of Histology and Embryology, Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
437145@mail.muni.cz

Lesáková Petra
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
437104@mail.muni.cz

Luza Bernadette
Center for Molecular Biology, University of Vienna
Dr. Bohr Gasse 9, 1030 Vienna, Republic of Austria
bernadette.luza@gmx.at

Madeja Zofia
Department of Genetics and Animal Breeding, Poznan University of Life Sciences
Wołyńska 33, 60-637 Poznan, Republic of Poland
zmadeja@up.poznan.pl

Magnin Valentina
University of South Bohemia in České Budějovice
Branišovská 1160/31, 370 05 České Budějovice, Czech Republic
valentina.magnin@gmail.com

Macháčová Simona
Institute of Molecular Genetics CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
simona.machacova@img.cas.cz

Machoň Ondřej
Institute of Experimental Medicine CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic,
ondrej.machon@iem.cas.cz

Marônek Martin
Institute of Molecular Biomedicine
Sasinkova 4, 811 08 Bratislava, Slovak Republic
martin.maronek@gmail.com

Mihály József
Biological Research Centre, Szeged
Temesvari krt. 62., 6726 Szeged, Republic of Hungary
mihaly.jozsef@brc.mta.hu

Modliński Jacek A.
Institute of Genetics and Animal Breeding, Polish Academy of Sciences
Postepu 36A, 05-552 Jastrzebiec, Republic of Poland
amsa@wp.pl

Mršťáková Simona
Institute of Molecular Genetics CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
simona.mrstakova@img.cas.cz

Müller Dalma
Eötvös Loránd University
Pázmány Péter sétány 1/C, 1117
Budapest, Republic of Hungary
dalma.muller2@gmail.com

Murín Matej
Institute of Animal Physiology and Genetics CAS, v. v. i.
Rumburská 89, 277 21 Liběchov, Czech Republic
murin@iapg.cas.cz

Nevoral Jan
Faculty of Medicine in Pilsen - Charles University
Alej Svobody 1655/76, Severní Předměstí, 323 00 Plzeň, Czech Republic
jan.nevoral@lfp.cuni.cz

Nickl Petr
Institute of Experimental Medicine CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
petr.nickl@iem.cas.cz

Nkubito Claudia
Institute of Genetics, Biological Research Centre, HAS
Temesvari krt 62., 6726 Szeged, Republic of Hungary
bencemelinda@gmail.com

Nováková Barbora
Ceitec - Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
451444@mail.muni.cz

Nováková Monika
Veterinary Research Institute
Hudcova 70/36, Medlánky, 621 00 Brno, Czech Republic
novakova@vri.cz

Olbertová Kristýna
Institute of Animal Physiology and Genetics CAS, v. v. i.
Veveří 967/97, Veveří, 602 00 Brno, Czech Republic
437234@mail.muni.cz

Olexiková Lucia
NPPC-Research Institute for Animal Production
Hlohovecká 2, 95141 Lužianky, Slovak Republic
olexikova@vuzv.sk

Paclíková Petra
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
394465@mail.muni.cz

Pasovská Martina
Institute of Experimental Medicine CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
martina.pasovska@gmail.com

Pawlak Piotr
Poznan University of Life Sciences
Wojska Polskiego 28, 60-637 Poznan, Republic of Poland
piotrpaw@up.poznan.pl

Pejtsik Diána
Eötvös Loránd University, Faculty of Science
Temesvári krt. 62, 6726 Szeged, Republic of Hungary
pejtsikdiana@gmail.com

Pergner Jiří
Institute of Molecular Genetics CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
jiri.pergner@img.cas.cz

Pirity Melinda Katalin
Biological Research Centre - Hungarian Academy of Sciences
Temesvári krt. 62., H-6726 Szeged, Republic of Hungary
pirity.melinda@brc.mta.hu

Pisko Jozef
Centre of Biosciences, Slovak Academy of Sciences, Institute of Animal Physiology
Šoltésovej 4-6, 040 01 Košice, Slovak Republic
pisko@saske.sk

Pospíšilová Anna
Charles University in Prague
Viničná 1594/7, Nové Město, 128 00 Praha, Czech Republic
pospia@natur.cuni.cz

Procházka Radek
Institute of Animal Physiology and Genetics CAS
Rumburská 89, 277 21 Liběchov, Czech Republic
prochazka@iapg.cas.cz

Prokešová Šárka
Czech University of Life Sciences
Kamýcká 129, Suchdol, 165 00 Praha, Czech Republic
cherry.07@seznam.cz

Pryszcz Leszek
IIMCB
Ks. Trojdena, Warsaw, Republic of Poland, 02-109
lpryszcz@iimcb.gov.pl

Psutková Viktorie
Charles University
Viničná 1594/7, Nové Město, 128 00 Praha, Czech Republic
psutkovv@natur.cuni.cz

Putnová Barbora
Institute of Animal Physiology and Genetics CAS, v. v. i.
Veveří 967/97, Veveří, 602 00 Brno, Czech Republic
putnovab@vfu.cz

Rabata Anas
Department of Histology and Embryology, Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
ar2004@hotmail.com

Radaszkiewicz Katarzyna
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
katarzynajonek@gmail.com

Radaszkiewicz Tomasz
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
radaszkiewicztomasz@gmail.com

Radonová Lenka
Veterinary Research Institute
Hudcova 296/70, Medlánky, 621 00 Brno, Czech Republic
radonova@vri.cz

Sampino Silvestre
Institute of Genetics and Animal Breeding, Polish Academy of Sciences
ul. Postepu 36A, 05-552 Jastrzebiec, Republic of Poland
s.sampino@ighz.pl

Serati Federica
University of Pavia
via Adolfo Ferrata 9, 27100 Pavia, Italy
fede.s33@libero.it

Šindelka Radek
Institute of Biotechnology, CAS
Průmyslová 595, 252 50 Vestec, Czech Republic
sindelka@ibt.cas.cz

Sinka Rita
University of Szeged Department of Genetics
Kozepfasor 52, 6726 Szeged, Republic of Hungary
rsinka@bio.u-szeged.hu

Smutná Tereza
Institute of Animal Physiology and Genetics CAS
Rumburská 89, 277 21 Liběchov, Czech Republic
terka.smutna12@gmail.com

Soukup Vladimír
Charles University in Prague
Viničná 1594/7, Nové Město, 128 00 Praha, Czech Republic
soukup@natur.cuni.cz

Sovadinová Iva
Masaryk University, Faculty of Science
Kamenice 753/5, 625 00 Brno, Czech Republic
i.sova@seznam.cz

Stachowiak Anna
Institute of Genetics and Animal Breeding of the Polish Academy of Sciences
Postepu 36A, 05-552 Jastrzebiec, Republic of Poland
a.stachowiak@ighz.pl

Steffal Pavel
University of South Bohemia in České Budějovice
Branišovská 1160/31, 370 05 České Budějovice, Czech Republic
steffal.pavel@gmail.com

Steklíková Klára
Institute of Experimental Medicine CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
klara.steklikova@iem.cas.cz

Sumbal Jakub
Department of Histology and Embryology, Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic jakub.sumbal@gypri.cz

Sutus Enikő
Biological Research Centre - Hungarian Academy of Sciences
Temesvári krt 62, 6726 Szeged, Republic of Hungary
sutus.eniko@brc.mta.hu

Suwinska Aneta
Department of Embryology, University of Warsaw
Miecznikowa 1, 02-095 Warsaw, Republic of Poland
asuwinska@biol.uw.edu.pl

Svitok Pavel
GYN – FIV a.s., Centre for Gynecology and Assisted Reproduction
Záhradnícka 42, 82108 Bratislava, Slovak Republic
mario@skitravel.sk

Svobodová Tereza
CEITEC - Cellular Imaging (CELLIM) - Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
svobodova@vri.cz

Svoradová Andrea
Constantine the Philosopher University, Faculty of Natural Sciences, Nitra, Slovakia
andrea.svoradova@ukf.sk

Szabó Viktória
Biological Research Centre - Hungarian Academy of Sciences
Temesvári krt. 62, H-6726 Szeged, Republic of Hungary
szabo.viktoria@brc.mta.hu

Szarka-Kovács Alexandra Brigitta
Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences
Temesvári körút, 6726 Szeged, Republic of Hungary
szarka.brigitta@brc.mta.hu

Škultéty Michal
CEITEC - CELLIM, Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
michal.skultety@ceitec.muni.cz

Šmarda Jan
Department of Experimental Biology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
smarda@sci.muni.cz

Šolc Petr
Inst. of Animal Physiology and Genetics CAS
Rumburská 89, 277 21 Liběchov, Czech Republic
solc@iapg.cas.cz

Šranko Patrik
GYN – FIV a.s., Centre for Gynecology and Assisted Reproduction
Záhradnícka 42, 82108 Bratislava, Slovak Republic
slovakia@skitravel.sk

Šumberová Veronika
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
veronika.sumberova@gmail.com

Šupák Marek
Veterinary Research Institute
Hudcova 296/70, Medlánky, 621 00 Brno, Czech Republic
marek.supak@gmail.com

Takács Bertalan
Institute of Genetics, Biological Research Centre Hungarian Academy of Sciences
Temesvári krt. 62. , 6726 Szeged, Republic of Hungary
takacs.bertalan119@gmail.com

Tetková Anna
Institute of Animal Physiology and Genetics CAS
Rumburská 89, 277 21 Liběchov, Czech Republic
tetkova@iapg.cas.cz

Tomková Mária
Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
majkat.93@gmail.com

Toralová Tereza
Institute of Animal Physiology and Genetics AS CR, v.v.i.
Rumburská 89, 277 21 Liběchov, Czech Republic
toralova@iapg.cas.cz

Trachtulec Zdenek
Institute of Molecular Genetics CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
zdenek.trachtulec@img.cas.cz

Trebichalská Zuzana
Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
trebich.zuzana@gmail.com

Tur Irfan
Veterinary Research Institute
Hudcova 296/70, Medlánky, 621 00 Brno, Czech Republic
tur@vri.cz

Türmer Katalin
University of Pécs Medical School
Department of Biophysics
Szigeti Street 12., 7624 Pécs, Republic of Hungary
katalin.turmer@aok.pte.hu

Varga Zsombor
ELTE Eötvös Loránd University
Pázmány P. stny. 1/C, 1117 Budapest, Republic of Hungary
varga.zsombor95@gmail.com

Vašíček Jaromír
Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
jaromir.vasicek@gmail.com

Večeřa Josef
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
vecera@sci.muni.cz

Vedelek Viktor
University of Szeged
Kozepfasor 52, 6726 Szeged, Republic of Hungary
ugu@veta.hu

Vincze Katalin
Eötvös Loránd University
Viola str. 16-18., 1094 Budapest, Republic of Hungary
vinczekatalin.elte@gmail.com

Virnicchi Giorgio
University of South Bohemia in České Budějovice
Branišovská 1160/31, 370 05 České Budějovice, Czech Republic
giorgio.virnicchi@gmail.com

Vodička Petr
Institute of Animal Physiology and Genetics CAS
Rumburská 89, 277 21 Liběchov, Czech Republic
vodicka@iapg.cas.cz

Vondálová Blanářová Olga
Section of Animal Physiology and Immunology, Faculty of Science, Masaryk University
Kamenice 5, 625 00 Brno, Czech Republic
olgablanarova@seznam.cz

Vrlíková Lucie
Institute of Animal Physiology and Genetics Rumburská 89, 277 21 Liběchov, Czech Republic
vrlikova@iach.cz

Winata Cecilia
International Institute of Molecular and Cell Biology in Warsaw
Ks. Trojdena 4, 02-109 Warsaw, Republic of Poland
cwinata@iimcb.gov.pl

Winiarczyk Dawid
Institute of Genetics and Animal Breeding of the Polish Academy of Sciences
Postepu 36A, Jastrzebiec, 05-552 Magdalenka Republic of Poland
d.winiarczyk@ighz.pl

Zahradníček Oldřich
Institute of Experimental Medicine CAS
Vídeňská 1083, Krč, 142 00 Praha, Czech Republic
oldrich.zahradnicek@iem.cas.cz

Zietek Marta
Institute of Genetics and Animal Breeding of the Polish Academy of Sciences
Postepu 36A, 05-552 Jastrzebiec, Republic of Poland
m.zietek@ighz.pl

Zikmund Tomáš
CEITEC Brno University of Technology
Purkyňova 656/123, 612 00 Brno, Czech Republic
tomas.zikmund@ceitec.vutbr.cz

Żyżyńska-Galeńska Krystyna
Institute of Genetics and Animal Breeding of the Polish Academy of Sciences
Jastrzebiec ul. Postepu 36a, 05-552 Magdalenka, Republic of Poland
k.zyzynska@ighz.pl

Authors and co-authors of the presentations

Abbreviations:
T – talk
P – poster
bold – presenting author