

## Inhibition by Drebrin of the Actin-Bundling Activity of Brain Fascin, a Protein Localized in Filopodia of Growth Cones

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**Abstract:** The purification of drebrin, an actin-binding protein that is specifically expressed in embryonic rat brain, was described previously. During the purification of drebrin, we found that an actin-binding protein of 54 kDa was also expressed at high levels in embryonic brain, and this protein was identified by immunoblotting as fascin. To explore the roles of fascin in brain development, we purified fascin from brains of infant rats and characterized it. We found that the actin-binding activity of fascin was strongly inhibited by drebrin. Fascin caused formation of actin bundles, a process that was inhibited in the presence of drebrin, as confirmed by electron microscopy and a low-speed centrifugation assay. In PC12 cells, fascin was localized in the filopodia of growth cones, whereas drebrin was localized in the basal region of growth cones. Our results suggest that fascin might play an important role in the organization of actin in filopodia and that this organization might be regulated by drebrin.  
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The motility of growth cones plays an essential role in the outgrowth of dendrites and axons from nerve cells. The tip of each growth cone contains highly dynamic bundles of actin known as filopodia, and it has been suggested that the filopodia regulate the motility of the growth cones (Smith, 1994; Zheng et al., 1994). Disruption of filopodia by cytochalasin causes the retraction of growth cones in cultured cells (Yamada et al., 1970; Marsh and Letourneau, 1984; Forscher and Smith, 1988; Fan et al., 1993; Neely and Gesemann, 1994). Moreover, treatment of neurons with cytochalasin causes navigational errors in limb buds of the grasshopper (Bentley and Toroian-Raymond, 1986) and in brain of *Xenopus* (Chien et al., 1993). These results suggest that the actomyosin system might be responsible for the motility and pathfinding ability of growth cones. Details of the mechanisms that regulate the organization of actin during the outgrowth of neurites remain, however, unclear.

Fascin is an actin-bundling protein that was originally isolated from sea urchin eggs. It has an apparent molecular mass on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of 58 kDa (Kane, 1975, 1976). It binds to actin filaments with a stoichiometry of one molecule of fascin to four or five actin monomers and appears to have two actin-binding sites per molecule (Bryan and Kane, 1978). A cDNA for fascin has been cloned (Bryan et al., 1993), and the amino acid sequence deduced from the cDNA is homologous to that of the product of the *singed* gene of *Drosophila*, which is responsible for the development of bristles and is involved also in oogenesis (Cant et al., 1994). The deduced amino acid sequence of fascin was also found to be homologous in part to the amino acid sequence of a 55-kDa actin-bundling protein from HeLa cells (Yamashiro-Matsumura and Matsumura, 1985, 1986), suggesting that the 55-kDa protein from HeLa cells might be the human homologue of fascin. The cDNAs of the vertebrate homologue of fascin have also been cloned from human lymphocytes (Mosialos et al., 1994), human teratocarcinoma (Duh et al., 1994), *Xenopus* intermediate pituitary (Holthuis et al., 1994), and mouse brain (Edwards et al., 1995).

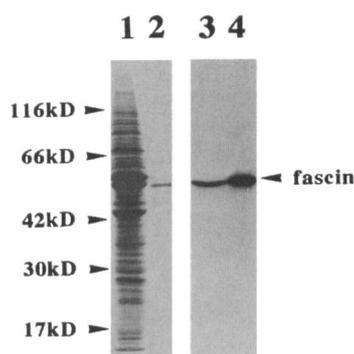
Drebrin was first identified in chicken brain, and its expression was shown to be closely related to the development of brain (Shirao and Obata, 1985). Transfection of fibroblasts with cDNA for drebrin induced dendrite-like structures, suggesting that drebrin might be related to the outgrowth of neurites (Shirao et al., 1992, 1994). We showed previously that drebrin has actin-binding activity and that it strongly inhibits the binding of tropomyosin and  $\alpha$ -actinin to actin (Ishikawa et al., 1994). We suggested that drebrin might regulate the organization of actin in the outgrowth of

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Abbreviation used: SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.



**FIG. 1.** Purification of brain fascin: a blotted membrane, after SDS-PAGE, stained with Coomassie Brilliant Blue (lanes 1 and 2) and the corresponding immunoblot (lanes 3 and 4) stained with fascin-specific monoclonal antibody followed by horseradish peroxidase-conjugated anti-mouse antibodies. Color reaction was performed with the ECL system (Amersham). Lanes 1 and 3, crude extract of the brain of a 7-day-old rat; lanes 2 and 4, purified fascin from rat brain.

neurites by modifying the activities of other actin-binding proteins (for review, see Shirao, 1995).

In this report, we show that drebrin inhibits the actin-binding and actin-bundling activities of fascin *in vitro*. Furthermore, we demonstrate that fascin is localized in filopodia, whereas drebrin is located at the basal region of growth cones. These results are discussed in terms of the possible roles of fascin and drebrin in the organization of actin filaments during the outgrowth of neurites.

## MATERIALS AND METHODS

### Proteins

Fascin was purified from rat brains by the methods of Yamashiro-Matsumura and Matsumura (1985) with some modifications, as follows. Seven-day-old rats were killed, and their brains were dissected out, rinsed in phosphate-buffered saline, frozen, and stored in liquid nitrogen until use. Frozen brains were homogenized in a Teflon homogenizer in 2 volumes of extraction buffer that contained 100 mM NaCl, 1 mM MgCl<sub>2</sub>, 10 mM EGTA, 14 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml of leupeptin, and 20 mM Tris-HCl (pH 7.6). The homogenate was centrifuged at 100,000 g for 1 h, and the supernatant was used as the crude extract of brain (Fig. 1, lane 1). The crude extract was mixed with 24 μM skeletal muscle actin, incubated for 30 min on ice, and centrifuged again at 100,000 g for 1 h. The pellet was resuspended in and dialyzed against 5 mM NaHCO<sub>3</sub> plus 14 mM 2-mercaptoethanol with three changes of the dialysis solution over the course of 3 days. The dialysate was clarified by centrifugation at 14,000 g for 20 min and applied to a column (1.0 cm i.d. × 10 cm) of DEAE-Toyopearl 650S (Toyo Soda, Tokyo, Japan) that had been incorporated into an HPLC system (model 655A; Hitachi, Tokyo) and preequilibrated with buffer A that contained 14 mM 2-mercaptoethanol and 20 mM Tris-HCl (pH 7.6). The column was washed with 2 column volumes of buffer A and developed with a linear gradient of NaCl (0–500 mM) in buffer A. The fractions that contained fascin

were concentrated by ultrafiltration with a Centricon 30 apparatus (Amicon, Danvers, MA, U.S.A.). The concentrate was applied to a column (1 cm i.d. × 30 cm) of Superose 12HR (Pharmacia, Piscataway, NJ, U.S.A.) that had been equilibrated with buffer A plus 100 mM NaCl. The fractions containing fascin were concentrated by a Centricon 30 apparatus and used as purified fascin (Fig. 1, lane 2).

Drebrin was purified from brains of newborn rats as described previously (Ishikawa et al., 1994). Actin was purified from chicken skeletal muscle as described previously (Matsumura and Lin, 1982).

### Actin-binding assay

Fascin (0–3.7 μM) and drebrin (0–1.9 μM) at various concentrations were mixed with a 7.1 μM solution of actin filaments in 100 mM NaCl and 20 mM Tris-HCl (pH 7.6). After a 30-min incubation at room temperature, each reaction mixture was centrifuged in an LP42TI rotor (Beckman, Palo Alto, CA, U.S.A.) at 140,000 g for 40 min. Concentrations of proteins in supernatants and resuspended pellets were determined by SDS-PAGE and subsequent densitometry.

### Actin-bundling assay

Actin-bundling activity was measured by the following three methods.

(a) *Low-speed centrifugation assay.* Fascin (0–3.7 μM) and drebrin (0–1.9 μM) at various concentrations were incubated with a 7.1 μM solution of actin filaments in 100 mM NaCl plus 20 mM Tris-HCl (pH 7.6) for 30 min at room temperature, and the mixtures were then centrifuged at 8,000 g for 20 min. The amounts of actin in both supernatants and resuspended pellets were determined by SDS-PAGE and densitometry.

(b) *Fluorescence microscopy.* Actin filaments were labeled with rhodamine-phalloidin (Molecular Probes, Eugene, OR, U.S.A.) as previously described (Okagaki et al., 1991) and used as labeled actin. A 7.1 μM solution of actin filaments in which 1% of the actin was labeled was incubated with 1.4 μM fascin in the presence or absence of 1.1 μM drebrin for 30 min at room temperature. Solutions were then observed with a fluorescence microscope (Axioplan; Zeiss, Oberkochen, Germany) equipped with a ×100 oil lens (Plan-NEOFLUAR; Zeiss).

(c) *Electron microscopy.* A 7.1 μM solution of actin filaments was mixed with fascin at 1.4 μM in the presence or absence of 1.1 μM drebrin for 30 min at room temperature. Then the filaments were stained with 1% uranyl acetate and observed with an electron microscope (JEM-100C; Nihon Denshi, Tokyo) at a magnification of ×2,000 and ×10,000.

### Cell culture and immunofluorescence staining

PC12 cells were maintained in Dulbecco's modified Eagle's medium that contained 10% fetal bovine serum in an atmosphere of 5% CO<sub>2</sub> and 95% air at 37°C. PC12 cells were induced to differentiate by removal of fetal bovine serum from the medium and addition of 20 ng/ml of nerve growth factor (2.5S; Wako, Osaka, Japan).

Immunofluorescence staining was performed as described elsewhere (Yamashiro-Matsumura and Matsumura, 1986). Cells were fixed with absolute methanol for 5 min at –10°C, washed three times with phosphate-buffered saline, and permeabilized with absolute acetone (–10°C). For unknown reasons, such fixation in methanol was not suitable for staining with rhodamine-phalloidin. Therefore, we fixed the cells with a 3.7% solution of formaldehyde in phosphate-buffered saline instead of methanol for rhodamine-phalloidin staining.

### Other procedures

Protein concentrations were determined by the method of Bradford (1976), with bovine serum albumin as the standard. SDS-PAGE was performed as described by Blattler et al. (1972) on 12.5% polyacrylamide gels with the buffer system described by Laemmli (1970).

## RESULTS

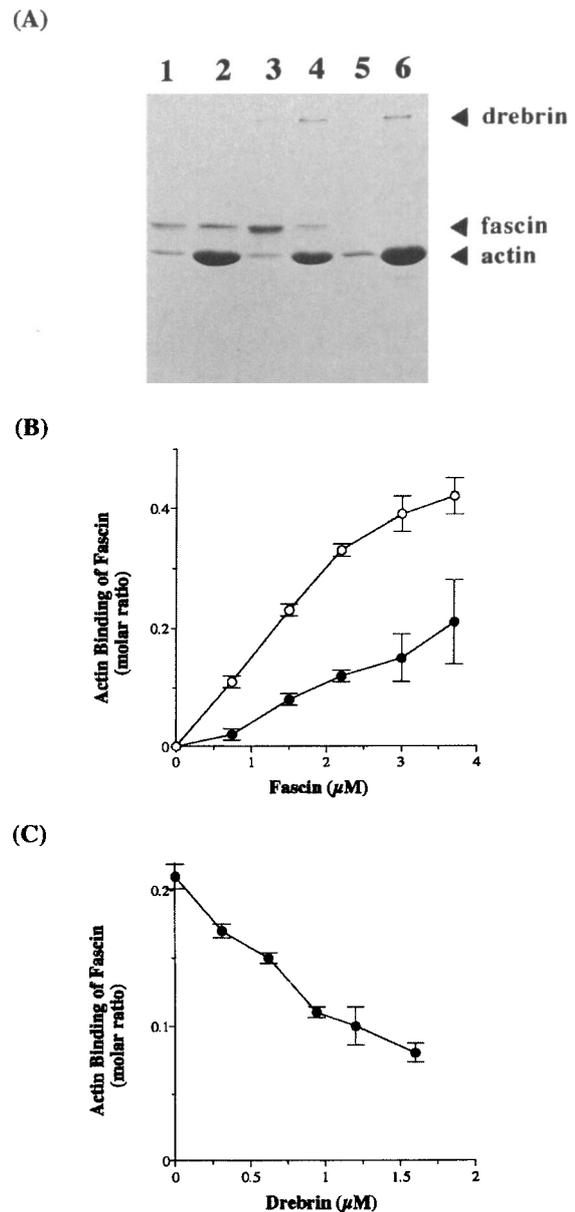
### Inhibition of the actin-binding activity of brain fascin by drebrin

Brains of 7-day-old rats were used as starting material for the purification of fascin because the amount of fascin in brains at this stage is relatively high (data not shown). As shown in Fig. 1 (lane 2), we purified the 54-kDa protein to homogeneity using a modified version of the method for purification of fascin from HeLa cells (Yamashiro-Matsumura and Matsumura, 1985). The protein reacted with monoclonal antibody raised against fascin from HeLa cells (Fig. 1, lane 4), indicating that it was brain fascin. About 0.2 mg of brain fascin was obtained from 20 g of brains.

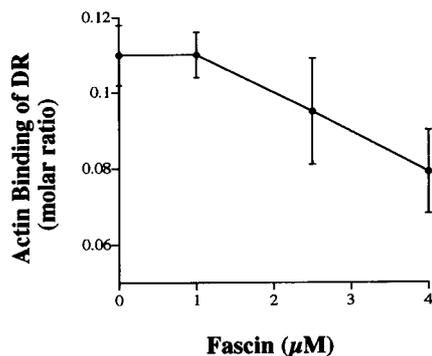
We examined the binding to actin filaments of brain fascin by high-speed centrifugation. As shown in Fig. 2B, brain fascin bound to actin filaments with a stoichiometry of one molecule of fascin to two or three molecules of actin. The stoichiometry of the binding of sea urchin fascin and of human fascin to actin was reported to be 1:4–5 (Bryan and Kane, 1978; Yamashiro-Matsumura and Matsumura, 1985). This discrepancy may be due to the differences in determination of protein concentration.

Next, we examined the effect of drebrin on the actin-binding activity of brain fascin because drebrin has been shown to modulate the actin-binding activities of other actin-binding proteins, such as tropomyosin and  $\alpha$ -actinin (Ishikawa et al., 1994). When we mixed fascin at 2.2  $\mu\text{M}$  with actin filaments, 56% of the added fascin coprecipitated with actin filaments (Fig. 2A, lane 2). This value dropped to 21% in the presence of 1.6  $\mu\text{M}$  drebrin (Fig. 2A, lane 4), indicating that drebrin inhibited the actin-binding activity of fascin. To confirm this result, we examined the binding of fascin to actin filaments at various concentration of fascin in the presence of 1.9  $\mu\text{M}$  drebrin (Fig. 2B). The amounts of precipitated fascin were lower than those in the absence of drebrin (Fig. 2B). At 3.7  $\mu\text{M}$  fascin, the actin binding of fascin was inhibited by 50% by drebrin at 1.9  $\mu\text{M}$ . We also examined the binding of fascin to actin filaments at various concentrations of drebrin (Fig. 2C). When we mixed actin filaments with brain fascin at 1.5  $\mu\text{M}$  in the absence of drebrin, fascin bound to actin filaments at a molar ratio of 0.21. This value gradually decreased to 0.08 as we increased the concentration of drebrin.

Do fascin and drebrin competitively bind to actin filaments? The binding of drebrin to actin filaments seems to be inhibited by fascin (compare lanes 3 and 5 in Fig. 2A). To confirm this result, we examined



**FIG. 2.** The actin-binding activity of fascin is inhibited by drebrin. **A:** Fascin (2.2  $\mu\text{M}$ ) and drebrin (1.6  $\mu\text{M}$ ) were mixed with a 7.1  $\mu\text{M}$  solution of actin filaments for 30 min at room temperature, and then the mixture was centrifuged at 140,000  $g$  for 40 min. Both supernatants and pellets were examined by SDS-PAGE. Lanes 1 and 2, supernatant and pellet, respectively, observed in the presence of fascin; lanes 3 and 4, supernatant and pellet, respectively, in the presence of fascin and drebrin; and lanes 5 and 6, supernatant and pellet, respectively, in the presence of drebrin. **B:** Fascin at various concentrations (0–3.7  $\mu\text{M}$ ) and drebrin (1.9  $\mu\text{M}$ ) were mixed with a 7.1  $\mu\text{M}$  solution of actin filaments. Subsequent procedures were the same as those described in A. Amounts of proteins were determined by densitometry. Molar ratios of pelleted fascin to actin filaments are plotted against the concentration of fascin: in the absence (○) and in the presence (●) of drebrin. Data are mean  $\pm$  SD (bars) values ( $n = 3$ ). **C:** Fascin at 1.5  $\mu\text{M}$  and drebrin at various concentrations (0–1.6  $\mu\text{M}$ ) were mixed with a 7.1  $\mu\text{M}$  solution of actin filaments. Molar ratios of pelleted fascin to actin filaments are plotted against concentration of drebrin. Data are mean  $\pm$  SD (bars) values ( $n = 3$ ).



**FIG. 3.** Actin-binding activity of drebrin (DR) is inhibited by fascin. Fascin at various concentrations (0–4.0  $\mu\text{M}$ ) and DR (0.94  $\mu\text{M}$ ) were mixed with a 7.1  $\mu\text{M}$  solution of actin filaments. Subsequent procedures were the same as those described in the legend to Fig. 2. Amounts of proteins were determined by densitometry. Molar ratios of pelleted DR to actin filaments are plotted against the concentration of fascin.

the binding of drebrin at various concentrations of fascin (Fig. 3). When we mixed actin filaments with drebrin at 0.94  $\mu\text{M}$  in the absence of fascin, drebrin bound to actin filaments at a molar ratio of 0.11. This value gradually decreased to 0.079 as we increased the concentration of fascin. These results suggest that fascin and drebrin competitively bind to actin filaments.

#### Inhibition of the actin-bundling activity of brain fascin by drebrin

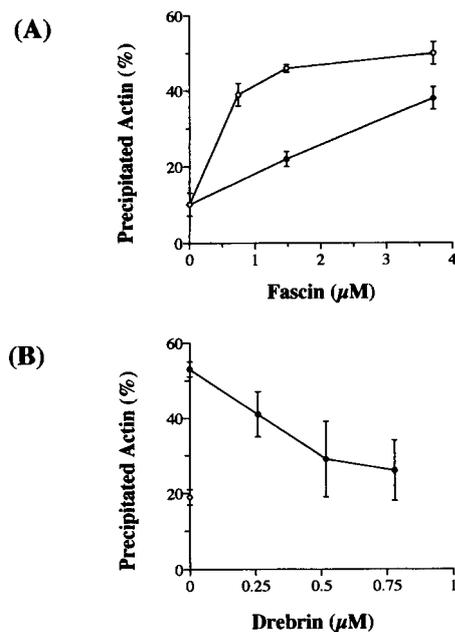
One of the most important functions of fascin seems to be its actin-bundling activity. Fascin seems to have two actin-binding sites per molecule, causing the formation of tight, compact bundles of actin (Bryan and Kane, 1978). We examined the effects of drebrin on the actin-bundling activity of fascin by a low-speed centrifugation assay. As shown in Fig. 4A, the amount of pelleted actin gradually increased as the amount of fascin was increased. About 50% of the actin in the assay was pelleted when fascin was added at 3.7  $\mu\text{M}$ , indicating that fascin has actin-bundling activity. In the presence of 1.9  $\mu\text{M}$  drebrin, this value decreased (Fig. 4A), suggesting that drebrin inhibited the actin-bundling activity of fascin. To confirm these results, we examined the amount of actin that was pelleted at a constant concentration of fascin in the presence of various concentrations of drebrin (Fig. 4B). In the presence of 1.5  $\mu\text{M}$  fascin, 53% of the actin was pelleted. This value gradually decreased as the amount of drebrin in the assay increased. At 0.8  $\mu\text{M}$ , drebrin almost completely eliminated the actin-bundling activity of fascin.

Next, we examined the actin bundles formed by fascin directly by fluorescence microscopy. When actin filaments labeled with rhodamine-phalloidin were observed under a fluorescence microscope, a brighter background but no bundles or filaments could be seen (data not shown). In the presence of fascin, we ob-

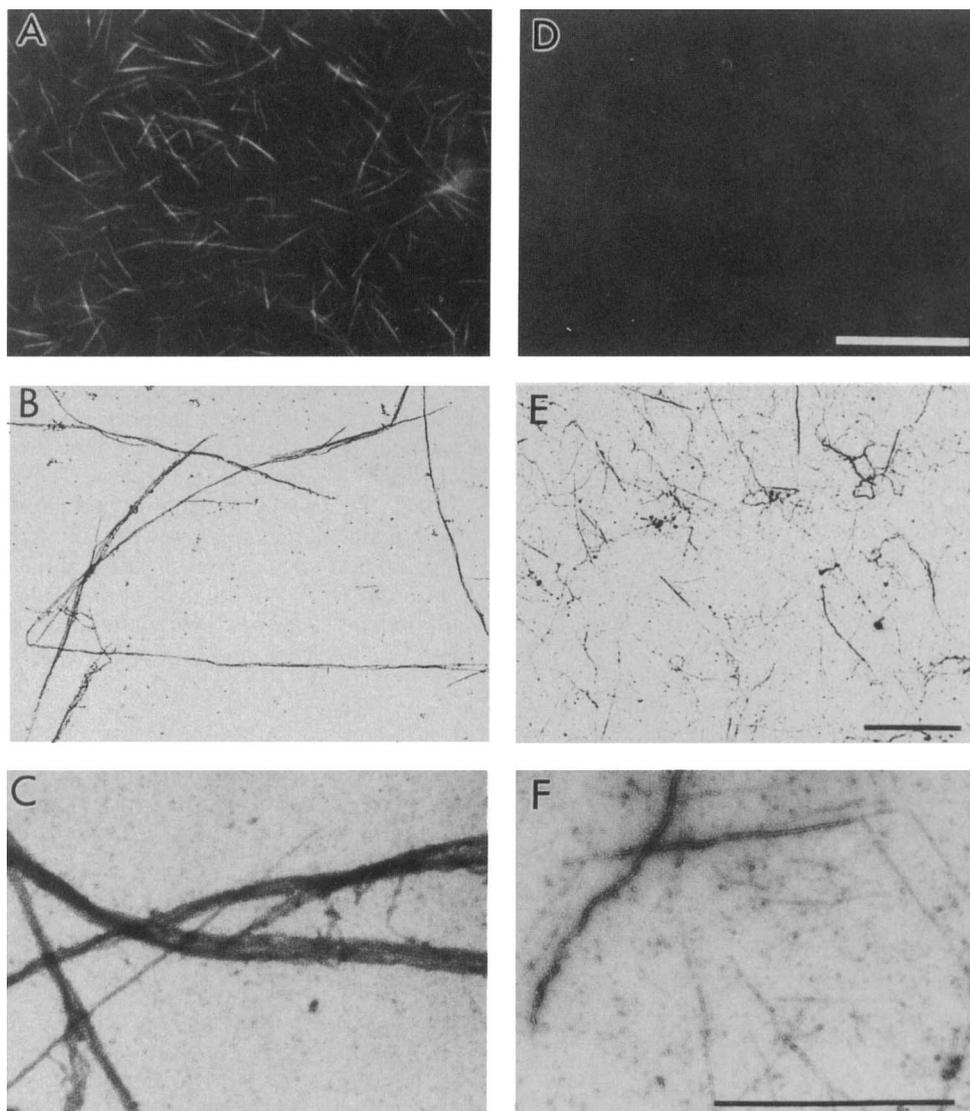
served bright bundles of actin, which seemed to be straight and uniform (Fig. 5A). In contrast, these bundles disappeared completely in the additional presence of drebrin (Fig. 5D), a result that was compatible with the results of the low-speed centrifugation assay. These results were confirmed by electron microscopy. In the absence of drebrin, fascin generated tight, compact bundles of actin (Fig. 5B and C). The diameters of the bundles seemed to be <0.2  $\mu\text{M}$ . These bundles became looser and then disappeared in the presence of drebrin (Fig. 5E and F).

#### Localization of fascin and drebrin in PC12 cells

We showed previously that drebrin was localized at the same sites as actin filaments in neuroblastoma cells (Asada et al., 1994) and in fibroblasts transfected with cDNA for drebrin (Ishikawa et al., 1994; Shirao et al., 1994). To determine whether drebrin was colocalized with actin filaments in growth cone, we examined the localization of drebrin in differentiated PC12 cells (Fig. 6). Bright bundles of actin could be seen in the filopodia of growth cones, in neurites, and in cell bodies (Fig. 6A, C, F, and I). Furthermore, patchy staining



**FIG. 4.** The actin-bundling activity of fascin is inhibited by drebrin. **A:** Fascin at various concentrations (0–3.7  $\mu\text{M}$ ) and drebrin (1.9  $\mu\text{M}$ ) were mixed with a 7.1  $\mu\text{M}$  solution of actin filaments for 30 min at room temperature, and then the mixture was centrifuged at 8,000 g for 20 min. Both supernatants and pellets were analyzed by SDS-PAGE, and amounts of pelleted actin were determined by densitometry and plotted against the concentration of fascin: in the absence (○) and in the presence (●) of drebrin. **B:** Fascin (1.5  $\mu\text{M}$ ) and a 7.1  $\mu\text{M}$  solution of actin filaments were mixed with drebrin at various concentrations (0–0.8  $\mu\text{M}$ ). Subsequent procedures were the same as described in A. Amounts of pelleted actin were plotted against the concentrations of drebrin: (○) indicates the amount of actin pelleted in the absence of fascin.



**FIG. 5.** Direct visualization of actin bundles generated by fascin in the absence (**A–C**) and in the presence (**D–F**) of drebrin. **A** and **D**: A  $7.1 \mu\text{M}$  solution of actin filaments labeled with rhodamine-phalloidin was incubated with  $1.4 \mu\text{M}$  fascin in the absence (**A**) or presence (**D**) of  $1.1 \mu\text{M}$  drebrin, and then actin filaments were observed by fluorescence microscopy. Bar =  $20 \mu\text{m}$ . **A** and **D** are the same magnifications. **B**, **C**, **E**, and **F**: A  $7.1 \mu\text{M}$  solution of actin filaments was incubated with  $1.4 \mu\text{M}$  fascin in the absence (**B** and **C**) or presence (**E** and **F**) of  $1.1 \mu\text{M}$  drebrin. Then filaments were fixed with 1% uranyl acetate and observed with an electron microscope. Bars =  $2 \mu\text{m}$ . **B** and **E** are the same magnifications as are **C** and **F**.

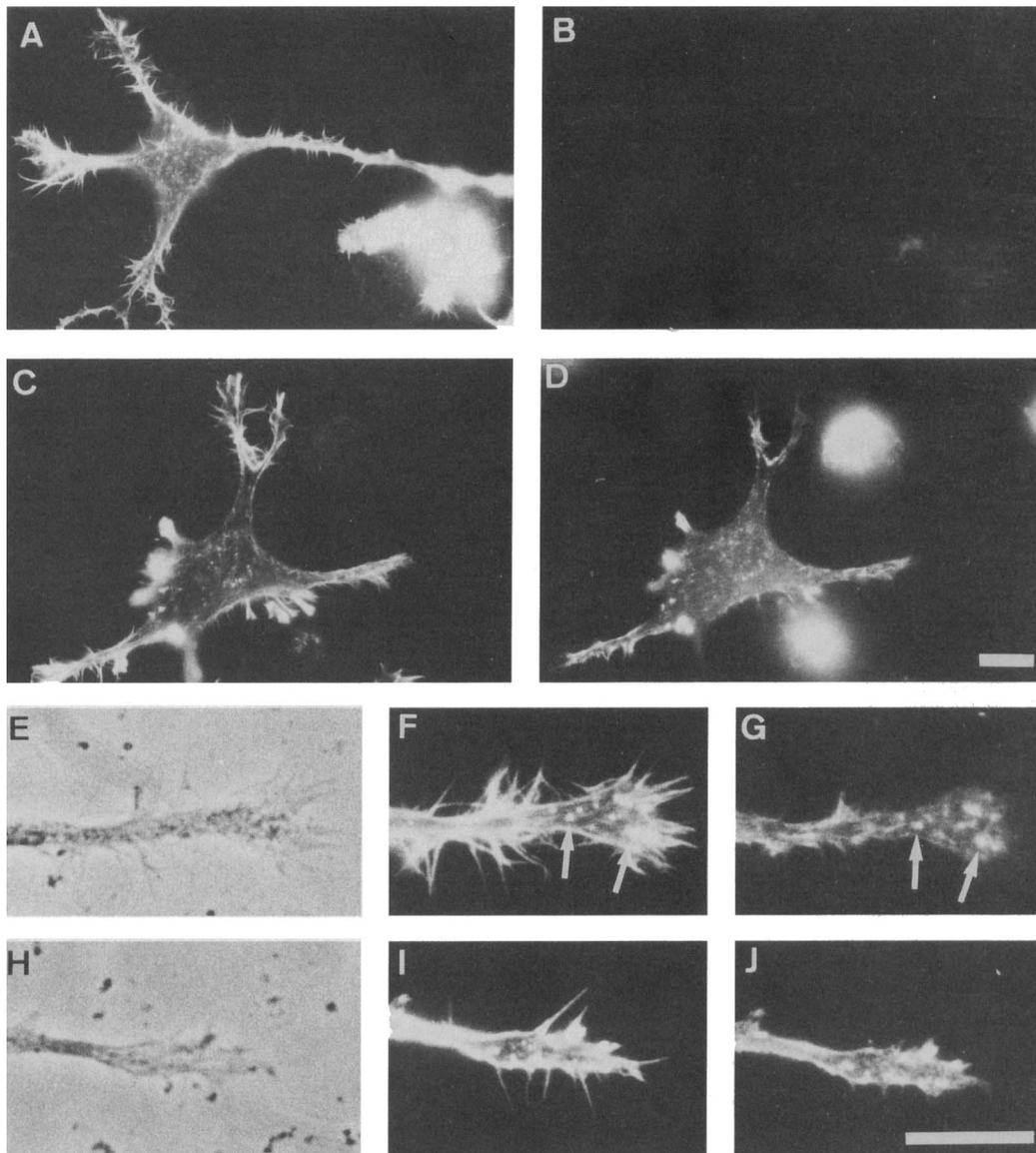
was observed in neurites and in the basal region of growth cones (arrows). Drebrin was colocalized with these patchy structures but was not seen in the filopodia (Fig. 6G and J).

Fascin has been found in the filopodia of coelomocytes (Otto et al., 1979) and fibroblasts (Yamashiro-Matsumura and Matsumura, 1986). As shown in Fig. 7C and F, fascin was localized in the filopodia of growth cones. By contrast, drebrin was not found in filopodia but was found in the basal region of growth cones (Fig. 7B and E). Thus, fascin and drebrin are differentially distributed in growth cones. Nonetheless, in the bottom regions of some filopodia, however, both

fascin and drebrin were found together (arrows in Fig. 7E and F).

## DISCUSSION

We purified a 54-kDa protein from the brains of infant rats. Four criteria allowed us to identify this protein as brain fascin: (a) its cross-reactivity with fascin-specific antibodies (Fig. 1), (b) the similarity of its molecular mass to that of fascin, (c) its actin-binding and actin-bundling activities (Figs. 2, 4, and 5), and (d) its pattern of elution from a DEAE column, which was similar to that of fascin.



**FIG. 6.** Distribution of drebrin in growth cones of PC12 cells. **A, C, F, and I:** Actin stained with rhodamine-phalloidin. **B:** Immunofluorescence staining with preimmune serum as a control. **D, G, and J:** Immunofluorescence staining with drebrin-specific polyclonal antibodies [RDE1 (Ishikawa et al., 1994)]. **E and H:** Phase images. Bars = 10  $\mu\text{m}$ . Note that drebrin is colocalized with actin in a patchy structure (arrows) but is not seen in the filopodia.

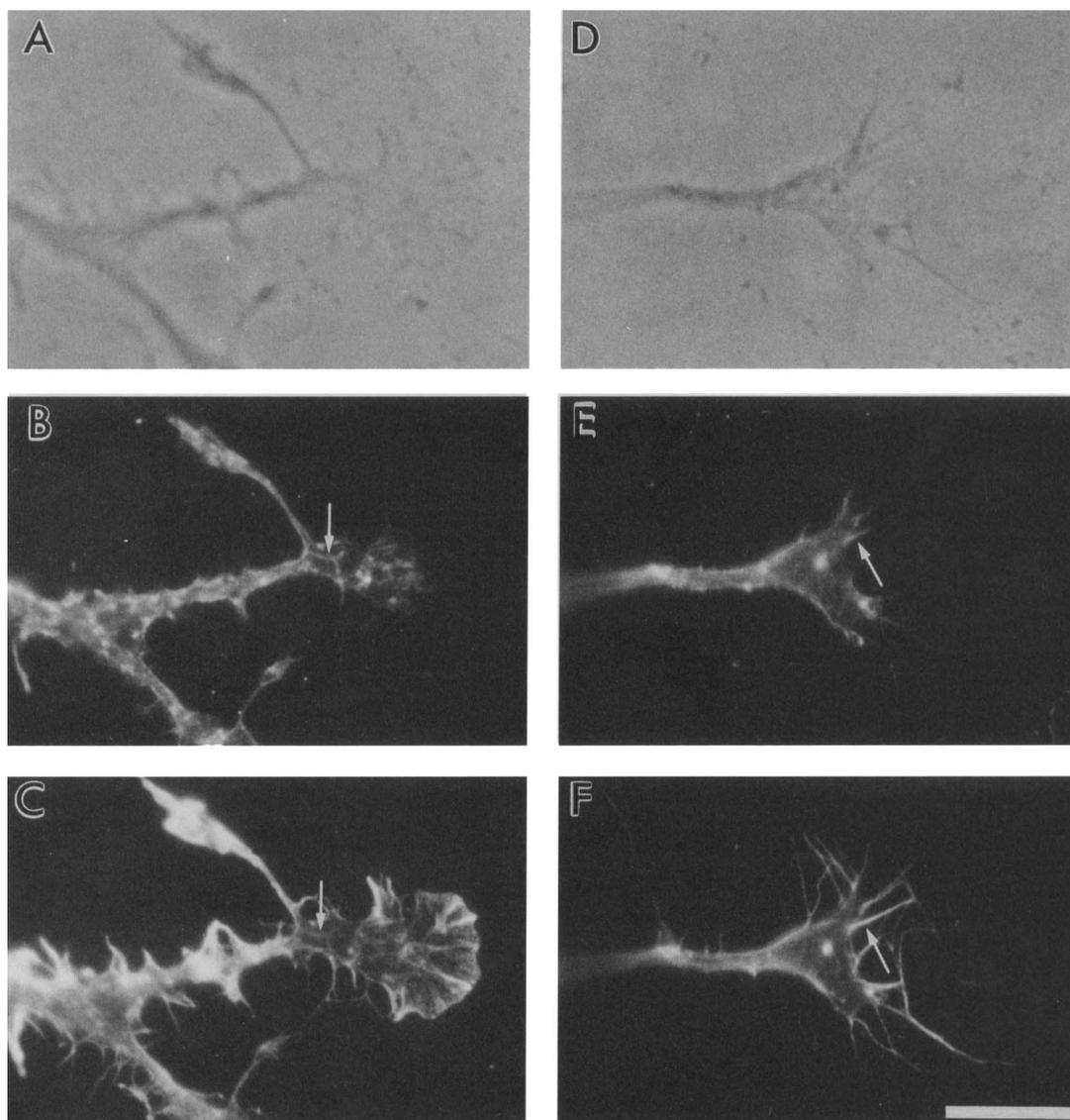
The modulatory effects of drebrin on the activities of fascin were examined, and we found that drebrin and fascin bound competitively to actin filaments (Figs. 2 and 3): The actin-bundling activity of fascin was strongly inhibited by drebrin (Figs. 4 and 5).

Two actin-binding proteins with molecular masses similar to that of fascin have been purified from brain: a 53-kDa protein from porcine brain (Maekawa et al., 1983) and a protein designated GP-55 from rat brain (Saborio et al., 1985). GP-55 seems to be different from fascin because it is concentrated in the Golgi apparatus and not in filopodia. By contrast, the bio-

chemical characteristics of the 53-kDa protein, including actin-bundling activity, pI, and competition for binding to actin with tropomyosin, are quite similar to those of fascin. It seems likely that the 53-kDa protein from porcine brain is fascin.

#### Regulation of cytoskeletal proteins in the brain during development

Many proteins that are specifically expressed in association with the outgrowth of neurites have been reported (for review, see Skene, 1989). Although the functions of most of these proteins are unclear, some proteins have been suggested to play important roles in



**FIG. 7.** Differential localization of fascin and drebrin in growth cones of PC12 cells. **A** and **D**: Phase-contrast images. **B** and **E**: Immunofluorescence staining with drebrin-specific polyclonal antibodies RDE1. **C** and **F**: Immunofluorescence staining with fascin-specific monoclonal antibody (Yamashiro-Matsumura and Matsumura, 1986). Bars = 10  $\mu\text{m}$ . Note that fascin and drebrin are found together in the basal regions of growth cones (arrows in B and C) and bottom regions of some filopodia (arrows in E and F).

the outgrowth of neurites. Two actin-binding proteins, namely, actin-depolymerizing factor (Bamburg and Bray, 1987) and drebrin (Shirao and Obata, 1985), are dominantly expressed at embryonic and neonatal stages, when the dendrites and axons of nerve cells are in the process of vigorous extension. In this report, we found that fascin is also expressed at high levels in a crude extract of embryonic brain (data not shown). As the actomyosin system regulates the motility of neurites (Yamada et al., 1970; Marsh and Letourneau, 1984; Forscher and Smith, 1988; Fan et al., 1993; Neely and Gesemann, 1994), these three actin-binding proteins might well play important roles in the organization of actin during the outgrowth of neurites.

#### Possible role of fascin in growth cones

In the growth cones of growing neurites, the filopodia are continuously extended and retracted (Bray and Chapman, 1985; Harris et al., 1987), and actin bundles are probably formed and destroyed again within a few minutes. It is widely accepted that the organization of actin is regulated by various kinds of actin-binding protein. Although many actin-binding proteins have been purified from brains, fascin (Fig. 7) and  $\alpha$ -actinin (Sobue and Kanda, 1989) are the only actin-bundling proteins that have been found in filopodia to date. The cross-linking distance in actin bundles generated by fascin is 11 nm (Bryan and Kane, 1978), whereas in bundles generated by  $\alpha$ -actinin it

is 36 nm (Meyer and Aebi, 1990). The space between actin filaments of filopodia is 13 nm (DeRosier and Edds, 1980), so it seems probable that fascin plays a central role in the bundling the actin filaments in filopodia.

How is the actin-bundling activity of fascin regulated? In this report, we demonstrated the inhibition by drebrin of the actin-bundling activity of fascin (Figs. 4 and 5). We also showed that, in some roots of filopodia, both drebrin and fascin can be found together (Fig. 7). At this point, we have no evidence that this region of a filopodium is about to disappear, but it is tempting to speculate that actin filaments become more loosely arranged on the binding of drebrin. Once bundles of actin filaments have been loosened, they might be easily attacked by actin-destabilizing proteins such as actin-depolymerizing factor, which has been reported in growth cones (Bamburg and Bray, 1987).

#### Possible role of drebrin in outgrowth of the neurites

What is the function of drebrin in the outgrowth of neurites? We showed previously that drebrin strongly inhibits the actin-binding activities of both tropomyosin and  $\alpha$ -actinin (Ishikawa et al., 1994). Tropomyosin and  $\alpha$ -actinin may stabilize actin filaments by protecting them from severance by gelsolin (Ishikawa et al., 1989a,b) and actin-depolymerizing factor (Bernstein and Bamburg, 1982). Therefore, we postulated that drebrin might destabilize actin filaments by freeing actin filaments of tropomyosin and  $\alpha$ -actinin, thereby maintaining the actin filaments in a dynamic state suitable for the outgrowth of neurites. As indicated in the previous paragraph, drebrin also destroys the actin bundles formed by fascin. Thus, drebrin might function as a disrupter of the organization of actin filaments in the extension of neurites. A mechanism for reconstruction of the disrupted bundles of actin filaments remains to be demonstrated.

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