

# ACTIN IN *XENOPUS* OOCYTES

## I. Polymerization and Gelation in vitro

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### ABSTRACT

It has been found that a high-speed supernatant fraction from *Xenopus* oocytes extracted in the cold will form a clear, solid gel upon warming. Gel formation occurs within 60 min at 18°–40°C, and is, at least initially, temperature reversible. Gelation is strictly dependent upon the addition of sucrose to the extraction medium. When isolated in the presence of ATP, the gel consists principally of a 43,000-dalton protein which co-migrates with *Xenopus* skeletal muscle actin on SDS-polyacrylamide gels, and a prominent high molecular weight component of approx. 250,000 daltons. At least two minor components of intermediate molecular weight are also found associated with the gel in variable quantities. Actin has been identified as the major constituent of the gel by ultrastructural and immunological techniques, and comprises roughly 47% of protein in the complex. With time, the gel spontaneously contracts to form a small dense aggregate. Contraction requires ATP. In the absence of exogenous ATP, a polypeptide which co-migrates with the heavy chain of *Xenopus* skeletal muscle myosin becomes a prominent component of the gel. This polypeptide is virtually absent from gels which have contracted in ATP-containing extracts. It has also been found that Ca<sup>++</sup> is required for gelation in oocyte extracts. At both low and high concentrations of Ca<sup>++</sup> (defined as a ratio of Ca<sup>++</sup>/EGTA in the extraction medium), gelation is inhibited.

KEY WORDS actin · gelation · contraction · calcium · *Xenopus* · oocytes

Variations in cytoplasmic consistency often accompany important cellular events. For example, during fertilization and cleavage, eggs of several marine invertebrates undergo distinct changes in consistency (9). Similarly, consistency changes are known to occur during the hormonally induced meiotic maturation of amphibian oocytes (21). Our interest in oocyte maturation in *Xenopus laevis* has led us to seek a molecular and functional understanding of these cytoplasmic changes.

The dynamic structural properties of the cytoplasm are evident in a number of other cell types, as well. In particular, amoebae and slime molds undergo reversible sol-gel transformations in localized regions of the cytoplasm during movement (for review, see references 1 and 14). Motile extracts of these cells exhibit viscosity changes in vitro (23, 33, 35, 37) and have been used to advantage in studying structural transformations at a molecular level. Experimental evidence linking consistency changes in vitro with the assembly and interaction of contractile protein filaments (11, 23, 24, 33, 34) has suggested that the

dynamic organizations of these proteins may regulate the structural properties of the cytoplasm. We have therefore begun to investigate the possible involvement of contractile proteins in consistent changes in *Xenopus* oocytes.

Recent studies on the gelation of crude cell-free extracts (6, 12, 25, 30, 36) have suggested a means of approaching this question. Gel formation *in vitro* has been described for a number of different cell types and involves the temperature-dependent polymerization and cross-linking of actin filaments. The gelation of crude extracts would appear to mimic at least some of the known structural properties of the cytoplasm. It was felt that this approach might provide a useful method for identifying contractile proteins and for studying the factors which might control their interaction in *Xenopus* oocytes.

Using methods analogous to those devised by Kane (12) for sea urchin eggs, we have been able to induce the gelation of actin in cell-free extracts of *Xenopus* oocytes. Analysis of the gelled material has indicated that it is composed principally of F-actin and a polypeptide of approx. 250,000 daltons. In this paper, we explore the conditions for the extraction and gelation of these and accompanying proteins. We also describe the effects of  $Ca^{++}$  and ATP on the gelation phenomenon and on a contraction or shrinkage of the cross-linked gel.

Despite the demonstration in this paper that oocyte actin is able to polymerize *in vitro*, in the accompanying report (22) we show that most of the oocyte actin is not polymerized *in vivo*. A preliminary report of the work presented here has appeared (5).

## MATERIALS AND METHODS

### *Isolation of Xenopus Oocytes*

Female *Xenopus laevis* were obtained from the South African Snake Farm (Fishoek, South Africa). Mature animals were sacrificed, and the ovaries, containing oocytes in all stages of development, were excised and placed in amphibian Ringer's solution. Ovaries were cut into small pieces and treated with collagenase at a concentration of 0.2% (wt/vol) in 0.1 M phosphate buffer, according to Schorderet-Slatkine and Drury (26). When the ovarian connective tissue matrix had broken down, follicles released into the medium were collected and washed in several changes of Ringer's solution. Oocytes were then allowed to fall through a  $1.8 \times 100$ -cm column of Ringer's solution. With this procedure, oocytes in different stages of growth could be separated

from one another. In these studies, only the largest (stages 5 and 6) oocytes were used (7).

### *Preparation of Cell-Free Extracts*

Oocytes were washed quickly in two changes of distilled water, and aliquots containing 2.5 ml of loosely packed cells were transferred to 15-ml Sorvall centrifuge tubes (DuPont Instruments-Sorvall, DuPont Co., Wilmington, Del.). Cells were washed once in 10 ml of ice-cold homogenization buffer containing 0.9 M sucrose, 10 mM dithiothreitol (DTT), 1 mM ATP, 1 mM EDTA, and 20 mM Tris-HCl (pH 7.2). (ATP, DTT, and EDTA are not essential for gelation.) Oocytes were then compressed into a tight pellet by centrifugation at 1,000 g for 5-10 min, at 0°-4°C, in a Sorvall HB-4 rotor. After pipetting off as much of the supernate as possible, 4.8 ml of cold homogenization buffer was added to the cell pellet. Oocytes were then homogenized in the cold in a loose-fitting Dounce Teflon homogenizer (Kontes Co., Vineland, N. J.). When all of the cells had broken, the homogenate was centrifuged at 20,000 g for 15 min, at 2°C. A small hole was cut in the bright-yellow lipid fraction at the top of the extracts, and the postmitochondrial supernate was collected with a Pasteur pipette. The supernate from this preliminary low-speed centrifugation was then recentrifuged at 100,000 g for 60 min, at 2°C, in a Spinco Type 40 rotor (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.). The clear supernate from this spin was collected and the pH readjusted to 7.4 with the slow addition of 0.2 N NaOH. This fraction was designated "the high speed extract."

### *Polyacrylamide Gel Electrophoresis*

Polyacrylamide gel electrophoresis was carried out on sodium dodecyl sulfate (SDS) slab gels as described by Studier (31). The discontinuous gel system of Laemmli (15) was used with modification according to Anderson et al. (2). The percentage of acrylamide used in each case is noted in the text. Protein samples were solubilized before electrophoresis by heating to 100°C for 2 min in sample buffer containing 50 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 0.002% bromphenol blue, and 0.1 M DDT (16). Molecular weights were determined from standard curves derived from skeletal muscle myosin heavy chain (200K), bovine serum albumin (68K), actin (43K), carbonic anhydrase (32K), lactoglobulin (18K), and lysozyme (14K). SDS-gels were fixed and stained in 50% methanol-10% acetic acid, containing 0.1% Coomassie Brilliant Blue, after the completion of electrophoretic runs. For de-staining of gels, 40% methanol-7.5% acetic acid was used.

### *Preparation of Muscle Actin and Heavy Meromyosin*

*Xenopus* skeletal muscle actin was prepared from acetone powders of muscle by the method of Spudich

and Watt (29) with two cycles of polymerization and sedimentation from 0.8 M KCl.

Rabbit skeletal muscle myosin was the generous gift of Dr. Carl Moos (State University of New York at Stony Brook). Heavy meromyosin was prepared by digestion of myosin at a concentration of 14 mg/ml with trypsin treated with L-(1-tosylamido 2-phenyl)ethyl chloromethylketone (TPCK-trypsin, Sigma Chemical Co., St. Louis, Mo.), as described by Woodrum et al. (38).

### *Electron Microscopy*

Samples were placed on carbon-coated Formvar grids and stained with 1% uranyl acetate. Electron micrographs were made with a JEOL model JEM 100B electron microscope operated at 60 kV.

### *Protein Determinations*

Determinations of total protein were made using Lowry protein assays with bovine serum albumin as a standard (17).

Quantitative scanning densitometry of Coomassie Blue-stained gels was used to determine the relative amount of actin and other polypeptides in the isolated gel material from *Xenopus* extracts. Individual tracks were cut from slab gels with a sharp knife and scanned with a Gilford (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) scanning spectrophotometer at a wavelength of 595 nm. The area under the peaks was determined by planimetric integration, and the amount of each polypeptide relative to the total protein was calculated from the ratio of a given polypeptide peak to the total area under the peaks. This approximation is based on the assumption that the average amount of dye bound per unit weight by all other polypeptides is the same as that bound by any given polypeptide.

## RESULTS

### *Gelation in Extracts of Xenopus Oocytes*

Cell-free extracts were prepared by homogenization of oocytes in low ionic strength sucrose solutions in the cold. Upon warming in a test tube, the high-speed supernate from such extracts forms a clear, solid gel in which liquid flow is completely restricted; gelled extracts will fragment into large chunks if the tube is quickly tilted, or inverted.

Gelation normally occurs within 30 min at 18°–40°C, and is initially temperature reversible. However, with standing in the warm, the gelled extracts will not dissolve if returned to the cold.

Gel formation occurs in the absence of exogenous K<sup>+</sup>, divalent cations, or ATP. However, sucrose is absolutely required for gelation. Consistent results have been obtained with concentra-

tions of 0.9 M sucrose in the extracting media. This concentration is considerably greater than isotonic for these cells. Extracts prepared in the absence of sucrose fail to gel. The addition of solid sucrose to these extracts before warming permits normal gelation, however. Thus, the structural components of the gel are soluble in the absence of sucrose but do not interact to form a gel.

### *Analysis of the Structural Components of the Gel*

The gelled material can be separated from the soluble components of the extract by centrifugation at 25,000 g for 15 min. By this procedure, the gel is compressed into a dense pellet which can be recovered intact. The polypeptide composition of such gel pellets has been analyzed by SDS-polyacrylamide gel electrophoresis. As seen in Fig. 1, the gel is composed principally of a 43,000-dalton polypeptide, which co-migrates with *Xenopus* skeletal muscle actin, and a prominent high molecular weight component of approx. 250,000 daltons. These components are highly enriched in the gel (tracks 4 and 5) relative to their concentration in the high-speed extract (track 1). Quantitative scanning densitometry of stained SDS-polyacrylamide gels (Fig. 2) has been used to determine the relative contribution of these and other polypeptides to the total gel protein (Table I). Roughly 46.6% of the Coomassie Blue-stainable material of the gel co-migrates with actin, and about 4.3% is contributed by the high molecular weight component, the respective polypeptides being present at a molar ratio of approx. 66:1. The gel itself comprises about 3% of the total protein of the extract.

In addition to the 43,000- and 250,000-dalton polypeptides, at least two minor components with molecular weights of 53,000 and 68,000 daltons are usually concentrated in the gelled material. It might be pointed out that Kane has found a prominent 58,000-dalton polypeptide in association with gelled extracts of sea urchin eggs (12, 13). With respect to polypeptides of about 50,000 daltons, the gelled material of *Xenopus* oocytes is clearly different from that of sea urchin eggs. In *Xenopus*, the 53,000-dalton polypeptide represents, on the average, less than 2% of the total stainable protein of isolated gels.

The pelleted gel material can be solubilized in low ionic strength buffers which depolymerize

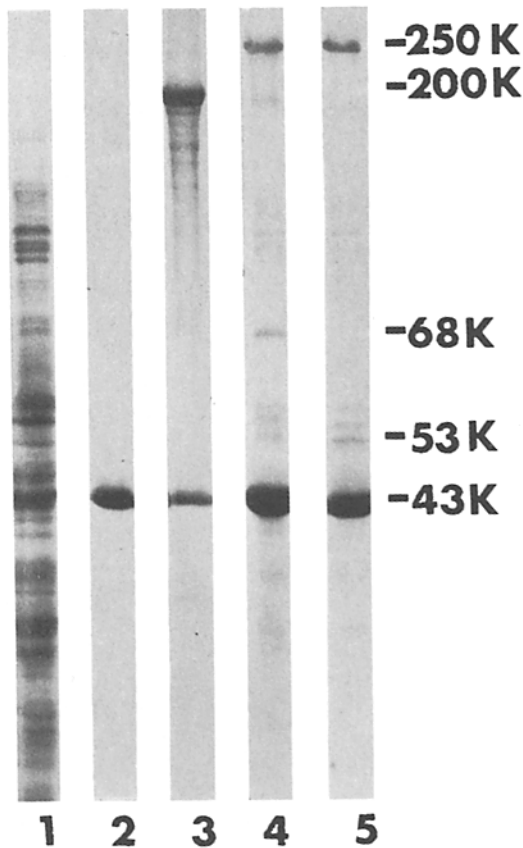


FIGURE 1 Electrophoretic analysis of isolated gel material from cell-free extracts of *Xenopus* oocytes. Tracks were cut from a 10% SDS-polyacrylamide slab gel stained with Coomassie Blue. (1) High-speed extract of *Xenopus* oocytes; (2) purified *Xenopus* skeletal muscle actin; (3) crude *Xenopus* skeletal muscle actomyosin; and (4-5) isolated gel material from warmed extracts of *Xenopus* oocytes (extracts were prepared from oocytes of two different animals). 15-30  $\mu$ g of protein were applied to each slot.

muscle actin, or by the addition of high concentrations of salt. Negatively stained preparations of gels taken apart in 0.6 M KCl have been examined by electron microscopy. Randomly oriented 60-80 Å filaments are clearly visible in such preparations (Fig. 3a). These filaments may be decorated with muscle heavy meromyosin to form characteristic arrowhead-shaped complexes indicative of actin (Fig. 3b).

Actin was further identified in the gel complex by assaying its ability to cross-react with antiserum against purified muscle actin. The isolated gel was dried on a microscope slide, fixed in acetone, and

reacted sequentially with rabbit antiserum against actin and fluorescein-labeled goat anti-rabbit IgG. A strong cross-reaction was observed with the gel. When the primary antiserum was first absorbed with an excess of purified *Xenopus* skeletal muscle actin, the cross-reaction was completely abolished.

Thus, by physical, ultrastructural, and immunological criteria, actin is the major component of the isolated gel.

#### Effects of ATP on Gelled Extracts

When ATP is included in the extracting media, the gelled material undergoes a slow, spontaneous contraction during which time the soluble components of the extract are excluded from the gel.

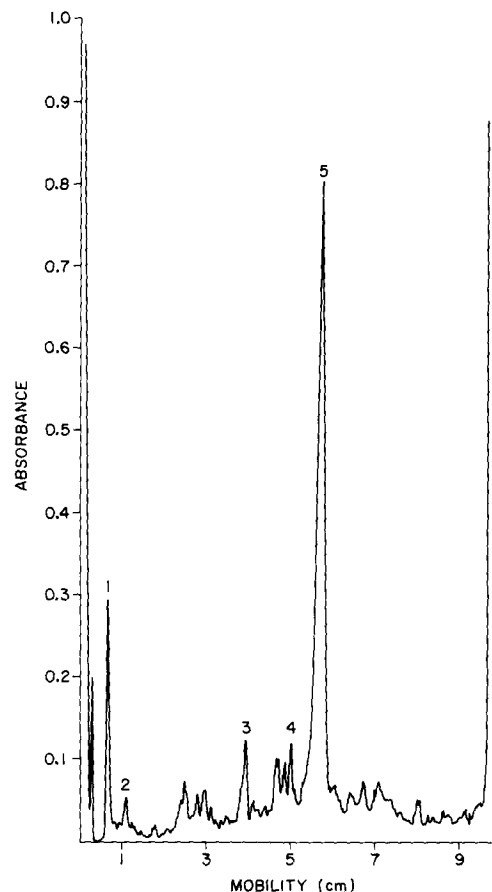


FIGURE 2 Densitometric scan of Coomassie Blue-stained polypeptides of the isolated oocyte gel, fractionated on a 10% SDS-polyacrylamide gel. The material was isolated from an extract incubated for 60 min at 34°C. (1) 250,000 daltons; (2) 200,000 daltons; (3) 68,000 daltons; (4) 53,000 daltons; and (5) 43,000 daltons.

TABLE I  
*Polypeptide Composition of Gelled Material Isolated  
 from Extracts of Xenopus Oocytes*

Polypeptide	Total protein* %
Actin	46.6 ± 9.8
53,000 daltons	1.7 ± 0.2
68,000 daltons	0.8 ± 0.5
200,000 daltons (myosin?)	0.6 ± 0.2
250,000 daltons	4.3 ± 1.3

\* Numbers represent the percent of total stainable protein ± SD ( $n = 7$ ) as determined by integration of densitometer scans of 10% SDS-polyacrylamide gels.

Within 6–8 h the gel forms a small opaque aggregate as seen in Fig. 4. In the absence of exogenous ATP, no significant volume change occurs, suggesting that contraction of the gel may be an energy-requiring process.

It was of interest to compare the molecular composition of the gelled material before and after contraction. As judged by electrophoretic analysis, no detectable differences in the polypeptide composition of the two fractions could be seen (Fig. 5).

A distinct difference in the polypeptide composition of gels prepared in the presence and absence of ATP was, however, found. Initial experiments revealed the presence of a prominent 200,000-dalton component in the gelled material prepared without exogenous ATP (Fig. 6a). This polypeptide co-migrated with the heavy chain of *Xenopus* skeletal muscle myosin.

Attempts to repeat these results met with variable success. However, in time-course studies, it was found that the myosin-like component always associates with the gel, provided the extracts are incubated for a sufficient length of time. An example of such a time-course study is presented in Fig. 6b. It can be seen that gels prepared in the absence of exogenous ATP contain virtually no material at the position of myosin heavy chain after 3 h of incubation at 34°C. However, by 8 h, a prominent 200,000-dalton polypeptide is associated with the uncontracted gel. This polypeptide is completely absent from the fully contracted, ATP-containing controls. It is possible that the interaction of the myosin-like component with the gel proteins is initially inhibited by endogenous ATP in the extract. Hydrolysis of the ATP through time could slowly lead to binding of the myosin-like component and result in the formation of a "rigor"-type complex in the gel.

The 200,000-dalton myosin-like polypeptide may comprise as much as 5% of the total stainable protein of the gel. It would appear from Fig. 6a that the 53,000-dalton component of the isolated gel is significantly reduced upon binding of the 200,000-dalton polypeptide. However, because of the variability in the amount of this component in gels prepared in the presence of ATP, additional studies will be required to determine whether this change is meaningful.

### *Effects of Ca<sup>++</sup> on Gelation*

Gelation in extracts of other cell types is inhibited by Ca<sup>++</sup> (6, 12, 25, 30). The level of free Ca<sup>++</sup> in these systems has been controlled by the addition of EGTA to the extracting media. In contrast, gelation in extracts of *Xenopus* oocytes occurs in the absence of a chelating agent such as EGTA.

When EGTA was added to *Xenopus* extracts, gelation was in fact inhibited, suggesting that Ca<sup>++</sup> might be required for gel formation.

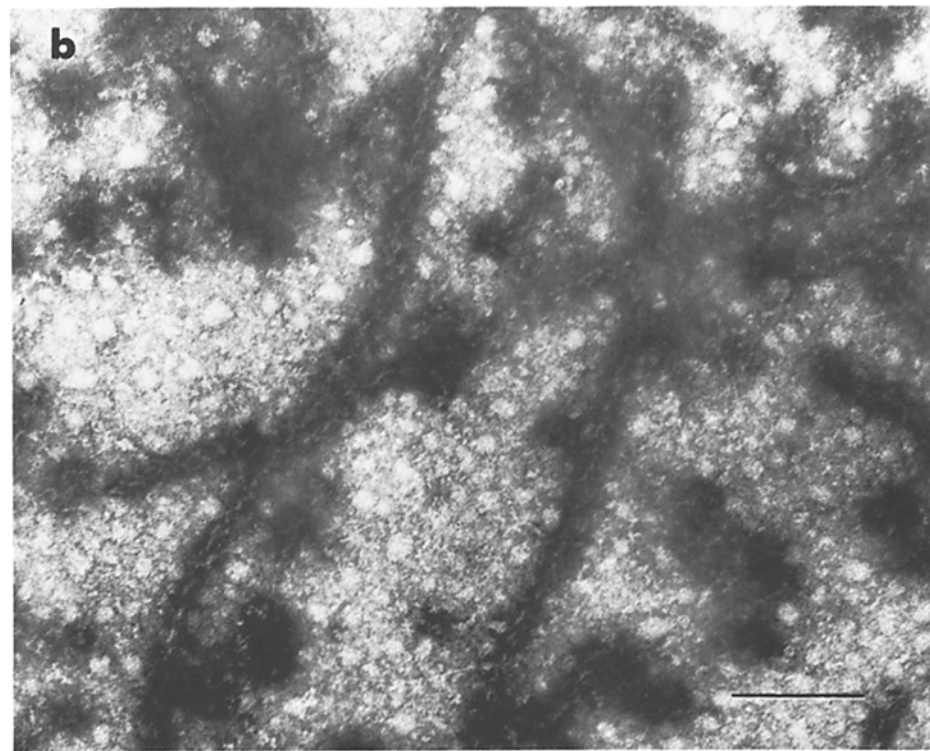
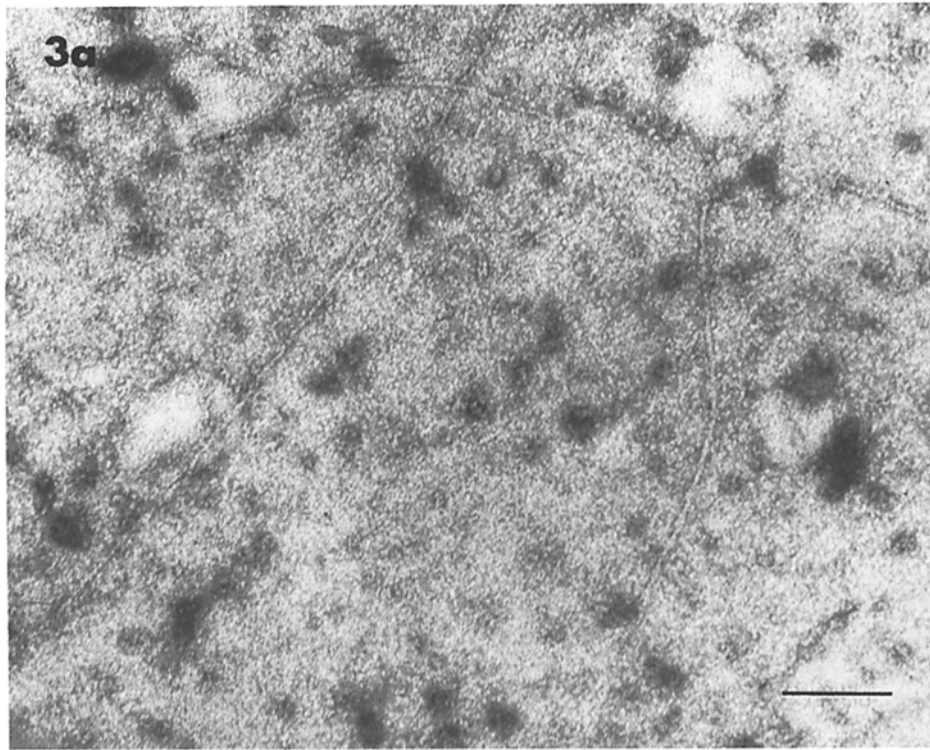
In order to examine this point, Ca<sup>++</sup> was added back to EGTA-containing extracts before warming. The pH of these extracts was readjusted to 7.4 after the addition Ca<sup>++</sup>. At a final concentration in the extract of 1.67 mM EGTA, gelation (measured as sedimentable material) was almost completely abolished. The addition of Ca<sup>++</sup> reversed the inhibition. In Fig. 7 (tracks 1–3), it can be seen that gelation was roughly proportional to the ratio of Ca<sup>++</sup>/EGTA up to a ratio of 0.5, which was optimal for gel formation.

As in other systems, gelation in extracts of *Xenopus* oocytes is also inhibited by high levels of Ca<sup>++</sup>. At a ratio of 0.75 Ca<sup>++</sup>/EGTA, gel formation was markedly reduced in these extracts (Fig. 7, track 4). Under these conditions, a visible precipitate usually forms over the course of several hours. This insoluble material is often selectively enriched in an actomyosin-like complex (Fig. 7, track 5), suggesting a different organization of contractile proteins in the presence of high levels of Ca<sup>++</sup>.

## DISCUSSION

### *Gelation*

Gel formation in crude extracts of *Xenopus* oocytes involves both the assembly and cross-linking of actin filaments. In this respect, it is thought to be directly analogous to gelation in extracts of other cell types (6, 12, 25, 30, 36). It



**FIGURE 3** Electron micrographs of isolated gel material dissolved in 0.6 M KCl, 20 mM Tris-HCl (pH 7.4), and 1 mM DTT. Preparations were negatively stained with uranyl acetate. (a) Single filaments present in the dissolved oocyte gel material. (b) Filaments after the addition of rabbit skeletal muscle heavy meromyosin. Note the "arrowhead" complexing. Bar, 0.2  $\mu$ M.

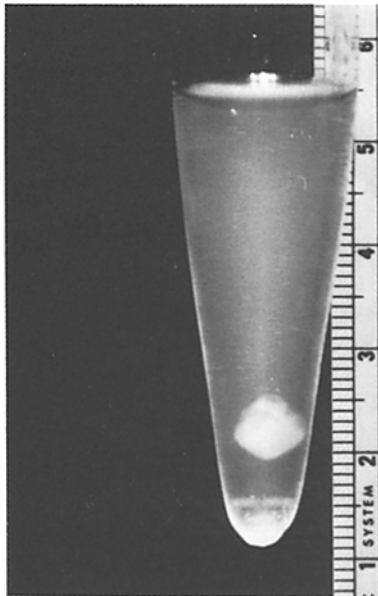


FIGURE 4 The appearance of a gelled extract 10 h after incubation at 34°C. The contracted gel is visible near the bottom of the tube.

is likely that the prominent 250,000-dalton polypeptide associated with the gelled material of these extracts is similar, if not identical, to the high molecular weight actin-binding protein in gels of sea urchin egg (12) and rabbit macrophage (30) extracts. Stossel and Hartwig (30) have found that the macrophage actin-binding protein can promote gelation of purified macrophage or muscle actin, suggesting that the high molecular weight component is involved in the cross-linking of actin filaments in cell-free extracts. However, in view of findings which indicate that other proteins are also capable of inducing gelation of purified F-actin (19, 20), we cannot be certain that the high molecular weight polypeptide in *Xenopus* extracts is essential for gel formation. It may be relevant to point out that purified *Acanthamoeba* actin is apparently capable of undergoing gelation by itself (25).

High concentrations of sucrose are required for gelation in extracts of *Xenopus* oocytes. Similarly, either sucrose or glycerol is necessary for gel formation in extracts of certain other cell types (12, 25, 30, 36), and it has been suggested that the effect of sucrose may reflect an alteration in the physical properties of G-actin which permit it to polymerize in the absence of bound divalent cation and nucleotide (30). On the other hand, it

has been found that sucrose inhibits the extent of gelation in extracts of *Dictyostelium* (6). Thus, at present, the role of sucrose in gelation is unclear, and further study will be required before it is fully understood.

It has been proposed that the gelation of crude cell-free extracts may correspond to the assembly of a dynamic, contractile gel reticulum within the cell (25). Under this assumption, gel formation would be expected to be sensitive to factors that can influence the structural properties of the cytoplasm in vivo. There is considerable evidence

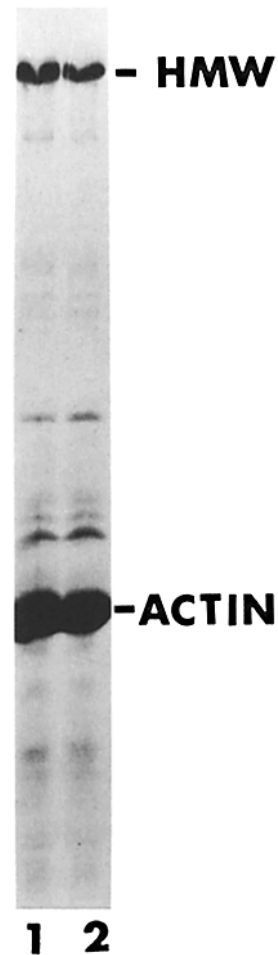


FIGURE 5 Electrophoretic analysis of gelled extracts before and after extraction. (1) Uncontracted gel isolated by centrifugation (extract incubated for 60 min at 34°C); (2) contracted gel aggregate isolated 8 h after incubation at 34°C. HMW denotes the high molecular weight binding protein. Polypeptides were fractionated on a 10% SDS-polyacrylamide slab gel.

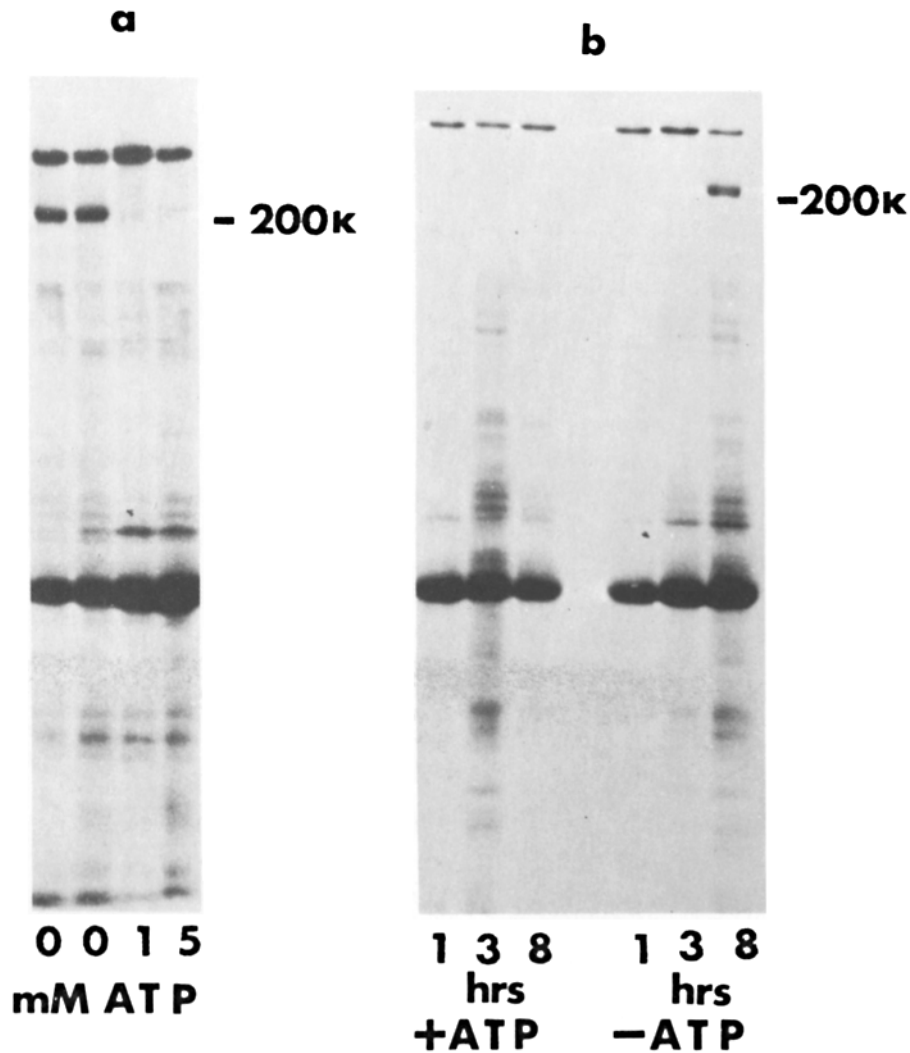


FIGURE 6 Electrophoretic analysis of gelled material from oocyte extracts prepared in the presence and absence of exogenous ATP. (a) Gels isolated from extracts prepared in the presence of varying concentrations of exogenous ATP (the gelled material was isolated 3 h after incubation of extracts at 34°C.); and (b) gels isolated from extracts at different times after incubation at 34°C (these represent aliquots of extracts prepared from oocytes of the same animal extracted in the presence or absence of exogenous ATP). Polypeptides were fractionated on a 10% SDS-polyacrylamide slab gel.

that  $\text{Ca}^{++}$  can affect the structural (and contractile) properties of the cytoplasm of intact cells. Gross consistency changes can be induced in *Xenopus* oocytes by the removal of divalent cations from the medium in which they are isolated. Under these conditions, the cytoplasm undergoes a marked "softening" (21). The microinjection of low concentrations of  $\text{Ca}^{++}$  into these cells causes a hardening of the cytoplasm, while the introduction of higher concentrations causes a further

softening (21). In amphibian eggs, the microinjection of  $\text{Ca}^{++}$  into the cortical cytoplasm will induce the formation of a cleavagelike furrow (10). Formation of a normal cleavage furrow in fertilized eggs can be inhibited by the introduction of EGTA-containing buffers into the cell (3). The contractile ring which forms in association with the furrow is known to have the properties of an endothermic gel (18) and is composed of actin-containing microfilaments (27). Contractions of



the egg cortex in response to wounding of the plasma membrane also require  $\text{Ca}^{++}$  (8). These contractions are associated with the appearance of dense arrays of microfilaments in the region of the wound (4). These studies indicate that  $\text{Ca}^{++}$  can alter the structural organization of the cytoplasm, and that these structural changes are often associated with microfilament formation. Indeed, correlations between the appearance of microfilaments in vivo and the presence of  $\text{Ca}^{++}$  have suggested that  $\text{Ca}^{++}$  may play a role in actin filamentogenesis (28). The fact that  $\text{Ca}^{++}$  can promote gelation in extracts of *Xenopus* oocytes is at least consistent with this idea.

The gelation of oocyte extracts involves both

the assembly and interaction of actin filaments; thus,  $\text{Ca}^{++}$  might be required in either or both of these processes. It is considered unlikely, however, that  $\text{Ca}^{++}$  is required for the cross-linking of actin filaments in the gel. When gelled material isolated from these preparations is dissolved in 0.6 M KCl, actin filaments are present in solution (Fig. 3). These filaments are capable of re-forming a gel if the dissolved material is dialyzed against low ionic strength buffers. Since  $\text{Ca}^{++}$  levels would be expected to be quite low under these conditions, junctions between filaments can probably form in the absence of free  $\text{Ca}^{++}$ . Therefore,  $\text{Ca}^{++}$  is probably required for the assembly of actin filaments in crude oocyte extracts. While it is

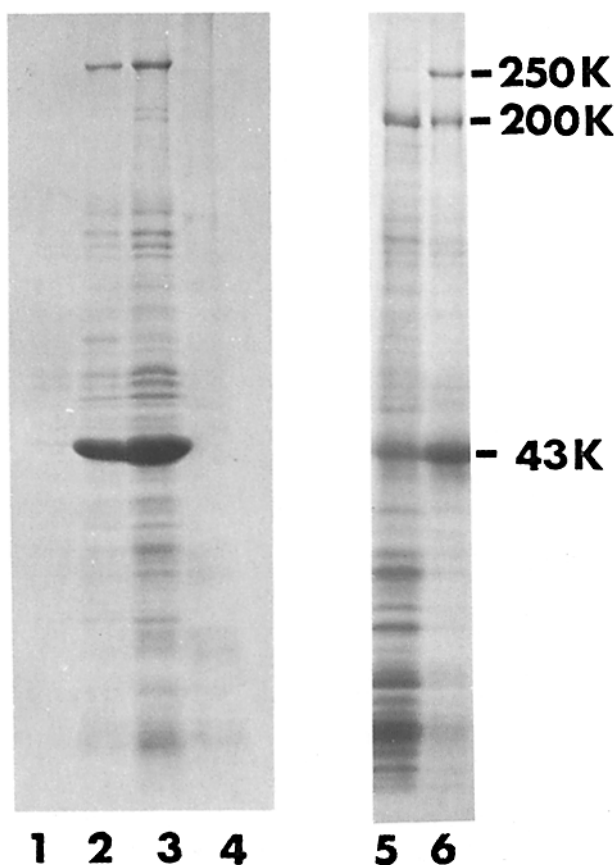


FIGURE 7 Electrophoretic analysis of gelled material from oocyte extracts prepared in the presence of varying ratios of  $\text{Ca}^{++}$ /EGTA. The following ratios are shown: (1) 0; (2) 0.25; (3) 0.5; (4) 0.75 (samples were prepared from the same extract containing a final concentration of 1.67 mM EGTA). The gelled material was isolated by centrifugation 2 h after incubation of extracts at 34°C. (5) Insoluble proteins isolated from extracts prepared at a ratio of 0.75  $\text{Ca}^{++}$ /EGTA 6 h after incubation at 34°C; (6) gel material isolated from extracts prepared in the absence of exogenous ATP. Polypeptides were fractionated on a 7–17% SDS-polyacrylamide gradient slab gel.

conceivable that some low level of  $\text{Ca}^{++}$  may be needed simply to maintain actin polymerizability, the requirement for  $\text{Ca}^{++}$  may reflect a significant regulatory control over the assembly of actin filaments in these cells.

Studies on cell-free extracts of amoebae have also raised the possibility that  $\text{Ca}^{++}$  may regulate actin filament assembly. Taylor et al. (34) have found that  $\text{Ca}^{++}$  can promote the formation of actin filaments in cytoplasmic extracts of *Amoeba proteus* and in plasmalemma-ectoplasm "ghosts" of *Chaos carolensis*, suggesting that the level of free  $\text{Ca}^{++}$  in these cells may control transformations of actin from nonfilamentous to stable filamentous states.

There is little evidence that  $\text{Ca}^{++}$  can regulate the polymerization of purified cytoplasmic actins. Nevertheless, it is possible that potential regulatory factors which may control filament assembly in crude extracts (and perhaps in vivo) are lost upon isolation of the proteins.

### Contraction

An understanding of the contractile properties of gelled cytoplasmic extracts may well provide insight into certain motility phenomena in vivo. Stossel and Hartwig (30) have found that myosin is required for the contraction of purified macrophage actin-actin binding protein gels, and, more recently, Condeelis and Taylor (6) have demonstrated that contractions in gelled extracts of *Dictyostelium* also require myosin. Thus, gel contractions may result from actomyosin interactions analogous to those that occur in muscle.

Contractions in gelled extracts of *Acanthamoeba* (25) and *Dictyostelium* (6) are stimulated by  $\text{Ca}^{++}$ . In that  $\text{Ca}^{++}$  can influence contractility in vivo (3, 8, 10, 32), its ability to regulate contraction in cytoplasmic extracts may be significant. Condeelis and Taylor (6) have suggested that  $\text{Ca}^{++}$ -stimulated contraction in gelled extracts of *Dictyostelium* may result from structural alterations in the gel. It has been found that the addition of micromolar  $\text{Ca}^{++}$  to these extracts leads to solation and the release of free actin filaments from the gel matrix (6). It has been argued that this structural change may permit the interaction of myosin with actin filaments (in myosin-containing extracts), and may result in contraction of the gel. These protein interactions are thought to be sterically hindered in the cross-linked gel (6).

Elevated levels of  $\text{Ca}^{++}$  (measured as the ratio

of  $\text{Ca}^{++}/\text{EGTA}$ ) inhibit gelation in extracts of *Xenopus* oocytes. The effect of high concentrations of  $\text{Ca}^{++}$  in this system cannot, therefore, be interpreted with respect to contraction. However, the fact that an actomyosin-like complex can sometimes be isolated from extracts prepared in the presence of high levels of  $\text{Ca}^{++}$  (Fig. 7) suggests that a change in the organization of contractile proteins may take place under these conditions.

At  $\text{Ca}^{++}$  levels that permit gelation, the gelled material of oocyte extracts undergoes a slow decrease in volume over time. Exogenous ATP is required for this contraction, suggesting that it could be an energy-requiring process. Nevertheless, we find very little material co-migrating with the heavy chain of *Xenopus* skeletal muscle myosin associated with either the expanded or fully contracted gel in the presence of ATP (Fig. 5). The absence of a myosin-like component in the contracted gel might be explained by a cycling of actin-myosin interactions within the gel complex. Thus, at any one time, one might not expect to find much myosin associated with the complex. It is clear that a myosin-like polypeptide (200,000 daltons) is at least capable of interacting with the gel, since it becomes prominent in the complex in the absence of exogenous ATP (Fig. 6). However, at present, we cannot exclude the possibility that the slow contraction of gelled oocyte extracts results from the accumulation of nonspecific bonds between filaments, leading to a progressive shrinkage of the gel.

We are grateful to Dr. Fredrick Miller of our Department of Pathology for his gifts of rabbit antiserum against actin. The help of Dr. Jules Elias, Department of Pathology, in designing and executing the immunological characterization experiments is also gratefully acknowledged.

This investigation was supported by a United States Public Health Service Biomedical Research Support grant 5S07RR0767-11 to the State University of New York at Stony Brook.

Received for publication 2 June 1977, and in revised form 3 January 1978.

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