JCB Online Supplemental Material

Rogers et al. http://www.jcb.org/cgi/doi/10.1083/jcb.200303023

Supplemental materials and methods

Actin dynamics in living S2 cells

To visualize actin dynamics in living cells, we generated a stable cell line carrying an inducible EGFP–actin construct. EGFP–actin5c was constructed by polymerase chain reaction splicing by overlap extension (PCR SOEing) using an EST clone for full-length *Drosophila* actin 5C (clone SD12460; Invitrogen) and a vector carrying the EGFP sequence (BD Biosciences; CLONTECH Laboratories, Inc.) as templates. Primers flanking EGFP were designed to introduce an EcoRI site at the 5′ end of the chimera, while primers flanking actin5c were used to introduce a NotI site at the 3′ end. The chimeric PCR product was then digested with these two restriction enzymes and cloned into the EcoRI and NotI sites on the pMT/His-V5B vector (Invitrogen). S2 cells were cotransfected with pMT-GFP-actin5c and pCoHYGRO selection plasmid using Cellfectin reagent (Invitrogen), and stable cells were obtained using hygromycin selection according to the manufacturer's instructions. Before an experiment, EGFP–actin expression was induced with 500 μm copper sulfate for 3–8 h, and cells were plated into con A–treated glass bottom microwell dishes (MatTek Corporation). Cells were imaged using a cooled CCD camera, as described in the Materials and methods. Time-lapse images were acquired at a rate of every 5 s for a period of up to 10 min. Image sequences were imported into ImageJ to generate movies, measure the rates of actin retrograde flow, and generate kymographs.