Gelsolin–Actin Interaction and Actin Polymerization in Human Neutrophils

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Abstract. The fraction of polymerized actin in human blood neutrophils increases after exposure to formyl-methionyl-leucyl-phenylalanine (fmlp), is maximal 10 s after peptide addition, and decreases after 300 s. Most of the gelsolin (85 ± 11%) in resting ficoll-hypaque (FH)-purified neutrophils is in an EGTA resistant, 1:1 gelsolin-actin complex, and, within 5 s after 10⁻⁷ M fmlp activation, the amount of gelsolin complexed with actin decreases to 42 ± 12%. Reversal of gelsolin binding to actin occurs concurrently with an increase in F-actin content, and the appearance of barbed-end nucleating activity. The rate of dissociation of EGTA resistant, 1:1 gelsolin–actin complexes is more rapid in cells exposed to 10⁻⁷ M fmlp than in cells exposed to 10⁻⁹ M fmlp, and the extent of dissociation 10 s after activation depends upon the fmlp concentration. Furthermore, 300 s after fmlp activation when F-actin content is decreasing, gelsolin reassociates with actin as evidenced by an increase in the amount of EGTA resistant, 1:1 gelsolin–actin complex. Since fmlp induces barbed end actin polymerization in neutrophils and since in vitro the gelsolin–actin complex caps the barbed ends of actin filaments and blocks their growth, the data suggests that in FH neutrophils fmlp-induced actin polymerization could be initiated by the reversal of gelsolin binding to actin and the uncapping of actin filaments or nuclei. The data shows that formation and dissociation of gelsolin–actin complexes, together with the effects of other actin regulatory proteins, are important steps in the regulation of actin polymerization in neutrophils. Finally, finding increased amounts of gelsolin–actin complexes in basal FH cells and dissociation of the complex in fmlp-activated cells suggests a mechanism by which fmlp can cause actin polymerization without an acute increase in cytosolic Ca²⁺.

Binding of chemotactic factors, such as the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fmlp), to receptors on the neutrophil plasma membrane triggers a variety of motile responses including locomotion, phagocytosis, projection of pseudopodia, aggregation, and secretion (35). Actin polymerizes rapidly upon stimulation of neutrophils by the chemotactic peptide (11, 14, 15, 16, 32, 42), and considerable evidence indicates that this polymerization is essential for motility (1, 7, 18, 48). Stimulation of human neutrophils causes increased phosphoinositide turnover, diacylglycerol accumulation (9, 41), and a transient elevation of the intracellular free Ca²⁺ and cAMP concentrations (31, 35, 44). These changes are possible signal mechanisms for transmitting membrane receptor perturbations into actin assembly. The increase in intracellular Ca²⁺ is not necessary for the actin polymerization response (2, 17, 34, 36), but still has a modulatory effect on actin polymerization after fmlp exposure (2).

Several actin-associated proteins control actin assembly in vitro and may, through intermediary signals, link fmlp binding to the neutrophil surface to intracellular actin polymerization (24, 39). The studies presented here focus on the role of one of these actin-associated proteins, gelsolin, in regulating neutrophil actin polymerization. Gelsolin is of interest because it is regulated in vitro by calcium and by membrane polyphosphoinositides and because it can theoretically promote actin assembly or disassembly, depending on the influence of these effectors (20, 22, 23, 26, 46, 47). In vitro calcium rapidly causes gelsolin to mediate F-actin disassembly (4, 22) by noncovalent severing of actin filaments, a process that drastically shortens filaments and that solvates cross-linked actin networks (22). In the presence of micromolar calcium and G-actin, gelsolin more slowly associates with two actin monomers and nucleates them for assembly in the slow growing direction, the direction defined as pointed by the arrowhead conformation that myosin head frag-
Materials and Methods

Preparation of Human Neutrophils

Human neutrophils were prepared from peripheral blood by Howard's modification (42) of Boyum's technique (3). Blood samples were drawn from volunteers, anticoagulated with EDTA (7 mM final concentration), and sedimented in 5% wt/vol Dextran 70 in normal saline (Pharmacia Fine Chemicals, Piscataway, NJ) for 1 h to produce leukocyte-rich plasma. Red blood cells remaining were lysed in 0.86% NH₄Cl, resuspended in PBS (pH 7.4) and mixed leukocytes in Ca²⁺ and Mg²⁺-free Hank's balanced salt solution were fractionated on ficoll-diatrizoate gradients at 25°C for 45 min. Platelets prepared by gel filtration had high complex levels that fell during incubation at 37°C. Thereafter, thrombin or ADP induced transient complex formation in concert with actin polymerization (29). The study described here extends these investigations to the human neutrophil, a cell type extensively characterized with respect to signal response coupling. In addition, the formation of barbed end nucleation activity in neutrophil extracts, recently described by Carson et al. (6), was correlated with the state of gelsolin–actin complexes.

Preparation of Triton-insoluble Cytoskeletons and Triton-soluble Supernatants

These preparations were made as previously described (10, 42). Briefly, cells in HBSSG⁺⁺ were incubated for 10 min at 37°C and then exposed either to fmlp (Sigma Chemical Co., St. Louis, MO) or to DMSO <0.0001 vol % (the vehicle used to dissolve fmlp) for various times by adding an equal volume of HBSSG⁺⁺ containing DMSO or fmlp. Reactions were terminated by addition of an equal volume of lysing buffer (2.0% Triton X-100, 80 mM EGTA, 20 mM imidazole HCl, 7 mM diisopropylfluorophosphate (pH 7.15)). In preliminary experiments (Figs. 3 and 4), cells were vortexed and immediately centrifuged at 15,700 g for 1 min to separate Triton-soluble supernatants and Triton-insoluble cytoskeletons. Initially, the supernatant fluids were frozen in liquid nitrogen for detection of gelsolin–actin complexes or were analyzed for total polypeptide composition by SDS-PAGE. In subsequent more detailed experiments, cells were incubated for 10 min with fmlp, centrifuged, and the supernatants were immediately used to quantify gelsolin–actin complexes and cytoskeleton-associated actin as described below. No differences were found between fresh and frozen specimens.

To eliminate variations between cell preparations determinations of F-actin content, nucleating activity, gelsolin–actin complex, and cytoskeleton-associated actin were measured concurrently on the same cell preparation. For F-actin content and nucleating activity 0.5-ml aliquot of cells was activated by brisk addition of 0.5 ml of 2x final fmlp or DMSO in HBSSG⁺⁺ and fixed with formalin at indicated time intervals. The zero time point represents simultaneous addition of formalin and fmlp. The order of timed samples was varied to assure reproducibility. Each timed sample was then split into two aliquots, one for F-actin determination and one for nucleating activity from fixed cells.

For gelsolin–actin complex analysis and cytoskeleton-associated actin (CAA) after 10-min incubation at 37°C a separate 0.125-ml aliquot of cells was activated by addition of 0.125 ml of 2x fmlp or DMSO in HBSSG⁺⁺ and, at 2-s intervals, 0.250 ml of lysis buffer containing diisopropylfluorophosphate was briskly added, and then separated into Triton-soluble supernatants and Triton-insoluble cytoskeletons as noted above. Order of samples with respect to duration of exposure to fmlp was varied to assure reproducibility.

Measurement of CAA

Triton-insoluble cytoskeletons pelleted from 2.5 × 10⁶ neutrophils were dissolved in solubilizing buffer (10% SDS, 4 M urea, 20% glycerol, 2% mercaptoethanol, Tris-HCl, pH 8.0) at 100°C for 10 min. 30- or 100-μl samples of solubilized cytoskeletons and whole cells were run on 5–15% SDS-PAGE. In subsequent more detailed experiments, cells were incubated for 10 min with fmlp, centrifuged, and the supernatants were immediately used to quantify gelsolin–actin complexes and cytoskeleton-associated actin as described below. No differences were found between fresh and frozen specimens.

Using and optimizing the assay described here, the following reagents were utilized: human neutrophils, anticoagulated with EDTA (7 mM final concentration), and volunteers. Blood samples were drawn from volunteers, anticoagulated with EDTA (7 mM final concentration), and sedimented in 5% wt/vol Dextran 70 in normal saline (Pharmacia Fine Chemicals, Piscataway, NJ) for 1 h to produce leukocyte-rich plasma. Red blood cells remaining were lysed in 0.86% NH₄Cl, resuspended in PBS (pH 7.4) and mixed leukocytes in Ca²⁺ and Mg²⁺-free Hank's balanced salt solution were fractionated on ficoll-diatrizoate gradients at 25°C for 45 min. Platelets prepared by gel filtration had high complex levels that fell during incubation at 37°C. Thereafter, thrombin or ADP induced transient complex formation in concert with actin polymerization (29). The study described here extends these investigations to the human neutrophil, a cell type extensively characterized with respect to signal response coupling. In addition, the formation of barbed end nucleation activity in neutrophil extracts, recently described by Carson et al. (6), was correlated with the state of gelsolin–actin complexes.
Figure 2. Partitioning of gelsolin between Triton-insoluble cytoskeletons and Triton-soluble supernatants in fmlp-activated neutrophils. Neutrophils (2 × 10^6 cells) were exposed to control solvent (D) or 5 × 10^{-7} M fmlp for the indicated times at 37°C, then lysed with Triton lysis buffer, and separated into insoluble cytoskeletons and soluble supernatants. Cytoskeletons and supernatants from 2 × 10^6 cells were run on 5-15% SDS-PAGE, transferred to nitrocellulose, and stained with goat anti–rabbit gelsolin and HRP-tagged anti-goat IgG. Shown are immunoblots from a representative experiment. MW, molecular weight markers; WC, 2 × 10^6 intact neutrophils.

Howard et al. Gelsolin and Actin in Neutrophils 1985
expressed as moles of actin per mole of gelsolin at each indicated time, such that a value of 1.0 means all gelsolin is bound to actin in an EGTA-resistant, 1:1 complex.

Results

Time Course of Formyl Peptide-induced Actin Polymerization in Neutrophils at 37°C

Activation of ficoll-Hypaque (FH)-purified neutrophils with maximal doses of fmlp (>10^{-7} M) caused rapid polymerization of actin of a magnitude similar to that previously reported (16, 45). Peptide-induced polymerization was documented both as increases in CAA in operationally defined Triton-insoluble residues and as increases in F-actin content quantified by NBD phallacidin binding (see Fig. 1). Increase in both were maximal within 10 s after exposure to 10^{-7} M fmlp, remained static for at least 30 s, and after 300 s declined toward basal values. These results predict that if gelsolin-actin interactions modulate actin polymerization, alterations in gelsolin-actin interactions should occur within the first 10 s after fmlp activation at 37°C.

Gelsolin–Actin Interactions: Partitioning of Gelsolin

As shown in Fig. 2, practically all gelsolin was in the Triton-soluble supernatant of control cells and little or none remained in the Triton insoluble cytoskeleton. After fmlp activation of the neutrophil, gelsolin remained in the Triton-soluble supernatant and did not partition into the Triton-insoluble cytoskeleton after fmlp activation. These results show that gelsolin is available in the Triton-soluble supernatant for analysis.

Gelsolin–Actin Interactions: Quantity of EGTA-resistant, 1:1 Gelsolin–Actin Complex in fmlp-activated Neutrophils

Shown in Figs. 3 and 4 B are results of gelsolin-actin complex determination in five replicate samples of FH neutrophils from one individual on a single day. Fig. 3 shows gels of proteins immunoadsorbed onto monoclonal antigelsolin-coated beads. Note that (a) the intensity of the 90-kD gelsolin band is constant in all samples before and after activation, and (b) in gels of immunoprecipitates from fmlp-activated cells, the intensity of the 43-kD actin band decreases. As shown in Fig. 4 B, the actin/gelsolin ratio is 0.62 ± 0.08 (mean ± SD, n = 5) in control cells and 0.19 ± 0.07 (mean ± SD, n = 5) in cells activated with 5 × 10^{-7} M fmlp for 3–5 s. The results suggest that in FH-purified neutrophils, fmlp causes a decrease in the amount of gelsolin bound to actin in an EGTA-resistant, 1:1 complex.

Similarly, Fig. 4 A shows results from six trials with FH-purified neutrophils from six different donors on six different days. In control cells, the actin/gelsolin ratio is 0.85 ± 0.11 (mean ± 1 SD, n = 6) and within 3–5 s after addition of 5
Kinetics and fmlp Concentration Dependence of Gelsolin–Actin Interactions in FH-purified Neutrophils

To define the kinetics of change in gelsolin–actin interactions and the relationship of gelsolin–actin interactions to actin polymerization in fmlp-activated neutrophils more clearly, we simultaneously determined the F-actin content and the amount of EGTA-resistant, 1:1 gelsolin–actin complex in FH-purified neutrophils activated with fmlp. Fig. 5 A shows the SDS-PAGE of Triton soluble cytoskeletons and Fig. 5 B shows the SDS-PAGE of proteins immunoabsorbed from Triton-soluble supernatants with antigelosin-coated beads. There is a rapid increase in the amount of CAA after fmlp activation as evidenced by increased intensity of the 43-kD bands (Fig. 5 A). The increase in CAA is maximal by 10 s, remains elevated at 40 s and 300 s. In parallel, (see Fig. 5 B) proteins immunoabsorbed onto antigelsolin-coated beads reveal a relatively constant intensity of the 90-kD gelosin band as well as the IgG heavy chain and the IgG light chain. In contrast, a progressive decrease in the intensity of the 43-kD actin band is observed. The decrease in the actin band is maximal 10 s after fmlp activation and becomes more intense 40 and 300 s after fmlp-activation. These qualitative results suggest that in the fmlp-activated, FH-purified cell gelsolin binding to actin decreases and that reversal of gelsolin binding to actin accompanies fmlp-induced actin polymerization.

Quantitative changes in the actin/gelsolin ratio (a reflection of the fraction of gelsolin bound to actin in an EGTA-resistant, 1:1 gelsolin–actin complex) and quantitative changes in the F-actin content of cells exposed to $10^{-7}$ M and $10^{-9}$ M fmlp are shown in Fig. 6. Immunoprecipitates from control cell extracts exhibit an actin/gelsolin ratio of $0.82 \pm 0.11$ (mean $\pm 1$ SD, $n = 4$) that is constant for 300 s and no change in F-actin content during 300 s. In immunoprecipitates of extracts from both $10^{-9}$ M and $10^{-7}$ M fmlp-activated neutrophil, the actin/gelsolin ratio declines to $0.11 \pm 0.04$ (mean $\pm 1$ SD, $n = 3$) and $0.25 \pm 0.10$ (mean $\pm 1$ SD, $n = 4$) after a 4- and 8-s exposure to $10^{-7}$ M and $10^{-9}$ M fmlp. 300 s after exposure to either $10^{-7}$ M or $10^{-9}$ M fmlp, the actin/gelsolin ratio rebounds towards higher values ($0.47 \pm 0.14$ and $0.70 \pm 0.20$; mean $\pm 1$ SD, $n = 4$) and the F-actin content turns toward baseline values. These results (a) confirm that in extracts of resting FH-purified neutrophils, much of the cytosolic gelsolin is bound to actin in an EGTA-resistant, 1:1 gelsolin–actin complex; (b) show that within 4–8 s after fmlp-activation EGTA-resistant gelsolin binding to actin in the extracts is reversed and that at longer time periods (≥300 s) gelsolin reassociates with actin in the extracts. These changes in gelsolin–actin interactions are a mirror image of changes in the F-actin content of neutrophils and occur concurrently with the polymerization and depolymerization of actin in fmlp-activated cells.

Comparisons of temporal changes of actin/gelsolin ratio in FH neutrophils activated with $10^{-7}$ M and $10^{-9}$ M fmlp also suggest that the kinetics and extent of reversal of gelsolin binding to actin depend upon fmlp concentration. As can be seen in Fig. 6, both the rate and extent of the reversal of EGTA-resistant gelsolin binding to actin are slightly less in extracts derived from $10^{-9}$ M fmlp compared with $10^{-7}$ M fmlp. Specifically, a significant decrease in the actin/gelsolin ratio is detectable after 4 s exposure to $10^{-7}$ M fmlp and after $\geq 6$ s exposure to $10^{-9}$ M fmlp. As shown in Fig. 7, the extent of reversal of EGTA-resistant gelsolin binding to actin in neutrophil extracts 10 seconds after fmlp activation of FH neutrophils is proportional to the fmlp concentration. A decrease in actin/gelsolin ratio is observed in extracts of cells exposed to $>10^{-10}$ M fmlp. Results at the $10^{-10}$ M fmlp concentration are equivocal; however, the actin/gelsolin ratio is clearly decreased at concentrations of fmlp $>5 \times 10^{-10}$ M.
Appearance of Barbed End Nucleating Activity and gelsolin-actin complex in neutrophils exposed to $10^{-7}$ M fmlp; B shows the same parameters in neutrophils exposed to $10^{-9}$ M fmlp.

Relationship of Gelsolin-Actin Interactions to the Appearance of Barbed End Nucleating Activity

As shown in Fig. 8, concurrent with the reversal of EGTA-resistant gelsolin binding to actin, there is rapid appearance of increased amounts of barbed end nucleating activity in neutrophil extracts. The amount of the barbed end nucleating activity is greater in the presence of $10^{-7}$ M than in the presence of $10^{-9}$ M fmlp; however, in both cases, the nucleating activity increases as the EGTA-resistant binding of gelsolin to actin is reversed. The temporal appearance of barbed end nucleating activity coincides with the progressive reversal of gelsolin binding to actin and the increase in F-actin content (Fig. 6) at both fmlp concentrations. Furthermore, at times >40 s after fmlp activation when gelsolin reassociates with actin, a decline in the barbed end nucleating activity is observed. These data are consistent with the notion that gelsolin binding to the barbed end of actin filaments in neutrophils regulates their availability for monomer addition and growth in the FH neutrophil and that uncapping of barbed ends of actin filaments in neutrophils is an important step in regulation of actin filament growth.

Discussion

The studies presented in this paper describe the temporal relationship between changes in gelsolin–actin interactions, the appearance of barbed end nucleation sites for actin polymerization and the assembly of a detergent-resistant actin skeleton in ligand-activated neutrophils. The results show that (a) in basal FH-purified neutrophils, most gelsolin is tightly complexed with actin; (b) in fmlp-activated FH neutrophils, gelsolin–actin complexes rapidly dissociate and then slowly reform as actin accumulates in the Triton-insoluble cytoskeleton; (c) the barbed end nucleating activity and the F-actin content of activated neutrophils increases as the amount of gelsolin–actin complex decreases. These results are generally consistent with a mechanism for fmlp-induced actin polymerization that involves the formation and dissociation of gelsolin–actin complexes to yield actin oligomers that provide barbed ends for nucleation of actin assembly.

Any model that describes the regulation of actin polymerization in neutrophils must take these observations into consideration and must also explain the inconsistencies we observe between the expected behavior of gelsolin in vitro and the observed behavior of gelsolin in fmlp-activated cells. The model should explain why gelsolin, an actin binding protein, is not bound to the Triton-insoluble cytoskeleton; how, if gelsolin is a Ca$$^{++}$$-regulated actin binding protein, its binding to actin is reversed; why cytoskeletal actin accumulation is halted after 10 s when nuclei are still available; and, why the extent of decrease in gelsolin–actin complex is not equal to the extent of increase in nucleating activity. One possible model for actin polymerization in neutrophils is shown in Fig. 9. In this model, the creation of and elongation of nuclei are the central focus, and three independently reversible events that regulate gelsolin–actin interactions, polymerizable monomer concentration and cross-linking of oligomers are included.

The critical observation of our studies with neutrophils and previous studies with macrophages and platelets (10, 29) is that 1:1 gelsolin–actin complexes in cells can dissociate after activation. Dissociation of gelsolin–actin complexes in neutrophils occurs at a time when the cytoplasmic Ca$$^{++}$$ is increased which theoretically should cause formation of gelsolin–actin complexes (4, 31, 47). The dissociation of complexes is most likely explained by alterations in phospholipid metabolism in the cell that can be related to another pathway of gelsolin's regulation. In addition to causing actin polymerization, fmlp causes an increase in phosphatidylinositol turnover and PIP$_2$ resynthesis in neutrophils (9). The actin binding affinity of several actin binding proteins is modulated by phosphatidylinositol metabolites (5, 20, 27), and, of direct relevance to gelsolin–actin interactions, the EGTA-resistant, 1:1 gelsolin–actin complex can be dissociated by PIP and PIP$_2$ in micelles or vesicles in vitro (20, 23). Formyl peptide could therefore initiate actin polymerization in FH neutrophils at 37°C by stimulating resynthesis of PIP$_2$, which reverses gelsolin binding to actin, uncaps barbed-end actin nuclei, and thereby allows rapid monomer addition to the barbed end of the nuclei. This concept is included in the model to explain the dissociation of gelsolin–actin complexes in cells. The regulation of gelsolin's association with actin by polyphosphoinositides allows actin assembly to occur at the membrane–cytoplasm interface and are consistent with ultrastructural immunohistochemical studies that suggest that gelsolin delivers actin oligomers to the membrane after ligand-stimulated cell activation (13).

The proposed model also takes into account the observation that in the Triton-treated cell gelsolin, whether com-
plexed to actin or not, is released into the Triton-soluble supernatant and is not bound to the Triton-insoluble cytoskeleton. This observation is consistent in all cell types studied to date (10, 29) and likely reflects the fact that the net effect of gelsolin binding to actin is to produce shortened actin filaments (47). The Triton-insoluble cytoskeleton is an operationally defined structure that does not contain all the F-actin in the cell. This cytoskeleton rather contains actin in the form of cross-linked, stable filaments that are resistant to depolymerization by dilution. Since cross-linking of filaments is extremely dependent upon filament length, and since gelsolin binding shortens actin filaments, and prevents filament annealing by blocking the barbed ends of F-actin, it is not surprising that both free gelsolin and gelsolin bound to actin oligomers are excluded from the Triton-insoluble cytoskeleton. Therefore, in the proposed model actin filament, cross-linking and/or annealing can explain gelsolin exclusion from the cytoskeleton. In addition, cross-linking of filaments to cytoskeleton may deplete nuclei from the supernatant solution because cytoskeletal integration of free filaments produced by reversal of gelsolin binding decreases the available number of nuclei and thereby dampens the polymerization signal, as shown below.

The incorporation of nuclei into the cytoskeleton and the involvement of profilin may also partly explain two additional experimental observations: (a) the lack of direct quantitative relationship between the fourfold decrease in gelsolin-actin complex and the 1.5-fold increase in nucleation activity (Fig. 8A); and (b) the failure of actin to continue polymerizing despite the dissociation of gelsolin–actin complexes 10 s after activation (Figs. 6A and 8A). If gelsolin–actin interactions alone regulated actin polymerization by controlling nucleation, then actin should continue to polymerize after 10 s of activation and a direct quantitative relationship between the decrease in gelsolin–actin complex and the increase in nucleation activity should exist. The opposite is true in experimental observations. The failure of nuclei to continue to elongate after 10 s of activation can be ascribed, as shown in the model, to effects of other regulatory proteins; i.e., profilin, which regulates the ability of monomers to polymerize. In the basal cell, profilin does not sequester enough monomer to regulate actin polymerization alone (37). However, 10 s after fmlp activation when a significant fraction (75%) of monomer is integrated into Triton-insoluble filaments, profilin could sequester enough monomer to limit polymerization despite availability of nuclei or could contribute to the disappearance of oligomers that are depolymerized by dilution during Triton extraction. Detailed kinetic analysis of fmlp-induced actin polymerization (43) also suggests that a process like monomer regulation and, other than nucleation, becomes rate limiting as the F-actin content approaches a maximum in activated neutrophils.

The contrasts between gelsolin–actin interactions in platelets (29) and those reported here in neutrophils also yield interesting insights into the role of gelsolin in regulating actin polymerization. In both thrombin-activated platelets and simultaneous with F-actin content (shown in Fig. 6) and gelsolin–actin complex. Nucleating activity was measured spectrofluorometrically by monitoring rabbit skeletal muscle pyrenyl-actin polymerization initiated by fixed neutrophils. Values shown are mean ± 1 SD from four trials. A shows nucleating activity and gelsolin–actin interactions in neutrophils exposed to $10^{-9}$ M fmlp; B shows the same parameters in neutrophils exposed to $10^{-9}$ M fmlp.
the fmlp-activated neutrophil dissociation of gelsolin-actin complexes precede the polymerization of actin. However, the amount of EGTA-resistant 1:1 gelsolin-actin complex are drastically different in resting platelets and FH-purified neutrophils. In nonstimulated platelets Lind et al. found a small fraction (33%) of gelsolin complexed with actin and suggested that during the Ca**+ transient that follows thrombin activation, Ca**+-dependent binding and severing of filaments create gelsolin-actin complexes and nuclei; then actin polymerizes as gelsolin-actin complexes dissociate (29). In contrast, in resting FH-purified neutrophils, the majority (82%) of gelsolin is complexed with actin, and fmlp activation dissociates the complexes, unmasks nuclei, and permits actin polymerization. On the surface, the differences between gelsolin–actin interactions in platelets and neutrophils suggest that the role of gelsolin in regulating cytoskeletal organization is drastically different or even contradictory. However, on closer examination, the differences are more apparent than real and offer new insights into gelsolin–actin interactions in cells. The observations on gelsolin–actin interactions in platelets and neutrophils can be rectified by the fact that during purification the basal FH-purified neutrophil is “activated.” The reasons for this activation are not all known, but one factor could be the exposure of cells to endotoxin and/or FH during cell preparation (12, 19). FH neutrophils have a 46% higher basal F-actin content (mean ± SD for F-actin content 82.0 ± 3.5, n = 4) exhibit a polarized shape and produce more superoxide than neutrophils prepared under endotoxin free conditions (mean ± SD for F-actin content = 56.1 ± 7.9, n = 8) (12, 19). Furthermore, the fraction of gelsolin complexed with actin in the basal endotoxin free cell (34 ± 4%, n = 14) is lower than in the FH cell and similar to that in platelets (our unpublished observation). These data (a) show that in the basal FH-purified neutrophil gelsolin is in a form consistent with the cell having experienced a Ca**+ transient during preparation; (b) suggest how gelsolin could be involved in regulating actin polymerization in FH cells even though a Ca**+-transient is not acutely necessary for polymerization (2, 17, 34, 36; and c) minimize the apparent differences in the role of gelsolin in regulating actin polymerization in platelets and neutrophils.

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