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Isoform-dependent Regulation of Drebrin Dynamics in Dendritic Spines

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Abstract—Dendritic spines have stable filamentous actin (F-actin) and dynamic F-actin. The formation of stable F-actin plays a pivotal role in spine formation. Drebrin binds to and stabilizes F-actin in dendritic spines. Interestingly, the conversion of the drebrin E isoform to drebrin A occurs in parallel with synapse formation, suggesting that this conversion promotes synapse formation via F-actin accumulation. In this study, we measured the dynamics of GFP-tagged drebrin E (GFP-DE) and drebrin A (GFP-DA) in cultured hippocampal neurons by fluorescence recovery after photobleaching analysis. We found that GFP-DA has a larger stable fraction than GFP-DE. The stable drebrin fraction reflects its accumulation in dendritic spines, therefore the isoform conversion may increase the amount of stable F-actin in dendritic spines. The stable fraction was dependent on the drebrin A-specific sequence "Ins2", located in the middle of the drebrin protein. In addition, F-actin depolymerization with latrunculin A significantly reduced the stable GFP-DA fraction. These findings indicate that preferential binding of drebrin A to F-actin than drebrin E causes higher stable fraction of drebrin A in dendritic spines, although the F-actin-binding ability of purified drebrin E and drebrin A are comparable. Therefore, we suggest that a drebrin isoform conversion from drebrin E to drebrin A in dendritic spines results in the accumulation of drebrin-bound stable F-actin, which plays a pivotal role in synapse formation.

Key words: actin cytoskeleton, dendritic spine, drebrin E, drebrin A, fluorescence recovery after photobleaching, synapse formation.

INTRODUCTION

Filamentous actin (F-actin) is a major component of the dendritic spine cytoskeleton. F-actin in dendritic spines is stable and resistant to actin-depolymerizing drugs, such as cytochalasin D (Allison et al., 2000). However, spines are motile and change their shape in an F-actin-dependent manner (Fischer et al., 1998). This led to the hypothesis that both stable and dynamic F-actin exist in dendritic spines (Halpain, 2000). The presence of stable and dynamic actin has been demonstrated using fluorescence recovery after photobleaching (FRAP) (Star et al., 2002) and photoactivatable GFP-fused actin (Honkura et al., 2008; Frost et al., 2010). However, the molecular mechanisms regulating stable and dynamic F-actin are poorly understood.

Drebrin is an actin-binding protein that stabilizes F-actin by structural alterations (Sharma et al., 2011, 2012; Mikati et al., 2013; Grintsevich and Reisler, 2014). Drebrin-bound F-actin is resistant to cytochalasin D in cultured fibroblasts (Ikeda et al., 1995). These results suggest that drebrin determines the stability of F-actin in dendritic spines. During development, clusters of drebrin-bound F-actin appear in dendritic filopodia, the precursors of dendritic spines (Takahashi et al., 2003). Drebrin has two major isoforms, drebrin A (DA) and drebrin E (DE). The conversion from DE to DA occurs in parallel with synapse formation. We have shown that DA accelerates F-actin clustering in dendritic spines (Takahashi et al., 2003). Furthermore, using FRAP, we showed that the stable drebrin fraction reflects the clustering of drebrin-bound F-actin in dendritic spines (Takahashi et al., 2009). Therefore, studying the stable fraction of individual drebrin isoforms in dendritic spines is important to elucidate the molecular mechanism regulating the amount of stable F-actin.

In this study, we examined the dynamics of GFP-tagged DE and DA in the dendritic spines of cultured hippocampal neurons using FRAP. We found that the stability of the two drebrin isoforms was different in dendritic spines. The stable DA fraction was significantly larger than the stable DE fraction. Pharmacological

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Abbreviations: DA, drebrin A; DE, drebrin E; DIV, day(s) *in vitro*; F-actin, filamentous actin; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; Jasp, jasplakinolide; LatA, latrunculin A; sDA, s-drebrin A.

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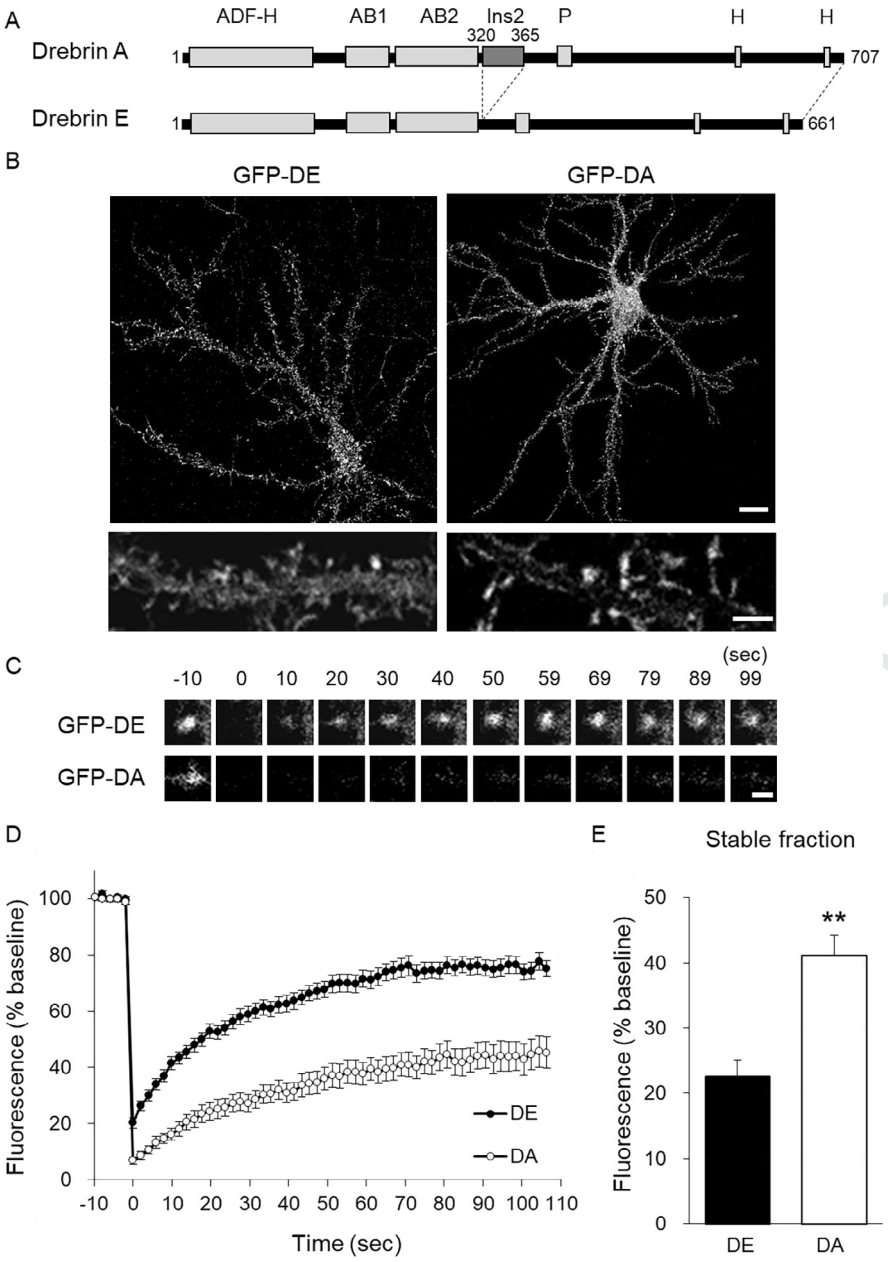

Fig. 1. Stable GFP-drebrin E (DE) fraction is smaller than the stable GFP-drebrin A (DA) fraction in dendritic spines of cultured hippocampal neurons. (A) Domain structure of DA and DE. ADF-H: ADF homology domain, AB1: Actin-binding region 1, AB2: Actin-binding region 2, Ins2: drebrin A-specific sequence, P: Proline-rich region. H: Homer-binding motif. (B) Localization of GFP-DA and GFP-DE in cultured hippocampal neurons at 15–17 days *in vitro*. Top panels show gray-scale images of GFP signal in cultured neurons transfected with GFP-DE (left) and GFP-DA (right). Bottom panels show higher magnification images. Scale bar = top panel, 20 μm; bottom panel, 2 μm. (C) Representative time-lapse sequences during fluorescence recovery after photobleaching (FRAP) of neurons expressing GFP-DE or GFP-DA. Fluorescence recovery is an index of drebrin turnover. Scale bar = 1 μm. (D) FRAP curves of GFP-DE and GFP-DA. Fluorescence recovery of GFP-DE was faster than that of GFP-DA. (E) The stable GFP-DE fraction was significantly smaller than stable GFP-DA fraction. Twenty-five spines were examined for GFP-DE and 29 spines were examined for GFP-DA. $^{**}p < 0.01$.

experiments indicated that this difference is mediated by the affinity of the drebrin isoform for F-actin. Thus, preferential binding of DA to F-actin than DE may cause higher stable fraction of DA in dendritic spines, although the F-actin-binding ability of purified DE and DA are comparable. These results suggest that the amount of stable drebrin-bound F-actin in dendritic spines is increased by conversion of DE to DA.

MATERIALS AND METHODS

Animals

Animal experiments were performed according to the guidelines of the Animal Care and Experimentation Committee (Gunma University, Showa Campus, Maebashi, Japan) and conformed to NIH guidelines for the use of animals in research. Every effort was made to minimize animal suffering and to reduce the number of animals used. Pregnant Wistar rats were obtained from Charles River Japan Inc. (Yokohama, Japan). Animals were maintained under standard white cyclic lighting, with free access to food and water.

HIPPOCAMPAL CELL CULTURE

Experiments were performed on Banker-style low-density hippocampal neurons (Goslin et al., 1998; Takahashi et al., 2003). After deep anesthesia, embryos were removed from timed pregnant Wistar rats at embryonic day 18 and decapitated. Hippocampi were dissected from embryonic brains, and dispersed into a single-cell solution by trituration. Cells were plated on coverslips coated with poly-L-lysine, and then incubated in Minimum Essential Medium (MEM; Thermo Fischer Scientific, Waltham, MA, USA) supplemented with fetal bovine serum for 3 h. The coverslips were transferred to a dish containing supporting glial cells attached to the bottom. Supporting glial cells were maintained in serum-free MEM containing B27 supplement (Thermo Fischer Scientific), 0.6% glucose, and 1 mM sodium pyruvate. Cytosine β-D-arabino-furanoside (5 μM; Sigma, St. Louis, MO, USA) was added to the cultures at 4 days *in vitro* (DIV) to inhibit glial proliferation. Supporting glial cells were prepared from the cerebral cortex of newborn rats (Goslin et al., 1998). For pharmacological experiments, latrunculin A (LatA) was obtained from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan)