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Review

MAGUKs in synapse assembly and function: an emerging view

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Abstract. Neuronal morphogenesis, synaptogenesis and synaptic plasticity are fundamental aspects of nervous system development. Much of our current understanding of how each of these processes contributes to the establishment and maintenance of neural circuitry has come from a molecular description of specific classes of key molecules. With regard to synapse assembly and function, a family of membrane-associated guanylate kinase homologs (MAGUKs) have emerged as central organizers of multicomponent protein signaling complexes. In particular MAGUKs appear to play fundamental roles in the transport, anchoring and signaling of specific subclasses of synaptic receptors and ion channels. In this review, we will focus on the role that subfamilies of MAGUKs play during the formation, maintenance and plasticity of the vertebrate central nervous system glutamatergic synapse.

Key words. MAGUKs; synapses; PDZ domain; glutamate receptors; cytoskeleton; synaptic plasticity.

Introduction

Synapses of the mammalian central nervous system (CNS) are specialized sites of cell-cell contact designed for the rapid and efficient transmission of signals between neurons and their targets. Synapses are asymmetric cellular junctions composed of a presynaptic bouton, a synaptic cleft and a postsynaptic reception apparatus. The presynaptic bouton is about 1 μm in size, filled with several hundred small (30–40 nm) synaptic vesicles (SVs). Depending on synapse type, these vesicles will be filled with specific neurotransmitters, such as glutamate, acetylcholine, dopamine, glycine or gamma amino butyric acid (GABA). The depolarization of the presynaptic terminal by an invading action potential causes SVs to dock and fuse with a specialized region of the presynaptic plasma membrane called the active zone (AZ) and release their transmitter into the synaptic cleft (a small space between the pre- and postsynaptic plasma membranes ~20 nm across). Associated with the postsynaptic plasma membrane and situated opposite to the presynaptic AZ is a second membrane specialization called the postsynaptic density (PSD). The PSD is a protein-rich region into which neurotransmitter receptors are clustered at high density. This arrangement has the advantage that the fusion of a single SV can deliver sufficient neurotransmitter to bathe and activate the postsynaptic neurotransmitter receptors within milliseconds of the action potential arriving in the presynaptic bouton. Electrophysiological studies have shown that synaptic transmission at individual synapses is tightly regulated by levels of electrical activity. For example, presynaptic activity temporally correlated with the strong depolarization of a postsynaptic neuron can lead to the strengthening of synapses (termed synaptic potentiation), while correlated and noncorrelated pre- and postsynaptic action potential firing or lower synaptic activity (low-frequency stimulation) can lead to the weakening of

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synapses (termed synaptic depression) and even synapse elimination. Depending on the synapse type, the strengthening or weakening of CNS synapses involves either increasing or decreasing (respectively) the number of transmitter receptors clustered within the postsynaptic plasma membrane [1] or changes in the probability of presynaptic neurotransmitter release [2].

Much effort over the last 15 years has focused on understanding how changes in synaptic strength, measured electrophysiologically, correlate to changes in the molecular composition of synapses. Initially, research has centered on identifying structural components of the PSD and AZ with the hope that this information would provide clues to questions such as what makes a glutamate synapse different from a GABAergic or cholinergic synapse. Of these, the most progress has been made at glutamatergic synapses, where over 200 proteins have been identified [3, 4]. These studies indicate that synapses of different types are defined in part by unique sets of proteins. However, they also suggest that different complex ensembles of proteins perform a myriad of synaptic functions. Defining these ensembles and assigning specific functions has become a major focus of research in synaptic biology. Clues to how protein ensembles are created and function at synapses have come largely through the analysis of the superfamily of membrane-associated guanylate kinases (MAGUKs) and the collection of proteins that interact with them (fig. 1). These proteins have emerged as central organizers of not only synapses, but other cellular junctions. Furthermore they have been implicated in a variety of functions from suppressing tumor formation to organizing signaling complexes, and more recently in membrane protein trafficking [5–12]. In this review, we will focus on the roles played by MAGUKs in the assembly and function of synapses. For reviews on the role of these proteins in nonneuronal cells and tumorigenesis, we suggest [7, 8, 10, 12, 13].

Structural features of MAGUKs

MAGUKs represent a growing superfamily of multi-domain proteins that are related by the presence of a shared set of structural domains (see fig. 1). Domains common to

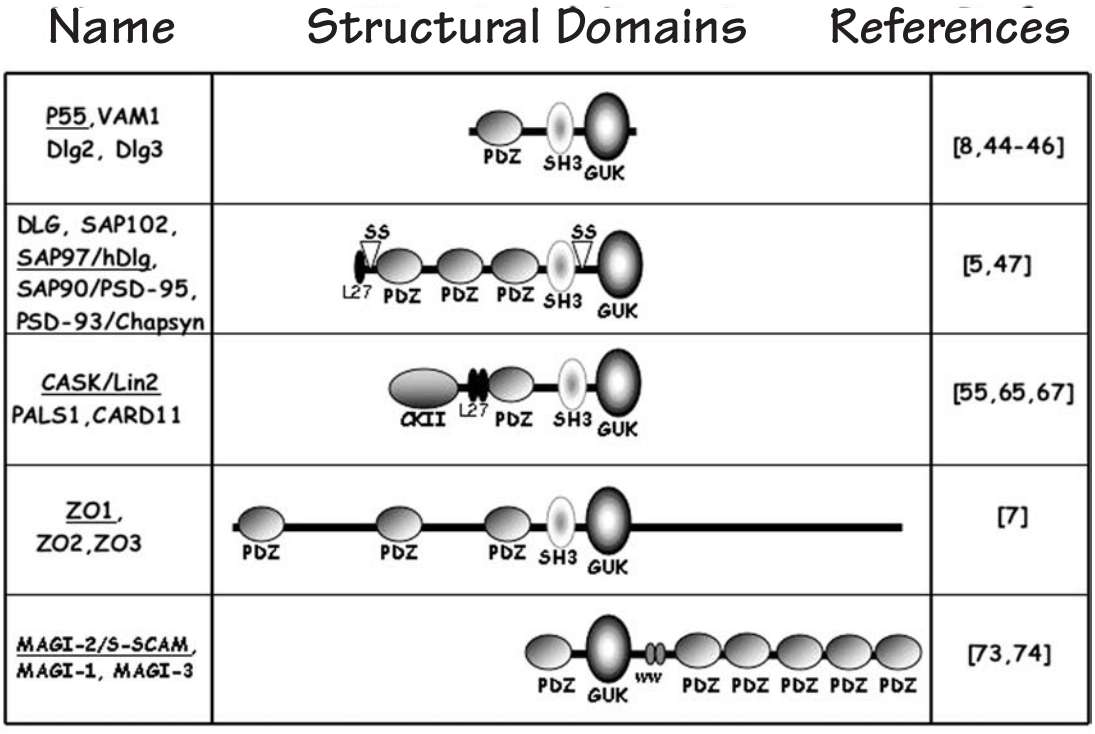

Figure 1. Domain organization of synaptic MAGUKs. Five subfamilies of MAGUKs have been characterized, and the domain organization of representive members from each family is shown. These proteins have evolved different modular domains that confer specific protein-protein interactions. The guanylate kinase (GUK) domain is a central feature of this family. This domain is catalytically inactive and has evolved as a protein-protein interacting domain. All members contain one or more PSD-95/SAP90, DLG, ZO1 (PDZ) domains that directly bind the carboxyl-terminal tails of a variety of proteins including kinases, channels and cytoskeletal proteins. MAGUK family members also possess an Src homology domain 3 (SH3), with the exception of MAGI/S-SCAM. This domain can form intramolecular interactions with the GUK domains as well as bind proteins containing PXXP sequences. The L27 domain is present in SAP97/Dlg, CASK and SAP90/PSD-95 and appears to allow these molecules to undergo homo- or hetero-interactions. The Ca2+/calmodulin kinase II (CaMKII) domain in CASK has been found to bind members of the Mint family. The regions marked SS in the Dlg family represent alternative slicing sites where short peptide sequences (1–33 aa) can be inserted changing the properties of these molecules (see text).

most MAGUKs include the PSD-95/SAP90-DLG-ZO1 (PDZ) domain, a src homology 3 (SH3) domain and a guanylate kinase-like domain (GUK). In addition MAGUKs have been found to contain regions homologous to calcium/calmodulin-dependent protein kinase II (CaMKII), WW domains and L27 domains. MAGUKs have been identified not only in vertebrate species but also in flies and worms and can be organized into five subfamilies based on the arrangement and composition of these structural elements (see below; fig. 1). In what follows, we describe the basic features of the core PDZ, SH3 and GUK domains; a discussion of the other domains/regions will be deferred to sections on specific MAGUKs.

GUK domains

The hallmark of all MAGUKs is the presence of a GUK domain. The domain (~200 aas) shares strong structural similarities with soluble guanylate kinases, enzymes that convert GMP to GDP at the expense of ATP. However, the GUK domains in MAGUKs do not appear to exhibit any guanylate kinase activity in vitro [14–17]. This is consistent with sequence and crystallographic studies that indicate that the P-loop involved in ATP binding is absent in these proteins [14, 15, 18]. Several GUK domains have been tested and shown to bind GMP but not ATP, though the importance of nucleotide binding remains enigmatic [14], as it is not required for function [16] or the binding to GUK domain binding partners [17]. Based on crystallographic studies, it is clear that the GUK domains in these proteins closely resemble the overall structure of authentic guanylate kinases [18]. This suggests that evolutionarily MAGUKs have captured the structural features of guanylate kinases for their own purpose. By all accounts this new function is one involved in forming a selected set of protein-protein interactions which facilitate the attachment of specific MAGUKs to the cortical cytoskeleton, to the microtubule and actin-based transport machinery and/or molecules involved in signal transduction (more later).

PDZ domains

The PDZ domain has become rather famous in the last few years based on its ability to bind short peptide sequences, mostly found at the C-terminal tails of interacting proteins [19–21]. X-ray crystallographic studies of SAP90/PSD-95 and CASK (two MAGUKs) have shown that PDZ domains are composed of two α helixes and six β strands [22, 23]. The C-terminal target peptide sequences bind the groove between the second α helix and second β strand with the GLGF motif, providing a carboxylate binding loop. Binding partner specificity is mostly due to amino acid substitutions within the PDZ domain and the (–3), (–2) and (0) residues in the C-terminal (T/S)XV peptide motif within the PDZ ligand [24]. The specificity of PDZ-peptide interactions appears to be independent of the residue at the (–1) position since its side chain does not interact with the PDZ domain [22, 23, 25]. Additional information in the specificity in PDZ binding can also lie up to 8–10 residues from the C-terminus as seen when GluR1 binds SAP97/hDlg [26]. Interestingly, PDZ domains can also interact with internal T/SXV motifs [27] and non-carboxyl-terminal cyclic peptides [28]. For instance, an internal sequence from the neuronal-nitric oxide synthase (nNOS) PDZ domain binds the peptide binding groove of the syntrophin PDZ domain [29]. Other examples include the PDZ domain in actinin-assoicated LIM protein (ALP), which binds the spectrin-like motif in α-actinin-2 [30], and the PDZ domains in the protein tyrosine phosphatase PTP-BL and reversion-induced LIM protein (RIL) that bind to internal protein segments in the LIM domain in RIL [31]. These data demonstrate that PDZ domains are more versatile than initially thought.

SH3-GUK intramolecular interactions

As with the GUK and PDZ domains, the SH3 domain in MAGUKs is a site of protein-protein interaction. It is structurally related to the SH3 domain in Src and Sem5 [32, 33]. In one example, the SH3 domain in SAP90/PSD-95 has been shown to bind PXXP sequences in its interacting protein KA2 [34]. However, in most cases, the SH3 domains in MAGUKs interact with binding partners in a nonclassical PXXP manner [35–37]. For example, the SH3 domains in SAP90/PSD-95, SAP97/hDlg and CASK (calmodulin-associated serine kinase) have been found to interact with their own GUK domains via an intramolecular interaction [35–38]. This interaction is thought to hold the SH3-GUK domains in a 'closed' state. Crystallographic studies indicate that of the six β strands that make up classical SH3 domains, four are contributed by the SH3 domain and two by the GUK domain in these proteins [32, 33]. Based on biochemical studies and sequence analysis the SH3-GUK intramolecular interaction is a conserved feature of the MAGUK family [35–38].

At present, it is unclear what regulates the open/closed states of the SH3-GUK intramolecular interaction. X-ray crystallographic studies suggest that the hook region may perform such a regulator function [32, 33]. The hook region in MAGUKs was originally defined as sequences situated between the SH3 and GUK domains of DLG (see fig. 1) [39]. In some MAGUKs, the hook region is a site of alternative splicing that introduces short peptide segments endowing each isoform with slightly different properties (see below). With regards to the regulation of the SH3-GUK interaction, there is a calmodulin (CaM) binding site present in the hook domains of DLG family members which binds CaM in a calcium-dependent manner [40, 41]. Whether CaM binding in fact pushes the SH3-GUK interaction into an open state is not known.

One limitation in these and analogous studies is the lack of accurate assays that can detect the conformational state of the SH3-GUK domains. Indirect assays have sought to assess whether intermolecular binding partners have access to either the SH3 or GUK domains. This strategy has shown that peptide binding to the PDZ domains can promote the association of, for example, MAP1A to the GUK domain in PSD-93/Chapsyn-110 [42]. Presumably, this would regulate the association of PSD-93/Chapsyn-110 to dendritic microtubules. A second identified regulator of the SH3-GUK intramolecular interaction is the N-terminus of SAP97/hDlg (S97N). This domain is predicted based on modeling to fold back and bind its own SH3 domain [36]. This intramolecular interaction can negatively regulate GKAP binding to the GUK domain of SAP97/hDlg [36], as well as access of the KA2 subunit of the kainate receptor to the SH3 domain in SAP97/hDlg [34]. Interestingly, deleting the sixth (F) β strand present in the GUK domain of SAP90/PSD-95, which presumably puts the SH3-GUK domains in an 'open' state, promotes increased surface shaker K+ channel clustering [38]. Taken together, these data indicate that as in other molecules, such as Src, intramolecular interactions oriented around the SH3 domain in MAGUKs determine the open and closed states of these proteins. Such mechanisms could be fundamental in regulating when and where specific binding partners are able to plug into the multidomain structure of MAGUKs and in this way regulate the assembly of macromolecular complexes.

Members of the MAGUK superfamily

As mentioned above, MAGUKs can be divided into at least five subfamilies based on the organization of their different structural domains. The simplest of these belong to the p55 family of MAGUKs (fig. 1) (see [8]). These proteins contain a single PDZ, an SH3 and GUK domain. These molecules were first characterized in erythrocytes and lymphocytes and shown to build protein complexes with members of the glycophorin-C family of single-pass transmembrane proteins as well as protein 4.1, a member of the ezrin, moesin and radixin family of actin/spectrin-binding proteins [43]. The p55 subfamily, which includes VAM1, DLG2 and DLG3, is quite old and appears to be critical for the integrity of cortical cytoskeletal structures such as those associated with the erythrocyte plasma membrane [8, 44–46]. Specific functions in neurons have not been described.

The DLG subfamily of MAGUKs has been studied the most extensively. These proteins contain three PDZ domains, an SH3 domain and a C-terminal GUK domain (fig. 1). The first characterized was the *Drosophila* tumor suppressor gene DLG [13, 47]. It was subsequently found to be a component of the *Drosophila* neuromuscular junction (NMJ) [6, 48]. Mutations in *dlg–/–* cause dramatic defects in the formation of septate junctions between epithelial cells [47, 49] and of the larval NMJ [48] (see below). Four vertebrate homolog of DLG have been described, SAP90/PSD-95, SAP102, SAP97/hDlg and Chapsyn-110/PSD93 [5], all of which have been localized to CNS glutamatergic synapses (see [9, 50]) as well as at cholinergic synapses of the CNS [51] and NMJ [52]. Of these, SAP97/hDlg is the most broadly expressed and is found in nearly every tissue and cell type. Members of this family interact with a range of proteins, including ion channels, adhesion molecules, other cytoskeletal proteins as well as subunits of the glutamate receptors (see below). This has led to the hypothesis they are important in assembling receptor-associated signaling complexes at synapses and other membrane specializations [9, 53, 54]. As discussed later, these proteins also appear to play roles in membrane trafficking.

The third family of MAGUKs includes the proteins CASK, PALS1 and CARD11 (fig. 1). Similar to the DLG family, this family is built on the p55 backbone with a C-terminal PDZ, SH3 and GUK domain (in that order), but also contains unique sets of N-terminal domains. For example, the N-terminal third of CASK contains a CaMKII-like domain, as well as two L27 domains that are situated between the CaMKII and PDZ domains [55, 56]. As with the GUK domains, the CaMKII domain in CASK is catalytically inactive and has evolved as a protein-protein interaction domain capable of associating with members of the lin10/X11a/Mint1 family [57] as well as Caskin [58]. CASK was initially identified in *Caenorhabditis elegans* as a gene (Lin-2) involved in epidermal growth factor (EGF)-receptor localization and cell fate determination during the induction of the vulva epithelium [16, 59]. It was simultaneously described in vertebrate neurons as a protein interacting with neurexin [55], proteins involved in synapse formation and calcium channel clustering (see below). In this situation, as in vertebrate cells, CASK/lin2 forms a tripartite complex with the Lin-10 (X11a/Mint1) and Lin-7 (Veli/Mals) gene products [57, 59]. The L27 motif (first identified in Lin-2 and Lin-7) in CASK/lin2 was initially characterized as the Lin-7/Veli/Mals binding site [57] and subsequently to bind the N-terminus of SAP97/hDlg [56], a GluR1 binding protein [60]. In *C. elegans*, the tripartite Lin-2/Lin-7/Lin-10 complex has been implicated in the delivery and anchoring of the EGF receptor to the epithelial lateral membrane. These and other studies (see below) suggest a role for CASK in membrane trafficking [58, 61, 62] as well as the assembly of cellular junctions between epithelial cells and synapses (see below) [63, 64]. In contrast to CASK, PALS1 and CARD11 contain a unique region (U1) and CARD (caspase recruitment domain), respectively, as well as L27 domains, all situated N-terminal of the PDZ-SH3-GUK domains (fig. 1)

[65]. PALS1 has been implicated in building multi-component protein complexes at the epithelial tight junction [66], while CARD11/CARMA1 is a lymphocyte-specific MAGUK apparently involved in NF-κ-B signaling [67, 68]. Neither PAL1 nor CARD11/CARMA1 will be discussed further.

The fourth subfamily of MAGUKs include the Zonal Occludence (tight junction) proteins ZO1, ZO2 and ZO3 (fig. 1). As with the DLG family, the ZO1 subfamily contains three N-terminal PDZ domains, an SH3 domain, a GUK domain as well as a C-terminal extension of varying lengths [7]. In these molecules, the structure of the enzymatically inactive GUK domain has diverged the most from authentic guanylate kinases [69]. With regard to tight-junction biology, this family has been studied extensively and shown to interact with a large collection of molecules, including occludin, the claudins, each other, the actin cytoskeleton by its C-terminal tail as well as signaling molecules (see [69, 70]). As with other MAGUKs, members of the ZO1 family appear to be critical for assembling proteins complexes and in particular for organizing membrane fenestration within the tight junction created by occludin and claudins [69]. In nonepithelial cells, lacking tight junctions, ZO1 localizes to cadherin-based adherence junctions where it directly binds α-catenin [71]. In neuronal cells, ZO1 has been localized to puncta adhaerentia junctions and synaptic junctions formed between mossy fiber terminals and the shaft of CA3 dendrites [72] (see below).

The fifth MAGUK family contains proteins such as MAGI and S-SCAM. These are inverted MAGUKs with their GUK domains situated at the N-terminus followed by two WW domains and five PDZ domains (fig. 1) [73, 74]. Structurally, these GUK domains are quite similar to those in the DLG family and are able to interact with similar proteins, including members of the GKAP/SAPAP family [74] and BEGAIN [75, 76] (see below). There are three MAGI isoforms (MAGI-1, 2 and 3). Like the DLG family, MAGIs are broadly expressed in both neuronal and nonneural cells [73, 76]. Of these, MAGI-2, also called S-SCAM, is neuron specific, where it has been localized to the PSD of excitatory glutamatergic synapses [74, 77]. In contrast, MAGI-1 and MAGI-3 are mostly nonneuronal and have been localized to tight junctions between epithelial cells [73, 78]. Similar to SAP90/PSD-95, MAGI-2/S-SCAM has been shown to interact with a number of PSD proteins, including N-methyl-D-aspartate (NMDA) receptors, suggesting possible organization roles within the PSD (see below).

Synapse assembly

As discussed in detail in many recent reviews [79–81], synapse assembly involves a series of steps initiated by axo-dendritic contact, resulting in the formation of fully functional pre- and postsynaptic compartments. Membrane trafficking of synaptic proteins is thought to be a critical first step that results in the differential transport of pre- and postsynaptic molecules into the correct cellular compartments, e.g. axons and dendrites, respectively. Initial contact, stabilized by the clustering of cell-cell adhesion molecules, promotes the subsequent recruitment and clustering of cortical cytoskeletal proteins that further stabilize the adhesion site and provide a platform for the recruitment of voltage- and ligand-gated ion channels. Temporal studies of synaptogenesis have shown that the presynaptic active zone forms first, as soon as 20 min after touching [82], possibly by the fusion of an active zone precursor vesicle that carries many of the essential active zone proteins in a quantal fashion [83, 84]. Postsynaptic assembly occurs on a somewhat slower and protracted time scale requiring the delivery of PSD proteins via numerous types of vesicular intermediates, rather than a discrete set of postsynaptic precursor vesicles carrying most of the PSD proteins [85]. As such, these data indicate that the presynaptic active zone and postsynaptic density use distinct modes of assembly. With regard to MAGUKs, most studies, as discussed below, indicate that they are not only important structurally for the integrity of synapses but also in the trafficking of multi-component receptor and ion channel complexes from the cell soma to synapses.

Many critical findings regarding the role of MAGUKs in the assembly and function of synapses have come from studies on DLG at the *Drosophila* NMJ. For example, the analysis of mutant *dlg* alleles have demonstrated that DLG is necessary for the assembly and function of synapses on body wall muscles [6, 48]. Spatially, DLG has been localized at the cytoplasm face of the presynaptic plasma membrane as well as the postsynaptic subsynaptic reticulum (SSR) [86]. Developmentally, it initially appears within presynaptic boutons at sites of cell-cell contact with muscle cells. Its appearance at the postsynapse is delayed somewhat but increases as the SSR grows [86]. Since its initial cloning, a number of DLG binding partners have been identified [87–91]. For example, the first two PDZ domains in DLG were quickly recognized as binding sites for the C-terminal tails of Shaker-type voltage-gated K+ channel [87] as well as the cell adhesion molecule Fasciclin II (FasII) [88, 92]. Deleting these tails from FasII and Shaker disrupted their synaptic localization [87]. Similarly, in a *dlg–/–* background both proteins are mislocalized [87, 88, 92], indicating that DLG plays a fundamental role in their recruitment to synapses. The postsynaptic recruitment of DLG itself is rather complex, involving a multi-step process [93]. That is, rather than being directly recruited to synapses from the cytosol, DLG appears to initially associate with a muscle subcortical compartment, followed by the plasma membrane and only later with the NMJs [93].

These data indicate that a series of membrane trafficking steps, perhaps via its association with proteins like Shaker and FasII, brings DLG progressively to synapses. This conclusion is consistent with deletion studies in which the first two PDZ domains in DLG, as well as the hook region and GUK domain, were found to be critical for synaptic localization [88, 93, 94]. Intriguingly, synaptic activity is also thought to play a role in the synaptic localization of DLG via the activation of CaMKII. In an elegant study by Dr Budnik and colleagues, the expression of constitutively active CaMKII was found to increase the levels of DLG at the NMJ [95]. CaMKII is thought to act by phosphorylating a serine residue in PDZ1 of DLG, which presumably regulates the binding of PDZ1 binding partners [95]. While a formal link between synaptic activity, CaMKII activation and DLG localization is still missing, these studies indicate that DLG plays fundamental roles in the assembly and function of synaptic junctions and that this MAGUK is keenly attuned to synaptic activity (see [11, 90]).

Vertebrate MAGUKs in synapse assembly

The basic features ascribed to DLG at the *Drosophila* NMJ include possible roles in organizing multi-component protein complexes and ensuring that they are correctly targeted and anchored to either the pre- and/or postsynaptic plasma membrane (see fig. 2). The data also indicate that DLG may play a role in the transport of these complexes from intracellular ER stores to the synapse. Unresolved issues include the exact nature and diversity of the protein complexes assembled by DLG. As discussed below, vertebrate neurons express a collection of MAGUKs (CASK, ZO1, MAGI-2, SAP90/PSD-95, SAP97/hDlg, SAP102 and PSD-93/Chapsyn-110) that appear to retain many of these basic features of DLG, yet

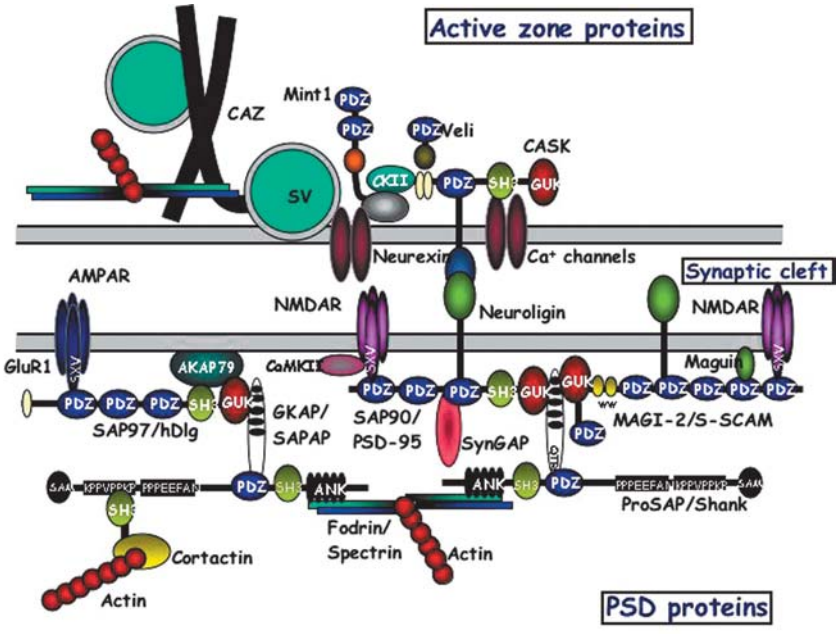

Figure 2. Molecular complexes assembled by MAGUKs at excitatory synapses. Excitatory synapses have been shown to contain a myriad of proteins localized at the active zone and/or the postsynaptic density. MAGUK proteins have been localized at both the pre- and the postsynapse, where they are thought to form complexes with other synaptic proteins. One of the best-characterized presynaptic MAGUKs is CASK. It is thought to build a multimeric complex together with Veli and Mints and subunits of the N- and P/Q-type voltage-gated Ca2+ channels. This MAGUK can also associate with the cell adhesion molecule neurexin-beta. Whether it is directly or indirectly associated with other components of the cytoskeleton assembled at active zones (CAZ) is unclear. Within the postsynapse, MAGUKs, including SAP90/PSD-95, SAP102, SAP97/hDlg and MAGI-2, have been found to form complexes with both NMDA and AMPA type glutamate receptors. For example, the PDZ domains in SAP90/PSD-95 have been found to interact with the C-terminus of NMDA receptor subunits (NMDAR). This facilitates the formation of a signaling complex that includes the Ca2+/calmodulin-kinase II (CaMKII) as well as the Ras-GAP (SynGAP). Similarly, SAP97/hDlg can interact with the cytoplasmic tails of GluR1 subunits of the AMPA receptor (AMPAR), localizing these receptors within the PSD. The guanylate kinase (GUK) domain in SAP90/PSD-95 and SAP97/hDlg provide additional links to the postsynaptic cytoskeletal matrix via its binding to GKAP/SAPAPs. GKAP in turns binds via its C-terminal tail to the PDZ domain in ProSAP/Shank family members. ProSAP/Shank also contains several ankyrin (Ank) repeats, an SH3 domain, a SAM domain and a proline-rich region. The ankyrin repeats have been shown to interact with the actin cytoskeletal protein Fodrin/Spectrin. These interactions help to link the PSD to the plasma membrane. ProSAP also uses its proline-rich domain to bind the SH3 domain in other F-actin-binding proteins such as cortactin as well as the EVH domain in Homer. This latter interaction provides a link between the NMDAR signaling complex and the metabotrophic glutamate receptor (mGluRs) coupled to intracellular stores of calcium. MAGI-2 is also thought to form macromolecular complexes with both NMDAR as well as the cell-adhesion molecules neuroligin and MAGUIN.

through variation in sequence and domain organization have evolved to allow a broad range and diversity of protein complexes to be assembled. Recent data on these vertebrate MAGUKs also indicate a fundamental role for these molecules in protein trafficking. Highlights of these features are discussed below.

ZO1 in mossy fiber synapse assembly

As mentioned above, most available data on ZO1 are oriented around its role in the formation of tight junctions between epithelial cells (see [69, 70]). However, there is one interesting example suggesting a role for ZO1 in synaptogenesis. In particular, ZO1 has been localized to puncta adhaerentia junctions and synaptic junctions formed between mossy fiber terminals and the shaft of CA3 dendrites [72] (see below). The former resemble adherent junctions between epithelial cells with a highly organized actin cytoskeletal matrix. ZO1 recruitment to puncta adhaerentia junctional sites is mediated by its association with the calcium-independent cell adhesion molecule nectin and the actin binding protein afadin [72]. Nectin and afadin have been found to play important roles in organizing cadherin/catenin-based adhesion in a number of systems [96]. At mossy fiber synapses, they are initially associated with the nascent synapse and become restricted to the puncta adhaerentia junctions as the mossy fiber bouton and multi-headed spine develop [96]. Disrupting nectin function interferes with synapse formation, supporting a role for cadherin-mediated adhesion both in the formation of puncta adhaerentia junctions and synaptic function [96]. How ZO1 participates in this transition is not known, but a function linking cadherin-mediated adhesion and the underlying actin cytoskeleton is likely.

CASK in ion channel trafficking

As discussed above, CASK is a structurally unique MAGUK with an ability, similar to DLG family members, to build multi-component protein complexes within neuronal cells. Spatially, it has been localized to the pre- and postsynapse as well as with intracellular membranes within neurons [64], suggesting a broad set of functions for CASK in these subcellular compartments. Within nerve terminals, CASK has been found in a complex with not only Veli and Mint but also with N- and P/Q-type calcium channels [97]. This interaction has been suggested to be important for the synaptic targeting of these channels to presynaptic active zones [62]. Similar results were found in Lymnaea, where CASK was found be essential in the localization of N-type calcium channels to active zones [98]. While CASK may play a trafficking function for these calcium channels (see below), its ability to promote the assembly of actin while in a complex with protein 4.1 and neurexin 1β suggests that the CASK/Veli/Mint complex may physically link calcium channels to adhesion molecules within the active zone [99].

In addition to assembling multimeric protein complexes at synaptic junctions, several lines of evidence indicate that CASK may be involved in the formation of these complexes on vesicular membranes during their transport to synapses. First, as mentioned above, CASK immunoreactivity is found associated with intracellular membranes [64]. Second, CASK together with Mint-1 has been found in a multimeric complex with NR2B subunits of the NMDA receptor and the kinesin microtubule motor KIF17 [61]. Such a complex might be predicted to transport NMDA receptors to the postsynapse. However, genetic evidence does not support a role for Mint-1, in the synaptic targeting of NMDA or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [100], confusing the hypothesized role of CASK in the transport of these receptors. Nonetheless, the ability of the CASK/Veli/Mint complex to associate with both CAMs and ion channels in association with vesicular membrane is consistent with additional roles for CASK in membrane trafficking.

MAGI proteins in NMDA receptor clustering

Although the structural organization of the MAGI subfamily is quite different from other MAGUKs (e.g. with its GUK domain at the N-terminus), it appears to share many properties with the DLG family members SAP90/PSD-95 and SAP102 in terms of its putative binding partners. For example, the GUK domain in MAGI-2 can bind the cytoskeletal proteins GKAP/SAPAP similar to SAP90/PSD-95. Moreover, the second PDZ domain in MAGI-2 can interact with the neuronal cell adhesion molecule, neuroligin [74], as well as GDP/GTP exchange factor (GEF) for Rap1 [101], while the fifth PDZ binds subunits of the NMDA receptor [74], as SAP90/PSD-95. Unique binding partners for PDZ 3, 4 and 5 in MAGI-2 include a protein lipid phosphatase (PTEN) [102], the PH domain-containing protein MAGUIN 1 [103], δ-catenins [104, 105] and activin receptor [106].

One important issue regarding MAGI-2/S-SCAM function at synapses is what role it plays in the hierarchy of PSD assembly. Domain mapping of MAGI-2/S-SCAM has shown that the C-terminal PDZ domain of MAGI-2/S-SCAM is required for its synaptic targeting [105]. Interestingly, this region binds the C-terminus of β-catenin, indicating that β-catenin, within a N-cadherin complex, might recruit MAGI-2/S-SCAM to synapses [105]. Consistent with this hypothesis, overexpression of the C-terminus of β-catenin, excluding the armadillo repeats required for its own synaptic targeting, blocks the synaptic targeting of MAGI-2/S-SCAM but not β-catenin [105]. Surprisingly, the synaptic recruitment of NMDA receptors was not affected, indicating that the ability of MAGI-

2/S-SCAM to bind these glutamate receptors is not essential for their synaptic localization. One issue that has not been extensively explored is whether, like other MAGUKs, MAGI-2/S-SCAM is also involved in functions beyond organizing the PSD, e.g. the trafficking of multi-component protein complexes. At present, time-lapse imaging of MAGI-2/S-SCAM is lacking. Nonetheless, studies on members of the kinesin family of microtubule-dependent motors have shown that the second and third PDZ domains in MAGI-2/S-SCAM can bind the C-terminal tail of KIF1Bα [107]. These data suggest that MAGI-2/S-SCAM may be a kinesin receptor for specific vesicular cargos. The isolation and characterization of these MAGI-2/S-SCAM complexes associated with membranes would be a way to open this emerging area of MAGUK research.

The SAP90/PSD-95, SAP102, SAP97/hDlg and Chapsyn-110/PSD93 subfamily

Structural diversity

Regarding synapse assembly and function, vertebrate homologues of DLG (SAP90/PSD-95, SAP102, SAP97/hDlg and Chapsyn-110/PSD93) are the most well studied. These proteins appear to have retained many of the basic features of DLG, while acquiring some new facets. For example, all four share the same domain topology as DLG, including the three PDZ domains, an SH3 domain and GUK domain (fig. 1). However, a careful analysis of their deduced amino acid sequence reveals that each (except possibly SAP97/hDlg) is the most divergent from DLG within sequences that flank these core domains. The most remarkable are those sequences situated N-terminal to the first PDZ domain and between the SH3 and GUK domains (see [5]). This simple observation suggests that these regions are likely to impart unique properties to these mammalian family members. This concept of conserved yet divergent function is exemplified by studies on these domains in vertebrate neurons. For example, the N-termini of SAP90/PSD-95, SAP102, SAP97/hDlg and PSD-93/Chapsyn-110 have all been found to be critical for subcellular targeting, yet via distinct molecular mechanisms. For SAP90/PSD-95, synaptic targeting requires dual palmitoylation of a pair of N-terminally situated cysteines [108]. This modification is also critical for the surface clustering of ion channels in heterologous cells [109, 110], indicating that this lipid modification may direct SAP90/PSD-95 into synapses [108, 111]. Intriguingly, PSD-93/Chapsyn-110 is also modified on a pair of N-terminal-situated cysteines by palmityolation [112]. However, in this case synaptic targeting does not depend on these residues, but on sequences situated within the first 30 residues of PSD-93/Chapsyn-110 [112]. This observation is somewhat puzzling as this region of SAP90/PSD-95 and PSD-93/Chapsyn-110, while not identical, displays significant homology [109, 112]. This suggests that yet-to-be-identified binding partners for these flanking sequences also play a role in the synaptic targeting of these two MAGUKs.

While cysteine residues are also found in the N-termini of SAP102 and SAP97/hDlg, neither is lipid modified [112], indicating that as with PSD-93/Chapsyn-110 their synaptic localization depends on other mechanisms. A synaptic targeting signal in SAP102 has been mapped to its N-terminus through chimeric studies with SAP90/PSD-95 [112]. Interestingly, these cysteines were not found to be important for synaptic targeting but for binding zinc [112]. Though the function of this motif in SAP102 is not known, similar zinc-finger motifs present in rabphilin, RIM, Piccolo and Bassoon function as protein-protein interacting sites (see [113]). Similar to SAP102, the N-terminus of SAP97/hDlg (referred to as the S97N domain) is quite long (187 residues) [114]. It is composed of at least three distinct functional elements, one involved in subcellular targeting [115], one in homo-multimerization [116, 117] and one in regulating intra- and intermolecular interactions [34, 36]. It is also a site of alternative splicing [114, 117, 118]. This can lead to the insertion of two different proline-rich sequences (I1A and I1B) found to bind the SH3 domains in three tyrosine kinases, Lck [118] Crk and Abl [117], and to influence homo-multimerization of SAP97/hDlg containing these inserts [117]. With regard to subcellular targeting, a L27 or MRE motif (present in the first 65 residues of SAP97/hDlg) appears to be the most important, at least in nonneural cells, where it has been shown to function in the retention of SAP97/hDlg and ion channels within the endoplasmic reticulum [119, 120] and the targeting of SAP97/hDlg to the epithelial lateral membrane [56, 115, 121]. Binding partners important for these diverse functions include CASK [56, 122] (see above), myosin VI (a microfilament dependent motor protein) [123] and Hrs (an endosomal ATPase that regulates vesicular protein sorting) [120]. Since its initial characterization in SAP97/hDlg, similar L27 domains have been found in splice variants of SAP90/PSD-95 [120] and DLG [56, 124]. As in SAP97/hDlg, the L27 domain in SAP90/PSD-95 can bind the L27 domain in CASK as well as Hrs, suggesting that this motif endows this isoform of SAP90/PSD-95 with properties similar to SAP97/hDlg, including an association with endosomal membrane and synaptic targeting [120].

Taken together, these data on functional diversity in the N-termini of MAGUKs demonstrate that sequence variation is an important ingredient in creating molecular diversity. While most studies to date have concentrated on defining features unique to the N-termini of these proteins, additional sequence variation in the form of small peptide inserts has been observed in all family members. In most cases, binding partners for these in-

serts have not been identified. One exception is the I3 insert situated between the SH3 and GUK domains of SAP97/hDlg. This has been found to be a protein 4.1 binding site [121, 125] that not only facilitates the synaptic recruitment of SAP97/hDlg but also its association with the actin cytoskeleton within dendritic spines [126]. Clearly, only the surface has been scratched with regard to the molecular and functional diversity that can be created by this family of proteins.

Assembling macromolecular signaling complexes

As modular proteins, it has often been hypothesized that the most likely function of the SAP90/PSD-95 subfamily of MAGUK is as central organizers of vertebrate CNS synapses. To a large extent this conclusion is based on the spatial distribution of these proteins, their range of binding partners and genetic studies. However, it is fair to say that there are large gaps in our knowledge in particular of the in vivo molecular complexes assembled by each family member. With this caveat in mind, below is a summary of the properties assigned to SAP97/hDlg, SAP90/PSD-95, SAP102 and PSD-93/Chapsyn-110.

As a group, all have all been localized to either the pre- and/or postsynaptic face of excitatory and in some cases inhibitory CNS synapses (see [9, 50, 51]). Binding partners for the PDZ domains include both voltage- and ligand-gated ion channels as well as cell adhesion molecules. Examples include the Shaker voltage-gated K^+ channels [127], subunits of the NMDA [128, 129], AMPA [60] and kainate [130] receptors, neuroligin [131] and the ras-GAP, synGAP [132, 133]. Again note that with few exceptions all four proteins have the ability to bind all of these proteins in vitro and in heterologous cells. A variety of proteins have also been found to interact with the GUK domains in these proteins. The first characterized were components of the neuronal cytoskeleton. For example, within the dendrites of Purkinje cells PSD-93/Chapsyn-110 was found to bind the microtubule-associated protein MAP1A [42]. At synapses, all four are capable of binding the postsynaptic GKAP/ SAPAP family [77, 134, 135], which in turn associate with the ProSAP/Shank family of cytoskeletal scaffold proteins [136, 137]. These latter interactions are thought to provide additional points of attachment to the postsynaptic cytoskeleton. In addition, the ProSAP and Shank proteins associate via Homer to metabotrophic glutamate and inositol-1,4,5-triphosphate (IP3) receptors, thereby linking synaptic signaling to the regulated release of calcium from intracellular stores [138]. Additional GUK domain binding partners include the Rap1-GAP, SPAR, a protein involved in the regulation of spine morphogenesis/actin assembly [139], as well as the PKA adaptor protein AKAP79 [32, 140].

Together these studies indicate that the vertebrate homologues of DLG are also involved in building macromolecular signaling complexes at vertebrate CNS synapses. However, as mentioned above, many of the details regarding where, when and with whom remain mostly unanswered. This is in part due to the difficulties in defining precisely which of these complexes are built by SAP97/hDlg, SAP102, SAP90/PSD-95 and/or Chapsyn-110/PSD-93. For example, early studies on SAP90/PSD-95 provided compelling evidence that this MAGUK formed complexes with NR2 subunits of the NMDA receptor both in vitro and in vivo [52, 128, 141, 142]. However, NMDA receptors were found to cluster normally in mice lacking functional SAP90/PSD-95 [143], suggesting that this MAGUK was not necessarily involved in scaffolding NMDA receptors within PSDs. Intriguingly, the overexpression of SAP90/PSD-95 does not affect the number of postsynaptic NMDA receptors, but does increase AMPA-type glutamate receptors [111]. As discussed below, the latter is achieved by the mutual association of SAP90/PSD-95 and AMPA receptors with the protein Stargazin [111]. These findings indicate that the ultimate clues to MAGUK function in vivo will not necessarily come through the identification of binary interactions made by these proteins but through the elucidation of each MAGUK multi-component complex.

Role of synaptic proteins in trafficking

An important issue regarding MAGUK-mediated complex assembly is whether it occurs only at synapses or whether it could occur earlier, during protein synthesis and maturation. At best this question is only partially answered. Initial studies on the NMDA receptor revealed that prior to its synaptic insertion, it is present in a complex with CASK, Mint and Kif17a (see below) [61]. However this complex did not include SAP90/PSD-95, perhaps for the reason mentioned above. In contrast, SAP97/hDlg has been found associated with immature forms of the AMPA receptor in the endoplasmic reticulum as well as with maturing post-Golgi forms [144]. Similarly, SAP102 has been found associated with both immature and mature NMDA receptors and sec8 as they transit through the endoplasmic reticulum to the plasma membrane (see below) [145]. These data indicate that MAGUKs not only associate with at least some binding partners during their biogenesis, but may also participate in their trafficking between different membrane compartments. This concept is consistent with studies in *Drosophila* showing that DLG is critical in the membrane trafficking of Shaker K^+ channels in CNS axons [94].

Mechanistic clues to the role of MAGUKs in the membrane trafficking of these channels are beginning to emerge from a number of different biochemical studies. For example, studies on SAP97/hDlg have found that its

GUK domain directly binds to the cargo binding domain of the microtubule-dependent motor GAKIN [146, 147], while its N-terminus directly interacts with the cargo binding domain of the actin-dependent motor myosin VI [123]. Moreover, the microtubule-dependent motor KIF1Bα has been found to interact via its C-terminal tail with the PDZ domains in this group of proteins [107]. Indirect interactions with motor proteins have also been described. For example, Mint-1 (a component of the CASK complex) directly binds the microtubule-dependent motor Kif17 [61], while GKAP (a GUK binding partner) binds a dynein/myosin V light chain (DLC) [148]. Together these data indicate that these MAGUKs may provide critical links between motor proteins and the vesicular cargos they carry to and from synapses. Genetic and imaging studies will be required to explore these issues further.

MAGUKs and synaptic plasticity in the mammalian CNS

Synaptic plasticity is defined as an activity-dependent change in synaptic strength. Some forms of synaptic plasticity [termed long-term potentiation (LTP), and long-term depression (LTD)] are expressed via the rapid insertion or removal of the AMPA-type glutamate receptors from the postsynaptic plasma membrane (for review see [149, 150]). AMPA receptor insertion appears to occur primarily extrasynaptically via the exocytosis of vesicular membranes [151] carrying distinct receptor/protein complexes (e.g. SAP97/GluR1-containing AMPA receptors [144]; Stargazin/GluR2-containing AMPA receptors [152]). Lateral diffusion followed by anchoring via postsynaptic scaffold protein, such as SAP90/PSD-95 [152], is ultimately thought to promote glutamate receptor clustering at high density within the PSD [153]. AMPA receptor removal from synapses is envisioned [128] to occur via a clathrin/dynamin-dependent endocytic process, involving the ATPase N-ethylmaleimide-sensitive fusion protein (NSF), and the PDZ domain-containing proteins GRIP (glutamate receptor interacting protein) and PICK (protein that interacts with C kinase) [154–156] (fig. 3). Although changes in synaptic transmission efficacy are often realized by the trafficking of AMPA receptors into and out of synapses, recent studies indicate that AMPA receptor dynamics are also regulated both by AMPA receptor-interacting proteins, and indirectly by the activation of synaptic scaffolding proteins and signaling molecules associated with other receptor complexes (e.g. ionotropic, metabotropic and tyrosine kinase-linked receptors). In this section, we will review how MAGUK proteins could influence the changes in glutamate receptor dynamics that occur during synaptic function and plasticity via their known interactions with these receptors.

Glutamate receptor recycling and synaptic maintenance

As discussed above, individual MAGUKs have been described to exist in distinct complexes with different glutamate receptor subtypes. For example, SAP97/hDlg has been identified as a binding partner for GluR1 subunits of the AMPA receptor [60], a glutamate receptor implicated in dynamic changes in synaptic strength such as occurs with LTP and LTD (see [149]). In contrast, SAP102 [129], MAGI-1/S-SCAM [74] and CASK [61] have been found in complexes with NMDA receptors, glutamate receptors known to be crucial for LTP and LTD. Finally, while SAP90/PSD-95 was initially found as a component of the NMDA receptor complex [128], it has also been characterized in a complex with Stargazin and to influence the delivery of synaptic AMPA receptor [152]. However, what relevance do these interactions have to synaptic plasticity?

With regard to the AMPA receptor, the current literature supports possible roles for SAP97/hDlg both in the trafficking and recruitment of GluR1-containing AMPA receptors to synapses. For example, biochemical studies have shown that a majority of the GluR1-containing AMPA receptors bound to SAP97/hDlg are associated with ER and Golgi membranes and that they appear to dissociate from SAP97/hDlg prior to receptor anchoring in the synaptic junctions [144]. This conclusion is consistent with light microscopy studies of dissociated hippocampal neurons showing that a majority of SAP97/hDlg immunoreactivity is present in the cytoplasm with less than one third being present at synapses [144]. Such data would suggest that SAP97/hDlg has functions outside the synapse, perhaps in receptor trafficking from the soma to the plasma membrane. However, there are a number of factors confusing this issue, making this interpretation less than conclusive. For example, SAP97/hDlg has been found to exist in at least seven different isoforms (see [117]) that arise via alternative splicing of exons that introduce short peptide sequences either into the N terminus (I1A, I1B) (see above) or Hook region (I2–I5). Initial experiments indicate that they may be differentially distributed [117, 121, 126]. Additionally, electron microscopy studies show that SAP97/hDlg is localized within the PSD of asymmetric synapses in cortical slices, where it was colocalized with GluR1 by double-label immunogold electron microscopy [157, 158]. Clearly, these data indicate that SAP97/hDlg performs tasks other than just trafficking receptors to the plasma membrane.

Studies addressing these questions are just beginning to appear. For example, in cultured cortical and hippocampal neurons, SAP97/hDlg colocalizes with both GluR1 and NR1 at synaptic sites [126, 144, 157]. In addition, the recruitment of GluR1-containing AMPA receptors into synapses has been shown to be dependent on the interac-

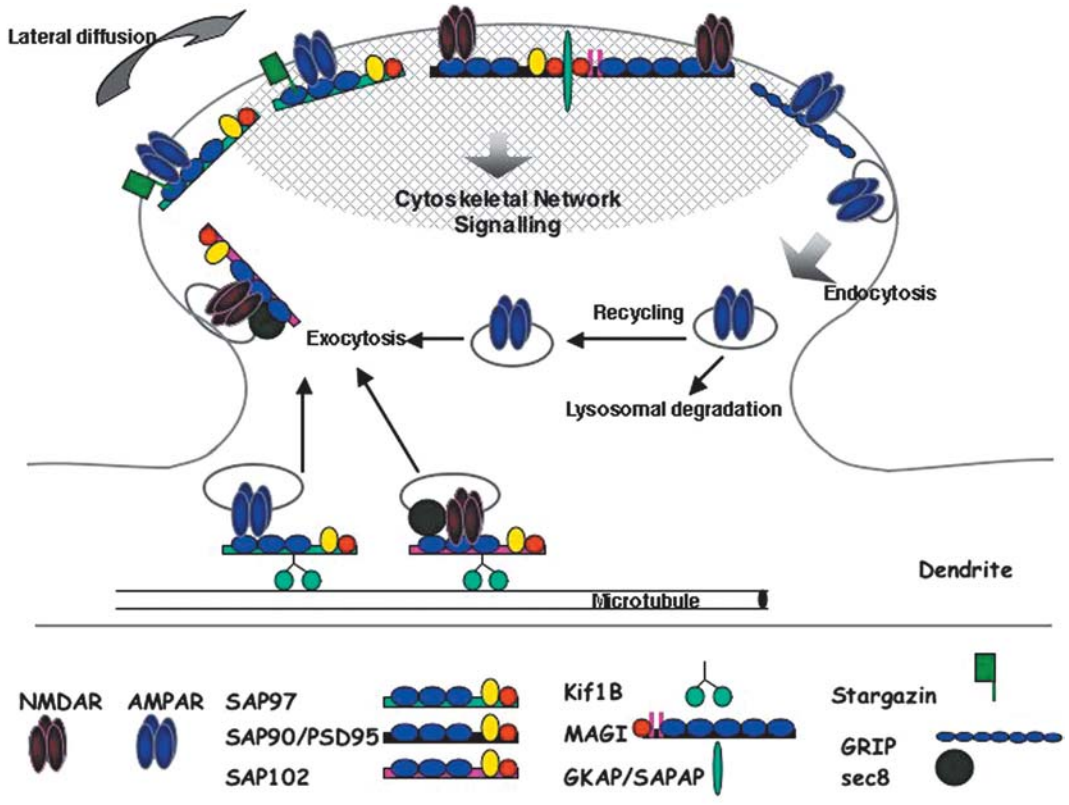

Figure 3. Schematic diagram of glutamate receptor recycling at the postsynaptic plasma membrane. PSD proteins provide a scaffold for the localization, clustering and maintenance of glutamate receptors within the postsynaptic density, and enable rapid changes in the level of AMPA receptor expression at the postsynapse. The dynamic insertion and internalization of AMPA receptors depicted here displays the plastic nature of protein-protein interactions within the PSD, suggesting the PSD is in fact a highly changeable scaffold that can be regulated in an activity-dependent manner. Of note, both SAP97/hDlg and SAP102 have been implicated in the trafficking of glutamate receptors along microtubules using the microtubule-dependent motor such as Kif1Ba. They are also thought to facilitate the delivery of these receptors into the plasma membrane. Lateral diffusion of these receptors into the PSD would result in their stable anchoring via MAGUKs such as SAP90/PSD-95 as well as MAGI-2/S-SCAM. Finally, activity-dependent mechanisms are likely to affect the turnover rates of these receptors at synapses. Here, clathrin/dynamin-mediated endocytosis would drive the retrieval of these receptors from the plasma membrane. Proteins such as GRIP (with six PDZ domains) and PICK1, a PKC substrate, have been implicated in this step of the pathway.

tions between the C-terminus of GluR1 and group 1 PDZ domain proteins [159], a site through which GluR1 and SAP97/hDlg interact [26]. These results are indicative of an important role of SAP97/hDlg in synaptic delivery of GluR1 subunits of the AMPA receptor. Interestingly, GFP-SAP97 expressed in hippocampal cultures exhibits a robust synaptic distribution with a high concentration in dendritic spines [126]. This distribution is isoform specific and is observed only in SAP97/hDlg isoforms containing I3 but not I2 inserts. The latter I2 isoform exhibits a diffuse somato-dendritic pattern [126]. The recruitment of the I3 isoform into spines, in contrast to SAP90/PSD-95, was sensitive to Latrunculin-A, a F-actin depolymerization drug [126], indicating that SAP97/hDlg is probably associated with the actin-rich dendritic spine and that it is not necessarily a tightly associated component of the PSD. This conclusion corresponds well to the biochemical properties of synaptic SAP97/hDlg in vivo [60]. Functionally, the overexpression of SAP97/hDlg with I3 inserts, but not I2, cause an enlargement of the dendritic spine heads as well as an increase in synaptic GluR1 surface expression. Physiologically, this was associated with an increase in mini-excitatory postsynaptic current (mEPSC) frequency but not amplitude. These data indicate that these GluR1-containing receptors are not necessarily being delivered to the postsynaptic plasma membrane but perhaps only to the neck of the dendritic spine. Taken together, these data imply that SAP97/hDlg performs roles in the delivery and possibly in the retention of AMPA receptor at synapses.

What about a role for MAGUKs in the retrieval of AMPA receptors from the postsynaptic membrane? At present, the removal of synaptic AMPA receptors is thought to occur through the differential sorting of receptor between recycling pools and degradative pathways [160]. Following AMPA receptor removal from the synaptic membrane in dissociated hippocampal cultures, the recently internalized GluR1-containing AMPA receptors rarely colocalized with SAP97/hDlg expression [144]. Moreover, neither early nor late endosomes showed any SAP97/ hDlg

expression following GluR1 internalization. These data indicate that SAP97/hDlg does not appear to play a role in the removal process of AMPA receptors from the synaptic membrane nor their translocation to the endocytic pathway. Clearly, future studies will need to focus on defining the specific protein complexes assembled by individual SAP97/hDlg isoforms and the roles each plays in the stepwise synaptic trafficking of AMPA receptors.

Interestingly, some of the properties exhibited by recombinant SAP97/hDlg are also seen in neurons overexpressing SAP90/PSD-95. For example, the overexpression of both cause increases in the size of the spine head, but only SAP90/PSD-95 causes an increase in mEPSC amplitude [111]. This increase has been shown to require the tetraspan transmembrane protein Stargazin, which can bind via its C-terminal tail to the PDZ domains in SAP90/PSD-95 as well as other members of this MAGUK family (fig. 3). At this time, researchers argue that Stargazin helps deliver AMPA receptors to the plasma membrane and that SAP90/PSD-95 provides an anchoring site for these receptors within the PSD. This concept is supported by data showing that the C-terminal tail of Stargazin is not necessary for delivering AMPA receptors to the plasma membrane but rather into the synapses. Moreover, the palmitoylation of SAP90/PSD-95 and its recruitment into synapses was found to be important for this process [152]. However, it is currently unclear how, within the PSD, SAP90/PSD-95 promotes the anchoring of AMPA receptors, as Stargazin is not part of this synaptic complex, and AMPA receptors have not been found to directly bind SAP90/PSD-95 [60].

At this point, it fair to conclude that these two MAGUKs are involved in the insertion and retention of synaptic AMPA receptor; however, many of the details are still missing. For example, in addition to the issues just raised, are Stargazin and SAP97-AMPA receptor complexes different? Do they work in unison, in parallel or in series? Resolution of these issues will clearly require further studies incorporating such techniques as dynamic imaging of these molecules during synaptic plasticity.

NMDA receptor dynamics

The dynamics of NMDA receptor insertion into the synapse has been considerably less well studied. Early developmental studies suggested that most nascent synapses are initially formed as silent synapses, possessing NMDA receptors but not AMPA receptors [161], and then based on neuronal activity can be activated and further potentiated [162–164]. This concept is consistent with dynamic imaging studies showing that NMDA receptors are present at nascent synaptic sites [82, 165] and suggest that there are NMDA receptor-dependent processes that ultimately lead to the recruitment of AMPA receptors (see [166]). It therefore has been of some interest to examine the dynamic events associated with the synaptic recruitment of NMDA receptors and their associated proteins. Initial studies have focused on two MAGUKs, SAP90/PSD-95 and MAGI/S-SCAM, both found to bind the C-terminal tails of NR2 subunits of the NMDA receptors [74, 128]. Interestingly, SAP90/PSD-95 exhibits a different time course of recruitment to nascent synapses than that seen for NMDA receptors [82, 85, 167, 168]. Furthermore, NMDA receptor clustering is frequently observed to occur at synapses without the presence of SAP90/PSD-95 or MAGI-1/S-SCAM. This is consistent with previous experiments showing that SAP90/PSD-95 knockout mice still displayed normal synaptic NMDA receptor clustering [143]. Thus, it would appear that SAP90/PSD-95 plays a limited role in localization of NMDA receptors within the postsynaptic membrane. Instead, as discussed above, it would appear that SAP90/PSD-95 is perhaps performing a more fundamental role in synaptic AMPA receptor recruitment.

If not SAP90/PSD-95, then which proteins are involved in trafficking of NMDA receptors to synapses? Three candidates are SAP102, CASK and SAP97/hDlg. SAP102 was initially identified as an NMDA receptor interacting protein, where it was found to bind the cytoplasmic C-terminal tail of NR2 subunits [129, 169]. In contrast to the delayed expression of SAP90/PSD-95 (postnatal day 10–15), SAP102 is expressed during early neonatal brain development in a pattern that mirrors the expression of NR1 and NR2B subunits of the NMDA receptor. Similar to SAP97/hDlg, SAP102 is not concentrated solely within the PSD of asymmetric synapses [129], but is also found distributed within the somatodendritic compartment of neurons [50, 169]. Recent studies by Wenthold and colleagues have shown that sec8, a component of the exocyst complex [145], cannot only plug into the PDZ domains in SAP102 but forms a trimeric complex with NMDA receptors containing primarily NR2B subunits. Intriguingly, sec8 and the sec8-SAP102 interaction was found to be essential for NMDA receptors containing NR2B subunits to exit the endoplasmic reticulum and reach the plasma membrane. These data suggest that similar to SAP97/hDlg, SAP102 is intricately involved in glutamate receptor trafficking. This concept is compatible with biochemical data showing that both of these MAGUKs are able to directly interact with the cargo binding domains of two different classes of microtubule-dependent motor proteins, Kif1Bα [107] and GAKIN [147].

The two other MAGUKs found to interact with NMDA receptors are CASK and SAP97/hDlg. As described above, the former is linked to NR2B subunits of the NMDA receptors by Mint-1 and appears to be associated with vesicular membranes, as the complex contains the microtubule dependent motor Kif17 [61], indicating that CASK may participate in the trafficking of NMDA receptor from the

soma to synapse. NMDA receptor complexes with SAP97/hDlg have also been described. In this case, binding of SAP97/hDlg to the C-terminal tail of NR2A was regulated by CaMKII phosphorylation of serine residues in PDZ1 [170]. This complex was not detected in triton-insoluble synaptic junctional fractions, indicating that like AMPA receptors, SAP97/hDlg may be involved in the trafficking of NMDA receptors to synapse. Clearly future studies are necessary to unravel whether NMDA receptors are transported to synapses together with other synaptic proteins via a single vesicular transport intermediate, or whether multiple vesicular types comprising distinct protein complexes are required for this task. If studies on the trafficking of proteins through the endoplasmic reticulum-Golgi are any clue, there are likely to be many vesicular cargos rather than just one.

As with AMPA receptors, NMDA receptors are thought to undergo a dynamic process of removal from the synaptic membrane. Mechanistically, NMDA receptors have been found to undergo rapid dynamin-dependent endocytosis upon glycine priming [171] and prolonged agonist application [172], and in addition can freely diffuse between synaptic and extrasynaptic sites [173]. At present, it would appear that SAP90/PSD-95 is crucial for this synaptic regulation, as uncoupling of NR1 from SAP90/PSD-95 is necessary for NR1 endocytosis [174]. Thus, NMDA receptors may only be dynamic following unbinding from SAP90/PSD-95, which is acting as an anchoring or 'stabilizing' protein, linking NMDA receptor function to the PSD. Plasticity of NMDA receptor expression is particularly intriguing, as any change in NMDA receptor number or function will have profound effects on the ability of a neuron to undergo further NMDA receptor-dependent plasticity. Having such receptors anchored at the PSD and only subject to down-regulation under certain conditions would ensure that the synapse protects its ability to undergo NMDA receptor-dependent plasticity.

Physiological roles of MAGUKs during synaptic plasticity

As discussed above, it is currently unclear which MAGUKs are actually responsible for the synaptic clustering of AMPA and NMDA receptors. One important approach will be to examine the effects of deleting or mutating each MAGUK on synaptic structure and physiology. Unfortunately, at this stage the literature is not well developed. Partial knockout mice have been generated for SAP90/PSD-95, PSD93 and SAP97/hDlg [143, 175, 176]. Mutant mice that lack full-length SAP90/PSD-95 show strikingly enhanced NMDA receptor-dependent LTP, no LTD and impaired spatial learning. However, synaptic NMDA receptor-mediated currents are not affected, nor is synaptic morphology [143]. SAP90/PSD-95 overexpressing neurons exhibit potentiated AMPA receptor-mediated transmission, and display an increased probability of undergoing long-term depression [177]. This shift in synapse weight results from an increase in the number of synapses expressing AMPA receptors (i.e. a decrease in the number of silent synapses) rather than an increased number or function of AMPA receptors at active synapses. Thus, the role of SAP90/PSD-95 at the synapse appears crucial to coupling NMDA receptors to synaptic plasticity and learning, but not for NMDA receptor localization or function. Knockouts of PSD-93/Chapsyn-110 tell a different story, as mutant mice lacking PSD-93/Chapsyn-110 show completely normal physiological synaptic function at cerebellar Purkinje cell synapses [175]. This result suggests that this MAGUK, the only one expressed at this synapse, does not play any role in development or maintenance of synaptic function. However, a recent study examining PSD-93/Chapsyn-110 function in the forebrain and spinal cord dorsal horn has shown that mutant mice lacking PSD-93/Chapsyn-110 exhibited a reduction in the surface expression of NR2A and NR2B NMDA receptor subunits and also decreased NMDA receptor-mediated postsynaptic currents [178]. Moreover, these mice showed blunted NMDA receptor-dependent persistent pain. This study demonstrates the importance of the interaction between a glutamate receptor binding protein with its partner not only for normal synaptic functioning but also for higher brain functions such as pain response.

A partial knockout mouse for SAP97/hDlg has been generated; this mutant mouse expresses a truncated form of SAP97/hDlg that has the three PDZ domains but lacks the SH3, GUK and hook region [176]. Mutants display in utero growth retardation, severe craniofacial abnormalities and die perinatally. Synaptically, this partial deletion was found to have no effect on the synaptic clustering of glutamate receptors [158]. However, as the SH3-GUK domains do not appear to be necessary for synaptic targeting of SAP97/hDlg [126] and it is the PDZ domains that are critical for its interactions with AMPA receptor [60], a full knockout of this molecule will be necessary to fully understand its role in the development and maintenance of synapses.

Plasticity of the PSD

In order for a synapse to grow and remodel, not only do glutamate receptors need to undergo dynamic changes in surface expression, but the PSD itself must be subjected to dynamic changes in protein composition. This concept may appear somewhat counterintuitive, as examining the PSD by electron microscopy has led many to conclude that the PSD is a stable, unplastic structure. However, some plasticity must be present to enable this structure to

allow for the dynamic exchange of its different receptors. Initial clues to this question have come from both biochemical and imaging studies. For example, Mike Ehlers has elegantly shown that global changes in synaptic activity lead to changes in PSD protein turnover and ubiquitination [179]. Intriguingly, different PSD proteins appeared to be 'coregulated' in their expression patterns, such that specific protein 'sets' were either increased or decreased in a stable and reversible manner in response to altered activity. Coregulated protein ensembles could result from the activity-regulated removal of scaffold proteins that subsequently destabilize groups of proteins. Obviously, given the known ability of MAGUKs to act as scaffolding proteins, these molecules could be key candidates for regulating such en masse movement. Of the MAGUKs measured, SAP90/PSD-95 and SAP102 showed activity-dependent regulation, such that SAP90/PSD-95 and NR2A were enriched in active synapses and reduced in inactive synapses. The NR2B subunit and SAP102 showed reciprocal expression levels. Increasing synaptic activity led to ubiquitination and accelerated total protein turnover at the PSD, but this was not the case for all proteins (for example, PSD-95 and NR2A). Thus, changes in activity can lead to bulk turnover of PSD proteins, which may serve to maintain synaptic identity in the face of protein turnover. However, not all PSD components were subject to multi-protein coregulation; for example, neither SAP97/hDlg nor PSD93/Chapsyn-110 were altered by global changes in synaptic activity. It will be important to determine whether changes in PSD protein composition is a mechanism employed by heterologous synapses when changes in activity occur at the single synapse level.

Progress in the dynamic imaging of green fluorescent protein (GFP)-tagged PSD proteins supports the concept that the core components of a PSD are undergoing dynamic, activity-dependent changes. Much of this literature has concentrated on SAP90/PSD-95, as it is considered to be such a core part of the PSD. These studies have shown that the concentration of SAP90/PSD-95 at synapses is regulated in an activity-regulated manner [168, 180]. Interestingly, a high level of SAP90/PSD-95 dynamics was observed at spine precursors, suggestive that these immature structures are the primary sites of PSD formation and remodeling at nascent synapses [168]. In more mature synapses, the large majority of spines are stable over time, but ongoing turnover and structural alterations certainly do occur [168, 180]. These authors suggest that synaptic maintenance is a more subtle process than synaptic formation, enabling mature synaptic structure to be maintained in the face of homeostatic activity. However, changes in synaptic activity can strongly influence PSD dynamics, influencing turnover and replacement of PSD proteins at mature synapses [180]. Imaging of other PSD proteins has shown large differences in their turnover rates. For instance, Homer/Zip-45 is highly dynamic, turning over in the course of minutes [167], while SAP90/PSD-95 and the GUK domain binding protein SAPAP1 turnover with half-lives of hours [76]. These data suggest that some proteins such as SAP90/PSD-95 and SAPAP1 perform basic structural roles within the PSD, for example maintaining synapse size under homeostatic conditions, thus allowing other molecules to perform more dynamic aspects of synaptic function. Conceivably, exchanging these platform molecules in groups, as suggested by the data collected by the Ehlers study [179], would allow the synapse to change its fundamental properties as activity dictates.

Concluding remarks

During the last decade, tremendous progress has been made in identifying protein constituents of synaptic junctions. The detailed analysis of families of synaptic scaffolding molecules, such as the MAGUKs, are providing fundamental clues not only into how synapses are assembled, but also how the dynamic regulation of ensembles of molecules can lead to profound changes in synaptic strength and plasticity. Important areas of future research will include defining not only the interactions that different synaptic molecules can make, but more important, the multimeric complexes that they are able to form in spatially restricted patterns and how such complexes influence synaptic output. There is also a crucial need for studies that are able to combine dynamic imaging of individual and groups of molecules with the physiological properties of neurons and their synapses. Such studies should allow us to determine how the multitude of PSD components can be independently regulated whilst still maintaining synaptic function and plasticity.

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