A two-component cytoskeletal system of *Xenopus laevis* egg cortex: concept of its contractility

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ABSTRACT The cortex of *Xenopus laevis* eggs comprises two components: the plasma membrane with underlying microfilaments (external layer) and the cytoplasmic matrix with embedded pigment granules (internal layer). Both components of the egg cortex are capable of contracting under the influence of calcium ions. The cortex of the fully grown oocyte does not have the ability to contract, but acquires it during progesterone-stimulated maturation, when the oocyte is transformed into an egg. It has been proposed, on the basis of the data on the cortex cytoskeletal organization, that the submembranous microfilaments form an anisotropic network in the oocytes, which is transformed into an isotropic, randomly organized network in the egg. The latter is capable of contractile acts. Reorganization of the cytoskeleton in the internal cortex layer leads to the formation of the actin contractile gel. Data are provided on the role of actin-associated proteins in changes of organization of the actin cortical cytoskeleton. Mechanisms underlying the different sensitivity of microfilaments of the internal and external layers to cytochalasin B, as well as the coordinated (in time) development of the contractility in these layers, are discussed. The model proposed for development of the cortical contractility during oocyte maturation (Ryabova *et al.*, 1994a) is considered on the basis of a two-component cytoskeletal system.

KEY WORDS: *amphibians, oocyte maturation, cortex contractility, cytoskeleton, actin*

Introduction

The cortex of animal somatic cells comprises the membrane and underlying microfilaments. The cortex of the somatic cells is multi-functional. Exo- and endocytosis, intracellular contacts, and the distribution of receptors, as well as the capacity to form a contractile ring during cytokinesis, i. e., the capacity to generate contractile processes, are all functions of the cortex.

In comparison to somatic cells, amphibian oocytes have specific features of cortical organization, both morphological and functional. Holtfreter (1943a,b) was the first to describe the superficial layer of the egg (cells of the early embryo) capable of contractions and translocations: the surface coat. This layer was characterized by the presence of pigment granules as markers. Contractions of this layer provide epibolic movements and translocations of ectodermal pigmented cells.

The contractile properties of the amphibian egg cortex play an important role not only in the formation of a cleavage furrow (cytokinesis), but also in the entry of spermatozoon into the egg, movement of pronuclei (Elinson, 1987), extrusion of polar bodies, and wound healing on the egg surface (Bluemink, 1972).

Experiments on the cortex grafting allowed Curtis (1960), to put forward a suggestion about the morphogenetic activity of the cortex. Some recent data also suggest the morphogenetic potential of the cortex (Dent *et al.*, 1992).

Structural organization of the cortex

Electron microscopy data (Grey *et al.*, 1974; Franke *et al.*, 1976; Selman and Perry, 1970) suggest that the pigmented cortex of the amphibian oocytes and eggs has a complex structure, including a plasma membrane with underlying microfilaments. This is an analog of the somatic cell cortex, whose thickness, as in the somatic cells, is 0.1 μm. The plasma membrane forms numerous outgrowths: microvilli with characteristic parallel microfilaments. A net of microfilaments is located under smoothed regions of the membrane, which is often called «felt». Subcellular organelles, that is, pigment and cortical granules, numerous membrane vesicles, reticulum, ribosomes, and mitochondria (except yolk plates), are embedded in a deeper, relatively dense cytoplasm or cytoskeletal matrix. This layer is 3-7 μm thick (Fig. 1). Such a layer is absent in the vegetal hemisphere, and yolk plates can be in direct contact with the submembranous microfilaments.

The pigmented egg cortex is capable of contraction under the influence of Ca^{2+}, as was shown for wound (incision) healing on the egg surface (Bluemink, 1972). The thickness of contracted

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pigmented layer reaches 15-30 μm. However, in the fully grown oocyte, this layer does not respond to any stimuli by contraction.

Merriam *et al.* (1983) demonstrated differences in sensitivity to cytochalasin B between two layers of the egg cortex: the superficial layer formed by the plasma membrane and underlying microfilaments, and the deeper layer formed by the cytoplasmic matrix with embedded pigment and cortical granules. At the time, the cytoskeletal composition of the cytoplasmic matrix had not been studied. Contraction induced in the deeper layer is not suppressed by cytochalasin B, while the superficial layer loses its contractile properties in the presence of this drug. Thus, it was shown that the pigment concentration in the animal pole its movement to the region of prospective cleavage furrow proceed normally in the presence of cytochalasin B, while the furrowing is fully blocked due to a destroyed layer of submembranous microfilaments.

On the basis of these data, a hypothesis of a two-component system of cortical contractions in *X. laevis* egg was put forward. According to the terminology of the authors (Merriam *et al.*, 1983), the superficial part of the cortex is the true cortex or the cortex *per se*, while the deeper part is the subcortical matrix.

Since this terminology was not widely accepted and most researches of the early amphibian development define the «cortex» as the entire superficial egg layer, including both submembranous microfilaments and the pigmented matrix (Franke *et al.*, 1976; Gall *et al.*, 1983; Klymkowsky *et al.*, 1987), we propose to call the superficial part of the cortex, i. e., the plasma membrane with underlying microfilaments, the external or membrane-containing layer, and its deeper part, i. e., the cytoskeletal matrix with embedded pigment and cortical granules, the internal or pigment-containing layer (Ryabova, 1995). This terminology stresses the unity of these two components of the cortex, since they respond to external factors, including Ca^{2+}, by contraction as a whole (Fig. 2).

According to Merriam *et al.*, 1983, the external layer is responsible for spermatozoon entry, formation of a contractile ring during cytokinesis, formation of microvilli, and endo- and exocytosis. Functions of this layer are inhibited by cytochalasin B.

The functions of the internal layer comprise exocytosis of the cortical granules, epibolic movements, and isometric contractions of the pigmented egg surface observed within 5-6 min after the spermatozoon entry and within 70 min after fertilization (Elinson, 1975). The internal layer is involved in the formation of the grey crescent and rotation of the cortex, although the mechanisms underlying cortical contractions and rotation are different (Elinson and Rowning, 1988). Wound healing and formation of the cleavage furrow are accompanied by the concentration of the pigment and cytoskeletal matrix, suggesting the involvement of the internal layer in these processes. The functions of this layer are not suppressed by cytochalasin B.

Differences in the sensitivity of the external and internal layers of the cortex to cytochalasin B suggest differences in their molecular organization and mechanisms regulating the assembly and orientation of the cytoskeletal components responsible for contractile acts.

It is well known that the contractile act is based on the interaction between actin and myosin. But the published data concerning the «preparation» of actin and myosin molecules for interaction in non-muscle cells and, specifically, in the amphibian egg cortex, are scarce. On the basis of the two-component composition of the cortex and recent data about proteins and structures responsible for non-muscle contractility and identified in the cortex of the amphibian oocytes and eggs, we will try to understand and interpret the development of contractility during oocyte maturation in terms of the changes occurring in the cortex.

External membrane-containing layer of the cortex: Supramolecular structure and mechanisms of transformation

The external layer of the oocyte (egg) cortex is represented by the plasma membrane with structurally connected microfilaments.

The cortex of the fully grown oocyte, including its external layer, is not capable of contraction. At this stage, actin, as the main component of the filamentous layer associated with the membrane, provides mechanical tension and rigidity to the oocyte surface.

For many cell types, it was shown that the anisotropic state or parallel arrangement of unipolar actin filaments is characteristic of rigid cell structures, while the isotropic (without obvious orientation) arrangement is characteristic of contractile structures (Schliwa, 1986).

Microfilaments extending from the numerous, relatively long microvilli that cover the surface of the fully grown oocyte can play the role of skeleton, maintaining the anisotropic organization of the entire system of the cortical submembranous microfilaments. Indeed, the microvilli are anisotropic structures, since the microfilaments that form them are parallel and unipolar; bundles of microfilaments extending from the long microvilli toward the ooplasm are also comparatively long and, thus, can fulfill the mechanical function, i. e., maintain the anisotropy of the entire submembranous system of microfilaments.

The experimental data available suggest anisotropic distribution of submembranous filaments in the surface regions between the microvilli. Thus, it was shown that, in the isolated *X. laevis* cortex, the submembranous microfilaments are located perpendicularly to the plasma membrane and, correspondingly, are parallel to each other in all cortex regions (Ryabova, 1991a) (Fig. 3A). There are data that the microfilaments preexisting in the cortex of some cell types before cytokinesis, at least their major part, are attached to the membrane with their barbed end (Begg *et al.*, 1978), and the polarity of the microfilaments attached to the end of microvilli and plasma membrane is identical (Gall *et al.*, 1983). This suggests the unipolarity of the entire system of submembranous microfilaments of the cortex in the fully grown oocyte. Thus, the distribution of submembranous microfilaments can determine the rigidity of the external cortex layer and its incapacity for contractile acts.

During progesterone-stimulated maturation, the surface of the oocyte changes: it becomes relatively smooth, since the microvilli markedly shorten by the end of oocyte maturation (Ryabova, 1983; Bement and Capco, 1990; Bement, 1992). Reduction of the microvilli appears to impart to the entire submembranous system of microfilaments a higher degree of isotropy, since the cortex loses the scaffolding that maintained the unipolarity of the submembranous microfilaments. In the egg, the major part of submembranous microfilaments lose, with all probability, strict orientation and represent a random isotropic network, which is a structural basis of contractility (Gall *et al.*, 1983; Maupin and

Pollard, 1986). It was also shown that the isolated cortex of the *X. laevis* egg contains a network of chaotically arranged microfilaments (Ryabova, 1991a) (Fig. 3B). Figure 4 (a,b) schematically shows the transformations of submembranous microfilaments described above.

Microfilaments of the isotropic random network can have different polarity with reference to the membrane: microfilaments with opposite polarity were found in the cortex of sea urchin eggs (Ishidate and Mabuchi, 1988).

Since the bundles and network of microfilaments are interchangeable, one can propose that some unipolar microfilaments of the microvilli transform in elements of the isotropic contractile network (Ryabova, 1983). The isotropic network of microfilaments is extremely labile and capable, in turn, of transformation in bundles under the influence of various external factors.

Both the rigid and contractile state of the system of submembranous microfilaments, as well as the transition between these states, are maintained and regulated by many factors. It is known that the maturation-promoting factor (MPF) is formed in the ooplasm under the influence of progesterone, which has a kinase activity (cyclin-p 34 cdc2-kinase is a part of the MPF protein complex). Phosphorylation of cytokeratins and myosin in the maturing *X. laevis* oocytes by MPF-dependent kinases has been reported (Klymkowsky *et al.*, 1991; Kelley *et al.*, 1995). The data of Bement and Capco (1990) on the regulatory role of protein kinase C in development of contractility and formation of cleavage furrow in the *X. laevis* eggs allow a suggestion that phosphorylation of actin-binding proteins (actin?) changes the supramolecular organization of the actin-containing structures. Hence, protein kinases that phosphorylate actin-binding proteins can be considered as such a factor.

The appearance and disappearance of actin-binding proteins can also regulate reorganization of the actin network. Several groups of proteins modulating and regulating the supramolecular organization of actin in the cell have been identified (Mabuchi, 1990).

(1) Proteins binding monomeric actin (profilin, actoforin).
(2) Proteins capping the barbed end of filaments and displaying severing activity (gelsolin).
(3) Proteins that cross-link filaments (α-actinin, spectrin, tropomyosin).

There is also a group of proteins mediating attachment and interaction of actin filaments with the membrane: vinculin, talin, α-actinin.

The following proteins were found in the cortex of the amphibian oocytes and eggs: α-actinin, tropomyosin, spectrin (Campanella and Gabbiani, 1980; Campanella *et al.*, 1990) in *Discoglossus*; and spectrin (Giebelhaus, et al. 1987; Ryabova *et al.*, 1994c), vinculin and talin (Evans *et al.*, 1990; Ryabova and Vassetzky, 1996) in *Xenopus*.

However, the role of these proteins in regulation of submembranous microfilaments of the oocytes and eggs has not been studied, one can only speculate about and the involvement

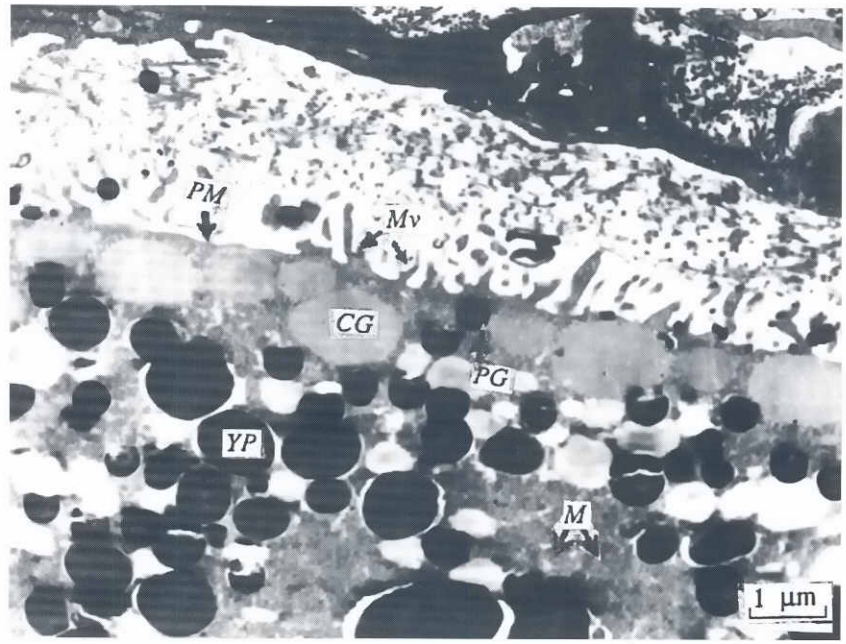

Fig. 1. Section of a fully grown oocyte. *(electron microscopy). CG, cortical granules; M, mitochondria; Mv, microvilli; PG, pigment granules; PM, plasma membrane; YP, yolk plates.*

of these proteins in the transformation of actin-containing cortical structures. Spectrin found in the cortex of the fully grown oocyte provides for rigid cross-linking between the submembranous microfilaments and maintains their anisotropic orientation. This protein is not found in the cortex at the end of maturation, i. e., in the egg (Ryabova *et al.*, 1994c). Apparently, reorientation of the submembranous microfilaments leading to the formation of an isotropic network can be realized only in the absence of spectrin. Alpha-Actinin and tropomyosin form weak complexes with actin filaments, which do not prevent their spatial reorganization (Pollard *et al.*, 1990). Therefore, we can expect the presence of these proteins at all stages of oocyte maturation. These proteins can, however, play a special role in maintaining the three-dimensional structure of the isotropic network and provide its lability in the egg, when the cortex has already acquired the capacity for contraction.

Vinculin, which is present in the cytoskeletal fraction only in the egg, appears to be involved in the attachment of microfilaments to the plasma membrane. The molecular organization of the site of attachment of microfilaments to the membrane in the oocyte should differ from that in the egg, since vinculin is absent in the cytoskeletal system of the oocyte (Evans *et al.*, 1990; Ryabova and Vassetzky, 1996). Vinculin was found only in the cytoskeletal fraction of the ooplasm, while, in the oocyte, it is present in the cytoplasmic fraction, which is soluble in non-ionic detergents. Different organization of the site of attachment can determine the ability or inability of the microfilaments for contractile acts.

The external layer of the *X. laevis* cortex is sensitive to cytochalasin B at all stages of oocyte maturation (Merriam *et al.*, 1983; Ryabova *et al.*, 1986). Irrespective of how the submembranous microfilaments are attached to the membrane, this means that the barbed end of submembranous microfilaments is not capped by cytoplasmic proteins and can interact with cytochalasin B.

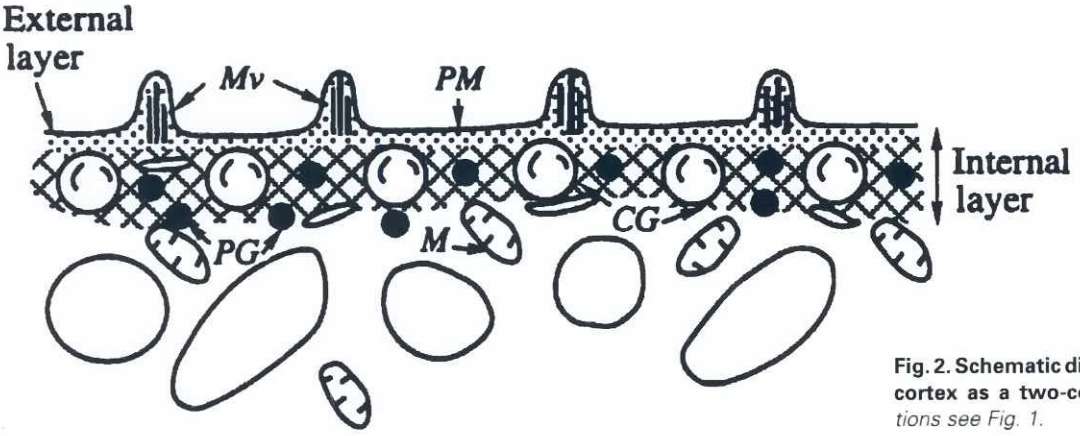

Fig. 2. Schematic diagram of the structure of oocyte cortex as a two-component system. *For designations see Fig. 1.*

Thus, the isotropic network of submembranous actin filaments, with a non-capped barbed end attached to the plasma membrane by means of vinculin, is capable of contraction. Thus, the system of actin filaments is ready to interact with myosin in this conformational orientation or in the other, which is acquired by the filaments very rapidly, directly under the influence of factors stimulating contraction.

A question naturally arises: what is the mechanism of the contractile act if the isotropic network of microfilaments associated with the plasma membrane constitutes its morphological basis?

It was shown on models of contractile processes in the cortex of amphibian oocytes and eggs, wound healing (Bluemink, 1972; Ryabova, 1983), formation of the contractile ring in the bisected oocytes stimulated by progesterone (Capco *et al.*, 1992) and formation of the cleavage furrow (Maupin and Pollard, 1986; Mabuchi, 1990), that bundles of actin filaments or aggregates of parallel filaments with opposite polarity are always formed in the region of contraction (actin filaments involved in the contractile act never form anisotropic structural complexes). Contraction is generated by interaction between antiparallel microfilaments and myosin. It is essential that actin is polymerized in the cortex before the contractile act, i.e., microfilaments preexist, they are not formed *de novo* in the cortex, and they are arranged in bundles from the isotropic network only during contraction.

Reorganization of preexisting actin filaments in bundles can be triggered by Ca^{2+}. Indeed, relatively thick bundles of actin microfilaments can be seen in the cortex isolated from the egg during cortical contraction or in a Ca^{2+}-containing medium (Fig. 3C). Rapid changes in the system of actin filaments are realized on the basis of weak bonds between the filaments, just as between the filaments and plasma membrane.

Myosin 2 is involved in contraction. In the given case, this is the most probable form of myosin, its isozyme being found in the muscle and most non-muscle cells. Each head has a site of binding with actin and a catalytic region for ATP hydrolysis. The tail is associated with the other myosin tails to form bipolar myosin filaments, so that the myosin heads can cross-link opposite polarized actin filaments and stretch them with reference to each other. In most cases, phosphorylation of the myosin light chain stimulates interaction of myosin heads with actin, leading to ATP hydrolysis and generation of strength and mobility. Myosin was found in the ooplasm and cortex of the *X. laevis* oocytes and eggs (Ryabova *et al.*, 1994b).

The corresponding scheme reflects rearrangement of the system of submembranous microfilaments during oocyte maturation, including the contractile act specific for the system of microfilaments in the mature egg (Fig. 4). It is evident that spatial reorganization of submembranous filaments present in the fully grown oocyte is essential for acquisition by the egg cortex of contractility, i. e., capacity for contraction. Spatial reorganization of the submembranous microfilaments in the mature egg is also a prerequisite of the contractile act. This ideas make it possible to explain the previously incomprehensible data, specifically the results of Christensen *et al.* (1984) concerning the incapacity of isolated cortices of the *X. laevis* oocytes to contract in a solution of myosin, unlike the isolated egg cortices.

Internal pigment-containing layer of the cortex: supramolecular structure and mechanisms of transformation

The internal cortex layer in the fully grown oocyte is a rigid cytoskeletal network (matrix), comprising intermediate filaments (Gall *et al.*, 1983; Godsave *et al.*, 1984; Klymkowsky, et al., 1987), microtubules (Gall *et al.*, 1983) and microfilaments (Franke *et al.*, 1976; Gall *et al.*, 1983; Ryabova, 1991a,b). Figure 5 shows that the pigment granules, the most characteristic element of the internal cortex layer, are embedded in polymerized actin (Ryabova and Vassetzky, 1993).

The anisotropic state of the cytoskeletal system of the internal layer can be the entire complex of cortical cytoskeletal elements. Gall *et al.* (1983) noted that distribution of actin filaments in the cortex of the *Xenopus* oocytes is directly related to the distribution of keratin filaments. The keratin filaments form a skeleton that sets the pattern for distribution of the microfilaments. The microfilaments of the internal layer form two types of structure: separate, relatively long microfilaments and a network of microfilaments or a microfilamentous matrix (Gall *et al.*, 1983; Ryabova, 1991b) (Fig. 6).

During oocyte maturation, the cytoskeletal system of the internal cortex layer, like that of the external one, tends to the isotropic state: the keratin filaments are destroyed and only the actin-containing homogenous matrix, comprising, in all probability, relatively short actin filaments, is preserved as a contractile element. Spectrin disappears. It is likely that the transformations of the

cytoskeletal structures observed in the internal cortex layer lead to gel formation. It is known that gel formation depends on the concentration of cross-links and the mean length of the gel structural elements, in this case microfilaments. An increase in the density of cross-links between the microfilaments and in the mean length of filaments converts the system to a more rigid phase. Spectrin and separate, relatively long microfilaments of the internal cortex layer can ensure the rigid state of the actin system, while disappearance of these microfilaments can convert the rigid system in a contractile gel.

During oocyte maturation, the ooplasm is hydrated (Dettlaff, 1988). This may be very important in the actin gel formation. It is known that changes of water concentrations in solutions of amphophilic compounds, including most biological molecules, lead to phase transitions.

Thus, we can assume that the three-dimensional actin gel is the morphological basis of contractility of the internal cortex layer. There are many data on the capacity of gels for interaction with myosin. However, it is not known how the state of the gel and its contractile activity are related, i. e., how actomyosin interactions are realized in the gel system. However, it is known that, if the degree of cross-linking of the actin filaments in the network is very high, myosin is incapable of interaction with such a network (Schliwa, 1986). This is observed in the isolated cortex of the fully grown oocyte in a medium containing myosin: the actin matrix densely cross-linked by spectrin cannot contract (Christensen *et al.*, 1984). If the degree of cross-linking of the structural elements of the actin gel decreases below a certain critical value, the gel is transformed in sol and loses the capacity for interaction with myosin (Schliwa, 1986). Hence, organization of the pigment-contacting actin gel in the egg cortex is maintained by a certain critical concentration of actin-binding proteins, except spectrin. The nature of these proteins is not known. It is clear, however, that these are not submembranous proteins, as the internal layer of the cortex is located at a sufficient distance from the membrane. Thus, differences in supramolecular mechanisms underlying the formation of contractility in the external and internal cortex layers can be essential: the role of submembranous proteins realizing the structural connection of the microfilaments with the plasma membrane may be essential in the external layer, while, in the internal, other actin-binding proteins cross-linking the filaments are significant.

The concept of the internal cortex layer as an actin gel, i.e., a three-dimensional network of actin filaments, suggests that their rapidly growing (barbed) ends are capped by actin-binding proteins. Thus, the resistance of the internal cortex layer to cytochalasin B is observed.

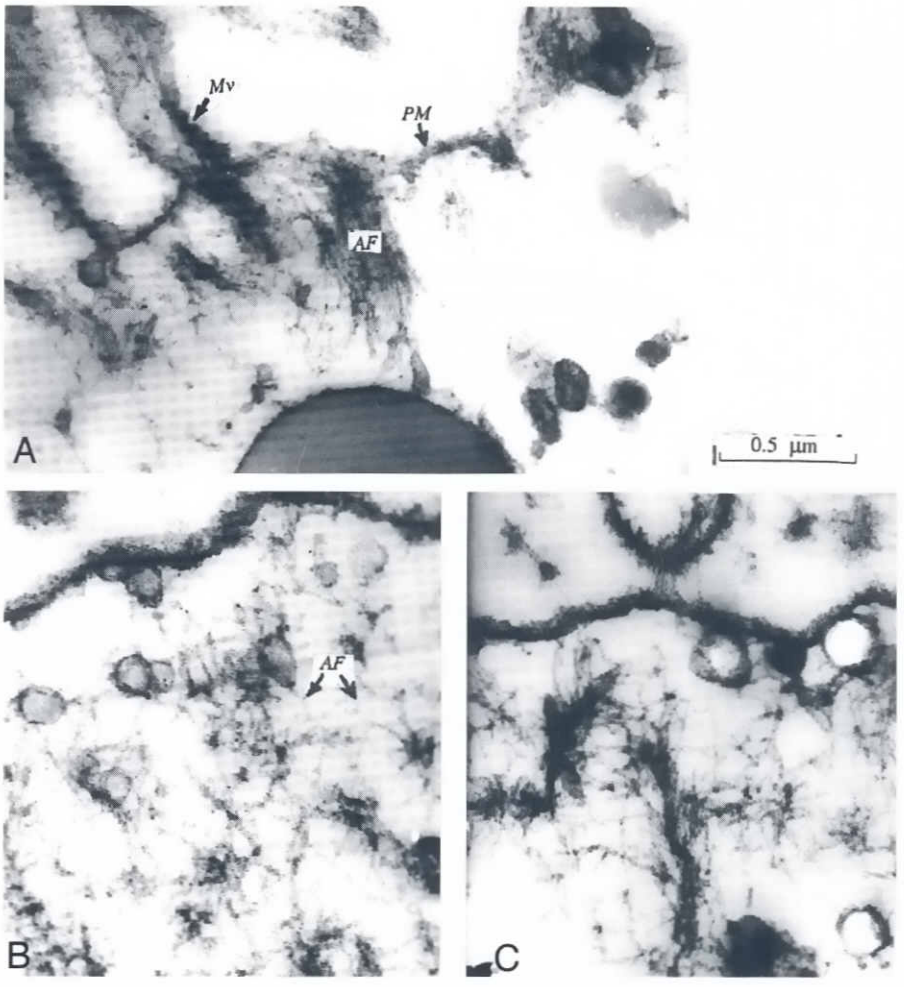

Fig. 3. Microfilaments of the cortex external layer at different stages of oocyte maturation, including the contractile act: (**A**) *microfilaments form an anisotropic network in a fully grown oocyte;* (**B**) *microfilaments form an isotropic network in an egg;* (***C***) *microfilaments form bundles during the contractile act. Bar, 0.5 μm. Designations: AF, actin filaments; BAF, bundles of actin filaments. For all other designations see Fig. 1.*

The cortex (predominantly, its internal layer) can be thought of as a peripheral part of the fully grown oocyte connected by a system of cytoskeletal structures with deep regions of the ooplasm, together with which it forms a rigid complex incapable of contraction. During oocyte maturation, cytoskeletal structures connecting the cortex with the ooplasm are destroyed (keratin filaments) or reoriented (microfilaments) in a way such as to liberate the cortex from the rigid connection with the ooplasm. As a result, the cortex acquires the capacity to move (contract) with reference to the ooplasm (Ryabova, 1993; Ryabova *et al.*, 1994a).

Thus, we propose that the internal layer of the egg cortex is an actin gel embedding pigment and cortical granules, with which myosin (myosin 2) is co-localized. This gel is capable of free movement (contraction), with reference to the ooplasm, because it loses the rigid cytoskeletal connection with the latter. The gel, i. e., the internal pigment-containing layer of the cortex, is capable of contraction, irrespective of the external membrane-containing layer. Thus, during formation of the cleavage furrow in the

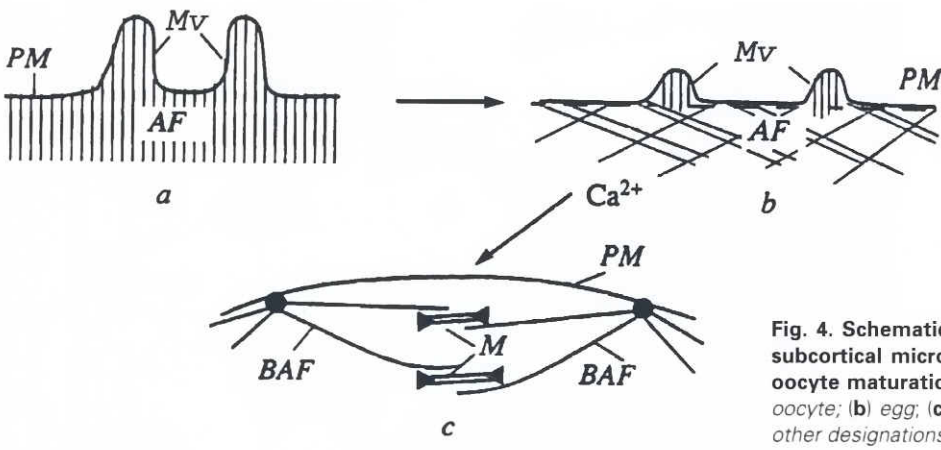

Fig. 4. Schematic diagram of transformation of the system of subcortical microfilaments in the cortex external layer during oocyte maturation, including the contractile act: (**a**) *fully grown oocyte;* (**b**) *egg;* (**c**) *contractile act. Designations: M, myosin. For all other designations see Figs. 1 and 3.*

presence of cytochalasin B, the external cortex layer is destroyed, while the internal one continues to contract (Merriam *et al.*, 1983). At the same time, both cortex layers are capable of simultaneous contraction under the influence of Ca^{2+}: in the area of incision on the cortex of the common frog egg, the external layer contracts, as shown by protuberances formed by the plasma membrane, and the inner layer contracts, as shown by the flow of pigment granules to the area of incision (Bluemink, 1972; Ryabova, 1983).

During oocyte maturation, the contractile potential of the cortex increases, as shown by Dettlaff (1966), who described the pattern and rate of wound healing on the oocyte surface at different stages of its maturation, and Capco *et al.*, (1992), in experiments on bisected maturation oocytes.

We relate the increase of the contractile potential of the cortex to its liberation from the rigid connection with the ooplasm (Ryabova, 1993; Ryabova *et al.*, 1994a). It is possible that the gel formation in the internal cortex layer and its liberation are causally related rather than simply coincide in time. The liberation of the cortex starts in the equatoril and spreads toward the animal pole. The process of gel formation in the inner layer appears to spread in a similar manner. The entire internal cortex layer becomes a gel by the end of maturation that is capable of contraction in experiments with bisected eggs under the influence of Ca^{2+}.

Temporal ratio of development of contractility in the internal and external cortex layers

Maturation of the ooplasm, including its superficial layer, i. e., cortex, is initiated by the hypophysial hormone progesterone. The progesterone receptors, located on the external surface of the oolemma, bind to the ligand and trigger the chain of subsequent events: conformational changes in the membrane, the appearance of maturation promoting factor and cascade of secondary messengers, specifically protein kinase C, which is involved in acquisition of the capacity for contraction by the cortex (Bement and Capco, 1991).

We propose that the spreading of progesterone-induced conformational membrane changes is a rapid process and, as a consequence, the alteration of the membrane form mediated by microfilaments is also a rapid process. In this case, the external cortex layer should acquire the capacity for contraction directly after the hormonal effect, i. e., markedly earlier than the final maturation of the ooplasm. However, this requires experimental testing.

In the inner cortex layer, the development of contractility, i. e., acquisition of the gel properties by this layer, strictly corresponds to the timing of ooplasm maturation, since, morphologically, this layer is a part of ooplasm.

If development of the contractility in the external and internal cortex layer proceeds asynchronously, the expression of this asynchrony can be expected under the experimental conditions. Indeed, if the oocyte is dissected in any direction just after the progesterone stimulation, the cortex forms a contractile ring along the section edge (Capco, personal communication). The contractile ring is destroyed under the influence of cytochalasin B. Hence, the external cortex layer is involved in formation of the contractile

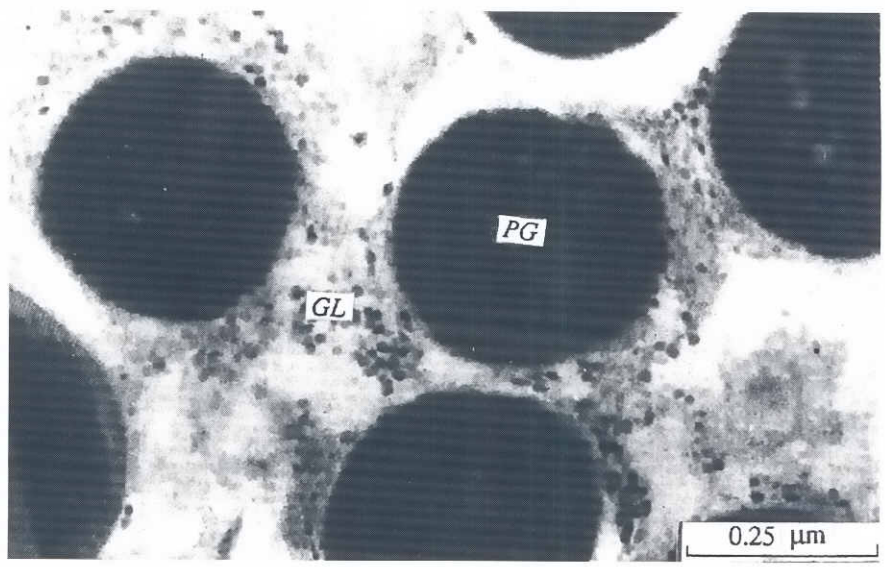

Fig. 5. Polymerized actin in the cortex intrnal layer. *Phalloidin conjugated with the colloid ,gold particles specifically reacts with the polymerized actin. Designations: GL, gold label. For all other designations see Fig. 1.*

ring. The capacity of the cortex for formation of a contractile ring in any area suggests that development of contractility in the external layer is a rapid process.

As the ooplasm matures, the internal cortex layer is liberated from the rigid connection with the ooplasm and acquires the features of the gel capable of contraction. Contractions of the internal layer enhance the contractile potential of the cortex as a whole, which by the end of maturation comprises the contractile activity of external and internal layers. The contractile potential of the internal layer is so high at the end of maturation that, in the case of bisected eggs, the cortex forms a cap in the presence of cytochalasin B when the external cortex layer is destroyed (Merriam *et al.*, 1983).

Direction of cortical contractions

In the skeletal and smooth muscle cells, contractions are generated in one direction only: in the first case, the sarcomere is contracted, and, in the second case, the entire cell is contracted. Non-muscle cells also generate linear forces of contractions but the contractile proteins are arranged in a three-dimensional complex (network), so that the force generated inside this complex will result from complex three-dimensional deformations. Therefore, the contractile complex of non-muscle cells can change very rapidly in space and time. This is also true for the contractile complex of the maturing oocyte or egg. The direction of the resultant of cortical contractions in the egg is determined also as a result of the interaction between the forces generated by the external and internal layers. Thus, in the case of bisected oocytes directly after hormonal stimulation, the resultant of cortical contractions is directed from the animal pole and, at the end of maturation, toward the pole. In different cases, directions of the cortical contractility may vary: local excitation at the site of needle pricking or spermatozoon entry, wound healing on the surface, formation of contractile rings on bisected oocytes, and epibolic movements of the cortex. Thus, the contractile system of the egg cortex is characterized by a high degree of plasticity, thereby providing for a number of embryological processes.

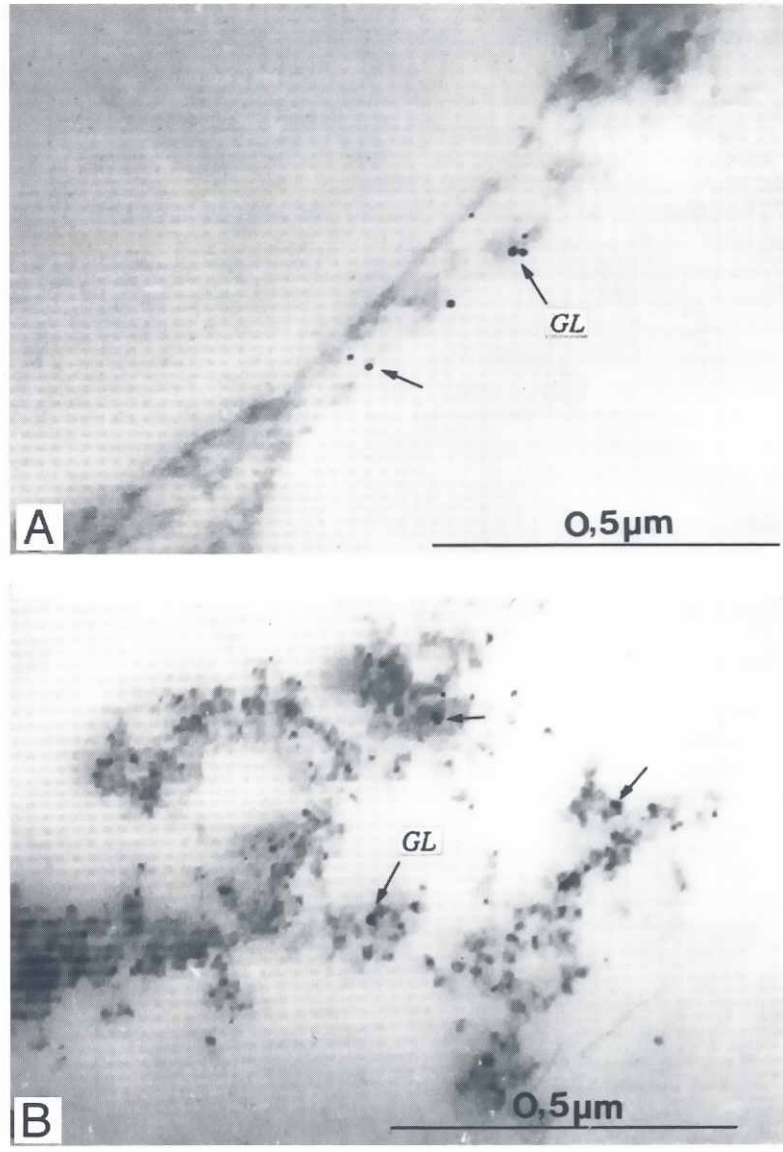

Fig. 6. Actin filaments of the cortex internal layer of an oocyte (a) and egg (b). Immunoelectron microscopy. *Gold labels indicate the actin nature of the filamentous structures.*

Conclusion

Thus, we considered the two-component contractile system of the cortex of *X. laevis* oocyte during its maturation in the light of available data on the cortical cytoskeleton of the cell and supramolecular mechanisms underlying actomyosin interaction.

The cortex of the amphibian egg differs from the cortex of the other cells by the presence of the internal pigment-containing layer capable of contraction. The existence of this layer in the egg is related, most likely, to specific features of the structure and functioning of the egg and embryo. First, such a large cell as an amphibian egg requires a potent cortex capable of maintaining the form and spatial orientation of the ooplasm as a whole. Even when the cortex loses its rigidity, it continues to fulfil this function. Second, the oocyte as a large cells requires a high contractile potential, which is also provided by the internal cortex layer, to realize cytokinesis. Third, the internal cortex layer due to its contractile properties provides a number of specific functions of the egg and embryo: exocytosis of cortical granules, translocation of pigment and morphogens (Ryabova *et al.*, 1994b), and formation of the dorsoventral axis and epibolic movements. The distribution of the main cytoskeletal structures and proteins in the amphibian oocytes and eggs is characterized, as a rule, by the presence of the cortical gradient, i. e., predominant localization of the cytoskeletal elements in the cortex region, and specifically in its internal layer (Campanella and Gabbiani, 1980; Ryabova *et al.*, 1994c). At the same time, there are many data suggesting a structural connection of the proteins and mRNAs with various cytoskeletal elements (Toh *et al.*, 1980; Perry and Capco, 1988; Ryabova *et al.*, 1994b). In this respect, the hypothesis of Curtis

(1960) concerning the morphogenetic function of the cortex based on the results of the internal cortex layer grafting becomes understandable.

Thus, the two-component cytoskeletal system of the cortex in the *X. laevis* (amphibian) egg determines not only specific features of its functioning and development of cortical contractility, but also the morphogenetic potential of the cortex.

We did not consider cortical microtubules, which may be involved, together with the actomyosin system, in the ooplasmic translocations. Thus, rotation of the cortex after fertilization is blocked by colchicine (Manes *et al.*, 1978), which destroys the transient system of parallel microtubules «paving the way» to the formation of the grey crescent (Elinson and Rowning, 1988). Although the mechanisms of ooplasmic translocations, based on the system of microtubules and on the actomyosin interactions, are different, one can propose that a certain state (maturity) of the actomyosin complex of the egg is a prerequisite for rotation of the cortex.

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