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Tetrahymena Eukaryotic Translation Elongation Factor 1A (eEF1A) Bundles Filamentous Actin through Dimer Formation

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Eukaryotic translation elongation factor 1A (eEF1A) is known to be a multifunctional protein. In *Tetrahymena*, eEF1A is localized to the division furrow and has the character to bundle filamentous actin (F-actin). eEF1A binds F-actin and the ratio of eEF1A and actin is approximately 1:1 (Kurasawa *et al*., 1996). In this study, we revealed that eEF1A itself exists as monomer and dimer, using gel filtration column chromatography. Next, eEF1A monomer and eEF1A dimer were separated using gel filtration column, and their interaction with F-actin was examined with cosedimentation assay and electron microscopy. In the absence of Ca^{2+}/calmodulin (CaM), eEF1A dimer bundled F-actin and coprecipitated with F-actin at low-speed centrifugation, but eEF1A monomer did not. In the presence of Ca^{2+}/CaM, eEF1A monomer increased, while dimer decreased. To examine that Ca^{2+}/CaM alters eEF1A dimer into monomer and inhibits bundle formation of F-actin, Ca^{2+}/CaM was added to F-actin bundles formed by eEF1A dimer. Ca^{2+}/CaM separated eEF1A dimer to monomer, loosened F-actin bundles and then dispersed actin filaments. Simultaneously, Ca^{2+}/CaM/ eEF1A monomer complexes were dissociated from actin filaments. Therefore, Ca^{2+}/CaM reversibly regulates the F-actin bundling activity of eEF1A.

Key words: actin, Ca^{2+}, calmodulin, cytoskeleton, eukaryotic translation elongation factor 1A (eEF1A), *Tetrahymena*.

Cytokinesis is the final step in the cell cycle. In cytokinesis, the contractile ring constricts the cell surface at a cellular equator and forms a division furrow and then divides the cell into two daughter cells (*1–3*). The contractile ring is composed of actin filaments and myosin II (*4*). The dynamic organization of actin filaments in the contractile ring is regulated by a small GTPase Rho-mediated signal (*5*) and a number of actin-modulating proteins (*6–9*). Myosin II induces contraction in the contractile ring (*4*). However, how the contractile ring was formed at the division furrow is not well understood.

The contractile ring structure of *Tetrahymena*, which is similar to those in animal cells, is composed of actin filaments and lateral strips on the contractile ring actin filaments (*10–13*). Some proteins, division-related proteins are localized in the division furrow, for example, profilin as an actin polymerization factor (*14*), p85 as a division plane determination factor (*15*), calmodulin (CaM) as Ca^{2+}-binding protein (*15*, *16*), eukaryotic translation elongation factor 1A (eEF1A) (*16*) and fimblin (*17*, *18*) as actin filaments bundling factors.

In particular, eEF1A is a very interesting protein. It is a very abundant cytoplasmic protein conserved in all species in eukaryotes and prokaryotes, and catalyzes the GTP-dependent binding of aminoacyl-tRNAs to their respective mRNA anticodons at the A site of ribosomes, in the peptide elongation phase of protein synthesis (*19–21*). Several reports indicated that eEF1A has a second role, as a regulator of microtubule rearrangements (*22–26*). Moreover, it has been shown that eEF1A is involved in the regulation of the actin cytoskeleton (*27–30*). Because eEF1A binds to actin filaments with relatively high affinity, eEF1A could be a potent regulator of the dynamics of the actin cytoskeleton. In *Dictyostelium*, eEF1A has a role in β-actin mRNA anchoring to the actin cytoskeleton (*31*). Furthermore, binding between eEF1A and actin is sensitive to cellular parameters such as nucleotide concentration and pH (*32–34*). Since eEF1A participates in cytoskeletal regulation as well as protein synthesis, eEF1A is a multifunctional protein.

Tetrahymena eEF1A bundles rabbit skeletal muscle F-actin as well as *Tetrahymena* F-actin (*35*). Using *Tetrahymena* eEF1A, *Tetrahymena* actin and recombinant *Tetrahymena* CaM, Kurasawa *et al*. demonstrated that Ca^{2+}/CaM bound to eEF1A and completely inhibited the formation of F-actin bundles by eEF1A, but did not affect the formation of the eEF1A/F-actin complexes (*36*). CaM is a 17 kDa calcium-binding protein, which together with p85, regulates the initiation of cytokinesis (*15*), and co-localizes with eEF1A at the division furrow in *Tetrahymena* (*16*). In addition, Ca^{2+} plays an important role in cytokinesis, since an elevation of Ca^{2+} concentration was seen in the equatorial region just before and after the formation of the cleavage furrow (*36–38*). These results lead us to the hypothesize that eEF1A induces the formation of F-actin bundles in the contractile ring and that the F-actin bundling activity of eEF1A is regulated by Ca^{2+}/CaM.

An eEF1A dimer model has been proposed by Kurasawa *et al*. to explain the F-actin bundling induced by eEF1A.

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They suggested that actin filaments were cross-linked by dimers of eEF1A because the binding ratio of *Tetrahymena* eEF1A to skeletal muscle F-actin was approximately 1:1 and eEF1A has just one actin-binding domain which is homologous to the actin-binding domain of depactin (*36*). However, it is not proved that eEF1A forms dimer or that eEF1A dimers bundle F-actin.

In this study, we found that eEF1A forms dimer, using gel filtration column chromatography, and that eEF1A dimer bundled F-actin but eEF1A monomer did not. Ca^{2+}/CaM reversibly regulates F-actin bundling activity of eEF1A, since Ca^{2+}/CaM separated eEF1A dimer into monomer and Ca^{2+} chelation formed eEF1A dimer from monomer. These results suggest that eEF1A dimer cross links F-actin, and that Ca^{2+}/CaM induces monomerization of eEF1A and inhibits bundle formation of F-actin.

MATERIALS AND METHODS

Cell Culture—Tetrahymena pyriformis (strain W) was cultivated as described previously (*39*).

Electrophoresis and Immunoblot Analysis—SDS-PAGE was performed on 8% or 10% running gel with 3% stacking gel according to Laemmli (*40*). Gels were stained with silver or Coomassie Brilliant Blue R-250. Immunoblot analysis was carried out according to the method of Towbin *et al*. (*41*), using an alkaline phosphatase–conjugated goat anti rabbit IgG (Tago, Burlingame, USA) as a second antibody (*16*).

Preparation of eEF1A, CaM and Skeletal Muscle Actin—Tetrahymena eEF1A was purified as described previously (*42*). *Tetrahymena* CaM was bacterially expressed as a glutathione S-transferase fusion protein and was purified with a glutathione affinity column (*43*). Rabbit skeletal muscle actin was purified according to the procedure of Pardee and Spudich (*44*).

Molecular Weight Determination—Purified eEF1A was dialyzed against MES buffer [10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES), 0.75 mM 2-mercaptoethanol (2-ME), 2 mM $MgCl_2$, 80 mM KCl, 5% glycerol, 0.01 mM *p*-toluenesulfonyl-L-lysine chloromethyl ketone (TLCK), 5 μg/ml leupeptin, 0.3 mM phenylmethylsulfonyl fluoride (PMSF), pH 6.8] for 9 h at 4°C. 3 μM eEF1A was incubated for 1 h at 26°C, and then applied to the gel filtration column (Superdex 200, Amersham Pharmacia Piscataway, USA). To examine the effects of Ca^{2+}/CaM on eEF1A, purified eEF1A and CaM were individually dialyzed against MES buffer containing 1 mM $CaCl_2$. We gently mixed 3 μM eEF1A and 3 μM CaM and incubated for 1 h at 26°C, and then applied to the gel filtration column. The elution volume of each peak of eEF1A and CaM were measured. Using each elution volume, native molecular weights of eEF1A and CaM were determined with a Gel Filtration Column Calibration Kit (Amersham Pharmacia).

Low-Speed and High-Speed Cosedimentation Assay—F-actin was dialyzed against the MES buffer containing 40 mM KCl and 0.5 mM ATP. We dialyzed 10 μM eEF1A against MES buffer, and incubated for 1 h at 26°C. To separate the monomer and dimer fractions, 10 μM eEF1A was applied to the gel filtration column. Concentrations of eluted fraction were set to 1 μM. Each fraction was mixed with 1 μM F-actin in the MES buffer and incubated at 26°C for 30 min. The mixtures were centrifuged at low-speed (12,000 × *g* for 30 min), or at high-speed (200,000 × *g* for 30 min) and their supernatant and pellet were analyzed by SDS-PAGE.

Negative Staining Electron Microscopy—eEF1A and F-actin were mixed and incubated at 26°C for 30 min. The mixture was mounted on a carbon-coated grid and negatively stained with 4% uranyl acetate. The grids were observed with a JEOL JEM-1010 electron microscope at an accelerating voltage of 80 kV.

RESULTS

eEF1A Forms Dimer—We measured the native molecular weight of eEF1A under the conditions in which eEF1A bundles rabbit skeletal muscle F-actin, using the gel filtration column. Two peaks were detected (Fig. 1B), and each

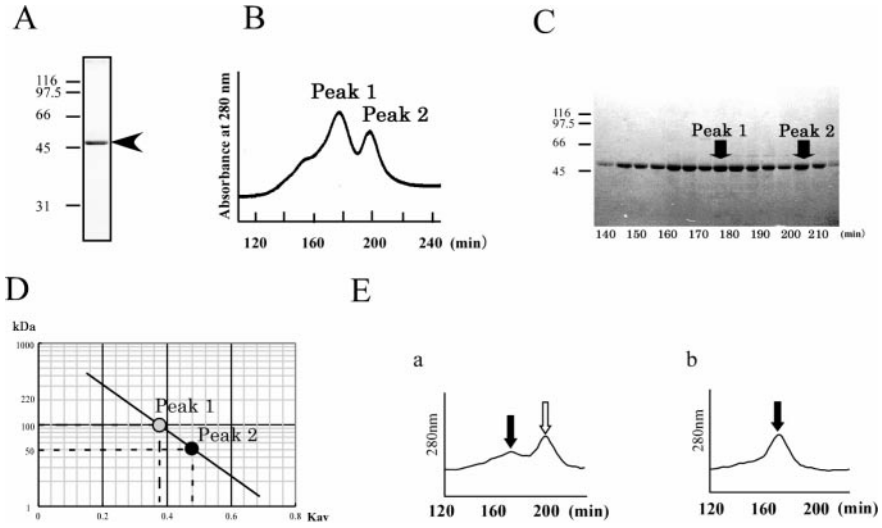

Fig. 1. ***Tetrahymena* eEF1A forms monomer and dimer.** (A) Purified *Tetrahymena* eEF1A. Molecular mass markers are on the left. (B) Elution profile of eEF1A from the gel filtration column. After dialyzing eEF1A against MES-buffer, it was incubated at 26°C for 1 h and separated with the gel filtration column. The two peaks were called peak 1 and peak 2 from the left for convenience. (C) The 10% SDS-PAGE pattern of each fraction eluted from 140 min to 210 min. Molecular mass markers are on the left. (D) Native molecular weights of two peaks of eEF1A. Kav values for the two peaks of eEF1A were calculated and plotted on the calibration curve. Gray and black circles indicate peak 1 (eEF1A dimer) and peak 2 (eEF1A monomer), respectively. (E) Elution profile of eEF1A monomer (a) and dimer (b) fraction from the gel filtration column at 26°C for 30min. White arrow is monomer peak and black arrows are dimer peaks.

peak included only eEF1A (Fig. 1C). Using a Gel Filtration Column Calibration Kit, native molecular weights of eEF1A of peaks 1 and 2 were determined to be 100 and 50 kDa (Fig. 1D). Therefore peak 1 and peak 2 were in agreement with a dimer and a monomer, suggesting that eEF1A forms a dimer. Next, we examined the stability of eEF1A dimer formation. We re-separated the monomer fraction (peak 2) and dimer fraction (peak 1) by the gel filtration column after incubating each fraction for 30 min at 26°C. When the monomer fraction was re-chromatographed, the monomer peak (white arrow) and the dimer peak (black arrow) were detected (Fig. 1E, a). On the other hand, when the dimer fraction was re-chromatographed, only dimer peak was detected (Fig. 1E, b). These results showed that eEF1A monomer formed a small amount of dimer by itself for 30 min, and that eEF1A dimer is stabile.

Furthermore, we examined the binding affinity of eEF1A monomer and eEF1A dimer to F-actin by high speed cosedimentation assay. In high-speed centrifugation, non-bundled F-actin precipitated, and eEF1A monomer and dimer co-precipitated with F-actin. However, without F-actin, eEF1A monomer and eEF1A dimer did not precipitate (Fig. 2A). These results show that eEF1A monomer as well as dimer can bind to F-actin. The binding ratio of eEF1A monomer : actin and eEF1A dimer : actin were 1:1, respectively (Fig. 2B, a and b). Scatchard plot analysis indicated that the dissociation constants (K_d) of monomer and dimer against F-actin were 0.9 μM and 1.2 μM, respectively (Fig. 2C, a and b).

F-Actin Bundle Formation by eEF1A Dimer—F-actin bundling activity of eEF1A monomer or dimer was examined by low-speed cosedimentation assay and electron microscopy. In low-speed centrifugation, only bundled F-actin precipitated, while non-bundled F-actin, eEF1A monomer and eEF1A dimer hardly precipitated (Fig. 3A, lanes 2, 4 and 6). eEF1A monomer did not precipitate with F-actin, while eEF1A dimer coprecipitated with F-actin (Fig. 3A, lanes 8 and 10). The ratio of eEF1A to actin in the precipitate was 1:1 (Fig. 3A, lane 10).

To investigate the effect of eEF1A monomer and dimer on F-actin bundling, F-actin mixed with eEF1A monomer or dimer was analyzed by electron microscopy after negative staining. In F-actin alone, thin and curved fibers were observed (Fig. 3B, a). In the mixture of F-actin and eEF1A monomer, straight F-actin (Fig. 3B, b) and partially bundled F-actin was observed (Fig. 3B, c). eEF1A monomer bound actin filaments and straightened curved filaments. Since eEF1A monomer formed a small amount of dimer by itself during incubation (Fig. 1E, a), the dimer bundled F-actin. In the mixture of F-actin and eEF1A dimer, many thick bundles of F-actin were observed (Fig. 3B, d). These data indicate that eEF1A dimer has F-actin bundling activity, and that eEF1A monomer binds F-actin, but can hardly form F-actin bundles.

Effects of pH and Ca^{2+}/CaM on eEF1A Dimer Formation—It was reported that the bundling of F-actin was regulated by pH (*34*) and Ca^{2+}/CaM (*35*). We thought that pH and Ca^{2+}/CaM regulate eEF1A dimer formation. To examine the effect of pH on eEF1A dimer formation, eEF1A was dialyzed to three kinds of MES buffer (pH 5.7, 6.9 and 8.0), and the native molecular weight of eEF1A was investigated using the gel filtration column. Although eEF1A dimer formation was induced at pH 6.9 more

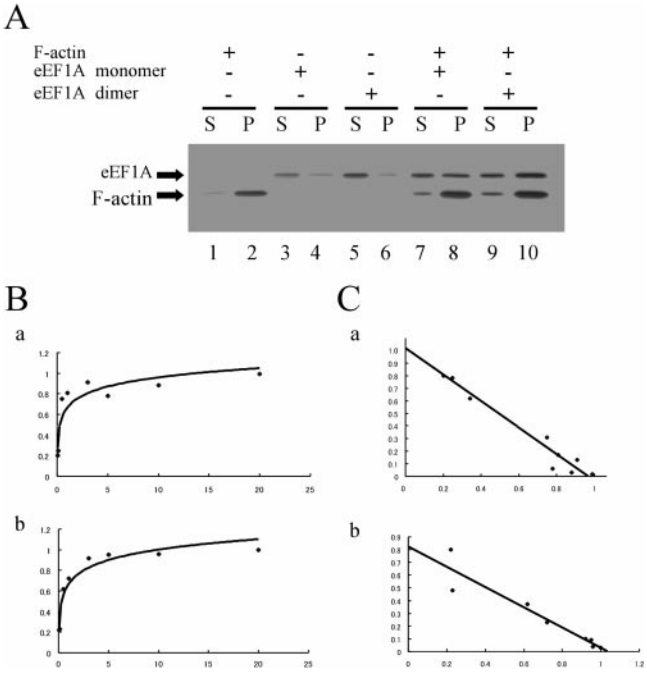

Fig. 2. **The binding affinity of eEF1A monomer and dimer to F-actin.** (A) High speed cosedimentation assay. eEF1A was separated into monomer and dimer fractions, each fraction and 1 μM F-actin were mixed and incubated at 26°C for 30 min, and then centrifuged at 200,000 × *g* for 30 min. The supernatants (S) and pellets (P) of the centrifugation were analyzed by SDS-PAGE using 10% gel. Lanes 1 and 2: S and P of F-actin alone; lanes 3 and 4: S and P of eEF1A monomer alone; lanes 5 and 6: S and P of eEF1A dimer alone; lanes 7 and 8: S and P of a mixture of F-actin and eEF1A monomer; lanes 9 and 10: S and P a mixture of F-actin and eEF1A dimer. (B) The binding affinity of eEF1A monomer (a) and dimer (b) to F-actin. Cosedimentation assay of eEF1A monomer or dimer (final concentration at 2 μM) with F-actin (final concentration at 0.05, 0.1, 0.5, 1, 3, 5, 10, and 20 μM) was performed. The pellets were analyzed by SDS-PAGE, and amounts of eEF1A and actin in pellets were measured from strength of Coomassie Brilliant Blue R-250 staining in protein bands. *X* axis is concentration of F-actin. Y axis is the molecule number of eEF1A binding to one molecule of actin. (C) Scatchard plot analysis to calculate K_d value of eEF1A monomer (a) and dimer (b) against F-actin. *X* axis is the molecule number of eEF1A binding to one molecule of actin. *Y* axis is the molecule number of eEF1A binding to one molecule of actin/the molecule number of actin.

than at pH 5.7 and pH 8.0, eEF1A dimer formation was not critically affected by pH (Fig. 4, A–C).

Next, to examine the effect of Ca^{2+}/CaM on eEF1A dimer formation, we measured native molecular weight of eEF1A in the presence of Ca^{2+} and CaM on a gel filtration column. In the presence of 1 mM $CaCl_2$, the elution profile of eEF1A did not change (Fig. 4D). The elution profile of CaM alone showed one peak, with a molecular weight of 17 kDa (Fig. 4E). The mixture of CaM and eEF1A was applied to the gel filtration column in the presence of 1 mM $CaCl_2$. The elution profile showed that eEF1A monomer increased, while dimer decreased (Fig. 4F). These results indicate that Ca^{2+}/CaM separates eEF1A dimer into monomer, and suggest that Ca^{2+}/CaM regulates the monomer-dimer exchange of eEF1A.

F-actin Bundling Activity of eEF1A Is Reversibly Regulated by Ca^{2+}/CaM—In a previous study, Kurasawa

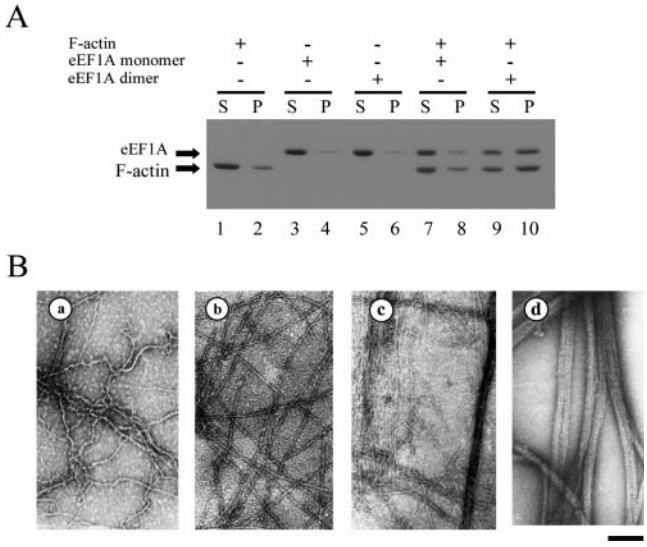

Fig. 3. **eEF1A dimer bundled F-actin.** (A) Low speed cosedimentation assay. eEF1A was separated into monomer and dimer fractions, each fraction and 1 μM F-actin were mixed and incubated at 26°C for 30 min, and then centrifuged at 12,000 × *g* for 30 min. The supernatants (S) and pellets (P) of the centrifugation were analyzed by SDS-PAGE using 10% gel. Lanes 1 and 2: S and P of F-actin alone; lanes 3 and 4: S and P of eEF1A monomer alone; lanes 5 and 6: S and P of eEF1A dimer alone; lanes 7 and 8: S and P of a mixture of F-actin and eEF1A monomer; lanes 9 and 10: S and P of a mixture of F-actin and eEF1A dimer. (B) Electron microscopy with negative of staining. 2 μM eEF1A monomer or 2 μM eEF1A dimer was mixed with 3 μM F-actin in the MES buffer and incubated at 26°C for 30 min, and then each mixture were observed by electron microscopy with negative staining. a: F-actin alone; b and c: the mixture of F-actin and eEF1A monomer; d: the mixture of F-actin and eEF1A dimer. The bar represents 200 nm.

et al. showed that Ca^{2+}/CaM bound eEF1A and F-actin bundling activity of eEF1A was completely inhibited by Ca^{2+}/CaM (*36*). However, eEF1A and Ca^{2+}/CaM co-localized in the division furrow during cytokinesis in *Tetrahymena* (*16*). Because eEF1A bundles F-actin and Ca^{2+}/CaM regulates F-actin bundling activity of eEF1A, the monomer-dimer exchange of eEF1A may be involved in the organization of the contractile ring. To investigate how Ca^{2+}/CaM regulates F-actin bundling activity of eEF1A, we performed two experiments.

First, a mixture of eEF1A, CaM, F-actin and 1 mM Ca^{2+} was observed by electron microscopy after negative staining. There were many straight actin filaments, but no F-actin bundles (Fig. 5A, b). To chelate Ca^{2+}, 3 mM EGTA was added to the mixture and the mixture was observed by electron microscopy. Removal of Ca^{2+} induced formation of F-actin bundles (Fig. 5A, d). Furthermore, using low speed and high-speed centrifugation, we examined how Ca^{2+}/CaM regulates F-actin bundling activity by eEF1A. In the presence of Ca^{2+}, eEF1A and CaM did not precipitate but F-actin was precipitated by high-speed centrifugation (Fig. 5A, a). These results show that, in the presence of Ca^{2+}, eEF1A and CaM did not bind F-actin and eEF1A could not bundle F-actin. Therefore we suggested that Ca^{2+}/CaM decreases binding affinity of eEF1A to F-actin. In the absence of Ca^{2+}, F-actin and eEF1A co-precipitated by low-speed centrifugation, but CaM did not (Fig. 5A, c).

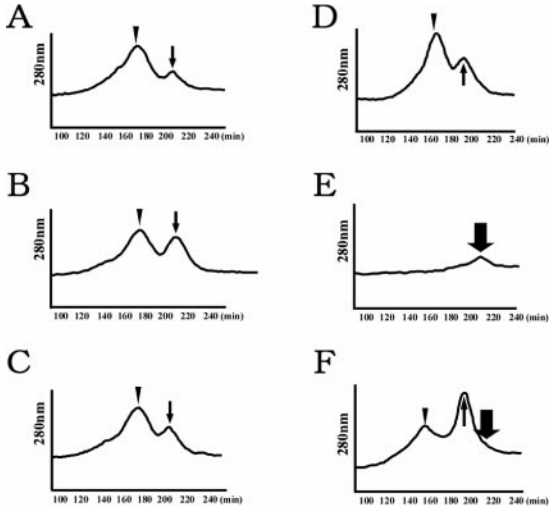

Fig. 4. **Effects of pH and Ca^{2+}/CaM on eEF1A dimer formation.** (A–C) After dialyzing 3 μM eEF1A against one of three kinds of MES buffer (pH 5.7, 6.9 and 8.0), eEF1A was separated into monomer and dimer by Superdex 200 column chromatography. (A) pH 5.7, (B) pH 6.9, (C) pH 8.0. (D–F) Elution profiles of Superdex 200 column chromatography in the MES buffer including 1mM $CaCl_2$. (D) Elution profile of eEF1A. (E) Elution profile of Ca^{2+}/CaM. (F) Elution profile of the mixture of eEF1A and Ca^{2+}/CaM. Arrowheads show eEF1A dimer, small arrows show eEF1A monomer and big arrow show Ca^{2+}/CaM.

These results show that, in the absence of Ca^{2+}, eEF1A bundled F-actin and CaM did not bind eEF1A.

Secondly, the mixture of eEF1A CaM, F-actin and 1 mM EGTA was observed by electron microscopy. Many thick bundles were observed in the mixture (Fig. 5B, a). Next, 3 mM $CaCl_2$ was added to the mixture and the mixture was observed by electron microscopy. Addition of Ca^{2+} induced dispersion of F-actin bundles (Fig. 5B, b). In particular, the tips of F-actin bundles were loosened and spread out, although many thick bundles were not loosened completely. Accordingly, we suggest that F-actin bundling activity of eEF1A is reversibly regulated by Ca^{2+}/CaM.

DISCUSSION

This study showed that eEF1A forms monomer and dimer. eEF1A monomer and dimer have activity of binding to F-actin and each K_d against F-actin are almost equal. However, eEF1A dimer has F-actin bundling activity but eEF1A monomer does not. Ca^{2+}/CaM changes eEF1A dimer into monomer, and Ca^{2+}/CaM induces to loosen F-actin bundles. To chelate Ca^{2+} helps the formation of F-actin bundles. Therefore, F-actin bundling activity of eEF1A is reversibly regulated by Ca^{2+}/CaM. Since CaM and eEF1A colocalized in the division furrow during cytokinesis (*16*), Ca^{2+}/CaM may regulate F-actin bundling in the contractile ring.

eEF1A Dimer Bundles F-actin—Previously, Kurosawa *et al*. demonstrated that Ca^{2+}/CaM binds to eEF1A and inhibits the formation of F-actin bundles by eEF1A (*36*). However, the association of Ca^{2+}/CaM with eEF1A shows any serious influence on the eEF1A/F-actin binding. In this study, we found that Ca^{2+}/CaM decreased the affinity of eEF1A to F-actin and removed eEF1A from F-actin in this

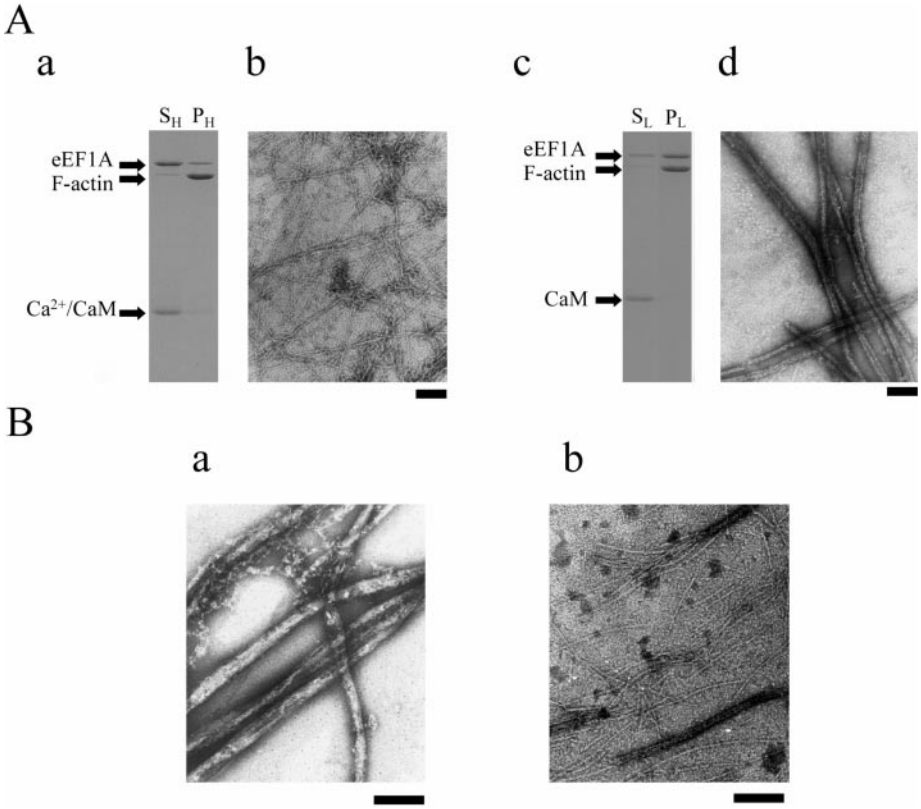

Fig. 5. **F-actin bundling activity of eEF1A is reversibly regulated by Ca^{2+}/CaM.** (A) The change of F-actin bundling activity of eEF1A by chelating Ca^{2+}. The mixture of F-actin, eEF1A and CaM in the MES buffer containing 1 mM Ca^{2+} was examined by the high-speed centrifugation (a) and observed by electron microscopy with negative staining (b). The supernatants (S_H) and pellets (P_H) were analyzed by SDS-PAGE using 15% gel. To chelate Ca^{2+}, 3 mM EGTA was added to the mixture, and then the mixture was examined by the low-speed centrifugation (c) and by electron microscopy (d). The supernatant (S_L) and pellets (P_L) were analyzed by SDS-PAGE. (B) The change of F-actin bundling activity of eEF1A by adding Ca^{2+}. The mixture of F-actin, eEF1A and CaM in the MES buffer containing 1 mM EGTA was observed by electron microscopy (a). 3 mM Ca^{2+} was added to the mixture, and then the mixture was observed by electron microscopy (b).

study. The difference between Kurasawa's results and ours is caused by the difference in salt concentration. Kurasawa *et al*. used MES buffer containing 10 mM KCl, but in this study, we used MES buffer containing 40 mM KCl. In MES buffer containing 40 mM KCl, Ca^{2+}/CaM decreases the affinity of eEF1A to F-actin and eEF1A/Ca^{2+}/CaM complex cannot bind to F-actin. Thus, we propose a new model for the Ca^{2+}/CaM regulation of F-actin bundling by eEF1A dimer (Fig. 6).

In the absence of Ca^{2+}, CaM can not bind eEF1A, eEF1A forms dimer and eEF1A dimers bundle F-actin. In the presence of Ca^{2+}, Ca^{2+}/CaM complex binds eEF1A and converts eEF1A dimer to monomer, and then F-actin bundles are loosened. At the same time, Ca^{2+}/CaM decreases the binding affinity of eEF1A monomer to F-actin, and eEF1A/Ca^{2+}/CaM complex is dissociated from F-actin. On the other hand, Liu *et al*. proposed that a single eEF1A molecule should be sufficient to cross link two actin filaments, since one eEF1A molecule should have weak and strong actin binding sites (*31*). The binding ability of the weak actin binding site of eEF1A is one fifth of that of the strong actin binding site, therefore the question remains whether eEF1A monomer can bundle F-actin and or not. Under the conditions in which eEF1A induced F-actin bundling in *Tetrahymana*, we found that eEF1A formed dimer and that eEF1A dimer could bundle F-actin but eEF1A monomer could not. Since eEF1A is a conservative protein in evolution, we speculate that the monomer-dimer exchange of eEF1A may regulate F-actin bundling in mammalian cells.

The Function of eEF1A in Division Furrow—eEF1A and fimbrin, which are actin-bundling proteins, colocalized at

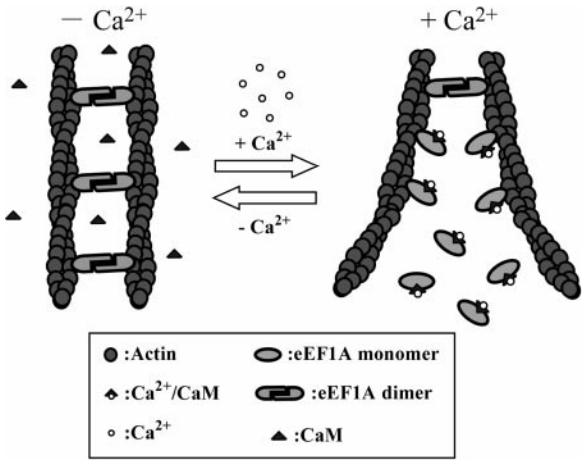

Fig. 6. **A schematic illustration of model for the Ca^{2+}/CaM regulation of F-actin bundling by eEF1A dimer.**

the division furrow in *Tetrahymena* (*16*, *17*). Although *Tetrahymena* eEF1A disperses in cytoplasm during interphase, eEF1A comes to localize in the division furrow during cytokinesis (*16*). We speculate that the conversion of eEF1A monomer to dimer correlates with the appearance of eEF1A at the division furrow in dividing cells, and that this localization change of eEF1A may be deeply involved in cytokinesis. At the time of contractile ring formation, eEF1A localizes to the division plane, forms dimer and bundles F-actin. However, we think that the actin filament bundle formed by eEF1A cannot constrict in the contractile ring, because the bundle is still thick (Figs. 3 and 5). Recent

studies have shown that the contractile ring is a dynamic structure in which actin filaments continuously assemble and disassemble (*2*, *3*, *45*). Arp2/3 complex, formins, profilin, and ADF/cofilin have been identified as regulatory proteins that control contractile ring dynamics during cytokinesis (*45–48*). In *Tetrahymena*, Ca^{2+}/CaM converts eEF1A dimer to monomer and loosens the F-actin bundle; then myosin and other actin-regulating proteins can directly interact with contractile ring actin filaments. As a result, the contractile ring constricts and cytokinesis progresses.

Multifunctions of Tetrahymena *eEF1A*—eEF1A is a multifunctional protein. How are the multiple functions of eEF1A controlled? Edmonds *et al*. proposed that the rise of pH controls interaction between F-actin and eEF1A, since elevation of pH inhibited binding of eEF1A with F-actin (*32*). It is known that cytoplasmic alkalinization increases protein synthesis in several cell types (*49*). Changes in cytoplasmic pH (pH_i) and protein synthesis are synchronous with the cell cycle in many cell types (*50*). Although we thought that pH has regulated two functions of eEF1A, F-actin binding and protein synthesis, we found that pH had no effect on eEF1A monomer-dimer exchange (Fig. 4). Ca^{2+}/CaM converts eEF1A dimer to monomer (Fig. 4), and eEF1A dimer forms F-actin bundles but eEF1A monomer can not (Fig. 3). In translation, eEF1A may function as a monomer and form a complex with eEF1B. Therefore, eEF1A monomer-dimer exchange by Ca^{2+}/CaM may be an important control mechanism for regulation of eEF1A multiple functions, such as regulator in the actin cytoskeleton and as an elongation factor.

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