# ACTIN-BASED RETROGRADE/CENTRIPETAL FLOW IN SEA URCHIN COELOMOCYTES: EFFECT OF PHOSPHATASE INHIBITION ON CYTOSKELETAL ORGANIZATION AND CELL CENTER DYNAMICS

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Actin-based retrograde/centripetal flow is widespread in nucleated cells and involves the continual movement of the lamellipodial plasma membrane and the underlying actin cytoskeleton from the cell periphery towards the cell center. It has been hypothesized to play a fundamental role in cell motility, with one piece of supporting evidence being that the rate of actin-based retrograde flow in *Aplysia* neuron growth cones is inversely related to the rate of growth cone advance. The process has also been suggested to be involved in the cell's sampling of the local environment for directional cues, in the transduction of guidance signals, and in morphogenesis. The sea urchin coelomocyte represents an exceptional experimental model for studying the process of retrograde flow. Previous studies have suggested that flow in these discoidal shaped cells involves a two part mechanism: actin polymerization at the cell periphery coupled with acto-myosin contraction at the cell center (Henson et al., 1999, *Mol. Biol. Cell* 10:4075-4090). Part of the evidence for this hypothetical mechanism derives from pharmacological experiments. Cytochalasin D, a drug which inhibits actin polymerization, stops flow at the cell periphery, but not the center. The kinase inhibitors staurosporine and KT5926, which inhibit acto-myosin contraction, arrest flow in the center and not the periphery.

We have extended previous experiments by testing for the effects of phosphatase inhibition on the flow process. Given that kinase inhibition arrests central flow, we hypothesized that phosphatase inhibition would impact the cell center through stimulation of hyper-contractility. Isolated coelomocytes were treated with 1 nM - 1 $\mu$M Calyculin A, with the response monitored by digitally-enhanced video microscopy, staining for F-actin with fluorescent phalloidin and immunofluorescent labeling for actin, myosin II and depactin. In general the pattern of flow in treated cells was altered to include the development of arcs and rivulets in the peripheral actin cytoskeleton (Figure 1). In the cell center, the perinuclear actin and myosin ring appeared to undergo contraction as compared with control cells. In addition, confocal microscopic XY scans and Z scans revealed that actin containing membranous folds accumulated in the cell center (with some cells exhibiting an actual twisting of this material) which resulted in a significant increase in the height of this region (Figure 1). This result suggested that phosphatase inhibition was fundamentally altering the dynamics of the cell center and therefore may be interfering with acto-myosin contraction, actin depolymerization and/or recycling mechanisms. Support for this interpretation comes from the fact that depactin, the

echinoderm analogue of the ADF/cofilin family of actin depolymerizing proteins, immunolocalizes to the center of flowing cells. Taken together these results suggest that phosphorylation status has a major impact on actin cytoskeletal organization as well as the structure and dynamics of the cell center. Future experiments will concentrate on the identification of coelomocyte proteins which become hyperphosphorylated following Calyculin A treatment.

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Figure 1: Confocal microscopic images of control and Calyculin A treated coelomocytes stained for F-actin using fluorescent phalloidin. Note the extensive rivulets present in the treated cell as well as the accumulation and twisting of actin-containing material in the cell center. Magnification = 1000X.