Published in final edited form as:

Curr Top Dev Biol. 1992 ; 26: 35–52.

Actin and Actin-Associated Proteins in *Xenopus* Eggs and Early Embryos: Contribution to Cytoarchitecture and Gastrulation

Elaine L. Bearer

Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912

I. Introduction

Actin is intimately involved in several of the events on which development depends. Some of these events are known to occur in embryos of many widely divergent species. The amphibian embryo has been widely used as a model system for the exploration of cell biological events that contribute to development, in part because it is a vertebrate system that can be fertilized *in vitro*, and is easily manipulated during the early stages of embryogenesis. This article will review the role of the actin-based cytoskeleton of amphibian eggs during the major events of embryogenesis. In addition, some new information on novel actin-binding proteins that may contribute to the structuring of that cytoskeleton will be provided.

II. Oogenesis

It is now apparent from observations in a variety of species that the cytoplasm of the oocyte is highly structured (Bearer, 1991a). In *Drosophila*, maternally synthesized mRNA and protein are asymmetrically distributed, and that distribution is essential for the establishment of the embryonic axes (Nüsslein-Volhard and Roth, 1989). In *Caenorhabditis elegans*, positional information encoding posteriority is redistributed after fertilization in a process that requires actin (Strome and Hill, 1988), and in ascidian embryos a similar process depends on an actin-dependent cortical contraction that also redistributes cues involved in establishing the posterior parts of the future embryo (reviewed in Jeffrey and Swalla, 1990).

A. The Mitochondrial Cloud

In stage I previtellogenic oocytes of the frog, *Xenopus laevis*, a tight ball of cytoplasm termed the mitochondrial cloud is readily observed in either whole mounts or sections of ovaries (Heasman *et al.*, 1984; see Fig. 4A). The formation of this structure identifies the vegetal hemisphere. In addition to mitochondria, it contains the major cytoskeletal proteins, tubulin, intermediate filaments (Wylie *et al.*, 1985), and actin (T. Whitfield, C. C. Wylie, and E. L. Bearer, unpublished observations), as well as two of the putative factors involved in the future developmental program: the germplasm, containing polar granules, that becomes segregated into the future germ cells during the cleavage stages (Heasman *et al.*, 1984), and Veg-1 mRNA, encoding a member of the transforming growth factor *B* (TGF-*B*) family (Weeks and Melton, 1987; Yisraeli and Melton, 1988).

Later in oogenesis, the mitochondrial cloud fragments and the same constituents are then found in a shell just within the plasma membrane of the vegetal half of the embryo.

Colchicine, which depolymerizes the microtubule system, inhibits the accumulation of the Veg-1 transcript in this shell during stages III and IV of oogenesis, while cytochalasin, which blocks actin polymerization, causes the message to be released from the vegetal shell in stage V or VI oocytes (Yisraeli *et al.*, 1989). It is not yet clear whether this localization of Veg-1 message in the vegetal half is necessary for the future establishment of the embryonic axes. Although the cytochalasin experiments support the notion that actin filaments play a part in retaining the Veg-1 message in its proper location, it is not known how they do this. Other proteins are likely to be involved, either directly as attachment links between the message and the filaments, or indirectly as organizers of the filament system itself. Furthermore, it is not understood why the actin is localized there, whether it is monomeric or polymerized in filaments in this particular place within the cytoplasm (Dent and Klymkowsky, 1988; Franke *et al.*, 1976).

B. Nuclear Actin

In addition to its cytoplasmic roles, actin is also present in the nucleus of the *Xenopus* oocyte (Clark and Merriam, 1977) and may be involved in chromosome condensation, which occurs during oocyte maturation and results in the meta-phase II-arrested state of the mature and fertilizable egg (Rungger *et al.*, 1979). Anti-actin antibodies affect transcription of lampbrush chromosomes in oocytes of the urodele, *Pleurodeles waltii* (Sheer *et al.*, 1984), and thus this nuclear actin may also play a role in transcription during oogenesis.

III. Fertilization and Early Cleavage

During fertilization of most species, the sperm entry into the egg cytoplasm, the exocytosis of cortical granules, and the meeting of sperm and egg pronuclei all appear to require actin filaments (Stewart-Savage and Grey, 1982; reviewed in Vaquier, 1981).

A. Cortical Contractions

In *Xenopus*, the pigmented animal hemisphere contracts isometrically within 5–6 min of sperm entry (Elinson, 1975). The contractile cortex of the zygote is 0.5–3 μm thick, excludes yolk platelets, and contains cortical granules, pigment granules, and actin filaments (Merriam *et al.*, 1983; Christensen *et al.*, 1984). Within 20–30 min the center of gravity is shifted to the vegetal pole, so that all fertilized eggs are oriented with the animal side up, while unfertilized eggs orient randomly, often on their sides. The first contraction relaxes and microtubule networks form in the vegetal hemisphere that are necessary for the subsequent rotation, which sets up the gray crescent and specifies the embryonic axes (see Houliston and Elinson, Chapter 4 in this volume). A second contraction of the pigmented cortex occurs approximately 70 min after fertilization, relaxing as the first cleavage furrow forms. Cytochalasin B has been shown to have no effect on these contractions in *Xenopus*, affecting only the formation of the cleavage furrow. However, by electron microscopy it appears that cytochalasin B does not completely depolymerize the actin filaments even when injected into the egg during these contractions (Luchtel *et al.*, 1976). Thus, the developmental significance of these contractions and their dependence on actin have not been defined. That depolymerization of actin filaments may be permissive for cortical

rotation is implied by the result that cytochalasin treatment induces formation of a morphological gray crescent in unactivated eggs lying on their sides (Manes *et al.*, 1978).

Overaged, unfertilized eggs will undergo a similar contraction followed by a stretching of the animal pole cortex over the vegetal pole reminiscent of epiboly, the movements of the surface epithelium during gastrulation (E. L. Bearer, personal observations; Holtfreter, 1943). This behavior suggests that the cytoskeletal machinery necessary for this movement is present and preprogrammed in the egg.

While the animal hemisphere is contracting, the actin network of the vegetal pole may depolymerize, because after fertilization Veg-1 message is released from its tight shell in the cortex of the vegetal hemisphere, but remains in the vegetal cytoplasm just as it does in mature oocytes treated with cytochalasin B (Weeks and Melton, 1987). In contrast, the polar granules and germplasm, perhaps retained by vimentin-like intermediate filaments, remain in the cortex of the vegetal pole, aggregate, and are divided equally to the first four daughter cells, and unequally in subsequent divisions until they become segregated into the future germ cells (Heasman *et al.*, 1984).

B. Biochemistry of the Contractile Machinery

The contraction of the animal cortex has been studied in some detail biochemically. Extracts of mature eggs can be induced to form a gel in the presence of 0.6 M sucrose. The gel will contract with calcium, with or without ATP (Clark and Merriam, 1978). It contains a 43-kDa protein (actin) and a 250-kDa protein, as well as 53- and 68-kDa proteins in lower amounts. Only in the absence of exogenous ATP is a 200-kDa putative myosin found associated with the contractile gel. Cortices of eggs and embryos can be dissected off the underlying cytoplasm (Franke *et al.*, 1976), or eggs can be cut in half (Christensen *et al.*, 1984). In either of these preparations, the contraction can be elicited and the necessary components extracted and reconstituted. Cortices from oocytes prior to maturation (prior to attaining metaphase II arrest) are refractile to such contractions. A *Xenopus* myosin II-like molecule was found to be soluble under these conditions but necessary for the contractions (Christensen *et al.*, 1984).

The animal cap contraction ultimately results in the formation of the first cleavage furrow, which first becomes visible approximately 75 min after fertilization over the animal hemisphere. The subsequent cleavages all depend on actin for both the formation of the cleavage furrow and the proper segregation of cytoplasmic components. In addition, the cells appear to adhere to each other via an actin-based process, because cytochalasin treatment results in the cells becoming dissociated even within an intact vitelline membrane.

IV. Gastrulation

A. Cell Shape Changes and Cell Movements

The microtubule-dependent rotation of the cortex over the underlying cytoplasm results in an asymmetric distribution of unknown factors that defines the dorsal lip of the blastopore and the embryonic axis. Whatever these asymmetrically distributed factors might be, their continued localization in that region of the embryo probably depends both on proper

segregation of the factors into daughter cells and on some sort of cytoskeletal stabilization. By midblastula transition when the zygotic genome is activated, during interphase of nuclear cycle 9, the cells are apparently programmed to begin the movements of gastrulation. These movements are symmetrical in embryos that were prevented from rotating by UV irradiation (Malacinski *et al.*, 1977; Gerhart *et al.*, 1989), producing ventralized embryos; they are also inaccurate in embryos in which transcription is blocked, suggesting that maternal cues and maternally deposited proteins as well as newly synthesized zygotic gene products are necessary for gastrulatory movements to occur.

The first cells to change shape are the surface cells of the gray crescent, in the marginal zone at the boundary between the large, yolky cells of the vegetal pole and the smaller animal pole cells (Keller, 1981; Hardin and Keller, 1988). These cells have been named “bottle cells” because of their characteristic shape. Initially, these cells remain vegetal to the involuting marginal zone surface epithelium, but they are ultimately also carried inside and contribute to the anterior-most tip of the archenteron (Keller, 1981). During the next two stages of embryogenesis, a wave of bottle cell formation encircles the equator, spreading laterally from either side of the dorsal lip and meeting at the ventral lip to form the complete ring of the blastopore.

During the complex movements that follow, two events stand out: migration of the deep cells of the marginal zone, and spreading of the surface cells of the animal cap. The deep cells originate in the marginal zone of the dorsal lip of the blastopore, in the region between the lateral floor of the blastocoel cavity and the gray crescent. They ultimately contribute to mesodermal structures. Early in gastrulation, the deep cells of the dorsal lip of the blastopore migrate up over the roof of the blastocoel, apparently on a fibronectin substrate (Winklbauer, 1990, and references therein). These migrations appear to involve complex cell shape changes (Winklbauer, 1990) with protrusions of filopodia rather than the conventional leading edges observed in cultured fibroblasts. In contrast, the surface cells apparently retain some of their cell-cell contacts and move collectively as a sheet, which ultimately spreads out as a single layer over the entire surface of the embryo, engulfing the vegetal mass. Only a narrow band of surface cells at the equator actually involutes and these form the inner surface of the roof of the archenteron. The yolky vegetal cells are apparently passively carried along by these movements.

Further evidence that these movements are a combined result of maternal and zygotic elements has been shown by the behavior of cells taken from different parts of the embryo at different stages of development and observed *in vitro* (Nakatsuji, 1986; Winklbauer, 1988). When cells from the animal cap are plated on fibronectin-coated coverslips, they do not migrate until after stage 8. The inner cells from the equatorial region are the earliest to migrate and move more vigorously than cells from other parts of the embryo. The surface epithelium has different adhesion properties than the endodermal cells of the vegetal pole. Although vegetal pole cells adhere to fibronectin, they do not migrate.

B. Actin-Binding Proteins

Because these movements rely on actin, it becomes particularly interesting to determine what molecules regulate the actin filament network such that it can be at the right place and

the right time for stabilization of the Veg-1 message in the vegetal shell, and also be involved in such different concerted cell movements as the shape changes of the surface epithelium and the cell migrations of the deep cells. Some of the factors that regulate actin polymerization should contribute to both processes, while others must be specific, for each process is different.

To date, only a few of the protean numbers of actin-binding proteins have been conclusively found in *Xenopus* oocytes and embryos. These include gelsolin (Ankenbauer *et al.*, 1988), spectrin (Giebelhaus *et al.*, 1987), and myosin (Christensen *et al.*, 1984). Gelsolin is an actin filament-severing and barbed end-binding protein that nucleates filaments from the pointed, or slow-growing, end. In the oocyte, gelsolin apparently binds monomers and prevents the vast pool of actin (4.1 mg/ml) from polymerizing inappropriately (Merriam and Clark, 1978). In addition to these actin-binding proteins, vinculin and talin, cytoskeletal proteins involved in the formation of adhesion plaques, have been found in frog eggs and embryos (Evans *et al.*, 1990), as well as homologs of the integrin (fibronectin) receptors (DeSimone and Hynes, 1988). Talin is known to bind directly to the fibronectin receptor (Horwitz *et al.*, 1986). Thus its contribution to the fibronectin-based migrations of the deep cells over the blastocoel roof may shed light on its involvement in cellular migrations in other systems. That these proteins are present in the egg suggests that they are stockpiled for future use during gastrulation, and that they are not the key elements whose synthesis is triggered by maternal cues in the deep cells.

C. Actin-Associated Proteins Involved in Cell Migrations

It seems likely that key cytoskeletal regulatory elements exist and are turned on in specific cells prior to or during midblastula transition. These key elements would be responsible for initiating the cytoskeletal changes necessary for the different types of cell shape changes that result in the different types of movements of gastrulation. For example, simplistically, if the cell shape changes involved in epiboly relied on a contractile protein, perhaps calcium channels regulating the action of that protein, or the protein itself, would be synthesized only in sufficient levels to respond to contractile stimuli in the surface cells of the animal cap. Or, if actin polymerization-depolymerization is a necessary part of inner cell migrations, then those proteins involved in such reorganizations of the actin cytoskeleton would be specifically induced in deep cells and not in vegetal cells. Conversely, those actin-binding proteins involved in maintenance of the cell cortex or other housekeeping-type actin filament behavior would be stockpiled in the egg, and not differentially expressed just prior to gastrulation. Alternatively, key proteins regulating these disparate actin behaviors may be stockpiled in the egg, either localized in particular regions consistent with their future contribution or becoming localized in those regions during the rotations, contractions, or early cleavages or embryogenesis.

D. MAb 2E4 Antigen

To test this idea, the author has investigated the distribution of an actin-associated protein recognized by the monoclonal antibody, 2E4 (MAb 2E4), in eggs and early embryos. This antibody was originally raised against proteins extracted from human blood platelets that were retained on an F-actin column and eluted with ATP (Bearer, 1991b). The antibody

stains the leading edge of migrating chick embryo fibroblasts, and the antigen-antibody complex associates with the barbed ends of purified actin filaments *in vitro* in an ATP-sensitive manner (Bearer, 1991b,c). Because of its association with the barbed end of actin filaments, which is thought to be the end that elongates through polymerization to form filaments, and because of its location in cells at sites where such polymerization is occurring, the antigen may be involved in regulating actin filament polymerization. The antibody recognizes a 43-kDa protein in blots from a wide variety of species from fruit flies to humans. This band comigrates and copurifies with actin, but can be separated from the bulk of actin in the presence of urea at low pH (Bearer, 1991b).

In polyethylene glycol-embedded sections of stage 9 *Xenopus laevis* embryos, MAb 2E4 stains most prominently the deep cells of the marginal zone (arrow in Fig. 1A). There is some staining of cell margins over the entire animal cap, but the large yolky cells in the vegetal pole are only lightly stained. In contrast, an anti-actin antibody of the same isoform as MAb 2E4, used as a positive control, stains cells equally throughout the embryo (Fig. 1B). In two other embryos viewed at higher magnification (Fig. 1C and D), MAb 2E4 staining again appears more prominent on one side of each of the cells it stains. Both surface epithelium and deep cells in the marginal zone are strongly stained, as well as one side of deep cells in the animal cap. Again, staining of vegetal cells is not observed. A single small cell at the crotch of the blastocoel is also strongly positive (Fig. 1C, arrow).

When the mesoderm is dissected from stage 25 neurulas, cut into small pieces, and cultured on matrigel-coated coverslips, individual cells will crawl out of the mass of tissue. Cells in such cultures were fixed and stained with both MAb 2E4 (Fig. 2A) and fluorescein-conjugated phalloidin, which stains actin filaments (same cell, Fig. 2B). Comparisons of such double-labeled cells reveal that MAb 2E4 antigen appears as punctate dots that overlie the ends of actin filament bundles in the pseudopods projecting from the cell body. Punctate or granular staining by MAb 2E4 is also present in the body of the cell, but the actin filament staining is too dense for a correlation to be made. Microspikes extending from the side of the cell are also stained by both MAb 2E4 and phalloidin, and a granular staining of MAb 2E4 is observed along bundles of actin filaments in two cells at the lower edge of these micrographs. Because long filament bundles are made up of interdigitating shorter filaments, this punctate staining could reflect the ends of filaments within a bundle.

MAb 2E4 recognizes a single band of apparent M_r 43K in homogenates of human blood platelets and chick embryo fibroblasts (Bearer, 1991b). However, in cytoskeletal extracts of stage 10 or 11 embryos made according to a protocol that extracts the actin-binding protein gelsolin from *Xenopus* oocytes (Ankenbauer *et al.*, 1988), MAb 2E4 blots to five bands of approximate molecular weights of 130K, 95K, 48K, 43K, 25K (Fig. 3A, lane 1: Coomassie-stained gel; lane 2: MAb 2E4-stained Western blot). To see which of these five protein species were responsible for the restricted staining pattern, polyclonal mouse antibodies were made to each band cut from a preparative "curtain" gel (James and Elgin, 1986). These antibodies were then tested by blotting to fresh extract to see whether they recognized bands consistent with the immunogen (Fig. 3B), and by indirect immunofluorescence to see if they recognized the same structures stained by MAb 2E4.

Two of the antisera recognized bands of the same molecular weight as the immunogen—band 2, 95kDa and band 3, 48kDa doublet (Fig. 3B, lanes 2 and lanes 3). All three other antisera recognized both a 75kDa band and a 25kDa band, as well as another band that was unique to each antiserum, i.e., band 1 (130k immunogen) blots to a 43kDa band (Fig. 3B, lanes 1), band 4 (43kDa immunogen) blots to a >250kDa band (Fig. 3B, lanes 4), and band 5 (25kDa immunogen) blots to a 130kDa band (Fig. 3B, lanes 5). This complicated blotting pattern suggests that, while the 95kDa and 48kDa species are distinct, the 130kDa, 75kDa, 43kDa, and 25kDa bands are related to each other antigenically. A 75kDa band was not seen in the MAb 2E4 blot, perhaps because this is a proteolytic fragment that does not contain the antigenic epitope recognized by the monoclonal antibody.

The three antisera that blotted to similar bands also gave similar staining patterns on stage 1 oocytes. In plastic sections of stage I oocytes, the mitochondrial cloud is readily identified by Nomarski optics (Fig. 4A, arrow), while the nucleus (Fig. 4A–C, “n”) is also recognizable. In serial sections stained with MAb 2E4, the mitochondrial cloud is more brightly fluorescent than the cytoplasm, and the nucleus is not stained at all (Fig. 4B). Both nucleus and mitochondrial cloud are brightly stained by anti-actin antibodies (not shown). In other serial sections of the same oocyte, antisera 1, 4, and 5 stained the mitochondrial cloud like MAb 2E4, but stained the nucleus as well (antiserum 1 is shown in Fig. 4C). Antiserum 4 also stained the vitelline membrane during its formation later in oogenesis. In contrast, antiserum 3 stained small granular material in the cytoplasm only, and antiserum 2 did not stain oocytes.

The most remarkable staining pattern of the 2E4 monoclonal antibody was the staining pattern in the late blastula (Fig. 1). This pattern was mimicked by antiserum 3. By indirect immunofluorescence performed on plastic sections, antiserum 3 recognized the cells in the crotch of the blastocoel, and one side of the cells over the animal cap (Fig. 5A). In these preparations, the pattern of staining was granular. In whole mounts of gastrulating embryos (stage 10) stained with antiserum 3 by the indirect immunoperoxidase method and clarified by *n*-butanol, a dark reaction product is readily observed in the deep cells beneath the dorsal lip of the blastopore (Fig. 5B). At this time point, bottle cells are at the leading margin of the involuting tissue (Hardin and Keller, 1988), which is the point of darkest staining by antiserum 3. In an embryo observed lying on its side, oriented with the dorsal lip toward the top of the photograph and the animal cap to the left (Fig. 5C), a line of reaction product is seen (small arrows) that extends up to the floor of the blastocoel (larger arrow). The region just vegetal to the blastocoel floor is more darkly stained, and the vegetal pole is not stained. At this time point, actual involution has extended only about half way to the roof of the blastocoel, and thus the staining precedes involution. The roof of the blastocoel has collapsed in this embryo. Bottle cells would not yet have formed in the ventral lip, and no cell movements would have begun. No reaction product is seen in this region, either (Fig. 5C, arrowhead). Parallel staining of embryos from the same preparation, in which the primary antibody was omitted, have no reaction product detectable deep to the dorsal lip, although the pigment line can be seen to identify it. A nonspecific reaction product is present at the vegetal pole (Fig. 5D). Higher magnifications of other embryos at the same

stage of gastrulation reveal the intense staining of the deep cells in the dorsal lip (Fig. 5E and F).

To study the expression pattern of the 48-kDa doublet recognized by antiserum 3, extracts were made of eggs and embryos, the proteins separated by polyacrylamide gel electrophoresis, and the gel either stained with Coomassie to visualize the protein bands (Fig. 6A) or transferred to nitrocellulose and blotted with antiserum 3. Five eggs or embryos were used for each lane. The 48-kDa antigen doublet was not detected until stage 5 of embryogenesis. Both bands of the doublet were equally intense relative to each other at each stage. It was found that some protein is also present in the oocyte, but it is not extracted under the conditions that extract it from the embryo, and is detected only in the pellet.

V. Conclusion

This chapter has reviewed the contribution of actin to the events of oogenesis and early embryogenesis in eggs and embryos of the frog *X. laevis*. This contribution includes the organization of the mitochondrial cloud and the maintenance of the vegetal shell of cytoskeletal components that limits the diffusion of Veg-1 mRNA during oogenesis, the cortical contractions that occur following fertilization, the localization of as yet undefined developmental cues in appropriate places within the cytoplasm and their appropriate apportionment to daughter cells during cytokinesis, and the cell shape changes and motility that produce gastrulation.

In addition, preliminary results have been presented showing that a group of proteins antigenically related to an actin-associated antigen, the MAb 2E4 antigen, is localized in regions of the egg and early embryo where actin filaments play an incisive role in these events. Finally, it has been shown that at least one of these proteins, the 48-kDa doublet recognized by antiserum 3, is preferentially expressed prior to midblastula transition in the subpopulation of cells that begin the movements of gastrulation—the deep cells of the dorsal lip of the blastopore.

Many more questions remain to be answered, both about these antigens and about the role of actin itself, in the formation and movements of the components of the mitochondrial cloud during oogenesis, and the regulation of cell motility on which gastrulation depends. For example, does actin depolymerize in the vegetal pole on fertilization? What effect does the apical contraction, occurring after fertilization, have on the subsequent rotation that determines the dorsal lip and the anterior–posterior axis of the embryo? Are there key regulatory proteins that mediate cell motility? Is their synthesis one of the triggers for subsequent cell movements? If so, how does their presence or activity affect actin filament dynamics in the cell?

Acknowledgments

I am indebted to Christopher Wylie for my introduction to *Xenopus* embryos, and to him and Tania Whitfield for the preliminary staining of embryos and eggs with MAb 2E4. I also wish to thank Ray Keller for advice and discussions. This work is supported by a grant from the American Cancer Society, California chapter (#SS44-89), and the Frederick Bang Award, Marine Biology Lab, Woods Hole, Massachusetts.

References

- Ankenbauer T, Kleinschmidt JA, Vanderkerckhove J, Franke WW. Proteins regulating actin assembly in oogenesis and early embryogenesis of *Xenopus laevis*: Gelsolin is the major cytoplasmic actin-binding protein. *J. Cell Biol.* 1988; 107:1489–1498. [PubMed: 2844829]
- Bearer EL. Actin in *Drosophila* embryos: Is there a relationship to positional cue localization? *BioEssays.* 1991a; 13(4):199–204. [PubMed: 1859400]
- Bearer EL. A 43 kD protein associated with the leading edge of migrating cells. 1991b Submitted for publication.
- Bearer EL. Direct observations of actin filament severing by gelsolin, and binding by gCap 39 and CapZ. *J. Cell Biol.* 1991c (in press).
- Christensen K, Sauterer R, Merriam RW. Role of soluble myosin in cortical contractions of *Xenopus* eggs. *Nature (London).* 1984; 310:150–151. [PubMed: 6738711]
- Clark TG, Merriam RW. Diffusible and bound actin in nuclei of *Xenopus laevis* oocytes. *Cell (Cambridge, Mass.).* 1977; 12:883–891.
- Clark TG, Merriam RW. Actin in *Xenopus* oocytes. I. Polymerization and gelation *in vitro*. *J. Cell Biol.* 1978; 77:427–447. [PubMed: 565781]
- Dent, JA.; Klymkowsky, MW. Wholmount analysis of cytoskeletal reorganization and function during oogenesis and early embryogenesis in *Xenopus*. In: Shatten, H.; Shatten, G., editors. *Cell Biology of Fertilization*. Orlando, Florida: Academic Press; 1988.
- DeSimone DW, Hynes RO. *Xenopus laevis* integrins: Structural conservation and evolutionary divergence of integrin B subunits. *J. Biol. Chem.* 1988; 263:5333–5340. [PubMed: 2833505]
- Elinson RP. Site of sperm entry and cortical contraction associated with egg activation in the frog. *Rana pipiens*. *Dev. Biol.* 1975; 47:257–268. [PubMed: 1081960]
- Evans JP, Page BD, Kay BK. Talin and vinculin in the oocytes, eggs and early embryos of *Xenopus laevis*: A developmentally regulated change in distribution. *Dev. Biol.* 1990; 137:403–413. [PubMed: 2105901]
- Franke WW, Rathke PC, Seib E, Trendelenburg MF, Osborn M, Weber K. Distribution and mode of arrangement of microfilamentous structures and actin in the cortex of the amphibian oocyte. *Cytobiologie.* 1976; 14:111–130. [PubMed: 1034572]
- Gerhart J, Keller R. Region-specific cell activities in amphibian gastrulation. *Annu. Rev. Cell Biol.* 1986; 2:206–229.
- Gerhart J, Danilchik M, Doniach T, Roberts S, Rowning B, Stewart R. Cortical rotation of the *Xenopus* egg: Consequences for the anteroposterior pattern of embryonic development. *Development.* 1989; (Suppl.):37–51. 1989. [PubMed: 2699856]
- Giebelhaus DH, Zelus BD, Henschman SK, Moon RT. Changes in the expression of a-Fodrin during embryonic development of *Xenopus laevis*. *J. Cell Biol.* 1987; 105:843–853. [PubMed: 3040772]
- Hardin J, Keller R. The behavior and function of bottle cells during gastrulation of *Xenopus laevis*. *Development (Cambridge, UK).* 1988; 103:211–230.
- Heasman J, Quarmby J, Wylie CC. The mitochondrial cloud of *Xenopus* oocytes: The course of germline granule material. *Dev. Biol.* 1984; 105:458–469. [PubMed: 6541166]
- Holtfretey J. Properties and functions of the surface coat in amphibian embryos. *J. Exp. Zool.* 1943; 93:251–323.
- Horwitz A, Duggan K, Buck C, Beckerle MC, Burridge K. Interaction of plasma membrane (ibronectin receptor with talin, a transmembrane linkage. *Nature (London).* 1986; 320:531–533. [PubMed: 2938015]
- James TC, Elgin S. Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene. *Mol. Cell. Bioi.* 1986; 6(11):3862–3872.
- Jeffrey WR, Swalla BJ. The myoplasm of ascidian eggs: A localized cytoskeletal domain with multiple roles in embryonic development. *Semin. Cell Biol.* 1990; 1:373–381. [PubMed: 2102391]
- Keller RE. An experimental analysis of the role of bottle cells and the deep marginal zone in gastrulation of *Xenopus laevis*. *J. Exp. Zool.* 1981; 216:81–101. [PubMed: 7288390]

- Keller RE. The cellular basis of gastrulation in *Xenopus laevis*: Active, postinvolution convergence and extension by mediolateral interdigitation. *Am. Zool.* 1984; 24:589–603.
- Luchtel D, Bluemink JG, De Laat SW. The effect of injected cytochalasin B on filament organization in the cleaving egg of *Xenopus laevis*. *J. Ultrastruct. Res.* 1976; 54:406–419.
- Malacinski GM, Brothers AJ, Chung HM. Destruction of the neural induction system of the amphibian egg with ultraviolet radiation. *Dev. Biol.* 1977; 56:24–39. [PubMed: 557008]
- Manes ME, Elinson RD, Barbieri FD. Formation of the amphibian grey crescent: Effects of colchicine and cytochalasin B. *Wilhelm Roux's Arch. Dev. Biol.* 1978; 185:99–104.
- Merriam RW, Clark TG. Actin in *Xenopus* oocytes II. Intracellular distribution and polymerizability. *J. Cell Biol.* 1978; 77:439–447. [PubMed: 565782]
- Merriam RW, Sauterer RA, Christensen K. A subcortical, pigment-containing structure in *Xenopus* eggs with contractile properties. *Dev. Biol.* 1983; 95:439–446. [PubMed: 6825942]
- Nakatsuji N. Presumptive mesodermal cells from *Xenopus laevis* gastrulae attach to and migrate on substrata coated with fibronectin or laminin. *J. Cell Sci.* 1986; 86:109–118. [PubMed: 3654774]
- Nüsslein-Volhard C, Roth S. Axis determination in insect embryos. *Ciba Found. Symp.* 1989; 144:37–55. discussion 55–64, 92–98. [PubMed: 2673683]
- Rungger D, Rungger-Braendle E, Chaponnier C, Gabbiani G. Intranuclear injection of anti-actin antibodies into *Xenopus* oocytes blocks chromosome condensation. *Nature (London)*. 1979; 282:320–321. [PubMed: 574192]
- Sheer U, Hinssen H, Franke WW, Jockusch BM. Microinjection of actin-binding proteins and actin antibodies demonstrates involvement of nuclear actin in transcription of lampbrush chromosomes. *Cell (Cambridge, Mass.)*. 1984; 39:111–122.
- Stewart-Savage J, Grey RD. The temporal and spatial relationships between cortical contractions, sperm tail formation, and pronuclear migration in fertilized *Xenopus* eggs. *Wilhelm Roux's Arch. Dev. Biol.* 1982; 191:241–245.
- Strome S, Hill DP. Early embryogenesis in *C. elegans*: The cytoskeletal and spatial organization of the zygote. *BioEssays*. 1988; 124:1–8.
- Vacquier VD. Dynamic changes of the egg cortex. *Dev. Biol.* 1981; 84:1–26. [PubMed: 6265303]
- Weeks DL, Melton DA. A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF- β . *Cell (Cambridge, Mass.)*. 1987; 51:861–867.
- Winklbauer R. Differential interaction of *Xenopus* embryonic cells with fibronectin *in vitro*. *Dev. Biol.* 1988; 130:175–183. [PubMed: 3181626]
- Winklbauer R. Mesodermal cell migration during *Xenopus* gastrulation. *Dev. Biol.* 1990; 142:155–168. [PubMed: 2227092]
- Wylie CC, Brown D, Godsave SF, Quarmby J, Heasman J. The cytoskeleton of *Xenopus* oocytes and its role in development. *J. Embryol. Exp. Morphol.* 1985; 89(Suppl.):1–15. [PubMed: 2420913]
- Yisraeli JK, Melton DA. The maternal mRNA Vgl is correctly localized following injection into *Xenopus* oocytes. *Nature (London)*. 1988; 336:592–595. [PubMed: 3200307]
- Yisraeli JK, Sokol S, Melton DA. The process of localizing a maternal messenger RNA in *Xenopus* oocytes. *Development*. 1989; (Suppl.):31–36. 1989. [PubMed: 2636138]

Fig. 1.

Polyethylene glycol-embedded sections of stage 8 and 9 *Xenopus* embryos stained with MAb 2E4 or an anti-actin monoclonal antibody according to Wylie *et al.* (1985). (A) MAb 2E4-stained section of a stage 9 embryo. bc, Blastocoel cavity; v, vegetal mass. Arrow indicates bright staining by the antibody in the presumptive dorsal lip. (B) Anti-actin monoclonal antibody stains a stage 8 embryo diffusely, with more concentrated staining at the cortex of individual cells. bc, Blastocoel cavity; a, animal pole; v, vegetal pole. Bar in (A) (= 0.4 mm) gives magnification for (A) and (B). (C and D) Higher magnification of

sections of other embryos stained with MAb 2E4. Brightest staining is seen in the cells of the presumptive mesoderm, the deep cells of the marginal zone. The basal edge of cells over the animal cap are also stained. bc, Blastocoel cavity; a, animal cap; v, vegetal mass. Arrow in (C) points out a single small cell, lying on the floor of the blastocoel, that stains strongly. Bar in (D) (0.22 mm) gives magnification for (C) and (D).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2. Mesodermal cells from a stage 25 embryo crawling on matrigel-coated coverslips, fixed in 4% paraformaldehyde and stained by indirect immunofluorescence with MAb 2E4 and phalloidin. (A) MAb 2E4 stains in a punctate pattern both the cytoplasm and the pseudopods (large open arrows). Microspikes or filopodia extending from the lateral cell borders are also weakly stained (small arrows). (B) The same cell also stained with phalloidin displays fine filaments in the pseudopods (open arrows). The ends of these filaments appear to coincide with the points stained by MAb 2E4. Microspikes or filopodia extending from the lateral margin also stain (small arrows). Bar = 25 μ m.

Fig. 3.

MAB 2E4 recognizes several bands in blots of cytoskeletal extracts made from stage 10 embryos according to Ankenbauer *et al.* (1988). Lane 1, Coomassie-stained gel; lane 2, Western blot stained with MAb 2E4. Molecular weights for the Coomassie gel are indicated to the left. Amido-black staining of a strip of adjacent nitrocellulose was used to identify bands stained by the antibody. These are indicated by the lines connecting the gels. The bands were numbered and used as immunogens for injection into five separate mice. The identifying numbers are given to the right of lane 2. (B) Western blots of extracts of stage 10 *Xenopus* embryos with antisera resulting from immunizing five mice with individual bands corresponding to those recognized by MAb 2E4 and cut from the preparative gel shown in (A), lane 1. Lane AB: Amido-black staining of transfer blot. Lanes 1: 1/50, 1/100, 1/500 dilutions of antisera resulting from band 1; Lanes 2: 1/50, 1/100, 1/500 dilutions of antisera resulting from band 2; Lanes 3: 1/50, 1/100, 1/500 dilutions of antisera resulting from band 3; Lanes 4: 1/50, 1/100, 1/500 dilutions of antisera resulting from band 4; Lanes 5: 1/50,

1/100, 1/500 dilutions of antisera resulting from band 5. Arrowheads to the right of the blot indicate bands recognized by the antisera. Note that the 75- and 25-kDa bands are stained in lanes 1, 4, and 5, while the 95-kDa band is unique to antiserum 2. Antiserum 3 was so strong that it spread across the blot, obscuring whether the other antisera also cross-react with that band in this blot. In other blots, the other antisera did not appear to recognize that 48-kDa doublet. Molecular weights are indicated to the left.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 4.

Plastic-embedded sections of stage 1 oocytes. (A) Nomarski optics identify the mitochondrial cloud (arrow) and the nucleus (n). (B) MAb 2E4 stains the mitochondrial cloud (arrow) and the cytoplasm weakly. The nucleus is not stained. (C) Antiserum 1 (like antisera 4 and 5) stains both the cloud (arrow) and the nucleus. The cytoplasm is also lightly stained. Bar = 25 μ m.

Fig. 5.

Staining pattern of antiserum 3 on *Xenopus* embryos. (A) Plastic-embedded section of a stage 9 embryo stained with antiserum 3 indirect immunofluorescence. The angle of the blastocoel cavity (bc) is shown, revealing staining of individual cells of the presumptive mesoderm, and staining of the deep cells over the animal cap. Bar = 0.1 mm. (B–F) Whole

mounts of stage $10\frac{1}{2}$ embryos stained with immunoperoxidase method and clarified with butanol/isobutane so that the deep cells of the involuting marginal zone are revealed. (B) Dark reaction product (small arrows) is seen in the involuting marginal zone, perhaps in highest concentration in the bottle cells at the leading margin of involution. (C) A different embryo stained the same way, viewed on its side. A line of reaction product (small arrows) leads up to the floor of the blastocoel (open arrow). The marginal zone around the embryo is stained, while the vegetal cells are not visible because they are not stained, and no ventral lip is apparent yet (arrowhead). (D) A control embryo incubated with secondary antibody only. No reaction product is seen in the involuting marginal zone (small arrows). Bar = 1 mm. (E and F) Higher magnifications of two more embryos, showing similar staining of the involuting cells of the dorsal lip of the blastopore (small arrows). Bar = 0.5 mm.

Fig. 6. A developmental profile of the expression in eggs and embryos of the 48-kDa doublet recognized by antiserum 3. (A) Coomassie-stained 10% polyacrylamide SDS gel of cytoskeletal extracts and (B) Western blot with antiserum 3 of Lane 1, ovaries; lane 2, laid eggs; lane 3, 30 min after fertilization; lane 4, stage V embryos; lane 5, stage VI embryos; lane 6, stage 7 embryos; lane 7, stage 8 or 9 embryos; lane 8, stage 10 embryos. Five embryos were extracted for each lane. Molecular weights are given to the left.