

Cecilia Aquino Perez, M. Sc.

Doctoral thesis abstract

In this doctoral thesis we aimed to find and study novel mechanisms regulating cell cycle phase transitions in non-stressed conditions and in context of the cell response to various types of stress. First, we focused on studying Polo-like kinase 3 that has previously been implicated in activation of the cell cycle checkpoint after DNA damage. For this, we employed CRISPR/Cas9-mediated gene editing to knock-out PLK3 in RPE cells while in parallel performing RNA interference assays and submitting the cells to different types of stress. The main observation was that in both systems PLK3 was dispensable for response to DNA damage, hypoxia and osmotic stress. Through mass spectrometry analysis of purified EGFP-PLK3 we identified PP6 and its regulatory subunits PPP6R1 and PPP6R3 as novel PLK3 interactors. We observed that PLK3 is phosphorylated in its conserved residue Thr-219 and that PP6 depletion boosted PLK3 phosphorylation status but did not affect its kinase activity. The possible regulation of PLK3 through PP6 is interesting and its biological relevance will be addressed by future research. Next, we performed a transcriptomic analysis in human RPE-FUCCI cells aiming to identify new regulators of the cell cycle. We selected Family with sequence similarity 110 member A (FAM110A) for further characterization as the protein was highly expressed in G2 cells and localized to the mitotic spindle and spindle poles throughout mitosis. Through siRNA-mediated depletion of FAM110A we observed an impairment in chromosomal congression, delayed Metaphase-to-Anaphase transition and misorientation of the mitotic spindle. By implementing a mass spectrometry analysis of purified mitotic FAM110A we identified the CK1 family isoforms CK1 ϵ and CK1 δ , alongside with the cytoskeletal proteins tubulin, actin, α/β -catenin and α -actinin as potential interactors. Through *in vivo* and *in vitro* assays, we showed that CK1 δ interacted with FAM110A through its C-terminal domain. In addition, CK1 phosphorylated Ser-252-255 residues, which proved to be necessary for FAM110A's binding to tubulin and for chromosomal alignment during metaphase. Following with other cytoskeletal interactors, FAM110A interaction with actin proved to be relevant for chromosomal congression and during actin dynamics during mitosis. These observations pave an interesting path for future studies over FAM110A potential cytoskeletal cross-talk regulation roles during mitosis.