Contractile Ring Formation in Xenopus Egg and Fission Yeast

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ABSTRACT. How actin filaments (F-actin) and myosin II (myosin) assemble to form the contractile ring was investigated with fission yeast and Xenopus egg. In fission yeast cells, an aster-like structure composed of F-actin cables is formed at the medial cortex of the cell during prophase to metaphase, and a single F-actin cable(s) extends from this structure, which seems to be a structural basis of the contractile ring. In early mitosis, myosin localizes as dots in the medial cortex independently of F-actin. Then they fuse with each other and are packed into a thin contractile ring.

At the growing ends of the cleavage furrow of Xenopus eggs, F-actin at first assembles to form patches. Next they fuse with each other to form short F-actin bundles. The short bundles then form long bundles. Myosin seems to be transported by the cortical movement to the growing end and assembles there as spots earlier than F-actin. Actin polymerization into the patches is likely to occur after accumulation of myosin. The myosin spots and the F-actin patches are simultaneously reorganized to form the contractile ring bundles.

The idea that a Ca signal triggers cleavage furrow formation was tested with Xenopus eggs during the first cleavage. We could not detect any Ca signals such as a Ca wave, Ca puffs or even Ca blips at the growing end of the cleavage furrow. Furthermore, cleavages are not affected by Ca-chelators injected into the eggs at concentrations sufficient to suppress the Ca waves. Thus we conclude that formation of the contractile ring is not induced by a Ca signal at the growing end of the cleavage furrow.

Key words: contractile ring/fission yeast/Xenopus egg/actin/myosin

Animal cells and primitive eukaryotic cells such as fission yeast and cellular slime mold amoeba divide by the contraction of the contractile ring formed at the division plane of the cell. It has been proposed that the position of the division plane where the contractile ring assembles is determined by astral or central microtubules of the mitotic apparatus (Rappaport, 1965; 1986; Hiramoto, 1971; Cao and Wang, 1996; Wheatley and Wang, 1996). Thus a signal for cleavage may be transmitted from the microtubules to the cell cortex, and it is able to induce assembly of actin filaments (F-actin) and myosin II (myosin) into the contractile ring. However, the entity of the signal has not been clarified so far. Involvement of Rho-mediated cleavage signaling pathways has recently been proposed (Kishi et al., 1993; Mabuchi et al., 1993), while that of Ca ions as a mediator has classically been considered. Here we investigated the latter possibility using Xenopus laevis eggs and describe it in the second chapter. How F-actin and myosin assemble to form the contractile ring is another problem which has not been well understood. We investigated the process of contractile ring formation in the fission yeast Schizosaccharomyces pombe and Xenopus egg in detail using optical sectioning and three-dimensional reconstruction fluorescence microscopy and confocal microscopy, respectively. The results are described in the first chapter.

I. Assembly of The Contractile Ring

Introduction

The fission yeast cell has three simple F-actin structures: cortical patches, cables and rings (Marks and Hyams, 1985;
Kanbe et al., 1990; Arai et al., 1998; Fig. 1). The F-actin patches localize to both growing ends of the cylindrical cell during interphase. It is suggested that the F-actin patches play a role in polarized cell growth (Kobori et al., 1989; Ishiguro and Kobayashi, 1996). The localization of F-actin patches has been shown to be controlled by small GTP-binding proteins and various actin-modulating proteins (for review see Gould and Simanis, 1997; Ishiguro, 1998; Le Goff et al., 1999). The F-actin cables run longitudinally during interphase and seem to be linked to some F-actin patches. During mitosis, the F-actin patches disappear from the ends of the cell, and the F-actin ring is formed at the medial region of the cell. It has not been known how the actin filaments accumulate at the medial region and form the ring structure. As the ring shrinks during cytokinesis, septum invaginates centripetally from the cell surface.

Myosin-II is believed to be a molecular motor that generates a force for contraction of the contractile ring through interaction with F-actin (Mabuchi and Okuno, 1977; DeLozanne and Spudich, 1987; Knecht and Loomis, 1987). Localization of myosin changes dynamically during mitosis: it is diffusely distributed in the prometaphase cytoplasm, but during metaphase to anaphase, it is recruited to the medial cortex (Fujiwara and Pollard, 1976; DeBiasio et al., 1996; Sanger and Sanger, 2000). In fission yeast, two myosin-II heavy chains, Myo2 and Myp2/Myo3, have been identified as components of the F-actin ring (Kitayama et al., 1997; May et al., 1997; Bezanilla et al., 1997; Motegi et al., 1997; Balasubramanian et al., 1998). Myo2 is essential for cell growth, while Myo3 is conditionally essential. We have

![Diagram of F-actin ring formation](image)

**Fig. 1.** A model for F-actin ring formation in the wild type *S. pombe* cell. F-actin cables are elongated between both ends of the cell during interphase. As positional signal(s) is generated from unseparated SPBs and/or nucleus at pre-prophase, formation of an aster-like structure is initiated from a spot during prophase. During metaphase, the aster formation and accumulation of the F-actin cables progress at the medial region. A leading F-actin cable extends from the aster and encircles the cytoplasm at the equator to form a primary F-actin ring. Thick F-actin ring is formed during anaphase by packing and fusion of the accumulated F-actin cables with the leading cable. During cytokinesis, the F-actin ring initiates contraction.
found that Myo2 and Myo3 function in a cooperative manner in the formation of the F-actin ring (Motegi et al., 1997; 2000). There are two myosin-II light chains, Cdc4 (McColum et al., 1995) and Rlc1 (Naqvi et al., 2000; Le Goff et al., 2000). Cdc4 is essential for cell growth, while Rlc1 is not essential. These light chains bind to both heavy chains (Motegi et al., 2000; Le Goff et al., 2000).

Xenopus egg is also a good system for studying the mechanism of contractile ring formation. In amphibian eggs, the cleavage furrow first appears at the animal pole and advances toward the vegetal hemisphere, being formed continuously at the growing ends. Therefore, the sequences of the contractile ring formation can be investigated intensively at the growing end of the cleavage furrow, while the central region of the cleavage furrow is suitable for investigating the mechanism of the contraction. Thus, reorganization of actin and myosin into the contractile apparatus during furrow formation of Xenopus egg was investigated by examining the cytoskeletal changes at the growing end of the cleavage furrow.

**Assembly of F-actin into the contractile ring in fission yeast**

Optical sectioning and three-dimensional reconstruction fluorescence microscopy have confirmed (Arai and Mabuchi, 2002; Fig. 1) that in an interphase S. pombe cell which passed through “new end take off” (Marks and Hyams, 1985), several F-actin cables run in the longitudinal direction of the cell from one end of the cell to the other, and a number of short F-actin cables are also observed. F-actin patches are concentrated at both growing ends. During prophase to early prophase when two closely associated SPBs are seen on the nucleus, some F-actin cables are seen to adjoin the SPBs. During prophase to metaphase, a novel F-actin structure which we call the aster-like structure is formed at the medial region of the cell. The center of this structure is located in between separated SPBs suggesting a possibility that this structure is formed being influenced by SPBs. Single F-actin cables extend from the aster and encircle the cell at the equator. We call these F-actin cables “leading F-actin cables”.

During early anaphase, F-actin cables at the medial region seem to associate with the leading cable. A fairly distorted portion is seen at one side of the F-actin ring in contrast to the other side, which is derived from the aster-like structure. The F-actin ring becomes smooth during late anaphase. The F-actin patches are scarcely seen especially at the medial region of the cell during this stage.

**Myosin-II heavy chains are required for the formation of the F-actin contractile ring and localized to the ring**

To investigate the localization of both Myo2 and Myo3 in same fission yeast cells, the myo3 null cells carrying pGFP81myo3 were stained with anti-Myo2 antibodies (Motegi et al., 2000). The staining pattern of Myo2 seems to be the same as the fluorescence pattern of GFP-Myo3 in mitotic cells, that is, both myosin heavy chains appear at the division site almost at the same time.

We investigated how Myo2 assembles into the medial ring during mitosis, because the function of Myo2 may be more important for cytokinesis than that of Myo3. We examined wild type cells by immunofluorescence microscopy (Fig. 2), or living synchronously dividing cdc25-22 myo2 null cells expressing GFP-Myo2 (Motegi et al., 2000). At early mitosis, GFP-Myo2 is detected as dots and projections of the Myo2 f-actin fibers becomes visible in the same area, which seem to be connected to the dots. These structures form a network that surrounds the cell at the medial region. The network is then packed into a sharp ring. This process takes place within about 20 minutes.

During pro- to metaphase, the Myo2 dots do not seem to localize to the aster-like structure of the F-actin cables. However, the dots and the projections tend to align along the F-actin cables during anaphase. When the continuous F-actin ring is established in the next stage in the division plane, Myo2 is colocalized to the F-actin ring. Similar behavior of myosin is observed when Rlc1 is monitored by observing GFP-rlc1 expressing cells during cytokinesis (Le Goff et al., 2000).

**F-actin is not required for accumulation of the Myo2 dots in fission yeast**

The localization of Myo2 is not seen in mitotic cdc4 mutant cells (McColum et al., 1995; Chang et al., 1996). However, in cdc3, cdc8, and cdc12 cells (Balasubramanian et al., 1992 and 1994; Chang et al., 1996), the Myo2 dots appear in the wide medial region of the cells (Motegi et al., 2000) as in wild type cells. Similar accumulation of the Myo2 dots around the division plane is seen in mitotic cdc25-22 cells in which F-actin had been disrupted during G2/M arrest by using Lat-A. This is confirmed by observing cdc25-22 myo2 null cells expressing GFP-Myo2: the Lat-A-treated cells display the GFP-Myo2 dots in the medial cortex. Thus, it is suggested that F-actin cables and proper localization of F-actin patches are not required for the appearance of Myo2 dots at the medial cortex of the cell.

**F-actin organization at the growing end of the cleavage furrow during the first cleavage of Xenopus egg**

F-actin structures in the cleavage furrow have been studied with cortex isolated from Xenopus eggs at various stages by rhodamine-phalloidin staining and by injection of fluorescently labeled G-actin during the first cleavage (Noguchi and Mabuchi, 2001; Fig. 3). At the growing end of early furrow (stage 1) where no contraction along its longitudinal axis is detected, F-actin appears as patches, the diameter of which was 0.5 to 1 μm. Timing of emergence seems to be
different among the patches even in a small area of the cortex. It took 30 to 40 seconds for the full growth of the patch from emergence to reaching maximum fluorescence. These observations suggest that actin polymerization takes place to form the patches.

In the following stage (stage 2), the cleavage furrow initiates contraction along its longitudinal axis. At the growing end of the furrow, the F-actin patches are connected with each other to form short F-actin bundles of about 2.8 μm long. When rhodamine-labeled G-actin is injected near the growing end of a cleavage furrow, it is incorporated into all the F-actin patches near the injection site and also into some short actin bundles within 30 sec. In the central region of the cleavage furrow, a number of long F-actin bundles are observed. These bundles are aligned in parallel to the longitudinal axis of the furrow: they are considered to be structural units of the contractile ring. As the furrow region becomes wider during stages 2 and 3, the number of the long F-actin bundles increases. The width of a belt of the long F-actin bundles reaches 100 μm at stage 3. In the next stage, the belt suddenly becomes tightly packed so that its width is less than 20 μm. This change seems to coincide with deepening of the furrow and with addition of a large amount of new cell membrane in the cleavage furrow. Finally, at stage 5, the two growing ends merge at the vegetal pole to form a complete ring structure that encircles this large cell.

**Cortical movement at the growing end of the cleavage furrow and accumulation of myosin**

The accumulation of materials into the cleavage furrow may be accounted for by a movement of cortex from surrounding region (Koppel et al., 1982; Wang et al., 1994). This was substantiated by visualization of cortex by attaching fluorescently labeled WGA beads on the cortex (Noguchi and Mabuchi, 2001). The cortex moves centripetally in the peri-cleavage furrow region, which is 20 to 30 μm in width, at a speed of about 16 μm/min. However, accumulation of F-actin is shown to initiate later and to be steeper than that of cortex. This result, together with that of the microinjection of rhodamine-G-actin, suggests that actin polymerizes after the cortex is accumulated in the cleavage furrow region. On the other hand, immunofluorescent staining with anti-myosin II serum reveals that accumulation of myosin II in the cleavage furrow occurs earlier than that of F-actin, and well coincides with that of cortex. This suggests that myosin may be transported with the cortex.

Myosin first forms spot structures at the growing end of the cleavage furrow. The size of the myosin spots is about 0.5 μm which is closer to “minimyosin filament” seen in the lamellipodia of moving fibroblast (Verkhovsky et al., 1995). Then newly emerging F-actin patches colocalize with the myosin spots. The myosin spots are arranged into tandem arrays along both the short and the long F-actin bundles in interior regions of the cleavage furrow.

**Discussion**

**Process of F-actin ring formation**

It was suggested that formation of the aster-like structure of F-actin cables and extension of the leading cable from this structure are important steps in the contractile ring formation in fission yeast. The formation of these structures is a novel finding in *S. pombe*. The formation of the aster-like structure seems to be initiated near the SPBs during early mitosis. It is tempting to speculate that the SPBs control the formation of the aster-like structure. This idea is consistent with the proposal that the positional signal(s) for the F-actin ring formation is generated from the nucleus in the middle of the cell (Chang and Nurse, 1996), probably through SPBs during prophase (Bähler et al., 1998) in *S. pombe*. Actually, it has been reported that Mid1 which has been considered to determine the site of the F-actin ring formation (Chang et al., 1996; Sohrmann et al., 1996; Edamatsu and Toyoshima, 1996), localizes on the nuclear membrane during interphase and re-localizes to the broad medial region of the cells during prophase (Sohrmann et al., 1996; Bähler et al., 1998). Function of Plol, a polo-like kinase, is required for this re-localization, and Plol localizes to the SPBs during...
prophase to anaphase (Bähler et al., 1998). Therefore, it is likely that these factors are involved in the signaling pathway for the F-actin ring formation. Although we do not know yet whether these factors themselves are relevant to the formation of the aster-like structure, some signal(s) from the nucleus may mark a spot on the medial cortex at pre-prophase or early prophase, and the formation of the aster-like structure may initiate from this spot.

In higher animal cells, the small G-protein Rho has been considered to be a member of the cleavage signaling pathways (Kishi et al., 1993; Mabuchi et al., 1993). Diaphanous/formin family proteins have been proposed to be downstream effector proteins of Rho and to be involved in the cleavage signaling (Watanabe et al., 1996). Cdc12 is a Diaphanous/formin family protein in fission yeast and has been shown to be essential for F-actin ring formation (Chang et al., 1996). It has been shown that GFP-Cdc12 forms a spot at the medial region of the cell during early mitosis, and it extends a strand that encircles the cell at the medial region (Chang, 1999). This resembles the extension of the leading F-actin cable from the aster-like structure.

Since Cdc12 binds profilin (Cdc3) (Chang et al., 1997) which has also been shown to be necessary in cytokinesis (Balasubramanian et al., 1994), and profilin is considered to be able to enhance actin polymerization (Pantaloni et al., 1993), it could be that Cdc12 induces actin polymerization from the center of the aster-like structure to form the leading F-actin cable. It remains to be clarified whether the actin cables of the aster-like structure including the leading cable are formed through polymerization from monomers or through clustering of preexistent F-actin cables.

We revealed reorganization of actin at the growing end of the cleavage furrow of Xenopus eggs during early stages of cytokinesis. In the early cleavage furrow or at the growing end of the furrow, F-actin patches are formed. Actin polymerization is shown for the first time to be involved in this process. In this respect, again it is important to investigate whether actin polymerization takes place during formation of the contractile ring in fission yeast or other cells. In cultured mammalian cells, recruitment of preexistent F-actin to the cleavage furrow has been reported (Cao and Wang, 1996a). The other interesting question is “what is the

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**Fig. 3.** Early stage (stages 1 and 2) of formation of the contractile ring in Xenopus egg. Myosin II is transported from outside of the furrow to the growing end of the cleavage furrow possibly by cortical movement which occurs in the peri-cleavage furrow region, and forms dots. F-actin then accumulates at the myosin dots to form clusters which we call F-actin patches. This process involves actin polymerization. The F-actin patches then line up in the longitudinal direction and fuse with each other to form short bundles. The short bundles are then linked with each other at the ends to form long bundles. Myosin dots are aligned on these bundles.
F-actin patch?” Are the F-actin patches at the growing end of the cleavage furrow of Xenopus eggs and those in fission yeast during interphase analogous apparatus? We need to know how F-actin are arranged in these structures.

The patches are fused with each other and obviously organized into short F-actin bundles (Fig. 3). Then, the short F-actin bundles are reorganized into the long F-actin bundles in the central region of the furrow. The long F-actin bundles are finally packed into the thin contractile ring at stage 4. The formation of the long bundles from the short bundles or packing of the long bundles into the tight contractile ring may share a similar process with the packing of accumulated cables to form the tight F-actin ring in fission yeast. In case of the sea urchin egg too, F-actin becomes accumulated cables to form the tight F-actin ring in fission yeast. In case of the sea urchin egg too, F-actin becomes accumulated cables to form the tight F-actin ring in fission yeast. Therefore, a basically similar molecular mechanism may underlie these processes in these different kinds of cells.

Cortical movement at the division site

The cortical movement in the Xenopus egg prior to cytokinesis occurs only in the peri-cleavage furrow zone (Fig. 3). Similar movements of the cortex around the cleavage furrow have been observed in sea urchin eggs by means of attachment of carbon particles on the surface (Dan, 1943), and in cultured mammalian cells by attachment of fluorescently labeled lectins (Koppel et al., 1982; Wang et al., 1994). Since the cortical movement is one of the earliest events in the cleavage furrow formation, the peri-cleavage furrow zone could be the cortical region that is responding to the signal for the cleavage furrow formation from the mitotic apparatus. It is tempting to speculate that a role of the cleavage signal in animal cells is to stimulate the equatorial area of the cortex to induce the cortical movement in order to accumulate further signaling molecules and materials necessary to construct the contractile ring. One of these materials seems to be myosin-II as discussed below.

Assembly of myosin-II in the cleavage furrow and role of myosin-II in the formation of the contractile ring

Myo2 or Rlc1 appears as dots in the medial cortical region of the fission yeast cell during early mitosis. Then, it forms a dot-fiber network which is then packed into a tight ring structure. The assembly of the Myo2 dots at the division site is an F-actin-independent process, while the packing of the wide cortical network into the ring is F-actin-dependent. The facts that the tail region of Myo2 can assemble at the division site (Naqvi et al., 1999) and an ATPase dead Myo2 cannot assemble F-actin cables into the F-actin ring (Naqvi et al., 1999) strongly support this idea. In Xenopus eggs, myosin also appears at the growing end of the cleavage furrow as dots earlier than F-actin does. The dots are later aligned on the F-actin bundles. Similar dot-like appearance of myosin in the early cleavage furrow have been observed in cultured fibroblastic cells (DeBiasio et al., 1996). The dot-fiber network of myosin has also been reported in cultured cells (DeBiasio et al., 1996) and sea urchin eggs (Schroeder and Otto, 1988; Mabuchi, 1990). These features in the appearance of myosin in the division site seem to be common in eukaryotic cells since these are seen in the very different cell species. However, there could be a difference among various cells in the manner of assembly of myosin: that is, in fission yeast, the myosin assembly as dots is completely an F-actin-independent process, while in Xenopus egg, myosin seems to be accumulated at the division site via the cortical flow which may utilize F-actin (Noguchi and Mabuchi, 2001).

The size of the myosin spots appeared at the growing end of the Xenopus cleavage furrow is close to that of the cytoplasmic “myosin spots” seen in the active lamella undergoing protrusion of fibroblastic cells (Verkhovsky et al., 1995). These spots have been found to be clusters of short myosin filaments called “minifilaments”. Fishkind et al. (Fishkind, D. J., Herman, I. M., Manubay, C. M. and Wang, Y. L. 1996. Mol. Biol. Cell, 7 Supplement, 560a.) have found that the minifilaments are localized to the cleavage furrow of tissue cultured cells. The myosin spots in the Xenopus egg cleavage furrow may also consist of these minifilaments. It is of interest to conjecture whether the Myo2 spots in the division site of fission yeast also represent minifilaments. We do not know whether Myo2 or Myo3 forms filaments in the cell since these myosin II heavy chains contain a number of proline residues which are not present in the conventional myosin II heavy chains.

Although myosin assembles independently of F-actin at the division site during early stage of the contractile ring formation, the myosin structures are colocalized with F-actin structures during a later stage when the contractile ring F-actin bundles are formed in both fission yeast and Xenopus eggs. In fission yeast, the motor activity of myosin has been shown to be necessary in the later stage. Therefore, myosin and F-actin interact with each other to establish the contractile ring structure. Since contraction of the contractile ring is initiated during this stage (Mabuchi, 1994; Noguchi and Mabuchi, 2001), sliding between F-actin and myosin filaments may take place in this process as proposed previously (Mabuchi, 1990).

II. Cleavage Signaling: Are Ca Ions Involved in This Process?

Introduction

In large eggs which undergo unilateral cleavage, it has been speculated that localized elevation of cytosolic free
calcium ion concentration ([Ca\textsuperscript{2+}]i) at the growing end of the cleavage furrow triggers cleavage furrow formation by the activation of myosin ATPase activity through phosphorylation by the myosin light chain kinase. However, data regarding the relevance of Ca waves in cleavage furrow formation is contradictory or incomplete. In fish eggs, it has been suggested that the cleavage furrow formation is accompanied by a localized Ca wave which propagates in the subcortical layer along the growing end of the cleavage furrow (Fluck et al., 1993; Chang and Meng, 1995; Webb et al., 1998). However, in these studies, the actual positions of the furrow ends have not been identified when the Ca waves have been observed. In Xenopus eggs, a Ca wave occurs at the growing end of the cleavage furrow. However, timings of the signal appearance differ among the reports: they are not restricted to the Whitaker, 1998). Thus, involvement of Ca wave in cytokinesis remains uncertain.

Ca waves do not appear at the growing end of the cleavage furrow

We made simple but important improvements in observing Ca signals in Xenopus eggs. We removed the fertilization membrane and viewed the egg from the animal hemisphere. In order to detect any Ca waves which may propagate along the cleavage furrow, we simultaneously visualized the cleavage furrow by staining the egg with a low concentration of rhodamine-WGA. We injected Calcium-green dextran as a Ca indicator, and imaged [Ca\textsuperscript{2+}]i with time-lapse recording using a confocal microscope (Noguchi and Mabuchi, 2002). No Ca wave was observed at the growing end of the cleavage furrow in any of the eggs examined (more than 200 furrows) before and during initiation of cleavage and during advancement of cleavage.

Neither Ca puff nor Ca blip occurs at the growing end of the cleavage furrow

We next investigated smaller Ca signals such as Ca puffs and/or Ca blips at the growing end of the cleavage furrow (Noguchi and Mabuchi, 2002). We used a highly sensitive fluorescent Ca indicator, Fluo-4 (Thomas et al., 2000) and the subcortical layer of the egg was scanned at a high speed and at a higher magnification. Injection of poorly metabolizable derivative of inositol 1,4,5-trisphosphate, 3FIP (3-deoxy-3-fluoro-D-my-o-inositol 1,4,5-trisphosphate) induces Ca puffs and Ca blips in the subcortical layer. Thus the system we used is sensitive enough to detect these Ca signals, and the Xenopus egg possesses such Ca signaling mechanism. Then we imaged each growing end at a rate of 0.78 sec/frame for more than 20 sec in which a cleavage furrow advances about 50 μm. Almost no microspike is detected at the growing end of the cleavage furrow in any of the eggs examined (0 to 3 signals/egg. n = 15). However, in all of these eggs, a number of Ca blips and Ca puffs are detected after microinjection of 3FIP (30 to 60 signals/egg). Therefore, no significant Ca microspike occurs at the growing end of the furrow. Moreover, the 3FIP3 injection revealed that while Ca releasing sites are present around the growing end of the cleavage furrow, they are not noticeably concentrated there. Furthermore, no significant Ca microspike is detected in the contractile ring region. Combined with the results of the Ca wave imaging, we conclude that no localized Ca signal accompanies the growing furrow end during the first cleavage of Xenopus eggs.

Two Ca waves appear during late stages of cytokinesis

Although we could not detect a Ca wave at the growing end of the cleavage furrow, we were able to detect other Ca waves (Noguchi and Mabuchi, 2002; Fig. 4). One type of Ca wave (wave 1) appears a few minutes after the initiation of the membrane insertion in the deepened furrow region. It propagates only in the region of the newly inserted membranes and lasts for five minutes, but never propagates into the old membrane region. Therefore, wave 1 is likely to be the counterpart of the “furrow deepening wave” which has been observed in zebrafish egg (Chang and Meng, 1995; Webb et al., 1998).

A second Ca wave (wave 2) appears after the completion of the first cleavage. It begins mostly at the animal pole and travels along the border of the old and the new membrane regions. It emerges sporadically and does not propagate for a long distance. Based on these features, it is likely that wave 2 corresponds to the Ca wave previously observed along the cleavage furrow in Xenopus egg (Muto et al., 1996). The speed of both wave 1 and wave 2 is 1.66 μm/sec, suggesting that they belong to the slow Ca wave class (Jaffe and Creton, 1998).

Injection of Ca chelators does not affect cleavages

Injection of EGTA at an intracellular concentration of 0.9 mM quickly and effectively lowers the [Ca\textsuperscript{2+}]i and suppresses both waves 1 and 2. It does not affect either the first or the second cleavage. The injection of dibromoBAPTA also clearly suppresses both waves although its ability to lower the [Ca\textsuperscript{2+}]i is a little weaker than that of EGTA. The first cleavage occurs normally. The second cleavage is usually
inhibited in the blastomere on the injected side, while it occurs normally in the blastomere on the other side. These results confirm that neither Ca wave 1 nor 2 is involved in cytokinesis. In addition, dibromoBAPTA induces aberrant and irreversible cortical contraction of the *Xenopus* egg.

**Discussion**

**Ca signal is not involved in the furrow formation at the growing end of the cleavage furrow**

Myosin II is activated by phosphorylation of its regulatory light chain at the activation sites. It has been shown by double staining with antibodies against phosphorylated myosin light chain and myosin heavy chain (Matsumura et al., 1998; Hori-Murata et al., 1998), that phosphorylation and assembly of myosin occur in close time periods in dividing cultured cells. Biochemical experiments have shown that myosin II is kept in inactive state during mitosis and activated through the phosphorylation after the end of mitosis in *Xenopus* egg and cultured cells (Satterwhite et al., 1992; Yamakita et al., 1994). There has been a speculation that free Ca ions activate myosin light chain kinase through binding to calmodulin prior to cytokinesis, and then myosin light chain kinase activates myosin by phosphorylation of the regulatory light chain. Here we demonstrated that Ca wave, Ca puff and even Ca blip which may be the smallest unit of Ca release in the cell (Parker et al., 1996; Bootman et al., 1997; Sun et al., 1998) do not accompany cleavage furrow formation in *Xenopus* eggs. Furthermore, suppression of the Ca waves 1 and 2 by lowering the [Ca**2+**]i does not affect the progression of the cleavage furrow. Therefore, a Ca signal is not likely to be directly involved in the formation of the contractile ring in *Xenopus* eggs, although we could not deny the possibility that a [Ca**2+**]i might change deep inside of the egg. Therefore, the above speculation may not be applicable to the formation of the contractile ring at the growing end of the cleavage furrow in *Xenopus* egg. Another possible mechanism of myosin activation through the low molecular weight GTPase Rho is more likely to be involved in the cleavage signaling (Kishi et al., 1993; Mabuchi et al., 1993), since the pathway containing Rho may not utilize free Ca ions as an activator.

**Properties of wave 1 and wave 2**

It was demonstrated that the two slow Ca waves, wave 1 and wave 2, detected along the cleavage furrow during the 1st cleavage are not required for cytokinesis in *Xenopus* egg. Wave 1 restrictively propagates within the new membrane region. The new membranes may be immature just after its formation as compared to the old membranes and the compositions of phospholipids and proteins might be different from those of the old ones. This is the first report that a Ca wave selectively propagates in a different area of the cell because of the possible differences in the membrane. In contrast, wave 2 propagates along the border of the old and the new membrane regions after cleavage. It is known that tight junctions are formed between two blastomeres along this region after the first cleavage is accomplished (Wang et al., 1997). Moreover, [Ca**2+**]i elevates during tight junction formation in tissue cultured cell (Merzdorf et al., 1998). Therefore, wave 2 might possibly be involved in tight junction formation between the blastomeres.
Contractile Ring Formation in Frog Egg and Fission Yeast

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