

Chapter 3

Biochemistry of Drebrin and Its Binding to Actin Filaments

Ryoki Ishikawa

Abstract Drebrin is an actin-binding protein mainly expressed in developing neurons and dendritic spine in mature neurons. To understand the functions of drebrin in vivo, we must understand its molecular properties. In this chapter, I will focus on the purification and characterization of drebrin in vitro. Drebrin binds to F-actin with a stoichiometry of 1:5~6 with a K_d of $1\sim3 \times 10^{-7}$ M and strongly inhibits the binding of other actin-binding proteins such as tropomyosin, caldesmon, fascin, α -actinin, and cofilin. It also inhibits the activities of myosin-II and myosin-V. These results are discussed in terms of the possible roles of drebrin in the stability, dynamics, and organizations of actin structures in neuronal cells.

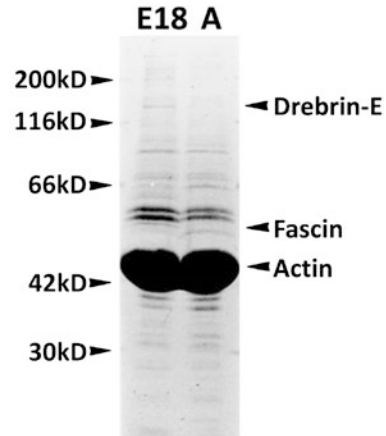
Keywords Actin • Myosin-II • Myosin-V • Tropomyosin • Caldesmon • Fascin • α -Actinin • Cofilin

3.1 Purification of Drebrin

In the early 1990s, we started to search for actin-binding proteins specifically expressed in the embryonic rat brain using the F-actin co-sedimentation method. The total cell extract of embryonic day 18 (E18) or adult rat brain was ultracentrifuged at $100,000 \times g$ for 1 h. Many structures composed of multi-molecules such as F-actin sediment under this centrifugal force. Exogenous F-actin was added to the supernatant, and the sample was ultracentrifuged again. Proteins co-sedimented with F-actin were analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue. As shown in Fig. 3.1, two bands with masses of approximately 140 and 55 kDa were obtained from E18 brain samples. Using immunoblotting, we identified the 55 kDa band as fascin, a filopodial actin-bundling protein, but we could not find a good candidate for the 140 kDa band, and so we purified the protein. We

R. Ishikawa
School of Nursing, Gunma Prefectural College of Health Science,
Maebashi, Gunma 371-0052, Japan
e-mail: ryoki@gchs.ac.jp

Fig. 3.1 Actin-binding fractions from rat brain. Brains were homogenized in 100 mM KCl, 1 mM MgCl₂, 5 mM EGTA, 5 mM DTT, and 20 mM Tris-Cl (pH 7.5) and centrifuged at 100,000 × *g* for 1 h. The supernatant was recovered, mixed with 24 μM chicken skeletal muscle F-actin for 30 min, then centrifuged again at 100,000 × *g* for 1 h. The sediment was analyzed by SDS-PAGE. E18, fraction from embryonic day 18 rat. A fraction from adult rat



found that this protein was heat stable: after 5 min of incubation in a boiling water bath followed by centrifugation, the protein was recovered in the supernatant. The protein was further purified by ammonium sulfate fractionation, DEAE column chromatography, and superose 6HR column chromatography. The purity of the 140 kDa band after passage through the superose 6HR column was more than 95% as judged by SDS-PAGE. Twenty grams of E18 rat brain yielded 0.05 mg of purified 140 kDa protein (Ishikawa et al. 1994). The protein was identified by partially digesting it with V8 protease in SDS-PAGE, blotting it onto a PVDF membrane, and cutting out and sequencing the resulting 30 kDa band. The partial amino acid sequence thus obtained, LSGHFENQKVMYGF, was identical to the amino acid sequence of rat drebrin (residues 54–67). Furthermore, antibody raised against chicken drebrin (M2F6; Shirao and Obata 1986) reacted with this protein. The precise position of the protein band on an SDS gel coincided with drebrin-E and not with drebrin-A. Thus, we concluded that the 140 kDa protein was drebrin-E.

We failed to purify drebrin-A from adult rat brain. As shown in Fig. 3.1, the drebrin-A band was not detected in adult rat brain extract when the SDS-PAGE gel was stained with Coomassie Brilliant Blue. Does drebrin-A lose the ability to bind to F-actin? When drebrin-A was detected by immunoblotting, centrifugation of the heat-stable fraction of adult rat brain at 140,000 × *g* resulted in the recovery of drebrin-A to the supernatant. In contrast, drebrin-A co-sedimented with F-actin under the same centrifugation conditions (Shirao et al. 1994), indicating that drebrin-A has actin binding activity following its extraction in soluble form. In the absence of exogenous F-actin, most of the drebrin-A in the sample sedimented after centrifugation at 100,000 × *g* for 1 h (Hayashi et al. 1996). It seems likely that only a small fraction of drebrin-A was extracted in the soluble form; therefore, purifying drebrin-A from brain tissue would require identifying conditions under which most drebrin-A would be extracted in the soluble form. Consequently, we decided to express drebrin in a bacterial expression system and purify it. We first tried using a normal expression system, pET19b (Novagen) as a vector and BL21(DE3) as a host,

but the expression levels of both drebrin-A and drebrin E were very low, and we failed to purify either protein. Because drebrin has a domain containing ten consecutive proline residues (residues 364–373 in drebrin-E and 410–419 in drebrin-A), it is probable that tRNAs recognizing proline codons may be a limiting factor. We therefore changed the host to BL21(DE3) codon plus RP that contained an extra copy of the poly *pro-L* gene. This gene encodes the tRNA that recognizes the proline codon CCC. Using this expression system, we obtained sufficient quantities of drebrin-A in the bacterial cell extract (Ishikawa et al. 2007). Other hosts such as Rossetta 2™ are also available for expression (Sharma et al. 2011). The protein was purified using the same methodology as used to purify drebrin from brain tissue. An 8 liter bacterial culture provided 2~3 mg of purified drebrin-A.

3.2 Actin-Binding and Actin-Bundling Properties of Drebrin

We reported that drebrin-E purified from brain tissue bound to F-actin with a stoichiometry of 1:5 (drebrin-E/actin) and an apparent dissociation constant (K_d) of 1.2×10^{-7} M (Ishikawa et al. 1994). Bacterially expressed drebrin-A also bound to F-actin with a stoichiometry of 1:5~6 with a K_d of 2.7×10^{-7} M (Ishikawa et al. 2007). The bacterially expressed N-terminal half of drebrin (residues 1–300, common to drebrin-E and drebrin-A) contains the actin-binding domain and showed similar affinity for F-actin, with a K_d of 2.0×10^{-7} M, although with a different stoichiometry of 1:3 (Grinstevich et al. 2010). Therefore, it seems likely that the F-actin-binding properties of drebrin-E and drebrin-A are the same or very similar. As discussed below, however, 6 nM of drebrin strongly inhibited the sliding velocity of F-actin on myosin-II (Hayashi et al. 1996), and 10 nM of drebrin-E strongly inhibited the binding of myosin-V to F-actin (Kubota et al. 2010). Furthermore, the addition of 10 nM tetramethylrhodamine-labeled drebrin-E to 23 nM F-actin fixed on a glass surface revealed that F-actin (23 nM) strongly and uniformly binds fluorescently labeled drebrin-E (Kubota et al. 2010). All these results suggest that the K_d of drebrin from F-actin is less than 10 nM. Our F-actin co-sedimentation assay was quantified by SDS-PAGE and subsequent Coomassie Brilliant Blue staining, followed by densitometry; however, it is difficult to detect nanomolar levels of protein using this method. Therefore, the true affinity of drebrin to F-actin might be much higher than that indicated by the F-actin co-sedimentation assay.

Does drebrin have actin-bundling activity? Drebrin-E purified from brain tissue did not exhibit any actin-bundling activity, as confirmed by electron microscopy and a low-speed centrifugation assay (Ishikawa et al. 1994). In contrast, some bacterially expressed drebrin-E and drebrin-A did exhibit actin-bundling activity. SDS-PAGE indicated that the drebrin fraction collected after DEAE column chromatography was more than 95% pure. Application of this fraction to a superose 6HR column provided two major peaks which both showed as a single drebrin band on SDS-PAGE. The first peak, which was collected in the void volume, had actin-bundling activity, while the second peak, which was eluted at 24~28 min at the flow

rate of 0.5 mL/min, did not have actin-bundling activity, as confirmed by a low-speed centrifugation assay (unpublished result). Because drebrin corresponding to the first peak did not sediment after centrifugation at $100,000 \times g$ for 40 min, it seems likely that drebrin forms oligomers but not aggregate. We do not currently know whether oligomer formation by drebrin is an artifact of being synthesized in a bacterial expression system. Therefore, the second peak has been used as “purified drebrin” in the experiments we have published to date.

Molecular dissection of drebrin revealed two actin-binding domains, and the fragment containing both domains had actin-bundling activity as confirmed by low-speed centrifugation and electron microscopy (Worth et al. 2013). Furthermore, the exposure of F-actin to drebrin reacted with cdk5, an enzyme that phosphorylates S142 in drebrin, or the S142D mutant of drebrin, resulted in the formation of tight bundles of F-actin, indicating that drebrin has actin-bundling activity under specific conditions (Worth et al. 2013; also see Sect. 3.3 for details).

3.3 Competitive Binding of Drebrin for F-actin with Other Actin-Binding Proteins

Tropomyosin is a rod-shaped protein consisting of two α -helical polypeptides that form a coiled coil structure. Tropomyosin binds to thin filaments in skeletal muscle cells and to stress fibers in smooth muscle and non-muscle cells and may stabilize actin structures by protecting F-actin from actin-destabilizing proteins such as gelsolin (Ishikawa et al. 1989a, b) and ADF/cofilin (Bernstein and Bamburg 1982). We found that drebrin strongly inhibited tropomyosin binding to F-actin, and this was confirmed by an F-actin co-sedimentation assay (Ishikawa et al. 1994). Similar concentrations of drebrin and tropomyosin (1.1 μM drebrin-E, 1.7 μM tropomyosin, and 7.1 μM actin) resulted in a 95% reduction in the amount of tropomyosin bound to F-actin, whereas drebrin-E binding to F-actin was only weakly inhibited by tropomyosin. Even a ten times higher concentration of tropomyosin (0.67 μM drebrin-E versus 7.6 μM tropomyosin at an actin concentration of 7.1 μM) reduced the amount of drebrin-E bound to F-actin by only 70%. These results may reflect the relatively higher affinity of drebrin for F-actin compared to the affinity of tropomyosin for F-actin. We concluded that drebrin and tropomyosin competitively bind to F-actin *in vitro*. Furthermore, transfection of drebrin-E into CHO-K1 cells caused the dissociation of tropomyosin from microfilaments (Ishikawa et al. 1994), consistent with the results obtained in *in vitro* experiments.

Caldesmon is a rod-shaped, calmodulin-binding protein expressed in smooth muscle and in some non-muscle cells such as neurons. Because tropomyosin and caldesmon mutually enhance F-actin binding (Ishikawa et al. 1989a, b, 1998), we also examined whether drebrin affects the actin-binding of caldesmon in the presence or absence of tropomyosin. As shown in Fig. 3.2a, the amount of caldesmon bound to F-actin gradually decreased as the concentration of drebrin-A increased (open circles). This inhibition was partially negated in the presence of tropomyosin

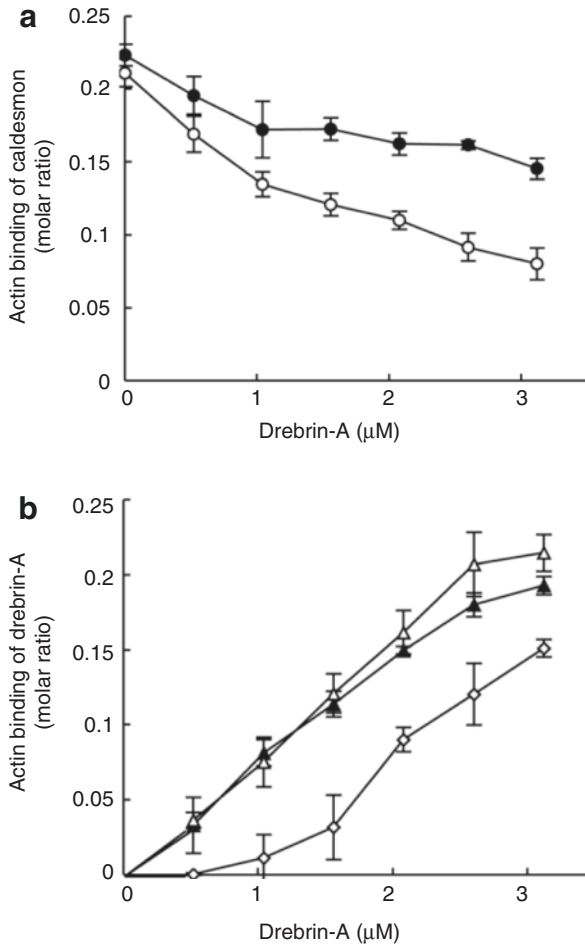


Fig. 3.2 Caldesmon and tropomyosin bind competitively with drebrin-A to F-actin. Different concentrations (0–3.1 μM) of drebrin-A were mixed with 7.1 μM F-actin in the presence of 2.3 μM smooth muscle caldesmon and/or 3.0 μM smooth muscle tropomyosin in 100 mM KCl and 20 mM Tris-Cl (pH 7.5) for 30 min, then centrifuged at $100,000 \times g$ for 40 min. The supernatant and the sediment of each sample were separated and analyzed by SDS-PAGE. The amounts of each protein were determined by densitometry. **(a)** Actin binding of caldesmon was plotted against drebrin-A concentration. Open circles, in the presence of 2.3 μM caldesmon, closed circles, in the presence of 2.3 μM caldesmon and 3.0 μM tropomyosin. **(b)** Actin binding of drebrin-A was plotted against drebrin-A concentration. Closed triangles, in the presence of 2.3 μM caldesmon, open triangles, in the presence of 3.0 μM tropomyosin, open diamonds, in the presence of 2.3 μM caldesmon and 3.0 μM tropomyosin

(closed circles). Like tropomyosin, however, caldesmon had little effect on actin binding by drebrin-A when similar amounts of drebrin-A and caldesmon were present (compare Fig. 3.2b, closed triangles, and Fig. 3.1 in Ishikawa et al. 2007), whereas the presence of tropomyosin and caldesmon together inhibited drebrin

binding to F-actin (Fig. 3.2b, open diamonds). These results could be explained by the following: (1) both tropomyosin and caldesmon compete with drebrin to bind to F-actin, (2) the affinity of tropomyosin or caldesmon alone for F-actin is too weak to inhibit drebrin binding to F-actin, and (3) the presence of tropomyosin and caldesmon together mutually stimulates their affinity for F-actin, and this enhanced affinity for F-actin is sufficient to inhibit drebrin binding to F-actin.

Fascin is a globular actin-binding protein with a molecular mass of 53–55 kDa that bundles F-actin tightly and localizes in microspikes and filopodia in cultured cells. As shown in Fig. 3.1, more fascin in the soluble fraction from E18 rat brain retained the ability to bind actin compared to fascin in the soluble fraction from adult brain. However, the concentration of fascin in the soluble fraction from E18 rat brains co-sedimented with F-actin was similar to that from the brains of 5-day-old rat, and brain weight of 5-day-old rat was much larger than that of E18 rat. We therefore purified brain fascin from the brains of 5-day-old rats and examined the effects of drebrin-E on the actin-binding and actin-bundling activity of fascin. An actin co-sedimentation assay revealed that actin binding of fascin was strongly inhibited by drebrin-E, while drebrin binding to F-actin was partially inhibited by fascin (Sasaki et al. 1996), suggesting that fascin and drebrin competitively bind to F-actin. We also found that the actin-bundling activity of fascin was inhibited by drebrin-E and confirmed this finding by a low-speed centrifugation assay and electron microscopy (Sasaki et al. 1996).

α -Actinin is a rod-shaped actin-cross-linking protein composed of two identical polypeptides aligned antiparallel to each other which localizes in the z-band in skeletal muscle, stress fibers, and adhesion plaques in non-muscle cells. We found that drebrin-E inhibited both the actin-binding and actin-cross-linking activity of α -actinin (Ishikawa et al. 1994).

Cofilin is an actin-binding protein with a molecular weight of ~19 kDa that has G-actin-binding activity, F-actin-binding activity, weak F-actin-severing activity, and F-actin-destabilizing activity. Drebrin and cofilin competitively bind to F-actin and inhibited the F-actin-severing activity of cofilin (Grinstevich and Reisler 2014).

3.4 Modulation of Myosin Activity by Drebrin

A variety of actin-binding proteins, such as tropomyosin (Yamaguchi et al. 1984), caldesmon (Ngai and Walsh 1984), and fodrin (Wagner 1984), were reported to modulate energy consumption through the actin-activated ATPase activity of myosin-II. We therefore examined whether drebrin also modulates myosin activity and found that the actin-activated ATPase activity of smooth muscle myosin-II (Hayashi et al. 1996) and skeletal muscle myosin-II (Fig. 3.3a) was inhibited by drebrin. An *in vitro* motility assay revealed that a low concentration (6 nM) of drebrin-E reduced the average sliding velocity of F-actin on smooth muscle myosin-II from 0.34 $\mu\text{m/s}$

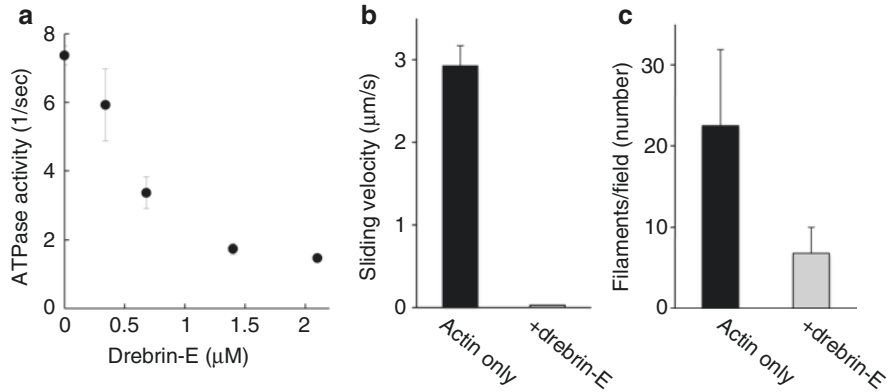


Fig. 3.3 Drebrin inhibits actin-activated ATPase activity (a), in vitro sliding on F-actin (b), and F-actin binding (c) of skeletal muscle myosin II. (a) 5.8 μM F-actin and different concentrations of drebrin-E were mixed in 25 mM KCl, 2.5 mM MgCl_2 , 20 mM DTT, and 20 mM Tris-Cl (pH 7.5) for 30 min, and then 0.11 μM skeletal muscle myosin II and 1 mM ATP were added. ATPase activities were determined by a NADH-coupling ATP-regeneration system. (b, c) 5.8 μM F-actin (a mixture of rhodamine-phalloidin-labeled F-actin: unlabeled F-actin = 1:500) was incubated with 1.4 μM drebrin-E for 30 min at room temperature, then perfused into a flow chamber coated with 0.11 μM skeletal muscle myosin II in 25 mM KCl, 2.5 mM MgCl_2 , 1 mM ATP, 20 mM DTT, and 20 mM Tris-Cl (pH 7.5) plus anti-bleach materials. Samples were observed with a total internal reflection fluorescence microscope. The average sliding velocity of the filaments (b) and the number of filaments attached to the glass surface 2–3 min after perfusion (c) were plotted

(without drebrin) to 0.10 $\mu\text{m/s}$ (Hayashi et al. 1996) and that saturation levels of drebrin-E (F-actin concentration versus drebrin-E concentration of 5.8 μM :1.4 μM) completely inhibited the sliding of F-actin on skeletal muscle myosin-II (Fig.3.3b). One possible interpretation of these results is that drebrin may interfere with the power producing step of myosin-II, thus reducing the sliding velocity. We also found that the number of F-actin filaments attached to the myosin-II-coated glass surface decreased in the presence of drebrin-E (Fig. 3.3c), indicating that drebrin inhibits myosin-II binding to F-actin. We conclude that drebrin may modulate myosin-II activity not only by inhibiting the power producing step of myosin-II but also by inhibiting myosin-II-binding to F-actin, thus reducing the energy consumption of myosin-II.

Myosin-V is a key unconventional myosin in mammalian brain and play roles in membrane traffic in nerve cells (Vale 2003). We found that drebrin-A inhibited the actin-activated ATPase activity of myosin-V (Ishikawa et al. 2007). The number of F-actin filaments attached to a glass surface decorated with myosin-V decreased in the presence of drebrin-A, but once they attached to the surface, the average sliding velocities of F-actin filaments remained unchanged in the presence or absence of drebrin-A (Ishikawa et al. 2007). These results were obtained by an in vitro motility assay, in which multi-myosin-V molecules simultaneously attached to a single F-actin filament. Unlike myosin-II, however, myosin-V is a processive motor, so the

movement of a single myosin-V molecule along F-actin filaments can be observed. The running velocities of a Q-dot-labeled myosin-V molecule on F-actin in the presence or absence of drebrin-E were the same, around $0.7 \mu\text{m/s}$ (Kubota et al. 2010), compatible with the results obtained by an *in vitro* motility assay. On the other hand, the average run-length of a single myosin-V “walk” decreased, from $\sim 0.8 \mu\text{m}$ in the absence of drebrin to $\sim 0.4 \mu\text{m}$ in the presence of 10 nM drebrin-E (Kubota et al. 2010). The binding frequency of myosin-V to F-actin was also decreased under these conditions (Fig. 3.2 of Kubota et al. 2010). How to evaluate these results?

Four states of single myosin-V head have been identified at different stages of the ATP hydrolysis cycle: ATP-bound form, ADP/Pi-bound form, ADP-bound form, and no-nucleotide form. Both the ATP-bound and ADP/Pi-bound forms are short-lived and have weak affinity for F-actin. The ADP-bound form is a rate-limiting step and is present for around 70% of the ATPase reaction. The no-nucleotide form is short-lived. The ATP-bound form and no-nucleotide form have strong binding affinity for F-actin. According to the hand over hand model, the leading head in the ADP/Pi-bound form binds to F-actin, while the trailing head in the ADP-bound form strongly attaches to F-actin (Fig. 3.4 step 2). Pi is released from the leading head, and the head binds strongly (Fig. 3.4 step 3). ADP is released from the trailing head (Fig. 3.4 step 4), then ATP binds to the trailing head, and the head detaches from F-actin (Fig. 3.4 step 5). The trailing head swings forward to become a leading head, while the former leading head binds tightly to remain in the same position, resulting in the myosin-V body moving one step forward (Fig. 3.4 step 6). During or after this process, ATP is dephosphorylated to ADP/Pi (Fig. 3.4 step 7). This cycle continues until the ATP binds to the trailing head before the leading head binds to F-actin. As long as one of the two feet strongly binds to F-actin, myosin-V can walk on F-actin, whereas when both feet are simultaneously weakly bound, myosin-V detaches from F-actin.

We postulate that drebrin inhibits step 2, namely, binding of the leading head to F-actin, but does not affect the other steps. If this is the case, the lifetime of the ADP/Pi-bound form should increase, but the speed of movement will be essentially unaffected because the rate-limiting step is not affected. Furthermore, because the average overlap time when both feet are tightly bound to F-actin becomes shorter, the probability that the trailing head detaches before the leading head binds increases, and so the run-length of myosin-V becomes shorter. Thus, the total run distance of myosin-V becomes shorter and the energy consumption (ATPase activity) decreases. Indeed, the amount of myosin-V that co-sedimented with F-actin decreased with increasing concentration of drebrin-E in the presence of ATP, whereas the amount of myosin-V co-sedimenting with F-actin remained unchanged in the presence of ADP (Kubota et al. 2010). This strongly suggests that binding of the ADP-bound form of myosin-V is not modulated by drebrin.

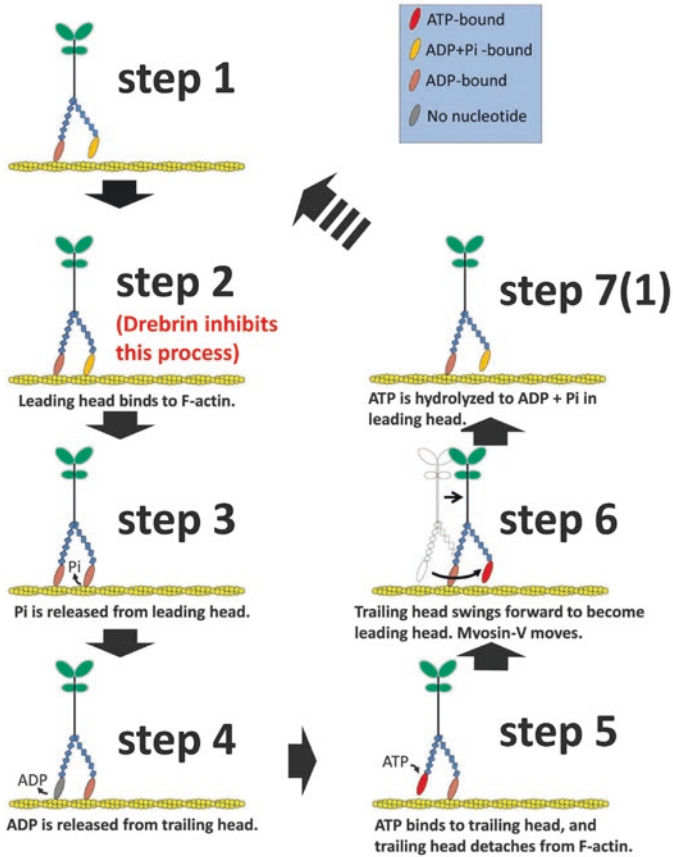


Fig. 3.4 Hand over hand motion of myosin-V

3.5 Conclusion

In this chapter, we showed that drebrin inhibits the actin-binding and actin-bundling activities of a wide variety of actin-binding proteins. Drebrin also inhibits the activity of myosin-II and myosin-V. Therefore, the expression of drebrin should cause drastic structural changes in actin organization.

In non-muscle cells such as neurons, a variety of actin-binding proteins differentially localize in and form specific actin structures such as stress fibers, filopodia, lamellipodia, and adhesion plaque. For example, drebrin-E concentrates in the basal region of filopodia, adhesion plaque, and actin arch, but not in the filopodia tip or lamellipodia in the growth cone of cultured neurons (Sasaki et al. 1996; Mizui et al. 2009). How does this “intracellular differentiation” in actin structures occur? We do not have a clear answer at this point, but the competitive binding between actin-

binding proteins to F-actin should affect intracellular differentiation because they cannot simultaneously occupy the same position on F-actin filaments. Tropomyosin (Yang et al. 1979), cofilin (Hawkins et al. 1993; Hayden et al. 1993), and drebrin (Sharma et al. 2012) are known to bind to F-actin cooperatively, resulting in the formation of clusters on F-actin filaments (Schmidt et al. 2015; Ngo et al. 2015; Sharma et al. 2012). It is tempting to speculate that the initial point of intracellular differentiation in actin structures may be the spontaneous binding of such cooperative actin-binding proteins to F-actin. If a single molecule of drebrin, tropomyosin, or cofilin binds to F-actin, a cluster of these proteins will form and grow as long as the expression level of drebrin, tropomyosin, or cofilin is adequate and the F-actin filaments are bare. Spontaneous binding of competitors should slow or stop the growth of the cluster or cause the cluster on F-actin to break down, depending on the protein's expression level and affinity for F-actin. Rearrangements such as severing, polymerization, depolymerization, annealing, or cross-linking may accelerate the assembly and compartmentation of specific actin-binding proteins to form specific actin structures. Therefore, drebrin may be an important factor for the intracellular differentiation of actin structures.

Does the expression of drebrin cause the actin structure to be static or dynamic? At least in part, drebrin may cause the actin structure to be more dynamic because drebrin inhibits the binding of actin-stabilizers such as tropomyosin and caldesmon, both of which protect F-actin from gelsolin severing (Ishikawa et al. 1989a, b), in contrast to drebrin which does not protect F-actin from gelsolin severing (Ishikawa et al. 1994). However, drebrin also inhibits the binding of actin destabilizers such as cofilin (Grinstevich and Reisler 2014) or the highly mobile actin-bundler fascin (Sasaki et al. 1996). Furthermore, the inhibition of myosin-II and myosin-V activities by drebrin decreases the sliding speed of myosin-II (Hayashi et al. 1996) and the run length of myosin-V (Ishikawa et al. 2007; Kubota et al. 2010), possibly resulting in decreased cell motility compared to the absence of drebrin. The binding of drebrin to F-actin may also increase the stability of F-actin (Mikati et al. 2013). Therefore, it seems likely that the overall effect of drebrin expression on actin structure may be to make the cells more static.

References

- Bernstein BW, Bamberg JR (1982) Tropomyosin binding to F-actin protects the F-actin from disassembly by brain actin-depolymerizing factor (ADF). *Cell Motil* 2:1–8
- Grinstevich EE, Reisler E (2014) Drebrin inhibits cofilin-induced severing of F-actin. *Cytoskeleton* 71:472–483
- Grinstevich EE, Galkin VE, Orlova A et al (2010) Mapping of drebrin binding site on F-actin. *J Mol Biol* 398:542–554
- Hawkins M, Pope B, Maciver SK et al (1993) Human actin depolymerizing factor mediates a pH-sensitive destruction of actin filaments. *Biochemistry* 32:9985–9993
- Hayashi K, Ishikawa R, Ye LH et al (1996) Modulatory role of drebrin on the cytoskeleton within dendritic spines in the rat cerebral cortex. *J Neurosci* 16:7161–7170

- Hayden SM, Miller PS, Brauweiler A et al (1993) Analysis of the interaction of actin depolymerizing factor with G- and F-actin. *Biochemistry* 32:9994–10004
- Ishikawa R, Yamashiro S, Matsumura F (1989a) Differential modulation of actin-severing activity of gelsolin by multiple isoforms of cultured rat cell tropomyosin: Potentiation of protective ability of tropomyosin by 83-kDa nonmuscle caldesmon. *J Biol Chem* 264:7490–7497
- Ishikawa R, Yamashiro S, Matsumura F (1989b) Annealing of gelsolin-severed actin fragments by tropomyosin in the presence of Ca^{2+} : potentiation of the annealing process by caldesmon. *J Biol Chem* 264:16764–16770
- Ishikawa R, Hayashi K, Shirao T et al (1994) Drebrin, a development-associated brain protein from rat embryo, causes the dissociation of tropomyosin from actin filaments. *J Biol Chem* 269:29928–29933
- Ishikawa R, Yamashiro S, Kohama K et al (1998) Regulation of actin binding and actin bundling activities of fascin by caldesmon coupled with tropomyosin. *J Biol Chem* 273:26991–26997
- Ishikawa R, Katoh K, Takahashi A et al (2007) Drebrin attenuates the interaction between actin and myosin-V. *Biochem Biophys Res Commun* 359:398–401
- Kubota H, Ishikawa R, Ohki T et al (2010) Modulation of the mechano-chemical properties of myosin V by drebrin-E. *Biochem Biophys Res Commun* 400:643–648
- Mikati MA, Grinstevich EE, Reisler E (2013) Drebrin-induced stabilization of actin filaments. *J Biol Chem* 288:19926–19938
- Mizui T, Kojima N, Yamazaki H et al (2009) Drebrin E is involved in the regulation of axonal growth through actin-myosin interactions. *J Neurochem* 109:611–622
- Ngai PK, Walsh MP (1984) Inhibition of smooth muscle actin-activated myosin Mg^{2+} -ATPase activity by caldesmon. *J Biol Chem* 259:13656–13659
- Ngo KX, Koder N, Katayama E et al (2015) Cofilin-induced unidirectional cooperative conformational changes in actin filaments revealed by high-speed atomic force microscopy. *elife* 4:e04806
- Sasaki Y, Hayashi K, Shirao T et al (1996) Inhibition by drebrin of the actin-bundling activity of brain fascin, a protein localized in filopodia of growth cones. *J Neurochem* 66:980–988
- Schmidt WM, Lehman W, Moore JR (2015) Direct observation of tropomyosin binding to actin filaments. *Cytoskeleton* 72:292–303
- Sharma S, Grinstevich EE, Phillips ML et al (2011) Atomic force microscopy reveals drebrin induced remodeling of F-actin with subnanometer resolution. *Nano Lett* 11:825–827
- Sharma S, Grinstevich EE, Hsueh C et al (2012) Molecular cooperativity of drebrin₁₋₃₀₀ binding and structural remodeling of F-actin. *Biophys J* 103:275–283
- Shirao T, Obata K (1986) Immunohistochemical homology of 3 developmentally regulated brain proteins and their developmental change in neuronal distribution. *Dev. Brain Res* 29:233–244
- Shirao T, Hayashi K, Ishikawa R et al (1994) Formation of thick, curving bundles of actin by drebrin A expressed in fibroblast. *Exp Cell Res* 215:145–153
- Vale RD (2003) The molecular motor toolbox for intracellular transport. *Cell* 112:467–480
- Wagner PD (1984) Calcium-sensitive modulation of the actomyosin ATPase by fodrin. *J Biol Chem* 259:6306–6310
- Worth DC, Daly C, Geraldo S et al (2013) Drebrin contains a cryptic F-actin-bundling activity regulated by Cdk5 phosphorylation. *J Cell Biol* 202:793–806
- Yamaguchi M, Ver A, Carlos A et al (1984) Modulation of the actin-activated adenosinetriphosphatase activity of myosin by tropomyosin from vascular and gizzard smooth muscles. *Biochemistry* 23:774–779
- Yang Y-Z, Korn ED, Eisenberg E (1979) Cooperative binding of tropomyosin to muscle and acanthamoeba actin. *J Biol Chem* 254:7137–7140