Role of the MAGUK Protein Family in Synapse Formation and Function

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ABSTRACT: Synaptic function is crucially dependent on the spatial organization of the presynaptic and postsynaptic apparatuses and the juxtaposition of both membrane compartments. This precise arrangement is achieved by a protein network at the submembrane region of each cell that is built around scaffold proteins. The membrane-associated guanylate kinase (MAGUK) family of proteins is a widely expressed and well-conserved group of proteins that plays an essential role in the formation and regulation of this scaffolding. Here, we review general features of this protein family, focusing on the discs large and calcium/calmodulin-dependent serine protein kinase subfamilies of MAGUKs in the formation, function, and plasticity of synapses. © 2011 Wiley Periodicals, Inc. Develop Neurobiol 72: 57–72, 2012

Keywords: **MAGUK; DLG; synapse; CASK; PSD-95**

INTRODUCTION

Chemical synapses are cellular domains that allow rapid and efficient transmission of the signals between a neuron and its target. This arrangement requires precise apposition between the two cells involved, where the presynaptic bouton is in close coordination with the postsynaptic neuron. In the presynaptic bouton, the regions of release of the synaptic vesicles—called active zones—are spatially localized opposite to the receptors fields in the postsynaptic membrane. This precise spatial localization is achieved as a result of a network composed by scaffold and cytoskeletal proteins, where receptors, channels, adhesion, and transduction proteins are attached (Sheng, 2001; Zhai et al., 2001). The composition and regulation of this scaffold are essential for the synaptic transmission and for the activity-induced changes that every synapse is constantly undergoing.

In the last decade, much has been learnt about the molecular processes that take place during development to form new synapses (Craig et al., 2006; Prokop and Meinertzhagen, 2006; Südhof and Malenka, 2008). Additionally, the regulation of the short- and long-term changes that affect the synaptic transmission during the lifetime of a mature neuronal circuit has been thoroughly studied (Glanzman, 2010). Although synaptic plasticity is a general characteristic of all chemical synapses, research efforts have focused on glutamatergic excitatory synapses mainly. In this context, a large body of data on the assembly, function, and regulation of synapses has come from studies on the family of scaffold proteins named MAGUK (membrane-associated guanylate kinase, Funke et al., 2005). The MAGUK family, in particular the discs large (DLG) subfamily, is extensively expressed in the brain and well conserved throughout evolution, and it is present in all Metazoans (te Velthuis et al., 2007). Here, we review recent work revealing the role of MAGUKs with a focus on the DLG and calcium/calmodulin-dependent serine protein ki-

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Table 1 MAGUKs Subfamilies

MAGUK Subfamily	Vertebrate Proteins (alternative name)	*Drosophila* Proteins (alternative name)
MPP1	MPP1 (P55)	
MPP2-7	MPP2 (DLG2), MPP3 (DLG3), MPP4 (DLG6), MPP7	Metro (Skiff)
MPP5	MMP5 (PALS1)	Stardust
DLG	DLG1 (SAP-97), DLG2 (PSD-93), DLG3 (SAP-102, NE-Dlg), DLG4 (PSD-95)	DLG1 (DLGS97 and DLGA)
DLG5	DLG5	DLG5 (CG6509)
ZO	TJP1-3 (ZO1-3)	Polychaetoid
CASK	CASK	dCASK (CAKI)
CARMA	CARD11 (CARMA2), CARD10 (CARMA3), CARD14 (CARMA2)	
MAGI	MAGI	MAGI
CACNB	Voltage-dependent L-type calcium channel β subunit	Calcium channel β (Caβ)

nase (CASK) subfamilies on the formation, function, and plasticity of synapses.

MAGUK PROTEIN FAMILY

The proteins of the MAGUK family have been classified phylogenetically in 10 subfamilies by comparison of the genomic sequences of the core PDZ-SH3-GUK region and the supplemental domains that they possess (Table 1 and Fig. 1). The 10 subfamilies are: CASK, membrane protein palmitoylated 1 (MPP1), MPP2-7, MPP5, zona occludens (ZO), caspase recruitment domain containing MAGUK protein (CARMA), DLG, discs large 5 (DLG5), calcium channel β subunit (CACNB), and MAGUK with an inverted repeat (MAGI) (de Mendoza et al., 2010). Recent data suggest that all subfamilies except CACNB and MAGI are monophyletic and that these two subfamilies are either product of convergence or a sister group of the "core MAGUKs" (de Mendoza et al., 2010). The simplest member of the MAGUK family is P55, a member of the MPP family, which has a characteristic module shared by all MAGUKs

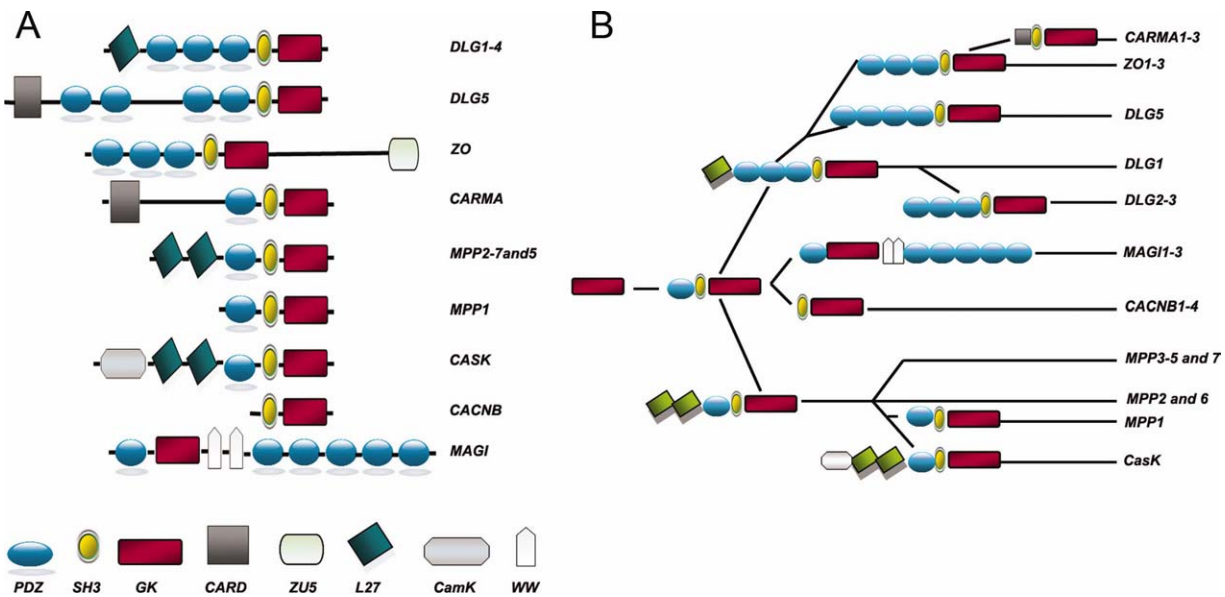

Figure 1 (A) Domain organization of the MAGUK subfamilies, proteins belonging to each family can be found in Table 1. MPP proteins 2–5 and 7 belong to different subfamilies, but share the same domain organization. (B) Model of the evolution of the MAGUK family proposed by te Velthuis et al. (2007). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

consisting of one PDZ (PSD-95, DLG, ZO-1) domain (found in all MAGUKs with the exception of CACNB subfamily), one Src Homology 3 (SH3) domain (except in MAGI subfamily), and a catalytically inactive guanylate kinase (GK) domain with homology to yeast GK. On the evolutionary model proposed by te Velthuis et al. (2007), the more ancient subfamily is the MPP family, corresponding to the central core of PDZ-SH3-GUK domains with two L27 domains. DLG and ZO subfamilies as well as DLG5 subfamily would have evolved by PDZ domain duplication events. The phylogenetic data suggest that the CARMA subfamily is more recent and evolved from the ZO subfamily [see Fig. 1(B), te Velthuis et al., 2007]. Although MAGUK family was thought to be exclusive to Metazoans, recent genomic data show that MAGUKs can be found in the common ancestor of Metazoans, the protist *Capsaspora owczarzaki* suggesting an earlier origin for this protein family (de Mendoza et al., 2010).

Members of the subfamilies DLG, CASK, MPP, CACNB, and MAGI are expressed in the central nervous system (CNS) where they play various roles in the formation and function of synapses. (Laura et al., 2002; Jing-Ping et al., 2005; Deng et al., 2006; Gosens et al., 2007). Members of the ZO subfamily are not expressed in neurons but are present in the brain, where they are important for the formation and maintenance of the blood–brain barrier (Wolburg and Lippoldt, 2002). By contrast, members of the CARMA family mediate antibody recognition in hematopoietic cells and inflammation in a variety of tissues (McAllister-Lucas et al., 2010).

MAGUK PDZ Domains

The PDZs are modular protein–protein interaction domains of about 100 amino acids that are often found at the plasma membrane and are usually involved in signal transduction, playing a central role in assembling signaling complexes (Kim and Sheng, 2004). The interaction between PDZ domains and their target proteins is mainly through the C-terminal peptide motif of the ligand proteins. However, binding to internal polypeptides and lipids and heterodimerization of PDZ domains have been demonstrated as well (Sierralta and Mendoza, 2004; Funke et al., 2005). The PDZ domains are usually classified in three types based on the consensus sequence of the last four amino acids in the C-terminal tail they bind to (Harris and Lim, 2001). However, a classification in 16 different types, including larger stretches of aminoacids, has been proposed recently (Tonikian et al., 2008). Most MAGUKs contain Type I PDZ domains, but CASK, members of MPP subfamily and MAGI, bear Type II PDZ domains.

In the DLG subfamily, all three PDZs are Type I (binding motif is S/TXV), but they have specific partners. For instance, only the first two PDZs of the DLG family bind the specific peptide motif found at the C terminus of Shaker-type K^+ channels and *N*-methyl-D-aspartic acid (NMDA) type glutamate receptor (Kim et al., 1995; Kornau et al., 1995; Niethammer et al., 1996; Tejedor et al., 1997). However, the sole presence of the consensus sequences in the potential partner does not ensure the binding. Thus, while the subunit NR2A of NMDA receptor displays a strong interaction with the PDZ 1 and 2 domains of PSD-95 and SAP-97, the NR1-3 and NR1-4 subunits do not show any interaction despite the presence of a T/SXV motif in their C-terminal tail sequences (Bassand et al., 1999).

MAGUK SH3 Domains

The interaction between SH3 domains and their targets is less well characterized than the MAGUKs PDZ domains. Usually, the SH3 domain binds proline-rich sequences (PXXP) in target proteins, but binding partners for MAGUK's SH3 domains have not been found. *In vitro* and *in vivo* studies suggest that the SH3 and GUK domains form an intramolecular interaction that blocks the PXXP motif recognition, thus preventing the SH3 domain to interact with polyproline sequences in other proteins (McGee and Bredt, 1999; McGee et al., 2001). In the human DLG (hDLG) this intramolecular interaction can be modulated by two proline-rich, alternatively spliced insertions in the N-terminus, which form a double binding site that is capable of interacting with several SH3 domains *in vitro* (McLaughlin et al., 2002). For instance, the N-terminal part of SAP-97 binds the SH3 segment of PSD-95 suggesting an heteromeric interaction; this association could be involved in PSD-95 induced dendritic clustering and trafficking of GluR-A AMPA receptors (Cai et al., 2006).

MAGUK L27 Domains

The subfamilies CASK, DLG (DLG1, 2, and 4), and MPP (2, 5, and 7) contain an N- terminal domain named L27 (found in Lin2 and Lin7), which allows heterointeractions and/or homointeractions (Feng et al., 2004; Li et al., 2004; Petrosky et al., 2005). By alternative splicing, the members of the DLG subfamily (except SAP-102), can express either a prototypic

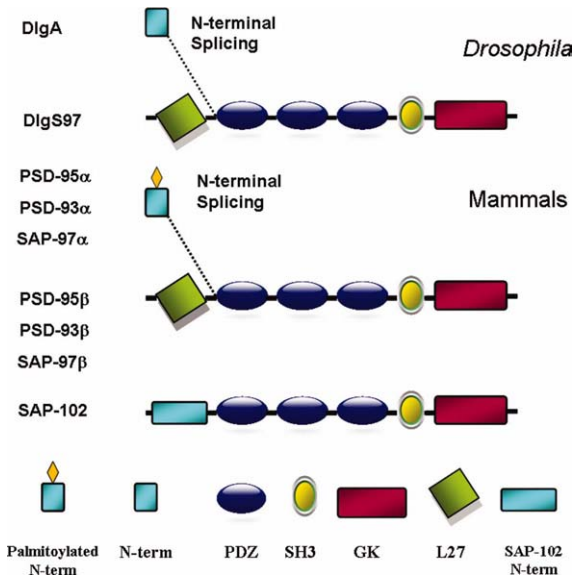

Figure 2 Domain organization and splice variants in the DLG subfamily. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

N-terminal L27 domain (β-isoforms: PSD-95β, SAP-97β, and PSD-93β; DLGS97 in *Drosophila*, reviewed in Thomas et al., 2010) or a putative palmitoylation motif of N-terminal cysteines (Fig. 2; α-isoforms). Although PSD-95 and PSD-93 main isoform is the α-isoform, SAP-97 is expressed mostly as β-isoform. β-Isoforms can homomultimerize and heteromultimerize with other proteins via their L27 domain, for example, the first L27 domain of mLin-2/CASK interacts with the L27 domain of SAP-97β; in turn, the second L27 domain of mLin-2/CASK binds to the L27 domain of Lin-7 (VELI/MALS-1-3/mlin-7) (Chetkovich et al., 2002; Lee et al., 2002; Feng et al., 2004). This tripartite complex SAP-97β/CASK/VELI in mammals has been implicated in the transport of glutamate receptors and potassium channels at the postsynapse, and in vesicle exocytosis at presynaptic sites (Setou et al., 2000; Lee et al., 2002; Wu et al., 2002; Leonoudakis et al., 2004). In *Drosophila*, *in vitro* studies have demonstrated an interaction between the L27 domain of dCASK and DLGS97 (Lee et al., 2002). On the other hand, a complex has been recently shown in the *Drosophila* larval neuromuscular junction (NMJ) between DLGS97, the MPP7 ortholog, Metro and dLin7 formed through their L27 domains (Bachmann et al., 2010).

MAGUK GK Domains

GK domains catalyze the reversible phosphate transfer from ATP to GMP and contain a binding site for the GMP substrate, which is structurally different to the sites of adenylate kinases and G-proteins (Stehle and Schulz, 1990). However, the molecular modeling of a MAGUKs GK domain has provided evidence that, although catalytically active in origin, it gradually lost its enzymatic activity (Kuhlendahl et al., 1998; te Velthuis et al., 2007). Thus, the GK domain evolved to a protein–protein interaction module that, even in the absence of any detectable catalytic activity, is able to bind nucleotides, perhaps inducing conformational changes that regulate its function (Li et al., 2002). In a unique mechanism, CASK GK domain has been shown to translocate to the nucleus and to be important in the formation of a nuclear complex with Trb-1 and CINAP, participating in NR2B transcriptional regulation (Hsueh et al., 2000; Reese et al., 2007; Huang and Hsueh, 2009).

In *Drosophila*, a well-studied interaction is the one between the protein GUK-holder and the GK domain of DLG that allows the recruitment of the PDZ-containing protein Scribble (Mathew et al., 2002; Qian and Prehoda, 2006). Known mutations that truncate the GK domain of DLG have important defects in synaptic structure and function (Budnik et al., 1996).

SH3-HOOK-GK Interaction Domains

In vitro and crystallographic studies of purified PSD-95 and SAP-97 proteins suggest that SH3 and GK domains can sustain an intramolecular interaction that acts as a regulator of GK domains in their binding to other proteins (Shin et al., 2000; Wu et al., 2000; McGee et al., 2001; Qian and Prehoda, 2006). The *in vivo* importance of this intramolecular interaction has been confirmed in *Drosophila,* where mutant DLG proteins that are unable to form this intramolecular interaction exhibit defects in the neuroblast asymmetric division, a known role of DLG (Newman and Prehoda, 2009). Recently, it was proposed that SH3 modulates GK by an allosteric mechanism and not by occluding the GK binding surface as proposed originally (McGee et al., 2001; Marcette et al., 2009). The SH3-HOOK-GK domain is present in all MAGUK members, except in the MAGI subfamily that lacks the SH3 domain; crystallographic studies of the SH3-GK fragment of PSD-95, plus biochemical studies and sequence alignment of SH3-GK modules from different MAGUKs, suggest that this intramolecular interaction is general in the MAGUK family (McGee et al., 2001; Tavares et al., 2001). Furthermore, several MAGUK proteins, such as PSD-95, PSD-93, SAP-97, CASK, p55, and DLG form intermolecular interactions between these domains (McGee and Bredt, 1999; Nix et al., 2000;

Shin et al., 2000). An additional mechanism of regulation is the described interaction between the L27 domain of SAP-97 and the HOOK region that is able to release the GK domain and to engage in intermolecular interactions (Wu et al., 2000; Sabio et al., 2005). This intramolecular interaction could explain the differences in the conformation adopted by SAP-97 and PSD-95, which despite high similarity in the stability and sequence of their SH3-HOOK-GUK domain show specific structural organization and folding dynamics. Thus, SAP-97 is a more rigid and compact structure, while PSD-95 shows a more flexible conformation (Vandanapu et al., 2009).

CAMK Domains

Proteins of the CASK subfamily contain an N-terminal Ca^{+2}/calmodulin-dependent (CaM)-kinase domain. CASK CaM-kinase domain was presumed catalytically inactive, because it is devoid of enzymatic activity *in vitro* and it lacks the canonical Mg^{+2} binding motif (D-F-G, motif that most kinases bear, Hata et al., 1996). Recently, however, it was found that, unlike other kinases, the CaMK domain of CASK is active in absence of Mg^{+2} and constitutively binds nucleotides and catalyzes its autophosphorylation (Mukherjee et al., 2008).

MAGUKs IN SYNAPSE FORMATION AND FUNCTION

Expression Pattern of MAGUK Proteins in the Nervous System

DLG and CASK subfamilies are the most studied MAGUKs due to their clear role in synapse formation and function. Although members of both subfamilies are expressed in epithelial tissues and in the peripheral nervous system, our focus here will be on their function in the CNS.

The DLG subfamily of proteins (see Fig. 2) is expressed in the CNS at all stages of development (see Fig. 3) and, although all members can be found presynaptically and postsynaptically, some of them are mainly found in the postsynaptic compartment in excitatory synapses and restricted to the postsynaptic density (PSD), specially PSD-95 (Kim and Sheng, 2004; Funke et al., 2005). Also, there are differences among DLG proteins in their temporal and spatial expression. PSD-95 exhibits a low expression during embryonic and early postnatal development in most brain regions, but it is enhanced during postnatal development reaching a maximum expression in adults (see Fig. 3, Hsueh and Sheng, 1999; Al-Hallaq et al., 2001). PSD-93 shows an expression profile similar to PSD-95 in the hippocampus, with low expression at early postnatal stages, increasing later in development (Sans et al., 2000). On the contrary, SAP-102 has a high expression in the hippocampus during the first postnatal week, remain stable by postnatal day P35 and decreases in the adult stage (Müller et al., 1996; Sans et al., 2000). SAP-97 presents an expression pattern opposite to PSD-95/PSD-93 in the hippocampus and other brain tissues, where it decreases its expression levels from the embryo to adult stages suggesting that SAP-97 participates in developmental process of the nervous system (Cai et al., 2008).

By contrast, CASK expression does not show significant differences during embryo to adult development in the rat brain (Hsueh and Sheng, 1999). CASK, however, presents a striking regulation in its subcellular distribution during the development. Thus, it shifts its localization from axonal distribution during postnatal week 2 to somatodendritic localization in adult brain (Hsueh and Sheng, 1999). In addition, CASK is also present in nonsynaptic membranes and intracellular compartments (Hsueh et al., 1998).

In *Drosophila,* there is only one DLG gene, which expresses two proteins. One of them, DLGA, is expressed in the nervous system and epithelia. The other variant, DLGS97, is expressed only in the nervous system and the NMJ. It has been shown that DLGA is highly expressed in the nervous system of the embryo. In the larvae, the expression level is similar to DLGS97, while in the adult DLGA is downregulated and DLGS97 becomes the major isoform (Mendoza-Topaz et al., 2008). As mentioned before, vertebrate members of the DLG subfamily express in the same two protein variants (with and without L27 domains). However, to our knowledge, a differential expression (temporal or spatial) has not been studied for any of these genes. In *Drosophila,* CASK homolog dCASK is expressed mainly, but not exclusively, in the nervous system during all development (Martin and Ollo, 1996). The distinct spatiotemporal regulation of the proteins of the DLG subfamily during the development could reflect different functions in synapse development and physiology.

Subcellular Localization of DLG Proteins

It is accepted that PSD-95, PSD-93, and SAP-102 are mainly postsynaptic proteins, while SAP-97 is presynaptic and postsynaptic (Aoki et al., 2001). Studies using EM have shown that PSD-95, PSD-93, and SAP-102 are localized on PSDs associated with

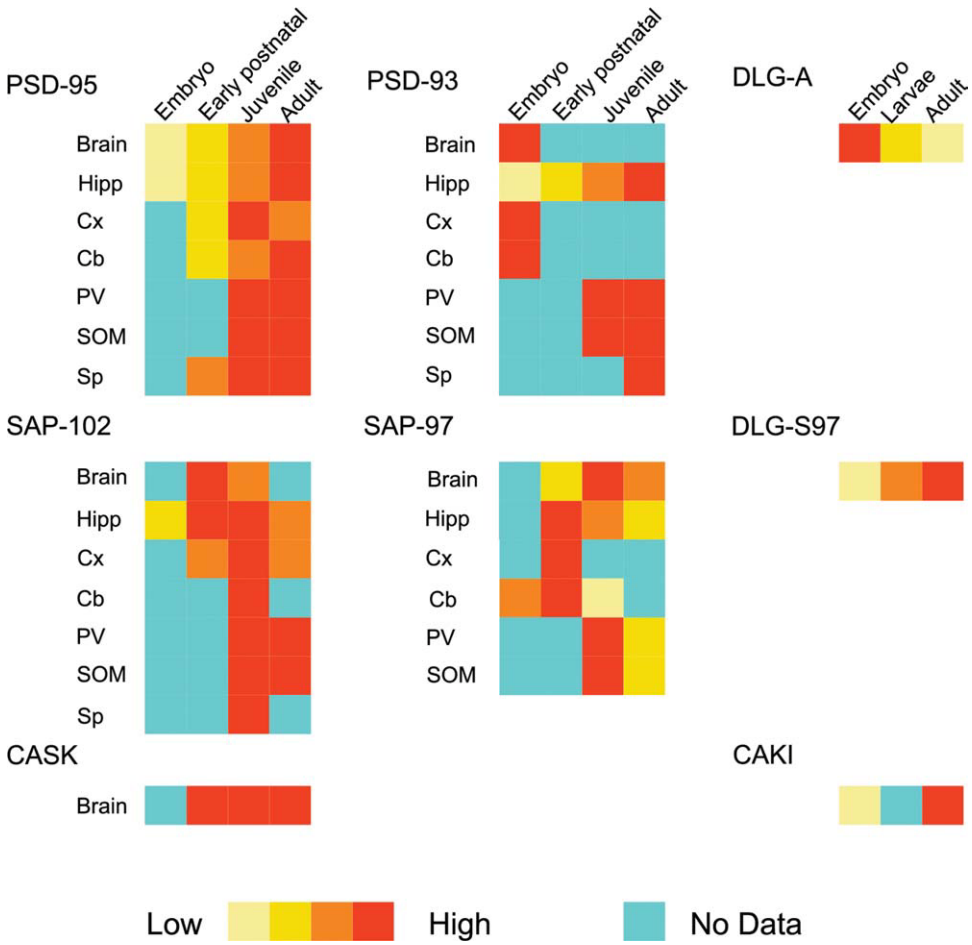

Figure 3 Expression pattern of synaptic MAGUKs in mammalian and insect nervous system. The expression is shown using color code, indicating the relative expression during development, based on the literature. Blue squares indicate no data available for the particular developmental stage. The insect data were obtained from complete animals for embryo expression and from brain tissue in larval and adult stages. Hipp (Hippocampus), Cx (Cortex), Cb (Cerebellum), PV (parvalbumin positive interneurons of the visual cortex), SOM (somatostatin positive interneurons of the visual cortex), Sp (spinal cord).

asymmetric axo-spinous junctions, the same sites where the glutamate receptors are primarily localized (Table 2). The presence of PSD-95 over axo-dendritic and axo-somatic (presumably inhibitory) synapses is rare. However, PSD-95 is found in axons, albeit localized mostly at nonsynaptic sites. PSD-93, which is similar to PSD-95, not only localizes over the PSD in dendrites, but also in presynaptic sites and nonsynaptic portions of the axon (Aoki et al., 2001).

SAP-97 is localized presynaptically and postsynaptically. In some instances, SAP-97 immunostaining appears along the neck of the spinous plasma membrane, leaving the PSD unlabeled (Aoki et al., 2001). In the PSD, SAP-97 is mostly distributed near edges next to the cleft as well as forming clusters away from the cleft. By contrast, PSD-95 and PSD-93 are distributed evenly throughout the PSD (DeGiorgis et al., 2006). Interestingly, it appears that a variation in MAGUKs localization among adjacent PSDs occurs. Thus, some PSDs lacking PSD-95, PSD-93, or SAP-102 are observed in EM preparations (Aoki et al., 2001), suggesting that those PSDs lacking one DLG protein may utilize one of the other two.

PSD-95 is highly stable within synaptic spines, more than other postsynaptic proteins such as CAMKIIa, CAMKIIb, GluR2, or Stargazin and also more stable than other MAGUKs, including SAP-102 (Zheng et al., 2010). This stabilization is blocked by activation of NMDA receptor under an experimental protocol to ellicit long-term depression (Sturgill et al., 2009).

Table 2 Subcellular Distribution of the DLG Subfamily of Proteins

	Subecelular Localization (Neurons)
Mammals	
PSD-95	Mainly postsynaptic, also found in presynaptic terminal and nonsynaptic sites
PSD-93	Mainly postsynaptic, also found in presynaptic terminal and nonsynaptic sites
SAP-102	Mainly postsynaptic, also found in presynaptic terminal and nonsynaptic sites
SAP-97	Presynaptic/postsynaptic, also found in presynaptic terminal and nonsynaptic sites
CASK	Somatodendritic compartment presynaptic/postsynaptic
Drosophila	
DLG-A	Presynaptic/postsynaptic, mainly perisynaptic
DLG-S97	Presynaptic/postsynaptic, mainly perisynaptic
CASK	Presynaptic/postsynaptic

In *Drosophila*, both DLGA and DLGS97 are expressed in motor neurons and the muscle cells at the NMJ (Mendoza-Topaz et al., 2008). In the adult brain, DLGS97 localizes in all neuropiles, overlapping with the label of Bruchpilot, a central component of active zones (Albornoz et al., 2008).

Thus, different proteins of the DLG subfamily show distinct subcellular localization and participate in the formation of protein complexes in the presynaptic and postsynaptic sites.

MAGUKs in Synaptogenesis

Synaptogensis is a highly regulated process implicated in the building of neural circuits. The expression of a number of MAGUK proteins early during synaptogenesis suggests that they are involved in the regulation of this process. Although it is accepted that the initial steps of the synaptogenesis do not involve the DLG subfamily of proteins, it is also clear that in mammals as well as in *Drosophila* NMJ, DLG proteins are necessary for the clustering and stabilization of the glutamate receptors once the presynaptic and postsynaptic sites have been contacted and stabilized by adhesion proteins (Chen and Featherstone, 2005; Waites et al., 2005).

The participation of DLG and CASK subfamilies in the maturation of the mammalian synapses has been suggested by overexpression experiments. PSD-95 and SAP-97 overexpression increase the size of the spines and the formation of multi-innervated spines in hippocampal neurons. Interestingly both effects are abolished by nNOS blockage, implicating a pathway where PSD-95/SAP-97 is upstream of nNOS during synapse formation (Nikonenko et al., 2008; Poglia et al., 2010). The dendrite growth promoted by SAP-97 overexpression requires the binding to GluR1 (Zhou et al., 2008). Additionally, SAP-97 overexpression and to a lesser extent PSD-95 or SAP-102, enhance the expression of presynaptic proteins such as Synaptophisin, Synapsin, and Bassoon (Regalado et al., 2006). Also, the overexpression of several PSD-95 interacting proteins has an effect in spine morphogenesis. For instance, overexpression of Preso, a protein with WW, Ferm, and PDZ domains, increases the number of dendritic spines; whereas Preso knockdown decreases them (Lee et al., 2008). Another PSD-95 interactor, the adhesion molecule Netrin G ligand (NGL), is also involved in synapse development. Incubation with a NGL-2 soluble fragment or knocking down of NGL-2 using an RNAi reduces the number of excitatory synapses (Kim et al., 2006).

Although overexpression experiments have shed light about the function of MAGUK proteins in synapse development, loss of function experiments have not produced consistent results. Knockout (KO) mutant mice for PSD-95, PSD-93, or SAP-102 do not have defects in synapse development (Migaud et al., 1998; McGee et al., 2001; Cuthbert et al., 2007). PSD-95 mice carrying a targeted mutation that introduces a stop codon in the third PDZ domain show only an altered density of dendritic spines in the hippocampus (Vickers et al., 2006). In the case of SAP-97, KO mice display cleft palate and die at birth (Caruana and Bernstein, 2001). Neuronal cultures of mutant animals do not show any defect in the glutamate receptor distribution. However, a detailed examination of the synapse ultrastructure is still pending (Klocker et al., 2002). Moreover, acute knockdown using shRNA against PSD-95 does not produce defects in dendritic spine density in primary hippocampal cultures (Elias et al., 2006). As it has been demonstrated that PSD-93 and PSD-95 can compensate for each other and that even SAP-102 can compensate some defects observed in PSD-95/PSD-93 KO neurons, the lack of defects in KO mice can be explained by functional redundancy (Elias et al., 2006; Howard et al., 2010).

On the other hand, CASK has been implicated in synaptogenesis due to its recruitment to developing terminals by adhesion molecule SynCAM. This

recruitment is dependent on Cdk5 phosphorylation of CASK (Samuels et al., 2007).

In vivo studies in *Drosophila* have shown that MAGUKs participate in synapse formation. Using the larval NMJ, a glutamatergic synapse, it has been demonstrated that DLG is localized at both sides of the synapse, similar to SAP-97 protein in vertebrates (Lahey et al., 1994; Budnik, 1996). Taking advantage of the lack of molecular redundancy, several studies have shown that *dlg* mutant larvae display defects in synaptic bouton morphology (Budnik et al., 1996; Mendoza-Topaz et al., 2008). Interestingly, splice variants mutants for *dlgA* (α form) and *dlgS97* (β form) exhibit similar defects in several aspects of bouton morphology such as active zone length and bouton size (Mendoza-Topaz et al., 2008). The more important defects in the morphology of the NMJ displayed in the highly hypomorphic mutant $dlg^{XI\text{-}2}$ (affecting all splice variants) that includes a reduced subsynaptic reticulum (a postsynaptic structure involved in a variety of roles, including postsynaptic protein localization and local translation), are rescued by the presynaptic expression of the mammalian homologs SAP-102 and SAP-97, but not by PSD-95. This indicates a notable structural conservation throughout evolution (Thomas et al., 2010).

Drosophila larval NMJ has been used as a model of the development and function of mammalian central synapse for several years. It is important to acknowledge, however, that some features of vertebrate central synapses are not well conserved in *Drosophila* NMJ. The main difference with respect to vertebrate MAGUKs is that DLG is not localized in the synaptic compartment as in mammals, precluding a direct function in receptor clustering. Instead, DLG localizes in a perisynaptic region, where a ternary complex formed by DLGS97, Metro, and dLin7 is implicated in delimitating receptor fields during NMJ development. Thus, in *dlg* mutants as well as in *metro* and *dlin-7* mutants the receptors fields are larger than in wild type (Mendoza-Topaz et al., 2008; Thomas et al., 2010). It is not clear yet, if this function is conserved in vertebrates, but it has been shown that the mutation of the three homologs of Lin-7 in mammals (MALS) leads to an increase in synaptic size in microisland neuronal cultures (Olsen et al., 2005).

A characteristic of the DLG gene in *Drosophila* is that the two main transcripts have different expression patterns and specific mutants for them are available (Albornoz et al., 2008; Mendoza-Topaz et al., 2008). *dlgS97* mutants are viable, while *dlgA* mutants are lethal during larval stages, similar to *dlg* mutants. Adult *dlgS97* mutants exhibit significant defects in complex behaviors like phototaxis, circadian rhythms, and courtship behavior, confirming a major role of DLG in the function and regulation of the synapse *in vivo* (Mendoza-Topaz et al., 2008).

MAGUKs in Glutamate Receptor Trafficking

At the vertebrate PSD, MAGUK proteins play important roles in receptor clustering and trafficking. Both of these processes are essential for efficiency and plasticity in glutamatergic synapses.

PSD-95 is the most studied MAGUK for its role in the clustering and trafficking of glutamate receptors, especially NMDA-R (Elias and Nicoll, 2007). However, PSD-95 is also implicated in the trafficking of AMPA receptors, although not through direct interactions but mediated by transmembrane AMPA-R regulatory proteins (TARPs) (Chen et al., 2000; Nicoll et al., 2006).

Several lines of evidence implicate SAP-97 in receptor trafficking. As stated above, SAP-97, PSD-95, and PSD-93 occur as two splice variants (α and β). SAP-97α is mostly localized at the PSD, while SAP-97β is found in the perisynaptic region. Both of them are able to bind GLUR1-AMPA receptors. The available evidence suggests that the ratio between these two isoforms can regulate the distribution of GLUR1-AMPA receptors and, hence, the synaptic strength (Waites et al., 2009). Since it has been shown that coexpression of a dominant negative form of Myosin VI, which disrupts the interaction between SAP-97 and Myosin VI, decreases the insertion of AMPA-R in the postsynaptic membrane in response to transient activation of NMDA receptors, it is thought that the motor protein Myosin VI regulates the interaction between AMPA-R and SAP-97 (Nash et al., 2010). Remarkably, the acute overexpression of SAP-97β promotes the traffic of AMPA and NMDA receptors to the synapse in pyramidal immature neurons (from P1-P2 brain slices) but not in mature neurons (from P8-P9 brain slices). However, chronic overexpression *in vivo* along development enhances the synaptic transmission in mature neurons (P8, Howard et al., 2010). Thus, these findings suggest that SAP-97β plays a role in receptor trafficking during development rather than in adult plasticity.

SAP-97 is known to bind the NMDA receptor subunits NR2A and NR2B, through its PDZ binding domain, mediating their retention in the endoplasmic reticulum (Jeyifous et al., 2009). The phosphorylation of SAP-97 by CAMKII on serine 39 allows the release from the endoplasmic reticulum to the postsynaptic compartment, while the further phosphoryla-

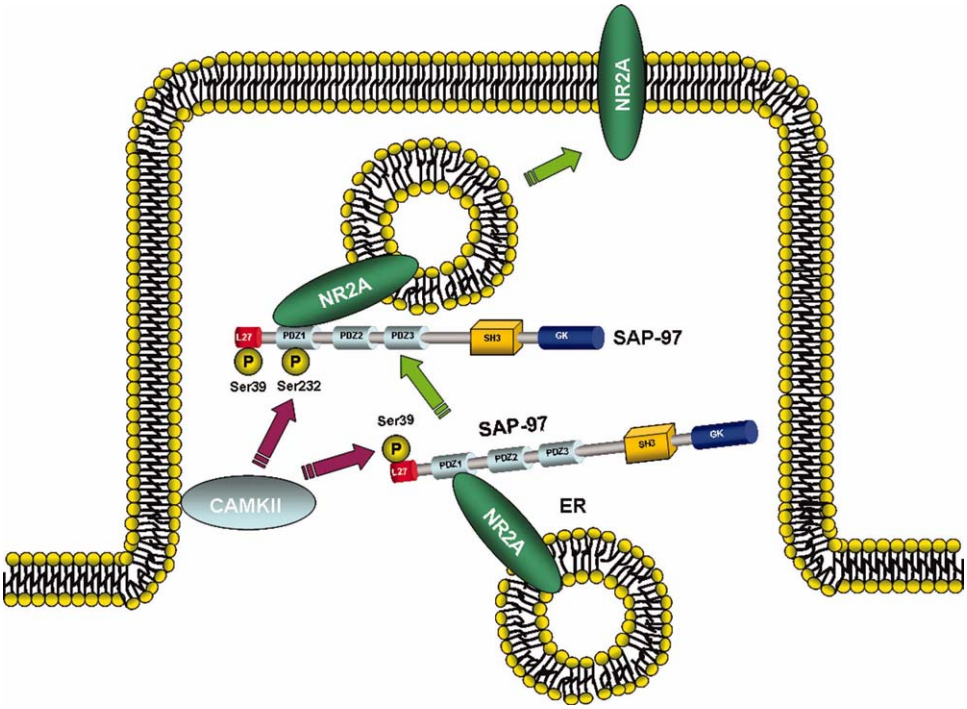

Figure 4 Example of regulation of receptor trafficking by MAGUK proteins. SAP-97 is able to bind NR2A subunit of NMDA receptor through its first PDZ domain. It has been shown that SAP-97 can retain NR2A in the endoplasmic reticulum. After phosphorylation of the Serine 39 on the L27 domain of SAP-97, the complex is released from the ER and moves to the PSD. Posterior phosphorylation of the Serine 232 in the PDZ1 domain disrupts the complex, and produces the insertion of NR2A subunit into the PSD membrane; see text for details. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tion on serine 232 of SAP-97 induces the disruption of SAP-97-NR2A complex and mediates the delivery of NR2A to the synaptic membrane (Fig. 4, Mauceri et al., 2007).

Localization of Kv4.2 potassium channels in dendritic spines is also regulated by SAP-97. It has been shown that SAP-97 (and also PSD-95) binds Kv 4.2 through its PDZ-1 and PDZ-3 domains, and that the CAMKII phosphorylation of SAP-97 also regulates the trafficking of this channel. Interestingly, the phosphorylation in this case was shown to be dependent on Ca^{2+} release from the endoplasmic reticulum (Gardoni et al., 2007).

In mammals, the redundancy could indicate cooperativeness among the DLG subfamily members. For instance, the frequency of spontaneous postsynaptic currents was diminished only in the double KO PSD-95/PSD-93, with lower GluR1 and GluR2 content in the PSD-enriched fraction, but normal NR1 levels of expression. Also, the expression levels of SAP-102 in the double KO mice were higher, indicating a compensatory mechanism. On the other hand, although an shRNAi against SAP-102 has no effect in synaptic transmission in mature neurons, it decreases the transmission in the double KO background (AMPA and NMDA currents, Elias et al., 2006). In addition, despite the lack of defects in synaptic transmission in SAP-97 KO mice, the overexpression of SAP-97 is able to compensate the defects in synaptic transmission in the PSD-93/PSD-95 double KO (Howard et al., 2010). Thus, these results indicate that different MAGUK proteins can compensate each other in the function and trafficking of glutamate receptors.

In *Drosophila*, there is little evidence of a role of DLG in glutamate receptor trafficking. Two kinds of glutamate receptors are expressed in *Drosophila*; one of them contains GluRIIA subunit, while the other contains a GluRIIB subunit. The DLG loss-of-function condition produces loss of GluRIIB but not of GlutRIIA from the synaptic compartment, suggesting a role for DLG in the stabilization or trafficking of GLURIIB (Chen and Featherstone, 2005).

MAGUKs and Synaptic Function

The efficacy of synaptic transmission and its regulation during plasticity depends on complex processes in presynaptic and postsynaptic elements. However,

the presynaptic function of MAGUKs has been scarcely investigated. Indeed, electrophysiological studies in the larval NMJ of *Drosophila dlg* mutants show that these mutants exhibit larger amplitudes of evoked junction currents and this phenotype can be rescued by the presynaptic expression of DLG protein, but not by postsynaptic expression (Budnik et al., 1996). It is possible that DLG proteins regulate the trafficking and clustering of ionic channels and thus, the excitability of the presynaptic terminals affecting the amount of neurotransmitter released. For instance, in *Drosophila* is well known the influence of Shaker K^+ channels, a DLG binding protein, in the neurotransmitter release and the short-term synaptic plasticity. This phenomenon is due to indirect regulation of the Ca^{2+} entrance to the terminal (Jan et al., 1977; Delgado et al., 1994). Another interesting possibility is that DLG proteins regulate the Ca^{2+} dynamics by its interaction with the plasma membrane Ca^{2+} ATPases (PMCAs). PMCA constitutes a ubiquitous, high affinity system for the expulsion of Ca^{2+} from the cell and it is thought to be responsible for the long-term setting and maintenance of intracellular Ca^{2+} levels. Yeast two hybrid and coimmunoprecipitation assays have shown that the C-terminal tail of PMCA4b interacts with the PDZ1 and 2 domains of PSD-95, PSD-93, SAP-97, and SAP-102, whereas only the first three bind to the tail of PMCA2b (Kim et al., 1998; DeMarco and Strehler, 2001). These interactions may play a role in the recruitment and maintenance of the PMCA at specific membrane domains involved in local Ca^{2+} regulation. Besides excitability and Ca^{2+} dynamics, MAGUKs could also regulate the cycle of vesicles. For instance, *Drosophila dlg* mutants show an increased size of synaptic vesicles (Karunanithi et al., 2002; Mendoza-Topaz et al., 2008). Also in mutants for the protein Scribble whose localization is regulated by DLG, there is a clear increase in the number of vesicles of the reserve pool and defects in the replenishment of the ready releasable pool. The main defect of these mutants is the loss of both facilitation and post-tetanic potentation (Roche et al., 2002).

Another MAGUK member that has been associated with the regulation of the presynaptic function is CASK. CASK is located at the presynaptic membrane bound to the cytoplasmic C-terminus of β-neurexins (Martinez-Estrada et al., 2001; Sun et al., 2009). In mammals and *Drosophila*, CASK has been associated with the regulation of neurotransmitter release (Montgomery et al., 2004). Using the yeast two-hybrid technique in COS7 cell cultures, the interaction between the GUK domain of CASK and Rabphilin3a was determined (Zhang et al., 2001). Rabphilin3a is a presynaptic protein involved in synaptic vesicle exocytosis; interestingly, as CASK also binds β-neurexins through its PDZ domain, these results suggest a possible role of rabphilin3a-CASK- β-neurexins in the presynaptic vesicles exocytosis (Zhang et al., 2001). Also, CASK interacts with α_2-liprin through the CaMK domain and the first L27 domain, Cdk5 phosphorylates these domains, reducing this interaction (Samuels et al., 2007). The multiplex α_2-liprin-CASK-Veli could control the release of neurotransmitters, recruiting synaptic release machinery to presynaptic active zones (Olsen et al., 2005, 2006). These interactions suggest that CASK is involved in synaptic vesicle exocytosis (Zhang et al., 2001).

In *Drosophila*, adult flies mutant for *CASK* (*dCASK*) display a reduced locomotor behavior and altered courtship conditioning as well as defects in synaptic function (Martin and Ollo, 1996; Zordan et al., 2005). Additionally, it has been reported a genetic interaction between *Drosophila Neurexin* (*dNrx*) and *dCASK* that it is associated with synaptic defects in the larval NMJ (Sun et al., 2009). Electrophysiological studies of *dCASK* mutants show altered neurotransmitter release at the NMJ, with a fourfold increase in the frequency of the spontaneous evoked postsynaptic potentials (mEPP), increased mean amplitude, multiquantal release, and conserved quantal amplitude. These results suggest that dCASK protein is involved in vesicle release control.

MAGUKs in Learning and Memory

The role of NMDA and AMPA receptors at glutamatergic synapse and its implication in synaptic plasticity is well established as well as the importance of these factors in memory and learning process (Genoux and Montgomery, 2007). Therefore, many molecules that have direct or indirect relation with these receptors might be involved in plasticity, as well as in learning and memory mechanism (Ehrlich and Malinow, 2004; Funke et al., 2005). As expected, the DLG subfamily of proteins, which interacts directly or indirectly with glutamate receptors, has been involved in synaptic plasticity (Cuthbert et al., 2007; Howard et al., 2010). In addition, DLG subfamily also plays a role in learning and memory through its association with other synaptic molecules such as the dopamine receptor D1, which binds PSD-95. This association blocks the ability of the D1 receptor to bind NMDA-R, generating a multiprotein complex that functionally uncouples D1 receptor trafficking and signaling from modulation by NMDA (Zhang et al., 2007). In the same way, SAP-102 is

associated with NR2B-containing receptors and it is important for normal NMDA-induced activation of ERK pathway, having an effect in LTP normal induction (Sans et al., 2000; Cuthbert et al., 2007). The importance of these interactions is demonstrated by the learning defect shown by the PSD-95 mutant mice (Migaud et al., 1998; Komiyama et al., 2002).

The suggested kinase activity of CASK as well as its possible regulation by Ca^{2+} and Calmodulin makes it a good candidate to be involved in plasticity. In *Drosophila*, a mechanism of regulation of CAMKII by CASK playing a permissive role for the auto-phosphorylation of CAMK has been described, also suggesting an important role in plasticity for CASK (Hodge et al., 2006).

MAGUKs in Disease

It is not surprising that mutations on some MAGUK genes are involved in synaptic-related diseases. Two studies report that mutations in the gene *SAP-102* are found in patients with X-linked mental retardation (Tarpey et al., 2004; Zanni et al., 2010). In some of them, the mutations introduce a premature stop codon up stream or within the third PDZ domain and produce truncated proteins that are not able to bind the NMDA receptor and other proteins implicated in the NMDAR pathway. Additionally, a mutation in a splicing site was found. This mutation produced a frame shift leading to a premature stop codon proximal to the third PDZ domain. The disruption of the ability to bind the NMDA receptor with further defects in trafficking could be the cause of intellectual disabilities observed.

Mutations in *CASK* have been found in patients with defects in brain development. Five individuals with X-linked syndromes, which include microencephaly, disproportionate brainstem, cerebellar hypoplasia, and severe mental retardation, were found to bear mutations in the *CASK* gene. One of them was caused by a paracentric inversion of the X chromosome affecting this gene (Najm et al., 2008). A missense mutation in *CASK*, which partially skips exon two, has been found to participate in FG syndrome (X-Linked syndrome also causing mental retardation) in an Italian family (Piluso et al., 2009). These conditions are likely to involve defects in synaptogenesis, where CASK plays an important role.

Parkinson's disease is characterized by the loss of dopaminergic neurons in the *substantia nigra* leading to the motor symptoms observed in the patients, being L-DOPA (a precursor of Dopamine) the most effective treatment. Dysfunction of NMDA receptor complex has been implicated in the L-DOPA induced dyskinesia that occurs in a percentage of the patients under this treatment (Bibbiani et al., 2005). It has been reported that NR2B localization is altered in rat models of dyskinesia induced by L-DOPA. Interestingly, in animals with nondyskinetic behavior, the treatment with a peptide (TAT-NR2B C-tail), which blocks the interaction between NR2B and MAGUKs, shifts the phenotype to dyskinetic. This suggests that the interaction with MAGUK proteins is important for NMDA function in the context of the disease (Gardoni et al., 2006).

Other pathologies like Alzheimer's disease, Huntington and neuronal injury caused by cerebral ischemia have also been related to MAGUK function; these topics are addressed elsewhere (Gardoni, 2008; Gardoni et al., 2009).

Thus, MAGUK proteins are involved in several diseases, likely related to their function in synaptogenesis and receptor trafficking of NMDA receptor in particular.

CONCLUDING REMARKS

In the last years, substantial progress has been attained unveiling the participation of MAGUK proteins in synaptic physiology. The main function in nervous system is clearly the assembly of synaptic complexes that allow the regulation of several aspects of synapse metabolism such as synaptogenesis, receptor trafficking, synaptic function, and plasticity. The importance of MAGUK in these processes is exemplified by the wide spectrum of nervous system diseases that involve mutations in MAGUK proteins. A research area that remains poorly explored is the determination of specific components of synaptic complexes for the different MAGUKs *in vivo*. Until now, mainly PSD-95 complex has been studied, and as it is clear that different MAGUKs have different subcellular distribution, they could form complexes with different properties.

An important issue to address in the future is the study of MAGUK proteins like SAP-97 and CASK in the formation of presynaptic complexes. This aspect of synaptic physiology has received less attention than its postsynaptic counterpart. For instance, it will be interesting to determine whether the discovery of new functions of NMDA receptor in the presynaptic compartment might involve the participation of SAP-97 as a scaffold protein (McGuinness et al., 2010).

The function of MAGUKs reveals how the structure and the combinatorial use of a handful of modu-

latory elements give rise to complexity at different subcellular, cellular, and systems level.

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