Ph.D. Thesis Review

The thesis of Mgr. Jarmila Princová „New interconnections between lipid metabolism and chromatin regulation" aimed, based on its preliminary scope as published on the online faculty information system, to

- *elucidate how fatty acid synthesis contributes to the regulation of chromatin structure, maintenance of genome integrity, and proper chromosome segregation*
- *afford unique insight into novel interconnections between two important cellular processes with fundamental relevance for carcinogenesis: cellular metabolism and chromatin regulation*

To achieve this goal, the author's work focused on two sets of questions: how the addition of ammonia contributes to the alleviation of mitotic defect in specific mutants related to lipid metabolism, and how impaired fatty acid metabolism is coupled to specific chromatin modifications and resistance to oxidative stress.

Form and elaboration

The thesis is written in a clear language with minimal frequency of minor formal omissions. For example, a word is probably missing in one of introductory sentences (p. 1: "*Although a mere change in lipid composition of cells and especially of biological membranes has an effect on stress resistance [3,4], the regulation is often more complex* and *can be achieved, for example, by gene expression changes based on altered lipid metabolism [6].*") and several highly specialized abbreviations are used but not explained in the text (p. 10: the author explains SAM, but not SAH, p. 13: H3K9, p. 52: 3-MB-PP1), leaving the reader fully reliant on the List of abbreviations at the beginning of the thesis. By the way, FA is in fact defined in the text, but only at the 13th occurrence of "fatty acid".

The text is arranged in a standard graphic layout and accompanied by illustrative figures. However, I have several objections to the integration of the graphic layout and the factual content of the text. In general, when reading the thesis I had the feeling that it was written in a hurry and with minimal effort, which unfortunately had a negative impact on the final form of the work. Although I am convinced that the topic of the work is quite close to my own specialization, I was forced to go back repeatedly while reading the thesis, to search in the text and to consult numerous external sources. It was virtually impossible for me to read the work continuously as a compact body with a clear message.

First of all, as mentioned above, specific objectives that were to be achieved are missing in the text of the thesis. Fortunately, I have an access to the online faculty information system, so I could find these.

The structure of the Introduction chapter is highly asymmetrical and disjointed. While section 1.1 is less than 2 pages long, section 1.2 contains 22 pages. This makes section 1.1 somehow stand out from the otherwise compact rest of the chapter, which certainly does not correspond to its significance. In an introductory paragraph to the part 1.2 (p. 3), the author says: "*Disturbances or changes to the lipid metabolism have been shown to affect the oxidative stress response using several molecular mechanisms including regulation of lipid droplets and stress-gene induction by regulating chromatin modifications, which will be covered in this chapter.*" It is not clear from the text why these particular two mechanisms were chosen. A short introduction to a broader context is really missing here. It would have been sufficient, especially after the illustrative initial examples of the link between lipid metabolism and oxidative stress leading to serious diseases mentioned by the author in section 1.1, that perhaps the most prominent/best studied manifestation here is the imbalance in fatty acid levels. That excess fatty acid removal is mainly through the formation of triacylglycerols (hence lipid droplets in 1.2.1) or the acyl-CoA beta-oxidation in the mitochondrial matrix. By the way,

why is beta-oxidation not given its own section? After all, the author needs it when she explains the main sources of acetyl-coenzyme A later on... (p. 12)? Another possible fate of excess FFA, which leads to the formation of toxic lipids such as lysophospholipids, ceramides or diacylglycerols, is not mentioned at all. OK, it is usually a minor one. A few words about the subcellular compartmentalization of lipid metabolism (including biosynthesis, transport and degradation) would not hurt either: arguments involving ER stress or mitochondrial dysfunction, for example, would be immediately easier and self-explanatory. Last but not least, why is neither the description of the transcription factor Cbf11 nor the MAP kinase Sty1 included in the Introduction? Both seem to be central to the published data.

The Results section represents a mixture of the results already published, which the author presents in the form of reprints of respective scientific articles (pars 3.1, 3.2 and 3.4), and a short summary of the unpublished results (3.3 and 3.5). Personally, I very much missed such a summary of the published data as well. Instead I had to read entire publications, in each case including a separate introduction and discussion, and then try to reconnect with the (largely missing) story of the thesis. As a consequence the whole thesis gave me a very fragmented impression.

The significance of section 3.6, which provides only a reference to an unpublished manuscript, is elusive. It is unfortunate if we only learn that the data obtained elsewhere are important for the discussion but have no opportunity to see them. This also directly contradicts the author's statement in the preface to Chapter 3: *"My unpublished findings regarding lipid metabolism and its impact on chromatin biology and stress response will be presented in this chapter together with my published research papers obtained during my PhD studies."* The findings mentioned in 3.6, whether we consider them published or not, are not presented at all.

Minor comments:

- There is an error in the numbering of subchapters in the Introduction chapter. Namely section 1.3.1 should probably be labelled 1.2.2.
- English version of the Abstract ends with the sentence: *"Overall, we have elucidated how fatty acid synthesis contributes to the regulation of chromatin modifications, maintenance of genome integrity, and oxidative stress resistance."* Compare it to the translation of the Czech version: *"This work has contributed to elucidating how fatty acid synthesis regulates chromatin modifications, maintenance of genome integrity and cellular resistance to oxidative stress."*

Science

The thesis includes three publications published in peer-reviewed impacted journals. Two of them (Publication #1 and Publication #3) are original research articles, one (Publication #2) is a methodological article/published protocol. The author of the thesis is the first author of Publication #2 and Publication #3. Publication #3 has been published in a prestigious journal *PLoS Genetics*. No data from the preprint mentioned in the part 3.6 of the Results was included in the thesis and therefore could not be evaluated.

Without any doubts, the results of author's experimental work represent (in the context of the published articles) an important and valuable contribution to the understanding of how lipid metabolism on the one hand, and gene expression and chromatin integrity on the other can be interconnected. Personally, I would not count the removal of the 'cut' phenotype among the modes of chromatin regulation and would be satisfied with the explanation that it is due to insufficient biosynthesis of phospholipids that are missing in the construction of the new nuclear envelope. However, this does not change the fact that the author's findings are interesting and potentially

important. And the finding that yeast cells defective in fatty acid synthesis show increased expression of a subset of stress-responsive genes, which is associated with increased histone acetylation in the corresponding gene promoters and leads to increased cellular resistance to oxidative stress, is simply impressive. In Publication #3, the author not only identified a specific link between lipid metabolism and chromatin regulation, but also described it in molecular detail.

Conclusions

Formally, I do not consider this thesis a win. I am convinced that if the author had paid more attention to its elaboration, the result would have been much better.

In terms of originality, the author's ability to conduct research and achieve scientific results, as well as the author's contribution to published studies, the thesis meets the requirements for a creative scientific work. For this reason in particular, I believe that the author should be awarded the degree of PhD.

Prague, Apr 25, 2023

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Questions

(answering these questions will not affect my evaluation of the thesis. It should only serve as a basis for discussion after the presentation of the candidate)

1. In Publication #1, you report an increase in lipid droplet content, indicative of increased availability of phospholipids, in exponentially growing WT cells following the NH_4Cl treatment. In the Discussion, you only speculate on the possible mechanism behind this phenomenon. Have you considered that NH_4^+ supply can be a bottleneck of ethanolamine/choline biosynthesis, which is a prerequisite for the production of the most abundant phospholipid species?
2. Have you or somebody else tested to what extent the lipidome of the 'cut' mutant cells changes when switching from EMM to YES media and/or when NH_4Cl is added?
3. Based on your findings, could you comment on the potential application of small metabolites, e.g. in alleviating insulin resistance in type 2 diabetes or other manifestations of prolonged inflammation associated with excessive ROS production?
4. The absence of the phosphatidylinositol-specific phospholipase C Plc1 in budding yeast has been found to lead to depletion of the acetyl-CoA pool and consequently to overall histone hypoacetylation (Galdieri et al., *J Biol Chem* 288(39):27986-98, 2013). And the *plc1*Δ mutant showed reduced resistance to oxidative stress induced by 0.8 mM H_2O_2 (Cooper et al., *Mol Cell Biol* 19(5):3338-48, 1999). In Publication #3, you mention the opposite effect also observed in *S. cerevisiae*, in cells lacking the gene encoding the acetyl-CoA carboxylase homologous to Cut6, in which acetyl CoA levels are elevated (Galdieri and Vancura, 2012; cited in the thesis). Do you think that the effects observed in Publication #3 are more gene-

specific compared to the above examples and do not just reflect variation in acetyl CoA content in selected mutants?