Chapter 14
Homer, Spikar, and Other Drebrin-Binding Proteins in the Brain

Hiroyuki Yamazaki and Tomoaki Shirao

Abstract Drebrin is a major F-actin-binding protein in the brain. In the past two decades, many drebrin-binding proteins in addition to F-actin have been identified in several research fields including neuroscience, oncology, and immunology. Among the drebrin-binding proteins, there are various kinds of proteins including scaffold proteins, nuclear proteins, phosphatases, microtubule-binding proteins, G-actin-binding proteins, gap junction proteins, chemokine receptors, and cell-adhesion-related proteins. The interaction between drebrin and its binding partners seems to play important roles in higher brain functions, because drebrin is involved in the pathogenesis of some neurological diseases with cognitive defects. In this chapter, we will first review the interaction of Homer and spikar with drebrin, particularly focusing on spine morphogenesis and synaptic function. Homer contributes to spine morphogenesis by cooperating with shank and activated Cdc42 small GTPase, suggesting a novel signaling pathway comprising Homer, drebrin, shank, and Cdc42 for spine morphogenesis. Drebrin sequesters spikar in the cytoplasm and stabilizes it in dendritic spines, leading to spine formation. Finally, we will introduce some other drebrin-binding proteins including end-binding protein 3 (EB3), profilin, progranulin, and phosphatase and tensin homologue (PTEN). These proteins are involved in Alzheimer's disease and cancer. Therefore, further studies on drebrin and its binding proteins will be of great importance to elucidate the pathologies of various diseases and may contribute to their medical treatment and diagnostics development.

Keywords Homer • Spikar/ZMYND8 • EB3 • Profilin • Progranulin • PTEN • Ras • Dendritic spine • Drebrin-binding protein

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T. Shirao, Y. Sekino (eds.), *Drebrin*, Advances in Experimental Medicine and Biology 1006, DOI 10.1007/978-4-431-56550-5_14

14.1 Introduction

Drebrin is a typical F-actin-binding protein in the dendritic spines in the brain of vertebrates. Additionally, flies and nematodes express a drebrin-like protein, although they contain a characteristic SH3 domain in the C-terminal region (Butkevich et al. 2015). They may be considered partly homologues to drebrin and mouse actin-binding protein 1 (mAbp1/SH3P7) (Lappalainen et al. 1998; Yamazaki et al. 2001). Therefore, the interaction between drebrin and its binding partners seems critical for higher brain functions such as learning and memory, which are observed in vertebrates. We elucidated various drebrin characters as an F-actin-binding protein between the 1980s and the 1990s (Shirao and Obata 1985; Ishikawa et al. 1994); however, there have only been a few reports about drebrin-binding proteins, even after the discovery that drebrin is a component of the actin cytoskeleton. In 1996, we found myosin and gelsolin in the drebrin-bound actin complex using co-immunoprecipitation analysis with anti-drebrin antibody (mAb M2F6) (Hayashi et al. 1996). Soon after, Mammoto et al. have found that the G-actin-binding protein, profilin, binds to drebrin (Mammoto et al. 1998). In the twenty-first century, the identification reports of novel drebrin-binding proteins including Homer and spikar rapidly increased (Fig. 14.1). Homer is involved in various

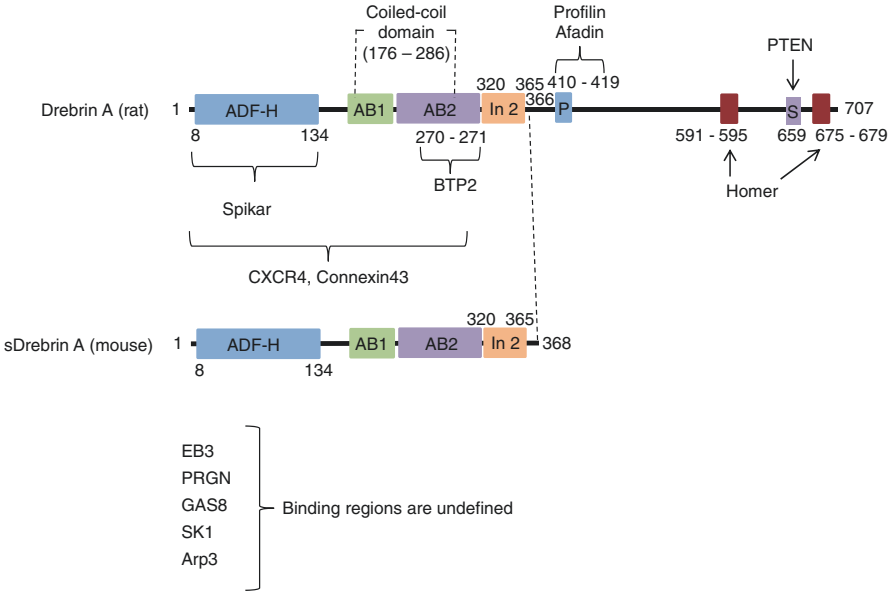

Fig. 14.1 Schematic representation of drebrin and the binding sites of drebrin-binding proteins. Spikar binds to the ADF-H domain in the N-terminal region. Homer binds to the conserved sequence, PPXXF, in the C-terminal region. Profilin and afadin bind to the proline-rich region. CXCR4 binds to the N-terminal region (1–271). PTEN regulates the phosphorylation of Ser 659 of rat drebrin A (Ser 647 in the human homologue) by activity-dependent phosphatase activity. The binding sites on drebrin of EB3, connexin 43, progranulin, GAS8, SK1, and Arp3 are unknown. *ADF-H* ADF homology domain, *AB*1 Actin-binding region 1, *AB*2 Actin-binding region 2, *In*2 Ins 2 (drebrin A-specific sequence), *P* Proline-rich region

brain functions including synaptic functions, neuronal development, neurological diseases, and behavior (for review, see Szumlinski et al. 2006; Foa and Gasperini 2009; Pouliquin and Dulhunty 2009; Luo et al. 2012). Spikar is a transcription cofactor that is also involved in dendritic spine formation. We identified spikar as a drebrin-binding protein (Yamazaki et al. 2014), which was registered in the DDBJ/EMBL/GenBank databases in 2001. In this chapter, we first focus on Homer and spikar and introduce their function in synapse formation. In the latter half, we introduce other drebrin-binding proteins reported in the past two decades and discuss the relationship and implications of their interactions regarding brain functions and disorders.

14.2 Homer

Homer protein family members are encoded by three genes (*Homer1*, *2* and *3*), and many splicing variants have been identified (Shiraishi-Yamaguchi and Furuichi 2007). The subtypes are also called Vesl, PSD-Zip45, and Cupidin. Each Homer gene expresses long- and short-type Homers; the long types are Homer1b–d, Homer2a and b, and Homer3a and b, and the short types are Homer1a (Vesl-1s), Homer2c and d, and Homer3c and d. Short-type Homers lack self-assembly region, which contains coiled-coil domain and leucine zipper motif. Homer1a is a short-type protein that was originally identified as an immediate early gene induced by seizures in rat hippocampus (Kato et al. 1997; Brakeman et al. 1997). Homer1a protein was also isolated as a metabotropic glutamate receptor (mGluR)-binding protein during the same period (Brakeman et al. 1997). Since then, several binding partners of Homer have been identified. It has been elucidated that the Ena/VASP homology 1 (EVH1) domain of Homer recognizes a proline-rich consensus sequence (PPXXF or FPPPP) in several proteins including mGluR, Shank, inositol trisphosphate receptor (IP3R), transient receptor potential canonical (TRPC) channels, dynamin3, and drebrin (Xiao et al. 1998; Tu et al. 1999, 1998; Yuan et al. 2003; Gray et al. 2003; Shiraishi et al. 2003). Homer forms a mesh-like matrix structure with shank, which causes spine enlargement and spine recruitment of several proteins including IP3R, postsynaptic density (PSD)-95, F-actin, guanylate kinase-associated protein (GKAP), and glutamate receptors (Sala et al. 2001; Hayashi et al. 2009).

The drebrin E and drebrin A isoforms, both of which have two PPXXF motifs in their C-terminal region, have been shown to bind to Homer2b (Shiraishi-Yamaguchi et al. 2009). Double mutation of the PPXXF motifs causes loss of the Homer2b-binding activity of drebrin, although it is not clear which motif is crucial for the drebrin-Homer2b binding (Shiraishi-Yamaguchi et al. 2009). The long subtype Homer proteins form an intermolecular disulfide bond between cysteine residues of C-terminal coiled-coil domain by oxidation, resulting in dimerization (Nepliouev et al. 2011). Disruption of the disulfide bond with a reducing reagent such as dithiothreitol (DTT) attenuates the binding of Homer and drebrin (Nepliouev et al. 2011). Another assay using Homer1a showed that either of the drebrin mutants, F543A or F621A, could disrupt the interaction between drebrin and Homer1a (Nepliouev et al. 2011), indicating that both of the PPXXF motifs of drebrin bind to Homer.

Thus, it has been suggested that drebrin can bind to the long subtype Homer dimers more efficiently than to the short subtype Homer. Because the EVH1 domains of Homer are highly conserved between Homer family proteins, drebrin is supposed to be able to bind to Homer3 as well. Conversely, the brain-specific short-type drebrin isoform, s-drebrin A, contains actin-binding regions but no PPXXF motif (Jin et al. 2002), suggesting that s-drebrin A regulates the actin cytoskeleton in the spine without being implicated in a Homer-protein complex (Fig. 14.1).

What is the physiological role of the interactions between drebrin and Homer? Although Homer can bind to several PSD proteins, it localizes not only at the PSD but also in the cytoplasmic area of dendritic spines (Shiraishi et al. 2004), where it seems to interact with IP3R on the endoplasmic reticulum (ER). Drebrin is concentrated in the cytoplasmic area of dendritic spines rather than in the PSD area (Kobayashi et al. 2007). Thus, drebrin may interact with Homer in the center of the spines. Drebrin attenuates the interaction between actin and myosin V (Ishikawa et al. 2007), and myosin Va, concentrated at the tip of the ER tubule, plays a pivotal role in the ER targeting. Therefore, it has been suggested that drebrin is involved in the localization of the Homer-IP3R-ER complex at the center of dendritic spines (Fig. 14.2).

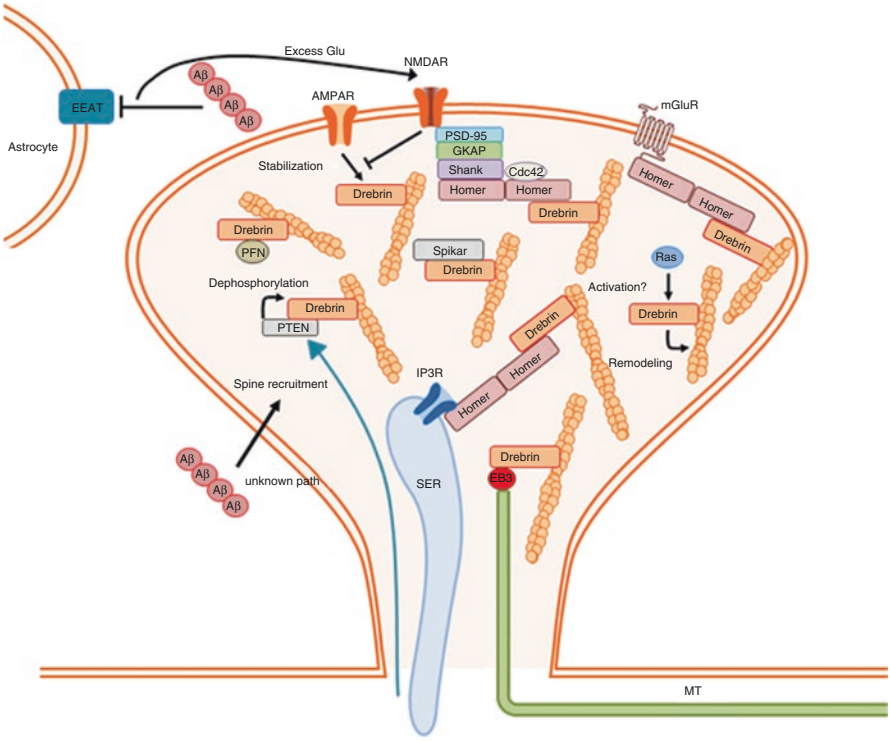

Fig. 14.2 Schematic diagram of presumable drebrin-complex and the signaling mechanism. Amyloid-beta recruits PTEN into dendritic spine, and then PTEN dephosphorylates drebrin. Ca^{2+} influx through NMDA receptor induces drebrin exodus that is the translocation of drebrin-F-actin complex from spine to dendrite (Mizui et al. 2014). In contrast, AMPA-receptor activity stabilizes drebrin within spines. Biou et al. (2008) suggested that drebrin remodels F-actin in the downstream of Ras signaling. Drebrin-EB3 complex plays a role as an adaptor between F-actin and microtubules

Drebrin is known to be involved in spine morphogenesis and formation (Takahashi et al. 2003; Ivanov et al. 2009). Homer2 interacts with activated Cdc42, and a Homer2 mutant lacking the Cdc42-binding region impaired synapse formation and function (Shiraishi-Yamaguchi et al. 2009). Cdc42 and its regulators such as guanine nucleotide exchange factors (GEFs) play important roles in spine morphogenesis and formation (Moon and Zheng 2003; Chen et al. 2012). The Cdc42-binding region of Homer is separate from the EVH1 domain, which may enable drebrin association with the Homer-Cdc42 complex. These data raise the possibility of a novel signaling pathway for spine morphogenesis involving drebrin and activated small G proteins.

14.3 Spikar/ZMYND8

Spikar (also called KIAA1125, CTCL, tumor antigen Se14-3, ZMYND8, Prkcbp1, or RACK7) was first isolated as a drebrin-binding protein by a yeast two-hybrid screening of a rat brain library (Yamazaki et al. 2014). Previously, this gene was reported as a protein kinase C-binding protein (Fossey et al. 2000). Many splice variants of spikar are found in various database sites including NCBI. We have classified spikar into three major isoforms: spikar A, spikar B, and spikar delta-C (Fig. 14.3). Spikar A is similar to spikar B but lacks 20 residues in the N-terminal and 28 residues in the C-terminal region. Spikar delta-C lacks the C-terminal region of spikar A/B and contains a specific sequence in its C-terminal. There are no reports about the function of spikar delta-C so far.

Spikar contains a PHD domain, a bromodomain, a PWWP domain, a MYND domain, and a nuclear-receptor recognition motif (LSYLL) (Fig. 14.3). The PHD

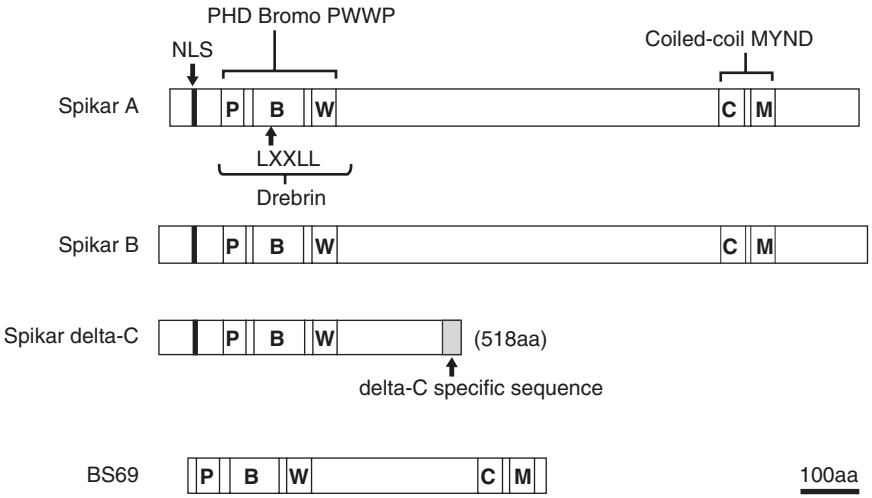

Fig. 14.3 Schematic diagram of spikar isoforms and BS69. *P* PHD domain, *Bro* Bromodomain, *W* PWWP domain, *C* Coiled-coil, *M* MYND domain

domain consists of two zinc fingers, and many PHD-containing proteins associate with chromatin and regulate its activities (Bienz 2006; Baker et al. 2008). The bromodomain associates with acetylated lysine residues found in the C-terminal region of histones (Sanchez and Zhou 2009). Bromodomain-containing proteins are associated with obesity, inflammation, cancer, and neurological disorders (Padmanabhan et al. 2016; Sanchez and Zhou 2009; Belkina and Denis 2012) and have become new therapeutic targets for these diseases. The nuclear-receptor recognition motif of spikar in the bromodomain binds to nuclear receptors including thyroid hormones and steroid receptors (Savkur and Burris 2004). The PWWP domain is named after a conserved Pro-Trp-Trp-Pro motif; however, the first three amino acids are not strictly conserved (Stec et al. 2000; Qin and Min 2014). The PWWP motif of spikar is Pro-Phe-Trp-Pro. The PWWP domain has a DNA- and histone-binding ability (Qiu et al. 2002; Wu et al. 2011). Like the PHD domain, the MYND domain contains a zinc finger motif, which can fold two zinc atoms (Lutterbach et al. 1998). The most studied MYND domain-containing proteins are the SMYD family proteins, which consist of SMYD1–5 and function as a lysine methyltransferase (Spellmon et al. 2015). Taken together, spikar is likely a nuclear protein.

BS69 is likely to be a spikar family protein, because it contains a PHD domain, a bromodomain, a PWWP domain, and a MYND domain in the same order within the molecule. BS69 is an adenovirus E1A-binding protein that interacts with the nuclear-receptor corepressor, N-CoR, and functions as a transcription corepressor (Masselink and Bernards 2000). However, we found that spikar functions as a coactivator of thyroid hormone receptor, glucocorticoid receptor, and estrogen receptor (Yamazaki et al. 2014). Interestingly, it has recently been reported that spikar acts as a transcription corepressor of histone H3 lysine 4 (H3K4) demethylase (*JARID1D*) (Li et al. 2016). Moreover, spikar is associated with the nucleosome remodeling and deacetylase (NuRD) complex in the nucleus, where it plays a role as a DNA damage response (DDR) factor, recruiting the NuRD complex to damaged chromatin, to repress transcription and promote double strand break repair by homologous recombination (Kloet et al. 2015; Gong et al. 2015; Adhikary et al. 2016).

The drebrin-binding region of spikar was assessed by a yeast two-hybrid assay and a GST pull-down assay, which showed that the N-terminal region of spikar interacts with the ADF-H domain of drebrin (Yamazaki et al. 2014). The ADF-H domain is found in all drebrin isoforms. In addition, the drebrin-binding region of spikar is found in spikar A, spikar B, and spikar delta-C. In the drebrin-binding region of spikar, there are nucleosome-associating domains, suggesting that drebrin may interfere with the association between spikar and the nucleosome.

Spikar is ubiquitously expressed in various tissues and concentrated in the cellular nuclei, although the expression level is higher in the spleen and thymus (Yamazaki et al. 2014). Spikar contains a nuclear localization signal (NLS, KKKKK) in the N-terminal region and is localized in the nucleus, but a spikar mutant (mNLS-spikar) with a mutated NLS (KTTKK) does not localize in the nucleus. In the rat brain, spikar is highly expressed in the cerebellum, although it is expressed throughout the brain (Yamazaki et al. 2014). In cultured astrocytes, how-

ever, spikar was hardly detected by western blotting. However, immunocytochemical studies and observation of GFP-spikar localization indicated that spikar is concentrated in the nucleus in HEK293 cells and cultured neurons (Yamazaki et al. 2014). Interestingly, in neurons, spikar is found in the cytoplasm in addition to the nucleus. Particularly, spikar is localized in dendritic spines, where drebrin is concentrated (Yamazaki et al. 2014). Furthermore, spikar tends to localize in drebrin-rich spines (Yamazaki et al. 2014). These findings suggest that spikar is localized in dendritic spines in a drebrin-dependent manner. In contrast, the localization of drebrin in dendritic spines was unaffected in spikar-knockdown neurons (Yamazaki et al. 2014). In addition to the postsynaptic localization, our subcellular fractionation data suggest that spikar is localized in presynapses associated with synaptic vesicles (Fig. 14.4).

Knockdown of spikar using shRNA resulted in decreased spine density without alteration of spine morphology (Yamazaki et al. 2014). We conducted rescue experiments using wild-type spikar and mNLS-spikar, which showed that not only wild-type spikar but also mNLS-spikar were able to recover the spine density (Yamazaki et al. 2014). Importantly, the fact that cytoplasmic spikar could rescue the spine density indicates that spikar plays multifunctional roles in both the nucleus and cytoplasm. In HEK293 cells, GFP-spikar was localized in the nucleus and rarely observed in the cytoplasm. In contrast, in HEK293 cells overexpressing drebrin, GFP-spikar tended to localize in the cytoplasm in addition to the nucleus (Yamazaki et al. 2014). Moreover, in drebrin-knockdown neurons, the spine localization of spikar was weak (Yamazaki et al. 2014). These observations indicate that the cytoplasmic localization of spikar, in particular the spine localization in neurons, depends on drebrin. Live-cell imaging of spikar-knockdown cultured neurons showed that spikar plays a role in spine maintenance and de novo spine sprouting (Yamazaki et al. 2014). Conversely, overexpression of mNLS-spikar increased the spine and filopodium number. Furthermore, drebrin knockdown abolished the effect of the mNLS-spikar overexpression. These data suggest that the localization and the function of spikar depend on drebrin (Yamazaki et al. 2014). Because drebrin accumulates in filopodia from an early stage of neuronal development (Takahashi et al. 2003), we propose that drebrin sequesters and stabilizes spikar in dendritic spines and contributes to spine formation and maintenance (Fig. 14.4). This idea is consistent with the fact that the spine localization of drebrin does not depend on spikar.

Several studies have shown a relationship between spikar and cancer. For example, spikar expression is upregulated in squamous cell cancer and cervical intraepithelial neoplasia (Bierkens et al. 2013). In colorectal cancer, a high mutation frequency was found in the spikar gene (Park et al. 2002). Additionally, spikar plays a role in promoting tumor angiogenesis (Kuroyanagi et al. 2014). Because drebrin is highly expressed in some cancer cells and is involved in tumor formation and invasion (Vaskova et al. 2011; Terakawa et al. 2013; Lin et al. 2014; Mizutani et al. 2014; Xu et al. 2015), the interaction between drebrin and spikar may be critical for cancer growth. Investigation of the role of the interaction between drebrin and spikar in cancer cells may provide new insight into the mechanism of cancer migration and growth.

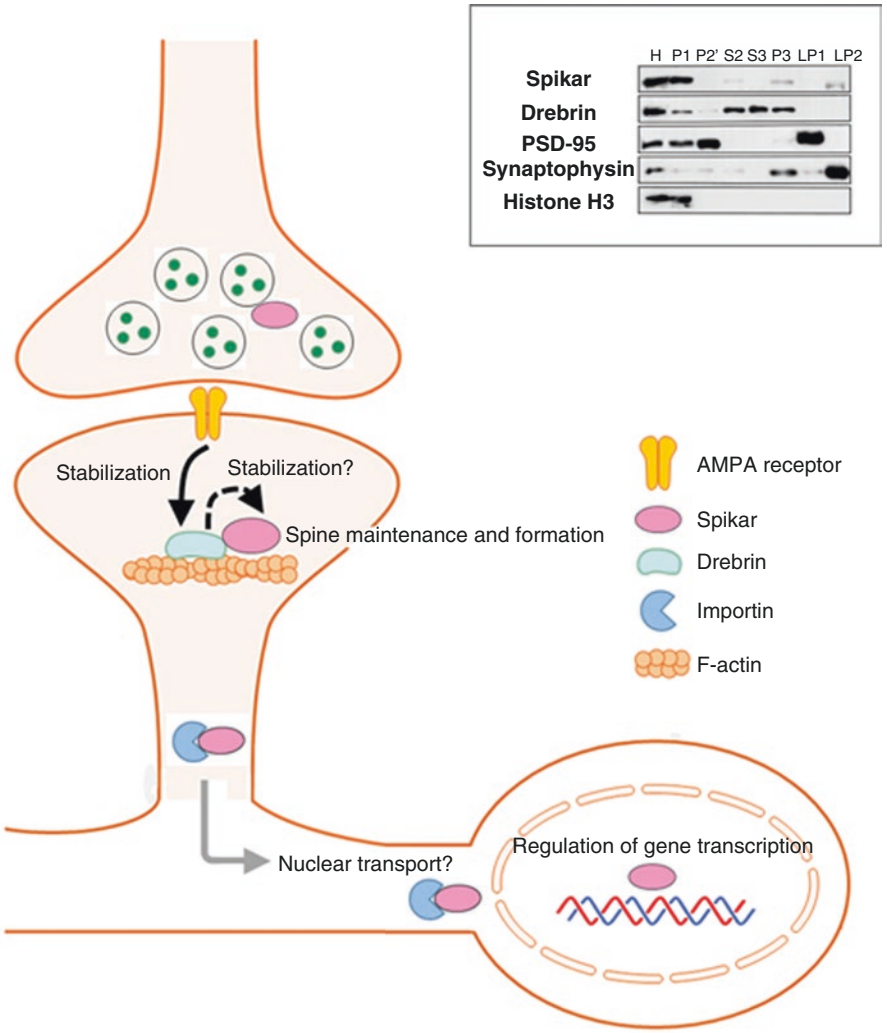

Fig. 14.4 Schematic diagram illustrating the anchoring of spikar in dendritic spines and its function in the nucleus. AMPA-receptor activity stabilizes drebrin in dendritic spines. Drebrin sequesters spikar in dendritic spines, and then spikar accumulates in drebrin-rich spines. Spikar function depends on drebrin. mNLS-spikar concentrates in dendritic spines, suggesting that spikar transports into the nucleus from dendritic spines with the importin complex. In the nucleus, spikar is involved in the regulation of gene transcription. Inset immunoblot image shows the subcellular distribution of spikar. The protein extract (20 μg protein) from each fraction was analyzed by western blotting with antibodies against spikar, drebrin, PSD-95, synaptophysin, and histone H3. The fractions were as follows: *H* homogenate, *P*1 cell nuclei and debris, *P*2 synaptosomal fraction, *S*2 non-synaptosomal fraction, *S*3 cytosolic fraction, *P*3 microsomal fraction, *LP*1 synaptosomal plasma-membrane fraction, *LP*2 synaptic vesicle fraction

14.4 EB3

EB3 was isolated as a molecule that binds to the C-terminal region of APCL, which is a brain-specific homologue of the tumor suppressor gene APC, by yeast two-hybrid screening. The name is based on its significant homology to the microtubule-binding protein, EB1 (Su et al. 1995). EB1 and EB3 are expressed ubiquitously in many tissues, although EB3 is particularly highly expressed in the brain and skeletal muscle (Nakagawa et al. 2000). EB3 and EB1 bind to the plus-tip of microtubules and regulate its dynamics (Straube and Merdes 2007). Drebrin was found in a protein complex that was pulled down with GST-EB3 from the growth cone cytosol but not with GST-EB1 (Geraldo et al. 2008). This indicates that drebrin specifically binds to EB3 but not to EB1. The binding site for drebrin is in the middle region of EB3, between the microtubule-biding region and the coiled-coil region. This middle region is absent from the EB1 sequence (Geraldo et al. 2008). In growth cones, drebrin interacts with EB3 at the tips of microtubules in the proximal region of the filopodia. Drebrin knockdown or overexpression of dominant negative EB3 causes an inhibition of neurite elongation. These findings indicate that the drebrin-EB3 interaction is critical for growth cone mobility by regulating the association between actin-filaments and microtubules.

Recently, we and others have shown that CDK5 phosphorylates drebrin at Ser 142 (pan drebrin) and Ser 342 (drebrin A) (Tanabe et al. 2014; Worth et al. 2013). Gordon-Weeks and colleagues proposed a model in which the phosphorylation at Ser 142 causes a conformational change of drebrin, consequently exposing the coiled-coil domain and EB3 binding region to the outside of the molecule (see Fig. 4.3 in Chap. 4) (Worth et al. 2013). It has been suggested that phosphorylated drebrin can more easily interact with EB3 than nonphosphorylated drebrin. Additionally, the phosphorylation-induced conformational change of drebrin enables bundling of two discrete actin-filaments. However, at present, this model needs to be verified by more studies.

While it has previously been believed that microtubules are not present in dendritic spines, imaging technology using fluorescent proteins and the RNAi technique have revealed that dynamic microtubules can transiently protrude into dendritic spines and regulate their development (Gu et al. 2008). The protrusion of microtubules into spines depends on Ca^{2+} influx through synaptic NMDA receptors (Merriam et al. 2013). Conversely, excessive Ca^{2+} influx induced by 50 μM glutamate removes EB3 from the growing microtubule plus-ends in dendrites (Kapitein et al. 2011). In addition, we have reported that the Ca^{2+} influx through NMDA receptors induced drebrin exodus from dendritic spines (Mizui et al. 2014). Therefore, drebrin may function as an adaptor that links the EB3 complex of microtubule plus-ends to the actin cytoskeleton in dendritic spines and enables a transient protrusion of microtubules into dendritic spines (Fig. 14.2). It is consistent with a recent report that drebrin knockdown decreased spine invasion of microtubules (Merriam et al. 2013).

The interaction of EB3 and drebrin has also been suggested in non-neuronal cells. Drebrin E is localized in the apical domain of columnar epithelial cells (Bazellières et al. 2012). Drebrin knockdown influences the apicobasal elongation during cell development, a phenotype that is observed in EB3-knockdown cells (Bazellières et al. 2012). The drebrin-EB3 complex regulates the cooperation between the actomyosin apical network and the apico-lateral microtubule network. Disruption of this interaction results in destabilization of the actin-based terminal web.

14.5 Profilin

Profilin is a classical actin-binding protein, which was isolated as an inhibitor molecule of actin polymerization in 1977 (Carlsson et al. 1977). Profilin sequesters G-actin, which results in inhibition of actin polymerization (Carlsson et al. 1977; Mockrin and Korn 1980). However, profilin also has an actin polymerization function (Tilney et al. 1983; Pring et al. 1992; Pantaloni and Carlier 1993). The interaction between the profilin I isoform and drebrin was first identified by affinity column chromatography with GST-profilin from rat brain cytosol (Mammoto et al. 1998) and was confirmed by a membrane overlay assay. Because profilin I can be purified with poly-proline agarose beads (Tanaka and Shibata 1985), it is likely to bind to the proline-rich region of drebrin (Fig. 14.1). Profilin II is specifically expressed in the brain (Honore et al. 1993), although both profilin I and profilin II are found in the brain. The interaction between profilin II and drebrin has not been reported, yet; however, drebrin is expected to bind to profilin II, because profilin II binds to several ligands (poly-proline, actin, phosphoinositides) that profilin I binds to (Lambrechts et al. 2000). Profilin II accumulates in dendritic spines after induction of long-term potentiation (LTP) in vitro and in vivo (Ackermann and Matus 2003). In addition, it has been indicated that emotional excitation such as fear conditioning drives profilin into dendritic spines in rat amygdala (Lamprecht et al. 2006). Similarly, drebrin immunoreactivity increased in the middle molecular layer of the hippocampus concomitantly with enhancement in F-actin content after in vivo LTP induction (Fukazawa et al. 2003). These observations suggest that the interaction between drebrin and profilin is enhanced after LTP induction.

14.6 Progranulin

Progranulin, also known as granulin-epithelin precursor, proepithelin, acrogranin, and GP88/PC-cell-derived growth factor, is a ubiquitously expressed, secreted protein throughout the body, which was originally identified as a growth factor-like molecule (Shoyab et al. 1990; Anakwe and Gerton 1990; Plowman et al. 1992;

Bhandari et al. 1993; Zanocco-Marani et al. 1999; Zhu et al. 2002; Bateman and Bennett 2009). Progranulin is a multifunctional protein that is involved in several types of physiological phenomena including inflammation, cell proliferation, wound healing, development, and tumorigenesis (Konopka et al. 2014; Bateman and Bennett 2009). The interaction between progranulin and drebrin has recently been discovered in the course of cancer research. Drebrin was found in the eluted proteins of a pull-down assay with recombinant progranulin and protein extracts of 5637 bladder cancer cells (Xu et al. 2015). Drebrin modulated the progranulin-induced actin cytoskeleton remodeling and motility of cancer cells. Drebrin-depletion in UMUC-3 bladder cancer cells inhibited tumor formation in implanted mice in vivo (Xu et al. 2015).

The best-known important characteristic of progranulin in neurology is its implication in frontotemporal dementia (FTD) and Alzheimer's disease (AD). Mutations in the *progranulin* gene are found in familial FTD patients (Baker et al. 2006), and the mutations are risk factors for AD (Carecchio et al. 2009; Perry et al. 2013). Progranulin knockdown in cultured neurons results in a decrease in dendritic spine density and an increase in filopodium-like protrusion (Tapia et al. 2011). Interestingly, drebrin is decreased in the brain of patients with Alzheimer's disease (Harigaya et al. 1996) and Down syndrome (Shim and Lubec 2002). In addition, the loss of drebrin function by an antisense oligonucleotide treatment caused a spine density decrease (Takahashi et al. 2006). Therefore, drebrin may be involved in a progranulin-related signal transduction pathway for spine formation and development.

14.7 PTEN

Phosphatase and tensin homologue (PTEN), which is a tumor suppressor, functions as a phosphatase for phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and a protein phosphatase (Song et al. 2012). Drebrin was found in PTEN-immunoprecipitates from rat brain and liver by mass spectrometry analysis (Kreis et al. 2013). PTEN dephosphorylates drebrin at Ser 647 (Ser 659 in rat drebrin A) in an activity-dependent manner (Kreis et al. 2013). The Ser residue is conserved among chicken, human, rabbit, mouse, rat, and xenopus (Kreis et al. 2013). It has recently been reported that PTEN is essential for Alzheimer's disease pathology (Knafo et al. 2016). As mentioned above, drebrin is decreased in Alzheimer's disease. Furthermore, amyloid-β oligomer induced PTEN recruitment into dendritic spines (Knafo et al. 2016) and drebrin decreased in dendritic spines (Lacor et al. 2007; Ishizuka et al. 2014). Moreover, PTEN is known to be essential for NMDA-receptor-dependent long-term depression (Arendt et al. 2014). It has been reported that over-activation of NMDA receptor induces calpain-mediated proteolysis of drebrin (Chimura et al. 2015). Taken together, the dephosphorylation of drebrin at Ser 659 by PTEN may be involved in the pathology of Alzheimer's disease, possibly through drebrin degradation.

14.8 Ras

Ras is a small G protein family, which consists of three major members: H-Ras, N-Ras, and K-Ras (Ellis et al. 1981; Shimizu et al. 1983). They were first identified as oncogenes, and its signal transduction pathways including extracellular and intracellular signals have been studied in detail (Malumbres and Barbacid 2003). In addition to tumor formation driven by Ras mutation, Ras proteins are engaged on several vital phenomena such as cell proliferation and differentiation (Feramisco et al. 1984; Hagag et al. 1986). In synapses, Ras is involved in neurotransmitter release and synaptic plasticity such as LTP and LTD (Brambilla et al. 1997; Jovanovic et al. 2000; Komiyama et al. 2002; Kim et al. 2003; Hou and Klann 2004; Schenk et al. 2005; Qin et al. 2005; Li et al. 2006; Rumbaugh et al. 2006; Banko et al. 2006). The activity of Ras is regulated by exchanging GTP binding (active form) or GDP binding (inactive form), and this conversion is mediated by their upstream regulators GAP/GEF (Shih et al. 1980; Calés et al. 1988; Adari et al. 1988; West et al. 1990; Crechet et al. 1990; Huang et al. 1990). Among a number of downstream signaling pathway of Ras, MAPK and PI3K cascades are assumed to be associated with synaptic function such as memory formation (Atkins et al. 1998; Blum et al. 1999; Chen et al. 2005; Peineau et al. 2007; Kim et al. 2011; Choi et al. 2014).

It is not clear whether drebrin forms a protein complex with Ras. However, Biou et al. (2008) have reported that Ras modulates drebrin function in dendritic spines. The overexpression of constitutively active Ras resulted in a similar phenotype as the drebrin overexpression in cultured neurons, and the effect on dendritic spine morphology was dependent on drebrin (Biou et al. 2008). Additionally, dominant negative Ras abolished the phenotype of drebrin overexpression (Biou et al. 2008). The downstream effectors of Ras play significant roles in spine growth and morphogenesis. For instance, PI3K regulates the activity of Rho family small G proteins, which are closely associated with dendritic spine morphogenesis (Sjolander et al. 1991; Rodriguez-Viciana et al. 1994; Nakayama et al. 2000; Penzes et al. 2001; Tashiro and Yuste 2008). Moreover, constitutively active Ras binds to Rac GEF Tiam1, and the interaction is essential for Rac1 activation (Yamauchi et al. 2005). Taken together, these findings suggest that Ras activation is involved in drebrin-mediated dendritic spine morphogenesis.

14.9 Other Drebrin-Binding Proteins

In addition to the drebrin-binding proteins described above, there are various other studies about drebrin-related proteins, which form protein-complexes with drebrin (Fig. 14.1). Rufy3, GAS8, SK1, Arp3, and myosin IIB were found in immunoprecipitated complexes with drebrin (Wei et al. 2014; Zhao et al. 2009; Yagoub et al. 2014; Li et al. 2011; Cheng et al. 2000); however, so far it is unknown whether drebrin directly binds to these proteins. Nonetheless, it is known that drebrin binds

to connexin 43 (Butkevich et al. 2004), which is a well-known component of gap junctions in glial cells (Rash et al. 2000), and stabilizes gap junctions to the submembrane actin cytoskeleton (Butkevich et al. 2004). Afadin is a cell-adhesion-related protein, which binds to the poly-proline region of drebrin with its PR1-2 region (Rehm et al. 2013). CXCR4, a chemokine receptor, binds to the N-terminal region (1–271) of drebrin (Perez-Martinez et al. 2010). In T lymphocytes, drebrin recruits CXCR4 to the immune synapses upon super-antigen stimulation (Perez-Martinez et al. 2010). The relationships between drebrin and connexin 43, afadin, and CXCR4 are reviewed in detail in other chapters of this book.

14.10 Conclusion

In this chapter, we reviewed drebrin-binding proteins with regard to neuronal functions. Identification of novel drebrin-binding proteins has increased across various disciplines in recent years, although the implications of their association with drebrin have not been fully determined. In some cases, such as connexin 43, drebrin acts like a stabilizer for binding partners on the actin cytoskeleton, and its knockdown leads to alteration of their subcellular localization. Based on these observations, drebrin may also function as a scaffold protein tethering its binding partners to the actin cytoskeleton in the cytoplasmic area of dendritic spines. We suppose that this interaction is regulated by synaptic activity. Because drebrin is involved in the mechanism of neurological diseases, further studies on the drebrin interactome will be of great importance to determining the pathologies of various diseases and may contribute to their medical treatment and diagnostics development.

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