Association of Gelsolin with Actin Filaments and Cell Membranes of Macrophages and Platelets

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Abstract. Recent evidence that polyphosphoinositides regulate the function of the actin-modulating protein gelsolin in vitro raises the possibility that gelsolin interacts with cell membranes. This paper reports ultrastructural immunohistochemical data revealing that gelsolin molecules localize with plasma and intracellular membranes, including rough endoplasmic reticulum, cortical vesicles and mitochondria of macrophages, and blood platelets. Anti-gelsolin gold also labeled the surface and interior of secondary lysosomes presumably representing plasma gelsolin ingested by these cells from the lung surface by endocytosis. Gelsolin molecules, visualized with colloidal gold in replicas of the cytoplasmic side of the substrate-adherent plasma membrane of mechanically unroofed and rapidly frozen and freeze-dried macrophages, associated with the ends of short actin filaments siting on the cytoplasmic membrane surface. A generalized distribution of gelsolin molecules in thin sections of resting platelets rapidly became peripheral, and plasmalemma association increased following thrombin stimulation. At later times the distribution reverted to the cytoplasmic distribution of resting cells. These findings provide the first evidence for gelsolin binding to actin filament ends in cells and indicate that gelsolin functions in both cytoplasmic and membrane domains.

The actin-associated protein gelsolin extracts easily from cell debris in aqueous homogenates, and low concentrations (μM) of Ca²⁺ activate gelsolin for binding to actin (Yin and Stossel, 1979, 1980). Immunofluorescence microscopy has revealed a diffuse distribution of gelsolin in the cell periphery (Yin et al., 1981a; Carron et al., 1986; Cooper et al., 1988). These data led to the conclusion that gelsolin is a "cytosolic" protein, a location appropriate for its actin nucleating, filament severing, and end-blocking functions to control cortical cytoplasmic actin assembly. The recent discovery, however, that polyphosphoinositides, phosphatidylinositol monophosphate, and phosphatidylinositol 4,5-bisphosphate modulate the actin-binding, filament-severing, and end-binding functions of gelsolin in vitro (Janneny and Stossel, 1987; Janneny et al., 1987) has therefore pointed to a greater complexity. If polyphosphoinositides regulate gelsolin function in vivo, gelsolin must interact with membranes where these phospholipid molecules reside. We have, therefore, examined the distribution of gelsolin by immunoelectron microscopy of lung macrophages and of platelets activated under the conditions reported by Lind et al. (1987) to elicit reversible complexing of gelsolin to actin.

Materials and Methods

Preparation of Cells

Platelets. Nine volumes of normal human blood were drawn into 1 vol of Aster-land anticoagulant, transferred to plastic centrifuge tube, and centrifuged at 200 g for 10 min at 4°C. Platelets were isolated from the plasma layer by gel filtration through a Sephadex 2B column equilibrated with 160 mM NaCl, 2 mM MgCl₂, 0.5 mM Na₂HPO₄, 10 mM Hepes, and 10 mM glucose containing 3 mg/ml of BSA (RIA grade).

Gel-filtered platelets were incubated for 60 min at 37°C to insure a resting state. In electron micrographs, 89% of the cells in the resting population were discoid in shape and lacked surface projections (n = 237). To determine the distribution of gold particles in resting cells only platelets discoid in electron micrographs were used (see below). Resting platelets were activated by the addition of 1 U of thrombin per milliliter for the times of 15 and 30 s, and 1, 5, and 20 min. 90% of the cells treated with thrombin for 15 s were activated as judged by the formation of pseudopodia (n = 250). Gold particles were quantitated only in activated cells displaying pseudopodia or filapodia on their surfaces. These activation times were terminated by the immediate fixation of the cells (see below).

Macrophages. Lung macrophages were obtained from rabbits injected 2–3 wk previously with 1 ml of complete Freund’s adjuvant by intratraheal lavage with 154 mM NaCl (saline). Harvested cells were washed twice by centrifugation in saline and resuspended in Krebs-Ringer phosphate (KRP) buffer lacking added calcium. A 10% suspension (vol/vol) of macrophages in KRP was incubated for 15 min at 37°C before fixation.

Preparation of Cell Sections for the Electron Microscope

Embedding and Sectioning of Cells. Resting and thrombin-activated platelets and macrophages were fixed by the addition of 10 vol of 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 containing 5 mM CaCl₂ for 1 h at room temperature. To form a tissue mass for embedding and sectioning the cells were sedimented into pellets while in fixative. Cell pellets were de-
hydrated in ethanol, embedded in Lowicryl K4M, and sectioned with a diamond knife (Roth, 1982).

Labeling of Sections with Protein A-Gold. Gold particles, 8 nm in diameter, were prepared with tannic acid according to the method of Slot and Geuze (1985). Gold particles were coated with *Staphylococcus aureus*-protein A (Pharmacia Fine Chemicals, Piscataway, NJ) as described by Slot and Geuze (1985). Polyclonal goat affinity-purified anti-rabbit macrophage actin-binding protein and antigelsolin IgG have been characterized previously (Stendahl et al., 1980; Yin et al., 1981a). Affinity-purified rabbit anti-goat IgG was purchased from Cappel Laboratories (Malverne, PA).

To label the sectioned cells with gold, cut sections were placed on the surface of 10-μl drops of 15 μg/ml of antigelsolin or actin-binding protein IgG in PBS containing 1% BSA, pH 8.2 (PBS-BSA) and incubated for 16 h at 4°C. The sections were then washed thrice with PBS-BSA, washed thrice by transferring them to drops of PHEM buffer containing 1% BSA for 5 min each. They were then transferred to 10-μl drops of 15 μg/ml of antigelsolin or actin-binding protein and antigelsolin IgG have been characterized previously by drawing along the membrane with a pen having an point width of 80 nm. A qualitative association of gold particles with internal cell membranes was apparent (see Results) but was not quantitated. Although these gold particles appear to be associated with the boundaries of intracellular granules and vesicles, their relationship to (distance from) the limiting membrane could not be established, e.g., there was only limited preservation of internal membranes in Lowicryl sections of cells fixed only with glutaraldehyde. Such particles were included, however, in the total number of gold within a defined zone.

All gold particles within a defined zone of a cell were counted and the area of the zone determined by tracing its boundaries on the surface of a GTCO digitizing pad. Areas, total particles, and particles per μm² of cytoplasm, as well as the number of gold particles associated with a length of membrane (per μm) were determined as previously described using a DEC 11/73 computer (Hartwig and Shevlin, 1986) and are expressed as mean ± SEM. Data means were compared using a two-tailed, unpaired t test. All gold particles within 35 cells were counted for resting cells and each time point after thrombin stimulation. The ratio of membrane bound to total gold particles was determined in micrographs by counting membrane associated and total gold in sections containing complete platelets. Cells were photographed at random in the electron microscope.

Macrophages. The distribution of gold particles in sectioned macrophages were determined in similar fashion to platelets. Gold falling within the following regions were quantitated: (a) membrane associated; (b) the most cortical 85 nm of cytoplasm; (c) an area near the cell center but not within nuclei; (d) nuclear; and (e) within lysosomes. Gold was quantified from 15 macrophage sections.

Results

Localization of Gelsolin and Actin-binding Protein in Macrophages

Fig. 1 shows representative electron micrographs of Lowicryl-embedded macrophages labeled with affinity-purified goat anti-gelsolin IgG, rabbit anti-goat IgG, and protein A-coated 8-nm gold particles. Antigelsolin gold label was observable in several compartments of the cell. Some appeared to reside within the expanse of cortical cytoplasm not obviously apposed to or within definable organelles. Others, often in aggregates, containing two to six particles, were also associated with plasma membrane and intracellular membranes, such associations being defined as gold particles residing <1 diam of membrane. Still others localized on rough endoplasmic reticulum inside secondary lysosomes (Nichols, 1976) often containing lung surfactant and on the edges of mitochondria (Figs. 1 and 3). Very few gold particles were within the nucleus (Figs. 2 and 3). The central cytoplasm labeled less than the cell cortex. No gold labeling was observed when gelsolin-absorbed IgG or preimmune IgG was used as the primary IgG.

The electron micrographs in Fig. 4 compare the pattern of gelsolin labeling with that of actin-binding protein. Anti-actin-binding protein label was visually more striking than gelsolin staining because it occurred in clusters containing five to eight gold particles, and the clusters concentrated in the most cortical zones of cytoplasm. The predilection of gold label for membranes or the inside of lysosomes observed for gelsolin was not evident with actin-binding protein staining. Clusters of gold particles against actin-binding protein.
Electron micrographs showing the distribution of antigelsolin gold staining in macrophages. Glutaraldehyde-fixed cells were embedded in Lowi-cryl K4M, sectioned, and sequentially treated with goat antigelsolin IgG, rabbit anti-goat IgG, and 8-nm colloidal gold particles coated with S. aureus-protein A. Representative regions from the cortex of two macrophages are shown. The insets show low magnifications indicating the cortical regions shown at higher magnification. Gelsolin locates both in the cytoplasm and near the plasma membrane of these cells. In general, single or double gold particles are found in the cytoplasm. Gelsolin gold label near the membrane is in larger aggregates. Bars, 0.2 μm; (inset) 2 μm.

Figure 2. Distribution of gelsolin and actin-binding protein in macrophages. Histogram quantitating the distribution of gelsolin and actin-binding protein in macrophage sections. The concentration of gold particles was determined as described in Materials and Methods. These data are expressed as the mean ± SEM of gold particles in the five zones from 30 cells. ■, gelsolin; □, actin-binding protein.

The distribution of gelsolin and actin-binding protein label in the sections was analyzed by morphometry (Fig. 2). This analysis focussed on the apportionment of label between plasma membrane and nonplasmalemmal localizations. This quantitative analysis demonstrated that antigelsolin label was 1.75-fold more concentrated on membrane than in the most cortical cytoplasm (0–85-nm zone of cytoplasm) whereas actin-binding protein label at the membrane and in the most cortical cytoplasm was equivalent: when the concentration of anti-actin-binding protein gold particles on the membrane was compared to that in the most cortical cytoplasm no difference between the two was found (Fig. 2). The concentration of anti-actin-binding protein label in the most cortical cytoplasm (0–85-nm zone), however, was 3.6-fold greater than in the cell center. A 3.9-fold-increase in the total concentration of gold label for actin-binding protein in the most cortical cytoplasm relative to gelsolin may reflect both an increased concentration of this protein in the cell cortex and...
the greater size and the extended nature of actin-binding protein molecules.

**Membrane-associated Gelsolin Is Also Bound to Short Actin Filaments**

Mechanical cleavage opens cells by ripping off pieces of apical membrane and permits viewing of the cytoplasmic side of the plasma membrane and specific labeling of intracellular structures. A network of filaments very similar in structure to the cortical networks revealed by detergent permeabilization (Hartwig and Shevlin, 1986) is visible at the edges of unroofed macrophages ripped open in the process of spreading on the surface of a glass coverslip. The filaments have 10-nm diam and the beaded substructure of actin and acquire a characteristic fluted appearance (Heuser and Kirschner, 1980; Hartwig and Shevlin, 1986) when reacted with myosin S1 (Fig. 5 b). The actin network extends from the upper edge of the cell, where the upper membrane was mechanically removed, to the cytoplasmic side of the substrate-adherent membrane (Fig. 5 a). The more central regions of these un-
Figure 6. Stereo-paired micrographs showing that gelsolin locates on the ends of short filaments and on the cytoplasmic membrane surface of mechanically opened macrophages. Gelsolin antigenic sites were visualized in the electron microscope by treatment with goat anti-gelsolin IgG followed by 8-nm gold particles coated with rabbit anti-goat IgG. (a) Stereo-paired micrographs showing the cytoplasmic side of...
roo ted cells have, in contrast, only a sparse actin filament coating. The periphery and center of substrate-attached plasma membrane, therefore, differ in actin filament density.

Figs. 6 and 7 show a comparison of staining by antigelsolin and anti-actin-binding protein in actin filament-rich marginal and filament-poor central zones of unroofed macrophages. Figs. 6 b and 7 a show regions near the margin of unroofed cells with a thick three-dimensional actin filament network. Viewing in stereo reveals that gold label against gelsolin does not bind filaments in the network coating the membrane surface but rather binds at points where filaments extend from the network and approach the cytoplasmic side of the membrane. In many cases, the antigelsolin gold label aggregate into clusters or piles making the gold appear separate from the membrane. Such piles may also represent points where the plasma membrane enfolds to contact actin filaments. Since gelsolin does not, however, label filaments within the three-dimensional network of unroofed cells, it must be binding to filament ends coming from the network that approach the cytoplasmic face of the plasma membrane (Fig. 6). Actin-binding protein staining, on the other hand, locates as previously reported (Hartwig and Shevlin, 1986) almost exclusively within the network, at points of filament intersection and only rarely at the ends of filaments contacting membrane (Fig. 7). The binding of gelsolin but not actin-binding protein to filament ends on short filaments lying parallel to the membrane is most clearly demonstrated in Figs. 6 a and 7 b which show central regions of the cytoplasmic side of membrane that contain only a sparse actin filament covering. As shown in Fig. 6, gold particles identifying gelsolin antigenic sites locate at the ends of many short actin filaments. Binding of gold particles is limited to one end of each filament: gold decorates either the end of the filament that attached to the membrane or its unattached end, but never the two ends of the same filament. Gold particles binding to bare regions of the cytoplasmic surface membrane were occasionally observed although gold associated with filament ends was much more common. Antigelsolin gold also coated the surface of some internal cell membranes such as rough endoplasmic reticulum and vesicles lying on the substrate adherent cell surface. It also stains the ends of actin filaments attaching to them (data not shown) as expected from gold-stained sections of macrophages. As shown in Fig. 7 b, gold directed against actin-binding protein did not bind regions of adherent membrane containing sparse filaments.

Intracellular Distribution of Gelsolin in Resting, Activated, and Postactivated Platelets

Fig. 8 (a–d) compares the intracellular distribution of gelsolin in electron micrographs of sections of resting platelets (Fig. 8 a) or of platelets that have been reacted with 1 U/ml of thrombin of various times (Fig. 8, b–d). The discoid shape of gel-filtered platelets untreated by thrombin confirmed that these cells were unactivated (Fig. 8 a) and 89% of the platelets retained their discoid shape in these thin sections. Likewise, the formation of pseudopods and filopods in thrombin-treated cells were consistent with the conclusion that thrombin had a stimulating effect (Fig. 8, b–d) and 90% of cells showed protrusions after thrombin treatment for 15 s. In resting cells, antigelsolin label, generally in small aggregates of two to three gold particles, was dispersed throughout the cytoplasm. It was excluded from the marginal microtubule band and from the bulk of intracellular granules. The exception was a population of densely staining bodies that labeled (Fig. 8, a–c). As simplified above, some of these may correspond to mitochondria (Fig. 8, b–d). After activation of platelets, antigelsolin label increased in concentration, was clustered into larger aggregates of particles, and became more peripheral and membrane associated (Fig. 8, b–d). Mitochondria also labeled with gold on their surfaces (Fig. 8, b and d).

Fig. 10 summarizes a morphometric analysis of gold labeling. Two major changes in the pattern of immunogold labeling against gelsolin are apparent when platelets were activated. First, the number of gold particles bound increased more than twofold in activated relative to resting cells; there was a marked increase in the total number of gold particles in all zones of cells treated with thrombin for 15 s and 1 min (Figs. 10 A and 11 A) relative to cells at rest. The increased gold labeling at 15 s was statistically significant (p < 0.001). The label density, however, returned to resting levels after 5 min of thrombin treatment, indicating that the increase in gold density at the early times of activation was related to cell activation. Second, after normalizing for the increased gold number in stimulated cells there was a shift in the distribution of gold to the membrane during cell activation (Figs. 10 C and 11 B). The percent of the total antigelsolin gold associated with the membrane of resting and cells treated with thrombin for 15 and 60 s was 4.0, 6.5, and 6.3 %, respectively (Fig. 11 B). Membrane-associated gold (percent of total) label against gelsolin, therefore, increased by 58–63 %, 15–60 s after thrombin stimulation. This net increase in membrane-gold reversed after longer incubations with thrombin (Fig. 11 B). This decrease in membrane-gold after prolonged exposure to thrombin correlated with the loss of many surface extensions from the platelets (Fig. 8 d).

Fig. 9 shows electron micrographs of sections cut from these same platelet-containing blocks but reacted against anti-actin-binding protein IgG instead of antigelsolin IgG. As observed in macrophages, anti-actin-binding protein label was more clustered and peripheral than gelsolin label and many of the gold clusters were periodically bound just beneath the plasma membrane (Fig. 9 a). There was little staining of intracellular granules. After activation, the staining pattern at the membrane did not change although there was a movement of actin-binding protein from the cell center to the cortical cytoplasm (Fig. 9, b–d).

These impressions were quantified by morphometry. Figs. 10, B and D, and 11 show that the distribution of immunogold macrophage membrane covered with a thick actin network. Gold particles, when viewed in stereo, locate preferentially at the filament-membrane interface, at points where individual filaments intersect the cytoplasmic side of the membrane (white arrows). (B) Stereo-paired micrographs showing a more central region from an unroofed macrophage. This region has only a sparse filament coat. Gold particles are selectively bound on the ends of short filaments (white arrows) that attach to the membrane. Occasionally gold aggregates also decorate bare membrane. Bar, 0.1 μm.
Figure 7 Localization of actin-binding protein within the filament networks covering the adherent membrane from mechanically unroofed macrophages. (a) Stereo-paired electron micrographs showing the distribution of actin-binding protein in a filament-rich region on the cytoplasmic side of an unroofed cell. Gold particles bind within the filament network, at points of filament-filament intersections. They are
Figure 8. Immunogold localization of gelsolin in Lowicryl sections from resting and activated platelets. (a) Representative section through a resting platelet. (b) Platelet treated with thrombin for 15 s, then fixed. (c) Platelet treated with thrombin for 1 min, then fixed. (d) Platelet incubated with thrombin for 20 min. Bars, 0.2 μm.

occasionally bound on the ends of filaments intersecting the membrane. (b) Stereo-paired micrographs from a filament-sparse region of an unroofed cell showing that anti-actin-binding protein gold label does not bind to filament ends intersecting the cytoplasmic side of the membrane. Bar, 0.1 μm.
reactive with actin-binding protein was unchanged at the membrane when platelets were activated (Fig. 10, B and D): there was no significant alteration in the number of gold particles associated with membrane between 0 and 1 min of thrombin treatment ($p \geq 0.82$). A significant shift in anti-actin-binding protein gold into the most 160 nm of cortical cytoplasm came after cell stimulation ($p \leq 0.002$). This difference disappeared after 1 min of continued stimulation,
Figure 11. Effect of platelet activation on gelsolin and actin-binding protein associated with membranes. Histograms comparing membrane-associated gold label for gelsolin against actin-binding protein in resting and activated platelets. (A) Total number of gold particles within 10 nm of the plasma membrane and total length of plasma membrane in resting and cells activated for 0.25, 0.5, 1.0, 5.0, and 20.0 min was determined. Data is expressed as gold number per μm of plasma membrane. (B) Data of A expressed as a percentage of total cellular gold at the membrane for resting and platelets activated for 15 s, 1, and 20 min. This normalizes for increased gold label at the 15-s and 1-min time points. ■, gelsolin; □, actin-binding protein.

Figure 10. Effect of thrombin stimulation on the distribution of gelsolin and actin-binding protein in platelets. Histograms comparing the distribution of gelsolin to actin-binding protein in the cytoplasm and on the membrane of resting and thrombin-activated platelets. (A and B) Gold concentration in various regions of the cell after antigelsolin or anti-actin-binding protein labeling procedures, respectively. (C and D) Data from A and B when expressed as a percentage of total label in all cellular compartments. This compares gold label between the different regions of the cells but normalizes for the increased antigelsolin gold in the activated cells. Total gold in sections through 100 platelets was analyzed to derive these data. The data is expressed as mean ± SEM. and the gold particle density/μm² failed to differ statistically from the resting cell values. The zones that describe the cell center did not acquire significant changes throughout cell activation. A population of gold bound near plasma membrane in both resting and activated cells. The percent of the total membrane-associated gold directed against actin-binding protein in resting and cells stimulated with thrombin for 15 and 60 s was 8, 6, and 7.5%, respectively (Fig. 11 B).

Discussion

This study presents the first ultra-structural evidence for gelsolin binding to actin filaments in cells and for the as-
Gelsolin purifies from cell extracts either as free molecules or in complexes with actin (Kurth et al., 1983; Chaponnier et al., 1987; Kurth and Bryan, 1984; Lind et al., 1987), and it was important to determine whether membrane-associated gelsolin bound actin filaments. Gelsolin, therefore, was stained in mechanically unroofed macrophages. Since these cells were unroofed in physiological buffers containing 10 mM EGTA, artificial binding of free gelsolin to actin in these samples should have been minimal. Gelsolin located in the proximity of the ends of short actin filaments adherent to the cytoplasmic side of membranes from unroofed cells. Gold particles were either near the membrane-attached end of filaments passing out of the three-dimensional filament network or on the ends of individual short filaments lying on the cytoplasmic surface of the plasma membrane. Filaments labeled with gold at one of their ends but never both. Whether these short actin filaments connect to the membrane by gelsolin or along their sides by other proteins (Wueste-hube and Luna, 1987) remains to be determined. Antigelsolin IgGs bind only to the "barbed" end of myosin S1 decorated gelsolin-capped actin filaments in vitro (Yin et al., 1981b), which implies that the gelsolin and the membrane converge at the barbed actin filament end. The filaments are therefore positioned such that if membrane polyphosphoinositides dissociate a gelsolin block at the barbed or fast-exchanging actin filament end, the fast-growing ends of actin filaments can serve as nuclei for actin monomers for promoting growth of the submembrane actin network in response to cell perturbation. The preponderance of label associating with actin filaments suggests that the affinity of gelsolin-actin for poly-phosphoinositides may be greater than that of free gelsolin, although differences in reactivity of the antibodies could also be an explanation. Biochemical experiments will be required to decide this question.

Gelsolin staining at the plasma membrane was more clustered than in the cytoplasm. These larger gold clusters may correspond to aggregates of gelsolin molecules, either free or in association with actin filaments. In many cases, two or more filaments end near the same spot on the membrane and have ends that label with clustered gold particles. It is likely, therefore, that each end is capped by gelsolin and that the clusters observed in cell sections correspond to these multiple filament-membrane attachment sites. This localization of gelsolin on filament ends is the first demonstration that gelsolin binds to the ends of actin filaments in the cell.

Antigelsolin staining also decorated the cytoplasmic surface of certain intracellular membranes, in particular, rough endoplasmic reticulum and mitochondria. In replicas, staining of both the limiting membrane of these structures and the ends of filaments attaching to them were observed. Staining of rough endoplasmic reticulum presumably reflects the location of newly synthesized gelsolin. The labeling of mitochondria is surprising and may indicate that gelsolin plays a role in the intracellular movement of these organelles. The interior of secondary lysosomes in macrophages also stained. This staining may represent extracellular gelsolin that is internalized together with lung surfactant (Nichols, 1976). Immunogold labeling with antigelsolin IgG contrasts with that of anti-actin-binding protein IgG. Actin-binding protein is a large (540-kD) extended molecule that cross-links actin filaments into three-dimensional networks in vitro. As expected from earlier work with detergent-permeabilized cells (Hartwig and Shevlin, 1986), gold directed against actin-binding protein heavily labeled the cortical cytoplasm of macrophages in thin sections and located at filament intersections within the cortical actin network of unroofed macrophages. Anti-actin-binding protein staining did not, however, concentrate near the macrophage plasma membrane nearly to the extent as antigelsolin label and was only rarely found on points where filaments attached to macrophage membrane. The cytoplasmic side of the plasma membrane in resting and activated platelets did label to some extent for actin-binding protein. This result is consistent with biochemical studies showing that 10% of total platelet actin-binding protein links the integral membrane glycoprotein lb to actin filaments (Fox, 1985; Okita et al., 1985; Ezzell et al., 1988).

Nature of the Interaction of Gelsolin with Membrane

Gelsolin Relocation during Platelet Activation

Platelet stimulation with thrombin causes rapid actin assembly. Gelsolin, in glutaraldehyde-fixed cells, but not actin-binding protein, moved to the plasma membrane shortly after platelets were activated by thrombin and returned to a cytoplasmic location at later times. The labeling of gelsolin, but not actin-binding protein, also increased twofold in acti-
vated platelets. The increase in gelsolin staining may reflect a conformational change in gelsolin molecules or the exposure of domains previously hidden by binding to other proteins or lipids.

The findings bear on the mechanism of gelsolin's action in agonist-stimulated cells. It is widely believed that thrombin stimulates results in a rapid cleavage of platelet membrane phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol (Rittenhouse, 1983). Soluble inositol 1,4,5-triphosphate, in turn, mediates cytosolic calcium release (Berridge, 1984). Since the majority of gelsolin molecules in resting platelets are not complexed to actin (Kurth and Bryan, 1984), cellular activation and elevation of cytosolic calcium concentrations ≥0.1 μM would be expected to activate gelsolin molecules to bind and form tight complexes with actin, a prediction verified experimentally (Kurth and Bryan, 1984; Lind et al., 1987). The present study has established that some of these complexes are gelsolin molecules at the ends of short actin filaments. Such actin oligomers could diffuse to the plasma membrane and attach via the binding site for polyphosphoinositides on gelsolin molecules (Yin et al., 1988) or to other integral membrane proteins that function in this capacity (Wuestehube and Luna, 1987). In this study, gelsolin moved to the platelet membrane within 15 s after treatment with thrombin. We did not, however, look at earlier times after thrombin treatment to see if a more rapid redistribution of gelsolin occurred. This rapid movement of gelsolin to the membrane parallels changes in cell shape, the formation of gelsolin–actin complexes in these cells (Lind et al., 1987), a fall and rise in phosphatidylinositol 4,5-bisphosphate concentration (Rittenhouse, 1983), and rises in cytosolic calcium after treatment with thrombin. Since our studies were on glutaraldehyde-fixed cells, however, which may not rapidly fix cells in the process of activating, it is not possible to precisely relate the time course of gelsolin movement to the release of these intracellular messengers. The subsequent attachment of gelsolin to membrane phosphatidylinositol 4,5-bisphosphate regenerates after its initial fall would, in turn, release gelsolin from the actin oligomers (Janney et al., 1987) explaining the dissociation of tight actin–gelsolin complexes after their accumulation in thrombin-stimulated platelets and provide nuclei for rapid subsequent actin filament assembly.

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