Abstracts

Session 1 – Hormonal regulation

Apocrine secretion in *Drosophila* melanogaster: novel secretory and transport mechanism - implications for entire metazoa and clues for rare diseases

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While apocrine secretion was among the earliest secretory mechanisms to be described, its underlying basis remains poorly understood. We reappraised our understanding of apocrine mechanism using insights about apocrine secretion from the salivary glands of *Drosophila*, in which molecular genetic analyses have provided a glimmer of hope for elucidating the mechanistic aspects of this fundamental process. In contrast to the well-defined process of exocytosis, apocrine secretion is non-vesicular transport and secretory pathway that entails the loss of part of the cytoplasm. It often involves apical protrusions and generates cytoplasmic fragments inside a secretory lumen. In its most intense phase this process is accompanied by the release of large fragments of cellular structures and entire organelles that include mitochondria, Golgi, and portions of the ER, among others. Proteomic analyses revealed that the secretion is composed of hundreds to thousands of membranous, cytoskeletal, microsomal, mitochondrial, ribosomal, and even nuclear as well as nucleolar proteins. Strikingly, although many nuclear proteins are released, the nuclear DNA itself remains intact. In spite of this complexity, it appears that several protein components of apocrine secretion are identical, regardless of the location of the apocrine gland even in evolutionary distant organisms. This type of secretion appears to be common to many, if not all, barrier epithelial tissues including mammalian skin derivatives and the epididymis, and is implicated also in lung/bronchi and intestinal epithelium. Our wide screen in D. melanogaster identified nearly 30 novel genes involved in this non-canonical process. Majority of them are unknown, so-called CG genes. Apocrine secretion is a mechanism that in eukaryotic metazoans provides the *en masse* delivery of a very complex proteinaceous mixture from polarized epithelial tissues to allow for communication at exterior interfaces. Astonishingly, drastic changes in apocrine secretion are manifested in association with more than 2 dozens of skin, breast and salivary gland diseases including cylindromatosis, hemosiderotic dermatofibroma, bilateral axilliar metachronous apocrine tumors, pigmented apocrine hydrocystoma, apocrine adenoma, fibroadenomatic carcinoma, microcapilary mucinous cystadenoma, parotic sclerotizing polycystic adenosis, many of which belong to the category of rare diseases. Thus, deciphering this novel signaling pathway can shed more light also on etiology and pathogenesis of these diseases, improve their diagnosis and help to develop therapy.

Drosophila-based, high-throughput screening for environmentally safe endocrine disruptors

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Insects are essential for sustaining ecosystems and agriculture, yet some species threaten human health as they spread devastating diseases. The capability to control insect pests and disease vectors in a selective manner that is safe to human health and environment is therefore a critical task. Juvenile hormone (JH) is vital to insect development. Being unique to arthropods, JH signaling is a suitable target for controlling insect pests, parasites, and disease vectors (such as mosquitoes or blood-sucking bugs) without affecting the health of humans and other vertebrates. Our discovery of the JH receptor (JHR) has enabled identification of new and environmentally safe JHR agonists for selective insect control. Using reporters of JH activity based initially in *Drosophila* cell lines, we have used advanced, fully automated high-throughput screening for endocrine disruptors of insect development. Initial screens of a diversity library of ~90 thousand compounds have yielded tens of hits with activity near or exceeding that of existing JH mimics. Follow-up high-throughput screens were devised based on JHR proteins from seven target insect species including mosquitoes or bed bugs. In this way, we have obtained the first species-selective JH mimics. These compounds are agonists of the JHR, and some of them indeed disrupt development of the targeted species without affecting others. Some of them act in nanomolar doses while display no cytotoxicity in human cell lines. This research leads to potential new means for controlling disease vectors and pests without negative impact on beneficial species and human health.

Acromegaly in insects: Analysis of IGF in insects using published nextgen transcriptomes

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Acromegaly is a rare, chronic, progressive disease characterized by an excess secretion of insulin-like growth factor. Insects have been shown to be useful models for the understanding of insulin physiology and hence it seemed of interest to see whether or not they might could be similarly useful for IGF. I therefore reanalyzed published genomes and transcriptomes for the presence of insulin and IGF related hormones (IIRHs). Four different types of IIRHs are present in insects. These include three hormones that are produced by genes located next to one another in the genomes of cockroaches and termites, but which in many species are dispersed within their genomes. One of these three IIRHs, which has been called gonadulin, is likely an ortholog of relaxin. Although its primary sequence is very different from that of relaxin, the sequences of their receptors shows them to be evolutionarily related. The second hormone is an unambiguous IGF, while the third is an ortholog of *Drosophila* insulin-like peptide 7. The fourth type of hormone I have called sirps, for short IGF-related peptides, as their sequence homology to IGF and their use of the IGF receptor suggest that they must have evolved from IGF. In insects IGF is produced mainly by the fat body, that performs functions of both the vertebrate liver and adipose tissue. Acromegaly in insects is probably best approximated by physogastry in which female insects increase the size of their abdomen when maturing eggs, a phenomenon that is extreme in queens of the higher termites. Interestingly, in transcriptomes of such queens the number of IGF transcript sequences is very high. Although this correlation is not necessarily causal, this seems to be an interesting starting point for further research.

The role of RNA interference pathways in antiviral immunity

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To combat viruses, invertebrates such as insects rely on their innate immune system, since they do not possess an antibody-based adaptive immune system. In recent years, evidence accumulated highlighting the importance of small regulatory RNA pathways in protecting insects against viruses. These small RNAs do not code for proteins but act at the post-transcriptional level via a process generally designated as "RNA interference" (RNAi).
In general, RNAi is triggered by double-stranded RNA molecules, which are further converted into small RNAs that then induce a sequence-dependent knockdown of the RNA target. Given the high diversity of insect species, several characteristics of the RNAi process, such as dsRNA degradation, cellular uptake and systemic signal spreading, appear to show species-dependent variation. In this invited lecture, recent progress on the mechanisms of RNAi and the role(s) of the associated small RNA mediated pathways in insect antiviral immunity are being summarized.
Acknowledgements: I am very grateful to the Research Foundation of Flanders (FWO) (G093119N) and the Special Research Fund of KU Leuven (C14/19/069) for financial support.

Session 2 -Metabolism and stress response

Regulators of *Drosophila* neutral lipid metabolism

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Lipid metabolism dysfunction causes widespread human health threats such as obesity or atherosclerosis. But defects in lipid homeostasis also trigger rare diseases such as neutral lipid storage disease or lipodystrophy by impairment of evolutionarily conserved genes, which contribute to pathophysiology via incompletely understood mechanisms.
Our laboratory has a long-standing interest in the functional characterization of regulatory and enzymatic lipid metabolism genes using the *Drosophila melanogaster* model.
Adipokinetic hormone (Akh) is the central systemic regulator of triacylglycerol (TG) catabolism, which employs canonical G protein-coupled receptor signaling in the central nervous system and in the adipose tissue to coordinate behavior with energy supply in periods of scarcity. Akh is exclusively expressed in the

Akh-producing cells (APC) of the corpora cardiaca and operates in a release-controlled mode of action. We employed a genetic screen aimed at identifying new Akh regulators in APCs involved in translating metabolic need to Akh production and release.
Sterols are a second class of major neutral lipids with diverse important functions e.g. as membrane components or steroid hormones. Similar to TGs, excess sterols are being stored as steryl esters (SE) in intracellular lipid droplets, which serve as sterol reservoir of particular importance for the sterol auxotroph insects. Little is known about the fly enzymes, which catalyze the reversible interconversion between free sterols and SEs. We show that *Drosophila* Hormone-sensitive lipase (Hsl) hydrolyses a broad range of neutral lipid substrates *in vitro* including TGs and SEs. *In vivo*, Hsl acts as major SE hydrolase, which controls interorgan sterol homeostasis and mother-toembryo sterol allocation to optimize fecundity under dietary sterol limitation. Sterol Oacyltransferase (SOAT) activity antagonizes Hsl-mediated SE breakdown to build up SE stores. We generated a fly SOAT mutant and present its initial characterization.

Mutation in *Drosophila* concentrative nucleoside transporter 1 alters spermatid maturation and mating behavior

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Concentrative nucleoside transporters (Cnts) are unidirectional carriers that mediate the energy-costly influx of nucleosides driven by the transmembrane sodium gradient. Cnts are transmembrane proteins that share a common structural organization and are found in all phyla. Although there have been studies on Cnts from a biochemical perspective, no deep research has examined their role at the organismal level. Here, we investigated the role of the *Drosophila melanogaster cnt1* gene, which is specifically expressed in the testes. We used the CRISPR/Cas9 system to generate a mutation in the *cnt1* gene. The *cnt1* mutants exhibited defects in the duration of copulation and spermatid maturation, which significantly impaired male fertility. The most striking effect of the *cnt1* mutation in spermatid maturation was an abnormal structure of the sperm tail, in which the formation of major and minor mitochondrial derivatives was disrupted. Our results demonstrate the importance of *cnt1* in male fertility and suggest that the observed defects in mating behavior and spermatogenesis are due to alterations in nucleoside transport and associated metabolic pathways. Since nucleoside transporters are also expressed in the blood barrier of the human testis, our study may indicate a similar physiological function of *cnt* in humans.

This work was supported by the European fund for regional development “Interreg Austria/Czech 549 Republic” (REGGEN-ATCZ207) and the junior grant project GACR (19-13784Y to LK).

Using 13C methionin tracing to examine Ahcy role in *Drosophila melanogaster* immunity

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Immune system activation is an energy demanding process requiring metabolic changes on the organismal level to ensure enough energy for immune cells to successfully face the pathogen. Our laboratory uses *Drosophila melanogaster* larvae infected with parasitoid wasp *Leptopilina boulardi* to induce immune system activation and examine subsequent systemic metabolic changes and metabolism changes of the immune cells themselves.
Activated immune cells produce extracellular adenosine as a systemic metabolism regulator that is inhibiting glucose uptake by non-immune tissues. Thus, there is a high level of glucose available for activated hemocytes. What is the metabolic origin of this extracellular adenosine in hemocytes?
In mammals, S-adenosylmethionine (SAM) cycle accelerates in activated immune cells. This metabolic pathway is responsible for the methylation of diverse biomolecules and produces S-adenosylhomocysteine (SAH) which is immediately converted to adenosine and homocysteine by S-adenosylhomocysteine hydrolase (Ahcy). Therefore, we have considered Ahcy as a possible source of extracellular adenosine and we have already obtained data confirming this hypothesis. 13C methionine tracing shows that SAM cycle activity increases also in activated *D. melanogaster* hemocytes.
Our data further imply that adenosine is also highly used intracellularly. The first step of the SAM cycle is a unique reaction in which the adenosyl moiety of ATP is consumed along with methionine to synthesize SAM. We propose that a part of the adenosine pool produced by Ahcy is recycled back to ATP through the cooperation of adenosine kinase, adenylate kinase, and glycolysis, as losing all of the adenosine would be a huge energy wasting since all used ATP would have to be refilled by de novo purine synthesis. Our current effort is to examine this complex metabolic network showing adenosine as a crucial molecule affecting not only systemic energetic metabolism and supporting immune response but also participating in the intracellular energy balance of activated immune cells.

Session 3 – Immune system

Insulin resistance in peripheral organs mediates immune response via JAK/STAT signaling

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JAK/STAT is a highly conserved pathway, which plays a key role in immune response. In the case of Drosophila larvae, JAK/STAT signaling is involved in differentiation of hemocytes into lamellocyte to fight against parasitic wasp infection (*Leptipilina boulardi*). This, in part, is mediated by the secretion of cytokines Upd2 and Upd3 from hemocytes, which activates JAK/STAT signaling in skeletal muscles; required for the efficient encapsulation and melanization of the wasp egg. Deletion of Upd2 and Upd3 leads to significant reduction in lamellocyte number, and therefore efficient immune response.

In times of food scarcity, or high metabolic demand tissues compete for the nutrients available. Parasitic wasp infection leads to a redistribution of nutrients from surrounding tissues, such as muscles and fat body,

to provide sufficient energy for immune response. This supports the 'selfish' or 'privileged' immune system hypothesis, as the immune cells are prioritized over other tissues to aid the survival of the animal. Reduction in carbohydrate uptake in peripheral tissues during infection is mediated by suppression of insulin signaling (IS) in these non-essential organs. Our findings show lower levels of pAkt in the larval fat body and skeletal muscles during infection, indicating IS suppression. We propose the reduction in IS observed is a result of Insulin resistance (IR) in these tissues. Furthermore, we suggest this IR occurs via Upd2 and Upd3 mediated activation of JAK/STAT in muscles leading to a systemic metabolic reprogramming during infection.

These studies in Drosophila shine a light on the complexities of immune and insulin signaling pathways; leading to the identification of potential new drug targets to treat metabolic disorders such as Diabetes.

Modified binding site of insect chitinase-like proteins is important for their function

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Chitinase-like proteins (CLPs) are diverse group of proteins belonging to glycoside hydrolase family 18, which can be found from human, across mammals to invertebrates. CLPs function is often associated with inflammation and tissue remodeling. Surprisingly, recent discoveries even show that insect CLPs from the family of IDGFs (Imaginal Dish Growth Factors) can modulate immune response of mammals and influence infectivity of viral disease transferred by insect vectors.
Here we compare binding sites of human CLPs and IDGFs from insects. We focused on IDGF2 protein from model organism *Drosophila melanogaster*, which is the best characterized member of the IDGF family. Our work shows that insect IDGF2 acquired new binding pocket and it does not bind to N-acetylglucosamine.
Our work brings new insights about insect CLPs binding abilities, which are crucial for their function as modulators of host response in insects as well as mammals and human.

Yeast glucan particles for macrophage-specific regulation of cellular metabolism in *Drosophila*

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Although adaptations in macrophage cellular metabolism allow for their functional versatility, their metabolic polarization in the wrong context underlies many human diseases. Inhibition of certain metabolic pathways is therefore considered an appropriate therapeutic strategy to regulate macrophage function. Although many promising compounds bearing this potential have been identified by in vitro screening, their administration requires macrophage-specific delivery and validation in vivo.
We have established yeast-derived glucan particles (GPs) as a highly specific macrophage delivery tool with negligible immunogenicity and toxicity in a *Drosophila* experimental model. We revealed that GPs can be used in *Drosophila* for macrophage-specific delivery of transcription factors and metabolic inhibitors that effectively affect macrophage function. GPs can be easily modified with either fluorescent tags or paramagnetic nanoparticles, allowing their tracking and isolation of treated cells for subsequent analyses. We show that *Drosophila* represents a suitable experimental organism for primary testing of macrophage-specific delivery tools and therapeutic treatments.

Macrophage-induced insulin resistance is an adaptive strategy for lipoprotein mobilization upon bacterial infection

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Activation of the immune system must be tightly coordinated with systemic metabolic changes that redirect nutrients from stores to the immune system. Satisfying the nutritional requirements induced by the macrophage metabolic polarization is essential to limit the pathogen burden. However, the factors regulating this interplay remain unclear. We revealed that metabolic polarization of pro-inflammatory macrophages leads to the production of the insulin/IGF antagonist ImpL2 in infected *Drosophila*. Macrophage-derived IMPL2 abrogates fat body insulin signaling and induces FOXO-driven lipoprotein mobilization. Enhanced lipolysis elicits substantial infiltration of this tissue by macrophages. The increase in circulating lipoproteins and their utilization by macrophages is essential for infection resistance. An analogous mechanism can also be found in mammals, where infection-activated macrophages produce the ImpL2 homolog IGFBP7 in a HIF-1α-dependent manner. Moreover, Igfbp7 manipulation in liver macrophages affects apolipoprotein expression in mouse hepatocytes and the release of LDL and VLDL from human liver spheroids.

Omics-based approach to study honey bee (Apis mellifera) response to parasitic mite Varroa sp. and associated pathogens

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Honey bees (*Apis mellifera L.*) are the most important managed pollinators worldwide, and according to the estimates, about two-thirds of crops used in human food production are dependent on pollination. A long history of domestication and international transport of *A. mellifera* has resulted in a cosmopolitan distribution of the bees, but unfortunately, also its pathogens and parasites, which are considered to be one of the main factors behind honey bee losses. Therefore, the global decline of the honey bee population poses a significant social, economic, and scientific concern. Despite years of intensive research, the complex mechanisms of *Varroa*-honey bee interaction are still not untangled. Therefore, we employed a unique combination of transcriptomic, proteomic, metabolomic, and functional analyses to reveal new details about the effect of *Varroa* mites and naturally associated factors, including viruses, on honey bees. We focused on differences between *Varroa*-parasitised and unparasitised ten-day-old worker bees collected before overwintering from the same set of colonies reared without anti-mite treatment. Significant changes were observed at all organismal levels in immunity, oxidative stress response, olfactory recognition and other specific processes. Moreover, the supplementary comparison to honey bees collected from colonies with standard anti-*Varroa* treatment provides insights into the effect of a pyrethroid flumethrin. Recent findings of omics analyses help reveal new details of honey bee response to parasitic mites and suggest new ways to control diseases of this beneficial insect.

Our work is supported by the Ministry of Agriculture of the Czech Republic grant NAZV no. QK1910286; SFI Research Infrastructure Call 2012 (12/RI/2346 (3)) and Projects of Large Research, Development and Innovations Infrastructures "e-Infrastruktura CZ" (e-INFRA LM2018140).

Session 4- General physiology and human related diseases

Rare human circadian clock diseases in perspective of clock evolution in general and insect models in particular

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Nearly all the environment on our planet is periodically changing during the day. Therefore, not surprisingly, organisms have evolved the internal time-measuring devices, so-called circadian clocks that regulate metabolism, physiology, and behavior in synchrony with the external light/dark regime. Thus, organisms can effectively anticipate regular changes and maximize their fitness, whereas malfunction of the clock has a severe negative impact. In humans, several clock diseases were reported and mapped at the gene level, often with the help of model organisms including *Drosophila*. Here, we will provide a comprehensive evolutionary analysis of clock setups across Bilateria. The human clock setup will be compared in the context of clock evolution in basal Deuterostomia and vertebrates, including major genome duplications and gene losses. Furthermore, the dawn of next-generation sequencing and genomics allowed us to provide a solid comparison of the human clock with major changes in clock setup in insects, where we have mapped major lineage-specific changes in the clock genes. Finally, we will describe unique insect clock mutants that have some implications on clock evolution in mammals including humans.

Snazarus, the *Drosophila* homolog of the SCAR20 (spinocerebellar ataxia, autosomal recessive 20) - linked SNX14 maintains membrane balance in fruitfly nephrocytes

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Keywords: SCAR20, Snazarus, nephrocyte, membrane recycling, endosome

The endomembrane system of eukaryotic cells represent an intricate network the members of which are connected with each other via vesicular transport processes and permanent physical contacts. This complex, dynamic system plays a pivotal role in cell physiology and its proper function requires the concerted action of several proteins. Main research focus of our group is the investigation of genes and proteins involved in vesicular trafficking routes chanelling to lysosomes, the central degradative organelles of cells.
Members of the Sorting nexin (Snx) protein family play important roles in numerous locations of the endolysosomal system. All Snx proteins contain the lipid-binding PX-domain that enables their membrane association where they utilize other protein domains to take part in versatile molecular events. However, exact cellular functions of many Snx proteins are currently unknown. Most Sorting nexins are evolutionarily conserved, offering the possibility to investigate their functions in model organisms.
We use various fruitfly tissues to study the molecular functions of the less characterized Snx proteins in the endolysosomal system. Our current focus is the investigation of the function of *Snazarus* (*Snz*), a known membrane contact site protein whose mammalian homologue, SNX14 is involved in a rare human hereditary neurodegenerative disease, SCAR20 (spinocerebellar ataxia, autosomal recessive 20). Loss-of-function mutations of the human SNX14 gene lead to cerebellar atrophy (due to the loss of Purkinje-cells), intellectual disability and coarse facial features.
Our results show that loss of Snazarus function leads to striking alterations in the endosomal system of the highly endocytic larval nephrocytes. Importantly, our findings highlight the involvement of Snz in the function of recycling endosomes, a previously unknown aspect of the protein's function.

Eusocial insects as model systems to study roles of telomerase in lifespan extension

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Telomerase activity and telomere restoration in certain somatic cells of human adults maintain the proliferative capacity of these cells and contribute to their regenerative potential, and telomerase activity and telomere length are commonly considered lifespan predictors. Eusocial insects provide excellent model systems for aging research based on their extraordinary caste-related lifespan differences that contradict the typical fecundity/lifespan trade-off. In agreement with the common presumption, telomerase activity is upregulated in the reproductive, long-lived individuals of eusocial insects such as queens and kings,

proposing that telomerase activity acts as a key factor in their extended longevity. But, as documented by the presence of telomerase in somatic tissues of numerous invertebrate and vertebrate species, the connection between telomerase activity and the predicted lifespan is not clear. Here, I ask whether somatic telomerase activity in eusocial reproductives may serve its non-canonical function to protect its individuals against the exacerbated metabolic stress upon reproduction and be a reflection of a more common phenomenon among species. I propose a hypothesis that the presence of telomerase activity in somatic cells reflects a different reproduction strategy of the species.

An Efficient Method for Quantifying the Degree of Neurodegeneration in an Insect Brain

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For decades, the fruit fly *Drosophila melanogaster* has served as a model organism for studying the molecular and genetic basis of many human diseases, including neurodegeneration. In previous studies, the degree of neurodegeneration of the insect brain was determined by subjectively assessing the extent of damaged areas on paraffin sections of the brain. We have developed a method that allows accurate quantification of the damaged areas of the brain. This method is based on the use of whole adult *Drosophila* brain preparations counterstained with a fluorescently labeled secondary antibody and then visualized under a confocal laser scanning microscope. We subjected the obtained series of Z-axis images to a thorough analysis in the program Fiji (ImageJ), using a combination of different features that accurately determined the damaged brain area in each image of the Z-stack series. By subsequently including the confocal layer thickness parameter, the volume of damaged tissue can be determined with high accuracy. Another way to quantify the volume of neuronal tissue degeneration was to reconstruct the affected part of the brain using a 3D model with Imaris software. We correlated the obtained values of the damaged area with the volume of the whole brain to quantify the percentage of neurodegeneration. The data obtained from the degenerative brains and the control brains were statistically analyzed using a Student's t-test.

The silk proteins of the Mediterranean flour moth *Ephestia kuehniella*, a pest belonging to the Pyralidae family

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Many lepidopteran caterpillars produce labial gland secretions to form silk cocoon, a strategy to protect the immobile pupae. Here we analyzed the soluble proteins from the cocoons of the Mediterranean flour moth *Ephestia kuehniella*, a pest belonging to the Pyralidae family. Combining high-throughput RNA sequencing and tandem mass spectrometry, we confirmed that existence of fibroin heavy chain, fibroin light chain, and P25 (fibrohexamerin) proteins. We also identified that *E. kuehniella* silk contains multiple sericins, mucins, protease inhibitors, and several other proteins. We further characterized the spatial expression of several silk genes in different regions of silk glands, and found genes that display identical expression pattern across species. Finally, by comparing the data with those of the greater wax moth *Galleria mellonella*, we attempted to characterize the conserved silk components in the Pyralidae family.

Mitochondria at the crossroad of cellular metabolism and cell death signaling

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Mitochondria in eukaryotic cells likely evolved from bacterial endosymbiont about 1.5 billion years ago and in majority of today's organisms mitochondria represent major hub for cellular metabolism as well as for several modalities of regulated cell death (RCD). Thus, mitochondria participate in both carcinogenesis and cancer treatment shifting a balance either to cell proliferation/survival or to cancer cell death. Among RCD-linked signaling, the intrinsic or mitochondria-dependent apoptosis plays the major (though not the only one) role in cancer cells demise. Apparently, the prime activators/regulators of mitochondrial apoptotic signaling as well as modulators of mitochondrial metabolism are with mitochondria largely associated proteins from the Bcl-2 family. Targeting of anti-apoptotic Bcl-2 family proteins such as Bcl-XL or Bcl-2 itself by selective drugs (BH3 mimetics, e.g. FDA-approved venetoclax) and mitochondrial metabolism affecting compounds (e.g. respiratory complex I inhibiting MitoTam) appears to be an effective approach for nowadays cancer therapy and their synergistic effect might lead to potent and effective elimination of cancer cells.

Drug repurposing screen in *Drosophila melanogaster* to develop new treatment for Dynamin 2 caused centronuclear myopathy

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Centronuclear myopathies (CNM) are a group of congenital myopathies where cell nuclei are abnormally located in the center of muscle cells. The clinical condition is characterized by severe muscle weakness and atrophy of the skeletal muscles. Three groups of genetic abnormalities associated with CNM have been identified so far, out of which the autosomal dominant mutations in the *Dynamin 2* gene (DNM2) on chromosome 19 is the best studied. DNM2 linked CNM (AD-CNM) is a rare human disease, the incidence is approximately 7 per million.

Dynamins are actin-binding, microtubule associated GTPases that play essential role in endocytosis and cell motility through mediating membrane scission. In mammals, three different dynamin genes have been identified. DNM1 is neuron-specific, DNM2 is expressed in most cells, DNM3 is expressed in the testis. Mutations in the auto-inhibitory surface of DNM2 increase the stability of the oligomer, thereby increase its GTPase activity, which then leads to CNM. To date there is no curative treatment available for AD-CNM patients.

Chin and co-workers have shown previously that the overexpression of the mutant forms of human DNM2 causes myopathy in *Drosophila melanogaster* (Chin et al. 2015 Hum. Mol. Gen.). Therefore, we decided to set up a screening platform that allows the large scale in vivo repurposing type of drug screening to develop new treatment for AD-CNM. We established more than 30 transgenic *Drosophila* lines which upon induction express wild type and mutant forms (R465W, A618T, S619L, G537C, K562E) of human DNM2. The overexpression of the mutant proteins caused disoriented movement in both larval and adult stages, pupal deformities and lethality. By monitoring pupal lethality, we screened a drug library of 2342 drugs, and identified 37 that rescued the lethality at pupal stage. Eight out of the 37 drugs proved to be effective in the second round of the experiment, where the minimal effective concentration could be also determined. We strongly believe that our results are potentially beneficial to develop novel therapeutic approaches for DNM 2-caused CNM.

This work was supported by NKFIH (National Research, Development and Innovation Office) through the National Laboratory for Biotechnology program, grant NKFIH-871-3/2020

High-throughput drug screening sheds light on novel treatment strategies for amyotrophic lateral sclerosis

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There are several rare human diseases that result in deteriorating quality of life, or even death, however, there are no treatment plans for many of these ailments. By high-throughput screening for clinically approved drugs, it is possible to find active ingredients that can be used to treat these rare illnesses. Amyotrophic lateral sclerosis (ALS) is a currently incurable neurodegenerative disease which causes the degeneration of motor neurons and results in death 3-5 years after its onset. The disease is associated with several genetic mutations, including amino acid changes in the nuclear localization signal (NLS) of the *fused in sarcoma* (fus) gene. Under normal conditions, the Fus protein is localized in the nucleus, however, mutations in its NLS region result in the cytoplasmic accumulation of the protein and the formation of aggregates. Since Fus is required for RNA metabolism, its accumulation outside the nucleus causes perturbations in RNA quality control, leading to the onset of progressive neurodegeneration.
Using Drosophila melanogaster with mutated fus alleles as our model, we have performed high-throughput screening of approximately 2400 drugs which are already in clinical use for the treatment of other diseases. During our first screen, we have identified 48 potential candidates which alleviate the symptoms of the disease. We are currently conducting in-depth dose-dependency experiments, in the hopes of finding novel treatments for ALS.

This work was supported by NKFIH (National Research, Development and Innovation Office) through the National Laboratory for Biotechnology program, grant NKFIH-871-3/2020.

Information-based selection of *Drosophila melanogaster* genes to develop models for drug repurposing in human

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The list of side effects on a patient information leaflet in every medicine package leaves no doubt that the vast majority of drugs act in multiple ways. Drug repurposing screens therefore represent an attracting approach to find new applications for FDA approved or investigational chemicals. These screens make drug discovery rapid and cost-effective, as the repurposed drug has already been found to be convincingly safe in humans, and the process of authorization is in most cases completed. Drug repositioning screens can be carried out by using cell cultures with the setback that cultured cells greatly differ from multicellular organisms. The use of mice can help to overcome this problem but it is an expensive solution and not suitable for larger screens. The golden mean could be the use of lower animals such as *Drosophila melanogaster* that can be screened cost-efficiently.
Despite the obvious difference between human and *Drosophila*, the fly is considered a feasible model organism to study human genetic diseases. Approximately 75% of human genes that are linked to disorders, have functional homolog in *Drosophila*. The already established fly disease models proved to be powerful tools to characterize the molecular processes behind the given disease. An excellent example for that is

cancer biology, as several human tumor suppressors and oncogenes were first described in the fly. But beside cancer pathways, many physiological and neurological properties are also conserved in *Drosophila*. Our intention was to find *Drosophila* genes that have human orthologs and could be used as models for drug reprofiling screens. These undertakings carry high risk as finding effective compound in a size-limited drug library is not granted and even a good hit could turn out to be ineffective in mammalian cells, thus the careful selection of the ortholog is important. Therefore, we set up a criteria framework, which was used to sort all known human disease genes. In our pipeline we used evolutionary conservation data, genetic and physical interaction data, and human disease information. The best ranking hits were selected and some of the screens are in progress using the most promising genes.

This work was supported by NKFIH (National Research, Development and Innovation Office) through the National Laboratory for Biotechnology program, grant NKFIH-871-3/2020.

Identification of potential new agents for the treatment of human lamin-associated skeletal myopathies in Drosophila melanogaster model system

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Skeletal myopathies are rare hereditary human diseases that are caused by the dominant mutations of the *LMNA* gene encoding the A type of lamin proteins. Lamins are important components of the nuclear lamina, which has fundamental role in the nucleus, such as distribution and stabilization of the nuclear pore complexes, nucleus positioning, chromatin spatial organization, and also chromosome segregation, signal transduction, and cytoskeleton assembly. Due to their multiple and important roles in the cell, their mutations contribute to a wide variety of serious diseases in human.
The commonly used genetic model organism, Drosophila melanogaster or fruit fly has the homologues of the human Lamin genes therefore, it serves as an excellent model organism to investigate the function of these genes. The human Lamin A, and its Drosophila homologue Lamin C, are highly conserved; 70% of the amino acids correlated with the human skeletal myopathies are present also in the fruit fly. An experimental system, which models the human lamin associated skeletal myopathies, has been already established in Drosophila. This system allows the exploring of new molecular pathways involved in the pathomechanism of the myopathies, and also the identification of potential new drugs which affect these processes. Our aim was to test the effect of a panel of 2342 drugs, already successfully used in the treatment of other diseases, on the available Drosophila laminopathy model system, in order to identify potential new agents for the treatment of human lamin associated skeletal myopathy. The results of the screen will be presented.

This work was supported by NKFIH (National Research, Development and Innovation Office) through the National Laboratory for Biotechnology program, grant NKFIH-871-3/2020

Acute myeloid leukemia model in *Drosophila melanogaster*

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Since signaling pathways and transcription factors regulating hematopoiesis are evolutionary conserved, Drosophila melanogaster is an ideal model organism to study blood cell associated tumor formation. The main regulatory elements of human hematopoiesis are present in Drosophila with a reduced genetic complexity, and the immune cells, i.e. hemocytes show remarkable similarities both in function and in differentiation to vertebrate myeloid cells. Mutations in JAK/STAT signaling lead to leukemia, myeloproliferative neoplasms (MPNs) and solid tumors in human. The Drosophila genome contains a single JAK gene called *hopscotch* (*hop*). The gain-of-function mutation in the hop gene ($hop^{Tum\text{-}l}$) results in leukemia-like symptoms in the larvae: hemocytes overproliferate and lamellocytes differentiate in uninduced larvae, which leads to melanotic nodules and to the disruption of the lymph gland, the main hematopoietic organ. As the mechanistic link between JAK/STAT activity and tumor formation is clear both in flies and humans, Drosophila allows for fast, high-throughput drug-screens to help the integration of new therapeutic agents in cancer research.

This work was supported by NKFIH (National Research, Development and Innovation Office) through the National Laboratory for Biotechnology program, grant NKFIH-871-3/2020.

Effects of the membrane and its biophysical properties on binding and membrane crossing of materials

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Binding of ligands to transmembrane receptors and transmembrane crossing of lipid soluble materials are often taken for granted, and the subtle effects of the membrane are often overlooked. The presentation will focus on two such remarkable and somewhat unexpected membrane effects.
Binding of growth factors to their receptors is the first step in transmembrane signaling. We investigated the concentration profile of epidermal growth factor (EGF) in the vicinity of the membrane in EGF receptor (EGFR)-expressing cells. We observed that the concentration of the ligand exhibited a peak close to the membrane (0-5 □m), which was abolished after cell fixation, and it was also absent in giant plasma membrane-derived vesicles prepared from the same kind of cells. If the cells also expressed ErbB2, another member of the EGFR family, an additional peak 10-20 □m from the membrane appeared, which was caused by a local decrease in the diffusion coefficient of EGF, likely due to the deposition of extracellular matrix.

Cell penetrating peptides (CPP) are promising tools for delivery of drugs to cells. Penetratin, a widely studied representative of CPPs, is readily endocytosed, and while it can traverse the intact plasma membrane or the membrane of endosomes, this latter step is the bottleneck in its cellular delivery. The dipole potential is a neglected, but very strong intramembrane, positive potential, which is due to the preferential orientation of the dipole moments of lipid carbonyl groups and interfacial water molecules. We showed that decreasing the dipole potential by treating cells with phloretin or statins (inhibitors of cholesterol biosynthesis) significantly enhances the accumulation of penetratin in the cytosolic compartment. The presented results provide a strong motivation to consider the contribution of the biophysical properties of the plasma membrane to cellular effects.

Characterization of driver mutations in the erb-b2 receptor tyrosine kinase 2 (ERBB2) gene

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The epidermal growth factor family of receptor tyrosine kinases (ErbBs) can play a crucial role for cell fate decisions by regulating cell proliferation, survival, differentiation and migration in the human body during embryonic development, as well as throughout adult life. We adapted duplex sequencing, an ultra-deep and highly accurate sequencing method that generates sequence information on both DNA strands (group into the duplex consensus sequence) to screen driver mutations in *ERBB2* in the male germline. We identified mutations that are found at increased frequencies in some sperm donors. Two of these mutations occur also in a large number of different donors suggesting that these novel variants are important driver mutations in the male germline. Further, we characterized the clonal expansion of candidate mutations in two post mortem testes from 0 70- and 73-years old donor by droplet digital PCR (ddPCR), along with the analysis of the functional changes in the downstreamactivation of the ERBB2 signaling pathway by biophysical methods. This unexpected enrichment of mutations in sperm and testis might have unknown consequences in the offspring from these fathers.

Grants:
FWFP30867000, REGGEN ATCZ207

Research using *Drosophila* genetic models of intellectual disabilities (ID) in Fragile X (FSX) may influence future Public Health measures to deal with disorders such as Autism Spectrum Disorder (ASD).

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During the past 25 years, research employing molecular genetic analysis of *Drosophila* ID models have resulted in a large body of information on genes associated with these disorders, their mechanisms of action and possible actions to restore normal behavioral activity. While a single allele may cause disabilities such as that related to Fragile X syndrome (FXS) have yielded potential applications for interventions and treatment, this has not been the case for disorders associated with complex multigene interactions in Autism Spectrum Disorder (ASD). Individuals with FSX are frequently codiagnosed with ASD with FSX being the

leading known genetic cause of ASD. More current studies utilizing new genetic and epigenetic tools show promise for future progress in interventions and treatments. Examples include understanding the action of a number of gene mutations associated with ASD, development of multigene methodologies and the effect of gut microbiome and diet in nerve development and behavior. The latter type of studies have been of value in our understanding of a number of diseases such as diabetes and behavior in ASD diagnosed children. Insights and progress using gut microbiome/nutritional factors in *Drosophila* models may result in potential new treatments including public health interventions of ASD.

Association between Ultrasonography Fetal Anomalies and Autism Spectrum Disorder

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Background: Multiple evidence support the prenatal predisposition of autism spectrum disorder (ASD). Nevertheless, robust data about abnormalities in fetuses later developing into children diagnosed with ASD are lacking. Prenatal ultrasound is an excellent tool to study abnormal fetal development as it frequently used to monitor fetal growth and identify fetal anomalies throughout pregnancy.

Methods: We conducted a retrospective case-sibling-control study of children diagnosed with ASD (cases); their own typically developing, closest-in-age siblings (TDS); and typically developing children from the general population (TDP), matched by year of birth, sex and ethnicity to investigate the association between ultrasonography fetal anomalies (UFAs) and ASD. The case group was drawn from all children diagnosed with ASD enrolled at the Azrieli National Center of Autism and Neurdevelopment Research. Fetal ultrasound data from the fetal anatomy survey were obtained from prenatal ultrasound clinics of Clalit Health Services (CHS) in southern Israel.

Results: The study comprised 659 children: 229 ASD, 201 TDS, and 229 TDP. UFAs were found in 29.3% of ASD cases vs. only 15.9% and 9.6% in the TDS and TDP groups (aOR=2.23, 95%CI=1.32-3.78, and aOR=3.50, 95%CI=2.07-5.91, respectively). Multiple co-occurring UFAs were significantly more prevalent among ASD cases. UFAs in the urinary system, heart, and head&brain were the most significantly associated with ASD diagnosis ($aOR_{Urinary}$ =2.08, 95%CI=0.96-4.50 and $aOR_{Urinary}$=2.90, 95%CI=1.41-5.95; aOR_{Heart}=3.72, 95%CI=1.50-9.24 and aOR_{Heart}=8.67, 95%CI=2.62-28.63; and $aOR_{Head\&Brain}$=1.96, 95%CI=0.72-5.30 and $aOR_{Head\&Brain}$=4.67, 95%CI=1.34-16.24; vs. TDS and TDP, respectively). ASD females had significantly more UFAs than ASD males (43.1% vs. 25.3%, p=0.013) and a higher prevalence of multiple co-occurring UFAs (15.7% vs. 4.5%, p=0.011). No sex differences were seen among TDS and TDP controls. ASD fetuses were characterized by a narrower head and a relatively wider ocular-distance vs. TDP fetuses (OR_{BPD}=0.81, 95%CI=0.70-0.94, and $aOR_{Ocular\text{-}Distance}$=1.29, 95%CI=1.06-1.57). UFAs were associated with more severe ASD symptoms.

Conclusions: Our findings shed important light on the abnormal multiorgan embryonic development of ASD and suggest fetal ultrasonography biomarkers for ASD.

CRISPR/Cas9-mediated gene knock-out and gene editing as a biotechnological approach to chicken resistance to viral diseases

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The enveloped viruses penetrate the host cells via specific receptor molecules and blocking these entry routes might prevent the virus-caused diseases. CRISPR/Cas9 editing of genes encoding the receptors is a way how to mount the anti-virus resistance. As a proof of the principle, we present here the artificially created resistance to avian leukosis virus in chicken.

Avian sarcoma and leukosis virus (ASLV) diversified into seven subgroups (A, B, C, D, E, J, and K) present as either exogenous or endogenous viruses in domestic chicken. These subgroups are classified by the subgroup-specific receptor usage. ALV subgroups enter the cell through Tva, a member of the family of low-density lipoprotein receptors, Tvb, a tumor necrosis factor receptor-related protein, Tvc, a butyrophilin family protein with two immunoglobulin-like domains, or Tvj identified as the chicken Na+/H+ exchanger type 1 (chNHE1) with twelve transmembrane segments and prominent extracellular loop 1.

For all ASLV receptors, virus-resistant alleles exist, mostly due to the frame shift mutations or amino-acid substitutions. For example, single W38 deletion or substitution makes the NHE1 receptor molecule resistant to virus entry. Some of ASLV-resistant receptor alleles segregate in domestic chicken and can be used for breeding the ASLV-resistant lines. Resistant alleles for NHE1, however, have not been found in chicken.

Using the CRISPR/Cas9 gene editing tools, we introduced frame shift indel mutations into the endogenous copies of tva, tvc, and tvj receptor genes. These mutations abrogated the receptor functions and conferred the resistance to the respective virus subgroup in cell culture.

Ultimately, we prepared a chicken line with W38 deletion within the endogenous NHE1 and chicken line with frame-shift knock out of tva gene using the CRISPR/Cas9 and demonstrated that theses chicken lines are resistant to ALV-J/ALV-A infection in vivo. The new techniques of gene knock-out and gene editing in chicken employed in this work will be described. Our original methods of orthotopic transplantation of primordial germinal cells into adult recipients improved efficiency of gene modification and enabled to skip the chimeric G0 stage. This approach might become the state-of-art for biotechnological use of chicken in virology, immunology and developmental science.

This research was funded by the Czech Science Foundation (grant no. 15-23993S) and Ministry of Agriculture of the Czech Republic (grant no. QK1810344).

Title: A DNA origami-based biointerface for probing the spatial requirements of cell surface receptor triggering

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The nanoscale spatial organization of ligands and receptors is emerging as an important theme in regulating cell behavior yet inherently challenging to investigate. We have developed a cell-responsive biomimetic interface based on laterally mobile functionalized DNA origami platforms to probe the spatial requirements for receptor-mediated signaling. Applying this biointerface to study the molecular mechanisms of T-cell activation we determine the nanoscale ligand and receptor organization that serves as a basis for productive signaling.

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