Laboratory of Cell Differentiation

Chemical genetics, haematopoietic and neural cell differentiation, signalling pathways, nuclear receptors

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The main interest of the laboratory lies in the molecular mechanism of cell fate determination. In the lab we have established *in vitro* systems to study the self-renewal and differentiation of haematopoietic, neural and mesenchymal stem cells. We use growth factors and small molecules as tools to manipulate these systems. More recently, we have initiated more systematic search for such tools using chemical biology/genetics approaches.

Nuclear receptors function as ligand-dependent transcription factors to regulate gene transcription in response to specific physiological stimuli such as steroids, retinoids, thyroid hormone and vitamin D. Thyroid hormone receptors, activated in response to thyroid hormone (T3), are known to modulate the level of serum cholesterol via complex regulatory pathways. By screening for T3-regulated genes we have identified Disp3, a sterol-sensing domain-containing protein that is related to the Dispatched family of proteins. DISP3 is predominantly expressed in specific cell types of the brain, retina and testis and localizes within the endoplasmic reticulum, and was found to co-localize with cholesterol. Ectopic expression of DISP3 in fibroblasts resulted in elevated cholesterol levels combined with an altered cholesterol distribution. We proposed that DISP3 represents a new molecular link between thyroid hormone and cholesterol metabolism in the brain
(Zikova *et al.* 2009). Moreover, we have identified two neural stem cell lines that highly express Disp3. Disp3 expression is positively regulated by T3 treatment, and upon differentiation the level of Disp3 dramatically changes, suggesting that Disp3 may modulate self-renewal or differentiation. Brain tumours such as medulloblastoma are believed to arise from neural precursor cells. Analysis of a small number of primary human tumours revealed very high expression of Disp3, suggesting an important role for this protein in their pathogenesis. We have performed RNAi and overexpression studies and found out that Disp3 is able to modulate the cell fate of neural stem cells and their progeny.

We have also identified, cloned and characterized the first non-mammalian Tpo, chicken thrombopoietin, and its receptor c-Mpl. Discovery of chicken Tpo and c-Mpl will greatly facilitate future studies regarding thrombocytic differentiation and haematopoietic stem cell development. Moreover, we have introduced an experimental model of chicken bi-potent thrombo/erythropoietic progenitors that can be used to identify key regulators of cell fate determination (Bartunek *et al.* 2008). In addition, we have extended our studies to vertebrate hematopoietic development by introducing a new model organism in our laboratory – the zebrafish – and we established the first *ex vivo* cultures of haematopoitic cells (Stachura *et al.* 2009).

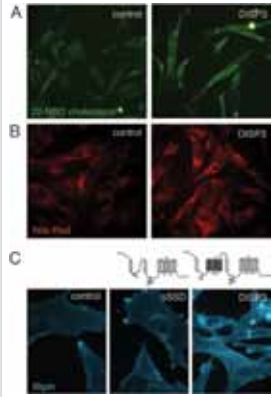

Fig. 1. Ectopic expression of DISP3 leads to accumulation of lipid droplets. (A) Accumulation of exogenous NBD-cholesterol in lipid droplets in control and DISP3-expressing cells, (B) Control and DISP3 cells stained by Nile Red to evaluate lipid droplet formation. Note the increased number of lipid droplets in DISP3-expressing cells. (C) Cholesterol accumulation and relocalization is compromised in DSSD-DISP3-expressing cells. CEF cells stably transfected with either an empty vector, vector encoding DSSD-DISP3, or wt DISP3 were analysed by filipin staining.

Fig. 2. Zebrafish as a model to study vertebrate haematopoiesis. Doublehemizygous transgenic zebrafish Tg(gata1::DsRed); Tg(mpx::EGFP) at 4 days post fertilization with single haematopoietic cells fluorescently labelled (red, erythroid cells, green, myeloid cells/neutrophils).

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- AS CR, IAA500520705 – Characterization of the new member of sterol-sensing-domain protein family and its role in lipid metabolism, 2007-2009, M. Zíková
- GA CR, GA310/08/0878 – The role of the cells prion protein in erytroidnic differentiation: possible link to peripheral pathogenesis of prion diseases, 2008-2012, P. Bartůněk
- GA CR, GA204/09/1905 – Disp3: A potential role in the self renewal and differentiation of neural stem cells, 2009-2012, P. Bartůněk
- GA CR, GAP305/10/0953 – New regulators of megakaryocyte and erythroid lineage commitment, 2010-2013, P. Bartůněk
- Ministry of Industry and Trade, FT-TA5/136 – System for identification of biologically active compounds: Focused combinatorial libraries, P. Bartůněk

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