SPREADING OF SEA URCHIN (STRONGYLOCENTROTUS DROEBACHIENSIS) COELOMOCYTES: DYNAMICS OF THE ACTIN CYTOSKELETON AND THE EFFECTS OF ELEVATED INTRACELLULAR CALCIUM.

John H. Henson and David Nesbitt
Department of Biology, Dickinson College, Carlisle, PA 17013

Cell shape changes in the majority of cell types are mediated by the actin cytoskeleton (actin filaments and the associated actin binding proteins). The elaborate actin cytoskeleton of the sea urchin coelomocyte provides an excellent model experimental system for studying the molecular mechanisms underlying these shape changes. Coelomocytes play important roles in coelomic fluid clotting and the phagocytosis of foreign matter, and can be induced to synchronously undergo an actin-mediated shape change from a lamellipodial to a filopodial form. Although several studies have focused on filopodial formation in coelomocytes (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), very little is known concerning the generation of lamellipodia from preexisting filopodia. The generation of lamellipodia has a general significance in cell motility since they are the primary organelles of locomotion in most tissue cells. Therefore, we have focused on the examination of the dynamics of actin and myosin II organization during the filopodial to lamellipodial shape change exhibited by spreading filopodial cells. This shape transformation involves the generation of lamellipodia and the dismantling of filopodia. The importance of intracellular calcium levels in this process was also addressed in experiments involving the treatment of spreading cells with the calcium ionophore A23187. The studies involved a combination of high resolution, digitally enhanced video microscopy, the fluorescent detection of filamentous actin and the immunofluorescent detection of myosin II.

For observation of the substrate induced filopodial to lamellipodial shape change, 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). The cells were then transformed to the filopodial form by reducing the NaCl concentration of the CCM to 0.3 M. The filopodial cells were allowed to settle onto a poly-L-lysine coated coverslip and the process of lamellipodial generation and filopod disruption was observed. For video microscopic observation the coverslips were mounted onto a slide by means of a vaseline well which had spaces for the perfusion of solutions. The cells were viewed with a Nikon 60 X (N.A. 1.4) planapochromatic phase contrast objective lens and video images collected using a MTI newvicon camera. The video signal was digitized by a Hamamatsu Argus 10 digital image processor which allowed for digital contrast enhancement, frame averaging and background subtraction. Video enhanced images were recorded on a Mitsubishi super VHS editing VCR. For the staining of filamentous actin, cells were treated with a fixation solution consisting of 0.5% glutaraldehyde plus 100 mM lysolecithin in CCM and then stained with rhodamine phalloidin (RdPh). For the localization of myosin II, cells were fixed with 3% formaldehyde, 0.1% glutaraldehyde in CCM, postfixed in 100% acetone at -20°C, and then stained with a polyclonal antiserum raised against the heavy chain of sea urchin egg myosin II followed by a fluorescein conjugated secondary antibody.

Video enhanced phase contrast microscopy of the spreading process (see figure 1) indicated that lamellipodia arise near the cell center and spread radially out to engulf the substrate attached filopods. RdPh staining of spreading cells (fig. 1 E) indicates that the lamellipodia are filled with a network of actin filaments. With respect to filopodial dissolution, many filopods appear to unravel along points distil to the advancing edge of the lamellipod (arrow in fig. 1 A), while others appear to remain as intact phase dense tracts within the cytoplasm. RdPh staining suggests that these

phase dense tracts consist of persistent filopod derived actin bundles. This was confirmed in experiments in which the same cell was video taped and then fixed and stained with RdPh. This type of procedure allowed for the direct correlation between live cell behavior and the organization of filamentous actin.

The process of lamellipodial spreading and the dismantling of filopodia can be completely reversed by treating cells with 10-20 μM of the calcium ionophore A23187 (fig. 1 C, D). The ionophore-mediated elevations in intracellular calcium cause the cessation of lamellipodial spreading, the formation of actin bundles at the periphery and within the cytoplasm, and the eventual elongation of the peripheral filopodia. Internal bundles of actin filaments are evident as phase dense tracts within the lamellipodial cytoplasm (fig. 1 D). RdPh staining allowed for a comparison between the filamentous actin distribution in spread cells in the presence or absence of ionophore treatment (fig. 1 E, F). This staining indicated the dramatic rearrangement of actin which occurs in cells exposed to elevations of intracellular calcium. The peripheral network of relatively short filaments present in the lamellipodia of spread cells is transformed into bundled arrays of long filament. The ionophore mediated actin reorganization takes place only in calcium supplemented (1 mM) CCM, suggesting that intracellular calcium stores alone are not mediating this effect.

The dismantling of the filopod core bundles of actin filaments, the generation of lamellipodia and the bundling of actin in response to ionophore treatments all would be expected to involve the action of actin binding proteins (ABP) in the coelomocytes. Filament severing proteins would be expected to play a part in dismantling of filopodia, while filament cross linking proteins might be present in the actin filament networks within lamellipodia. The ABP complement of these cells is relatively unknown, particularly in comparison to the wealth of information available on the ABPs present in the sea urchin egg and early embryo. Fascin is an ABP which is known to bundle actin within the microvilli of sea urchin embryos and the filopodia of the coelomocytes. However, this bundling process shows no calcium sensitivity in vitro suggesting that the ionophore elicited effects in coelomocytes are not being mediated entirely by this protein. One major ABP which exhibits calcium sensitive associations with actin in many cells is myosin II. We have raised a monospecific polyclonal antiserum against sea urchin egg myosin II heavy chain (see western blot in fig. 2) in order to begin immunofluorescent localization studies using this probe. In preliminary double label experiments utilizing RdPh and anti-myosin, (fig. 2 A, B) myosin is seen to codistribute with actin particularly at the cell periphery and along stress fibers. We are currently in the process of labeling spreading cells with this antiserum in an attempt to determine if myosin is crucial for lamellipodial advancement or if it is involved in the selective stabilization of filopodia.

Figure 1: Video enhanced phase contrast microscopy of a spreading filopodial coelomocyte treated with calcium ionophore (panels A - D), and rhodamine phalloidin (RdPh) staining of filamentous actin in spread cells (panels E and F). Panel A: This cell has already spread considerably. Note that lamellipodia may spread from the cell center or arise directly from a filopod (arrow). Panel B: The cell center derived lamellipodia have advanced and fused with those derived from the dismantling of filopods. Phase dense tracks in the cytoplasm mark the presence of former filopods.The arrow indicates a lamellipod which is actively spreading. Panel C: Immediately following treatment with the calcium ionophore the leading edge of the formerly advancing lamellipod (arrow) acquires filopodial extensions. Panel D: After extended exposure to the ionophore the cytoplasm of the cell becomes crisscrossed with phase dense tracks and the remaining filopodia have undergone significant elongation. Panel E: RdPh staining of spread cell showing actin network in the lamellipodia and the filopod derived actin bundles in the cytoplasm. Panel F: RdPh staining of ionophore treated cell showing the extensive cytoplasmic actin bundles and the absence of the actin filament networks. Magnification = 1,000 X. Time in minutes and seconds is given in the upper right of each panel.

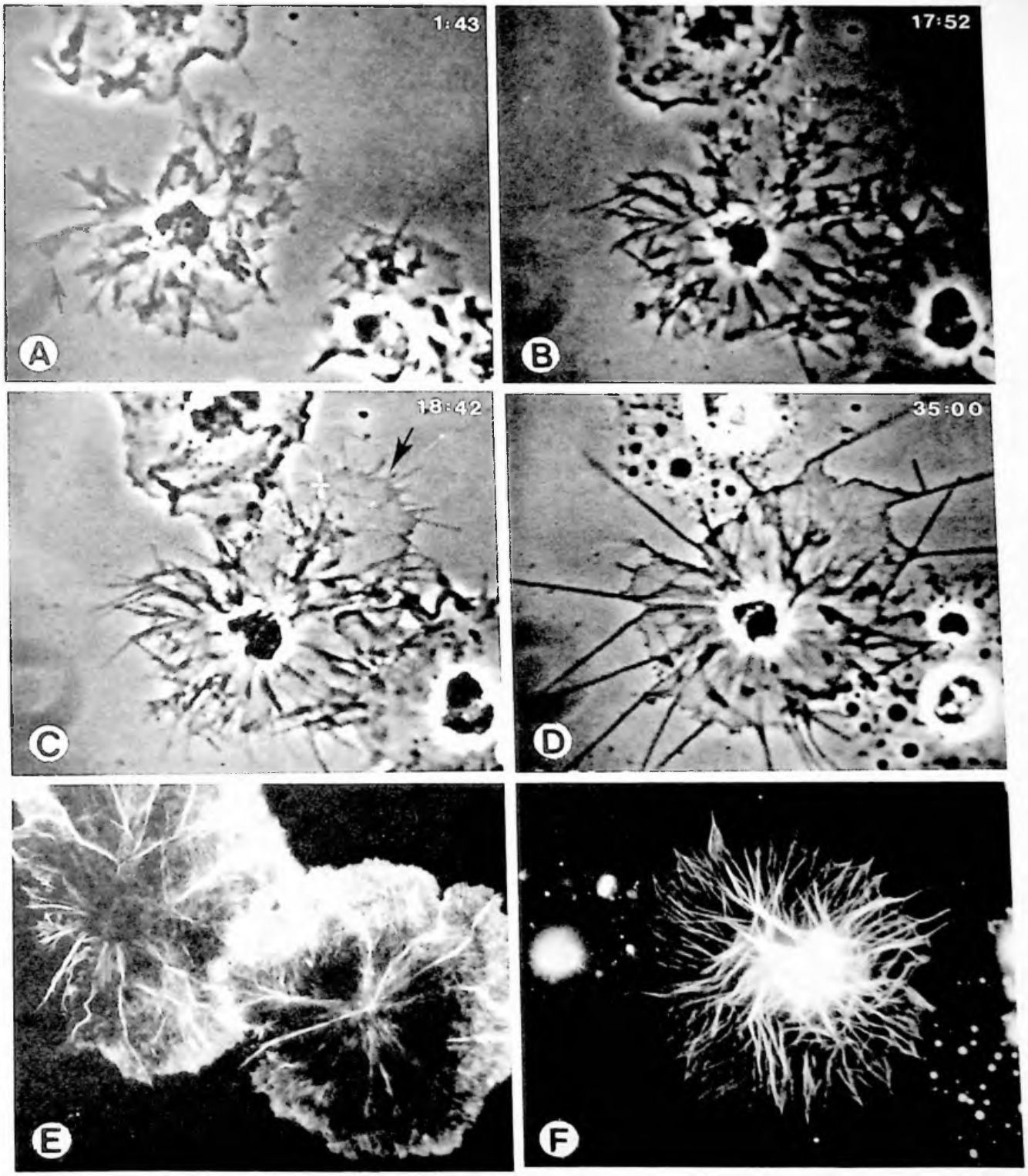

FIGURE 1

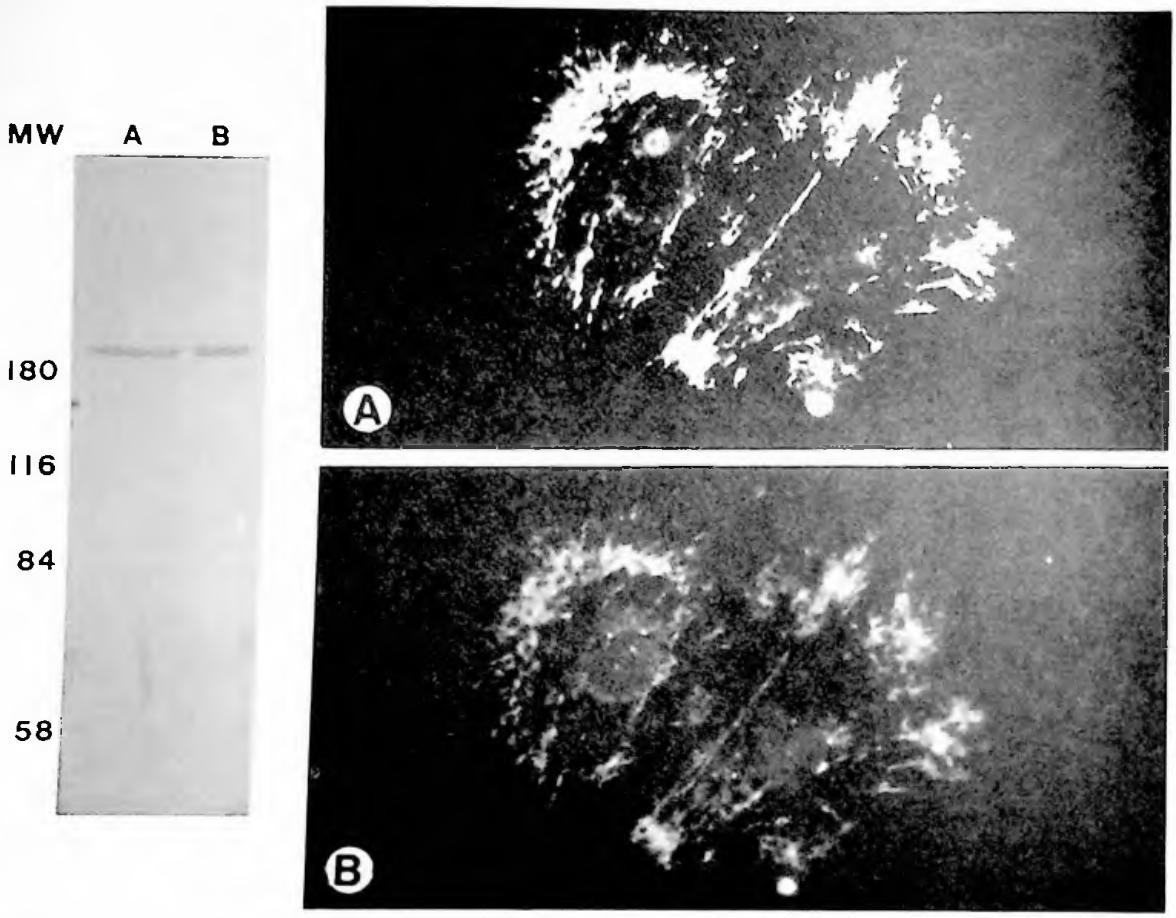

Figure 2: Western blot of anti-sea urchin myosin II antiserum against lysates of sea urchin eggs (lane A) and sea urchin coelomocytes (lane B). The single immunoreactive species present in the blots migrates at the appropriate molecular weight (200 kDa). Panels A and B: Double label of coelomocytes with RdPh (A) and anti-myosin II (B) reveals that the two proteins appear to codistribute particularly at the cell periphery, in filopods and in stress fibers. Magnification = 1,000 X.

The preliminary results reported here indicate that the process of spreading of filopodial coelomocytes involves a dramatic rearrangement of this cell's extensive actin cytoskeleton. This process offers a wealth of opportunities for examination of the reorganization of actin filaments and the in vivo functions of actin binding proteins. Future experimentation will involve the immunolocalization of major actin binding proteins during this process as well as the correlation of cell spreading behavior with ultrastructure as revealed by whole mount negative stain transmission electron microscopy. The results of these studies should provide insights into how actin mediates motility and shape changes in a variety of cell types.

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