

## Chapter 2

# Scaffold Proteins at the Postsynaptic Density

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**Abstract** Scaffold proteins are abundant and essential components of the postsynaptic density (PSD). They play a major role in many synaptic functions including the trafficking, anchoring, and clustering of glutamate receptors and adhesion molecules. Moreover, they link postsynaptic receptors with their downstream signaling proteins and regulate the dynamics of cytoskeletal structures. By definition, PSD scaffold proteins do not have intrinsic enzymatic activities but are formed by modular and specific domains deputed to form large protein networks. Here, we will discuss the latest findings regarding the structure and functions of major PSD scaffold proteins.

Given that scaffold proteins are central components of PSD architecture, it is not surprising that deletion or mutations in their human genes cause severe neuropsychiatric disorders including autism, mental retardation, and schizophrenia. Thus, their dynamic organization and regulation are directly correlated with the essential structure of the PSD and the normal physiology of neuronal synapses.

**Keywords** BMP signaling • Development • Glutamate receptors • Neuromuscular junction • Wnt signaling

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## 2.1 The MAGUK Family

### 2.1.1 Structural Organization of the MAGUK Proteins

Genetic and biochemical studies over the past 20 years have identified the membrane-associated guanylate kinases (MAGUKs) as ubiquitous scaffolding molecules concentrated at sites of cell-cell contact such as synapses (Craven and Brecht 1998; Kornau et al. 1997; Sheng and Sala 2001; Sheng and Kim 2002). MAGUK members include SAP90/PSD-95, SAP102, SAP97, Chapsyn 110/PSD93, and p55 (SAP = synapse-associated protein). They represent a superfamily of multidomain proteins that are related by the presence of a shared set of structural domains. The defining feature of MAGUKs is the presence of a region of approximately 300 amino acids at the C-terminus with homology to yeast guanylate kinase (GK), which catalyzes the ATP-dependent phosphorylation of GMP to GDP. Curiously, the GK domain in MAGUKs is catalytically inactive (Olsen and Brecht 2003), but it is always accompanied by either a preceding SH3 (Src homology 3) domain or followed closely by a WW (two conserved Trp residues) motif. Also, MAGUKs always contain PDZ (PSD-95/DLG/ZO-1) domains (in most cases three), and all these modular motifs in MAGUKs mediate protein-protein interactions.

PDZ domains typically bind specific C-terminal sequences in target proteins (Kim et al. 1995; Kornau et al. 1995). However, some PDZ domains can heterodimerize (Brennan et al. 1996a). Several structures of isolated PDZ domains by x-ray crystallography and three-dimensional nuclear magnetic resonance (NMR) spectroscopy reveal that PDZ domains are compact and modular. Interestingly, a number of recent studies have demonstrated that two or more PDZ domains connected in tandem often display target-binding properties that are distinct from those of each isolated domain or even the simple sum of the isolated PDZ domains (Long et al. 2003). For PSD-95, the linking sequence between the first two PDZ domains is formed by five residues, rigid and highly conserved, suggesting that it might reduce interdomain movement rather than simply function as a passive linker. The structure of the PDZ1 and 2 tandem showed that the two PDZ domains indeed contact each other in a side-by-side manner and that two of their target-binding grooves point in directions that are favorable for binding to the tails of multimeric transmembrane proteins extending from the membrane surface into the cytoplasm including NMDA receptors (NMDARs). Mutations that increase the length of the interdomain linker impaired the supramodular nature of PDZ1 and 2 of PSD-95 which then displayed weaker binding to dimeric targets and a decreased capacity in clustering (Long et al. 2003).

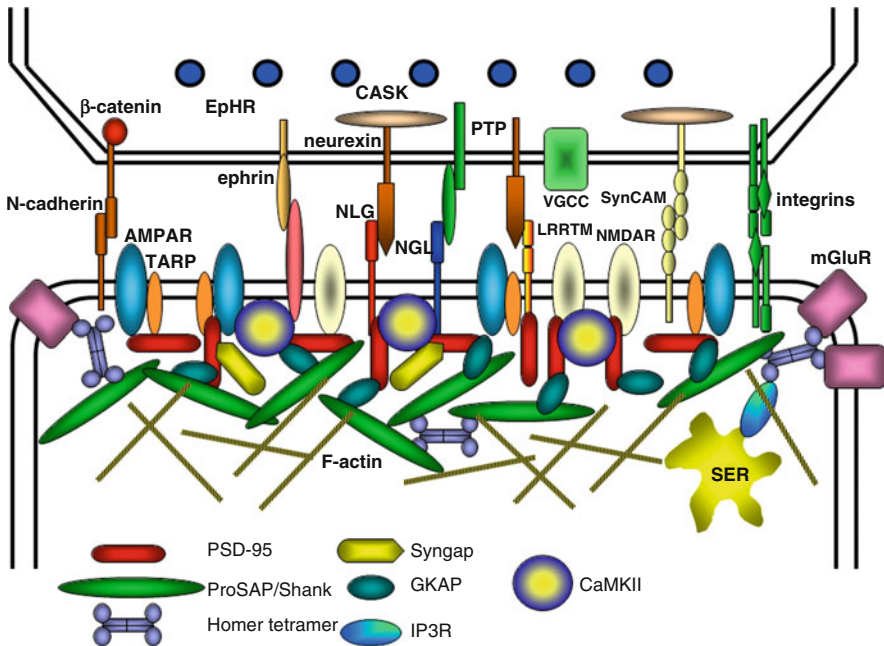
Like PDZ domains, SH3 domains are protein-protein interaction modules that commonly occur in proteins with widely divergent functions (Kuriyan and Cowburn 1997). SH3 domains typically bind to polyproline motifs (ProXXPro); however, MAGUK SH3 domains rarely bind to such ProXXPro-containing sequences. One of the few is the proline-rich C-terminus of  $\alpha$ -secretase ADAM10

that binds to the SH3 domain of SAP97 (Marcello et al. 2007). Conversely, numerous ligands bind with high affinity to the GK domain of MAGUKs. These ligands include, for example, guanylate kinase-associated proteins (GKAPs) (Kim et al. 1997; Takeuchi et al. 1997). In addition to their interaction with downstream signaling proteins, GK domains in MAGUKs bind to their SH3 motifs, preferentially in an intramolecular fashion (Shin et al. 2000; McGee et al. 2001). As for the PDZ1 and 2, the crystal structures of the PSD-95-SH3-GK tandem revealed that the SH3 domain and the GK domain pack extensively with each other to form an integral structural unit as an integral supramodule required for the proper functioning of PSD-95. Indeed, disruption of the SH3-GK interaction compromised PSD-95-mediated clustering properties (Hsueh and Sheng 1999), and mutations that disrupt SH3-GK packing in the only PSD-95 family MAGUK in *Drosophila melanogaster* (DLG1) resulted in a tumorigenic phenotype of larval imaginal discs (Woods et al. 1996).

### **2.1.2 Interactions and Functional Properties of MAGUK Family Members at the PSD**

Mammalian brain is the tissue expressing the greatest diversity of MAGUK proteins. In each synapse, a typical PSD is composed of a huge complex protein network consisting of several hundred different proteins whereas MAGUK family members are of crucial importance. They are localized at CNS glutamatergic synapses (Garner et al. 2000; Aoki et al. 2001) as well as at cholinergic synapses (Conroy et al. 2003). As modular proteins, it has often been hypothesized that the most likely function of PSD-MAGUKs is being central organizers of vertebrate CNS synapses. They are, in fact, key scaffold proteins determining the steady state as well as the activity-dependent changes of glutamate receptor numbers in excitatory synapses (Elias and Nicoll 2007) (Fig. 2.1).

PSD-95 (also named SAP90), a major complex of the PSD fraction, can be seen as the prototypical best characterized MAGUK protein of the PSD. It is now clear that the most important function of PSD-95 is to organize signaling complexes at the postsynaptic membrane. However, the amount of PSD-95 can also regulate the balance between the number of inhibitory and excitatory synapses (Levinson and El-Husseini 2005). PSD-95 interacts with a large variety of molecules and thus, by physically bringing together cytoplasmic signal transduction proteins and surface receptors, may facilitate the coupling of various signaling cascades within the PSD. More than 15 years ago, the first and second PDZ domains of PSD-95 (PDZ1 and 2) were described to bind the extreme C-termini of Kiv1.4 of the Shaker K<sup>+</sup> channels (Kim et al. 1995) and of NR2A/B subunits of the NMDARs (Cho et al. 1992; Kistner et al. 1993; Kornau et al. 1995; Niethammer et al. 1996). Since then, a whole variety of proteins associated to the distinct domains of PSD-95 has emerged. Here is a list of the most relevant and recently described ones.



**Fig. 2.1** The diagram shows a schematic organization of the protein network in the PSD. Only major families and certain classes of PSD proteins are shown. The interaction between them is schematically indicated

The other glutamate receptor that directly binds to PSD-95 is the kainate receptor whose KA2 subunit was shown to be linked at the SH3-GK domains of PSD-95 (Garcia et al. 1998). The second major class of transmembrane proteins that bind to PSD-95 are the synaptic cell adhesion molecules (SynCAMs). The neuroligin transmembrane ligand, neuroligin, binds to the third PDZ domain of PSD-95 (PDZ3) (Irie et al. 1997; Song et al. 1999). Neuroligin (NLGN) is an adhesion molecule with the ability to induce synapse formation. Interestingly, a significant subset of SynCAMs including NGLNs, synaptic-adhesion-like molecules (SALMs), ADAM22, and leucine-rich repeat transmembrane proteins (LRRTMs) associated with PSD-95, suggesting that they may act in concert to couple trans-synaptic adhesion to the molecular organization of synaptic proteins. Thus, PSD-95 may be one of the central organizers that recruits diverse proteins to sites of synaptic adhesion, promotes trans-synaptic signaling, and couples neuronal activity with changes in synaptic adhesion (Han and Kim 2008; Margeta and Shen 2010).

Importantly, PSD-95 greatly influences synaptic transmission and plasticity mainly because it recruits the stargazin tetraspanning membrane protein to synapses via binding to its C-terminus with PDZ1 and 2. Stargazin and its relatives are associated with AMPA receptors (AMPArs) and are essential for their surface expression, surface diffusion, synaptic accumulation, and function (Chen et al. 2000;

Tomita et al. 2005). These data may explain why PSD-95 overexpression potentiates AMPAR-mediated excitatory postsynaptic currents (EPSCs), but not the currents of the directly linked NMDAR (Elias et al. 2006; Sumioka et al. 2010). The role of PSD-95 in regulating AMPAR number at the PSD can also be mediated by the interaction with other proteins modulating AMPAR internalization. Han et al. showed that a regulated interaction of the endocytic adaptor RalBP1 with the small GTPase RalA and PSD-95 controls NMDAR-dependent AMPAR endocytosis during LTD. NMDAR activation brings RalBP1 close to PSD-95 to promote the interaction of RalBP1-associated endocytic proteins with PSD-95-associated AMPARs (Han et al. 2009). Similarly, Bhattacharyya et al. suggest that interaction of PSD-95 and calcineurin with A kinase anchoring protein AKAP150 is critical for NMDAR-triggered AMPAR endocytosis and LTD (Bhattacharyya et al. 2009). How these two mechanisms are functionally connected remains to be determined.

The second PSD-95 PDZ domain (PDZ2) can also bind to the PDZ domain in neuronal nitric oxide synthase (nNOS) (Brenman et al. 1996a, b). nNOS is a  $\text{Ca}^{2+}$ -calmodulin-activated enzyme that produces the nitric oxide involved in neurotransmission and excitotoxicity. Interestingly, the ternary NMDAR-PSD-95-nNOS complex may functionally couple NMDAR gating to nNOS activation, as it is suggested by the observation that disrupting the NMDAR-PSD-95 interaction with a synthetic peptide that mimics the last nine residues of NR2B or a synthetic compound that blocks the interaction between PSD-95 and nNOS reduces NMDAR-induced excitotoxicity in vitro and in vivo without affecting NMDAR function (Aarts et al. 2002; Zhou et al. 2010). Recently, it has been shown that the interaction with nNOS is required for the ability of PSD-95 to regulate synaptogenesis and multi-innervated dendritic spines suggesting a physiological role for the NMDAR-PSD-95-nNOS complex at synapses (Nikonenko et al. 2008).

Several other binding partners of PSD-95 are scaffold proteins and regulators or effectors of small GTPases. The synaptic GTPase-activating protein for Rac, SynGAP, can interact with all three PDZ domains of PSD-95 via its C-terminus (Chen et al. 1998; Kim et al. 1998). The SH3-GK of PSD-95 also binds the spine-associated Rap-Gap SPAR (Pak et al. 2001), AKAP (Bhattacharyya et al. 2009), SPIN90/WISH (Kim et al. 2009), MAP1a (Reese et al. 2007), Preso, and other scaffold proteins such as the four members of the GKAP family (Kim et al. 1997; Takeuchi et al. 1997). Most of these interactions have been implicated in the regulation of both the size and the number of spines and synapses (Brenman et al. 1998; Kim et al. 1998; Colledge et al. 2000; Pak et al. 2001; Vazquez et al. 2004). Finally, a nuclear protein, AIDA-1d, has been identified to interact with PDZ1 and 2 of PSD-95 and to shuttle between the synapse and the nucleus. Synaptic activity induces a  $\text{Ca}^{2+}$ -independent translocation of AIDA-1d to the nucleus, where it couples to Cajal bodies and increases nucleolar numbers and protein synthesis thus linking synaptic activity and protein biosynthetic capacity (Jordan et al. 2007).

It is now important to underline individual localization and expression characteristics of PSD-MAGUK family members. Each of the MAGUK proteins shows a different distribution in respect to subcellular compartments of the brain.

PSD-95 and PSD-93 are highly enriched in the postsynaptic density (PSD), especially due to their high palmitoylation degree (El-Husseini et al. 2000). SAP102 and SAP97 are found in dendrites and axons and are abundant in the cytoplasm as well as at synapses. Further, PSD-MAGUK proteins exhibit a distinct developmental expression pattern: SAP102 is highly expressed and functionally dominates in early postnatal development, whereas PSD-95 and PSD-93 predominate at later stages (Sans et al. 2000; Elias et al. 2006). Regarding 3D-structure, there are further differences among the PSD-MAGUKs. Negative stain images of PSD-95 and SAP97 suggest that these two highly related proteins are in fact adopting different shapes. PSD-95 monomers are relatively compact whereas SAP97 monomers are relatively extended rod shapes that tend to dimerize (Nakagawa et al. 2004). However, both proteins contain alternative N-termini, expressing either an L27 domain (beta-isoform) or double cysteines that are normally palmitoylated (alpha-isoforms) (Schlüter et al. 2006).

In vivo, MAGUK family members apparently interact with different, but overlapping, sets of proteins with PSD-95 and PSD-93 being preferentially associated with the NR2A and SAP102 with the NR2B subunit of the NMDAR (Sans et al. 2000). This phenomenon suggests that the properties of the NR2B-SAP102 complex may be different from those of the NR2A-PSD-95/PSD-93 complex and that the functional properties of synaptic NMDARs may depend on the prevalence of one or the other (Kim et al. 2005). However, also PSD-93 and PSD-95 may have opposite roles in regulating LTP (Carlisle et al. 2008). SAP102 and SAP97 are involved in the trafficking of NMDARs and AMPARs, respectively. By interacting with the PDZ-binding domain of Sec8, SAP102 can associate with the exocyst complex and regulate the delivery of NMDARs to the surface of neuronal cells (Sans et al. 2003). SAP97 directly interacts with the AMPAR GluR1 subunit (Leonard et al. 1998), and the fact that the SAP97-GluR1 complex has been found early in the secretory pathway indicates that SAP97 can regulate the trafficking of GluR1 (Sans et al. 2001). CaMKII phosphorylation of SAP97 in the N-terminal L27 domain promotes the synaptic targeting of SAP97 and GluR1 (Mauceri et al. 2004). To some extent like PSD-95, the overexpression of SAP97 increases the number of synaptic AMPARs, induces spine enlargement, and increases the frequency of miniature EPSCs (mEPSCs) (Rumbaugh et al. 2003; Nakagawa et al. 2004; Howard et al. 2010). The abundance of PDZ scaffold proteins in synapses with overlapping targets for interaction raises questions regarding the specificity redundancy of the scaffolds. For example, PSD-95, PSD-93, SAP102, and SAP97 are expressed in excitatory synapses, and each of these MAGUKs can mediate the trafficking of glutamate receptors at different developmental stages (Sans et al. 2000; Elias et al. 2006). Knockout studies in mice have revealed that MAGUKs have in part the ability to functionally compensate for each other (Migaud et al. 1998), but only a correct highly interconnected MAGUK system is assuring appropriate glutamate receptor expression and localization at synapses (Elias and Nicoll 2007).

### **2.1.3 *Synaptic Localization and Spatial Regulation of the MAGUK PSD-95***

Spatially, PSD-95 is closely associated to membrane receptors and ion channels and seems to be arranged perpendicular to the PSD membrane (Chen et al. 2008). On the ultrastructural level, the PSD can anatomically be divided into three layers: the first layer mainly contains membrane receptors, ion channels, and CAMs, with NMDARs at the center and AMPARs at the periphery; the second layer is enriched with MAGUK proteins, in particular PSD-95, which are closely coupled to the membrane receptors and ion channels; the third layer is comprised of ProSAP/Shank and GKAPs (see the following paragraphs) (Petrálie et al. 1994; Valtschanoff and Weinberg 2001). Synaptic localization of PSD-95 depends on the palmitoylation of two N-terminal cysteines (Cys3 and Cys5) (Craven et al. 1999), and synaptic activity induces the removal of PSD-95 by depalmitoylation of the two Cys residues (El-Husseini et al. 2002). A set of enzymes capable of inducing PSD-95 palmitoylation has recently been identified, but some controversy remains as to which of them is specific for PSD-95, and only one of these, the palmitoyl transferase DHHC2, seems to be regulated by synaptic activity (Fukata et al. 2004; Huang et al. 2004; Noritake et al. 2009). PSD-95 can be degraded through the ubiquitin-proteasome pathway by means of direct ubiquitylation (Colledge et al. 2003) or indirectly, via the ubiquitylation and degradation of its interacting protein SPAR (Pak and Sheng 2003). Two different phosphorylation sites have been identified on PSD-95 with opposite effects. Phosphorylation of serine 295 by JNK-1 enhances the synaptic accumulation of PSD-95 (Pavlowsky et al. 2010), while trafficking of PSD-95 to synapses is inhibited by activity-dependent CaMKII phosphorylation at serine 73 (Steiner et al. 2008). In general, the activity-dependent accumulation, dispersal, or degradation of PSD-95 is often associated with an increase or loss of synaptic AMPARs, strengthened or weakened synapses, and changes in glutamate-receptor-induced intracellular signaling such as CREB and MAPK phosphorylation (Ehlers 2003).

## **2.2 The ProSAP/Shank Family**

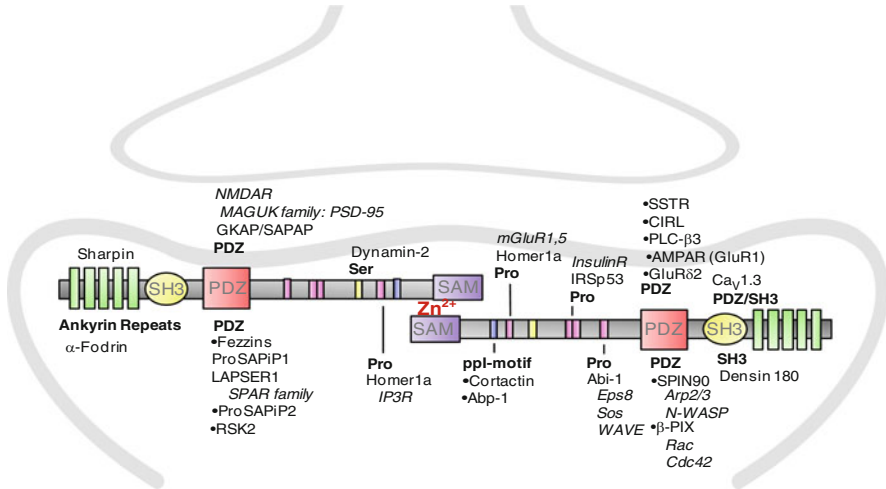
### **2.2.1 *Molecular Composition and Expression Profile of the ProSAP/Shank Family***

The ProSAP/Shank family of scaffold proteins consists of three members all highly enriched in the PSD and localized at the interface between membrane receptors and cytoskeletal elements: Shank1 (also named Shank1a, Synamon, or SSTRIP), ProSAP1/Shank2 (also named CortBP1), and ProSAP2/Shank3 (Boeckers et al. 1999a, b; Naisbitt et al. 1999; Yao et al. 1999). These molecules contain multiple



domains which are essential for various protein-protein interactions within the PSD: N-terminal ankyrin repeats, an SH3 domain, a PDZ domain, a proline-rich domain, and a sterile alpha motif (SAM) domain. The name ProSAP (*proline-rich synapse-associated protein*) derives from proline-rich clusters that are conserved among all family members (Boeckers et al. 1999a, b), while the term Shank reflects the SH3 domain and multiple *ankyrin* repeats (Naisbitt et al. 1999). ProSAPs/Shanks are large proteins with a molecular mass of more than 180 kDa (Boeckers et al. 1999a). All three share 63–87% amino acid identity while SH3, PDZ, and SAM domains are conserved at the highest level. Shank1 is only expressed in brain (Yao et al. 1999); ProSAP1/Shank2 also appears in non-neuronal tissue like pancreas, pituitary, lung, liver, kidney, and testis (Redecker et al. 2001, 2003; McWilliams et al. 2004, 2005; Dobrinskikh et al. 2010); and ProSAP2/Shank3 has been detected in almost every tissue examined (Lim et al. 1999). Interestingly, all three ProSAP/Shank family members show a distinct expression pattern in *Xenopus laevis* embryos indicating a functional role of this protein family not only in the adult organism but also in embryonic development (Gessert et al. 2011). Within the nervous system, ProSAP/Shank expression is not limited to cortical areas but has further been detected in glial cells (Redecker et al. 2001), at olfactory cilia membranes (Saavedra et al. 2008) and at postsynaptic specializations of retinal (Brandstätter et al. 2004), and various peripheral synapses (Raab et al. 2010). However, all three family members are highly expressed in the hippocampus and cortex. In the cerebellum, Shank1 and ProSAP1/Shank2 primarily appear in Purkinje cells, while ProSAP2/Shank3 is only found in the granular cell layer (Boeckers et al. 1999a, b, 2004). On the subcellular level, ProSAPs/Shanks are not localized directly underneath the postsynaptic membrane but extend up to 120 nm deep inside the PSD (Naisbitt et al. 1999; Tao-Cheng et al. 2010). Alternative splicing events regulate ProSAP/Shank domain composition (Boeckers et al. 1999b; Lim et al. 1999). Shank2E, for example, one of the two major alternative splice variants of ProSAP1/Shank2, is only expressed in epithelial cells and contains an SH3 domain and N-terminal ankyrin repeats like ProSAP2/Shank3 and Shank1 (McWilliams et al. 2004). The other major alternative splice variant of ProSAP1/Shank2 called ProSAP1A misses the ankyrin repeats, but still includes the SH3 domain (Boeckers et al. 1999a, b). Further, there is knowledge of an alternatively spliced Shank1 lacking the SAM domain called Shank1b (Sala et al. 2001). Interestingly and contrary to Shank1 and ProSAP1/Shank2, tissue-specific expression of the ProSAP2/Shank3 gene is exclusively regulated by DNA methylation (Ching et al. 2005; Beri et al. 2007). A recent study further demonstrates that methylation of the ProSAP2/Shank3 gene predominantly happens at intragenic CpG island promoters. Thus, alternative transcripts are generated and expressed differentially not only in a tissue- and cell-type-specific manner but even within the same cell types from distinct brain regions (Maunakea et al. 2010) (Fig. 2.2).





**Fig. 2.2** The diagram shows the domain composition of ProSAP/Shank family members at the PSD (the three major domains, SH3, PDZ, and SAM, are clearly depicted). For each domain/interacting motif, direct binding partners as well as indirect ones (*italicized* letters) are listed

### 2.2.2 Synaptic Recruitment and Assembly of ProSAP/Shank Family Members

Proper formation and maturation of a synapse requires the specific localization of proteins at both sites of the contact. A noticeable feature of all three ProSAP/Shank mRNAs is their strong dendritic localization, which – in response to the appropriate stimuli – makes local translation directly at the site of spines and synapses probable (Bockers et al. 2004; Falley et al. 2009). The postsynaptic recruitment of ProSAP/Shank family members depends on certain amino acid sequences within these molecules called synaptic targeting signals. Shank1, for example, only requires an intact PDZ domain for synaptic localization (Sala et al. 2001; Romorini et al. 2004). In contrast, C-terminal elements of ProSAP1/Shank2 and ProSAP2/Shank3 including the SAM domain, the ppI motif, and a serine-rich stretch of approximately 50 amino acids (Okamoto et al. 2001; Boeckers et al. 2005; Grabrucker et al. 2009) are responsible for synaptic targeting. Interestingly, high turnover rates of ProSAP/Shank family members have been observed at single PSDs in immature neuronal cultures (Bresler et al. 2004). Additional *in vitro* studies have revealed that ProSAP1/Shank2 and ProSAP2/Shank3 are core elements of newly formed PSDs at nascent synapses, while Shank1 is only recruited during the later process of synapse maturation (Sala et al. 2001; Boeckers et al. 2005; Grabrucker et al. 2011). These observations are supported by the fact that transient expression of ProSAP2/Shank3 is sufficient to induce functional dendritic spines in aspiny cultured cerebellar granule cells while Shank1 is not capable to induce the same effect (Roussignol et al. 2005). Recent studies show that the SAM domains of ProSAP1/Shank2 and

ProSAP2/Shank3 are not only crucial for postsynaptic targeting but, due to their oligomerization ability, further mediate assembly of large ProSAP/Shank sheets via zinc ions thus forming a postsynaptic platform (PSP) (Baron et al. 2006; Gundelfinger et al. 2006; Grabrucker et al. 2011). Zinc ions are located in and released from presynaptic vesicles of glutamatergic terminals, can bind glutamate receptors at hippocampal synapses, and enter the postsynaptic compartment (Assaf and Chung 1984); (Li et al. 2001). Whereas  $Zn^{2+}$  binding regulates the packaging density of ProSAP2/Shank3 (Baron et al. 2006), Shank1 seems to stabilize synapses in a  $Zn^{2+}$ -insensitive mechanism (Grabrucker et al. 2011). Sharpin, whose C-terminal part has been shown to interact with the ankyrin repeats of Shank1, can dimerize through its N-terminal half further cross-linking ProSAPs/Shanks (Lim et al. 2001). Shank1 is also able to multimerize via homomeric attachment of the ankyrin repeats and the SH3 domain (Romorini et al. 2004). All these mechanisms of gigantic ProSAP/Shank multimerization are key events in forming a polymeric network structure that – together with postsynaptic Homer – resides at the core of the PSD (Hayashi et al. 2009). Interestingly, the oligomerization state of certain ProSAP/Shank interaction domains such as the PDZ domain is known to regulate the binding affinity of partner ligands thus rendering ProSAP/Shank platforms even more diverse in respect to multiprotein complex formation at the PSD (Iskenderian-Epps and Imperiali 2010). Among PSDs of cultured neurons, continuous loss and redistribution has been shown for ProSAP2/Shank3, a phenomenon that was independent from protein synthesis or degradation and could be accelerated by electrophysiological stimulation (Tsurriel et al. 2006). This might result from activity-dependent changes of ProSAP/Shank levels and/or their subcellular distribution mediated by secondary modifications such as palmitoylation or phosphorylation. In fact, phosphorylation sites have already been found in ProSAP2/Shank3 (Jaffe et al. 2004), and the ribosomal S6 kinase (RSK) has been identified to interact with and phosphorylate Shank1 and ProSAP2/Shank3 (Thomas et al. 2005).

### ***2.2.3 Clustering of Receptor Complexes at the PSD by ProSAPs/Shanks***

Located underneath the postsynaptic membrane, ProSAP/Shank scaffolds are primarily involved in the recruitment, clustering, and functional coupling of transmembrane proteins like postsynaptic glutamate receptors while interaction can happen directly or indirectly via adaptor proteins. Among the latter are the GKAPs. These molecules are attached to the ProSAP/Shank PDZ domain via their C-termini and via their N-termini associated with MAGUKs such as PSD-95 (Boeckers et al. 1999a; Naisbitt et al. 1999). Interestingly, the GluR1 subunit of the AMPAR directly binds to the PDZ domain of all ProSAP/Shank family members via its C-terminal PDZ domain-binding motif (Uchino et al. 2006). Binding partner

of the proline-rich stretch right next to the serine-rich region of Shank1 and ProSAP2/Shank3 is Homer1a, a protein which clusters metabotropic glutamate receptors (mGluRs) mGluR1a and mGluR5 at the PSD via their C-termini and further interacts with the inositol trisphosphate receptor (IP<sub>3</sub>R) of the spine apparatus (Tu et al. 1999; Sala et al. 2001, 2005). In this context, it has been shown that a ternary complex composed of ProSAP1/Shank2, its PDZ domain interaction partner phospholipase  $\beta$ -3, and Homer1b contributes to mGluR-evoked calcium mobilization (Hwang et al. 2003). Moreover, the GluR $\delta$ 2 subunit has been introduced as interactor of the Shank1 and ProSAP1/Shank2 PDZ domains selectively at the PSDs of parallel fiber-Purkinje cell synapses in the cerebellum (Uemura et al. 2004). A short serine-rich sequence of Shank1 and ProSAP1/Shank2 proteins further mediates direct association with the proline-rich region of dynamin-2, a PSD molecule participating in membrane turnover and glutamate receptor recycling (Okamoto et al. 2001; Boeckers et al. 2002). All those interactions define the crucial role of the ProSAP/Shank-based scaffolding network in cross-linking distinct glutamate receptor subtypes to each other and to intracellular calcium stores. However, not only glutamate receptors but also other G-protein-coupled receptors and voltage-gated ion channels depend on ProSAP/Shank presence at the PSD. Among the G-protein-coupled receptors, the neurotransmission-related somatostatin receptor 2 (SSTR2) has been identified as being clustered at the PSD by all three ProSAP/Shank family members via PDZ domain interaction as well as the calcium-independent receptor (CIRL) for  $\alpha$ -latrotoxin that binds to the PDZ domains of Shank1 and ProSAP1/Shank2 and may take part in cell adhesion (Zitzer et al. 1999; Kreienkamp et al. 2000). Furthermore, two studies have shown that the voltage-gated L-type calcium channel Ca<sub>v</sub> 1.3 binds to the SH3 and/or the PDZ domains of Shank1 and ProSAP2/Shank3, interactions that tend to be important for linking calcium influx to pCREB signaling (Olson et al. 2005; Zhang et al. 2005).

#### ***2.2.4 Association of ProSAPs/Shanks with the Postsynaptic Cytoskeleton***

The dynamical interplay of the postsynaptic ProSAP/Shank protein scaffold with the cytoskeleton of the dendritic spine is accomplished via proteins directly attached to or indirectly involved in the regulation of actin. In this context, it is important to mention three key in vitro studies. The first one demonstrates that single overexpression of a Shank1 isoform (Shank1b) in hippocampal neurons is capable to promote the maturation and growth of preestablished dendritic spines (Sala et al. 2001). The second one shows that the cortactin-binding site and the ankyrin repeat regions of ProSAP2/Shank3 are both indispensable for proper spine maturation thus clearly implicating concerted involvement of ProSAP/Shank-binding actin-associated proteins in the formation of plastic spines and functional synapses (Roussignol et al. 2005). Interestingly, the third study has implicated

ProSAP1/Shank2 as part of a transient postsynaptic-signaling complex whose further members include PSD-95 and GKAP and which regulates activity-dependent spine growth (Steiner et al. 2008).

The interaction of ProSAPs/Shanks with Densin-180 antagonizes dendritic branching in order to promote the development of functional spines and synapses (Quitsch et al. 2005). While physically linked to F-actin of dendritic spines,  $\alpha$ -fodrin interacts with the N-terminal ankyrin repeats of Shank1 and ProSAP2/Shank3 via one of its spectrin motifs (Bockers et al. 2001) and is further processed in a calmodulin-dependent manner whenever intracellular calcium levels are elevated followed by the reorganization of cytoskeletal elements within spines. ProSAP-interacting protein 2 (ProSAPiP2), one of the most recently identified molecules binding to the PDZ domain of ProSAP2/Shank3 might also be involved in the attachment and modulation of cytoskeletal elements due to its actin binding properties (Liebau et al. 2009). The C-terminal ppI motif of ProSAP1/Shank2 and ProSAP2/Shank3 mediates interaction with the SH3 domain of two proteins that are tightly attached to the actin cytoskeleton, cortical-actin-binding protein (cortactin) and actin-binding protein1 (Abp1) (Du et al. 1998; Boeckers et al. 1999b; Qualmann et al. 2004). Abp1 has been shown to regulate spine morphology by controlling actin polymerization within spine heads (Haeckel et al. 2008), while cortactin has long been known as an effector of activity-dependent, actin-based spine morphology regulation (Hering and Sheng 2003). Moreover, SH3 protein interacting with Nck, 90 kDa (SPIN90), a well-known binding partner of F-actin and of actin regulators like the Arp2/3 complex and N-WASP, especially promotes Shank1b-mediated spine enlargement by interaction with the Shank1 C-terminus (Kim et al. 2009). In addition to all proteins that directly interact with actin, further ProSAP/Shank binding partners exist that are indirectly involved in actin-based cytoskeletal rearrangements within dendritic spines, mainly via small-GTPase-dominated signaling pathways. The signal transduction molecule  $\beta$ PIX is among them, interacting with the PDZ domains of all ProSAP/Shank family members and contributing to cytoskeletal reorganization within dendritic spines as being a guanine nucleotide exchange factor (GEF) for the Rac1 and Cdc42 small GTPases (Park et al. 2003). The latter two molecules further induce the binding of insulin receptor substrate IRSp53 to two N-terminally positioned, consecutive proline-rich clusters of Shank1 and ProSAP2/Shank3, respectively, thereby implying the involvement of ProSAP/Shank platforms in insulin-dependent remodeling of the postsynaptic cytoskeleton (Bockmann et al. 2002; Soltau et al. 2002, 2004). Another study has identified Abelson interacting protein 1 (Abi-1) as interaction partner of the ProSAP2/Shank3 proline-rich clusters and implies that it controls actin assembly within developing dendritic spines by regulating Rac-dependent pathways in a complex together with Eps8, Sos1, and WAVE1 (Proepper et al. 2007). Furthermore, proteins of the SPAR family (SPAR, SPAR2), which crucially regulate the actin cytoskeleton within dendritic spines by activating the small GTPases Rap1 and Rap2, are cross-linked to the PDZ domain of ProSAP2/Shank3 via the Fezzin family members ProSAP-interacting protein 1 (ProSAPiP1),

postsynaptic density protein containing leucine-zippers, 70 kDa (PSD-Zip70), and LAPSER1 (Maruoka et al. 2005; Wendholt et al. 2006; Schmeisser et al. 2009).

To summarize, the ProSAP/Shank platform is a core element of the PSD which mainly clusters postsynaptic receptor complexes by cross-linking them to the actin cytoskeleton of dendritic spines. Because of their gigantic multimerization ability and various protein-protein interaction domains, ProSAP/Shank scaffolds serve as a meshwork for the integration of multiple other molecules into the PSD. By reorganizing cytoskeletal elements, ProSAPs/Shanks have further emerged to be essential modulators of activity-dependent remodeling of synaptic contacts in the mammalian nervous system.

## 2.3 Other Major Scaffold Proteins

A number of studies has provided quantitative information on the stoichiometry of proteins in the PSD using several approaches including EM combined with quantitative immunoblotting (Chen et al. 2005), quantitative mass spectroscopy (MS) (Cheng et al. 2006), and green fluorescent protein (GFP)-based quantitative fluorescence calibration (Sugiyama et al. 2005). The PSD can be biochemically isolated by extracting synaptosome preparations with nonionic detergents, such as Triton X-100, which does not solubilize the PSD.

An average PSD of 360 nm diameter might contain a total molecular mass of  $1.10 \pm 0.36$  gigadaltons (GDa) (Chen et al. 2005), for example, there would be 10,000 proteins of approximately 100 kDa on average. These studies have also definitively demonstrated that scaffold proteins are major components of the PSD. However, the most abundant proteins are two enzymes: CaMKII and SynGAP together represent more than 8% of the PSD protein mass (Cheng et al. 2006). Although it remains a mystery, why two enzymes should be so plentiful in the PSD, some recent evidences suggest that CaMKII and SynGAP could play a structural as well as a regulatory role in synaptic homeostasis. For example, CaMKII $\beta$  binds to F-actin and several other abundant PSD proteins (Colbran and Brown 2004) while autophosphorylated CaMKII $\alpha$  acts as a scaffold to recruit proteasomes to dendritic spines (Bingol et al. 2010). Because SynGAP, in addition to its RasGAP activity, contains multiple protein-protein interaction motifs, one cannot exclude that it may also have a scaffolding function in the PSD (Rama et al. 2008). Among the more classical scaffold proteins, PSD-95 was found to be highly enriched and much more abundant than its closest relatives PSD-93 and SAP102 (see previous paragraph).

At the PSD, MAGUKs (PSD-95) bind to GKAPs which interact with ProSAPs/Shanks that in turn bind the Homers. According to the quantitative MS studies described above, GKAP family proteins are approximately equimolar with ProSAP/Shank family proteins, but only about 30–40% as abundant as PSD-95 family proteins, and twice as abundant as Homer family proteins (Cheng et al. 2006). If an average PSD contains 300 molecules of PSD-95, we can assume the following stoichiometry: 400 MAGUK family members, 150 GKAP family members, 150

ProSAP/Shank family members, and 60 Homer family members. Indeed, using a quantitative fluorescence imaging approach in cultured hippocampal neurons (Sugiyama et al. 2005), Okabe and colleagues have obtained similar quantitative results for PSD-95, but higher values for ProSAP/Shank family proteins (310 copies) and Homer family proteins (340). These differences might be explained by the distinct metrology used for quantification on the one hand and by the different synaptic protein expression in cultured neurons versus adult brain on the other hand. It is important to mention that the numbers are higher in large PSDs and lower in small PSDs, but their stoichiometry seems to be preserved among each PSD, suggesting the presence of a possible “master” organizing scaffold protein.

Despite those quantitative discrepancies, it is clear that the MAGUK(PSD-95)-GKAP-ProSAP/Shank-Homer platform accounts for a substantial proportion of the total protein mass within the PSD and represents the core scaffold structure of the PSD (Sugiyama et al. 2005).

### ***2.3.1 The GKAP Family***

The four members of the GKAP (also named GKAP/SAPAP) family of proteins were originally identified as proteins interacting with the GK domain of PSD-95 (Kim et al. 1997; Takeuchi et al. 1997). The N-terminal domain of GKAP binds to PSD-95 while the rest of the protein exhibits binding domains for synaptic scaffolding molecule (S-SCAM), nArgBP2, and dynein light chain, thus suggesting a function as a scaffold protein that links PSD protein complexes to motor proteins (Naisbitt et al. 2000). The very C-terminal part further interacts with the PDZ domain of ProSAPs/Shanks. It has recently been demonstrated that GKAP is a specific substrate of one E3 ubiquitin ligase, the RING finger-containing protein TRIM3. TRIM3 stimulates ubiquitylation and proteasome-dependent degradation of GKAP and the associated protein Shank1. The suppression of endogenous TRIM3 results in increased accumulation of GKAP and Shank1 at synapses and prevents the loss of GKAP induced by synaptic activity (Hung et al. 2010). Interestingly, degradation of GKAP and ProSAP/Shank occurs during memory consolidation and reconsolidation (Lee et al. 2008).

### ***2.3.2 The Homer Family***

The Homer proteins are encoded by three genes (Homer1-3) and structurally formed by an N-terminal Ena/VASP homology 1 (EVH1) domain followed by a coiled-coil domain that mediates dimerization with other Homer proteins. The Homers were originally discovered by cloning of Homer1a, a splice variant of the Homer1 gene, which is regulated as an immediate early gene (IEG). Homer1a is rapidly upregulated in neurons in response to synaptic activity induced by seizure or

during induction of LTP and is selectively induced in cells of the hippocampus when rodents engage in exploratory behavior (Brakeman et al. 1997; Kato et al. 1998; Fagni et al. 2002). The Homer1 gene encodes for two additional and longer transcripts, Homer1b and Homer1c, which are more similar to the other Homer genes, Homer2 and Homer3, that have also been reported to encode for several transcripts, but none of them is induced by neuronal activity (Soloviev et al. 2000; Xiao et al. 2000). The EVH1 domain of Homer1 binds to a PPXXF or very similar sequence motif in ProSAP/Shank, mGluR1/5, the inositol-1,4,5-trisphosphate (IP<sub>3</sub>) receptor, the ryanodine receptor, different members of the TRPC family of ion channels, PLC $\beta$  (Nakamura et al. 2004; Hwang et al. 2005), selective L-type Ca<sup>2+</sup> channel isoforms (Yamamoto et al. 2005; Huang et al. 2007), and oligophrenin (Govek et al. 2004). Through their ability to self-associate, Homer isoforms containing the coiled-coil domain (called “CC-Homer” or Homer1b in the case of the Homer1 gene) can physically and functionally link the proteins and receptors that bind to the EVH1 domain. This scaffold-like activity has well been demonstrated for the ability of Homer to facilitate a physical association between type I mGluRs and the IP<sub>3</sub>R or TRPC1 and the IP<sub>3</sub>R. In each case, this association is required for the mGluRs and for the TRP channel to respond to signals (Yuan et al. 2003; Sala et al. 2005). Homer1a only contains the EVH1 domain, but lacks the coiled-coil domain; it functions as a natural dominant negative because it cannot dimerize. As on the mRNA level, Homer1a expression is induced by synaptic activity; it might function as a regulator of synaptic structure and activity (Sala et al. 2003). More recently, Homer1a has been implicated in the regulation of homeostatic scaling by regulating agonist-independent signaling of group I mGluRs, a process which scales down the expression of synaptic AMPARs (Hu et al. 2010). These data suggest that behind their function as scaffolds, Homer proteins exhibit a specialized signaling function at the synapse by regulating the activity of type I mGluR.

### 2.3.3 *The GRIP Family*

The four previously discussed PSD scaffold protein families (MAGUKs, GKAPs, ProSAPs/Shanks, Homers) are mostly directly or indirectly linked to the NMDAR complex, while the other major ionotropic glutamate receptors, the AMPARs, are classically linked to a different set of scaffold proteins including GRIP/ABP (encoded by the two distinct genes GRIP1 and ABP/GRIP2) and the protein interacting with C kinase 1 (PICK1). These interactions may account for the dynamic behavior of AMPARs at synapses. Several evidences suggest that GRIP proteins are involved in the synaptic trafficking and/or stabilization of AMPARs and other interacting proteins. However, GRIP, being formed by seven PDZ domains, interacts with many proteins, including Eph receptors and their ephrin ligands, RasGEF, liprin- $\alpha$ , PICK1, the transmembrane protein Fraser syndrome 1 (FRAS1), metabotropic and kainate-type glutamate receptors, the cadherin-associated protein neural-plakophilin-related arm protein (NPRAP), and the



metalloproteinase membrane-type 5 MMP (MT5-MMP) (Dong et al. 1997; Bruckner et al. 1999; Dong et al. 1999; Ye et al. 2000; Hirbec et al. 2002; Setou et al. 2002; Wyszynski et al. 2002; Takamiya et al. 2004; Hoogenraad et al. 2005; Monea et al. 2006; Silverman et al. 2007). Thus, not surprisingly, GRIP proteins can participate in synaptic and neuronal functions not only by interacting with AMPARs but also by interacting with Eph receptors and their ephrin ligands, a signaling complex known to be involved in dendritic spine morphogenesis and hippocampal synaptic plasticity (Hoogenraad et al. 2005). GRIP interaction with motor proteins (directly with conventional kinesin KIF5 or indirectly with KIF1A via liprin- $\alpha$ ) further suggests that this protein family may also contribute to the transport of AMPARs to the synapse (Shin et al. 2003; Hoogenraad et al. 2005). GRIP is widely expressed in different tissues and in neurons, and it is present in both axons and dendrites (Wyszynski et al. 2002). Therefore, the function of GRIP has to be considered beyond the regulation of AMPARs. This is supported by the fact that GRIP1 knockout mice show hemorrhagic blisters and embryonic lethality (Bladt et al. 2002; Takamiya et al. 2004). Only the GluR2/3 subunits of the AMPAR specifically bind to the PDZ5 domain of GRIP, although the PDZ4 domain is also required for strengthening this interaction. Several studies have demonstrated the role of GRIP in AMPAR trafficking to synapses using primary neuronal cultures. A more conclusive work by Takamiya et al. showed that the genetic ablation of both *GRIP* genes blocks LTD expression in cerebellar neurons while single deletion of either isoform allows LTD to occur, suggesting the ability of the two proteins to functional compensate each other at least in part (Takamiya et al. 2008). Finally, certain splice variants of GRIP can be palmitoylated thereby regulating its association with the plasma membrane and localization at the synapse (DeSouza et al. 2002). In contrast, nonpalmitoylated GRIP mostly associates with intracellular membranes (Fu et al. 2003). To conclude, these differentially modified subpopulations of GRIP might control synaptic and intracellular pools of AMPARs, respectively.

### 2.3.4 *PICK1*

The BAR (bin/amphiphysin/rvs) and PDZ-domain-containing protein PICK1 (protein interacting with C-kinase 1) directly binds to the GluR2/3 subunits of AMPARs (Dev et al. 1999; Xia et al. 1999). PICK1 is present at synaptic and nonsynaptic sites in neurons, and its PDZ domain shows relatively promiscuous binding. In addition to PKC $\alpha$  and GluR2/3, it has many other binding partners (both pre- and postsynaptic), including the netrin receptor UNC5H (Williams et al. 2003), various metabotropic glutamate receptor subtypes (Hirbec et al. 2002; Perroy et al. 2002), the dopamine plasma membrane transporter, and the erythroblastic leukemia viral oncogene homologue 2 (ErbB2) receptor tyrosine kinase (Jaulin-Bastard et al. 2001; Torres et al. 2001). PICK1 plays a clear and important role in AMPAR surface expression, trafficking, and synaptic targeting (Jin et al. 2006; Hanley 2008).

Recently, Anggono et al. demonstrated that PICK1 participates in homeostatic plasticity by regulating the subunit composition, abundance, and trafficking of GluR2-containing AMPARs (Anggono et al. 2011). However, PICK1 negatively regulates Arp2/3-mediated actin polymerization thus influencing both NMDA-induced AMPAR internalization and dendritic spine morphology (Rocca et al. 2008; Nakamura et al. 2011). The pleiotropic role of PICK1 can probably explain the finding that loss of PICK1 has no significant effect on synaptic plasticity in juvenile mice but impairs some forms of LTP and multiple distinct forms of LTD in adult mice, suggesting that PICK1 is selectively required for hippocampal synaptic plasticity and learning in adult rodents (Volk et al. 2010).

The role of GRIP and PICK1 in the stabilization of synaptic versus intracellular AMPAR pools has been studied extensively (Daw et al. 2000; Kim et al. 2001; Chung et al. 2003; Hirbec et al. 2003; Seidenman et al. 2003). However, not all the controversies have been resolved, also because of overlapping specificities of PDZ domain interactions. The peptides that are typically used to interfere with the PDZ interactions of GRIP and PICK1 are probably not highly specific for these proteins or for GluR2/3 interactions. Recent experiments in knockout mice are promising to elucidate the specific functions for GRIP and PICK1 (Takamiya et al. 2008; Anggono et al. 2011).

## **2.4 Postsynaptic Scaffolds and Their Relation to Neuropsychiatric Disorders**

As several neuropsychiatric disorders have directly been linked to altered synaptic morphology and function throughout recent years, future therapeutic implications will require an in-depth understanding of the molecular mechanisms underlying the disruption of subcellular structures at the synapse like the active zone or the PSD. Referring to the latter, mislocalization and dysregulation of postsynaptic scaffold proteins are crucial events during the pathophysiological course of several so-called synaptopathies like distinct forms of autism, schizophrenia, or dementia.

### ***2.4.1 The MAGUK Family and Other Scaffold Proteins***

Considering that PSD-95 is the most abundant scaffold protein in the PSD, it is not surprising that its human gene *DLG4* was extensively studied for the presence of specific polymorphisms and mutations to be associated with mental diseases. However, up to now, only one preliminary study suggests the association between *DLG4* gene variation, autism spectrum disorders (ASDs), and Williams' syndrome (Feyder et al. 2010), and perhaps a polymorphism in the promoter gene is linked to schizophrenia (Cheng et al. 2010). Interestingly, alterations in PSD-95 expression

have been found in patients with Alzheimer's disease (AD) and in a mouse model of Fragile X syndrome, a genetically linked autistic-like disorder (Zalfa et al. 2007; Leuba et al. 2008; Zhu et al. 2010). Moreover, mental retardation is clearly associated with the human DLG3 gene that encodes SAP102 (Tarpey et al. 2004; Zanni et al. 2010). The mutations identified by Tarpey et al. introduce premature stop codons within or before the third PDZ domain, and it is likely that these alterations impair the ability of SAP102 to interact with the NMDAR and/or other proteins involved in downstream NMDAR signaling pathways.

The first evidence for a major role of SAPAP3 in brain function was demonstrated by Feng's lab. Mice deficient for SAPAP3 developed an obsessive-compulsive disorder (OCD)-like phenotype, including compulsive grooming and increased anxiety (Welch et al. 2007), while multiple rare SAPAP3 missense variants in humans have been found associated with trichotillomania (TTM) and OCD (Züchner et al. 2009). Further genetic studies should provide more insights into SAPAP3 mutations in humans and TTM or OCD. Similarly, research should provide information whether there is an association between a Homer2 gene mutation and schizophrenia or development and maintenance of alcohol- and substance-use disorders which have been found in KO mice (Szumlinski et al. 2004) but not in humans (Szumlinski et al. 2006).

### ***2.4.2 The ProSAP/Shank Family***

Haploinsufficiency of the ProSAP2/Shank3 gene as underlying cause for the Phelan-McDermid syndrome (also named PMS, 22q13.3 deletion syndrome) provides the most direct link between the loss of a postsynaptic scaffold protein and a disorder whose major clinical features include neuropsychiatric symptoms, among them global developmental delay, absent or severely delayed speech, muscular hypotonia, and "autistic-like" behavior (Bonaglia et al. 2001; Phelan et al. 2001; Wilson et al. 2003; Manning et al. 2004). To exclude that the disruption of genes other than ProSAP2/Shank3, but also located on 22q13.3, is the genetically determined reason for the above-described neuropsychiatric symptoms, researchers have defined a minimal critical region on the chromosome including ProSAP2/Shank3 (Bonaglia et al. 2001; Wilson et al. 2003; Delahaye et al. 2009). A balanced translocation between chromosome 12 and 22 with the breakpoint in the ProSAP2/Shank3 gene (Bonaglia et al. 2001) and another, recurrent breakpoint within intron 8 of the ProSAP2/Shank3 gene exclusively affecting the latter, clearly supported its crucial role in the molecular pathology of PMS (Bonaglia et al. 2006). Further studies have identified de novo mutations in the ProSAP2/Shank3 (Durand et al. 2007; Moessner et al. 2007; Gauthier et al. 2009) and ProSAP1/Shank2 (Berkel et al. 2010) genes in individuals diagnosed with ASD. These mutations might all have a severe impact on the molecular setup of the whole postsynaptic protein platform in the affected patients by disrupting its physiological properties which are crucial for normal synaptic homeostasis and the balance between excitation and

inhibition. Considering the fact that alterations in other synaptic proteins like presynaptic neurexins (Kim et al. 2008; Ching et al. 2010) or postsynaptic neuroligins (Jamain et al. 2003) have been identified in autistic individuals and which are directly attached to the ProSAP/Shank platform via protein-protein interactions, a synaptic NRXN-NLGN-ProSAP/Shank pathway has been proposed whose dysregulation might be one of the core pathophysiological causes in the development of ASDs (Bourgeron 2009). In this context, some *in vivo* data have already been collected by analysis of transgenic mouse models harboring a targeted disruption of this pathway on the level of the ProSAP/Shank platform, like the Shank1 knockout mouse which exhibits smaller dendritic spines, weaker synaptic transmission (Hung et al. 2008), and reduced motor functions (Silverman et al. 2011), or the ProSAP2/Shank3 haploinsufficiency mouse that shows delayed synaptic development, a decrease in synaptic transmission and reduced social sniffing and ultrasonic vocalizations (Bozdagi et al. 2010). Furthermore, mice with distinct deletions of the ProSAP2/Shank3 gene exhibit self-injurious repetitive grooming and deficits in social interaction most probably due to defects at striatal synapses and corticostriatal circuits (Peça et al. 2011).

Schizophrenia has emerged to be another neuropsychiatric disorder related to ProSAP/Shank malfunction as revealed by *de novo* mutations in the ProSAP2/Shank3 gene in patients ascertained for this neuropsychiatric disease (Gauthier et al. 2010). However, both individuals identified in this study exhibited impaired intellectual abilities even before the diagnosis of schizophrenia. Most interestingly, a very recent study from the same consortium (Hamdan et al. 2011) found truncating and/or splicing mutations in several synaptic scaffold proteins including ProSAP2/Shank3 in patients with nonsyndromic intellectual disability (NSID) thus again supporting the crucial role of scaffolds like ProSAP/Shank for the proper development and maintenance of higher brain function.

Recent investigations have additionally implicated ProSAP/Shank platform disassembly in neurodegeneration. Accumulation of soluble  $\beta$ -Amyloid oligomers in rat frontocortical cell culture, in the cortex of transgenic Alzheimer's Disease (AD) mouse models, and in the cortex of AD patients is accompanied by a reduction of Shank1 (Roselli et al. 2009; Pham et al. 2010) and ProSAP2/Shank3 (Gong et al. 2009; Pham et al. 2010) while ProSAP1/Shank2 levels seem to be upregulated (Gong et al. 2009). In this context, it is important to mention that ProSAP/Shank platforms are organized and stabilized via zinc at their C-terminal SAM domains (Baron et al. 2006; Gundelfinger et al. 2006; Grabrucker et al. 2011). Zinc is a metal ion that has the capacity to directly bind to  $\beta$ -Amyloid (Matsubara et al. 2003) and is known to promote its aggregation (Friedlich et al. 2004; Miller et al. 2010). As  $\beta$ -Amyloid accumulation within the synaptic cleft has previously been suggested to contribute to the development of cognitive impairment in AD by trapping synaptic zinc rather than through direct neuronal toxicity (Deshpande et al. 2009; Adlard et al. 2010), one could imagine a synaptopathic mechanism involving  $\beta$ -Amyloid accumulation entrapment of synaptic zinc and a disruption of the postsynaptic ProSAP/Shank platform.

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