

Ph.D. Thesis Review

The thesis of Mgr. Veronika Biddle „Analysis of functional interactions of phospholipids in the cell nucleus” addressed the following questions:

1. Which tools are applicable for detection of nuclear phosphoinositides?
2. What is the distribution of phosphoinositides within the nucleus, in particular PI(4)P and PI(4,5)P₂?
3. What are the binding partners of PI(4)P and PI(4,5)P₂ in the cell nucleus?
4. How is PI(4,5)P₂ involved in DNA transcription?
5. What is the function of PI(4)P in the cell nucleus?

(p. 21 of the thesis)

To answer these, a number of sophisticated experiments were performed, in which the author of the thesis significantly participated. The experimental approaches included modern methods of fluorescence and electron microscopy, molecular biology, protein expression, purification and analysis or lipid mass spectrometry, and they brought many original and valuable results.

Form and elaboration

The thesis is written in good English containing minimal frequency of typos. Text is subdivided into standard chapters, using four levels of decimal classification. In fact, three would be probably enough, as the only use of the fourth level is in the part 1.2.1.1, which is the only subsection of 1.2.1. Similarly, the list of abbreviations could be shorter, if items mentioned only once in the text (PI3KC2 β , TBP, WT1 and others) were not abbreviated. General discussion of selected results of three published original papers and one manuscript is concise and insightful.

Chapter 1 is well composed and introduces the reader in a comprehensible form to the field of interest. In contrast to the title of the thesis, the Introduction describes solely the nuclear phosphoinositides and does not contain any information concerning other species of nuclear lipids. Apparently, the final thesis is more narrowly focused than was its initial idea. This is correct of course, however the literature showing the necessary context does exist and should be included, at least for the basic orientation of the reader. For example, nuclear lipid droplets are only briefly mentioned in the Discussion (p. 26). They however contain a significant part of the nuclear lipids. When comparing them to the newly described NLIs, nLDs are unknown to the reader. (Minor point: the lipid composition of the hydrophobic core of nLDs is not described correctly (*“TAGs and cholesterol”*, p. 26). According to the cited reference [81], it should rather be *“cholesterol esters, cholesterol and TAGs”*).

All the information contained in Chapter 8 (Attachments) can also be found in Chapter 3 (Research papers). At both locations, the author brings the list of publications, which are attached to the thesis. At none of these locations, however, these publications were connected with the list of references (Chapter 7), in which the three already published papers of the author are also listed as [69, 72, 73]. Then, the sentences which cite these references and miss an explicit notion of the authorship (*“we observed”*, *“we found”*) are confusing to the reader. For example, reading the sequence: *“We showed that PI(4)P specifically localises to nuclear lamina, nucleolus, nuclear speckles and small nucleoplasmic foci [72]. PI(4,5)P₂ localises to nucleolus, nuclear speckles and small nucleoplasmic foci as well [73].”* (p. 24) the reader does not know, whether the two sentences compare two different original findings of the author (parts of the thesis), or whether the second sentence just cites the finding (of somebody else, maybe) which has been published earlier. In this particular example, it was the former case. But in the same paragraph, one can find yet another case: *“Through the use of transmission electron microscopy, we detected PI(4)P predominantly in dense fibrillar component (DFC) and smaller portion also localises to a granular component (GC) of nucleoli [72]. On the contrary, nucleolar PI(4,5)P₂ signal*

is denser in fibrillar centre (FC) and DFC [74]." Here the second sentence refers to the results of a study which has not been co-authored by the author of this thesis. This makes extremely difficult to distinguish between the discussion of the author's original findings and references to the work of others. The confusion could be easily prevented if short result summary was included in the Chapter 3.

Science

The most important question of whether the thesis research generated significant new knowledge in a scientific area, has to be answered positively. Experimental results of the author discussed in the thesis have been published in three peer-reviewed publications. V. Biddle is the first author of one of these. Another co-authored original paper has been prepared for publication and presented in the thesis as a submission-ready manuscript. In other words, most of the results of the thesis have already been subjected to an unbiased review and found valuable and worth of publishing. Based on the list in Chapter 3, the participation of V. Biddle on these results was significant.

Conclusions

Concerning the originality, the author's ability to perform research and to achieve scientific results, as well as the author's contribution to the published studies, the thesis satisfies the conditions of a creative scientific work. Based on these reasons, I believe the author should be awarded the PhD degree.

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Jan Malinsky
Institute of Experimental Medicine CAS
Víteňská 1083
142 20 Praha 4

Questions

1. How do the PIP localization tools discussed in part 4.1 of the thesis (and Publication 1, [69]) interfere with the nuclear function of the lipids? Could it be, for example, that nucleoplasmic foci enriched with the label are in fact induced rather than detected by the label expression in the observed system? (I am quite sure this question had to be answered during the review process of the Publication 1, so please, just summarize the controls which have been performed with this respect.)
2. I googled the term "Nuclear lipid islet", but with the exception of the information related to the Publication 2 (Sobol et al., 2018, [73]) I could not find any notion about this structure. I have read carefully Publication 2, with a special emphasis on Fig. 2, showing the results related to the morphology and lipid composition of NLIs. As I understand it, the triacylglycerols are not a major lipid component of the structure (Fig. 2H). You documented there that besides TAGs, NLIs contain also PC, PE, SM, ceramide and cholesterol. Do you have any model of the arrangement of these lipids within the structure of a diameter 40 – 100 nm?