In reviewing work on cytoplasmic contractile proteins and the contributions made to this field by The Journal of Cell Biology, some perspective is gained by first pointing out that most cell biologists were not aware of the existence of these important cellular constituents more than 12 years ago. Times have changed, and today cytoplasmic contractile protein research is one of the busiest areas in cell biology. My purpose here is to highlight some of the important events in the growth of this field and to forecast some future trends. More exhaustive coverage of the field is found in recent books (1, 2) and review articles (3–7). Closely related historical reviews on cellular motility by R. D. Allen and muscle by Franzini-Armstrong and Peachy are included in this volume.

Without question, the most important landmark in this field was the independent purification of actin and myosin from the slime mold Physarum by Hatano and co-workers (8, 9) in Japan and by Adelman and Taylor (10) in the United States in the late 1960s (Fig. 1). To be sure, there were earlier reports by Loewy (11) and others (12) describing “actomyosin-like” proteins in nonmuscle cells, but all of these preparations were too crude to be characterized convincingly. However, once highly purified contractile proteins were available, it was straightforward to establish that they shared many important features with their muscle counterparts and to make a strong argument that they participate in cellular motile mechanisms.

A second major event was the publication in The Journal of Cell Biology in 1969 of a paper by Ishikawa et al. (13) that described a morphological technique for identifying actin filaments in cells by electron microscopy. Their technique was simply to treat glycerated cells with muscle heavy meromyosin that decorated cytoplasmic thin filaments with arrowhead-shaped complexes (Fig. 2), identical with those originally observed along heavy meromyosin-decorated pure actin filaments (Fig. 3) by Huxley (14). Armed with this technique, morphologists found actin virtually everywhere in nature (reviewed in reference 3). More recently a second generation of morphologists has used fluorescent antibodies to localize actin (15), myosin (16), and additional accessory proteins (17) in many cell types.

This work has led to a large number of studies characterizing the cytoplasmic contractile protein molecules and their distributions in cells. Other lines of investigation in this area have included efforts to demonstrate the involvement of the contractile proteins in specific cellular movements and in cytoplasmic structure.

**Cytoplasmic Contractile Protein Characterization**

**ACTIN:** Actin is the most thoroughly characterized cytoplasmic contractile protein. Initial reports were concerned with establishing the existence of actin in various nonmuscle cells and tended to include only a superficial characterization of several different properties. In the last five years, the sophistication of the analysis has increased considerably, so that knowledge about some cytoplasmic actins now approaches what we know about muscle actin.

The concentration of actin varies among different cell types, but it is always one of the most, if not the most, abundant cellular proteins (Table 1). In highly motile cells it constitutes 10–15% of the total protein and is present in concentrations of 100–250 μM. Although it is clear that there is a vast excess of actin over myosin in nonmuscle cells, it is by no means clear...
FIGURE 2 Electron micrograph of the actin filaments of the intestinal epithelial cell brush border decorated with myosin subfragment-1 arrowheads. This method was introduced by Dr. Hal Ishikawa in 1969 and improved by adding tannic acid to the fixative by Dr. David Begg, who contributed this micrograph.

FIGURE 3 Electron micrographs by the author of (a) a negatively stained Acanthamoeba actin filament and (c) a negatively stained Amoeba proteus actin filament decorated with muscle heavy meromyosin. Models of (b) an actin filament and (d) an actin filament decorated with myosin heads from the work of Moore et al. (J. Mol. Biol. 50:279 [1979]).

what all this extra actin is doing in the cell. A leading speculation, discussed in detail below, is that the bulk of the actin is used as a structural protein.

Actins from all major branches of the phylogenetic tree have now been sequenced (18–20). About 95% of the residues are identical in muscle and cytoplasmic actins, but as a group all of the cytoplasmic actins are more similar to each other than they are to muscle actin. This suggests that there have been different evolutionary pressures on muscle and cytoplasmic actins. The N-terminal is the most variable region of the actin molecule, and minor sequence and compositional differences in the first few residues account for the distinctive isoelectric points of the three different isoactin classes found in vertebrates (19). The unusually basic isoelectric point of Acanthamoeba actin (21) is attributable to a histidine at position 228 where the other acts have a neutral residue.1


<table>
<thead>
<tr>
<th></th>
<th>Total protein</th>
<th>Concentration</th>
<th>Actin: myosin ratio</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>(µmol/kg)</td>
<td></td>
</tr>
<tr>
<td>Actin</td>
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<tr>
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<tr>
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<td></td>
</tr>
<tr>
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<td>1.3</td>
<td></td>
</tr>
<tr>
<td>-II</td>
<td>1.2</td>
<td>2.3</td>
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The three-dimensional structure of the actin molecule is not yet known. However, both two- and three-dimensional actin crystals of muscle and nonmuscle actins are now being studied by electron microscopy and X-ray diffraction (22, 23, 23a) so this information should soon be available.

Given the highly conserved nature of the actin molecule, it is not surprising that all actin filaments are indistinguishable by electron microscopy (see reference 3) (Fig. 3). As established by X-ray diffraction of live muscle (24) and confirmed by electron microscopy of many different actin specimens, actin filaments consist of a double-helical array of more or less globular actin molecules (14). The polymer is 6 nm wide, the molecules are 55 nm long, and the helix repeats every 37 nm. The similarities among various actin filaments extend beyond the double-helical structure of the filaments to the myosin-binding sites. They must be nearly identical, because all known actins bind myosin in precisely the same way to form polarized arrowhead-shaped complexes which repeat with every turn of the underlying actin helix (see reference 3) (Fig. 3). As stressed by Huxley (14), the polarity of actin filaments revealed by myosin decoration is an essential feature for tension generation. In every case studied, the force generated by actin-myosin interaction pulls the actin filament in the direction pointed by the arrowheads.

In those cases where cytoplasmic actin polymerization has been studied in detail (21, 25, 26), the process has been shown to be generally similar to actin polymerization in muscle. The only substantial differences were found under nonphysiological conditions. Actin polymerization involves three major steps (27). First, several monomers bind together to form a short oligomer. This slow step provides a nucleus. Next, monomers rapidly add to the nucleus to elongate the filament. Elongating filaments grow in both directions with a strong bias toward the "barbed" end (the end with the arrowhead barbs if the filament were decorated with myosin) (28). The third step is the annealing of two filaments end-to-end to form a longer filament. Filaments will grow until the concentration of monomer is reduced to the so-called "critical concentration" of monomer that remains in apparent equilibrium with any amount of filament. Rather than a true equilibrium, this is more likely to be a steady state with net actin monomer addition at one end and net loss from the other end (29). The regulation of the polymerization process is discussed below.

All actins are capable of binding myosin reversibly and in the presence of adenosine triphosphate (ATP) of activating the myosin Mg-ATPase activity (see reference 3). This cyclic interaction of myosin, actin, and ATP is thought to be the physiologically relevant, force-generating enzyme activity of the proteins. When cytoplasmic actins were compared with muscle actin for the ability to stimulate the muscle myosin ATPase, all of the actins gave the same Vmax, but muscle actin had a higher affinity for the myosin than did the cytoplasmic actins (26).

As mentioned above, the classic method for identifying actin filaments in cells is decoration with myosin fragments (either heavy meromyosin or subfragment-1). Studies of many cells have shown that most 6-nm filaments in cells are composed of actin (13) (Fig. 4). Some cytoplasmic actin filaments are preserved in thin sections of fixed cells, but there is concern that the conventional fixation process, especially extended exposure to OsO4, fragments actin filaments to form "microfilament networks" (30). Negative staining (31) and freeze drying (32), which preserve straight actin filaments, are two alternate methods for preparing cells for electron microscopy. They promise to be extremely useful in mapping out associations among the fibrous elements of the cytoplasm, although they are currently limited to extracted cell models.

Fluorescence microscopy with labeled antibody (15) or labeled heavy meromyosin (33) allows one to evaluate quickly the overall distribution of actin in the whole cell. As much for aesthetic reasons as anything else, considerable attention has focused on the so-called "stress fibers", which are prominent in some tissue culture cells (Fig. 5). These fibers are composed of actin filament bundles with associated myosin (16) and accessory proteins (17). They are potentially contractile (34) and may be involved with cytoplasmic retraction in some forms of cell movement. However, most cells with prominent stress fibers exhibit little locomotion (34a) (Fig. 5). On the other hand, rapidly motile cells have a diffuse distribution of actin and myosin.

A promising method for studying actin distribution in living cells is microinjection of actin labeled with a fluorescent dye (35-37). Image intensification of the fluorescence allows direct observation of actin dynamics. Alternatively, the injected cell can be fixed and the actin distribution observed by conventional means.

Actin filaments are highly concentrated in the peripheral cytoplasm (cortex) of many cells, which suggests that they may be attached to the plasma membrane. This was shown to be true by isolating membranes from cells such as Acetabularia (38) and the intestinal absorptive cell (39) and by showing biochemically and microscopically that actin filaments are...
FIGURE 5 Tissue culture cells stained with fluorescent antibodies. (a) PtK-2 cells stained with purified anti-actin showing continuously labeled stress fibers. (b) HeLa cells stained with purified anti-platelet myosin showing punctate labeling of stress fibers. (c, d) Phase-contrast time lapse videotape records of the movements of living chick embryo cells taken 30 min and 1 min before fixation and staining with fluorescent anti-actin. (e, f) Fluorescence micrographs of the two cells indicated in (d) showing the diffuse staining of these migrating cells. Micrographs by Dr. Ira Herman.

attached. Most importantly, the barbed end of the actin filaments always seems to be attached to the cytoplasmic surface (Fig. 2) (34, 39, 40). Consequently, any tension generated by myosin interaction with these filaments will exert a “pull” on the plasma membrane, just as in the case of the Z line in striated muscle.

In spite of the profound functional significance of these membrane attachments, essentially nothing is known about their molecular basis. Early enthusiasm about the Z-line protein α-actinin being involved directly with attachment (39) has not been substantiated, although both α-actinin (17, 41, 42) and a 130,000-mol wt protein (43) are found near actin attachment sites in tissue culture cells and the zonula adherens of epithelial cells.

MYOSIN: The work on cytoplasmic myosins lagged behind studies of cytoplasmic actin for a number of years. Initial problems were the small amount of myosin in most cells and the lack of simple purification procedures. Moreover, no morphological work was possible until antibodies were developed, because myosin cannot be identified in nonmuscle cells by conventional light or electron microscopy. Now there are reliable purification procedures (for example, see reference 44) and methods for localization (16).

In contrast with muscle, where myosin is the major protein, myosin is a minor protein in nonmuscle cells (Table I). This is appropriate for myosin, the force-generating, energy-transducing enzyme in these contractile systems, because the forces required for cellular motility are orders of magnitude less than those developed by muscle.

Unlike the actins, which are all rather similar, the myosins are remarkably variable. Even though the myosins from various muscles are all the same size and shape, they differ in primary structure and enzyme activity (45). Nonmuscle cells even have myosins with different sizes and shapes (Table II) (38, 46–49), and at least one cell, Acanthamoeba, has multiple myosins (46–48). Given this diversity, one might ask what defines myosin? I feel that the essential features of myosin are the ability to bind reversibly to actin filaments and actin-activated ATPase activity. Other features, such as the capacity to form bipolar filaments, are common to most myosins, but probably are not essential.

All myosins consist of “heavy chains” and “light chains” (Table II). In most cases, one end of the two heavy chains forms an α-helical-coiled coil “tail”; the remaining part of the heavy chain, together with the light chains, form two globular

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<th>Table II</th>
<th>Cytoplasmic Myosin Classes</th>
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<tbody>
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<td></td>
<td>Molecular weight</td>
</tr>
<tr>
<td>Metazooan</td>
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</tr>
<tr>
<td>Slime mould</td>
<td>~500,000</td>
</tr>
<tr>
<td>Acanthamoeba myosin-I</td>
<td>~180,000</td>
</tr>
<tr>
<td>Acanthamoeba myosin-II</td>
<td>~420,000</td>
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Pollard Cytoplasmic Contractile Proteins 159s
"heads" (Fig. 6) (50, 51). In both muscle (50) and nonmuscle cells (52) the heads contain the catalytic site for ATP hydrolysis and the actin-binding site. The tails form the backbone of myosin filaments.

Except for the globular *Acanthamoeba* myosin-I, which has no tail (46), all muscle and cytoplasmic myosins form bipolar filaments under physiological conditions (Fig. 6) (48, 49, 53). Although they differ in size, all of these myosin filaments have the same geometrical features. In the center is a bare zone, the same length as the myosin tail, composed of an antiparallel overlapping array of myosin tails. This bare zone is flanked by terminal regions of variable length where the myosin heads protrude from the surface of the filament at 15-nm intervals (24). Thus myosin filaments have a plane of mirror symmetry in the center of the bare zone. The thickness of the filament depends on how many heads are present in each 15-nm interval (53). The filaments formed by most purified cytoplasmic myosins are small compared with the myosin-thick filaments in muscle, which are composed of 300-400 myosin molecules (54) and are 18 nm wide and about 1.5 μm long. Platelet myosin filaments, for example, are composed of about 30 myosin molecules and are 10-11 nm wide and 0.3 μm long (53). *Acanthamoeba* myosin-II filaments are even smaller (48).

Such myosin filaments are not seen in electron micrographs of nonmuscle cells. It has been argued (53) that this is the consequence of the low concentration of myosin and the small size of the filaments, but it could equally well be that little of the myosin is usually assembled into filaments. Early results from electron-microscope localization of myosin with ferritin-labeled antibodies suggest that all of these factors contribute to the apparent absence of myosin filaments (54a).

**Participation of Contractile Proteins in Cellular Movements**

Establishing mechanisms for the generation of cellular motile force has been difficult compared with that for muscle, where direct physiological studies of contractile protein activity manifested as tension generation or shortening are straightforward. The biochemical properties of the cytoplasmic contractile proteins make it seem obvious almost from the beginning that they must be responsible for cellular movements, but even the obvious must be proven. In fact, very few cellular movements have been shown to be powered by the contractile proteins. The following discussion is divided into two sections: first, the arguments that the cytoplasmic contractile proteins can generate tension and motion, and second, the evidence that they are responsible for a specific movement—cytokinesis.

**The Arguments:** One suspects from the parsimony of nature and the similarity of the structures of actin and myosin filaments in all cells, that the well-studied mechanism generating force in muscle is universal. Because the myosin heads at each end of the filaments are oppositely polarized, one filament can cross-link two or more oppositely polarized actin filaments. In muscle, a structure of this kind is repeated with crystalline precision many times in parallel and in series to make sarcomeres and myofibrils (24). No actomyosin contractile structures with such clearly defined geometry have been identified in nonmuscle cells, but filaments of cytoplasmic actin and myosin can form loosely organized networks with the same essential geometrical features (52, 53). This shows on mechanical grounds, at least, that the contractile proteins of nonmuscle cells can generate tension and motion by the same sliding filament mechanism used in muscle.

The most highly ordered cellular actomyosin structures appear to be the brush border of some epithelial cells and the stress fibers of tissue culture cells. In the brush border, a large number of microvilli protrude from the surface. Each microvillus contains a bundle of actin filaments attached at their barbed ends to the tip of the microvillus (39). The pointed ends of the filaments are in the terminal web, where they are associated with myosin (55). Geometrically, the structure is similar to a sarcomere folded in the middle of the A band. The natural motion of microvilli is not known, but a sliding-filament mechanism can account for the movement of the microvillar bundles into the terminal web in demembranated models treated with ATP (56). Stress fibers are less ordered. They consist of a bundle of parallel actin filaments (57) with intermittent concentrations of myosin (16), α-actinin (17), tropomyosin (17), and a new actin-binding protein called filamin by some (58). However, the detailed arrangement of these proteins in stress fibers has not been established, so that the mechanism of their contraction (34) is unknown.

**Figure 6** Platelet myosin. (a) An electron micrograph of a shadowed platelet myosin molecule contributed by Dr. K. Burridge. (b) A drawing of a platelet myosin molecule showing the constituent polypeptides. (c) An electron micrograph of a negatively stained bipolar filament formed from purified platelet myosin taken by the author. *l* is the length of the bare zone, *D* is the diameter of the bare zone, arrowheads mark some of the myosin heads. (d) A two-dimensional model of a platelet myosin filament showing the dimensions of the filament in nanometers from the work of Nieder- man and Pollard (53).
In addition to these structural considerations, the following facts strengthen the argument that the cytoplasmic contractile proteins generate forces for cellular movements by a mechanism similar to that of muscle. It is possible to construct functional actin-actin (59), myosin-myosin (60), and actin-myosin hybrids (review in reference 3) from quite diverse muscle and nonmuscle cells. For example, any myosin will bind to any actin, and any actin will activate myosin ATPase (26), which show that the essential features of the active sites are conserved. Furthermore, actomyosin threads (61) and gels (62) have been formed from a number of purified myosins and actins and shown to contract upon addition of ATP.

The Evidence: Cytokinesis is the most thoroughly studied example of a cellular movement believed to be powered by actin and myosin. Elegant micromanipulation studies (63) established that the cleavage furrow itself develops enough tension to bring about the deformation of the cell during cytokinesis. In the base of the furrow is a narrow band of parallel actin filaments, called the contractile ring, which encircles the equator of the cell (64). The volume of a contractile ring decreases during cytokinesis, which suggests that it disassembles (65). Both myosin (16) and α-actinin (66) are present in the cleavage furrow and, in many cells, are more highly concentrated there than elsewhere in the cytoplasm (Fig. 7). Surprisingly, actin does not seem to be concentrated in the furrow, compared with other regions of the cell cortex (Fig. 7) (67, 68), which suggests that the unique feature of the contractile ring is the parallel alignment of the filaments. It has been suggested that tension on preexisting random actin filaments brought about by contraction confined to the equatorial region might align the filaments and form the contractile ring (66).

Microinjection studies have provided direct evidence for the participation of myosin and actin in cytokinesis. Injection of myosin antibodies into living echinoderm eggs inhibited cytokinesis (Fig. 8) (69), presumably by inactivating myosin. The injected cells survived and continued nuclear division, but cytokinesis was permanently blocked. Microinjection of frog eggs with heavy meromyosin inactivated with a sulfhydryl reagent also blocked cytokinesis (70), presumably by binding permanently to actin filaments and interfering with their interactions with cellular myosin.

Although these experiments provide evidence for an actomyosin purse-string mechanism of cytokinesis, the details of the mechanism are unclear. No other cellular movement is understood in even this much detail.

Regulation of Cellular Contractile Protein Function

The regulation of the contractile apparatus must be a challenging task for the cell. Like muscle, nonmuscle cells must turn the contractile machinery off and on, but on top of that nonmuscle cells must also specify when and where actin and myosin filaments assemble and disassemble. I will cover filament assembly mechanisms before considering the regulation of motility.

There are a number of reasons to believe that cells must have mechanisms that specify the number, the sites of assembly, and lengths of their actin filaments, in addition to having some way to regulate the interaction of the filaments with each other, with membranes, and with other cellular structures, such as microtubules. For example, the ephemeral contractile ring in cytokinesis organizes, exerts tension on the plasma membrane, contracts, and disappears in a matter of minutes (65). Other filamentous structures, such as the brush border of intestinal or renal tubular epithelial cells, are much more stable, but some mechanism is responsible for the precisely ordered arrays of membrane-associated actin filaments, which are all the same length (39).

The total amount of actin filament in a cell is probably determined by the concentration of available actin, but not all of the actin is available for polymerization. Some of it seems to be sequestered. For example, some sperm have a cup of actin complexed with other proteins stored for eventual polymerization during the acrosomal reaction (71). In other cells, a substantial fraction of the actin is bound to a 16,000-mol wt protein, profilin, which inhibits the nucleation of actin polymerization (72, 72a). Together with a limited number of polymer initiation sites, profilin could maintain the total number and overall lengths of the filaments.

A more specific way to determine the length would be to
capped one or both ends of the filaments, to prevent subunit addition or loss. Such a capping protein has been purified from *Acanthamoeba* (72b). It consists of 28,000- and 31,000-mol wt subunits and blocks monomer addition at the “barbed” end of actin filaments. A protein named β-actinin may cap the “pointed” end of muscle actin filaments (73), but its mechanism and the identification of its subunits are not settled. Physarum actin filaments. A protein named fl-actinin may cap the subunits and blocks monomer addition at the “barbed” end of cap one or both ends of the filaments, to prevent subunit addition at the final site. For example, the filaments in intestinal epithelial cells seem to grow in the pointed direction from dense membrane plaques, which eventually become the tips of the microvilli (76). In some sperm, the actin filament bundle in the acrosomal process grows in the barbed direction from a filamentous structure named the actomere (77). The molecular components of these and other nucleating structures are not known. The only nucleating molecule known is the *Acanthamoeba*-capping protein (72b). At very low concentrations (1/1000 actin molecules), it initiates actin polymerization and, because it also blocks growth in the barbed direction, determines both the site and direction of polymerization.2

Before anyone investigated the regulation of cytoplasmic actin-myosin interaction, it was shown by experiments with glycerinated cells (78), caffeine-treated slime-mold fragments (79), and demembranated cytoplasmic models (80) that the free Ca++ concentration controlled cytoplasmic contraction and streaming. The threshold Ca++ concentration for movement was in the micromolar range. The source of Ca++-stimulating movements in living cells has not been established. Because Ca++ also stimulates contraction in muscle, the first thoughts about mechanisms regulating cellular motility turned to the vertebrate skeletal-muscle paradigm of tropomyosin and troponin (81). This is a negative control mechanism, because it turns off the spontaneous interaction of actin and muscle myosin, which occurs regardless of the Ca++ concentration. Tropomyosin-troponin regulate contraction by blocking the actin-myosin interaction when the Ca++ concentration is low. When the Ca++ concentration is in the micromolar range, the inhibition is removed and the actin, myosin, and ATP continue their cyclic interaction until the Ca++ concentration falls and the block is restored.

When Cohen and Cohen (82) purified tropomyosin from platelets, it seemed as though the earlier history of similarities to muscle would be repeated in nonmuscle cells. Next, brain tropomyosin (83) was shown to form a functional, Ca++-regulated hybrid with muscle troponin, actin, and myosin. When a tropomin-C-like molecule was found in brain (84) and adrenal gland (85), only troponin-T and troponin-I were missing to reconstitute the whole regulatory system. But cytoplasmic troponin-T and troponin-I have not been found and may not exist. Instead, something new and much more interesting emerged: positive regulation of cytoplasmic contractile systems.

The first example of a positive regulatory mechanism was found in *Acanthamoeba* (86). When myosin-I was first purified, it was found to lose its actin-activated ATPase during the final steps of the purification. This lost activity was restored by adding back a partially purified 95,000-mol wt “cofactor” protein. From the stoichiometry of the reactants, it was speculated that the cofactor protein acted on the myosin rather than upon actin. A crude cofactor protein was also found in macrophages (87).

Independently, Adelstein and his colleagues found that platelet myosin light chains were phosphorylated and that the phosphorylated form of myosin had much higher actin-activated ATPase activity than the dephosphorylated form (88). In the initial experiments, Ca++ and cAMP had no effect on the kinase or the actomyosin ATPase, so it was not clear that myosin phosphorylation had anything to do with control of cellular movements. However, through subsequent experiments with both smooth muscle and nonmuscle cells, it was shown that Ca++ regulates the light-chain kinase through the calcium-binding protein calmodulin, the tropomin-C-like protein purified previously (89, 90). Actually, calmodulin seems to have been discovered several times. Calmodulin binds Ca++ and then binds to and activates the light-chain kinase, which, in turn, activates the myosin. Activation of contraction by light-chain phosphorylation has been shown directly in synthetic platelet actomyosin threads (61), so this mechanism, coupled with appropriate phosphatases, should be capable of turning cellular contractions on and off.

It has now been shown that the macrophage cofactor protein is a light-chain kinase (91), but the *Acanthamoeba* cofactor turned out to be a heavy-chain kinase (92). Dephosphorylated myosin-I has little actin-activated ATPase activity, whereas the phosphorylated form is highly active.

It is conceivable that myosin phosphorylation alone can account for the regulation of cytoplasmic contractility. However, the function of cytoplasmic tropomyosin has not been established, and it is possible that there are additional unrecognized mechanisms controlling cellular contraction.

**Actin as a Structural Protein**

Since the 1830s, when Dujardin proposed the existence of a...
gelatinous contractile material in the cytoplasm, there have been repeated suggestions that the cellular contractile machinery also plays an essential structural role (see reference 93 for a review). By the 1930s, it was generally accepted that living cytoplasm is a gel-like material which varies in consistency with time, depending on the activity of the cell. Some believed that the gel was essential for motility. The direct demonstration of this connection at the molecular level came from experiments on amoeba cytoplasmic extracts. Thompson and Wolpert (94) found that cold, cell-free extracts of *Amoeba proteus* streamed when supplied with ATP and warmed to room temperature. Pollard and Ito (95) showed that the extracts increased in viscosity at the time they were warmed and that this consistency change is correlated with the formation of innumerable 6-nm filaments from a soluble precursor. These 6-nm filaments were later identified as actin filaments. It was argued that the polymerization and depolymerization of the actin might control cytoplasmic consistency.

In subsequent experiments on demembranated amoeba cytoplasm, Taylor et al. (80) found that conditions which influence actin-myosin interaction also affected the viscoelastic properties of the cytoplasm. They suggested modulation of actin-myosin interaction as an addition factor in the determination of cytoplasmic consistency.

A series of papers from several laboratories (62, 96–101), have established that the components of many cells solubilized by homogenization in the cold can form a solid gel when warmed to room temperature. From the work of the author (44), it was discovered that cold, cell-free extracts of *Amoeba proteus* streamed when supplied with ATP and warmed to room temperature (Fig. 9). Initially, these experiments were carried out qualitatively by tipping test tubes, but now the kinetics of the gelation reaction can be followed with a sensitive low-shear, falling-ball viscometer (101). Generally, it has been found that gelation requires Mg-ATP and is inhibited by micromolar free Ca$^{2+}$. Actin filaments are the major component of these gels, and under appropriate conditions myosin associates with the gel to cause contraction.

Because the gels have a much higher viscosity than actin alone, efforts have been made to isolate cross-linking molecules from these extracts (Table III). The first was actin-binding protein from macrophages (102). This large protein cross-links actin filaments to form a homogeneous gel. Independently, a similar protein was purified from smooth muscle and named filamin (58). Small- and medium-size gelation factors have been isolated from *Acanthamoeba* (101, 103).

Sea urchin eggs are particularly interesting, because the gels differ in structure from the others mentioned above. The sea urchin extract forms actin filament bundles, rather than isotropic actin filament networks. The bundles are held together by a 58,000-mol wt protein named fascin, which is spaced at 11-nm intervals along the bundles (102). Similar bundles with 10-nm periodicity are found in sea urchin egg microvilli (104).

There is general agreement that the Ca$^{2+}$ concentration controls, at least in part, the gelation process, but there is little agreement about the mechanism. The simplest case is that cross-linking by one of the gelation factors is inhibited by Ca$^{2+}$. This is true for a 110,000-mol wt gelation factor from ascites tumor cells (105). Another possibility is that additional proteins participate, as suggested for macrophages (75). There the Ca$^{2+}$-requiring protein gelsolin inhibits gelation by reducing the length of the filaments and by thus increasing the concentration of actin-binding protein required for gelation. The actin filament-capping protein (72b) may be an additional factor in the gelation process because it strongly inhibits the self-association of actin filaments, which normally contribute to the stabilization of the gels (106).

These cross-linked actin networks are thought to be responsible for the gelatinous nature of the cytoplasmic matrix and are likely to be a component of the latticework of cytoplasmic matrix fibers, which Porter, Buckley, and others (107, 108) have referred to as microtubules. With improved methods involving extraction, quick-freezing, drying, and metal coating (32), the elements of this lattice have now been shown to include actin filaments and intermediate filaments. Almost certainly this actin filament network is responsible for limiting the Brownian movement of the organelles and probably contributes to the maintenance of cellular shape and the nonhomogeneous distribution of intracellular components. The gel may also be important to the cell as a scaffolding for certain enzymes (109) and may bind the "free" polyribosomes (110).

In addition to these associations of actin filaments with each other, they may also interact with microtubules (111). This association is weak and requires the microtubule-associated proteins found on the surface of the tubules. The functional significance, if any, of this interaction is not established. It could be purely structural, as suggested by electron microscopy.

![Figure 9](https://example.com/f9.png)

**Figure 9** Gelation and contraction of a 140,000-g supernate of an *Acanthamoeba* homogenate induced by warming the cold extract to room temperature. From the work of the author (44).
of critical point-dried specimens (107, 108) or the contractile proteins attached to microtubules may power microtubule-dependent movements (111).

**Drugs That Act on Cytoplasmic Contractile Proteins**

Since the pioneering work of Carter (112) and Schroeder (113), we have known that micromolar or submicromolar concentrations of cytochalasins inhibit some cellular movements. The cytochalasins are a group of closely related ~500-mol wt organic molecules produced by molds and distinguished by alphabetical designations. Cytochalasin B has been used the most widely. Cytochalasin D has the highest potency in inhibiting cellular movements (114).

There was early speculation (115) that actin (“microfilaments”) is the target of the cytochalasins, but the first experiments with purified actin (116) failed to reveal a direct effect of micromolar cytochalasin B on polymerization. It was found that some cytochalasins inhibit glucose transport into cells (117), although this could not be correlated with the effects on motility (114).

More recently, it was shown that submicromolar concentrations of cytochalasin B, D, and E inhibit gelation of cytoplasmic extracts (93, 98, 101, 118) and reconstituted systems of purified actin with various cross-linking molecules (12, 101, 106). This has been traced to a direct substoichiometric effect on actin filament network formation, which occurs when a few molecules of cytochalasin bind to an actin filament consisting of hundreds of actin molecules (106, 119). Two mechanisms have been suggested: either cytochalasin reduces the length of the filaments (119) or it inhibits the self-association of the filaments (106). These same concentrations of cytochalasin also reduce the rate of actin filament growth (106, 120–122) by blocking monomer addition at the barbed end of the filament (106), so the cytochalasin-binding site(s) is most likely at the barbed end of the filament. Under physiological conditions, micromolar cytochalasin B strongly inhibits actin filament network formation and gelation (106, 118) with only minimal effects on polymerization rate (106), so it seems that the inhibition of cellular motility is caused by the structural change.

Phalloidin is a second alkaloid that reacts directly with actin. This molecule is a product of the poisonous mushroom, *Amanita phalloides*. It binds to actin molecules in actin filaments and stabilizes the filaments (123) under a variety of conditions where actin filaments usually depolymerize, including exposure to DNase I (124), 0.6 M KI (125), OsO$_4$ (126), ultrasonication (125), and high temperature (127). This stabilization probably accounts for the abundance of actin filaments in electron micrographs of phalloidin-treated cells (128). Although phalloidin does not readily enter cells, it can be microinjected (129). This treatment inhibits cellular locomotion in tissue-cultured cells (129) and amoebae (130). Presumably, this means that depolymerization of actin filaments is required in some way for normal locomotion, but other explanations are also conceivable. Phalloidin can be attached to fluorescein and has been used to stain actin filaments in tissue-culture cells (131).

**Future Trends**

The major question in motility is: How is tension generated during actin-myosin interaction? It seems to me that research on muscle is more likely to answer this fundamental question than is work on cellular motility. However, cellular systems continue to reveal fascinating new insights into contractile proteins, which have escaped attention in muscle, so that one cannot exclude the possibility that research on cellular motility will contribute to this area. For example, much of the actin crystallography is being done on cytoplasmic actins.

In nonmuscle cells, much of the future work on contractile proteins will focus in two areas: (a) investigation of the mechanisms which control assembly and tension generation, and (b) elucidation of the macromolecular anatomy of contractile protein systems. By macromolecular anatomy, I mean definition of the molecular associations. This would include, for example, a detailed structural analysis of the actin filament and how the various associated proteins bind to the filament. In particular, this analysis will require definition of the molecular interactions, which link the components of the system together and to other cellular constituents.

Both of these issues promise to be extremely complex. One can safely predict that a number of new proteins will have to be discovered and characterized before a detailed understanding of either regulation or molecular anatomy will be realized. I also predict that no single approach will provide the answers. It is, after all, the phenomena observed in living cells that have prompted the molecular analysis of cellular contractile systems during the last 15 years. It is tempting to accept the resulting biochemical purification and characterization of the components as the culmination of the work. Although this is (and will continue to be) essential for progress, one will always have to return to the intact, preferably living, cell to test the ideas generated in the biochemistry laboratory. This two-way street between the living cell and molecular analysis is what cell biology is all about.

**REFERENCES**