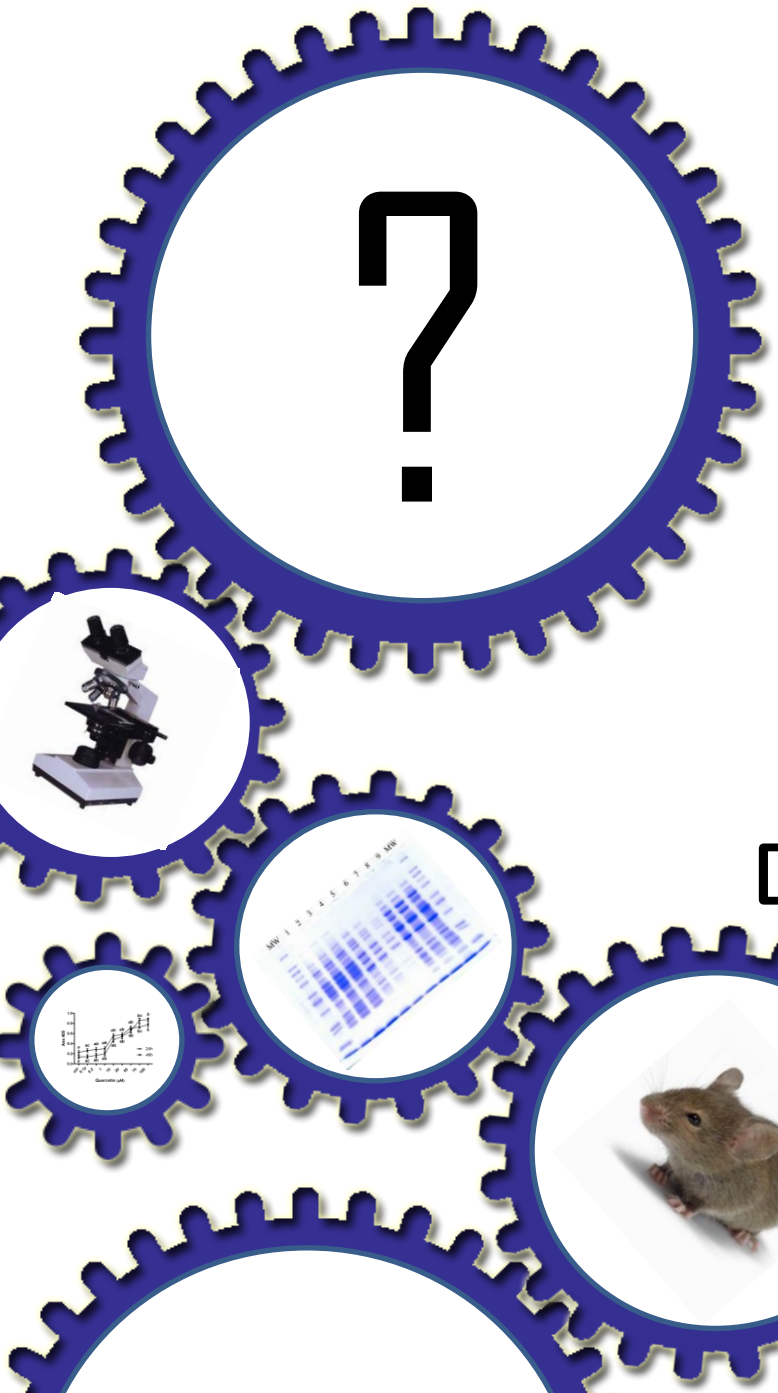
4th PhD conference

3rd June 2011

Abstract book



What will be the next step in your PhD...?

Career development lecture

Radka Kolenovská

Keynote lecture

Heather Marlow

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Programme

9:00 – 9:15 Opening session

Václav Hořejší and Michaela Liegertová

9:20 – 10:20 Sesion I

9:20-9:40 **Samira Hozeifi (**Department of RNA Biology**)**
"SNAP tag, one for all and all for one."
9:40-10:00 **Antonio Pombinho (**Department of Cell Differentiation**)**
"High-Throughput Screening"
10:00-10:20 **Lucie Tůmová (**Department of Cellular and Developmental Biology**)**
"Searching for a weapon in the war against colorectal cancer"

10:20-10:45 Coffee break

10:45 – 11:50 Session II

10:45-11:05 **Matyáš Šíma (**Department of Molecular and Cellular Immunology**)**
"Genetic Control of Resistance to Trypanosoma brucei brucei Infection in Mice"
11:05-11:25 **Jana Oujezdská (**Department of Immunobiology**)**
"Tracking of maternal cells in embryonic tissues."
11:25-11:50 **Alžběta Kalendová (**Department of Biology of the cell nucleus**)**
"Express yourself!"

12:00-13:00 Lunch

13:00 – 14:15 Session III

13:00-13:20 **Petr Pajer (**Department of Molecular Virology**)**
"Looking for the reasons of cancer existence"
13:20-13:55 **Juraj Piško (external PhD talk)**
"What the bleep is our sleep?"
13:55-14:15 **Ondřej Štěpánek (**Department of Molecular Immunology**)**
"Scientific projects: A lesson from Underpants Gnomes"

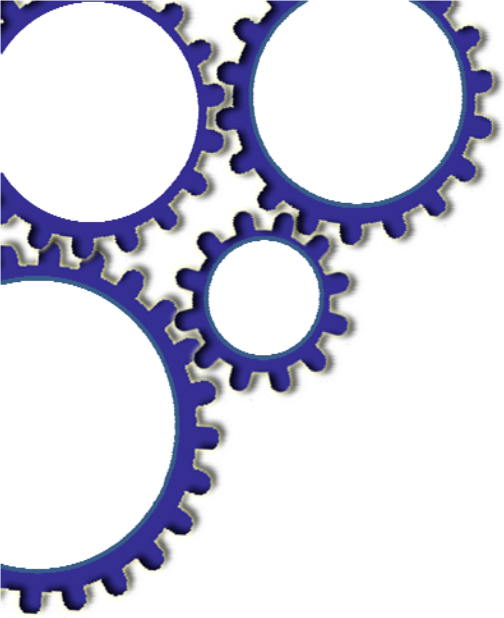
14:15-14:40 Coffee break

14:40 – 15:30 Session IV

14:40-15:30 **Key-note lecture: Heather Marlow (EMBL)**
"The evolution of animal body plans with regard to nervous system centralization"
15:30-16:20 **Career development lecture: Radka Kolenovská (ORION Clinical Services)**
"Development of Original Medicines – the Marvellous World of Clinical Research"

16:30-17:00 Best presentation evaluation

17:00-0:00 Party



Talks

SNAP-tag, one for all and all for one

Samira Hozeifi

To study proteins' function few questions should be addressed: "When? Where? With whom?". Tags are one of the best tools that would help us to answer these questions.

SNAP-tag is the first multi-purpose tag. It is a 20kDa mutant of a DNA repair protein, which reacts specifically with its substrate, benzylguanine (BG).

SNAP-tag accepts many different substrates and allows the attachment of wide range of fluorophores and affinity labels (BG-biotin) or beads (BG-sepharose) in vivo or in vitro. SNAP-tag answer the above questions as follows:

When?: SNAP-tag Labeling could be readily turned on or off by adding its substrates or inhibitors to study the dynamic processes.

Using different colorful substrates at different time points, one could distinguish between older and newly synthesized tagged proteins.

Where?: SNAP-tag has no limitation with respect to cellular localization and deep tissue visualization would be possible. Its substrates are inert, cell permeable and not losing the signal compare to organic fluorophores.

With whom?: SNAP-tag could be used to confirm the "specific" protein-protein interaction by pull down assay, using microplate assay and also FRET.

Furthermore, CLIP-tag, sibling of SNAP-tag, react with benzylcytosine (BC) that make simultaneous labeling of proteins possible.

Altogether SNAP-tag is the one that could guide us to answer all these questions without going through cycles of cloning and lead us to understand the proteins' function.

High-Throughput Screening

Antonio Pombinho

A huge and ever increasing number of small molecules needs to be tested for their bioactivity. Methods for analyzing these compounds are trying to follow the increasing complexity of our understanding of biological processes. Therefore, highly reproducible, automated and miniaturized assays, for drug or simply for biological probes discovery, are nowadays constantly evolving.

In this presentation, we will go briefly through the steps that lead to a new drug discovery, from compound choice and management, assay development and data storage, to compound validation, passing through some recent and interesting instrumentation developments.

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Searching for a weapon in the war against colorectal cancer

Lucie Tůmová

Canonical Wnt signaling pathway is one of the most important pathways involved in cell differentiation and proliferation. This pathway is crucial not only in the embryonic development but also in renewal of adult tissues. Intestinal epithelium is the fastest self-renewing tissue in a human body. Aberrant activation of the Wnt signaling pathway in the intestinal stem cells leads to development of colorectal cancer, which is the third most common type of cancer in the Western world.

We tested a group of given small molecules *in vitro* as potential antagonists of canonical Wnt signaling pathway in different cell lines. Several of these compounds decreased presence of nuclear β-catenin, the hallmark of the active Wnt/ β-catenin pathway, in mouse L cells. These compounds were also able to down regulate the Wnt target genes Axin2 and SP5 in human colorectal cancer lines DLD-1 and LS174T.

We employed $APC^{CKO/CKO}$xLGR5-$creERT^2$ mouse strain to test ability of a novel synthetic compound JW55 to restrain tumor growth and development in the mouse intestines due to the canonical Wnt signaling pathway inhibition. After 21 days of daily feeding with JW55, mice developed smaller number and size of tumors in small intestines and colon compared with the control group. Effectiveness of this compound suggests that JW55 could be useful for future pharmaceutical applications.

Genetic Control of Resistance to *Trypanosoma brucei brucei* Infection in Mice

Matyáš Šíma

Trypanosoma brucei brucei infects livestock, with severe effects in horses and dogs. Mouse strains differ greatly in susceptibility to this parasite. However, no genes controlling these differences were mapped. In our study we obtain the first identification of chromosomal loci (*Tbbr1 – 4*) controlling susceptibility to *T. b. brucei* infection. While mapping in F_2 hybrids of inbred strains usually has a precision of 40 – 80 Mb, in RC strains we mapped *Tbbr2* to a 2.15 Mb segment containing only 26 genes, which will enable an effective search for the candidate gene (Šíma et. al., Plos Neglected Tropical Diseases, in press). From used methods I chose mouse blood collecting from the saphenous vein and estimating of cytokines in mouse serum by Bender MedSystems: FlowCytomix™ Technology to talk about them in more detail.

Tracking of maternal cells in embryonic tissues

Jana Oujezdská

During normal pregnancy bidirectional cell trafficking occurs between the mother and the fetus. Some of the maternal cells that cross the placental barrier and enter embryonic structures persist in offspring till late adulthood. This phenomenon is referred to as maternal microchimerism. In addition to several studies describing maternal microchimerism in murine late gestation fetuses (E12-E18), Bertrand et al have identified maternal macrophages invading embryonic structures at early developmental stages (E7.5).

Several potential biological functions of maternal cells in developing embryo or fetus have been proposed. These include elimination of preteratogenic cells followed by protection from abnormal development or shaping the lymphocyte repertoire formation in the developing thymus.

Previously maternal cells have been detected in embryonic tissues using several approaches including PCR, flow cytometry and immunohistochemistry. However different methods led to inconsistent results.

We have used flow cytometry as well as *in vitro* confocal microscopy to track transgenic maternal cells engrafting early murine embryos. Using this approach we were able to determine the kinetics of maternal cell migration towards embryonic tissues as well their surface marker expression.

Express yourself!

Alžběta Kalendová

Protein expression and purification is an important field in research. Frequently, it is essential to have a high amount of protein of interest with high purity, since it is a starting material to study its properties and behaviour under specific conditions. However, it is not easy to reach this state. There are many expression systems available (bacterial, yeast, insect, mammalian, cell-free etc.) possessing various advantages and limitations. It is therefore important to select the most appropriate method based on the protein character and purpose of its use. Once the protein is expressed successfully, it requires to be purified efficiently in as little steps as possible.

My presentations will give a brief overview of methods for expression and purification currently available for those who are interested and also for those who are not.

Looking for the reasons of cancer existence

Petr Pajer

Any scientific experimentation should be motivated by the effort to answer some fundamental question. For example “What is the reason of cancer existence?”

Initially, we have established an animal model of various tumors induced by insertional mutagenesis. Early infection of young chickens with MAV retrovirus results in the development of multiple clonal tumors – nephroblastomas, lung angiosarcomas, liver carcinomas. We have identified tens of candidate genes responsible for induction of these tumors and revealed the probable molecular basis of their genesis. We have also compared the identified genes with those mutated in related animal models and spontaneously arisen human tumors. Our observations are satisfactorily consistent with current conceptions of molecular carcinogenesis. So, could our results help answer the originally proposed question? Not really, I’m afraid.

Current scientific concepts are almost exclusively oriented towards optimization research. In contrast to the true basic (breakthrough) research, it can be easily planned, monitored, quantified and funded. But the optimization research is hardly capable of solving fundamental problems as exemplified by the above-mentioned question.

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What the bleep is our sleep?

Juraj Piško, M.D.

The brain and neural functions still belong to the most mysterious phenomena of physiology and pathophysiology. And one of neurally regulated processes – sleep - is one of the most challenging enigmas even in the 21th century.

Despite multiple experiments, animal models, studies of human sleep disorders and relatively deep knowledge on the molecular level, we still don´t know its precise function. For a long time, sleep was considered to be a passive state - simple deficiency of wakefulness due to exhaustion of an organism. However, pioneering studies of several great neuroscientists of 20. century revealed that sleep is actively driven by certain neural centres and is eliminated by lesions in specific brain areas. Furthermore, brain activity during REM stage of sleep is as high or even higher than in our wakeful state.

There are many theories that try to explain the role of sleep in our life – e.g. protection from potentially dangerous dark phase of the day; energy saving; recovering from preceding physical, behavioral and metabolic activity, memory consolidation; mental and emotional processing and (especially in the case of REM sleep) the role in neural development. However, none of these hypotheses has been convincingly proven and there are several arguments against each of them.

The only thing that we know for sure is: sleep is essentially important for our life. Animals that are experimentally deprived of sleep and humans suffering from severe familiar insomnia are condemned to death in a quite short time. However, causes of their death are not consistent and don´t elucidate the role of sleep at all. Furhermore, human studies revealed that not only shortened sleep (less than 6 hours) but even prolonged sleep (more than 9 hours) is associated with higher morbidity and mortality. And functions of mental events during our sleep, ie. dreams, are far from being understood. The aim of my talk is to summarize current important knowledge of the sleep physiology and to review the most significant theories about its role in our life.

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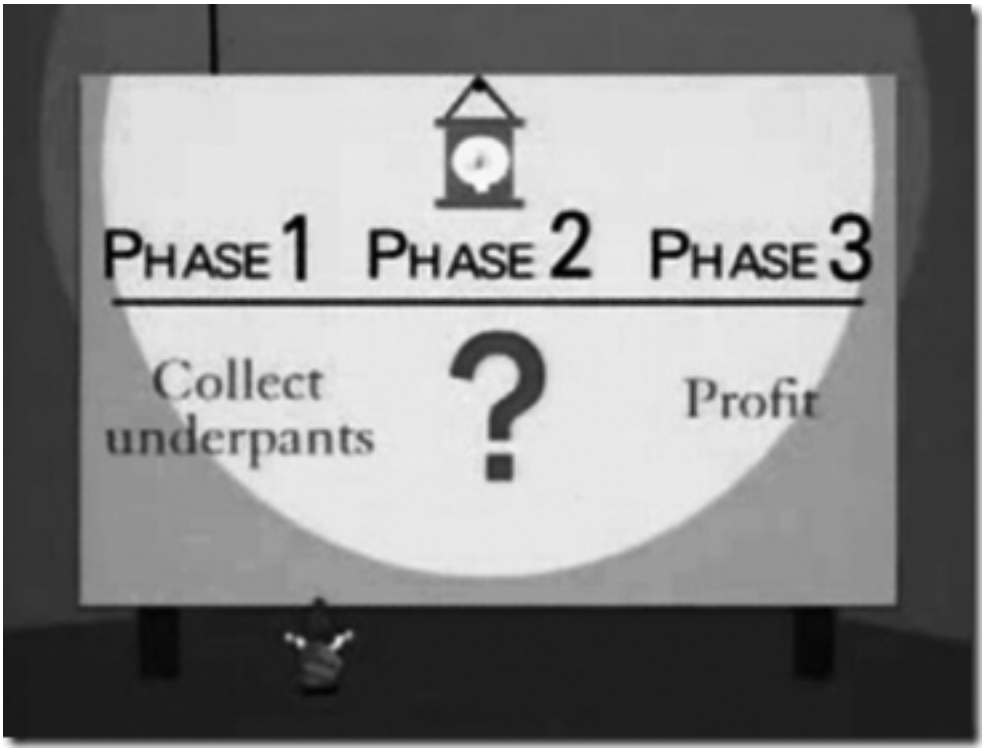
Scientific projects: A lesson from Underpants Gnomes

Ondřej Štěpánek



The evolution of animal body plans with regard to nervous system centralization

Heather Marlow (EMBL)

The evolution of animal body plans is hypothesized to have been driven in large part by novel morphological innovations such as brain and through-gut evolution. These innovations allowed animals to move into new ecological niches and drove further radiation of the many forms making up the Bilaterian (bilaterally symmetrical) animals.

Today, many examples of these bilaterally symmetrical animals persist, but only a few groups have been studied in great detail at the molecular level. By examining many representatives of these distantly related groups as well as their earlier branching "radially" symmetrical relatives, we gain better insight into the characters present in the last common ancestor of the Bilaterians.

Particularly, we are interested in studying the emergence and elaboration of the centralized nervous system. The first animals with nervous systems had loosely organized nerve nets and many species which exist today retain this simple organization. Within the Bilateria, centralization into condensed nerve cords has occurred in many species in addition to cephalization, the movement of these neural elements to the anterior end of the animal.

We have focused on the annelid worm model Platynereis dumerilii and compare this model to other Bilaterian animals such as Drosophila and vertebrates to understand what elements existed in the earliest brains. In addition, we work with the non- Bilaterian model sea anemone Nematostella vectensis to understand what cell types existed in the early nerve nets prior to the condensation of these cells into brains and nerve cords. By examining these distantly related animals we can infer what features of the nervous systems were present at each major node, or branching point, in the metazoan animal tree and where major morphological innovations have occurred.

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