Journal of Neurochemistry, 2007, **102**, 324–332 doi:10.1111/j.1471-4159.2007.04662.x

MINI REVIEW

More than just synaptic building blocks: scaffolding proteins of the post-synaptic density regulate dendritic patterning

John P. Vessey and Daniela Karra

Department of Neural Cell Biology, Center for Brain Research, Medical University of Vienna, Vienna, Austria

Abstract

The dendritic arbor is responsible for receiving and consolidating neuronal input. Outgrowth and morphogenesis of the arbor are complex stages of development that are poorly understood. However, recent findings have identified synaptic scaffolding proteins as novel regulators of these important events. Scaffolding proteins are enriched in the post-synaptic density where they bind and bring into close proximity neurotransmitter receptors, signaling molecules, and regulators of the actin cytoskeleton. This property is important for dendritic spine morphogenesis and maintenance in the mature neuron. Scaffolding proteins are now being described as key regulators of neurite outgrowth, dendritic development, and pattern formation in immature neurons. These proteins, which include post-synaptic-95, Shank and Densin-180, as well as many of their interacting partners, appear to regulate both the microtubule and actin cytoskeleton to influence dendrite morphology. Through a large array of protein–protein interaction domains, scaffolding proteins are able to form large macromolecular complexes that include cytoskeletal motor proteins as well as microtubule and actin regulatory molecules. Together, the new findings form a persuasive argument that scaffolding proteins deliver critical regulatory elements to sites of dendritic outgrowth and branching to modulate the formation and maintenance of the dendritic arbor.

Keywords: dendrite, dendritic arbor, dendritic development, post-synaptic density, PSD-95, scaffolding proteins.

J. Neurochem. (2007) **102**, 324–332.

Dendrites receive and consolidate neuronal input. Unlike axon development and guidance, dendrite morphogenesis and patterning has only recently become a focus of study. During development, the dendritic arbor is highly dynamic and is continuously forming new branches as well as maintaining or eliminating existing ones (Jan and Jan 2003; Landgraf and Evers 2005). Although significant advances have been made in the identification of specific genes regulating dendritic morphogenesis and pattern formation in *Drosophila* (Gao *et al.* 1999; Parrish *et al.* 2006), the precise molecular mechanisms that govern these processes in flies and mammals remain poorly understood. The search for novel regulators of dendritic patterning has recently turned up unexpected results. Surprisingly, well-known proteins with scaffolding functions at the post-synaptic density (PSD), such as PSD-95, Shank, and Densin-180, have recently been identified as important regulators of early neurite outgrowth and dendritic patterning. Prior to these findings, other proteins enriched in the PSD had been shown to influence dendritic development. Most notably, calcium/calmodulin-dependent protein kinase II, important for activity-dependent synaptic plasticity, has been found to limit dendritic outgrowth in various neuronal subtypes (Zou and Cline 1999; Pattinson *et al.* 2006). The absence of calcium/calmodulin-dependent protein kinase II leads to aberrant branching and enlarged dendritic arbors. This review, however, will focus on scaffolding proteins of the PSD now found to influence dendritic pattern formation. This represents a novel role for this class of proteins, a role performed in

Received February 8, 2007; revised manuscript received April 3, 2007; accepted April 5, 2007.

Address correspondence and reprint requests to John P. Vessey, Department of Neural Cell Biology, Center for Brain Research, Medical University of Vienna, A-1090, Vienna, Austria.

E-mail: john.vessey@meduniwien.ac.at

Abbreviations used: Abp1, actin-binding protein 1; ER, endoplasmic reticulum; GRIP1, glutamate receptor interacting protein 1; IP_3R, inositol (1,4,5)-trisphosphate receptor; LRR, leucine-rich repeat; mGluR, metabotropic glutamate receptor; Abi-1, Abelson interacting protein 1; PDZ, PSD-95 discs large zona occludens-1; PP1, protein phosphatase 1; PSD, post-synaptic density; SH3, Src homology 3.

subcellular domains not previously assumed to be under the influence of such proteins.

Since the initial isolation of PSD scaffolding proteins, studies have largely focused on their roles at the mature synapse where they fulfill many functions, including the organization of post-synaptic signaling complexes, regulation of synaptic transmission, and formation and maintenance of dendritic spines (Kim and Sheng 2004). As an example, PSD-95, perhaps the best studied of these molecules, is important at the synapse because of its ability to bind and anchor the NMDA receptor (Kornau *et al.* 1995), among many other proteins at the PSD. Functional information regarding scaffolding proteins was highlighted by mice mutant for PSD-95. These mice, although demonstrating enhanced long-term potentiation, were impaired in spatial learning tasks (Migaud *et al.* 1998). These findings were among the first to indicate that scaffolding proteins exert important effects at the synapse and are critical for hippocampal-dependent memory formation.

It now appears that PSD-95 and other scaffolding proteins play an equally important role outside of the synapse. A recent paper by Charych *et al.* (2006) demonstrated that PSD-95 acts as a negative regulator of dendritic branching. This finding is reminiscent of results obtained with studies of Densin-180 and Shank, two additional members of the PSD (Quitsch *et al.* 2005). Together, with data encompassing other scaffolding proteins, a novel regulatory role for PSD proteins in developing neurons is now becoming apparent. These new findings primarily come from experiments using cultured dissociated neurons. These *in vitro* systems allow for sensitive morphological analyses not easily performed *in vivo*. Cultured neurons also allow for greater resolution of the subcellular localization of scaffolding proteins, a property which ultimately led to the observations of non-synaptic pools of these proteins in both mature and developing neurons. From these observations arose the hypothesis that scaffolding proteins must regulate certain non-synaptic properties of neurons. It is likely that these proteins modulate dendritic patterning by regulating the cytoskeleton and by facilitating transport of larger macromolecular complexes. Although much remains to be discovered in regards to this novel role, it is now evident that synaptic scaffolding proteins have a much broader role in neuronal biology than previously assumed. In this review, we will summarize the data surrounding the role of PSD scaffolding proteins in dendritic branching as well as propose one potential mechanism by which they exert their influence on neuronal morphology.

Protein–protein interaction domains important for dendritic patterning

Post-synaptic scaffolding proteins achieve their structural and organizational function by direct protein–protein interactions with many synaptically localized molecules. This occurs via the presence a variety of protein interactions motifs including PSD-95 discs large zona occludens-1 (PDZ), Src homology 3 (SH3), guanylate kinase-like, leucine-rich repeat (LRR), and sterile alpha motif domains (Kim and Sheng 2004). New data suggest that these binding motifs are important for the role of scaffolding proteins in regulating dendrite morphology. The biochemical properties as well as the three dimensional structure of the PDZ domain are well known (Fanning and Anderson 1996; Sheng and Sala 2001; van Ham and Hendriks 2003). PDZ domain-containing proteins often have more than one PDZ domain, with the PSD protein glutamate receptor interacting protein 1 (GRIP1) containing as many as seven (Dong *et al.* 1997). Utilizing the PDZ domain and other protein–protein interacting motifs, synaptic scaffolding proteins are able to form large macromolecular complexes at the PSD. Proteins that bind the PDZ domain typically do so via a motif encompassing the last four amino acids at their C-terminus (Songyang *et al.* 1997). At the synapse, many classes of proteins contain PDZ domains. Extracellular adhesion molecules, ionotropic and metabotropic glutamate receptors (mGluRs), scaffolding proteins, and actin-binding proteins (Abp) are but a small subset of these (Kim and Sheng 2004). The PDZ domain is therefore necessary for synaptic signaling to exert its influence on the dynamics of the actin cytoskeleton found within dendritic spines. Recently, interest in this domain as a potential drug target for disease pathways has arisen (Dev 2004).

PSD-95, cypin, and snapin

Post-synaptic density-95 was recently identified as a novel regulator of dendritic patterning (Charych *et al.* 2006). Since the initial identification of PSD-95 (Sampedro *et al.* 1981), studies of this protein have largely focused on its role at the synapse and PSD of mature neurons where it has been identified as a regulator of synapse maturation. It achieves this by inducing clustering of post-synaptic elements and maturation of pre-synaptic components (El-Husseini *et al.* 2000). The fact that a large pool of non-synaptic PSD-95 can be observed in developing neurons upon immunostaining led to the hypothesis that PSD-95 may have an unidentified role in the immature neuron (Okabe *et al.* 2001; Prange and Murphy 2001; Charych *et al.* 2006).

Over-expression of PSD-95 in immature neurons led to dramatic alterations of the dendritic arbor. Primary dendrites were shorter, there were fewer secondary dendrites and overall dendritic complexity was reduced. Conversely, when PSD-95 protein levels were knocked down via antisense oligonucleotides, secondary dendrite numbers increased, resulting in a more complex dendritic arbor (Charych *et al.* 2006). Based on these findings, it appears that non-synaptic PSD-95, expressed in developing neurons, acts as a negative regulator of dendritic branching.

Previous work from the same laboratory had identified the cytoplasmic PSD-95 interacting protein, cypin, as a positive regulator of dendritic branching. Cypin is a guanine deaminase that contains a class 1 PDZ domain-binding motif in its C-terminus (Firestein *et al.* 1999). Over-expression of cypin causes an increase in primary and secondary dendrite numbers. This effect is dependent on the deaminase domain as over-expression of a guanine deaminase activity deficient mutant resulted in a decrease in branching in a dominant-negative fashion (Akum *et al.* 2004). In the more recent study, coexpression of both PSD-95 and cypin not only led to an attenuation of the effects induced by cypin alone, but also to a decrease of secondary dendrites. Based on these morphological studies, it would appear that cypin antagonizes the actions of PSD-95 and that as PSD-95 blocks all of the effects of cypin, it must act downstream of its interacting partner (Charych *et al.* 2006).

In light of these conclusions, an interesting question about the stoichiometric relationship between PSD-95 and cypin arises. Are the expression patterns of these proteins developmentally regulated in a manner that gives preference to the actions of one over the other at certain time points of neuronal maturation? This model becomes more complex when one considers a cypin interactor, snapin. Snapin, which interacts with cypin via an atypical protein–protein interaction site, not through a PDZ domain, is largely restricted to the cell body where it inhibits cypin action and hence blocks microtubule assembly (Chen *et al.* 2005). Like PSD-95, overexpression of snapin reduces dendritic complexity, although in a different manner. Snapin reduces primary dendrite numbers (Chen *et al.* 2005), whereas PSD-95 reduces primary branch points (Charych *et al.* 2006). It will be interesting to determine if these three proteins exist in a complex that acts as a regulator of dendritic morphology at sites of both neurite outgrowth and branching.

Cypin has been shown to influence microtubule dynamics (Akum *et al.* 2004) and indirect interactions between PSD-95 and cytoskeletal elements via the microtubule-binding protein cysteine-rich interactor of PDZ3 (CRIPT) have been previously demonstrated (Passafaro *et al.* 1999). It has therefore been proposed that the effects exerted by PSD-95, cypin, and snapin on dendritic patterning may occur via alterations of the microtubule network. Although yet to be confirmed in neurons, over-expression of PSD-95 in COS7 cells significantly altered the organization of microtubules in a striking way. Instead of originating from one central microtubule-organizing center, the filaments appeared disorganized and, in some instances, surrounded perinuclear PSD-95 aggregates (Charych *et al.* 2006). This disruption of microtubules upon overexpression of PSD-95 may point to the mechanism by which PSD-95 may alter the branching pattern of dendrites. This is also an intriguing finding as the position of the microtubule-organizing center has been implicated in determining which neurite will eventually become the axon (de Anda *et al.* 2005). Perhaps there is a yet to be identified role for PSD-95 in determining neuronal polarity and axonal outgrowth at even earlier time points in maturation. It is also interesting that PSD-95 should act as a stop signal for dendritic branching around the time when spine development is about to begin. It will be interesting to see if PSD-95 plays multiple roles in the mature neuron or rather switches from a branching inhibitor to a modulator of spine morphology and function.

Shank, densin-180, and δ-catenin

Like PSD-95, the Shank proteins are a major component of the PSD (Naisbitt *et al.* 1999; Sheng and Kim 2000; Boeckers *et al.* 2002). There are three Shank genes, all of which are expressed in the brain and their protein products found at the synapse. Shank proteins interact with a variety of other proteins via PDZ domains, proline-rich regions, sterile alpha motif domains, ankyrin repeats, and SH3 domains (Sheng and Kim 2000). Shank interactors include mGluRs (Tu *et al.* 1999), Densin-180 (Quitsch *et al.* 2005), cortactin (Naisbitt *et al.* 1999; Boeckers *et al.* 2002), α-fodrin (Böckers *et al.* 2001), Abp1 (Qualmann *et al.* 2004), and Abelson-interacting protein 1 (Abi-1) (Proepper *et al.* 2007). Cortactin, α-fodrin, and Abp1 are F-actin binding proteins and therefore provide a direct link between the Shank proteins and the actin cytoskeleton. From electron microscopic studies, it appears that the Shank proteins are located in the middle of the PSD, in a perfect position to link apical transmembrane receptors with the more basal actin cytoskeleton (Valtschanoff and Weinberg 2001; Baron *et al.* 2006). In mature neurons, Shank is able to induce the formation of functional synapses in aspiny cerebellar neurons, perhaps via its ability to bring both receptors and cytoskeletal elements into close proximity (Roussignol *et al.* 2005). Shank proteins are also responsible for shaping the morphology of dendritic spines via the recruitment of Homer in both developing and mature neurons (Sala *et al.* 2001), highlighting their ability to regulate neuronal morphology.

Although expression of Shank proteins increases with neuronal maturity (Sala *et al.* 2001), they are expressed in all developing neurons and are found associated with cortactin (Du *et al.* 1998; Naisbitt *et al.* 1999). This expression pattern indicates that this family of scaffolding proteins also has additional roles outside of the PSD. Similar to PSD-95 and its binding partners, Shank proteins do not act alone in regulating dendritic morphology. They exert their effects through an interaction with Densin-180, another key component of the PSD (Apperson *et al.* 1996; Quitsch *et al.* 2005). Densin-180 is a member of the LRR and PDZ family of scaffolding proteins characterized by a LRR near the N-terminus and one or more PDZ domains in the C-terminus (Bilder *et al.* 2000; Walikonis *et al.* 2001). It was found that upon overexpression of Densin-180, developing neurons form irregular and

substantially more complex dendritic arbors. These neurons also failed to develop synapses, as defined by dendritically localized PSD-95 clusters. The region of Densin-180 responsible for this effect contains a LRR close to the N-terminus, as overexpressed constructs lacking this domain did not induce an increase in arborization. Acting antagonistically, Shank proteins prevented the alterations in neuronal morphology induced by the overexpression of Densin-180 when coexpressed (Quitsch *et al.* 2005).

The mechanism by which this antagonism occurs may involve another binding partner of Densin-180, δ-catenin, which has been shown to interact with the C-terminal PDZ domain of Densin-180 (Martinez *et al.* 2003; Quitsch *et al.* 2005). Immunoprecipitation and pull-down experiments have demonstrated that Shank proteins interact directly with Densin-180 and when this interaction persists, δ-catenin cannot bind (Quitsch *et al.* 2005). Although a clear functional link has yet to be demonstrated, evidence suggests that Densin-180 and δ-catenin act together to induce the increase in arborization found in overexpression studies. Similar increases in dendritic arborization that were found upon overexpression of Densin-180 were obtained when δ-catenin was overexpressed in neurons. δ-Catenin-induced branching of processes as well as elongation of existing dendrites via modulation of the actin cytoskeleton (Martinez *et al.* 2003). Of these three interacting partners, Shank, Densin-180, and δ-catenin, evidence suggests that δ-catenin, through an established protein–protein interaction with cortactin and regulation of the RhoA GTPase (Martinez *et al.* 2003), is the effector protein of dendritic patterning. The role of both the Shank proteins and Densin-180 would appear to be regulatory in nature with Shank acting as a stop signal for δ-catenin activity.

Recently, a novel-binding partner of Shank3, Abi-1, a protein regulating Rac-dependent pathways and thereby being involved in actin reorganization (Stradal *et al.* 2001; Leng *et al.* 2005), has been reported (Proepper *et al.* 2007). The C-terminal SH3 domain of Abi-1 is responsible for the interaction with the conserved proline-rich region of Shank3. Moreover, using Abi-1 deletion constructs the authors demonstrate that Abi-1 SH3 domain is necessary and sufficient to localize Abi-1 to the PSD in mature neurons, suggesting that Abi-1 is being recruited to the PSD via its interaction with Shank3 (Proepper *et al.* 2007). In contrast to its distribution in mature neurons, Abi-1 localizes in dendrites and is enriched in the growth cone of young neurons (Courtney *et al.* 2000; Proepper *et al.* 2007). Similar expression patterns have been reported for Shank proteins (Du *et al.* 1998). For functional analyses RNAi was used to specifically down-regulate Abi-1, and an increase in the number of dendrites and branching points in young hippocampal neurons was observed. In contrast, overexpression of Abi-1 results in a simplified dendritic tree, implicating a putative role of Abi-1 in regulating dendrite morphology (Proepper *et al.* 2007). Further studies of the novel Abi-1-Shank interaction would be necessary to determine if the two proteins cooperate to antagonize the activity of δ-catenin and thereby regulate dendrite morphology.

Homer

The Homer family of proteins also coordinates the shaping of the PSD through its scaffolding functions. Unlike PSD-95 and the Shank proteins, Homer does not contain a PDZ motif. Rather, all members of the family possess a coiled-coil domain with two leucine zipper motifs in their C-termini. This region facilitates the dimerization between Homer monomers, which is necessary for their role as a scaffolding molecule (Sun *et al.* 1998; Tadokoro *et al.* 1999; Duncan *et al.* 2005). At the synapse, Homer proteins bind extra- and intracellular receptors as well as other scaffolding molecules (Kim and Sheng 2004). Coimmunoprecipitation and colocalization studies identified an association between Homer and mGluRs found at dendritic spines (Brakeman *et al.* 1997; Kato *et al.* 1998; Xiao *et al.* 1998) as well as intracellular inositol (1,4,5)-trisphosphate receptors (IP_3Rs) (Tu *et al.* 1998), which are found on the membranes of dendritically localized endoplasmic reticulum (ER) (Bannai *et al.* 2004; Fukatsu *et al.* 2004). Homer proteins also bind to Shank and actin. Both Homer and Shank proteins act cooperatively to facilitate the accumulation of actin at synapses, which is important in the regulation of dendritic spine morphology (Shiraishi *et al.* 1999; Usui *et al.* 2003).

Like the Shank family, Homer proteins are expressed at all stages of neuronal development (Shiraishi *et al.* 2004). They have been demonstrated to be important in axonal path finding (Foa *et al.* 2001), indicating that roles outside of the mature synapse also exist for this family of proteins. Preliminary evidence suggests that Homer proteins also play a role in the development of the dendritic arbor. Overexpression of Homer 1c causes a reduction of dendritic branching in cerebellar Purkinje neurons (Tanaka *et al.* 2006). Unlike the data already discussed, in which changes of dendritic morphology appear to act via manipulation of the actin cytoskeleton, this effect appears to be mediated through modulation of intracellular Ca^{2+} signaling. Neurons overexpressing Homer 1c displayed larger and more frequent Ca^{2+} transients when stimulated with mGluR and IP_3R agonists (Tanaka *et al.* 2006). Increases in local Ca^{2+} concentrations have been implicated in the stabilization of developing dendrites (Lohmann *et al.* 2002; Lohmann and Wong 2005) and ER has been found to be enriched at dendritic branch points (Walton *et al.* 1991; Terasaki *et al.* 1994). It will be interesting to see if Homer 1c leads to an increase in intracellular IP_3R clustering at sites of dendritic ER or rather alters the kinetics of existing receptors. It will also be important to test if the loss of Homer 1c leads to an increase in branching via a reduction in local Ca^{2+} fluxes.

GRIP1

The GRIP1 is a PDZ domain-containing scaffolding protein found in abundance at the PSD of both glutamatergic and GABAergic synapses (Dong *et al.* 1997; Li 2005). It was first identified by its specific interaction with the C-terminus of the GluR2 and GluR3 subunits of AMPA receptors (Dong *et al.* 1997). GRIP1 has largely been studied in relation to synaptic trafficking and stabilization of AMPA receptors (Dong *et al.* 1997; Wyszynski *et al.* 1999). Beside this interaction, several binding partners have been identified for the various PDZ domains of GRIP1, including EphB receptor tyrosine kinases as well as ephrin-B ligands (Torres *et al.* 1998; Bruckner *et al.* 1999). Interestingly, the microtubule-based motor protein kinesin heavy chain (also termed KIF5) also interacts with a region of GRIP1 termed L2, which is located between the PDZ 6 and PDZ 7 domains (Setou *et al.* 2002).

In a recent study, GRIP1 protein was implicated in dendrite morphogenesis. GRIP1 knockdown via RNAi in mature rat hippocampal neurons resulted in a retraction of dendritic processes and a loss of most primary dendrites and higher order branches. GRIP1 down-regulation did not effect the branching of the axon, and spared one major primary dendrite. This single dendrite, however, did not branch and lacked secondary dendrites (Hoogenraad *et al.* 2005). A similar phenotype was detected in immature neurons, implicating that GRIP1 is essential for the development and the maintenance of the dendritic arbor. In order to map the crucial domains that cause this effect, the authors' generated deletion constructs of GRIP1. They identified two regions, L2 and PDZ6, of GRIP1 that when absent, cause the mutant protein to act as a dominant-negative. The latter is known to bind EphB receptors (Torres *et al.* 1998; Bruckner *et al.* 1999). In immature GRIP1-deficient neurons, EphB2 failed to target to dendrites and the plasma membrane, and was found to accumulate in the Golgi, suggesting a role for GRIP1 in EphB2 vesicle trafficking from the Golgi to the dendrite (Hoogenraad *et al.* 2005).

Moreover, in the absence of GRIP1, the distribution of the motor protein KIF5 was altered by an increased localization in the cell body. Over-expression of dominant-negative constructs consisting of the GRIP1-binding domain of KIF5 or the L2 domain of GRIP1 led to a decrease of primary dendrites and an accumulation of exogenous EphB2 in the Golgi region, indicating that the GRIP1-KIF5 interaction is necessary for dendrite morphogenesis and for the normal targeting of EphB2 to the dendritic surface (Hoogenraad *et al.* 2005). The authors therefore propose that GRIP1 serves as an adapter between KIF5 and EphB2 and thereby regulates receptor trafficking. The next step involves an EphrinB and EphB2 interaction which then activates the downstream EphB2 signaling pathway that is required for dendrite morphogenesis.

Neurabin

Neurabin was first purified as a novel neural tissue-specific F-actin binding protein from rat brain (Nakanishi *et al.* 1997). A closely related protein, Spinophilin, was identified as a novel protein phosphatase-1 (PP1) binding protein that localizes to dendritic spines (Allen *et al.* 1997). Neurabin has a similar structural organization to Spinophilin, with both proteins containing an N-terminal F-actin binding domain, a central PDZ domain, a PP1-binding motif, and predicted coiled-coil domains at the C-terminal region (Allen *et al.* 1997; Nakanishi *et al.* 1997; Satoh *et al.* 1998; McAvoy *et al.* 1999). Most studies have focused on the role of Neurabin and Spinophilin in dendritic spine morphogenesis. The F-actin binding domain is required to promote dendrite and spine maturation (Zito *et al.* 2004; Terry-Lorenzo *et al.* 2005). In the mature neuron, Neurabin is found in the PSD (Muly *et al.* 2004) where it acts as a scaffolding protein and recruits, among other proteins, PP1 (Terry-Lorenzo *et al.* 2002) and the Rac-specific guanine exchange factor Tiam1 (Buchsbaum *et al.* 2003). Neurabin and Spinophilin single knockout mice show defects in synaptic plasticity, indicating an important role at the synapse (Feng *et al.* 2000; Allen *et al.* 2006).

Interestingly, in addition to the Neurabins regulating dendritic spine morphology, there are indications that they might be involved in dendritic outgrowth and patterning. First, Neurabin is concentrated in the lamellipodia of growth cones in developing neurons (Nakanishi *et al.* 1997). Second, blocking the expression of endogenous Neurabin leads to an inhibition of neurite formation in cultured hippocampal neurons (Nakanishi *et al.* 1997; Orioli *et al.* 2006). Third, recent data has shown that the sole *Caenorhabditis elegans* homolog of Neurabin (NAB-1) physically interacts with the SAD-1 kinase and that this interaction mediates neuronal polarity by regulating axon-dendrite determination in a variety of neurons (Hung *et al.* 2007). The authors suggest a possible role of mammalian Neurabins prior to dendritic spine maturation and a potentially conserved role for the Neurabin protein family during early neurite differentiation (Hung *et al.* 2007). It will be interesting to see if the mammalian homologs to NAB-1 fulfill similar roles in early dendritic patterning.

Synthesis and conclusions

At the synapse, activity can induce lasting changes in dendritic spine morphology. These changes are in part brought about by rearrangement of the actin cytoskeleton, a process that requires the contribution of scaffolding proteins found at the PSD (Schubert and Dotti 2007). These scaffolding proteins link the post-synaptic neurotransmitter receptors with actin-interacting proteins by binding both classes of molecules and bringing them into close proximity

Table 1 A summary of PSD scaffolding proteins with identified roles in dendritic patterning and the specific protein-interacting partners important for these effects

PSD-scaffolding Protein	Primary role in dendritic patterning	Protein interactors	References
PSD-95	Regulates primary dendrite length as well as primary and secondary dendritic branching	Cypin Snapin	Akum *et al.* 2004; Chen *et al.* 2005; Charych *et al.* 2006
Densin-180	Regulates primary dendritic outgrowth and dendritic branching	Shank δ-Catenin	Martinez *et al.* 2003; Quitsch *et al.* 2005
Shank	Antagonizes Densin-180 and δ-catenin to regulate primary dendritic outgrowth	Densin-180	Naisbitt *et al.* 1999; Qualmann *et al.* 2004; Quitsch *et al.* 2005; Proepper *et al.* 2007
	Interacts with Abi-1 to regulate primary dendritic outgrowth and dendritic branching	δ-Catenin Abi-1 Cortactin Abp1	
Homer	Alters dendritic arbor complexity via regulating dendritic Ca^{2+} homeostasis	IP_3R	Tanaka *et al.* 2006
GRIP1	Regulate primary dendrite length and number	EphB2 KIF5	Setou *et al.* 2002; Hoogenraad *et al.* 2005
Neurabin	Promotes initial neurite extension	PP1 Tiam1	Terry-Lorenzo *et al.* 2002; Buchsbaum *et al.* 2003; Hung *et al.* 2007

PP1, protein phosphatase-1; PSD, post-synaptic; GRIP1, glutamate receptor interacting protein 1; KIF5, kinesin heavy chain; Abp, actin-binding protein 1; IP_3R, inositol (1,4,5)-trisphosphate receptor; Abi-1, Abelson-interacting protein.
See the text for specific details.

to one another (Kim and Sheng 2004). Evidence for a role of PSD scaffolding proteins in another form of morphological regulation, dendrite development and pattern formation has been presented and discussed (For a summary, see Table 1). Although not confined to manipulation of both the actin and microtubule networks, it would appear that this mechanism is the primary manner in which scaffolding proteins exert their influence on the dendritic arbor. Synaptic versus non-synaptic effects of scaffolding proteins could potentially be explained by the organization of the cytoskeleton in neurons. Whereas microtubules are found in the dendritic shaft and excluded from the spine head, actin and actin stress fibers are found in both compartments. Signaling pathways that are regulated by scaffolding proteins that converge on microtubules would exclude effects on the dendritic spines.

Non-synaptic PSD-95 and two interacting partners, cypin and snapin, act in concert to rearrange microtubules and hence alter dendritic outgrowth and branching. Cypin would appear to be the effector molecule in this complex as it directly binds tubulin heterodimers and promotes microtubule assembly, whereas snapin act as an inhibitor of cypin (Akum *et al.* 2004; Chen *et al.* 2005; Charych *et al.* 2006). These three proteins likely operate in a stoichiometric manner to ultimately determine dendritic pattern formation. Similarly, Shank and Densin-180 act together to regulate the activity of δ-catenin, a cortactin-binding protein (Quitsch *et al.* 2005). Cortactin binds and cross-links actin filaments in a tyrosine phosphorylation-dependent manner (Huang *et al.* 1997). Therefore, Densin-180 and δ-catenin act in a complex to coordinate actin rearrangement with Shank operating as a negative regulator when bound to Densin-180 (Quitsch *et al.* 2005). Cytoplasmic calcium signaling has been linked to changes in the dendritic cytoskeleton of neurons by regulating Rho family GTPases (Konur and Ghosh 2005). Preliminary evidence links Homer to alterations in IP_3R-mediated Ca^{2+} fluxes in neurons, again leading to alterations in the morphology of the dendritic arbor (Tanaka *et al.* 2006). Much more remains to be elucidated regarding the manner in which Homer acts to influence dendritic branching via modulation of Ca^{2+} fluxes from intracellular stores.

Dendritic pattern formation requires the activity of regulatory molecules at specific sites, such as points of primary dendritic outgrowth and at subsequent branch points (Jan and Jan 2003). As an example, targeting of Kalirin-7, a GDP/GTP exchange factor for Rac1, by PDZ-containing proteins, is critical for its precise function. At the PSD, Kalirin-7 acts to increase spine volume, whereas if confined to the cell soma via removal of its PDZ domain, it induces the formation of aberrant neurites (Penzes *et al.* 2001). Both actions are the result of manipulation of the actin cytoskeleton and are regulated by PDZ motif containing proteins such as PSD-95. These effector molecules must be delivered to these sites in a regulated manner and scaffolding proteins have been linked with motor protein complexes (Kneussel 2005). Specific interactions between motor proteins and scaffolding proteins have been described for PSD-95 and KIF1Bα (Mok *et al.* 2002), PSD-95 and myosin-V and

dynein (Naisbitt *et al.* 2000) as well as GRIP1 and KIF5 (Setou *et al.* 2002). It is now apparent that large macromolecular complexes containing scaffolding proteins, cytoskeletal regulatory proteins, and cytoskeletal motors are responsible for the effects found on dendritic patterning. Within this complex, the scaffolding proteins would appear to play multiple roles as adaptor molecules, regulators of effector proteins and as targeting elements for other proteins involved in cytoskeletal regulation. Although not limited to regulating dendritic pattern formation in the developing neuron, it would appear that this is the primary role of scaffolding proteins found outside of the mature synapse.

Acknowledgements

This work was supported by the Sonderforschungsbereich (SFB446), Hertie-Stiftung, and the Schram-Stiftung (all to Dr Michael A. Kiebler). The authors wish to thank Drs Paolo Macchi and Ralf Dahm for critical reading of the manuscript and for helpful insight and discussion. The authors also wish to thank Dr Michael Kiebler for his insight, guidance, and encouragement. Without his support and excellent supervision, this work would not have been possible.

References

Akum B. F., Chen M., Gunderson S. I., Riefler G. M., Scerri-Hansen M. M. and Firestein B. L. (2004) Cypin regulates dendrite patterning in hippocampal neurons by promoting microtubule assembly. *Nat. Neurosci.* **7**, 145–152.

Allen P. B., Ouimet C. C. and Greengard P. (1997) Spinophilin, a novel protein phosphatase 1 binding protein localized to dendritic spines. *Proc. Natl Acad. Sci. USA* **94**, 9956–9961.

Allen P. B., Zachariou V., Svenningsson P. *et al.* (2006) Distinct roles for spinophilin and neurabin in dopamine-mediated plasticity. *Neuroscience* **140**, 897–911.

de Anda F. C., Pollarolo G., Da Silva J. S., Camoletto P. G., Feiguin F. and Dotti C. G. (2005) Centrosome localization determines neuronal polarity. *Nature* **436**, 704–708.

Apperson M. L., Moon I. S. and Kennedy M. B. (1996) Characterization of densin-180, a new brain-specific synaptic protein of the *O*-sialoglycoprotein family. *J. Neurosci.* **16**, 6839–6852.

Bannai H., Inoue T., Nakayama T., Hattori M. and Mikoshiba K. (2004) Kinesin dependent, rapid, bi-directional transport of ER sub-compartment in dendrites of hippocampal neurons. *J. Cell Sci.* **117**, 163–175.

Baron M. K., Boeckers T. M., Vaida B., Faham S., Gingery M., Sawaya M. R., Salyer D., Gundelfinger E. D. and Bowie J. U. (2006) An architectural framework that may lie at the core of the postsynaptic density. *Science* **311**, 531–535.

Bilder D., Birnbaum D., Borg J. P. *et al.* (2000) Collective nomenclature for LAP proteins. *Nat. Cell Biol.* **2**, E114.

Böckers T. M., Mameza M. G., Kreutz M. R., Bockmann J., Weise C., Buck F., Richter D., Gundelfinger E. D. and Kreienkamp H. J. (2001) Synaptic scaffolding proteins in rat brain. Ankyrin repeats of the multidomain Shank protein family interact with the cytoskeletal protein alpha-fodrin. *J. Biol. Chem.* **276**, 40104–40112.

Boeckers T. M., Bockmann J., Kreutz M. R. and Gundelfinger E. D. (2002) ProSAP/Shank proteins – a family of higher order organizing molecules of the postsynaptic density with an emerging role in human neurological disease. *J. Neurochem.* **81**, 903–910.

Brakeman P. R., Lanahan A. A., O'Brien R., Roche K., Barnes C. A., Huganir R. L. and Worley P. F. (1997) Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* **386**, 284–288.

Bruckner K., Pablo Labrador J., Scheiffele P., Herb A., Seeburg P. H. and Klein R. (1999) EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains. *Neuron* **22**, 511–524.

Buchsbaum R. J., Connolly B. A. and Feig L. A. (2003) Regulation of p70 S6 kinase by complex formation between the Rac guanine nucleotide exchange factor (Rac-GEF) Tiam1 and the scaffold spinophilin. *J. Biol. Chem.* **278**, 18833–18841.

Charych E. I., Akum B. F., Goldberg J. S., Jornsten R. J., Rongo C., Zheng J. Q. and Firestein B. L. (2006) Activity-independent regulation of dendrite patterning by postsynaptic density protein PSD-95. *J. Neurosci.* **26**, 10164–10176.

Chen M., Lucas K. G., Akum B. F., Balasingam G., Stawicki T. M., Provost J. M., Riefler G. M., Jornsten R. J. and Firestein B. L. (2005) A novel role for snapin in dendrite patterning: interaction with cypin. *Mol. Biol. Cell* **16**, 5103–5114.

Courtney K. D., Grove M., Vandongen H., Vandongen A., LaMantia A. S. and Pendergast A. M. (2000) Localization and phosphorylation of Abl-interactor proteins, Abi-1 and Abi-2, in the developing nervous system. *Mol. Cell Neurosci.* **16**, 244–257.

Dev K. K. (2004) Making protein interactions druggable: targeting PDZ domains. *Nat. Rev. Drug Discov.* **3**, 1047–1056.

Dong H., O'Brien R. J., Fung E. T., Lanahan A. A., Worley P. F. and Huganir R. L. (1997) GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature* **386**, 279–284.

Du Y., Weed S. A., Xiong W. C., Marshall T. D. and Parsons J. T. (1998) Identification of a novel cortactin SH3 domain-binding protein and its localization to growth cones of cultured neurons. *Mol. Cell Biol.* **18**, 5838–5851.

Duncan R. S., Hwang S. Y. and Koulen P. (2005) Effects of Vesl/Homer proteins on intracellular signaling. *Exp. Biol. Med. (Maywood)* **230**, 527–535.

El-Husseini A. E., Schnell E., Chetkovich D. M., Nicoll R. A. and Bredt D. S. (2000) PSD-95 involvement in maturation of excitatory synapses. *Science* **290**, 1364–1368.

Fanning A. S. and Anderson J. M. (1996) Protein–protein interactions: PDZ domain networks. *Curr. Biol.* **6**, 1385–1388.

Feng J., Yan Z., Ferreira A., Tomizawa K., Liauw J. A., Zhuo M., Allen P. B., Ouimet C. C. and Greengard P. (2000) Spinophilin regulates the formation and function of dendritic spines. *Proc. Natl Acad. Sci. USA* **97**, 9287–9292.

Firestein B. L., Brenman J. E., Aoki C., Sanchez-Perez A. M., El-Husseini A. E. and Bredt D. S. (1999) Cypin: a cytosolic regulator of PSD-95 postsynaptic targeting. *Neuron* **24**, 659–672.

Foa L., Rajan I., Haas K., Wu G. Y., Brakeman P., Worley P. and Cline H. (2001) The scaffold protein, Homer1b/c, regulates axon pathfinding in the central nervous system *in vivo*. *Nat. Neurosci.* **4**, 499–506.

Fukatsu K., Bannai H., Zhang S., Nakamura H., Inoue T. and Mikoshiba K. (2004) Lateral diffusion of inositol 1,4,5-trisphosphate receptor type 1 is regulated by actin filaments and 4.1N in neuronal dendrites. *J. Biol. Chem.* **279**, 48976–48982.

Gao F. B., Brenman J. E., Jan L. Y. and Jan Y. N. (1999) Genes regulating dendritic outgrowth, branching, and routing in *Drosophila*. *Genes Dev.* **13**, 2549–2561.

van Ham M. and Hendriks W. (2003) PDZ domains-glue and guide. *Mol. Biol. Rep.* **30**, 69–82.

Hoogenraad C. C., Milstein A. D., Ethell I. M., Henkemeyer M. and Sheng M. (2005) GRIP1 controls dendrite morphogenesis by regulating EphB receptor trafficking. *Nat. Neurosci.* **8**, 906–915.

Huang C., Ni Y., Wang T., Gao Y., Haudenschild C. C. and Zhan X. (1997) Down-regulation of the filamentous actin cross-linking activity of cortactin by Src-mediated tyrosine phosphorylation. *J. Biol. Chem.* **272**, 13911–13915.

Hung W., Hwang C., Po M. D. and Zhen M. (2007) Neuronal polarity is regulated by a direct interaction between a scaffolding protein, Neurabin, and a presynaptic SAD-1 kinase in *Caenorhabditis elegans*. *Development* **134**, 237–249.

Jan Y. N. and Jan L. Y. (2003) The control of dendrite development. *Neuron* **40**, 229–242.

Kato A., Ozawa F., Saitoh Y., Fukazawa Y., Sugiyama H. and Inokuchi K. (1998) Novel members of the Vesl/Homer family of PDZ proteins that bind metabotropic glutamate receptors. *J. Biol. Chem.* **273**, 23969–23975.

Kim E. and Sheng M. (2004) PDZ domain proteins of synapses. *Nat. Rev. Neurosci.* **5**, 771–781.

Kneussel M. (2005) Postsynaptic scaffold proteins at non-synaptic sites. The role of postsynaptic scaffold proteins in motor-protein-receptor complexes. *EMBO Rep.* **6**, 22–27.

Konur S. and Ghosh A. (2005) Calcium signaling and the control of dendritic development. *Neuron* **46**, 401–405.

Kornau H. C., Schenker L. T., Kennedy M. B. and Seeburg P. H. (1995) Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* **269**, 1737–1740.

Landgraf M. and Evers J. F. (2005) Control of dendritic diversity. *Curr. Opin. Cell Biol.* **17**, 690–696.

Leng y., Zhang J., Badour K., Arpaia E., Freeman S., Cheung P., Siu M. and Siminovitch K. (2005) Abelson-interactor-1 promotes WAVE2 membrane translocation and Abelson-mediated tyrosine phosphorylation required for WAVE2 activation. *Proc. Natl Acad. Sci. USA* **102**, 1098–1103.

Li R. W. (2005) GRIP1 in GABAergic synapses. *J. Comp. Neurol.* **488**, 11–27.

Lohmann C. and Wong R. O. (2005) Regulation of dendritic growth and plasticity by local and global calcium dynamics. *Cell Calcium.* **37**, 403–409.

Lohmann C., Myhr K. L. and Wong R. O. (2002) Transmitter-evoked local calcium release stabilizes developing dendrites. *Nature* **418**, 177–181.

Martinez M. C., Ochiishi T., Majewski M. and Kosik K. S. (2003) Dual regulation of neuronal morphogenesis by a delta-catenin-cortactin complex and Rho. *J. Cell Biol.* **162**, 99–111.

McAvoy T., Allen P. B., Obaishi H., Nakanishi H., Takai Y., Greengard P., Nairn A. C. and Hemmings H. C. Jr (1999) Regulation of neurabin I interaction with protein phosphatase 1 by phosphorylation. *Biochemistry* **38**, 12943–12949.

Migaud M., Charlesworth P., Dempster M. *et al.* (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**, 433–439.

Mok H., Shin H., Kim S., Lee J. R., Yoon J. and Kim E. (2002) Association of the kinesin superfamily motor protein KIF1Balpha with postsynaptic density-95 (PSD-95), synapse-associated protein-97, and synaptic scaffolding molecule PSD-95/discs large/zona occludens-1 proteins. *J. Neurosci.* **22**, 5253–5258.

Muly E. C., Allen P., Mazloom M., Aranbayeva Z., Greenfield A. T. and Greengard P. (2004) Subcellular distribution of neurabin immunolabeling in primate prefrontal cortex: comparison with spinophilin. *Cereb. Cortex* **14**, 1398–1407.

Naisbitt S., Kim E., Tu J. C., Xiao B., Sala C., Valtschanoff J., Weinberg R. J., Worley P. F. and Sheng M. (1999) Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron* **23**, 569–582.

Naisbitt S., Valtschanoff J., Allison D. W., Sala C., Kim E., Craig A. M., Weinberg R. J. and Sheng M. (2000) Interaction of the postsynaptic density-95/guanylate kinase domain-associated protein complex with a light chain of myosin-V and dynein. *J. Neurosci.* **20**, 4524–4534.

Nakanishi H., Obaishi H., Satoh A., Wada M., Mandai K., Satoh K., Nishioka H., Matsuura Y., Mizoguchi A. and Takai Y. (1997) Neurabin: a novel neural tissue-specific actin filament-binding protein involved in neurite formation. *J. Cell Biol.* **139**, 951–961.

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

Orioli D., Colaluca I. N., Stefanini M., Riva S., Dotti C. G. and Peverali F. A. (2006) Rac3-induced neuritogenesis requires binding to Neurabin I. *Mol. Biol. Cell* **17**, 2391–2400.

Parrish J. Z., Kim M. D., Jan L. Y. and Jan Y. N. (2006) Genome-wide analyses identify transcription factors required for proper morphogenesis of *Drosophila* sensory neuron dendrites. *Genes Dev.* **20**, 820–835.

Passafaro M., Sala C., Niethammer M. and Sheng M. (1999) Microtubule binding by CRIPT and its potential role in the synaptic clustering of PSD-95. *Nat. Neurosci.* **2**, 1063–1069.

Pattinson D., Baccei M., Karadottir R., Torsney C., Moss A., McCutcheon J., Giese K. P. and Fitzgerald M. (2006) Aberrant dendritic branching and sensory inputs in the superficial dorsal horn of mice lacking CaMKIIalpha autophosphorylation. *Mol. Cell Neurosci.* **33**, 88–95.

Penzes P., Johnson R. C., Sattler R., Zhang X., Huganir R. L., Kambampati V., Mains R. E. and Eipper B. A. (2001) The neuronal Rho-GEF Kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic morphogenesis. *Neuron* **29**, 229–242.

Prange O. and Murphy T. H. (2001) Modular transport of postsynaptic density-95 clusters and association with stable spine precursors during early development of cortical neurons. *J. Neurosci.* **21**, 9325–9333.

Proepper C., Johannsen S., Liebau S., Dahl J., Vaida B., Bockmann J., Kreutz M. R., Gundelfinger E. D. and Boeckers T. M. (2007) Abelson interacting protein 1 (Abi-1) is essential for dendrite morphogenesis and synapse formation. *EMBO J.* **26**, 1397–1409.

Qualmann B., Boeckers T. M., Jeromin M., Gundelfinger E. D. and Kessels M. M. (2004) Linkage of the actin cytoskeleton to the postsynaptic density via direct interactions of Abp1 with the ProSAP/Shank family. *J. Neurosci.* **24**, 2481–2495.

Quitsch A., Berhorster K., Liew C. W., Richter D. and Kreienkamp H. J. (2005) Postsynaptic shank antagonizes dendrite branching induced by the leucine-rich repeat protein Densin-180. *J. Neurosci.* **25**, 479–487.

Roussignol G., Ango F., Romorini S., Tu J. C., Sala C., Worley P. F., Bockaert J. and Fagni L. (2005) Shank expression is sufficient to induce functional dendritic spine synapses in aspiny neurons. *J. Neurosci.* **25**, 3560–3570.

Sala C., Piech V., Wilson N. R., Passafaro M., Liu G. and Sheng M. (2001) Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* **31**, 115–130.

Sampedro M. N., Bussineau C. M. and Cotman C. W. (1981) Postsynaptic density antigens: preparation and characterization of an antiserum against postsynaptic densities. *J. Cell Biol.* **90**, 675–686.

Satoh A., Nakanishi H., Obaishi H. *et al.* (1998) Neurabin-II/spinophilin. An actin filament-binding protein with one pdz domain localized at cadherin-based cell-cell adhesion sites. *J. Biol. Chem.* **273**, 3470–3475.

Schubert V. and Dotti C. G. (2007) Transmitting on actin: synaptic control of dendritic architecture. *J. Cell Sci.* **120**, 205–212.

Setou M., Seog D. H., Tanaka Y., Kanai Y., Takei Y., Kawagishi M. and Hirokawa N. (2002) Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature* **417**, 83–87.

Sheng M. and Kim E. (2000) The Shank family of scaffold proteins. *J. Cell Sci.* **113**, 1851–1856.

Sheng M. and Sala C. (2001) PDZ domains and the organization of supramolecular complexes. *Annu. Rev. Neurosci.* **24**, 1–29.

Shiraishi Y., Mizutani A., Bito H., Fujisawa K., Narumiya S., Mikoshiba K. and Furuichi T. (1999) Cupidin, an isoform of Homer/Vesl, interacts with the actin cytoskeleton and activated rho family small GTPases and is expressed in developing mouse cerebellar granule cells. *J. Neurosci.* **19**, 8389–8400.

Shiraishi Y., Mizutani A., Yuasa S., Mikoshiba K. and Furuichi T. (2004) Differential expression of Homer family proteins in the developing mouse brain. *J. Comp. Neurol.* **473**, 582–599.

Songyang Z., Fanning A. S., Fu C., Xu J., Marfatia S. M., Chishti A. H., Crompton A., Chan A. C., Anderson J. M. and Cantley L. C. (1997) Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* **275**, 73–77.

Stradal T., Courtney K. D., Rottner K., Hahne P., Small J. V. and Pendergast A. M. (2001) Ahe Abl interactor proteins localize to sites of actin polymerization at the tips of lamellipodia and filopodia. *Curr. Biol.* **11**, 891–895.

Sun J., Tadokoro S., Imanaka T., Murakami S. D., Nakamura M., Kashiwada K., Ko J., Nishida W. and Sobue K. (1998) Isolation of PSD-Zip45, a novel Homer/vesl family protein containing leucine zipper motifs, from rat brain. *FEBS Lett.* **437**, 304–308.

Tadokoro S., Tachibana T., Imanaka T., Nishida W. and Sobue K. (1999) Involvement of unique leucine-zipper motif of PSD-Zip45 (Homer 1c/vesl-1 L) in group 1 metabotropic glutamate receptor clustering. *Proc. Natl Acad. Sci. USA* **96**, 13801–13806.

Tanaka M., Duncan R. S., McClung N., Yannazzo J. A., Hwang S. Y., Marunouchi T., Inokuchi K. and Koulen P. (2006) Homer proteins control neuronal differentiation through IP(3) receptor signaling. *FEBS Lett.* **580**, 6145–6150.

Terasaki M., Slater N. T., Fein A., Schmidek A. and Reese T. S. (1994) Continuous network of endoplasmic reticulum in cerebellar Purkinje neurons. *Proc. Natl Acad. Sci. USA* **91**, 7510–7514.

Terry-Lorenzo R. T., Elliot E., Weiser D. C., Prickett T. D., Brautigan D. L. and Shenolikar S. (2002) Neurabins recruit protein phosphatase-1 and inhibitor-2 to the actin cytoskeleton. *J. Biol. Chem.* **277**, 46535–46543.

Terry-Lorenzo R. T., Roadcap D. W., Otsuka T., Blanpied T. A., Zamorano P. L., Garner C. C., Shenolikar S. and Ehlers M. D. (2005) Neurabin/protein phosphatase-1 complex regulates dendritic spine morphogenesis and maturation. *Mol. Biol. Cell* **16**, 2349–2362.

Torres R., Firestein B. L., Dong H., Staudinger J., Olson E. N., Huganir R. L., Bredt D. S., Gale N. W. and Yancopoulos G. D. (1998) PDZ proteins bind, cluster, and synaptically colocalize with Eph receptors and their ephrin ligands. *Neuron* **21**, 1453–1463.

Tu J. C., Xiao B., Yuan J. P., Lanahan A. A., Leoffert K., Li M., Linden D. J. and Worley P. F. (1998) Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. *Neuron* **21**, 717–726.

Tu J. C., Xiao B., Naisbitt S. *et al.* (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **23**, 583–592.

Usui S., Konno D., Hori K., Maruoka H., Okabe S., Fujikado T., Tano Y. and Sobue K. (2003) Synaptic targeting of PSD-Zip45 (Homer 1c) and its involvement in the synaptic accumulation of F-actin. *J. Biol. Chem.* **278**, 10619–10628.

Valtschanoff J. G. and Weinberg R. J. (2001) Laminar organization of the NMDA receptor complex within the postsynaptic density. *J. Neurosci.* **21**, 1211–1217.

Walikonis R. S., Oguni A., Khorosheva E. M., Jeng C. J., Asuncion F. J. and Kennedy M. B. (2001) Densin-180 forms a ternary complex with the (alpha)-subunit of Ca^{2+}/calmodulin-dependent protein kinase II and (alpha)-actinin. *J. Neurosci.* **21**, 423–433.

Walton P. D., Airey J. A., Sutko J. L., Beck C. F., Mignery G. A., Sudhof T. C., Deerinck T. J. and Ellisman M. H. (1991) Ryanodine and inositol trisphosphate receptors coexist in avian cerebellar Purkinje neurons. *J. Cell Biol.* **113**, 1145–1157.

Wyszynski M., Valtschanoff J. G., Naisbitt S., Dunah A. W., Kim E., Standaert D. G., Weinberg R. and Sheng M. (1999) Association of AMPA receptors with a subset of glutamate receptor-interacting protein *in vivo*. *J. Neurosci.* **19**, 6528–6537.

Xiao B., Tu J. C., Petralia R. S., Yuan J. P., Doan A., Breder C. D., Ruggiero A., Lanahan A. A., Wenthold R. J. and Worley P. F. (1998) Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. *Neuron* **21**, 707–716.

Zito K., Knott G., Shepherd G. M., Shenolikar S. and Svoboda K. (2004) Induction of spine growth and synapse formation by regulation of the spine actin cytoskeleton. *Neuron* **44**, 321–334.

Zou D. J. and Cline H. T. (1999) Postsynaptic calcium/calmodulin-dependent protein kinase II is required to limit elaboration of presynaptic and postsynaptic neuronal arbors. *J. Neurosci.* **19**, 8909–8918.

