

Dynamics of Myosin II during Mitosis and Cytokinesis of *Drosophila* S2 Cells

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Myosin II is one of the earliest components of the contractile ring to appear in the equatorial cortex during mitosis. In *Drosophila* S2 cells, myosin is initially localized to the equatorial cortex by signals from rho1 and rho kinase. A second rho1-dependent pathway that leads to formation of a specific actin filament assembly involving formin and profilin is necessary to stabilize the myosin at the equatorial cortex (Dean, S.O., Rogers, S.L., Stuurman, N., Vale, R.D. and Spudich, J.A., *Proc Natl Acad Sci USA* 102:13473-13478, 2005). We have used FRAP analysis to examine the dynamics of the turnover of myosin II in the equatorial cortex at the earliest stage of recruitment, after stabilization into a contractile ring, and at various times after cytokinesis proceeds. We compared these dynamics to that of a “myosin II cloud” found in the spindle region just prior to initial myosin recruitment to the equatorial cortex. We found that the recovery rate of GFP-tagged myosin II regulatory light chain (GFP-RLC) in the equatorial cortex after photobleaching is slower than that of the myosin cloud, and it dramatically decreases upon the metaphase-anaphase transition, and further decreases as constriction of the contractile ring occurs. Cells containing GFP-RLC-E20E21 (a phospho-mimic form of the RLC) show delayed fluorescence recovery at all stages compared to wild type cells, indicating the importance of rho kinase phosphorylation control on the myosin dynamics. FRAP of these cells was also used to explore whether the origins of the myosin in the contractile ring are from cortical flow or from the equatorial cytoplasm.

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