SCAR-Arp2/3 polarise the actomyosin cortex in Drosophila neuroblasts

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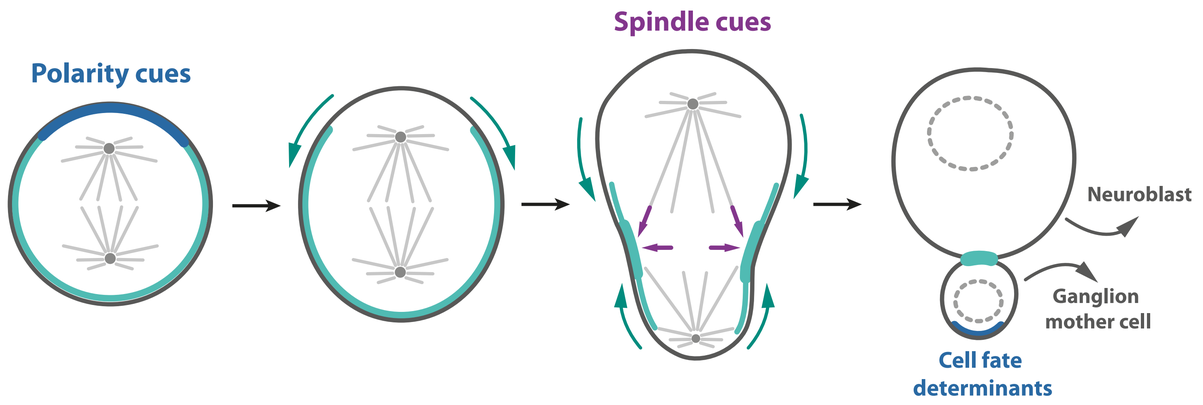
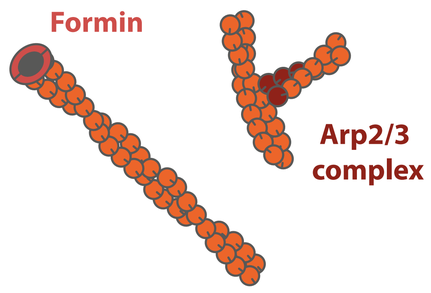
Introduction

While the Formin-nucleated actomyosin cortex has been shown to drive the changes in cell shape that accompany cell division in both symmetric and asymmetric cell divisions, it is not clear whether or not Arp2/3-nucleated branched actin filament networks also play a role. In order to look for mitotic roles of the Arp2/3 complex, here we use Drosophila neural stem cells as a model system, called neuroblasts. These cells are unusual in that they divide asymmetrically to produce a large and small daughter cell with different fates. Polarity cues are required to start a myosin flow at anaphase onset which leads to apical cortical expansion and subsequent shifting of the plane of division towards the basal side of the dividing cell. Spindle cues are necessary for proper constriction of the furrow and completion of division.

With this work we demonstrate that the Arp2/3 complex has a role in aiding cortical remodelling in dividing neuroblasts and in carrying on proper asymmetric division.



Question



The mitotic actin cortex is nucleated by Formin. What is instead the role of the branched-actin network nucleated by Arp2/3 in dividing neuroblasts?

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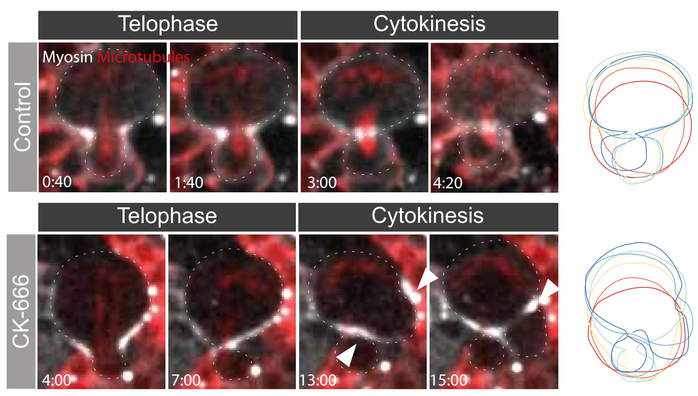

Inhibiting the Arp2/3 complex lead to cortical defects at cytokinesis. Cells were treated with Arp2/3 chemical inhibitor CK-666. Microtubule marker is cherry::Jupiter, expressed with UAS/Gal4. Myosin marker is Sqh::GFP (knock in). Scale bar: 5 µm. Time in mm:ss, relative to anaphase onset.

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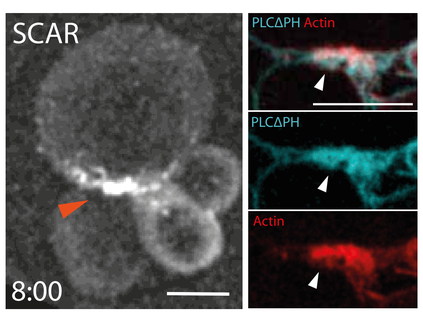
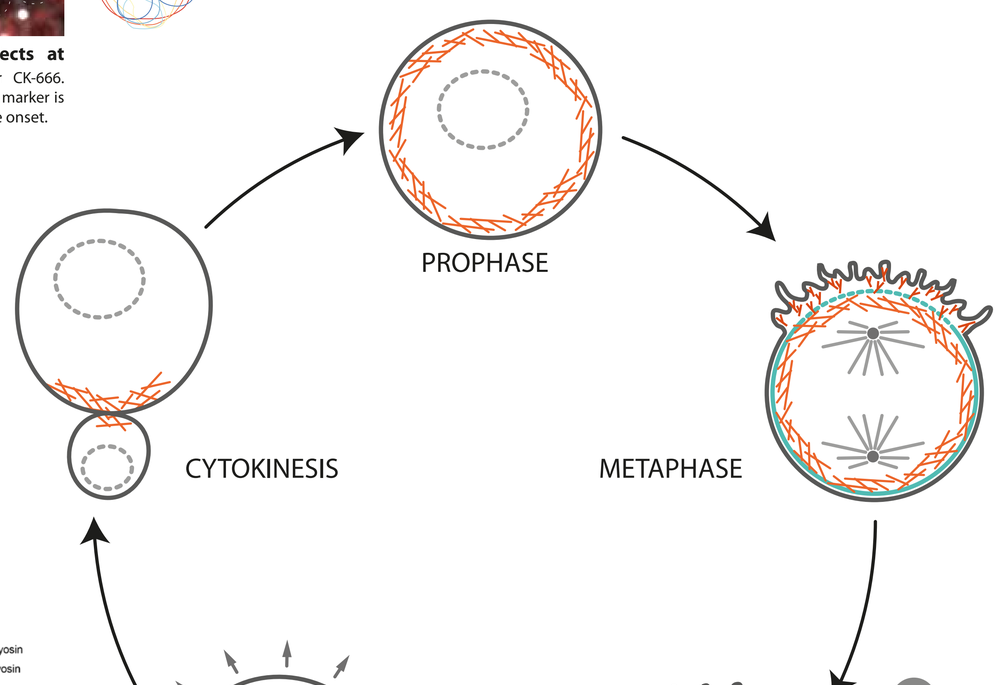


SCAR and actin are also enriched at the furrow at cytokinesis. Montage shows cell from dissociated brain. PLCΔ PH::GFP binds phospholipid PIP2. Actin probe is LifeAct::GFP. All markers are expressed with UAS/GAL4 system. Scale bar: 5 µm. Time in mm:ss, relative to anaphase onset.



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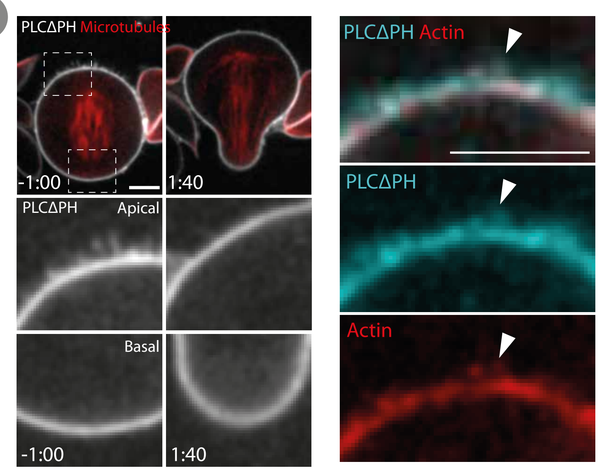

Actin-rich filopodia-like membrane protrusions are visible at the apical side of the neuroblast at metaphase, and disappear after cortical expansion. PLCΔPH::GFP binds phospholipid PIP2. Microtubule marker is cherry::Jupiter. Actin probe is LifeAct::GFP. All markers are expressed with UAS/GAL4 system. Scale bar: 5 µm. Time in mm:ss, relative to anaphase onset.

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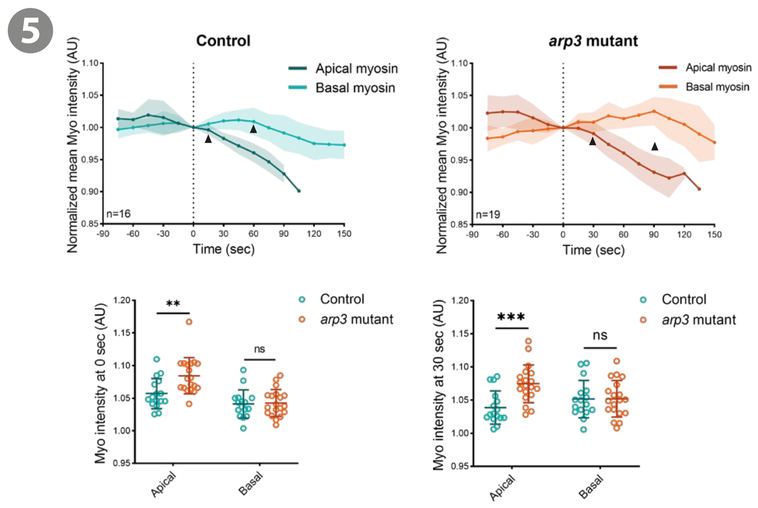

***arp3* mutant cells show more myosin at the apical side of the neuroblast in metaphase and apical myosin clearance is delayed.** Myosin intensity was measured at the apical and basal side of neuroblasts during metaphase-anaphase transition. Absolute intensity was normalized by subtracting background. Graphs on top show myosin intensity changes with time, and arrows point to when myosin starts to be cleared from the apical and basal side of cells. Graphs on bottom show myosin intensity at anaphase onset and 30 seconds later at apical and basal side of neuroblast.

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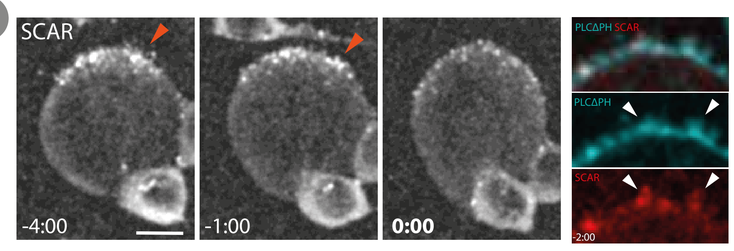

Arp2/3 nulceator promoting factor SCAR localizes asymmetrically at the apical side of the neuroblast in metaphase, and it is enriched at membrane protrusions. Montage shows cell from dissociated brain. PLCΔPH::GFP binds phospholipid PIP2. PLCΔPH::GFP and SCAR:GFP markers are expressed with UAS/GAL4 system. Scale bar: 5 µm. Time in mm:ss, relative to anaphase onset.

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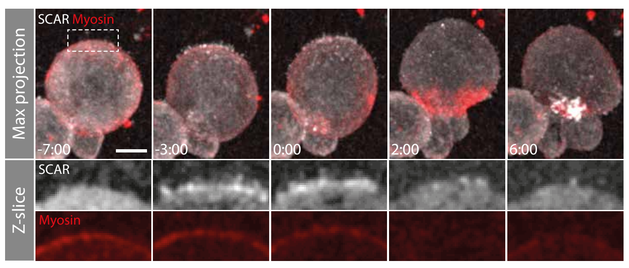

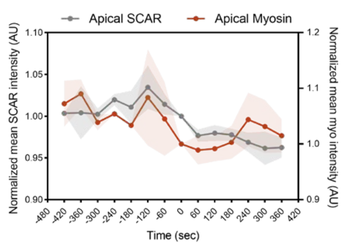

SCAR and myosin follow the same dynamics and are both cleared from the apical cortex at anaphase onset. Montage shows cell from dissociated brain. SCAR::GFP is expressed with UAS/GAL4 system. Myosin marker is Squash::GFP (knock-in). Scale bar: 5 µm. Time in mm:ss, relative to anaphase onset.

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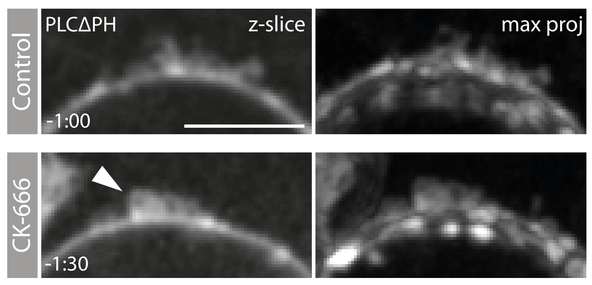
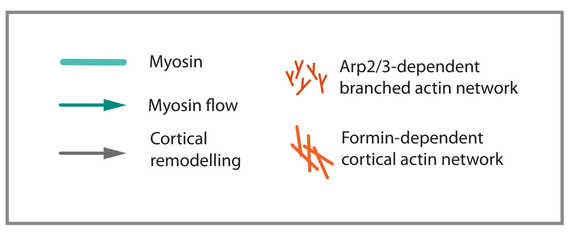


Chemical Arp2/3 inhibitor CK-666 leads to disorganized membrane protrusions. PLCΔPH::GFP binds phospholipid PIP2. Marker is expressed with UAS/GAL4 system. Scale bar: 5 µm. Time in mm:ss, relative to anaphase onset.



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Conclusion

Remodeling of the cortex during cell division is carried out by rearrangements of the actin cytoskeleton, which in turn is mainly nucleated by Formins in mitosis. Cells that undergo asymmetric divisions face additional challenges as they divide, since they have to coordinate shape changes with polarity establishment and fate determinant segregation. With this work, we have shown that another actin nucleator, the SCAR-Arp2/3 complex, is involved in the precise regulation of cortical dynamics. Here we show that SCAR and Arp2/3 are recruited to the apical side of the neuroblast in prophase-metaphase where they nucleate branched actin network that supports the formation of filopodia-like membrane protrusions. At the same time, this network of branched actin limits the accumulation of Myosin at apical pole of the cell which helps breaking the symmetry of the actomyosin cortex at the beginning of anaphase. As a result, the loss of Arp2/3 function leads to a disorganized apical membrane and to excessive myosin at anaphase onset. This in turn leads to affected dynamics of cortical expansion, and possibly to cortical instabilities and defects at cytokinesis.

Therefore, this work help to shed light on particular mechanisms that cells undertake to finely regulate cortical remodelling during cell division.

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