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Institute of Molecular Genetics

12th June 2015

IMG EMBO EXBIO Antibodies life technologies tataabiocenter DYNEX Roche

Programme

9:00-9:15	**Opening - Vaclav Horejsi**
9:15-9:35	Olga Ticha: Role of Tcf3 and Tcf4 transcription factors during neurogenesis
9:35-9:55	Radim Zidek: Wnt signalling in *Platynereis*' neurodevelopment
9:55-10:15	Matyas Sima: How to sequence a mouse gene
10:15-10:35	Sarka Techlovska: Metabotropic Glutamat Receptor 1 splice variants subunit composition
10:35-11:00	**Coffee break**
11:00-12:00	**EMBO YOUNG INVESTIGATOR LECTURE** **Julien Vermot: Mechanical forces in the developing embryonic cardiovascular network**
12:00-13:00	**Lunch**
13:00-13:20	Monika Horazna: MSX1 function in intestinal tumorigenesis
13:20-13:40	Edita Krizova: Danger face of R-loops
13:40-14:00	Helena Fabryova: Colugos acquired lentiviruses before it was cool
14:00-14:10	**Coffee break**
14:10-14:30	Albert Font Haro: Sensing viruses: Dichotomy of immunoreceptor signaling in plasmacytoid dendritic cells
14:30-14:50	Tomas Paulenda: Why ORMDL and asthma
14:50-15:10	Matous Voboril: Emerging new role of TLRs in immunity and tolerance
15:10-15:20	**Voting for the best presentation**
15:30-00:00	**Best presentation announcement, closing remarks & party**

Olga Ti cha
Kozmik La b

Role of Tcf3 and Tcf4 transcription factors during neurogenesis

The canonical Wnt signaling plays a key role in maintenance of the balance between proliferation and differentiation of neural progenitors during embryonal and adult neurogenesis. Transcription factors Tcf/Lef are likely to have a central role in shaping a cell-type specific response to Wnt signalling. In neurogenesis, Wnts act as a morphogenetic gradient by decelerating precocious differentiation. In the adult brain Wnt signaling is active in adult stem cell niches (ASCN) - in the dentate gyrus and in the subventricular zone (SVZ). Our unpublished data show specific expression of Tcf3 in both areas of ASCNs and that of Tcf4 protein in the SVZ. Furthermore, we localized Tcf3 and Tcf4 proteins in the embryonic cortex in a concentration gradient which is reciprocal to the gradient of the Wnt activity. We propose that TCF4 and TCF3 act cooperatively in the embryonal cortex and fine tune the transcriptional response to Wnt signaling by its negative regulation. To elucidate the role of Tcf3 and Tcf4 during adult and embryonic neurogenesis we will employ conditional knock-out experiments using Cre/LoxP system. We also plan to create a new forebrain specific Cre line utilizing forebrain-specific enhancer of the FoxG1 gene.

Radim Zidek
KOzmik lab

Wnt signalling in *Platynereis'* neurodevelopment

In neural development, Wnt signalling specifies posterior end of a body, lateral regions of neural plate, they decide between proliferation and differentiation and guide growing axons. Marine annelid *Platynereis dumerilii* is a representative of the clade Lophotrochozoa, a large phylogenetic group which has been previously under-represented among model organisms. We characterize the involvement of Wnt signalling in neurodevelopment of this marine worm and look for conserved regulation of neural patterning genes. We manipulate Wnt/β-catenin signalling by cultivating the larvae in presence of its chemical activators or inhibitors and, by in-situ hybridization, we then visualize expression patterns of selected transcription factors crucial for neuroectoderm patterning. Here, I demonstrate that the manipulations successfully change levels of β-catenin and the expression of pax6 and pax3/7 in ventral neuroectoderm, as well as the apical domain of nk2.1, are greatly reduced upon over-activation of canonical Wnt signalling. Interestingly, both activation and inhibition lead to a loss of comissures between the pair of ventral nerve cords. We have also isolated and characterized the sequence of the single *Platynereis'* Tcf, the effector of Wnt/β-catenin pathway. Pdu-Tcf behaves similar to LEF1, an activator of gene expression, in mammalian cells in-vitro and it is expressed broadly during larval development.

Maty As Sima
Lipoldo V a lab

How to sequence a mouse gene

Using genetic mapping in F2 hybrids between differentially susceptible mouse strains (BALB/c and CcS-11) we were able to identify locus on chromosome 7 which affects response to tick born encephalitis. This locus co-localizes with gene Irf3 which codes a protein acting as a molecular switch for antiviral activity. We hypothesized that Irf3 can play role in different susceptibility of tested mice. To test if Irf3 is our gene of interest we decided to sequence this gene in examined strains. The gene length is 5200 bp and we performed sequencing of whole gene by sequencing PCR products covering entire gene. In my presentation I will try to describe whole procedure of preparation of samples for sequencing along with all obstacles and troubleshooting which we discovered during setting of the method in our laboratory. I will also touch how to analyze the data.

Sarka Techlovska
Blahos la B
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Metabotropic Glutamat Receptor 1 splice variants subunit composition

Glutamate mediates excitatory neuronal signalization in mammalian brain. Among the receptors which are able to recognize glutamate belong ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) that belong to G-protein coupled receptors (GPCRs) superfamily. Function-related, specific feature of GPCRs that were described in last decade, is, among others formulation of receptor multimeric complexes. Metabotropic glutamate receptors are involved in physiological processes such as learning and memory. Metabotropic glutamate receptor 1 is located on the postsynaptic neuronal portions, where it affects synaptic plasticity and plays a role in the feedback inhibition of neurotransmitter release. Metabotropic glutamate receptor 1 is in vivo present in mammalian brain in several splice variants. The long mGluR1a was described to forms covalently linked homodimers. We discovered heteromers between the long mGluR1a and short variant mGluR1b in vivo. Moreover, we revealed that the mGluR1b is trafficked to the synapses only when covalently connected to mGluR1a. Therefore, mGluR1a/b heterodimers are a novel receptors found on postsynaptic portions of the neurons.

Julien Vermot

EMBO

Mechanical forces in the developing embryonic cardiovascular network

Mechanical forces are fundamental to cardiovascular development and physiology. The interactions between mechanical forces and endothelial cells are mediated by mechanotransduction feedback loops. Defects in these processes can cause catastrophic developmental abnormalities, in particular in the cardiovascular system where blood flow is generating shear forces essential for cardiogenesis. We are interested in understanding how hemodynamic forces modulate cardiovascular function and morphogenesis. Overall, we aim to **unravel the biological links between mechanical forces, mechanotransduction and endothelial cell responses.** We will discuss these fundamental questions and approaches we develop in the lab in the context of blood vessels and heart valve development in zebrafish embryo. We will also address how to image and quantify mechanical forces in vivo using optical tools. This work helps to better understand the origins of congenital diseases such as cardiomyopathies, valvulopathies and ciliopathies as well as abnormal conditions like atherosclerosis and tumor spreading.

Institut Génétique Biologie Moléculaire Cellulaire
Illkirch-Graffenstaden, France

Matou S Voboril
Filipp La b
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Emerging new role of TLRs in immunity and tolerance

Tolerance to “self” is the fundamental property of the immune system and its breakdown can lead to autoimmune diseases. In order to eliminate self-reactive T-cells during their development in the thymus, Aire promotes the expression of peripheral self-antigens in medullary thymic epithelial cells. Recently, Aire was suggested to fulfil a similar function in the rare subclass of lymph node cells. Although the detection, characterization and function of these Aire-expressing cells have been intensively investigated, their role and properties in inflammatory processes is completely unknown. The results of our recent experiments show the expression of TLRs and their signaling adaptors by Aire-expressing cells in lymph nodes and demonstrate, for the first time, changes in the tolerogenic capacity of particular type of Aire-expressing cell after TLR2 and TLR9 stimulation. This phenomenon brings completely new insight into the role of TLRs signaling in context of peripheral tolerance during the inflammation.

Mo Ni ka Horazna
Kor In ek lab

MSX1 function in intestinal tumorigenesis

The canonical Wnt signaling pathway is essential for cell fate decision during embryogenesis and adult tissue homeostasis. Disruption or misregulation of Wnt signaling underlies onset of various diseases including colorectal cancer. A key regulator of the pathway is the tumor suppressor APC (Adenomatous Polyposis Coli). APC inactivation causes hyperproliferation of intestinal stem cells subsequently leading to neoplasia formation.In order to identify genes affected by loss of Apc, we performed expression profiling of the intestinal epithelium in mice with the inactivated gene. The transcriptional factor Msx1 (MSH homeobox 1) belonged to the genes displaying significantly increased expression after Apc inactivation. The role of the MSX1 protein in colorectal cancer has not been elucidated yet, although the MSX1 expression is observed to be remarkably increased in human colorectal adenocarcinoma and in intestinal tumors of Apc-deficient mice. The importance of the Msx1 gene in the mouse intestine was further supported by in situ hybridization of Msx1 mRNA which revealed Msx1 expression in both intestinal crypts and tumors. Moreover, the Msx1 mRNA expression in distinct cellular populations of mouse intestinal epithelium correlated with expression of intestinal stem cell markers, which suggests an important role of Msx1 in the tissue.

Edi Ta Krizova

Barte K lab

Danger face of R-loops

The cellular genome has to challenge number of extracellular threats as well as intracellular dangers of structures referred as R-loops. R-loops represent co-transcriptional RNA:DNA hybrids where nascent RNA transcript hybridizes with DNA template. The non-template strand remains unpaired and is predisposed to mutations, DNA breaks and recombination. Moreover, unscheduled R-loop formation and its stabilization results in the replication fork stalling and collapse. Stabilized R-loops are therefore viewed as highly genotoxic structures that might contribute to transformation of pre-cancer to fully malignant cells. Although R-loops are intensively studied, the mechanisms preventing their formation and contributing to their resolution remain poorly understood. We aim to identify proteins associated with R-loops under conditions leading to replication-transcription interference and to determine the role of identified proteins in genome integrity maintenance. Between a few known R-loop resolving proteins belongs RNaseH1, an enzyme specifically hydrolyzing the RNA strand in RNA:DNA hybrid. To gain a tool for R-loops isolation we established a stabile cell line expressing GFP-tagged RNAseH1 with mutated catalytic domain. Such modified enzyme is able to recognize and bind R-loops but not to degrade them. This cellular system will simplify R-loop isolation by chromatin immunoprecipitation and associated proteins will be identified by mass spectrometry.

Hele Na Fabryova

Hejn Ar lab

Colugos acquired lentiviruses before it was cool

Mammalian genomes contain a large fraction of endogenous retroviral sequences that are formed by germline infiltration of various retroviruses. We performed a computational screening of 104 publicly available vertebrate genomes to identify a novel endogenous retrovirus. We stumbled upon endogenous lentivirus in Malayan colugo (*Galeopterus variegatus*). So far known endogenous lentiviruses were identified in ferret, lemur, and rabbit. This is the first report of an endogenous lentivirus in an Asian mammal and potentially closest relative of primates. The assembled proviruses contain many defects, solo LTRs are present in the germline and we detected the provirus in the related species *Cynocephalus variegatus* (the only additional extant species in the order Dermoptera). These facts indicate that *Galeopterus variegatus* genomes might contain the oldest described endogenous lentivirus.

Tomas Paulenda
Draber Pe. Lab

Why ORMDL and asthma

Orosomucoid (ORM) family proteins have been discovered many years ago in yeasts. Increased interest in these proteins started after discovery of the ORM1-like 3 (ORMDL3) human ortholog and even more after finding of ORMDL3 polymorphisms to be associated with childhood onset asthma. ORMDL proteins are ubiquitously expressed in human tissues. Interestingly, they share high sequence similarity in all vertebrates, suggesting their physiological importance. At present it is known that ORM proteins in yeasts are involved in regulation of unfolded protein response (UPR), cell wall stress, nutrient conditions and most importantly in regulation of sphingolipid biosynthesis. In mouse and human tissues, ORMDL proteins function as regulators of Ca^{2+} signaling, UPR, and sphingolipid biosynthesis. However, it is unlikely that decreased sphingolipid content is responsible for childhood onset asthma because all three ORMDL proteins must be down-regulated before the sphingolipid pathway is changed, whereas only ORMDL3 protein is overexpressed in asthma patients. Discovery of new biochemical pathways in which ORMDL proteins are involved is crucial for understanding the role of ORMDL3 in asthma

Albert Font Haro
Hejnar lab

Sensing viruses: Dichotomy of immunoreceptor signaling in plasmacytoid dendritic cells

Recent studies reported that production of IFN-I and other cytokines by plasmacytoid dendritic cells (pDC) triggered by TLR9 agonists is abrogated by crosslinking of C-type lectin regulatory receptors (RR), such as BDCA2 or DCIR. We have recently shown that hepatitis C virus particles inhibit, via binding of E2 glycoprotein to RRs, production of IFN-I in pDCs exposed to HCV-infected hepatocytes, and induce in pDCs a rapid phosphorylation of Akt and Erk1/2, in a manner similar to the cross-linking of BDCA-2 or DCIR. The crosslinking of RR is characterized by phosphorilation of Syk, BLNK, Mek-1 and Erk1/2. Here we addressed the question whether pharmacologic targeting of RRs signaling pathway can restore the functionality of pDC abrogated by ligation of RR, and what is the underlying mechanism of this abrogation. To this end we specifically inhibited Syk and Mek-1 in pDC concomitantly exposed to TLR and RR agonists. Inhibitors of syk at subliminal concentrations restored partially IFN-alpha production, while inhibitors of Mek-1 restored the production of IFN-I and IL-6 blocked by ligation of RRs. Pharmacologic targeting of RRs pathway may constitute an attractive new approach to study mechanisms of modulation of pDC activation in pathophysiological conditions.

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Group In volvement in PhD Co nference