Drebrin-induced Stabilization of Actin Filaments*

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Background: Drebrin is a mammalian neuronal protein that binds to and organizes filamentous actin (F-actin) in dendritic spines.

Results: Drebrin protects actin filaments from depolymerization and rescues their formation in different cases of longitudinal and lateral contact perturbation.

Conclusion: Drebrin stabilizes actin filaments through its effect on their interstrand and intrastrand contacts.

Significance: We elucidate the mechanism by which drebrin governs F-actin dynamics in dendritic spines.

Drebrin is a mammalian neuronal protein that binds to and organizes filamentous actin (F-actin) in dendritic spines, the receptive regions of most excitatory synapses that play a crucial role in higher brain functions. Here, the structural effects of drebrin on F-actin were examined in solution. Depolymerization and differential scanning calorimetry assays show that F-actin is stabilized by the binding of drebrin. Drebrin inhibits depolymerization mainly at the barbed end of F-actin. Full-length drebrin and its C-terminal truncated constructs were used to clarify the domain requirements for these effects. The actin binding domain of drebrin decreases the intrastrand disulfide cross-linking of Cys-41 (in the DNase I binding loop) to Cys-374 (C-terminal) but increases the interstrand disulfide cross-linking of Cys-265 (hydrophobic loop) to Cys-374 in the yeast mutants Q41C and S265C, respectively. We also demonstrate, using solution biochemistry methods and EM, the rescue of filament formation by drebrin in different cases of longitudinal interprotomer contact perturbation: the T203C/C374S drebrin protomers, its shorter fragments, DrbABD3 (amino acids 233–300/317 containing the actin binding domain (ABD) (23)), Drb1–300 (amino acids 1–300 containing the ADF homology domain (ADF-H), ABD, and the helical charged motif), and Drb2–252 (amino acids 2–252 containing the ADF-H and helical charged motif and truncated after a predicted coiled-coil region). A schematic of the drebrin molecule is shown in Fig. 7.

The function of cells depends on the maintenance of their morphology, their motility, and the ability to adapt their shape in response to external stimuli. Dendritic spines are dynamic structures, and their shape, size and density have been shown to change during development and adulthood (1). During development, dendritic protrusions initiate out as filipodia, which mature into spines (2). These changes are associated with learning, aging, and diseases such as mental retardation (3, 4). Indeed, morphological studies of spines in dementia patients show a correlation between brain dysfunction and abnormal spine morphology (5, 6). This highlights the crucial need for studying the mechanisms of spine maintenance to understand higher brain functions such as memory and learning. F-actin is one of the major cytoskeletal components of spines (7–9) and is involved in organizing the dendritic pool of actin (1). Drebrin protein levels were found to be decreased in Alzheimer’s disease (14) and Down syndrome patients (15, 16). Furthermore, down-regulation of drebrin A expression in developing hippocampal neurons suppresses the accumulation of F-actin within dendritic spines (11). A natural interest then arises in the mechanism by which drebrin governs F-actin dynamics and structure in its role in spine plasticity.

Drebrin binds to F-actin with a stoichiometry of 5:1 (actin: drebrin protomers, Kd of ~0.12 μM) (17, 18) and competes with and inhibits the activity of F-actin-binding proteins such as α-actinin, tropomyosin, fascin, and myosin (17, 19, 20). In this study we employed drebrin A, a neuron-specific isoform that is expressed predominantly in the adult brain (12, 21, 22), and its shorter fragments, DrbABD3 (amino acids 233–300) containing the actin binding domain (ABD) (23), Drb1–300 (amino acids 1–300 containing the ADF homology domain (ADF-H), ABD, and the helical charged motif), and Drb2–252 (amino acids 2–252 containing the ADF-H and helical charged motif and truncated after a predicted coiled-coil region). A schematic of the drebrin molecule is shown in Fig. 7.

Recent atomic force microscopy analysis showed that binding of drebrin A to actin filaments increases their persistence length and helical pitch (~40 nm versus 36 nm for “bare” actin) (18), revealing its effect on the structure of F-actin. This stimulated a more detailed probing of the structural effects of drebrin on actin filaments to elucidate the mechanism by which

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§ The abbreviations used are: Drb, drebrin; ABD, actin-binding domain; ADF-H, actin depolymerization factor homology; CP, capping protein; Lat-A, latrunculin; DSC, differential scanning calorimetry; B-end, barbed-end; P-end, pointed end; GC, gelsolin-capped; TM, tropomyosin.
these two spine-resident proteins regulate spine plasticity and affect brain functions. Here we examine the overall effect of drebrin on F-actin stability and the intrastrand and interstrand contacts in the lateral and longitudinal interfaces of F-actin.

**EXPERIMENTAL PROCEDURES**

**Proteins**—Actin from rabbit back muscle was prepared as described by Spudich and Watt (24). Actin was kept on ice in G buffer (5.0 mM Tris, (pH 8.0), 0.2 mM CaCl₂, 0.2 mM ATP, 5 mM β-mercaptoethanol) and used within 2 weeks from its purification. Skeletal actin was labeled with pyrene-maleimide as described before (25). Pyrene-maleimide was obtained from Molecular Probes (Eugene, OR).

Grimeysin-cleaved G-actin was obtained as described previously (26). Ca-G-actin (2.0 mg/ml) was digested at an enzyme: protein mass ratio of 1:50 overnight on ice. Because actin cleaved with *Escherichia coli* protease (ECP) (between Gly-42 and Val-43) is resistant to further proteolysis by this protease, protease inhibitor was not added.

Yeast actin mutants were purified in the Ca-G-actin form by DNase I affinity chromatography (25). 25% sucrose was added to the DNase I column elution buffer for protein stability. DNase I was purchased from Bio-World. Yeast actin was stored in a Ca²⁺-G-buffer (5 mM Tris-HCl (pH 8.0) containing 0.2 mM CaCl₂, 0.2 mM ATP, and 1 mM DTT). Actin was further purified by passage over Sephacryl S-200 equilibrated in Ca-G buffer to eliminate residual actin oligomers. Full-length drebrin A, Drb-FL, was purified according to Sharma *et al.* (18), and the shorter drebrin constructs were purified according to Grintsevich *et al.* (23).

**Depolymerization Assays**—Actin depolymerization assays using Lat-A or by dilution were performed at 25 °C by adding 20 μM Lat-A to 2 μM actin labeled with pyrene maleimide (25% or 5% labeled) or by diluting actin to below its barbed-end Cc ([actin] = 0.08 μM) in 50 mM KCl, 2 mM MgCl₂, 5 mM β-mercaptoethanol, 0.2 mM ATP, 5 mM Tris (pH 8.0) in the presence or absence of saturating amounts of drebrin. Actin was first polymerized using KMH buffer (50 mM KCl, 2 mM MgCl₂, 1 mM DTT, 0.2 mM ATP, 10 mM Heps (pH 7.4)) for 1 h at 25 °C and then incubated at 2 μM with 10 μM heterodimeric capping protein (CP) for 10 min at 25 °C before initiating depolymerization. Gelsolin was purified using methods published previously (27). Gelsolin-actin seeds were formed by incubating 10 μM skeletal actin with 5 μM gelsolin overnight on ice. Latrunculin (Lat-A) was purchased from Sigma. Actin depolymerization was followed by a decrease in pyrene fluorescence, with the excitation wavelength set at 365 nm and the emission set at 407 nm, using an Alphascan fluorimeter (Photon Technology International). Rates were calculated by measuring the slope of the decay in fluorescence over the first 60 s of the reaction (ΔFluo/sec). Samples were then incubated overnight at 4 °C. The next day, their fluorescence intensity was recorded, they were spun at high speed (TLA100, 95,000 rpm, 4 °C, 20 min), and both the supernatants and pellets were loaded on an SDS-PAGE gel. The total change in fluorescence (Fmaxday1 – Fminday2) was measured, and it corresponded to the amount of actin in the supernatant (quantified from the SDS gel using ScionImage software). Subsequently, initial rates of depolymerization were converted to micromolar/second. Binding densities for Drb-FL in Fig. 1A were calculated on the basis of $K_d = 0.12 \, \mu M$ and a binding stoichiometry of 1:5 (DrbFL:actin). For Drb1–300, in Fig. 2C, binding densities report the amount of drebrin pelleted along with the remaining F-actin (Drb density = [Drb]pellet/ ([F]pellet/3) × 100%. The control experiments testing the efficiency of capping (for both CP and gelsolin) were performed as follows. 2 μM actin labeled with pyrene maleimide actin was polymerized by addition of 2 mM MgCl₂ and 50 mM KCl, and the change in fluorescence was followed. When a plateau was reached, 1 μM profilin-bound G-actin labeled with 5% pyrene maleimide (1:10 actin:profilin ratio) was added to detect any additional increase in fluorescence (indicating elongation from uncapped barbed ends because profilin actin is not incorporated into filaments from the pointed ends). No elongation was detected. The positive control was performed with uncapped filaments as follows. 2 μM actin labeled with 5% pyrene maleimide actin was polymerized by addition of 2 mM MgCl₂ and 50 mM KCl, and the change in fluorescence was followed. Once a plateau was reached, 1 μM profilin-bound G-actin labeled with 5% pyrene maleimide (1:5 actin:profilin ratio) was added to the mixture, and an increase in fluorescence was detected because of elongation at the barbed ends.

**Differential Scanning Calorimetry**—Differential scanning calorimetry (DSC) experiments were carried out in an N-DSC II calorimeter (CSC). Mg-ATP-G-actin in G buffer (2 mM Tris, 0.2 mM CaCl₂, 0.2 mM ATP, 0.1 mM tris-(2-carboxyethyl)phosphine (TCEP), 0.4 mM EGTA, 0.1 mM MgCl₂ (pH 8)) was polymerized with 2 mM MgCl₂ and 100 mM KCl. Final protein mixtures contained 10 mM Pipes, 1 mM Tris, 0.2 mM CaCl₂, 0.2 mM ATP, 0.1 mM tris-(2-carboxyethyl)phosphine, 2.0 mM MgCl₂, and 100 mM KCl (pH 7.5).

**Disulfide Cross-linking**—Prior to the cross-linking reactions, DTT (or βME) was removed from actin by passing it over a Sephadex G-50 column equilibrated with 5 mM HEPES, (pH 7.5), 0.2 mM CaCl₂, and 0.2 mM ATP. The eluted G-actin was polymerized for 30 min with 3.0 mM MgCl₂ at room temperature. DrbABD233–300 was passed through a Zeba-spin desalting column to exchange the buffer into 5 mM HEPES (pH 7.5), 0.2 mM CaCl₂, 0.2 mM ATP, and 3 mM MgCl₂. Disulfide cross-linking was catalyzed by addition of 4 μM CuSO₄ to actin at room temperature. Samples of the reaction were then taken at different time points (0–60 min), at which reactions were stopped with 10 mM N-ethylmaleimide. 10 μM BSA was added to the samples after the reaction was stopped with N-ethylmaleimide and before mixing the different aliquots with sample loading buffer and loading them on SDS electrophoresis gels. All samples were analyzed by SDS electrophoresis gels to determine the extent of cross-linking. Scion Image was used to quantify band intensities (I), which were normalized to the monomer band at time 0′ (I0) to generate decay plots (I/I0) using Sigma Plot software.

**Actin Polymerization**—Actin polymerization was assessed by following the increase in light scattering as a function of time with a PTI fluorometer set at 350 nm for the excitation and emission wavelengths. Polymerization of actin was induced by addition of 2 mM MgCl₂ and 50 mM KCl, except for the T203C/C374S mutant, which was polymerized by the addition of 3 mM MgCl₂.
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MgCl₂ alone because of its sensitivity to KCl. Actin polymerization data are offset to time zero. For experiments at 25 °C, the desired drebrin construct was mixed with Mg-G-actin in Mg-G buffer (10 mM Hepes (pH 7.5), 0.2 mM ATP, 4 mM EGTA, 0.1 mM MgCl₂, and 1 mM dithiothreitol). Temperature was regulated with a thermostated water bath and set at 25 °C unless stated otherwise.

Electron Microscopy—The undiluted samples from each experiment were applied to carbon-coated grids and stained with 1% uranyl acetate. The grids were examined in a JEM1200-EX electron microscope (JEOL) at an accelerating voltage of 80 keV and a nominal magnification of 80,000.

RESULTS

Drebrin Stabilizes Actin Filaments—Prior studies revealed a structural effect of drebrin on actin filaments. Here, full-length neuron-specific Drebrin-A (Drb-FL) and its N-terminal construct (sequence 1–300, Drb1–300) were employed to test their effect on actin depolymerization. On the basis of previous reports (23, 18), Drb1–300 binds to skeletal F-actin with an affinity close to that Drb-FL but with a lower binding stoichiometry (each Drb1–300 molecule binds at a ratio of 1:3 drebrin:actin protomer, whereas Drb-FL binds at a mole ratio of 1:5 drebrin:actin). This indicates that Drb1–300 represents the “actin-binding core” of drebrin, making it an important tool to study F-actin-drebrin interactions.

To probe for the stabilization of F-actin by drebrin, we examined filament depolymerization in pyrene assays in the presence of a G-actin-sequestering agent, Lat-A (Fig. 1). We tested the effect of increasing concentrations of Drb-FL on the depolymerization of uncapped actin filaments and filaments capped at the barbed end with heterodimeric CP. In uncapped filaments, barbed-end (B-end) depolymerization predominates because it is significantly faster than the pointed-end (P-end) one (28, 29). Drb-FL significantly decreased the depolymerization rates of uncapped filaments, reaching 88% inhibition at full saturation. Best fit of the data shows that 50% inhibition of depolymerization of uncapped F-actin is achieved at a low binding density of drebrin (~18%) (Fig. 1A).

In addition, high-speed cosedimentation revealed that, in the presence of both Lat-A and Drb-FL, actin filaments do not depolymerize fully (in contrast to the no-drebrin control), even after 18 h of incubation at 4 °C (Fig. 1B). The nature of this effect is not yet clear. To rule out Drb-FL-induced nucleation of Lat-A-bound G-actin, we performed control experiments in which 2 μM G-actin mixed with a 10-fold excess of Lat-A was left overnight in polymerizing conditions at 4 °C in the presence and absence of Drb-FL. Neither sample showed any pelleted actin when spun at high speeds the next day (data not shown), suggesting that Drb-FL did not allow nucleation of Lat-A bound G-actin.

Depolymerization at the P-end of filaments capped with CP was slow (compared with uncapped ones), and any further decrease in rate because of the protection by Drb-FL could have been too small to detect (Fig. 1A). The simplest way to assess more accurately the effect of drebrin on P-end depolymerization was to increase the observed rates of depolymerization by increasing the number of free P-ends. To this end, we employed another B-end capper, gelsolin. For this assay we polymerized actin filaments from gelsolin seeds (see “Experimental Procedures”). This allows controlling the number of P-ends because filaments will polymerize preferably from preformed gelsolin-actin seeds. As expected, at an actin:gelosin ratio of 130:1, the observed depolymerization from P-ends was faster than in CP-capped filaments. Using this assay, we detected the inhibition of P-end depolymerization by Drb-FL. The degree of inhibition was smaller than for uncapped filaments. Fig. 1C shows representative plots of total (from both barbed and pointed ends) pyrene-F-actin disassembly and gelsolin-capped (GC) F-actin (from P-ends) disassembly in the presence of Drb-FL. Drb-FL causes ~88% inhibition (Fig. 1C, upper panel, bar plot) of total depolymerization versus 48% inhibition of P-end depolymerization at the same binding density (90%) (Fig. 1C, lower panel, bar plot). As revealed by high-speed cosedimentation analysis, the binding of Drb-FL to F-actin at equilibrium was not affected by gelsolin capping (data not shown). Also, it is worth mentioning that elongation of CP- or gelsolin-capped F-actin from the profilin-G-actin complex was not detected in control experiments, confirming that drebrin does not displace either capping protein from the barbed end (data not shown). An additional indication of actin stabilization by drebrin was provided by DSC assays. The Tₘ of F-actin was increased by 0.5 °C in the presence of saturating amounts of Drb-FL (Fig. 1D).

Lat-A assisted F-actin depolymerization experiments were repeated with the shorter drebrin construct, Drb1–300, and depolymerization was again observed from uncapped filaments and GC-filaments. Based on the determined depolymerization rates the inhibition of F-actin depolymerization by Drb1–300 was ~40%, and it was similar for the gelsolin-capped (GC) and non-capped filaments at the same Drb1–300 binding density (~90%) (Fig. 2A&B). In addition, complete depolymerization of filaments was also inhibited in the presence of Drb1–300, as there was less actin found in the supernatant of F-actin solutions pelleted after overnight incubation with Lat-A at 4 °C (Fig. 2C).

We also performed depolymerization experiments in the presence of Drb-FL and Drb1–300 (5 and 11-fold excess over their Kₘ, respectively) using the standard dilution-induced method where F-actin is diluted below its Cc (80 nm). Both types of depolymerization assays (dilution-based and Lat-A assisted) yielded very similar results (data not shown). Overall, full-length drebrin showed a stronger F-actin stabilizing effect on uncapped filaments than the Drb1–300 construct at 90% binding density. The differences in the inhibition of depolymerization between Drb-FL and Drb1–300 were rather unexpected considering similar affinities of both constructs for F-actin (see “Discussion” for details).

In addition, equimolar amounts of Drb1–300 also increased the Tₘ of F-actin by 0.5 °C in DSC assays (Fig. 2D), which is consistent with the overall F-actin stabilization. The relatively small, but reproducible effect of drebrin on the melting temperature of F-actin can be attributed to the fact that Drb1–300 itself undergoes a thermal unfolding with a peak maximum at 59.7 °C, i.e. 10 °C below that of F-actin (data not shown). Considering the fact that there would be a small overlap between the Drb1–300 and F-actin melting peaks, it is possible that only
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A fraction of drebrin remains bound to the filaments under the conditions of our DSC experiments. Thus, it is likely that the stabilizing effect of drebrin is underestimated in this assay.

Effects of the Actin-binding Domain (DrbABD) of Drebrin on Longitudinal and Lateral Contacts in F-actin—Our previous study on the actin-binding domain of drebrin, DrbABD,
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FIGURE 2. Drebrin–300 inhibits F-actin depolymerization from both barbed and pointed ends and increases the thermal stability of F-actin. A, top panel, 2 μM 25% pyrenyl-labeled skeletal F-actin was depolymerized in the presence of Lat-A (20 μM) only (trace 1) or with LatA and 2 μM Drebrin–300 (trace 2). Bottom panel, gelsolin-capped 2 μM 5% pyrenyl-labeled skeletal F-actin depolymerized in the presence of Lat-A (20 μM) only (trace 1) or with Lat-A and 2 μM Drebrin–300 as well (trace 2). B, the bar plot shows the depolymerization inhibition percentage by 2 μM Drebrin–300 on uncapped F-actin (black bar) and on GC-F-actin (gray bar). C, concentrations of G-actin versus Drebrin–300 binding density after 2 μM F-actin was depolymerized by Lat-A (20 μM), alone or with different amounts of Drebrin–300 present, overnight at 4 °C. The inset shows the SDS-PAGE patterns of the high-speed centrifugation supernatants of F-actin ± Drebrin–300. A Drebrin–300 and an actin standards (Drebrin std and Actin std) at three different concentrations (1, 2, and 4 μM for both) were loaded for quantification purposes. D, DSC scans of 25 μM skeletal F-actin alone (thin trace) and in the presence of 2 μM Drebrin–300 (thick trace) and 8.3 μM Drebrin–300 (dashed trace). Similar results were reproduced over two independent experiments. Representative plots are shown. MHC, molecular heat capacity.
revealed that it bridged two actin protomers and was the strongest actin-interacting module within the drebrin molecule (23), which was in good agreement with the reported in vivo data (30). The fact that DrbABD bridges two adjacent actin protomers calls for assessing its effect on interprotomer contacts and its contribution to overall F-actin stabilization. We showed that both drebrin constructs act as the actin binding domain, DrbABD (residues 233–317) and DrbABD233–300 (residues 233–300), and have very similar binding affinities to F-actin (23). Conveniently, DrbABD233–300 does not contain any endogenous cysteines, which makes it easier to assess its effect on interprotomer contacts in F-actin via actin cross-linking studies. Copper-catalyzed cross-linking has been used previously to examine the longitudinal and lateral interfaces in F-actin, employing the double cysteine actin mutants Q41C and S265C, respectively (31). In these actins, a cysteine is introduced at either residue 41 or 265 while retaining the endogenous cysteine 374. Here we used a similar approach and monitored the protomer-protomer cross-linking reactions in these mutant actin filaments via SDS-PAGE, where actin monomer depletion because of F-actin cross-linking was monitored. Different gel patterns were obtained in the presence and absence of DrbABD233–300. DrbABD233–300 slowed the longitudinal cross-linking between actin residues Q41C (D-loop) and Cys-374 (C terminus) (Fig. 3A), whereas it accelerated the lateral cross-linking between the residues Cys-265 (hydrophobic plug) and Cys-374 (Fig. 3B). The inhibition or acceleration of disulfide formation in the presence of drebrin could be due to the change in the mean distance between the residues on adjacent actin protomers in F-actin. In pyrene actin fluorescence assays, Drb-
ABD showed an insignificant inhibition of depolymerization (~5% calculated at full saturation), suggesting that it does not stabilize actin filaments (data not shown).

Our results suggested that drebrin may stabilize the lateral interface in F-actin, which needed to be confirmed with the full-length protein and/or its “actin-interacting core,” Drb1–300. To this end, we employed acts with perturbed lateral or longitudinal interfaces and monitored the effect of longer constructs (Drb-FL, Drb1–300, and Drb2–252) on the intra-protomer contacts in F-actin.

Effects of Drebrin on the Polymerization of Actin with an Impaired Ability to Form Longitudinal Contacts—Substituting Thr-203 to cysteine and Cys-374 to a serine (TC/CS-actin) in yeast actin inhibits its spontaneous filament formation upon salt addition (32). Similarly, cleaving skeletal actin with grime-lysin (previously known as E. coli protease ECP) between residues 42 and 43 inhibits its polymerization (26, 33, 34). On the basis of the Fujii et al. model (35), these residues are part of the longitudinal interface in F-actin, and the (Thr-203) mutation inhibits actin polymerization, most likely by weakening longitudinal contacts among actin protomers. The polymerization activity of TC/CS actin can be restored by cofilin, which bridges two adjacent actin protomers by interacting with subdomain 1 (SD1) and SD3 on the “upper protomer” and SD2 on the lower one (36). To test whether drebrin could “correct for” a weakened longitudinal F-actin interface, TC/CS actin was polymerized in the presence of saturating amounts of Drb-FL or Drb1–300. Light scattering experiments show that, like cofilin (32), both constructs rescue the polymerization of this actin mutant (Fig. 4), although many rescued filaments are short. We con-
firmed the effect of drebrin on the longitudinal interface using grimelysin-cleaved skeletal actin. Again, the increase in light scattering signal suggested that addition of Drb-FL, Drb1–300, or Drb2–252 rescues the polymerization of grimelysin cleaved-actin, and EM images confirmed the formation of actin filaments (Fig. 5, A–F). However, the addition of DrbABD to the cleaved actin does not rescue its polymerization because there was no observed increase in light scattering, and actin filaments were not present in the EM images, which is consistent with our depolymerization results (Fig. 5, A and C).

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**FIGURE 5.** Drebrin-induced Stabilization of F-actin. The experiments were done in triplicate with all drebrin constructs mentioned and yielded similar results. A representative plot is shown for each construct. A, light scattering results showing the polymerization of 5 μM cleaved Mg2⁺ actin with 2 mM MgCl₂ and 50 mM KCl alone (trace 1) and with the additional presence of 50 μM DrbABD (trace 2), 25 μM Drb2–252 (trace 3), 1.7 μM Drb1–300 (trace 4), and 1 μM Drb-FL (trace 5). Polymerization of 5 μM uncleaved skeletal actin is shown in trace 6. A.U., arbitrary unit. B, EM image of aggregates of cleaved actin polymerized with 3 mM MgCl₂ only. C, EM image of actin polymerized in the presence of DrbABD. D, EM image of actin filaments obtained in the presence of Drb2–252. E, EM image of actin filaments formed in the presence of Drb1–300. F, EM image of actin filaments polymerized with 2 mM MgCl₂ and 50 mM KCl in the presence of 1 μM Drb-FL.

**Drb-FL and Drb1–300 Restore the Polymerization of the GG-Actin Mutant**—The mutation of two actin residues, Val-266 and Leu-267, at the amino-terminal end of the hydrophobic loop, was shown previously to cause a polymerization defect in this yeast actin mutant, GG-actin (37). According to the model by Fujii et al. (35), the hydrophobic plug contacts four regions on the opposite strand, including amino acids 201–203 and 39–42 of one subunit and amino acids 170–174 and 285–286 of an adjacent subunit. Mutating both Val-266 and Leu-267 to glycines reduces the hydrophobicity of the plug by two thirds and disrupts the contacts needed for actin polymerization (35). This mutant was used for prior tropomyosin studies and is a suitable tool for testing the effects of interacting factors on the lateral interface in F-actin (37). As shown in Fig. 6, the addition of salts to GG-actin causes a slight but steady increase in light scattering. EM shows that this change in signal is due to actin aggregation rather than its polymerization into stable filaments (Fig. 6C). Under the same conditions, the addition of saturating amounts of either Drb-FL or Drb1–300 induces a stable increase in light scattering, and the EM shows that this change in signal reflects actin filament formation (Fig. 6, D–F). EM images show that the addition of Drb1–300 induces the formation of long filaments, whereas Drb-FL samples show filaments along with some bundle and/or aggregate formation, which might be an artifact of the negative staining.

Previously documented rescue of GG-actin mutant polymerization by tropomyosin exhibited cold sensitivity because of the hydrophobic nature of the contacts (37). We observed a similar effect with drebrin. As shown in Fig. 6, A and B, GG-actin was polymerized in the presence of the Drb-FL, Drb1–300, or Drb2–252 construct until the light scattering signal reached a plateau, indicating a completion of the drebrin-induced GG-actin polymerization. The temperature was then lowered to 4 °C over a 30-min period. The light scattering of the sample decreased during that time, indicating actin depolymerization. EM images showed few small aggregates and some remaining
filaments in the sample (data not shown). A subsequent increase in temperature caused repolymerization of actin into filaments. The light scattering signal increased again (Fig. 6, A and B), and EM images confirmed the presence of actin filaments. The addition of DrbABD to GG-actin causes a small increase in light scattering, pointing to the formation of higher-order actin oligomers (Fig. 6B). These structures are, however, not temperature-sensitive because the signal remains almost unchanged, even after the sample is brought to 4 °C (Fig. 6B). We conclude that DrbABD does not restore the polymerization of GG-actin mutant, unlike the longer drebrin constructs.

**DISCUSSION**

Using depolymerization assays, DSC, and polymerization deficient actins as tools, we demonstrated that drebrin binding inhibits F-actin depolymerization and facilitates the assembly of polymerization-impaired actin by stabilizing both lateral and longitudinal contacts in filaments. We also clarified the domain requirements for the observed effects.

*Drebrin Inhibits Depolymerization of Actin Filaments from B-ends Stronger Than from P-ends*—Drb-FL causes a 90% decrease in the depolymerization rates of uncapped filaments, in which B-end depolymerization predominates. This points to a strong barbed end protection by Drb-FL because the B-end is
the faster depolymerizing end of the filament. Using gelsolin-capped filaments, we were able to estimate the effect of drebrin on pointed end depolymerization. Saturating concentrations of Drb-FL and Drb1–300 caused an ∼50% and ∼40% decrease in the depolymerization rate of GC filaments, respectively. This observation implies a lower affinity of drebrin to the P-ends compared with the B-ends.

Drb1–300 caused a similar ∼40% decrease in depolymerization rates of both capped and uncapped filaments. The Drb1–300 construct showed a weaker inhibition of uncapped filament depolymerization than Drb-FL despite the fact that their affinities for F-actin are similar (K_d = 0.2 and 0.12 μM, respectively, for Drb1–300 and Drb-FL). The simplest explanation would be that the weakly interacting C-terminal part of Drb-FL reduces the protomer off rate at the B-end, further contributing to the overall inhibition of actin depolymerization (Fig. 7, A–C).

As shown in Fig. 7 (top right corner), the drebrin molecule consists of an ADF-H domain, a helical/charged motif, an actin-binding domain (DrbABD), and an intrinsically disordered C-terminal part. Structural information available on F-actin-ADF/cofilin interactions (38–40), together with drebrin-actin interface mapping results (23), suggest that the C-terminal part of drebrin may be oriented toward the B-end of the filament (as shown in Fig. 7). Because the actin binding “core” of drebrin is localized within the N-terminal part of the molecule, we speculate that the dissociation of the actin monomers will shorten the lifespan of the drebrin-F-actin complex more drastically at the P-end than at the B-end (Fig. 7, D–F, versus A–C). Several possible scenarios can explain the lower affinity of Drb-FL to the P-end of filaments (Fig. 7).

1) Assuming that drebrin binding sites on F-actin do not overlap, five actin protomers are required for the binding of one Drb-FL molecule (Fig. 7, F–D). This would imply that, because of a steric clash, Drb-FL is not able to rebind with high affinity to a shorter filament stretch (less than five protomers) after its dissociation from the filament. In such a case, the dissociation of the first terminal actin protomer (of five) at the P-end of the Drb-FL binding site impairs the binding sites for ADF-H, which is critical for filament binding (on the basis of the data reported for N-terminal truncations of homologous Abp1 (41)). Dissociation of a second subunit from the P-end will only allow for the binding of DrbABD, which did not inhibit depolymerization in our assays. Loss of another protomer at the P-end will abolish drebrin binding, allowing for the F-actin depolymerization step with the rate characteristic of the uncomplexed F-actin.

2) Assuming that Drb-FL is able to interact with filament segments shorter than five protomers, unless precluded by a steric clash, the scenario shown in Fig. 7, G and H, is feasible. Because saturating amounts of Drb-FL and Drb1–300 show similar levels of P-end protection (Figs. 1C, lower panel, bar plot, and 2B), it is possible that the intrinsically disordered C-terminal region does not clash with the neighboring drebrin molecule when its 1–300 region is interacting with the last three to four protomers (of five forming the Drb-FL binding site) at the P-end (Fig. 7). Upon dissociation of the first and second actin protomers from the P-end, Drb-FL might be able to rebind to the remaining three- or four-protomer stretch with its C-terminal region pushed aside. However, it is expected that drebrin will be unable to interact with the two remaining actin

FIGURE 7. Effect of drebrin on B-end and P-end actin depolymerization. Center, schematic representation of an actin filament in complex with full-length drebrin. Individual actin protomers are represented by spheres. Protomers proposed to interact with the actin-binding core of drebrin (seq. 1–300) are shown in red. Protomers bound/protected by the C-terminal part of drebrin molecule are colored in light blue. For simplicity, only one strand is shown in A–I. On the basis of the presented results and the available interface mapping data, drebrin is proposed to bind to the filament with its C-terminal sequence oriented toward the B-end. A–C represent the scenario of B-end depolymerization and the subsequent effect on drebrin binding. D–F depict the scenario of P-end depolymerization and the subsequent effect on drebrin binding. G–I represent another P-end depolymerization possibility when drebrin-binding sites partially overlap on F-actin. See details in text (“Discussion”). A schematic representation of the drebrin molecule is shown in the inset. The ADF-H, helical charged motif, and AB domain of drebrin (DrbABD) are represented by rectangles. The C-terminal part of the molecule (predicted to be unstructured) is represented by a curvy line. Numbers correspond to the K_d, affinity rates of both capped and uncapped filaments. The Drb1–300 construct showed a weaker inhibition of uncapped filament depolymerization than Drb-FL despite the fact that their affinities for F-actin are similar (K_d = 0.2 and 0.12 μM, respectively, for Drb1–300 and Drb-FL). The simplest explanation would be that the weakly interacting C-terminal part of Drb-FL reduces the protomer off rate at the B-end, further contributing to the overall inhibition of actin depolymerization (Fig. 7, A–C).

As shown in Fig. 7 (top right corner), the drebrin molecule consists of an ADF-H domain, a helical/charged motif, an actin-binding domain (DrbABD), and an intrinsically disordered C-terminal part. Structural information available on F-actin-ADF/cofilin interactions (38–40), together with drebrin-actin interface mapping results (23), suggest that the C-terminal part of drebrin may be oriented toward the B-end of the filament (as shown in Fig. 7). Because the actin binding “core” of drebrin is localized within the N-terminal part of the molecule, we speculate that the dissociation of the actin monomers will shorten the lifespan of the drebrin-F-actin complex more drastically at the P-end than at the B-end (Fig. 7, D–F, versus A–C). Several possible scenarios can explain the lower affinity of Drb-FL to the P-end of filaments (Fig. 7).
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protomers (of five protomers) at the P-end because of a steric clash with the N-terminal part of a neighboring drebrin molecule (Fig. 7) and will dissociate from the P-end.

According to the scheme proposed in Fig. 7, A–C, no steric clash occurs at the B-end because of the arrangement of drebrin molecules. Dissociation of the first three actin protomers will still allow for a weak binding of the ADF-H of drebrin and its helical/charged motif to the B-end and the inhibition of depolymerization.

It should be noted that the scenarios proposed above do not take into account the “on” and “off” rates of the interaction of drebrin with actin filaments (yet to be determined). The simplified scheme in Fig. 7 presents a case in which the five-promoter actin stretch adjacent to the terminal drebrin binding site at the ends is always occupied by another drebrin molecule. However, this will only be true in case of very slow Drb-FL off rates from actin filaments. If drebrin off rates are fast, the binding modes proposed in scenarios 1 and 2, and at the B-end, will alternate with high affinity drebrin binding modes, because of the dissociation of the neighboring drebrin molecule, which in turn will cause transient availability of longer undecorated F-actin stretches at the filament ends.

In light of the differences in drebrin stabilization effects on P-end and B-ends, we considered several factors that contribute such differences. 1) A reduced affinity of Drb-FL for P-ends could also be due to a difference in its affinity for ADP vs ADPactin. However, our unpublished results indicate that drebrin does not have a preference for a specific nucleotide state of filament (Grintsevich EE et al., to be published elsewhere), excluding this possibility. 2) Facilitated cofilin binding to GC-actin filaments suggests that gelsolin alters the morphology of F-actin in a cooperative manner (42). It is possible that gelsolin-induced allosteric changes can lead to a weaker binding of drebrin to GC-F-actin. Co-sedimentation of Drb-FL with gelsolin-capped and uncapped actin filaments showed no differences in drebrin binding. Measurements of drebrin binding kinetics to GC-F-actin will be needed to rule out the less likely possibility that both “on” and “off” rates of drebrin interaction with GC-actin filaments are changed.

At a cellular level, a different affinity of drebrin for the two filament ends will translate into the P-end of drebrin-decorated F-actin being available to interact with P-end capping proteins. For example, tropomodulins (Tmods) regulate the dynamics of the slow-growing pointed end of the actin filament and require tropomyosins (TM) for optimal function as TMs greatly enhance Tmods pointed-end capping activity (43, 44). However, drebrin binding to actin filaments may interfere with the subsequent binding and effect of B-end cappers and/or elongation factors. In line with this possibility, a potential cross-talk between neuronal B-end capper Eps8 and drebrin E was reported recently (45). Moreover, our findings are consistent with an earlier report (46) showing that drebrin-expressing fibroblasts were resistant to cytochalasin D, which causes depolymerization of actin filaments trough B-end capping and monomer sequestration. Inhibition of depolymerization could be a mechanism used by drebrin to protect actin filaments and, thereby, the size and morphology of dendritic spines.

The effect of Drebrin on Longitudinal Contacts in F-actin and Domain Requirements—Drb-FL and its actin-binding core, Drb1–300, both rescue the polymerization of the TC/CS yeast actin mutant (impaired polymerization because of the mutation in the SD4 of actin), as observed by light scattering and EM. A similar effect was observed with grimelysin-cleaved actin. Drb FL, Drb1–300, and Drb2–252 all induced the formation of actin filaments (Fig. 5, A and D–F). However, the strongest actin interacting unit of the drebrin molecule, DrbABD, failed to rescue, on its own, the polymerization of grimelysin-cleaved actin (Fig. 5, A and C). These results are in line with the Cu2+ catalyzed cross-linking of the yeast actin mutant Q41C, in which we observed an inhibition of cross-linking between residues 41 (in SD2) and the native Cys-374 on F-actin in the presence of DrbABD233–300. The inhibition of disulfide formation by DrbABD233–300 suggests an increase in the mean distance between Cys-374 and the DNase I binding loop on adjacent actin protomers in F-actin. It is tempting to speculate that the observed inhibition of Cys-41-Cys-374 cross-linking by DrbABD233–300, together with its inability to rescue the polymerization of actins with a perturbed longitudinal interface, can be explained by DrbABD-induced “under-twisting” of F-actin (the helical pitch is 40 nm versus 36 nm in bare actin (18)). It is possible that this “stretched” conformation is stabilized by binding of N-terminal drebrin domains (ADF-H and predicted coil-coiled region, seq. 2–252) to the filament. However, it is also possible that the inhibition of cross-linking by DrbABD233–300 may be caused by steric hindrance because of the proposed binding to subdomain 2 of actin of DrbABD (23).

The Effect of Drebrin on Lateral Contacts in F-actin and Domain Requirements—An increase in the cross-linking rate between Cys-265 and Cys-374 in the presence of DrbABD233–300 suggested its stabilization of interstrand contacts in F-actin. We employed the GG-yeast actin mutant to test this possibility. This particular mutant has two hydrophobic residues, Val-266 and Leu-267, substituted to glycines and a high critical concentration > 20 μM (37). Similar to the effect of drebrin on the longitudinal interface of F-actin, the N-terminal constructs Drb1–300 and Drb2–252, and full-length protein rescued the polymerization of GG-actin. DrbABD alone was not sufficient to rescue actin polymerization (Fig. 6).

The observed filament stabilization would be consistent with drebrin binding in the area of interstrand contacts and restoring the impaired interface and/or compensating for the missing contacts by binding to several protomers. According to the mapping reported previously (23), DrbABD is centered on SD2 of actin and makes an interstrand contact only in a minor binding mode. Together with its inability to protect actin filaments from depolymerization and rescue the polymerization of GG-actin, this suggests that the enhanced cross-linking in the presence of DrbABD233–300 most likely reports on its allosteric effect on the lateral interface in F-actin. Taking into account the reported data on ADF/cofilin interactions with actin filaments, the N-terminal ADF-H domain of drebrin is an unlikely candidate for direct stabilization of the lateral interface (36). Moreover, control experiments performed with the GG-mutant in the presence of yeast cofilin showed no rescue of its polymerization (data not shown). This leaves the helical/charged region

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(amino acids134–233) of drebrin as a possible candidate for direct stabilization of the lateral interface of actin. Our hypothesis is that the drebrin molecule wraps around the filament, “stapling” two protofilaments by its helical/charged motif. High-resolution EM reconstruction and detailed interface mapping is needed to confirm or rule out this hypothesis. It should be noted that the time scales of GG-actin polymerization rescue by drebrin are similar to those reported previously for several tropomyosins (37). In the case of TMs, it was shown that the rescue efficiency is proportional to the number of quasirepeats in the TM molecule. As shown in Fig. 6, we observed a similar effect with Drb-FL addition (5:1 stoichiometry), yielding faster polymerization kinetics than Drb1–300 (3:1 stoichiometry) or Drb2–252 (2:1 stoichiometry).

Lowering the temperature of GG-actin filaments to 4 °C in the presence of drebrin constructs caused their partial depolymerization but not as complete as reported previously for TM. Lack of complete GG-actin depolymerization at 4 °C in the presence of drebrin indicates that its overall stabilization effect (taking into consideration the additional stabilizing effects on the longitudinal contacts of F-actin reported earlier) is stronger than that of TMs tested in the same system. Repolymerization of GG-actin in the presence of drebrin was not detected below 20 °C, indicating a similar temperature sensitivity to tropomyosins in stabilization of the lateral interface of actin.

Our combined results provide evidence for filament stabilization of actin by drebrin and analyze its structural basis. These results point to the likely role and mode of action of drebrin in preserving the integrity of F-actin structures in dendritic spines. In conclusion, drebrin inhibits F-actin depolymerization from both B- and P-ends. The P-end of actin is protected by drebrin to a lesser degree than the B-end. Results obtained with the Drb-FL and Drb1–300 constructs suggest that the intrinsically disordered C-terminal part of the drebrin molecule is responsible for the stronger inhibition of B-end than P-end depolymerization (Figs. 1C and 2B). Despite being the strongest binding module within the drebrin molecule, DrbABD alone does not rescue the polymerization of actins with an impaired ability to form an interface between protomers.

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