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Review of Mgr. Radek Šindelka's Ph.D. thesis Gene expression in early development of *Xenopus laevis*

The primary objectives of Ph.D. studies of Mgr. Radek Šindelka summarized in the submitted Ph.D. thesis were:

- application of quantitative real-time reverse-transcription polymerase chain reaction (qPCR) to determine profiles of transcripts critical for early stages of *Xenopus* development, a model organism used in the laboratory of Radek's mentor and his Ph.D. thesis supervisor Prof. Jiří Jonák, and
- attempt to elucidate their biological role in these processes.

Clearly, Radek has been very successful in the first part; so much so that this success obviously led to a thematic shift in later period of his Ph.D. studies. Although this shift clearly reduced a biological component of Radek's Ph.D. project, in my view, a rather technology development track, on which it stayed, has ultimately been perhaps even more fruitful than the second part of the original plan but certainly not less challenging. During this period Radek brought together his excellent knowledge of technology, in this case insight into implications of combination of sampling by cryostat with assaying obtained specimens with qPCR, and biology. Here he recognized an opportunity, which unique features of *Xenopus* egg stemming from its size, offers and use it to conceive the idea of a truly novel application - qPCR tomography. This new method undoubtedly represents a valuable addition to a toolbox of techniques used in today's molecular biology. Its practical impact lies, among other things, in its ability to detect transcripts in finer temporal/spatial resolution, a desired feature since it is clear that particularly nowadays it is not possible to start interpreting biology without detailed knowledge of behaviour *s.l.* of individual components of the system or model under study.

qPCR, the method Radek exploited the most during his Ph.D. studies, has a misleading reputation of being a very simple technique. However, this is not completely true and this misconception leads to frequent underestimation of its inherent problems and limitations. On the contrary, in its entirety qPCR is a multistep method with many caveats and subtleties, which – if not assessed and dealt with conscientiously – can have considerable negative impact on the outcome of the project. I appreciate that Radek addressed one of the most critical and burning components of qPCR, namely normalization of its results, in very early stages of his Ph.D. studies. Armed with better understanding he could perform much more competently later and could also implement more demanding approaches to qPCR data analysis. Manifestly, Radek's results show

that he has mastered this technique, and he could build upon these skills when he embarked on to more challenging projects, particularly in development of qPCR tomography.

Radek Šindelka's Ph.D. thesis is presented in the form of relatively brief (20 pages including references) introduction followed by a set of 6 articles with Radek being their principal author or co-author. From this set, 3 articles report on the primary experimental data and 3 are the review articles. In my view, the journals where Radek's articles with experimental data were published belong to the solid standard among scientific peer-reviewed journals and definitely represent the upper level of journals that publish techniques and methods orientated articles. The fact that these articles were accepted for publication there definitely demonstrates their worthiness for scientific community. As a matter of fact, *Nucleic Acids Research*, where the article on qPCR tomography with Radek as the first author has been published, is one of the leading open access journals printing articles dealing with novel methodologies.

Comparing Radek's achievements as an aspiring young scientist at the laboratory bench described in his Ph.D. thesis and quality of it Introduction chapter I need to state that he has fared much better at the former part. I understand that the latter part is meant to put the research articles presented in the second part of Ph.D. thesis in to the proper context but it should be able to exist independently. Unfortunately, this is not the case and I see it as an unnecessary flaw. In addition, my impression is that it was written under extreme time stress what caused that it did not get proper author's attention during preparation, editing and proof reading. Was there any particular reason for this haste? There is a striking difference between quality of the text of the thesis' Introduction and of the attached articles; it should get the same attention as the articles published in scientific press. I do not know if there is any upper limit of the page number for this part but, generally, it is written in the regrettable style that prefers brevity over clarity to detriment of the whole text as for example, in the chapter 1.4 (Quantitative real-time RT-PCR). It certainly makes no sense to repeat paragraphs from the very same text that is part of the articles section of the thesis (a rather exhaustive and authoritative review of qPCR by Kubista *et al.* with Radek among co-authors; Paper II) but I believe that here a better work could have been done. Several unnecessary simplifications together with inconsistencies in references to published literature, which definitely do not contribute to clarity of the text, and presence of several typographical errors are also annoying and improvement would be desired.

Overall assessment: despite of my objections concerning mainly the Ph.D. thesis' Introduction, which I consider minor, I believe that Mgr. Radek Šindelka has achieved goals set at the beginning of his Ph.D. studies and his Ph.D. thesis meets current formal requirements for such a document. I deem it suitable for defense and recommend accepting it. Assuming his success at this act I am convinced that Mgr. Radek Šindelka has shown qualities required from a successful Ph.D. student and with that has fulfilled all conditions demanded for completion of Ph.D. studies.

Questions for defense:

- It is stated that there is a specific distribution of mRNAs already before fertilization of a *Xenopus* oocyte. How is this distribution maintained?

- One of the consequences of the situation that transcription of zygotic genes is silenced till a fertilized oocyte divides into the early gastrula stage (~4000 daughter cells within approximately 6 hours) is the fact that maternal RNA and proteins must be allocated among them. Is there anything known about the "key" according to which this distribution takes place and how it is driven? How does transcriptional state of individual daughter cells after division look like?
- Small non-coding RNAs such as microRNAs have been implicated in the silencing process also in the presented Ph.D. thesis. Are any of the trancripts detected along the A-V axis identified as targets of microRNAs involved in regulation of developmental processes?
- Can any effect of stimulation of *X. laevis* eggs by hCG (human chorionic gonadotrophin) before IVF (*in vitro* fertilization) *per se* on intracellular mRNA gradients be excluded?
- I know that group of Prof. Jiří Jonák tested siRNA approach to knock down some of *Xenopus* transcripts. This approach is often used to see if there are any phenotypic changes caused by this "loss-of-function" mutation. Has this been applied to any transcript with identified A-V gradient and, if yes, what was the result?
- Has the assumption that total RNA is distributed homogenously within the cell been tested and how?
- What other systems are amenable to exploration by qPCR tomography?
- Would use of new-generation sequencing technology, for example, be appropriate approach to deepen our knowledge about transcripts distribution before/after fertilization but perhaps more importantly, would it contribute to better understanding of the whole process?

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