Cancer and Metastasis Reviews **11**: 69–77, 1992.

Actin structural proteins in cell motility

C. Casey Cunningham
Divisions of Experimental Medicine and Hematology/Oncology, Brigham & Women's Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA

Key words: actin-binding protein, cytoskeleton, cell motility, gelsolin, crosslinkers, severing proteins

Summary

The machinery for cell locomotion is based in a network of polymerized actin filaments supporting the peripheral cytoplasm. This network or 'gel' consists of actin filaments in a variety of configurations, including cables, loose bundles, and branching arrays; all formed by the interaction of actin-associated proteins with actin filaments. For cell locomotion to occur, this network must be reversibly disassembled or 'solated' to allow protrusion, then re-assembled to stabilize the resulting extension. Thus, proteins to promote both 'solation' and 'gelation' of actin are important for efficient cell locomotion. Because of their distribution, control, and *in vitro* effects on actin filaments, two such proteins, gelsolin and actin-binding protein (ABP) should play especially important roles in cell motility. Support for this premise is found in *in vivo* studies of mouse kidney fibroblasts which demonstrated increased translocational locomotion after cytoplasmic gelsolin expression was increased genetically and in melanoma cells missing actin-binding protein which behave as expected for a cell unable to achieve efficient actin gelation. Since malignant transformation is known to affect the expression and distribution of several of these actin structural proteins, including gelsolin, further investigations of the role these proteins play in cell motility will be important to the determination of tumor cell motility and hence metastatic propensity.

Introduction

The machinery for cell locomotion in eukaryotic cells resides in the peripheral cytoplasm, known as the cell cortex. Early cell biologists referred to this cortical cytoplasm as existing in a 'gelled' state [1], because of it's organelle-excluding appearance and apparent resistance to deformation. However, they also noted that with pseudopodial extension the cytoplasm locally became more liquid ('solated') to allow protrusion [2]. This gel-sol transformation underlies cytoplasmic protrusion, and hence cell locomotion, and subsequent investigations into actin and the actin associated proteins have attempted to provide a molecular explanation for these reversible changes in physical state. Actin, the principle component of the cortical cytoplasm, is a 42 kD globular protein possessing the unique ability to self associate into long polymers under intracellular conditions. However, actin polymerization does not occur with equal rapidity at both ends of an actin filament. Actin binding myosin fragments, designated S1 or heavy meromyosin, decorate actin filaments to give them the appearance of the feather of an arrow, and thus the ends are referred to as either 'barbed' or 'pointed'. Under physiologic conditions, actin monomers exchange much more rapidly at the barbed ends than at the pointed ends. The polymerized actin filaments invest the peripheral cortex with an extensive network, providing it with skeletal support and strength. Investigations of purified actin filament solutions *in vitro* have implied that these filaments alone cannot account for the elasticity and firmness of the cortical cell substance. Whereas pure actin filaments tend to line up in bundles of variable

length, *in vivo* actin filaments are found in a variety of configurations and structures. Tightly bundled actin filaments provide a core of support to filopodia and microvilli [3], looser bundles insert into the adhesion plaques which anchor the cell to its substrate [4], while broader structures such as lamellae are supported by a branched network of single filaments crossing at varying angles [5]. Other factors are therefore necessary for the 'gelation' of cortical cytoplasm [6].

In this review, we will primarily concentrate upon studies of mammalian cell actin-structural proteins as they relate to cell locomotion. However, work in other organisms can also provide valuable information since, though particular mechanisms and the role of specific proteins may vary, certain principles of motility and the cytoskeleton are true across phylogenetic boundaries.

Actin crosslinking proteins

The multiplicity of actin structures described above arises from the interaction of actin filaments with proteins, sometimes called gelation proteins because actin filament solutions acquire greater viscosity and firmness when joined together by them, which orient and tie together the filaments in the various configurations. There are several major crosslinkers in mammalian cells, but related proteins serving similar functions are also found in lower organisms and there is remarkable similarity across phylogenetic boundaries between many of the actin crosslinkers [7]. Most significantly, a 270 amino acid domain identified originally at the amino termini of the subunits of alpha-actinin, Dictyostelium ABP-120, and dystrophin [8–10] has since been similarly identified at the amino-terminus of ABP subunits [11] and the beta chains of spectrin [12]. All of these proteins have two actin binding domains, allowing them to bind to two separate filaments. Fimbrin has related regions of 240 amino acids, but these occur in the middle and at the c-terminus of the protein [13]. The presence of this shared putative actin binding domain in these five proteins suggests the use of a common mechanism for binding to actin, though more detailed information about this actin binding site is limited at present.

The actin crosslinking proteins can be grouped according to the configurations produced when they interact with actin filaments, which further depends on how the two actin binding domains, or heads, are separated. Fimbrin is a 70 kDa actin bundling protein recently found to be identical to t/l plastin and these isoforms are widely expressed. Fimbrin/plastin has no intervening structure between the actin binding domains and therefore gives rise to tightly bundled filaments. More loosely bundled actin cables result from proteins such as alpha-actinin which has a short stretch of predicted alpha-helical sequence between the heads, thus separating the filaments somewhat, though still linking them in a parallel fashion. Spectrin-fodrin and actin-binding protein (non-muscle filamin) are much larger molecules with longer intervening structures and the rod domains in these proteins also have binding sites for other proteins. Spectrin was first found in erythrocytes and is a heterodimer of 240 (alpha) and 220 kDa (beta) chains which appear to be fairly rigid in structure. Two paired parallel alpha and beta strands are joined end to end, with attachment sites for the proteins called ankyrin and band 4.1 located along the strands. In the red blood cell very short (0.2 μm) actin filaments joined by spectrin are crosslinked parallel to each other at some distance apart and are attached closely to the plasma membrane, forming a two-dimensional submembranous shell around the erythrocyte. Fodrin (non-erythroid spectrin) is an isoform of spectrin found in other types of cells and a spectrin-rich cortical shell resembling that of red blood cells has also been documented inside of resting human blood platelets [14].

In contrast to the two-dimensional nature of the spectrin/actin networks, actin-binding protein (ABP), is responsible for three-dimensional gelation of actin in the peripheral cytoplasm of mammalian cells [15–17]. First identified in lung macrophages [18], electron micrographs localize ABP in the peripheral cytoplasm of these cells to the branch points of orthogonal actin filament networks [5]. ABP is a dimer of two identical subunits,

each containing an actin binding head and a tail of beta-pleated sheet repeating regions. The tails are joined at the C-terminal end parallel to each other for a short distance, however the actin binding heads are not necessarily close to one another, due to two breaks in the repeating β-sheet sequences which act as 'hinge' regions so that the two actin binding domains can swivel apart at varying angles and thus crosslink actin filaments at up to right angles. A structurally similar protein isolated from chicken gizzard smooth muscle was named filamin [19], but this protein bundles actin filaments rather than crosslinking them, is therefore less efficient in actin gelation [15], and has recently been shown to have a peptide structure that is divergent from those of human uterine and platelet ABP [20]. We therefore reserve the name filamin for the avian smooth muscle isoform of ABP. Orthogonal crosslinking of actin filaments is the most efficient way to construct a stable network per mass of filament and explains ABP's efficiency in gelling a solution of actin filaments [21].

The importance of ABP for the normal gelation of peripheral cytoplasm, inferred from studies with ABP and actin *in vitro,* is affirmed by the characteristics of human melanoma cell lines which are deficient in this protein [22]. Three separate cell lines were isolated from three human tumors and found to completely lack detectable amounts of ABP. Cells from the three ABP-deficient lines had markedly decreased translocational locomotion in a chemotactic chamber when compared to ABP-expressing lines. More striking was the finding that these cells displayed near total and continuous blebbing of the cell surface membrane, suggesting that ABP is not only important for cell locomotion but provides stabilization for the plasma membrane. Blebbing of the plasma membrane is known to occur in conditions where the peripheral cytoskeleton or its connections to the plasma membrane would be disrupted, such as with mechanical, thermal, or ultrasonic stresses [23–25]. The exaggerated and persistent blebbing observed in genetically ABP-deficient cells implies that ABP is responsible for stabilizing the cells periphery through actions on actin, on the plasma membrane or both. Poor cortical gelation due to lack of efficient crosslinking of actin filaments is one explanation. However, ABP binds to the membrane glycoprotein Gp1b in platelets [26–28] and the high affinity IgG receptor (FcγR1) in myeloid cells [29] so connecting the actin cytoskeleton to the plasma membrane by binding of ABP to membrane glycoproteins could play an important role in anchoring the membrane. Thus, loss of ABP could result in a poorly attached plasma membrane with a consequent loss of stability and both membrane blebbing and poor motility. The primary importance of ABP for the abnormal phenotype of the ABP-deficient cells was unequivocally established by inducing normal levels of ABP expression in one of the deficient lines by genetic transfection which eliminated the constitutive blebbing and improved to normal the locomotory capacity of the genetically repaired cells [30].

Actin filament severing proteins

The combination of actin crosslinking proteins with actin explains many of the structures seen in the cell periphery. However, in order for movement to occur, this firm framework must be temporarily broken down (solated) to allow protrusion. This is accomplished with the reversible assembly of the actin network by proteins that regulate the linear assembly of actin. One class of such proteins has the ability to break (sever) the noncovalent bonds between monomers in filaments and remain tightly bound to the fast exchanging (barbed) ends of the severed filaments. There are at least six related proteins in the severing family (Table 1) and these share considerable amino acid sequence homology, particularly in the actin filament severing domains [31]. All six are activated for severing actin filaments by micromolar Ca^{2+} *in vitro.* Gelsolin, villin and severin have this severing activity inhibited by polyphosphoinositides (PPI's) such as phophatidyl inositol 4,5-bisphosphate (PIP_2) and phosphatidyl inositol 4-phosphate (PIP) [32–34]. Gelsolin and villin, in addition, have the ability to nucleate actin filaments to promote rapid polymerization of new filaments. These proteins are thus under the control of messengers believed impor-

tant in the signalling pathways of motile cells and are thus poised to play a key role in cell locomotion. However, villin is localized to the microvilli of the intestinal epithelia [35] and the related severing proteins adseverin, and scinderin are similarly restricted to adrenal medulla and chromaffin cells respectively [36, 37]. Therefore, in mammalian cells, gelsolin is the only severing protein currently known with the necessary wide distribution in cells to act as a central transducer of signal for surface motility.

Signal to pseudopod: A hypothesis

With the binding of chemoattractants to the appropriate receptor on the cell surface, a small influx of Ca^{2+} ions occurs. Simultaneously, phospholipase C is activated and cleaves PIP_2 in the membrane to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 causes the release of internal stores of Ca^{2+}. Gelsolin, normally bound by PIP_2, is now released and activated by Ca^{2+} to begin severing and capping actin filaments (Fig. 1). The result is a rapid disassembly of the actin network, allowing protrusion of a cytoplasmic extension such as a pseudopod. After attachment to the substrate, the extended cytoplasm must be 're-gelled' in order to provide a stable structure for the rest of the cell body to pull against. Therefore, in a 2nd phase of signalling, phosphotidylinositol 4-phosphate kinase regenerates PIP and PIP_2, and Ca^{2+} is sequestered or extruded. With the fall in Ca^{2+} and increase in PIP and PIP_2, gelsolin is inactivated and moves to the membrane bound to PIP_2. The newly freed actin filament ends can then rapidly add monomers and a phase of actin polymerization occurs. The newly elongated actin filaments are then crosslinked by crosslinking proteins such as ABP to reestablish the actin network of the resting cell and stabilize the cell protrusion.

According to this hypothesis, optimal locomotion depends upon the ability of a cell to both solate and gel its cytoplasm efficiently and therefore both actin severing and filament crosslinking proteins would be important to this balance. Studies of gelsolin in mammalian cells are consistent with this idea [38]. Full-length human cytoplasmic gelsolin cDNA was stably transfected into mouse kidney fibroblasts resulting in six clonal sublines overexpressing gelsolin in amounts varying from 25% to 125% above basal levels. All the gelsolin transfected cells had an increased rate of motility over control cells in two independent assays of locomotion, and this increase in motility was directly proportional to the amount of gelsolin overexpression in each assay (see Fig. 2). These results suggest that, at least in these fibroblasts, the level of gelsolin expression is rate limiting for cell motility. This does not imply, however, that complete ablation of gelsolin from these fibroblasts would result in a complete loss of motile ability. Various cytoskele-

Table 1. Actin filament severing proteins

Protein	$M_r \times 10^3$	Function	Regulation	Localization
Gelsolin	82	severs, caps, nucleates	activated by Ca^{2+}, inhibited by PPI's	ubiquitous
Villin	95	severs, caps, nucleates, bundles	activated by Ca^{2+}, inhibited by PPI's	intestinal microvilli
Adseverin/scinderin	74	severs, caps, nucleates	activated by Ca^{2+}, inhibited by PPI's	adrenal medulla/chromaffin cells
Severin	40	severs, caps	activated by Ca^{2+}, inhibited by PPI's	*Dictyostelium amoebae*
Fragmin	42	severs, caps, nucleates	activated by Ca^{2+}, inhibited by actin phosphorolation	*Physarum polycephalum*
Fragmin 60	60	severs, caps, nucleates	activated by Ca^{2+}	*Physarum polycephalum*

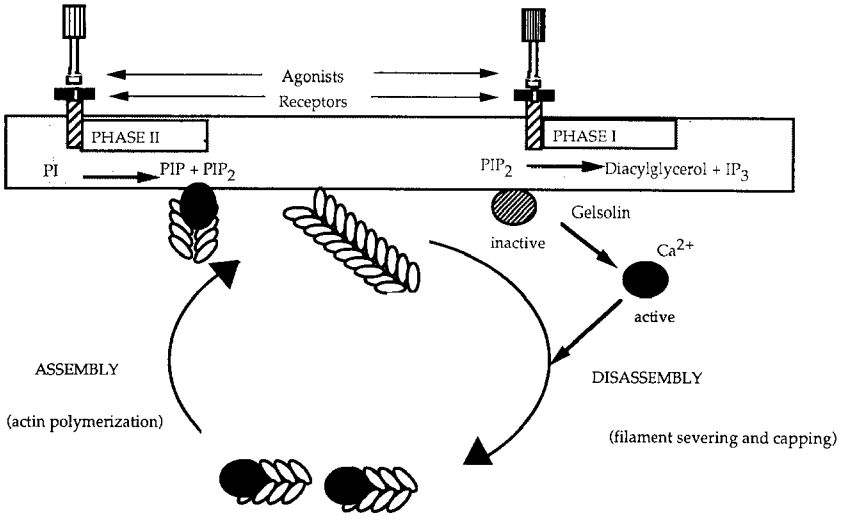

Fig. 1. Schematic of possible model for the receptor-response coupling underlying cell protrusion as explained in the text. The filaments polymerized during the assembly phase would then be crosslinked by proteins such as ABP to form a stable network. This model does not address the role of other actin-associated proteins which control the rate of linear filament assembly by sequestering monomers or capping filament ends, though these may be important to locomotion.

tal proteins have been genetically ablated in Dictyostelium discoideum amoebae and yet the organisms retained motile function. The gelsolin homologue in Dictyostelium is severin, yet its complete deletion did not significantly alter motility. Similarly, the loss of either of the actin crosslinkers alpha-actinin or ABP-120 still allowed the organisms to crawl, albeit with some reduced efficiency. Many actin-associated proteins therefore, may have overlapping functions, so that loss of a single protein does not result in complete loss of a particular function, such as gelation. Thus only partial alteration in motility occurs with the ablation of a particular gelation protein. Also, despite sequence similarity, the homologues of mammalian proteins found in Dictyostelium may not play precisely the same role in those organisms. For instance, severin does not nucleate the formation of new actin filaments as gelsolin does, and this may be an important component in cytoplasmic extension. This point is made again with the human melanoma cell lines which are completely deficient in actin-binding protein described above. Cells from these lines are still able to translocate, though with markedly reduced efficiency compared to other lines which express ABP.

Actin associated proteins and malignancy

Not all tumor cells have the same propensity to metastasize. Many factors underlie this difference but an important determinant of metastatic capability is the motile ability of the individual tumor cells, and this varies even among malignancies of the same tissue. One potential explanation for part of these differences lies in the effects of transformation on the actin cytoskeleton. When transformed by a variety of agents, many cell types have been shown to undergo loss of bundled actin filaments known as stress fibers and the organization of the actin filament array decreases generally [39–41]. In addition, the expression of several cytoskeletal proteins is altered. For example, several investigators have found that both the absolute amount of total actin and the relative ratio of the various actin isoforms changes in transformation [39, 40, 42, 43]. Gelsolin, in particular, has shown

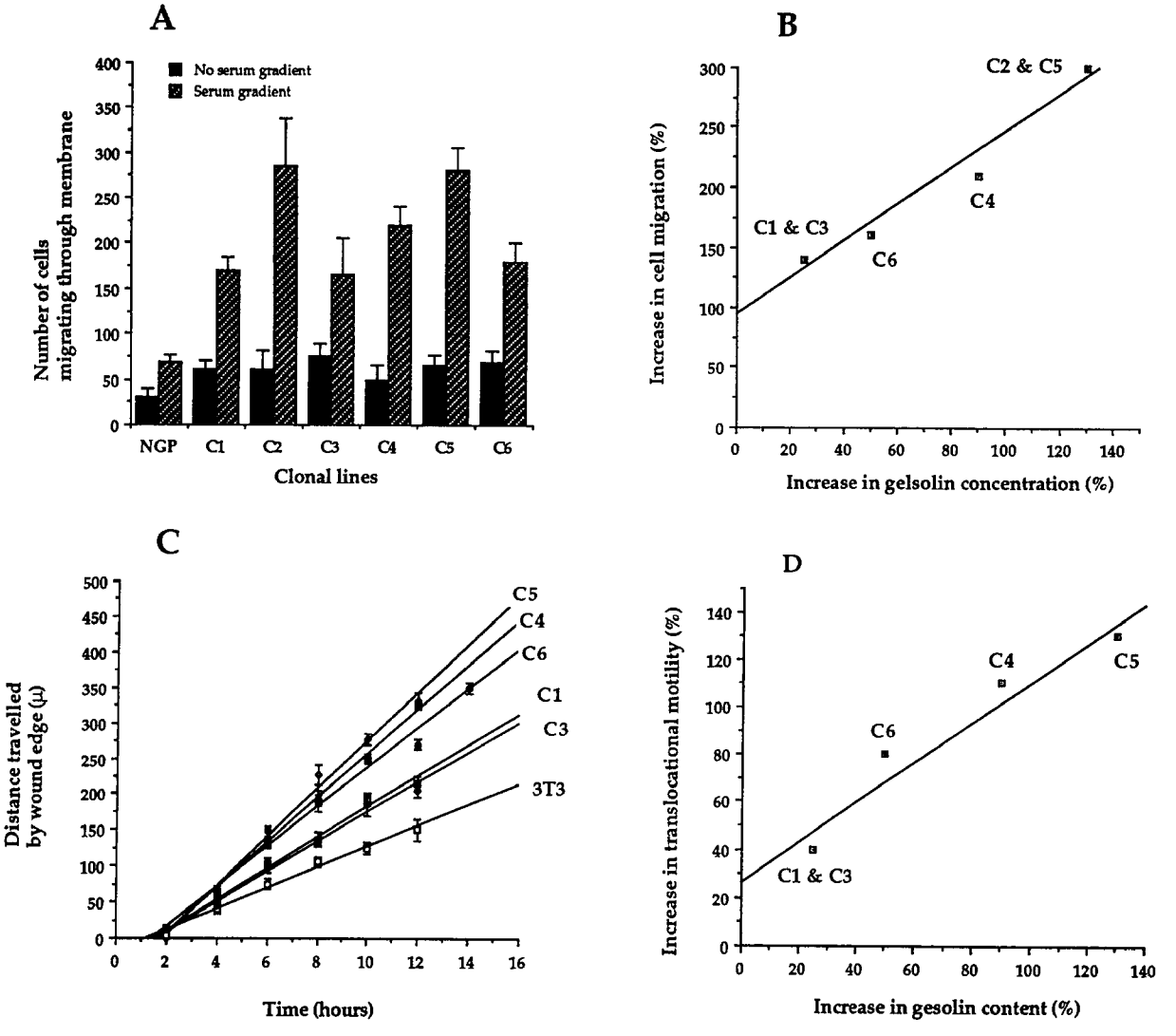

Fig. 2. Motility of gelsolin-transfected NIH-3T3 fibroblasts correlates directly with gelsolin expression. (A & B) Number of cells migrating through a two-compartment chamber in response to a gradient of serum for six lines (C1–C6) compared to control (NGP). The increase in motility is linear with gelsolin expression. (C & D) Rate of locomotion of the cells along the edge of a wound in a plate of confluent cells. Again the increase in motility is linear with gelsolin expression. (Figure reprinted with permission copyright 1991 by the AAAS.)

dramatic effects in expression with malignancy, being the most dramatically downregulated protein in fibroblasts transformed by either H-ras [44] or SV40 [45]. Interestingly, if the fibroblasts revert back to a nontransformed state, gelsolin levels increase markedly and these cells cannot then be re-transformed. There also is a direct correlation between gelsolin expression and stress fiber content and an inverse correlation between gelsolin and tumorigenic potential [46]. These changes extended to *in vivo* immunostained sections of human breast cancers where a sharp decrease in gelsolin staining compared to normal breast tissue was seen [47]. This suggests that gelsolin downregulation accompanies transformation generally. Paradoxically, in many cases transformation is accompanied by increased cell motility, again demonstrating the complexity of the balance between the various cytoskeletal proteins and cell locomotion.

Conclusions

1. Cells are supported by a network of polymerized actin filaments configured into a variety of structures by actin structural proteins which can bundle the filaments together into tight or loose cables, or crosslink them at high angles to form a stable framework. Actin-binding protein provides most of the high-angle filament crosslinking in mammalian cells.
2. In order for movement to occur, this framework must be reversibly disassembled and filament severing proteins are responsible for this action. Chief among these is gelsolin, which severs and caps existing filaments and nucleates new filaments, all under the control of Ca^{2+} and PPI's.
3. Efficient cell movement probably results from a balance between the forces deconstructing and constructing this framework, and protein functions overlap to some extent.
4. Malignant transformation alters the expression of some actin structural proteins as well as the organization of the cytoskeleton. The most prominently downregulated protein is gelsolin.

Key unanswered questions

1. What is the force driving protrusion of cytoplasmic extensions after the solation of the actin network? Several hypotheses have been put forth, including membrane bending, gel swelling, and contraction elsewhere in the cell.
2. What role do proteins which control actin monomer availability play in cell locomotion? Monomer sequestering proteins regulate actin polymerization, and this could have important effects on motility.
3. Do specific oncogenes alter the expression of specific cytoskeletal proteins and does this explain the differences in motility seen between tumor cells?
4. What is the mechanism by which transformation downregulates proteins such as gelsolin and could increased gelsolin expression lead to resistance to transformation?

Acknowledgements

This work is supported by NIH grants A1 08051, HL 19429, DK 38452, and HL 02158 and grants from the Whittaker Foundation and The Council for Tobacco Research.

References

1. Muller OF: Animalcula infusoria fluviatilia et marina, quae detexit systematice descipset et ad vivum delineari curavit. In: Molleri N, Copenhagen (ed) 10–11, 1786
2. Stossel TP, Janmey PA, Zaner KS: Cytomechanics. In: Bereiter-Han J, Anderson OR, Reif WE (ed) Cytomechanics. Springer, Berlin 131–153, 1987
3. Mooseker MS: Annu Rev Cell Biol 1: 209–241, 1985
4. Burridge K, Fath K, Kelly T, Nuckolls G, Turner C: Annu Rev Cell Biol 4: 487–525, 1988
5. Hartwig JH, Shevlin P: The architecture of actin filaments and the ultrastructural location of actin-binding protein in the periphery of lung macrophages. J Cell Biol 103: 1007–20, 1986
6. Stossel TP: Contribution of actin to the structure of the cytoplasmic matrix. J Cell Biol 99: 15s–21s, 1984
7. Schleicher M, Andre E, Hartmann H, Noegel AA: Actin-binding proteins are conserved from slime molds to man. Dev Genet 9: 521–30, 1988
8. Meyer RK, Aebi U: Bundling of actin filaments by alpha-actinin depends on its molecular length. J Cell Biol 110: 2013–24, 1990
9. Koenig M, Monaco AP, Kunkel LM: The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. Cell 53: 219–26, 1988
10. Noegel AA, Rapp S, Lottspeich F, Schleicher M, Stewart M: The Dictyostelium gelation factor shares a putative actin binding site with alpha-actinins and dystrophin and also has a rod domain containing six 100-residue motifs that appear to have a cross-beta conformation. J Cell Biol 109: 607–18, 1989
11. Gorlin Y, Yamin R, Egan S, Stewart M, Stossel T, Kwiatkowski D, Hartwig J: Human Endothelial Actin-binding Protein (ABP-280, Nonmuscle Filamin); A Molecular Leaf Spring. JCB 111: 1089–1105, 1990
12. Byers T, Husain-Chishti A, Dubreil R, Branton D, Goldstein L: Sequence similarity of the amino-terminal domain of *Drosophilia* beta spectrin to alpha-actinin and dystrophin. J Cell Biol 109: 1633–1641, 1989
13. de Arruda M, Watson S, Lin C-S, Leavitt J, Matsudaira P: Fimbrin is a homologue of the cytoplasmic phosphoprotein plastin and has domains homologous with calmodulin and actin gelation proteins. J Cell Biol 111: 1069–1079, 1990
14. Hartwig JH, Desisto M: The Cytoskeleton of the Resting Human Blood Platelet: Structure of the Membrane Skele-

ton and Its Attachment to Actin Filaments. JCB 112: 407–425, 1991
15. Brotschi EA, Hartwig JH, Stossel TP: The gelation of actin by actin-binding protein. J Biol Chem 253: 8988–93, 1978
16. Mimura N, Asuro, A: Actin-related gelation of Ehrlich tumour cell extracts is reversibly inhibited by low concentrations of Ca^{2+}. Nature 272: 273–276, 1978
17. Corwin HL, Hartwig JH: Isolation of actin-binding protein and villin from toad oocytes. Dev Biol 99: 61–74, 1983
18. Hartwig JH, Stossel TP: Isolation and properties of actin, myosin, and a new actinbinding protein in rabbit alveolar macrophages. J Biol Chem 250: 5696–705, 1975
19. Wang K, Ash JF, Singer SJ: Filamin, a new high-molecular-weight protein found in smooth muscle and non-muscle cells. Proc Natl Acad Sci USA 72: 4483–6, 1975
20. Hock RS, Davis G, Speicher DW: Purification of human smooth muscle filamin and characterization of structural domains and functional sites. Biochemistry 29: 9441–51, 1990
21. Janmey PA, Hvidt S, Lamb J, Stossel TP: Resemblance of actin-binding protein/actin gels to covalently crosslinked networks. Nature 345: 89–92, 1990
22. Cunningham CC, Byers HR, Hartwig JH, Stossel TP, Kwiatkowski DJ: Morphologic Properties of Three Actin-binding Protein (ABP, non-muscle filamin) – Deficient Human Malignant Melanoma Cell Lines are Consistent with Reduced Cortical Actin Gelation. J Cell Biol 109: 277a, 1989
23. Davies WA, Stossel TP: Peripheral hyaline blebs (podosomes) of macrophages. J Cell Biol 75: 941–55, 1977
24. Mesland DA, Los G, Spiele H: Cytochalasin B disrupts the association of filamentous web and plasma membrane in hepatocytes. Exp Cell Res 135: 431–5, 1981
25. Weiss E, Sterz I, Frimmer M, Kroker R: Electron microscopy of isolated rat hepatocytes before and after treatment with phalloidin. Beitr Pathol 150: 345–56, 1973
26. Fox JE: Identification of actin-binding protein as the protein linking the membrane skeleton to glycoproteins on platelet plasma membranes. J Biol Chem 260: 11970–7, 1985
27. Okita JR, Pidard D, Newman PJ, Montgomery RR, Kunicki TJ: On the association of glycoprotein Ib and actin-binding protein in human platelets. J Cell Biol 100: 317–21, 1985
28. Ezzell RM, Kenney DM, Egan S, Stossel TP, Hartwig JH: Localization of the domain of actin-binding protein that binds to membrane glycoprotein Ib and actin in human platelets. J Biol Chem 263: 13303–9, 1988
29. Ohta Y, Stossel TP, Hartwig JH: Ligand-Sensitive Binding of Actin-binding Protein (ABP) to Immunoglobulin G Fc Receptor I (FcγRI, CD64). Cell (in press): 1991
30. Cunningham CC, Gorlin JB, Kwiatkowski DJ, Hartwig JH, Janmey PJ, Byers HR, Stossel TP: Genetic Repair of Cortically Unstable Human Melanoma Cells Deficient in Actin-binding Protein. Science (in press): 1991
31. Andre E, Lottspeich F, Schleicher M, Noegel A: Severin, gelsolin, and villin share a homologous sequence in regions presumed to contain F-actin severing domains. J Biol Chem 263: 722–7, 1988
32. Janmey PA, Stossel TP: Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate. Nature 253: 362–4, 1987
33. Janmey PA, Matsudaira PT: Functional comparison of villin and gelsolin. Effects of Ca^{2+}, KCl, and polyphosphoinositides. J Biol Chem 263: 16738–43, 1988
34. Yin HL, Janmey PA, Schleicher M: Severin is a gelsolin prototype. Febs Lett 264: 78–80, 1990
35. Bretscher A, Weber K: Villin is a major protein of microvillus cytoskeleton which binds both G and F actin in a calcium-dependent manner. Cell 20: 839–847, 1980
36. Sakurai T, Kurokawa H, Nonomura Y: The Ca^{2+}-dependent Actin Filament-severing Activity of 74-kDa Protein (Adseverin) Resides in NTs NH_2-terminal Half. J Biol Chem 266: 4581–4585, 1991
37. Rodriquez DCA, Lemaire S, Tchakarov L, Jeyapragasan M, Doucet JP, Vitale ML, Trifaro JM: Chromaffin cell scinden, a novel calcium-dependent actin filament-severing protein. Embo J 9: 43–52, 1990
38. Cunningham CC, Stossel TP, Kwiatkowski DJ: Enhanced Motility in NIH 3T3 Fibroblasts That Overexpress Gelsolin. Science 251: 1233–1236, 1991
39. Leavitt J, Gunning P, Kedes L, Jariwalla R: Smooth muscle alpha-actin is a transformation-sensitive marker for mouse NIH 3T3 and Rat-2 cells. Nature 316: 840–2, 1985
40. Takahashi K, Heine UI, Junker JL, Colburn NH, Rice JM: Role of cytoskeleton changes and expression of the H-ras oncogene during promotion of neoplastic transformation in mouse epidermal JB6 cells. Cancer Res 46: 5923–32, 1986
41. Koffer A, Daridan M, Clarke G: Regulation of the Microfilament System in Normal and Polyoma Virus Transformed Cultured (BHK) Cells. Tissue & Cell 17: 147–159, 1985
42. Kakunaga T: Malignant transformation and alteration of actin-related proteins. Gan To Kagaku Ryoho 1987
43. Wang E, Goldberg AR: Changes in microfilament organization and surface topography upon transformation of chick embryo fibroblasts with Rous sarcoma virus. Proc Natl Acad Sci USA 73: 4065–9, 1976
44. Fujita H, Suzuki H, Kuzumaki N, Mullauer L, Ogiso Y, Oda A, Ebisawa K, Sakurai T, Nonomura Y, Kijimoto OS: A specific protein, p92, detected in flat revertants derived from NIH/3T3 transformed by human activated c-Ha-ras oncogene. Exp Cell Res 186: 115–21, 1990
45. Vandekerckhove J, Bauw G, Vancompernolle K, Honore B, Celis J: Comparative two-dimensional gel analysis and microsequencing identifies gelsolin as one of the most prominent downregulated markers of transformed human fibroblast and epithelial cells. J Cell Biol 111: 95–102, 1990
46. Banyard MR, Medveczyk CJ, Tellam RL: Microfilament organization correlates with increased cellular content of gelsolin. Exp Cell Res. 199, Mar 1990
47. Chaponnier C. Gabbiani G; Gelsolin modulation in epi-

thelial and stromal cells of mammary carcinoma. Am J Pathol 134: 597–603, 1989

Address for offprints:
C. C. Cunningham,
Division of Experimental Medicine, LMRC,
221 Longwood,
Boston, MA 02115, USA