

# Proteins Regulating Actin Assembly in Oogenesis and Early Embryogenesis of *Xenopus laevis*: Gelsolin Is the Major Cytoplasmic Actin-binding Protein

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**Abstract.** Oocytes, notably those of amphibia, accumulate large pools of nonfilamentous ("soluble") actin, both in the cytoplasm and in the nucleoplasm, which coexist with extensive actin filament arrays in the cytoplasmic cortex. Because the regulation of oogenically accumulated actin is important in various processes of oogenesis, egg formation, fertilization and early embryogenesis, we have purified and characterized the major actin-binding proteins present in oocytes of *Xenopus laevis*. Here we report that the major actin-binding component in the ooplasm, but not in the nucleus, is a polypeptide of  $M_r \sim 93,000$  on SDS-PAGE that reduces actin polymerization in vitro in a  $Ca^{2+}$ -dependent manner but promotes nucleation events, and also reduces the viscosity of actin polymers, indicative of severing activity. We have raised antibodies against the purified oocyte protein and show that it is

different from villin, is also prominent in unfertilized eggs and early embryos and is very similar to a corresponding protein present in various tissues and in cultured cells, and appears to be spread over the cytoplasm. Using these antibodies we have isolated a cDNA clone from a  $\lambda$ gt11 expression library of ovarian poly(A)<sup>+</sup>-RNA. Determination of the amino acid sequence derived from the nucleotide sequence, together with the directly determined sequence of the amino terminus of the native protein, has shown that this clone encodes the carboxy-terminal half of gelsolin. We conclude that gelsolin is the major actin-modulating protein in oogenesis and early embryogenesis of amphibia, and probably also of other species, that probably also plays an important role in the various  $Ca^{2+}$ -dependent gelation and contractility processes characteristic of these development stages.

O OGENESIS and early embryogenesis involve a number of diverse processes that depend on actin, be it polymerized into microfilaments, in mono- or oligomeric forms, or in complexes with other proteins. Apparently to accommodate the protein required for all these different functions, oocytes of many (probably all) species build up large stores of the nonmuscle  $\beta$ - and  $\gamma$ -type actin in both the cytoplasm ("ooplasm"), notably the cortical region (e.g., references 19, 21, 23, 35, 41, 42, 55; for review see 47), and the nucleoplasm, resulting in high concentrations of soluble actin of 4 mg/ml or more, at least in certain amphibian oocytes (e.g., references 10, 11, 24, 55; a considerable portion of the "nondiffusive" actin of reference 42 seems to be recovered in the soluble pool). This actin appears to contribute to diverse functions in oogenesis and egg maturation of various species ranging from an involvement in transcription (48), and meiotic divisions, notably chromosome condensation (46), to the formation of cortical microfilament bundles, including those attached to micro- and macrovilli and to the plaques of the adhaerens junctions with follicle cells (9, 17, 19, 41, 49). Actin and actomyosin have also been described as being involved, probably together with other proteins and

in response to changes in concentrations of pH and divalent cations, in polar body formation, in various fertilization reactions such as cortical contractions, sperm uptake, exocytosis of cortical granules, elongation or retraction of microvilli, appearance and disappearance of microvilli, and establishment of the polarity of the early embryo (e.g., references 7, 9, 25, 38, 41, 47, 54). Moreover, maternally supplied actin is involved in formation of the "contractile rings" of the cleavage furrows during early embryogenesis in some species (e.g., references 18, 35, 49) and probably also contribute to the morphogenesis of the early (i.e., pregastrulation embryo) to the compaction process, and to the cell movements that accompany gastrulation (e.g., references 18, 29).

Amphibian oocytes, eggs, and embryos are classical objects of research on the cell and molecular biology of developmental processes, not only because of their large sizes and high rate of early development, but also because almost their entire early development (i.e., until the midblastula transition point) is effected by a maternally supplied pool of proteins and mRNAs. Hence, the large pool of maternal actin is likely to be involved in all the diverse processes of early embryogenesis and is differentially regulated, in patterns

characteristic of the specific embryonic cells, by proteins that modulate actin assembly. We have therefore identified and characterized the major actin-binding proteins found in oocytes of *Xenopus laevis*. In the present article, we report the major factor interfering with actin polymerization to be a cytoplasmic protein which turned out to be gelsolin. A nuclear actin-binding protein has been described elsewhere (2).

## Materials and Methods

### Fractionation of Protein from Ovarian Tissue

Adult female toads of *Xenopus laevis* obtained from the South African Snake Farm (Fish Hoek, Republic of South Africa) were killed by decapitation. The ovaries were removed, briefly washed first in SSC (150 mM NaCl, 15 mM sodium citrate) and then in "homogenization buffer" (buffer K: 82 mM KCl, 18 mM NaCl, 2 mM MgCl<sub>2</sub>, 1 mM  $\beta$ -mercaptoethanol, 0.2 mM CaCl<sub>2</sub>, 20 mM Tris-HCl, pH 7.4) containing 1 mM phenylmethylsulfonyl-fluoride, 5 mg/liter pepstatin and 5 mg/liter leupeptin (Sigma Chemical Co., St. Louis, MO). About 10 g ovary (wet weight) was used for a typical preparation. To the ovary material the 1.5-fold volume of buffer was added, and the tissue was homogenized by several up and down strokes using a motor-driven Dounce homogenizer (Kontes Glass Co., Vineland, NJ). The extract was centrifuged for 1 h at  $\sim$ 100,000 g at 4°C in a rotor (model SW 27; Beckman Instruments, Inc., Palo Alto, CA). To obtain an extract free from lipids and yolk, the centrifuge tube was pricked directly above the band representing the ribosomal fraction, and the clear extract was aspirated. The 100,000 g  $\times$  1-h supernatant ("postribosomal extract") was applied to a 2.5  $\times$  80 cm column of Sephadex G 150 (Pharmacia, Uppsala, Sweden) and eluted with buffer K. Fractions were tested by falling-ball viscosimetry for their ability to reduce the low shear viscosity (see below). Active fractions were pooled and applied to a 10 ml DNAase I-Sepharose affinity column (50 mg pancreatic DNAase I [Sigma Chemical Co.] bound to cyanogen bromide-activated Sepharose 4B, [Pharmacia]). Proteins not specifically bound were removed by washing with buffer K containing 1.0 M KCl. Subsequently, actin complexes bound to the column were eluted using buffer K containing 3 M guanidinium hydrochloride (Gu-HCl). Upon dialysis overnight against buffer K, the fractions were applied to a Mono Q anion exchange column (Pharmacia) which was eluted with a gradient of 0.1-1.0 M KCl. To dissociate actin from complexes with other proteins, the eluate in buffer K with Gu-HCl was dialysed overnight against buffer K containing 6 M urea. The dissociated proteins were then separated by Mono Q anion exchange chromatography as described above, with the exception that the elution buffer contained 6 M urea.

### Fractionation of Proteins from Other Cells and Tissues

Tissue pieces from heart muscle, leg skeletal muscle, and gastric submucosal smooth muscle were prepared from decapitated frogs (for details see reference 2), thoroughly washed in buffer K and homogenized with a rotating knife homogenizer (Fa. E. Buehler, Tübingen, FRG). Homogenates were centrifuged for 1 h at 100,000 g at 4°C and supernatant fractions (postribosomal extracts) were prepared essentially as described above.

Intestinal mucosa cells were detached from intestinal segments, after extensively rinsing with buffer K, in buffer K with 10 mM EDTA by gentle massaging (cf. reference 20). Detached cells were pelleted, washed twice in buffer K, and either directly dissolved in SDS-PAGE sample buffer, or used for the preparation of postribosomal extracts (see above).

Monolayer cultures of *Xenopus laevis* kidney epithelial (XLKE) cells (line A6) were grown and harvested as described (for references see 27, 31). Cell pellets were homogenized, and postribosomal extracts were obtained by centrifugation at 100,000 g for 40 min as described above.

Postribosomal extracts were also prepared from unfertilized eggs and embryos of stage 6.5 (morulae) according to Nieuwkoop and Faber (39).

### Sucrose Gradient Centrifugation

Postribosomal extracts (0.5 ml) from *Xenopus laevis* ovaries were layered on top of a 5-20% (wt/vol) sucrose gradient and centrifuged at  $\sim$ 100,000

1. *Abbreviation used in this paper:* buffer k, 82 mM KCl, 18 mM NaCl, 2 mM MgCl<sub>2</sub>, 1 mM  $\beta$ -mercaptoethanol, 0.2 mM CaCl<sub>2</sub>, 20 mM Tris-HCl, pH 7.4

g for 18 h, using BSA (4.3S), rabbit muscle aldolase (7.35 S), and catalase (11.3 S) as reference proteins in a parallel gradient (cf. reference 30). Fractions obtained were assayed for influences on actin polymerization by falling-ball viscosimetry as described above and by SDS-PAGE.

### Purification of Actin

Aceton dry powder from rabbit or *Xenopus* skeletal muscle was prepared according to the method of Seraydarian et al. (50), and actin was purified from this material essentially according to Spudich and Watt (52), with, however, the use of 0.8 M KCl instead of 0.6 M KCl before centrifugation. Actin concentrations were determined using an extinction coefficient of 0.62 at 290 nm for 1 mg/ml. Usually, actin was purified by gel filtration on a Sephadex G150 column equilibrated with 2 mM Tris-HCl, 0.2 mM CaCl<sub>2</sub>, 0.2 mM ATP, 0.02% NaN<sub>3</sub>, pH 8.0 (buffer A) and stored at 4°C.

### Viscosimetry

The high shear viscosity of skeletal muscle actin was determined in a Ostwald-type viscometer (Schott, Mainz, FRG) with a charge volume of 3.0 ml and outflow times (given for water) of  $\sim$ 15 s. Measurements were made every 2 min at 25°C. The viscosity was calculated according to Cooper and Pollard (12). Low shear viscosity was measured with a falling-ball viscometer (43). 20- $\mu$ l fraction sample was added to 170- $\mu$ l actin solution (0.3 mg/ml). Polymerization was started by adding 10- $\mu$ l solution containing 40 mM MgCl<sub>2</sub> and 20 mM ATP.

Protein concentrations were determined according to Bradford (4) using BSA as standard.

### Preparation of Antibodies

Guinea pigs,  $\sim$ 8 wk old, were subcutaneously injected with  $\sim$ 300  $\mu$ g purified protein, mixed with an equal volume of complete Freund's adjuvant, followed by an injection of 200  $\mu$ g of protein in incomplete adjuvant 3 wk later. Blood was collected 1 wk after the second injection. Serum and immunoglobulin G fractions were prepared by standard techniques. For comparison, antibodies against porcine villin (22) previously described (45) were used (kindly provided by Dr. D. Louvard, Institut Pasteur, Paris, France).

### Gel Electrophoresis, Peptide Mapping, and Amino Acid Sequence Analysis

Procedures used SDS-PAGE and two-dimensional gel electrophoresis that have been described (for literature see reference 1). Proteins separated by gel electrophoresis were transferred to nitrocellulose paper sheets and processed for immunoblotting with the modifications previously described (1) but omitting the "protein refolding step" in buffer containing 4 M urea. Antibodies bound were detected with <sup>125</sup>I-labeled protein A. Polypeptide spots were excised from gels and peptide maps were obtained as described by Elder et al. (16).

The amino-terminal amino acid sequence was determined on protein isolated from *Xenopus* ovary. The chromatographically homogeneous protein was further purified by SDS-PAGE on a 10% gel, electroblotted on glass fiber membranes coated with poly-(4-phenyl-N-methylpyridine), and subjected to sequence analysis, using a gas-phase sequencer (Applied Biosystems, Inc., Foster City, CA) as described (3).

### Separation of Nuclei and Ooplasm and Evaluation of Immunoblot Experiments

Pieces of *Xenopus* ovary were fixed in 10% TCA and follicle cells were manually removed. Nuclei ("germinal vesicles") were separated from ooplasm with watchmaker forceps. The proteins were analyzed by SDS-PAGE (18%), and the actin-modulating protein was identified by immunoblotting. For the determination of radioactivity bound to the nitrocellulose-blotted proteins, the corresponding strips were excised and radioactivity measured in a  $\gamma$  particle counter (LKB Instruments, Inc., Bromma, Sweden).

### Determinations of Actin-binding Protein in Subcellular Fractions

*Xenopus laevis* oocytes of stage VI were manually separated from the surrounding follicle epithelium with the use of watchmaker forceps (19) and

homogenized in buffer K at 4°C. The extract was centrifuged at 15,000 g for 20 min, and the supernatant ("mitochondrial supernatant") was separated from the pelleted material ("low speed pellet"). The supernatant was then centrifuged for 1 h at 100,000 g and 4°C, and the resulting supernatant ("postribosomal supernatant") separated from the pellet.

### Immunofluorescence Microscopy

Various tissues of *Xenopus laevis*, including intestine, ovary, skeletal muscle, and heart muscle, were immediately snap-frozen in isopentane that had been precooled in liquid nitrogen to  $\sim -130^\circ\text{C}$  (cf. reference 17). Immunofluorescence microscopy was performed on cryostat sections that had been fixed for 5 min in cold acetone ( $-20^\circ\text{C}$ ) or in 4 or 10% formaldehyde (freshly prepared from paraformaldehyde in PBS). The air-dried sections were incubated for 15 min with antiserum (usually diluted 1:10 in PBS) or with purified IgGs in PBS. The specimens were washed three times in PBS for 5 min each. Bound antibodies were visualized by reaction with FITC-conjugated or Texas red-labeled secondary antibodies (Dianova, Hamburg, FRG). Specimens were again washed in PBS, dipped in ethanol, air-dried, and finally mounted in Mowiol 4-88 (Hoechst, Frankfurt, FRG), and observed using a Zeiss photomicroscope III.

Cultured kidney epithelial cells of *Xenopus laevis* (XLKE, line A6) grown on cover slips were used for immunofluorescence microscopy of cultured cells.

### cDNA Cloning and Sequencing

A  $\lambda$ gt11 cDNA library prepared from *Xenopus laevis* ovary poly(A)<sup>+</sup> RNA was screened using the guinea pig antibodies mentioned above, and positive subclones were purified, subcloned into M13 mp18 and sequenced as described (31).

## Results

### Fractionation of Activities Interfering with Actin Polymerization

When gel filtration fractions from 100,000 g  $\times$  1 h supernatants ("soluble proteins") of *Xenopus laevis* ovaries were assayed for their effects of the *in vitro* polymerization of rabbit muscle actin a marked reduction of low shear viscosity was observed in peak I (Fig. 1). This assay was routinely used in the subsequent enrichment of the inhibitory factor. The inhibitory effect of another fraction (peak II, Fig. 1) was diminished when 2 mM MgCl<sub>2</sub> was used for the initiation of polymerization, instead of 100 mM KCl. This component will not be considered in the present study, but will be specifically dealt with elsewhere (cf. reference 2).

When the gel filtration fractions of peak I were pooled and fractionated further by DNAase I affinity column chromatography for actin complexes, the activity appeared with an actin-associated polypeptide of an SDS-PAGE mobility, at elevated ionic strength (cf. reference 1), corresponding to  $M_r \sim 105,000$  (Fig. 2 a). This polypeptide band was sometimes resolved into a doublet (data not shown). On conventional SDS-PAGE, it had a somewhat higher mobility with respect to the reference proteins used, migrating very close to rabbit muscle phosphorylase a, i.e.,  $M_r \sim 93,000$ . At this step of purification, the protein appeared in a mass ratio of  $\sim 0.9$  with respect to actin, as judged after Coomassie Blue staining (Fig. 2 a, lane 4), suggesting that most of it existed in actin complexes with a molar ratio of 1:2, as is typical of certain actin-binding proteins such as gelsolin in the presence of calcium (6, 8, 14, 28).

Using ion exchange chromatography on a MonoQ column the actin complex of this protein could be purified further (Fig. 2 a, lane 5), and the protein was finally separated from

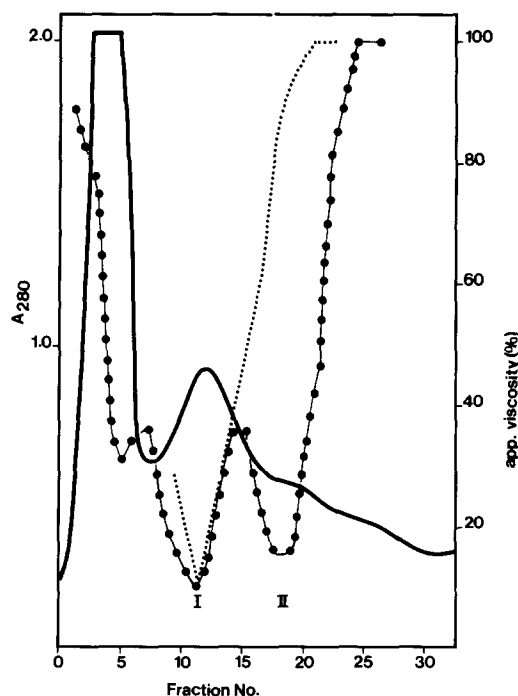


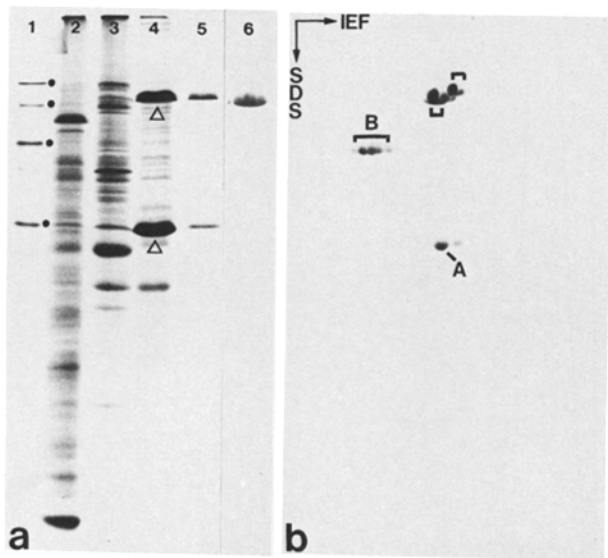
Figure 1. Fractionation of factors interfering with actin polymerization present in 100,000 g supernatant from whole *Xenopus* ovaries by gel filtration on a Sephadex G 150 column; 15 ml postribosomal supernatant, prepared as described in Materials and Methods, were loaded on the column and the protein eluted was measured by absorption at 280 nm (solid line). Individual fractions were assayed for their influence on the polymerization of rabbit skeletal muscle actin as measured by low shear viscosimetry. The polymerization of the G-actin solution was started by addition of KCl and ATP to final concentrations of 100 mM and 1 mM, respectively (continuous line with dots) or 2 mM MgCl<sub>2</sub> instead of KCl (finely dotted line). Active fractions, eluting in peak I (at a position corresponding to  $M_r$  180,000) were pooled and used for further purification. A second activity peak (peak II, eluting at the position of ovalbumin), vanished completely when actin polymerization was initiated by 2 mM MgCl<sub>2</sub> instead of 100 mM KCl.

actin by MonoQ chromatography in the presence of 6 M urea (Fig. 2 a, lane 6). Two-dimensional gel electrophoresis of the purified and still active protein, using the elevated ionic strength buffer system in the second dimension SDS-PAGE, revealed two components of very similar sizes and isoelectric pH values ( $\sim 5.40$  and  $\sim 5.45$ ), each of which appeared with a minor, more acidic satellite spot (Fig. 2 b), indicative of modification isoforms. This purified protein was used for immunization and further characterization.

Upon fractionation of the ovarian extracts by sucrose gradient centrifugation the bulk of the activity interfering with actin polymerization that corresponded to peak I of the gel filtration appeared with a mean peak value corresponding to 8.2S (data not shown; for details see reference 2).

### Effects on Actin Polymerization

To characterize the influence of the purified  $M_r \sim 93,000$  protein on the polymerization of skeletal muscle actin, high shear viscosity was measured in a Ostwald-type viscometer. When the polymerization was started by the addition of



**Figure 2.** Monitoring of the actin-modulating protein during purification. (a) SDS-PAGE (18%; for buffer system see references 2, 30) analysis of fractions obtained during the purification of the major actin-modulating activity from *Xenopus* ovaries (staining with Coomassie Blue). Lane 1, reference polypeptides (from top to bottom):  $\beta$ -galactosidase ( $M_r$  116,000), phosphorylase a ( $M_r$  93,000), BSA ( $M_r$  68,000), actin ( $M_r$  42,000); lane 2, total 100,000 g supernatant; lane 3, pooled peak I fractions obtained by gel filtration of the 100,000 g supernatant on Sephadex G 150 (cf. Fig. 1); lane 4, 3 M guanidinium-HCl (Gu-HCl) eluate obtained after chromatography of the pooled peak I fractions on DNase I agarose. The polypeptide of  $M_r \sim 105,000$  (in this gel system) coelutes with actin (open triangles); lane 5, peak activity fraction obtained after chromatography of the dialyzed Gu-HCl eluate (lane 4) on a MonoQ anion exchange column; lane 6, the purified protein, obtained by rechromatography of the MonoQ fractions (shown in lane 5), on a MonoQ column in the presence of 6 M urea. The purified protein showed a  $M_r$  of  $\sim 93,000$  in the SDS-PAGE system used here and its ratio to actin was somewhat higher after MonoQ separation (lane 5) than before (lane 4). We do not know whether this is a preparative artifact or reflects the enrichment of equimolar complexes formed in the oocyte. (b) Two-dimensional gel electrophoreses of the purified major actin-modulating protein of *Xenopus laevis* ovary (first dimension: isoelectric focussing; second dimension: SDS-PAGE). (B) BSA and rabbit muscle  $\alpha$ -actin (A) were used as markers in coelectrophoresis. The brackets denote two polypeptides each with an isoelectric variant.

2 mM  $MgCl_2$  and 1 mM ATP, effects of the protein on both the lag phase of polymerization and the final viscosity were noted (Fig. 3 a). The nucleation phase was markedly shortened and the end viscosity was considerably reduced. For example, at a molar ratio of 1:8 with respect to actin, the protein reduced the end viscosity by  $\sim 80\%$ .

The effect of the purified  $M_r \sim 93,000$  protein on preformed microfilaments is shown in Fig. 3 b. When the protein was added to actin polymerized by the addition of  $Mg^{2+}$  and ATP, which results in a steady state equilibrium of filaments and monomeric actin, a drastic drop in viscosity was observed (e.g.,  $\sim 60\%$  at a molar ratio of 1:8).

The activities of the  $M_r \sim 93,000$  protein and its complexes with actin were also influenced by the  $Ca^{2+}$  concentration (data not shown here; for details see reference 2). In the presence of  $Ca^{2+}$  the low shear viscosity of actin solu-

tions was dramatically reduced, for example, by  $\sim 60\%$  at a ratio of 1:1,400. In this assay the effect of preparations in which the molar ratio of this protein to actin was somewhat higher than 0.5 (cf. Fig. 2 a, lane 5) was less marked; at comparable molar ratios,  $Ca^{2+}$ -induced reduction of viscosity by only 20% (2). In the presence of 1 mM EGTA (i.e., at a  $Ca^{2+}/EGTA$  ratio of  $\sim 0.2$ ), the activity of the protein was strongly reduced and the complex was less sensitive to reduction of  $Ca^{2+}$  than the uncomplexed protein (2). However, when the actin-modulating protein was present at very high concentrations, its activity was not influenced by EGTA.

### Cytoplasmic Location and Concentration of the Actin-binding Protein

The intracellular distribution of the actin-binding protein in *Xenopus laevis* oocytes is illustrated in Fig. 4 a. Oocytes were fixed with TCA, followed by manual separation of nuclei ("germinal vesicles") and ooplasm, and the proteins recovered in either compartment examined by SDS-PAGE and subsequent immunoblot with antibodies raised against the purified  $M_r \sim 93,000$  protein (Fig. 4 a). The results showed that the nuclei contained only small amounts, if any, of the protein ( $\sim 2\%$ , as estimated from radioactivity determinations), in agreement with the results obtained by immunofluorescence microscopy (see below). It is quite possible that the minuscule amounts of nuclear protein detected in such immunoblots were due to contamination with juxtannuclear cytoplasmic proteins. Similar results were obtained when nuclei and ooplasm of unfixed oocytes were separated (data not shown).

The distribution of the  $M_r \sim 93,000$  protein in subcellular fractions as determined by SDS-PAGE and immunoblotting is also shown in Fig. 4 a (lanes 3-6). Relatively little of the protein was recovered from the low speed pellet, whereas the bulk of it was found in supernatant fractions.

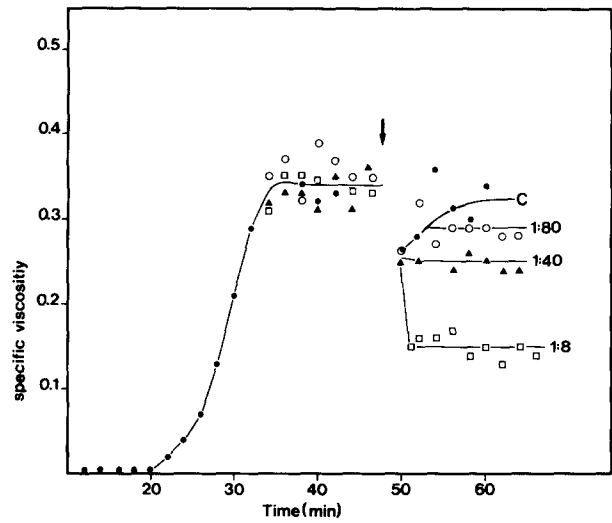
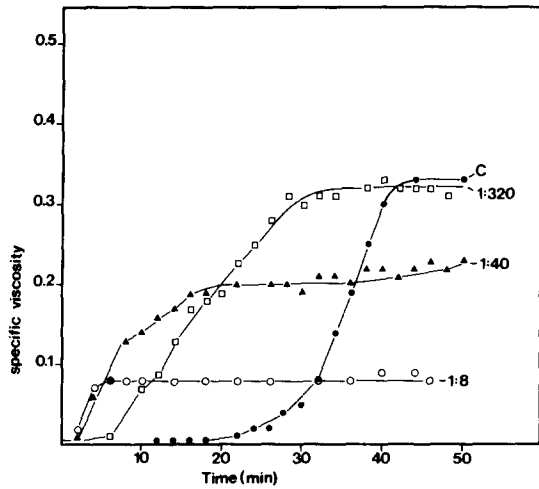
The total amount of the  $M_r \sim 93,000$  protein was estimated to be 125 ng per oocyte, the ratio of actin (cf. references 37, 42) to this protein as  $\sim 12$ , and the cytoplasmic concentration as  $\sim 0.19$  mg/ml.

### Presence of the Actin-binding Protein in Eggs and Embryos

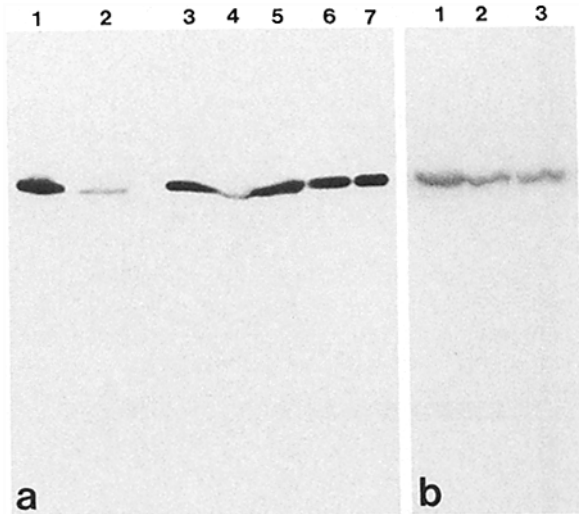
To elucidate the fate of the actin-binding protein during embryogenesis, unfertilized eggs, and embryos were analyzed by gel electrophoresis and immunoblotting (Fig. 4 b). These blots (Fig. 4 b, lanes 2 and 3) revealed a similar concentration of the protein in unfertilized eggs and in stage 6.5 morulae.

### Comparison of the Oocyte Protein with Actin-binding Proteins of Other Cells

Previously, Corwin and Hartwig (13) have described a  $M_r$  95,000 protein with  $Ca^{2+}$ -dependent, actin filament-severing activity in oocytes of the toad, *Bufo marinus*, which they reported to be villin. However, using villin antibodies that reacted with the brush border of amphibian intestine, we had not observed significant reactions with *Xenopus* oocytes and in view of the general experience that villin is expressed only in certain epithelial differentiation pathways (cf. references 5, 15, 51), we examined the possible relationship between the



**Figure 3.** Characteristics of purified major actin-modulating protein from *Xenopus* oocytes. (Left) Effects on the polymerization of rabbit skeletal muscle actin as measured in an Ostwald-type viscometer. The purified protein was added to an actin solution (final concentration: 0.3 mg/ml) in various molar ratios (indicated at the right hand end of each curve) and the kinetics of actin polymerization were compared with those of the control (C, only buffer was added to the actin solution). Polymerization was induced by addition of  $MgCl_2$  and ATP to concentrations of 2 and 1 mM, respectively. Molar ratios of actin to the  $M_r \sim 94,000$  protein (gelsolin is indicated). Note the earlier initiation of actin polymerization and also the marked reduction of end viscosity in the presence of the actin-modulating protein. (Right) Influence of the major actin-modulating protein from *Xenopus laevis* oocytes on preformed actin filaments. Skeletal muscle actin (0.3 mg/ml) was polymerized at 25°C by addition of 2 mM  $MgCl_2$  and 1 mM ATP. After reaching a steady state, the purified actin-binding protein was added in various molar ratios (indicated). The time of addition is marked by an arrow. The change in viscosity was measured using an Ostwald-type viscosimeter. Addition of the purified protein in a molar ratio of 1:8 reduced the steady state viscosity to about 50%. C, buffer control.

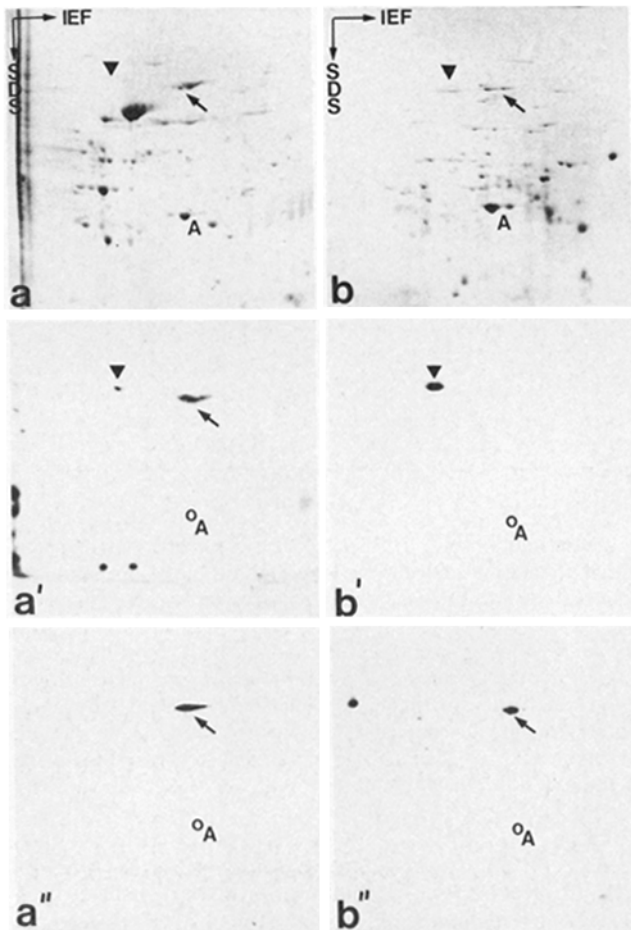


**Figure 4.** Detection of the major actin-modulating protein in different subcellular fractions of *Xenopus laevis* oocytes (a) as well as in eggs and early embryos (b) by SDS-PAGE and immunoblotting (only the autoradiograms are shown). (a) The amount of the protein of two manually isolated ooplasm (lane 1) as compared with that detected in 20 nuclei manually isolated from TCA-fixed oocytes (lane 2). Note the much higher concentration of the protein in the cytoplasm. In lanes 3-6 the protein present in soluble and pelletable fractions was compared with 0.25 µg purified protein (lane 7): lane 3, two total oocytes; lane 4, low speed pellet (10 min, 15,000 g, 4°C) of three homogenized oocytes; lane 5, supernatant fraction corresponding to lane 4; lane 6, high speed (1 h  $\times$  100,000 g, 4°C) supernatant corresponding to lanes 4 and 5. The proteins of the different fractions were separated by SDS-PAGE, transferred to nitrocellulose and probed using guinea pig antibodies raised against the

$M_r \sim 93,000$  oocyte protein and villin in extracts of ovaries (Fig. 5, a-a'') and intestinal mucosa (Fig. 5, b-b'') using antibodies that allowed us to distinguish the two proteins. As shown in Fig. 5, a-a', the villin antisera used reacted with a polypeptide that was much more basic and slightly lower in SDS-PAGE mobility (arrowhead in Fig. 5 a') as well as with the  $M_r \sim 93,000$  actin-binding protein (arrow in Fig. 5 a) whereas the antibodies specific for the latter protein reacted only with one component (arrow in Fig. 5 a''). In ovaries, the Coomassie Blue staining intensity of the villin candidate component was very low and the specificity of the antibody reaction difficult to assess. Both polypeptides, villin and the more acidic  $M_r \sim 93,000$  protein, were also identified in intestinal cells (Fig. 5, b-b''). At present, we have no explanation for the observation that the villin antisera used did not react with the intestinal  $M_r \sim 93,000$  polypeptide.

We therefore screened extracts from several other tissues and from cultured cells of *Xenopus laevis* for the presence of the  $M_r \sim 93,000$  actin-modulating protein, and found it in kidney epithelial cells of line A6 as well as in skeletal, cardiac, and smooth muscle tissues (data not shown; for details see reference 2). These results also showed that the polypeptide identified was among the more frequent proteins of diverse muscle tissues and indicated that it was an actin-binding protein of rather widespread cell-type occurrence.

purified major actin-modulating protein for *Xenopus laevis* ovary (see Fig. 2 a, lane 6). (b) Comparison of the amount of reactive protein present in *Xenopus laevis* ovaries (lane 1: 1 h  $\times$  100,000 g supernatant) with that present in fertilized eggs (lane 2, equivalent to lane 1) and in embryos of the morula stage (stage 6.5; lane 3).



**Figure 5.** Comparison of the major actin-modulating protein of  $M_r \sim 93,000$  from *Xenopus laevis* oocytes with villin by two-dimensional gel electrophoresis (as in Fig. 2 *b*) and immunoblotting. Proteins of postribosomal extracts from total *Xenopus* ovaries (*a*) and whole intestinal mucosa of *Xenopus* (*b*) were separated by two-dimensional gel electrophoresis and stained by Coomassie Blue (*a* and *b*) or processed, in parallel, for immunoblotting (autoradiograms are shown in *a'*, *a''*, *b'*, *b''*). After transfer of the proteins to nitrocellulose, polypeptides were reacted with rabbit antisera against porcine villin (*a'*, *b'*, immunoblots) and guinea pig antibodies against the purified  $M_r \sim 93,000$  actin-modulating protein from *Xenopus* ovaries (*a''*, *b''*). In (*a'*, *a''*, *b'* and *b''*) the position of actin (*A*) as determined after staining with Ponceau S is indicated. Note that the villin antiserum reacted with a polypeptide much more basic (*arrowheads*) than the  $M_r \sim 93,000$  polypeptide (*arrows*) detected with the specific antibodies. The villin antisera showed only a very weak reaction with a polypeptide at a similar position in *Xenopus laevis* ovary (*a'*; *arrowhead*) which, however, gave a peptide map completely different from that of the intestinal villin spot shown in *b*. The antibodies against the actin-modulating protein of *Xenopus laevis* oocytes reacted only with the polypeptide which was nearly isoelectric with actin (*a''* and *b''*; *arrow*), as expected from Fig. 2 *b*, and did not cross react with villin or the villin-related "spot" in ovaries. We have no explanation for the reactivity of villin antisera with the actin-modulating component of ovaries (*a'*), but not with that present in intestinal tissue (*b'*).

The relationship between the polypeptide reacting in the diverse cell types with the antibodies to the  $M_r \sim 93,000$  oocyte protein were also examined by peptide mapping. While the results (Fig. 6, *a*–*c*) confirmed the immunological

identification, minor differences observed between the polypeptides from different tissues could be taken as indications of cell-type-specific isoforms within a group of proteins or of the occurrence of certain cell-type-specific modifications.

### Immunolocalization

Immunofluorescence microscopy using the antibodies against the  $M_r \sim 93,000$  showed strong, mostly diffuse cytoplasmic staining in oocytes (Fig. 7, *a* and *b*) and also in cells of intestinal mucosa and submucosa (Fig. 7 *c*), showing some enrichment in the terminal web-brush border region of the intestinal epithelium (cf. reference 58) and on myofibrils in muscle. Nuclei were not significantly stained.

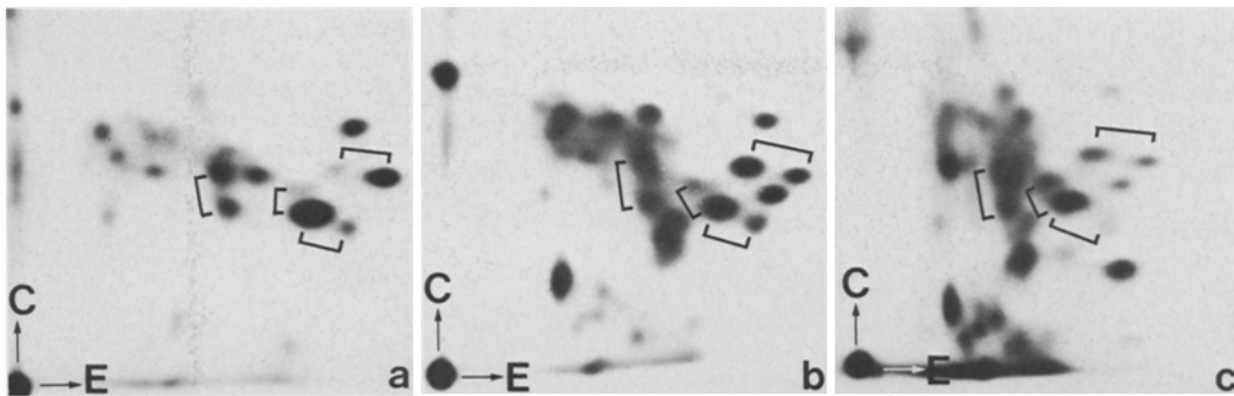
### Cloned cDNA and Amino Acid Sequences: Identification of the Protein as Gelsolin

Using the guinea pig antibodies against the  $M_r \sim 93,000$  actin-binding polypeptide doublet (see above) we isolated a cDNA clone from a *Xenopus laevis* ovary expression library with an insert of  $\sim 1.5$  kb and determined its nucleotide sequence (Fig. 8).<sup>2</sup> This sequence revealed a long open reading frame, followed by a noncoding region of 188 nucleotides presenting a polyadenylation signal located 16 nucleotides before a polyA stretch, of which 17 nucleotides were included in the clone. This indicated that the antibodies had reacted with the carboxy-terminal region of the protein. Computer search and comparison with the amino acid sequence deduced from this open reading frame with amino acid sequences of other proteins displayed a high degree of homology between this *Xenopus* protein and the sequence of human gelsolin (Fig. 9 determined from cDNA and genomic clones of references 32 and 33). To exclude the possibility of a "hybrid" protein, naturally occurring or resulting from a cloning artifact, and to distinguish between the various possible forms of cytoplasmic and secreted gelsolin, we also determined the amino-terminal sequence directly from the purified protein. The resulting amino-terminal sequence extending to residue 20 (Fig. 9) also showed very high degree of homology to those reported for cytoplasmic gelsolin of human, porcine, rabbit, and chicken origin (32, 33, 40, 57, 59). Of the 437 amino acid positions that could be directly compared with the corresponding human sequence, 316 (70%) were identical and another 40 were conservative exchanges.

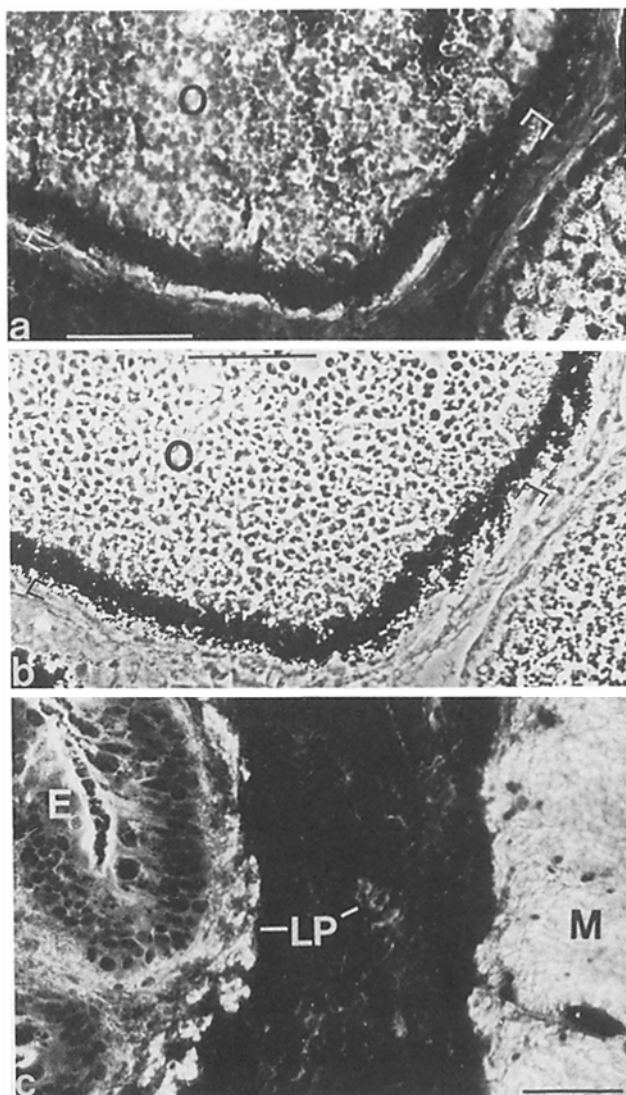
### Discussion

Our results show that gelsolin is a protein highly conserved during vertebrate evolution and is the predominant actin-binding and actin-modulating moiety in oocytes of *Xenopus laevis* and other amphibia (*X. borealis*; in the anuran species, *Pleurodeles waltlii*, the SDS-PAGE mobility of this polypeptide is considerably higher, corresponding to  $M_r \sim 85,000$ ; data not shown). Probably it is also present in oocytes and eggs of other vertebrates. The amount of gelsolin present in the amphibian ooplasm is relatively high, although it is still much below the concentration of the actin present. Therefore, we think that gelsolin is the most frequent component that can contribute to the astonishingly high concentrations

2. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number 400800.



**Figure 6.** Peptide maps of polypeptides reactive with antibodies against the actin-modulating  $M_r \sim 93,000$  protein from *Xenopus laevis* oocytes, identified by immunoblotting as shown in Fig. 8. Polypeptides were excised from gels, radioiodinated, digested with TPCCK-treated trypsin, and the resulting peptides were separated by electrophoresis (*E*) and chromatography (*C*) on cellulose plates. (*a*)  $M_r \sim 93,000$  polypeptide present in oocyte; (*b*) corresponding polypeptide of culture A6 cells; (*c*) corresponding polypeptide of heart muscle tissue. Peptide maps of the actin-modulating protein from A6 cells and heart muscle were very similar to each other, whereas the oocyte protein showed, besides similarities (*brackets*) a number of different peptides (for discussion, see text).



**Figure 7.** Immunofluorescence microscopy showing the distribution of the actin-modulating protein of  $M_r \sim 93,000$  in cryostat sections of *Xenopus laevis* oocytes (*a* and *b*) and intestine (*c*). The sections

of soluble actin in the ooplasm (for references, see Introduction), although we do not exclude contributions of less frequent and/or less active actin-binding proteins in the same compartment (for examples in oocytes of other species, see references 7, 9, 56). Recently, for example, a protein of the nonerythroid spectrin (fodrin) family has been identified in *Xenopus* oocytes (23).

We think that the  $M_r \sim 95,000$  protein of toad oocytes described as "villin" by Corwin and Hartwig (13) is probably gelsolin, which is known to be related to villin by size, amino acid sequence homology, and immunological cross-reactivity (cf. references 32, 36). Remarkably, the amino acid sequence homology in the "homology box" of the carboxy-terminal half ("fragment 51T") of chicken villin is even higher with *Xenopus* gelsolin (16 identical residues out of 30) than with human gelsolin (32). On the other hand, despite our failure to localize significant amounts of villin in oocytes, and the general experience in other species that villin expression is restricted to certain epithelial differentiations of advanced embryogenesis (5, 15, 51), we have to state that a minor polypeptide with similar electrophoretic coordinates as villin, reactive with a villin antiserum, occurs in some ovarian cells. This protein may well be located in follicular or interstitial cells, but at present we cannot formally exclude the possibility that oocytes contain, in addition to the abundant gelsolin, minor amounts of villin or a villinlike protein. Clearly, amphibian oocytes must contain some protein with an actin filament bundling activity as highly ordered microfilament bundles have been described in oocyte cortices (e.g., reference 19).

The gelsolin identified in amphibian oocytes and eggs is more acidic than that reported from mammalian cells, as judged from the isoelectric points in the presence of high

were fixed with 10% formaldehyde and treated with antibodies (antiserum diluted 1:10) against the actin-modulating protein (*a*). General staining of the oocyte cortex (demarkated by brackets) and ooplasm (*b*, phase-contrast image corresponding to *a*). *O*, ooplasm. In the intestine (*c*) epithelial cells (*E*) and smooth muscle (*M*) were stained intensely, whereas cells of the lamina propria (*LP*) were weakly, if at all, reactive. Bars, 50  $\mu\text{m}$ .

1 TTCATTCCAAGATGGGCTACCCAAAAAACCAGGTCCAAGTGCAGAGAGTGGCGAGACTCCTCTGTTAAACAGTTTTTCAAG  
 1 F I S K M G Y P K Q T Q V Q V L P E S G E T P L F K Q F F K  
 81 AACTGGCGAGACAAGGAGCAACTGACGGCATGGGTGTGCCTATGTCGCCAACACATTCGCCAAATTGAGAATGGCCTTCGATGTT  
 1 N W R D R K E A T D G M G V A Y V P N H I A K I E N V P F D V  
 181 ACTGTTCTACAGCAATCCCGCCATGGCTGCCAGCAGCGCATGGTGGATGGATCGGCAAAAAACAGATTTGGCGTATTGAAAAC  
 81 T V L H E S P A M A A Q H G M V D D G S G K K Q I W R I E N  
 271 TGTGAGAAAGTCCCGTTTTGGAATCGCATTACGGACAGTTTTATGGTGGAGACAGCTACATCATTGTATCATTATAAATCTGGTGG  
 91 C E K V P V L E S H Y G Q F Y G G D S Y I I L Y H Y K S G G  
 361 AAACAAGGCGAGATCATCTACATGGCAAGGGGATGATCAACAAGGACGAGATCACCGCTCGGCAATCCTGAGCGCCAGCTGGAC  
 121 K Q G Q I I Y T W G T Q G D D T K D E I T A S A I L S A Q L D  
 451 GAGGAGCTTGGTGGGCCAGTGCAGGTGCGGGTCTGCAGGGCAAGGAACCTGCCCATCTAATAAGCCTGTTCCGTTGAAAACTATG  
 151 E E L G G G P V Q V R V V Q G K E P A H L I S L F G G K P M  
 541 ATTATCTCAAAGGGGAACCTCCAGAGAAGCGGTGAGCAAAAGGATGCGAATGTCCTCTCTTCCAAGTCCGCACATCCTCTTCTGGA  
 181 I I Y K G G T S R E G G Q T K D A N V R L F Q V R T S S S G  
 631 TTCAGCAGAGCAGTAGAGTGGCAATACAGCTAGCAATCTGAACCTCAATGATCGCTTTGACTGACGACACCCTCTGCTTCCTACCTG  
 211 F S R A V L E V D T A S N L N S N D A F V L T T P S A S Y L  
 721 TGGGTTGGCAAGGTTCCACTAATGTGAAAAAAGATGGAGCCAAAGAACTACTAAAGATCTTGGGAGTCTCTGCGTCTGAGATACCAGAA  
 241 W V G Q G S T N V E K N G A K E L L K I L G V S A S E I P E  
 811 GGGCAGAAACTGATGACTTCTGGGGAGCTAGGCGGCAAGCAGACTACCGTACCTCCGCAAGACTAAAGGACAAGTTGAACGCCCAT  
 271 G Q E T D D F W G A L G K A D Y R T S A R L K D K L N A H  
 901 CCTCTCGTCTGTTTGCCTGTCTAACAAGACTGGCGCTTATTATTGAGGAGTTCAGGAGAAATCTCTCAGGATGATCGCCACA  
 301 P P R L F A C S N K T G R F I I E E V P G E I S Q D D L A T  
 991 GATGATGTCATGCTGTTGGATCTTGGGATCAGGTGTATGCTGGTGGTAAAGAGCCAGGAAGTGAAGAAAGAGCCATAGCA  
 331 D D V M L L D T W D Q V Y V W V G N E A Q E D E K K E A I A  
 1081 TCAGCCTACAAGTACATAGAGTGCACCTGCAAAACAGAGACAAGGAGGACTCCAGTTGCCATCACCAGCAAGGATTTGAGCCCCCACT  
 381 S A Y K Y I E S D P A N R D K R T P V A I T K Q G F E P P T  
 1171 TTCATAGGCTGGTCTCTGGTGGGAGGCTGATTACTGGGATGTCGACCCCTGGAGAGGGCAATGGCAGGTCTGAGCAGTTAATTGGAC  
 391 F I G W F L G W E A D Y W D V D P L E R A M A G L S S \*  
 1261 AAGCTGAACCCCTCCCGTGAATATCTCTGTGCCTTACCAGACTAGGAGATGTGCTCAGCCAGAGACTTCTCTAGCCCCACTTAGCTGTG  
 1351 CAATAACCAAGTGCCTCCCTCCCGTGTGACCTTGCCTTCTGACTATTGGAATATTTAAAGAAATAAGCAACCACATGTT  
 1441 CTAAAAAAAAAAAAAAAAAA

Figure 8. Nucleotide sequence of cDNA (clone  $\lambda$ gel X) selected from  $\lambda$ gt11 expression library of *Xenopus laevis* ovary (for details, see reference 31) and the amino acid sequence derived therefrom (one-letter code). The stop signal is denoted by an asterisk, the polyadenylation signal is underlined.

concentrations of urea (compare, for example, Fig. 2b of this study with Fig. 2 of reference 59). Obviously, our determination of the amino-terminal sequence of the *Xenopus* oocyte gelsolin excludes a plasma gelsolin with a "plasma extension", but it remains to be seen whether the gelsolin present in amphibian blood plasma is different from the homologous cytoplasmic gelsolin, as in several mammalian species (32, 33, 57, 59), or is identical to it, as it has been described in the chicken (40). In view of the recent finding of Kwiatkowski et al. (33) that diverse human cell types secrete

plasma gelsolin, we presently cannot exclude the possibility that oocytes may also secrete some plasma gelsolin. Like other authors investigating with mammalian gelsolin (cf. reference 59) we have also observed heterogeneities of *Xenopus* oocyte gelsolin with respect to sizes as well as peptide map and antibody reaction differences between gelsolin samples from different cell types. This may be due to limited proteolytic degradation or to posttranslational modifications (for examples of conformation-dependent epitope differences, see reference 27). Clearly, interpretations of possible cell-

1 MAPHRPAPALLCALSLALCALSLPVRAATASRGASQAGAPGGRVPEARPNMVMVEHPEFLKAGKEPGLQIWRVEKFDLVPVPTNLGYDFFTGDAYVILKT  
 \* \* \* \* \*  
 -----VXDHPFEKAGKDPGLQXWR-----  
 101 VQLRNGNLQYDLHYWLGNECSQDESAAAAIFTVQLDDYLNGRAVQHVQGFESATFLGYFKSGLKYKKGVAAGSGFKHVVNPVQVQLFQVKGRRVVR  
 -----  
 201 TEVPVSWESFNNGDCFILDLGNHQQWCSNSRYERLKAQVSKGIRDNERSGRARVHVSEEGTEPEAMLQVLGPKPALPAGTEDTAKEDAANRKLAKL  
 -----  
 301 YKVSNGAGTMSVSLVADENPFAGALKSEDCFLDHGKDGKIFVWKGKQANTEERKAALKTASDFITKMDYPKQTQVSVLPEGGETPLFKQFFKNWRDPD  
 \* \* \* \* \*  
 -----FISKMGYPKQTQVQLPESGETPLFKQFFKNWRDKE  
 401 QTDGLGLSYLSSHIANVERVPFDDAATLHTSTAMAAAGHGMDDGDTGQKGIWRIEGSKVVPVDPATYGGFYGGDSYIILYNYRHGGRRQQIYINWGAQSTG  
 \* \* \* \* \*  
 37 ATDGMVAVYPNHIAKINENVPDVTVLHESPAAMAAGHGMDDGSGKQGIWRIENCKEVPVLESHYGGFYGGDSYIILYHYKSGGKQGGIITYWGGDDSTK  
 -----  
 501 DEVAASA ILTAQLDEELGGTPVQSRVQVQKPEAHLMSLFGGKPMIYKGGTSREGGQTAPASTRLFQVRANSAGATRAVEVLPKAGALNSNDAFVLKTPS  
 \* \* \* \* \*  
 137 DEITASA ILSAQLDEELGGTPVQSRVQVQKPEAHLISLFGGKPMIYKGGTSREGGQTDANVRLFQVTRSSGFSRAVEVDNTASNLNSDAFVLKTPS  
 -----  
 601 AAYLWVGTGASEAEKTAQELLRLVRAQPVQVAEGSEPDGFWEALGGKAAAYRTSPRLKDKKMDAHPRLFACSNKIGRFVIEVPGELMQEDLATDDVML  
 \* \* \* \* \*  
 237 ASYLWVGGGSTNVEKNGAKELLLKILGVSASEIEGQETDDFWGALGGKADYRTSARLKD-KLNAAHPRLFACSNKTRGFIEVPGELISQDDLATDDVML  
 -----  
 701 LDTWDQVYVWVGKDSQEEKTEALTSAKRYIETDPANRDRRTPITVVKQGFEPSPVFWGLGWDYDYSVDPLORAMAELAA  
 \* \* \* \* \*  
 336 LDTWDQVYVWVNGEAGEDEKKEIASAYKIIESDPANRDKRTPVAITKQGFEPPTFIWGLQWEADYVDVPLERAMAGLSS

Figure 9. Comparison of the amino acid sequence determined from human plasma gelsolin cDNA (upper line; taken from reference 32) with portions of the amino acid sequence of the actin-modulating protein of  $M_r \sim 93,000$  from *Xenopus laevis* ovaries (lower line), specifically the amino-terminal sequence of the native protein (sequence underlined; X, amino acid not clearly identified) and the cDNA-derived amino acid sequence shown in Fig. 8. Identical residues are denoted by asterisks. The "villin homology box" of residues 414-444 (upper sequence) is discussed in the text.



type differences would profit from information on the organization of the gelsolin gene(s) of *Xenopus laevis*, a species in which the allelic complexity is greater than in humans (cf. references 32, 33) due to its tetraploid character.

The recovery of most of the oocyte gelsolin in complexes of  $M_r \sim 180,000$  (by gel filtration) sedimenting with  $\sim 8.2S$  in sucrose gradients that display molar ratios of almost 1:2 with respect to actin indicates that the bulk of the gelsolin occurs in 1:2 complexes with actin, as they are typical of gelsolin in the presence of  $Ca^{2+}$ , and that only little gelsolin is uncomplexed (for  $S$  values of pure gelsolin and of the 1:1 complex, see reference 28; see also references 6, 8, 14, 53; for dissociation rates of such complexes, see also reference 34). Apparently, such  $Ca^{2+}$ -dependent, "capping" complexes would be effective in controlling the growth of actin microfilaments. However, as our isolations were performed in buffers containing  $Ca^{2+}$  we cannot decide whether these gelsolin complexes were present in these oocytes or were artificially formed during preparation. Our findings of gelsolin-actin complexes also seem to explain the report of Merriam and Clarke (37) that the majority of the nonfilamentous oocyte actin from *Xenopus* oocytes is in complexes of apparent molecular masses of 100,000 or greater that cannot be brought to polymerization by addition of  $K^+$  and  $Mg^{2+}$  salts.

Gelsolin appears to be spread over the ooplasm, whereas only a little, if any, of this protein is contained within the confines of the nuclear envelope, suggesting that the protein does not considerably contribute to the regulation of the predominantly unpolymerized state of nuclear actin (for references see Introduction). Our results do not exclude the possibility that a certain cortical enrichment of gelsolin occurs, as described for various other cells (58).

From our data we propose that the delicate regulation of the storage of a large pool of soluble actin and of actin polymerization in the various regions of the ooplasm, with the coexistence of cortical bundles of actin filaments, involves gelsolin, probably in response to changes in the  $Ca^{2+}$  concentration. The same protein is also abundant in eggs and early embryos, indicating that gelsolin is the major component instrumental in the actin modulations involved in the morphological changes and the diverse functions in oogenesis, during oocyte maturation, in the egg, during fertilization, and early embryogenesis. Many of these processes have been reported to depend on changes in the concentrations of  $Ca^{2+}$  and protons (for references see 38, 47, 54). This points to a role of key importance for maternally supplied gelsolin, a protein well suited for both  $Ca^{2+}$ -dependent and  $Ca^{2+}$ -independent controls of various forms of actin assembly and also capable of responding to changes of pH (6, 26, 53, 57, 59), in the morphogenic and motile processes of fertilization, cleavage stages, and early embryonic differentiation during amphibian development (cf. references 17, 23, 29, 44, 47, 49, 54) and probably also the early embryogenesis of mammals.

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## References

1. Achtstätter, T., M. Hatzfeld, R. A. Quinlan, D. C. Parmelee, and W. W. Franke. 1986. Separation of cyokeratin polypeptides by gel electrophoresis and chromatographic techniques and their identification by immunoblotting. *Methods Enzymol.* 134:355-371.
2. Ankenbauer, T. 1988. Reinigung und Charakterisierung Actin-bindende Proteine der Amphibien-Oocyte. Ph.D. thesis, University of Heidelberg, Heidelberg, Federal Republic of Germany. 1-117.
3. Bauw, G., M. de Loose, D. Inzé, M. Van Montagu, and J. Vandekerckhove. 1987. Alterations in the phenotype of plant cells studied by  $NH_2$ -terminal amino acid-sequence analysis of proteins electroblotted from two-dimensional gel-separated total extracts. *Proc. Natl. Acad. Sci. USA.* 84:4806-4810.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
5. Bretscher, A., and K. Weber. 1979. Villin: the major microfilament-associated protein of the intestinal microvillus. *Proc. Natl. Acad. Sci. USA.* 76:2321-2325.
6. Bryan, J., and L. M. Coluccio. 1985. Kinetic analysis of F-actin depolymerization in the presence of platelet gelsolin and gelsolin-actin complexes. *J. Cell Biol.* 101:1236-1244.
7. Bryan, J., and R. E. Kane. 1982. Actin gelation in sea urchin egg extracts. *Methods Cell Biol.* 25:176-199.
8. Bryan, J., and M. C. Kurth. 1984. Actin-gelsolin interactions. Evidence for two actin-binding sites. *J. Biol. Chem.* 259:7480-7487.
9. Christensen, K., R. Sauterer, and R. W. Merriam. 1984. Role of soluble myosin in cortical contraction of *Xenopus* eggs. *Nature (Lond.)* 310:150-151.
10. Clark, T. G., and R. W. Merriam. 1977. Diffusible and bound actin in nuclei of *Xenopus laevis* oocytes. *Cell.* 12:883-891.
11. Clark, T. G., and R. W. Merriam. 1978. Actin in *Xenopus* oocytes. I. Polymerization and gelation in vitro. *J. Cell Biol.* 77:427-438.
12. Cooper, J. A., and T. D. Pollard. 1982. Methods to measure actin polymerization. *Methods Enzymol.* 85:182-210.
13. Corwin, H. L., and J. H. Hartwig. 1983. Isolation of actin-binding protein and villin from toad oocytes. *Dev. Biol.* 99:61-74.
14. Coué, M., and E. D. Korn. 1985. Interaction of plasma gelsolin with G-actin and F-actin in the presence and absence of calcium ions. *J. Biol. Chem.* 260:15033-15041.
15. Dudouet, B., S. Robine, C. Huet, S. Sahuquillo-Merino, L. Blair, E. Coudrier, and D. Louvard. 1987. Changes in villin synthesis and subcellular distribution during intestinal differentiation of HT29-18 clones. *J. Cell Biol.* 105:359-369.
16. Elder, J. H., R. A. Pickett, J. Hampton, and R. A. Lerner. 1977. Radioiodination of proteins in single polyacrylamide gel slices. *J. Cell Biol.* 252:6510-6516.
17. Ezzell, R. M., A. J. Brothers, and W. Z. Cande. 1983. Phosphorylation-dependent contraction of actomyosin gels from amphibian eggs. *Nature (Lond.)* 306:620-622.
18. Fleming, T. P., S. J. Pickering, F. Qasim, and B. Maro. 1986. The generation of cell surface polarity in mouse 8-cell blastomeres: the role of cortical microfilaments analysed using cytochalasin D. *J. Embryol. Exp. Morph.* 95:169-191.
19. Franke, W. W., P. C. Rathke, E. Seib, M. F. Trendelenburg, M. Osborn, and K. Weber. 1976. Distribution and mode of arrangement of microfilamentous structures and actin in the cortex of the amphibian oocyte. *Cytobiologie.* 14:111-130.
20. Franke, W. W., S. Winter, C. Grund, E. Schmid, D. L. Schiller, and E.-D. Jarasch. 1981. Isolation and characterization of desmosome-associated tonofilaments from rat intestinal brush border. *J. Cell Biol.* 90:116-127.
21. Gall, L., B. Picheral, and P. Gounon. 1983. Cytochemical evidence for the presence of intermediate filaments and microfilaments in the egg of *Xenopus laevis*. *Biol. Cell* 47:331-342.
22. Gerke, V., and K. Weber. 1983. Isolation and characterization of mammalian villin and fimbrin, the two bundling proteins of the intestinal microvilli. *Eur. J. Cell Biol.* 31:249-255.
23. Giebelhaus, D. H., B. D. Zelus, S. K. Henchman, and R. T. Moon. 1987. Changes in the expression of  $\alpha$ -fodrin during embryonic development of *Xenopus laevis*. *J. Cell Biol.* 105:843-853.
24. Gounon, P., and E. Karsenti. 1981. Involvement of contractile proteins in the changes in consistency of oocyte nucleoplasm of the newt *Pleurodeles waltlii*. *J. Cell Biol.* 88:410-421.
25. Hamaguchi, Y., and I. Mabuchi. 1988. Accumulation of fluorescently labeled actin in the cortical layer in sea urchin eggs after fertilization. *Cell Motil. Cytoskeleton.* 9:153-163.
26. Hwo, S., and J. Bryan. 1986. Immuno-identification of  $Ca^{2+}$ -induced conformational changes in human gelsolin and brevin. *J. Cell Biol.* 102:227-236.
27. Jahn, L., B. Fouquet, K. Rohe, and W. W. Franke. 1987. Cytokeratins in certain endothelial and smooth muscle cells of two taxonomically distant vertebrate species, *Xenopus laevis* and man. *Differentiation.* 36:234-254.
28. Janney, P. A., C. Chaponnier, S. E. Lind, K. S. Zaner, T. P. Stossel, and

- H. L. Yin. 1985. Interaction of gelsolin and gelsolin-actin complexes with actin. Effects of calcium on actin nucleation, filament severing, and end blocking. *Biochemistry*. 24:3714-3723.
29. Keller, R. E., M. Danilchik, R. Gimlich, and J. Shih. 1985. The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*. *J. Embryol. Exp. Morphol.* 89(Suppl.):185-209.
  30. Kleinschmidt, J. A., and W. W. Franke. 1982. Soluble acidic complexes containing histones H3 and H4 in nuclei of *Xenopus laevis* oocytes. *Cell*. 29:799-809.
  31. Kleinschmidt, J. A., C. Dingwall, G. Maier, and W. W. Franke. 1986. Molecular characterization of a karyophilic, histone-binding protein: cDNA cloning, amino acid sequence and expression of nuclear protein N1/N2 of *Xenopus laevis*. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:3547-3552.
  32. Kwiatkowski, D. J., R. Mehl, and H. L. Yin. 1988. Genomic organization and biosynthesis of secreted and cytoplasmic forms of gelsolin. *J. Cell Biol.* 106:375-384.
  33. Kwiatkowski, D. J., T. P. Stossel, S. H. Orkin, J. E. Mole, H. R. Colten, and H. L. Yin. 1986. Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain. *Nature (Lond.)*. 323:455-458.
  34. Lind, S. E., P. A. Janmey, C. Chaponnier, T.-J. Herbert, and T. P. Stossel. 1987. Reversible binding of actin to gelsolin and profilin in human platelet extracts. *J. Cell Biol.* 105:833-842.
  35. Luchtel, D., J. G. Bluemink, and S. W. de Laat. 1976. The effect of injected cytochalasin B on filament organization in the cleaving egg of *Xenopus laevis*. *J. Ultrastruct. Res.* 54:406-419.
  36. Matsudaira, P., R. Jakes, and J. E. Walker. 1985. A gelsolin-like  $Ca^{2+}$  dependent actin-binding domain in villin. *Nature (Lond.)*. 315:248-250.
  37. Merriam, R. W., and T. G. Clark. 1978. Actin in *Xenopus* oocytes. II. Intracellular distribution and polymerization. *J. Cell Biol.* 77:439-447.
  38. Merriam, R. W., R. A. Sauterer, and K. Christensen. 1983. A subcortical pigment-containing structure in *Xenopus* eggs with contractile properties. *Dev. Biol.* 95:439-446.
  39. Nieuwkoop, P. D., and J. Faber. 1975. Normal table of *Xenopus laevis* (Daudin). North-Holland Publishing Company, Amsterdam. 1-252.
  40. Nodes, B. R., J. E. Shackelford, and H. G. Leberer. 1987. Synthesis and secretion of serum gelsolin by smooth muscle tissue. *J. Biol. Chem.* 262:5422-5427.
  41. Otto, J. J., and T. E. Schroeder. 1984. Assembly-disassembly of actin bundles in starfish oocytes: an analysis of actin associated proteins in the isolated cortex. *Dev. Biol.* 101:263-273.
  42. Paine, P. L. 1984. Diffusive and nondiffusive proteins in vivo. *J. Cell Biol.* 99:188s-195s.
  43. Pollard, T. D. 1982. A falling ball apparatus to measure filament cross-linking. *Methods Cell Biol.* 24:301-311.
  44. Pollard, T. D., and J. A. Cooper. 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu. Rev. Biochem.* 55:987-1035.
  45. Pringault, E., M. Arpin, A. Garcia, J. Finidori, and D. Louvard. 1986. A human villin cDNA clone to investigate the differentiation of intestinal and kidney cells in vivo and in culture. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:3119-3124.
  46. Rungger, D., E. Rungger-Brändle, C. Chaponnier, and G. Gabbiani. 1979. Intracellular injection of anti-actin antibodies into *Xenopus* oocytes blocks chromosome condensation. *Nature (Lond.)*. 282:320.
  47. Sardet, C., and P. Chang. 1987. The egg cortex: from maturation through fertilization. *Cell Differ.* 21:1-19.
  48. Scheer, U., H. Hinssen, W. W. Franke, and B. M. Jockusch. 1984. Microinjection of actin-binding proteins and actin antibodies demonstrates involvement of nuclear actin in transcription of lampbrush chromosomes. *Cell*. 39:111-122.
  49. Selman, G. G., and M. M. Perry. 1970. Ultrastructural changes in the surface layers of the newt's egg in relation to the mechanism of its cleavage. *J. Cell Sci.* 6:207-227.
  50. Seraydarian, K., E. J. Briskey, and W. F. H. M. Mommaerts. 1967. The modification of actomyosin by  $\alpha$ -actinin. *Biochim. Biophys. Acta.* 133:399-411.
  51. Shibayama, T., J. M. Carboni, and M. S. Mooseker. 1987. Assembly of the intestinal brush border: appearance and redistribution of microvillar core proteins in developing chick enterocytes. *J. Cell Biol.* 105:335-344.
  52. Spudich, J. A., and S. Watt. 1971. The regulation of rabbit skeletal muscle contraction. *J. Cell Biol.* 24:4866-4871.
  53. Stossel, T. P., C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, K. S. Zaner. 1985. Nonmuscle actin-binding proteins. *Annu. Rev. Cell Biol.* 1:353-402.
  54. Vacquier, V. D. 1981. Dynamic changes of the egg cortex. *Dev. Biol.* 84:1-26.
  55. Vandekerckhove, J., W. W. Franke, and K. Weber. 1981. Diversity of expression of non-muscle actin in amphibia. *J. Mol. Biol.* 152:413-426.
  56. Wang, L.-L., and J. A. Spudich. 1984. A 45,000-mol-wt protein from unfertilized sea urchin eggs severs actin filaments in a calcium-dependent manner and increases the steady-state concentration of nonfilamentous actin. *J. Cell Biol.* 99:844-851.
  57. Weeds, A. G., J. Gooch, B. Pope, and H. E. Harris. 1986. Preparation and characterization of pig plasma and platelet gelsolins. *Eur. J. Biochem.* 161:69-76.
  58. Yin, H. L., J. H. Albrecht, and A. Fattoum. 1981. Identification of gelsolin, a  $Ca^{2+}$ -dependent regulatory protein of actin gel-sol transformation, and its intracellular distribution in a variety of cells and tissues. *J. Cell Biol.* 91:901-906.
  59. Yin, H. L., D. J. Kwiatkowski, J. E. Mole, and F. S. Cole. 1984. Structure and biosynthesis of cytoplasmic and secreted variants of gelsolin. *J. Cell Biol.* 259:5271-5276.