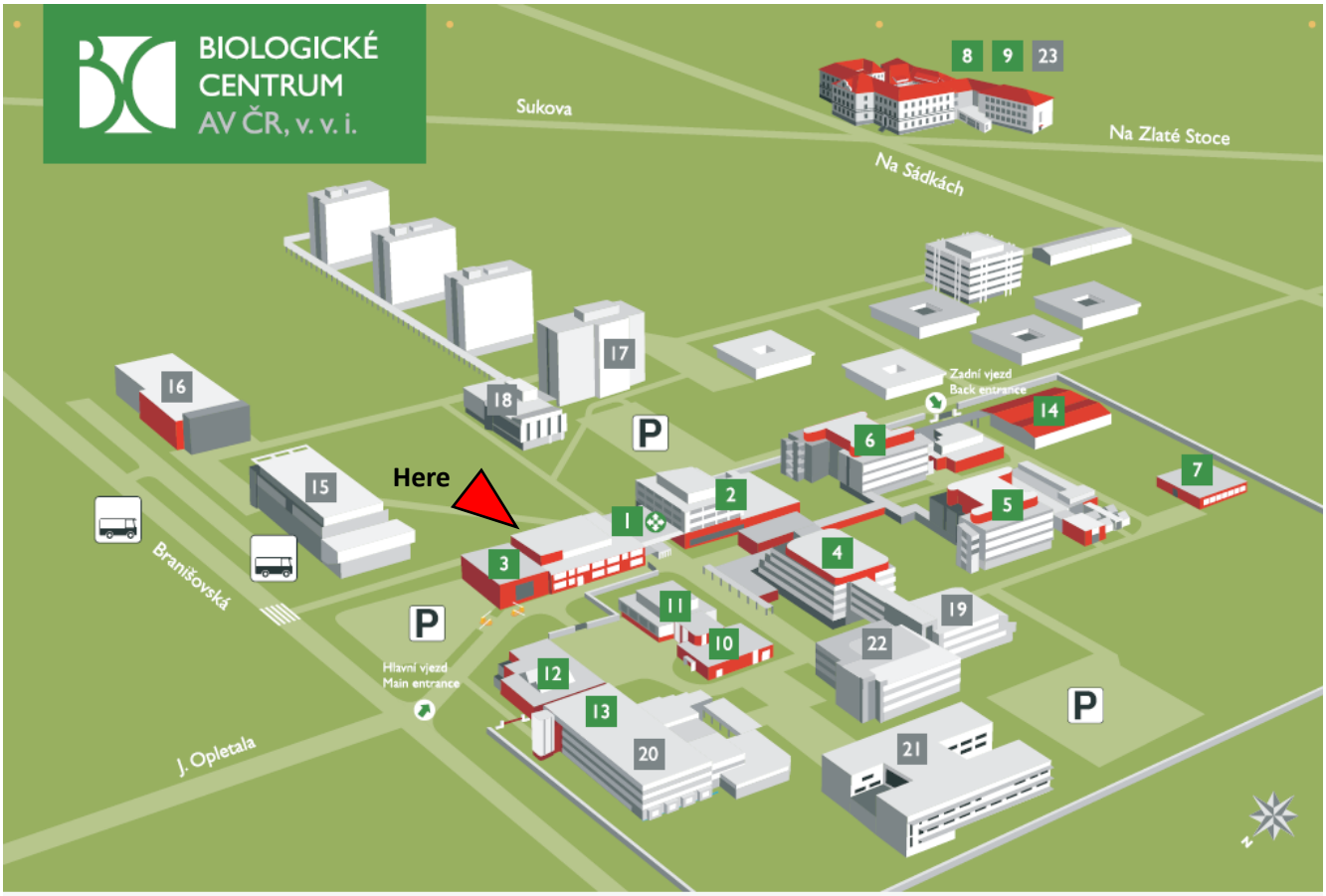

The international workshop on
"Frontiers In Insect Physiology"
June 12~14, 2019
Biology Centre CAS in Ceske Budejovice_Czechia

PROGRAMME

Place of the workshop

The workshop will take place at the Academic Congress Hall of the Biology Centre in Ceske Budejovice



	BUDOVY BIOLOGICKÉHO CENTRA AV ČR	BUILDINGS OF BIOLOGY CENTRE ASCR
1	Vstupní hala / Recepce	Entrance Hall / Reception
2	Ředitelství	Headquaters
	Středisko služeb	Servis Unit
3	Kongresové centrum	Congress Centre
4	Ústav molekulární biologie rostlin	Institute of Plant Molecular Biology
5	Entomologický ústav	Institute of Entomology
6	Parazitologický ústav	Institute of Parasitology
7	Laboratoř elektronové mikroskopie	Laboratory of Electron Microscopy
8	Hydrobiologický ústav	Institute of Hydrobiology
9	Ústav půdní biologie	Institute of Soil Biology
10	Úsek transferu technologií	Technology Transfer Office
	Garáže	Garages
11	Dílny	Workshops
12	Energocentrum	Energy Centre
13	Ubytovna / Dětská skupina Motýl	Dormitory / Motýl Children Group
14	Skladové haly	Storage Halls
	BUDOVY DALŠÍCH ORGANIZACÍ	**BUILDINGS OF OTHER ORGANIZATIONS**
15	JU | Rektorát / Filozofická fakulta	USB | Rector's Office / Faculty of Philosophy
16	JU | Akademická knihovna	USB | Academic Library
17	JU | Koleje	USB | Hall of Residence
18	JU | Aula	USB | Lecture Hall
19	JU | Přírodovědecká fakulta, budova A	USB | Faculty of Science, Building A
20	JU | Přírodovědecká fakulta, budova B	USB | Faculty of Science, Building B
21	JU | Přírodovědecká fakulta, budova C	USB | Faculty of Science, Building C
22	JU | Zemědělská fakulta	USB | Faculty of Agriculture
23	Centrum výzkumu globální změny AV ČR	Global Change Research Centre ASCR

Program of the workshop

Wednesday, June 12:

8:30-9:15	**breakfast and REGISTRATION**
9:15-15:10	
	Morning session - Insect hormones
9:15 - 9:20	**Michal Žurovec** - *Welcome note*
9:20 - 9:50	Marek Jindra
9:50-10:20	Robert Farkas
	Coffee break
10:30 - 11:00	Jozef Vanden Broeck
11:00 -11:30	Jan Veenstra
11:30-11:50	Ronald Kuhnlein
	Lunch
12:50 - 13:20	Martina Galikova
13:20 - 13:50	Heinrich Dircksen
13:50-14:10	Michal Zurovec
	Coffee break
	Afternoon session - metabolism and stress response
14:20-14:50	Valentina Magnin
14:50-15:10	Lenka Rouhova

Afternoon trip to Cesky Krumlov

Thursday, June 13:

8:15-9:00 **breakfast and discussions**

9:00-14:00

Morning session – metabolism and stress response

9:00-9:30 Tomas Dolezal
9:30-9:50 Gabriela Krejcova

Coffee break

10:00-10:20 Houda Ouns Maaroufi

Afternoon session - Immunity

10:20-10:40 Vaclav Broz
10:40-11:05 Paola Bellosta
11:05-11:25 Lucie Kucerova

Lunch

12:35-13:05 Istvan Ando
13:05-13:35 Gyöngyi Cinege
13:35-14:05 Pavel Hyrsl

Evening visit of Hluboka (castle or zoo)

Friday, June 14:

7:45-8:30 **breakfast and discussions**

8:30-11:30

Morning session - general physiology

8:30-8:55 Paola Bellosta
8:55-9:25 Mattias Behr
9:25-9:55 Gabor Juhasz

Coffee break

10:05-10:30 Ingrid Poernbacher
10:30-11:00 Peter Vilmos
11:00-11:30 Tomas Stetina

Afternoon trip to Trebon

Abstracts

Insect hormones

Developmental Signaling through the Juvenile Hormone Receptor

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Juvenile hormone (JH) maintains the status-quo character of insect larvae. It does so to optimize growth so that the body size attained is just right before a larva metamorphoses into an adult. When that size is reached, JH secretion ceases and the absence of JH allows metamorphosis to take place. If JH is artificially added at this time, metamorphosis fails as the animal repeats the larval stage and dies without reaching reproductive maturity. This is the effect of JH-mimicking insecticides that have been deployed since 1975 against disease vectors and pests in fields and households. JH is a sesquiterpenoid originating from an arthropod-specific branch of the mevalonate pathway; vertebrates do not synthesize JH.

Remarkably, the mode of action of JH, and of the JH-based insecticides, remained a mystery until our molecular and genetic evidence establishing an intracellular JH receptor (JHR)[1,2]. In *Drosophila* JHR is encoded by two paralogolous genes named *Met* and *gce*. Unlike all other known lipophilic animal hormones, which bind and activate zinc-finger transcription factors of the nuclear receptor (NR) family, the JHR is a bHLH-PAS protein. In its action JHR resembles another member of the bHLH-PAS family, the vertebrate Aryl hydrocarbon receptor AhR, aka 'dioxin receptor', activated by pollutants or endogenous ligands to the PAS domain. Likewise, binding of JH or its mimics to the PAS domain of Met or Gce stimulates its dimerization with another bHLH-PAS partner Taiman, leading to DNA binding and activation of target genes, some of which prevent metamorphosis.

In my talk, I will summarize what we have learned about the structure and function of JHR and about its roles in insect and particularly *Drosophila* development.

[1]*Charles J-P, et al. (2011) Proc Natl Acad Sci USA 108:21128-* (*https://doi.org/10.1073/pnas.1116123109*)

[2]*Jindra M, et al. (2015) PLoS Genet 11:e1005394 (https://doi.org/10.1371/journal.pgen.1005394)*

Novel properties and functions of ecdysone-regulated *Drosophila* salivary gland secretory (Sgs) glue proteins

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The salivary glue secretion (Sgs) produced by mature larvae of the fruitfly, *Drosophila melanogaster*, is a mixture of 8 unique proteins which, when solidify, serves to affix the freshly formed puparia to a substrate (*e.g*. glass wall in a culture vial) in an upright position. Salivary glands (SGs) produce a large amount of the mucinous glue-containing secretory granules which display a strong periodic acid-Schiff (PAS) positive reaction. Under transmission electron microscope, the Sgs proteins synthesized inside the SG cells tend to form Golgi-derived electron-dense secretory vesicles that then fuse into larger granules. Various authors have studied secretory granules in *D. melanogaster* and some in other species, and have described several different infrastructural elements within the granules. Towards the end of the last larval instar, the steroid hormone ecdysone is released into circulation and induces a complex response that leads to the initiation of metamorphosis. In the SGs, this is accompanied by a series of polytene chromosome puffs that reflect a cascade of transcriptional regulation, and the secretion of the glue by exocytosis. These 8 unique *Drosophila* proteins, varying between 7 and 140 kDa in size, are encoded by the genes named *Sgs-1*, *Sgs-3*, *Sgs-4*, *Sgs-5*, *Sgs-5bis*, *Sgs-7*, *Sgs-8*, and *Eig71Ee*. Although strong glycosylation was expected in most of the Sgs proteins even before their amino-acid sequence was known, only Sgs-3 initially showed motifs that conclusively supported the contention that it is heavily *O*-glycosylated. Later, detailed sequence analysis of Sgs-4 and Sgs-1 supported the view that they too are *O*-glycosylated (Furia *et al*., 1992; Roth *et al*., 1999). The Sgs-5 appears to have just single *N*-glycosylation and a single *O*-glycosylation sites, and the both Sgs-7 and Sgs-8 are not glycosylated at all. At the time of maximum synthesis, these Sgs proteins comprise for 25-30% of the total protein content of the salivary glands, with each salivary gland cell containing 2500-3000 individual secretory granules ranging from 0.2 to 2.5 or even 3.0 mm in diameter. Here we will present data on further physico-chemical properties of individual Sgs glue proteins, and we will indicate how these properties can affect function of these proteins and the glue as a whole.

In addition, for the first time here we provides an evidence that in contrast to previous expectations, into lumen exocytozed Sgs-glue has micellar fibrous-like spongious character that allows its fast hydration prior to expectoration. After Sgs-glue is released and solidified, its surface have uniformly smooth appearance, however its internal composition beneath this surface is rather structured and complex showing multilayered infrastructure resembling trabecular network. More interestingly, the released glue has ability to trap and immobilize various microorganisms including bacteria and yeasts, thus providing puparia the first line of defense against microbial invasion.

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Advances in elucidating the role of neuropeptides and their receptors in locust physiology

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Despite their diversity, some fundamental characteristics are shared by all animals. Like other metazoans insects are heterotrophs, implicating a need for the intake of food. Postembryonic developmental processes will essentially depend on this nutritional and energetic input. It is crucial that insects can rely on physiological mechanisms controlling and integrating these processes. Hormonal and neuronal signalling systems are playing an important role in this complex regulation.

In this presentation, we will consider neuropeptide-mediated signalling pathways that are implicated in the regulation of developmental-physiological processes in insects. In particular, we will discuss recent data obtained in locusts, which are swarming pests that irregularly devastate the agricultural production in large areas of the world. In a physiological and neurobiological context, locusts have proven to be interesting experimental research organisms. We have molecularly characterized several neuropeptide precursors and receptors in locusts, and will report on our recent physiological and molecular biological studies that further illustrate the important role of neuropeptide-mediated signalling systems. We have also evaluated the potential of peptide-mimetic analogues as molecular pharmacological tools to interfere with these signalling systems, as well as with the downstream physiological processes they are controlling. Moreover, RNA interference was employed as a highly efficient and robust method to post-transcriptionally silence the expression of peptide precursors and/or receptors.

The general aim of our work is to contribute to a better understanding of the regulation of insect postembryonic processes, as well as of the functional networking interactions between different regulatory pathways in an integrative, organismal context.

Acknowledgements:

We gratefully acknowledge the EU (Horizon-2020 project nEUROSTRESSPEP), the Research Foundation of Flanders (FWO-Flanders) and the Special Research Fund of KU Leuven (C14/15/050) for financial support.

Are insect diuretic peptides diuretic hormones ?

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A number of insect neuropeptides has been identified as being able to increase fluid secretion by the Malpighian tubules. These include a vasopressin-like peptide, various CRF-like peptides, leucokinins, periviscerokinins as well as calcitonin-like peptides. In *Drosophila* G-protein coupled receptors to yet other insect neuropeptides are expressed in the Malpighian tubules and at least some of the ligands for these receptors also increase fluid secretion. This raises the question as to how and why there are so many of these "putative diuretic hormones", or asked differently, how is the elimination of excess water really regulated in insects. In this talk I will try to convince you that many of these "putative diuretic hormones" are not diuretic hormones after all, and that their diuretic effects are really ancillary to their primary function.

Control of Drosophila storage lipid metabolism by Hormone-sensitive lipase

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Lipid storage and mobilization are essential cellular processes required for energy and membrane homeostasis and preventing lipotoxicity. Insects lipolytic enzymes control the release of specific lipid classes such as triacylglycerols and steryl esters from intracellular storage organelles called lipid droplets (LDs). We show that Drosophila Hormone-sensitive lipase (Hsl) is a multifunctional LD-associated lipid esterase. While mobilization of triacylglycerols and energy homeostasis are largely unimpaired in hsl mutants, the gene is required for efficient mobilization of free sterols from steryl ester (SE) storage pools. Specifically, impaired SE catabolism in hsl mutants causes progressive SE over-storage in developing embryos as well as in the adult fly fat body. Notably, Drosophila engages hsl-independent system(s) to mobilize SE pools in the absence of dietary sterol. Our data suggest a redundant control of SE catabolism via multiple enzymes and highlight an ancestral function of Hsl in this process.

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In search of a fly glucagon

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Stable levels of circulating sugars are in mammals maintained by the action of insulin and its antagonist, glucagon. Whereas the insulin pathway is highly conserved from worms to humans, most invertebrates do not have a homolog of glucagon. Nevertheless, the evolutionary conservation of insulin advocates for existence of its antagonist in invertebrates as well. In *Drosophila,* hyperglycemic functions are governed by the Adipokinetic hormone (AKH). This peptide, however, fulfills only a part of the functions of glucagon. We have recently discovered that AKH is a member of a novel broader signaling pathway involving a hormone called Ion transport peptide (ITP). Our genetic gain- and loss-of-function experiments show that ITP is a novel hyperglycemic hormone with additional functions similar to those of glucagon. ITP regulates secretion of AKH, but has also additional AKH-independent roles in processes governing development, reproduction, and lifespan. Altogether, our work describes a novel hormonal axis show that ITP is an important regulator of fly physiology with functions closely resembling those of mammalian glucagon.

Martina Gáliková[1,2], Peter Klepsatel[1] and Heinrich Dircksen[2]
[1]Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]Department of Zoology, Stockholm University, Stockholm, Sweden

Three different peptides arising by alternative splicing from a single ion-transport-peptide gene are differentially distributed in the nervous system of larval and adult Drosophila

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Ion transport peptide (ITP), discovered as antidiuretic hormone, is a member of the crustacean hyperglycemic hormone superfamily, which has been found in all investigated insects. In Drosophila melanogaster, three putative alternative splice-forms derive from a single 5-exon gene; they share the identical first half of the peptide but are very different in the C-terminal parts, i.e. a short form ITP (73aa) and two longer forms ITPL1 and ITPL2 (both 87aa). Isoform-specific antibodies show ITP to occur in 4 groups of neurosecretory cells and neurons throughout the central (CNS) and in lateral bipolar dendrite (LBD) putative sensory neurons of the peripheral nervous system (PNS; last segment), whereas the ITPL1/L2 occur only in LBD neurons of other segments innervating alary muscles of the heart. In larvae, ITP is only co-localised with allatostatins and CCAP in hindgut innervating neurons, but in adults also with CCAP in heart neurons and with other neuropeptides such as sNPF, NPF in CNS neurons. One large ITP-neuron type is a typical pars lateralis neurosecretory neuron innervating the corpora cardiaca (incl. adipokinetic hormone-cells) and corpora allata intrinsic cells. These neurosecretory ITP-neurons also

transiently innervate muscles to the so-called ptilinum, a structure used by pharate adults during head eclosion. An ITP-interneuron is identified in the adult clock system as "evening" clock-neuron in a similar position but different from the "morning" pdf-clock neurons. Interestingly, one long splice-form, ITPL1, also occurs in non-neuronal Inka-cells. Judging from the neuronal distribution in neurohaemal areas and innervation patterns of very different targets suggest several important functions of the peptide isoforms associated with moulting, homeostasis and heart beat control apart from the established biological clock function.

H. Dircksen, J. Strauss, Y. Liu, A. Mandali, D.R. Nässel

Functional analysis of adipokinetic hormone signaling in *B. mori*

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Insect adipokinetic hormones (AKHs) are well studied insect neuropeptides belonging to the AKH/RPCH (red-pigment concentrating hormone) family of energy-mobilising peptides. They are produced by the corpora cardiaca in insect brain and are involved in the activation of catabolic enzymes responsible for the mobilization of carbohydrates and lipids. Their physiological functions are mediated by adipokinetic hormone receptor (AKHR), which belongs to G-protein-coupled receptor (GPCR) family. Recent studies show that various insects can have more different AKH neuropeptide as well as AKH receptor paralogs encoded by separate genes. Recently, three distinct cDNAs encoding the AKH (AKH 1–3) as well as three genes for AKH receptors (AKHR1, AKHR2a and AKHR2b) have been cloned from the *B. mori* genome. In the present study we isolated and characterized a mutant in *Bombyx AkhR1* and characterized its effect on metabolism of carbohydrates and fats. *We also assessed* the *expression* pattern of AKHs and AKHRs, and examined effects of AKH1 -3 microinjection on the levels of carbohydrates as well as lipids in the hemolymph. We show that the function of AKH signaling in insects is well conserved.

Yoko Takasu, Tereza Konikova, Anna Zaloudikova, Yu-Hsien Lin, Hana Sehadova, Stepanka Tomkova, Dalibor Kodrik and Michal Zurovec

Metabolism and stress response

Mitochondrial dysfunction leads to major signalling changes driven by overactivation of the Tor pathway

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Although mitochondrial dysfunctions are the most frequent metabolic mutations present in human, manifested in a whole range of clinical disorders, the signalling networks triggered by malfunctioned mitochondria have not been well characterized. We have investigated the signalling changes caused by downregulation of the mitochondrial complex I subunit ND-49 (mammalian NDUFS2) in the Drosophila eye and wing disc. We identified the Tor pathway overactivation as the master regulator of further downstream signalling changes that include activation of the JNK, Notch and JAK/STAT pathways. The Tor mediated JNK activation is essential for the stimulation of compensatory apoptosis induced proliferation and for enhanced ROS production. The TOR pathway also directs a metabolic switch towards increased glycolysis by upregulation of lactate dehydrogenase expression. Our findings suggest a central role for Tor pathway in response to mitochondrial dysfunctions and they provide a rationale why the disease symptoms associated with respiratory dysfunctions are often alleviated by TOR inhibitors. We are searching for metabolic sensors that mediate the TOR activation as a response to mitochondrial complex I downregulation.

Valentina Magnin 1,2, Raquel Perez-Gomez 1,2, Vladimira Tuckova 2, Nora Hagleitner 2, Zorana Mihajlovic 1,2, Vera Slaninova 1,2, Sarah Bray 3 and Alena Krejci 1,2
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AdoR mutation influences fate of wts outgrowth clones

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(Ado) is a crucial metabolite and signaling molecule. Levels of extracellular adenosine can increase locally after stress or immune challenge and also in tumor microenvironment.In our previous work, we showed that mutation in the Drosophila adenosine receptor AdoR1 as well as silencing of the equilibrative

nucleoside transporter 2 (Ent2) by RNAi cause a severe reduction inthe frequency of hyperplastic wts and dco epithelial outgrowths. However, since we used spontaneous or chemically induced chromosomal recombination to create wts homozygote clones, itwas difficult to track the destiny and development of the clones over the time.Here we performed a detailed clonal analysis and followed the fate of the flippase-induced GFP-labeled mosaic clones from larvae to imagoes. Flippase system confirmed that the lack of AdoR signal can almost eliminate wts epithelial outgrowths in Drosophila wing and scutum as well as hyperproliferative non-differentiated wts cells in the Drosophila eye. But frequency of AdoR1 homozygote clones on heterozygote background was slightly reduced as well. We further followed the influence of AdoR mutation on apoptosis and proliferation rate.

Drosophila **model for immunometabolic interactions**

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Activated immune system demands energy. To ensure an adequate supply, the activated immune cells become privileged in terms of using organismal resources and they usurp nutrients by producing signals which reduce the metabolism of non-immune tissues. There are also mechanisms, which limit the privileged behavior of the immune system so that it does not become excessive and harmful to the host. Such mechanisms are essential for managing the trade-offs between various physiological systems within organism. The concept of privileged immunity will be presented together with data demonstrating molecular regulations of metabolism, from metabolism in immune cells to regulations of systemic metabolism, and their impacts on host immune response and physiology during various types of infection in Drosophila.

Activated *Drosophila* macrophages undergo transient metabolic remodeling towards aerobic glycolysis

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Macrophage-mediated phagocytosis and cytokine production represent the front lines of resistance to bacterial invaders. A key feature of this pro-inflammatory response in mammals is the complex remodeling of cellular metabolism towards aerobic glycolysis, which generates the energy and metabolites necessary for the bactericidal activity. Although the function of bactericidal macrophages is highly conserved, the metabolic remodeling of insect macrophages remains poorly understood. To address this deficiency, we used the adult fruit fly *Drosophila melanogaster* to investigate the metabolic changes that occur in macrophages during the acute and resolution phases of *Streptococcus*-induced sepsis. Our studies revealed that *Hypoxia inducible factor 1 α* (*Hif1α)* and *Lactate dehydrogenase* (*Ldh)* are crucial for macrophage activation, bactericidal function of the immune cells, and resistance to infection, thus revealing how the metabolic rewiring of macrophages is an essential step in host survival. These results demonstrate that molecular regulation of aerobic glycolysis induction and maintenance is conserved between insect and mammalian phagocytic cells. Further, we show that these metabolic rearrangements induce systemic

changes in carbohydrate metabolism by the action of *ImpL2*, which transcription is under the control of *Hif1α*. Moreover, these systemic changes are necessary for the fulfillment of the biosynthetic and energetic requirements of macrophage function and resistance to bacterial infection. Overall, our findings establish *Drosophila* as a powerful model for exploring the immuno-metabolic mechanisms that control functional polarization of macrophages and its impact on systemic metabolism.

Importance of concentrative nucleoside transporter (CNT2) in maintaining the gut homeostasis of *Drosophila*

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Nucleosides, as purines and pyrimidines, mediate fundamental biological effects to control growth and metabolism in all living systems. This balance of the nucleosides is primarily controlled by two types of nucleoside transporters; the concentrative nucleoside transporters (CNTs) and the equilibrative nucleoside transporters (ENTs) which are transmembrane proteins. In this project, we surveyed the physiological functions of CNT2 in the gut homeostasis. Our results showed that the *cnt2* mutation caused high lethality in the larval stage and a small pupal size, indicating that CNT2 is important for *Drosophila* development. In addition, loss of *cnt2* caused the decrease of the posterior midgut diameter and the increase of its epithelial layer thickness. These midgut phenotypes were accompanied with small cell size and high cell number due to the induction of stem cell proliferation. Feeding *cnt2* mutants with different nucleosides showed that uridine could rescue the *cnt2* mutation lethality, pupal size, as well as the gut phenotypes. This reflected that *cnt2* mutant phenotypes were caused by the disbalance of uridine concentration. Here we conclude that CNT2 is important in maintaining the larval development and the uridine transportation which is a key molecule controlling the gut homeostasis.

Immunity

Drosophila Imaginal disc growth factor 2 protects cells in vitro from serum deprivation

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Imaginal disc growth factors (IDGFs) are chitinase-like proteins (CLPs), which are able to recognize and bind carbohydrate structures. These secreted proteins were shown to enhance cell proliferation in vitro. With its crystal structure determined, IDGF2 is the best characterized member of the IDGF family.

We analyzed the effects of recombinant IDGF2 on the transcriptional profile of Cl.8+ cell line. The IDGF2 induced genes are mainly involved in innate immunity, morphogenesis, metabolic process, and transport of various substances. Consistently, IDGF2 expression is increased upon injury and bacterial infection. Moreover, IDGF2 was found to protect cells in vitro from the stress caused by serum deprivation and also partially rescue the cytotoxic effects of several xenobiotics.

Our findings suggest that IDGF2 has a cytoprotective role, helping cells to survive stress caused by a broad range of conditions. Having such an effect, IDGF2 has a potential to contribute to the development of serum-free media for cell cultures.

Broz Vaclav, Kucerova Lucie, Rouhova Lenka, Zurovec Michal

Adipose Chronic Inflammation in a *Drosophila* model for Obesity depends on eiger/TNFa signaling.

Paola Bellosta; University of Trento, Italy

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To better understand how macrophages are recruited to the adipose tissue in obesity, we are taking advantage of a conserved functional relationship in *Drosophila* between hemocytes (macrophage like cells) and adipocytes (larval fat body, FB). *Drosophila* FB- a metabolic tissue with similar physiological functions to the mammalian adipose tissue and liver- acts as a functional unit to control key metabolic processes and the native immune response, in addition to storing fats and sugars. By reducing the level of ecdysone, we were able to obtain larvae that continue to grow and reach an obese-like phenotype. These animals display phenotypes similar to these described in obesity, like a high content of triglycerides (TGAs) and of glucose from trehalose in the hemolymph, and cells or the FB that have acquired insulin resistance. Moreover, the obese larvae have a high number of hemocytes in their FB, resembling adipose macrophage migration, or ATM in human obese people. Moreover, we link dATM to a functional *eiger*/TNF signaling that was inhibited by the presence of polyphenols or antioxidants in the food. These data demonstrate for

the first time that immune cell infiltration into adipose tissue is an evolutionarily conserved process and provide an opportunity to develop a model in *Drosophila* to study the programs that are critical for immune cell recruitment to adipose tissue.

Paola Bellosta and Zhasmine Mirzoyan, Department of Cellular, Computational, and Integrate Biology-CIBIO, University of Trento Italy

Chitinase-like proteins work as clotting factors in *Drosophila melanogaster*

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Secreted glycoproteins from Drosophila IDGF family retain structural similarity to chitinases. They lack enzymatic activity but they possess modified binding domain for carbohydrates and possibly act in a similar manner as lectins. There are six members of IDGF protein family in Drosophila. We described previously that a null mutant in IDGF3 protein was sensitive to entomopathogenic nematode (EPN) infection and had serious clotting and wound healing defects. Here we examined role of other IDGF proteins in clot formation and wound healing. We also test for redundancy of IDGF proteins in clot formation and we searched for putative functional domains and positions of IDGF molecule.

New players in *Drosophila* innate immunity: The multinucleated giant hemocytes

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A novel type of encapsulating cells, the multinucleated giant hemocytes were identified originally in the ananassae subgroup of Drosophilidae. Their functions, origin and differentiation as well as ultrastructural features will be presented.

Cellular immune response involving multinucleated giant hemocytes in the Drosophilid Zaprionus indianus

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We have previously identified a novel cell type, the multinucleated giant hemocyte (MGH) in the ananassae subgroup of Drosophilidae. Here I present, that MGHs also differentiate in Zaprionus indianus, an invasive species belonging to the vittiger subgroup of the family, highly resistant to a large number of parasitoid wasp species. We have classified the hemocytes of Z. indianus based on blood cell type specific monoclonal antibodies, studied their function and analyzed their compartmental origin. Moreover electron microscopic analysis revealed a unique ultrastructure of the MGHs, which may furnish the giant hemocytes with a substantial metabolic advantage, hence contributing to the mechanism of the effective immune response.

Nematodes as a tool to study insect immunity

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Invertebrates and especially insects belong to the ecologically most successful organisms living on Earth. An adaptation to the antigen pressure of the environment (mainly to micro-organisms) depends on insect innate immunity. Invertebrates compensated the absence of complicated immune reactions by specific adaptations and functions of cellular and humoral parts of their immune system. Although an adaptive immunity in the form we know in vertebrates does not exist in invertebrates, there are advanced mechanisms modulating their immune response. Presented studies on fruit fly Drosophila melanogaster, wax moth Galleria mellonella and honey bee Apis mellifera describe cellular and humoral components of their immune system and methods for their measurement. In many experiments we used natural infection model combining two pathogens – bacteria Photorhabdus luminescens and nematode Heterorhabditis bacteriophora with their insect host. New mechanisms of insect immune response to nematobacterial pathogens were identified. Not surprisingly, among the genes significantly affected by the nematobacterial infection, mostly those related to immunity, cellular and developmental processes were found to be crucial, e.g. genes coding for members of coagulation cascade and recognition molecules.

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General physiology

Myc increases nutrients storage and autophagy in the fat body resulting in resistance to starvation.

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Regulation of animal growth requires a fine balance between mobilization and appropriate investment of storages (proteins and sugars). Studies in *Drosophila* identify several regulators of these metabolic processes many of which are concentrated in the fat body-FB, the tissue responsible for the storage of nutrients. The FB works also as a sensor for amino acid availability, where TOR signaling remotely regulates the release of Dilps to control animal growth. We were interested in understanding how Myc contributes to organismal growth when expressed in the FB, giving the fact that we found Myc downstream of TOR signaling to control protein synthesis and metabolism. Our data indicate that expression of Myc in FB modulates the storage of nutrients, allowing animal to better survive in starvation. In addition, Myc contributes to induce autophagy whit a mechanism that is dependent on lipid catabolism. These studies underline a conserved function for Myc in controlling metabolism that mimics to the switch of c-myc in pre-cancer cells necessary to drive cellular survival.

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Barriers in epithelial tissues

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Epithelial sheets form barriers that are crucial for organ formation and homeostasis. These barriers are formed by junctional complexes in interplay with cytoskeleton and extracellular matrices. In arthropods cuticles create first physical barriers at outermost epithelia where they protect animals against wounding, desiccation, and invading pathogens. However, underlying molecular mechanisms organizing cuticle assembly and disassembly are poorly understood. I will discuss conserved key factors coordinating apical chitin-matrix formation in Drosophila. We recently found that the chitin-binding protein Obstructor-A (Obst-A) is essential for proper localization of deacetylases and the GPI-linked Knickkopf protein that mature and stabilize newly synthesized cuticles. To ensure cuticle function in late embryos and during larval molting, Obst-A protein is apically secreted via Claudins at the Septate Junctions and internalized by Wurst/Clathrin-mediated endocytosis. The loss of Obst-A or its subcellular localization at the matrix disrupts normal cuticle packaging resulting in early larval lethality and extreme susceptibility against

external stresses. Importantly, extracellular Obst-A localization depends on specific Chitinases, indicating that these enzymes are not only required for chitin degradation, but for the initial control of proper chitin-matrix formation. Surprisingly, Obst-A controls ecdysone production by enabling proper growth of nerves at the surface of endocrine ring gland cells. As ecdysone regulates molting and expression of accompanied cuticle proteins, including Obst-A itself, it suggests that Obst-A could act in a feedback mechanisms to control growth, timing and molting during larval development. Altogether this defines Obst-A as major cuticle regulator of cuticle formation which is unique among known cuticle components.

Autophagic and endocytic vesicle fusions in Drosophila

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My group is interested in autophagy and related lysosomal degradation pathways, including endocytosis, phagocytosis and crinophagy. We identified many new regulators of autophagy in Drosophila via genome-wide RNAi screening in starved mosaic larvae, which led us to investigate vesicle fusions as one of the main topics in my lab. My group identified the autophagosomal SNARE Syntaxin 17 and its partners (Takats 2013 JCB), HOPS tethering complex (Takats 2014 MBoC), and small GTPases Rab7 (Hegedus 2016 MBoC), Rab2 (Lorincz 2017 JCB) and Arl8 (Boda 2019 BBA) as required for autophagosome-lysosome fusion. We also showed that the HOPS-related tethering complex CORVET functions in endosome maturation and phagosome-lysosome fusion, but only in those cell types that have high endocytic activity: larval nephrocytes and blood cells (Lorincz 2016 Elife). More recently, we deciphered the mechanisms of secretory granule-lysosome fusion aka. crinophagy (which takes place in all secretory cells) during the developmentally programmed glue granule production, secretion and degradation process in salivary glands (Csizmadia 2018 JCB).

Epithelial cells release adenosine to promote local TNF production in response to polarity disruption

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Disruption of epithelial integrity contributes to chronic inflammatory disorders through persistent activation of stress signalling. Here we uncover a mechanism whereby disruption of apico-basal polarity promotes stress signalling. We show that depletion of Scribbled (Scrib), a baso-lateral determinant, causes epithelial cells to release adenosine through equilibrative channels into the extracellular space. Autocrine activation of the adenosine receptor leads to transcriptional upregulation of TNF, which in turn boosts the activity of JNK signalling. Thus, disruption of cell polarity feeds into a well-established stress pathway through the intermediary of an adenosine signalling branch. Although this regulatory input could help ensuring an effective response to acute polarity stress, we suggest that it becomes deleterious in situations of low-grade chronic disruption by provoking a private inflammatory-like TNF-driven response within the polarity-deficient epithelium.

Physiological relevance of the localization of cytoskeletal proteins in the cell nucleus

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In recent years it has become clear that the main cytoskeletal components of eukaryotic cells are present also in the cell nucleus. However, there was no attempt so far to investigate the biological significance of this phenomenon therefore, we don't know today how essential is the nuclear localization of cytoskeletal proteins for the organism. The aim of our work is to understand the importance of nuclear actin and an actin-binding protein, Moesin of Drosophila melanogaster.

To investigate the physiological significance of nuclear actin and Moesin at the organism level, we built up two genetic systems in which the proteins are expressed with a Nuclear Export Signal (NES). The NES tag dramatically decreases the amount of the proteins in the nucleus without damaging their cytoplasmic functions. Our subsequent experiments revealed for the first time that the lack of actin or Moesin in the nucleus causes developmental and physiological defects which in turn reflects essential functions for the cytoskeletal proteins in the cell nucleus.

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Disruptions of mitochondrial integrity and function in response to cold in the larvae of Chymomyza costata

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Mitochondrial structural integrity and optimal functionality are generally considered as highly sensitive to extreme temperatures. Mitochondria in freeze-tolerant insects, however, can cope with extreme-cold stress (deep subzero temperatures, cellular freeze dehydration). Nevertheless, the mechanisms of mitochondrial stabilization in freeze-tolerant insects are poorly understood. We compared morphology (transmission electron microscopy), counts and location (Mitotracker™ Green staining), and function (citrate synthase activity, respiration) in mitochondria of fat body and hindgut tissues of differently acclimated larvae of drosophilid fly, *Chymomyza costata*. We show that mitochondria of warm acclimated, active larvae are sensitive to freezing stress, which causes loss of their function and physical disintegration. Entry into

diapause and subsequent cold acclimation resulted in stabilization of mitochondrial morphology and function upon freezing stress. Mitochondrial stabilization was also achieved by augmentation of larval diet with proline (chronic effect of proline), or incubation of larval fat body tissues *ex vivo* in a proline-rich medium (acute effect of proline). Hence, proline emerges as an important stabilizer of insect mitochondrial integrity and function upon cellular freeze dehydration.

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