ACTIN POLYMERIZATION AND INTERACTION WITH OTHER PROTEINS IN TEMPERATURE-INDUCED GELATION OF SEA URCHIN EGG EXTRACTS

R. E. KANE

From the Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96822

ABSTRACT

The gel which forms on warming the extracts of the cytoplasmic proteins of sea urchin eggs has been separated into two fractions, one containing F-actin and the other containing two proteins of 58,000 and 220,000 mol wt. When combined in 0.1 M KCl, even at 0°C, these components will form gel material identical to that formed by warming extracts. This gel is a network of laterally aggregated F-actin filaments which are in register and which display a complex cross-banding pattern generated by the presence of the other two proteins. Low concentrations of calcium block the assembly of these proteins to form this complex structure, which may play some cytoskeletal role in the cytoplasm.

This association of F-actin with the other proteins to form a gel is very likely the last step of the process occurring in warmed extracts. At low temperatures, gelation of extracts is limited by the relative absence of F-actin, as demonstrated by the inability to sediment it at 100,000 *g* and also by the fact that gelation occurs immediately if exogenous F-actin is added to cold extracts. The transformation of the G-actin present in the extract to the F-form is apparently repressed at low temperatures. This is shown directly by the failure of added G-actin to polymerize at low temperatures in the presence of extract. These observations resemble those which have been reported on preparations from amoeboid cells and may be significant in the involvement of actin and these other proteins in cell division and later developmental processes.

In a previous publication (10) it was demonstrated that warming an extract of the soluble cytoplasmic proteins of the sea urchin egg caused the formation of a gel in the solution, visible in the light microscope as a net of birefringent threads. The extraction medium contained sufficient glycerol to render it isotonic, EGTA to chelate the cell calcium, and buffer to control the pH during homogenization. Dialysis of the extract to remove all small molecular weight components reduced or eliminated this gelation on warming, but gelation could be restored by the addition of ATP and KCl. Gelation was not temperature reversible, as the gels were stable at 0°C once formed, but the material was soluble in high salt (0.5–1 M) solution after centrifugation from the extract. Subsequent reduction of the salt concentration of this dissolved gel solution to approx. 0.1 M by dilution or dialysis caused the reappearance of the gel, a process which does not require warming and which will occur at 0°C.

Sodium dodecyl sulfate (SDS) gel electrophoresis showed three major components to be present in this dissolved gel: a protein which co-migrated

with actin, a second protein of 58,000 mol wt which could be distinguished from tubulin, and a high molecular weight protein of 220,000 mol wt which did not co-migrate with myosin. The actin was seen to exist as F-actin filaments in negatively-stained preparations of gel dissolved in high salt, and the addition of 1 mM ATP to such solutions caused the actin filaments to form aggregates visible in the light microscope. These aggregates, previously referred to as fibrils (10), will now be termed bundles in conformity with the terminology of Morgan (12) and Spudich and Cooke (20). The bundles were highly birefringent due to the linear aggregation of the actin, but the filaments were not in register, and no indication of order was seen.

The formation of such actin bundles provides a simple method for the separation of the actin from the other two components of the gel; the first aim of this investigation was to determine whether the separated components would recombine to form a gel if mixed under appropriate conditions. If so, the parameters of the reaction can be determined by modification of the experimental conditions during recombination. A second aim was to determine the fine structure of the gel, which is better done using the material formed from the recombination of purified components rather than the more contaminated material formed in whole cell extracts. Finally, clarification of the nature of the reaction and the product in vitro should aid in interpreting the possible role of these components in the cell.

Actin has been identified in a variety of non-muscle cells (17) and in some cases constitutes a significant fraction of the total cell protein (1). It appears to have a role in cell motility, both in the classical case of the amoeba and in amoeboid motion in other cell types (see reference 3 for reviews). F-actin forms the thin (70 Å) filaments seen in amoeba cytoplasm (16), and these filaments have been observed to aggregate into bundles under conditions which induce motility in isolated amoeba cytoplasm (15, 24). These bundles have been isolated from amoeba (12) and are without linear order and appear similar to those which are formed in urchin egg preparations under the conditions described above. Similar bundles are formed by *Dictyostelium* actin under different ionic conditions (20), although additional components may be involved.

The biological significance of the gelation process and of the other two proteins involved is as yet unclear. Gelation has been induced in cytoplasmic extracts of *Acanthamoeba,* using methods similar to those developed in the urchin (14), but the constituent proteins are different and gelation would occur with purified actin in the presence of $MgCl_2$. A high molecular weight protein, termed actin-binding protein, has been found associated with actin in jelled cytoplasmic extracts of mammalian macrophages (7, 22); whether this protein is similar to the high molecular weight material seen in urchin cytoplasmic gels remains undetermined. The 58,000 mol wt protein which forms a major component of the urchin extract gel has not been seen as a significant component in these other cytoplasmic gel systems. A protein of this approximate size which interacts with actin has been observed in the acrosome of the sperm of *Limulus* (25), where it is involved in acrosomal extension via molecular rearrangement. More recently, an extract of a chick embryo fibroblast "skeleton" (prepared by removing most soluble components by Triton treatment) has been shown to contain a protein in this size range in addition to actin (2). Thus, further investigation of the copolymerization of actin and these other proteins in urchin extracts may yield useful information concerning the role of actin in a number of cell functions.

MATERIALS AND METHODS

Extracts of the soluble cytoplasmic proteins of unfertilized eggs of the Hawaiian sea urchin *Tripneustes gratilla* were prepared as described in detail previously (10). The eggs are homogenized in a medium containing 0.9 M glycerol, 5 mM ethyleneglycol-bis-[β-aminoethyl ether]*N,N'*-tetraacetic acid (EGTA) and 0.1 M piperazine-*N-N'*-bis[2-ethane sulfonic acid] (PIPES), pH 6.8, followed by centrifugation at 100,000 *g* to remove cytoplasmic particulates and insoluble material. The supernate is dialyzed against a large volume of 0.01 M PIPES, pH 6.8 for 24 h at 0°C. The only modification that has been made in this procedure since the previous report is the inclusion of 0.1 mM EGTA in the dialysis medium. Extracts are usually dialyzed in the approximate ratio of 20 ml to 1 liter so that the EGTA present in the extract contributes 0.1 mM EGTA to the medium, but the inclusion of a fixed amount of EGTA eliminates the effect of different dialysis volumes and gives more uniform results. Preparation of gels also follows the published procedure (10): 1 mM ATP and 0.01–0.02 M KCl are added to the dialyzed extract, and it is warmed to 35–40°C for 30–60 min. The resulting gel material is centrifuged from the extract and dissolved in high salt solution; in these experiments, 0.5 M KCl has been routineiy used. The volume of salt solution used to dissolve the gel

is normally one-tenth the volume of extract from which the gel originated, and after centrifugation to remove any insoluble material the usual yield is approx. 1.5 ml of solution with a protein concentration of 1–2 mg/ml. 1 mM ATP causes much of the actin present to aggregate as microscopic bundles during storage at 0°C. Separation of the actin by this procedure is the starting point for the experiments to be described here. The complete procedure is given in Scheme I (details in text).

G-actin was prepared by dialyzing an F-actin solution for 2–3 days against a solution containing 5 mM PIPES, 0.2 mM ATP, and $MgCl_2$, pH 6.8, with daily changes of the dialysis medium. After dialysis, the solution was centrifuged at 100,000 *g* for 3 h to sediment any F-actin remaining. 1 mM NaN_3, was added to all protein solutions to be held in storage.

Photomicrographs were made with a Zeiss universal microscope, using Zeiss phase contrast, interference contrast, and polarization optics. For electron microscopy, protein preparations were negatively stained with 1% uranyl acetate and examined and photographed in a Philips 201 electron microscope operated at 60 kV.

SDS-acrylamide gel electrophoresis was done on 5% gels, using the methods of Weber and Osborn (27). Protein determinations were made by the method of Lowry et al. (11), using a serum albumin standard.

ATP, GTP, all buffers, and protein molecular weight standards were obtained from Sigma Chemical Co., St. Louis, Mo. Rabbit muscle actin, sea cucumber (*Holothuria atra*) myosin, and beef brain tubulin were the gifts of Dr. Richard Himes of the Department of Biochemistry, University of Kansas, Lawrence, Kansas.

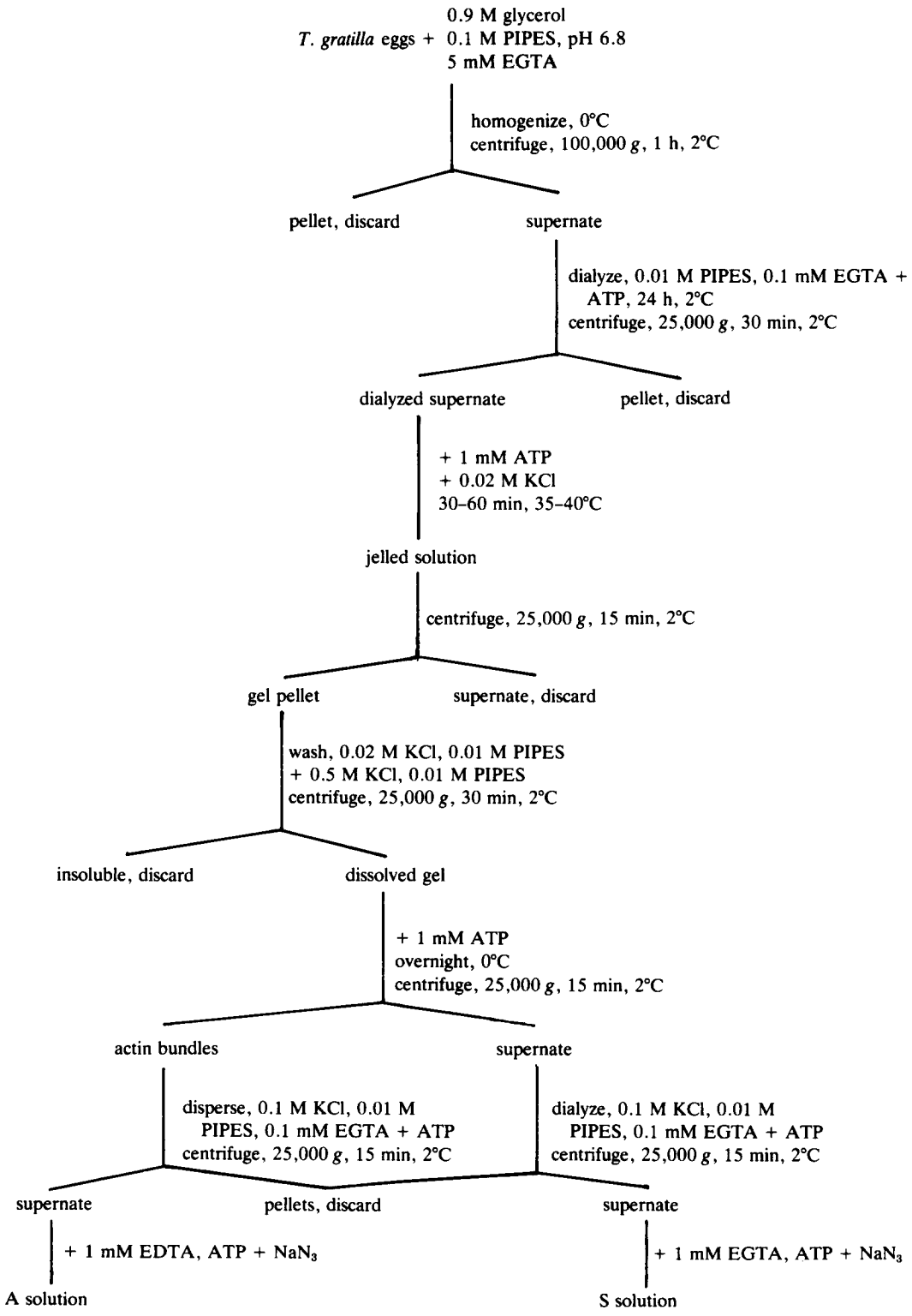
RESULTS

Separation and Recombination of the Gel Components

Adding 1 mM ATP to the gel material dissolved in high salt solution causes a slow appearance of microscopic bundles of F-actin filaments in the solution. These aggregates form over a period of several hours, and the solution is usually held at 0°C overnight before further processing. Centrifugation at 25,000 *g* for 15 min sediments these actin bundles; the supernate contains the other proteins of the original gel polymer plus some residual actin. After sedimenting the actin bundles, most of the actin remaining in the supernate is removed by dialysis against 0.1 M KCl, 0.01 M PIPES, pH 6.8, containing 0.1 mM ATP and EGTA. The actin recombines with the other proteins in the supernate to form a gel at this ionic strength, and after several hours of dialysis this gel is removed by centrifugation at 25,000 *g* for 15 min and discarded. While the salt concentration of the supernate is being reduced to 0.1 M in this manner, the pellet of actin bundles is dispersed in the same 0.1 M KCl solution by gentle homogenization, and any insoluble material is removed by the same centrifugation procedure. The F-actin is stable at this ionic strength, but will not aggregate into bundles below 0.25–0.3 M salt. The final products are an F-actin solution (A) and a supernatant solution (S), both in 0.1 M KCl, whose compositions by SDS-gel electrophoresis are shown in Fig. 1 *a–c*. Actin is the only significant component of the A solution, while the S solution contains major proteins of 58,000 and 220,000 mol wt, along with some unreactive actin which will not sediment at 100,000 *g* and remains as G-actin through the procedure. This is confirmed by electron microscopy, as the A solution consists of F-actin filaments, while the S solution contains only globular material.

This procedure separates the F-actin from the other protein components of the gel, and it is then possible to determine whether gel material will reassemble spontaneously on their recombination. Considerable variability occurred in early experiments, due to the fact that the assembly of the recombined components to form a gel is blocked by low concentrations of calcium, as is gelation in cell extracts (10). EGTA was then maintained at 0.1 mM through the preparative procedure and was added to the final A and S solutions at 1 mM. Under these conditions, material similar in microscope appearance to that formed on warming the extracts appears a few minutes after combining the A and S solutions at room temperature (24°C) or at 0°C (Fig. 2). The preparative procedure results in solutions with protein concentrations of the order of 1 mg/ml, with the concentration of the A solution approximately twice that of the S; a mixture of A to S in the ratio of 1:2 is most effective for gelation. If 1 mM sodium azide is added to retard bacterial growth, the solutions are stable indefinitely at 0°C and can be combined to form a gel after weeks of storage. As might be expected from the composition of the protein solutions used, the gel formed by recombination has the same components in the same ratio as the original extract gel (Fig. 1 *d*).

The recombination of these components to form gel is blocked by free calcium concentrations in the range of $5–10 \times 10^{-7}$ M, as determined through the use of calcium-EGTA mixtures. Gel material formed by recombination will also disintegrate and disappear if the calcium concentration


0.9 M glycerol
T. gratilla eggs + 0.1 M PIPES, pH 6.8
5 mM EGTA
homogenize, 0°C
centrifuge, 100,000 g, 1 h, 2°C
pellet, discard
supernate
dialyze, 0.01 M PIPES, 0.1 mM EGTA + ATP, 24 h, 2°C
centrifuge, 25,000 g, 30 min, 2°C
dialyzed supernate
pellet, discard
+ 1 mM ATP
+ 0.02 M KCl
30–60 min, 35–40°C
jelled solution
centrifuge, 25,000 g, 15 min, 2°C
gel pellet
supernate, discard
wash, 0.02 M KCl, 0.01 M PIPES
+ 0.5 M KCl, 0.01 M PIPES
centrifuge, 25,000 g, 30 min, 2°C
insoluble, discard
dissolved gel
+ 1 mM ATP
overnight, 0°C
centrifuge, 25,000 g, 15 min, 2°C
actin bundles
supernate
disperse, 0.1 M KCl, 0.01 M PIPES, 0.1 mM EGTA + ATP
centrifuge, 25,000 g, 15 min, 2°C
dialyze, 0.1 M KCl, 0.01 M PIPES, 0.1 mM EGTA + ATP
centrifuge, 25,000 g, 15 min, 2°C
supernate
pellets, discard
supernate
+ 1 mM EDTA, ATP + NaN3
+ 1 mM EGTA, ATP + NaN3
A solution
S solution

SCHEME I

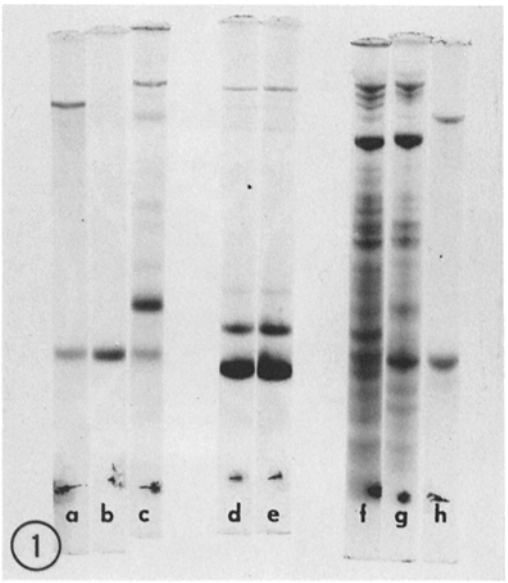


FIGURE 1 SDS-polyacrylamide gel electrophoresis. (*a*) Standard containing rabbit muscle actin and sea cucumber myosin; (*b*) isolated actin of A solution; (*c*) supernatant solution S; (*d*) gel formed by recombination of components after 30 min at 0°C; (*e*) gel formed by recombination of components after 7 h at 24°C; (*f*) 100,000 *g* pellet from extract centrifuged at 0°C; (*g*) 100,000 *g* pellet from depleted extract centrifuged at 30°C; and (*h*) actin and myosin standard for *f* and *g*.

of the solution is raised to this value. The gelation of dialyzed cell extracts was found previously to be blocked at 0.1 mM calcium (10); the use of calcium-buffered solutions showed that the minimum calcium concentration for the prevention of gelation in extracts is also $5\text{–}10 \times 10^{-7}$ M.

Negative staining of the gel material which appears after mixing A and S solutions demonstrates that the gel is formed of linear aggregates of F-actin filaments (Fig. 3). A faint and variable crossbanding is visible, but these aggregates display little evidence of ordered structure. However, gel samples negatively stained after several hours at 24°C or 1 day or more at 0°C appear very regularly crossbanded in the electron microscope (Fig. 4), although no change in appearance occurs at the light microscope level. As in the case of actin paracrystals (8), there is an alternation of regions where the actin filaments appear either narrow and sharp or wider and more fuzzy, depending on whether the constituent helical strands overlap or are seen side by side. The pattern in the gel material is more complex, however, in that there appears to be an area of increased thickness or density near the center of the narrow region of the actin filaments, and the region where the filaments are less sharp appears to be subdivided into two bands (Fig. 5). The repeat period is approx. 365 Å, and the apparent center-to-center spacing between the filaments in these arrays is also very regular and is approx. 80 Å. The change from a less ordered to a more ordered structure is not accompanied by any change in the protein composition of the gel (Fig. 1 *d* and *e*), indicating that a reorganization of existing molecules is involved and not the addition of new units from the surrounding medium.

Actin Polymerization as the Basis of Extract Gelation

The demonstration that separated protein fractions rapidly assemble to form a visible gel on recombination at 0°C indicates that the gelation of dialyzed extracts on warming must proceed by a different and possibly more complex mechanism. No gel forms in extracts after extended storage at 0°C in the presence of ATP and KCl, and such extracts take several hours to form a gel at room temperature; only at 35°–40°C does visible gel material appear in a short time. If components of either the A or S fraction of the gel are limiting in the extract, this can be determined by adding each of these two fractions separately to the extract at 0°C. Since the salt concentration of the dialyzed extract is very low and that of the protein fractions is 0.1 M, a 1:1 mixture will have a salt concentration of 0.05 M, which should allow gelation. No effect is seen on mixing the S fraction with extract at 0°C, but mixing the A fraction with the extract causes the immediate formation of large quantities of gel. Thus the unavailability of F-actin apparently prevents the gelation in the dialyzed extracts at 0°C.

F-actin could be rendered unavailable for gelation in the extract by a variety of mechanisms, the simplest being that the actin in the extract is in the form of G-actin and is transformed to F-actin on warming, followed by its combination with the other components to form gel material visible in the light microscope. This possibility can be investigated by centrifugation of the extract at sufficient force to sediment any F-actin present. The pellet resulting from centrifugation of dialyzed extract for 3 h at 100,000 *g* at 0°C contains relatively little actin (Fig. 1 *f*), and the extract gels normally after such centrifugation. Thus, the presence of actin in

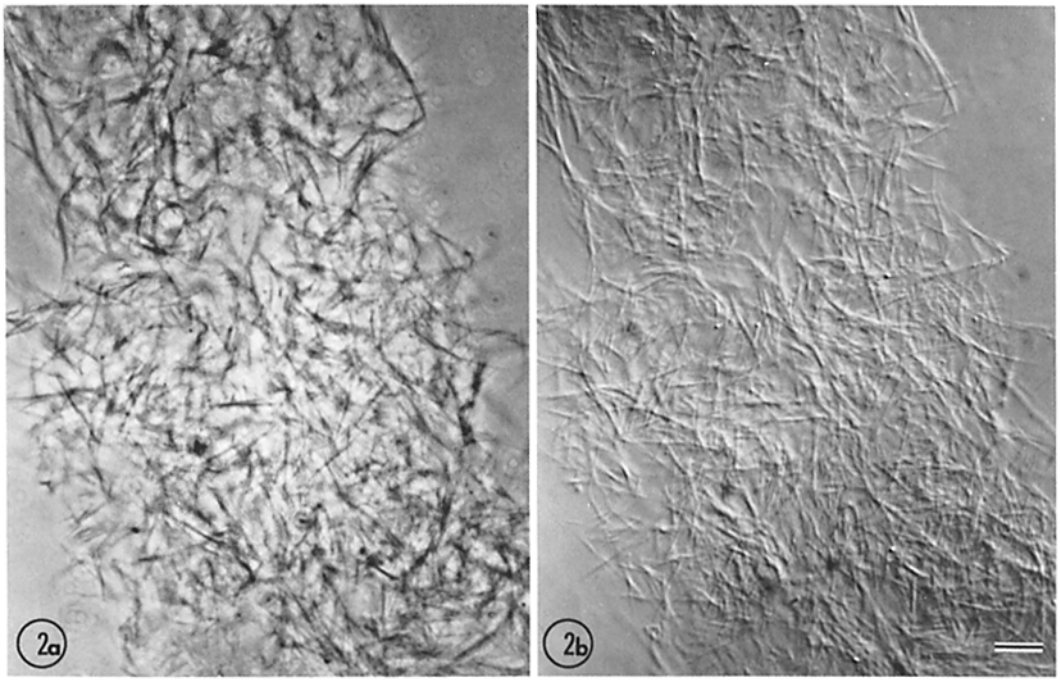


FIGURE 2 Photomicrographs of gels formed by recombination of components in 0.1 M KCl at 24°C. (*a*) Phase contrast; and (*b*) interference contrast. Both × 600. Reference mark = 10 μm.

the G form with a G–F transformation during warming appears to be a possible mechanism for gelation in extracts.

Such a G–F transformation is difficult to demonstrate independently of gelation in extracts, for any F-actin formed during warming combines immediately with the other components to form a gel. However, the amount of actin present in the extract is in excess of that which can combine with the 58,000 and 220,000 mol wt components, as can be shown by the following experimental sequence. A dialyzed extract prepared by the usual methods is warmed in the presence of ATP and KCl, and the resulting gel is removed. The supernatant solution after gel removal, termed a depleted extract, is returned to 0°C, and 1 mM ATP is added. This depleted extract forms no gel on mixing with F-actin solution, demonstrating that one or more of the S-fraction components has been removed by gelation. This extract will also not respond to the addition of S fraction at 0°C, indicating that no F-actin is available for combination. However, if such extract is mixed with S fraction and raised to 35°–40°C for 30–60 min, a small amount of typical gel material appears in the solution. This suggests that some G-actin remains in the depleted extract after gelation and that this G-actin is polymerized to F-actin on warming and can then combine with the added S-fraction components. This can be shown more directly by adding S fraction to aliquots of the depleted extract over a warming and cooling cycle. Addition of S fraction to depleted extract does not result in gel material until after 30–60 min on warming, demonstrating that the G–F transformation requires the same time period as in the original extract; and, in addition, gelation on adding S fraction declines after cooling and disappears after several hours at 0°C. The G–F transformation is thus not only stimulated by warming, but reverses on cooling, so that the actin of extracts stored at 0°C is always in the form of G-actin.

This transformation of G- to F-actin in depleted extracts on warming can also be demonstrated by warming such an extract to 35°–40°C for 30 min to polymerize the actin and then centrifuging at 100,000 *g* for 3 h at 25°–30°C. The actin remains polymerized at this temperature, and a significant actin band is seen in the pellet (Fig. 1*g* and *h*). The polymerization of actin in a regular extract can be shown by utilizing the calcium blockage of gelation. 0.1 mM calcium is added to an extract,

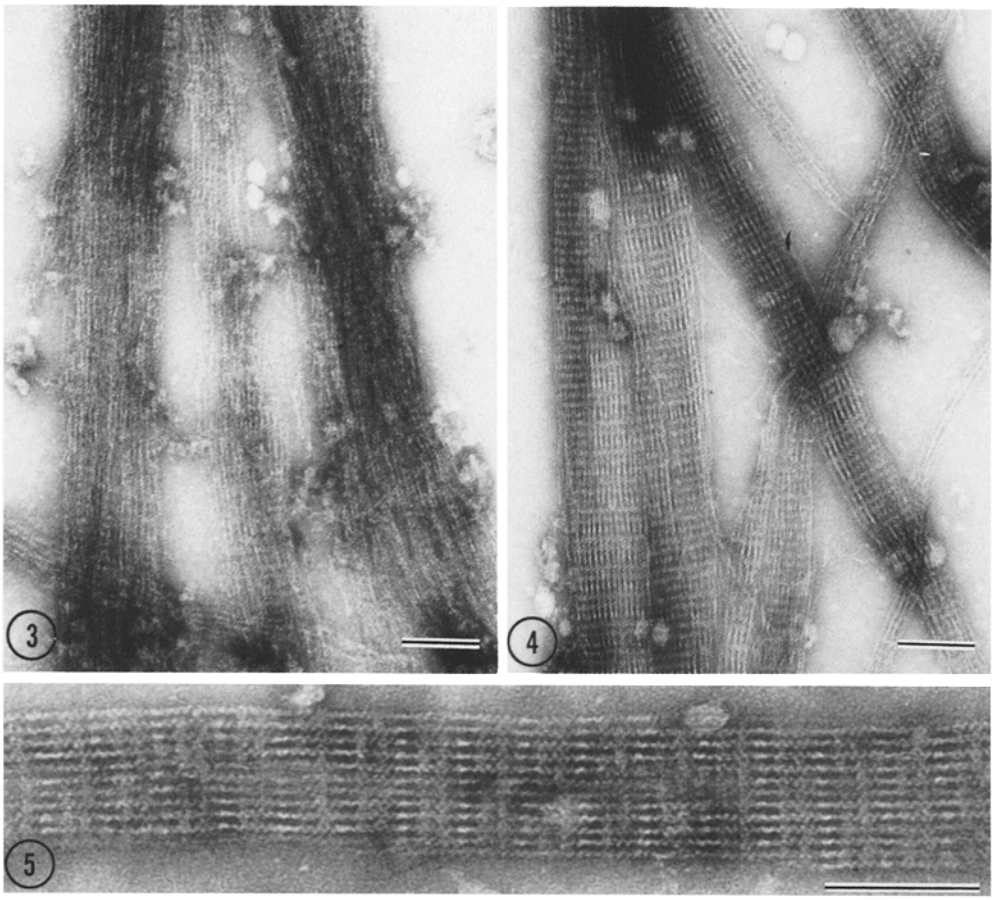


FIGURE 3 Gel material formed by recombination of components, negatively stained after 1 h at 24°C. × 102,500. Reference mark = 0.1 μm.

FIGURE 4 Gel material formed by recombination of components, negatively stained after 24 h at 0°C. × 102,500. Reference mark = 0.1 μm.

FIGURE 5 Same material as Fig. 4 × 205,000. Reference mark = 0.1 μm.

and the extract is warmed and centrifuged at 100,000 *g* for 3 h at 25°–30°C. No gel forms in the presence of calcium, but actin polymerizes to the F form and is present in the pellet.

It has been reported that cytoplasmic actin, unlike muscle actin, displays a marked temperature dependence of polymerization in KCl, which is abolished in the presence of 2 mM $MgCl_2$ (5). The addition of 2 mM $MgCl_2$ to egg extracts causes the rapid appearance of a structureless precipitate, which contains actin but does not resemble the gel formed on warming.

Experiments with G-actin

To investigate the behavior of G-actin in this system, samples of F-actin prepared as described previously were depolymerized by dialysis. Samples of approx. 1 ml were dialyzed against 200 ml of 5 mM PIPES, pH 6.8, 0.2 mM ATP and $MgCl_2$ at 0°C for 3 days, with daily changes of the dialysis medium. After completion of dialysis, the solution was centrifuged at 100,000 *g* for 3 h; no F-actin was visible by negative staining of the resulting solution (Fig. 6). The G-actin polymerizes rapidly at 24°C to F-actin in the presence of 1 mM ATP and 0.05–0.1 M KCl (Fig. 7); this F-actin behaves indistinguishably from the original F-actin with regard to gelation on mixing with S fraction or extract. Polymerization of the G-actin also occurs in the presence of ATP and millimolar magnesium or calcium.

More directly related to conditions in the ex-

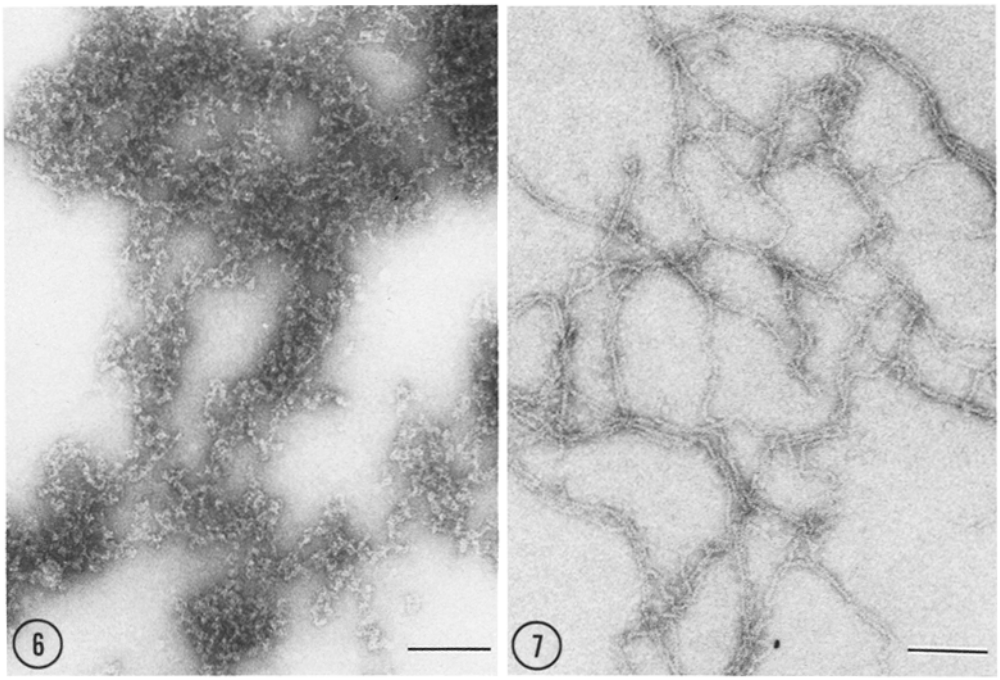


FIGURE 6 Depolymerized actin prepared by dialysis. × 102,500. Reference mark = 0.1 μm.

FIGURE 7 Same material as Fig. 6, polymerized by the addition of 0.1 M KCl. 1 h, 24°C. × 102,500. Reference mark = 0.1 μm.

tract is the interaction of G-actin with the components of the S fraction. If G-actin in low salt solution is mixed with S fraction containing sufficient KCl to provide a final concentration of 0.05–0.1 M and if ATP is maintained at 1 mM, a gel appears in approx. 30 min at 24°C. The time for gelation is approximately halved in the presence of 1 mM $MgCl_2$, which presumably acts to accelerate the G- to F-actin transformation which must be the first step of this reaction. Polymerization occurs more slowly at 0°C, visible gel material appearing approx. 2 h after combining the components. The reaction time at 0°C is also shortened in the presence of magnesium, in agreement with the reported effect (5) of magnesium on the temperature dependence of the polymerization of cytoplasmic actin. In all cases, the fine structure of the gel is identical to that of the gel formed directly from F-actin and undergoes similar changes with time.

The observation that dialyzed extracts, which the evidence indicates contain G-actin plus the 58,000 and 220,000 mol wt components, never form gel during extended storage at 0°C indicates that the transformation of G- to F-actin is inhibited in such preparations, and it is this inhibition which is apparently overcome by warming. This effect of extract on actin polymerization can be demonstrated directly by combining G-actin and extract. A 1:1 mixture of dialyzed extract containing KCl and ATP with G-actin solution increases the actin concentration of the extract approximately five times over its usual value, yet such preparations often form no gel at 0°C or form only a small amount after periods of 24 h or more. Since extract will react immediately with F-actin, the rate of actin polymerization in such mixtures must be greatly reduced. A measure of the effect of extract on actin polymerization is provided by the observation that a mixture of G-actin and S components forms a gel in 2 h or less under similar conditions.

DISCUSSION

In the first experiment of this sequence, gel material appeared in an urchin egg extract of unknown composition, by an unknown mechanism which required warming to 35°–40°C, a temperature considerably above physiological for the sea urchin. Rather than attempting to determine the

nature of the reaction occurring under such complex circumstances, it seemed more reasonable to separate the gel from the rest of the extract, determine its composition, and then investigate whether the reaction which occurs in the extract can be duplicated in a system containing only these components. The information obtained in this manner can then be utilized to analyze the reaction in the extract and ultimately that in the cell.

The fortuitous discovery that the addition of ATP to gel material dissolved in high salt solution causes the F-actin present to aggregate into microscopical bundles provides a simple method for the separation of the actin from the other two proteins which make up the gel. This bundle-forming process is of intrinsic interest because of its apparent similarity to the formation of actin bundles or fibrils in the cytoplasm of amoeboid cells (15, 16), but in the present experiments it is of value primarily as a method of preparing actin. The two fractions which result, one containing F-actin and the other the 58,000 and 220,000 mol wt components, are stable indefinitely in 0.1 M KCl, but on combination, even at 0°C, form gel material identical to that formed in warmed extracts. This reaction is spontaneous on the recombination of F-actin with the other gel components and does not require warming or the participation of any other component of the extract.

In the light microscope the first material to appear after the recombination of the components consists of fine threads or fibrils at the limit of resolution; these rapidly associate to form a gel network. Electron microscopy demonstrates that these threads are formed by the side-by-side aggregation of F-actin filaments, but this process differs from actin bundle formation in two significant ways. These multicomponent fibrils formed on mixing the two fractions adhere randomly to each other, giving rise to the gel network, and they also gradually develop a highly ordered structure evident from the banding pattern; neither of these processes occurs during bundle formation. The banding pattern is at first irregular and varies in different areas of the gel, but in time it becomes uniform and regular throughout all the material. The evidence indicates that this takes place by the reorganization of existing material as the protein composition of the gel does not change during this process.

The axial periodicity of this material indicates that the F-actin filaments are ordered as they are in actin paracrystals, but the more complex banding pattern must be due to the other proteins present. In related observations (9), the cross striations of actin paracrystals have been attributed to the presence of tropomyosin and troponin. EM observation allows only descriptions of the patterns; the molecular basis of the pattern can be elucidated by image analysis, including optical diffraction which has proven successful in studies of paracrystals of F-actin-tropomyosin-troponin (4, 13, 21, 26). Such a study is presently under way by D. DeRosier, E. Mandelkow, A. Silliman, L. G. Tilney, and R. E. Kane. This study is being done parallel with those on the material of the *Limulus* sperm acrosome described by Tilney (25). This latter material contains actin and a protein of 55,000 mol wt in approximately equimolar ratio and has a pattern different from that observed in the sea urchin gel material.

It may be significant that the calcium concentration which controls the assembly of these components into a complex structure is in the same range as that which induces contraction in amoeba cytoplasm preparations (23). The report (2) that the skeleton remaining after extraction of vertebrate cells with Triton has a protein composition similar to that of the urchin gel material suggests that these proteins may be involved in such a cytoskeleton. If this is the case, the induction of contraction might require a modification of these structural units, and it appears that both can be effected by calcium concentrations in the physiological range.

The formation of gel material through the combination of F-actin with the other protein components is very likely the last step of the reaction which takes place on warming the extracts, as shown by the immediate gelation which occurs on adding F-actin to such extracts. It is the lack of F-actin in extracts, demonstrated by the absence of actin from the 100,000 *g* pellets, which prevents their gelation at 0°C. When these extracts are warmed, G-actin is transformed to F, slowly at 24°C and more rapidly at 35°–40°C, resulting in the formation of gel. Thus, is appears that the G–F transformation is inhibited at low temperatures and that warming causes gelation by overcoming this inhibition. Although a temperature dependence of polymerization of cytoplasmic actin has been reported (5), the inhibition of polymerization in extracts appears to be due to more than this, as seen by comparing the results of mixing (at 0°C) G-actin with S solution and with extract. In

the former case, gel forms in a few hours due to the transformation of G- to F-actin, while in the latter case gel may appear in a day or more, if at all. The experiments with depleted extracts demonstrate additionally that if the F-actin formed on warming is not "trapped" into a temperature-insensitive gel, it reverts to G-actin on cooling to 0°C, indicating that the process is not merely the heat denaturation of an inhibitor of polymerization.

Related observations on the effects of temperature have been made in a number of other biological systems. In the pioneering work of Thompson and Wolpert (24) on isolated amoeba cytoplasm, motility was induced only when preparations cooled to 4°C were warmed to room temperature in the presence of ATP, and this motility was correlated with the appearance of fibrillar material. Pollard and Ito (15) observed that the induction of motility on warming such preparations was associated with the appearance of 70 Å filaments which then aggregated to form microscopical bundles. These 70 Å filaments were identified as F-actin by the addition of muscle heavy meromyosin (16) and must arise via a G–F transformation in the cytoplasm. A similar dependence of actin polymerization on temperature has been observed in mammalian macrophages (7, 22). These results indicate that G-actin is present at low temperatures and is transformed reversibly to F-actin on warming in a variety of cell types. This temperature effect is useful in the study of actin polymerization in vitro, but the nature of the intracellular process involved in controlling the state of actin remains unknown.

The participation of actin in cellular structure and motility may be controlled at another level by calcium, which these experiments have shown to influence the assembly of F-actin with other proteins to form possible cytoskeletal units and which will also induce cytoplasmic contraction (23). The cell divisions and later developmental stages of the urchin embryo must involve control mechanisms of this kind. Studies of the cleavage furrow of the sea urchin egg (19) have shown that changes in the form and distribution of fibrous material may occur over relatively short periods of the cell cycle. Amoeboid motion plays a major role in the cell movements at gastrulation of the sea urchin embryo (6), and experimental procedures which induce amoeboid movement in uncleaved marine eggs have been reported (18). The balance between pools of unpolymerized and polymerized actin and its combination with other proteins will presumably vary with division cycles and stage of development; these differences may be detectable using the extraction methods described here. This may provide a useful approach to the study of the role of these proteins in cell division and development.

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