Polarity and Reorganization of the Endoplasmic Reticulum during Fertilization and Ooplasmic Segregation in the Ascidian Egg

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Abstract. During the first cell cycle of the ascidian egg, two phases of ooplasmic segregation create distinct cytoplasmic domains that are crucial for later development. We recently defined a domain enriched in ER in the vegetal region of *Phallusia mammillata* eggs. To explore the possible physiological and developmental function of this ER domain, we here investigate its organization and fate by labeling the ER network in vivo with $DiIC_{16}(3)$, and observing its distribution before and after fertilization in the living egg. In unfertilized eggs, the ER-rich vegetal cortex is overlaid by the ER-poor but mitochondria-rich subcortical myoplasm. Fertilization results in striking rearrangements of the ER network. First, ER accumulates at the vegetal-contraction pole as a thick layer between the plasma membrane and the myoplasm. This accompanies the relocation of the myoplasm toward that region during the first phase of ooplasmic segregation. In other parts of the cytoplasm, ER becomes progressively redistributed into ER-rich and ER-poor microdomains. As the sperm aster grows, ER accumulates in its centrosomal area and along its astral rays. During the second phase of ooplasmic segregation, which takes place once meiosis is completed, the concentrated ER domain at the vegetal-contraction pole moves with the sperm aster and the bulk of the myoplasm toward the future posterior side of the embryo. These results show that after fertilization, ER first accumulates in the vegetal area from which repetitive calcium waves are known to originate (Speksnijder, J. E. 1992. *Dev. Biol.* 153:259–271). This ER domain subsequently colocalizes with the myoplasm to the presumptive primary muscle cell region.

THE ER is a key cellular organelle involved in the synthesis of membrane lipids and proteins, secretion, and calcium homeostasis. Morphologically, it is organized as a continuous and extensive network of tubules and cisternae with typical three-way junctions. A number of recent studies have revealed that it is a highly dynamic structure which interacts with a variety of cellular constituents, including microtubules and actin filaments (Terasaki et al., 1986; Allen and Brown, 1988; Dabora and Sheetz, 1988; Kachar and Reese, 1988; Lee and Chen, 1988; Vale and Hotani, 1988; Lee et al., 1989; McCauley and Hepler, 1990; Hepler et al., 1990; Knebel et al., 1990; Terasaki, 1990; Allan and Vale, 1991; Terasaki and Jaffe, 1991).

Extensive ER networks have been described in eggs from a variety of species. Most work has concentrated on ER in the peripheral layer of the egg, the cortex. The cortical ER forms a two-dimensional network parallel to the plasma membrane with connections to the internal ER network (Campanella and Andreucetti, 1977; Sardet, 1984; Luttmer and Longo, 1985; Sardet and Chang, 1987; Henson et al., 1989; Speksnijder et al., 1989*a*; Terasaki et al., 1991; Houliston and Elinson, 1991). In sea urchin and ascidian eggs, this cortical ER is capable of calcium uptake and release and contains a high-capacity, low-affinity calcium-binding protein as well as a high concentration of calcium (Oberdorf et al., 1986; Payan et al., 1986; Oberdorf et al., 1988; Henson et al., 1989; Gualtieri and Sardet, 1989; Sardet et al., 1992). Recently we demonstrated that the cortical ER network is a target of inositol 1,4,5-trisphosphate (IP_3) mediated calcium release, and could be implicated in the propagation of waves of elevated free calcium (Terasaki and Sardet, 1991). These observations suggest that the ER network may play a key role in the regulation of the cytosolic calcium concentration in the egg at fertilization and during the cell cycle (e.g., Whitaker and Patel, 1990; Jaffe, 1991).

A particularly interesting aspect of ER organization in the egg cortex is its polar distribution and attachment to the plasma membrane along the animal-vegetal axis. Such polarity was first described in the *Xenopus* egg (Gardiner and Grey, 1983; Campanella et al., 1984), where differences in the density of ER-plasma membrane junctions were hypothesized to be linked to the preference for sperm entry in the

animal hemisphere, and to the decreased velocity of the calcium wave at fertilization in the vegetal hemisphere (Kline and Nuccitelli, 1985; Busa and Nuccitelli, 1985). Similarly, polarization of the ER has been demonstrated in anurans (Campanella et al., 1988).

Recently, we described a marked polarization in the density and configuration of the ER network in isolated cortices of the unfertilized egg from the ascidian *Phallusia mammillata*. The cortex in the vegetal hemisphere of this egg is characterized by a network of ER made of tightly knit tubules and cisternae, whereas the animal hemisphere contains a much less dense cortical network consisting only of tubules (Sardet et al., 1992). This vegetal ER-rich cortex is also enriched in actin filaments, but is poor in microtubules (Sardet et al., 1992). Furthermore, it is spatially associated with the subcortical domain of mitochondria-rich myoplasm in the vegetal hemisphere of the unfertilized egg (Sardet et al., 1992). This myoplasm is of great interest because after fertilization it is translocated to a region of the egg destined to become muscle and is thought to contain muscle-specific factors (Conklin, 1905; Whittaker, 1987; Venuti and Jeffery, 1989; Satoh et al., 1990). Using thin-section electron microscopy, we have further noted that after fertilization, a thick layer of ER accumulated between the plasma membrane and the myoplasm in the vegetal-contraction pole region (Gualtieri and Sardet, 1989). These observations prompted us to investigate the organization of the ER network in the ascidian egg and its reorganization after fertilization in more detail.

Recent developments in fluorescence microscopy (e.g., White et al., 1987) and vital staining of intracellular membranes with carbocyanine dyes (Terasaki et al., 1984; Terasaki, 1989; Terasaki et al., 1991) have made it possible to follow ER dynamics in living cells (Terasaki et al., 1984, 1986; Allan and Brown, 1988; Kachar and Reese, 1988; Terasaki, 1989, 1990; Knebel et al., 1990; McCauley and Hepler, 1990). We have developed a technique based on the spreading of dye molecules through the membranes of the ER after microinjection of an oil droplet saturated with dye (Terasaki and Jaffe, 1991). ER is labeled preferentially because it is the only organelle with some continuity in the cell (Terasaki and Jaffe, 1991). This staining method is thus more specific than methods based on diffusion of dyes through the plasma membrane, and should be applicable to all cells that can be microinjected, including large yolky cells such as eggs.

In an effort to understand the possible function of the vegetal ER domain in the *Phallusia mammillata* egg, we have used this technique to examine the organization and dynamics of the ER network in relation to the polarity of the unfertilized egg and to the cortical and cytoplasmic movements that accompany ooplasmic segregation (Conklin, 1905; Reverberi, 1971; Sawada, 1988; Jeffery and Bates, 1989; Sardet et al., 1989) in the fertilized egg.

Materials and Methods

Biological Material

Phallusia mammillata were collected in Sète on the Mediterranean coast of France, and kept in aquaria with running sea water. Gametes were obtained by dissection; concentrated sperm were kept at 4°C, and the eggs were rinsed several times with filtered sea water including 10 mM TAPS, pH 8.0 (TAPS SW). Eggs could be stored up to 24 h at 15–18°C. They were dechorionated by digestion with 0.1% trypsin in TAPS SW for 2–3 h, and then rinsed extensively. Dechorionated eggs were kept in gelatin-coated dishes to prevent sticking and lysis, and were inseminated with sperm preactivated by incubation with chorionated eggs as described by Sardet et al. (1989).

Labeling of the ER

The ER network of the isolated cortex of the *Phallusia* egg can be visualized after incubation with the membrane dyes $DiOC_6(3)$ and $DiIC_{16}(3)$ (Gualtieri and Sardet, 1989; Sardet et al., 1992). Incubation of whole eggs with $DiOC_6(3)$ or $DiOC_7(3)$, which have been used to label ER in animal and plant cells (Terasaki et al., 1984; Lee and Chen, 1988; McCauley and Hepler, 1990; Knebel et al., 1990) does not label ER selectively, because most of this dye is taken up by yolk granules. In contrast, incubation with $DiIC_{16}(3)$ results in plasma membrane labeling only, because this dye cannot cross the egg plasma membrane.

Selective labeling of the ER was achieved by microinjecting a saturated solution of $DiIC_{16}(3)$ (Molecular Probes, Inc., Eugene, OR) in Wesson oil (see Terasaki and Jaffe, 1991) using Hiramoto's low pressure method as described by Kiehart (1982). Unfertilized eggs were injected with the dye at random positions relative to their animal-vegetal axis, and held in position by slightly compressing them between two coverslips, a procedure which does not affect normal development (e.g., see Speksnijder et al., 1990*a*). At the site where the injected oil droplet comes in contact with membranes of the ER, the dye moves from the oil into the ER membrane and spreads within this membrane. Because the ER is a continuous network that extends throughout the egg, the dye spreads through the whole ER and is seen to fill it in ~30 min. In contrast, labeling of other membranous organelles remains limited to the area of immediate contact with the oil droplet.

Confocal Laser Scanning Microscopy

Labeled eggs were examined with a confocal laser scanning microscope (CLSM)[1] using an Argon laser at 514 nm (model MRC-500, Bio-Rad Microscience Division, Hemel Hempstead, Hertfordshire, UK) and a Zeiss Axioplan microscope equipped with the following objectives: a Plan Neofluar 40x/NA 0.75, a Plan Neofluar 40x/NA 1.30 (oil), and a Plan Apochromat 63x/NA 1.40 (oil) (Carl Zeiss Nederland, Weesp, The Netherlands). The laser beam was attenuated with a neutral density filter to 1% or 3% of its intensity so as to reduce photo-damage, and the pinhole position used was 3–5. Images were obtained using the fast scan rate and the Kalman integration algorithm, and were stored on a Panasonic Optical Memory Disc Recorder, which allowed for time-lapse analysis of the temporal aspects of ER organization in addition to the three-dimensional spatial information provided by the CLSM.

After the dye was allowed to spread throughout the unfertilized egg, which takes ~30 min, the distribution and dynamics of the ER network were studied by making z-series (through focus) and t (time)-series. Sperm was then added between the coverslips and the changes after fertilization were recorded in the same manner.

Photographs were made from the optical memory disc recorder images displayed on a video monitor. Because the video output from the CLSM does not provide a square image, the objects appear somewhat elongated in vertical direction. This slight distortion is most apparent in images of the unfertilized egg (Fig. 1 *A*; Fig. 3 *A*, *top*).

Electron Microscopy

Unfertilized and fertilized eggs were dechorionated with trypsin and fixed according to Gualtieri and Sardet (1989) in 4% glutaraldehyde, 0.2 M NaCl, 0.35 M sucrose, 5 mM $CaCl_2$, 0.2 M Na-cacodylate buffer pH 7.2 for 30 min to which 0.05% OsO_4 was added for the first 10 min. The eggs were rinsed in 0.2 M Na-cacodylate pH 7.2, postfixed in 1% OsO_4, 0.8% $K_4Fe(CN)_6$ for 1 h, rinsed in distilled water, dehydrated in a graded series of ethanol, and embedded in Spurr's plastic.

Results

ER Organization in the Unfertilized Ascidian Egg

The unfertilized egg of *Phallusia mammillata* displays an

1. *Abbreviations used in this paper*: CLSM, confocal laser scanning microscope.

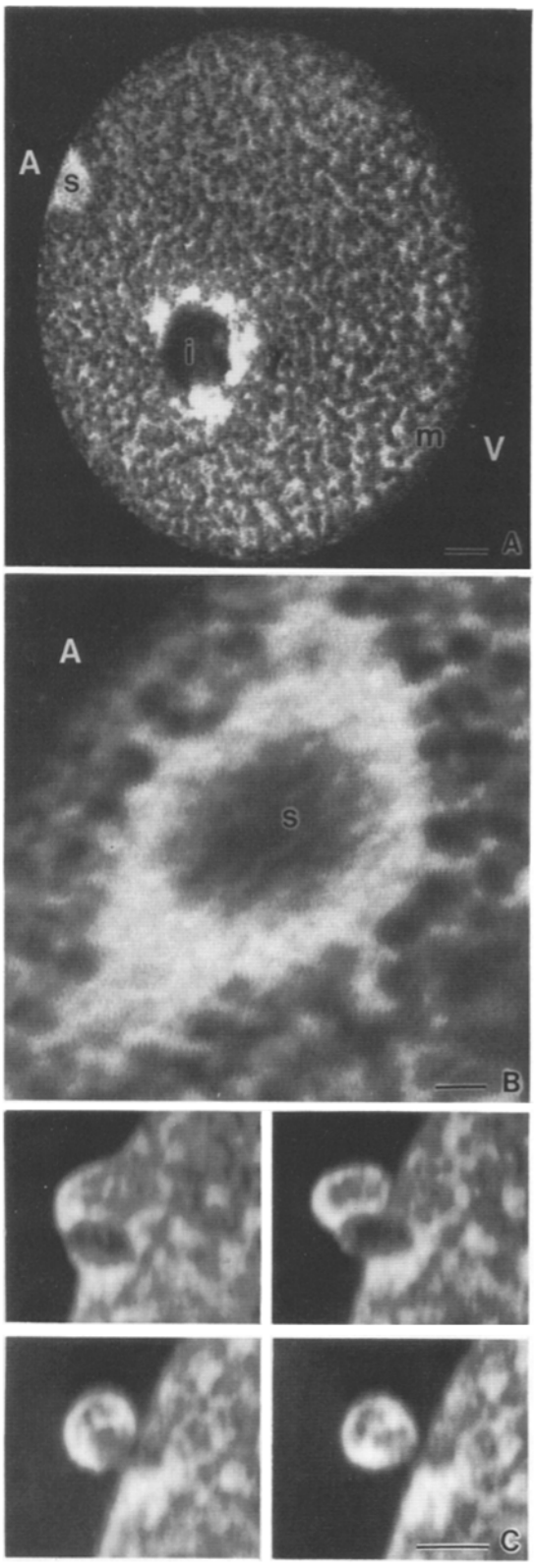


Figure 1. Labeling of the ER network of the ascidian egg. (*A*) Unfertilized egg of the ascidian *Phallusia mammillata* injected with $DiIC_{16}(3)$ to visualize the ER. This median section taken with a CLSM ~30 min after injection shows the meiotic spindle area (*s*) at the animal pole (*A*), the oil droplet from which the dye has diffused into the ER at the site of injection (*i*) and the ER-poor myoplasm (*m*). The brightly fluorescent ER-rich microdomains increase in size from the animal (*A*) toward the vegetal (*V*) pole (see also Fig. 3 *A*, *top*). Bar, 10 μm. (*B*) Higher magnification of the meiotic spindle area at the animal pole (*A*); the spindle (*s*) is surrounded by an ER-rich zone. Bar, 1 μm. (*C*) First polar body formation at ~6 min after fertilization. The spindle lies perpendicular to the egg surface. Bar, 10 μm.

animal-vegetal polarity manifest by the position of the meiotic spindle at the animal pole and the localization of a subcortical myoplasmic domain in the vegetal hemisphere (Sardet et al., 1989; Gualtieri and Sardet, 1989; Speksnijder et al., 1990*a*; Sardet et al., 1992). Observations with a CLSM on 10 unfertilized eggs (five different egg batches) with their ER labeled using $DiIC_{16}(3)$, show those two features clearly. At low magnification, it is apparent that the meiotic spindle area at the animal pole is enriched in ER compared with the surrounding cytoplasm (Fig. 1). In contrast, the subcortical myoplasmic domain in the vegetal hemisphere is relatively poor in ER (Fig. 1 *A*; Fig. 3 *A*, *top*), except for occasional strands with a high density of ER that extend from the vegetal cortex through the myoplasmic domain into the cytoplasm (Fig. 2 *C*). The ER network in the rest of the cytoplasm is organized in ER-poor areas, characterized by sparse tubules and sheets, and ER-rich microdomains from which other organelles such as yolk granules are excluded. In those ER-rich microdomains, the structural organization of the ER network cannot be clearly resolved by the CLSM (Fig. 2). The size of these ER-rich microdomains is somewhat variable from egg to egg, but in most cases a polarity is observed in their distribution, i.e., the larger sized ones predominate in the vegetal hemisphere, whereas the smaller ones are found in the animal hemisphere (Fig. 1 *A*; Fig. 3 *A*, *top*). Another typical characteristic of the cytoplasmic ER visible in thin sections for electron microscopy is the presence of tubules or sheets opposed tightly to large portions of yolk vesicle membranes (not shown).

An interesting feature of the unfertilized egg is the organization of the cortical ER network. By focusing on the animal part of the equatorial region of eggs positioned with their animal-vegetal axis parallel to the focus plane of the microscope, a clear border zone becomes visible, which shows the transition from a very dense network of tightly knit tubules and cisternae to a much less dense network consisting mainly of sparse tubules (Fig. 2, *A* and *B*). The dense cortical ER network is always located in the vegetal hemisphere of the egg. This agrees with our previous light and electron microscopic observations on isolated cortices and thin sections of whole *Phallusia mammillata* eggs (Gualtieri and Sardet, 1989; Sardet et al., 1992). The position of the transition between the two types of cortical ER corresponds to the edge of the underlying myoplasmic domain (Fig. 2 *C*). This suggests that there exists a colocalization of the subcortical myoplasmic domain and the dense cortical ER network in the unfertilized egg.

The continuity between the cortical and the cytoplasmic ER can be visualized by making a z-series from the upper egg surface into the cytoplasm (Fig. 2 *C*). These show that the fine network of tubules and cisternae is limited to the cortical zone situated immediately beneath the plasma membrane. Areas with densely concentrated ER can be seen to extend from the cortex deeper into the cytoplasm. These larger patches of dense ER are probably lost during the cortex isolation procedure, which would account for the presence of large holes in the ER network that we have observed

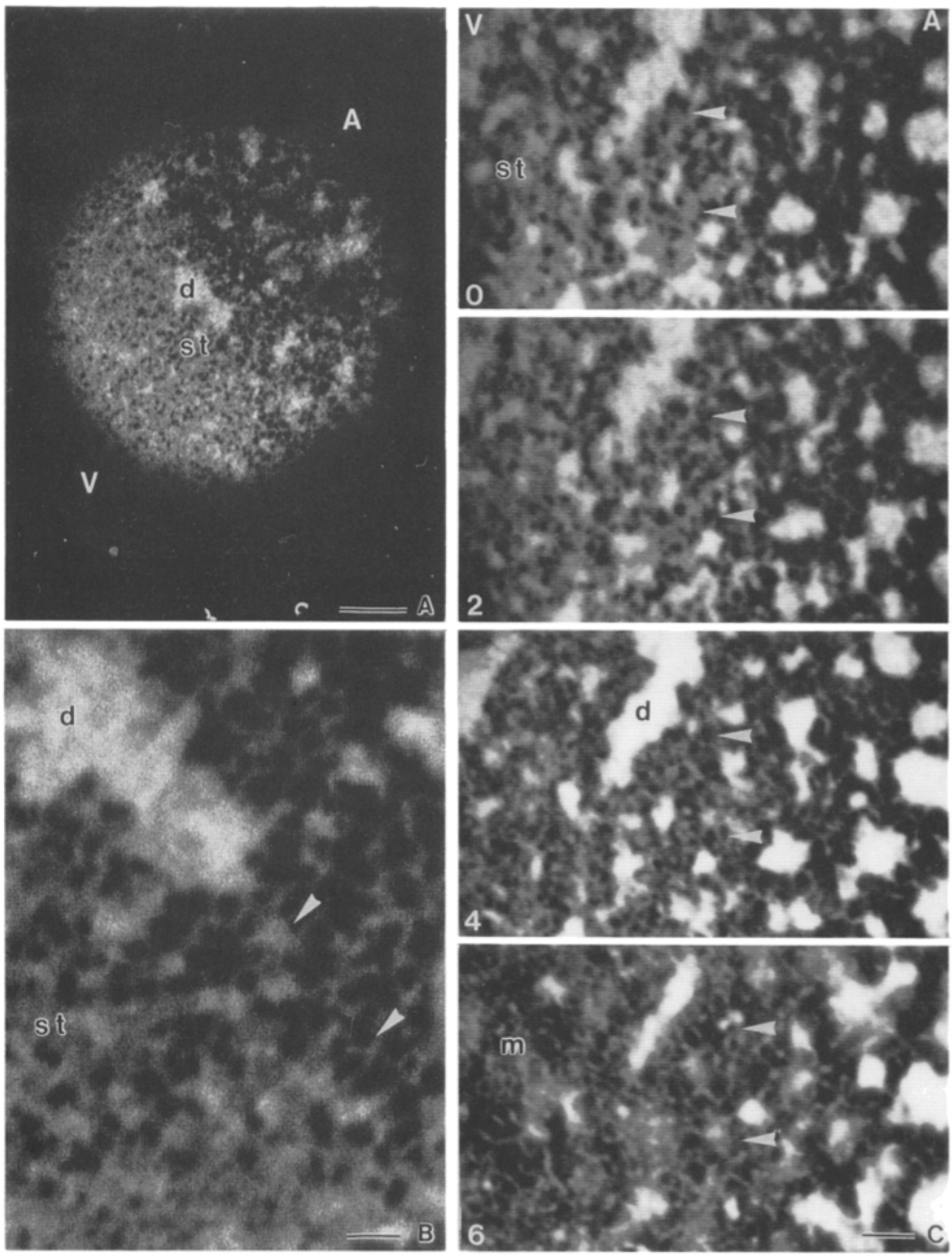


Figure 2. Polarity of the cortical ER network in the unfertilized egg. (*A*) Upper focus level of an egg oriented with its animal-vegetal (*A* and *V*) axis parallel to the focal plane showing the cortical ER network in the equatorial region of the egg. A dense network of sheets and tightly knit tubules (*st*) is present in the vegetal cortex (*bottom left*), whereas only sparse tubular ER and occasional ER-rich microdomains (*d*) are present in the animal cortex. Bar, 10 μm. (*B*) Higher magnification of the cortical transition zone (*arrowheads*) in the same egg. Bar, 2 μm. (*C*) Optical (*z*) sections (2 μm apart) through the cortical and subcortical transition zone (*arrowheads*). The vegetal (*V*) cortex is characterized by a dense ER network (*st*, *top*) which overlies the mitochondria-rich but ER-poor myoplasm (*m*, *bottom*). Strands of ER-rich microdomains (*d*) extend through the cytoplasm. Numbers at bottom left of each image indicate its *z*-position (in μm). Bar, 1 μm.

in cortices isolated from the *Phallusia* egg (Sardet et al., 1992).

The above described distribution of the ER network in the unfertilized egg was found to be similar in all eggs studied and to be independent of the site of injection recognizable by the position of the injected oil droplet.

Reorganization of ER after Fertilization

The changes in the organization of the cortical and the cytoplasmic ER network at fertilization were monitored by focusing on the median plane of unfertilized eggs with their animal-vegetal axis parallel to the focal plane, and recording successive images at regular intervals after sperm addition. This approach revealed that during the wave of cortical contraction that sweeps over the egg surface after fertilization, ER becomes concentrated rapidly into a cortical location at the vegetal-contraction pole simultaneous with the segregation of the subcortical myoplasm (Fig. 3 *A*). This contraction wave lasts ~2 min in *Phallusia mammillata* (Sardet et al., 1989; Speksnijder et al., 1990*a*). At the end of it, a brightly fluorescent layer ~2-6 μm thick is found at the contraction pole (Fig. 3 *A*), indicating that a very dense region of ER tubules and sheets has formed between the mitochondria-rich myoplasmic domain and the plasma membrane. This was confirmed by thin section electron microscopy (Figs. 3, *B–D*, and 4).

The ER in the rest of the cytoplasm also undergoes dramatic changes, first noticeable 4–5 min after fertilization. At this time, the size of the ER-rich microdomains increases progressively to up to 8 μm in diam (Fig. 3 *A*). Similarly, large ER-poor microdomains are formed that consist mainly

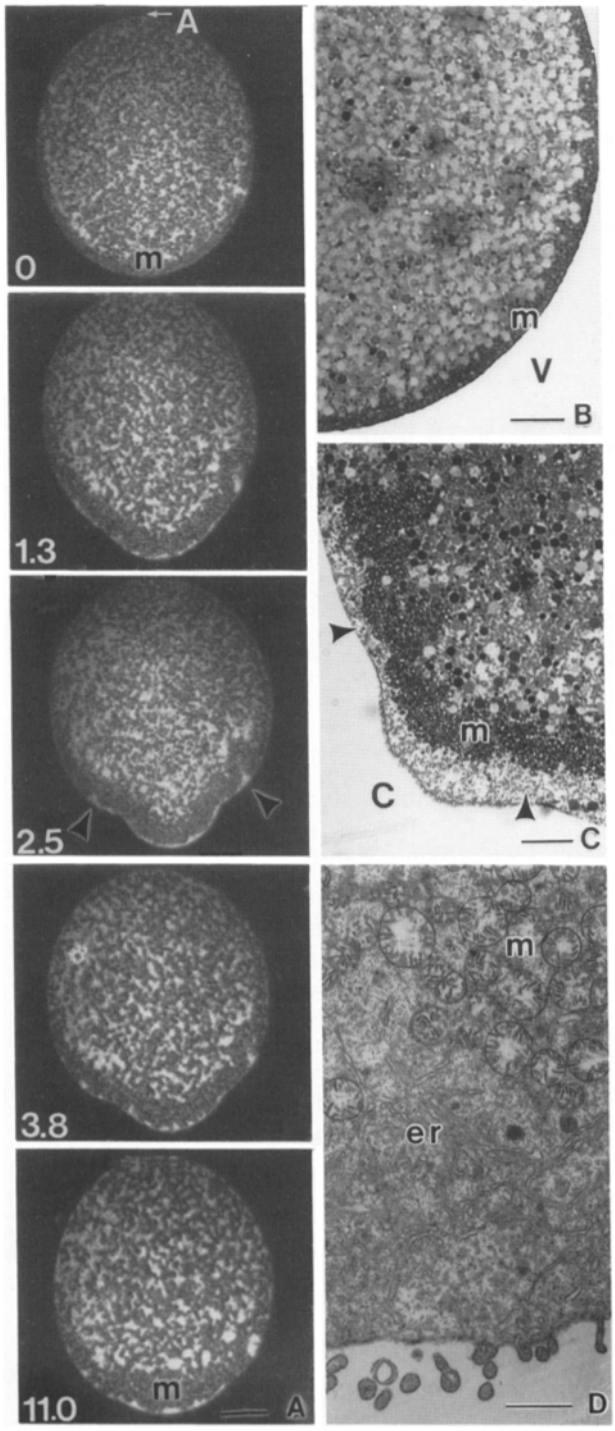


Figure 3. Reorganization of the ER network after fertilization. (*A*) Time series of a median optical section of an egg oriented with its animal-vegetal axis parallel to the focal plane (*A*; animal pole). The series starts with an unfertilized egg at the top showing the subcortical ER-poor myoplasm (*m*) in the vegetal hemisphere. After fertilization, the myoplasm thickens and ER accumulates in the vegetal-contraction pole area (*arrowheads*). Note that the size of the ER-rich microdomains increases progressively. Numbers at left bottom indicate time after fertilization in min. Bar, 20 μm. (*B–C*) Thick sections of plastic embedded eggs. (*B*) Vegetal hemisphere of an unfertilized egg with the myoplasm (*m*) closely apposed to the plasma membrane. Bar, 10 μm. (*C*) View of the vegetal-contraction pole (*C*) at 5 min after fertilization. A 2–6-μm thick layer (*arrowheads*) appears between the folded myoplasm (*m*) and the plasma membrane of the vegetal-contraction pole constriction in which the ER has accumulated. Bar, 10 μm. (*D*) Electron micrograph of a thin section of an egg at 5 min after fertilization showing the accumulation of ER (*er*) at the vegetal-contraction pole between the myoplasm (*m*) and the plasma membrane which displays large microvilli. Bar, 2 μm.

of vesicular organelles such as yolk granules. The size increase of the ER microdomains probably lies behind the coarser texture noticeable by differential interference contrast optics ~5 min after fertilization (see Fig. 1, *I–L* in Sardet et al., 1989).

Once these microdomains have increased in size, the organization of the cytoplasmic ER does not change much, except in the region of the sperm aster. The sperm aster is first clearly visible 10–13 min after fertilization as ER accumulates at the periphery of its small spherical centrosomal region (Fig. 5 *A*). As the aster grows, more ER accumulates along the astral rays. During meiosis, between first and second polar body formation, oscillatory contractions cross the egg as a result of repetitive calcium waves (Sardet et al., 1989; Speksnijder et al., 1990*b*). During this period, cytoplasmic ER microdomains can be seen to move back and forth along the animal-vegetal axis of the egg.

ER Movements during the Second Phase of Ooplasmic Segregation

After the completion of meiosis and the extrusion of the first (Fig. 1 *C*) and second polar bodies (at ~6 and 23 min after fertilization, respectively), the bulk of the myoplasm moves with the sperm aster toward the equator of the egg and becomes located at the future posterior pole of the embryo (Conklin, 1905; Reverberi, 1971; Whittaker, 1987; Jeffery and Bates, 1989; Sardet et al., 1989). During this process, the centrosomal area of the male aster changes shape from a sphere to a disk (Fig. 5). The layer of cortical ER at the vegetal-contraction pole was found to move with the bulk of the myoplasm to an equatorial position. During this movement, it narrows and thickens progressively, becomes elongated in the direction of its movement, and is visible as a brightly fluorescent, fan-shaped mass with its tip pointing toward the centrosomal region and its base in the vegetal hemisphere (Fig. 5 *B*). This ER mass appears to be continuous with the ER in the astral region, occupying the areas within the folds of the myoplasm (Fig. 5). Toward the end of the movement, distinct streaks of ER extending from the equatorial cortex to the male pronucleus become visible (Fig. 5 *A*). These probably correspond to the ropelike structure visible in differential interference contrast optics described previously (see Fig. 1, *R–T* in Sardet et al., 1989). This streak extends into the interior of the egg as the male and female pronuclei move inward (Fig. 5 *A*).

A total of 17 eggs from seven egg batches were used to study the different aspects of ER dynamics after fertilization, and each developmental event was recorded at least three times. Six of the 17 recorded eggs continued to divide, five were polyspermic, one failed to cleave, and development of the remaining five eggs was not recorded. No differences in ER dynamics were observed between those different groups, except for the recruitment of ER in more than one aster in

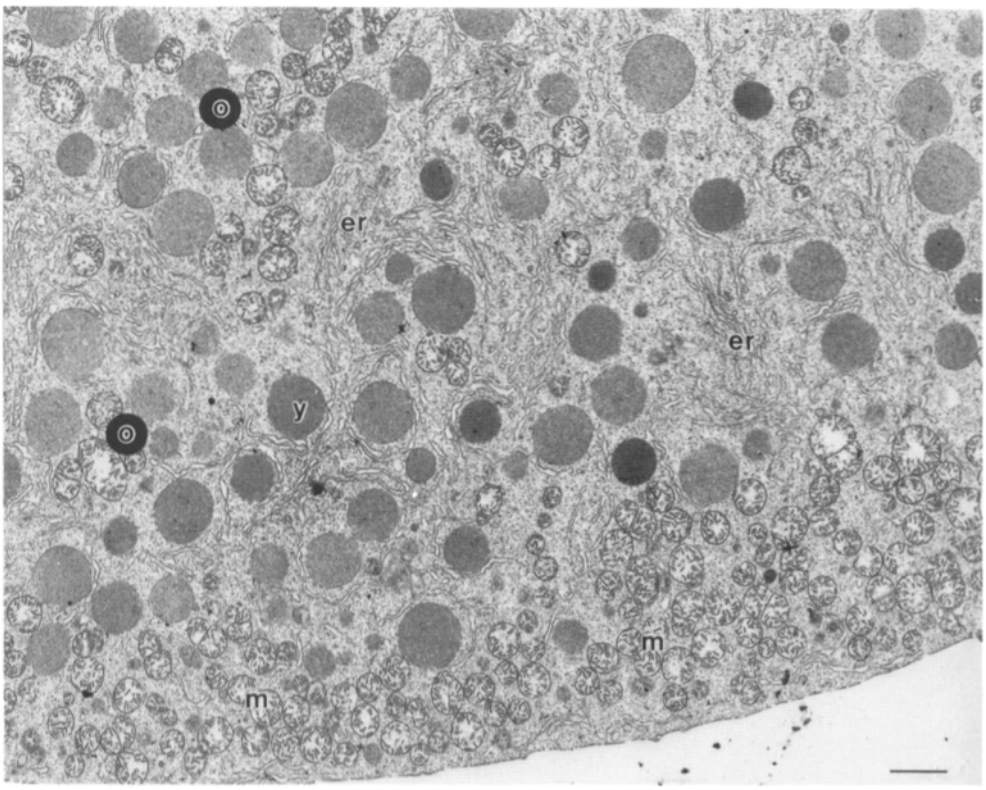


Figure 4. Ultrastructural organization of the ER microdomains in the fertilized egg (20 min post-fertilization). View of the subequatorial region showing the mitochondria-rich myoplasm (*m*), yolk granules, as well as ER-rich (*er*) and ER-poor (*o*) microdomains. Bar, 2 μm.

the polyspermic eggs. A schematic drawing summarizing the main events described above is given in Fig. 6.

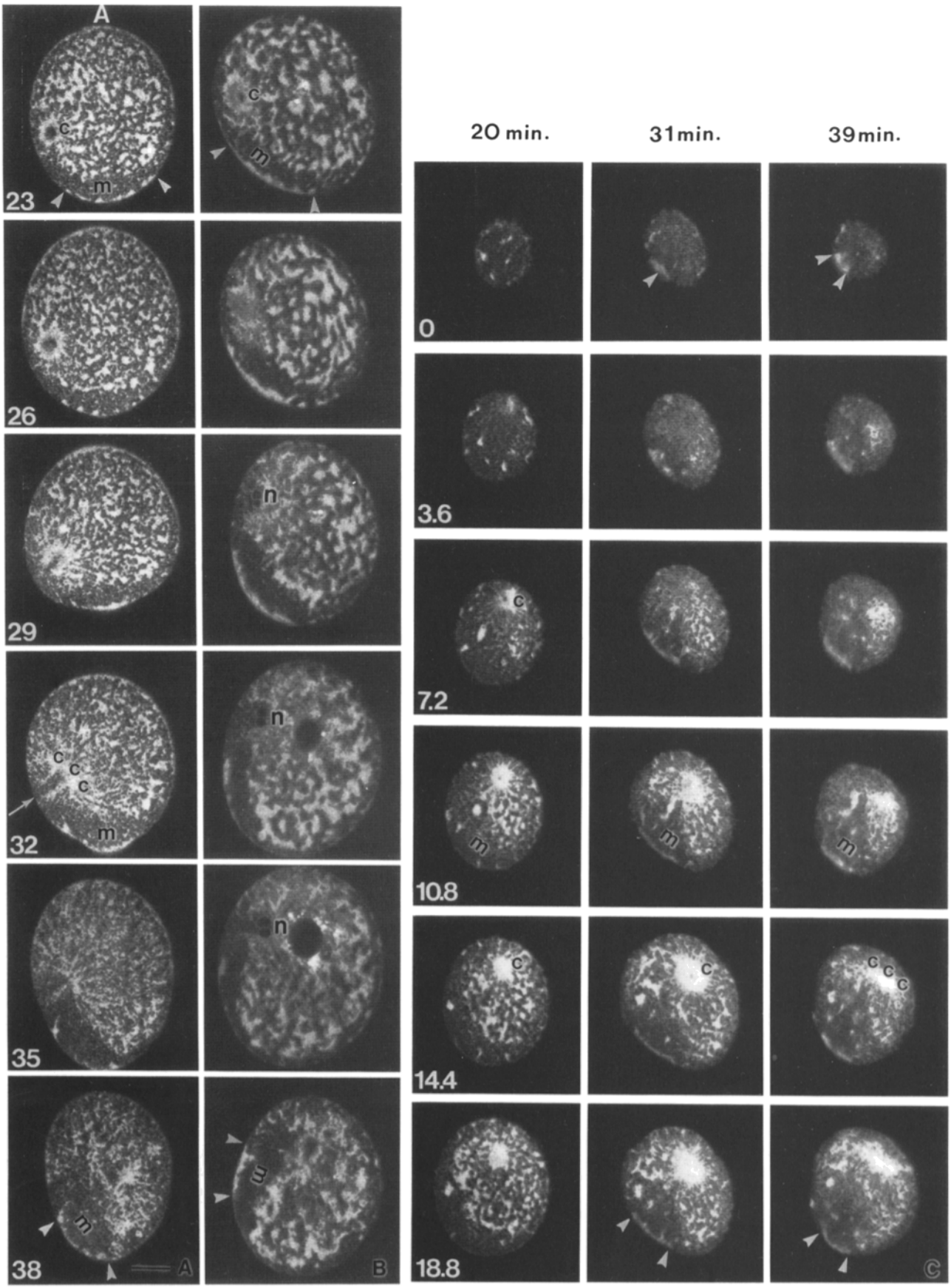
Discussion

The present study demonstrates that microinjection of $DiIC_{16}(3)$ dissolved in oil combined with confocal laser scanning microscopy is an ideal means to study the polarity and dynamics of the ER in the living egg of *Phallusia mammillata*. Our main finding is that the ER network is already polarized in the unfertilized egg, and that fertilization results in a striking reorganization and relocalization of the ER: part of the ER network first becomes rapidly (within 2 min) concentrated in the vegetal-contraction pole area and subsequently translocates toward the male centrosomal area and equator of the egg, whereas the remaining cytoplasmic ER aggregates into microdomains of increasing sizes.

ER Network is Already Polarized in the Unfertilized Egg

In the unfertilized sea urchin egg, a continuous ER network of tubules and cisternae is distributed homogeneously throughout the entire egg (Terasaki and Jaffe, 1991). The situation is very different in the unfertilized ascidian egg, where a distinct polarity in the distribution of the ER is observed. This adds the ER to the list of organelles and cytoskeletal components that are distributed in a polar fashion along the animal-vegetal axis of these eggs (Sawada, 1988; Swalla et al., 1991; Sardet et al., 1989, 1992). The polarity in the cytoplasmic ER is manifest by the ER-rich region around the meiotic spindle, the presence of ER-rich microdomains of increasing size toward the vegetal pole, and the low abundance of ER in the layer contributed by the subcortical, mitochondria-rich myoplasmic domain. The concentration of ER in the meiotic spindle region was expected on basis

Figure 5. ER movements during the second phase of ooplasmic segregation as observed with the CLSM. Bar, 20 μm. (*A*) Time series of a median section passing through the animal-vegetal axis of the egg and the sperm centrosomal region (*c*). The myoplasm (*m*) moves toward the centrosomal region (*c*) which changes its shape from a sphere to a disk (*ccc*), and a streaklike structure (*arrow*) that extends from the vegetal-posterior cortex toward the centrosomal region becomes visible. The ER domain originally located in the vegetal-contraction pole region (*arrowheads*) migrates together with the subcortical myoplasm. Time in min after fertilization is indicated at the bottom left of each image. (*A*) Animal pole. (*B*) Similar sequence seen from a different angle; the focal plane passes through the closely apposed male and female pronuclei (*n*), which together move to the egg center (*bottom*). The dark area shown in the fourth and fifth image represents the injected oil droplet from which all the dye has diffused into the ER. The cortical ER (*arrowheads*) can again be seen to move with the myoplasm (*m*). (*C*) Three series of successive z-sections taken at ~20, 31, and 39 min after fertilization. The series start at the surface (*top*) near the male centrosomal area (*c*) at the future posterior pole, and step through the myoplasm (*m*) toward the vegetal pole. Numbers in the left row indicate the z-positions (in μm) of the images. As time progresses, the male centrosomal area changes from a sphere to a disk (*ccc*). The ER domain thickens and assumes the shape of a fan (*arrowheads*) with its tip in the centrosomal area and its base in the vegetal hemisphere.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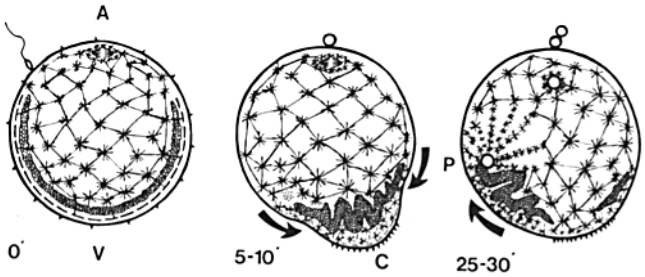

Figure 6. Schematic drawing of the changes in the organization of the ER network and the myoplasm during ooplasmic segregation in the ascidian *Phallusia mammillata*. ooo, myoplasm; "", ER-rich microdomains; →, bulk movement of the ER domain. (*A*) animal pole; (*V*) vegetal pole; (*C*) vegetal-contraction pole. In the unfertilized egg (0'), the vegetal hemisphere is endowed with a subcortical myoplasmic domain as well as dense layer of cortical ER. The cytoplasmic ER is organized in microdomains that increase in size toward the vegetal hemisphere. Sperm entry, which occurs preferentially in the animal hemisphere, triggers a wave of cortical contraction that carries the subcortical myoplasm to a vegetal-contraction pole (*C*) during the first phase of ooplasmic segregation. At 5–10 min after fertilization, ER is accumulated between the plasma membrane and the segregated myoplasm, and in the cytoplasm, the ER-rich microdomains have increased in size. At 25–30 min after fertilization, during the second phase of ooplasmic segregation, the ER domain moves with the bulk of the myoplasm as a fan-shaped mass toward the male centrosomal area, and becomes located at the future posterior pole of the embryo (*P*).

of earlier studies using thin-section electron microscopy and immunolocalization (for review, see Hepler, 1989). This, as well as the concentration of the ER observed in the sperm aster and the mitotic spindle after fertilization, is possibly mediated by molecular motors capable of moving ER along microtubules towards their minus ends (Allan and Vale, 1991). The nonrandom distribution of other cytoplasmic ER into clearly delineated microdomains that increase in size toward the vegetal pole is a new and unexpected finding. The mechanism of such domain formation is not known but may similarly involve motor molecules that promote movement and growth of ER along cytoskeletal components (Kachar and Reese, 1988; Dabora and Sheetz, 1988; Vale and Hotani, 1988; Allen and Vale, 1991).

The polarity in the ER network is most prominent in the egg cortex. In the vegetal part underlying the mitochondria-rich myoplasm, a layer of tightly knit tubules and sheets is found, whereas the cortical ER in the animal hemisphere is much less dense and consists of tubules mainly. Our previous work (Sardet et al., 1992) has shown that this ER polarity corresponds to a polarized distribution of cytoskeletal elements in the isolated cortex. In the ER-rich vegetal area, actin filaments are numerous, whereas microtubules are rare. The opposite is found in the animal cortex where only sparse ER tubules are present. Double-labeling studies show that microtubules often run along ER tubules over considerable distances. We found, however, that colchicin does not disrupt the polarity of the ER cortical network, whereas cytochalasin does (Sardet et al., 1992), which suggests that actin filaments are instrumental in maintaining the polarity of the cortical ER network as they are in maintaining the polarity of sperm entry in the egg at fertilization (Speksnijder et. al., 1989*b*).

ER Accumulates at the Vegetal-contraction Pole during the First phase of Ooplasmic Segregation

Actin filaments also play an important role in the rapid and dramatic reorganization of the egg that follows fertilization. Sperm entry results in a wave of calcium release which, in turn, triggers the first phase of ooplasmic segregation. A cortical contraction wave originating from the animal pole propagates toward the vegetal pole region, and ends in a constricted region close to but not necessarily coincident with the vegetal pole. We have named this region the vegetal-contraction pole area (Speksnijder et al., 1990*a*). During this contraction wave, which lasts ~2–3 min, the subcortical myoplasmic layer becomes concentrated in the vegetal-contraction pole region. The driving force for this contraction is provided by actin filaments, because it can be blocked by treatment with cytochalasin, but not with colchicin (Zalokar, 1974; Sawada, 1988; Jeffery and Bates, 1989; Sawada and Schatten, 1989; Speksnijder et al., 1990*a*,*b*). The present study shows that concomitantly with this actin-driven concentration of the myoplasm at the vegetal-contraction pole, ER accumulates into a subcortical domain between the myoplasm and the plasma membrane. The role of actin in this ER movement appears to be quite different from the one described in characean algae (Kachar and Reese, 1988), where ER is thought to move across polarized actin filaments attached to the plasma membrane. In the *Phallusia* egg, however, the cortical actin network accumulates with the ER at the vegetal-contraction pole. Because actin filaments in the *Phallusia* egg cortex interact extensively with the ER network (Sardet et al., 1992), we would suggest that the ER accumulation may be the result of an actino-myosin driven concentration of the ER network. Alternatively, this newly formed vegetal ER domain may be recruited in part from the deeper cytoplasmic ER, to which it is connected by single strands and occasional bundles of ER tubules and cisternae.

Several experimental observations suggest that this ER domain at the vegetal-contraction pole may be the source of the repetitive calcium waves that sweep through the egg after the first phase of ooplasmic segregation and originate mainly from this region (Speksnijder et al., 1990*b*). When the cortical contraction that concentrates the ER network and the myoplasm at the vegetal-contraction pole is blocked with cytochalasin, the repetitive calcium waves no longer start in this region, but instead tend to originate from the region of sperm entry, usually in the animal hemisphere of the egg (Speksnijder et al., 1990*b*). This suggests that during the cortical contraction some component necessary for the initiation of the repetitive calcium waves becomes localized in the vegetal-contraction pole area. The nature of this component was investigated by centrifuging unfertilized eggs such that their cytoplasmic components, including the myoplasm, are displaced. Such treatment, which does not affect the polar distribution of cortical components such as the ER network, does not alter the starting position of the calcium waves (Speksnijder, 1992), suggesting that a component that is initially localized in the egg cortex and subsequently becomes concentrated in the vegetal-contraction pole region after the first phase of ooplasmic segregation, is involved in initiating the repetitive calcium waves. We thus suggest that the ER domain at the vegetal-contraction pole is a likely candidate for this calcium wave pacemaker, in which case the

ER is instrumental in creating an oscillating gradient of calcium in the egg, with calcium periodically increased in the vegetal-contraction pole region (Speksnijder et al., 1990*b*).

Accumulated ER is Relocalized during the Second Phase of Ooplasmic Segregation

The second phase of ooplasmic segregation takes place after meiosis and the repetitive calcium waves have ceased. This phase is slower than the first one, lasting ~20 min, and can be blocked with colchicin (but not with cytochalasin), suggesting that unlike the first phase it is a microtubule-dependent movement (Sawada and Schatten, 1988, 1989). During this movement, the bulk of the myoplasm relocates from the vegetal-contraction pole to a more equatorial position (Sardet et al., 1989). We now show that the ER domain that accumulated between the myoplasm and the plasma membrane during the first phase of ooplasmic segregation, moves simultaneously with the myoplasm and the sperm aster toward the equator, and then toward the center of the egg.

This movement of the myoplasm and the ER domain in ascidian eggs is reminiscent of the cortical rotation movement during the first cell cycle in fertilized frog eggs (reviewed in Houliston and Elinson, 1992). They both comprise a translocation of the egg surface with attached cortical actin layer relative to the inner cytoplasm, occur in the vegetal hemisphere of the fertilized egg, are involved in defining the embryonic axes, and are dependent on intact microtubules, but not actin filaments (Manes et al., 1978; Vincent et al., 1987; Jeffery and Bates, 1989; Sawada and Schatten, 1989; Houliston and Elinson, 1992). In frog eggs, the rotation involves the singular movement of the vegetal cortex containing actin and ER along a parallel array of stationary microtubules in a plus-end directed fashion possibly using kinesin as a molecular motor (Houliston and Elinson, 1991). In ascidian eggs, however, the translocation movement is probably more complex because it occurs in two steps with distinctly different speeds, suggesting that different mechanisms may be at work. During the first step, myoplasm and ER move together with the sperm aster along the egg surface toward the equator at a speed of 3–6 μm/min (Sardet et al., 1989). During this step, the ER domain becomes elongated in the direction of its movement and assumes the shape of a fan, which suggests that it may be pulled by the sperm aster. The force generating mechanism of this step may depend on microtubule-associated motor molecules such as kinesin that generate plus-directed movement between astral microtubules and the cortex. Alternatively, force may be generated by microtubule motors that promote sliding between two populations of microtubules (see Vallee and Shpetner, 1990), some of which would be part of the sperm aster while others would be anchored to the cortex. During the second step, the sperm aster moves at ~10 μm/min to its final location toward the center of the egg, whereas the myoplasm and the ER domain move at ~26 μm/min in the same direction. During the latter step, the ER mass becomes narrower and thicker. This suggests that in addition to the pulling force exerted by the sperm aster, the ER could be concentrated toward the center of the aster via movement along astral microtubules using a minus-end directed motor molecule such as dynein (Vallee, 1991). Clearly, the microtubule-mediated movement of the ER domain during the second phase of ooplasmic segregation is complex and probably involves multiple mechanisms and motors (see e.g., Allan and Vale, 1991; Schroer and Sheetz, 1991; Gill et al., 1991).

Developmental Function of the ER Domain

With respect to the possible developmental consequences of our observations, we would point out that in addition to the mitochondria-rich myoplasm, the posterior blastomeres, which are the major precursors of the primary muscle cell lineage, will inherit most of the ER domain. This would probably endow them with a large intracellular store of releasable calcium. Furthermore, it has been shown that cellular factors involved in gastrulation and dorsal determination are transiently localized in the vegetal pole area between the first and second phase of ooplasmic segregation, since gastrulation and axis formation are blocked when this region is deleted (Bates and Jeffery, 1987). Although the latter experiments were done on the eggs of a different ascidian species, it is likely that a large part of the ER domain and its associated molecules were removed. Recent research has identified a localized maternal mRNA encoding a cytoskeletal protein that may be involved in axis determination (Jeffery, 1990*a*, *b*). Future research may elucidate if the ER accumulation in the vegetal-contraction pole region plays a role in this process.

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