



## Opponent's review

### PhD thesis of Cecilia Aquino Perez, M. Sc.

**Title:** "New molecular mechanisms involved in cell cycle control"

#### Topicality of the research:

During her PhD studies, Cecilia Aquino Perez aimed on understanding of basic molecular mechanisms controlling cell cycle progression. She concentrated on characterization of cellular response to various stress stimuli, including DNA damage, osmotic shock, hypoxia and irradiation, and the role of PLK3 in these processes. She also searched for genes differentially expressed during cell cycle, with possible function in cell cycle regulation. Among these, she and her colleagues focused on FAM11A and described a function of this protein in mitosis. In the same study, they uncovered the regulatory role of casein kinase I in controlling FAM11A localization and function. Regulation of cell cycle is still not fully understood, and many details, which might have important role in controlling cell cycle events, are yet waiting to be discovered. To understand the cell cycle regulatory mechanisms is very important, since uncontrolled division and loss of checkpoint control are characteristic features of cancer cells. Until now, research focused on cell cycle regulation, not only aid to our understanding of basic aspects of this activity typical for living cells, but it also equipped researchers and medical specialist with multiple substances and inhibitors, which are being used in therapy of cancer. It is therefore vital to continue in such research and this is why Cecilia's work is of high importance.

#### Introduction of the problem and relevant literature:

The introduction section of the thesis covers comprehensively recent literature and main hypotheses concerning cell cycle progression, and various aspects of control mechanisms of cell cycle in somatic cells, are discussed here. Cecilia also described the role of main cell cycle regulators, such as kinases, cyclins, CDKs and phosphatases, and especially those, which are functionally or mechanistically relevant to the topic of her PhD. My minor criticism here would be the length of this section, as it is 48 pages long. It should be perhaps more focused on the subject.

#### Methods and techniques used in the study:

To obtain her results, Cecilia used a large variety of molecular biology and cell biology techniques, including immunodetection, molecular cloning, cell culture, gene targeting using CRISPR-Cas9 approach, knockdown by RNAi, flow cytometry and cell sorting, qPCR, cell cycle synchronization, time lapse microscopy, immunoprecipitation and mass spectrometry, and many others. From this overview it is clear that Cecilia is familiar with large cohort of laboratory techniques. This will be a significant benefit for her future career in science, if she chooses this path.

#### Main objectives:

The main objectives of her studies were characterization of the function of PLK3 kinase in DNA damage and stress response, and identification of genes differently expressed during cycle stages, with potential impact on cell cycle regulation.

**Characterization of the results and achievements:**

The results of Cecilia's work were published in two scientific journals, namely in Cells and EMBO Reports, in both instances she is the first author. Both are very good journals with IF 6.7 and 8.8 respectively. In the first paper published in Cells, Cecilia and her colleagues discovered that PLK3 is not required for stress response in human cells, as deleting the gene showed no impact on cellular response, which was in contrast to previously published studies. In this study, they also identified a novel interacting partner of PLK3, PP6, which is involved in regulation of PLK3 by dephosphorylating its Thr219. In her more recent paper in EMBO reports, Cecilia and her co-workers focused on transcripts differentially expressed between cells in G1 and G2 phase of the cell cycle. Important aspect of this study was that the untransformed human cells used for identification of differentially expressed genes were not synchronized and instead, cell sorting, based on FP reporters, was used to obtain cells in desired cell cycle stage. Among transcripts which were known to be differentially expressed between G1 and G2, they identified FAM110A, with higher expression in G2. They showed that this protein is required for alignment of chromosomes, for control of metaphase to anaphase transition and for the length of mitosis, and also for spindle positioning. They also discovered that casein kinase 1, which was found in complex with FAM110A, is required for its phosphorylation and full functionality. The published results are of high scientific standards and brought novel and important information about the cell cycle control.

**Questions for the candidate:**

1. In the manuscript published in Cells, Cecilia and her co-workers excluded the requirements of PLK3 for stress checkpoint in human transformed cells and suggested that it is not essential for this type of response in human. Is it possible that PLK3 might be required for the stress response in untransformed cells?
2. For the second manuscript, authors used cell sorting instead of synchronization to obtain cell populations in different cell cycle stages. Are there any data showing that this procedure is in fact less stressful than the synchronization?
3. Depletion of FAM110A caused cell cycle arrest with chromosome alignment defects. Was any experimental work done to address the nature of this arrest in terms of SAC activity and APC/C activation?

**Conclusion and recommendation:**

In my opinion Cecilia Aquino Perez demonstrated her ability to carry complex experimental work, critical and independent thinking and prepared well written and informative thesis. The experimental work involved sizeable number of techniques, experiments were well planned and executed. With collaboration of her colleagues the results were published in prestigious journals and they will certainly have impact on scientific community. Therefore, I recommend proposed thesis for defence and the candidate for receiving PhD degree.

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VÝZKUMNÝ ÚSTAV  
VETERINÁRNÍHO  
LÉKAŘSTVÍ, v. v. i.

Výzkumný ústav  
veterinárního lékařství, v. v. i.,  
Veterinary Research Institute  
Hudcova 296/70  
621 00 Brno

Sekretariát:  
+420 5 3333 2501

Operátor:  
+420 5 3333 1111

[vri@vri.cz](mailto:vri@vri.cz)

[www.vri.cz](http://www.vri.cz)

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Martin Anger, DVM, PhD.

Director of OU CEITEC VRI

Head of department of Genetics and reproductive  
biotechnologies

CEITEC - Veterinary Research Institute

Hudcova 70, 621 00 Brno, Czech Republic

Tel: +420 533 331 411, e-mail: [anger@vri.cz](mailto:anger@vri.cz)

