Contribution of Actin to the Structure of the Cytoplasmic Matrix

THOMAS P. STOSSEL
Hematology/Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston, MA 02114

The realization that actin is a constituent of nonmuscle cells (1) and the identification of actin filaments in the periphery of such cells (2) opened up a new world of molecular biology. For the first time it became possible to explain in detail a body of phenomena described over two centuries concerning important aspects of cell shape, movement, and consistency. Although actin comprises only one of several intracellular fiber systems of cells, it is an extremely important one. This essay briefly reviews some present concepts of the contribution of actin to the "cytoplasmic matrix."

The appreciation of actin's existence in nonmuscle cells immediately provided some intuitively attractive ideas concerning the behavior of such cells. First, actin is a globular monomer that assembles reversibly to form long fibers, and this assembly increases the apparent viscosity of an actin solution (3). Therefore, changes in the state of assembly in different parts of a cell could account for differences in cytoplasmic consistency. Second, long actin fibers, if sufficiently stiff and especially if organized as bundles, could maintain the cell or parts of cells in a particular configuration. Third, actin, working together with myosin, could generate the contractility observed in these cells. Fourth, actin fibers could act as cables to tie parts of the cell, including its investing membrane, together. These four basic ideas, which concern "mechanical" properties of actin fibers, continue in their broadest sense to be valid. However, it has become apparent that the functions of actin, influenced by a seemingly endless number of "actin-binding proteins," are extraordinarily complex. Although this complexity seems formidable and confusing at first glance, it actually is a testimony to the marvellous versatility of this highly conserved protein (4) and to nature's ingenuity in the engineering of cytoplasm.

Research on actin in cytoplasmic structure and function has been advancing on two fronts. On one, investigators study the details of actin assembly and rheology in vitro and the influence of purified actin-associated proteins on these properties. The rheological behavior of crude cytoplasmic extracts has often provided the direction for such research. On the other, cell biologists examine the morphology of actin in cells in the light and the electron microscope and also determine the location of the actin-associated proteins in the cell.

Morphology of Actin in the Cell

The most striking feature immediately apparent from microscope studies is the apparent concentration of actin filaments in the periphery of cells. This distribution is the rule in both freely mobile ameboid cells and in tissue cells with fairly fixed shapes. Hence the cell cortex, called the ectoplasm by light microscopists, is a principal domain of actin fibers in the cytoplasmic matrix.

The actin filament architectures revealed by electron microscopy are of several types. One of the most extensively studied is the parallel bundle of actin filaments. Such bundles are evident in the brush border microvilli of epithelial cells, in the stereocilia of cochlear hair cells, in the cortical microvilli of fertilized eggs and oocytes, in the acrosomal processes of marine sperm, and in the processes extending from the surfaces of a variety of cells, including blood platelets, sea urchin coelomocytes, and mammalian cells cultivated in vitro (Fig. 1). These bundles are nearly crystalline in structure and display periodicities that facilitate detailed optical analysis. They have a unidirectional polarity, as heavy meromyosin or myosin subfragment 1 labeling has shown, in which the characteristic myosin fragment arrowheads point toward the center of the cell. Although the physiologic purposes of actin bundles of this type are hardly known, some role in stabilizing regions of the cell seems most logical. Other contributors to this supplement review the compositional and structural details of such bundles (5, 6).

Actin fibers also organize in parallel as bundles known as *stress fibers* on the ventral surface of mammalian fibroblasts and epithelial cells in culture and of certain endothelial cells in situ (7–9) (*d* in Fig. 1 *B*). The actin filaments of stress fibers seem less tightly packed and less optically ordered than the bundles mentioned above, and they need not display a unidirectional polarity (10, 11). Similar actin bundles are also visible in electron micrographs of the cytoplasm of amebas (12).

Similar to stress fiber bundles are arrays of actin filaments that orient as *annular rings* around the whole of the cell or its parts. The entire circumference of the contractile ring of dividing cells contains an annulus of actin filaments in which

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 NO. 1 PT. 2 JULY 1984 15s–21s

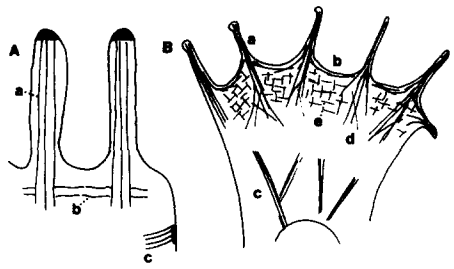

FIGURE 1 Schematic depictions of actin configurations in the periphery of prototypes of fixed and motile cells as viewed through the microscope. (*A*) Brush border epithelial cell. Microvilli filled with a core bundle of actin filaments (*a*) that project into the terminal web zone within which high molecular weight proteins (e.g., fodrin) crosslink the bases of the bundles (*b*). In the microvilli, the bundles contain, among other proteins, villin and fimbrin, proteins that bundle actin filaments in vitro. Tropomyosin is bound to the actin filaments in the bundles in the terminal web region. An annulus of actin filaments (c) invests the periphery of the terminal web and is connected to specialized regions of the plasma membrane. (*B*) A moving tissue culture cell that extends an anterior lamellipod and from which extend microspikes filled with actin filaments arrayed as parallel bundles (*a*). These actin bundles contain actin-associated proteins such as α-actinin and actin-binding protein. Parallel actin filaments also line the leading edge of the cell between the microspikes (*b*) and form stress fiber bundles on the cell's ventral surface (*c*). The stress fibers contain myosin, α-actinin, tropomyosin, and actin-binding protein molecules. Actin fibers of various lengths overlap extensively in the cortical region between the bases of the microspike bundles (*d*). An orthogonal network of short actin fibers occupies large expanses of the cell periphery (e).

neighboring filaments may be of opposite polarity (13). A similar structure encases the apical surface of brush border epithelial cells just proximal to the microvilli (5, 14) (*a* in Fig. 1*A*). Such bundles presumably have a role in contractile events associated with cytokinesis and brush border motility. Another "loose bundle" of actin filaments exists at the peripheral margin of the leading edge of blood platelets and of tissue culture cells and orients in parallel to the ruffling membrane (15, 16) (*b* and *d* in Fig. 1 *B*).

Actin fibers in the cell also orient at various angles to one another. Actin filaments splaying out from stress fibers and from the base of projections extending from the cell form oblique angle relationships with one another. In cultured fibroblasts and epithelial cells these fibers can be quite long, and they appear to overlap one another extensively (*d* in Fig. 1 *B*). This actin configuration has been called the *peripheral weave* (15, 16).

Although the actin bundles and long fibers that overlap are impressive in appearance, a substantial majority of the actin filaments in the cell cortex consists of short fibers that seem to form a relatively isotropic structure. This system of filaments was originally called a *microfilament network*, and is the main actin filament organization that cytochalasins disrupt (17). The peripheral part of Porter's microtrabecular network may in part be composed of such actin filaments (18). At least some of these filaments intersect in a perpendicular fashion to form T- and X-shaped junctions (19, 20). In the case of T-shaped structures, the polarity of the actin filament forming the base of the "T" is such that myosin fragment arrowheads point toward the vertex of the joint (19, 20). These *orthogonal networks* of actin filaments comprise much of the periphery of motile cells such as amebas, macrophages, leukocytes, and blood platelets and of mammalian tissue culture cells transformed by oncogenic viruses.

This extensive isotropic matrix of actin filaments could account for a *gel* structure long believed to exist in peripheral cytoplasm (21). A gel is definable as a solution of relatively low solute concentration that has elastic properties resembling those of a solid. Long actin fibers linked together in oblique or right-angle relationships can create such an "isotropic" gel. The pore size of the gel could determine the exclusion of organelles of certain sizes while permitting the diffusion of smaller particles and solutes. Such a cortical gel could exert tension on parts of the cell and possibly affect the cell's response to osmotic stresses (22). The perpendicular branching of actin fibers in this matrix would allow the maximum extension of the cortex with a minimum mass of actin protein and would also maximize the pore size of the matrix for diffusion. Disruption of the network could be responsible for the transition of the cytoplasmic matrix from the gel to a more fluid state in which organelles flow more freely.

Actin configurations involving monomers, oligomers, or very short filaments may also exist within the cytoplasmic matrix but are barely visible or invisible with the microscope techniques presently available. For example, actin oligomers are part of the two-dimensional network that laminates the inner surface of erythrocytes (23), and there is evidence that small filaments may also be part of the structure of neuronal processes (24), or, a controversial point, may reside in the mitotic spindle (25).

The question must always be asked whether the actin morphologies observed in the microscope are accurate reflections of "reality" or are distortions resulting from the techniques used to prepare the images. However, the structures described above have for the most part been seen with a variety of different microscopy technologies and, as elaborated below, can be reconstructed with actin and other proteins in vitro. These artificial assemblies are amenable to physical characterization, which can provide independent evidence of the structural configurations of the proteins. Therefore, I believe we can be reasonably optimistic that the actin images visualized in cells with the microscope are a first approximation of actin's structure in the living cell. Furthermore, the investigation of actin in its pure state and together with actin-modulating proteins allows us to begin to establish the mechanical properties of actin fibers within the cell, properties that can only by guessed at on the basis of morphology alone.

Mechanical Properties of Actin In Vitro

We now have a very sophisticated understanding of the behavior of pure actin. The ability of the globular actin monomer to assemble reversibly via a nucleation step into polarized double helical filaments, the opposite ends of which differ in their binding affinities for actin monomers and for actin-associating proteins, is part of the secret of actin's versatility (Fig. 2). As a monomer, actin can theoretically diffuse easily among different regions of the cell and, as a polymer, serve the mechanical functions of the cell. The nucleation step in assembly and differences in affinities for actin monomers and for actin-associating proteins at the opposite ends of the filaments allow for the fine regulation of actin filament length and number. The extent of nucleation can determine the number of polymers over which actin monomers distribute, thereby controlling the polymer length distribution. Blocking of the "high affinity" end of actin filaments increases

the free monomer concentration and shortens the filaments. Blocking of the "low affinity" end would have the opposite effect. The capacity of actin fibers to break and then anneal spontaneously permits fragmentation of actin fibers to serve as a mechanism for rapid and efficient changes in actin filament length and number (26–30). For the purposes of this review, however, I emphasize the effects of these changes on the mechanical properties of actin fibers.

It is now clear that actin filaments in solvents of a composition believed to approximate the intracellular environment behave as a system of relatively stiff rods that overlap one another (31) (Fig. 2). This conclusion is based upon a number of characteristic rheologic properties of actin filament solutions. First, the viscosity of actin is highly shear dependent (32, 33). As the shear increases, the viscosity falls, because the flow conditions tend to disentangle the fibers, thereby reducing their tendency to hinder one another's diffusion. Second, for a given rate of shear, the viscosity is directly proportional to the filament concentration and also to the fiber length, because the degree of filament overlap and hence of constraints to diffusion vary directly with these parameters (Fig. 3). Third, the flow of actin fibers at a given stress is also time dependent, because the filaments initially resist deformation but eventually achieve a state of steady flow (31). A solution of pure actin filaments, some of which are several micrometers in length (34), is extremely viscous, and the diffusion of the individual long filaments is very limited (35). However, strictly speaking, the pure actin filaments do not form a true gel. Many individual fibers are extensively overlapped but not permanently "crosslinked" as in a true gel. For this reason and on the basis of information given in the next paragraph, pure actin is unlikely to explain the gel-like properties of cortical cytoplasm. With respect to the problem of the "reality" of morphology, it is reassuring that pure actin filaments visualized in three-dimensional space with the aid of stereo pair electron micrographs resemble overlapping straight rods (36, 37).

With information now available, it is possible to predict the viscosity of an actin filament solution provided that the rate of shear, the average filament length, and the filament concentration are known (31). Because it now also is becoming possible to determine approximately the concentration and length of actin fibers in cytoplasm, some initial estimates as to the viscosity of peripheral cytoplasm can be attempted on the basis of the assumption that only pure actin filaments compose this cytoplasm (31). If the concentration of actin filaments in cortical cytoplasm is on the order of 10 mg/ml (38), if the average intracellular actin filament length is about 100 nm (39), and if the applied shear is on the order of 0.3 s^{-1}, the passive stress applied by the flow of extracellular medium past a cell moving at a rate of 20 μm/min, the viscosity of the cortical actin would be on the order of 275 cP, or approximately that of 80% glycerol. Because the shear stresses experienced by cortical cytoplasm likely are higher than that proposed, it can be inferred that actin filaments alone could not account for the high apparent viscosity of the isotropic matrix of peripheral cytoplasm, which is sufficient to exclude cytoplasmic organelles and sustain contractile tension. Therefore, actin-modulating proteins that physically tie actin filaments together must be responsible for some of the mechanical properties of actin filaments in the isotropic cortical actin matrix of the cytoplasm.

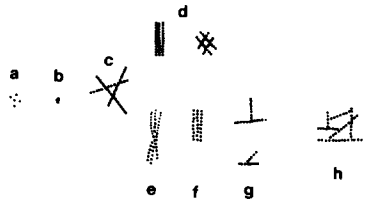

FIGURE 2 Configurations of actin in vitro. Actin can exist as a monomer (*a*), an oligomeric nucleus (*b*), and as extensively overlapping semiflexible rods (*c*). Under nonphysiologic ionic conditions the actin fibers can align into linear paracrystals or polygonal nets (*d*). Under the influence of actin-associated proteins, actin fibers can form loose bundles resembling stress fibers (*e*) or highly ordered bundles (*f*) or can branch at oblique or right angles (*g*), or, when sufficiently crosslinked by the appropriate proteins, can form a three-dimensional gel (*h*).

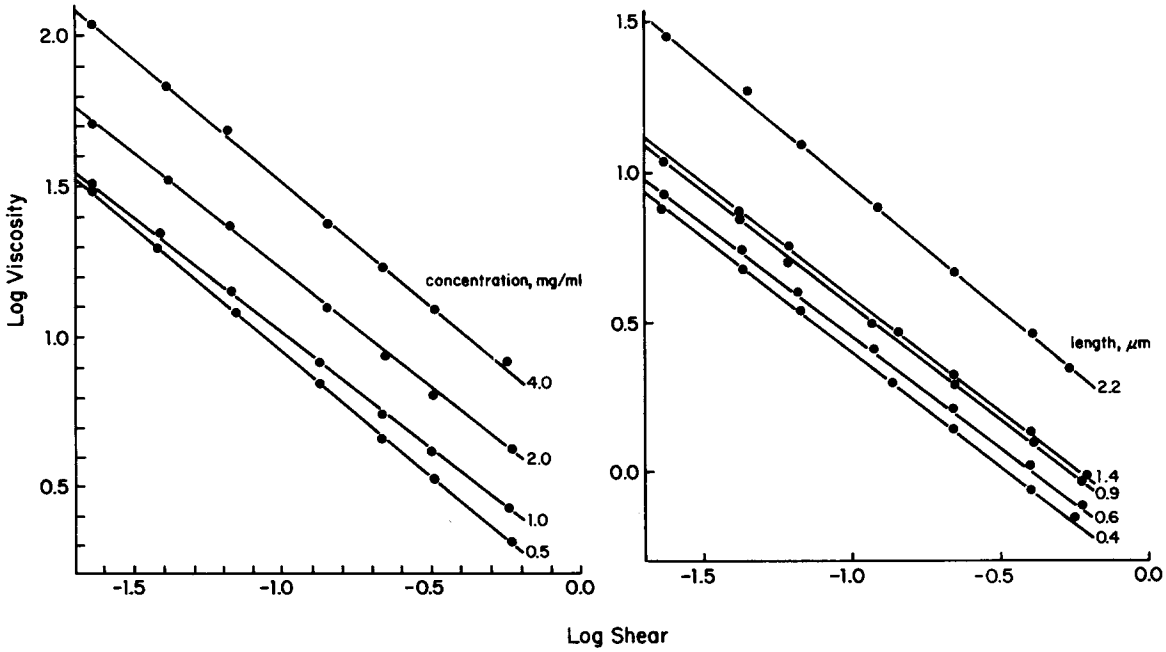

FIGURE 3 Effects of shear, concentration, and filament length on the absolute viscosity of actin filaments.

Even if actin alone cannot account for the gel properties of the isotropic cytoplasmic matrix, changes in the number and length of actin filaments could create marked alterations in cytoplasmic flow. For example, an organelle propelled by a fluid flow created by some contractile force through an actin matrix would slow down if the viscosity of the matrix increased. Because of the decrease in rate of flow and the shear dependence of actin's viscosity, the apparent viscosity of the matrix would increase. Because the absolute viscosity of actin fibers varies with the negative 0.8 power of the shear (40), small variations in fiber length or concentration at a given shear rate would markedly influence the apparent viscosity of the system (Fig. 3).

The comments above concerning the rheological properties of actin filaments refer to concentrations at which fibers overlap significantly but are not excessively concentrated. At sufficiently high concentrations polymers begin to align in parallel, and this sufficient concentration is inversely related to the polymer length and flexibility (41). At present there is no information concerning the effects of very high actin protein concentrations on actin filament configurations, although there is no reason to suppose that actin behaves differently from synthetic polymers studied in the past. Although many investigators have documented that nonphysiological solvent conditions such as low pH and high magnesium or other charged molecule concentrations cause actin bundles and nets to form (42), the effect of high concentrations of actin in more "physiological" media have not been examined. Again, however, it can be expected that the behavior of concentrated actin filaments alone will be under some modification by actin modulating proteins in the cell.

Actin-associating Proteins Affecting the Mechanical Properties of Actin

These proteins play on the intrinsic versatility of actin by interacting with a remarkable number of mutually accommodating binding sites on actin molecules. The effects of these proteins on actin in vitro may not necessarily occur in the cell, but it is not unreasonable to consider that they do.

The actions of some known actin-modulating proteins are summarized in Table I. Many such proteins participate in regulating the assembly of actin and the number or length of filaments. One class, the prototype for which is profilin, acts by *sequestering actin monomers*, thereby preventing the spontaneous nucleation of actin that leads to filament formation.

TABLE I

Actin-Associating Proteins of Nonmuscle Cells

General class	Name of protein	Cell of origin	References
Actin monomer-sequestering proteins	Profilin	Ubiquitous?	15, 65–68
	Depactin	Marine eggs	69, 70
Actin filament-nucleating, end-blocking, and severing* proteins	Gelsolin*	Mammalian cells	71–76
	Villin*	Epithelial cells; toad oocytes	5, 77–79, 104
	"Capping" proteins	Amebas, brain	80–84
	Fragmin*	*Physarum*	85, 86
	Severin*	*Dictyostelium*	87, 88
	β-Actinin‡	Kidney cells	89
	Acumentin‡	Mammalian macrophages, leukocytes	90–92
Inhibit actin filament fragmentation	Tropomyosins	Ubiquitous?	93–97
Actin filament-crosslinking proteins forming actin networks of various degrees of isotropy	Myosin	Ubiquitous	98, 99
	Actin-binding protein	Mammalian and avian cells; toad oocytes	100–104
	Spectrin	Erythrocytes	23
	Fodrin	Ubiquitous?	105–107
	"220 kdalton" protein	Marine eggs	108
	"120 kdalton" protein	*Dictyostelium*	109
Actin filament-crosslinking proteins forming actin bundles	Fascin	Marine eggs, coelomocytes	108
	Fimbrin	Vertebrate cells	110, 111
	α-Actinins	Ubiquitous?	112–117a
	"53 KDa" protein	Mammalian brain	118
Proteins proposed to link actin fibers to other structures			
To plasmalemma	Spectrin	Erythrocytes	23
	Fodrin?	Ubiquitous?	105–107
	Vinculin	Vertebrate cells	64
	Vitamin D–binding protein	Mammalian lymphocytes	119
	Actin-binding protein	Mammalian and avian cells; toad oocytes	120
To microtubules	Microtubule-associated proteins	Ubiquitous?	25

* Indicates that the protein has both end-blocking and severing activity.
‡ Block the "low affinity" end of actin filaments. The other proteins block the "high affinity" ends.

Because the nucleation of pure actin is very efficient under the solvent conditions in the cell and since the total actin concentration in the cell is high, most of the cellular actin would be filamentous if nucleation were not prevented. Therefore, the presence of monomer-sequestering agents would allow a large pool of actin to diffuse about the cell. An equilibrium between the high-affinity end of actin filaments and the profilin molecule may be sufficient to determine the extent to which profilin can liberate actin monomers to grow on filaments.

After cells are disrupted under solvent conditions favoring actin assembly, invariably the fraction of total cell-derived actin that is not in the form of long filaments is unexpectedly high (43). Furthermore, the unpolymerized actin extracted from the cells is complexed to profilin (e.g., 44, 45). Moreover, a significant fraction of fluorochrome-labeled actin inserted into cells by microinjection has a relatively high diffusion coefficient and exchanges with the actin in bundles (46, 47). These findings suggest that monomeric actin can be complexed to profilin in the cytoplasm as well as in vitro.

Another class of proteins promotes the *nucleation of actin monomers* (possibly by stabilizing spontaneously forming nuclei) and *blocks the ends of actin filaments*, thereby regulating filament growth and the equilibrium of actin monomers between profilin and actin filaments. The stimulation of nucleation results in a shortening of the filament length distribution, because the actin monomer pool becomes distributed over a larger number of filaments. β-Actinin, an *Acanthamoeba* protein and other cell "capping" proteins, and acumentin are examples of this class. Some of these proteins that nucleate actin growth and block filament ends bind to the actin with such high affinity that they can actively *sever actin filaments* into fragments. These actin-severing proteins, specifically gelsolin, fragmin, villin, and severin are regulated by calcium concentrations in the submicromolar range, providing a linkage between cell activation and signaling mechanisms and mechanical events. Because these severing proteins all bind to the end of actin filaments with the highest affinity for actin monomers, they prevent the addition of actin monomers to the filaments from the profilin-actin complex. Actin in cell extracts prepared with calcium-containing solutions (to make the final free calcium concentration submicromolar) shifts its partitioning from filaments to profilin, and oligomeric actin in the extracts is complexed to gelsolin (e.g., 45, 48). The findings are consistent with the idea that the interactions between actin, gelsolin, and profilin that occur in vitro also take place within the cell. The fragmenting action of these severing proteins can be partially inhibited by tropomyosins, asymmetrical proteins that lie in the groove of actin filament helices.

A third class of proteins *links actin filaments together* into bundles or gels. Because most such proteins can increase the viscosity of actin filament solutions, they have often been called gelation factors. However, many of these proteins actually link actin filaments into a side-to-side configuration and are therefore more accurately termed bundling proteins (examples of which include villin, fimbrin, and α-actinins). Fascin, villin, and fimbrin reside in filament bundles observed in microvilli extending from cells, and α-actinin is localizable to stress fibers (e.g., 49–51). Therefore, it is possible that these bundling proteins have a role in forming actin bundles in vivo.

A few proteins link actin filaments together such that the filaments can overlap at oblique or at right angles that more nearly approximate isotropic gels as defined above. Actin-binding protein, spectrin, myosin, and fodrin are examples of this type of protein, although at sufficiently high concentrations they can also cause actin fibers to form bundles. Actin-binding protein is unique in promoting the perpendicular branching of actin filaments. Since such configurations of actin are observed in electron micrographs of the isotropic matrix of cells and because actin alone does not gel, it can be inferred that the proteins that induce such isotropic branching of actin fibers in vitro are responsible for the isotropic actin matrix in the cell. Moreover, the polarity of actin filaments at T-shaped junctions in the cell is consistent with the polarity of actin filaments observed in T-shaped intersections of actin assembled with actin-binding protein in vitro (52).

The distinction between bundling and isotropic gelation can be made qualitatively with the electron microscope or quantitatively with methods that determine the quantity of added modulating protein required to increase the sedimentability of actin or to produce an abrupt increase in actin's viscosity, a transition known as the *gel point*. An isotropic gelling protein efficiently recruits actin fibers into a network, whereas a bundling agent has a tendency to form redundant crosslinks between fibers already aligned in parallel. Therefore, proteins that promote isotropic actin gelation have lower minimum gelling concentrations than proteins that produce actin bundles. Proteins that produce isotropic actin gels are very large, flexible molecules capable of forming highly extended conformations in solution, examples being actin-binding protein, myosin, spectrin, and fodrin. Differences in the extensibility and/or flexibility of these proteins may account for their variations in crosslinking efficiency (53). Proteins that promote the formation of parallel actin bundles are smaller and take the configuration of globular monomers or of rigid rods, such as fascin, fimbrin, and α-actinin. The α-actinins are also interesting for the fact that their actin crosslinking properties are inhibited by calcium concentrations in the submicromolar range.

In the case of flexible polymers, gelation occurs when enough fibers are chemically crosslinked to create a continuous molecule that extends completely from one boundary of the polymer system to the other (54). However, obliquely or perpendicularly branching fibers of actin might be so entangled, and their diffusion so constrained, that the system might behave as a gel even if not chemically linked into the kind of complete network characteristic, for example, of vulcanized rubber.

Just as changes in actin fiber length can profoundly affect the flow of objects through a solution of linear actin polymers as described above, variations in filament length can regulate the gelation of crosslinked actin networks. The gel point of a crosslinked polymer system is fixed at a critical crosslink concentration, Vc, which is equal to the polymer mass, C, divided by the weight-average filament length, $L\bar{w}$ (54). The validity of this relationship for actin networks has been experimentally established, and the ability of actin fragmentation, which decreases $L\bar{w}$ without proportionally affecting C, to increase Vc has also been demonstrated (53, 55–57). Therefore, the critical nature of the transition between sol and gel states permits filament fragmentation to have important effects on the system's consistency, if the gel point is crossed. The many actin fragmenting proteins found in cells suggest that this mechanism for changing the consistency of the actin-

rich matrix may be an important one.

Although the analysis of the *dimensions* of the cortical actin gel is only beginning (e.g., 39), it is interesting that the spacing between actin filaments in the network may be relatively large, i.e., on the order of 100 nm. If this is the case, there is reasonable freedom for the diffusion of small molecules and of even very small organelles within this network, assuming they are not physically bound to the fibers. Even if bound significantly, the bulk of water and ions present in relatively high concentrations would be free to diffuse without hindrance. This conclusion is consistent with estimates of the microscopic viscosity of cytoplasm (58) and indicates that the cytoplasm is amazingly crowded with fibers yet perhaps can also accommodate considerable space for solutes.

The *extension* of the cytoplasmic matrix can be related to the assembly of actin filaments. Filament growth may occur in the form of linear bundles or as the expansion of an isotropic gel. Whether such growth itself directly provides partially or totally the force for propulsion of the cell membrane or whether other forces, such as an osmotic drive, extend the boundaries of the cell, with the actin matrix following to stabilize the extension, is unclear (59).

The *contractility* of actin in cytoplasm must depend upon the activity of myosin molecules that probably are organized as filaments within the cytoplasmic matrix. Although contractility is not the subject of this supplement, it is worth noting that if myosin aggregates are responsible for generating contractile forces within the cell, the state of assembly of the cytoplasmic actin matrix could determine the efficiency by which this contractile force is propagated within the cell (60–63). Furthermore, myosin molecules and filaments may contribute to the rigidity of the cytoplasmic actin matrix.

A final class of actin-associating proteins is responsible for the *linkage of actin fibers to other structures*, such as the plasma membrane, to other organelles and to other fiber systems within the cells. Some thoughts and experiments bearing on these associations are summarized in other essays of this supplement (23, 25, 63, 64). It may also be important to consider that the complex branching actin matrix could entangle itself with other fiber systems, with large membrane molecules, and with organelles. Therefore, although it is likely that specific chemical interactions mediate associations between actin fibers and other cellular structures, the possible importance of physical constraints that may in turn be regulated by small changes in actin fiber dimensions should not be overlooked.

This research was supported by grants HL 19429 and 09321 from the U. S. Public Health Service, the Council for Tobacco Research, The Muscular Dystrophy Association, and the Edwin S. Webster Foundation.

REFERENCES

1. Hatano, S., and F. Oosawa. 1966. Isolation and characterization of plasmodium actin. *Biochim. Biophys. Acta.* 127:488–498.
2. Ishikawa, H., R. Bischoff, and H. Holtzer. 1969. Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* 43:312–328.
3. Straub, F. B. 1942. Actin. *Stud. Med. Szeged.* 2:3–15.
4. Vanderkerckhoeve, J., and K. Weber. 1978. At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino terminal peptide. *J. Mol. Biol.* 126:783–802.
5. Mooseker, M. S., E. M. Bonder, K. A. Conzelman, D. J. Fishkind, C. L. Howe, and T. C. S. Keller, III. 1984. Brush border cytoskeleton and integration of cellular functions. *J. Cell Biol.* 99(1, Pt. 2):104s–112s.
6. Tilney, L. G., and M. S. Tilney. 1984. Observations on how actin filaments become organized in cells. *J. Cell Biol.* 99(1, Pt. 2):76s–82s.
7. Buckley, I. K. 1981. Fine structural and related aspects of nonmuscle-cell motility. *In* Cell & Muscle Motility. R. M. Dowben and J. W. Shay, editors. Plenum Publishing Corp., New York. 135–203.
8. Wong, A. J., T. D. Pollard, and I. Herman. 1983. Actin filament stress fibers in vascular endothelial cells in vivo. *Science (Wash. DC).* 219:867–869.
9. Byers, H. R., and K. Fujiwara. 1982. Stress fibers in cells in situ: immunofluorescence visualization with antiactin, antimyosin, and anti-alpha actinin. *J. Cell Biol.* 93:804–811.
10. Begg, D. A., R. Rodewald, and L. I. Rebhun. 1978. The visualization of actin filament polarity in thin sections. Evidence for the uniform polarity of membrane-associated filaments. *J. Cell Biol.* 79:846–852.
11. Nagai, R., Y. Yoshimoto, and N. Kamiya. 1978. Cyclic production of tension force in the plasmodial strand of *Physarum polycephalum* and its relation to microfilament morphology. *J. Cell. Sci.* 33:205–225.
12. Sanger, J. M., and J. W. Sanger. 1980. Banding and polarity of actin filaments in interphase and cleaving cells. *J. Cell Biol.* 86:568–575.
13. Schroeder, T. E. 1981. Interrelations between the cell surface and the cytoskeleton in cleaving sea urchin eggs. *In* Cytoskeletal Elements and Plasma Membrane Organization. G. Poste and G. L. Nicolson, editors. Elsevier/North Holland Biomedical Press, New York. 170–216.
14. Burgess, D. R. 1982. Reactivation of intestinal epithelial cell brush border motility: ATP-dependent contraction via a terminal web contractile ring. *J. Cell Biol.* 95:853–863.
15. Lindberg, U., A. S. Hoglund, and R. Karlsson. 1981. On the ultrastructural organization of the microfilament system and the possible role of profilactin. *Biochimie (Paris).* 63:307–323.
16. Small, J. V. 1981. Organization of actin in the leading edge of cultured cells: influence of osmium tetroxide and dehydration on the ultrastructure of actin networks. *J. Cell Biol.* 91:695–705.
17. Wessells, N. K., B. S. Spooner, J. F. Ash, M. O. Bradley, M. A. Ludueña, E. L. Taylor, J. T. Wrenn, and K. T. Yamada. 1971. Microfilaments in cellular and developmental processes. *Science (Wash. DC).* 171:135–143.
18. Porter, K. R. The cytomatrix: a short history of its study. *J. Cell Biol.* 99(1, Pt. 2):3s–12s.
19. Schliwa, M., and J. Van Blerkom. 1981. Structural interactions of cytoskeletal components. *J. Cell Biol.* 90:225–235.
20. Schliwa, M. 1982. Action of cytochalasin D on cytoskeletal networks. *J. Cell Biol.* 92:79–91.
21. Muller, O. F. 1786. Animalcula infusoria fluviatilia et marina, quae detexit systematice descipset et ad vivum delineari curavit. N. Molleri, Copenhagen. 10–11.
22. Porter, K. R., and J. B. Tucker. 1981. The ground substance of the living cell. *Sci. Am.* 22:57–67.
23. Marchesi, V. T. 1983. The red cell membrane skeleton: recent progress. *Blood.* 61:1–11.
24. Lasek, R. J., J. A. Garner, and S. T. Brady. 1984. Axonal transport of the cytoplasmic matrix. *J. Cell Biol.* 99(1, Pt. 2):212s–221s.
25. Pollard, T. D., S. C. Selden, and P. Maupin. 1984. Interaction of actin filaments with microtubules. *J. Cell Biol.* 99(1, Pt. 2):33s–37s.
26. Oosawa, F., and S. Asakura. 1975. Thermodynamics of the Polymerization of Protein. Academic Press, Inc., New York.
27. Tobacman, L. S., and E. D. Korn. 1983. The kinetics of actin nucleation and polymerization. *J. Biol. Chem.* 258:3207–3214.
28. Wegner, A. 1982. Kinetic analysis of actin assembly suggests that tropomyosin inhibits spontaneous fragmentation of actin filaments. *J. Mol. Biol.* 161:217–227.
29. Frieden, C., and D. W. Goddette. 1983. Polymerization of actin and actin-like systems: evaluation of the time course of polymerization in relation to the mechanism. *Biochemistry.* 22:5836–5843.
30. Cooper, J. A., E. L. Buhle, Jr., S. B. Walker, T. Y. Tsong, and T. D. Pollard. 1983. Kinetic evidence for a monomer activation step in actin polymerization. *Biochemistry.* 22:2193–2202.
31. Zaner, K. S., and T. P. Stossel. 1983. Physical basis of the rheologic properties of F-actin. *J. Biol. Chem.* 258:11004–11009.
32. Kasai, M., H. Kawashima, and F. Oosawa. 1960. Structure of F-actin solutions. *J. Polymer Sci.* 44:51–69.
33. Maruyama, K., M. Kaibara, and E. Fukada. 1974. Rheology of F-actin. 1. Network of F-actin in solution. *Biochim. Biophys. Acta.* 371:20–29.
34. Kawamura, M., and K. Maruyama. 1970. Electron microscopic particle length of F-actin polymerized in vitro. *J. Biochem (Tokyo).* 67:437–457.
35. Tait, J. F., and C. Frieden. 1982. Chemical modification of actin. Acceleration of polymerization and reduction of network formation by reaction with *N*-ethylmaleimide, or 7-chloro-4-nitro-2,1,3-nitro-2,1,3-bezoxadiazole. *Biochemistry.* 21:6046–6053.
36. Heuser, J. E., and M. W. Kirschner. 1980. Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. *J. Cell Biol.* 86:212–234.
37. Niederman, R., P. C. Amrein, and J. H. Hartwig. 1983. Three-dimensional structure of actin filaments and of an actin gel made with actin-binding protein. *J. Cell Biol.* 96:1400–1413.
38. Davies, W. A., and T. P. Stossel. 1977. Peripheral hyaline blebs (podosomes) of macrophages. *J. Cell Biol.* 75:941–955.
39. Niederman, R., J. Hartwig, L. Peters, and T. P. Stossel. 1983. The 3-D organization of actin filaments in motile cytoplasm. *J. Cell Biol.* 97(2, Pt.2):274*a*. (Abstr.)
40. Zaner, K. S., and T. P. Stossel. 1982. Some perspectives on the viscosity of actin filaments. *J. Cell Biol.* 93:987–991.
41. Flory, P. J. 1956. Statistical thermodynamics of semiflexible chain molecules. *Proc. R. Soc. Lond. A.* 234:60–72.
42. Hanson, J. 1973. Evidence from electron microscope studies on actin paracrystals concerning the origin of the cross-striation in the thin filaments of vertebrate skeletal muscle. *Proc. R. Soc. Lond. B.* 183:39–58.
43. Bray, D., and C. Thomas. 1976. Unpolymerized actin in fibroblasts and brain. *J. Mol. Biol.* 105:527–544.
44. Carlsson, L., F. Markey, I. Blikstad, and U. Lindberg. 1979. Reorganization of actin in platelets stimulated by thrombin as measure by the DNase I inhibition assay. *Proc. Natl. Acad. Sci. USA.* 76:6376–6380.
45. Markey, F., T. Persson, and U. Lindberg. 1981. Characterization of platelets extracts before and after stimulation with respect to the possible role of profilactin as microfilament precursor. *Cell.* 23:145–153.
46. Wang, L-L., and J. Bryan. 1981. Isolation of calcium dependent platelet proteins that interact with actin. *Cell.* 25:637–649.
47. Kreis, T., B. Geiger, and J. Schlessinger. 1982. Mobility of microinjected rhodamine actin within living chicken gizzard cells determined by fluorescence photobleaching recovery. *Cell.* 29:835–845.
48. Wang, Y-L., F. Lanni, P. L. McNeil, B. R. Ware, and D. L. Taylor. 1979. Mobility of

cytoplasmic and membrane-associated actin in living cells. *Proc. Natl. Acad. Sci. USA.* 79:4660–4664.
49. Otto, J. J., R. E. Kane, and J. Bryan. 1979. Formation of filopodia in coelomocytes: localization of fascin, a 58000 dalton actin crosslinking protein. *Cell.* 17:285–293.
50. Bretscher, A., and K. Weber. 1979. Villin: the major microfilament-associated protein of the intestinal microvillus. *Proc. Natl. Acad. Sci. USA.* 76:2321–2325.
51. Bretscher, A., and K. Weber. 1980. Fimbrin: a new microfilament-associated protein present in microvilli and other cell surface structures. *J. Cell Biol.* 86:335–340.
52. Hartwig, J. H., J. Tyler, and T. P. Stossel. 1980. Actin-binding protein promotes the bipolar and branching polymerization of actin filaments. *J. Cell Biol.* 87:841–848.
53. Hartwig, J. H., and T. P. Stossel. 1981. Structure of macrophage actin-binding protein molecules in solution and interacting with actin filaments. *J. Mol. Biol.* 145:563–581.
54. Flory, P. J. 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca, NY.
55. Hartwig, J. H., and T. P. Stossel. 1979. Cytochalasin B and the structure of actin gels. *J. Mol. Biol.* 134:539–553.
56. Yin, H. L., K. S. Zaner, and T. P. Stossel. 1980. Ca control of actin gelation. Interaction of gelsolin with actin filaments and regulation of gelation. *J. Biol. Chem.* 255:9494–9500.
57. Nunnally, M. H., L. D. Powell, and S. W. Craig. 1981. Reconstitution and regulation of actin gel-sol transformation with purified filamin and villin. *J. Biol. Chem.* 256:2083–2086.
58. Clegg, J. S. 1984. Intracellular water and the cytomatrix: some methods of study and current views. *J. Cell Biol.* 99(1, Pt. 2):167s–171s.
59. Oster, G. F., A. S. Perelson, and L. G. Tilney. 1982. A mechanical model for elongation of the acrosomal process in thyone sperm. *J. Math. Biol.* 15:259–265.
60. Stendahl, O. I., and T. P. Stossel. 1980. Actin-binding protein amplifies actomyosin contraction, and gelsolin confers calcium control on the direction of contraction. *Biochem. Biophys. Res. Commun.* 92:675–681.
61. Kane, R. E. 1983. Interconversion of structural and contractile actin gels by insertion of myosin during assembly. *J. Cell Biol.* 97:1745–1752.
62. Taylor, D. L., and M. Fechheimer. 1982. Cytoplasmic structure and contractility: the solation–contraction coupling hypothesis. *Phil. Trans. R. Soc. Lond. B.* 299:185–197.
63. Condeelis, J. S. 1984. Properties of the 120,000- and 95,000-dalton actin-binding proteins from *Dictyostelium discoideum* and their possible functions in assembling the cytoplasmic matrix. *J. Cell Biol.* 99(1, Pt. 2):119s–126s.
64. Geiger, B., Z. Avnur, G. Rinnerthaler, H. Hinssen, and V. J. Small. 1984. Microfilament-organizing centers in areas of cell contact: cytoskeletal interactions during cell attachment and locomotion. *J. Cell Biol.* 99(1, Pt. 2):83s–91s.
65. Ozaki, K., H. Sugino, T. Hasegawa, S. Takahashi, and S. Hatano. 1983. Isolation and characterization of *Physarum* profilin. *J. Biochem. (Tokyo).* 93:295–298.
66. Tobacman, L., S. L. Brenner, and E. D. Korn. 1983. Effect of *Acanthamoeba* profilin on the pre-steady state kinetics of actin polymerization and on the concentration of actin at steady state. *J. Biol. Chem.* 258:8806–8812.
67. Tseng, P. C-H., and T. D. Pollard. 1982. Mechanism of action of *Acanthamoeba* profilin: demonstration of actin species specificity and regulation by micromolar concentrations of $MgCl_2$. *J. Cell Biol.* 94:312–318.
68. Fattoum, A., C. Roustan, J. Feinberg, and L.-A. Pradel. 1980. Biochemical evidence for a low molecular weight protein (profilin-like protein) in hog thyroid gland and its involvement in actin polymerisation. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 118:237–240.
69. Mabuchi, I. 1983. An actin-depolymerizing protein (depactin) from starfish oocytes: properties and interactions with actin. *J. Cell Biol.* 97:1612–1621.
70. Hosoya, H., I. Mabuchi, and H. Sakai. 1982. Actin modulating proteins in the sea urchin egg. I. Analysis of G-actin-binding proteins by DNase I-affinity chromatography and purification of a 17,000 molecular weight component. *J. Biochem.* 92:1853–1862.
71. Yin, H. L., and T. P. Stossel. 1979. Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein. *Nature (Lond.).* 281:583–586.
72. Yin, H. L., J. H. Hartwig, K. Maruyama, and T. P. Stossel. Ca control of actin filament length. Effects of macrophage gelsolin on actin polymerization. *J. Biol. Chem.* 256:9693–9697.
73. Yin, H. L., J. H. Albrecht, and A. Fattoum. 1981. Identification of gelsolin, a Ca-dependent regulatory protein of actin gel-sol transformation, and its intracellular distribution in a variety of cells and tissues. *J. Cell Biol.* 91:901–906.
74. Petrucci, T. C., C. Thomas, and D. Bray. 1983. Isolation of a Ca-dependent actin-fragmenting protein from brain, spinal cord, and cultured neurones. *J. Neurochem.* 40:1507–1516.
74a. Nishida, E., Y. Ohta, and H. Sakai. 1983. The regulation of actin polymerization by the 88K protein/actin complex and cytochalasin B. *J. Biochem. (Tokyo).* 94:1671–1683.
75. Lind, S. E., H. L. Yin, and T. P. Stossel. 1982. Human platelets contain gelsolin. *J. Clin. Invest.* 69:1384–1387.
76. Kurth, M. C., L-L. Wang, J. Dingus, and J. Bryan. 1983. Purification and characterization of a gelsolin-actin complex from human platelets. Evidence for Ca-insensitive functions. *J. Biol. Chem.* 258:10895–10903.
77. Glenney, J. R., Jr., P. Kaulfus, and K. Weber. 1981. Actin assembly modulated by villin: Ca-dependent nucleation and capping of the barbed end. *Cell.* 24:471–480.
78. Craig, S. W., and L. D. Powell. 1980. Regulation of actin polymerization by villin, a 95,000 dalton cytoskeletal component of intestinal brush borders. *Cell.* 22:739–746.
79. Hesterberg, L. K., and K. Weber. 1983. Demonstration of three distinct calcium-binding sites in villin, a modulator of actin assembly. *J. Biol. Chem.* 258:365–369.
80. Isenburg, G., U. Aebi, and T. D. Pollard. 1980. An actin-binding protein from *Acanthamoeba* regulates actin filament polymerization and interactions. *Nature (Lond.).* 288:455–459.
81. Maruta, H., G. Isenburg, T. Schreckenbach, R. Hallman, G. Risse, T. Shibayama, and J. Hesse. 1983. Ca-dependent actin-binding phosphoprotein in *Physarum polycephalum.* I. Ca/actin-dependent inhibition of its phosphorylation. *J. Biol. Chem.* 258:10144–10150.
82. Bamburg, J. R., H. E. Harris, and A. G. Weeds. 1980. Partial purification and characterization of an actin-depolymerizing factor from brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 121:178–182.
83. Isenburg, G., R. Ohnheiser, and H. Maruta. 1983. 'Cap 90', a 90-KDa Ca-dependent F-actin-capping protein from vertebrate brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 163:225–229.
84. Grumet, M., and S. Lin. 1981. Purification and characterization of an inhibitor protein with cytochalasin-like activity from bovine adrenal medulla. *Biochim. Biophys. Acta.* 678:381–387.
85. Hasegawa, T., S. Takahashi, H. Hayashi, and S. Hatano. 1980. Fragmin: a calcium ion sensitive regulatory factor on the formation of actin filaments. *Biochemistry.* 19:2679–2683.
86. Hinssen, H. 1981. An actin modulating protein from *Physarum polycephalum.* I. Isolation and purification. *Eur. J. Cell Biol.* 23:225–233.
87. Brown, S. S., K. Yamamoto, and J. A. Spudich. 1982. A 40,000-dalton protein from *Dictyostelium discoideum* affects assembly properties of actin in a Ca-dependent manner. *J. Cell Biol.* 93:205–210.
88. Yamamoto, K., J. D. Pardee, J. Reidler, L. Stryer, and J. A. Spudich. 1982. Mechanism of interaction of *Dictyostelium* severin with actin filaments. *J. Cell Biol.* 95:711–719.
89. Maruyama, K., and H. Sakai. 1981. Cell beta-actinin, an accelerator of actin polymerization, isolated from rat kidney cytosol. *J. Biochem. (Tokyo).* 89:1337–1340.
90. Southwick, F. S., and T. P. Stossel. 1981. Isolation of an inhibitor of actin polymerization from human polymorphonuclear leukocytes. *J. Biol. Chem.* 256:3030–3036.
91. Southwick, F. S., and J. H. Hartwig. 1982. Acumentin, a protein in macrophages which caps the "pointed" end of actin filaments. *Nature (Lond.).* 297:303–307.
92. Southwick, F. S., N. Tatsumi, and T. P. Stossel. 1982. Acumentin, an actin-modulating protein of rabbit pulmonary macrophages. *Biochemistry.* 24:6321–6326.
93. Cohen, I., and C. Cohen. 1972. A tropomyosin-like protein from human platelets. *J. Mol. Biol.* 68:383–387.
94. Terossian, D., S. D. Fuller, M. Stewart, and A. G. Weeds. 1981. Porcine platelet tropomyosin. Purification, characterization and paracrystal formation. *J. Mol. Biol.* 153:147–167.
95. Cote, G. P., and L. B. Smillie. 1981. The interaction of equine platelet tropomyosin with skeletal muscle actin. *J. Biol. Chem.* 256:7257–7261.
96. Bernstein, B. W., and J. R. Bamburg. 1982. Tropomyosin binding to F-actin protects the F-actin from disassembly by brain actin-depolymerizing factor (ADF). *Cell Motility.* 2:1–8.
97. Fattoum, A., J. H. Hartwig, and T. P. Stossel. 1983. Isolation and some functional properties of macrophage tropomyosin. *Biochemistry.* 22:1187–1193.
98. Abe, S-I., and K. Maruyama. 1974. Dynamic viscoelastic study of acto-heavy meromyosin in solution. *Biochim. Biophys. Acta.* 160:160–172.
99. Trinick, J., and G. Offer. 1979. Cross-linking of actin filaments by heavy meromyosin. *J. Mol. Biol.* 133:549–556.
100. Stossel, T. P., and J. H. Hartwig. 1975. Interactions of actin, myosin and an actin-binding protein of rabbit alveolar macrophages. Macrophage myosin Mg-adenosine triphosphatase activity requires a cofactor for activation by actin. *J. Biol. Chem.* 250:5706–5712.
101. Schollmeyer, J. V., G. H. R. Rao, and J. G. White. 1978. An actin-binding protein in human platelets. Interactions with alpha actinin on gelation of actin and the influence of cytochalasin B. *Am. J. Pathol.* 93:433–446.
102. Rosenberg, S., A. Stracher, and R. C. Lucas. 1981. Isolation and characterization of actin and actin-binding protein from human platelets. *J. Cell Biol.* 91:201–211.
103. Schloss, J. A., and R. D. Goldman. 1979. Isolation of a high molecular weight actin-binding protein from baby hamster kidney (BHK-21) cells. *Proc. Natl. Acad. Sci. USA.* 76:4484–4488.
104. Corwin, H. L., and J. H. Hartwig. 1983. Isolation of actin-binding protein and villin from toad oocytes. *Dev. Biol.* 99:61–74.
105. Levine, J., and M. Willard. 1981. Fodrin: axonally transported polypeptides associated with the internal periphery of many cells. *J. Cell Biol.* 90:631–643.
106. Glenney, J. R., Jr., P. Glenney, and K. Weber. 1983. The spectrin-related molecule, TW-260/240, cross-links the actin bundles of the microvillus rootlets in the brush borders of intestinal epithelial cells. *J. Cell Biol.* 96:1491–1496.
107. Glenney, J. R., Jr., and P. Glenney. 1983. Fodrin is the general spectrin-like protein found in most cells whereas spectrin and the TW protein have a restricted distribution. *Cell.* 34:503–512.
108. Bryan, J., and R. E. Kane. 1978. Separation and interaction of the major components of sea urchin actin gel. *J. Mol. Biol.* 125:207–224.
109. Condeelis, J., S. Geosits, and M. Vahey. 1982. Isolation of a new actin-binding protein from *Dictyostelium discoideum. Cell Motility.* 2:273–285.
110. Bretscher, A. 1981. Fimbrin is a cytoskeletal protein that crosslinks F-actin in vitro. *Proc. Natl. Acad. Sci. USA.* 78:6849–6853.
111. Glenney, J. R., Jr., P. Kaulfus, P. Matsudaira, and K. Weber. 1981. F-actin binding and bundling properties of fimbrin, a major cytoskeletal protein of the microvillus core filaments. *J. Biol. Chem.* 256:9283–9288.
112. Mimura, N., and A. Asano. 1979. Ca-sensitive gelation of actin filaments by a new protein factor. *Nature (Lond.).* 282:44–48.
113. Burridge, K., and J. R. Feramisco. 1981. Non-muscle alpha-actinins are calcium-sensitive actin-binding proteins. *Nature (Lond.).* 294:565–567.
114. Pollard, T. D. 1981. Purification of a calcium-sensitive actin gelation protein from *Acanthaemoeba. J. Biol. Chem.* 256:7666–7670.
115. Rosenberg, S., A. Stracher, and K. Burridge. 1981. Isolation and characterization of calcium-sensitive alpha-actinin-like protein from human platelet cytoskeletons. *J. Biol. Chem.* 256:12986–12991.
116. Condeelis, J., and M. Vahey. 1982. A calcium- and pH-regulated protein from *Dictyostelium discoideum* that cross-links actin filaments. *J. Cell Biol.* 94:466–471.
117. Fechheimer, M., J. Brier, M. Rockwell, E. J. Luna, and D. L. Taylor. 1982. A calcium- and pH-related actin binding protein from *D. discoideum. Cell Motility.* 2:287–308.
117a. Bennett, J. P., K. S. Zaner, and T. P. Stossel. 1984. Isolation and some properties of macrophage alpha actinin. Evidence that it is not an actin gelling protein at physiologic temperature. *Biochemistry.* In press.
118. Maekawa, S., S. Endo, and H. Sakai. 1983. Purification and partial characterization of a new protein in porcine brain which bundles actin filaments. *J. Biochem. (Tokyo).* 94:1329–1337.
119. Petrini, M., D. L. Emerson, and R. M. Galbraith. 1983. Linkage between surface immunoglobulin and cytoskeleton of B lymphocytes may involve Gc protein. *Nature (Lond.).* 306:73–74.
120. Fox, J. E. B., A. K. Baughan, and D. R. Phillips. Direct linkage of GP_{IB} to a M_r 250K polypeptide in platelet cytoskeleton. *Blood.* 62:255A. (Abstr.)