Chapter 10
Drebrin and Spine Formation

Hideto Takahashi and Yusuke Naito

Abstract Dendritic spines are the postsynaptic receptive regions of most excitatory synapses in the central nervous system. Thus, spines are supposed to act as a fundamental unit for information processing of the brains. Previous studies have demonstrated the roles of drebrin in the formation of dendritic spines and in the recruitment of synaptic proteins to postsynaptic sites. Further, a live imaging study has revealed the unique dynamics of drebrin in dendritic spines, which help to understand how drebrin is involved in dendritic spine formation. This review will provide a basic knowledge about dendritic spine and overview recent progresses in understanding of the roles of drebrin in dendritic spine morphogenesis and synaptogenesis.

Keywords Dendritic spine • Dendritic filopodia • Actin cytoskeleton • Postsynaptic density (PSD) • Activity dependent • Protein trafficking • FRAP

10.1 The Biology of Dendritic Spine

10.1.1 Dendritic Spine

In the neurons of the adult brains, the most glutamatergic presynaptic terminals innervate dendritic spines, tiny protoplasmic protrusions emerging from dendritic shafts of the target neurons (Harris and Kater 1994; Rochefort and Konnerth 2012; DeFelipe 2015). Dendritic spines constitute fundamental neuronal contact sites for excitatory synapses. Spines are composed of three major components: a base connected to a dendritic shaft, a neck, and a head which connects with an axon. Spines are typically less than 2 μm in length and possess a spine head approximately 1 μm in width. The morphology of dendritic spines varies largely among spines and within one spine over time. Based on their morphology in fixed brain tissues and fixed cultured neurons, dendritic spines are generally classified into three

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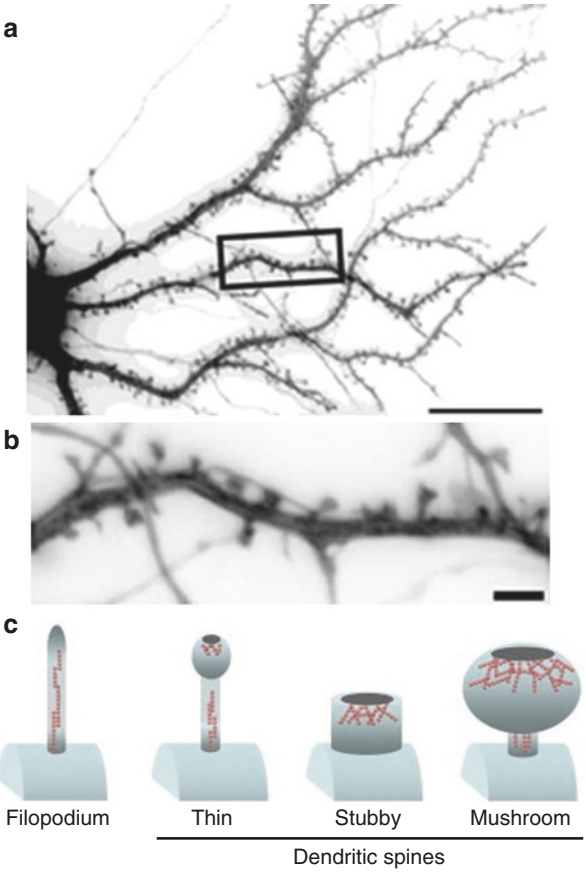

Fig. 10.1 (**a**, **b**) A microscope image of a hippocampal neuron in low-density cultures at 21 days in vitro by Dil labeling. Many mushroom-shaped spines emerging from dendritic shaft are observed in (**b**). Scale bars: 20 μm in (**a**) and 2 μm in (**b**). Adapted from Shirao and González-Billault (2013) with permission. (**c**) Schematic representation of morphologies of dendritic protrusions. There are two types of dendritic protrusions, dendritic filopodium, a long thin headless protrusion, and dendritic spine, which has bulbous head. Dendritic spines also exhibit three different types: thin, stubby, and mushroom type. *Gray disks* represent the PSD structure and chains of *red circles* represent F-actin. Adapted from Sekino et al. (2007) with permission

morphological types: (1) thin spines, which have a small head and narrow neck; (2) stubby spines, which don't have neck; and (3) mushroom-shape spines, which have a relatively large head and short neck (Fig. 10.1). Time-lapse imaging studies have shown that one spine is not static and takes transition among these morphological states, suggesting that observed spine shapes are snapshots of morphologically dynamic spines (Dailey and Smith 1996; Ziv and Smith 1996; Dunaevsky et al. 1999). Importantly, the size and shape of dendritic spines are changed in response to synaptic activity and experiences, reflecting the nature of synaptic plasticity. For example, long-term potentiation (LTP) is associated with the increase in spine head volume (Matsuzaki et al. 2004; Maletic-Savatic et al. 1999) and the length of spine neck (Araya et al. 2006). In contrast, long-term depression is accompanied by the shrink of dendritic spines (Zhou et al. 2004). Thus, the morphological plasticity of dendritic spines is supposed to be the cellular basis of synaptic plasticity underlying learning and memory. This idea also suggests the structure-function relationship of dendritic spines, which will be more discussed later.

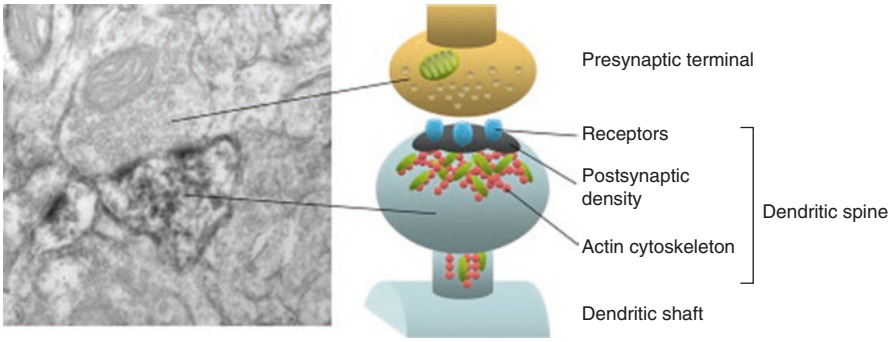

Fig. 10.2 Ultrastructure of dendritic spine synapse. The *left* panel shows an immunno-electron microscopic image for drebrin at an asymmetric synapse. Drebrin is enriched in dendritic spine but not presynaptic terminal. The *right* schematic image shows basic components of an asymmetric synapse. Dendritic spine consists of an actin cytoskeleton with actin-binding proteins and a PSD structure scaffolding neurotransmitter receptors. Adapted from Sekino et al. (2007) with permission

10.1.2 Structural Elements of Dendritic Spines: Actin Cytoskeleton and PSD

Dendritic spines are composed of two major structural elements: actin cytoskeleton and postsynaptic density (PSD) (Kennedy 1997; Sekino et al. 2007) (Fig. 10.2). Actin is a highly abundant protein (typically 5–10% of total proteins) in eukaryotic cells including neurons. Individual actin molecules have two states, a monomeric state as a globular actin (G-actin) and a polymeric state (Kabsch and Vandekerckhove 1992). Polymerization of monomeric actins results in the formation of filamentous actin (F-actin). G-actin molecules reversibly bind to the end of F-actin. Thereby, actin has dynamic equilibrium between G-actin and F-actin, which is one of key factors that determine actin cytoskeleton in dendritic spines (Okamoto et al. 2004). F-actin generally forms two types of structures, F-actin bundles and F-actin networks (Svitkina 2013). Cells including neurons express many F-actin binding proteins, which determine and/or modify F-actin bundles and networks. In general, F-actin regulates cell structures by modulating its bundle and network structures. For example, cellular projections including filopodia and microvilli contain F-actin bundles, whereas the peripheral submembranous region of cells mainly contains F-actin networks. In neuron, F-actin is enriched in dendritic spines (Matus et al. 1982; Cohen et al. 1985). Electron microscopic studies have shown that a spine head contains the network (lattice structure) of F-actin, whereas a spine neck contains F-actin bundles like filopodia (Landis and Reese 1983). In addition, F-actin generally regulates the motile force of cells through the modulation of polymerization and depolymerization states of F-actin and the activity of myosin ATPase (Pollard and Borisy 2003). Time-lapse imaging studies using green fluorescent protein (GFP)-tagged actin have revealed that dendritic spines undergo rapid continuous motility based on actin dynamics (Star et al. 2002). The reagents that change actin

polymerization states such as cytochalasin D and latrunculin A alter spine motility. Interestingly, spine motility is also regulated by glutamate receptor activations (Fischer et al. 2000), suggesting that spine motility may contribute to the plasticity of excitatory synapses. In dendritic spines, many F-actin binding proteins including drebrin exist and regulate the shapes, size, and dynamics of dendritic spines.

The PSD is an electron-dense highly organized protein structure attached to the postsynaptic membrane of dendritic spines (Kennedy 1997) (Fig. 10.2). PSD is composed of various scaffold proteins such as membrane-associated guanylate kinases (MAGUKs) and many PSD-95/Dlg/ZO-1 (PDZ)-containing proteins (Kim et al. 1995; Kim and Sheng 2004). PSD-95 is a member of MAGUK family and a typical PDZ protein abundant in PSD (Cho et al. 1992; Kistner et al. 1993). PSD-95 directly binds to NMDA-type glutamate receptor subunit GluN2 (Niethammer et al. 1996) and indirectly binds to AMPA-type glutamate receptor via transmembrane AMPA receptor regulatory proteins (TARPs) such as stargazin (Tomita et al. 2005, 2004; Chen et al. 2000), suggesting that PSD-95 plays roles in regulating the trafficking and anchoring of glutamate receptors. PSD-95 is further involved in dendritic spine morphology. Overexpression of PSD-95 in cultured neurons enlarges dendritic spine (El-Husseini et al. 2000). Overexpression studies have also shown that other PSD proteins such as Homer and Shank regulate spine morphology (Sala et al. 2001). Thus, PSD proteins are also a key element that controls dendritic spine structure. Interestingly, many PSD proteins are physically and functionally associated with F-actin. Thus, it has been suggested that actin cytoskeleton and PSD cooperatively, rather than independently, regulate dendritic spine morphology.

10.1.3 Structure-Function Relationship of Dendritic Spines

Many evidences about the morphological changes of dendritic spines in synaptic plasticity have suggested structure-function relationship of dendritic spines (Kasai et al. 2003). Several previous studies have revealed that the size of dendritic spines is closely correlated with their synaptic function. An electromicroscopic study with postembedding immunogold labeling has shown that smaller spines with less than about 180 nm of PSD lack AMPA receptors (Takumi et al. 1999). These smaller spines are called “silent synapses,” which do not generate excitatory postsynaptic potentials under basal stimulation. In addition, the size of PSD in dendritic spines is correlated with the number of AMPA receptors, supporting that larger spines are more functional in terms of AMPA receptor-mediated excitatory postsynaptic potentials. Another study using a glutamate uncaging technique has further revealed that spine head volume is correlated with the expression of functional AMPA receptors (Matsuzaki et al. 2001). Such structure-function relationship of dendritic spines suggests that the molecular and cellular mechanisms that control spine structure are important for our understanding of how functional neuronal circuits in the brains develop and are modified by experiences for higher brain functions such as cognition, learning, and memory (Kasai et al. 2003, 2010).

10.1.4 Dendritic Spine Morphogenesis: The Role of Dendritic Filopodia

How do dendritic spines form during neuronal development? Immature neurons, especially before synaptogenesis period, do not have dendritic spines. Instead, they possess dendritic filopodia, thin headless protrusions emerging from dendritic shaft (Yoshihara et al. 2009; Ebrahimi and Okabe 2014). The major difference of dendritic filopodia from spines is the lack of a bulbous head. As neurons mature, dendritic filopodia disappear, and dendritic spines emerge. The developmental transition from dendritic filopodia to spines is supposed to be crucial for the development of functional excitatory synapses on target neurons. The developmental transition further suggests that dendritic filopodia may act as a precursor of dendritic spines. This possibility is supported by several time-lapse imaging studies (Dailey and Smith 1996; Ziv and Smith 1996; Dunaevsky et al. 1999). A majority of dendritic filopodia rapidly extends and retracts with short-term lifetime (around 10 min) in an early developmental stage. As innervated axons increase along dendrites during development, some filopodia can make physical contact with axons and are stabilized by the association of functional presynaptic terminals. This observation suggests that dendritic filopodia can be transformed into dendritic spines after the physical contact with axons. What molecular mechanism governs the transformation of dendritic filopodia to spines? A previous time-lapse imaging has shown that the initiation of spine morphogenesis precedes synaptic accumulation of PSD-95 (Okabe et al. 2001). Further, PSD-95 mutant mice exhibit normal dendritic spine morphogenesis (Migaud et al. 1998). Thus, PSD-95 would be not required for spine morphogenesis during development. On the other hand, the pharmacological experiment has shown that depolymerization of F-actin by latrunculin A causes the disassembly of some synaptic structural elements (Allison et al. 2000). Further, the important evidence is that dendritic filopodia are actin-rich structure like spines (Fischer et al. 1998; Dunaevsky et al. 1999). Therefore, actin-based mechanisms would be crucial for the developmental transition from dendritic filopodia to dendritic spines.

10.2 The Roles of Drebrin in Dendritic Spine Morphogenesis

As described in the previous chapters (see Chaps. 3 and 7), drebrin is an F-actin-binding protein and enriched in dendritic spines (Figs. 10.2 and 10.3). Drebrin has two major isoforms, drebrin E and drebrin A based on alternative splicing of exon 12a. The expression of drebrin A isoform is upregulated at synaptogenesis period. Further, the amount of drebrin positively correlates with spine head size in the adult mouse cerebral cortex (Kobayashi et al. 2007). Together, drebrin would be one of the best candidates as an actin-based molecular mechanism that regulates dendritic spine morphogenesis during development. A cell-biological study on drebrin has

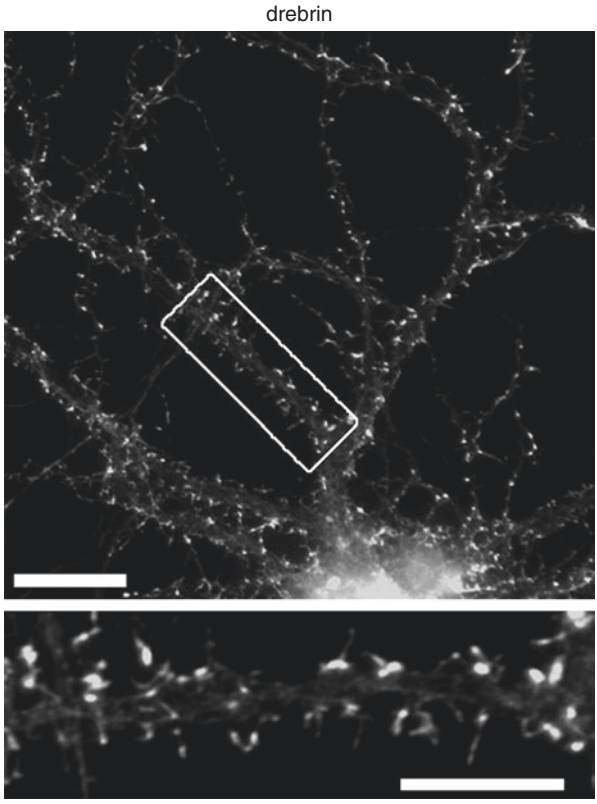

Fig. 10.3 Drebrin immunolabeling of a cultured hippocampal neuron at 15 days in vitro by mouse monoclonal anti-drebrin antibody M2F6. Drebrin clusters are observed at dendritic spines. The lower panel presents the boxed region in the upper panel. Scale bars: 20 μm (*upper*), 10 μm (*lower*). Adapted from Takahashi et al. (2009) with permission

demonstrated that the cluster formation of drebrin occurs in dendritic filopodia, especially at the contact site between dendritic filopodia and axons (Takahashi et al. 2003). In addition, drebrin A knockdown experiments have revealed that drebrin clustering in dendritic filopodia is required for dendritic spine morphogenesis during development and synaptic accumulation of PSD-95 (Takahashi et al. 2003, 2006). The following paragraphs will focus on describing the developmental changes of drebrin localization in dendritic filopodia and the roles of drebrin in dendritic spine morphogenesis during development.

10.2.1 Drebrin Clustering in Dendritic Filopodia

As dendritic filopodia and spines both are actin-rich structure, developmental transition from dendritic filopodia to spines can be visualized by F-actin labeling of hippocampal neurons in low-density culture using fluorescent-conjugated phalloidin

(Takahashi et al. 2003). At 7 days in vitro (DIV), almost all of F-actin-positive dendritic protrusions are observed as long, thin, and headless (filopodia-like) protrusions. At 14 DIV, the dendritic protrusions show the mixed population of dendritic filopodia and spines, including long and thin protrusions, with or without a small head, and mushroom-type or stubby spine like protrusions. At 21 DIV, almost all of the dendritic protrusions exhibit mushroomlike or stubby shapes with enriched F-actin levels. Thus, F-actin labeling of cultured hippocampal neurons is useful for observing the developmental shift from dendritic filopodia to spines. This observation is also consistent with the previous studies using fluorescent dyes (Papa et al. 1995; Ziv and Smith 1996).

How is drebrin distribution changed in association with the developmental changes of F-actin-positive dendritic protrusions? Double labeling for drebrin and F-actin has shown interesting changes in drebrin distribution in dendritic shaft and protrusions. At 7 DIV, the intense immunoreactivity of drebrin with F-actin staining is detected at the submembranous regions along dendrites. The distributions of drebrin and F-actin in dendritic filopodia exhibit the fibrous staining pattern. At 21 DIV, most of the drebrin immunoreactivity is detected as clusters colocalized with F-actin in dendritic spines. At dendritic shaft, drebrin immunoreactivity is not detectable although F-actin staining remained there. These data support that drebrin is enriched in dendritic spines and also indicate that drebrin distribution is not completely overlapped with F-actin distribution although drebrin binds to F-actin. At 14 DIV, transitional distribution of drebrin can be detected. The detailed analysis of drebrin-F-actin labeling at 14 DIV has revealed that there are two types of drebrin distribution in dendritic filopodia, diffuse pattern and cluster pattern. Thus, dendritic filopodia can be classified into either (1) cluster-type filopodia or (2) diffuse-type filopodia in terms of the existence of drebrin clusters (Takahashi et al. 2003). According to the quantitative analysis, at 7 DIV, approximately 90% of dendritic filopodia is classified as diffuse-type filopodia, and the rest is classified as cluster-type filopodia. From 7 to 14 DIV, the population of diffuse-type and cluster-type filopodia is drastically changed. At 14 DIV, the number of cluster-type filopodia doubles that at 7 DIV and accounts for 50% of total filopodia. In contrast, the number of diffuse-type filopodia at 14 DIV drops by half compared to that at 7 DIV. Thus, drebrin-F-actin labeling reveals the developmental transition from diffuse-type filopodia to cluster-type ones. The transition of diffuse-type filopodia to cluster-type ones was also observed by the time-lapse imaging of GFP-tagged drebrin A (Fig. 10.4). Previous time-lapse studies have shown that some filopodia are very dynamic, whereas other filopodia are converted to stable spines (Dailey and Smith 1996; Ziv and Smith 1996; Dunaevsky et al. 1999). In addition, the dynamics of dendritic filopodia and spines are stabilized during development (Ziv and Smith 1996). The diffuse-type filopodia may represent the dynamic filopodia, whereas the cluster-type filopodia may represent the transitional stage to stable spines.

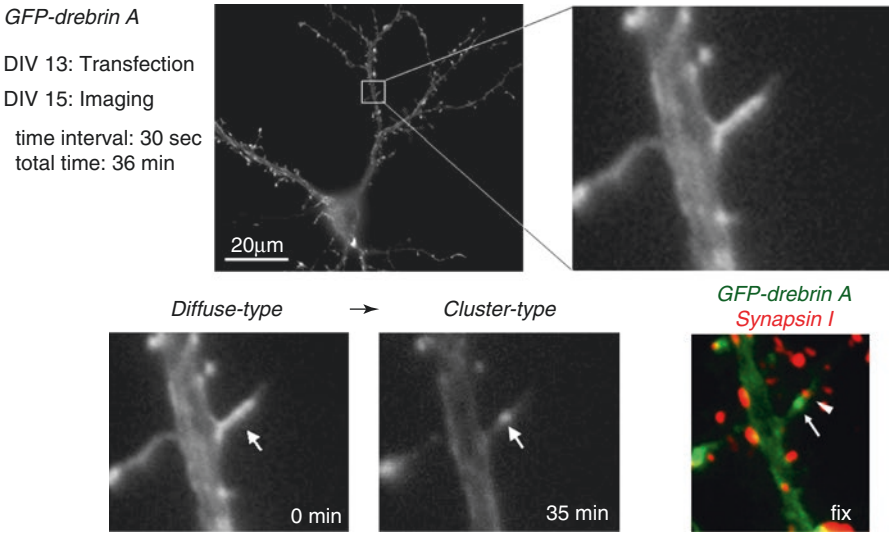

Fig. 10.4 The time-lapse imaging of GFP-tagged drebrin A. GFP-tagged drebrin A was transfected into hippocampal neurons at 13 days in vitro. Two days after the transfection, GFP signal was imaged every 30 s for 35 min by confocal microscopy. The bottom panel shows that drebrin A was diffusely distributed throughout a filopodium (an *arrow* in the *left bottom* panel) in the beginning of the imaging. 35 min after starting the imaging, the diffuse pattern of drebrin A distribution shifted to a cluster pattern (an *arrow* in the *middle bottom* panel). This drebrin A cluster was juxtaposed with presynaptic maker synapsin I (*arrowhead* in the *right bottom* panel)

10.2.2 Drebrin Clustering at Synaptic Contact of Dendritic Filopodia

Drebrin clustering in dendritic filopodia is detected from synaptogenesis period, suggesting that drebrin clustering is associated with synaptic contact of dendritic filopodia (Takahashi et al. 2003). The quantitative analysis of triple labeling of cultured hippocampal neurons for drebrin, F-actin, and synapsin I, a presynaptic vesicle protein, has shown that a majority of cluster-type filopodia is associated with synapsin I clusters, indicating that cluster-type filopodia have synaptic contact. In contrast, most of diffuse-type filopodia is not associated with synapsin I clusters. The important observation is that drebrin clusters in the synaptic cluster-type filopodia are always juxtaposed with synapsin I clusters, demonstrating the tight association of drebrin clustering in dendritic filopodia with presynaptic contact. These data suggest that drebrin clusters in filopodia are precursors of postsynaptic structures of spine synapses. Further, it has been suggested that drebrin clustering would be triggered by synaptic contact. The molecular and cellular mechanisms that trigger drebrin clustering will be discussed later.

10.2.3 Drebrin Clustering vs PSD-95 Clustering in Dendritic Filopodia

The major structural components of dendritic spines are actin cytoskeleton and PSD (Kennedy 1997; Sekino et al. 2007). Given drebrin clustering at the contact site between dendritic filopodia and presynaptic terminals, it is interesting to address whether the formation of PSD at the contact sites also occurs together with drebrin clustering. Triple labeling for F-actin, synapsin I, and PSD-95 or drebrin has revealed that almost all of dendritic spines with presynaptic contacts (synaptic spines) contain both PSD-95 and drebrin clusters (Takahashi et al. 2003). In contrast, only about half (57%) of dendritic filopodia with presynaptic contacts (synaptic filopodia) contains PSD-95 clusters, but almost all (86%) of synaptic filopodia contains drebrin clusters (Takahashi et al. 2003). These quantitative data indicate that postsynaptic clustering of drebrin in dendritic filopodia may be followed by that of PSD-95. After dendritic filopodia contact with axons, actin reorganization tends to precede PSD formation in dendritic filopodia, supporting that dendritic filopodia with drebrin clusters (cluster-type filopodia) would be a precursor of dendritic spines (protospines). According to a previous electromicroscopical study (Fiala et al. 1998), some filopodia possess nascent synapses containing PSD structure. A time-lapse imaging study has also shown that filopodia with PSD-95 clusters directly transform into mature spines during development (Marrs et al. 2001). Therefore, in developing neurons, most of filopodia containing both drebrin and PSD-95 clusters may exhibit the transitional stage into mature spines although it is also possible that some filopodia with drebrin and PSD-95 clusters represent temporal snapshots of mature spines.

10.2.4 Drebrin Clustering Is Required for PSD-95 Clustering

The evidence that drebrin clustering precedes PSD-95 clustering in dendritic filopodia raises an interesting question whether drebrin clustering is required for PSD-95 clustering. As described in Chap. 2, drebrin has two major alternative splicing isoforms, drebrin E and drebrin A. Notably, the upregulation of drebrin A expression occurs in synaptogenesis period, in which drebrin clustering is also observed (Hayashi et al. 1998). These data suggest that developmental upregulation of drebrin A is essential for drebrin clustering and the initial step of synaptogenesis. To suppress the drebrin A upregulation, the antisense oligonucleotide that targets drebrin A-specific nucleotide sequence has been developed (Takahashi et al. 2003). The drebrin A antisense oligonucleotides selectively suppress the expression of drebrin A but not that of drebrin E or actin. As expected, drebrin A knockdown by the antisense oligonucleotides indeed inhibits drebrin clustering in dendritic protrusions

(Takahashi et al. 2003). Drebrin A knockdown significantly reduces the number of cluster-type filopodia but increases that of diffuse-type filopodia, indicating that drebrin A knockdown inhibits the developmental transition from diffuse-type filopodia to cluster-type ones (Takahashi et al. 2003). How about PSD-95 clustering in the case of drebrin A knockdown? Drebrin A knockdown significantly suppresses synaptic clustering of PSD-95. In addition, the rescue of drebrin A expression into the knocked-down neurons restores synaptic clustering of PSD-95 (Takahashi et al. 2003). Together drebrin clustering triggered by drebrin A upregulation is crucial for synaptic clustering of PSD-95 during development.

10.2.5 Drebrin Clustering Is Required for Dendritic Spine Morphogenesis

Drebrin A knockdown in cultured neurons also impairs dendritic spine morphogenesis. Drebrin A knockdown significantly reduces the number and the width of dendritic spines (Takahashi et al. 2006). In consequence, drebrin A knockdown causes filopodia-like thin dendritic protrusions. Given that drebrin A is necessary for drebrin clustering and PSD-95 clustering, it has been suggested that drebrin clustering is crucial for the morphogenesis of mature dendritic spines with PSD structure. Together, the following model of how drebrin is involved in dendritic spine morphogenesis during development has been proposed (Fig. 10.5). At the early stage of synaptogenesis, dendrites are bristled with many dendritic filopodia in which drebrin is diffusely distributed (diffuse-type filopodia). Diffuse-type filopodia are supposed to be very dynamic with short-term life span. After diffuse-type filopodia

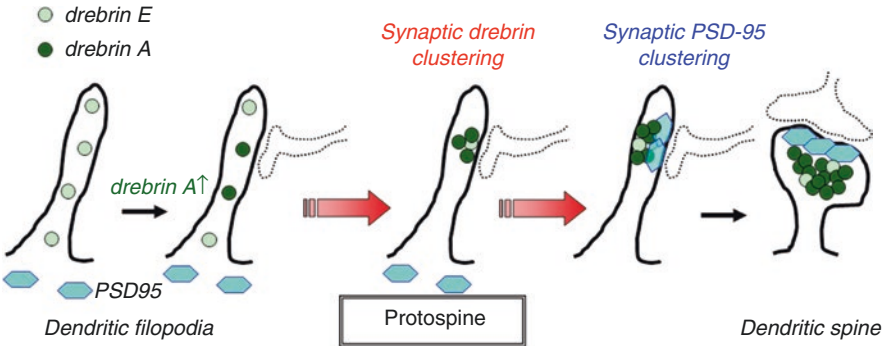

Fig. 10.5 Schematic summery of dendritic spine morphogenesis from dendritic filopodia. At early of synaptogenesis, drebrin E is gradually replaced by drebrin A, which is developmentally upregulated, in filopodia. Once a presynaptic terminal contacts with a filopodium, drebrin A is accumulated at nascent contact sites. At this stage, the filopodia with drebrin clusters (cluster-type filopodia) don't have PSD-95 clusters. Afterward, PSD-95 is further accumulated depending on drebrin clustering. Consequently, the filopodia with drebrin clusters transform into mature spines. Therefore, the filopodia that have drebrin clusters but not PSD-95 ones are suggested to be "protospines"

contact with an axon, drebrin is accumulated with F-actin at a postsynaptic site in the filopodia. As a result, the diffuse-type filopodia are transformed to be cluster type as a protospine. The drebrin clustering with F-action requires developmental upregulation of drebrin A expression. In the cluster-type filopodia, PSD-95 is then accumulated depending on the cluster formation of drebrin with F-actin. Finally, the cluster-type filopodia are transformed to mature dendritic spines, which contain both drebrin-based actin components and PSD structure (Fig. 10.5). Thus, the drebrin-F-actin complex anchors the postsynaptic components and governs the morphological maturation of dendritic spines.

10.2.6 Can Drebrin A Promote Dendritic Spine Morphogenesis?

The developmental upregulation of drebrin A is required for synaptic clustering of PSD-95 and dendritic spine morphogenesis during development. These findings suggest that forced expression of drebrin A in immature neurons might promote dendritic spine morphogenesis and/or synaptic clustering of PSD-95. However, the experiments of drebrin A overexpression do not support these possibilities (Mizui et al. 2005). The overexpression of drebrin A in immature hippocampal neurons from 7 to 9 DIV does not induce the morphologically mature types of dendritic spines. Instead, drebrin A overexpression induces extremely large dendritic

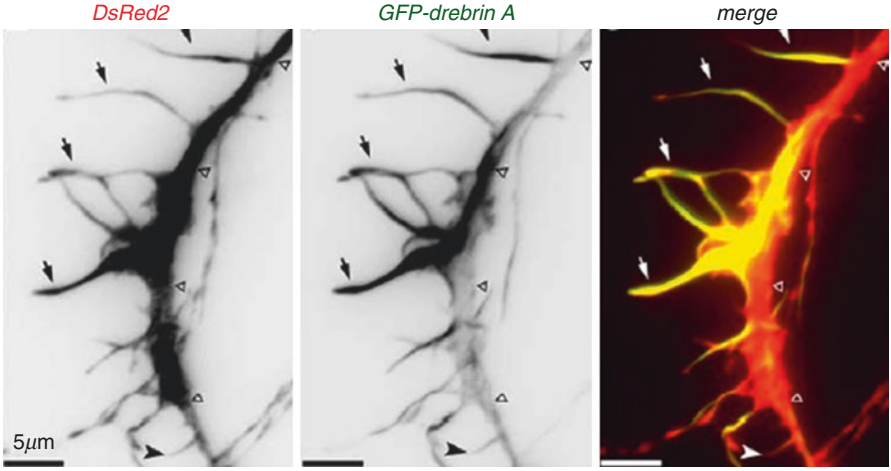

Fig. 10.6 "Megapodia" induced by drebrin A overexpression. GFP-tagged drebrin A vector was transfected to cultured hippocampal neuron at 7 days in vitro together with DsRed2 vector to visualize dendritic morphology. Neurons expressing GFP-drebrin A and DsRed2 were imaged 2 days after transfection. GFP-drebrin A (*green*) was accumulated in dendritic protrusions and formed large abnormal headless protrusions named "megapodia" (*arrow*). Scale bars: 5 μm. Adapted from Mizui et al. (2005) with permission

protrusions without head (Fig. 10.6). The large headless protrusions contain the large amount of drebrin A, F-actin, and PSD-95**.** However, drebrin A and PSD-95 are diffusely distributed and not clustered in the large protrusions. Further, the large protrusions induced by drebrin A do not contain MAP2, indicating that they do not correspond to the branch of dendrites. Thus, the large headless and F-actin-rich dendritic protrusions are called as "megapodia" meaning large filopodia (Fig. 10.6). The induction of megapodia by drebrin A overexpression suggests that drebrin A upregulation is necessary but not sufficient for spine morphogenesis. Given that drebrin clustering occurs locally at the axon-dendritic filopodia contact sites, it is possible that drebrin clustering is regulated by not only drebrin A upregulation but also by local signaling from presynaptic terminals such as the release of neurotransmitters and/or growth factors from presynaptic terminals and/or transsynaptic adhesion signals.

10.3 Activity-Dependent Modulation of Drebrin Dynamics at Dendritic Spines

Many previous studies have shown that synaptic activity regulates the formation, maintenance, and plasticity of dendritic spines (Kasai et al. 2010; Saneyoshi et al. 2010; Papa and Segal 1996; Nimchinsky et al. 2002), suggesting that drebrin-based actin cytoskeleton in dendritic spines is highly regulated by synaptic activity. In the following paragraphs, activity-dependent clustering and dynamics of drebrin will be discussed.

10.3.1 Synaptic Activity Controls Drebrin Clustering During Development

Pharmacological perturbation of neuronal activity has revealed the involvement of synaptic activity in drebrin clustering during development. Chronic treatment (8 to 15 DIV) of hippocampal neurons in cultures with tetrodotoxin, which blocks action potentials, significantly decreases the number of drebrin clusters along dendrites (Takahashi et al. 2009). This finding supports the idea that spontaneous synaptic activity positively regulates drebrin clustering during development. What if neuronal activity is increased? Chronic treatment with the $GABA_A$-receptor blocker picrotoxin, which enhances excitatory synaptic transmission, significantly increases the number of drebrin clusters (Takahashi et al. 2009). Thus, spontaneous synaptic activity, especially its excitatory components, promotes drebrin clustering. Given the phenotypes of drebrin A overexpression in immature neurons, these results also suggest that not only developmental upregulation of drebrin A but also spontaneous synaptic activity is necessary for drebrin clustering during development and consequently for dendritic spine morphogenesis.

10.3.2 AMPA Receptor Activity Regulates Synaptic Clustering of Drebrin

In the brains, excitatory synaptic transmission is generally mediated by presynaptically released glutamate and postsynaptic glutamate receptors. Pharmacological treatments with subtype-specific blockers of glutamate receptors have demonstrated that AMPA receptor activity is involved in drebrin clustering in spine morphogenesis (Takahashi et al. 2009) (Fig. 10.7). Cultured hippocampal neurons were chronically treated with subtype-specific blockers of glutamate receptors

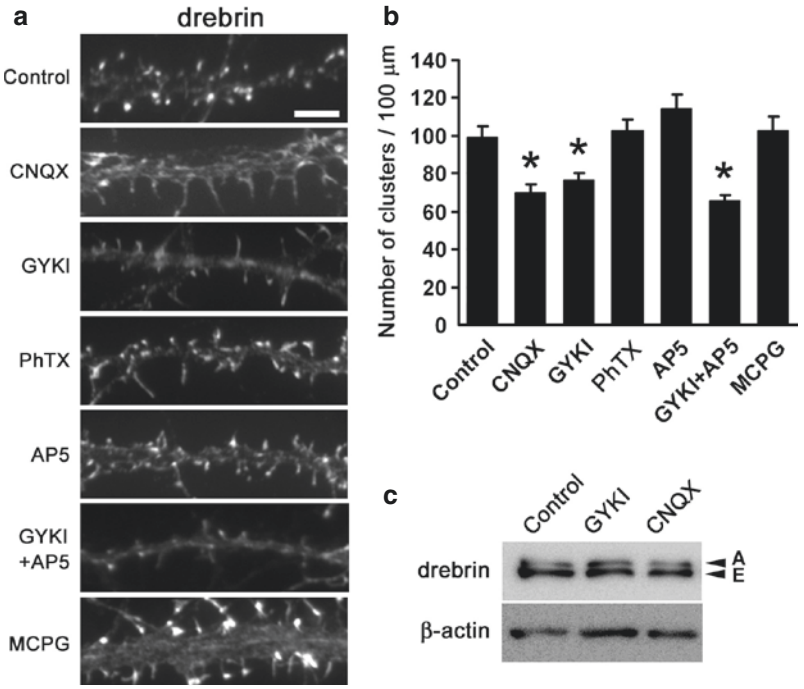

Fig. 10.7 The involvement of AMPA-type glutamate receptor (AMPAR) activity in drebrin clustering on dendritic spines. (**a**) Hippocampal neurons were treated with the following pharmacological agents from 8 days in vitro for 7 days: the AMPAR and kainite-receptor blocker CNQX (40 µM), the specific AMPAR blocker GYKI (10 µM), the Ca^{2+}-permeable (GluA2-subunit-lacking) AMPAR blocker philanthotoxin (PhTX, 10 µM), the NMDA-type glutamate receptor blocker AP5 (50 µM), GYKI (10 µM) plus AP5 (50 µM), and metabotropic glutamate receptor blocker MCPG (100 µM). Scale bar: 5 µm. (**b**) Quantitative analysis of drebrin clusters after these treatments. CNQX, GYKI, and GYKI plus AP5 treatments significantly suppressed drebrin clustering (*$P < 0.01$ versus control), indicating that Ca^{2+}-impermeable AMPAR regulates drebrin clustering. (**c**) Western blot data show that GYKI and CNQX treatments, blocking AMPAR or kainite receptor, do not affect the expression level of drebrin protein. Adapted from Takahashi et al. (2009) with permission

including (1) CNQX, a competitive inhibitor for AMPA receptors and kainite receptors; (2) GYKI, a noncompetitive inhibitor specific for AMPA receptors; (3) philanthotoxin, a blocker for only Ca^{2+}-permeable AMPA receptors; (4) AP5, an NMDA-type glutamate receptor blocker; and (5) MCPG, a selective blocker of metabotropic glutamate receptors. Interestingly, chronic treatment with CNQX or GYKI, which can block AMPA receptor activation, significantly diminishes drebrin clustering on dendrites. Philanthotoxin does not affect drebrin clustering, indicating that Ca^{2+}-impermeable AMPA receptors regulate drebrin clustering. Drebrin clustering is not affected by AP5 or MCPG. Thus, the activation of NMDA receptors and metabotropic glutamate receptors is dispensable for drebrin clustering. The suppression of drebrin clustering by AMPA receptor inhibition is not due to the alteration of drebrin expression level because the Western blot analysis has shown that the chronic treatment with CNQX and GYKI does not affect total expression level of drebrin protein (Fig. 10.7c). Further, according to double immunolabeling with drebrin and synapsin I, the chronic treatment with GYKI does not affect the clustering of synapsin I despite the decrease in drebrin cluster number (Takahashi et al. 2009). The selective diminishment of drebrin clustering is thus not due to secondary effects of impaired formation of presynaptic contact sites. According to more detailed analysis of drebrin clustering in dendritic filopodia by triple labeling for drebrin, synapsin I, and F-actin, the inhibition of AMPA receptor activation with GYKI suppresses drebrin clustering on dendritic protrusions, especially at synaptic sites, without affecting it on dendritic shafts. Notably, the enhancement of AMPA receptor activity with the AMPA receptor desensitization blocker, cyclothiazide (CTZ), increases total drebrin cluster density on dendritic protrusions in the presence of NMDA receptor blocker AP5 without affecting synapsin I cluster density (Takahashi et al. 2009). Together, the spontaneous activation of Ca^{2+}-impermeable (GluA2-containing) AMPA receptors specifically facilitates drebrin clustering on dendritic protrusions without affecting the formation of presynaptic contact sites.

10.3.3 Chronic Inhibition of AMPA Receptors Impairs Dendritic Spine Morphogenesis During Development

Given that AMPA receptors regulate drebrin clustering in dendritic protrusions and that drebrin clustering governs dendritic spine morphogenesis, it has been suggested that AMPA receptor activity also regulates spine morphogenesis during development through drebrin. As expected, chronic treatment of hippocampal neurons with AMPA receptor blocker GYKI indeed impairs spine morphogenesis and instead induces filopodia-like thin spines, some of which are malformed with Y-shaped or multibranched structures (Takahashi et al. 2009). In contrast, chronic treatment with

other blockers such NMDA receptor blocker AP5 or metabotropic glutamate receptor blocker MCPG does not significantly affect spine morphogenesis (Takahashi et al. 2009). Thus, AMPA receptor activity conducts normal spine morphogenesis presumably through regulating drebrin clustering in dendritic filopodia and spines. These results are consistent with those by using hippocampal slices in culture showing that chronic blockage of AMPA receptor activation reduces the density and length of dendritic spines in hippocampal CA1 pyramidal neurons (McKinney et al. 1999; Mateos et al. 2007). Further, chronic treatment with GYKI diminishes synaptic clustering of PSD-95 in dendritic spines but not that on dendritic shaft (Takahashi et al. 2009). These data also support a model by which spontaneous AMPA receptor activity induces drebrin clustering, which consequently promotes the formation of PSD-95 clusters in spines, thereby enabling the normal development of dendritic spines.

10.3.4 AMPA Receptor Activity Stabilizes Drebrin in Dendritic Spines

Activity-dependent clustering of drebrin would be due to the changes of drebrin dynamics in dendritic filopodia and spines. Fluorescent recovery after photobleaching (FRAP) analysis is a useful and widely used approach to examine the turnover of synaptic proteins within individual spines in real time. FRAP analysis using cultured hippocampal neurons transfected with GFP-tagged drebrin A (GFP-drebrin A) has revealed an intriguing activity-dependent turnover of drebrin in dendritic spines (Takahashi et al. 2009) (Fig. 10.8). Under normal physiological condition that allows spontaneous synaptic activity, GFP-drebrin A shows relatively dynamic turnover in dendritic spines. In spines at hippocampal neurons at 2 weeks in vitro, about 23% of drebrin is immobile as a stable fraction, whereas the rest of drebrin (about 75%) is mobile. The exchange rate of drebrin mobile fraction follows a time constant of approximately 6 min (Takahashi et al. 2009). Thus, about a quarter of total drebrin within a single spine is stabilized under spontaneous neuronal activity. In addition, FRAP analysis has demonstrated the interesting roles of AMPA receptor activity in drebrin turnover in dendritic spines. The treatment with CNQX decreases the stable fraction of GFP-drebrin A to approximately 9% without affecting the exchange rate. Conversely, the treatment with AMPA or CTZ in the presence of AP5, which enhances only AMPA receptor activity, increases the fraction of stable drebrin without changing its exchange rate (Takahashi et al. 2009). AP5 treatment itself has no effect on the stable drebrin fraction but decreases the time constant for drebrin turnover to about 4 min. These results indicate that the activity of AMPA receptors, but not that of NMDA receptors, contributes to stabilize drebrin in spines.

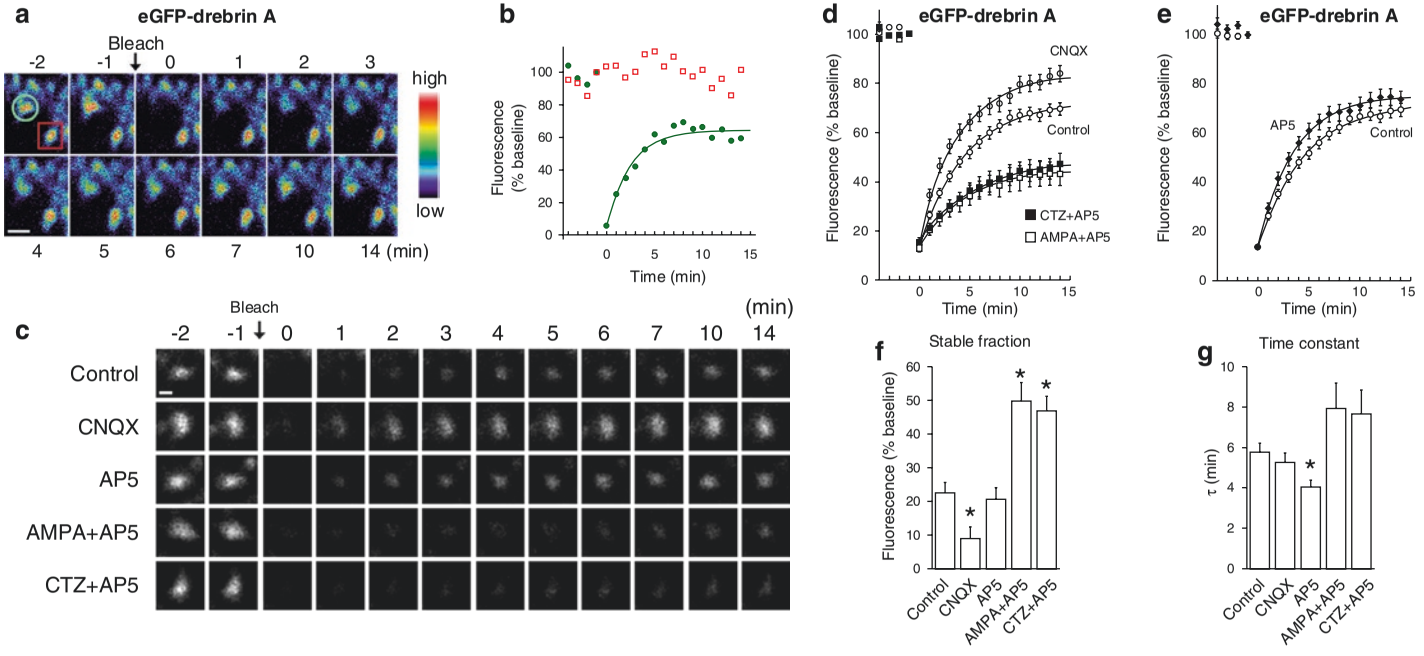

Fig.10.8 AMPAR activity increases stable fraction of drebrin in dendritic spines. (**a**) A GFP-drebrin A transfected neuron was analyzed by time-lapse imaging during fluorescence recovery after photobleaching (FRAP). GFP-drebrin A in a single spine (*green circle*) was selectively and completely photobleached by a small fast light pulse, whereas a neighboring cluster (*red square*) was unaffected. The subsequent images show gradual recovery of the GFP-drebrin A signal. Scale bar: 3 μm. (**b**) Normalized fluorescent intensity in the photobleached spine and the neighboring spine. The green curve was derived from the equation $F(t) = 1 - fs - fm*\exp(-t/\tau)$, where fs, fm, t, and τ are the stable fraction, dynamic fraction, time (minutes), and time constant, respectively. (**c**) The representative time-lapse sequences during FRAP of GFP-drebrin A of the vehicle control neurons and those in the presence of CNQX (40 μM), AP5 (100 μM), AMPA (1 μM) plus AP5 (100 μM), or CTZ (100 μM), a positive allosteric modulator of the AMPARs and kainate receptors (100 μM) plus AP5 (100 μM). Scale bar: 1 μm. (**d**, **e**) FRAP curves of drebrin in the indicated conditions. (**f**, **g**) The comparison of stable fraction (**f**) and time constant (**g**) of drebrin under each condition. AMPAR blockade by CNQX decreases the size of the stable drebrin fraction. By contrast, AMPAR activation by AMPA, or CTZ plus AP5, raises the stable drebrin fraction (**f**). Thus, AMPAR activity positively regulates the stable fraction of drebrin. NMDA receptor blockade by AP5 shortens the time constant of dynamic drebrin without affecting the stable drebrin fraction, indicating spontaneous NMDA receptor activity regulates the transport efficiency of mobile drebrin into spines (**g**). Adapted from Takahashi et al. (2009) with permission

10.3.5 An Increase in Stable Drebrin Is Accompanied by Increased Drebrin Clustering

An interesting question is whether the increase in the stable drebrin fraction corresponds with the increase in drebrin clustering. FRAP analysis of GFP-tagged actin has shown that short-term (15- to 30-min) treatment with cytochalasin D, an F-actin capping reagent, increases the stable fraction of actin in dendritic spines (Star et al. 2002). Short-term treatment with cytochalasin D to nonneuronal cells also induces the aggregation of drebrin-associated F-actin in the cytoplasm (Asada et al. 1994), presumably by changing the turnover of drebrin-F-actin complexes. These data suggest that cytochalasin D would be useful to examine what happens to drebrin clustering when the stable drebrin fraction is changed. Notably, FRAP analysis has shown that a 1-h cytochalasin D treatment increases stable drebrin fraction threefold (Takahashi et al. 2009). The short-term cytochalasin D treatment also promotes the clustering of drebrin with F-actin on dendrites (Takahashi et al. 2009). Thus, the increased fraction of stable drebrin is tightly accompanied by the enhancement of drebrin clusters. Given that AMPA receptor activity increases the stable fraction of drebrin in dendritic spines, AMPA receptor activity enhances drebrin stability in spines and consequently promotes drebrin clustering in spines. Taken together, AMPA receptor-dependent stabilization of drebrin in dendritic spines is an activity-dependent cellular mechanism underlying spine morphogenesis during development.

10.3.6 How Does AMPA Receptor-Mediated Drebrin Stabilization Contribute to Spine Morphogenesis and Plasticity?

According to previous studies on drebrin in nonneuronal cells (see also Chap. 6), drebrin-bound F-actin is resistant to cytochalasin D. Interestingly, F-actin in dendritic spines is also resistant to cytochalasin D. Given that dendritic spines contain co-clusters of drebrin and F-actin, these evidences suggest that drebrin stabilizes actin cytoskeleton in spines by providing their F-actin with cytochalasin D resistance. In addition, time-lapse imaging studies have shown that dendritic filopodia have short lifetime, whereas dendritic spines are maintained for a long term (Dailey and Smith 1996; Marrs et al. 2001; Grutzendler et al. 2002; Portera-Cailliau et al. 2003). Spine head volume is correlated with the amount of functional AMPA receptors (Matsuzaki et al. 2001). As drebrin contents are also correlated with spine head volume (Kobayashi et al. 2007), it has been further suggested that the rapid turnover of filopodia is due to an insufficient amount of stable drebrin, whereas spine persistence is due to an AMPA receptor-regulated abundance of stable drebrin. Thus, AMPA receptor-mediated drebrin stabilization may regulate the turnover of dendritic filopodia and spines and determine structure-function relationship of spines

by stabilizing their actin cytoskeleton. Importantly, AMPA receptor-mediated drebrin stabilization would also contribute to synaptic plasticity. The induction of long-term potentiation (LTP) is accompanied by the enlargement of spine head (Matsuzaki et al. 2004; Okamoto et al. 2004; Honkura et al. 2008) and synaptic accumulation of drebrin and F-actin in input-specific manner (Fukazawa et al. 2003), suggesting that AMPA receptor-mediated drebrin stabilization could be involved in the enlargement of stimulated spines. How do AMPA receptors increase stable drebrin? Channel activation of AMPA receptors not only induces ion influx but also activates intracellular signaling pathways such as mitogen-activated protein kinase (MAPK), protein tyrosine kinase Lyn, and Rho GTPase (Wang and Durkin 1995; Wang et al. 1997; Hayashi et al. 1999; Kim et al. 2004). Interestingly, a previous study has revealed that drebrin physically and functionally interacts with Ras GTPase in spine plasticity (Biou et al. 2008). Therefore, it would be interesting to address how AMPA receptor-induced signaling pathways mediate drebrin-based actin reorganization for spine formation, maintenance, and/or plasticity.

10.3.7 Impairment of Activity-Dependent Drebrin Clustering and Dendritic Spine Pathology

Abnormal spine morphology is accompanied by many neurodevelopmental disorders such as Fragile X syndrome and neurodegenerative diseases such as Alzheimer's disease (Hering and Sheng 2001; Fiala et al. 2002). It has been well established that drebrin downregulation is a pathognomonic feature of Down syndrome and Alzheimer's disease (Harigaya et al. 1996; Shim and Lubec 2002; Calon et al. 2004; Counts et al. 2006). Previous studies involving a mouse model of Alzheimer's disease have shown that a decrease in AMPA receptors at the synapse is followed by a decrease in the proportion of drebrin-positive spines (Chang et al. 2006; Mahadomrongkul et al. 2005). Normal spine morphogenesis is impaired by declustering of drebrin using drebrin A antisense oligonucleotides (Takahashi et al. 2003) and also by drebrin knockdown using RNA interference (Biou et al. 2008). Further AMPA receptor blockade results in malformed spines like filopodia-like thin spines with Y-shape and multibranched protrusions (Takahashi et al. 2009). It has been thus suggested that the impairment of AMPA receptor-mediated drebrin clustering in spines would be one of the pathogenesis of spine abnormality in the neurological conditions.

10.4 Drebrin Involvement in Activity-Dependent Synaptic Clustering of NMDA Receptors

It has been well known that NMDA receptor blockade induces synaptic accumulation of NMDA receptors as homeostatic scaling of NMDA receptors (Rao and Craig 1997; Perez-Otano and Ehlers 2005). In general, homeostatic synaptic plasticity is

supposed to be crucial for maintaining the excitability of neurons within the dynamic range (Turrigiano 2008). As described above, FRAP analysis has revealed that NMDA receptor blockade decreases the time constant of dynamic drebrin without affecting the stable drebrin fraction (Takahashi et al. 2009), indicating that spontaneous NMDA receptor activity regulates the transport efficiency of drebrin into spines. In the following, the involvement of drebrin in activity-dependent trafficking of NMDA receptors will be discussed.

10.4.1 Drebrin A Is Required for Activity-Dependent Synaptic Accumulation of NMDA Receptors

PSDs contain high concentrations of NMDA receptors as well as PSD-95 (Kennedy 2000). PSD-95 binds to the NMDA receptor subunits GluN2A and GluN2B (Niethammer et al. 1996). As described above, drebrin A upregulation is required for synaptic clustering of PSD-95. Further, the inhibition of NMDA receptors changes the exchange rate of mobile drebrin fraction (Takahashi et al. 2009). Therefore, it is possible that drebrin A regulates synaptic targeting of NMDA receptors via PSD-95. Originally, NMDA receptors are not highly clustered at synapses under the condition that allow spontaneous neuronal activity. Drebrin A knockdown by antisense oligonucleotides does not affect synaptic clustering of NMDA receptors in cultured hippocampal neurons under such condition. On the other hand, NMDA receptor blockage induces significant accumulation of NMDA receptors at synaptic sites (Rao and Craig 1997). Interestingly, drebrin A knockdown suppresses synaptic clustering of NMDA receptors induced by NMDA receptor blocker AP5 (Takahashi et al. 2006). Thus, drebrin A knockdown selectively blocks activity-dependent synaptic clustering of NMDA receptors (Fig. 10.9). Curiously, drebrin A knockdown increases PSD-95 clustering on dendrites when neurons are treated with AP5 (Takahashi et al. 2006), suggesting that synaptic clustering of NMDA receptors accelerated by AP5 treatment is independent of the molecular interaction between PSD-95 and NMDA receptors. Previous studies have demonstrated the involvement of cAMP-dependent protein kinase (PKA) in the activity-dependent synaptic clustering of NMDA receptors (Crump et al. 2001). Notably, PKA activation by IBMX, which inhibits cAMP phosphodiesterase and consequently increases cAMP levels to activate PKA, is sufficient to induce synaptic clustering of NMDA receptors (Crump et al. 2001). Thus, it would be interesting to examine whether and how PKA regulates drebrin clustering and dynamics, e.g., through drebrin phosphorylation. As described above, FRAP analysis has shown that NMDA receptor activity controls the exchange rate of mobile drebrin fraction without affecting stable drebrin fraction. Therefore, it has been suggested that mobile drebrin fraction contributes to the transport of NMDA receptors into spines.

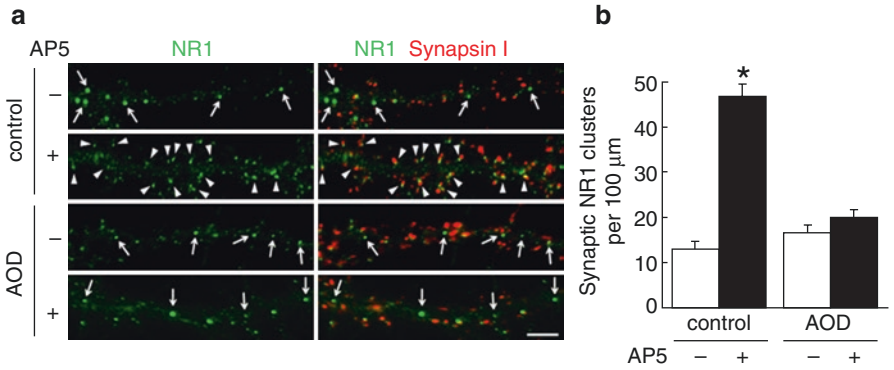

Fig. 10.9 The suppression of drebrin A expression inhibits activity-dependent synaptic targeting of NMDA-type glutamate receptor (NMDAR). (**a**) Cultured hippocampal neurons were treated with antisense oligonucleotides against drebrin A-specific exon (AOD) at 12 days in vitro for 2 days and further incubated in the medium with or without 50 μM NMDAR blocker AP5 for additional 2 days. The neurons were then immunostained for common NMDAR subunit GluN1 (*green*) and synapsin I (*red*). In control neurons, AP5 treatment increased synaptic clusters of GluN1 (NR1) associated with synapsin I clusters (*second top*, *arrowheads*). In contrast, in AOD-treated neurons, AP5 treatment had no significant effect on GluN1 clustering (*bottom*). *Arrows* in all panels indicate non-synaptic GluN1 clusters. Scale bar, 5 μm. (**b**) Quantitative analysis of total and synaptic NMDAR clusters. Note that AP5 enhances NMDAR clustering in the control neurons, whereas AP5 does not enhance it in drebrin A knockdown neurons. Adapted from Takahashi et al. (2009) with permission

10.4.2 In Vivo Role of Drebrin A in Activity-Dependent Synaptic Targeting of NMDA Receptors

The involvement of drebrin A in activity-dependent synaptic targeting of NMDA receptors has been also demonstrated by the analysis of drebrin A knockout (DAKO) mice. As only unique exon (exon 12a) of drebrin gene is deleted in DAKO mice, DAKO mice lose drebrin A but retain drebrin E, thus maintaining same expression level of total drebrin to wild-type mice (Aoki et al. 2009). In wild-type mice, the in vivo application of AP5 to cortical surface induces rapid accumulation of NMDA receptors in dendritic spines within 30 min. In contrast, the AP5 treatment of DAKO mice does not induce NMDA receptor accumulation in spines (Aoki et al. 2009). These data indicate that drebrin A is involved in the rapid form of activity-dependent homeostatic accumulation (synaptic scaling) of NMDA receptors in dendritic spines of adult cortex in vivo. Similar to in vitro results, the basal level of NMDA receptors in spines in DAKO mice is not significantly different from that in wild-type mice (Aoki et al. 2009), supporting that drebrin A is dispensable for basal targeting of NMDA receptors in dendritic spines.

10.5 Conclusion

Drebrin clustering is crucial for dendritic spine morphogenesis and synaptic clustering of PSD-95 during development. Drebrin clustering requires not only the developmental upregulation of drebrin A but also excitatory synaptic activity mediated by AMPA receptors. AMPA receptor activity promotes drebrin clustering by increasing the stable drebrin faction in dendritic spines. In contrast, NMDA receptor activity controls the turnover speed of mobile drebrin. Further, drebrin A is required for activity-dependent synaptic targeting of NMDA receptors but not for their basal targeting. These findings highlight the pivotal roles of drebrin on dendritic spine formation and synaptic plasticity.

References

Allison DW, Chervin AS, Gelfand VI, Craig AM (2000) Postsynaptic scaffolds of excitatory and inhibitory synapses in hippocampal neurons: maintenance of core components independent of actin filaments and microtubules. J Neurosci 20(12):4545–4554

Aoki C, Kojima N, Sabaliauskas N, Shah L, Ahmed TH, Oakford J, Ahmed T, Yamazaki H, Hanamura K, Shirao T (2009) Drebrin a knockout eliminates the rapid form of homeostatic synaptic plasticity at excitatory synapses of intact adult cerebral cortex. J Comp Neurol 517(1):105–121. doi:10.1002/cne.22137

Araya R, Jiang J, Eisenthal KB, Yuste R (2006) The spine neck filters membrane potentials. Proc Natl Acad Sci U S A 103(47):17961–17966. doi:10.1073/pnas.0608755103

Asada H, Uyemura K, Shirao T (1994) Actin-binding protein, drebrin, accumulates in submembranous regions in parallel with neuronal differentiation. J Neurosci Res 38(2):149–159. doi:10.1002/jnr.490380205

Biou V, Brinkhaus H, Malenka RC, Matus A (2008) Interactions between drebrin and Ras regulate dendritic spine plasticity. Eur J Neurosci 27(11):2847–2859. doi:10.1111/j.1460-9568.2008.06269.x

Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, Rostaing P, Triller A, Salem N Jr, Ashe KH, Frautschy SA, Cole GM (2004) Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. Neuron 43(5):633–645. doi:10.1016/j.neuron.2004.08.013

Chang EH, Savage MJ, Flood DG, Thomas JM, Levy RB, Mahadomrongkul V, Shirao T, Aoki C, Huerta PT (2006) AMPA receptor downscaling at the onset of Alzheimer's disease pathology in double knockin mice. Proc Natl Acad Sci U S A 103(9):3410–3415. doi:10.1073/pnas.0507313103

Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS, Nicoll RA (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature 408(6815):936–943. doi:10.1038/35050030

Cho KO, Hunt CA, Kennedy MB (1992) The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. Neuron 9(5):929–942

Cohen RS, Chung SK, Pfaff DW (1985) Immunocytochemical localization of actin in dendritic spines of the cerebral cortex using colloidal gold as a probe. Cell Mol Neurobiol 5(3):271–284

Counts SE, Nadeem M, Lad SP, Wuu J, Mufson EJ (2006) Differential expression of synaptic proteins in the frontal and temporal cortex of elderly subjects with mild cognitive impairment. J Neuropathol Exp Neurol 65(6):592–601

Crump FT, Dillman KS, Craig AM (2001) cAMP-dependent protein kinase mediates activity-regulated synaptic targeting of NMDA receptors. J Neurosci 21(14):5079–5088

Dailey ME, Smith SJ (1996) The dynamics of dendritic structure in developing hippocampal slices. J Neurosci 16(9):2983–2994

DeFelipe J (2015) The dendritic spine story: an intriguing process of discovery. Front Neuroanat 9:14. doi:10.3389/fnana.2015.00014

Dunaevsky A, Tashiro A, Majewska A, Mason C, Yuste R (1999) Developmental regulation of spine motility in the mammalian central nervous system. Proc Natl Acad Sci U S A 96(23):13438–13443

Ebrahimi S, Okabe S (2014) Structural dynamics of dendritic spines: molecular composition, geometry and functional regulation. Biochim Biophys Acta 1838(10):2391–2398. doi:10.1016/j.bbamem.2014.06.002

El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, Bredt DS (2000) PSD-95 involvement in maturation of excitatory synapses. Science 290(5495):1364–1368

Fiala JC, Feinberg M, Popov V, Harris KM (1998) Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J Neurosci 18(21):8900–8911

Fiala JC, Spacek J, Harris KM (2002) Dendritic spine pathology: cause or consequence of neurological disorders? Brain Res Brain Res Rev 39(1):29–54

Fischer M, Kaech S, Knutti D, Matus A (1998) Rapid actin-based plasticity in dendritic spines. Neuron 20(5):847–854

Fischer M, Kaech S, Wagner U, Brinkhaus H, Matus A (2000) Glutamate receptors regulate actin-based plasticity in dendritic spines. Nat Neurosci 3(9):887–894. doi:10.1038/78791

Fukazawa Y, Saitoh Y, Ozawa F, Ohta Y, Mizuno K, Inokuchi K (2003) Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. Neuron 38(3):447–460

Grutzendler J, Kasthuri N, Gan WB (2002) Long-term dendritic spine stability in the adult cortex. Nature 420(6917):812–816. doi:10.1038/nature01276

Harigaya Y, Shoji M, Shirao T, Hirai S (1996) Disappearance of actin-binding protein, drebrin, from hippocampal synapses in Alzheimer's disease. J Neurosci Res 43(1):87–92. doi:10.1002/jnr.490430111

Harris KM, Kater SB (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. Annu Rev Neurosci 17:341–371. doi:10.1146/annurev.ne.17.030194.002013

Hayashi K, Suzuki K, Shirao T (1998) Rapid conversion of drebrin isoforms during synapse formation in primary culture of cortical neurons. Brain Res Dev Brain Res 111(1):137–141

Hayashi T, Umemori H, Mishina M, Yamamoto T (1999) The AMPA receptor interacts with and signals through the protein tyrosine kinase Lyn. Nature 397(6714):72–76. doi:10.1038/16269

Hering H, Sheng M (2001) Dendritic spines: structure, dynamics and regulation. Nat Rev Neurosci 2(12):880–888. doi:10.1038/35104061

Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GC, Kasai H (2008) The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron 57(5):719–729. doi:10.1016/j.neuron.2008.01.013

Kabsch W, Vandekerckhove J (1992) Structure and function of actin. Annu Rev Biophys Biomol Struct 21:49–76. doi:10.1146/annurev.bb.21.060192.000405

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure-stability-function relationships of dendritic spines. Trends Neurosci 26(7):360–368. doi:10.1016/S0166-2236(03)00162-0

Kasai H, Fukuda M, Watanabe S, Hayashi-Takagi A, Noguchi J (2010) Structural dynamics of dendritic spines in memory and cognition. Trends Neurosci 33(3):121–129. doi:10.1016/j.tins.2010.01.001

Kennedy MB (1997) The postsynaptic density at glutamatergic synapses. Trends Neurosci 20(6):264–268

Kennedy MB (2000) Signal-processing machines at the postsynaptic density. Science 290(5492):750–754

Kim E, Sheng M (2004) PDZ domain proteins of synapses. Nat Rev Neurosci 5(10):771–781. doi:10.1038/nrn1517

Kim E, Niethammer M, Rothschild A, Jan YN, Sheng M (1995) Clustering of Shaker-type K+ channels by interaction with a family of membrane-associated guanylate kinases. Nature 378(6552):85–88. doi:10.1038/378085a0

Kim SJ, Jeon S, Shin EY, Kim EG, Park J, Bae CD (2004) AMPA, not NMDA, activates RhoA GTPases and subsequently phosphorylates moesin. Exp Mol Med 36(1):98–102. doi:10.1038/emm.2004.14

Kistner U, Wenzel BM, Veh RW, Cases-Langhoff C, Garner AM, Appeltauer U, Voss B, Gundelfinger ED, Garner CC (1993) SAP90, a rat presynaptic protein related to the product of the Drosophila tumor suppressor gene dlg-A. J Biol Chem 268(7):4580–4583

Kobayashi C, Aoki C, Kojima N, Yamazaki H, Shirao T (2007) Drebrin a content correlates with spine head size in the adult mouse cerebral cortex. J Comp Neurol 503(5):618–626. doi:10.1002/cne.21408

Landis DM, Reese TS (1983) Cytoplasmic organization in cerebellar dendritic spines. J Cell Biol 97(4):1169–1178

Mahadomrongkul V, Huerta PT, Shirao T, Aoki C (2005) Stability of the distribution of spines containing drebrin A in the sensory cortex layer I of mice expressing mutated APP and PS1 genes. Brain Res 1064(1-2):66–74. doi:10.1016/j.brainres.2005.10.012

Maletic-Savatic M, Malinow R, Svoboda K (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. Science 283(5409):1923–1927

Marrs GS, Green SH, Dailey ME (2001) Rapid formation and remodeling of postsynaptic densities in developing dendrites. Nat Neurosci 4(10):1006–1013. doi:10.1038/nn717

Mateos JM, Luthi A, Savic N, Stierli B, Streit P, Gahwiler BH, McKinney RA (2007) Synaptic modifications at the CA3-CA1 synapse after chronic AMPA receptor blockade in rat hippocampal slices. J Physiol 581(Pt 1):129–138. doi:10.1113/jphysiol.2006.120550

Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci 4(11):1086–1092. doi:10.1038/nn736

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature 429(6993):761–766. doi:10.1038/nature02617

Matus A, Ackermann M, Pehling G, Byers HR, Fujiwara K (1982) High actin concentrations in brain dendritic spines and postsynaptic densities. Proc Natl Acad Sci U S A 79(23):7590–7594

McKinney RA, Capogna M, Durr R, Gahwiler BH, Thompson SM (1999) Miniature synaptic events maintain dendritic spines via AMPA receptor activation. Nat Neurosci 2(1):44–49. doi:10.1038/4548

Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, Grant SG (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature 396(6710):433–439. doi:10.1038/24790

Mizui T, Takahashi H, Sekino Y, Shirao T (2005) Overexpression of drebrin A in immature neurons induces the accumulation of F-actin and PSD-95 into dendritic filopodia, and the formation of large abnormal protrusions. Mol Cell Neurosci 30(4):630–638

Niethammer M, Kim E, Sheng M (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci 16(7):2157–2163

Nimchinsky EA, Sabatini BL, Svoboda K (2002) Structure and function of dendritic spines. Annu Rev Physiol 64:313–353. doi:10.1146/annurev.physiol.64.081501.160008

Okabe S, Miwa A, Okado H (2001) Spine formation and correlated assembly of presynaptic and postsynaptic molecules. J Neurosci 21(16):6105–6114

Okamoto K, Nagai T, Miyawaki A, Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat Neurosci 7(10):1104–1112. doi:10.1038/nn1311

Papa M, Segal M (1996) Morphological plasticity in dendritic spines of cultured hippocampal neurons. Neuroscience 71(4):1005–1011
Papa M, Bundman MC, Greenberger V, Segal M (1995) Morphological analysis of dendritic spine development in primary cultures of hippocampal neurons. J Neurosci 15(1 Pt 1):1–11
Perez-Otano I, Ehlers MD (2005) Homeostatic plasticity and NMDA receptor trafficking. Trends Neurosci 28(5):229–238. doi:10.1016/j.tins.2005.03.004
Pollard TD, Borisy GG (2003) Cellular motility driven by assembly and disassembly of actin filaments. Cell 112(4):453–465
Portera-Cailliau C, Pan DT, Yuste R (2003) Activity-regulated dynamic behavior of early dendritic protrusions: evidence for different types of dendritic filopodia. J Neurosci 23(18):7129–7142
Rao A, Craig AM (1997) Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. Neuron 19(4):801–812
Rochefort NL, Konnerth A (2012) Dendritic spines: from structure to in vivo function. EMBO Rep 13(8):699–708. doi:10.1038/embor.2012.102
Sala C, Piech V, Wilson NR, Passafaro M, Liu G, Sheng M (2001) Regulation of dendritic spine morphology and synaptic function by Shank and Homer. Neuron 31(1):115–130
Saneyoshi T, Fortin DA, Soderling TR (2010) Regulation of spine and synapse formation by activity-dependent intracellular signaling pathways. Curr Opin Neurobiol 20(1):108–115. doi:10.1016/j.conb.2009.09.013
Sekino Y, Kojima N, Shirao T (2007) Role of actin cytoskeleton in dendritic spine morphogenesis. Neurochem Int 51(2–4):92–104. doi:10.1016/j.neuint.2007.04.029
Shim KS, Lubec G (2002) Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer's disease and Down syndrome. Neurosci Lett 324(3):209–212
Shirao T, González-Billault C (2013) Actin filaments and microtubules in dendritic spines. J Neurochem 126(2):155–164
Star EN, Kwiatkowski DJ, Murthy VN (2002) Rapid turnover of actin in dendritic spines and its regulation by activity. Nat Neurosci 5(3):239–246. doi:10.1038/nn811
Svitkina TM (2013) Ultrastructure of protrusive actin filament arrays. Curr Opin Cell Biol 25(5):574–581. doi:10.1016/j.ceb.2013.04.003
Takahashi H, Sekino Y, Tanaka S, Mizui T, Kishi S, Shirao T (2003) Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. J Neurosci 23(16):6586–6595
Takahashi H, Mizui T, Shirao T (2006) Down-regulation of drebrin A expression suppresses synaptic targeting of NMDA receptors in developing hippocampal neurones. J Neurochem 97(Suppl 1):110–115. doi:10.1111/j.1471-4159.2005.03536.x
Takahashi H, Yamazaki H, Hanamura K, Sekino Y, Shirao T (2009) Activity of the AMPA receptor regulates drebrin stabilization in dendritic spine morphogenesis. J Cell Sci 122(Pt 8):1211–1219. doi:10.1242/jcs.043729
Takumi Y, Ramirez-Leon V, Laake P, Rinvik E, Ottersen OP (1999) Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. Nat Neurosci 2(7):618–624. doi:10.1038/10172
Tomita S, Fukata M, Nicoll RA, Bredt DS (2004) Dynamic interaction of stargazin-like TARPs with cycling AMPA receptors at synapses. Science 303(5663):1508–1511. doi:10.1126/science.1090262
Tomita S, Adesnik H, Sekiguchi M, Zhang W, Wada K, Howe JR, Nicoll RA, Bredt DS (2005) Stargazin modulates AMPA receptor gating and trafficking by distinct domains. Nature 435(7045):1052–1058. doi:10.1038/nature03624
Turrigiano GG (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135(3):422–435. doi:10.1016/j.cell.2008.10.008
Wang Y, Durkin JP (1995) alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, but not N-methyl-D-aspartate, activates mitogen-activated protein kinase through G-protein beta gamma subunits in rat cortical neurons. J Biol Chem 270(39):22783–22787

Wang Y, Small DL, Stanimirovic DB, Morley P, Durkin JP (1997) AMPA receptor-mediated regulation of a Gi-protein in cortical neurons. Nature 389(6650):502–504. doi:10.1038/39062

Yoshihara Y, De Roo M, Muller D (2009) Dendritic spine formation and stabilization. Curr Opin Neurobiol 19(2):146–153. doi:10.1016/j.conb.2009.05.013

Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 44(5):749–757. doi:10.1016/j.neuron.2004.11.011

Ziv NE, Smith SJ (1996) Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. Neuron 17(1):91–102