

REGULATION OF MOTILITY IN NONMUSCLE CELLS

SARAH E. HITCHCOCK

From the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

In the past several years actomyosin contractile systems have been related to many types of cell movement and other functions such as cytoplasmic streaming, cytokinesis, blood clot retraction, and phagocytosis. The evidence for the presence of actin and myosin in nonmuscle cells is now widespread and has been the subject of a recent review (85) and two recent published symposia (31, 43). Regulation of these processes is complicated and probably involves more than one mechanism. In vivo and in vitro studies have shown that calcium may regulate some of these motile systems in a way similar to calcium regulation in muscle: MgATP (adenosine 5'-triphosphate) and micromolar concentrations of free calcium ion are required for functional actin-myosin interaction (contraction) to take place. This may be a target for some of the intracellular calcium. The purpose of this review is to evaluate recent advances in our understanding of the regulation of these cellular motile processes. The evidence for calcium control of motility in vivo and in cellular models will be presented. This will be followed by a discussion of the types of regulation that have been found or might be expected on the basis of existing studies of muscle and nonmuscle cells. Emphasis will be placed on work that has been published since the 1974 Pollard and Weihing review (85).

STUDY OF REGULATION OF MOTILITY USING CELL MODELS

A calcium requirement for cellular motility was first shown in glycerinated fibroblasts by Hoffmann-Berling (42). He had previously shown that

glycerinated fibroblasts developed tension and shortened in the presence of exogenous ATP. When "relaxing grana" (vesicles of the sarcoplasmic reticulum) from muscle or fibroblasts were added, the shortening was reduced, but addition of calcium to 10^{-4} M restored contraction. At that time the free calcium concentration could not be determined.

Membrane-bound plasmodial fragments of the slime mold *Physarum* were isolated by treatment with caffeine and showed calcium-dependent cytoplasmic movement (38, 40). These fragments exhibited fountain streaming, rotation, and contraction. When ethyleneglycolbis(β -aminoethylether)-*N,N'*tetraacetic acid (EGTA) was added to chelate calcium, movement of the granular cytoplasm ceased and the cytoplasm expanded to fill the whole volume of the fragment. When calcium was added, contraction began again. The threshold calcium concentration for this process was between 10^{-7} and 10^{-5} M, in the same range as for muscle contraction. Contraction could also be seen if calcium was injected with a micropipette. More recently, Ridgway and Durham (89) have correlated the local intracellular calcium concentration with streaming in *Physarum*. They injected aequorin (a protein which emits light on binding calcium) into plasmodia and found that the calcium-mediated light output and the electrical potential varied cyclically with time. By observing the direction of the streaming, they could show that contraction occurred in the region of elevated calcium concentration.

Cohen and De Vries (20) have measured the

isometric tension of blood clot retraction involving platelets. They showed that the tension produced by a clot exhibited the same calcium dependence as the MgATPase of extracted actomyosin with half maximal activity at 5×10^{-7} M Ca^{++} . Although this calcium dependence was observed during the period of tension development, it was lost after full tension was attained.

Both Mooseker (69) and Rodewald et al. (92) have reported MgATP-dependent contraction of isolated brush borders from intestinal epithelium. Mooseker found a calcium requirement for this contraction with a threshold of 10^{-6} M. Rodewald et al. did not have calcium dependence in their system. The microvillar bundles (which contain actin but not myosin, see below) retracted into and through the terminal web region which contains actin and myosin. In these isolated preparations, the contraction was not reversible.

In order for calcium to be a regulatory ion in vivo there must be a calcium-sequestering (and -releasing) system. Vesicles that will accumulate calcium have been isolated from a number of sources (reviewed in reference 85). Membranous vesicles from platelets have been isolated that showed MgATP-dependent, oxalate-stimulated calcium uptake and could concentrate calcium 500-fold compared to the medium (90). Calcium uptake was inhibited by Salyrgan but not affected by sodium azide. These and other controls indicated that the microsomal fraction was similar in many ways to vesicles of the sarcoplasmic reticulum from muscle (37). Rose and Loewenstein (93) have studied the calcium ion distribution in the cytoplasm of *Chironomus* salivary gland cells by injecting aequorin and visualizing the calcium-dependent fluorescence with a TV-image intensifier system. They found that when calcium was injected, the fluorescence was confined to the vicinity of the micropipette. This restriction depended on energy metabolism since cyanide and ruthenium red resulted in diffuse and prolonged aequorin fluorescence. This showed that calcium can be effectively compartmentalized in the cytoplasm and suggested that mitochondria may be involved in local sequestering of calcium (since cyanide and ruthenium red inhibit calcium uptake in mitochondria but not sarcoplasmic reticulum; see references 59, 116, and 118). It has been suggested that mitochondria play a role in calcium homeostasis (see papers in reference 15), but the control of release of calcium from mitochondria is not understood. It is also possible that the plasma

membrane could be involved in calcium homeostasis by having areas which are differentially permeable to calcium as has been shown in developing fucoid eggs (91).

CALCIUM REGULATION OF STREAMING OF ISOLATED CYTOPLASM

Isolated cytoplasm has also been used as an in vitro model for studying cell motility, and several experiments have provided convincing evidence that actin and myosin are major contractile elements. Streaming of isolated cytoplasm was first described in the amoeba *Chaos carolinensis* by Allen et al. (5). In 1963, Thompson and Wolpert (109) demonstrated ATP-induced motility in extracts of *Amoeba proteus*. They showed filamentous material in the extracts, but it was left for Pollard and Ito (81) to show that motile extracts contained two classes of filaments (thick and thin). The thin (7-nm) filaments, like actin, bound heavy meromyosin (82). These groups also showed that it was necessary to warm the extracts to have streaming. This observation will be discussed in greater detail later.

A specific calcium requirement was first shown by Taylor et al. (106) in their study of cytoplasmic streaming in the amoeba *Chaos carolinensis*. They showed that isolated cytoplasm could be cycled through rigor, contracted, and relaxed states by altering the free calcium and ATP concentrations. In the presence of calcium and MgATP, the cytoplasm formed pseudopodium-like looped structures which showed directional streaming and contracting fibrils. The cytoplasm showed some of the physical properties expected of muscle fibrils: the cytoplasm in the rigor state had fibrils which, when stretched, increased in birefringence and often fractured, and which, when released, recoiled elastically. In relaxation solution, the cytoplasm lost its viscoelasticity and birefringence, and the fibrils did not cohere or fracture when stretched. When the fibrils were examined in the electron microscope, they were found to contain what appeared to be actin and myosin filaments. Calcium- and MgATP-dependent movement of granules has been shown in isolated cytoplasm of mammalian fibroblasts (45).

CALCIUM-SENSITIVE GELATION, CONTRACTION, AND ATPase OF CRUDE EXTRACTS

The occurrence of gelation and contraction of crude cytoplasmic extracts has been used recently

as an in vitro analogue for study of the gel-sol transformation that takes place in the cytoplasm of amoeboid cells. The difference between the extracts used for studies of gelation and contraction described here and those in the previous section is that they usually represent the supernatant from a high-speed centrifugation of cells which have been extracted in the cold with a low-ionic-strength buffer often containing glycerol or sucrose. The membrane fractions for the most part have been removed. Several laboratories have used this type of preparation for study of these phenomena, and many of the results in this interesting and promising area must be regarded as preliminary at this time. The investigators have used different sources of material and slightly different conditions and methods of preparation and analysis. Consequently, it is impossible to know whether the differences in the results are fundamental to the material studied or whether they are artifactual, i.e., due to slight differences in ionic strength, pH, or other such factors. An attempt to evaluate these experiments in detail is beyond the scope of this review. Nevertheless, it is becoming clear that there are three separable parts of the process: polymerization of actin to form filaments, gelation of actin filaments, and contraction of the gel which requires actin and myosin or a myosin-like protein. Regulation of filament formation and the possible involvement of specific proteins in actin filament formation and gel formation will be discussed later in the review.

Kane (46) was the first to report gelation and contraction in this type of extract of unfertilized sea urchin eggs. His extract, in the presence of millimolar concentrations of ATP and 20 mM KCl when brought from 4°C to 35°–40°C, became a solid gel which eventually contracted. Analysis of the centrifuged gel by sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis showed it to contain a 45,000 mol wt band (actin) and a 58,000 mol wt band which was in a constant ratio to the actin, <1 mol/actin monomer, but the precise stoichiometry was not determined. The pellet also contained a variable amount of a 220,000 mol wt band which was larger than the sea urchin muscle myosin heavy chain. Work recently reported (47) showed that recombination of egg F-actin with a fraction containing the 58,000 and 220,000 mol wt components resulted in gelation. The actin filaments in the gel formed ordered paracrystalline arrays similar to F-actin paracrystals but with a different banding pattern. The

gelation process required low calcium concentrations and was inhibited by free calcium ion concentrations above $5\text{--}10 \times 10^{-7}$ M, and excess Mg over the ATP concentration inhibited gelation. High KCl concentrations (0.6–1 M) dissociated these proteins from the actin.

Similar temperature- and ATP-dependent phenomena have been observed in *Acanthamoeba* (65, 80), macrophages (35, 103), *Amoeba proteus*, *Chaos carolinensis*, and *Dictyostelium discoideum* (22, 107, 108). Pollard (80) found that the initial gelation took place in the presence of MgATP, without calcium. The molecular arrangement of actin in the gel is not precisely understood. The rate of the subsequent contraction was accelerated by the addition of calcium to $2\text{--}4 \times 10^{-7}$ M. When the contracted pellet was examined electrophoretically, it was found to contain, besides actin, myosin, myosin-cofactor (a 97,000 mol wt protein required for actin-activated myosin ATPase in *Acanthamoeba* [83], prominent 280,000 mol wt and 50,000 mol wt bands. While actin alone could form a gel, myosin was required for contraction. Similar results were found in macrophages (103). However, the increase in turbidity (gelation) and final contraction occurred faster with EGTA present than in the presence of free calcium. Gels of the final contracted pellets showed actin, myosin, myosin-cofactor, 150,000 and 270,000 mol wt bands. The most cooperative calcium regulation of this process has been shown in extracts of amoebae by Condeelis and Taylor (22) and by Taylor et al. (107, 108). They found that the increase in turbidity associated with the appearance of actin filaments required micromolar calcium but that gelation proceeded at lower calcium concentrations ($\sim 10^{-8}$ M) and was inhibited by micromolar calcium concentrations. The gel was amorphous in structure. Contraction required the presence of myosin and micromolar calcium. Dodecyl sulfate acrylamide gels of contracted cytoplasmic gels contained actin, myosin, 95,000 mol wt, and low mol wt proteins (20,000–32,000), whereas the 48,000, 55,000, 220,000, 240,000, and 280,000 proteins remained in the supernate. The calcium requirement could be lost if the pH or ionic strength was increased or if sucrose was present. This observation may explain some of the discrepancies in results from different laboratories. Taylor believes that gelation and contraction are separate events and that contraction takes place when actin has transformed from the cytoskeletal form in the gel to the free F-actin

form when the gel breaks down (in the presence of calcium).

Cytochalasin B, at low concentrations (10^{-6} M) inhibits many types of cell movement which seem to be related to membrane-associated microfilament systems. The relationship of the action of the drug to the contractile proteins has not been understood. Two reports have shown that addition of micromolar concentrations of cytochalasin B to extracts or HeLa cells (121) or macrophages (36) inhibited actin gel formation of dissolved gels without (in certain conditions) depolymerizing or disrupting the actin filaments themselves. These investigators believe that they detected a reduction in the amount of high molecular weight protein associated with the actin, but, since the gels were of crude extracts with many protein components, these claims should only be made with guarded optimism.

In muscle, one of the standard ways to study calcium-dependent regulation is to measure the calcium sensitivity of the MgATPase of actomyosin. This is often satisfactory in studies of muscle because large amounts of material can be purified away from contaminating ATPases. Unfortunately, the same is not always true of nonmuscle preparations. While there have been successes and failures in measuring calcium-sensitive actomyosin ATPases, in many cases the results are difficult to interpret because the controls for mitochondrial, membrane-bound, and other ATPases were not included. Therefore, the added ATP can be hydrolyzed by contaminating ATPases as well as by actomyosin. Even the "contraction" analogues described earlier are subject to the same criticism because the final contraction is essentially a rigor state (no ATP).

STUDIES OF THE MOLECULAR BASIS OF REGULATION OF NONMUSCLE MOTILITY

The best understood regulatory systems are those of striated muscle: the troponin-tropomyosin actin-linked regulation and the myosin light chain-dependent regulation found in many invertebrate muscles (reviewed in references 28 and 56). In no case has a regulated actomyosin system been reconstituted with characterized components from nonmuscle cells. It seems likely that regulation of nonmuscle actomyosin-like systems involves a number of different mechanisms, and a given cell may employ one or more of them. Each of five

possible mechanisms will be reviewed in terms of evidence from muscle and nonmuscle systems.

Actin-Linked Regulation via Tropomyosin-Troponin or Other Actin-Binding Proteins

Vertebrate skeletal and cardiac as well as some invertebrate muscles have been shown to be regulated by the tropomyosin-troponin system first described by Ebashi (reviewed in references 28 and 56). The most extensively studied example is rabbit skeletal muscle, of which the amino acid sequences of the major components have been published, and much is known about the effect of calcium on the interactions among these proteins. Briefly, troponin is composed of 1 mol of each of three proteins: troponin-C (calcium-binding protein), troponin-I (inhibitory protein), and troponin-T (tropomyosin-binding protein). There is 1 mol of troponin per mol of tropomyosin and per seven actin monomers. It is generally believed that troponin, via tropomyosin, regulates the ability of the actins along the length of one tropomyosin (40 nm, seven actins) to interact with myosin.

Proteins similar to tropomyosin have been isolated and characterized from several nonmuscle vertebrate tissues, e.g., platelets (19), brain, neurons (25), brain synaptosomes (8), blood, pancreas (24), and fibroblasts (66). Although brain tropomyosin has a lower mol wt than muscle tropomyosin (30,000 vs. 35,000 apparent chain weight on SDS-acrylamide gels), Fine et al. (25) showed that it has a similar peptide map, binds to actin, and substitutes for muscle tropomyosin in a vertebrate skeletal muscle actomyosin regulatory system. Therefore, like actin, tropomyosin is an extremely highly conserved protein. The fact that these nonmuscle tropomyosins are six-sevenths of muscle tropomyosin in chain weight and would therefore span six instead of seven actins led McLachlan and Stewart (67) to postulate that the nonmuscle tropomyosin has six instead of seven 42-residue segments which represent gene duplication repeats in the molecule. The observation that brain tropomyosin binds less strongly to muscle actin than muscle tropomyosin (25) lends support to this suggestion.

The amount of tropomyosin in nonmuscle cells has not been quantitated, and it is not known whether enough of it is present to bind to all the actin present in these cells. A recent study by Lazarides (54) using indirect immune fluorescence showed that antibody to skeletal muscle tropo-

myosin stained tissue culture cells with a different pattern than anti-actin. He concluded that tropomyosin is not distributed uniformly with the actin. He suggested that it is not involved in all types of microfilament-dependent motility and may preferentially be associated with actin which contributes to the cytoskeleton. Tropomyosin has not been characterized from an invertebrate nonmuscle source, and at this time its existence there remains an open question. The presence of tropomyosin does not imply a requirement for troponin although the converse is true. (Troponin here is defined in terms of its tropomyosin-dependent effect on actin-myosin interaction.) Many invertebrate muscles and vertebrate smooth muscles lack troponin although tropomyosin is present in normal amounts.

Cohen et al. (21) were the first to provide evidence that there is actin-linked regulation in platelet actomyosin. They used the "actin competition test" which has been so useful in comparative studies of regulation in muscle (58). If an actomyosin or myofibril has actin-linked regulation, addition of excess pure actin will elevate the MgATPase in the absence of calcium since unregulated myosin will interact with the added pure actin but not with the regulated actin already in the myofibril. If there is myosin-linked regulation, the ATPase will remain low in the absence of calcium even with excess pure actin, and only the addition of calcium will increase the MgATPase activity. When excess pure rabbit muscle actin was added to a platelet actomyosin which gave 70% calcium sensitivity, the ATPase in the presence of EGTA was increased from 30% to 95% of the level of the original actomyosin in the presence of calcium (21). The ATPase activity of the platelet actomyosin with added actin in the presence of calcium was not measured. Since nonmuscle actomyosins are usually myosin-poor, this is probably not a crucial criticism. The results argue for the presence of an actin-linked regulatory system in platelets. Cohen et al. did not isolate a troponin-like protein although they obtained a crude platelet actin preparation which regulated muscle myosin.

More recently, a regulated actin complex has been isolated from platelets which confers calcium sensitivity to platelet or muscle actomyosins (88). This complex bound calcium with an affinity of 10^7 M⁻¹. Proteins in this complex cross-reacted with antibodies against muscle troponin-C (88) and tro-

ponin-I.¹ Since these results have not been published in full, it is not possible to evaluate them. Regulatory proteins have also been described in extracts of synaptosomes (87), brain (63), and leukocytes (98). However, none of these proteins has been characterized in detail.

Calcium-binding proteins similar to troponin-C have been isolated from a variety of sources: brain (14, 26, 60, 119), heart (101), and other neurosecretory tissues (53, 117). This protein is present in many invertebrate and vertebrate tissues. Fine et al. (26) showed that troponin-C-like protein from brain had an electrophoretic behavior similar to that of muscle troponin-C and, when combined with rabbit muscle troponin-T and -I, partially regulated the muscle actomyosin ATPase (40% calcium sensitivity vs. 75% in the muscle troponin control). Their protein was purified by preparative gel electrophoresis of 9 M urea extracts of whole embryonic chick brains. Therefore, it is not known whether Fine's protein is soluble in the cytoplasm or whether it is part of a troponin complex which is associated with actin filaments.

This question becomes an important one since it has been shown by a number of laboratories that a troponin-C-like protein (called modulator protein) is the calcium-dependent protein activator of the 3':5' cyclic nucleotide phosphodiesterase and adenylyl cyclase (reviewed in reference 117). It is a major protein in neurosecretory tissues (1-2% of the total soluble protein [119]) and will bind to phosphodiesterase in the presence but not in the absence of calcium (+EGTA [120]). The most extensive physical and chemical analyses have been carried out on the bovine brain and heart proteins (101, 119). The molecular weights, amino acid compositions, electrophoretic behavior, and ultraviolet absorption spectra of these proteins are similar to those of muscle troponin-C. There are four calcium-binding sites, two with $K_d = 1 \times 10^{-6}$ M and two $K_d = 8.6 \times 10^{-4}$ M. The most important criterion of all is an extremely high degree of sequence identity. With 60% of the brain protein sequence completed, Vanaman et al. (117) report that 60-70% of the residues are identical to those of the muscle troponin-C. The modulator proteins are extremely highly conserved since peptide maps of the bovine heart and brain proteins are nearly identical (101), and tryp-

¹ S. Puszkin, Mount Sinai School of Medicine, New York, N.Y. Personal communication.

tic digests of porcine and chicken brain proteins are identical (117). Although muscle troponin-C did not activate the phosphodiesterase (101), the brain modulator protein interacted with muscle troponin-I in a reconstituted actomyosin ATPase (7). It has not been shown to form a functional troponin-like complex with 1 mol each of troponin-I and -T. Therefore, the relationship of these proteins to troponin-like regulation of actomyosin interaction has not been shown.

Actin-linked regulation has been demonstrated in extracts of *Physarum*, but the proteins responsible for the calcium sensitivity have not been purified and characterized. Nachmias and Asch have isolated actomyosin which has a calcium-sensitive ATPase; when excess rabbit actin was added in the presence of EGTA, there was partial reversal of the inhibition of ATPase activity (73, 74). This suggests that an actin-linked system is involved. However, their recent results indicate that an actin-linked system is not the only form of regulation in *Physarum* since addition of muscle myosin to a calcium-sensitive actomyosin-containing pellet resulted in elevation of the ATPase in the presence of EGTA (71, 74). This suggests there may also be myosin-linked regulation. When small amounts of rabbit muscle myosin were added to a *Physarum* extract and centrifuged, the pellet was a calcium-sensitive hybrid. The calcium sensitivity was greatest when small amounts of myosin were added. Regulatory substances have been isolated from *Physarum* which conferred calcium sensitivity to superprecipitation of muscle actomyosin and which bound to muscle F-actin (48, 49, 105). However, the components of the extract have not been studied in detail. It is difficult to interpret the results with these relatively crude extracts until there is more information on the stoichiometry of the binding of these components to actin (or myosin).

Mockrin and Spudich (68) isolated a protein fraction from *Dictyostelium* (cellular slime mold) which bound to actin and made the actin-*Dictyostelium* myosin interaction calcium sensitive. It did not function with muscle myosin. The fraction which bound to G-actin (immobilized on a DNase I-agarose column) contained two major peptides, 30,000 and 15,000 mol wt on SDS gels, as well as other minor components. These workers did not determine which of these proteins bound to F-actin, and the stoichiometry of binding was not measured. This protein regulated with either mus-

cle or *Dictyostelium* actin, but required *Dictyostelium* myosin. One must question, as the authors did, whether its primary site of action is the actin or myosin. Application of the actin competition test described earlier might help to answer this question.

In summary, in nonmuscle cells there is some evidence that there is actin-linked regulation, but at this time there is no convincing evidence that a troponin complex like that found in skeletal muscle exists in nonmuscle cells.

Myosin-Linked Regulation via a Myosin Light Chain or Other Myosin-Binding Protein

Szent-Györgyi and his colleagues have shown that most invertebrate muscles have myosin-linked regulation (reviewed in reference 56). This type of regulation has been studied in detail in scallop muscle, and it has been shown that a myosin light chain is required for calcium binding and regulation by the myosin (50, 104). There is no evidence that this light chain is phosphorylated, although phosphorylation has been looked for. Myosin-linked regulation has also been shown to exist in vertebrate smooth muscle (13), but, as will be discussed later, this is probably due to a different mechanism. Aside from the indirect implications discussed above, this type of myosin-linked regulation has not yet been demonstrated in nonmuscle systems. The activity is at best extremely labile in muscle; it can be influenced by the ionic strength used in the ATPase assay (57), and it may have been overlooked for these reasons.

Cofactor proteins which are required for actin-activation of the myosin-ATPase have been described in *Acanthamoeba* (83) and in rabbit macrophages (102). These proteins are present in amounts far less than one per mole actin, but the stoichiometry in relation to myosin has not been determined. They do not appear to bind tightly to the myosin. Calcium has no effect on the cofactor-stimulated ATPase. Another unusual feature of the *Acanthamoeba* and macrophage systems is that the addition of muscle troponin-tropomyosin regulatory proteins resulted in inhibition of the actomyosin MgATPase both in the presence and in the absence of calcium (84, 102). The reason for this behavior is not understood. The muscle regulatory proteins showed normal calcium-sensitive actomyosin ATPase when combined with granulocyte or platelet myosin (reviewed in reference 85).

Protein Modification: Phosphorylation

Several contractile proteins have been shown to be phosphorylated: troponin-I and -T (77), the 20,000 mol wt class of light chains from muscle (LC₂, DTNB) (79), and the 20,000 mol wt light chain of platelet myosin (4, 23). Phosphorylation has been shown to affect activity in vitro in only two cases. Adelstein and Conti (4) showed that phosphorylated platelet myosin had higher actin activation than unphosphorylated myosin. The platelet light chain kinase was not calcium dependent (23), and the platelet myosin did not show calcium sensitivity when combined with actin (4). Aksoy et al. (6) and Górecka et al. (34) found that phosphorylation of the 20,000 mol wt light chain of chicken gizzard myosin was required for optimal actin activation. In contrast to the platelet kinase, the gizzard kinase required micromolar levels of calcium. Similar findings have been reported in guinea pig vas deferens (18). Although there are differences in the results from the two laboratories, they will not be discussed here. The resulting effect is that smooth muscle actomyosin preparations show a calcium-sensitive ATPase, but this sensitivity is at least partially due to the calcium-dependent enzymatic modification of the myosin by the kinase in the preparation rather than to a protein which is structurally part of the contractile apparatus such as tropomyosin, troponin, or a light chain. It is important to consider possibilities such as this when working with relatively crude extracts.

Although phosphorylation is the only protein modification reported to affect the actomyosin ATPase, it is possible that other types of modification such as acetylation, methylation, hydroxylation, or transamination could be involved in regulatory mechanisms.

Regulation of Filament Formation

Another way to regulate actomyosin-related motility is to regulate filament formation. There are instances in which filamentous structures are known to form rapidly and transiently. The contractile ring forms shortly before cytokinesis and disappears afterwards (96). Microfilament bundles form rapidly during spreading of fibroblasts and disappear in rounded-up cells (29, 30). In some sperm the acrosomal process is formed by rapid polymerization of actin, although there is no myosin in this structure (114).

While actin is known to be a major cytoplasmic protein (8.5% in fibroblasts, reference 11), many investigators have noticed that purification of filamentous actin from nonmuscle cells by the procedures developed for vertebrate skeletal muscle gives a low yield. Hatano (39) found that *Physarum* actin would form unusual polymers in the presence of magnesium. More recently, soluble forms of actin have been reported. It is not yet known whether the solubility of the actin is due to an intrinsic difference in the actin or is a result of interaction of actin with other proteins. Bray and Thomas (12) found that if cells were disrupted in a gentle manner shown not to damage muscle F-actin, much of the actin was soluble, in the monomeric state, and would not polymerize under conditions in which muscle G-actin did polymerize. At concentrations greater than 1 mg/ml (0.1%), it would polymerize to form apparently normal F-actin filaments and paracrystals. This threshold concentration was much higher than that needed for skeletal muscle actin. Adelman (3) reported that *Physarum* actin was soluble under conditions in which rabbit muscle actin polymerized. For polymerization of *Physarum* actin to proceed efficiently, ATP, CaCl₂, and KCl in a narrow concentration range were required. Molecular weight determination of the soluble form of *Physarum* actin showed it to be monomeric. This suggested that if its soluble state was a result of complex formation with another protein, it must be of low molecular weight. Platelet actomyosin has an even more unusual behavior (1, 2, 27). CaATP or MgATP will dissociate muscle actomyosin into myosin (6S) and F-actin. When CaATP was added to platelet actomyosin,² it dissociated into myosin and two fractions of actin: a monomeric form which polymerized (reversibly) into apparently normal filaments when ATP and divalent cations were removed and a polymeric form which depolymerized only under denaturing conditions. When MgATP was added to platelet actomyosin, it was dissociated into myosin and polymeric actin. Probst and Lüscher also reported a soluble form of actin (86).

These studies indicate that nonmuscle actin may differ in some way from skeletal muscle actin, at least as it is isolated from these cells. Highly purified *Dictyostelium* actin, on the other hand, shares

² In some papers on platelet actomyosin, another terminology is used: actomyosin = thrombosthenin; actin = thrombosthenin A; and myosin = thrombosthenin M.

the same polymerization properties as muscle actin (99). Purified *Acanthamoeba* actin and rabbit muscle actin differ, under some conditions, in their polymerization properties (32). They also differ in activation of the myosin heavy meromyosin and subfragment 1 MgATPases (33). Although both actins give the same V_{max} , a three-fold higher concentration of *Acanthamoeba* actin than muscle actin is required to obtain half-maximal activation of the myosin ATPase. Most of the properties examined of the two actins were similar, and these differences do not appear to be sufficient to explain the solubility of actin as it is extracted from the cell. It seems likely that additional proteins which bind to or modify actin must be involved.

The demonstration that in some cases nonmuscle actin will not polymerize readily implies that actin may exist in more than one form in the cells and is therefore a possible site for regulation of motility and cell structure. Several laboratories have shown that the gelation phenomenon described earlier involves actin filament formation. Thompson and Wolpert (109) and later Pollard and Ito (81) showed temperature- and ATP-dependent motility and filament formation in extracts of *Amoeba proteus*. Isenberg and Wohlfarth-Botterman (44) have isolated drops of cytoplasm from *Physarum* and have shown that with time the actin shifted from a nonfilamentous to a filamentous state. The cytoplasmic drop became more viscous and decreased in volume (contracted). The formation of filamentous actin was accelerated by EGTA (no Mg^{2+} or Ca^{2+}). While these studies found no evidence for a calcium requirement, more recently Taylor et al. (108) showed that the formation of filaments in extracts of *Amoeba proteus* depended on the temperature and the calcium ion concentration. The initial extract in rigor conditions (no ATP) extracted at 0°C and brought to room temperature had very few filaments. When relaxation solution was added (10^{-8} M Ca^{++} , MgATP) at room temperature, there was a slow increase in turbidity associated with an increase in the number of filaments. On increasing the calcium to 10^{-6} M, the turbidity increased further, many actin and myosin filaments were formed, and the gel contracted. This suggests that calcium, MgATP, and temperature regulate filament assembly. Similar phenomena have been described in sea urchin eggs (46), *Acanthamoeba* (80), and macrophages (103), but, since *Amoeba* showed the most effective calcium regu-

lation, it was selected to be discussed in greater detail. In the sea urchin, gelation proceeded better in the presence of EGTA (46).

Many of these investigators believe that a high molecular weight protein, greater in chain weight than the myosin heavy chain, is involved in the regulation of gelation. Negatively stained preparations of this protein showed hollow coils 12 nm in diameter (103). The contracted pellets of *Acanthamoeba* and macrophages were enriched for this high molecular weight protein, but Stossel and Hartwig (103) showed there was a very low stoichiometric ratio (about 1 per 100 actins). In contrast to these results, Condeelis and Taylor (22) and Taylor et al. (108) reported that contracted pellets of *Amoeba proteus* and *Dictyostelium discoideum* were not enriched for this high molecular weight protein. These proteins (220,000–280,000 mol wt) are typically called “actin binding proteins” and are often compared with spectrin from erythrocyte ghosts (see below) (10). At this time, little work has been reported on the interaction between purified protein and G- or F-actin, and the maximal binding and nature of the specificity are not known. Stossel and Hartwig (103) determined the amino acid composition of “actin binding protein” from macrophages and found it to be substantially different from that of spectrin (64). The amino acid composition of spectrin is more like that of platelet and muscle myosins than that of “actin binding protein” (9). Their protein appeared to be required for gelation of actin (103), but addition of spectrin to actin influenced the viscosity but not gelation (113). At this time, more extensive chemical work is needed before it will be known whether these proteins are homologous or whether they merely share the property of having a high molecular weight on SDS-acrylamide gels. Most SDS-acrylamide gels of these preparations contain many protein components, and it is not yet possible to decide which ones are involved in these processes. Maruta and Korn (65) have reported recently that the proteins from *Acanthamoeba* associated with gelation of purified F-actin are low in mol wt (23,000–38,000) and that the high mol wt protein (280,000 as identified by Pollard [80]) contained less than 3% of the gelation activity. These gelation factors bound to the actin, but only small amounts were required for gelation (0.002–0.02 mol/mol actin). It is possible that the preparations from other laboratories also contain these proteins but that they were regarded as low molecular weight contaminants or not de-

tected at all. Maruta and Korn (65) also found that a myosin-like protein, distinct from *Acanthamoeba* myosin previously described (84), was involved in contraction of the gel.

A possible mechanism for control of actin filament formation has been described in *Physarum*. In 1952, Loewy (62) reported that 5' AMP resulted in an increase in the viscosity of an actomyosin-type extract from *Physarum*. Recently, Nachmias (72) confirmed these results and showed that the increase in viscosity was due to formation of actin filaments. 5' AMP and 5' ATP were effective, but 3' AMP was not. She found that when muscle myosin was added to the actin-rich extract, a hybrid actomyosin was formed which contained components in addition to actin and myosin, including a 55,000 mol wt band (on SDS-acrylamide gels). The hybrid actomyosin had ATP phosphohydrolase activity which can convert 5' ATP into 5' AMP and pyrophosphate, and she believes that the 55,000 mol wt protein represents the enzyme and that it may have a role in filament assembly. Adelman (3) found that ATP was required for polymerization of actin in *Physarum* extracts which were relatively free of myosin, and that the ATP was rapidly hydrolyzed to AMP.

A soluble form of actin from spleen has been isolated and crystallized (16, 61). It was originally purified and characterized because of its ability to inhibit the activity of pancreatic deoxyribonuclease I and to form a stable 1:1 complex with the enzyme. Lazarides and Lindberg (55) showed the DNase I inhibitor to be a nonmuscle actin. DNase I will depolymerize muscle F-actin to form a complex of 1 DNase I: 1 G-actin like that originally described for the spleen actin (41). Recent analysis of the soluble spleen actin which crystallized showed that it was a complex of equimolar amounts of actin and a protein about 16,000 mol wt called profilin (16, 17). The complex was soluble (55,000–60,000 mol wt on Sephadex), but spleen actin not complexed with profilin polymerized to form filamentous actin. When profilin was combined with monomeric muscle actin in a buffer favoring depolymerization, subsequent addition of salt did not result in polymerization of actin. The control of the association between the two proteins is not yet understood. It is one of the most promising systems for study of regulation of filament formation since this soluble complex can be purified from the cell without the use of protein denaturants.

Another system which involves nonmuscle actin

is actin and spectrin interaction in erythrocyte ghosts (reviewed in reference 100). These proteins are commonly known as bands 1 and 2 (spectrin) and 5 (actin) and are found as a fuzzy layer on the cytoplasmic surface of the plasma membrane. The spectrin bands and actin are present in approx. a 1:1 molar ratio. They are removed by mild treatment in a slightly alkaline, low-ionic-strength medium, free of divalent cations. It is not yet clear whether these proteins are integral with the membrane or whether they coat the inside of the membrane by self-association into a network. However, the interaction between actin and spectrin does influence the state of the actin in this submembranous region.

While there is good evidence that band 5 is actin (97, 113), the relationship of spectrin to high molecular weight proteins in other cells is not clear. Ferritin-labeled antibody to spectrin stained the region under the plasma membrane in erythrocytes, but it was not detectable in nonerythrocytes (76). When stained with ferritin-labeled anti-uterine myosin, all cells except erythrocytes showed antibody under the plasma membrane and in the cytoplasm. However, in vitro antibody to myosin has a low affinity to spectrin although the same tests showed no cross-reaction between anti-spectrin and myosin (97). The antibody to spectrin reacted only with component 1 of spectrin. It is not known whether component 2 shares antigenic determinants with myosin since Sheetz et al. (97) were unable to determine whether anti-myosin reacted with component 1 or 2 of spectrin.

Biochemical studies of spectrin-actin interaction suggest that spectrin is different from myosin. The properties of the ATPase are not like those of myosin or actomyosin (52, 94), and the effect of spectrin on actin is different. When muscle G-actin was combined with spectrin isolated from fresh erythrocytes, the viscosity increased and the actin polymerized into filament bundles even though the ionic conditions were those favoring depolymerization of pure actin (78). When the polymerized protein was sedimented and analyzed by SDS-acrylamide gel electrophoresis, there were small amounts of spectrin. In contrast, myosin should bind extensively under these conditions. Spectrin from recently outdated erythrocytes inhibited polymerization of G-actin, but when it was added to F-actin, the viscosity was increased (103). The reason for the different results from studies of G-actin-spectrin interaction is not clear, but it is possible that working with outdated (sev-

eral days old) rather than fresh cells may present problems. In a recent study of the interaction of spectrin (from fresh erythrocytes) with spin labeled actin, it was found that spectrin did not affect the polymerization of actin (as determined by mobility of the spin label), but that the addition of spectrin to F-actin resulted in further immobilization of the spin label (51). Therefore, although it seems clear that spectrin must affect the state of actin in the cell and therefore probably the shape of the cell, experiments have not elucidated the nature of the interactions among these proteins.

Undoubtedly, the most dramatic instance of controlled polymerization of actin is the formation of the acrosomal process in certain sperm (111, 114). The acrosomal process in *Thyone* (sea cucumber) is essentially an actin paracrystal; but, before it forms, the actin is present as an amorphous material resembling G-actin anterior to the nucleus surrounding the acrosomal vacuole. This profilamentous actin was isolated from the cell by treatment with detergent, and on SDS-acrylamide gels there were three bands: actin, and two high mol wt bands (250,000 and 230,000), heavier than *Thyone* muscle myosin heavy chain. These bands had mol wt similar to those of the spectrin bands and, like spectrin, were extremely trypsin sensitive. However, in these cups the high molecular weight proteins appeared to be present in amounts less than 1 mol/mol actin. There was another low molecular weight protein that migrated with the dye front on 5% acrylamide gels which the authors presumed to be protamine from contaminating chromatin. An intriguing possibility is that this band is related to profilin isolated from spleen. The interpretation is that the high molecular weight proteins keep actin in a nonfilamentous state until the appropriate signal stimulates filament formation. At this time, the control mechanism is not known and the protein composition of the polymerized acrosomal process has not been determined. It will be interesting to learn which of these proteins remains with the actin after it has polymerized into filaments.

Interaction of Actin with Membranes

Many types of actin-myosin mediated motility in cells also involve membranes, and it is widely believed that it is necessary to have some component of the contractile apparatus anchored to a membrane to explain the movements that take place. Many investigators have isolated membrane fractions with associated actin (see papers in refer-

ence 31), and a recent study reported labeling of myosin in membranes (75). The membrane is another site in which regulation may take place, although there is no information about what controls the attachment of actin to membranes. It is not possible to review all the literature here, and I will instead discuss the work of Tilney and his colleagues on the subject. They have studied membrane-associated actin in microvilli of intestinal epithelial cells and in the acrosomal processes of sperm. While these systems lack myosin and therefore may not be entirely representative of actomyosin-related cell motile processes, they have been superbly suitable for demonstrating association of actin with membranes.

Mooseker and Tilney (70) showed that all the actin filaments in a microvillus had the same polarity: when decorated with heavy meromyosin, the arrowheads pointed away from the tip of the microvillus. They were attached to the membrane at the tip by a dense material and along the length of the microvillus by cross-bridges. Both the tip and the cross-bridges cross-reacted with anti- α -actinin which also cross-reacts with the Z-band of muscle (95). After microvilli were reduced by hydrostatic pressure, the first step in their reformation was accumulation of dense material on the cytoplasmic surface and filaments extended from the dense region into the cortical region of the cell (112). It was suggested that these dense regions were the assembly sites for the actin filaments.

Tilney (110) has shown that membrane specialization appears to be important in the accumulation of profilamentous actin during spermatogenesis in *Thyone*. In the earliest stages of development, the membrane of the acrosomal vacuole has a thickened surface which will lie posteriorly in the mature sperm. At the same time, a region of the nuclear membrane fuses, and this specialization programs the future location of the profilamentous actin (anterior to this region and posterior to the specialized region of the acrosomal vacuole). Tilney suggested that the high molecular weight proteins discussed earlier may be involved in control of the association.

Tilney and Mooseker (115) examined by freeze-fracture some of these membranes associated with actin filaments, and in all three cases (*Mytilus* and *Limulus* sperm and the tips of brush borders of intestinal epithelium) the cytoplasmic surfaces of the membranes were particle free, suggesting that particles are not involved in end-on attachment of actin filaments to membranes. They

concluded that if particles were involved in the direct association of actin with these membranes, they must be less than 100,000 in mol wt or they must be either integral or peripheral membrane proteins which would not be visualized by the freeze-fracture technique. Along the side of the microvillus there were particles on the membrane which may be associated with the cross-bridges that connect the actin filaments to the microvillar membrane along its length.

CONCLUSION

In this review I have tried to evaluate critically the information available about the regulation of motility and to give some idea of what types of regulation might be expected. These aims have been served at the expense of trying to write a comprehensive review, and some material relevant to the subject has been omitted in the interest of brevity.

The regulation of motility is one of the most active and interesting areas in the field of cell motility. In the next several years, many of the problems posed here will be solved, and we should know a great deal more about the role of contractile proteins in the structure and function of the cytoplasm. From what is known so far, it seems likely that there will be a variety of regulatory mechanisms different from those found in muscle, and more than one could be important in a given cell. For example, a cell could have two problems: first, local assembly of the filaments and, second, regulation of interaction between the filaments once they are formed, to result in contraction. In some cells reversibility is required (such as cytoplasmic streaming) whereas in others the contractile event happens only once (platelet contraction). In some cases, the contractile proteins may have a purely cytoskeletal role (sperm acrosomal process).

It remains to be established, beyond all doubt, that the actin- and myosin-containing filaments and fiber bundles are involved in cell motility, although there is good circumstantial evidence. One reason why this has been so difficult is that there is no model system that has been so useful as the myofibril has been to our understanding of muscle contraction. A reliable cellular model system is needed for testing the functions of these contractile proteins, and a successful effort to develop one would certainly be fruitful.

The power of observation is one of the most important tools a scientist has. While phenomena in cells and test tubes are interesting and the

description of them is valuable, I believe that this field has come to the stage where more analytical work is required, particularly of the biochemical type. Many microanalytical techniques are now available that are suitable for the analysis of these materials. For example, a protein should not be called an "actin binding protein" until the conditions, specificity, and stoichiometry of binding have been determined. The fact that proteins have similar molecular weights on SDS-acrylamide gels is insufficient reason to group them as a class of proteins. Other techniques for analysis should include two-dimensional gel electrophoresis, amino acid analysis, determination of immunochemical cross-reactivity, peptide mapping, sequence determination, and, of course, detailed comparative study of the properties of these proteins, such as that being done now with actin. A case in point is tropomyosin where homology has been established beyond doubt, even though the chain weight is different from that of the muscle protein.

I wish to thank Drs. M. Adelman, W. Lehman, U. Lindberg, V. Nachmias, S. Puszkin, L. Taylor, and D. Watterson for sending me manuscripts in advance of their publication. I am also grateful to Drs. R. Goldman and D. Hartshorne for their critical reading of the manuscript, and to Ms. Y. Francescon for typing it.

The material in this review was first presented as a paper at the National Science Foundation sponsored U.S.-Japan Cooperative Science Seminar on Cell Membranes and Calcium: Calcium in the Control of Cell Function, held in Boulder, Colorado, 12-15 September 1976.

Received for publication 1 February 1977.

REFERENCES

1. ABRAMOWITZ, J., A. STRACHER, and T. DETWILER. 1972. The differential effect of CaATP and MgATP on platelet actomyosin. *Biochem. Biophys. Res. Commun.* **49**:958-963.
2. ABRAMOWITZ, J. W., A. STRACHER, and T. C. DETWILER. 1975. A second form of actin: platelet microfilaments depolymerized by ATP and divalent cations. *Arch. Biochem. Biophys.* **165**:230-237.
3. ADELMAN, M. R. 1977. *Physarum* actin. Observations on its presence, stability, and assembly in plasmodial extracts and development of an improved purification procedure. *Biochemistry*. In press.
4. ADELSTEIN, R. S., and M. A. CONTI. 1975. Phosphorylation of platelet myosin increases actin-activated myosin ATPase activity. *Nature (Lond.)* **256**:597-598.

5. ALLEN, R. D., J. W. COOLEGE, and P. J. HALL. 1960. Streaming in cytoplasm dissociated from the giant ameba, *Chaos chaos*. *Nature (Lond.)* **187**:896-899.
6. AKSOY, M. O., D. WILLIAMS, E. M. SHARKEY, and D. J. HARTSHORNE. 1976. A relationship between Ca^{2+} sensitivity and phosphorylation of gizzard actomyosin. *Biochem. Biophys. Res. Commun.* **69**:35-41.
7. AMPHLETT, G. W., T. C. VANAMAN, and S. V. PERRY. 1976. Effect of the troponin C-like protein from bovine brain (brain modular protein) on the Mg^{++} -stimulated ATPase of skeletal muscle actomyosin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **72**:163-168.
8. BLITZ, A. L., and R. E. FINE. 1974. Muscle-like contractile proteins and tubulin in synaptosomes. *Proc. Natl. Acad. Sci. U. S. A.* **71**:4472-4476.
9. BOOYSE, F. M., T. P. HOVEKE, D. ZSCHOCKE, and M. E. RAFELSON, JR. 1971. Human platelet myosin. *J. Biol. Chem.* **246**:4291-4297.
10. BRAY, D. 1975. Sticky actin. *Nature (Lond.)* **256**:616.
11. BRAY, D., and C. THOMAS. 1975. The actin content of fibroblasts. *Biochem. J.* **147**:221-228.
12. BRAY, D., and C. THOMAS. 1976. Unpolymerized actin in tissue cells. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. *Cold Spring Harbor Conf. Cell Proliferation*. **3**:461-473.
13. BREMEL, R. D. 1974. Myosin-linked calcium regulation in vertebrate smooth muscle. *Nature (Lond.)* **252**:405-407.
14. BROSTROM, C. O., Y.-C. HUANG, B. M. BRECKENRIDGE, and D. J. WOLFF. 1975. Identification of a calcium-binding protein as a calcium-dependent regulator of brain adenylate cyclase. *Proc. Natl. Acad. Sci. U. S. A.* **72**:64-68.
15. CARAFOLI, E., F. CLEMENTI, W. DRABIKOWSKI, and A. MARGRETH, editors. 1975. In *Calcium Transport in Contraction and Secretion*. North Holland Publishing Company, Amsterdam.
16. CARLSSON, L., L.-E. NYSTRÖM, U. LINDBERG, K. K. KANNAN, H. CID-DRESDNER, S. LÖVGREN, and H. JÖRNVALL. 1976. Crystallization of a non-muscle actin. *J. Mol. Biol.* **105**:353-366.
17. CARLSSON, L., L.-E. NYSTRÖM, I. SUNDKVIST, F. MARKEY, and U. LINDBERG. 1977. Profilin, a low-molecular weight protein controlling actin polymerisability. International Symposium on Contractile Systems in Non-Muscle Tissues. North Holland Publishing Co., Amsterdam. In press.
18. CHACKO, S., M. A. CONTI, and R. S. ADELSTEIN. 1977. Effect of phosphorylation of smooth muscle myosin on actin activation and on Ca^{2+} regulation. *Proc. Natl. Acad. Sci. U. S. A.* **74**:129-133.
19. COHEN, I., and C. COHEN. 1972. A tropomyosin-like protein from human platelets. *J. Mol. Biol.* **68**:383-387.
20. COHEN, I., and A. DE VRIES. 1973. Platelet contractile regulation in an isometric system. *Nature (Lond.)* **246**:36-37.
21. COHEN, I., E. KAMINSKI, and A. DE VRIES. 1973. Actin-linked regulation of the human platelet contractile system. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **34**:315-317.
22. CONDEELIS, J. S., and D. L. TAYLOR. 1977. The contractile basis of amoeboid movement. V. The control of solation, gelation, and contraction in extracts from *Dictyostelium discoideum*. *J. Cell Biol.* In press.
23. DANIEL, J. L., and R. S. ADELSTEIN. 1976. Isolation and properties of platelet myosin light chain kinase. *Biochemistry*. **15**:2370-2377.
24. FINE, R. E., and A. L. BLITZ. 1975. A chemical comparison of tropomyosin from muscle and non-muscle tissues. *J. Mol. Biol.* **95**:447-454.
25. FINE, R. E., A. L. BLITZ, S. E. HITCHCOCK, and B. KAMINER. 1973. Tropomyosin in brain and growing neurons. *Nat. New Biol.* **245**:182-186.
26. FINE, R., W. LEHMAN, J. HEAD, and A. BLITZ. 1975. Troponin-C in brain. *Nature (Lond.)* **258**:260-262.
27. GALLAGHER, M., T. DETWILER, and A. STRACHER. 1976. Two forms of platelet actin that differ from skeletal muscle actin. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. *Cold Spring Harbor Conf. Cell Proliferation*. **3**:475-485.
28. GERGELY, J. 1976. Troponin-tropomyosin-dependent regulation of muscle contraction by calcium. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. *Cold Spring Harbor Conf. Cell Proliferation*. **3**:137-149.
29. GOLDMAN, R. D. 1975. The use of heavy mero-myosin binding as an ultrastructural cytochemical method for localizing and determining the possible functions of actin-like microfilaments in non-muscle cells. *J. Histochem. Cytochem.* **23**:529-542.
30. GOLDMAN, R. D., and D. M. KNIPE. 1973. Functions of cytoplasmic fibers in non-muscle motility. *Cold Spring Harbor Symp. Quant. Biol.* **37**:523-534.
31. GOLDMAN, R., T. POLLARD, and J. ROSENBAUM, editors. 1976. In *Cell Motility*. *Cold Spring Harbor Conf. Cell Proliferation*. Vol. **3**.
32. GORDON, D. J., E. EISENBERG, and E. D. KORN. 1976. Characterization of cytoplasmic actin isolated from *Acanthamoeba castellanii* by a new method. *J. Biol. Chem.* **251**:4778-4786.
33. GORDON, D. J., Y.-Z. YANG, and E. D. KORN. 1976. Polymerization of *Acanthamoeba* kinetics, thermodynamics, and copolymerization with muscle actin. *J. Biol. Chem.* **251**:7474-7479.
34. GÓRECKA, A., M. O. AKSOY, and D. J. HARTSHORNE. 1976. The effect of phosphorylation of gizzard myosin on actin activation. *Biochem. Bio-*

- phys. Res. Commun.* **71**:325-331.
35. HARTWIG, J. H., and T. P. STOSSEL. 1975. Isolation and properties of actin, myosin, and a new actin-binding protein in rabbit alveolar macrophages. *J. Biol. Chem.* **250**:5696-5705.
 36. HARTWIG, J. H., and T. P. STOSSEL. 1976. Interactions of actin, myosin, and an actin-binding protein of rabbit pulmonary macrophages. III. Effects of cytochalasin B. *J. Cell Biol.* **71**:295-303.
 37. HASSELBACH, W., and K. SERAYDARIAN. 1966. The role of sulfhydryl groups in calcium transport through the sarcoplasmic membranes of skeletal muscle. *Biochem. Z.* **345**:159-172.
 38. HATANO, S. 1970. Specific effects of Ca^{+2} on movement of plasmodial fragment obtained by caffeine treatment. *Exp. Cell Res.* **61**:199-203.
 39. HATANO, S. 1972. Conformational changes of plasmodium actin polymers formed in the presence of Mg^{++} . *J. Mechanochem. Cell Motility.* **1**:75-80.
 40. HATANO, S., and F. OOSAWA. 1971. Movement of cytoplasm in plasmodial fragment obtained by caffeine treatment. *J. Physiol. Soc. Jpn.* **33**:589-590.
 41. HITCHCOCK, S. E., L. CARLSSON, and U. LINDBERG. 1976. Depolymerization of F-actin by deoxyribonuclease I. *Cell.* **7**:531-542.
 42. HOFFMAN-BERLING, H. 1964. Relaxation of fibroblast cells. In *Primitive Motile Systems in Cell Biology*. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 365-375.
 43. INOUE, S., and R. E. STEPHENS, editors. 1975. *Molecules and Cell Movement*. Raven Press, New York.
 44. ISENBERG, G., and K. E. WOHLFARTH-BOTTERMANN. 1976. Transformation of cytoplasmic actin. *Cell Tiss. Res.* **173**:495-528.
 45. IZZARD, C. S., and S. L. IZZARD. 1975. Calcium regulation of the contractile state of isolated mammalian fibroblast cytoplasm. *J. Cell Sci.* **18**:241-256.
 46. KANE, R. E. 1975. Preparation and purification of polymerized actin from sea urchin egg extracts. *J. Cell Biol.* **66**:305-315.
 47. KANE, R. E. 1976. Actin polymerization and interaction with other proteins in temperature-induced gelation of sea urchin egg extracts. *J. Cell Biol.* **71**:704-714.
 48. KATO, T., and Y. TONOMURA. 1975. Ca^{2+} -sensitivity of actomyosin ATPase purified from *Physarum polycephalum*. *J. Biochem. (Tokyo)*. **77**:1127-1134.
 49. KATO, T., and Y. TONOMURA. 1975. *Physarum* tropomyosin-troponin complex. Isolation and properties. *J. Biochem. (Tokyo)*. **78**:583-588.
 50. KENDRICK-JONES, J., E. M. SZENTKIRALYI, and A. G. SZENT-GYÖRGYI. 1976. Regulatory light chains in myosins. *J. Mol. Biol.* **104**:747-775.
 51. KIRKPATRICK, F. H. 1976. Interaction of spectrin with muscle actin detected by spin labelling. *Biochem. Biophys. Res. Commun.* **69**:225-229.
 52. KIRKPATRICK, F. H., G. M. WOODS, R. I. WEED, and P. L. LACELLE. 1976. Fractionation of spectrin by differential precipitation with calcium. *Arch. Biochem. Biophys.* **175**:367-372.
 53. KUO, H. C. Y., and C. J. COFFEE. 1976. Purification and characterization of a troponin-C-like protein from bovine adrenal medulla. *J. Biol. Chem.* **251**:1603-1609.
 54. LAZARIDES, E. 1976. Two general classes of cytoplasmic actin filaments in tissue culture cells; the role of tropomyosin. *J. Cell Biol.* **70**(2, Pt. 2):359 a. (Abstr.).
 55. LAZARIDES, E., and U. LINDBERG. 1975. Actin is the naturally occurring inhibitor of deoxyribonuclease I. *Proc. Natl. Acad. Sci. U. S. A.* **71**:4742-4746.
 56. LEHMAN, W. 1976. Phylogenetic diversity of the proteins regulating muscular contraction. *Int. Rev. Cytol.* **44**:55-92.
 57. LEHMAN, W. 1977. Calcium ion-dependent myosin from decapod-crustacean muscles. *Biochem. J.* **163**. In press.
 58. LEHMAN, W., J. KENDRICK-JONES, and A. G. SZENT-GYÖRGYI. 1973. Myosin-linked regulatory systems: comparative studies. *Cold Spring Harbor Symp. Quant. Biol.* **37**:319-330.
 59. LEHNINGER, A. L., E. CARAFOLI, and C. S. ROSSI. 1967. Energy-linked ion movements in mitochondrial systems. *Adv. Enzymol. Relat. Areas Mol. Biol.* **29**:259-320.
 60. LIN, Y. M., Y. P. LIU, and W. Y. CHEUNG. 1974. Cyclic 3':5'-nucleotide phosphodiesterase purification, characterization, and active form of the protein activator from bovine brain. *J. Biol. Chem.* **249**:4943-4954.
 61. LINDBERG, M. U. 1966. Crystallization from calf spleen of two inhibitors of deoxyribonuclease I. *J. Biol. Chem.* **241**:1246-1248.
 62. LOEWY, A. G. 1952. An actomyosin-like substance from the plasmodium of a myxomycete. *J. Cell Comp. Physiol.* **40**:127-156.
 63. MAHENDRAN, C., and S. BERL. 1976. Isolation of a protein-complex regulating ATPase activity of brain actomyosin. *J. Neurochem.* **26**:1293-1295.
 64. MARCHESI, S. L., E. STEERS, V. T. MARCHESI, and T. W. TILLACK. 1970. Physical and chemical properties of a protein isolated from red cell membranes. *Biochemistry.* **9**:50-57.
 65. MARUTA, H., and E. D. KORN. 1977. Purification from *Acanthamoeba castellanii* of proteins that induce gelation and syneresis of F-actin. *J. Biol. Chem.* **252**:399-402.
 66. MASAKI, T. 1975. Tropomyosin-like protein in chick embryo fibroblasts. *J. Biochem.* **77**:901-904.
 67. McLACHLAN, A. D., and M. STEWART. 1976. The

- 14-fold periodicity in α -tropomyosin and the interaction with actin. *J. Mol. Biol.* **103**:271-298.
68. MOCKRIN, S. C., and J. A. SPUDICH. 1976. Calcium control of actin-activated myosin adenosine triphosphatase from *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. U. S. A.* **73**:2321-2325.
 69. MOOSEKER, M. S. 1976. Brush border motility, microvillar contraction in triton-treated brush borders isolated from intestinal epithelium. *J. Cell Biol.* **71**:417-433.
 70. MOOSEKER, M. S., and L. G. TILNEY. 1975. Organization of an actin filament-membrane complex. Filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. *J. Cell Biol.* **67**:725-743.
 71. NACHMIAS, V. T. 1975. Calcium sensitivity of hybrid complexes of muscle myosin and *Physarum* proteins. *Biochim. Biophys. Acta.* **400**:208-221.
 72. NACHMIAS, V. T. 1976. Adenosine 5'-phosphate and assembly of actin filaments in vitro. *J. Mol. Biol.* **107**:623-629.
 73. NACHMIAS, V., and A. ASCH. 1974. Actin mediated calcium dependency of actomyosin in a myxomycete. *Biochem. Biophys. Res. Commun.* **60**:656-664.
 74. NACHMIAS, V. T., and A. ASCH. 1976. A calcium-sensitive preparation from *Physarum polycephalum*. *Biochemistry.* **15**:4273-4278.
 75. OLDEN, K., M. WILLINGHAM, and I. PASTAN. 1976. Cell surface myosin in cultured fibroblasts. *Cell.* **8**:383-390.
 76. PAINTER, R. G., M. SHEETZ, and S. J. SINGER. 1975. Detection and ultrastructural localization of human smooth muscle myosin-like molecules in human smooth cells by specific antibodies. *Proc. Natl. Acad. Sci. U. S. A.* **72**:1359-1363.
 77. PERRY, S. V., and H. A. COLE. 1974. Phosphorylation of troponin and the effects of interactions between the components of the complex. *Biochem. J.* **141**:733-743.
 78. PINDER, J. C., D. BRAY, and W. B. GRATZER. 1975. Actin polymerization induced by spectrin. *Nature (Lond.)* **258**:765-766.
 79. PIRES, E., S. V. PERRY, and M. A. W. THOMAS. 1974. Myosin-light-chain kinase, a new enzyme from striated muscle. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **41**:292-296.
 80. POLLARD, T. D. 1976. The role of actin in the temperature-dependent gelation and contraction of extracts of *Acanthamoeba*. *J. Cell Biol.* **68**:579-601.
 81. POLLARD, T. D., and S. ITO. 1970. Cytoplasmic filaments of *Amoeba proteus*. I. The role of filaments in consistency changes and movement. *J. Cell Biol.* **46**:267-289.
 82. POLLARD, T. D., and E. D. KORN. 1971. Filaments of *Amoeba proteus*. II. Binding of heavy meromyosin by thin filaments in motile cytoplasmic extracts. *J. Cell Biol.* **48**:216-219.
 83. POLLARD, T. D., and E. D. KORN. 1973. *Acanthamoeba* myosin. II. Interaction with actin and with a new cofactor protein required for actin activation of Mg^{2+} adenosine triphosphatase activity. *J. Biol. Chem.* **248**:4691-4697.
 84. POLLARD, T. D., and E. D. KORN. 1973. The "contractile" proteins of *Acanthamoeba castellanii*. *Cold Spring Harbor Symp. Quant. Biol.* **37**:537-583.
 85. POLLARD, T. D., and R. R. WEIHING. 1974. Actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* **2**:1-65.
 86. PROBST, E., and F. LÜSCHER. 1972. Studies on thrombosthenin A, the actinlike moiety of the contractile protein from blood platelets. I. Isolation, characterization and evidence for two forms of thrombosthenin A. *Biochim. Biophys. Acta.* **278**:577-584.
 87. PUSZKIN, S., and S. KOCHWA. 1974. Regulation of neurotransmitter release by a complex of actin with relaxing protein from brain synaptosomes. *J. Biol. Chem.* **249**:7711-7714.
 88. PUSZKIN, S., S. KOCHWA, and R. E. ROSENFELD. 1975. Regulatory complex of human platelet actomyosin. *J. Cell Biol.* **67**(2, Pt. 2):346 a (Abstr.).
 89. RIDGWAY, E. B., and A. C. H. DURHAM. 1976. Oscillations of calcium ion concentrations in *Physarum polycephalum*. *J. Cell Biol.* **69**:223-226.
 90. ROBBLEE, L. S., D. SHEPRO, and F. A. BELAMARICH. 1973. Calcium uptake and associated adenosine triphosphatase activity of isolated brush platelet membranes. *J. Gen. Physiol.* **61**:462-481.
 91. ROBINSON, K. R., and L. F. JAFFE. 1974. Polarizing fucoid eggs drive a calcium current through themselves. *Science (Wash. D. C.)* **187**:70-72.
 92. RODEWALD, R., S. B. NEWMAN, and M. J. KARNOVSKY. 1976. Contraction of isolated brush borders from the intestinal epithelium. *J. Cell Biol.* **70**:541-544.
 93. ROSE, B., and W. R. LOEWENSTEIN. 1975. Calcium ion distribution in cytoplasm visualized by aequorin: diffusion in cytosol restricted by energized sequestering. *Science (Wash. D. C.)* **190**:1204-1206.
 94. ROSENTHAL, A. S., F. M. KREGENOW, and H. L. MOSES. 1970. Some characteristics of a Ca^{2+} dependent ATPase activity associated with a group of erythrocyte membrane proteins which form fibrils. *Biochim. Biophys. Acta.* **196**:254-262.
 95. SCHOLLMAYER, J. E., D. E. GOLL, L. G. TILNEY, M. MOOSEKER, R. ROBSON, and M. STROMER. 1974. Localization of α -actinin in nonmuscle material. *J. Cell Biol.* **63**(2, Pt. 2):304 a. (Abstr.).
 96. SCHROEDER, T. E. 1972. The contractile ring. II. Determining its brief existence, volumetric changes, and vital role in cleaving *Arbacia* eggs. *J. Cell Biol.* **53**:419-434.
 97. SHEETZ, M. P., R. G. PAINTER, and S. J. SINGER. 1976. Relationships of the spectrin complex of

- human erythrocyte membranes to the actomyosin of muscle cells. *Biochemistry*. **15**:4486-4491.
98. SHIBATA, N., N. TATSUMI, K. TANAKA, Y. OKAMURA, and N. SENDA. 1972. A contractile protein possessing Ca^{+2} sensitivity (natural actomyosin) from leucocytes. *Biochim. Biophys. Acta*. **256**:565-576.
 99. SPUDICH, J. A., and R. COOKE. 1975. Supramolecular forms of actin from amoebae of *Dictyostelium discoideum*. *J. Biol. Chem.* **250**:7485-7491.
 100. STECK, T. L. 1974. The organization of proteins in the human red blood cell membrane. *J. Cell Biol.* **62**:1-19.
 101. STEVENS, F. C., M. WALSH, H. C. HO, T. S. TEO, and J. H. WANG. 1976. Comparison of calcium-binding proteins. *J. Biol. Chem.* **251**:4495-4500.
 102. STOSSEL, T. P., and J. H. HARTWIG. 1975. Interactions between actin, myosin, and an actin-binding protein from rabbit alveolar macrophages. *J. Biol. Chem.* **250**:5706-5712.
 103. STOSSEL, T. P., and J. H. HARTWIG. 1976. Interactions of actin, myosin, and a new actin-binding protein of rabbit pulmonary macrophages. *J. Cell Biol.* **68**:602-619.
 104. SZENT-GYÖRGYI, A. G., E. M. SZENTKIRALYI, and J. KENDRICK-JONES. 1973. The light chains of scallop myosin as regulatory subunits. *J. Mol. Biol.* **74**:179-203.
 105. TANAKA, H., and S. HATANO. 1972. Extraction of native tropomyosin-like substances from myxomycete plasmodium and the cross reaction between plasmodium F-actin and muscle native tropomyosin. *Biochim. Biophys. Acta*. **257**:445-451.
 106. TAYLOR, D. L., J. S. CONDEELIS, P. L. MOORE, and R. D. ALLEN. 1973. The contractile basis of amoeboid movement. I. The chemical control of motility in isolated cytoplasm. *J. Cell Biol.* **59**:378-394.
 107. TAYLOR, D. L., J. A. RHODES, and S. A. HAMMOND. 1976. The contractile basis of amoeboid movement. II. Structure and contractility of motile extracts and plasmalemma-ectoplasm ghosts. *J. Cell Biol.* **70**:123-143.
 108. TAYLOR, D. L., J. S. CONDEELIS, and J. A. RHODES. 1977. The contractile basis of amoeboid movement. III. Structure and dynamics of motile extracts and membrane fragments from *Dictyostelium discoideum* and *Amoeba proteus*. *J. Supramol. Struct.* In press.
 109. THOMPSON, C. M., and C. M. WOLPERT. 1963. The isolation of motile cytoplasm from *Amoeba proteus*. *Exp. Cell Res.* **32**:156-160.
 110. TILNEY, L. G. 1976. The polymerization of actin. II. How nonfilamentous actin becomes nonrandomly distributed in sperm: evidence for the association of this actin with membranes. *J. Cell Biol.* **69**:51-72.
 111. TILNEY, L. G. 1976. The polymerization of actin. III. Aggregates of nonfilamentous actin and its associated proteins: a storage form of actin. *J. Cell Biol.* **69**:73-89.
 112. TILNEY, L. G., and R. R. CARDELL, JR. 1970. Factors controlling the reassembly of the microvillous border of the small intestine of the salamander. *J. Cell Biol.* **47**:408-422.
 113. TILNEY, L. G., and P. DETMERS. 1975. Actin in erythrocyte ghosts and its association with spectrin. *J. Cell Biol.* **66**:508-520.
 114. TILNEY, L. G., S. HATANO, H. ISHIKAWA, and M. S. MOOSEKER. 1973. The polymerization of actin: its role in the generation of the acrosomal process of certain echinoderms. *J. Cell Biol.* **59**:109-126.
 115. TILNEY, L. G., and M. S. MOOSEKER. 1976. Actin filament-membrane attachment: are membrane particles involved? *J. Cell Biol.* **71**:402-416.
 116. VALE, M. G. P., and A. P. CARVALHO. 1973. Effects of ruthenium red on Ca^{2+} uptake and ATPase of sarcoplasmic reticulum of rabbit skeletal muscle. *Biochim. Biophys. Acta*. **325**:29-37.
 117. VANAMAN, T. C., F. SHARIEF, J. L. AWRAMIK, P. A. MENDEL, and D. M. WATTERSON. 1977. Chemical and biological properties of the ubiquitous troponin-C like protein from non-muscle tissues, a multifunctional Ca^{2+} -dependent regulatory protein. *Curr. Top. Cell. Reg.* **II**: In press.
 118. VASINGTON, F. D., P. GAZZOTTI, R. TIOZZO, and E. CARAFOLI. 1972. The effect of ruthenium red on Ca^{2+} transport and respiration in rat liver mitochondria. *Biochim. Biophys. Acta*. **256**:43-54.
 119. WATTERSON, D. M., W. G. HARRELSON, JR., P. M. KELLER, F. SHARIEF, and T. C. VANAMAN. 1976. Structural similarities between the Ca^{2+} -dependent regulatory proteins of 3':5' cyclic nucleotide phosphodiesterase and actomyosin ATPase. *J. Cell Biol.* **251**:4501-4513.
 120. WATTERSON, D. M., and T. C. VANAMAN. 1976. Affinity chromatography purification of cyclic nucleotide phosphodiesterase using immobilized modular protein, a troponin C-like protein from brain. *Biochem. Biophys. Res. Commun.* **73**:40-46.
 121. WEIHING, R. R. 1976. Cytochalasin B inhibits actin-related gelation of HeLa cell extracts. *J. Cell Biol.* **71**:303-307.