Interconversion of Structural and Contractile Actin Gels by Insertion of Myosin During Assembly

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ABSTRACT   Extracts of the soluble cytoplasmic proteins of the sea urchin egg form gels of different composition and properties depending on the temperature used to induce actin polymerization. At temperatures that inactivate myosin, a gel composed of actin, fascin, and a 220,000-mol-wt protein is formed. Fascin binds actin into highly organized units with a characteristic banding pattern, and these actin-fascin units are the structural core of the sea urchin microvilli formed after fertilization and of the urchin coelomocyte filopods. Under milder conditions a more complex myosin-containing gel is formed, which contracts to a small fraction of its original volume within an hour after formation. What has been called "structural" gel can be assembled by combining actin, fascin, and the 220,000-mol-wt protein in 50-100 mM KCl; the aim of the experiments reported here was to determine whether myosin could be included during assembly, thereby interconverting structural and contractile gel. This approach is limited by the aggregation of sea urchin myosin at the low salt concentrations utilized in gel assembly. A method has been devised for the sequential combination of these components under controlled KCl and ATP concentrations that allows the formation of a gel containing dispersed myosin at a final concentration of 60-100 mM KCl. These gels are stable at low ($\sim$10 μm) ATP concentrations, but contract to a small volume in the presence of higher ($\sim$100 μm) ATP. Contraction can be controlled by forming a stable gel at low ATP and then overlaying it with a solution containing sufficient ATP to induce contraction. This system may provide a useful model for the study of the interrelations between cytoplasmic structure and motility.

Actin has been identified in cell types throughout the animal and plant kingdoms and is often present in sufficient amount to constitute a major cytoplasmic protein. The extent of its polymerization varies, but it seems likely that filamentous or F-actin forms the basic structural framework of most cells. Although it was originally isolated from motile cytoplasm (11) and attention was focused on its interaction with myosin as the basis of cell motility, a growing list of proteins that can cross-link actin filaments to form three dimensional gels has been identified in recent years (24). The interaction of actin with these proteins must give rise to many of the mechanical properties of the cytoplasm, while the interaction of actin with myosin is responsible for cell motility.

Alternation of the structural and contractile roles of actin may occur in the cytoplasm in such processes as cell shape change, the formation of surface and cortical specializations, and the translation of the cell relative to the substrate. Such interchanges of the cytoplasmic roles of actin have not yet been extensively studied and their mechanism and controlling factors are not well understood. The sea urchin egg provides a promising system for investigations in this area since sperm entry causes eggs to undergo, in a highly synchronous manner, a variety of cytoplasmic activities including exocytosis of granules in the cortical region, formation of microvilli, sequential cytokinesis, and the extensive cell shape changes and movements of embryogenesis. These cellular activities occur, to a varying extent, in many cell types and all would appear to involve actin in a structural and/or contractile role.

The sea urchin egg has already proven to be a valuable source of cytoplasmic extracts for investigations of the interaction of actin with other proteins in the cytoskeleton and in contractility. Actin is predominantly in the G form in the unfertilized egg, as polymerization to F-actin is inhibited and remains so in extracts of the soluble cytoplasmic proteins; addition of ATP and KCl to these preparations at 25°C, the normal phsyiological temperature of the Hawaiian sea urchin

used in these experiments, causes no significant actin polymerization over the course of several hours. However, warming to 40°C in the presence of ATP and KCl induces rapid polymerization, and the F-actin formed interacts with other cytoplasmic proteins to cause gelation of the extract in 30–60 min (13, 14). The gels that form are composed of actin and two additional proteins of 58,000 and 220,000-mol-wt. Actin is combined with the 58,000-mol-wt protein (now called fascin) to form hexagonally packed structures with a characteristic banding pattern, and these units are joined by the 220,000-mol-wt protein into a gel network (3, 4, 6). The cellular role of this actin-fascin unit was clarified when this banding pattern was observed in the structural core of the microvilli formed after fertilization of the egg (5) and also in the filopods of the sea urchin coelomocyte (7); fascin was later localized in these areas (22, 23). Optical diffraction and image reconstruction studies (26) provided further confirmation of this role of the actin-fascin unit as the microvillar core, and I have subsequently used the term structural gel for the material of this composition.

Subsequent experiments showed that a reduction in the temperature used to induce actin polymerization would give rise to gels of different composition and behavior. Structural gel was prepared at 40°C, a temperature later found to inactivate the myosin present in these extracts. Induction of actin polymerization by shorter exposure to 35°C retained myosin ATPase activity, and gels formed under these conditions contract to a small volume 20–30 min after formation in the presence of slighlty higher concentrations of ATP and KCl than used in the induction of structural gel. In addition to myosin and the proteins of structural gel, these contractile gels also contain proteins of 250,000 and 110,000 mol wt. Most interesting from a functional point of view was the observation that increased concentrations of ATP and KCl, which favor more rapid and complete contraction, also reduce the amount of fascin in the gel, suggesting that such shifts of the actin-associated proteins might mediate changes in the structural and contractile roles of actin (15).

Investigations of the contractile behavior of actin gels formed in these extracts are limited by the compromises that must be made between the conditions that favor maximum actin polymerization and those required to maintain myosin activity. A more direct experimental approach is via the assembly of contractile gels from components, as has been done in the case of structural gel, which can be formed by the combination of F-actin with the other gel components (14). The contracted material that forms in extracts is more complex than structural gel and contains several unidentified proteins; no attempt to duplicate this material was made in the experiments reported here, the aim instead being to use the minimum number of components necessary to form a gel capable of contraction. This requires the introduction of myosin into the three-dimensional gel formed when a solution of F-actin is combined with one containing fascin and the 220,000-mol-wt protein. This provides a relatively simple in vitro system for the investigation of the relations between gelation and contraction. More specifically, it may also serve as a useful model of the interchange of the structural and contractile roles of actin in the events following fertilization. Fascin localizes in the egg cortex soon after fertilization where it is known to combine with actin in the microvillar cores (23). Cortical actin must also interact with myosin in the formation of the cleavage furrow (21), so this in vitro system may aid in the understanding of these cellular processes.

MATERIALS AND METHODS

Extracts of the soluble proteins of unfertilized eggs of the Hawaiian sea urchin *Tripneustes gratilla* were prepared by methods described previously (13, 14). The washed and dejellied eggs are homogenized in a medium containing 0.9 M glycerol, 5 mM EGTA, and 0.1 M PIPES. After being centrifuged at 100,000 g for 1 h, the clear supernatant is removed and dialyzed overnight at 2°C to 10 mM PIPES, 0.1 mM ATP and EGTA, pH 6.9–7.0. After dialysis the extract is centrifuged at 25,000 g for 15 min at 2°C, and 1 mM EGTA and NaN₃ are added. Extracts are frozen in liquid nitrogen and stored at −80°C. Total protein concentration of the extracts is 10–12 mg/ml.

The proteins used in these experiments were prepared from extracts by methods that had been described in detail in previous reports. Sea urchin egg myosin was prepared by adding 8 mM ATP to extracts at 0°C (15), washing in 10 mM PIPES, and dissolving in 1 M KCl followed by adding glycerol to 50% and storing at −20°C. F-actin was prepared by the induction of aggregation by ATP in a solution of structural gel dissolved in 1 M KCl (13); the resulting aggregates were suspended in 50 mM KCl, 10 mM PIPES, pH 6.8. A solution of fascin and the 220,000-mol-wt protein was prepared by dialyzing the solution remaining after actin removal to 50 mM KCl and 10 mM PIPES and removing the precipitated material by centrifugation at 25,000 g for 15 min (14). Average protein concentrations were actin, 5 mg/ml, myosin, 3 mg/ml, and 58,000- and 220,000-mol-wt proteins, 1 mg/ml. A simple computer program was used that allows independent variation of the ratios of the protein components, maintains the appropriate KCl level at each step of assembly, and produces any desired volume at a given final protein and KCl concentration. The components are combined at 0°C in 15 ml conical tubes with rapid mixing. Aliquots are transferred to test tubes or centrifuge tubes appropriate to the sample size, allowed to remain at 0°C for 3–5 min, and then brought to room temperature, and timing is begun. Contraction time is estimated by eye or, in the case of the smallest (6 × 50 mm) tubes, in a 6 × binocular microscope. Contraction rate was determined by the measurement of two points in the contraction cycle, with 0 time defined as that of transfer from 0°C to room temperature: the time at the beginning of contraction, visible as the separation of the gel from the walls of the container; and the time for the completion of contraction, determined by comparison of the volume with that of a previously contracted sample of the same material. Completion time is generally two times the time of start of contraction, even though rates may vary by a factor of ten. Less accurate but reproducible estimates were also made of the time to reach three-fourths and one-half volume. Photomicrographs were made with a Zeiss universal microscope using Zeiss differential interference contrast.

Gelled material was sedimented by centrifugation at 20,000 g for 15 min at 24°C and F-actin at 150,000 g for 2 h at 24°C. SDS acrylamide gels were run at 7.5% using the procedure of Laemmli (19). The 0.75-mm slabs were stained for 1 hr in 0.1% Coomassie Brilliant Blue in 95% ethanol/water/glacial acetic acid (5:5:1 by volume) and destained by diffusion in 10% acetic acid. Protein measurements were made of pellets by the Hartree modification of the Lowry procedure (10), using a serum albumin standard, and of the supernatants by dye binding (2).

K⁺-EDTA ATPase activity was determined at 25°C in a medium containing 0.6 M KCl, 0.1 mM EDTA, 1 mM ATP, and 20 mM TRIS-HCl, pH 8.0. Inorganic phosphate liberated was measured by the method of Fiske and Subbarow (8). ATPase activity was also measured under the conditions used for contraction (60, 80, 100 mM KCl) in the absence and presence of actin at an actin/myosin ratio of 1:0.3 (details in text).

ATP, EGTA, EDTA, and PIPES buffer were obtained from the Sigma Chemical Co. (St. Louis, MO). Rabbit muscle myosin was the gift of Dr. Joseph Bryan, Department of Cell Biology, Baylor College of Medicine, Houston, Texas.

RESULTS

Assembly of Contractile Gels

Actin gel is formed by combining a solution of sea urchin F-actin with one containing the 58,000 and 220,000-mol-wt components at KCl concentrations of 50–100 mM, since actin and the other components are soluble at this salt concentration and the gel that forms is not, and appears as a network visible in the light microscope (14). The major obstacle to the inclusion of myosin in a gel of these components is the aggregation and precipitation of sea urchin egg myosin at this KCl concentration, a salt concentration of 0.45 M or above being required for myosin solubility (15). This limitation can be overcome by controlling the KCl and ATP levels during the sequential combination of these proteins, which allows

TABLE I

Assembly of Contractile Gel

Component	μl	KCl conc	ATP conc
		mM	
1 M KCl, 2 mM ATP	50	1,000	2.0
Actin, 5 mg/ml in 50 mM KCl	56	500	0.94
Myosin, 3 mg/ml in 0.5 M KCl-glycerol	28	500	0.75
Fascin-220,000-mol-wt protein, 1 mg/ml in 50 mM KCl	168	250	0.33
10 mM PIPES, pH 6.8	643	80	0.11
80 mM KCl	55	80	0.10
Total volume	1,000	—	—

Final solution: 80 mM KCl, 0.1 mM ATP. Actin concentration = 0.28 mg/ml, actin/myosin/58,000- and 220,000-mol-wt proteins = 1:0.3:0.6, total protein concentration = 0.53 mg/ml.

the distribution of unaggregated myosin in the gel as it forms.

The ratios of these proteins to be combined were estimated on the basis of previous experiments on the assembly of structural gel (14) and from observatons on the proteins present in contractile gels from extract (15); a weight ratio of actin/myosin/58,000- and 220,000-mol-wt proteins of 1.0:0.3:0.6 will be used as example. Direct combination of these protein solutions in this ratio results in a final KCl concentration of 100 mM and an amorphous protein precipitate. Formation of a gel network requires the sequential addition of components at 0°C with the control of KCl and ATP concentrations at each step followed by transfer to room temperature. Actin and myosin solutions are first combined to give a protein ratio of 1:0.3 in the presence of sufficient 1 M KCl to maintain a KCl concentration of 0.5 M in the mixture. To this mixture the fascin and 220,000-mol-wt protein solution is added, so that the ratio of these proteins to actin is 0.6. The KCl concentration at the completion of this addition is ~0.25 M, close to the solubility limit of the gel. Dilution to the desired final KCl concentration is then made by the addition of 10 mM PIPES buffer. Reduction in KCl concentration also reduces the protein concentration; 80 mM KCl proved a useful compromise in most instances. To reduce the association of actin and myosin during the sequential addition of components, ATP must be present; this is accomplished by calculating the amount of 0.1 M ATP required to establish the desired final ATP level (usually 0.1 mM, see below) and including this in the starting 1 M KCl solution. Table I summarizes the procedure for the assembly of a 1-ml sample under these conditions.

This mixture of components exists as a clear solution without visible structure at 0°C. Within a few minutes of reaching room temperature the solution become slightly turbid due to gel formation, and at ATP levels of 0.1 mM this gel separates from the walls of the container at 15–25 min and contracts to a few percent of its original volume in 30–50 min. The gelation-contraction sequence is similar in rate and appearance to that taking place when egg extract is warmed to induce actin polymerization, and the contracted material contains a similar quantity of protein. If a sample of this mixture is transferred from 0°C to a microscope slide at room temperature, a faint, irregular gel is seen to appear in the solution in a few minutes. This material gradually condenses to a network of interconnected linear elements (Fig. 1, *a–e*); this configuration is stable at very low ATP levels (<10

μM). At 0.1 mM ATP some filaments of this network shorten and thicken while others are stretched and broken as the material confined under the coverslip contracts from the edges towards the center (Fig. 1*f*) and finally forms a mass of dense, refractile granules.

Conditions for Gelation and Contraction

The contractile behavior of these reconstituted gels depends on the ratios of the protein components and on the KCl and ATP concentrations in the medium. At myosin ratios of <0.2, contraction slows and is often incomplete, while a minimum ratio of the 58,000- and 220,000-mol-wt gel proteins to actin of ~0.4 is necessary to form a gel of sufficient rigidity to remain intact during contraction. Experiments were carried out with protein ratios of 1:0.25:0.50 to 1:0.50:1.0, with acceleration of contraction occurring at higher myosin ratios and more dense gel forming at higher gel protein ratios. Variations in total protein concentration at a fixed ratio have little effect on contractile behavior, but below a lower limit of ~0.30 mg/ml the gels become extremely fragile. Increase in KCl concentration from 80 to 100 mM reduces the typical contraction time from 50 to 25 min, while reduction to 60 mM KCl slows it by the same factor and sometimes leads to incomplete contraction. The addition of magnesium also accelerates contraction, 1 mM Mg^{++} producing an effect similar to an increase of 20 mM in KCl concentration.

The interaction of myosin and the gel proteins with actin can be investigated separately by varying the ratios of each component to actin in the absence of the other. In actin/myosin combinations of 1:0.3, the proteins form a finely dispersed precipitate on reaching room temperature, but with increasing myosin, aggregates are formed, and in ratios of 1:0.6, the amount of myosin is sometimes sufficient to interconnect the actin filaments to a fragile mass that contracts irregularly. In the absence of myosin, the ratio of the gel proteins to actin determines the rate at which material visible in phase contrast appears in the solution. A ratio of 1:1 was previously reported to cause the rapid appearance of visible gel at both 0° and 24°C (14). The lower ratio of 1:0.6 used in the present experiments results in a solution that is optically clear at 0°C and that slowly forms visible gel over the course of 1 h when transferred to 24°C. Increase in ATP level from 0.1 to 1 mM delays the appearance of gel visible in phase contrast for several hours at 24°C. The formation of visible gel is not related to sedimentability, as a similar amount of protein is pelleted at 20,000 *g* immediately after being mixed at 0°C and after gel formation at 24°C.

The minimum ATP level sufficient to reduce the interaction of actin and myosin and prevent precipitation during the assembly process results in a final ATP concentration on the order of 10 μM. Under these conditions a gel network is formed upon transfer to room temperature but undergoes slight or no contraction. Incremental increases in the final ATP level from 10 to 100 μM increase the extent of contraction, with minimum final contraction volumes reached at 100 μM. Raising the ATP concentration to millimolar or above produces variable results. Contraction fails in some cases at millimolar ATP levels and the microscopic appearance of these samples, with no gel formed and only dense, unconnected granules appearing, indicated that the interaction of actin and myosin was occurring more rapidly than gelation. This conclusion was strengthened by the observation that 1 mM magnesium, which accelerates contraction in 100
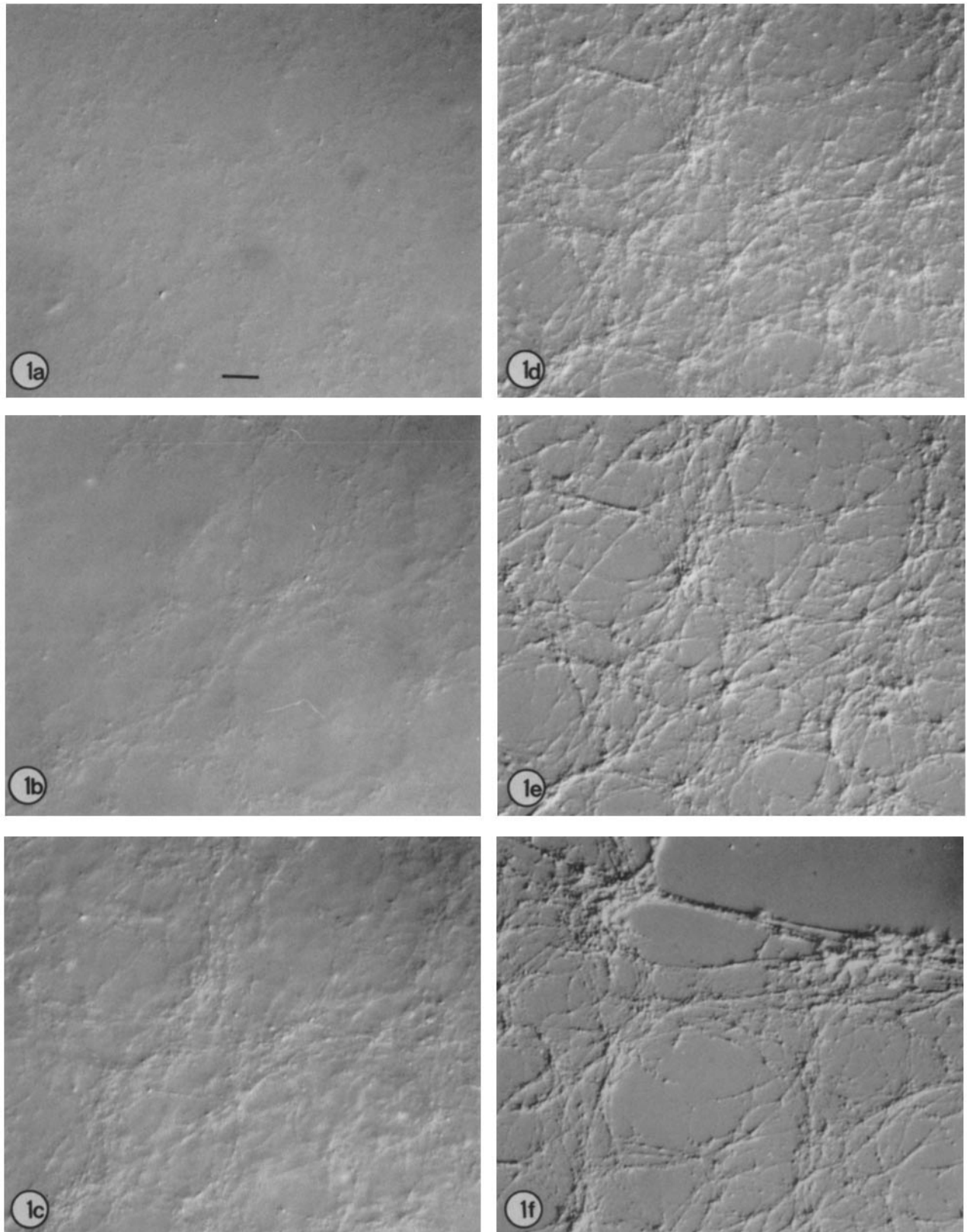
FIGURE 1 Differential interference contrast photomicrographs of combined gel components at room temperature, 12-min intervals. 1f moved to illustrate contracting edge. 80 mM KCl, 0.1 mM ATP. Bar, 20 μm. × 320.

μm ATP, routinely causes the formation of granular material rather than networks at higher ATP levels. This suggested that those samples that fail to form gel and contract at higher ATP levels might do so if the reaction were slowed; the addition of

1 mM EDTA to such samples results in the formation of gel networks and contraction. These variations in response to higher ATP levels are a function of individual sea urchin egg myosin preparations and their storage period.

Separate Induction of Gelation and Contraction

If the final ATP level of the combined components is low ($\sim 10\ \mu$M), gel networks form but undergo little or no contraction. These gels can subsequently be induced to contract by overlaying them with a solution of the same KCl concentration containing at least 100 μM ATP. The transformation from stable to contractile gel follows a characteristic sequence under these circumstances. The circular upper surface of the gel in contact with the overlay solution begins to contract, which reduces its diameter, and becomes visibly more dense. This reduction in diameter of the upper gel surface retracts it from the walls of the tube, allowing access of the overlay solution to additional gel surface and causing further contraction. This process continues until the complete separation of the gel from the walls and results finally in the reduction of the gel to a dense mass of small volume.

Composition of the Contracted Material

In sea urchin egg extracts contraction is accelerated and the protein content of the contracted material is reduced with increasing ATP and KCl, with a proportionately much greater reduction in the fascin content, resulting in its almost total elimination under some conditions (15). The composition of the contracted material formed by the reconstituted gels was examined to determine whether any specific protein changes are associated with changes in contraction rate or form.

The first SDS PAGE (Fig. 2) illustrates the components that are sedimented at 20,000 g after 1 h at 24°C in a combination of actin and myosin in the ratio of 1:0.3 (lane 1), actin and gel proteins in the ratio of 1:0.6 (lane 2), and actin, myosin, and the gel proteins in the ratios of 1:0.3:0.6 (lane 3), all in a solution containing 80 mM KCl and 0.1 mM ATP. Under these conditions the assembled proteins in sample 3 form a gel that contracts to a small pellet that can be

removed from the solution. However, no significant differences are observed between this contracted material and the total pellet recovered after 20,000 g sedimentation. For consistency, all samples were centrifuged. Myosin can be identified by means of its 200,000-mol-wt heavy chain in lanes 1 and 3. The material sedimented from a combination of actin and the gel proteins (lane 2) has the characteristic ratio of actin and fascin (see [3] for a detailed presentation) although the 220,000-mol-wt protein, which binds nonstoichiometrically, is present in much smaller amounts than in gel from extracts (15). The gel containing myosin shows no major change in fascin content after contraction (lane 3). Measurements of the protein recovered in the 20,000 g pellets (Table II) illustrate the relative effects of myosin and the gel proteins on actin sedimentability. Changes in the solution conditions that change the rate and form of contraction have no apparent effect on composition (Fig. 3). Samples with the same protein ratios and KCl concentrations, but with 1 mM ATP (lane 1), 1 mM ATP and Mg^{++} (lane 2), and 1 mM ATP and EDTA (lane 3) appear similar in composition, although contraction was complete in sample 3 at 1 h and had just begun in sample

TABLE II

Combination of Actin with Myosin and with the Gel Proteins

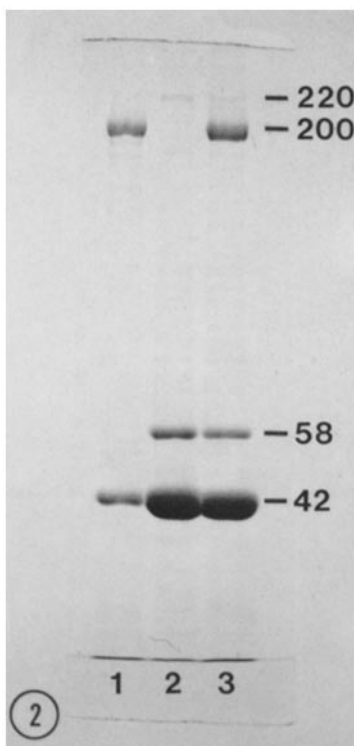| Composition			
Actin/ Myosin/Fascin– 220,000- mol-wt protein	Solution	Actin	Protein	20,000 g pellet
		$\mu g/ml$		
1.0:0.3:0	80 mM KCl, 0.1 mM ATP	280	364	109
1.0:0:0.6	80 mM KCl, 0.1 mM ATP	280	448	312
1.0:0.3:0.6	80 mM KCl, 0.1 mM ATP	280	532	383

FIGURE 2 SDS PAGE. 20,000 g pellets from: Lane 1, combination of 1:0.3 of actin/myosin; lane 2, combination of 1:0.6 of actin/gel proteins; lane 3, combination of 1:0.3:0.6 of actin/myosin/gel proteins. All in 80 mM KCl, 0.1 mM ATP.

FIGURE 3 SDS PAGE. 20,000 g pellets from combination of 1:0.3:0.6 of actin/myosin/gel proteins in presence of: Lane 1, 1 mM ATP; lane 2, 1 mM ATP and 1 mM MgCl$_2$; and lane 3, 1 mM ATP and 1 mM EDTA. All in 80 mM KCl.

1, and sample *2* formed only unconnected granules and not contractile gel. Increasing KCl concentration over the range of 60–100 mM KCl reduces the amount of protein sedimentable at 20,000 *g*, but a fraction of the protein lost from the low speed pellet with increasing KCl concentration is recovered at 150,000 *g* (Table III). SDS PAGE (Fig. 4) shows that there is no specific reduction in components with increasing KCl (lanes *1–3*). The material recovered at 150,000 *g* (lanes *4–6*) presumably exists in solution as F-actin filaments with bound myosin and fascin but was not assembled to gel that could be sedimented at the lower centrifugal force.

ATPase Activity and Contraction Rate

Measured ATPase values for sea urchin egg myosin and comparison with those of rabbit muscle myosin under the same conditions are given in Table IV. As reported previously (14) the K^+-EDTA ATPase of sea urchin egg myosin is lower than that of rabbit muscle myosin, but similar to that found by Mabuchi (20) for starfish egg myosin prepared by a different method. Both urchin egg and rabbit myosins have very low activity in 80 mM KCl, but differ in that rabbit myosin is activated 20 times at the actin/myosin ratio of 1:0.3 used in gel contraction, while urchin egg myosin activity is only doubled. These myosin ATPase activities in the presence of actin are reflected in the relative effectiveness of these myosins in gel contraction: a gel containing urchin egg myosin begins contraction at ~25 min and requires ~50 min for completion; if rabbit myosin is substituted at the same ratio, rapid contraction begins as the solution warms to room temperature and is complete in 3 min. The higher activity of rabbit myosin allows it to be substituted for urchin myosin in reduced amounts (ratios of 1:0.1) without major effects on contraction. The much greater ATPase activity of the rabbit myosin also requires an increase in ATP from 0.1 to 0.5 mM to prevent ATP depletion and incomplete contraction. The lower ATPase activity of sea urchin myosin is evident in the lower concentration (100–10 μM) at which ATP becomes limiting, and the extent of contraction, as measured by final volume, depends on ATP level.

These correlations of contraction rate and ATPase activity are not seen when the contraction rate of gels containing urchin egg myosin is modified by changes in the ionic conditions of the medium. Increases in KCl concentration from 60 to 80 mM and 80 to 100 mM each cause a reduction in gel contraction time of approximately one-half, but the measured actin-activated ATPase activities (Table V) show no such trend with changes in KCl concentration over this range. Measurement of the ATPase activity in the complete system during a normal gel contraction at 80 mM KCl and 0.1 mM ATP shows that the presence of the gel proteins in addition to actin and myosin causes a reduction of the already low

ATPase activity, and the inclusion of 1 mM $MgCl_2$, which accelerates contraction rate, causes no change in measured ATPase activity.

DISCUSSION

The minimum requirements for the formation of a contractile gel are actin filaments, protein(s) to cross-link these filaments into a three-dimensional network, and myosin to provide the force for contraction. In the sea urchin egg two proteins of 58,000 (fascin) and 220,000 mol wt are involved in actin gel formation (13, 14). Gels are formed on the combination of these proteins with actin at low (50–100 mM) KCl concentration; myosin can be added directly to such a gel by combining the proteins at a salt concentration at which they are souble and then diluting the solution so that the interaction of all proteins occurs simultaneously as the KCl level drops. Sufficient ATP must be present to prevent the immediate association of actin and myosin during dilution.

After being diluted to low salt conditions, the solution of combined proteins at 0°C has little or no visible structure at
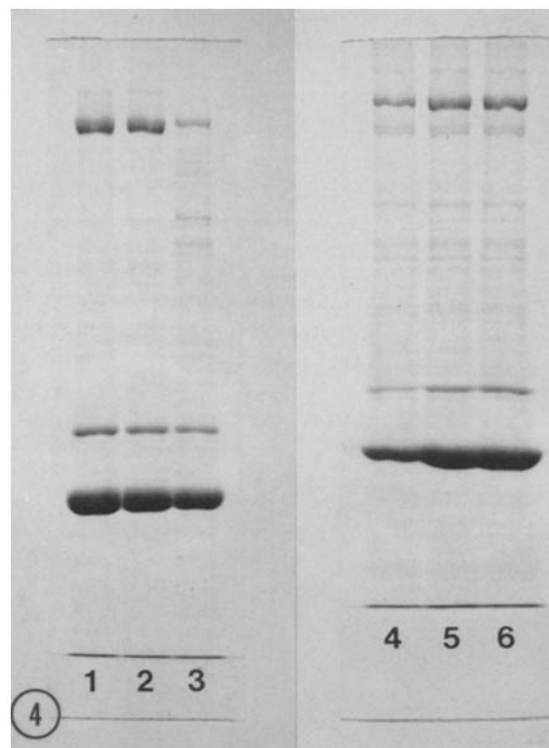

FIGURE 4   SDS PAGE. Combination of 1:0.3:0.6 of actin/myosin/gel proteins. Lanes *1–3*, 20,000 *g* pellets at 60, 80, and 100 mM KCl. Lanes *4–6*, 150,000 *g* pellets at 60, 80, and 100 mM KCl. All in 0.1 mM ATP.

TABLE III

Effect of KCl Concentration on Sedimentability

Composition						
Actin/Myosin/Fascin–220,000-mol-wt protein	Solution	Actin	Protein	20,000 g pellet	150,000 g pellet	150,000 g supernatant
			$\mu g/ml$			
1.0:0.3:0.6	60 mM KCl, 0.1 mM ATP	220	418	352	51	31
1.0:0.3:0.6	80 mM KCl, 0.1 mM ATP	220	418	311	72	35
1.0:0.3:0.6	100 mM KCl, 0.1 mM ATP	220	418	249	106	46

Table IV
Comparison of Sea Urchin Egg and Rabbit Muscle Myosin

Myosin	ATPase activity			
	K^+-EDTA	80 mM KCl 0 Actin	80 mM KCl actin:myosin, 1:0.3	Activation
	mmol P_i/min per mg myosin			
Sea urchin egg	259	7	12	2 ×
Rabbit muscle	892	17	348	20 ×

Table V
ATPase Activity of Sea Urchin Myosin

KCl	ATP	Protein ratio (Actin/Myosin/ 58,000- and 220,000- wt protein)	ATPase
			nmol/min per mg myosin
60	1	1:0.3:0	10
80	1	1:0.3:0	12
100	1	1:0.3:0	8
80	0.1	1:0.3:0.6	6
80	0.1, 1 mM Mg^{++}	1:0.3:0.6	6

the light microscope level, although actin is combined with the other proteins in a form that is sedimentable at 20,000 g. On transfer to 24°C a faint gel network becomes visible, similar in appearance to that formed by a combination of the urchin gel proteins with actin (14). The filaments of this network then increase in density, presumably by the transfer of F-actin from a less organized state to alignment along existing network elements, a process that requires the presence of myosin. At the low ATP levels necessary to prevent actomyosin aggregation during assembly (~10 μM), these networks are stable, but with increasing ATP, some network elements shorten while others are stretched and broken, determined by their angular distribution in local areas. Gels containing sea urchin egg myosin retract from the walls of the container and contract to a volume of a few percent of the orginal in <1 h if the ATP concentration is ~100 μM. The final volume is proportional to ATP leve in the range of 10-100 μM.

Gels in which rabbit muscle myosin has been substituted for urchin egg myosin contract at a much more rapid rate that correlates with the higher ATPase activity of the rabbit myosin, analogous to the correlation of speed of contraction with ATPase activity in a variety of vertebrate and invertebrate muscles (1). There is now convincing evidence that the ATPase activity of cytoplasmic and smooth muscle myosin in vertebrates is controlled by the reversible phosphorylation of a myosin light chain (25), and a similar mechanism has been demonstrated in echinoderm smooth muscle (18). The low values of the actin-activated ATPase activity of the sea urchin egg myosin measured here are characteristic of unphosphorylated cytoplasmic myosin, suggesting that an increase in ATPase activity by phosphorylation may also serve to regulate the activity of echinoderm cytoplasmic myosin.

Contraction of these reconstituted gels is accelerated by changes in KCl and MgCl₂ concentration without a proportional increases in ATPase activity. The contraction of an extract of sea urchin egg soluble cytoplasmic proteins (15) and of stable extract gels induced to contract by resuspension (16) is similarly accelerated by K^+ and Mg^{++}, and in these cases acceleration of contraction is accompanied by the reduction or almost total elimination of fascin from the contracted material. These observations would support a coupling of gel solation with contraction (12) as the interaction of actin with myosin in contraction is accompanied by the release of an actin cross-linking protein, and the ionic conditions in the medium thus might influence contraction through an effect on this release of fascin from the gel. However, this release of fascin is not an obligatory concomitant of contraction, as it does not occur in the reconstituted contractile system, and the loss of fascin that occurs during the contraction of the more complex gels formed in extract may involve additional and as yet unidentified cytoplasmic factors not present in the simplified reconstituted system.

Increasing ATP to millimolar levels slows the formation of gel, altering the relative rates to gelation and contraction and producing more variable results. A transformation from a loose flocculent precipitate to a more condensed granular form, similar to the process of superprecipitation of actomyosin solutions first described by Szent-Gyorgi (29), occurs if contraction begins before the gel network is completed. In the complete absence of the gel proteins, a ratio of myosin to actin of ≤0.6 results in the formation of a loosely aggregated precipitate that, if undisturbed, will contract to a small volume. At these higher ratios to actin, myosin serves to both link the actin filaments and provide the force for contraction. Actin cross-linking has also been demonstrated with heavy meromyosin, gelation resulting from the binding of the two heads of heavy meromyosin to different actin filaments (30). In actomyosin threads formed at high actin concentrations (4 mg/ml), the additional actin cross-linking provided by macrophage actin-binding protein has been shown to reduce the amount of muscle myosin required for contraction (27), and this amplification of contraction by increased actin cross-linking has been considered to argue against the coupling of gel solation with contraction (28). In this interpretation the reduction in actin lattice rigidity during contraction does not involve the reduction of actin–actin-binding protein cross-links, but rather the severing of the actin filaments between these cross-links through the action of the calcium-activated protein gelsolin (31). At the actin concentrations and low ratios of myosin to actin likely to be present in the cytoplasm, additional actin cross-linking, beyond that provided by myosin, will be required for the formation of a contractile gel network. Observations on these reconstituted gels from urchin eggs do not allow one to draw unequivocal conclusions regarding the mechanisms involved in modifying the gel network during contraction. Major changes in protein content do not occur, but the structure of the gel undegoes a reorganization in which the network of fine filaments characteristic of uncontracted gel gradually transforms to one with a small number of denser units, followed by the further thickening and shortening of these elements. The dependence of this process on myosin concentration and ATP level indicates that these visible changes may reflect an increase in myosin-actin links that results in the alignment of the actin filaments to form the thicker units that subsequently shorten as a result of the sliding forces generated.

The stable gel networks formed at low ATP concentrations retain the potential for contraction, which can be induced by small increases in ATP level. These are well within the phys-

iological range and suggested how cytoplasmic gelation and contraction might be regulated by ATP. Even in the simple in vitro system investigated here, the diffusion of ATP into these gels can result in the formation of complex shapes more suggestive of cellular events than the rapid and total contraction of gels assembled in the presence of sufficient ATP for immediate contraction.

The properties of gels assembled from components depend on the ratios of the other proteins to actin, ranging from a dense and noncontractile gel at higher ratios of the gel proteins and low myosin ratios to increasing rates of contraction and more fragile gels as myosin increases and gel proteins decrease. By analogy, variations in the behavior and properties of cytoplasm in different areas and at different times may reflect the local availability of proteins that can interact with actin, determined by protein localization or by factors controlling their interaction. Changes in the mechanical properties of the egg cortex during the cell cycle, for example, may be due to the localization of fascin in the cortex after fertilization (23), where it could contribute to cortical rigidity by binding with actin to form structural units as it does in the microvilli (5, 26). Contraction of the cleavage furrow must result from the interaction of cortical actin with myosin, which has been localized in the furrow region in other cell types (9). It should be noted in this regard that the insolubility of myosin at low salt concentrations that necessitates the preparation of contractile gel by the sequential addition of components is characteristic of sea urchin myosin only after its isolation. Myosin has previously been found to exist in cytoplasmic extracts, and presumably in the cell, in a form that is soluble in very low ionic strength (15). Myosin thus can be present in dispersed form in the cytoplasm and be available to interact directly with actin or actin gel networks in regions where contraction occurs.

A minimal system based on sea urchin actin, gel proteins, and myosin can display a variety of behavior under the influence of changes in the ATP level and in the ratios of the other proteins to actin. The cytoplasm contains a much larger number of proteins capable of interacting with actin along with control systems based on such factors as micromolar calcium concentrations and myosin phosphorylation; these factors must regulate the complex cell shape changes and movements of early embryogenesis.

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