F-actin ring formation and the role of F-actin cables in the fission yeast Schizosaccharomyces pombe

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Summary
Cells of the fission yeast Schizosaccharomyces pombe divide by the contraction of the F-actin ring formed at the medial region of the cell. We investigated the process of F-actin ring formation in detail using optical sectioning and three-dimensional reconstruction fluorescence microscopy. In wild-type cells, formation of an aster-like structure composed of F-actin cables and accumulation of F-actin cables were recognized at the medial cortex of the cell during prophase to metaphase. The formation of the aster-like structure seemed to initiate from branching of the longitudinal F-actin cables at a site near the spindle pole bodies, which had been duplicated but not yet separated. A single cable extended from the aster and encircled the cell at the equator to form a primary F-actin ring during metaphase. During anaphase, the accumulated F-actin cables were linked to the primary F-actin ring, and then all of these structures seemed to be packed to form the F-actin ring. These observations suggest that formation of the aster-like structure and the accumulation of the F-actin cables at the medial region of the cell during metaphase may be required to initiate the F-actin ring formation. In the nda3 mutant, which has a mutation in β-tubulin and has been thought to be arrested at prophase, an F-actin ring with accumulated F-actin cables similar to that of anaphase wild-type cells was formed at a restrictive temperature. Immediately after shifting to a permissive temperature, this structure changed into a tightly packed ring. This suggests that the F-actin ring formation progresses beyond prophase in the nda3 cells once the cells enter prophase. We further examined F-actin structures in both cdc12 and cdc15 early cytokinesis mutants. As a result, Cdc12 seemed to be required for the primary F-actin ring formation during prophase, whereas Cdc15 may be involved in both packing the F-actin cables to form the F-actin ring and rearrangement of the F-actin after anaphase. In spg1, cdc7 and sid2 septum initiation mutants, the F-actin ring seemed to be formed in order.

Key words: F-actin ring, F-actin cable, Aster-like structure, Deconvolution, 3D reconstruction

Introduction
In cytokinesis of animal cells, assembly of the actin-based contractile ring occurs in the cell cortex at the division plane during late anaphase to early telophase, and the cells divide through contraction of the contractile ring (Mabuchi, 1986). It has been proposed that positional signals for contractile ring assembly may be transferred from astral or central microtubules to the cell cortex (Rappaport, 1986). Furthermore, it has generally been accepted that various regulators including signaling or actin-binding proteins are involved in dynamic rearrangement of the actin cytoskeleton throughout the cell cycle. Although various proteins that are involved in cytokinesis have recently been identified, little is known about the molecular mechanism of the contractile ring assembly including determination of the division plane.

The fission yeast Schizosaccharomyces pombe has three simple F-actin structures: cortical patches, cables and rings (Marks and Hyams, 1985). Dynamic behaviour of these actin cytoskeletons during the cell cycle has been investigated (Marks and Hyams, 1985; Arai et al., 1998). 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 the deposition of cell wall materials and in the maintenance of 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 reviews, see 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. The F-actin cables are seen to attach to the F-actin ring during mitosis (Arai et al., 1998). It has been proposed that F-actin ring formation initiates before anaphase in S. pombe (Marks and Hyams, 1985), being different from animal cells (Mabuchi, 1994) or Schizosaccharomyces japonicus (Alfa and Hyams, 1990), in which the contractile ring is formed during late anaphase to telophase. Recently, it has been reported that the actin filaments first appear as a faint and broad F-actin ring in the wild-type S. pombe cell before anaphase. However, how the actin filaments accumulate at the medial region remains unknown. Then the ring becomes a sharp one as anaphase progresses (Arai et al., 1998; Bähler et al., 1998). As the ring shrinks during cytokinesis, septum invaginates centripetally from the cell surface. The F-actin patches relocalize near the septum region in this stage.

Several cell division mutants that cannot form the F-actin
Distinct events seem to occur in the early step of the F-actin ring formation: one is accumulation of Mid1 followed by the accumulation of F-actin as the medial broad ring, and then the ring narrows. The other is positioning of the Cdc12 as a spot in the medial cortex. The spot then forms the ring by extending a strand. However, the relationship between these events remains unclear. Furthermore, little is known about the process of structural rearrangement of F-actin during the F-actin ring formation including how actin filaments are arranged in the ‘broad F-actin ring’. Thus, we investigated the process of the F-actin ring formation by optical sectioning and three-dimensional (3D) microscopy in the wild-type S. pombe cells and some cytokinesis mutant cells. Our observations suggest that the F-actin ring formation is initiated by the accumulation of the F-actin cables and the formation of an aster-like structure composed of F-actin cables at the cell equator during prophase.

Materials and Methods

Yeast strains and growth conditions

All the strains were cultured in YPD liquid medium. JY heterozygous cells were used as wild-type cells and grown to exponential phase at 30°C. nda3-KM311 cold-sensitive mutant (National Collection of Yeast Cultures, Norwich, UK) were grown at 36°C, a permissive condition, and then incubated at 20°C for 8 hours, a restrictive condition. Temperature-sensitive mutants, cdc7-24, cdc12-112, cdc15-140, spo1-B8 (gifts from Viesturs Simanis, ISREC, Switzerland) and sid2-250 (a gift from Dannel McCollum, Massachusetts University, USA), were grown at 25°C and then incubated at 36°C, a restrictive condition, for 3-4 hours.

Fluorescence microscopy

Immunostaining was performed as previously described (Arai et al., 1998). Mitotic stages were defined by localization of spindle pole bodies (SPBs) according to Hagan (Hagan, 1998). Representative images are shown in the following figures. The SPBs were labeled with rabbit anti-Sad1 antibody (a gift from Iain Hagan) and then with rhodamine-conjugated anti-rabbit IgG antibody (Cappel, Durham, NC). For F-actin and nuclear staining, Bodipy (4,4-difluoro-4-boronic acid)-labeled phallacidin (Molecular Probes Inc., Eugene, OR) and 4',6-diamidino-phenylindole (DAPI) were used, respectively. For usual observations, the specimens were examined under a Zeiss Axioscope fluorescence microscope using a Plan Apochromat 63× lens and then photographed on Kodak T-Max films of ASA 400 (Fig. 6a-h). To obtain deconvoluted optical sections and 3D reconstructed images, we used a Delta Vision system (Applied Precision Inc., Issaquah, WA) with an Olympus IX70 fluorescence microscope equipped with a UPlanApo 100× lens according to the manufacturer’s protocol (Figs 1-9). Original fluorescence micrographs of serial optical sections were taken every 0.2 μm in the direction of Z-axis and digitized. The original images were deconvoluted, and then reconstructed to obtain 3D images of the cells. For the 3D reconstruction, we examined two calculation modes, ‘Max Intensity’ and ‘Additive’. In the former mode, major fluorophores were emphasized but minor ones were removed. The minor fluorophores were retained, however, in the latter mode. Because the Additive mode was suitable for visualization of F-actin structures, especially continuous F-actin cables, we used this mode to show whole F-actin cables in 3D images in this paper. To visualize F-actin structures more in detail, we showed deconvoluted serial sections.

Results

Because it was difficult to visualize the detailed structure of the actin cytoskeleton during F-actin ring formation in the S. pombe...
F-actin ring formation in fission yeast

In this stage, several F-actin cables ran in the longitudinal direction of the cell from one end of the cell to the other, and several short F-actin cables were also observed. F-actin patches were concentrated in both growing ends and at least one end of each F-actin cable was linked to an F-actin patch.

In the cells showing two SPB spots in one optical section (Fig. 2), a few major F-actin cables were seen to adjoin the SPBs at the mid region of the cells and branch in this region. The stage of these cells was considered to be pre-prophase to early prophase, for the following reasons. First, the length of these cells was in the range of 13-14 μm, which was the size of mitotic cells we used. Second, two adjacent SPBs were recognized in these cells in a 0.2 μm section. Third, maturation of duplicated SPBs, which are not separated yet, occurs in S. pombe cells during this stage, and the size of one matured SPB is about 0.2 μm (Ding et al., 1997). Although most of the F-actin patches were localized near the ends, a considerable number were seen in the cytoplasm away from the ends. Some of them were attached to the F-actin cables.

In a metaphase cell (Fig. 3), the density of the F-actin cables increased at the medial region of the cell. Most of these F-actin cables seemed to emanate radially from a focus forming an aster-like structure, whereas there were some cables that were not involved in this structure. A single F-actin cable extends from the aster and encircles the cell at the equator. We call this F-actin cable a ‘leading F-actin cable’. In Fig. 3A, this cable once branched into two cables and then they merged again. Two leading cables extending from the aster and moving in two opposite directions were occasionally seen (Fig. 3B). At the reverse side of the medial cortex, a clear longitudinal cable was often observed (Fig. 3). Serial sectioning showed that the accumulation of the F-actin cables and the formation of the aster-like structure occur near the cell surface but not in the inner cytoplasm.

Association of F-actin cables with the F-actin ring

In a cell at anaphase A (Fig. 4), a thicker F-actin ring was observed. A fairly distorted portion is seen at one side of the F-actin ring in contrast to the portion at the other side of the ring (Fig. 4d-f). This distorted portion may have been the aster-like structure at the previous stage. Heavy accumulation of F-actin cables was seen at the medial region, and they were linked to the F-actin ring. In a cell in anaphase B (Fig. 5a), a sharp and ‘straight’ F-actin ring was seen, to which some F-actin cables were always linked. In a late anaphase cell (Fig. 5b) in which SPBs were completely separated towards both ends, an F-actin ring accompanied by fewer F-actin cables was seen.

The F-actin patches showed relatively random distribution during prophase to mid anaphase, being sparse at the mid region as compared with interphase. However, the F-actin patches were scarcely seen, especially at the medial region of the cell during late anaphase.

As the cells initiated cytokinesis (Fig. 5c), the F-actin ring started to contract, and the F-actin cables became clearly visible again extending from the division site. The F-actin patches also reappeared at the medial region of the cells (Fig. 5c). In either a late cytokinesis cell (Fig. 5d) or separating daughter cells (Fig. 5e), many F-actin patches emerged around

Accumulation of F-actin cables at the medial region in wild-type cells during early mitosis

An interphase cell that passed through ‘new end take off’ (Marks and Hyams, 1985) showed a single spot of SPB (Fig. 1). In this stage, several F-actin cables ran in the longitudinal direction of the cell from one end of the cell to the other, and several short F-actin cables were also observed. F-actin patches were concentrated in both growing ends and at least one end of each F-actin cable was linked to an F-actin patch.

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the newly formed septa, and the F-actin cables elongated from the septa. Serial sections showed that the F-actin cables were linked to either the shrinking ring or the F-actin patches at the medial region (data not shown).

Broad F-actin ring in nda3 conditional mutant

The broad F-actin ring similar to the one seen in the wild-type cells during prophase (Arai et al., 1998) has also been observed in the nda3 cold-sensitive mutant at a restrictive temperature (Chang et al., 1996). This mutant is thought to be arrested at prophase, and duplicated SPBs cannot separate from each other on the nucleus (Hiraoka et al., 1984; Kanbe et al., 1990). At 2-4 minutes after release from the arrested condition, the SPBs initiate separation on the nucleus, and then chromosome segregation (~10 minutes after the release) and cytokinesis (~14 minutes after the release) proceed in order (Hiraoka et al., 1984; Kanbe et al., 1990). However, it has not yet been examined how this broad F-actin ring is related to that seen in the wild-type cells and reorganization of the actin cytoskeleton after the release.

We observed localization of F-actin in both the arrested nda3 mutant cells and the released cells using Bodipy-phallacidin staining. In the arrested cells showing condensed chromosomes, a broad F-actin ring and some elongated cables were seen as observed by conventional fluorescence microscopy (Fig. 6a,b). As analyzed by the optical sectioning and 3D reconstitution microscopy, an F-actin ring accompanied by several F-actin cables was visualized in the arrested cells (Fig. 6i). This appearance is very similar to that of early anaphase cell. These rings seemed to be loosely packed as compared to those seen in released cells (see below).

The F-actin rings were narrowed at 4 minutes after shift to permissive temperature (Fig. 6c,d). As analyzed by the 3D reconstruction microscopy (Fig. 6j), packed F-actin rings were clearly visible. These rings did not seem to be accompanied by the F-actin cables. F-actin patches appeared all over the cell cortex. After the completion of the chromosome segregation (Fig. 6e-h), the F-actin ring started to contract at ~10 minutes after the release.

Actin cytoskeleton in several temperature-sensitive cytokinesis mutants

Because we considered that cytokinesis mutants might have defects in organization of the F-actin cables, we examined actin cytoskeleton in some temperature-sensitive mutant cells. We examined the second mitosis after temperature shift to observe the exact phenotype of the mutant cells. Detailed mitotic stages were determined both by the extent of condensation of chromosomes and the position of the SPBs on the nuclei. During anaphase, two sister nuclei move towards both ends of the cell. However, they are pulled back towards the cell center after anaphase, and a septum is formed between the closely located nuclei during cytokinesis in the wild-type cell (Hagan, 1998). The SPBs always localize on the moving side of the nucleus. In most of cytokinesis mutant cells examined in this study, the sister nuclei separated after the first mitosis at a restrictive temperature were located close to each other near the center of the elongated cells. They stayed in these positions without a septum during the next interphase to metaphase. As a result, two spindles tended to elongate parallel to each other during the following anaphase in the second mitotic cycle.

F-actin structures in cdc12 and cdc15 mutant cells were analyzed by the optical sectioning microscopy. During interphase, F-actin cables seemed to be normally arranged in both the cdc12 and the cdc15 mutant cells (data not shown). In a cdc12-112 mutant cell at metaphase (Fig. 7A), two aster-like structures were seen in the medial cell cortex. However, no leading F-actin cable was recognized. During anaphase (Fig. 7B),
the aster-like structures were still recognizable and F-actin cables were accumulated at the medial region. However, again, no leading F-actin cable was seen, in contrast to anaphase wild-type cells in which the aster-like structure could not clearly be recognized; instead, a distorted (Fig. 4) or a packed (Fig. 5a) F-actin ring was observed. After anaphase (Fig. 7C), some F-actin cables were longitudinally elongated like interphase wild-type cells, and the accumulation of the F-actin cables at the medial region was not seen any longer.

In a cdc15-140 mutant cell at metaphase (Fig. 8A), two aster-like structures, each possessing a leading F-actin cable, were seen. During anaphase (Fig. 8B), two distorted F-actin rings, which seemed to be partially connected with each other, were formed. After anaphase (Fig. 8C), several longitudinal F-actin cables were clearly seen. These cables were elongated from a point at the medial cell cortex. A remnant of an F-actin ring, which did not seem to have shrunk after anaphase, was also seen at the cell equator, and it was linked to the medial point from which the F-actin cables were elongated. In the first mitotic cycle after shift to the restrictive temperature, only one aster-like structure was observed both in the cdc12 and cdc15 mutant cells (data not shown). Therefore, it is confirmed that one nucleus formed one aster-like structure, and that the two aster-like structures seen during the second mitosis were not fragments derived from one single aster-like structure through its degradation.

We further analyzed F-actin structures in spg1, cdc7 and sid2 mutant cells during and after the F-actin ring formation. These mutant cells showed similar phenotypes. During metaphase, we saw two aster-like structures near the nuclei. A leading cable seemed to extend from each of the aster-like structures (data not shown). Then, a single F-actin ring was formed in about half of the cells at late anaphase (Fig. 9a,b), whereas double rings were also observed in the remainder (Fig. 9c,d). We could never find cells that had no F-actin ring in this stage. Most of the double rings seemed to be connected to each other at one point. Moreover, one of the double rings was a complete ring, whereas the other one was incomplete (Fig. 9d). In the first division cycle after shift to the restrictive temperature, only one single F-actin ring was observed in these

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**Fig. 3.** F-actin structures in wild-type cells at metaphase. Green, red and blue represent F-actin, SPBs and DNA, respectively. (A) A cell in which a single leading cable extends from the aster-like structure. (B) A cell in which two leading cables extend from the aster-like structure. Rotating 3D images are shown in a and b (0° and 12°, respectively) of each panel. Direction of rotation is marked near a. (c) The F-actin cables in each cell. Thick lines represent the F-actin cables, including the aster at one side of the cell, whereas thin lines represent those at the reverse side of the cell. Deconvoluted serial sections are shown in d-o (A) or d-s (B). Pink arrows indicate an aster-like structure, and white arrows indicate leading F-actin cables. White arrowheads indicate longitudinal F-actin cable. Bars, 2 μm.
mutant cells during anaphase (data not shown). After anaphase, 24 out of 34 spg1 mutant cells, 22 out of 31 cdc7 mutant cells and 6 out of 32 sid2 mutant cells still possessed the F-actin ring – which did not seem to have contracted (Fig. 9g,h, right cell) – whereas the rest of the cells did not (Fig. 9e,f). As the SPBs were pulled back further towards the cell center, the persisted F-actin ring disappeared (Fig. 9g,h, left cell). Being distinct from the cdc15 cells at the same stage, the longitudinal F-actin cables were not seen in these mutant cells. Curiously, F-actin patches reappeared at the medial region (Fig. 9f,h), although septum formation was never observed in these cells.

**Discussion**

Mutual relationship among F-actin structures in *S. pombe* cells

*Schizosaccharomyces pombe* cells have three major actin cytoskeletons, namely the F-actin patches, the F-actin cables and the F-actin ring, which form during mitosis. Structural relationships among these cytoskeletons have not well been known. We have previously reported that the F-actin cables seem to be linked to some of the F-actin patches and to the F-actin ring (Arai et al., 1998). In this study, optical sectioning of the cell followed by deconvolution and 3D reconstruction allowed us a more detailed analysis of the actin cytoskeleton. The linking of the F-actin cables to some of the F-actin patches, at least at one end, was confirmed during interphase and early prophase. In addition, attachment of the patches on the cables was observed in metaphase cells. This is similar to *Saccharomyces cerevisiae*, in which the patches and the cables are often linked, as observed by 3D reconstruction (Karpova et al., 1998). Recently, it has also been reported that the actin patches move in a directed manner along the F-actin cables in *S. pombe* (Pelham and Chang, 2001). However, the F-actin ring is formed from the F-actin cables during mitosis in wild-type *S. pombe* cells (see below). Therefore, these three F-actin structures in *S. pombe* are closely related to each other. Because these cytoskeletons are dynamic structures such that they may disassemble and reassemble in the course of the cell cycle, and as only a small amount of G-actin seems to exist in the cell (R.A. and I.M., unpublished), it could be that F-actin interchanges between these cytoskeletons without depolymerization, or that very rapid depolymerization and repolymerization take place in the cell.

Protein constituents other than actin in these structures are reported to be different. Tropomyosin is present in both the F-actin cables and the F-actin ring, but is absent in the majority of the F-actin patches (Arai et al., 1998). By contrast, Arp3 is present in the F-actin patches but is absent from both the F-actin cable and the F-actin ring (McCollum et al., 1996; Arai et al., 1998). Type II myosin light chains (Cdc4 and Rlc1) (McCollum et al., 1995; Le Goff et al., 2000; Naqvi et al.,...
Formation of aster-like structure of F-actin cables
Major F-actin cables were branched near the SPBs at early prophase, and then an aster-like structure composed of the F-actin cables was seen in the middle region during metaphase. The branching of the F-actin cables seemed to be a first step in the formation of the aster-like structure. Because these events occur at a position very close to the SPBs, which are not yet separated from each other, it is tempting to speculate that the nonseparated SPBs control the formation of the aster-like structure during early prophase. The formation of this structure is a novel finding in *S. pombe* during early mitosis. It has also been reported that radial F-actin arrays, which are similar to the aster-like structure described here, are observed at a future site of new hypha formation and at a future elongation tip of each spore in the oomycete *Saprolegnia ferax* (Bachewich and Heath, 1998). The relationship between this structure and the SPBs/nucleus, however, has not been investigated.

Accumulation of F-actin cables at the medial region of the cell during metaphase
A broad and faint F-actin ring has been observed in early mitotic wild-type cells using conventional fluorescence microscopy (Chang et al., 1996; Arai et al., 1998; Bähler et al., 1998). In this study, the density of the F-actin cables increased in this region in metaphase wild-type cells. Thus, it is reasonable to consider that the broad F-actin ring visualized by the conventional microscopy actually represents the accumulated F-actin cables. Where do these F-actin cables come from? The first possibility is that the F-actin cables are originated from the center of the aster-like structure. However, our observation suggests that not all the cables emanated from the aster-like structure. A second possibility is that actin polymerization and subsequent bundling might have occurred in the broad medial region. However, we could not detect newly polymerized actin filaments, not yet bundled, in the metaphase wild-type, as analyzed by optical sectioning and 3D reconstruction microscopy. Thus, this theory is weakened, although the polymerized actin filaments could be removed from the image as background by the deconvolution process and only the strongly fluorescent F-actin cables could have remained. The third possibility is that the F-actin cables move from both sides of the cell to the medial region. This is probable because the density of the F-actin cables move from the ends to the medial region as mitosis progresses. It is necessary to live-record the movement of the cables to prove this hypothesis and to know how the cables move in the cell by using expression of GFP-actin fusion protein, for example.

Process of F-actin ring formation
A schematic model for the F-actin ring formation is presented in Fig. 10, which is based on the present observations. In this model, it is supposed that a positional signal(s) (see below) is generated from the SPBs or nucleus, or both, and reaches the cortex of the medial region of the cell at pre-prophase. The signal(s) induces the formation of the aster-like structure during early prophase. The accumulation of the F-actin cables is initiated during prophase.

During the formation of the aster-like structure, a leading F-actin cable seems to extend from this structure, and encircle the cell at the equator to form the primary F-actin ring during metaphase. F-actin cables accumulated in the medial region seem to be connected to the leading cable during early anaphase and then all the cable structures are packed to form the complete F-actin ring during late anaphase.

It has been shown that the type II myosin heavy chain Myo2 accumulates around the mid region independently of its head proteins, for example.

**Fig. 6.** F-actin structures in *nda3* mutant cells. *nda3* mutant cells were incubated at 20°C for 8 hours to arrest. For release experiment, once arrested, cells were re-incubated at 36°C and collected every 2 minutes. These cells were immediately fixed at each time, and then stained simultaneously with DAPI and Bodipy-phallacidin. (a-h) Photographs obtained by conventional fluorescence microscopy. Left, DAPI stainings; right, Bodipy-phallacidin stainings. (a,b) Arrested cells. (c-h) Released cells: 4 minutes (c,d), 10 minutes (e,f) and 14 minutes (g,h) after temperature shift-up. (i,j) 3D images. Green and blue represent F-actin and DNA, respectively. (i) Arrested cells. (j) Cells at 4 minutes after the release. Arrows indicate accumulated F-actin cables. Bar, 2 μm.
domain (Naqvi et al., 1999) and F-actin (Motegi et al., 2000) during early mitosis. Myo2 forms spot structures that do not colocalize with the aster-like structure. Subsequently, the Myo2 spots change into a spot-fiber network, and then the network is packed to form the ring along with the packing of the F-actin cables to form the F-actin ring. Colocalization of Myo2 with the F-actin cable is seen during this stage (Motegi et al., 2000), and this process may require ATPase activity of Myo2 (Naqvi et al., 1999) and the presence of F-actin (Motegi et al., 1997; Motegi et al., 2000).

Conversely, F-actin cables can accumulate in the mid region of the cell in the cdc12 mutant at the restrictive temperature (Motegi et al., 2000), although both Cdc4 (McCollum et al., 1995) and myosin II heavy chains (Myo2 and Myp2/Myo3) (Motegi et al., 1997; Motegi et al., 2000) are required for the formation of the F-actin ring. These observations suggest that the changes in F-actin organization at the mid region of the cell, which include the accumulation of F-actin cables and formation of the aster-like structure, and changes in myosin II spots at the mid region involve processes that are independent of each other. The next step to complete the F-actin ring formation may require the interaction of these structures.
Positional signals and the aster-like structure

Recently, it has been proposed 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) and that transduction of such signal(s) may be mediated by SPBs during prophase (Bähler et al., 1998) in *S. pombe*. In fact, it has been reported that Mid1, which has been considered to position the site of the F-actin ring formation (Chang et al., 1996; Sohrmann et al., 1996), localizes on the nuclear membrane during interphase and relocates to the broad medial region of the cells during prophase (Sohrmann et al., 1996; Bähler et al., 1998). For this relocation, function of Plo1 is required, and Plo1 localizes to the SPBs during prophase to anaphase (Bähler et al., 1998). Therefore, these factors are probably involved in the signaling pathway for the F-actin ring formation. However, it has not been clarified how the signal(s) is transferred from the nucleus or the SPBs, or both, to the cortex of the cell. Although the SPBs are attached on the nuclear membrane during mitosis in *S. pombe*: a part of the nuclear membrane just beneath the SPBs is collapsed at pre-prophase and then the SPBs are settled in the nuclear membrane (Ding et al., 1997). The partial collapse of the nuclear membrane at pre-prophase may be a trigger to generate signals for initiation of the F-actin ring formation. The unknown signal(s) from the SPBs may mark a spot on the medial cortex at pre-prophase or early prophase, and the formation of the aster-like structure may be initiated from this spot. The accumulated F-actin cables that are not involved in the aster-like structure may be regulated by other signals that localize to the broad medial region during prophase to anaphase, such as Mid1.

Possible role of Cdc12 in the ring formation

It has been reported that overexpressed Cdc12-GFP forms a spot and this spot moves in the cell during interphase, probably along microtubular tracks and F-actin tracks. The spot arrives at the medial region of the cell during prophase and a ring of Cdc12-
GFP is formed from it by the spot extending a Cdc12-GFP strand along the cell equator during prophase to metaphase (Chang et al., 1997; Chang, 1999). It has also been reported that a domain of Cdc12 possesses an ability to interact with components of SPBs (Petersen et al., 1998). It is possible that the Cdc12-GFP spot mediates the positional signal(s) from the SPBs to the medial cortex, or that the Cdc12-GFP spot recognizes the signal(s) that had been transferred on the medial cortex, and becomes the center of the aster-like structure. By contrast, this structure was observed in cdc12 mutant cells during prophase to metaphase in this study, suggesting that Cdc12 may not be necessary in the formation of the aster. However, it could be that the mutated Cdc12 still possesses function to form the aster-like structure. However, no leading F-actin cable was observed in the cdc12 mutant cells during mitosis, although the accumulation of the F-actin cables around the mid region occurred. This suggests that Cdc12 is required to form the leading F-actin cable and thereby to form the primary F-actin ring. The formation of the strand that encircles the cell equator from the Cdc12-GFP spot seems to be similar to the cell cortex from the aster-like structure. Thus, the leading F-actin cable may be formed by being influenced by the Cdc12 strand. It has been reported that Cdc12 interacts with Cdc3 profilin (Chang et al., 1997), and profilin is concentrated around the mid region during mitosis (Balasubramanian et al., 1994). Actin polymerization may be stimulated by Cdc12 via profilin in the formation of both the aster and the leading F-actin cable. It will be necessary in the future to show actual elongation of the leading F-actin cable from the aster-like structure and its relationship to the elongation of the Cdc12 strand in living cells by means of expression of GFP-fusion proteins. In addition, an activity of the accumulation of the F-actin cables in the mid region is retained during prophase to anaphase, and this accumulation is not very marked after anaphase in the cdc12 mutant cells. This transition of the F-actin cable arrangement does not seem to require the Cdc12 function.

Rng2, an IQGAP-like protein, has been considered to play
an important role in the signaling of the F-actin ring formation, as it localizes to the SPBs during interphase, and relocates to the medial ring during mitosis (Eng et al., 1998). rng2 null cells form an F-actin spot containing Cdc3 and calmodulin at the mid region, although they cannot form the F-actin ring. This spot formation is thought to be an intermediate step in the pathway of the F-actin ring formation (Eng et al., 1998). Moreover, rng2 mutation shows synthetic lethality with cdc12. Therefore, it would be important to investigate how Cdc12 and Rng2 are involved in the formation of the aster-like structure, and whether this structure and the F-actin spot are related.

Arrangement of F-actin cables in cdc15 mutant
In cdc15-140 mutant cells, the aster-like structure, the leading cable and the distorted F-actin ring were almost normally formed in order during mitosis, although cytokinesis never occurred subsequently. It has also been reported that the F-actin ring is formed in both cdc15-140 and cdc15-A5 mutant cells during mitosis, although both recruitment of the F-actin patches to the medial region and septation after the F-actin ring formation do not occur (Balasubramanian et al., 1998). On the contrary, it has been reported that the F-actin ring is formed only in a small population of mitotic cells of cdc15-140 and cdc15-287 (Fankhauser et al., 1995; Chang et al., 1997). Three different cdc15 mutant strains were used in these reports, including the present study. It may be that both the different mutations and minute differences in experimental conditions have resulted in the appearance of different phenotypes. In addition, the distorted F-actin ring may not have been regarded as the F-actin ring by some microscopic systems used for the above observation. From our detailed observation, the cdc15-140 mutant could form the distorted F-actin ring during anaphase, but the subsequent packing of the ring and its contraction did not occur. It is suggested that Cdc15 is required for packing of the accumulated F-actin cables to form the complete F-actin ring, which is able to contract.

In the cdc15-140 cells, a part of the F-actin ring still remained after anaphase, suggesting that these cells also have defects in the disassembly of the F-actin ring after anaphase. It has been reported that Cdc15 has a structural similarity to Imp2, which is required for F-actin ring disassembly and cell separation, and that a double mutant of cdc15-140 and imp2 shows synthetic lethality (Demeter and Sazer, 1998). These data suggest that Cdc15 is required for the rearrangements of actin cytoskeleton in the process of cytokinesis, including the packing of the F-actin cables during the formation of the F-actin ring and disassembly of the F-actin ring after the contraction.

Arrangement and function of F-actin ring in nda3 mutant
In the nda3 mutant, the formation of the F-actin ring and the accumulation of fine F-actin cables associated with the ring were observed at the restrictive temperature. The nda3 mutant cells are thought to be arrested at prophase, as duplicated SPBs cannot initiate separation on the nucleus because microtubules cannot elongate between these SPBs in this mutant (Hiraoka et al., 1984; Kanbe et al., 1990). However, in the arrested nda3 mutant cells, we could not detect the aster-like structure, which is normally formed during prophase in the wild-type cell, and the F-actin ring was similar to that of wild-type cells in early anaphase. Therefore, it could be that the mechanism by which the F-actin ring formation is controlled proceeded beyond prophase to early anaphase; the activity to accumulate the F-actin cables is probably stimulated and retained once cells enter prophase. As soon as the arrested cells were shifted to the permissive temperature, the tightly packed F-actin ring was formed, which was similar to the complete F-actin ring observed in the wild-type cells at late anaphase, which then contracted and cytokinesis progressed. Interestingly, the F-actin ring in the arrested nda3 mutant cells never contracted unless temperature was increased. These results may suggest that the completion of the F-actin ring formation is an essential step for the cell to enter cytokinesis, and that function of cytoplasmic microtubules is required for this process. However, nda3 mutant cells with mad2 defect can undergo septation (He et al., 1997). Because Mad2 has been known as a component of spindle check point (Li and Murray, 1991; He et al., 1997), the completion of the F-actin ring formation and the initiation of the ring contraction may be regulated by such a mitotic check point.

F-actin ring formation during cytokinesis in septum initiation mutants
At late anaphase, the single or double F-actin rings were formed in the spg1, cdc7 or sid2 mutant cells. In the latter case, one complete ring and one incomplete ring were connected to each other at one point. It is possible that the single ring was formed as a result of fusion of the double rings. The leading F-actin cable, the aster-like structure and the F-actin ring are formed in these mutant cells, indicating that the components of the septum initiation network (McCollum and Gould, 2001) are not involved in the formation of the F-actin ring.

Spg1 localizes only to the SPB(s) at all stages of the cell cycle and recruits Cdc7 to the SPB(s) during mitosis (Schmidt et al., 1997; Sohrmann et al., 1998). However, Sid2 localizes not only to the SPBs but also to the F-actin ring and both sides of the forming septum during late anaphase to cytokinesis (Sparks et al., 1999). It has also been reported that Sid2 is required for initiation of the F-actin ring contraction or septum formation, or both, as a downstream effector of the Spg1-Cdc7 pathway (Balasubramanian et al., 1998; Sparks et al., 1999). In fact, septum formation never occurred in these septum initiation mutant cells. We observed that the majority of the spg1 or cdc7 mutant cells possessed the F-actin ring after anaphase, whereas the ring disappeared earlier in most of the sid2 mutant cells. Thus, Sid2 may contribute to retaining the F-actin ring structure during contraction through localizing to this structure.

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