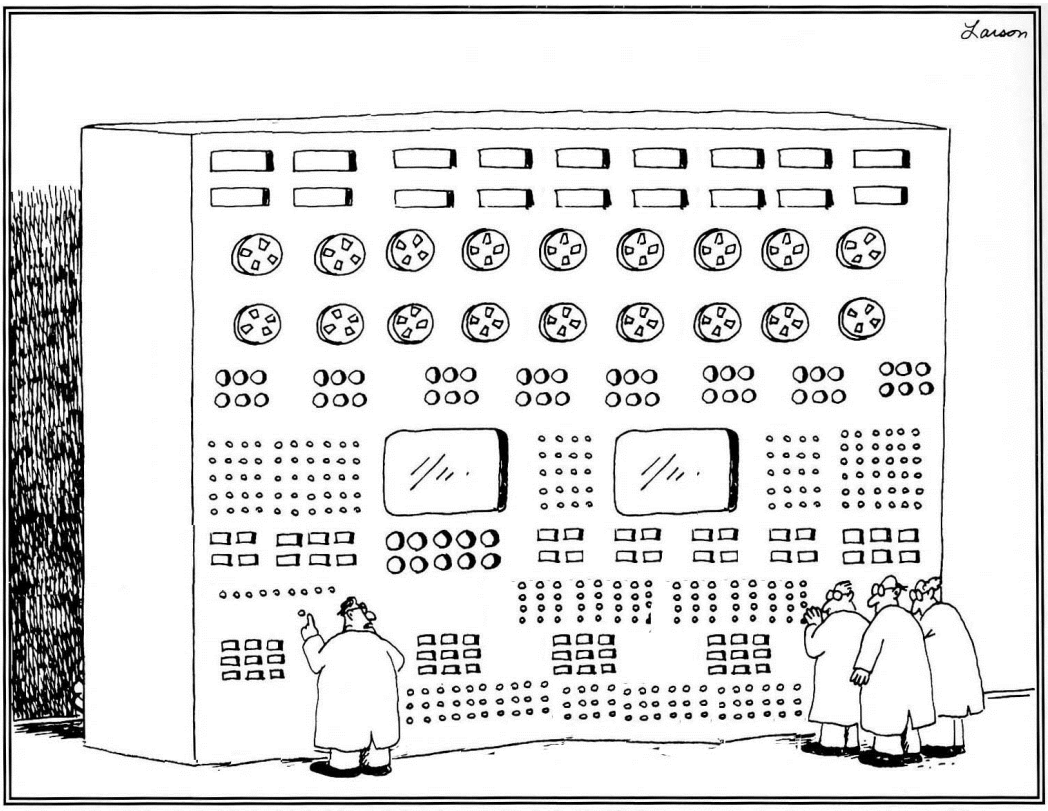
Molecular Methods in Insect Physiology

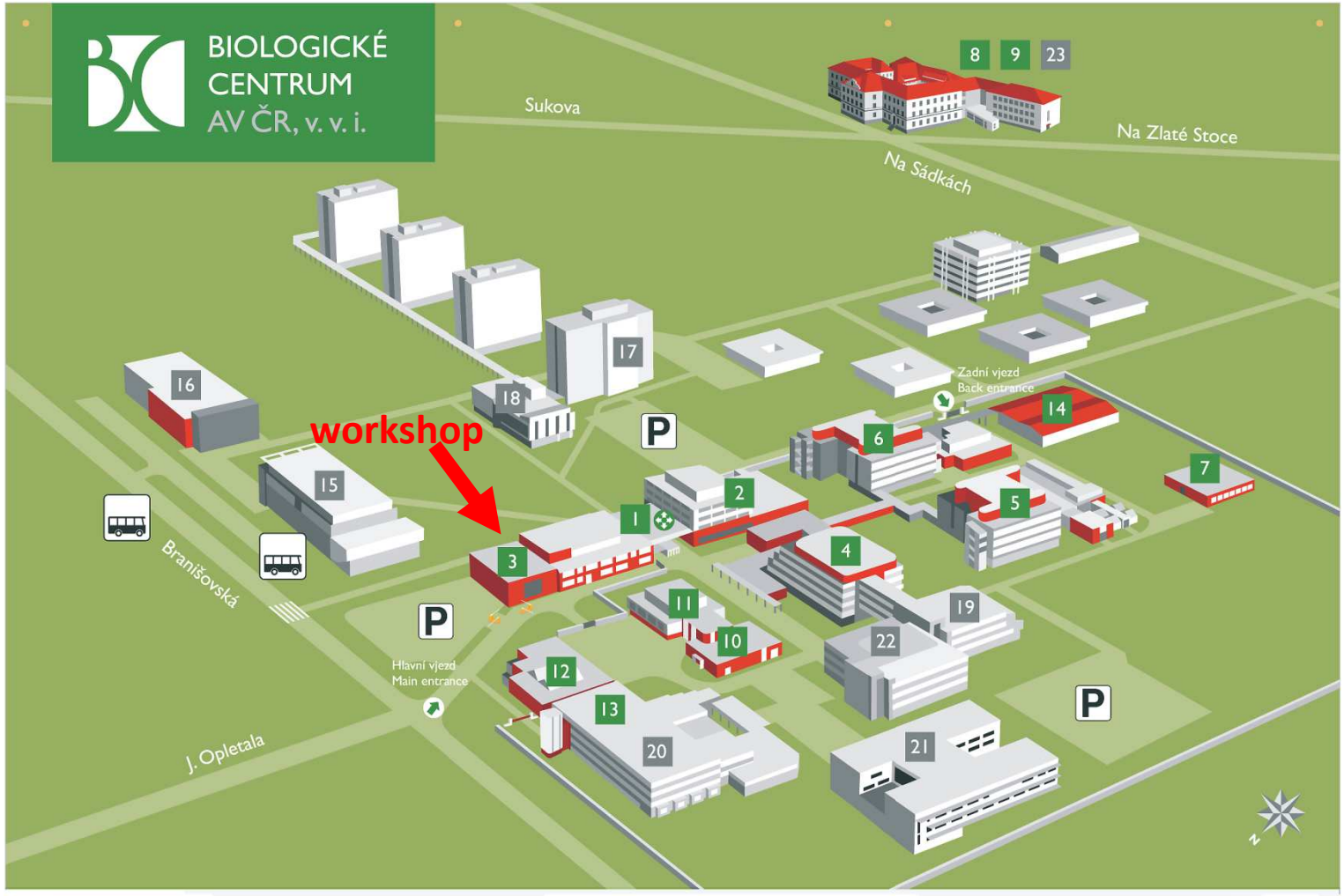
June 3 - 5, 2015, Ceske Budejovice, Czech Republic

PROGRAMME



"Wait a minute, gentlemen. ... Here's the 'on' switch over here."

The workshop is organized in frame of the project MODBIOLIN (GA 316304) supported from EU 7 th Framework Programme for Research and Development.



BUILDINGS OF BIOLOGY CENTRE ASCR

1 Entrance Hall / Reception
2 Headquaters
Servis Unit
3 Congress Centre
4 Institute of Plant Molecular Biology
5 Institute of Entomology
6 Institute of Parasitology
7 Laboratory of Electron Microscopy
8 Institute of Hydrobiology
9 Institute of Soil Biology
10 Technology Transfer Office
Garages
11 Workshops
12 Energy Centre
13 Dormitory / Motýl Children Group
14 Storage Halls

BUILDINGS OF OTHER ORGANIZATIONS

15 USB | Rector's Office / Faculty of Philosophy
16 USB | Academic Library
17 USB | Hall of Residence
18 USB | Lecture Hall
19 USB | Faculty of Science, Building A
20 USB | Faculty of Science, Building B
21 USB | Faculty of Science, Building C
22 USB | Faculty of Agriculture
23 Global Change Research Centre ASCR

Programme of the workshop:
„Molecular methods in Insect Physiology"
3. - 5. 6. 2015 in Congress Hall of the CAS,
Branisovska 31, Ceske Budejovice, Czech Republic

Wednesday, June 3:

9:15 - 16:15 **Morning session - Physiology**

9:15 - 9:20	**Michal Žurovec** - *Welcome note*
9:20 - 9:55	**David Doležel** - *Neuropeptides and neurohormones in linden bug, Pyrrhocoris apterus (Heteroptera)*
9:55 - 10:30	**Ronald Kühnlein** - *Adipokinetic Signaling in Drosophila Energy Homeostasis Control*
	Coffee break
10:45 - 11:15	**Jan Veenstra** - *Drosophila, the insect neuropeptide paradise*
11:15 -11:50	**Reinhard Predel** - *Single cell profiling of neuropeptides by mass spectrometry*
11:50 - 12:15	**István Kiss** - *Characterization of FMRF-related peptides and their receptors*
	Lunch
	Afternoon session - Methods
13:15 -13:45	**Yoko Takasu** - *Use of TALENs in Bombyx genome editing*
13:45 - 14:15	**Toshiki Tamura** - *Bombyx mori - knock-in experiments using oligos*
14:15 - 14:30	**Ligia Cota Vieira** - *Mutagenesis in Drosophila*
	Coffee break
14:40 - 15:20	**Marek Jindra** - *Juvenile hormone signaling: Finding a unique receptor for the unique hormone*
15:20 - 16:00	**Robert Farkaš** - *"Forgotten" aspects of cell death in salivary glands*
16:00 - 16:15	**Rodolphe W. Poupardin** - *Transcriptomic analysis of diapause induction in the malt fly Chymomyza costata*
	Evening visit of Hluboka (castle and restaurant)

Thursday, June 4:

8:45- 14:35 **Morning session - Immunity**

8:45 - 9:00	**Olympus** - Superresolution in confocal microscopy
9:00 - 9:35	**Pavel Hyršl/Pavel Dobeš** - *Immune response to nematobacterial infection*
9:35 - 10:10	**Ulrich Theopold** - *Cellular response to nematode infection and IDGF3*
10:10 - 10:25	**Václav Brož** - *Function of Drosophila IDGF2 protein*
	Coffee break
10:40 - 11:10	**Gábor Csordás** - *The plasticity of Drosophila melanogaster hemocytes*
11:10 - 11:45	**Viktor Honti** - *Comparative analysis of hemocyte differentiation and plasticity in Drosophila species*
11:45 - 12:20	**Bruno Lemaitre** - *The Drosophila antimicrobial response at the time of the Cas9/CRISPR gene targeting revolution*
	Lunch
13:20 - 14:00	**Tomáš Doležal** - *Extracellular Adenosine Mediates a Systemic Metabolic Switch during Immune Response*
14:00 - 14:35	**Michal Žurovec/Yu-Hsien Lin** - *Adenosine signaling*
14:35 - 14:55	**Renáta Novotná** - *CENTRAL EUROPE 2020 TRANSNATIONAL COOPERATION PROGRAMME*
	Afternoon trip to Cesky Krumlov

Friday, June 5:

8:40 - 12:30 **Morning session - Physiology/Models of human diseases**

8:40 - 9:00	**Pavel Poliak - BioTech** - *New tools in molecular biology*
9:00 - 9:35	**Philip Lehmann** - *Studying mechanisms of adaptation to seasonality in wild butterflies in the post-genomic era*
9:35 - 10:10	**Mathias Ziegler** - *The NAD metabolome: bioenergetics, signaling and biosynthesis*
10:10 - 10:35	**Lucie Kučerová** - *Diapause in D. melanogaster*
	Coffee break
10:50 - 11:25	**Mirka Uhlířová** - *Drosophila melanogaster as a model system for the study of developmental and tumor processes*
11:25 - 11:55	**Zoltan Asztalos** - *The Development of a Drosophila neuro-behaviour test battery*
11:55 - 12:30	**Alena Krejčí** - *Regulation of metabolism and growth by Notch signalling*
	Afternoon trip to Trebon

Big thanks to our Sponsors

BIOGEN
MOLEKULÁRNÍ BIOLOGIE A GENETIKA

Schoeller

BIO-RAD

OLYMPUS

www.BioTech.cz

VWR
We Enable Science

Bohemia genetics

PLAB

MERCK MILLIPORE

STINT
The Swedish Foundation for International Cooperation in Research and Higher Education

MODBIOLIN (GA 316304)

Workshop: Molecular Methods in Insect Physiology, 3-5 June 2015, České Budějovice

WEDNESDAY, JUNE 3, 2015

Neuropeptides and neurohormones in linden bug, Pyrrhocoris apterus (Heteroptera)

***David Doležel**, BC CAS, Institute Entomology, České Budějovice, Czech Republic (dolezel@entu.cas.cz)*

The linden bug, Pyrrhocoris apterus, served as a powerful model of insect endocrinology for decades. However, our knowledge of actual genes for neurohormones and their receptors was limited. Therefore we have used massively parallel sequencing and peptidomic approaches to identify genes, transcripts and corresponding processed peptides.

Adipokinetic signaling in Drosophila energy homeostasis control

***Ronald Kühnlein**, MPI, Göttingen, Germany, (rkuehnl@mpibpc.mpg.de)*

Body fat storage management is an essential component of energy homeostasis, which ensures insect survival during periods of scarcity or of excessive energy expenditure. Key to the mobilization of lipid stores during episodes of negative energy balance is the adipokinetic hormone (Akh) signalling pathway. This presentation will focus on the phenotypic analysis of
Drosophila melanogaster Akh signalling pathway loss-of-function mutants.

Drosophila, the insect neuropeptide paradise

***Jan Veenstra**, Univ. Bordeaux, France (jan.veenstra@u-bordeaux1.fr)*

The experimental tools available for Drosophila melanogaster has made this animal the most attractive model for elucidating the functional significance of specific neuropeptides. In the talk I try to illustrate this with two examples of neuropeptides effecting behavior and physiology respectively. I will try to illustrate why and how this model is so effective in elucidating the function of a neuropeptide for which no other information is available than its structure and its receptor.

Single cell profiling of neuropeptides by mass spectrometry

***Reinhard Predel**, University of Cologne, Germany (rpredel@uni-koeln.de)*

Analysis of cell-specific expression or cell-specific post-translational modifications of europeptides can contribute essentially to a better understanding of the complex functions of neuronal circuits. MALDI -TOF mass spectrometry is the method of choice to study such intercellular communication capabilities at the single neuron level. In the talk, examples of methods that are used for cell identification, dissection, and subsequent mass spectrometric analysis of peptidergic neurons are presented; with a focus on insect neurons.

Use of TALENs in Bombyx genome editing

***Yoko Takasu**, NIAS, Tsukuba-Ibaraki, Japan (takasu@affrc.go.jp)*

We recently established an efficient procedure for Bombyx gene knockout using TALENs. Our TALEN vector is optimized for Bombyx and the average efficiency of mutagenesis in somatic cells reaches 64%. With this system we are now trying to modify silk proteins, fibroin and sericin.

Construction of knock-in Bombyx mori using TALEN and ssDNA oligonucleotide

***Toshiki Tamura**, NIAS, Tsukuba-Ibaraki, Japan (ttamura@affrc.go.jp)*

To develop an efficient knock -in method in Bombyx mori, we studied a relation between the effect of ssDNA oligonucleotide sequence and TALEN target site. We found that the method is very efficient and enabled an insertion of new sequence up to 35 bp and a deletion of about 2 kb.

Mutagenesis in Drosophila

***Ligia Cota Vieira**, BC CAS, Institute Entomology, České Budějovice, Czech Republic (lvieira@entu.cas.cz)*

Transcription activator-like effector nucleases (TALENs) are highly efficient and have been extensively used for the generation of mutations in Drosophila melanogaster and other organisms. Since then, another system, using clustered regularly interspaced palindromic repeats (CRISPR), has been presented as an equally efficient option with a much lower cost. Our experience has shown that while the potential of CRISPR is undeniable, serious improvements are necessary to make it as reliable as TALENs.

Juvenile hormone signaling: Finding a unique receptor for the unique hormone

***Marek Jindra**, BC CAS, Institute Entomology, České Budějovice, Czech Republic (jindra@entu.cas.cz)*

Two non-peptide lipophilic hormones, ecdysone and juvenile hormone (JH), govern insect development. Finding a receptor for JH was lagging two decades behind finding the ecdysone receptor, and required both non-Drosophila models and fly genetics.

Characterization by RNAi silencing of FMRF-related peptides and their receptors

***Istvan Kiss**, BRC, MTA, Szeged (kiss43@brc.hu)*

Five neuropeptide genes are classified in the FMRF-related (FaRP) group: the Fmrf, dromyosuppressin (Dms), drosulfakinin (Dsk), neuropeptide F (npf) and short neuropeptide F (sNPF). In order to compare their effects on the locomotor activity of Drosophila adults, we made RNAi knockdown of the peptides and their specific receptor genes. In addition, we constructed Gal4 drivers with three distinct parts of the Fmrf gene's 5' regulatory sequence, and used them to ablate FMRF-positive neurons through rpr-indiced apoptosis. We examined the startle-induced changes in the flies' locomotor activity by measuring the mean velocity of movement (MVM). In general, the flies' locomotor activity was decreased by the RNAi knockdown induced in the CNS by the elav-Gal4 driver. The highest effects were observed when the DmsR-1 and DmsR-2 receptors or the Dsk and DskR-2 genes were silenced together. Male and female flies were not different in this respect. In the cell-ablation experiment, significant effects were observed in females when the UAS-rpr transgene was activated by the RS8-Gal4 or the RS17-Gal4 drivers, while the RS11-Gal4 was ineffective. Interestingly, the RS8-induced neural ablation had no effect ont he flies' negative geotaxis. These results confirm that the FaRP peptides and receptors are important regulators of the adult locomotor activity. In addition, with the new drivers we observed ectopic expression of the Fmrf gene in the imaginal discs.

"Forgotten" aspects of cell death in salivary glands

***Robert Farkaš**, Institute of Experimental Endocrinology, Slovak Academy of Sciences Bratislava, Slovak republic (ueenfark@savba.sk)*

In all metazoans programmed cell death (PCD) is a genetically encoded form of cell suicide that results in the orderly death of excessive, damaged or dangerous cells during normal development and adult life and thereby contributes to the maintenance of body homeostasis. During Drosophila metamorphosis in response to sequential ecdysone pulses, obsolete larval tissues are destroyed in a stage-specific manner as adult tissues and structures develop from small clusters of progenitor cells, resulting in the transformation of a larva into an adult fly. Here we analyze relationships between autophagic and apoptotic signaling pathways how they contribute to elimination of Drosophila larval salivary glands which serve as model organ to study metamorphic PCD.

Transcriptomic analysis of diapause induction in the malt fly Chymomyza costata

Rodolphe W. Poupardin, *BC CAS, Institute Entomology, České Budějovice, Czech Republic (rodolphe.poupardin@gmail.com)*

Diapause is an environmentally programmed and hormonally regulated period of dormancy which makes an important part of the life-cycle in many species of invertebrates. In this study, using a RNAseq approach, we focused on very early stages of diapause induction in the larvae of drosophilid fly, Chymomyza costata by characterizing global patterns of gene expression associated with photoperiodic induction of diapause.

Immune response to nematobacterial infection

***Pavel Hyršl**, Faculty of Science, Masaryk University, Kamenice 753/5, Brno, Czech republic (hyrsl@sci.muni.cz)*

Entomopathogenic nematodes and their associated bacteria comprise together highly pathogenic complex able to invade and kill insect host within two days. Both bacteria and nematodes produce variety of factors interacting with insect immune system that help to overcome host defences. The tripartite model (Drosophila, nematodes, bacteria) was established and used to show an immune function for candidate genes using different Drosophila mutants or RNAi lines with defects in clotting or other branches of the immune system. We demonstrated an immune function during nematode infection for known clotting enzymes and substrates, recognition molecules, eicosanoids etc. In conclusion, we show that the Heterorhabditis /Photorhabdus infection model is suitable to identify novel regulators of innate immunity and we bring evidence of coagulation immune function in insects against hematode infection.

Cellular response to nematode infection and IDGF3

***Ulrich Theopold**, Univ Stockholm, Sweden, (uli.theopold@molbio.su.se)*

We present new evidence for a protective function of the clot during nematode infections of insects. We also show that - similar to what has been proposed for mammals - a member of the Chitinase -like proteins in Drosophila appears to regulate wound healing.

Function of Drosophila IDGF2 protein

***Václav Brož**, BC CAS, Institute Entomology, České Budějovice, Czech Republic (vasa.broz@centrum.cz)*

IDGF2 is the prototypical member of Drosophila imaginal disc growth factor family described in 1999 by Kaz Kawamura. We characterized the effects of recombinant IDGF2 on tissue culture cells in vitro, as well its overexpression in vivo. We show that it has pleiotropic effects on cell survival and cell protection from starvation or toxic effects of some metabolites. We also show that in addition of being an immune-inducible gene, IDGF2 alone can prime the innate immunity response.

The cell mediated immunity of *Drosophila (two lectures from Dr. Ando laboratory)*

***Istvan Ando**, BRC Szeged, Hungary, (ando@brc.hu) **Gabor Csordas**, BRC Szeged, Hungary, (csordás.gábor@brc.mta.hu) **Viktor Honti,** BRC Szeged, Hungary, (honti.viktor@brc.mta.hu)*

Introduction

The aim of our studies is to explore basic mechanisms of blood cell development and cell mediated immunity to understand defense strategies utilized to eliminate invaders, using *Drosophila melanogaster* and other *Drosophila* species as model organisms. So far, we have developed tools for in *vivo* and *ex vivo* studies in the form of antibodies and fluorescent genetic markers to study the hematopoietic organs and the machinery of the immune system. The tools helped to define major classes and lineages of hemocytes, identified a major source of immune-responsive blood cells in the larva, and showed the plasticity of the phagocytic cell population in the cell-mediated immune responses. We further define immunological markers for functionally and developmentally distinct hemocyte subsets and develop *in vivo* genetic markers to trace hemocyte lineages and to analyze the blood cell compartments. The analysis of the compartmentalization and function of hematopoietic tissues and hemocytes helps to understand the communication between the blood cell compartments, e.g., the sessile hematopoietic tissue with the circulating blood cells as well as with other tissues in the course of the development and in the course of elimination of microbes and parasites.

Part 1 - The plasticity of Drosophila melanogaster hemocytes
Gabor Csordas

In the Drosophila larva, certain immune challenges, such as tumors or wasp parasitization, induce the massive differentiation of encapsulating blood cells, the lamellocytes. By utilizing an array of molecular markers and in vivo reporters, we found that these cells develop from macrophage -type hemocytes (the plasmatocytes), which were previously considered terminally differentiated effectors. Our current focus is to elucidate key factors, which are responsible for this transition.

Part 2 – Comparative analysis of hemocyte differentiation and plasticity in *Drosophila* species
Viktor Honti

We analyzed the larval effector hemocyte types of several *Drosophila* species, and found significant differences between the melanogaster and the ananassae subgroup. Instead of lamellocytes, encapsulation in *D. ananassae* and *D. bipectinata* larvae is achieved by a novel cell type, the multinucleated giant hemocyte. Here we summarize the analogies and differences of hematopoiesis and compartmental plasticity between *D. melanogaster* and *D. ananassae*.

The *Drosophila* antimicrobial response at the time of the Cas9/CRISPR gene targeting revolution

***Bruno Lemaitre**, Global Health Institute, Ecole Polytechnique Fédérale of Lausanne, Lausanne, Switzerland. (Bruno.lemaitre@epfl.ch)*

The application of *Drosophila* genetics to these mechanisms has generated insights into insect immunity and uncovered general principles of animal host defense. These studies have shown that *Drosophila* has multiple defense "modules" that can be deployed in a coordinated response against distinct pathogens. Today, *Drosophila* can be considered as having one of the best-characterized host defense systems among the metazoan. Until recently, a detailed understanding of the fly immune response was hampered by the difficulty of generating loss-of-function mutations as well as the technological limits of the RNAi approach. The Cas9/CRISPR revolution offers new opportunities to revisit in a systematic manner *Drosophila* immunity. At the interface between large-scale genomic studies that lack resolution and individual gene analysis that lack breadth, our laboratory has undertaken a meso-scale 'skilled' analysis of immune modules, notably by addressing the individual and overlapping function of large immune gene family. In this talk, I will summarize our current knowledge of the field and provide new insights recently gained in the laboratory.

Extracellular adenosine mediates a systemic metabolic switch during immune response

***Tomáš Doležal**, University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic, (dolezt04@prf.jcu.cz)*

We use parasitoid wasp infection of Drosophila larvae to study regulation of energy metabolism during immune response. We demonstrate that activated immune cells increase glycolysis and glucose/trehalose consumption and that they release adenosine as a mediator of systemic metabolic switch which is required for energy/nutrition to flow towards immune response. Our results experimentally demonstrate a trade-off between development and immune response.

Adenosine signaling

***Michal Žurovec/Yu-Hsien Lin**, BC CAS, Institute Entomology, České Budějovice, Czech Republic (zurovec@entu.cas.cz)*

Adenosine (Ado) is an ubiquitous metabolite, which plays a prominent role as a paracrine signal of metabolic imbalance within tissues. While adenosine signaling seems to be conserved among phyla, Drosophila and higher Diptera seem to have more genes involved in adenosine signaling than other insects. New reverse genetic tools allow examining the conservation of physiological functions by using targeted genome editing across species.

The Central Europe 2020 Programme – Opportunity for funding?

Renáta Novotná, *BC CAS, České Budějovice, Czech Republic (Renata.Novotna@bc.cas.cz)*

The Central Europe 2020 Programme is a continuation of the former Central Europe Transnational Programme, aiming at addressing challenges in the Central European region, namely in Austria, Croatia, the Czech Republic, Hungary, Poland, Slovakia, Slovenia and parts of Germany and Italy. Recommended number of partners is up 8 to 12 partners (not all have to be from Central Europe).

Studying mechanisms of adaptation to seasonality in wild butterflies in the post -genomic era

***Philipp Lehmann**, Univ Stockholm, Sweden, (philipp.lehmann@zoologi.su.se)*

Seasonal environments provide an excellent opportunity to study mechanisms behind adaptation, since many insects show strongly heritable patterns in various traits as a function of latitude. I will present an overview of a project studying how the butterflies Pieris napiand Pararge aegeria are adapted to seasonally variable environments in Europe. Some focal parts of the project are 1) the characterization of physiological development during pre-diapause and diapause termination, 2) eco-physiological constraints faced by the butterflies during diapause in different parts of their range and 3) ways to understand and study time-keeping mechanisms in non-model insects, utilizing the potential of forward genetics.

The NAD metabolome: bioenergetics, signaling and biosynthesis

***Mathias Ziegler**, Univ. Bergen, Norway (Mathias.Ziegler@mbi.uib.no)*

Besides being important in redox metabolism, NAD has emerged as versatile signaling molecule. NAD is used for protein mono- and poly-ADP-ribosylation, NAD-dependent deacetylation (by Sirtuins) and as precursor of intracellular calcium messengers. These processes include NAD degradation and necessitate continuous replenishment of cellular NAD pools. NAD biosynthetic pathways have therefore also moved into focus. During our studies we have developed a tool for "in situ" detection of NAD that can be adopted for use as a powerful protein subcellular localization assay.

Diapause in Drosophila melanogaster

***Lucie Kucerova**, Univ Stockholm, Sweden, (Luci.puci@seznam.cz)*

The fruit fly Drosophila melanogaster can enter reproductive diapause as a response to low temperatures and short day length. We have investigated genome wide transcriptional changes in diapausing D. melanogaster females. Many of the genes affected by diapause had previously been found to alter their expression in response to food deprivation and cold exposure, as well as they were identified to be part of latitude adaptations in D. melanogaster populations. We also identify a significant overlap in transcriptional changes with other diapausing Drosophila species from temperate regions. Taken together our data provide the first comprehensive analysis of transcriptional changes induced by diapause, and provide a framework for future investigations of dormancy in other organisms.

Transcriptional (dys)regulation of epithelial homeostasis

Mirka Uhlirova, *CECAD Cologne, University of Cologne, Germany (mirka.uhlirova@uni-koeln.de)*

Epithelia represent the defining tissue type in metazoans. Polarization of individual epithelial cells along the apico-basal axis is essential for the maintenance of epithelial function and homeostasis. Conversely, loss of polarity contributes to cancer development and metastasis formation. By combining unbiased genomic and genetic approaches in a *Drosophila* model, we have identified a novel polarity regulator and characterized a transcription factor network that promotes tumor malignancy in response to loss of polarity and oncogenic Ras signaling.

Regulation of metabolism and growth by Notch signalling

Alena Krejci*, University of South Bohemia, Faculty of Science / Institute of Entomology, Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czech Republic, (akrejci@prf.jcu.cz)*

Glycoytic switch is a characteristic feature of rapidly proliferating cells, like cells during development and during immune response or cancer cells, as well as of stem cells. Notch signalling is known to stimulate cell growth and division in various contexts and it is also needed for stem cell maintenance and for cell specification during immune response. Whether Notch regulates metabolic genes during these processes and whether they represent direct transcriptional Notch targets is not known. We show that genes mediating cellular metabolic changes similar to Warburg effect are direct transcriptional targets of Notch signalling. A short pulse of Notch activity is able to elicit long-lasting metabolic effects that are not dependent on the level of expression of the Notch receptor. Notch mediated metabolic shift helps to stimulate tissue growth during development and during Notch induced hyperplasia. Notch is active in other cells that undergo glycolytic switch and the direct regulation of metabolic genes may be a common mechanism that helps Notch to exert its effects in these tissues.