



## **Characterization of F-Actin Bundling Activity of Tetrahymena Elongation Factor 1 $\alpha$ Investigated with Rabbit Skeletal Muscle Actin**

Authors: Kurasawa, Yasuhiro, Watanabe, Yoshio, and Numata, Osamu

Source: Zoological Science, 13(3) : 371-375

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.13.371>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Characterization of F-Actin Bundling Activity of *Tetrahymena* Elongation Factor 1 $\alpha$ Investigated with Rabbit Skeletal Muscle Actin

Yasuhiro Kurasawa<sup>1</sup>, Yoshio Watanabe<sup>2</sup>, and Osamu Numata<sup>1\*</sup>

<sup>1</sup>*Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, and* <sup>2</sup>*Joubu University, Isesaki, Gumma 372, Japan*

---

**ABSTRACT**—Elongation factor 1 $\alpha$  (EF-1 $\alpha$ ) is an essential factor for protein synthesis in eukaryotes. Here, we demonstrated that *Tetrahymena* EF-1 $\alpha$  induced bundles of rabbit skeletal muscle F-actin as well as *Tetrahymena* F-actin *in vitro*, although *Tetrahymena* and skeletal muscle actins are different in some parts of their primary structures and in the binding abilities to some actin-binding proteins. Co-sedimentation experiments showed that the binding ratio of *Tetrahymena* EF-1 $\alpha$  to skeletal muscle F-actin in the bundles was 1 : 1. Electron microscopic observation showed that alkaline pH or high ionic strength reduced the bundling activity of *Tetrahymena* EF-1 $\alpha$  to some extent, although the EF-1 $\alpha$  seemed to be able to induce bundling of the F-actin within the range of physiological condition.

---

## INTRODUCTION

Translation elongation factor 1 $\alpha$  (EF-1 $\alpha$ ) catalyzes the GTP-dependent binding of aminoacyl-tRNA to the ribosome acceptor site in the peptide elongation phase of protein synthesis (Kaziro, 1978; Moldave, 1985). Several studies suggested that EF-1 $\alpha$  has the novel functions related to microtubules. In sea urchin, EF-1 $\alpha$  or EF-1 $\alpha$ -like protein associates with microtubules and is closely correlated to the nucleation of astral microtubules (Kuriyama *et al.*, 1990; Ohta *et al.*, 1990). On the other hand, in *Xenopus*, EF-1 $\alpha$  has the function of microtubule severing between interphase and mitosis (Shiina *et al.*, 1994). EF-1 $\alpha$  also participates in the regulation of another cytoskeletal protein, actin. An F-actin bundling protein ABP-50 from *Dictyostelium* (Demma *et al.*, 1990) has been identified as EF-1 $\alpha$  from cDNA sequencing data and detection of the activity of polypeptide chain elongation (Yang *et al.*, 1990). Upon addition of cAMP, a chemoattractant, EF-1 $\alpha$  becomes localized in the filopodia that are extended as a response to stimulation in *Dictyostelium* (Dharmawardhane *et al.*, 1991). Recent report indicated that pH regulates the F-actin binding properties of *Dictyostelium* EF-1 $\alpha$  (Edmonds *et al.*, 1995). In the previous paper (Kurasawa *et al.*, 1996), we demonstrated that *Tetrahymena* EF-1 $\alpha$  binds to *Tetrahymena* F-actin and induces F-actin bundles *in vitro*. The F-actin bundling activity was completely inhibited by Ca<sup>2+</sup>/calmodulin, whereas GTP/GDP had virtually no effect on the activity (Kurasawa *et al.*, 1996).

Actin is a highly conserved protein in eukaryotes, but *Tetrahymena* actin has some unusual properties. *Tetrahymena* actin lacks binding activities to phalloidin, muscle  $\alpha$ -actinin and tropomyosin, and fails to inhibit DNase I activity, although it shares essential properties with ubiquitous actins, such as K<sup>+</sup> or Mg<sup>2+</sup>-dependent polymerization into filaments, binding with muscle heavy meromyosin to form arrowheads, and slight but appreciable activation of myosin Mg<sup>2+</sup>-ATPase (Hirono *et al.*, 1989; Hirono *et al.*, 1990). From the analysis of amino acid sequences of several protozoan actin genes, six variable regions common to protozoan actins except for amoeba actins were found (Watanabe *et al.*, 1991). Hirono *et al.* (1992) suggested that unusual properties of *Tetrahymena* actin are mainly due to the presence of such variable regions. On the other hand, an actin-regulatory protein, profilin, from *Tetrahymena* is shown to have a potent inhibitory activity for *Tetrahymena* actin polymerization, but have only a slight inhibitory activity for the skeletal muscle actin polymerization (Edamatsu *et al.*, 1990). Namely, not only *Tetrahymena* actin but also *Tetrahymena* actin-binding protein(s) appear to be greatly diverged during evolution. These facts lead us to ascertain whether or not *Tetrahymena* EF-1 $\alpha$  interacts with the skeletal muscle F-actin as well as *Tetrahymena* F-actin.

In this paper, we demonstrate that *Tetrahymena* EF-1 $\alpha$  binds to and bundles skeletal muscle F-actin as well as *Tetrahymena* F-actin, although *Tetrahymena* actin has a very divergent primary structure and some unusual properties as compared with skeletal muscle actin. We also demonstrate the stoichiometry of the binding of the EF-1 $\alpha$  to F-actin and the effects of both pH and ionic strength on F-actin bundling

---

\* To whom correspondence should be addressed.

using skeletal muscle F-actin.

## MATERIALS AND METHODS

### Preparation of *Tetrahymena* EF-1 $\alpha$ and rabbit skeletal muscle actin

*Tetrahymena* EF-1 $\alpha$  was purified by the method described before (Takeda *et al.*, 1995). Rabbit skeletal muscle actin was purified according to the procedure of Spudich and Watt (1971).

### Gel electrophoresis

SDS-PAGE was performed on a 15% running gel with a 3% stacking gel according to Laemmli (1970). Gels were stained with Coomassie Brilliant Blue R-250.

### Protein concentration

Protein concentration was determined by the method of Bradford (1976) with bovine serum albumin as a standard.

### Co-sedimentation experiment

EF-1 $\alpha$  was dialyzed against MES buffer (10 mM MES, 0.75 mM 2-mercaptoethanol, 2 mM MgCl<sub>2</sub>, 5% glycerol, 0.01 mM N $\alpha$ -p-tosyl-L-lysine chloro-methyl ketone, 5  $\mu$ g/ml leupeptin, and 0.2 mM phenylmethylsulfonyl fluoride, indicated amount of KCl and indicated pH in each figure). Rabbit F-actin was dialyzed against the MES buffer containing 100 mM KCl and 0.5 mM ATP. EF-1 $\alpha$  and F-actin were mixed in the MES buffer and incubated at 26°C for 20 min or 30 min, then ultracentrifuged at 250,000 $\times$ g for 30 min. The supernatant and pellet of the ultracentrifugation were analyzed by SDS-PAGE. The quantities of EF-1 $\alpha$  and actin in individual fractions were determined with a scanning densitometer Bio Image (Millipore Investment Holdings Limited).

### Negative staining electron microscopy

EF-1 $\alpha$  and F-actin were mixed and incubated as in the co-sedimentation experiment except for the incubation time (indicated in each figure). The mixture was mounted on a carbon-coated grid and negatively stained with 4% uranyl acetate. Grids were examined with a JEOL 100CXII electron microscope at an accelerating voltage of 80 kV.

## RESULTS AND DISCUSSION

### *Tetrahymena* EF-1 $\alpha$ binds to rabbit skeletal muscle F-actin and induces F-actin bundles

We examined the interactions between *Tetrahymena* EF-1 $\alpha$  and rabbit skeletal muscle F-actin by both co-sedimentation experiment and electron microscopy. Co-sedimentation experiment showed that *Tetrahymena* EF-1 $\alpha$  co-precipitated with skeletal muscle F-actin (Fig. 1A, lane 4), whereas the EF-1 $\alpha$  alone did not precipitate (Fig. 1A, lane 2). Electron microscopic observation indicated that the EF-1 $\alpha$  induced tightly packed bundles of the F-actin (Fig. 1B-b). These results indicate that *Tetrahymena* EF-1 $\alpha$  binds to skeletal muscle F-actin as well as *Tetrahymena* F-actin and induces the formation of F-actin bundles. This suggests that *Tetrahymena* EF-1 $\alpha$  binds to a conserved region common between *Tetrahymena* and skeletal muscle actins. Thus, it is possible to speculate that both actin-binding region of EF-1 $\alpha$  and EF-1 $\alpha$ -binding region of actin have been conserved during evolution as their essential properties.

The binding ratio of EF-1 $\alpha$  to skeletal muscle F-actin was examined by co-sedimentation experiment. As the increase of EF-1 $\alpha$ , the EF-1 $\alpha$  which co-precipitated with skeletal muscle F-actin increased (Fig. 2A). The binding of *Tetrahymena* EF-1 $\alpha$  to F-actin was saturated at about one EF-1 $\alpha$  molecule per one actin molecule in the filament (Fig. 2B). Scatchard analysis of the binding of EF-1 $\alpha$  to F-actin gave a dissociation constant (Kd) of 3.4  $\mu$ M with a molar ratio at saturation of 1.09 : 1 (Fig. 2C).

In the previous report (Kurasawa *et al.*, 1996), we have proposed EF-1 $\alpha$  dimer model for the regulation of F-actin bundling activity of EF-1 $\alpha$  by Ca<sup>2+</sup>/calmodulin. In this model, we presumed that EF-1 $\alpha$  had only one actin binding domain

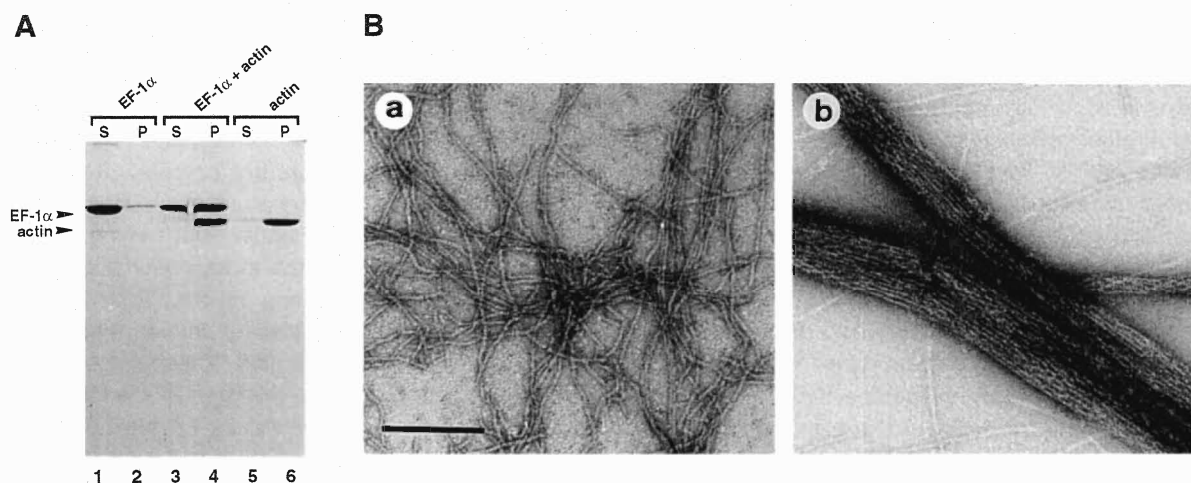
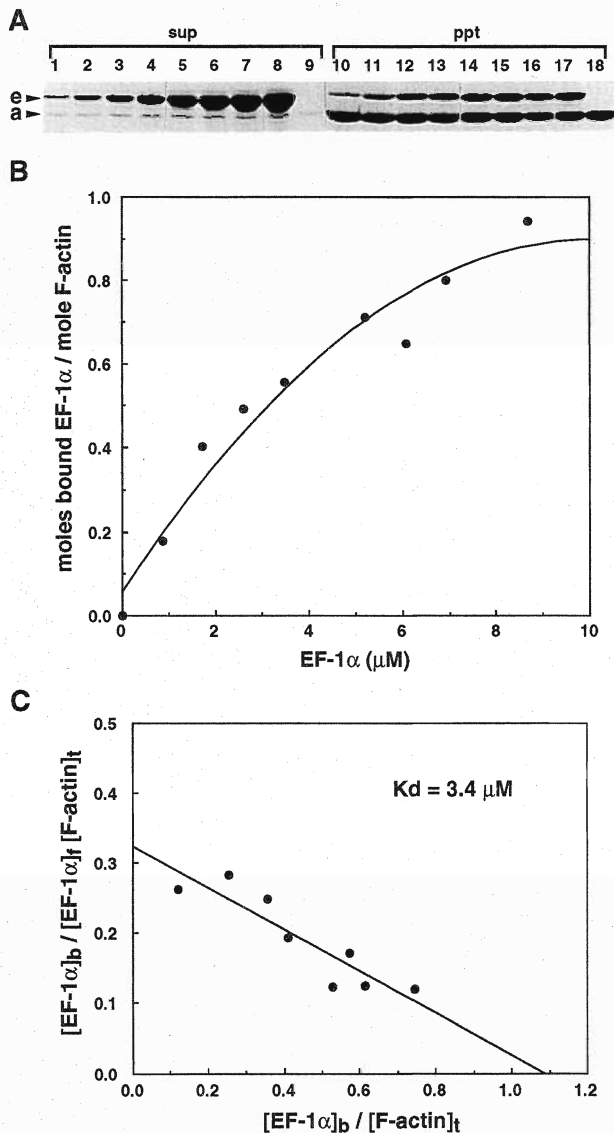


Fig. 1. Binding of *Tetrahymena* EF-1 $\alpha$  to skeletal muscle F-actin and the resultant bundle formation. (A) Two  $\mu$ M *Tetrahymena* EF-1 $\alpha$  and/or 2  $\mu$ M rabbit skeletal muscle F-actin in the MES buffer containing 40 mM KCl at pH 6.9 were incubated at 26°C for 30 min, then analyzed by co-sedimentation experiment. The supernatants (S) and pellets (P) of the ultracentrifugation were analyzed by SDS-PAGE. The samples containing EF-1 $\alpha$  alone (lanes 1 and 2), EF-1 $\alpha$  and F-actin (lanes 3 and 4), and F-actin alone (lanes 5 and 6) are indicated. Bands corresponding to EF-1 $\alpha$  and actin are shown. (B) Two  $\mu$ M F-actin alone (a) or a mixture of 2  $\mu$ M F-actin and 2  $\mu$ M EF-1 $\alpha$  (b) was incubated at 26°C for 20 min, then each of mixtures was analyzed by electron microscopy after negative staining. The bar represents 200 nm.



in a single molecule and anti-parallel dimer formation of EF-1 $\alpha$  was responsible for cross-linking the actin filaments, and that Ca<sup>2+</sup>/calmodulin inhibited such a dimer formation. This model expects 1 : 1 molar ratio of EF-1 $\alpha$  to actin. The molar ratio of EF-1 $\alpha$  to actin in the bundles measured in this study was about 1 : 1 and was consistent with the expected ratio in the dimer model. We are now investigating the possibility of the dimer forming activity of EF-1 $\alpha$  and its regulation by Ca<sup>2+</sup>/calmodulin.

#### *F-actin bundling activity of EF-1 $\alpha$ under several pH and ionic strength conditions*

By electron microscopy, we investigated the effects of pH and ionic strength on the F-actin bundling activity of *Tetrahymena* EF-1 $\alpha$  *in vitro*. Four electron micrographs indicate F-actin bundles formed by the EF-1 $\alpha$  at pH 5.7-7.5 (Fig. 3). When the F-actin and EF-1 $\alpha$  were incubated at pH 5.7 and pH 6.3, all actin filaments were included into bundles (Fig. 3A, B), while F-actin bundles except for a few single filaments were observed at pH 6.9 (Fig. 3C) and pH 7.5 (Fig. 3D). The ionic strength (0-120 mM KCl) has more striking effect on the F-actin bundling activity than pH. All actin filaments

Fig. 2. The binding ratio of EF-1 $\alpha$  to F-actin. (A) Increasing concentration of *Tetrahymena* EF-1 $\alpha$  and 3.3  $\mu$ M skeletal muscle F-actin in the MES buffer containing 40 mM KCl at pH 6.9 were incubated at 26°C for 20 min, then analyzed by co-sedimentation experiment. sup and ppt indicate the supernatant and pellet of each sample, respectively. The concentrations of EF-1 $\alpha$  incubated with F-actin were 0.87  $\mu$ M (lanes 1 and 10), 1.74  $\mu$ M (lanes 2 and 11), 2.61  $\mu$ M (lanes 3 and 12), 3.48  $\mu$ M (lanes 4 and 13), 5.22  $\mu$ M (lanes 5 and 14), 6.09  $\mu$ M (lanes 6 and 15), 6.96  $\mu$ M (lanes 7 and 16), 8.70  $\mu$ M (lanes 8 and 17), and 0  $\mu$ M (lanes 9 and 18). e, EF-1 $\alpha$ ; a, actin. (B) The binding curve of the data derived from Fig. 2A. (C) Scatchard analysis of the data derived from Fig. 2A. [EF-1 $\alpha$ ]<sub>b</sub>, the concentration of bound EF-1 $\alpha$ ; [EF-1 $\alpha$ ]<sub>f</sub>, the concentration of free EF-1 $\alpha$ ; [F-actin]<sub>t</sub>, the concentration of F-actin incubated with EF-1 $\alpha$ .

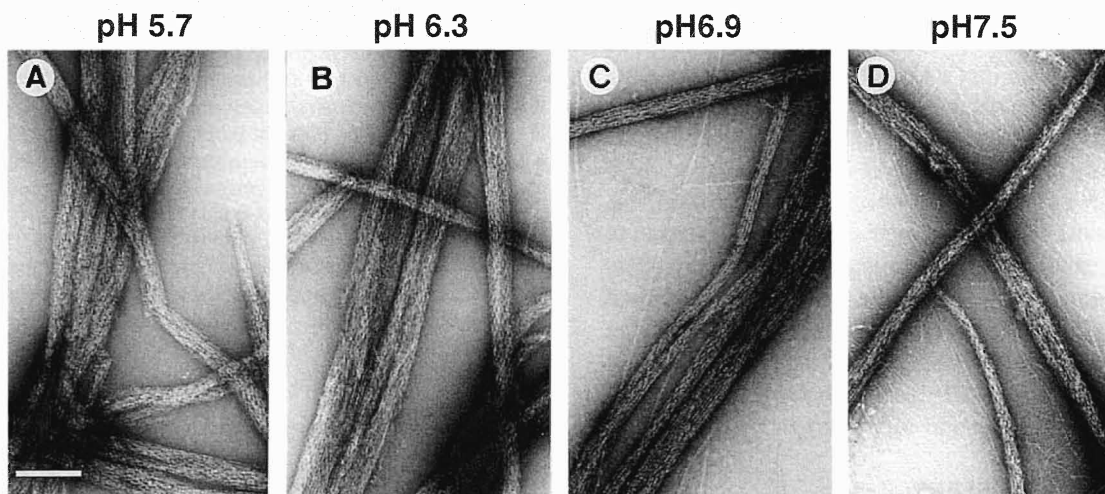


Fig. 3. The F-actin bundles formed by *Tetrahymena* EF-1 $\alpha$  at different pHs. Mixture of *Tetrahymena* EF-1 $\alpha$  and rabbit skeletal muscle F-actin in the MES buffer containing 40 mM KCl at pH 5.7 (A), pH 6.3 (B), pH 6.9 (C), or pH 7.5 (D) was incubated at 26°C for 10 min, and analyzed by electron microscopy after negative staining. The bar represents 200 nm.

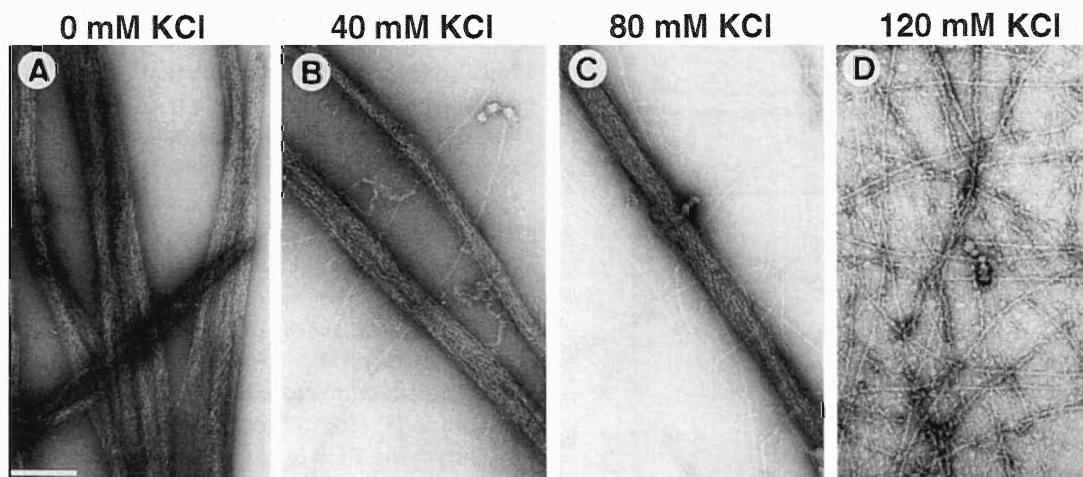


Fig. 4. The F-actin bundles formed by *Tetrahymena* EF-1 $\alpha$  in different KCl concentrations. Mixture of *Tetrahymena* EF-1 $\alpha$  and rabbit skeletal muscle F-actin in the MES buffer at pH 6.9 containing 0 mM (A), 40 mM (B), 80 mM (C), or 120 mM KCl (D) was incubated at 26°C for 10 min, and analyzed by electron microscopy after negative staining. The bar represents 200 nm.

were included into bundles under 0 mM KCl condition (Fig. 4A). As a increase of KCl concentration, F-actin bundles formed by EF-1 $\alpha$  decreased and single actin filaments increased (Fig. 4B, C, D). These results indicated that alkaline pH and high ionic strength reduced the F-actin bundling activity of *Tetrahymena* EF-1 $\alpha$ , although the EF-1 $\alpha$  seemed to be able to induce F-actin bundling within the range of physiological condition.

Edmonds *et al.* (1995) reported that the *in vitro* and *in vivo* association of *Dictyostelium* EF-1 $\alpha$  with F-actin was greatly affected by pH within the physiological range, and suggested that pH might be a potent modulator of actin organization in these cells. They also suggested that such a modulation might have important roles for the regulation of various phenomena in the *Dictyostelium* cell, because some phenomena including chemotaxis, protein synthesis, and determination of cell fate correlate with the intracellular pH. F-actin bundling activity of *Tetrahymena* EF-1 $\alpha$  also decreased in a high pH condition (pH 7.5), but such condition was not critical for the inhibition of bundling activity. On the other hand, high ionic strength (120 mM KCl) considerably inhibited the bundling activity of *Tetrahymena* EF-1 $\alpha$ . Because no rapid change of ionic strength in *Tetrahymena* cell is known, it is difficult to assume the ionic strength-dependent regulation of the bundling activity *in vivo*. Previously, we demonstrated that calmodulin clearly regulated the F-actin bundling activity of *Tetrahymena* EF-1 $\alpha$  in a Ca<sup>2+</sup> ion-dependent manner *in vitro* (Kurasawa *et al.*, 1996). We speculate that, in *Tetrahymena*, F-actin bundling activity of EF-1 $\alpha$  is mainly regulated by Ca<sup>2+</sup>/calmodulin rather than pH and ionic strength.

## REFERENCES

- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254
- Demma M, Warren V, Hock R, Dharmawardhane S, Condeelis J (1990) Isolation of an abundant 50,000-dalton actin filament bundling protein from *Dictyostelium* amoebae. *J Biol Chem* 265: 2286–2291
- Dharmawardhane S, Demma M, Yang F, Condeelis J (1991) Compartmentalization and actin binding properties of ABP-50: the elongation factor-1 alpha of *Dictyostelium*. *Cell Motil Cytoskeleton* 20: 279–288
- Edamatsu M, Hirono M, Watanabe Y (1990) Purification and characterization of *Tetrahymena* profilin. *Biochem Biophys Res Commun* 170: 957–962
- Edmonds BT, Murray J, Condeelis J (1995) pH regulation of the F-actin binding properties of *Dictyostelium* elongation factor 1 $\alpha$ . *J Biol Chem* 270: 15222–15230
- Hirono M, Kumagai Y, Numata O, Watanabe Y (1989) Purification of *Tetrahymena* actin reveals some unusual properties. *Proc Natl Acad Sci USA* 86: 75–79
- Hirono M, Tanaka R, Watanabe Y (1990) *Tetrahymena* actin: copolymerization with skeletal muscle actin and interactions with muscle actin-binding proteins. *J Biochem* 107: 32–36
- Hirono M, Sutoh K, Watanabe Y, Ohno T (1992) A chimeric actin carrying N-terminal portion of *Tetrahymena* actin does not bind to DNase I. *Biochem Biophys Res Commun* 184: 1511–1516
- Kaziro Y (1978) The role of guanosine 5'-triphosphate in polypeptide chain elongation. *Biochim Biophys Acta* 505: 95–127
- Kurasawa Y, Hanyu K, Watanabe Y, Numata O (1996) F-actin bundling activity of *Tetrahymena* elongation factor 1 $\alpha$  is regulated by Ca<sup>2+</sup>/calmodulin. *J Biochem* 119: 791–798
- Kuriyama R, Savereide P, Lefebvre P, Dasgupta S (1990) The predicted amino acid sequence of a centrosphere protein in dividing sea urchin eggs is similar to elongation factor (EF-1 $\alpha$ ). *J Cell Sci* 95: 231–236
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685
- Moldave K (1985) Eukaryotic protein synthesis. *Annu Rev Biochem* 54: 1109–1149
- Ohta K, Toriyama M, Miyazaki M, Murofushi H, Hosoda S, Endo S, Sakai H (1990) The mitotic apparatus-associated 51-kDa protein from sea urchin eggs is a GTP-binding protein and is immunologically related to yeast polypeptide elongation factor 1 $\alpha$ . *J Biol Chem* 265: 3240–3247

- Shiina N, Gotoh Y, Kubomura N, Iwamatsu A, Nishida E (1994) Microtubule severing by elongation factor 1 $\alpha$ . *Science* 266: 282–285
- Spudich JA, Watt S (1971) The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J Biol Chem* 246: 4866–4871
- Takeda T, Kurasawa Y, Watanabe Y, 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
- Watanabe Y, Hirono M, Takemasa T, Numata O (1991) Protozoan genes: as viewed from five *Tetrahymena* genes. *Japan J Protozool* 23: 1–11
- Yang F, Demma M, Warren V, Dharmawardhane S, Condeelis J (1990) Identification of an actin-binding protein from *Dictyostelium* as elongation factor 1 $\alpha$ . *Nature* 347: 494–496

(Received February 13, 1996 / Accepted March 19, 1996)