J. Biochem. **127**, 51–56 (2000)

Tetrahymena Elongation Factor-1α Is Localized with Calmodulin in the Division Furrow[1]

Osamu Numata,*,[2] Yasuhiro Kurasawa,*,[3] Kohsuke Gonda,* and Yoshio Watanabe†

**Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572; and †Jobu University, Isesaki, Gunma 372–8588*

Received June 24, 1999; accepted October 8, 1999

Translation elongation factor 1α (EF-1α) catalyzes the GTP-dependent binding of aminoacyl-tRNA to ribosomes. We previously reported that *Tetrahymena* EF-1α induced the formation of bundles of rabbit skeletal muscle filamentous actin (F-actin) as well as *Tetrahymena* F-actin [Kurasawa *et al.* (1996) *Zool. Sci. (Tokyo)* 13, 371–375], and that Ca^{2+}/calmodulin (CaM) regulated the F-actin–bundling activity of EF-1α [Kurasawa *et al.* (1996) *J. Biochem.* 119, 791–798]. In the present study, we investigated the binding between *Tetrahymena* EF-1α and CaM using a *Tetrahymena* EF-1α affinity column, and the localization of EF-1α and CaM by indirect immunofluorescence. Only CaM in the *Tetrahymena* cell extract bound to *Tetrahymena* EF-1α in a Ca^{2+}-dependent manner. In interphase *Tetrahymena* cells, EF-1α and CaM are colocalized in the crescent structure of the oral apparatus and the apical ring, while in dividing cells, they are colocalized in the division furrow. This is the first report describing the coexistence of EF-1α and CaM in the division furrow, suggesting that EF-1α and CaM are involved in the organization of contractile ring microfilaments during cytokinesis.

Key words: actin, calmodulin, cytokinesis, elongation factor 1α (EF-1α), *Tetrahymena*.

Translation elongation factor-1α (EF-1α) catalyzes the GTP-dependent binding of aminoacyl-tRNAs to the respective mRNA anticodons at the A site of ribosomes, in the peptide elongation phase of protein synthesis (*1–3*). Several recent reports indicated that EF-1α has a second role, *i.e.* as a regulator of microtubule rearrangements (*4–8*). Moreover, it was shown that EF-1α was also involved in the regulation of the actin cytoskeleton. ABP-50, a 50-kDa filamentous actin (F-actin)–bundling protein from *Dictyostelium discoideum* (*9*), was identified as EF-1α from cDNA sequencing data and the detection of polypeptides chain elongation activity (*10*). Stimulation of *Dictyostelium* cells with cAMP induced the concentration of EF-1α in actin bundles in the philopodia that were extended as a response to the stimulation (*11*). A 52-kDa F-actin–bundling protein from *Physarum polycephalum* was reported to react with antibodies raised against yeast EF-1α (*12*). The 52-kDa protein had two additional activities, microtubule-bundling and microtubules/F-actin co-bundling (*12*). Previously, we demonstrated that *Tetrahymena* EF-1α could also bind to F-actin and form F-actin bundles (*13–15*). Thus, EF-1α is regarded as a regulator in the organization of F-actin as well as microtubules.

EF-1α also participates in the regulation by Ca^{2+}/calmodulin (CaM). A carrot EF-1α homolog caused the bundling of microtubules *in vitro*, and its bundling activity was modulated by the addition of Ca^{2+} and CaM together (Ca^{2+}/CaM) (*16*). Kaur and Ruben demonstrated a direct interaction between CaM and the EF-1α from *Trypanosoma brucei* (*17*). Using EF-1α and actin purified from *Tetrahymena* and recombinant CaM, we demonstrated that Ca^{2+}/CaM completely inhibited the formation of F-actin bundles by EF-1α but did not affect the formation of the EF-1α/F-actin complex (*13*). These findings suggest that a Ca^{2+}/CaM-sensitive mechanism regulates the formation of F-actin bundles and microtubule bundles by EF-1α.

In *Tetrahymena*, F-actin bundles exist in the contractile ring, which is the constriction machinery in the division furrow during cytokinesis (*18–21*). The contractile ring is mainly composed of actin filaments and myosin. The force for the constriction is generated through the actin-myosin interaction (*22–25*). The formation and disappearance of the contractile ring are considered to be due to the actions of actin-modulating proteins. Some of these proteins, for example, profilin (*26*) and fimbrin-like protein (*27*), are localized in the division furrow in *Tetrahymena*. Intracellular Ca^{2+} is now thought to participate in the regulation of several aspects of cell division (*28*). The spatio-temporal pattern of Ca^{2+} transients on cytokinesis has been reported in medaka fish embryos (*29*), zebra fish embryos (*30*), and

[1]This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (06275101, 10213201).

[2]To whom correspondence and reprint requests should be addressed. Phone/Fax: +81-298-53-6648, E-mail: numata@sakura.cc.tsukuba.ac.jp

[3]Present address: Institute of Physical and Chemical Research (RIKEN), Tsukuba Life Science Center, 3–1–1 Koyadai, Tsukuba, Ibaraki 305–0047.

Abbreviations: CaM, calmodulin; CBB, Coomassie Brilliant Blue R-250; EF-1α, elongation factor 1α; F-actin, filamentous actin; FITC, fluorescein isothiocyanate; MES, 2-(*N*-morpholino)ethanesulfonic acid; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TLCK, N^{α}-*p*-tosyl-L-lysine chloro-methyl ketone.

Xenopus embryos (*31*). In *Xenopus* embryos (*31*), Ca^{2+} waves were observed along the cleavage furrows after cleavage furrow formation. These results lead us to the hypothesis that EF-1α induces the formation of F-actin bundles in the contractile ring and that the F-actin–bundling activity of EF-1α is regulated by Ca^{2+}/CaM.

To test this hypothesis, we examined EF-1α–binding proteins using a *Tetrahymena* EF-1α affinity column, and studied the intracellular localization of EF-1α in *Tetrahymena*. In this paper, we report that the binding of *Tetrahymena* EF-1α to CaM is Ca^{2+}-dependent. Immunofluorescence staining indicated that *Tetrahymena* EF-1α is diffusely distributed throughout the cytoplasm, and is localized in the oral apparatus and the apical region in interphase cells. Moreover, *Tetrahymena* EF-1α is localized with CaM in the division furrow during cytokinesis.

MATERIALS AND METHODS

Cell Culture—Cultivation of *Tetrahymena pyriformis* (strain W) and *T. thermophila* (B24964WT) was performed as described previously (*32*).

Electrophoresis and Immunoblot Analysis—SDS-PAGE was performed on a 10 or 15% running gel with a 3% stacking gel according to Laemmli (*33*). Native gel electrophoresis was performed on an alkaline glycerol gel (*34*). Gels were stained with silver or Coomassie Brilliant Blue R-250. To examine the specificity of the antibody, immunoblot analysis was carried out according to the method of Towbin *et al.* (*35*), using an alkaline phosphatase–conjugated goat anti–rabbit IgG (Tago, Burlingame, USA) as a second antibody.

Preparation of Tetrahymena EF-1α and CaM—Tetrahymena EF-1α was purified as described previously (*36*). *Tetrahymena* CaM bacterially expressed as a glutathione S-transferase fusion protein was purified by the method of Hanyu *et al.* (*37*). The GST-CaM fusion protein was expressed in *Escherichia coli*, strain JM109, and then purified on glutathione-Sepharose 4B (Amersham Pharmacia Biotech). To purify free CaM, GST-CaM–bound beads were incubated in PBS containing thrombin (50 cleavage units/ml beads; Amersham Pharmacia Biotech). A supernatant including CaM was obtained by centrifugation.

Preparation of a Tetrahymena Extract—T. pyriformis cells cultivated in 500 ml culture medium for 41 h were harvested by centrifugation at 1,200 ×*g* for 1 min. The cell pellet was washed twice with a cold NKC solution (34.2 mM NaCl, 1.07 mM KCl, 1.08 mM $CaCl_2$), and then divided into two parts. One part was resuspended in 2 volumes of extraction buffer [10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 10% glycerol, 1 mM $MgCl_2$, 5 μg/ml leupeptin, 10 μM *Nα-p*-tosyl-L-lysine chloro-methyl ketone (TLCK), 3.1 mM NaN_3, pH 7.0] containing 1 mM $CaCl_2$ (E-Ca buffer). The other part was resuspended in 2 volumes of extraction buffer containing 1 mM EGTA (E-EGTA buffer). Each suspension was sonicated for 4 min at an output setting of 8 using a cell distributor (Ultrasonics Inst., Tomy Seiko, Tokyo). Each sonicated suspension was centrifuged at 100,000 ×*g* for 1.5 h at 0°C. The resultant supernatants prepared in the presence of 1 mM $CaCl_2$ and 1 mM EGTA were designated as the Ca extract and EGTA extract, respectively.

Column Chromatography—The *Tetrahymena* EF-1α affinity column was prepared using HiTrap NHS-activated (Amersham Pharmacia Biotech, Uppsala, Sweden), according to the manufacturer's instructions. One milliliter of *Tetrahymena* EF-1α (2 mg/ml) was applied to a HiTrap NHS-activated Sepharose column. The column volume was 1 ml. The coupling efficiency was about 2 mg/ml resin. Coupling of BSA to the HiTrap NHS-activated was carried out in the same manner. Column chromatography was performed in the presence of 1 mM $CaCl_2$ or 1 mM EGTA. In the presence of 1 mM $CaCl_2$, 25 ml of the Ca extract was applied to a *Tetrahymena* EF-1α column or a control BSA column equilibrated with E-Ca buffer at the flow rate of 0.1 ml/min. After loading, each column was rinsed with E-Ca buffer containing 50 mM KCl (EK-Ca buffer) at 0.1 ml/min for 120 min. Then the columns were eluted successively with EK-Ca buffer containing 2 mM ATP, EK-Ca buffer containing 2 mM GTP, E-EGTA buffer containing 50 mM KCl (EK-EGTA buffer), E-Ca buffer containing 50 mM KCl, E-Ca buffer containing 0.3 M KCl, and E-Ca buffer containing 1.0 M KCl. In the presence of 1 mM EGTA, 25 ml of the EGTA extract was applied to an *Tetrahymena* EF-1α column equilibrated with E-EGTA buffer. After loading, the column was rinsed with EK-EGTA buffer at 0.1 ml/min for 120 min. Then the column was eluted successively with EK-EGTA buffer containing 2 mM ATP, EK-EGTA buffer containing 2 mM GTP, EK-Ca buffer, E-EGTA buffer containing 50 mM KCl, E-EGTA buffer containing 0.3 M KCl, and E-EGTA buffer containing 1.0 M KCl.

Polyclonal Antibodies—The purified *Tetrahymena* EF-1α was dialyzed against PBS, homogenized with an equal volume of complete Freund's adjuvant and then subcutaneously injected into rabbits. After one week, *Tetrahymena* EF-1α was homogenized with incomplete Freund's adjuvant and injected at weekly intervals. After the antigen had been injected 5 times, an anti–*Tetrahymena* EF-1α antiserum was obtained and then affinity purified on a *Tetrahymena* EF-1α–affinity column. A rabbit antiserum against *T. thermophila* CaM has been prepared using recombinant CaM expressed in *E. coli* (*38*).

Indirect Immunofluorescence—T. thermophila cells were washed twice in TPBS buffer (100 mM NaCl, 7.5 mM $Na_2HPO_4 \cdot 12H_2O$, 2.5 mM $NaH_2PO_4 \cdot 2H_2O$, pH 7.3) at 20°C and then left in TPBS buffer for 20 min at 20°C. The cells were then quickly air-dried on a slide and fixed with 0.2% formaldehyde in methanol for 4–5 min at –20°C. Next they were washed in phosphate-buffered saline (PBS), and preincubated in 0.1 M glycine in PBS at 4°C for 15 min and in 5% nonfat dried milk in PBS at 4°C for 30 min. Then they were incubated with the affinity purified rabbit anti–*Tetrahymena* EF-1α antibody (diluted 1:15 in 5% nonfat dried milk in PBS) or the rabbit anti–*Tetrahymena* CaM antibody (diluted 1:10) for 1 h at room temperature. The cell specimens were washed with PBS, and then incubated with a fluorescein isothiocyanate (FITC)–conjugated goat anti–rabbit IgG (diluted 1:100 in 5% nonfat dried milk in PBS; KPL, Maryland, USA) for 1 h at room temperature. After being washed, the specimens were mounted with 50% glycerol in PBS. These specimens were observed under a Karl Zeiss Axio fluorescence microscope and photographed on Tri-X Pan 400 film (Kodak, Rochester, USA).

RESULTS

Identification of Tetrahymena EF-1α–Binding Proteins—To investigate the rol of EF-1α as a regulator of actin filament organization, we examined EF-1α–binding proteins in *Tetrahymena*. Aliquotsof the *T. pyriformis* cell extract, prepared in a low ionic strength buffer containing 1 mM $CaCl_2$, were passed through the *Tetrahymena* EF-1α column and the control BSA column. Proteins absorbed to these columns were eluted successively with 2 mM ATP, 2 mM GTP, 1 mM EGTA, 50 mM KCl, 0.3 M KCl, and 1 M KCl, because EF-1α is a GTP-binding protein and the F-actin bundling activity of EF-1α is regulated by Ca^{2+}/CaM. Figure 1A shows an SDS-PAGE pattern for each eluate from the *Tetrahymena* EF-1α column. Proteins of apparent molecular weights of 78- and 74-kDa were eluted with 2 mM ATP (Fig. 1A, lanes 6 and 7), and a 16.7-kDa protein was the only protein eluted with 1 mM EGTA (Fig. 1A, lanes 10 and 11). The major proteins present in the *Tetrahymena* EF-1α column eluates were not significantly absorbed to the control BSA column (data not shown). On the other hand, when *Tetrahymena* cell extracts prepared in a low ionic strength buffer containing 1 mM EGTA were passed through the *Tetrahymena* EF-1α column, the 78- and 74-kDa proteins were absorbed to the column, but the 16.7-kDa protein was not (Fig. 1B). These results indicate that the binding of the 78- and 74-kDa proteins to *Tetrahymena* EF-1α is Ca^{2+}-independent and ATP-sensitive, while the binding between the 16.7-kDa protein and *Tetrahymena* EF-1α is Ca^{2+}-dependent.

The 16.7-kDa Protein Is Tetrahymena CaM—To determine whether or not the 16.7-kDa protein and CaM are identical, some properties of the 16.7-kDa protein and recombinant *Tetrahymena* CaM were compared. CaM is known to show a characteristic Ca^{2+}-dependent shift in electrophoretic mobility (*39*). The 16.7-kDa protein showed different mobility under Ca^{2+} and EGTA conditions (Fig. 2A). In the same gel, recombinant CaM also exhibited a Ca^{2+}-dependent shift in electrophoretic mobility (Fig. 2B), indicating that the 16.7-kDa protein has the ability to bind Ca^{2+} and to change the conformation. The rabbit anti–*Tetrahymena* CaM antibody crossreacted with the 16.7-kDa

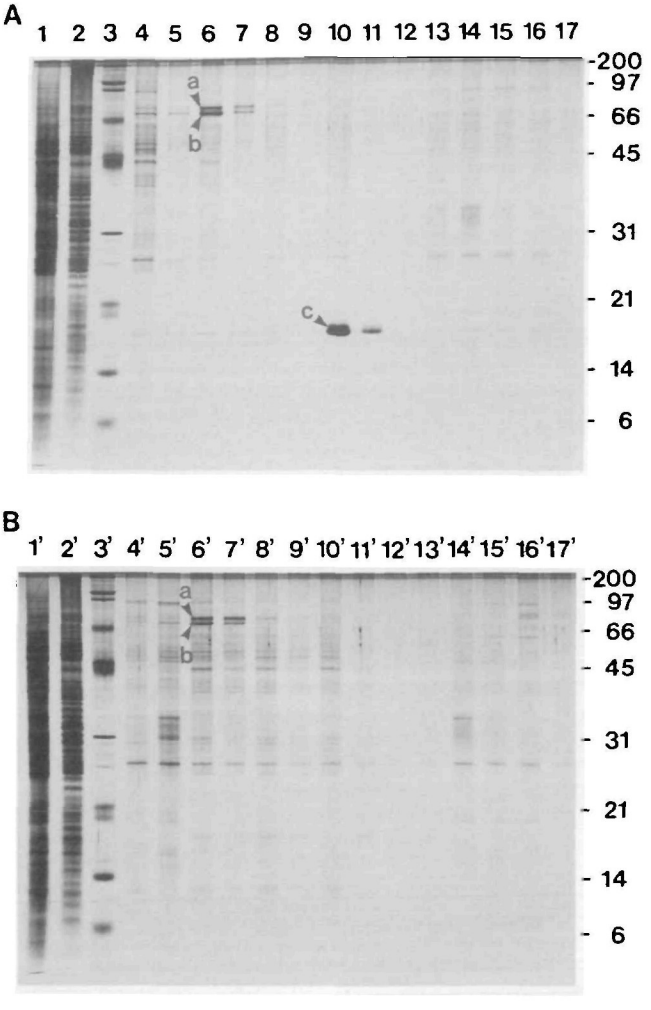

Fig. 1. **SDS-PAGE analysis of the proteins eluted from *Tetrahymena* EF-1α affinity columns loaded with a *Tetrahymena* cell extract.** A *Tetrahymena* cell extract prepared with E-Ca buffer (A) or E-EGTA buffer (B) was applied to a *Tetrahymena* EF-1α affinity column. Protein fractions eluted from each *Tetrahymena* EF-1α affinity column were run on a 15% gel. The gels were silver-stained. Lanes 1 and 1', *Tetrahymena* cell extract; lanes 2 and 2', precipitate of *Tetrahymena* cell lysate: lanes 3 and 3', molecular weight markers; lanes 4, 4', 5, and 5', flow-through fractions; lanes 6, 6', 7, and 7', 2 mM ATP-eluted fractions; lanes 8, 8', 9, and 9', 2 mM GTP-eluted fractions, lanes 10 and 11 in (A), 1 mM EGTA-eluted fractions; lanes 10' and 11' in (B), 1 mM $CaCl_2$-eluted fractions; lanes 12, 12', 13, and 13', 50 mM KCl-eluted fractions; lanes 14, 14', 15, and 15', 0.3 M KCl-eluted fractions; lanes 16, 16', 17, and 17', 1 M KCl-eluted fractions. The 2 mM ATP-eluted fraction (lanes 6, 6', 7, and 7') contained 78- and 74-kDa proteins. These proteins could bind to *Tetrahymena* EF-1α in the presence of 1 mM $CaCl_2$ (A) or 1 mM EGTA (B). One mM EGTA-eluted fraction (lanes 10 and 11 in A) contained a 16.7-kDa protein which could not bind to *Tetrahymena* EF-1α in the presence of 1 mM EGTA (lanes 10' and 11' in B). Arrowheads a, b, and c indicate the 78, 74, and 16.7-kDa proteins, respectively. The apparent molecular weights (kDa) are shown on the right.

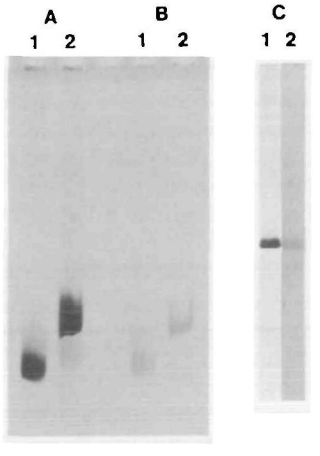

Fig. 2. **Ca^{2+}-dependent electrophoretic mobility shifts of the 16.7-kDa protein, and crossreaction between the anti–*Tetrahymena* CaM antibody and the 16.7-kDa protein.** The 1 mM EGTA-eluted fraction (A) and recombinant CaM (B) were electrophoresed on alkali-glycerol gels in the presence of 1.5 mM EGTA (1) or 1.5 mM $CaCl_2$ (2). (C) The 1 mM EGTA-eluted fraction was electrophoresed on a 15% gel (1) and then analyzed by immunoblotting using a rabbit anti–*Tetrahymena* CaM antibody (2).

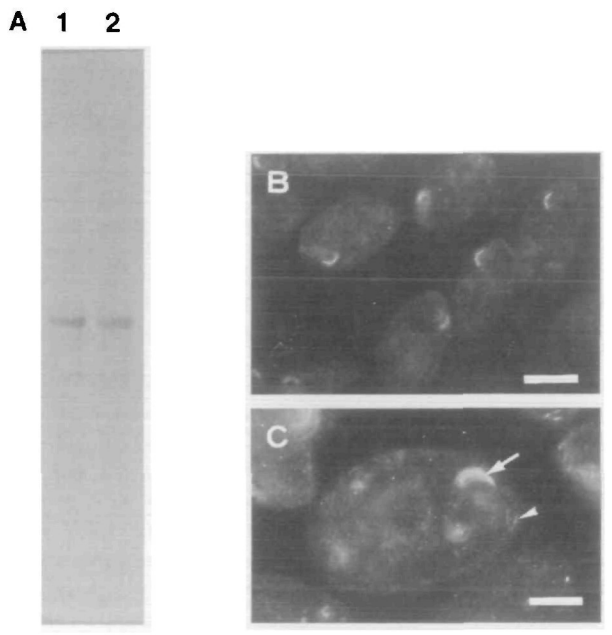

Fig. 3. **Specificity of anti-*Tetrahymena* EF-1α and localization of *Tetrahymena* EF-1α in interphase cells.** (A) A *T. pyriformis* cell extract (lane 1) and *T. thermophila* cell extract (lane 2) were resolved by 10% SDS-PAGE, and then analyzed by immunoblotting using an affinity-purified anti-*Tetrahymena* EF-1α antibody. (B and C) Interphase cells were stained with the anti-*Tetrahymena* EF-1α antibody. (B) and (C) are low and high magnification images, respectively. The arrow in (C) indicates the crescent structure of the oral apparatus, and the arrowhead indicates the apical region. The bars in (B) and (C) indicate 20 and 10 μm, respectively.

protein (Fig. 2C). These results show that the 16.7-kDa protein is *Tetrahymena* CaM.On the other hand, the N-terminal amino acid sequence of the 74-kDa protein was homologous to those of heat shock protein70 (hsp70) superfamily proteins (*40*), but that of the 78-kDa protein was not determined.

Localization of EF-1α in Tetrahymena thermophila—To examine the localization of EF-1α, we prepared a rabbit polyclonal antibody against *Tetrahymena* EF-1α. The specificity of the antibody, which was affinity-purified using *Tetrahymena* EF-1α, was analyzed by immunoblotting. As shown in Fig. 3A, the anti-*Tetrahymena* EF-1α antibody reacted specifically with EF-1α in *T. pyriformis* and *T. thermophila* cell extracts, respectively.

We examined the localization of EF-1α in *T. thermophila* cells. In interphase cells, immunofluorescence for *Tetrahymena* EF-1α appeared in the crescent structure of the oral apparatus and the apical region, and weak immunofluorescence was distributed throughout the cytoplasm, especially around the macronuclei (Fig. 3, B and C). In dividing cells, immunofluorescence for *Tetrahymena* EF-1α appeared in the division furrow from the early to the end stage of cytokinesis, in addition, as well as in the oral apparatus and the cytoplasm (Figs. 4 and 5A). At a very early stage of cytokinesis, a thin line of staining in the equatorial furrow region was found (Fig. 4A). From the middle to the late stage of cytokinesis, the fluorescence appeared as a thicker dotted line in the division furrow (Fig. 4, B–D). These staining patterns were not observed with preimmune serum (data not shown).

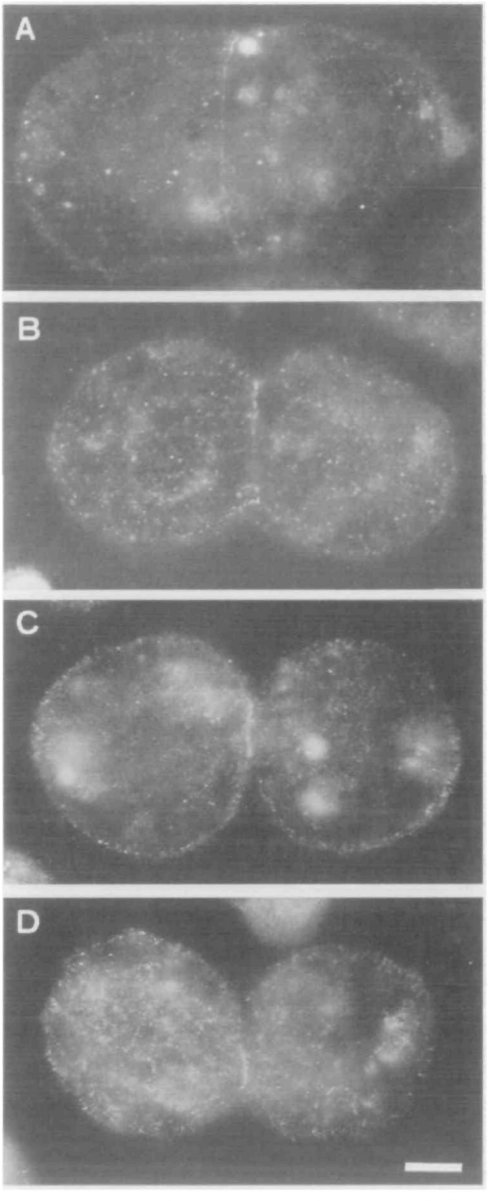

Fig. 4. **Localization of *Tetrahymena* EF-1α in dividing cells.** Dividing cells at various stages of cytokinesis (A–D) were stained with the anti-*Tetrahymena* EF-1α antibody. The micrographs (A–D) are arranged in order: early to late stages of cytokinesis. In the micrographs, the anterior portions of cells are aligned on the right. All cells are shown at the same magnification. The bar indicates 8 μm.

Comparison of the Localization of EF-1α with That of CaM in Dividing Tetrahymena Cells—*Tetrahymena* CaM was localized in the oral apparatus, cilia, basal bodies, apical ring and contractile vacuole pores in interphase *Tetrahymena* cells (*41*). To determine the *in vivo* relationship between EF-1α and CaM, this localization was examined by immunofluorescence staining using the rabbit anti-*Tetrahymena* EF-1α antibody and the rabbit anti-*Tetrahymena* CaM antibody. In interphase *Tetrahymena* cells, both *Tetrahymena* EF-1α and CaM were colocalized in the crescent structure of the oral apparatus and the apical ring (data not shown), while in dividing cells, both proteins were localized in the division furrow and the oral apparatus during cytokinesis (Fig. 5). Therefore, these results suggest

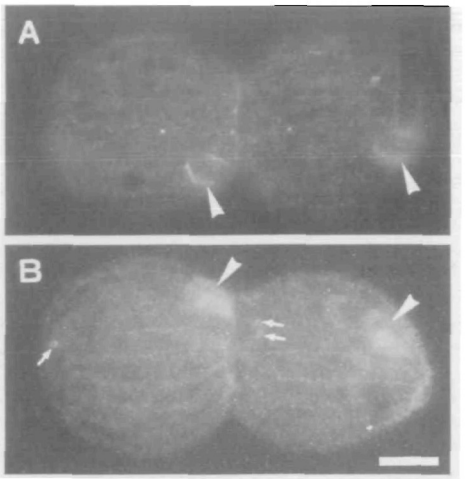

Fig. 5. **Localization of *Tetrahymena* EF-1α and CaM in dividing cells.** Dividing cells were stained with the anti-*Tetrahymena* EF-1α antibody (A) and anti-*Tetrahymena* CaM antibody (B). Arrows and arrowheads indicate the contractile vacuole pores and oral apparatus, respectively. In the micrographs, the anterior portions of cells are aligned on the right. Two cells are shown at the same magnification. The bar indicates 10 μm.

that CaM interacted with EF-1α *in vivo* as well as *in vitro*.

DISCUSSION

Previously, we reported that *Tetrahymena* EF-1α bound to F-actin and induced the formation of F-actin bundles *in vitro*, and that F-actin bundling activity was regulated by Ca^{2+}/CaM (*13*). In addition, a gel-shift assay involving native gel electrophoresis demonstrated Ca^{2+}-dependent direct binding between *Tetrahymena* EF-1α and CaM (*13*). Therefore, in the present study, we confirmed that only CaM in the *Tetrahymena* cell extract bound to *Tetrahymena* EF-1α in a Ca^{2+}-dependent manner (Figs. 1A and 2). However, actin and tubulin were not observed to bind to the *Tetrahymena* EF-1α affinity column. Since the *Tetrahymena* extract was prepared by centrifugation at 100,000 ×*g* for 1.5 h, almost all the F-actin and microtubules were probably removed from the extract.

By an immunofluorescence method, we demonstrated that *Tetrahymena* EF-1α was localized in the division furrow, besides being distributed diffusely in the cytoplasm (Fig. 4). This is the first report of the localization of EF-1α in the division furrow. Since *Tetrahymena* actin was localized there (*20*), EF-1α and actin might be colocalized in the division furrow during cytokinesis. Recently, we reported that the 71-kDa actin-binding protein is possibly a fimbrin homolog in *Tetrahymena* and that it induces the bundling of F-actin (*27*). Immunofluorescence for the fimbrin-like protein appeared as a thin dotted line in the equatorial furrow region at the early stage of cytokinesis. The dots of the fimbrin-like protein joined and a thick dotted line appeared in the division furrow, as the furrow constriction proceeded (*27*). At the late stage of cytokinesis, *Tetrahymena* EF-1α was localized as a thick dotted line in the division furrow (Fig. 4, C and D), suggesting that the localization here coincided with that of the fimbrin-like protein in terms of both appearance and timing.

In this study, we observed that CaM and EF-1α were localized in the division furrow (Fig. 5). Previously we reported that Ca^{2+}/CaM completely inhibited the F-actin–bundling activity of *Tetrahymena* EF-1α *in vitro* (*13*). On the other hand, the F-actin–bundling activity of the *Tetrahymena* fimbrin-like protein probably exhibits no Ca^{2+} sensitivity because of a lack of EF-hand Ca^{2+}-binding motifs (manuscript in preparation). Based on these findings, we suggest that the proteins in the division furrow play important roles in organizing F-actin into a bundle. The F-actin bundles formed by *Tetrahymena* EF-1α *in vitro* are similar to F-actin paracrystals, and are tighter than those formed by the fimbrin-like protein (*13, 27*). In such a tight bundle, myosin and other proteins can not interact with F-actin. Recently, Ca^{2+} waves were observed along the cleavage furrows in medaka fish embryos (*29*), zebra fish embryos (*30*), and *Xenopus* embryos (*31*). Therefore, we speculate that Ca^{2+}/CaM is a key factor in the formation of F-actin bundles in contractile rings. 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 binds directly to *Tetrahymena* EF-1α and the F-actin–bundling activity of *Tetrahymena* EF-1α is completely inhibited. As a result, it is possible that the tight F-actin bundles in contractile rings are loosened and that the *Tetrahymena* fimbrin–like protein maintains the loose F-actin bundles. Myosin and other proteins are able to interact with F-actin in the loose bundles; perhaps Ca^{2+}/CaM activates the myosin light chain kinase, and then constriction of the contractile ring occurs. Thus, regulation of the intracellular Ca^{2+} concentration may be essential for the organization of F-actin into bundles during cytokinesis. It is unclear how the free Ca^{2+} concentration changes and how free Ca^{2+} is associated with events in the cytokinesis of *Tetrahymena*.

REFERENCES

1. Kaziro, Y. (1978) The role of guanosine 5'-triphosphate in polypeptide chain elongation. *Biochim. Biophys. Acta* **505**, 95–127
2. Moldave, K. (1985) Eukaryotic protein synthesis. *Annu. Rev. Biochem.* **54**, 1109–1149
3. Riis, B., Rattan, S.I.S., Clark, B.F.C., and Merrick, W.C. (1990) Eukaryotic protein elongation factors. *Trends Biochem. Sci.* **15**, 420–424
4. Kuriyama, R. and Borisy, G.G. (1985) Identification of molecular components of the centrosphere in the mitotic spindle of sea urchin eggs. *J. Cell Biol.* **101**, 524–530
5. Kuriyama, R., Savereide, P., Lefebvre, P., and Dasgupta, S. (1990) The predicted amino acid sequence of a centrosphere protein in dividing sea urchin eggs is similar to elongation factor (EF-1α). *J. Cell Sci.* **95**, 231–236
6. Ohta, K., Toriyama, M., Endo, S., and Sakai, H. (1988) Mitotic apparatus-associated 51-kD protein in mitosis of sea urchin eggs. *Zool. Sci.* **5**, 613–621
7. Ohta, K., Toriyama, M., Miyazaki, M., Murofushi, H., Hosoda, S., Endo, S., and Sakai, H. (1990) The mitotic apparatus-associated 51-kD protein from sea urchin eggs is a GTP-binding protein and is immunologically related to yeast polypeptide elongation factor 1α. *J. Biol. Chem.* **265**, 3240–3247
8. Shiina, N., Gotoh, Y., Kubomura, N., Iwamatsu, A., and Nishida, E. (1994) Microtubule severing by elongation factor 1α. *Science* **266**, 282–285
9. 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
10. 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
11. Dharmawardhane, S., Demma, M., Yang, F., and Condeelis, J. (1991) Compartmentalization and actin binding properties of ABP-50: The elongation factor-1α of *Dictyostelium*. *Cell Motil. Cytoskeleton* **20**, 279–288
12. Itano, N. and Hatano, T. (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
13. 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
14. Kurasawa, Y., Watanabe, Y., and Numata, O. (1996) Characterization of F-actin bundling activity of *Tetrahymena* elongation factor 1α investigated with rabbit skeletal muscle actin. *Zool. Sci.* **13**, 371–375
15. Numata, O. (1996) Multifunctional proteins in *Tetrahymena*: 14-nm filament protein/citrate synthase and translation elongation factor-1α. *Int. Rev. Cyt.* **164**, 1–35
16. Durso, N.A. and Cyr, R.J. (1994) A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor-1α. *Plant Cell* **6**, 893–905
17. Kaur, K.J. and Ruben, L. (1994) Protein translation elongation factor-1α from *Trypanosoma brucei* binds calmodulin. *J. Biol. Chem.* **269**, 23045–23050
18. 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
19. 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
20. Hirono, M., Nakamura, M., Tsunemoto, M., Yasuda, T., Ohba, H., Numata, O., and Watanabe, Y. (1987) Localization and possible biological roles of actin in *Tetrahymena cells*. *J. Biochem.* **102**, 537–545
21. Watanabe, Y., Ohba, H., Hirono, M., and Yasuda, T. (1990) Analysis of furrow formation and furrowing during cell division in *Tetrahymena* using cell-division-arrest mutants. *Ann. N.Y. Acad. Sci.* **582**, 166–178
22. Mabuchi, I. (1986) Biochemical aspects of cytokinesis. *Int. Rev. Cytol.* **101**, 175–213
23. Salmon, E.D. (1989) Cytokinesis in animal cells. *Curr. Opin. Cell Biol.* **1**, 541–547
24. Satterwhite, L.L. and Pollard, T.D. (1992) Cytokinesis. *Curr. Opin. Cell Biol.* **4**, 43–52
25. Fishkind, D.J. and Wang, Y.L. (1995) New horizons for cytokinesis. *Curr. Opin. Cell Biol.* **7**, 23–31
26. Edamatsu, M., Hirono, M., and Watanabe, Y. (1992) *Tetrahymena* profilin is localized in the division furrow. *J. Biochem.* **112**, 637–642
27. 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
28. Hepler, P.K. (1994) The role of calcium in cell division. *Cell Calcium* **16**, 322–330
29. 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
30. Chang, D.C. and Meng, C. (1995) A localized elevation of cytosolic free calcium is associated with cytokinesis in the zebra fish embryo. *J. Cell Biol.* **131**, 1539–1545
31. 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
32. Watanabe, Y., Numata, O., Kurasawa, Y., and Katoh, M. (1994) Cultivation of *Tetrahymena* cells in *Cell Biol* (Celis, J.E., ed.) Vol. 1, pp. 398–404, Academic Press, California
33. Laemmli, U.K. (1970) Cleavage of structure proteins dividing the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
34. Perrie, W.T. and Perry, S.V. (1970) An electrophoretic study of the low-molecular weight components of myosin. *Biochem. J.* **119**, 31–38
35. Towbin, H.T., Stahelin, H.M., and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354
36. Takeda, T., Kurasawa, Y., Watanabe, Y., and Numata, O. (1995) Polymerization of highly purified *Tetrahymena* 14-nm filament protein/citrate synthase into filaments and its possible role in regulation of enzymatic activity. *J. Biochem.* **117**, 869–874
37. Hanyu, K., Takemasa, T., Numata, O., Takahashi, M., and Watanabe, Y. (1995) Immunofluorescence localization of a 25-kD *Tetrahymena* EF-hand Ca^{2+}-binding protein, TCBP-25, in the cell cortex and possible involvement in conjugation. *Exp. Cell Res.* **219**, 487–493
38. Gonda, K., Katoh, M., Hanyu, K., Watanabe, Y., and Numata, O. (1999) Ca^{2+}/calmodulin and p85 cooperatively regulate an initiation of cytokinesis in *Tetrahymena*. *J. Cell Sci.* **112**, 3619–3626
39. Head, J.F., Mader, S., and Kaminer, B. (1979) Calcium-binding modulator protein from the unfertilized egg of the sea urchin *Arbacia punctulata*. *J. Cell Biol.* **80**, 211–218
40. Numata, O., Kurasawa, Y., and Watanabe, Y. (1999) *Tetrahymena* elongation factor-1α binds to Hsp70 family proteins. *Zool. Sci.* **16**, 279–284
41. Suzuki, Y., Ohnishi, K., Hirabayashi, T., and Watanabe, Y. (1982) *Tetrahymena* calmodulin. Characterization of an anti-*Tetrahymena* calmodulin and the immunofluorescent localization in *Tetrahymena*. *Exp. Cell Res.* **137**, 1–14