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Biochemistry

Biochemistry of actomyosin-dependent cell motility (A Review)*

(actin/myosin/microfilaments)

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ABSTRACT **Actins and myosins similar to the major proteins of muscle are the major molecular components of intricate mechanochemical systems that perform numerous vital motility and structural functions in all eukaryotic cells. In this article, after a brief summary of the morphological distribution and ultrastructure of actin, myosin, and interrelated proteins of nonmuscle cells, our present knowledge of their biochemistry is critically appraised from the perspective that understanding complex cellular processes depends ultimately on the identification, purification, and biochemical characterization of the proteins involved. Although few conclusions are reached, possible molecular mechanisms for cellular regulation of actin polymerization, filament association, actomyosin ATPase activity, and mechanochemical coupling are discussed and a number of potentially fruitful directions for further research are suggested. These include comparative biochemical investigations and the study of the interaction of heterologous proteins, but particular emphasis is given to the need for quantitative studies at the molecular level of motility proteins purified from a single cellular source.**

Three mechanisms, ultrastructurally, biochemically, and mechanically distinct, have evolved for cell motility. Two of these, bacterial flagella (1, 2) and eukaryotic flagella (3–6), serve to move cells through fluid media, but they function differently. The third motile mechanism, that based on cytoplasmic microfilaments, is responsible for ameboid-type movement of a cell on a solid substratum. The major component of cytoplasmic microfilaments is the protein actin. Presumably, myosin (which is an ATPase that forms an enzymatically active complex with actin) provides the energy for the motility events, while a number of other proteins maintain the dynamic structural organization of the system and regulate its action. Similar systems in all eukaryotic cells (protist, plant, and animal, and including cells that also possess flagella) provide the mechanochemical basis for many other diverse cellular activities, including cytoplasmic streaming and saltatory movements, phagocytosis and secretory processes, cell division and possibly chromosome segregation, changes in cell shape, and probably even the regulation of the topographical distribution of membrane proteins and functional interconnections of the cell membrane with the nucleus (7–15). Some of these functions may have preceded in evolution the role of actin and myosin in ameboid movement. In their most recently evolved forms, actin and myosin comprise the highly structured contractile systems of muscles.

Actomyosin-based cell motility and related phenomena such as the cytoskeletal role of microfilaments and the interaction of microfilaments with cell membranes are increasingly the subjects of investigation at the cellular level and in crude cell extracts. I believe, however, that to understand the physiology of cellular events it is first necessary to understand their biochemistry. It is necessary, although often not easy, to isolate and purify the molecular components, to study their properties alone and in combination and then, finally, to reconstitute the physiological system. Thus, and only thus, can we unravel the complexities of cell biology. Studies on the intact system are essential to define the biological questions, but the answers will come largely from quantitative biochemistry.

In this article, I will briefly review the morphological distribution and ultrastructure of cytoplasmic microfilaments in nonmuscle cells and then selectively and critically appraise our present understanding of the biochemistry of actomyosin-dependent cell motility. I will reach the conclusion that we are now at a position to realize the complexity of the problem that lies ahead and to discern the directions for future research.

Morphology and ultrastructure

Electron microscopy of eukaryotic cells reveals microfilaments (5–7 nm wide), intermediate filaments (10 nm wide), and microtubules (25 nm wide). By their specific ability to bind heavy meromyosin, the microfilaments of more than 60 cells have been identified as actin filaments (14–16). Microfilaments are distributed primarily in the region immediately underlying the plasma membrane and in broad pseudopodal areas, and extend down all microprojections such as filopodia and microvilli. In tissue-cultured cells, microfilaments are observed (most graphically by immunofluorescence microscopy after reaction with anti-actin antibody) in either of two general distributions: (*i*) dispersed throughout the cytoplasm, in the perinuclear space, and associated with the leading ruffling areas of the cell surface of motile cells; and (*ii*) (most characteristically in well-spread, anchorage-dependent cells) as highly organized bundles of filaments, called stress fibers, generally submembranous and terminating away from the ruffling regions. The stress fibers disappear when cells are virally transformed (17). Microfilaments also form the transitory contractile ring of dividing cells (18), accumulate in large quantities at the plasma membrane in regions of active phagocytosis (19) (but may not be associated with phagolysosomes), and are observed in the region between the mitotic spindle poles and the chromosomes during mitosis (20, 21).

Microfilaments appear to be closely associated with the plasma membrane *in situ* and are greatly enriched in all preparations of purified animal cell plasma membranes (22, 23). The ultrastructural relationship between microfilaments and plasma membranes has been visualized best in situations such as the intestinal microvilli, where the microfilaments are especially well oriented: the microfilaments have the same polarity relative to the tip of the microvillus as do the thin filaments in skeletal muscle relative to the Z line (24).

Not all cytoplasmic actin is filamentous, however. Actin is present in high concentration in an apparently nonfilamentous state in the periacrosomal vesicle region of sea cucumber sperm before its rapid conversion to filaments in the acrosomal reaction (25), and the actin associated with the cytoplasmic surface of erythrocyte membranes may also be nonfilamentous (26). In addition to these localized concentrations of nonfilamentous

* By invitation. From time to time, reviews on scientific and technological matters of broad interest are published in the PROCEEDINGS.

Table 1. Partial amino acid sequence data for muscle and nonmuscle actins

Actin source	Amino acid residues																	
	10	16	17	106	129	176	228	266	271	278	286	294	296	298	305	216	357	364
Rabbit skeletal muscle	Cys	Leu	Val	Thr	Val	Met	Ala	Ile	Ala	Tyr	Ile	Ala	Asn	Met	Tyr	Ile	Thr	Ala
Human heart					Val				Ala	Tyr					Tyr	Ile	Ser	Ala
Bovine brain	Val/Ile	Met	Cys		Thr	Leu	Ala		Cys	Phe		Ala	Asn	Leu	Tyr	Ile	Ser	Ser
Human platelet					Thr				Cys	Phe					Tyr	Ile		
Acanthamoeba		Met		Val	Thr	Leu	His	Leu	Ala	Tyr	Val	Gly	Val	Leu	Phe	Leu	Ser	Ser

The data are from Elzinga and collaborators (47–49). *Acanthamoeba* actin was isolated by T. D. Pollard. The table shows only those residues where one or more of the four other actins for which partial sequence data are available differs from the totally sequenced rabbit skeletal muscle actin. Rabbit skeletal muscle actin contains 374 amino acid residues (50). Of the 79 amino acids that have been sequenced in human heart actin, only one is different (Thr → Ser, 357); 12 of the 159 sequenced amino acids of bovine brain actin, 2 of the 44 sequenced residues of human platelet actin (platelets show no differences from brain), and 14 of the 228 sequenced residues of *Acanthamoeba* actin are different. Bovine brain actin shows microheterogeneity at position 10.

actin in specific cells, all nonmuscle cells, as will be discussed later, seem to have specific mechanisms for maintaining actin in a nonpolymerized state.

In marked contrast to the almost universal occurrence of cytoplasmic actin microfilaments, thick filaments analogous to those formed by myosin in skeletal muscle have rarely, if ever, been observed in nonmuscle cells. Instead, immunofluorescence microscopy with anti-myosin antibodies indicates that myosin is closely associated with the actin microfilaments (27–29). The contractile rings of dividing cells react with anti-myosin, as do the stress fibers of cultured cells and the actin-containing region between chromosomes and mitotic spindles. Anti-myosin also diffusely stains the sub-plasma membrane region that typically contains a fibrous network of microfilaments too small to be visualized by light microscopy, but actin in the ruffling regions, microspikes, and regions of cell–cell contact may not have associated myosin (30). At least in some cells, myosin also seems to be associated with the plasma membrane (31), and antigenic sites of the myosin molecule seem to be accessible to antibody at the outer cell surface (32, 33). For the localization of motility proteins with fluorescent antibodies, cells are only lightly fixed with formaldehyde. This may not be sufficient to preserve the natural distribution of all cytoplasmic proteins.

Stress fibers of cultured mammalian fibroblastic cells also react with antibody raised against smooth muscle tropomyosin and α-actinin (34–36), apparently in alternating patches along the fibers (myosin also shows an intermittent distribution), and anti-α-actinin reacts with the actin microfilaments in the acrosomal process of horsehose crab sperm (37). Anti-α-actinin also reacts with the cytoplasmic surface of the intestinal microvillus membrane at the apparent points of attachment of the actin microfilaments (24). Tropomyosin may lend structural stability to the microfilaments (38, 39) in addition to functioning as a regulator protein (see below), and α-actinin, which is thought to be involved in the linkage of muscle thin filaments to the Z line of skeletal muscle (40, 41), may be a factor in coupling cytoplasmic microfilaments of nonmuscle cells to the plasma membrane. In this regard, as in others, nonmuscle cells may be more similar to smooth muscle cells (42) than to skeletal muscle.

A number of biochemical questions are raised by this brief summary of presently available morphological data. What regulates the ever-changing distribution of actin filaments within the cell and the state of polymerization of the actin? How are the microfilaments bound to the plasma membrane and to each other to form bundles of microfilaments? What controls the rapid dissociation of these linkages and their reformation? Is cytoplasmic myosin organized into bipolar filaments too small to be detected, or is the myosin of nonmuscle cells associated in some other form with the actin microfilaments? And, of course, the central question: how are the microfilaments employed to generate the many different kinds of movement with which they seem to be associated? It seem most likely that only traditional biochemistry blended with the most sophisticated ultrastructural techniques, a molecular cell biology, will answer these questions.

Biochemistry of nonmuscle actins

Actin has been isolated in reasonable purity from fewer than 15 cell types and reliably characterized, albeit still incompletely, in yet fewer instances. Actin accounts for as much as 20%–30% of the total protein of actively motile cells, such as amoebae and human blood platelets, and comprises about 1%–2% of the protein of tissues such as mammalian liver (14–16, 43–45). In our experience (44, 45), native actin can be recovered in very high purity in about 25% yield from many sources by a procedure involving chromatography on DEAE-cellulose, polymerization and depolymerization, and gel filtration of monomeric G-actin.

Nonmuscle actins closely resemble each other and rabbit skeletal muscle actin. All are proteins of about 42,000 daltons with the same electrophoretic mobility on dodecyl sulfate/polyacrylamide gels; each that has been examined contains 1 mol of bound adenine nucleotide per mol of protein; all have very similar amino acid compositions, including the presence of one residue of N^{τ}-methylhistidine, the function of which is unknown but, by inference from its evolutionary stability, must be important.

There are, however, significant chemical differences among the actins. Only actin from *Acanthamoeba castellanii* (43, 44), for example, has been found to contain N^{ϵ}-methyllysines, and all of the actins for which at least partial sequence data are available (46–49) are different, although the sequence as a whole is remarkably conserved. Only about 6% of the residues are different in actins from sources as evolutionarily distant as *Acanthamoeba castellanii* and rabbit skeletal muscle (Table 1). The sequence data now available for just a few actins allow for the remarkable possibility that all muscle actins may fall into one group and all vertebrate nonmuscle actins into another. Thus, the two muscle actins contain valine at position 129, alanine at position 271, and tyrosine at position 278, while the vertebrate nonmuscle actins contain threonine, cysteine, and phenylalanine, respectively, at these positions (Table 1). *Acanthamoeba* actin contains some of the residues of vertebrate muscle actins and some of the residues of vertebrate nonmuscle actins at these positions, and, in other positions, differs from both types of vertebrate actins.

Table 2. Comparison of the properties of nonmuscle and muscle actins

	Critical concentration, mg/ml							
	2 mM $MgCl_2$		0.1 M KCl		0.5 M KCl/ 1 mM $MgCl_2$	0.5 M KCl/ 1 mM $CaCl_2$		
Source of actin	25°	5°	25°	5°	25°	25°	5°	K_{app}, μM
Rabbit skeletal muscle	0.03	0.03	0.03	0.1	0.02	0.15	0.94	7.1
Human platelet	0.03	0.03	0.09	0.51	0.09	0.32	3.2	9.6
Rat liver	0.02	0.02	0.08	0.48				9.6
Chick embryo brain	0.02	0.02	0.07	0.5	0.01	0.22		9.6
Acanthamoeba castellanii	0.06	0.06	0.09	0.45	0.04	0.39		21.7

K_{app} is the concentration of F-actin required for half-maximal activation of the Mg^{2+}-ATPase of rabbit skeletal muscle heavy meromyosin in 2.5 mM $MgCl_2$/2.0 mM ATP/2.4 mM imidazole chloride, pH 7.0, at 24°. Data are from refs. 44, 45, and 56.

The sequence data also establish that a single species can possess more than one actin gene, because human heart and platelet actins are different. The further observation that bovine brain actin is microheterogeneous at position 10 suggests that there may even be two actin genes functioning in one cell. Recently, all nonmuscle vertebrate cells that have been examined (51–53) have been found to contain two forms of actin, β and γ, separable from each other by isoelectric focusing and differing from the more acidic α-actin, which is the only species detected in mammalian skeletal muscle. It is not known if the isoactins of a single cell represent different gene products [it seems unlikely, but not impossible, that the microheterogeneity at residue 10 of brain actin (Table 1) is the source of the two different isoelectric forms] or if they differ by a post-translational modification. It is also possible that the apparent multiplicity of isoelectric species is an experimental artifact. *Acanthamoeba* actin (δ) has an isoelectric point more alkaline than that of γ-actin (45) [possibly as a consequence of the substitution of histidine for alanine at position 228 (Table 1)].

All actins, both muscle and nonmuscle, polymerize at an appropriate ionic strength—for example, 2 mM $MgCl_2$ or 0.1 M KCl—to form F-actin, a 7-nm-wide, double-stranded helix with a half-pitch of 35 nm. In the process of polymerization, one molecule of bound ATP is converted to one molecule of bound ADP for every monomeric actin subunit. Polymerization is a two-step process of nucleation and elongation, the first step being rate-limiting and concentration-dependent (40). Nucleation occurs only above a certain concentration (the critical concentration) of actin, and F-actin (polymer) will always be in equilibrium with G-actin (monomer) at its characteristic critical concentration which is the equilibrium constant for the equation: $(actin)_n \rightleftharpoons (actin)_{n-1}$ + actin. The polymerization of any actin, in a specific solvent and at a specific temperature, is described by the critical concentration and by the reduced viscosity of the F-actin, i.e., the slope of the specific viscosity as a function of actin concentration.

Despite several reports to the contrary (54, 55), the polymerizations of all purified nonmuscle actins that have been studied are qualitatively similar to the polymerization of rabbit skeletal muscle actin (45, 56, 57). For each actin there is a critical concentration above which nucleation and elongation lead to the formation of polymeric F-actin. There are, however, significant quantitative differences. Although the reduced viscosities of all isolated nonmuscle actins known to be pure and native are experimentally indistinguishable (about 10 dl/g), all the nonmuscle actins appear to have higher critical concentrations than muscle actin when polymerized, in the absence of Mg^{2+}, in 0.1 M and 0.5 M KCl, and their critical concentrations are increased more than is muscle actin's, but not uniquely, by lowering the temperature (Table 2). Thus, for example, the high critical concentration of platelet actin in 0.5 M KCl/1 mM $CaCl_2$ (54) is shared by other nonmuscle actins (56), and even muscle actin has a relatively high critical concentration in this nonphysiological buffer. The nonmuscle actins polymerized similarly despite their individual differences in amino acid sequence. Almost certainly, the β- and γ-actins from a single source copolymerize *in vitro*; quantitative studies reveal only one polymerizing species and even *Acanthamoeba* (δ) and rabbit skeletal muscle (α) actins have been shown to form random copolymers (ref. 56; Y.-Z. Yang, E. D. Korn, and E. Eisenberg, unpublished observations).

If they behaved *in situ* as they do when purified, the nonmuscle actins would be almost entirely polymerized at the concentrations they occur in cells. However, crude extracts of *Acanthamoeba*, chick embryo brain, and human platelets (45, 55) can be prepared with concentrations of G-actin as high as 3–10 mg/ml even after extensive dialysis against 2 mM $MgCl_2$ (45), i.e., the G-actin is present at concentrations very much greater than its critical concentration. The conclusion is inescapable that these extracts must contain substances that interact with actin to prevent its polymerization (see below).

Other than its presumed structural (cytoskeletal) role, the only known function of F-actin (G-actin has no known specific biological function) is the activation of the Mg^{2+}-ATPase activity of myosin. Actin-activated myosin Mg^{2+}-ATPase can be characterized by a V_{max} (the ATPase activity per mole of myosin at infinite actin concentration) and a K_{app} (the concentration of F-actin required to reach half-maximal activity). Although all nonmuscle actins that have been purified have been shown to activate muscle myosin Mg^{2+}-ATPase, reliable quantitative data are available in only a few cases; it is necessary to obtain kinetic data at actin concentrations greater than K_{app}, and this has usually not been done. It is also important to use as enzyme either of two proteolytic fragments of muscle myosin, heavy meromyosin (two-headed) or subfragment-1 (single-headed), which, in contrast to native myosin, are soluble at the low ionic strengths that must be used, but are enzymatically very similar to intact myosin. The V_{max} will vary with the enzyme preparation and the assay conditions (it is particularly sensitive to ionic strength), but it is identical for all actins irrespective of source (44, 45). This is to be expected if, as has been proposed (58, 59), the rate-limiting step in the hydrolysis of ATP by actomyosin, at infinite actin concentration, is a change in myosin conformation that is independent of actin. When redrawn to conform to this generalization, apparently contradictory published curves (60) fit the experimental points equally well. However, consistently higher concentrations of the nonmuscle actins than of rabbit skeletal muscle actin are required to reach half-maximal activation (Table 2). This higher K_{app} is shared by human erythrocyte actin [when the published

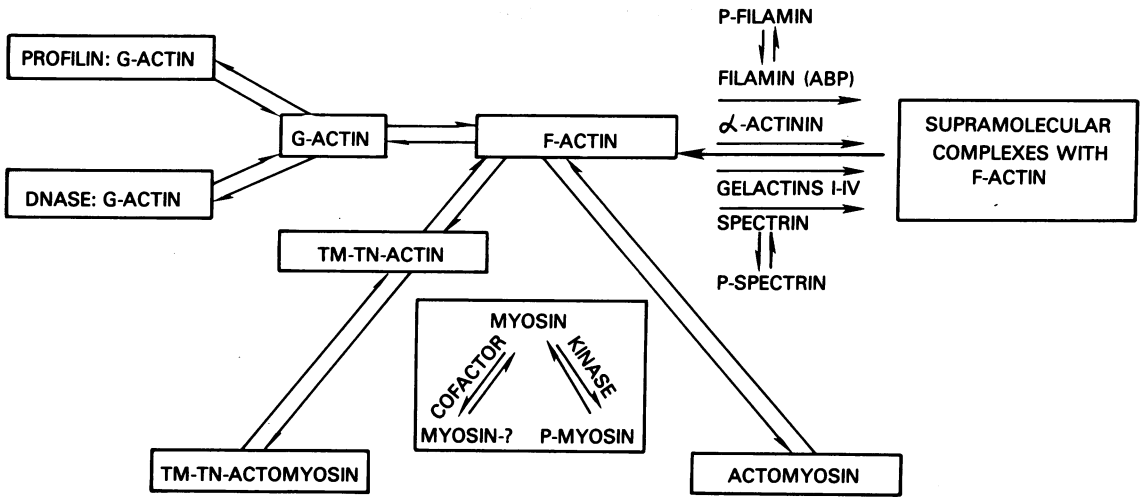

FIG. 1. Known or suspected interactions of actins, myosins, and associated proteins of nonmuscle cells. The evidence for these interactions comprises the major portion of this article. Not all of the proteins, and hence not all of the reactions, occur in any one cell. The interactions presented are neither entirely inclusive (i.e., there may be many other proteins not yet discovered that will interact with actin or myosin) nor necessarily exclusive (i.e., the proteins indicated may well undergo reactions not illustrated). TM, tropomyosin; TN, troponins; ABP, actin-binding protein; P-myosin, phosphorylated myosin; P-spectrin, phosphorylated spectrin; P-filamin, phosphorylated filamin (or ABP); myosin-?, the product of the interaction of macrophage cofactor protein with myosin.

curves for muscle and erythrocyte actin (60) are replotted to have the same V_{max}] and by *Dictyostelium* actin (61). Another measure of the interaction of F-actin and myosin is the stoichiometry of their interaction in the absence of ATP. Whenever it has been measured, nonmuscle actins, as do muscle actins, bind one myosin head per actin subunit.

Another interesting comparison has been made between *Acanthamoeba* and muscle actins (62). Under appropriate conditions, the stoichiometric complex of muscle tropomyosin and muscle F-actin (1:7) is only about 20%–40% as effective as muscle F-actin alone in activating myosin Mg^{2+}-ATPase. *Acanthamoeba* actin has a much lower affinity for muscle tropomyosin, but stoichiometric binding can be obtained by raising the Mg^{2+} concentration or ionic strength. When muscle tropomyosin does bind to *Acanthamoeba* actin, however, it has no effect or enhances the *Acanthamoeba* actin-activation of myosin Mg^{2+}-ATPase, i.e., the effect of muscle tropomyosin on *Acanthamoeba* actin is the opposite of its effect on muscle actin. In random copolymers of muscle and *Acanthamoeba* actins, the two actins maintain their differences; each is affected by tropomyosin as it is in its homopolymer (Y.-Z. Yang, E. D. Korn, and E. Eisenberg, unpublished observations). *Acanthamoeba castellanii* may not contain tropomyosin. Actin from human platelets, which do contain tropomyosin, is much more similar to muscle actin in its interaction with muscle tropomyosin.

These relatively few quantitative studies of nonmuscle actins are sufficient to indicate their general similarities to, as well as their significant differences from, muscle actin, and also to raise a number of questions for further experimentation. In what ways do β- and γ-actins differ, and do the differences have functional significance in the cell? If there are isoactins in the same cell, do they copolymerize *in situ* or are they in separate and distinct filaments? If the latter, do they function in different physiological processes and are they associated with different cellular organelles? By analogy to copolymers of muscle and *Acanthamoeba* actins, even in copolymers β- and γ-actins might express different biological properties. What sequence and structural differences are responsible for the differences in critical concentration of muscle and nonmuscle actins and the qualitative and quantitatve differences in their interactions with myosin and tropomyosin? Complete sequence data and forthcoming crystallographic information (63–65), combined with extensive comparisons of the physical and biochemical properties of the different actins, may allow assignment of actin functions to specific chemical sites. These studies are essential for our understanding of cell motility and they will also further elucidate the molecular interactions in muscle contractility.

Proteins of nonmuscle cells that interact with actin

The proteins that interact with actin will be discussed in three separate groups: those that are known to interact with G-actin (Fig. 1), those that are known to interact with F-actin (Fig. 1), and those that may associate in some way with assemblies of actin filaments (Fig. 1). Myosin and proteins that interact with actomyosin will be discussed later.

The presence in nonmuscle cell extracts of G-actin above its critical concentration implies the presence of one or more proteins that interact with actin to prevent its polymerization. Indeed, one such molecule (Table 3) has already been purified from spleen (63): a 16,000-dalton protein (profilin) that forms a 1:1 crystalline complex with G-actin. Wide distribution of profilin or similar proteins is suspected (63); a similar polypeptide may account for the nonpolymerized actin in the periacrosomal vesicle region of some invertebrate sperm (37). The only other protein known to interact with G-actin (Table 3) is DNase I (73), which forms a 1:1 crystalline complex (64) that has no DNase activity. The biological function of this interaction is unknown; it may be just an intriguing artifact, the occurrence of which led to the discovery of profilin. The potent DNase I inhibitor originally crystallized from calf spleen (74–76) proved to be an actin–profilin complex (63) which reacts with DNase to form an actin–DNase complex. If the reaction between actin and DNase is biologically significant, it could regulate either DNase function or actin polymerization in the cell.

Several proteins that interact with F-actin have been isolated from nonmuscle cells (Table 3); their possible interactions with G-actin are untested. "Actin-binding protein" (Table 3) has been purified from rabbit pulmonary macrophages (66). Polypeptides of similar molecular weight, about 250,000, have been detected in dodecyl sulfate/polyacrylamide electrophoretic gels of crude extracts of many cells but, until the proteins are purified and characterized, it is not justified to assume that

they are all actin-binding proteins. A protein (filamin) of similar molecular weight to actin-binding protein occurs in smooth muscle (77–79) but not in skeletal muscle. Anti-filamin antibodies react with the microfilament bundles of cultured mammalian cells, with microfilaments in microspikes, ruffling areas, and regions of cell–cell contact that do not react with anti-myosin, and, more diffusely, in regions of the cell that contain less-organized microfilaments (30), indicating that filamin and/or actin-binding protein (if they are different but immunologically crossreactive proteins) are associated with the actin filaments. Neither filamin nor actin-binding protein forms filaments alone. Both filamin and actin-binding protein bind to F-actin *in vitro* (66, 72, 77–80), and one study (80) shows a stoichiometry of one bound filamin dimer per 8–12 actin monomer subunits. Filamin has been shown to inhibit the ability of actin to activate myosin Mg^{2+}ATPase (72, 81); the physiological significance of this is unknown, but it may be that cellular aggregates of filamin (actin-binding protein) and actin filaments are restricted to a structural role if they cannot activate myosin ATPase.

It is still not clear whether filamin and actin-binding protein are identical proteins, if they are different proteins, both of which occur in nonmuscle cells, or, as seems most likely, if they are different but very similar and immunologically crossreactive proteins restricted to smooth muscle and nonmuscle cells, respectively. Reported differences in the amino acid compositions of filamin (78, 79) and actin-binding protein (82) may just represent tissue and/or species differences. Reports that cytochalasin B interferes with the gelation of actin-binding protein with actin (83, 84), combined with evidence that it does not interfere with the actin–filamin interaction (80, 81), suggest the two proteins may not be identical, but the reported effects of cytochalasin B on actin-binding protein may be due to interactions with other proteins in those partially purified extracts.

Spectrin, the major protein on the cytoplasmic surface of erythrocyte plasma membranes, almost certainly interacts with actin (26, 85, 86), although the available data are not quantitative and not absolutely conclusive.† Although spectrin and actin-binding protein are both of high molecular weight (Table 3), the two molecules are physically different and antigenically distinct. Spectrin is a multimeric aggregate of equal amounts of two different polypeptides (220,000 and 200,000 daltons), while actin-binding protein, at least by analogy to filamin (78, 79), is a dimer of two identical chains of 240,000–250,000 daltons. The smaller polypeptide of spectrin is enzymatically phosphorylated in the erythrocyte (87–89) and by partially purified erythrocyte protein kinases (90). The isolated protein may be an ATPase of very low specific activity (91, 92), although this still must be proved for homogeneously pure spectrin. ATPase activity has not been reported for actin-binding protein [or filamin (79)], but it can be phosphorylated in cultured fibroblasts (93). Spectrin (60), but not actin-binding protein, shows some antigenic crossreaction with smooth muscle myosin. Antibodies to smooth muscle myosin react very weakly with either one or both polypeptides of spectrin, but antibodies directed against the larger spectrin polypeptide do not react with myosin. Possibly, then, anti-myosin reacts specifically with the smaller of the two spectrin polypeptides, the one that can be phosphorylated. Antibodies directed against spectrin react with no cells other than erythrocytes (27, 94) despite the presence in these other cells of actin-binding protein and myosin.

† **Note Added in Proof.** Phosphorylated spectrin preferentially interacts with actin (180), which probably explains the variability of previous results using mixtures of the phosphorylated and nonphosphorylated forms.

Table 3. Proteins purified from nonmuscle cells and that interact with actin

Protein (ref.)	Source	Molecular weight	Subunits
	Interact with F-actin		
Actin-binding protein (66)	Macrophage		270,000
Spectrin (67–70)	Erythrocyte		220,000, 200,000
β Actinin (71)	*Physarum polycephalum*	86,000	43,000
Gelactin IV (72)	*Acanthamoeba castellanii*	78,000	38,000
Gelactin III (72)	*Acanthamoeba castellanii*	64,000	32,000
Gelactin II (72)	*Acanthamoeba castellanii*	55,000	28,000
Gelactin I (72)	*Acanthamoeba castellanii*	23,000	23,000
	Interact with G-actin		
DNase I (64, 73)	Pancreas	31,000	31,000
Profilin (63)	Spleen	16,000	16,000

Possible relationships between spectrin and myosin will be discussed more fully in the section on myosin biochemistry.

Recently, four low molecular weight gelation factors (gelactins), each of which independently causes F-actin to form gels, have been purified from *Acanthamoeba castellanii* (72). The gelactins are generally more active in gelling actin than are macrophage actin-binding protein and smooth muscle filamin. The gelactins also inhibit the ability of F-actin to activate myosin Mg^{2+}-ATPase (72). Similar low molecular weight gelation factors might well be present in other cells, including those that contain actin-binding protein and/or filamin. It is necessary, therefore, to develop quantitative assays for gelation and to account quantitatively for gelation activity during purification of proteins before deciding which proteins are responsible for the gelation of crude extracts of cells such as macrophage (82, 83) and HeLa (84), and, by inference, for at least some of the viscosity changes that occur in the cytoplasm of living cells.

Other proteins that interact with F-actin have been identified in nonmuscle cells but are not yet purified and characterized. A protein of similar molecular weight to muscle α-actinin is present in the filamentous bundle of the acrosomal process of horseshoe crab sperm (37), and this organelle and, as previously mentioned, several regions of intestinal microvilli and cultured fibroblastic cells react with anti-α-actinin. The horseshoe crab acrosomal process (37) and extracts of sea urchin eggs (95) contain proteins of about 55,000 daltons that clearly interact with F-actin. Preparations from sea urchin eggs containing the 55,000-dalton protein and a 200,000-dalton protein form gels with F-actin. One can only speculate if this widely distributed 55,000-dalton protein is related to the proteins of similar molecular weights that comprise neurofilaments (96) and tonofilaments (97).

It would be wrong, however, to conclude that every protein that induces actin to gel *in vitro* necessarily functions in that way in the cell. For example, muscle α-actinin forms gels with actin *in vitro* (98) but its role *in vivo* seems to be to bind actin filaments to the Z line (41). On the other hand, all proteins that interact with F-actin do not form gels. A β-actinin (Table 3) seems to have been isolated from *Physarum* (71). Although of lower molecular weight, this protein apparently shares with muscle β-actinin (99) the properties of accelerating the poly-

merization of G-actin, inhibiting the depolymerization of F-actin, inhibiting the gelation of F-actin antagonistically to α-actinin, and inducing formation of shorter actin filaments. β-Actinin may serve as a filament terminator *in vivo*. However, the possibility that *Physarum* β-actinin is just a dimer of denatured *Physarum* actin has not yet been eliminated (12).

Although the biochemical characterization of the several proteins discussed here has just begun, it is easy to imagine (Fig. 1) that the proteins that interact with G-actin and with F-actin may be components of complex regulatory mechanisms that control the state of polymerization of actin, the size and aggregation of actin filaments, the formation of stress fibers, the cellular distribution of actin, the association of actin with other organelles such as membranes and microtubules, and the ability of actin to activate myosin ATPase. (F-actin–gelactin and F-actin–filamin complexes are inactive, while F-actin–α-actinin is more active than actin alone.) Finally, if each of the many proteins known to interact with actin [and the known list could be extended to include possibly fibrin (100), aldolase (101), which forms paracrystals with F-actin (102), and several other enzymes of the glycolytic cycle (103)] binds to a specific site on the actin molecule, most of the actin molecule may be essential for function. This could explain the considerable conservation of the amino acid sequence of actin throughout evolution.

Biochemistry of nonmuscle myosins

The data summarized in this section and in Table 4 should be regarded only as a preliminary general description of the still very poorly defined myosins of nonmuscle cells. On the assumption that their properties will be very similar to those of skeletal muscle myosin, nonmuscle myosins have generally been isolated by extracting cells in 0.6 M KCl, precipitating actomyosin at low ionic strength, separating myosin from actin by ammonium sulfate precipitation, and subjecting the material solubilized in 0.5 M KCl to gel filtration on agarose columns. The typical myosin peak is then identified by its high molecular weight (450,000–500,000), polypeptide composition as revealed by dodecyl sulfate/polyacrylamide gel electrophoresis, and ATPase activity (Table 4). Most identified nonmuscle myosins probably consist of two heavy chains of about 200,000 daltons and two pairs of light chains of about 17,000 and 20,000 daltons, although the stoichiometry of the composition has seldom been determined. The light chains, by analogy with muscle myosin, are assumed to be associated with that end of the heavy chains, the globular region, that contains the actin-binding and ATPase sites. The rod portions of the heavy chains are involved in the association of myosin molecules, at low ionic strength, into bipolar filaments. The typical nonmuscle myosin, *in vitro*, forms bipolar filaments that are appreciably shorter (only about 300 nm) than those formed by muscle myosin (114, 126, 127).

The one known exception to these generalizations is *Acanthamoeba* myosin I (122, 123) which is the only nonmuscle myosin known to consist of only one heavy chain and two light chains (Table 4) for a total molecular weight of only about 180,000 daltons. *Acanthamoeba* myosin I apparently cannot form bipolar filaments. *Acanthamoeba castellanii* is also the only cell from which two myosins have been isolated. The recently isolated *Acanthamoeba* myosin II (124) is a more typical two-headed myosin (Table 4) capable of forming bipolar filaments (128) of even smaller dimensions than those formed by other nonmuscle myosins, as perhaps might be expected from its somewhat smaller molecular weight (Table 4). The relationship, if any, between the two myosins of *Acanthamoeba* is still unknown but clearly of great interest; one does not seem to be a proteolytic degradation product of the other (124), although that possibility has not been eliminated absolutely.

Physical properties alone, however, are insufficient to identify an ATPase as a myosin. A minimum additional requirement for a myosin is that it must bind to, and have its ATPase activity modified by, F-actin. Every nonmuscle myosin in Table 4 has been shown to form physical complex with muscle F-actin (Fig. 1). To various degrees depending on their sources, muscle myosins can be further characterized as ATPases actve in the presence of K^+ and EDTA, or Ca^{2+}, but with very low activity in the presence of Mg^{2+}. The Mg^{2+}-ATPase activity of skeletal muscle myosin is activated by F-actin; it is this actomyosin Mg^{2+}-ATPase that is the mechanochemically functional complex in muscle contraction. Therefore, most presumptive nonmuscle myosins have been assayed for ATPase activity under these various ionic conditions, and in the presence and absence of F-actin. The published results for different myosins are very different (Table 4). For some of the myosins, the observed K^+,EDTA-ATPase activity is greater than the Ca^{2+}-ATPase activity, for others it is the reverse; for some the Mg^{2+}-ATPase is activated by F-actin, for others not. When activation occurs the actomyosin Mg^{2+}-ATPase activity can be greater or less than the myosin Ca^{2+}-ATPase activity (Table 4). It is not clear, however, whether these variations are always due to inherent properties of the myosins, or if they may sometimes be due to differences in the assay conditions, or to the isolation of partially denatured or incompletely purified enzymes. In particular, it might be useful to assay proteolytic fragments of the nonmuscle myosins, analogous to muscle heavy meromyosin and subfragment-1, in order to avoid solubility problems. In any case, the variations in enzymatic activities recorded so far for nonmuscle myosins may be no greater than the range in activities of myosins isolated from different muscles (129).

Before any firm conclusions can be reached, however, about the range of properties of different nonmuscle myosins, the enzymes must be characterized more completely. In particular, it will be of great interest to search more carefully for the possible presence of multiple myosins in cells other than *Acanthamoeba*. Considerable CA^{2+}-ATPase activity and K^+,EDTA-ATPase activity are lost during the isolations of myosins from nonmuscle cells. The lost activity may represent nonmyosin ATPases, or just the inevitable loss of myosin identical to that which is isolated. it is worth pointing out, however, that enzymes with the properties of either *Acanthamoeba* myosin I (122) or *Acanthamoeba* myosin II (124) would not have been recovered in any of the purification procedures used to isolate myosins from other nonmuscle cells because neither of the *Acanthamoeba* myosins can be solubilized after precipitation in the presence of actin.

The possible relationship between erythrocyte spectrin and myosin can be considered in this context. Although the Ca^{2+}-ATPase activity of erythrocyte spectrin is very low, the highest value reported [0.006 μmol min^{-1} mg^{-1} (92)] is greater than the values reported for glial myosin (Table 4) and 50% as high as the activities of some muscle myosins (129). From its antigenic properties (see above), it is possible that one of the smaller of the two chains of spectrin resembles the heavy chains of myosins. Polypeptides of low molecular weight equivalent to the light chains of myosins have not been detected in spectrin, and it may be the absence of these light chains (130, 131) that accounts for the very low ATPase activity of spectrin and the absence of actin activation [actin activation of an uncharacterized erythrocyte ATPase has been reported (132) but never confirmed]. It is also possible that the apparent ATPase of spectrin is due to its phosphorylation and dephosphorylation by contaminating kinases and phosphatases that are known to

Table 4. Comparison of myosins of nonmuscle cells

Source (ref.)	Subunits*	ATPase, μmol min^{-1} mg^{-1} K^+,EDTA	Ca^{2+}	Mg^{2+}	Actin-Mg^{2+}	Comments
Human platelet (104–106)	200,000 (2) 19,000 (2) 16,000 (2)	0.9 1.0	0.38 0.41	0.002 0.006	0.029 0.170	Not phosphorylated Phosphorylated
Guinea pig leukocyte (107)	200,000	0.19	0.10	0.012	0.006	
Rabbit macrophage (108)	200,000 20,000 15,000	0.56	0.57	0.045	0.045 0.40	Minus cofactor protein Plus cofactor protein
Mouse fibroblast (109)	200,000	0.43		0.01	0.09	
Bovine medulla (110)	200,000 (2) 20,000 (2) 17,000 (2)	0.35	0.64	0	0.017	
Cat brain (111)			0.34	0.071	0.55	
Glial (112)	200,000 19,000 17,000	0.01	0.0002	0.001	0.0004	
Rat hepatocyte (113)	215,000 20,000	0.12		0.01	0.014	
Chick brain† (114)	200,000 23,000 (1) 20,000 (2.8) 17,000 (1.2)	0.49	0.49	0.015	0.04	
Squid brain (115)		0.17	0.075	0.003	0.025	
Starfish egg (116)	210,000 20,000 17,000	0.3	0.4	0.01	0.045	
Physarum polycephalum (117, 118)	225,000 (2) 21,000 (2) 17,000 (2)	0	2.0	0	0.44	
Dictyostelium discoideum (119, 120)	210,000 18,000 16,000	0.02	0.08	0.005	0.2	
Amoeba proteus (121)	225,000	0.01	0.14	0.02	0.12	
Chaos carolinensis (121)	225,000	0.01	0.1	0.03	0.18	
Acanthamoeba castellanii (76, 122–125)	I. 140,000 16,000 14,000 II. 170,000 17,500 17,000	3.2 0.12	0.38 0.83	0.04 0.08 0.036	0.08 1.23 0.060	Not phosphorylated Phosphorylated Plus or minus cofactor protein

The specific activities are the maximal reported values; none is a true V_{max}, i.e., the rate at infinite ATP concentration and, where applicable, infinite actin concentration. Assay conditions are usually not the same, and myosin and actomyosin ATPase are profoundly affected by ionic strength. The subunit composition of the light chains has often not been determined and the stoichiometry of the heavy and light chains has also not always been determined.

* Stoichiometry of subunits is given in parentheses.

† Chick fibroblast, platelets, liver, kidney, and sympathetic nerve myosins contain similar, but different, heavy chains of about 200,000 daltons. All these myosins contain 20,000- and 17,000-dalton light chains but only nerve also contains the 23,000-dalton light chain (114).

be present, and to use spectrin as a substrate, in isolated erythrocyte ghosts (87–90, 133). While recognizing the speculative nature of these remarks, it is interesting to consider the possibility that spectrin may represent an intermediate stage between nonmuscle myosins and actin-binding proteins that are similar to filamin.

Proteins that interact with nonmuscle actomyosins

It is generally assumed that actomyosin is the functional unit in nonmuscle cells as it is in muscle. It is natural, therefore, to expect that F-actin should activate the ATPase activity of nonmuscle myosins and that, under intracellular conditions, actomyosin will have more ATPase activity than myosin alone. However, this has not always been found (Table 4). In part, at least, this is now known to be due to the requirement for other proteins for actin activation of nonmuscle myosin Mg^{2+}-ATPase. One such discovery was the cofactor protein(s) of *Acanthamoeba castellanii* that is required for actin activation of *Acanthamoeba* myosin I (123) but which has no effect on actin activation of *Acanthamoeba* myosin II (123). A similar

observation was made later for the myosin of rabbit pulmonary macrophage (66). Macrophage myosin and *Acanthamoeba* myosin I are otherwise very different enzymes (Table 4). Very recently, the *Acanthamoeba* cofactor protein has been found to be a kinase that specifically catalyzes the phosphorylation of the 140,000-dalton heavy chain of *Acanthamoeba* myosin I (125). The Mg^{2+}-ATPase activity of the phosphorylated myosin is fully activated by F-actin in the absence of cofactor protein. The function of macrophage cofactor protein is still not known.

On the other hand, actin activation of the Mg^{2+}-ATPase activity of human platelet myosin (106, 134, 135) and myosins from other vertebrate nonmuscle cells (136, 137) occurs only after phosphorylation of their 20,000-dalton light chains by specific kinases. Phosphorylation of the corresponding light chain was subsequently found to stimulate actin activation of smooth muscle myosin (138–141) and, in this case, also to confer a requirement for Ca^{2+} to the actomyosin. Phosphorylation of the heavy chain, as well as the 20,000-dalton light chain, of fibroblast myosin was reported recently (142), but the effect of these phosphorylations on the myosin ATPase activity was not measured. Phosphorylation of fibroblast myosin heavy chain may be similar to the phosphorylation of spectrin (87–89). The discovery of these two regulatory mechanisms, heavy chain and light chain phosphorylation, should stimulate the research for similar, or different, mechanisms that may regulate actin activation of myosin in other nonmuscle and muscle cells.

Two forms of Ca^{2+} regulation of muscle contraction have been described (143): actin-linked and myosin-linked. In the former, a complex of tropomyosin and troponins I, T, and C binds to F-actin and prevents its interaction with myosin in the absence of Ca^{2+}; in the latter, one of the myosin light chains blocks interaction with F-actin in the absence of Ca^{2+} (it is not known if the phosphorylation of myosin light chain described above is one step in this mechanism or represents another type of myosin-linked Ca^{2+} regulation). In almost all muscle cells, the several Ca^{2+} regulatory mechanisms occur together but vertebrate skeletal muscle may have only the actin-linked process and molluscan muscle may exhibit only the myosin-linked process. Ca^{2+} regulation *in vitro* is manifested by a Ca^{2+} requirement for the Mg^{2+}-ATPase activity of actomyosin. Regulation of nonmuscle actomyosins is the subject of a recent review (144).

Direct evidence for tropomyosin in mammalian nonmuscle cells was provided by its isolation from human and calf platelets, calf brain and pancreas, and mouse fibroblasts (141–147). The nonmuscle tropomyosins are all of lower molecular weight than skeletal, smooth, and cardiac muscle tropomyosins, 30,000 [possibly 26,000 for bovine brain tropomyosin (148)] versus 35,000, and yield different, but similar, peptide maps. Nonmuscle and muscle tropomyosins have in common a COOH-terminal leucine residue and a blocked NH_2-terminus. Paracrystals formed by nonmuscle tropomyosins are very much like those formed by muscle tropomyosin.

The troponin complex from vertebrate skeletal muscle contains equimolar amounts of a 37,000-dalton troponin T (the tropomyosin-binding subunit), a 24,000-dalton troponin I (the inhibitory subunit), and an 18,000-dalton troponin C (the Ca^{2+}-binding subunit). The Ca^{2+} requirements of partially purified human platelet actomyosin (149) and rat brain actomyosin (150) have been attributed to mixtures of tropomyosin and three presumably troponin-like peptides with apparent molecular weights of 36,000, 18,000, and 14,000 (in platelets) and 38,000, 27,000, and 20,000 (in rat brain). More recently, a fraction with troponin-like properties has been partially purified from bovine brain (148). Its major components are of molecular weights 36,000, 17,500, and 14,500, similar to the values reported for the complex from human platelets, but significantly different from the molecular weights reported for the polypeptides from rat brain. Although it is tempting to assume that these proteins are the vertebrate nonmuscle cell equivalents of muscle troponins T, I, and C, none of the proteins has yet been purified. Therefore, it is not known if their properties individually will be coincident with those of the muscle troponins, or which of the nonmuscle proteins, in each cell, corresponds to which muscle troponin. Moreover, in contrast to the situation in muscle, one of the three polypeptides in each of the nonmuscle cells seems to be present in much less than equivalent concentration, and it is a different polypeptide in each case: the 18,000-dalton component in platelets, the 20,000-dalton component in rat brain, and the 36,000-dalton component in calf brain.

A most intriguing recent discovery is that the widely distributed Ca^{2+}-regulatory protein required for Ca^{2+} regulation of the activity of cyclic AMP phosphodiesterase is very similar, but not identical, in structure (151–153) and function (154–157) to muscle troponin C. Is it possible that this troponin C-like Ca^{2+}-regulatory protein interconnects cell motility systems with the numerous cellular activities that are regulated through cyclic AMP? Clearly, there is much to be done before the identities of these several proteins are established and their interactions with nonmuscle actomyosins are understood. Even less is known about Ca^{2+} regulation of protozoan actomyosins. Although there is evidence for myosin-linked Ca^{2+} regulation in *Dictyostelium discoideum* (120), and for both actin-linked and myosin-linked regulation in *Physarum polycephalum* (158–163), neither system has been sufficiently defined biochemically.

Molecular events of cell motility

Probably all contractile systems will share these features: (*i*) an on–off cycle in which two components interact, do work, and then separate; (*ii*) elasticity in one of the components, which is required for the interacting components to generate force; (*iii*) efficiency in the conversion of chemical energy to mechanical work. These three properties can be discussed most specifically in relation to the current thinking about the sliding-filament model for contraction of vertebrate skeletal muscle (164), but they are equally applicable to cell motility. (For reviews of the initial formulation of these ideas and their detailed development see refs. 165 and 166; for important modifications of the original ideas see ref. 167.)

It is generally believed that cross-bridges extend from the myosin thick filaments and attach to sites on the actin thin filament at some angle, say 90°, move to another angle, say 45°, release, and then cycle back to their original topographical position relative to another binding site on the actin filament. The presence of tropomyosin and troponin on the actin filament makes the binding of the cross-bridges dependent on the presence of Ca^{2+}, which is released from the sarcoplasmic reticulum as a consequence of membrane depolarization. In the absence of Ca^{2+}, the myosin cross-bridges will be free, in which state their ATPase activity is much less than that of actomyosin. According to theory, the elastic element in the myosin cross-bridge will generate positive force at the angle of initial binding, 90°. As the angle of the cross-bridge changes the positive force will diminish, becoming zero at a 45° angle. Because of the bipolarity of the myosin thick filament, cross-bridges at the two ends of the myosin filament will pull the separate sets of actin filaments to which they are attached towards each other, which, because the actin filaments are attached to the Z lines, will cause the sarcomere to shorten.

At every angle of binding between 90° and 45° a different equilibrium, determined by the relative rates of the on and off reactions, exists between bound and free cross-bridges. It is further suggested that at cross-bridge angles near 90°, where maximum positive force is generated, the on rate will be much faster than the off rate and most of the cross-bridges will be bound. At a 45° angle, where the negative force begins, the off rate will be very much faster than the on rate, so that the cross-bridges will be largely unbound and the negative force minimized. The maximal off rate of the cross-bridges is probably a major determinant of the maximal rate of muscle shortening. Every on–off cycle of each cross-bridge is thought to be coupled to the hydrolysis of one molecule of ATP. When an isotonic muscle shortens at maximal velocity it generates zero force and, therefore, it would be most efficient to minimize the hydrolysis of ATP by reducing the number of bound cross-bridges. Thus, when the muscle is shortening at maximal velocity, the on rate at 90° should be very much less than the off rate at 45° so that most of the myosin cross-bridges will be free. This theoretical prediction is now being actively investigated in several laboratories.

Steady state and presteady kinetic studies of the hydrolysis of ATP by rabbit skeletal muscle heavy meromyosin and subfragment-1 have now led to the identification of a number of intermediate steps at the molecular level that can be correlated with the proposed physiological events of isotonic muscle contraction (for review, see refs. 167 and 168). One representation of the enzymatic cycle is: M^*–ATP → M^{**}–ADP–P_i → M^+–ADP–P_i → AM^+–ADP–P_i → AM + ADP + P_i → AM–ATP → M^*–ATP, in which M^*, M^{**}, and M^+ are different conformational states of myosin, detected experimentally by changes in fluorescence, and A is actin. The actomyosin complex has been shown, by turbidity measurements, to dissociate and reassociate every time a molecule of ATP is hydrolyzed. This is the on–off cross-bridge cycle at the molecular level. The dissociation of actomyosin by ATP is found to be very rapid and its reassociation is very slow, apparently because of the slow conversion of myosin from a refractory state (perhaps M^{**}) that cannot bind actin to a nonrefractory state (perhaps M^+) to which actin can bind. According to this model, the slow formation of actomyosin *in vitro* (M^+–ADP–P_i → AM^+–ADP–P_i) corresponds to the slow formation of the cross-bridge between the actin and myosin filaments in muscle; the rapid dissociation of actomyosin (AM → AM–ATP → M^*–ATP) would follow the work stroke *in vivo*, i.e., the movement of the cross-bridge from an angle of 90° to an angle of 45° (which may occur in the enzymatic state of AM^+–ADP–P_i). The observation *in vitro* that, even at infinite concentration of actin, most of the myosin is free would correspond to the situation believed to exist *in vivo* where, at any instant in an isotonic contraction, most of the cross-bridges of the thick filaments are not attached to the thin filaments. The molecular mechanism for introducing maximal efficiency into the contractile system, therefore, may be the rate-limiting conversion of myosin from the refractory to the nonrefractory state.

There is no corresponding experimental biochemical model for the physiological situation of an isometric muscle contraction where the cross-bridges generate force without movement of the thin and thick filaments. In this case, it is proposed that each individual cross-bridge will go through its on–off cycle at a different fixed angle between 90° and 45°, depending on the position of the cross-bridge relative to the nearest binding site on the actin filament. Therefore, the on rate and off rate will be different for each cross-bridge, as will the equilibrium between free and bound cross-bridges. Overall, in contrast to the situation in an isotonic rapidly shortening muscle, in the isometric state most of the myosin cross-bridges are believed to be bound. Because no work is done in an isometric contraction, the ATPase activity is regulated at a low level, perhaps by the mean rate at which cross-bridges are released.

By simply substituting the plasma membrane (or microtubules or other organelles) for the Z lines of muscle, models at least superficially similar to the sliding filament model could be imagined to generate "isotonic" and "isometric" motile activity in nonmuscle cells. The best, but still insufficient, experimental evidence for a functioning sliding filament mechanism in nonmuscle cells is derived from studies of the contraction *in vitro* of isolated intestinal epithelial cell brush borders (169, 170). The polarized (24) microfilaments of each microvillus intercalate with microfilaments in the terminal web and these, in turn, seem to insert into the plasma membrane at the regions of the zonula adherens. Sliding of these two sets of microfilaments relative to each other, through the intermediacy of myosin filaments (which have not been discerned, however) could be the contractile mechanism in this model system.

But other physiological mechanisms can also be imagined by which the interaction of actin and myosin might be translated into cell motility: for example, one modeled after the interaction of microtubules and dynein ATPase in eukaryotic flagella (3–6, 171, 172). Multiple individual myosin molecules could be attached to one actin microfilament through the rod segments of their heavy chains, perhaps with the involvement of auxiliary proteins, leaving the myosin cross-bridge free to interact with another actin microfilament. Angular displacement of the myosin heads while they were temporarily attached to the second microfilament would, as in the traditional sliding filament model, result in the movement of one microfilament relative to the other. This model (72), for which there is as yet no experimental evidence, would not require the self-association of myosin molecules into bipolar filaments and would, therefore, be equally applicable to single-headed and two-headed myosins. The three requirements of an on–off cycle, elasticity, and efficiency would still apply, but they would be fulfilled by different biochemical mechanisms than in the sliding-filament model.

Because any myosin filaments in nonmuscle cells would be expected to be very small and sparse and because nonmuscle motile systems are, in general, not highly ordered, it is unlikely that electron microscopy or x-ray diffraction will provide significant additional insight into the mechanisms of cell motility at the molecular level. This emphasizes the need for quantitative biochemical studies. The physiological properties of a motility system are limited by, and in part can be inferred from, the biochemical properties of its molecular components. Ideally, therefore, for each nonmuscle system, the intermediates in the actomyosin ATPase cycle should be identified, their equilibrium concentrations determined, and their rates of interconversion quantified.

Is the V_{max} of the nonmuscle actomyosin ATPase very much greater than the V_{max} of free myosin ATPase? Is most of the nonmuscle myosin free or bound at infinite actin concentration in the presence of ATP? Does the nonmuscle myosin have refractory and nonrefractory states, and is the transition from one to the other rate limiting? Which steps in the enzymatic cycle are modulated by phosphorylation of myosin, cofactor proteins, and the other regulatory proteins? With the answers to these and similar questions, and with knowledge of the relative concentrations and distribution of actin and myosin in the cell, we should be able to deduce whether all motility activities in nonmuscle cells are essentially equivalent to isotonic and/or isometric muscle contraction, or whether other mechanisms need to be considered. At this time, none of the necessary bio-

chemical data have been obtained for any nonmuscle system. It will be feasible to obtain this information only for cells that can be obtained in relatively large numbers.

Nonmuscle cells contain much less myosin, relative to actin, than do skeletal muscle cells. This may be just because less force, and, therefore, fewer cross-bridges, is required for cell motility than for muscle contraction, but perhaps actin sometimes functions without myosin. Because of the coupled hydrolysis of one ATP per monomer, polymerization of actin has directionality (173); actin filaments decorated with heavy meromyosin elongate preferentially from the barbed, rather than from the pointed, end (174). Polymerization might be further regulated, and higher orders of aggregation induced, through the interaction of G- and F-actin with other proteins (Fig. 1), at least some of which can be phosphorylated, thus providing additional potential for introducing energy and control into the system. Such mechanisms might be sufficient to account, for example, for the rapid appearance and disappearance of the transitory microspikes that are common to many cells.

In fact, formation of the very long (up to 90 μm) acrosomal process in echinoderm sperm almost certainly results from just such changes in the polymerization state of actin with no involvement of myosin. The acrosomal process of sea cucumber sperm forms by the rapid, directional polymerization of actin (175) that was previously maintained in a nonpolymerized state by interaction with other proteins (25), possibly including profilin (63). In horseshoe crab sperm, on the other hand, the acrosomal process forms by a rapid transformation of the packing structure of preexisting actin filaments from a supercoiled bundle (37) into an elongated hexagonal paracrystal of microfilaments in which each actin subunit is linked to a 55,000-dalton protein (176). These phenomena, in addition to their intrinsic importance, are of potential significance as models of transformations that might occur in the organization of cytoplasmic actin in other cells.

Under appropriate conditions, for example, extracts of many cells will form gels, of which actin is the major component, when warmed to room temperature (76, 82–84, 177, 178), and there are several reports of gelation (177) or paracrystal formation (55, 57, 75) of incompletely purified F-actin. In general, these supramolecular assemblies result from F-actin interacting with one or more of the proteins discussed previously (Fig. 1 and Table 3). Such reactions *in situ* are probably very important in the formation of stress fibers and in controlling the local cytoplasmic viscosity. It may be more accurate, however, to consider all phenomena that involve actin, but not myosin-like ATPases, as processes that maintain, alter and regulate cell shape and structure, including assembly of the motility apparatus, rather than as motility processes *per se*. Cell extracts or reconstituted systems that contain myosin (72, 82, 83, 178, 179) will typically undergo syneresis following gelation. It is yet to be determined if this process will be of more help in understanding the molecular interactions of cell motility than the analogous events of superprecipition of muscle actomyosin have been in elucidating the mechanism of muscle contraction.

Concluding remarks

We know that all eukaryotic cells contain actin, myosin, and related proteins and that these function in many different motility activities that probably require the interaction of microfilaments with cell membranes, microtubules, and possibly other organelles. Contractile events similar to those of isotonic and isometric muscle contractions may occur in nonmuscle cells, but other mechanisms for cell motility are possible. Unfortunately, we still know very little about the biochemistry of nonmuscle actins and myosins and of the many associated proteins, and there are probably many more related proteins yet to be discovered. It is certain that the actomyosin-associated proteins are different in different cells and that their actins and myosins also differ significantly. There is certainly more than one actin in some cells and probably more than one myosin in at least one cell. The complexity of the cellular motility proteins is well illustrated by summarizing the information now available for *Acanthamoeba castellanti*. This small soil amoeba is known to contain one actin (Table 2), two myosins (Table 4), a heavy chain kinase required for actin activation of one of the myosins, and four gelactins (Table 3) that induce aggregation of F-actin (Fig. 1). In addition, *Acanthamoeba* must contain at least one protein capable of preventing the polymerization of G-actin under otherwise favorable conditions (45), yet-to-be-discovered Ca^{2+}-regulatory proteins, if the system is analogous to others, a phosphomyosin phosphatase, and mechanisms for triggering and regulating motility events in response to external and internal stimuli. Similar compilations could be made for platelets, macrophages, brain, erythrocytes and several other nonmuscle cells.

In view of the great complexity of the problem, progress in understanding cell motility will probably come more from the detailed and total description of the biochemistry of motility proteins in some of the presently better described systems than from the continued identification of similar proteins in additional systems, or from studies in intact cells and, particularly, crude cell extracts. Observations of living cells are required to delineate the cells' physiological capabilities and studies of fixed cells are necessary to determine the topographical distribution of motility proteins. The characteristics of crude cell extracts, however, are of value mostly only as a preliminary to further purification, because such preparations have lost the structural relationships of the cell and yet are still too complex and heterogeneous for studies at the molecular level. An understanding of motility activity must, in the last analysis, be based on the quantitative biochemistry of pure proteins.

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