

EVALUATION OF THE PH.D. THESIS OF

ANATOLY A. FILIMONENKO

on the

**DEVELOPMENT OF ULTRASTRUCTURAL METHODS AND THEIR APPLICATION IN STUDIES ON THE CELL NUCLEUS**

by Christian LANCTÔT, *oponent*, June 2014

*SUMMARY*

This work consisted of five subprojects. Three were devoted to the development and/or refinement of methods and two addressed biological questions. The aim of the first of the methodological projects was the implementation of an image analysis algorithm to assess the extent of co-localization of labels in electron microscopy images. The aim of the second was the development and validation of novel nanoparticles as labels in electron microscopy. The last methodological project was about the optimization of the cryosubstitution and embedding steps that are performed after high-pressure freezing of samples for electron microscopy. The two projects that were more biologically-oriented aimed at investigating the sites of DNA replication in the nucleus and the effect of transcriptional activation on the distribution of nuclear actin and myosin I, both at the ultrastructural level.

*STRENGTHS*

The candidate is undoubtedly highly skilled in all aspects of the preparation, immunolabeling and imaging of electron microscopy samples. His skills were successfully applied to a range of questions and problems. In particular, the use of nanoparticles of different shapes and sizes to reveal multiple targets under the electron microscope is a clever and useful addition to the existing labeling techniques. Similarly, the systematic localization of numerous proteins in replication bodies vs. replication foci allows a better understanding of the relationships that exist between these two dynamic structures. The thesis includes 8 peer-reviewed articles co-authored by the candidate (but, it should be noted, only 3 as first author, all published more than 7 years ago).

*WEAKNESSES*

The weaknesses of this dissertation are numerous. Before pointing the major ones out, I would like to mention that it is difficult for me to understand why the candidate did not

complete his Ph.D. studies much sooner. From his curriculum vitae and publication list, I gather that he has been working in the same laboratory since the year 1996. Did the candidate's Ph.D project really last 18 years and more? If yes, then I would express serious doubts about his ability to carry out a research project in an efficient and timely fashion.

The first weakness of this work lies in my opinion in its format. The introduction is only 6-page long; the discussion, at 9 pages, is barely longer. Considering the large amount of literature that exists on the subject, I think that electron microscopy methods could have been introduced and discussed much more extensively. Details of the chemical reactions and changes taking place during the different steps of the procedures could have been included. Results from the literature could have been explicated and compared, and not only cited. In addition, some passages included in these sections are taken from the Research papers. For instance, p.107 is repeated *verbatim* on p. 123 (this copying/pasting is a mark of poor scholarship). Overall, I am left with the clear impression that the dissertation is little more than a collection of already published papers. If the thesis is submitted as such (and I suppose it may well be), then I will note again that the candidate did not manage to get any paper in his thesis as first author since 2006.

The second weakness is the lack of a unifying theme. Electron microscopy methods are used throughout the work, but since they are varied in their design and in their application, I did not get a sense that the dissertation was presenting an in-depth analysis of the results that were obtained, or that these results were linked with each other. Again, a collection of papers that, apart from the use of electron microscopy, seem to have little in common.

The third weakness, and in my opinion the most important one, is the relative absence of critical thinking that is displayed in this work. This weakness is no doubt related to the choice the candidate made to have his dissertation consist mainly of published papers, since these must often present a polished aspect in order to be published. In this context, it is difficult for me to judge the ability of the candidate to criticize his own results, which should be the distinctive feature of a finishing Ph.D. student.

## QUESTIONS

1. The ultrastructure of DNA replication sites in the cell nucleus is studied in the *J. Struct. Biol.* (2004) and *Histochem. Cell Biol.* (2005) papers.

*What evidence, or lack thereof, is there that all of the replication bodies and foci have the same structure at any given time in S phase? How does this structure evolve during S phase? Were you able to observe the "domino effect" of DNA replication by electron microscopy? If not, which experiment could be performed to observe the dynamics of this process.*

2. The work on the ultrastructure of DNA replication sites was performed on saponin-permeabilized cells.

*Why did you choose to image permeabilized cells? What are the potential artefacts that are introduced by the procedure that was used? Compare your results with those obtained on intact cells.*

3. A comparison of samples embedded in various acrylic resins showed only slight differences in the efficiency of immunolabeling (*manuscript*)

*Which proportion of epitopes are indeed labeled in these experiments? Are there epitopes that would be chemically incompatible with the use of acrylic resins?*