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# The 135-kDa actin-bundling protein from lily pollen tubes arranges F-actin into bundles with uniform polarity

**Etsuo Yokota, Teruo Shimmen**

Department of Life Science, Faculty of Science, Himeji Institute of Technology, Harima Science Park City, Hyogo 678-1297, Japan

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**Abstract.** A plant 135-kDa actin-bundling protein (P-135-ABP) isolated from pollen tubes of *Lilium longiflorum* (Thunb.) binds stoichiometrically to F-actin filaments and bundles them in vitro (E. Yokota et al., 1998, Plant Physiol. 116: 1421–1429). To further understand the mechanism of actin-filament bundle formation by P-135-ABP, the polarity of each F-actin filament in bundles was examined using myosin subfragment 1 (S-1). Dissociation of F-actin filaments from bundles organized by P-135-ABP was induced by S-1. However, F-actin filaments that remained in a bundle and decorated by S-1 showed uniform polarity. These results indicate that P-135-ABP arranges F-actin filaments into bundles with uniform polarity and consequently plays a key role in the orientation of cytoplasmic streaming in pollen tubes.

**Key words:** Actin – Actin bundling protein – *Lilium* (pollen tube) – Pollen tube

Actin-filament bundles formed by several kinds of actin-cross-linking proteins have important roles in various cell functions (Furukawa and Fechheimer 1997; Tilney et al. 1998). In plant cells, actin-filament bundles are observed widely and, in many cases, act as tracks for transport of organelles and vesicles (Pierson and Cresti 1992; Nagai 1993; Shimmen and Yokota 1994). Typical and well known examples are the actin cables within *Nitella* and *Chara* cells. About 100 actin filaments of uniform polarity (Palevitz et al. 1974; Kersey et al. 1976) are packed in a single actin cable (Nagai and Rebhun 1966). In cells of higher plants, such as root hair cells of *Hydrocharis* and stamen hair cells of *Tradescantia*, actin-filament bundles are involved not only in cytoplasmic streaming but also in maintaining and stabilizing the structure of transvacuolar strands (Shimmen et al. 1995; Staiger et al. 1994). However, in contrast to non-plant cells, much less is known about the actin-bundling proteins responsible for organizing actin-filament bundles in plant cells. Only a fimbrin-like polypeptide in *Arabidopsis* (McCurdy and Kim 1998), elongation factor 1α in carrot cells (Yang et al. 1993) and P-135-ABP in lily pollen tubes (Yokota et al. 1998) have been identified biochemically or molecular biologically in higher-plant cells. Purified P-135-ABP is composed of a 135-kDa polypeptide, binds stoichiometrically to F-actin filaments and bundles them in vitro (Yokota et al. 1998). These data together with those from immunofluorescence-microscopy studies have indicated that P-135-ABP is a factor responsible for bundling actin filaments in lily pollen tubes (Yokota et al. 1998). In the present study, we show that P-135-ABP arranges F-actin into bundles of uniform polarity in vitro.

The plant actin-bundling protein P-135-ABP was purified from germinating pollen of lily, *Lilium longiflorum* (Thunb.), according to the method described previously (Yokota et al. 1998). Purified P-135-ABP was suspended in a solution containing 90 mM KCl, 1 mM EGTA, 2 mM $MgCl_2$, 50 μg/ml leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM DTT and 30 mM Pipes-KOH (pH 7.0). Myosin subfragment 1 (S-1) was prepared from rabbit skeletal muscle by the method of Margossian and Lowey (1982). Muscle actin was purified from chicken breast muscle according to Kohama (1981).

To the solution of purified P-135-ABP, F-actin was added and the mixture allowed to stand for 10 min at 20 °C. The final protein concentrations of P-135-ABP and F-actin were 14.7 μg/ml and 20 μg/ml, respectively. The mixture was placed on a carbon-coated copper grid, and actin-filament bundles were allowed to adhere to the carbon film. After 30 s, the grid was laid on a drop of 0.4 mg/ml S-1 in a solution containing 0.1 M KCl, 1 mM DTT and 20 mM Pipes-KOH (pH 7.0). After 7–15 s, the grid was removed from the S-1 drop and then laid immediately on a drop of 2% uranyl acetate for negative staining. Samples were examined with an electron microscope (model JEM-1200 EXII, Jeol) operated at 80 kV.

Protein concentrations were determined by the method of Bradford (1976).

Abbreviations: P-135-ABP = plant 135-kDa actin-bundling protein; S-1 = myosin subfragment 1

*Correspondence to*: E. Yokota;
E-mail: yokota@sci.himeji-tech.ac.jp; Fax: 81(7915)80175

We had already shown that P-135-ABP binds to F-actin filaments and cross-links them in a tight co-parallel fashion (Yokota et al. 1998). To determine the polarity of F-actin in bundles formed by P-135-ABP, we attempted to decorate F-actin filaments with S-1. When F-actin bundles were incubated with S-1 in a test tube for longer than 1 min, the bundles disappeared, and only dispersed individual F-actin filaments decorated with S-1 were present on carbon film (data not shown). This result suggests that the interaction of S-1 with F-actin is stronger than that of P-135-ABP with F-actin, and as a result, S-1 disorganizes F-actin bundles that have been formed by P-135-ABP. However, F-actin bundles were present when the S-1 treatment was for a short period of time as described below.

Figure 1 shows an actin-filament bundle incubated with S-1 for 7 s. The edge of the bundle where F-actin filaments were not packed tightly was suitable for observing S-1-decorated F-actin. In this region, F-actin filaments showed a uniform polarity: the arrowhead-like pattern formed on most F-actin filaments decorated with S-1 points to the right side in Fig. 1B.

Figure 2 shows an actin-filament bundle incubated with S-1 for 15 s. Compared with the bundle incubated with S-1 for 7 s (Fig. 1A), the bundle was thin and the filaments were only loosely associated. However, most F-actin filaments were decorated by S-1 and had the same polarity.

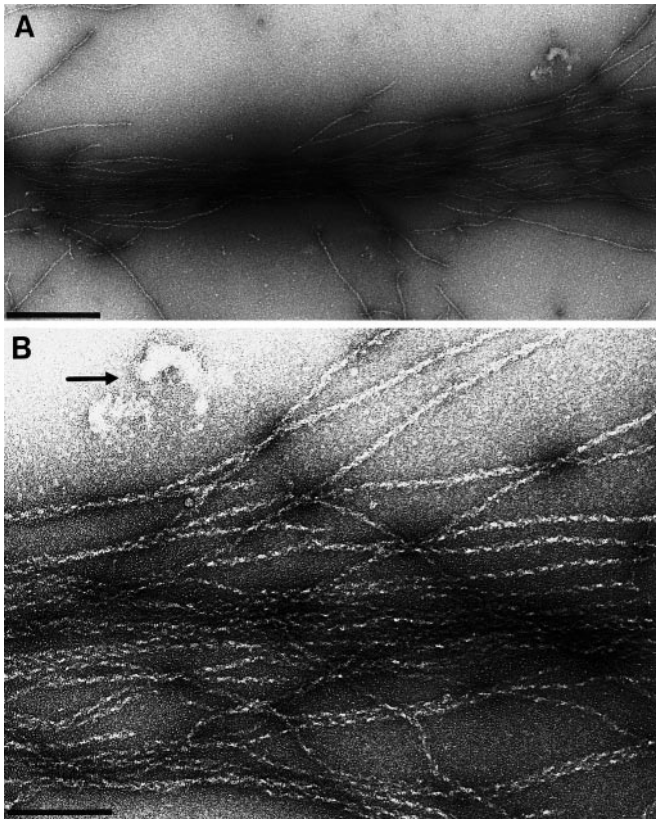


**Fig. 1A,B.** Negatively stained electron micrographs of an actin-filament bundle incubated with S-1 for 7 s. **A** Low magnification. **B** Higher magnification of an area (right side) of the bundle shown in **A**. *Arrow* in upper left-hand corner indicates orientation of S-1 arrowheads. Bars = 500 nm (**A**), 200 nm (**B**)

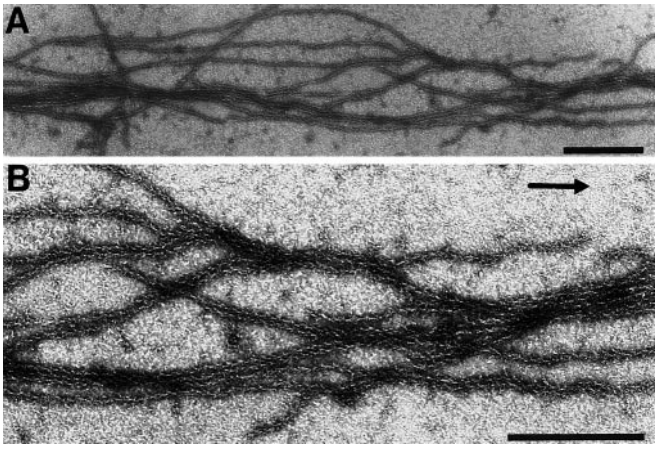


**Fig. 2A,B.** Negatively stained electron micrographs of an actin-filament bundle incubated with S-1 for 15 s. **A** Low magnification. **B** Higher magnification of an area (central region) of the bundle shown in **A**. *Arrow* in upper right-hand corner indicates orientation of S-1 arrowheads. Bars = 500 nm (**A**), 400 nm (**B**)

The present results clearly show that F-actin in a bundle formed by P-135-ABP has a uniform polarity. Except for actin cables in *Chara* and *Nitella* cells, little is known about the polarity of actin filaments in various bundles in plant cells. It has now been shown that P-135-ABP is generally distributed in the vegetative cells of higher plants as well as in pollen tubes and co-localizes with actin-filament bundles in root hair cells of *Hydrocharis* (Tominaga et al. 1999). These observations, together with the present results suggest that P-135-ABP arranges actin filaments into bundles with a uniform polarity and consequently determines the direction of cytoplasmic streaming, e.g. reverse fountain streaming in pollen tubes and root hair cells.

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