Abstract

Self-renewing tissues require a balance between proliferation and differentiation in order to maintain homeostasis. One hypothesis is that spindle positioning regulates this balance through equal or unequal distributions of cell fate determinants within the daughter cells, while also physically positioning the daughter cells in distinct fate domains. However, the mechanism of this regulation is not entirely clear. The Niessen laboratory previously identified Nuclear mitotic apparatus protein 1 (NuMA) S1221 as a target of aPKC using an unbiased phosphoproteomic screen, and demonstrated that the human equivalent phosphomimetic mutant NuMA-S1225D (NuMA-SD) is able to rescue spindle orientation, LGN localization, and daughter cell spreading in the absence of aPKC. Additionally, a pulldown utilizing this phosphomimetic mutant identified CLIP-associating protein 2 (CLASP2) as a highly enriched interaction partner in comparison to the phosphodeficient NuMA-S1225A (NuMA-SA) mutant. With the aim to better understand cell fate regulation in terms of spindle orientation, the role of the NuMA phosphorylation and CLASP2 interaction in spindle orientation was investigated. *In silico* experiments first identified the Tumor overgrowth gene 1 (TOG1) domain of CLASP2 as a potential interaction partner of phosphorylated NuMA and *in vitro* experiments confirmed this interaction. While loss of CLASP2 alone did not affect spindle angle, or bipolar LGN localization, NuMA-SD was unable to rescue the increased spindle from a combination of both aPKC^{iKO} and CLASP2^{KO}, confirming the importance of this interaction in planar spindle orientation. A comparison of MDCK Ctr, aPKC^{KO}, CLASP2^{KO}, and aPKC CLASP2^{dKO} cells identified a differential cortical localization of protein 4.1G in Ctr or CLASP2^{KO} versus aPKC^{iKO} or aPKC CLASP2^{dKO} cells, highlighting the presence of a potential rescue pathway. While the loss of 4.1G in combination with CLASP2 was sufficient to drive perpendicular spindles, an analysis of LGN localization showed no increase in monopolar LGN in the CLASP2 4.1G^{dKO} cells, suggesting that LGN and spindle orientation may be uncoupled in this case. Furthermore, reduced levels of LGN in CLASP2 4.1G^{dKO} cells showed no planar spindle rescue, indicating LGN may not be essential for perpendicular spindle orientation in this case. Since both spindle orientation and LGN localization were found to be unaffected upon loss of CLASP2 alone, the differential daughter cell spreading that was observed during live cell imaging was further investigated by identifying differences in post mitotic daughter cell β1 integrin expression in CLASP2^{KO} compared to Ctr. In combination, these results suggest a mechanism in which aPKC regulates NuMA/CLASP2 interaction to promote planar spindle, bipolar LGN, and equal daughter spreading. In the absence of this regulation, planar spindles and LGN localization (but not spreading) may still be maintained via a 4.1G dependent pathway.