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REVIEW

Determination of Division Plane and Organization of Contractile Ring in *Tetrahymena*

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ABSTRACT. **In the molecular mechanism of division plane determination and contractile ring formation, *Tetrahymena* 85kDa protein (p85) is localized to the presumptive division plane before the formation of the contractile ring. p85 directly interacts with *Tetrahymena* calmodulin (CaM) in a Ca^{2+}-dependent manner, and p85 and CaM colocalize in the division furrow. A Ca^{2+}/CaM inhibitor N-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide HCl (W7) inhibits the direct interaction between p85 and Ca^{2+}/CaM. W7 also inhibits the localization of p85 and CaM to the division plane, and the formation of the contractile ring and division furrow. In addition, p85 binds to G-actin in a Ca^{2+}/CaM dependent manner, but does not bind F-actin. *Tetrahymena* profilin is localized to division furrow and binds *Tetrahymena* elongation factor-1α (EF-1α). EF-1α, which induces bundling of *Tetrahymena* F-actin, is also localized to the division furrow during cytokinesis. The evidence also indicates that Ca^{2+}/CaM inhibits the F-actin-bundling activity of EF-1α, and that EF-1α and CaM colocalize in the division furrow. In this review, we propose that the Ca^{2+}/CaM signal and its target protein p85 cooperatively regulate the determination of the division plane and the initiation of the contractile ring formation, and that profilin and a Ca^{2+}/CaM-sensitive actin-bundling protein, EF-1α, play pivotal roles in regulating the organization of the contractile ring microfilaments.**

Key words: actin/Ca^{2+}/calmodulin/contractile ring/EF-1α/profilin/p85

In the cytokinesis of animal cells, first, a division plane is determined at the cellular equator and then an actomyosin-based contractile ring appears at the division plane. The contractile ring constricts, generates a division furrow, and divides the cell (Schroeder, 1968; Satterwhite and Pollard, 1992). The molecular mechanisms of division plane determination and contractile ring formation are not well understood.

The ciliated *Tetrahymena* is a good experimental system for studying the mechanism of cytokinesis; (i) *Tetrahymena* cells exhibit synchronous division induced by heat treatment (Zeuthen, 1964), and (ii) cell-division-arrest mutants (*cda* loci) have been isolated and partially characterized (Frankel *et al*., 1976a, 1976b, 1977, 1980a, 1980b; Tamura *et al*., 1984; Yasuda *et al.*, 1984; Ohba *et al*., 1986). One of the *cda* mutants, *cdaA1*, has been shown to have a defect in the formation of the division furrow (Frankel *et al.*, 1977, 1980a). We identified a single protein (Mr=85kDa, pI=4.7, designated as p85) which exhibits different mobility between wild type and *cdaA1* in two-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Ohba *et al.*, 1986). At the permissive temperature, p85 appeared at the presumptive division plane before the formation of the division furrow, and then was localized in the division furrow during cytokinesis in both wild type and *cdaA1* cells (Ohba *et al.*, 1986; Numata *et al.*, 1995). In *cdaA1* cells at the restrictive temperature, localization of p85 and formation of the division furrow were not observed (Ohba *et al.*, 1986). Therefore, it is thought that p85 is involved in the formation of the *Tetrahymena* division furrow.

The contractile ring structure in *Tetrahymena* is like that in animal cells, and it is composed of several division-associated structures (Yasuda *et al.*, 1980). One of these structures is the lateral stripe, which fastens the contractile ring microfilaments (Yasuda *et al.*, 1980,1984). The lateral stripe may be involved in the contractile ring organization. Since its components correspond to F-actin-bundling proteins, isolation and characterization of the actin-bundling proteins

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Abbreviations: CaM, calmodulin; G-actin, actin monomer; EF-1α, elongation factor-1α; EHT, the end of heat treatment; p85, *Tetrahymena* 85kDa protein; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; W7, N-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide HCl.

localized in the division furrow has long been an object of study. Recently, we succeeded in the isolation of two actin-bundling proteins, elongation factor 1α (EF-1α) (Kurasawa *et al*., 1996) and fimbrin (Watanabe *et al.*, 1998, 2000), from *Tetrahymena pyriformis*. In addition, another actin-binding protein, profilin, which regulates actin polymerization, is localized to division furrow in *Tetrahymena* (Edamatsu *et al*., 1992).

In this article, we review the relationship between p85 and calmodulin (CaM) during cytokinesis and properties of two actin-binding proteins, *Tetrahymena* profilin and EF-1α. Moreover, we suggest that Ca^{2+}/CaM and p85 cooperatively regulate the determination of the division plane, and that profilin and EF-1α have pivotal roles in the organization of the contractile ring.

I. Determination of Division Plane by Tetrahymena p85

The timing of the appearance of p85 at the division plane

Tetrahymena p85 is localized to the presumptive division plane before the division furrowing initiates. The timing of the appearance of p85 and actin at the division plane was determined utilizing the progression of micronuclear and macronuclear division as indicators (Gonda *et al*., 1999a). In *Tetrahymena thermophila*, micronuclear division occurs first. After micronuclear division, macronuclear division starts prior to the formation of the division furrow. When micronuclear division is finished, p85 appears at the division plane (Fig. 1A). At that time, macronuclear division

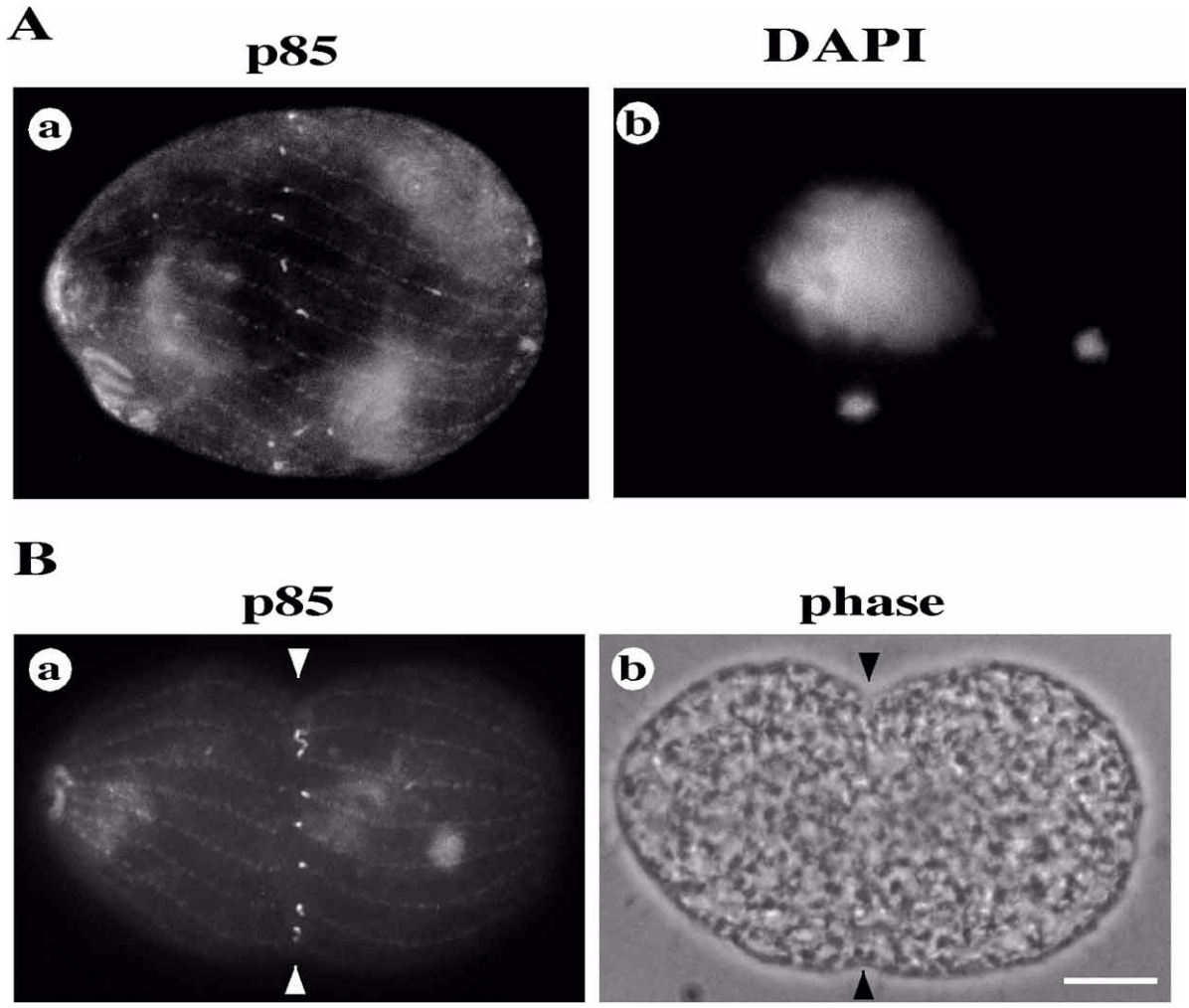

Fig. 1. *Tetrahymena* p85 and division furrow. (A) Immunofluorescent localization of p85 before the onset of division furrow constriction. (a) Staining for p85 with anti-p85 antiserum. (b) DNA staining with DAPI. (B) The division furrow is formed along the line of localization of p85 to the presumptive division plane. Cells just after the formation of the division furrow were prepared and subjected to immunostaining analysis. (a) and (b) show the same cell. (a) Immunofluorescence of p85. (b) Phase-contrast. Arrowheads: the site on the division furrow. Bar, 10 μm.

has yet to start (Fig. 1A, b) and fluorescence of actin is not observed at the division plane, indicating that p85 is localized to the division plane prior to macronuclear division and the appearance of actin.

Next, to clearly define the relationship between the site where the division furrow is formed and the dotted line of p85, cells just after the formation of the furrow were subjected to immunofluorescence staining with anti-p85 antiserum. This result showed that the division furrow forms along the line of localization of p85 (Fig. 1B). Thus, the appearance of p85 at the division plane is one of the earliest events in *Tetrahymena* cytokinesis, suggesting that the contractile ring is formed along the line of localization of p85 in the division plane.

Cloning and sequencing of the gene encoding p85

In order to further understand the molecular structure of p85, wild type p85 cDNA was cloned and sequenced (Gonda, *et al*. 1999b). The cDNA contained 2,632-nucleotides and one open reading frame of this cDNA encoded an 803-amino acid polypeptide with an estimated relative molecular mass of 86,036 (Gen Bank/EMBL/DDBJ; D84491) (Gonda *et al*., 1999b). The deduced amino acid sequence of wild type p85 contained two distinct repeat sequences, designated as Repeats I, II, III and Repeats A, B.

Searching the most recent databases failed to detect any significant sequence homology over the full length of p85, but showed sequence homologies to parts of several proteins (Gonda *et al.*, 1999b). Repeats I, II and III contained sequences similar to a CaM-binding site of yeast Ca^{2+}/CaM-dependent protein kinase Type II (CaM II kinase) (Pausch *et al.*, 1991) and a part of *Tetrahymena* actin (Cupples and Pearlman, 1986). Repeats A and B contained sequences similar to a part of human Ser/Thr protein kinase PCTAIRE-1 that was a cdc2 kinase homologue (Meyerson *et al.*, 1992).

Interaction between p85 and CaM

Sequence homology between p85 and a CaM-binding site of yeast CaM II kinase suggested that native p85 directly interacts with CaM. The purified p85 was mixed with GST-CaM-bound beads or GST-bound beads in the presence or absence of Ca^{2+}, and then co-sedimentation assay was performed. p85 was precipitated with GST-CaM-bound beads in the presence of Ca^{2+} (Fig. 2A, lane 3), but not its absence (Fig. 2A, lane 4). Furthermore, co-sedimentation of GST and p85 was not observed in the presence or absence of Ca^{2+} (Fig. 2A, lanes 1 and 2). These results suggest that p85 directly interacts with CaM in a Ca^{2+}-dependent manner.

To investigate the *in vivo* interaction between p85 and Ca^{2+}/CaM, the localization of p85 and CaM during the cell cycle was examined using indirect immunofluorescence. In interphase cells, p85 was localized to the oral apparatus (Ohba *et al*., 1986), while CaM was localized to contractile vacuole pores in addition to the oral apparatus, as in our previous study (Suzuki *et al.*, 1982). During cytokinesis, p85 and CaM colocalize in the division furrow (Fig. 2B). When the cells are fixed with 2 mM EGTA substituted for 1 mM $CaCl_2$, no localization of CaM in the division furrow is detected, suggesting that CaM is localized in the division furrow as a form of Ca^{2+}/CaM. These results indicate a cooperative role for p85 and Ca^{2+}/CaM in the formation of the division furrow.

To investigate whether Ca^{2+}/CaM is involved in the initiation of cytokinesis as well as the progression of division furrowing, the effects of the Ca^{2+}/CaM inhibitor W7 were examined (Gonda *et al*., 1999a). W7 is a most effective tool for clarifying Ca^{2+}/CaM signaling in cell functions, and has been used in the study of cell proliferation (Hidaka *et al.*, 1981), gene expression (Schafer *et al.*, 1996), and DNA repair (Lonn and Lonn, 1987). The effect of W7 on cytokinesis was tested using a synchronous cell division system. To observe the effect only on cytokinesis, W7 dissolved in dimethyl sulfoxide (DMSO) was added to the culture. W7 inhibited the formation of the division furrow (Fig. 2C). When the cells treated with 100μM W7 were transferred to fresh medium, they performed cytokinesis normally. Therefore, the effect of W7 on cytokinesis is reversible. In addition, W7 inhibited the direct interaction between p85 and Ca^{2+}/CaM depending on the dosages of W7, and also inhibited the localization of p85, CaM and actin to the division furrow (Gonda *et al.*, 1999a). These experiments suggest that W7 inhibits the direct interaction between p85 and Ca^{2+}/CaM and thereby prevents the localization of p85 to the presumptive division plane and some downstream events of p85, such as the formation of the contractile ring and division furrow (Fig. 2C).

Interaction between p85 and actin monomer

To investigate the role that p85 and Ca^{2+}/CaM might play in the formation of contractile ring microfilaments as a polymerization nucleus, interaction between p85 and actin filaments was examined using co-sedimentation assay. Co-sedimentation of p85 and actin filaments was not observed in the presence or absence of Ca^{2+}/CaM. Furthermore, co-sedimentation assay among p85, CaM, and G-actin was performed. G-actin and CaM were mixed with p85/anti-p85 antibody complex-bound beads, and then these mixture were centrifuged. The sample released from these beads were separated by SDS-PAGE and analyzed by immunoblotting with anti-p85 antibody. G-actin was precipitated with p85/anti-p85 antibody complex-bound beads in the presence of Ca^{2+}/CaM, but not in its absence. These results suggest that p85 directly interacts with G-actin in a Ca^{2+}/CaM-dependent manner.

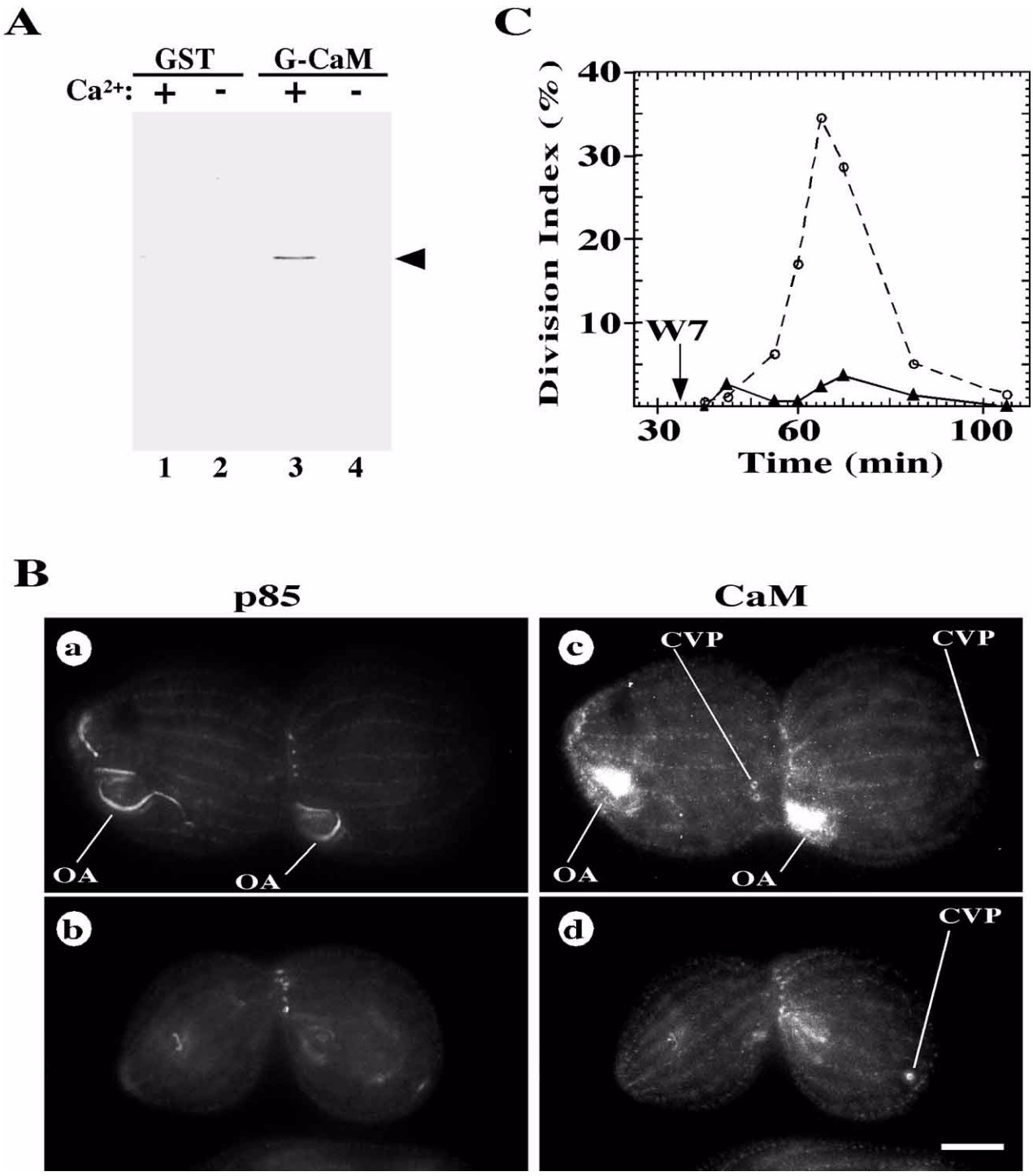

Fig. 2. Interaction between p85 and CaM. (A) Co-sedimentation assay of p85 and CaM. The purified p85 was mixed with GST-bound-beads (GST) or GST-CaM-bound-beads (G-CaM) in the presence (+) or absence (-) of Ca^{2+}, and then co-sedimentation assay was carried out. The samples released from beads were separated by 8% SDS-PAGE and analyzed by immunoblotting with anti-p85 antiserum (lanes 1–4). Arrowhead: p85. (B) Immunofluorescent localization of p85 and CaM during the cell cycle. (a), (c) Middle stage of cytokinesis. (b), (d) Late stage of cytokinesis. (a)-(d) Double immunofluorescence of p85 (a, b) and CaM (c, d). (OA) and (CVP) indicate the oral apparatus and the contractile vacuole pores, respectively. Bar, 10 μm. (C) W7 inhibits the formation of the division furrow in synchronously dividing cells. W7 dissolved in DMSO was added to the culture at 35 min after EHT, and then the cells forming the division furrow were enumerated. Open circles (○): the cells treated with DMSO alone. Closed triangles (▲): the cells treated with 100 μM W7 in DMSO. The ordinate: proportion of cells forming the division furrow. The abscissa: time (min) after EHT.

Regulation of the initiation and progression of cytokinesis by Ca^{2+}/CaM and p85

From the experimental results, we propose the following model which shows the regulation of cytokinesis by Ca^{2+}/CaM signaling and p85. We can dissect this model process into three stages: (1) determination of the division plane, (2) formation of the contractile ring, and (3) progression of the division furrowing. In this regard, W7 inhibits the direct interaction between p85 and Ca^{2+}/CaM and the localization of p85 at the division plane, suggesting that p85 directly interacts with Ca^{2+}/CaM and is then localized to the presumptive division plane prior to formation of the contractile ring (Fig. 1A). Thus, in the first stage, the division plane is possibly determined by the direct interaction of p85 and Ca^{2+}/CaM. Next, W7 inhibits the formation of the contractile ring and the division furrow, which are formed along the line of localization of p85 in the division plane (Fig. 1B). In addition, co-sedimentation assay indicated direct interaction between p85 and G-actin. Therefore, in the second stage, p85 and Ca^{2+}/CaM might play a role in the formation of contractile ring microfilaments as an actin polymerization nucleus. Finally, since Ca^{2+}/CaM signaling is thought essential to the constriction of the contractile ring, in the third stage, p85 and Ca^{2+}/CaM likely participate in the process of division furrowing.

Ca^{2+}/CaM is thought to be involved in the initiation and the progression of cytokinesis (Chang and Meng, 1995; Muto *et al*., 1996; Satterwhite and Pollard, 1992; Walsh, 1994). Although it is not known whether Ca^{2+}/CaM signaling relates to the regulation of cytokinesis in animal cells, Ca^{2+}/CaM signaling in fact does regulate cytokinesis in yeast (Lippincott and Li, 1998; Moser *et al*., 1997; Osuman and Cerione, 1998). From the model mentioned above, we propose that Ca^{2+}/CaM is one of the most important signals for cytokinesis, and that p85 and Ca^{2+}/CaM regulate the initiation of cytokinesis and may be involved in the progression of cytokinesis in *Tetrahymena*.

II. Organization of the Contractile Ring in Tetrahymena

Presence of profilin in the division furrow

An actin-binding protein, profilin, binds actin monomers and can prevent their incorporation into filaments. Conversely, however, profilin can promote monomer incorporation into filaments by stimulating the exchange of bound ADP to ATP. This exchange results in the formation of ATP-actin monomers, which are more rapidly assembled into filaments (Machesky and Pollard, 1993). In *Tetrahymena*, profilin is localized to division furrow (Edamatsu *et al*., 1992), suggesting that profilin may serve as a regulatory molecule that controls actin polymerization in the contractile ring.

Tetrahymena profilin is shown to colocalize with actin filaments in division furrow (Edamatsu *et al*., 1992). In general, profilin is known to interact only with G-actin but not F-actin. In fact, *Tetrahymena* profilin did not bind to *Tetrahymena* F-actin in an *in vitro* binding experiment (Edamatsu *et al*., 1992). It is unlikely that the colocalization is due to direct interaction between profilin and F-actin. Therefore, it is possible that a certain cytoskeletal component capable of anchoring profilin exists in the division furrow. To isolate the anchoring component, profilin-binding proteins were purified from *Tetrahymena* cell lysate, using a GST-profilin affinity column. The 1.0 M KCl fraction contained 100, 80, 70, 55, 49, 47, and 35 kDa proteins (Fig. 3). Since those proteins were not eluted from a control GST column, they seem to be specifically associated with the profilin column. From the N-terminal amino acid sequence, the 49 kDa protein was identified as *Tetrahymena* elongation factor 1α (EF-1α). Recently we found that *Tetrahymena* EF-1α binds F-actin and induces bundling of actin filaments (Kurasawa *et al*., 1996), and that EF-1α is localized to the division furrow (Numata *et al*,. 2000). These results suggest that profilin binds EF-1α and regulates the formation of contractile ring microfilaments.

F-actin-bundling activity of EF-1α regulated by Ca^{2+}/CaM

Recent direct and indirect evidence has indicated that EF-1α is an actin-bundling protein (Yamashiro-Matsumura and Matsumura, 1985; Demma *et al*., 1990; Yang *et al*., 1990; Itano and Hatano, 1991). To investigate whether *Tetrahymena* EF-1α interacts with F-actin, a co-sedimentation experiment was performed (Kurasawa *et al*., 1996). In the case of EF-1α alone, most of the EF-1α was not precipitated by high speed centrifugation. In the presence of F-actin, EF-1α was co-precipitated with F-actin. The binding of *Tetrahymena* EF-1α to F-actin saturates at a stoichiometric ratio of about 1 to 1. Electron microscopic observation demonstrated that *Tetrahymena* EF-1α in fact possesses F-actin-bundling activity (Fig. 4A, a). These results demonstrate that *Tetrahymena* EF-1α binds F-actin and induces bundling of F-actin.

Durso and Cyr (1994a, 1994b) reported that a carrot EF-1α homologue bundles microtubules *in vitro*, and moreover this bundling is modulated by the addition of Ca^{2+}/CaM. Kaur and Ruben (1994) demonstrated direct interaction between CaM and EF-1α from *Trypanosoma brucei*. Therefore, we examined whether Ca^{2+}/CaM can regulate the interaction between *Tetrahymena* EF-1α and F-actin. We examined the interaction among EF-1α, actin and CaM by co-sedimentation assay and electron microscopy (Kurasawa *et al*., 1996). In the presence of Ca^{2+}, CaM co-precipitates with EF-1α/F-actin complex, but not in the presence of EGTA. Since CaM with or without Ca^{2+} does not co-precipitate with F-actin alone, Ca^{2+}/CaM binds EF-1α within the

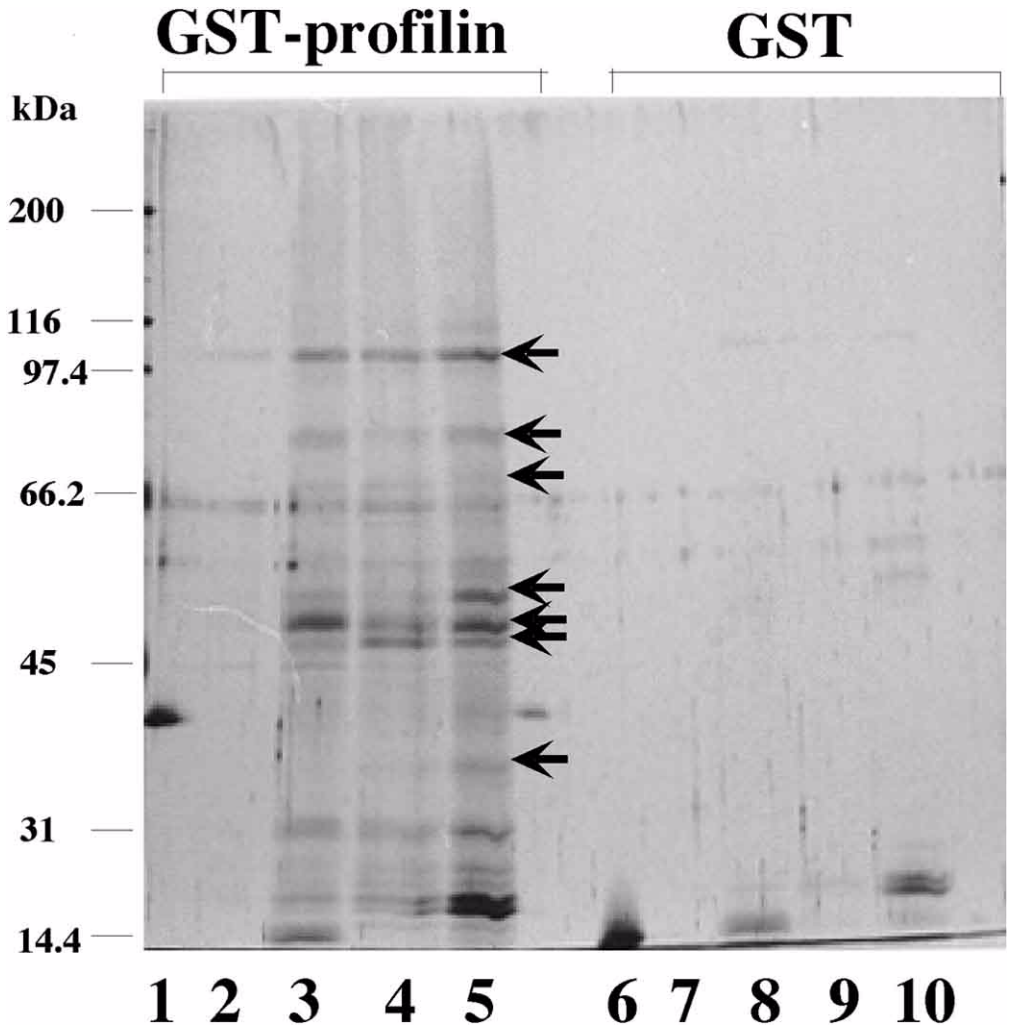

Fig. 3. The proteins eluted from a GST-profilin affinity column. A GST-profilin affinity column and a GST control column were loaded with a *Tetrahymena* cell extract. After loading, each column was rinsed with buffer E (20 mM HEPES, 100 mM KCl, 10μg/ml pepstatin A, 1 mM PMSF, 5μg/ml leupeptin, 0.1 mM TLCK, pH 7.5). Elution was carried out in four steps with buffer E containing 5 mM ATP, 0.4 M KCl and 1.0 M KCl, respectively. Lanes 1 and 6, GST-profilin and GST, respectively. Lanes 2 and 7, buffer E-washed fraction; lanes 3 and 8, 5 mM ATP-eluted fraction; lanes 4 and 9, 0.4 M KCl-eluted fraction; lanes 5 and 10, 1 M KCl -eluted fraction. In lane 5, there are many profilin-binding proteins. Arrows indicate the 100, 80, 70, 55, 49, 47, and 35 kDa proteins. The apparent molecular weights (kDa) are shown on the left.

EF-1α/F-actin complex. Electron microscopy showed that, in the absence of CaM, EGTA or Ca^{2+} has no effect on the F-actin-bundling activity (Fig. 4A, a and c), indicating that the bundling activity was Ca^{2+}-insensitive. In contrast, F-actin-bundling activity of EF-1α is completely inhibited in the presence of both Ca^{2+} and CaM (Fig. 4A, d), whereas CaM has no effect on the bundling activity in the presence of EGTA (Fig. 4A, b). These results strongly indicate that Ca^{2+}/CaM directly interacts with EF-1α and inhibits the F-actin-bundling activity of EF-1α.

Presence of EF-1α in the division furrow

We localized EF-1α by indirect immunofluorescence. In interphase cells, EF-1α is localized to the oral apparatus and the apical region. Weak immunofluorescence was also identified throughout the cytoplasm, especially around the macronuclei (Numata *et al.*, 2000). In dividing cells, EF-1α clearly localizes to the division furrow from the early to the end stage of cytokinesis, in addition to many dots in the cytoplasm (Fig. 4B) (Numata *et al.*, 2000). At a very early stage of cytokinesis, a thin line along the equatorial furrow region is stained (Fig. 4B, a). At late stage of cytokinesis, the fluorescence appears as a thicker line in the division furrow (Fig.4B, b).

Suzuki *et al.* localized *Tetrahymena* CaM in the oral apparatus, cilia, basal bodies, the apical region and the con-

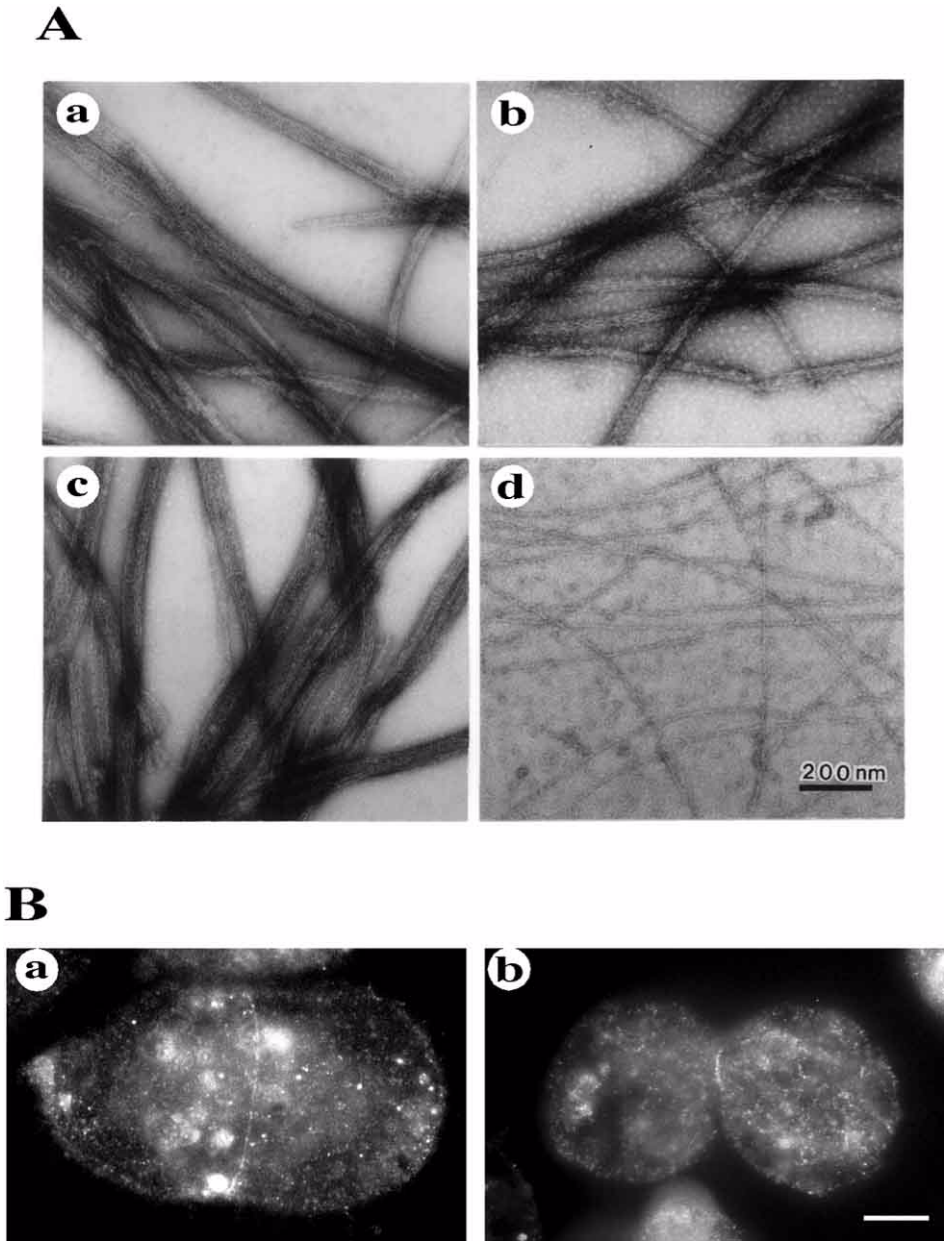

Fig. 4. The F-actin-bundling activity and localization of *Tetrahymena* EF-1α. (A) Regulation of the F-actin-bundling activity of EF-1α by Ca^{2+}/CaM. EF-1α preincubated without (a, c) or with CaM (b, d) was mixed with F-actin in the presence of 1 mM EGTA (a, b) or 1 mM $CaCl_2$ (c, d). Mixtures were incubated at 26° for 20 min and observed by electron microscopy after negative staining. (B) Localization of EF-1α in dividing cells. Dividing cells at early (a) and late (b) stages of cytokinesis were stained with the anti-*Tetrahymena* EF-1α antibody. Bar, 10μm.

tractile vacuole pores in the interphase cells (1982). To investigate *in vivo* the relationship between EF-1α and CaM, we examined these localizations by immunofluorescence. In dividing cells, both EF-1α and CaM are localized to the division furrow (Figs. 2B and 4B). These results indicate that CaM interacts with EF-1α *in vivo* as well as *in vitro*.

We showed that CaM and EF-1α localize to the division furrow (Figs. 2B and 4B), and Ca^{2+}/CaM completely inhibits the F-actin-bundling activity of *Tetrahymena* EF-1α *in vitro* (Fig. 4A). The F-actin bundles formed by *Tetrahymena* EF-1α *in vitro* are similar to the F-actin paracrystal (Fig. 4A). In such a tight bundle, myosin and other proteins seem unable to interact with F-actin. Recently, Ca^{2+} waves were observed along the cleavage furrows in medaka fish embryos (Fluck *et al.*, 1991), zebra fish embryos (Chang and Meng, 1995) and *Xenopus* embryos (Muto *et al.*, 1996).

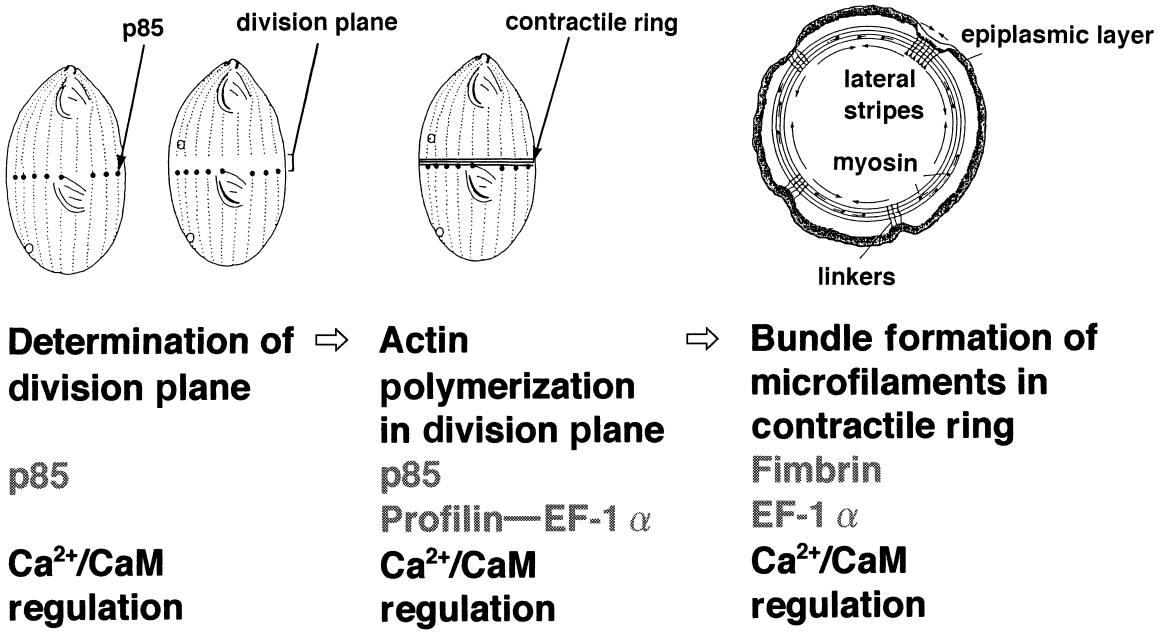

Fig. 5. The process of organization of the contractile ring in *Tetrahymena*. First, p85 is localized to the presumptive division plane, and then the division plane is formed. p85 and Ca^{2+}/CaM regulate determination of the division plane. Second, microfilaments are polymerized at the division plane. p85 directly binds actin monomer and may initiate actin polymerization. Profilin binds EF-1α and may regulate actin polymerization. Third, microfilaments are bundled into the contractile ring. EF-1α and fimbrin induce bundling of microfilaments. Ca^{2+}/CaM regulates F-actin-bundling activity of EF-1α. The right hand schematic drawing shows a cross section of the contractile ring.

Therefore, we speculate that Ca^{2+}/CaM may be a key factor in the formation of F-actin bundles in the contractile ring. In the absence of Ca^{2+}, CaM does not bind to *Tetrahymena* EF-1α, and EF-1α forms tight F-actin bundles. In the presence of Ca^{2+}, CaM directly binds to *Tetrahymena* EF-1α, and the F-actin-bundling activity of *Tetrahymena* EF-1α is completely inhibited. As a result, it is possible that tight F-actin bundles in the contractile ring are loosened, such that myosin and other proteins are able to interact with F-actin in the loose bundles, with Ca^{2+}/CaM possibly activating myosin light chain kinase, and causing the constriction of the contractile ring to occur. Thus, the regulation of the intracellular Ca^{2+} concentration may be essential for the formation of F-actin-bundles during cytokinesis.

We summarize the organization of the contractile ring in *Tetrahymena* (Fig. 5). p85 and Ca^{2+}/CaM regulate the determination of the division plane. p85 also directly binds actin monomer in a Ca^{2+}/CaM dependent manner. Therefore, p85 and Ca^{2+}/CaM may regulate the initiation of actin polymerization. Profilin binds to EF-1α and probably regulates actin polymerization. EF-1α is localized to the division furrow and induces bundling of microfilaments. Ca^{2+}/CaM regulates F-actin-bundling activity of EF-1α. We believe that Ca^{2+} plays a key role in the dynamic change of the localization of p85 and CaM during the cell cycle and the formation of F-actin bundles in the contractile ring. Therefore, Ca^{2+}/CaM is a key factor of "the organization of contractile ring". How the concentration of intracellular free Ca^{2+} changes during cytokinesis in *Tetrahymena*, and how Ca^{2+}/CaM and p85 regulate the initiation of contractile ring formation remain to be explored.

Conclusions

1. p85 directly interacts with *Tetrahymena* CaM in a Ca^{2+}-dependent manner, and colocalizes with CaM in the division furrow.
2. Ca^{2+}/CaM inhibitor W7 inhibits the interaction between p85 and Ca^{2+}/CaM, the localization of p85 and CaM to the division plane, and the formation of contractile ring and division furrow.
3. p85 directly interacts with *Tetrahymena* G-actin in a Ca^{2+}/CaM-dependent manner.
4. Profilin is localized to the division furrow and associates with EF-1α.
5. *Tetrahymena* EF-1α, which induces bundling of *Tetrahymena* F-actin, is localized to the division furrow.
6. Ca^{2+}/CaM inhibits the F-actin-bundling activity of EF-1α, and EF-1α and CaM are localized in the division furrow.

References

Chang, D.C. and Meng, C. 1995. A localization elevation of cytosolic free calcium is associated with cytokinesis in the *Zebrafish* embryo. *J. Cell Biol.*, **131**: 1539–1545.

Cupples, C.G. and Pearlman, R.E. 1986. Isolation and characterization of the actin gene from *Tetrahymena thermophila*. *Proc. Natl. Acad. Sci.*

USA, **83**: 5160–5164.

Demma, M., Warren, V., Hock, R., Dharmawardhane, S., and Condeelis, J. 1990. Isolation of an abundant 50,000-dalton actin filament bundling protein from *Dictyostelium* amoebae. *J. Biol. Chem.*, **265**: 2286–2291.

Durso, N.A. and Cyr, R.J. 1994a. A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor-1α. *Plant Cell*, **6**: 893–905.

Durso, N.A. and Cyr, R.J. 1994b. Beyond translation: elongation factor-1α and the cytoskeleton. *Protoplasma*, **180**: 99–105.

Edamatsu, M., Hirono, M., and Watanabe Y. 1992. *Tetrahymena* profilin is localized in the division furrow. *J. Biochem.*, **112**: 637–642.

Fluck, R.A., Miller, A.L., and Jaffe, L.F. 1991. Slow calcium waves accompany cytokinesis in medaka fish eggs. *J. Cell Biol.*, **115**: 1259–1265.

Frankel, J., Jenkins, L.M., Doerder, F.P., and Nelson, E.M. 1976a. Mutations affecting cell division in *Tetrahymena pyriformis*. I. Selection and genetic analysis. *Genetics*, **83**: 489–506.

Frankel, J., Jenkins, L.M., and Debaut, L.E. 1976b. Causal relations among cell cycle processes in *Tetrahymena pyriformis*. An analysis employing temperature-sensitive mutants. *J. Cell Biol.*, **71**: 242–260.

Frankel, J., Nelson, E.M., and Jenkins, L.M. 1977. Mutations affecting cell division in *Tetrahymena pyriformis*, syngen 1. II. Phenotypes of single and double homozygotes. *Dev. Biol.*, **58**: 255–275.

Frankel, J., Mohler, J., and Frankel, A.K. 1980a. Temperature-sensitive periods of mutations affecting cell division in *Tetrahymena thermophila*. *J. Cell Sci.*, **43**: 59–74.

Frankel, J., Mohler, J., and Frankel, A.K. 1980b. The relationship between the excess-delay phenomenon and temperature-sensitive periods in *Tetrahymena*. *J. Cell Sci.*, **43**: 75–91.

Gonda, K., Katoh, M., Hanyu, K., Watanabe, Y., and Numata, O. 1999a. Ca^{2+}/calmodulin and p85 cooperatively regulate an initiation of cytokinesis in *Tetrahymena*. *J. Cell Sci.*, **112**: 3619–3626.

Gonda, K., Nishibori, K., Ohba, H., Watanabe, A., and Numata, O. 1999b. Molecular cloning of the gene for p85 that regulates the initiation of cytokinesis in *Tetrahymena*. *Biochem. Biophys. Res. Commun.*, **264**: 112–118.

Hidaka, H., Sasaki, Y., Endo, T., Ohno, S., Fujii, Y. and Nagata, T. 1981. N-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide, a calmodulin antagonist, inhibits cell proliferation. *Proc. Natl. Acad. Sci. USA*, **78**: 4354–4357.

Itano, N. and Hatano, S. 1991. F-actin bundling protein from *Physarum polycephalum*: Purification and its capacity for co-bundling of actin filaments and microtubules. *Cell Motil. Cytoskeleton*, **19**: 244–254.

Kaur, K.J. and Ruben, L. 1994. Protein translation elongation factor-1α from *Trypanosoma brucei* binds calmodulin. *J. Biol. Chem.*, **269**: 23045–23050.

Kurasawa, Y., Hanyu, K., Watanabe, Y., and Numata, O. 1996. F-actin bundling activity of *Tetrahymena* elongation factor 1α is regulated by Ca^{2+}/calmodulin. *J. Biochem.*, **119**: 791–798.

Lippincott, J. and Li, R. 1998. Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis. *J. Cell Biol.*, **140**: 355–366.

Lonn, U. and Lonn, S. 1987. W-7, a calmodulin inhibitor, potentiates dacarbazine cytotoxicity in human neoplastic cell. *Inter. J. Cancer.*, **39**: 638–642.

Machesky, L.M. and Pollard, T.D. 1993. Profilin as a potential mediator of membrane-cytoskeleton communication. *Trends Cell Biol.*, **3**: 381–385.

Meyerson, M., Ender, G.H., Wu, C.-L., Su, L.-K., Gorka, C., Nelson, C., Harlow, E., and Tsai, L.-H. 1992. A family of human cdc2-related protein kinase. *EMBO J.*, **11**: 2909–2917.

Moser, M.J., Flory, M.R., and Davis, T.N. 1997. Calmodulin localizes to the spindle pole body of *Schizosaccharomyces pombe* and performs an essential function in chromosome segregation. *J. Cell Sci.*, **110**: 1805–1812.

Muto, A., Kume, S., Inoue, T., Okano, H., and Mikoshiba, K. 1996. Calcium waves along the cleavage furrows in cleavage- stage *Xenopus* embryos and its inhibition by heparin. *J. Cell Biol.*, **135**: 181–190.

Numata, O., Suzuki, H., Ohba, H., and Watanabe, Y. 1995. The mutant gene product of *Tetrahymena* cell-division-arrest mutant *cdaA1* is localized in the accessory structure of specialized basal body close to the division furrow. *Zool. Sci.*, **12**: 133–135.

Numata, O., Kurasawa, Y., Gonda, K., and Watanabe, Y. 2000. *Tetrahymena* elongation factor-1α is localized with calmodulin in the division furrow. *J. Biochem.*, **127**: 51–56.

Ohba, H., Ohmori, I., Numata, O., and Watanabe, Y. 1986. Purification and immunofluorescence localization of the mutant gene product of a *Tetrahymena cdaA1* mutant affecting cell division. *J. Biochem.*, **100**: 797–808.

Osuman, M.A. and Cerione, R.A. 1998. Iqgp1p, a yeast homologue of the mammalian IQGAPs mediates Cdc42p effects on the actin cytoskeleton. *J. Cell Biol.*, **142**: 443–455.

Pausch, M.H., Kaim, D., Kunisawa, R., Admou, A., and Thorner, J. 1991. Multiple Ca^{2+}/calmodulin-dependent protein kinase genes in a unicellular eukaryote. *EMBO J.*, **10**: 1511–1522.

Satterwhite, L.L. and Pollard, T.D. 1992. Cytokinesis. *Curr. Opin. Cell Biol.*, **4**: 43–52.

Schafer, H., Trauzold, A., Siegel, E.G., Folsch, U.R., and Schmidt, W.E. 1996. PRG1: a novel early-response gene transcriptionally induced by pituitary adenylate cyclase activating polypeptide in pancreatic carcinoma cell line. *Cancer Res.*, **56**: 2641–2648.

Schroeder, T.E. 1968. Cytokinesis: filaments in the cleavage furrow. *Exp. Cell Res.*, **53**: 272–276.

Suzuki, Y., Ohnishi, K., Hirabayashi, T., and Watanabe, Y. 1982. *Tetrahymena* calmodulin. Characterization of an anti-*Tetrahymen*a calmodulin and the immunofluorescent localization in *Tetrahymena*. *Exp. Cell Res.*, **137**: 1–14.

Tamura, R., Takahashi, M., and Watanabe, Y. 1984. Molecular mechanism of cell division in *Tetrahymena thermophila* I. Analysis of execution period of a division arrest ts-Mutant. *Zool. Sci.*, **1**: 62–73.

Walsh, M.P. 1994. Calmodulin and the regulation of smooth muscle contraction. *Mol. Cell. Biochem.*, **135**: 21–41.

Watanabe, A., Kurasawa, Y., Watanabe, Y., and Numata, O. 1998. A new *Tetrahymena* actin-binding protein is localized in the division furrow. *J. Biochem.*, **123**: 607–613.

Watanabe, A., Yonemura, I., Gonda, K., and Numata O. 2000. Cloning and sequencing of the gene for a *Tetrahymena* fimbrin-like protein. *J. Biochem.*, **127**: 85–94.

Yamashiro-Matsumura, S. and Matsumura, F. 1985. Purification and characterization of an F-actin-bundling 55-kilodalton protein from HeLa cells. *J. Biol. Chem.*, **260**: 5087–5097.

Yang, F., Demma, M., Warren, V., Dharmawardhane, S., and Condeelis, J. 1990. Identification of an actin-binding protein from *Dictyostelium* as elongation factor 1α. *Nature*, **347**: 494–496.

Yasuda, T., Numata, O., Ohnishi, K., and Watanabe, Y. 1980. A contractile ring and cortical changes found in the dividing *Tetrahymena pyriformis*. *Exp. Cell Res.*, **128**: 407–418.

Yasuda, T., Tamura, R., and Watanabe, Y. 1984. Molecular mechanism of cell division in *Tetrahymena thermophila*. Ultrastructure changes found in a division arrest ts-mutant. *Zool. Sci.*, **1**: 62–73.

Zeuthen, E. 1964. Synchrony of cell division and growth. E. Zeuthen, ed. John Wiley, New York, NY, pp.99–156.

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