IDENTIFICATION OF DISTINCT SUBSETS OF SEA URCHIN (STRONGYLOCENTROTUS DROEBACHIENSIS) COELOMOCYTES.

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The coelomic fluid of sea urchins contains a number of different cell types including flagellated vibratile cells, pigmented morulla cells, and bladder amoebocytes. This latter cell type is often referred to simply as coelomocytes and has been the most extensively studied. Coelomocytes appear to play important roles in coelomic fluid clotting and the phagocytosis of foreign matter, and can be induced to synchronously undergo actin-mediated shape changes between lamellipodial and filopodial morphologies (Edds, 1977. J. Cell Biol. 73:479-491; Otto and Bryan, 1979. Cell 17: 285-293; Henson and Schatten, 1983. Cell Motil. 3: 525-534; Hyatt et al., 1983. Cell Motil. 4: 57-71; Henson and Nesbitt, 1992. Bulletin, MDIBL 31: 3-6). To date, the coelomocyte subpopulation has been treated as a homogeneous group of cells. However, we have recently discerned that there exist at least two distinct subsets of coelomocytes based on a number of criteria including their overall morphology, relative degree of mobility, and distributions of microtubules, subcellular organelles and the microtubule motor protein kinesin (Henson et al., 1992. J. Cell Sci. 103: 309-320). These cell types appear to correspond to those recently separated out on sucrose step gradients by Edds (1991, J. Cell Biol. 115: 68 abstract). In this study we have examined the differences between these two subsets with reference to their ability to undergo lamellipodial to filopodial shape changes and in the distributions of actin filaments and the actin binding proteins myosin II, spectrin and filamin. These experiments involved video-enhanced microscopy, immunoblotting and light microscopic immunocytochemical localization.

Lamellipodial coelomocytes were collected from coelomic fluid in a low calcium anticoagulant, isolated by means of centrifugation onto a sucrose cushion and maintained in an isotonic coelomocyte culture medium (CCM = 0.5 M NaCl, 2.5 mM $MgCl_2$, 1 mM EGTA and 20 mM HEPES pH 7.4). Cells allowed to settle onto poly-L-lysine coated coverslips were viewed with a Nikon 60 X (N.A. 1.4) planapochromatic phase contrast objective lens and video images ed using a MTI newvicon camera. The video signal was digitized by a Hamamatsu Argus 10 digital image processor. Video-enhanced images were recorded on a Mitsubishi super VHS editing VCR. The two subsets of coelomocytes are not readily apparent in suspension culture, however they become clear once the cells are allowed to settle. Type 1 stationary cells spread onto the substrate as round flattend discs taking on the overall shape of a fried egg. The cytoplasm displays little in the way of particles or particle movement, and exhibits radial arrays of networked phase dense fibers (fig. 1). The type 2 motile cells rapidly spread lamellipodia, becoming polygonal in shape. The cytoplasm contains a large number of particles, many of which exhibit saltatory motion, and has parallel arrays of longitudinally oriented phase dense tracks.

Our previous studies have indicated that differences in intracellular particle movement seen in the two cell types is mirrored by differences in the distribution of microtubules: motile cells have extensive cytoplasmic arrays of microtubules while stationary cells contain only a sparse perinuclear array (see Henson et al., 1992 loc.cit.). We interpreted the differences in the organization of the phase dense fibers present in these two cell types as indicative of differences in actin filament organization. For the staining of filamentous actin, fixed cells were treated with rhodamine phalloidin. Stationary cells stained with phalloidin showed a radial array of short actin filaments arranged in networks which were particularly dense in the cortical region of the cell. This pattern is typical of published coelomocyte actin distributions. Motile cells stained with phalloidin exhibited a stress fiber pattern reminiscent of filamentous actin patterns in motile mammalian tissue culture cells, and unlike any of the published coelomocyte data. We hypothesized that differences in actin organization may be indicative of differences in the

expression and/or localization of actin binding proteins (ABPs) and therefore decided to examine the localization of the ABPs myosin II, spectrin and filamin. Western blots of coelomocyte (a mixture of both cell types) and egg high speed supernatants were probed with affinity purified polyclonal antibodies raised against antigens isolated from sea urchin eggs (spectrin and filamin antibodies were a kind gift of Dr. David Begg, University of Alberta). Figure 2 indicates the egg spectrin antibody recognized a closely spaced 235 and 240 kD doublet in both eggs and coelomocytes. The filamin antibody recognized a single 250 kD species in eggs but a widely spaced 220 and 250 kD doublet in coelomocytes. Filamin in many cells is known to consist of a homodimer of two 250-270 kD subunits. One possibility is that the filamin isoform in coelomocytes consists of a heterodimer. The myosin II antibody recognized a 200 kD protein in both samples. These three ABP antibodies displayed differential localization patterns in the two coelomocyte cell types. The stationary type 1 cells exhibited very little myosin II or spectrin staining, while filamin appeared to codistribute with the peripheral actin networks. The motile type 2 cells displayed an extensive amount of myosin II and filamin staining, concentrated in the stress fibers and at the periphery of lamellipodia. Spectrin staining of these cells gave two distinct patterns: a filamentous perinuclear pattern which did not colocalize with actin in cells double labeled with phalloidin; and a more general cytoplasmic array of punctate/vesicular staining. These latter spectrin localization patterns suggest that spectrin in coelomocytes may have a role in the movement of intracellular membrane bound organelles, a function it has recently shown to have in the sea urchin egg and early embryo (Fishkind et al., 1990. Dev. Biol. 142: 453-464; Fishkind et al., 1990. Dev. Biol. 142: 439-452).

The ability of the two cell types to undergo the lamellipodial to filopodial shape changes was tested in experiments involving the treatment of cells with hypotonic shock and with the calcium ionophore A23187 (see Henson and Nesbitt, 1992. Bulletin, MDIBL. 31: 3-6). Cells were either treated in suspension or after settling onto a coverslip. Type 1 stationary cells consistently transformed from the lamellipodial to filopodial morphology when exposed to either stimulus. The type 2 motile cells did not change shape with the treatments. Interestingly, in the presence of ionophore the motile cells frequently lysed due to massive contraction of the cytoplasm. This reaction may be representative of the differences in myosin II expression and distribution in the two cell types.

The results of these experiments clearly indicate the presence of two subsets of coelomocytes with fundamental differences in relative mobility, the capacity to change shape and in cytoskeletal organization. These differences offer an excellent opportunity for future study into the molecular mechanism of cell motility and shape changes. We currently are working to purify the two separate populations in an attempt to begin more extensive biochemical analysis of the differences in their complements of cytoskeletal proteins.

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Figure 1: Video-enhanced phase contrast micrograph of substrate attached coelomocytes showing both type 1 stationary (s) and type 2 motile (m) cells. Note radially arrayed networks of fibers in the stationary cells and the longitudinally arrayed parallel tracks in the motile cells. Magnification = 1,800 X.

Figure 2: Western blots of spectrin (panel 1) and filamin (panel 2) antibodies against high speed supernatants of homogenates of sea urchin eggs (lanes a) and coelomocytes (lanes b). Note that the spectrin antibody is also labeling proteolytic fragments of spectrin in the egg sample. 4 % SDS polyacrylamide gels were used in these immunoblots.

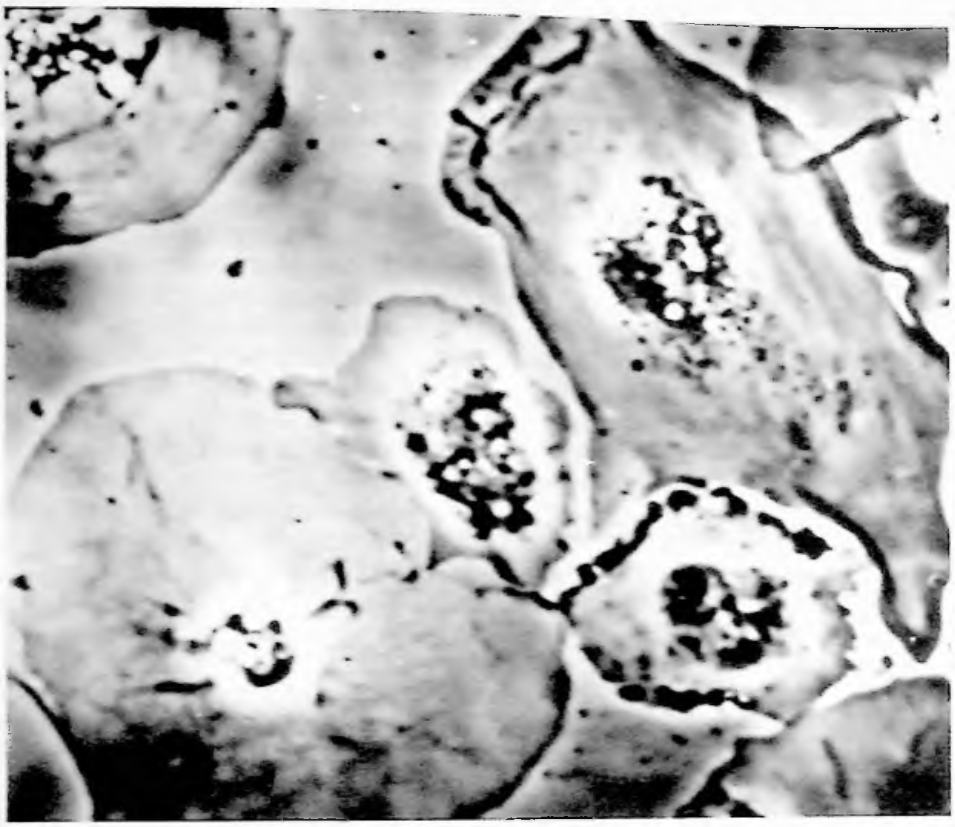

FIGURE 1

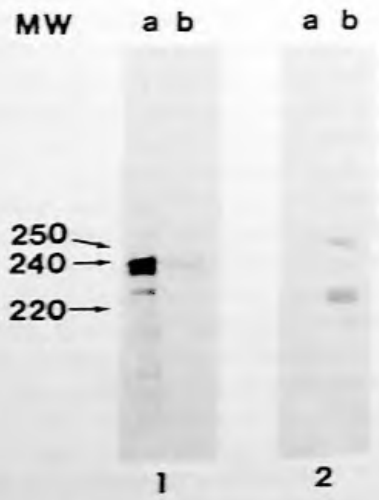

FIGURE 2