

## **Localization of myosin to the cleavage furrow of *Drosophila* S2 cells in the absence of cortical flow**

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Physiology Course 2006, Marine Biological Laboratory, Woods Hole, MA

The localization of myosin to the cleavage furrow establishes the contractile machinery for cell division. The most widely cited hypothesis for how myosin relocalizes during the metaphase-anaphase transition is that cortical flow drives myosin filaments from the poles to the equatorial region. Here we have investigated the mechanism of myosin localization during cytokinesis by visualizing myosin-GFP in *Drosophila* S2 cells spread on concanavalin A coated surfaces using total internal reflection microscopy. By this method, single myosin filaments on the cortex can be visualized and tracked with time. During interphase, prominent actin-mediated retrograde flow of myosin filaments is observed. However, when myosin is accumulating at the cleavage furrow during anaphase, we find that cortical myosin filaments are largely stationary and do not flow towards the equatorial region. Instead, the cleavage furrow accumulation of myosin results from two processes. First, cortical myosin filaments disappear from beneath the separating centrosomes during anaphase B, causing the depletion of myosin from the poles. This process does not require conventional asters, as acentrosomal poles (created by centrosomin RNAi) also caused disappearance of myosin filaments from the poles and resulted in a normal timing of cytokinesis. A similar result was observed with 3-D sectioning of dividing S2 cells by spinning disk confocal microscopy. Second, after the depletion of myosin at the poles had begun, new myosin filaments appeared at the equator, a process that was correlated with the appearance of stable microtubules at the equatorial cortex (observed by TIRF microscopy). Our observations suggest that high densities of dynamic microtubules cause the disassembly of myosin filaments, while low densities of dynamic microtubules coupled with the formation of stable microtubules promote the stabilization and potentially active transport of myosin filaments to the equatorial cortex.