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Biotechnology and Biomedicine Centre of the Academy
of Sciences and Charles University in Vestec



Study programme Developmental and Cell Biology

Faculty of Science, Charles University

Student Affairs Division,

Albertov 6, 128 00 Praha 2

Marcus Braun, Ph.D.
Laboratory of Structural
Proteins and their Complexes
BTÚ AV ČR, BIOCEV
Průmyslová 595, 25250 Vestec

325-873-772

marcus.braun@ibt.cas.cz

Praha, 17 May 2022

Opinion on the dissertation

Thesis author: **Cecilia Aquino**

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

Progression through the cell cycle is set by protein kinases, which control the transitions through the different phases of cell division. Some kinases are activated in response to cellular stress, activating cell cycle checkpoints, preventing cells from dividing. Mechanistically, one immediate way for a kinase to control cell division is to directly interfere with constituents of the cytoskeleton, for example, by modulating, through the addition of phosphate groups, the affinities of proteins crosslinking actin filaments - recently shown to be involved in cell division - with the microtubules.

In this bipartite thesis, the author first shows that Polo-like kinase 3 is irresponsive to cellular stress – a discrepancy with previous reports, which the author, after testing herself the antibodies used in the published studies, attributes to unspecificity of binding of all but one antibody used. Protein phosphatase 6, identified as interacting partner of through mass spectrometry, dephosphorylates the conserved Thr219, which, unlike reported for other family members, does, however, not modulate the enzymatic activity of the kinase. In the second part, through synchronization-free expression profiling, among 701 differentially expressed transcripts (G1 vs. G2), the author finds FAM110A (Family with sequence similarity 110 member A) - previously described to localize to centrosomes and spindle poles and to display cell cycle dependent expression - to impair chromosomal alignment, delay metaphase-to-anaphase transition, and affect spindle positioning. By mass spectrometry and immuno-precipitation, the author finds FAM110A binding to microtubules, actin and forming a complex with Casein Kinase I, which phosphorylates its C-terminal domain during mitosis. Wild-type FAM110A, but not the FAM110A-S252-S255A mutant deficient in CK1 phosphorylation, rescues from these effects after depletion of FAM110A, suggesting that that CK1 regulates chromosomal alignment by phosphorylating FAM110A, promoting its interaction with mitotic spindle.





This thesis presents a large volume of excellent and timely work, testing published results (on Polo-like kinase 3), screening for new proteins, in-depth describing one specific target of cell-cycle dependent kinases (FAM110A), and in doing so, touching on a topic currently under extensive debate in the cytoskeleton field, namely the importance of microtubule-actin direct, mechanical crosstalk. The work thus provides valuable insight into cell cycle progression, directly suggesting a follow-up project on the mechanics filament crosslinking and its phosphorylation dependent regulation. The presentation of the work is of a high quality and the text is clearly written. Overall, the presented work demonstrates the author's insight into the subject and his capability of independent scientific work. It is thus my pleasure to recommend this thesis by Cecilia Aquino for defense.

Questions

- 1) How common do you think it is that commercially available antibodies are unspecific? Do you think the unspecific binding, or non-recognition, is assay or cell-type specific, or would you think that the antibodies you tested just don't bind to PLK3 at all, no matter the conditions? How common to you think is it that false-positive results based on unspecific antibodies are published? Reading the literature, do you see people commonly present sufficient control experiments or did you become doubtful about many published results after your experience with PKL3?
- 2) During anaphase and telophase, but not during prophase and metaphase, FAM110A (in Fig. 27) seems to be localized not exactly at the spindle poles (no exact overlap with the γ -tubulin signal), but more inwards towards the spindle. Do you see this in other cells too, or is this apparent just in the example presented? Do you think you can localize the signal with enough precision to make a statement about this, and importantly, can you think of any technical explanation for the shifted positions of the signal, which would suggest it to be an artifact? Otherwise, the images suggest that FAM110A shifts in position depending in the stage of the cell cycle, hinting towards possible mechanical roles specific to anaphase and telophase. Would you agree?
- 3) The finding that FAM110A might crosslink microtubules and actin is intriguing. Can you please further hypothesize on the possible mechanics of the interaction, the CK1 phosphorylation and the function during cell division.
- 4) How conserved is FAM110A? It seems to be present in other mammals; is there any evidence of its presence in other species. And if not, are there related proteins within the human genome, possibly fulfilling similar functions, that might be conserved across species?

Prague, 17.05.2022

Marcus Braun