PhD thesis evaluation

Title: The Interplay Between the Notch Signaling Pathway and Cellular Metabolism
Author: Mgr. Věra Slaninová

Scientific quality: The presented thesis focuses on the crosstalk between the Notch signalling pathway and cellular metabolism. It uncovers direct regulation of several metabolic genes by Notch on one hand and mechanism through which metabolism can influence the outcome of Notch signalling on the other. In the first part of her thesis Věra Slaninová presents conclusive evidence that several metabolic genes including glucose transporter or lactate dehydrogenase are direct Notch target genes. She also shows that Notch activation induces metabolic shift towards glycolysis both in cultured cells and in Drosophila wing discs. Further she demonstrates that regulation of metabolic gene expression might be one of the mechanisms through which Notch signalling regulates growth of tissues such as wing disc. These findings were summarized in a paper in Open Biology where Věra Slaninová is the first author and to which she contributed substantial part of the data. In the second part of her thesis the author provides evidence that protein deacetylase Sirt1 promotes activation of the Notch pathway and that Su(H) is a Sirt1 target. These findings were included in a publication in Biochemical Journal where Věra Slaninová is the third author. The last experimental part of the thesis is comprised of unpublished data providing several indications that the activity of the Notch pathway is sensitive to the NAD+/NADH ratio. These findings offer possible mechanism linking metabolism and Notch pathway activity. Overall, the experimental data presented in the thesis are of high quality, they successfully passed the peer-review test and contributed novel insight into the field of metabolism-signalling crosstalk.

Formal quality: The thesis is written in a standard format, giving an overview of the studied topic in Introduction, then stating Aims, describing experimental approaches in Methods, presenting findings in Results, discussing them and then giving the necessary Bibliography. The Introduction gives an extensive but not overwhelming outline of the mechanisms of Notch signalling in development and disease, regulation of cellular metabolism and the interplay between signalling pathways, metabolism and growth. It gives even a Notch non-specialist a sufficient background to follow the experimental part. Aims are clearly stated, the Methods section represents a good guide for anyone who would like to repeat any of the presented experiments. I appreciate that in the Results section the author decided to present only the data that she really contributed to the publications instead of simple pasting of the papers. Unfortunately the presentation of the data is at several places much less careful than in the papers and I had to check the papers to find the necessary information. For example, in Fig.13 the orange columns represent the response of mutated Su(H) sites while the figure legend states "blue columns", the way the data were normalized is not clear from the legend, it is not clear what level of significance is represented by the asterisks. In Fig.25, the author says in the text that "…Ex527 and sirtinol increased the acetylation, particularly of the lower Su(H) band", while the data show change in acetylation of only the lower band etc. Overall the thesis suffers from high occurrence of typing errors and lack of editing, which spoils the good impression from the nice experimental work. Editing of the Bibliography section cannot be called anything else than careless.

Questions:
1) In Discussion you mention failure with the FlyFos system you planned to use to test the in vivo response of the Su(H) elements. How plausible is to mutate the Su(H) elements directly

in the Drosophila genome using CRISPR/Cas9? Would that be an alternative approach? Is there any other experimental approach that could be used instead of the FlyFos system?
2) In Fig.14C, do you have any explanation for the delay of the Impl3 peak after cycloheximide treatment?
3) In Fig.25 you demonstrate deacetylation of Su(H) by Sirt1. What is your explanation for the observation that application of Sirt1 activators affected both Su(H) forms while application of Sirt1 inhibitors affected only the faster migrating isoform? Why was it necessary to apply two different inhibitors or activators of Sirt1 in this experiment?
4) In Fig.3C of the Horvath et al. paper, m8 gene behaves differently in the Notch+SRT1720+2DG condition than the rest of the genes. Do you have any explanation for that?
5) What would be your experimental approach to find which lysines of Su(H) are deacetylated by Sirt1?

Conclusion: The presented thesis without doubt demonstrates that Věra Slaninová is able to independently conduct experiments, document the results, interpret them, discuss and present them to a wider scientific audience. The choice of *Drosophila* as an experimental model offered an extensive range of experimental approaches, ranging from genetics, biochemistry, bioinformatics to metabolic measurements giving Mgr. Slaninová an excellent background for any future scientific direction she may want to follow. In my opinion, all necessary requirements for this type of work were fulfilled and I recommend the thesis for defence.

Prague, January 9, 2017

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Objet: PhD thesis report for Ms Věra Slaninová, University of South Bohemia

Title of thesis: *The interplay between the Notch signaling pathway and cellular metabolism*

This PhD thesis was supervised by Dr. Alena Krejčí, at the Department of Entomology of the University of South Bohemia, České Budějovice.

Context. Intercellular signalling is a common feature of all metazoans. There are a few evolutionarily conserved signalling pathways which govern a wide range of cellular behaviours such as cell fate determination, motility, adhesion, proliferation... But while cell signalling can instruct the cell about its environment (biochemical composition, presence of other cells, intercellular messages), the response needs to be integrated within the responding cell with other cellular processes, and in particular cellular metabolism. Indeed, the metabolic state of the cell obviously determines and restricts the range of possible behaviours a cell can "afford".
Here Ms Věra Slaninová presents her results concerning the cross regulations between metabolism and the Notch signalling pathway in Drosophila cell lines and imaginal wing disc tissues, as well as in human endothelial cell lines. First she explores how Notch signalling directly controls the expression of several key enzymes during glycolysis and how Notch, through the control of the transcription repressor hairy, controls a general slow-down of the Krebs cycle. This work has led to a publication where Ms Věra Slaninová is first author. In a second part, she investigates if and how cellular metabolism, through metabolites sensors, and in particular Sirt1, could influence the outcome of the Notch signalling pathway. This has led to a paper on Sirt1 function in which Ms Věra Slaninová is third author.
The aims of this PhD: (i) identify the Notch transcriptional targets involved in the regulation of metabolism and to dissect the role of these genes in Notch driven tissues, (ii) test whether changes in NAD/NADH ratio affect Notch signalling, and (iii) investigate the role of the NAD+ sensor Sirt1 on the Notch pathway.
The contributions of Ms Věra Slaninová to each publication are clearly stated.

The Introduction is detailed presenting first the Notch signalling pathway, then the basics of cellular metabolism. The first part on Notch signalling is well presented and illustrated, even though the jumping from one model organism to another makes it sometimes a bit difficult to follow. The part on Notch and diseases could have gained a bit more structure and direction if the syndromes listed had been presented also for their metabolic part when that information is present and/or relevant. Ms Věra Slaninová mentions several times in that Introduction on Notch the existence of a "non-canonical" Notch signalling which has gained popularity with some authors, but which remains rather obscure to me. *I would have appreciated a better presentation of exactly what a non-canonical Notch signalling is, and a presentation in a more critical way of the arguments for it*. In a second part, cellular metabolism is very nicely presented. The level of details shows that Ms Věra Slaninová reached a high level of expertise in this field. However, not being a specialist in metabolism myself, the lack of figure support for this whole part, made it hard to read.

Results are very well presented. It is noteworthy that despite being part of accepted publications, most results are presented in more details and put nicely within the broader context of this PhD.
In a first part Ms Věra Slaninová investigated the impact of Notch signalling on the expression of key metabolic genes. The amount of work done is impressive and of very good quality. First using ChIP data and expression data obtained by other groups or by her lab prior to her joining, she selected a few metabolic genes that had the potential to be regulated by Notch. Following a rigorous pipeline of both transcriptional

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target validation (expression profiling following drug treatments, Notch dependent enhancer studies, in situ hybridization), and functional validation in cell lines and in overgrowing wing discs using RNAi-based knock-down, she provided compelling evidence that Notch directly regulates the expression of the glucose transporter Glut1, of the glycolysis enzyme Hex-A, and of the general tricarboxylic acid (TCA) cycle regulator hairy. These results are important and provide evidence that cells subjected to Notch signalling undergo a metabolic shift favouring glycolysis, and down-playing mitochondrial activity and Krebs cycle activity, in several Drosophila cell lines (S2 hemocytes and D8 muscle progenitors), in Drosophila tissues (over-proliferating wing discs), and also in human endothelial cells HMVEC. *Given the wide range of models tested, I feel that a reflection on the consistent effect of Notch on metabolic genes in very different tissues for which Notch has different outcomes (block of differentiation in D8, proliferation in wing discs, S2?), is missing. Do all the models tested, exhibit increased proliferation after Notch stimulation, which would explain their shift towards glycolysis? Is it expected that Notch would act similarly in other cell types?*

In a second part, Ms Věra Slaninová presents very briefly, her contribution to the study of the influence of the NAD+ sensor Sirt1 on Notch signalling. She was able to show that Sirt1 potentiates the activation of a Notch reporter by Nicd in cultured S2 Drosophila cells, and that the Notch pathway specific terminal transcription factor Su(H) is de-acetylated by Sirt1. It appears however that the in-vivo effects of Sirt1 are modest and the direct link between Su(H) acetylation and Notch pathway activity could not be definitively established. *Potential routes to investigate could have been proposed here in order to better understand the role of acetylation and NAD+ sensing on the Notch pathway.*

Finally, in a third part, Ms Věra Slaninová presents unpublished results towards understanding the role of the NAD+/NADH ratio on Notch signalling. This last part appears very preliminary and the lack of robust NAD+ or NADH reporters in-vivo make a lot of the results hard to interpret. *Nevertheless, I am wondering how one can explain that only some of the bona-fide E(spl) genes are affected in her experiments tampering with NAD+/NADH ratio? Are these the same in other cell lines?*

The **Discussion** is relatively short. Most of the technical and interpretation caveats are openly discussed, demonstrating a level of self-criticism and reflection that is at the basis of new hypothesis and further research. Personally, I would have liked if the discussion had been slightly longer, in particular *repositioning the interplay seen here between Notch and glycolysis/TCA cycle, within a broader perspective, such as the interplay of other signalling pathways with metabolism and their described consequences, or between Notch and other aspects of the cell metabolism, such as glutamin or lipid metabolisms*.

In summary, Ms Věra Slaninová has produced a huge amount of work combining very different approaches. The experimental work presented and discussed is very nice and interesting, bringing new evidence of the intricate relations between Notch signalling and metabolism. I, therefore, fully **recommend this thesis to be defended**.

Dr. Alexandre DJIANE
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