

A Dynamic Actin Cytoskeleton Is Essential to Cytoplasmic Streaming during *Drosophila* Oogenesis

M. E. Quinlan,¹ R. Loughlin,² M. Breckenridge,³ E. Kerkhoff,⁴ R. Mullins¹; ¹Cell and Molecular Pharmacology, UCSF and MBL, Woods Hole, MA, San Francisco, CA, ²Biophysics Graduate Group, UC Berkeley and MBL, Woods Hole, MA, Berkeley, CA, ³Department of Physiology and Biophysics, Case Western Reserve University and MBL, Woods Hole, MA, Cleveland, OH, ⁴Bayerisches Genomforschungsnetzwerk, Institut für funktionelle Genomik, Universität Regensburg, Regensburg, Germany

Developing *Drosophila* oocytes use both actin and microtubule cytoskeletal systems to define the dorsal-ventral and anterior-posterior axes of oocytes and developing embryos. In a late stage of oogenesis (10b) vigorous cytoplasmic streaming mixes the contents of maturing oocytes. Microtubules and kinesin motors drive streaming itself by a poorly understood mechanism. We found that taxol treatment potently inhibits streaming, indicating that, in addition to motor activity, microtubule dynamics are also necessary for streaming. Actin is implicated in timing because mutation of the actin binding proteins, Spir, Capu or Chickadee (profilin), or addition of actin-depolymerizing toxins produces premature onset of streaming. Spir and Capu are both essential polarity factors and actin nucleation factors, suggesting that continued production of the actin cytoskeleton is important. The role of actin during streaming is less well understood. We found that the actin filament stabilizer jasplakinolide inhibits streaming and causes a thickening of actin at the cortex, suggesting that actin dynamics continue to be important during streaming. It is likely that Spir, Capu and Chickadee collaborate to control the onset of streaming. Spir and Capu interact directly in vivo and in vitro and profilin strongly affects nucleation activity of formin proteins like Capu. The interaction between Spir and Capu is mediated by the KIND (Kinase Non-catalytic C-terminal Domain) domain of Spir and the FH2 (formin homology-2) domain of Capu – the nucleation domain of Capu. Spir and Capu modulate each other: Spir inhibits Capu while Capu enhances Spir's actin nucleation activity. This is the first report of actin nucleators directly regulating each other. We also found that Spir disappears from the cortex of the oocyte coincident with streaming onset. Together, these data suggest that regulation of the actin cytoskeleton by Spir establishes the timing of streaming

but another nucleation factor is apparently critical during streaming.