Biochimica et Biophysica Acta 1838 (2014) 589–594

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

The anchoring protein SAP97 influences the trafficking and localisation of multiple membrane channels ☆

Chantelle Fourie, Dong Li, Johanna M. Montgomery *

Department of Physiology, University of Auckland, New Zealand
Centre for Brain Research, University of Auckland, New Zealand

ARTICLE INFO

Article history:
Received 13 January 2013
Received in revised form 26 February 2013
Accepted 15 March 2013
Available online 25 March 2013

Keywords:
SAP97
Synapse
AMPA receptor
NMDA receptor
Potassium channel

ABSTRACT

SAP97 is a member of the MAGUK family of proteins that play a major role in the trafficking and targeting of membrane ion channels and cytosolic structural proteins in multiple cell types. Within neurons, SAP97 is localised throughout the secretory trafficking pathway and at the postsynaptic density (PSD). SAP97 differs from other MAGUK family members largely in its long N-terminus and in the sequences between the SH3 and GUK domains, where SAP97 undergoes significant alternative splicing to produce multiple SAP97 isoforms. These splice insertions endow SAP97 with differential cellular localisation patterns and functional roles within neurons. With regard to membrane ion channels, SAP97 forms multi-protein complexes with AMPA and NMDA-type glutamate receptors, and Kv1.4, Kv4.2, and Kir2.2 potassium channels, playing a major role in trafficking and anchoring ion channel surface expression. This highlights SAP97 not only as a regulator of neuronal excitability, synaptic function and plasticity in the brain, but also as a target for the pathophysiology of a number of neurological disorders. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

Contents

1. Introduction

Ultrastructural studies of excitatory glutamatergic synapses in the brain have revealed an electron-dense thickening in the postsynaptic membrane termed the postsynaptic density (PSD). Over 1400 proteins, involving 133 neurological and psychiatric diseases, have recently been identified in the human PSD [1]. Within the PSD, a high concentration of glutamate receptors, associated signalling proteins, and cytoskeletal elements are assembled by a number of synaptic scaffold proteins [2–5]. The PSD stabilizes alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-methyl D-aspartate receptors' (NMDARs) opposite presynaptic terminals, providing the platform for synaptic transmission and receptor movement in and out of the synaptic membrane during synaptic plasticity [3–7]. Among these scaffolding proteins in the PSD are the membrane-associated guanylate kinase homologs (MAGUKs), a protein family that are proposed to be central controllers of multi-protein signalling complexes [8]. The MAGUK family, including SAP97, PSD-95 (SAP90), PSD-93 (Chapsyn 110) and SAP102, share a common domain organization with three N-terminal PDZ domains, a Src-homology 3 (SH3) domain, and a carboxy terminal

☆ This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

* Corresponding author at: Department of Physiology, Centre for Brain Research, University of Auckland, Private Bag 92019, Auckland, New Zealand. Tel.: +64 9 923 9828; fax: +64 9 923 1769.
E-mail address: jm.montgomery@auckland.ac.nz (J.M. Montgomery).

0005-2736/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamem.2013.03.015

catalytically inactive guanylate kinase (GUK) domain [9]. Significant divergence among the MAGUK family members occurs within sequences between these core domains, especially those sequences at the N-terminus before the first PDZ domain, and between the SH3 and GUK domains [8]. Each family member therefore plays distinct regulatory roles in the targeting, trafficking and localisation of various membrane channels [8].

In this review we will focus purely on SAP97. SAP97 is the rat homologue of the *Drosophila* and human discs large tumour suppressor protein (hDlg). SAP97 contains the nucleotide and deduced amino acid sequence of a 97 kDa protein. It is widely expressed throughout the brain and in multiple organs, especially in epithelial cells in the body [10–13]. SAP97 is enriched in excitatory synapses, where it is strongly implicated in the trafficking and localisation of both NMDA and AMPA-type glutamate receptors, regulating synaptic plasticity and could be involved in multiple neurological diseases.

2. SAP97 structure and alternative splicing

Like the other MAGUKs, SAP97 is composed of three PDZ (PSD-95, Dlg, and ZO-1) repeats, a single SH3 domain, and an inactive GUK domain (Fig. 1). Additionally, SAP97 contains two regions of alternative splicing. One region is at the N-terminus and the other region is located between the SH3 and GUK domains (Fig. 1). In the N-terminal region of SAP97 there are two spliced insertions, I1A and I1B, located between the unique N-terminal domain of SAP97 and the first PDZ repeat. The N-terminal alternatively spliced region of SAP97 can bind SH3 domains from multiple proteins and also moderate SAP97 self-association [14]. The most prevalent isoform containing 1A, 1B, and I3 is thought to recruit distinct SH3-containing proteins to the sites of cell–cell contact, e.g. at synapses [14]. In addition to many insertions, SAP97 contains alternative N-termini expressing either double cysteines that normally are palmitoylated (αSAP97) or an L27 domain (βSAP97) (Fig. 1) [7,15,16]. The unique N-terminal domains bestow distinct subsynaptic SAP97 localisation, targeting αSAP97 to the PSD and βSAP97 primarily to perisynaptic regions [15]. βSAP97 has a longer N-terminal region featuring the L27 domain and is the most prominent in the brain [16]. The region between the SH3 and GUK domains of SAP97 contains four alternatively spliced insertions, which have been characterised I2, I3, I4 and I5 [11,14].

The alternatively spliced insertions result in the existence of multiple alternatively spliced SAP97 isoforms. These endow SAP97 with special functions but also result in the roles and functions of SAP97 being quite complicated to determine, especially when many earlier studies did not define which isoform was being characterised. The different intracellular distributions of SAP97 isoforms that have been characterised in both neurons and epithelial cells likely reflect their distinct functions within different cell types [14,17,18]. For example, insertion I3 is responsible for the localisation of SAP97 to the plasma membrane to the regions of cell–cell contact [14]. The I3 insert binds the actin/spectrin-associated protein, 4.1N, and this interaction is thought to be critical for the synaptic targeting of SAP97 and AMPA receptors [17,19]. Deleting the SH3 or GUK domains does not alter SAP97 localisation in spines, whereas deleting the hook region (ΔI3) that binds protein 4.1 reduces the targeting of SAP97 to spines [17]. The I2 insert does not bind protein 4.1 [11], and SAP97I2 has a diffuse expression pattern in the soma and dendrites of neurons with no localised concentration in dendritic spines [17]. The proposed role of the I2 insert is to target SAP97 to the nucleus [17,20,21], although I2 variants have also been described at the cell periphery and at the cell–cell contact points of differentiated cells [17,20]. To date, very little is known about I2, I4 and I5 inserts in neurons. All are known to be expressed in the brain [14] and in cultured hippocampal neurons [17], but whether they play a major role in regulating glutamatergic receptors as has been shown for SAP97-I3-containing isoforms remains to be determined.

3. SAP97 binding partners

SAP97 has been shown to interact with a number of binding partners through its protein–protein interaction domains including membrane ion channels and cytosolic structural proteins, and here we will describe a few of these binding interactions. The first identified binding partner of SAP97 was protein 4.1 [11,14], which as described above interacts with the I3 insert/HOOK domain of SAP97I3 situated between the SH3 and GUK domains. However, the unique N-terminus of SAP97 plays a major role in selecting binding partners and in SAP97 localisation at adhesion sites, as well as the ion channel clustering [22,23]. For example, the N terminus of SAP97 has a myosin VI binding site, and direct binding between myosin VI, SAP97 and the AMPA receptor subunit, GluA1, traffics AMPA receptors in a trimeric complex to and from the postsynaptic membrane [22]. SAP97 PDZ domains can directly interact with KIF1Bα (kinesin family member 1Bα), and S-SCAM (synaptic scaffolding molecule), forming protein complexes that may play an important role in the axonal transport of a number of SAP97- and S-SCAM-bound cargos, such as Kv1.4 potassium channel complexes [24–26]. In addition to binding KIF1B, SAP97 can also bind through its GUK domain to the microtubule-dependent motor KIF13B/GAKIN (kinesin family member 13B) [8,18,27].

SAP97 also has intramolecular interactions that can regulate its ability to bind other proteins. For example, the binding of GKAP (GUK-associated protein) to the GUK domain of SAP97 is negatively regulated by a series of intramolecular interactions between the SH3 and GUK domains [28]. However, the N-terminal sequences that precede PDZ1 facilitate GKAP binding through a U-shaped conformation state of SAP97. A unique feature of the existence of SAP97 in multiple conformations that depend on intramolecular interactions is how this could subsequently alter SAP97 recruitment and assembly at cell–cell contact sites in normal and disease states [27].

SAP97 also interacts with A-kinase-anchoring protein 79/150 (AKAP79/150) [29], a scaffold for PKA, PKC and the Ca^{2+}/calmodulin dependent protein phosphatase calcineurin (also known as PP2B). This interaction is proposed to bring these kinases and phosphatases close to their specific substrates at the synapse. For example, the SAP97–AKAP complex facilitates the phosphorylation of the glutamate receptor subunit GluA1 by PKA [29]. Therefore the SAP97–AKAP79 complex might be important for the recruitment of kinases and phosphatases to synaptic AMPARs. Phosphorylation of SAP97 by

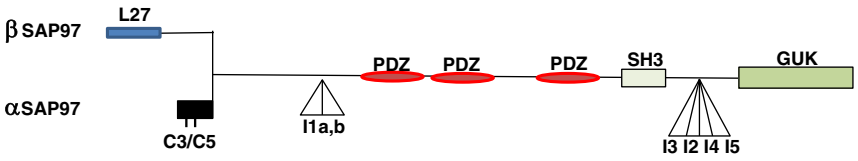

Fig. 1. Schematic diagram of the domain organization of SAP97 isoforms. Illustrated are the major protein–protein interaction sites of SAP97: three PDZ domains (red ovals), an SH3 domain (open rectangle) and a GUK domain (green rectangle). At the N-terminus are the α- and β-isoform inserts, with the former containing a palmitoylation motif (cysteine residues C3 and C5) and the latter an L27 domain. Alternatively spliced sequences (I1a, I1b, I2, I3, I4 and I5) are indicated at the N-terminus (I1a, I1b) and between the SH3 and GUK domains.
Diagram modified from [15].

CaMKII in the N-terminal L27 domain promotes synaptic targeting of SAP97, and of its binding partner GluA1 [30]. The L27 domain is involved in SAP97 heteromultimerization and homomultimerization with itself and other L27 domain-containing proteins, such as calmodulin-dependent serine protein kinase (CASK) [31]. CASK requires an L27 domain-mediated interaction to associate with the SAP97/NMDAR complex in hippocampal neurons [32]. Thus, L27 domains appear to play a central role in the higher order organization of multi-scaffold assemblies [33].

By far the majority of research that has examined the binding interactions of SAP97 has focused on its role in targetting and trafficking ion channel complexes. The major binding partners of SAP97 are AMPA and NMDA-type glutamate receptors, and K^+ channels [8]. These ion channels play major roles in regulating neuronal excitability, synaptic transmission and plasticity, and thus their targetting by SAP97 is of upmost importance for normal brain function.

4. SAP97 and AMPARs

MAGUKs generally interact with AMPARs in the PSD through AMPAR auxiliary subunits known as transmembrane AMPAR regulatory proteins (TARPs) [6,34]. SAP97 is the only member of the MAGUK family to interact directly with the AMPAR subunit GluA1 [35]. Because the recruitment of GluA1 is a critical step for inducing and maintaining forms of synaptic plasticity such as long-term potentiation (LTP), the interaction between GluA1 and SAP97 is one of the most interesting topics for many investigators to study MAGUKs in regulating AMPARs in synaptic plasticity. SAP97 directs GluA1 forward trafficking from the Golgi network to the plasma membrane. The minus-end directed actin-based motor, myosin VI and SAP97 are thought to form a trimeric complex with GluA1, with SAP97 acting as a molecular link between GluA1 and myosin VI to transport AMPA receptors to the postsynaptic plasma membrane [22].

However, the role of SAP97 in the trafficking and synaptic location of AMPARs is far from solved with multiple labs reporting different data. Early work showed that SAP97 only interacts with GluA1 early in the secretory pathway during its forward trafficking to the plasma membrane, suggesting that SAP97 acts on GluA1 solely before its synaptic insertion and that it does not play a major role in anchoring AMPARs at synapses [36]. Overexpression of βSAP97 was found to enhance the amplitude of AMPAR-mediated currents [6,37], rescue AMPAR currents reduced by RNAi-mediated knockdown of PSD-95 [16] and change the paired pulse ratio, indicating a change in presynaptic release probability through trans-synaptic signalling [6]. Expression of SAP97I3 also enlarges spines and increases mEPSC frequency [17]. Other studies indicated that overexpression of βSAP97 did not change AMPAR currents and loss of SAP97 did not change AMPAR EPSCs either [13,16,38,39]. Recently, overexpression of palmitoylated αSAP97I3 has been shown to promote synaptic strengthening by localising primarily in the PSD where it selectively regulates the synaptic pool of AMPA receptors [7,15]. In contrast, the L27-domain containing βSAP97I3 has been shown to selectively regulate the extrasynaptic pools of both AMPA and NMDA receptors and prevent NMDA receptor-dependent LTP [7]. Therefore N-terminal SAP97 isoforms appear to be able to regulate the ability to undergo plasticity by differentially controlling surface receptor distribution.

These contradictory data may be in part due to earlier studies not defining which splice isoforms of SAP97 were being characterised, or only describing one splice site insert between the SH3 and GUK domains but not other N-terminal splice inserts. The use of different experimental preparations and expression levels of SAP97 isoforms between studies also prevents direct comparisons. In addition, like other PSD-MAGUKs, SAP97 may be sufficient but not solely necessary for the trafficking of AMPARs to synapses [6,40]. Transgenic mice have provided limited insights into this as mice harbouring a truncation of SAP97 do not survive beyond birth because craniofacial deformities prevent feeding [41]. However, neurons grown in vitro from SAP97 mutant embryos develop normally and form synapses with normal levels of AMPAR subunits [13,16]. These findings raise the possibility that MAGUK family members share redundant functions in neurons and the loss of one can be partially compensated by other MAGUKs [16].

5. SAP97 and NMDARs

SAP97 is also implicated in targeting and trafficking NMDA receptor at synapses. Like other PSD-MAGUKs, SAP97 interacts directly via its PDZ domains with NMDAR PDZ-binding motifs [6,42]. SAP97 plays a role in regulating NMDA receptors through the GluN2A/2B subunits [43,44]. SAP97 directly associates with the GluN2A subunit of the NMDAR through its PDZ1 domain in a CaMKII regulated manner. CaMKII phosphorylates SAP97 on two major sites, Ser39 located in the N-terminal domain and Ser232 located in PDZ1 domain. CaMKII phosphorylation of SAP97-Ser39 releases the SAP97–GluN2A complex from the endoplasmic reticulum to drive SAP97 to the postsynaptic region [43]. NMDAR activation induces CaMKII-dependent phosphorylation of SAP97-Ser232 to disrupt the SAP97–GluN2A interaction [45]. Thus, CaMKII-dependent phosphorylation of SAP97 controls both GluN2A trafficking and insertion at synapses [43].

SAP97 is also important for the targeting of NMDARs to dendritic spines in hippocampal neurons. A novel secretory pathway for NMDAR trafficking requiring SAP97 has been described [32]: βSAP97 and CASK are required for the sorting of NMDARs from AMPARs in the somatic ER, causing a majority of NMDARs to bypass somatic Golgi and traffic via dendritic Golgi outposts to synapses [32]. When this alternative pathway is unavailable, NMDARs can traffic to synapses via the conventional secretory pathway, as evidenced by the accumulation of NMDARs in somatic Golgi during the knockdown of βSAP97 or CASK. Intriguingly, SAP97 only appears to utilise the Golgi outpost secretory pathway when in a complex with NMDARs, as it does not traffic with AMPARs through this novel pathway [32]. This trafficking pathway could allow NMDARs to be more efficiently targeted to synapses and may also enable local regulation of NMDAR insertion near to synaptic sites (Fig. 2).

In immature neurons, SAP97 preferentially interacts with GluN2A compared with GluN2B, and overexpression of SAP97 during early development can increase synaptic NMDAR number [6]. In utero, overexpression of SAP97 can drive the developmental NMDAR subunit switch, inducing larger NMDAR current amplitudes and faster NMDAR current kinetics. These data indicate a potential role for SAP97 in controlling both the number, the subunit composition and consequently the channel kinetics of synaptic NMDARs [6].

Similar to studies on SAP97 and AMPARs, multiple studies have examined the role of SAP97 in regulating synaptic NMDARs (e.g. [6,7,16,37]). Again, conflicting data has been produced on the effects of βSAP97 and its deletion or knockdown on NMDAR-mediated currents. The complex splicing of SAP97 at multiple sites likely underlies these differing results, particularly the I3/I4/I5 splice inserts which have not been independently characterised in neurons, and it is not clear which of these inserts were present in all studies. Targeted knockdown of βSAP97 alone has shown that in the absence of βSAP97, synaptic AMPARs and NMDARs are increased whereas extrasynaptic receptors are decreased. This suggests that a major role of endogenous βSAP97 is to exclude receptors from the synapse and negatively regulate synaptic plasticity [7]. Deletion of all SAP97 isoforms revealed that glutamatergic synaptic transmission and LTP were normal in mature neurons [6]. Therefore, the roles of SAP97 isoforms in regulating NMDAR-dependent functions can be compensated by other PSD-MAGUKs.

6. SAP97 and potassium channels

In addition to the multiple roles of SAP97 in binding potassium and sodium channel subtypes in cardiac cells to regulate cardiac

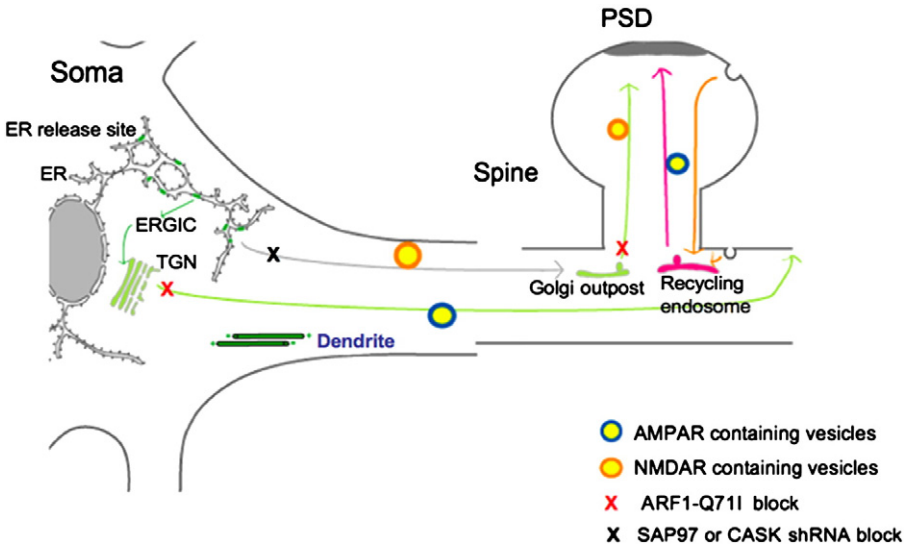

Fig. 2. Model of the alternative secretory pathway in neurons through which SAP97 traffics with NMDA receptors. SAP97, CASK and NMDA receptors are proposed to exit the endoplasmic reticulum and bypass the ERGIC pathway. The SAP97/CASK/NMDA receptor complex travels along dendrites in mobile ER-derived vesicles. NMDARs are then inserted into synapses via Golgi outposts. Blocking trans-Golgi network exit via ARF-Q71I block fails to prevent NMDAR but not AMPAR trafficking to synapses, however downregulation of SAP97 or CASK reduces synaptic NMDARs. SAP97 is therefore thought to associate with NMDARs during and/or after their assembly in the ER and this interaction prevents their ability to transit from ER exit sites to the somatic Golgi.
Diagram is from [32].

action potentials and delayed rectifier currents [46–48], SAP97 also plays a major role in directing the trafficking, localisation and anchoring of potassium channels in neurons. Voltage-gated potassium channels (Kv channels) were first discovered in electrophysiological experiments carried out in squid axons [49]. Later, in *Drosophila*, electrical recordings from the Shaker mutant produced a unique phenotype at the neuromuscular junction that could be mimicked with a potassium channel blocker [50]. It was later shown that the Shaker gene encodes several voltage-gated potassium channel α-subunits [51]. There are twelve families of Kv channels (Kv1–12) and their diversity arises from the fact that Kv channels may be homotetramers or heterotetramers of different subunits within the same family [52]. Their function is diverse and includes maintaining membrane potential and modulating electrical excitability in neurons and muscle. Another type of potassium channel, inwardly rectifying potassium (Kir) channels, consists of seven Kir subfamilies, Kir1–7 [53]. Inwardly rectifying potassium (Kir) channels are characterised by their large inward currents at potentials negative to the potassium equilibrium potential and small outward currents at potentials that are more positive. Kir channels are also important in maintaining resting membrane potential, repolarisation of the action potential and mediating cell excitability. Both Kv and Kir channels have been shown to be associated with SAP97, which mediates channel localisation and trafficking.

Binding of Kv channels (like NMDA receptors) is mediated by their C-terminal tail binding to the PDZ1 and PDZ2 domains in the N-terminus of SAP97 and PSD95 [54]. Both SAP97 and PSD95 bind Shaker-type voltage-gated K + channel subunits (Kv1) with high affinity and also mediate their clustering [24,54]. Evidence for a SAP97/Kv1 interaction comes from COS cells where co-immunoprecipitation studies have shown that Kv1.4 antibodies co-immunoprecipitate SAP97 with Kv1.4 and similarly SAP97 antibodies co-immunoprecipitate SAP97 with Kv1.4 [24]. Also, the co-transfection of SAP97 and Kv1.4 in COS cells results in their colocalisation via a direct interaction between the C-terminus of the Kv1.4 channel and SAP97 and leads specifically to their cytoplasmic distribution [24,25].

Interestingly, the efficiency of the interaction between SAP97 and Kv1 subunits does not correlate with channel surface expression efficiency (unlike with PSD95) in COS cells [25]. For example, the SAP97 clustering efficiency is very similar for Kv1.1, Kv1.2 and Kv1.2 + Kvβ2 channels but these channels have dramatically different cell surface expression efficiencies. Further analysis showed that as well as increasing the cytoplasmic distribution of the Kv1 channel, SAP97/Kv1 clustering also dramatically reduces the surface expression of these channels [25]. The clustering efficiency of SAP97 with Kv1 channels closely reflects the magnitude of decrease in cell surface expression, indicating that this interaction with SAP97 leads to the intracellular retention of Kv1 channels. Furthermore, immunostaining for ER proteins showed that SAP97 retains Kv1.4 channels in an ER derived compartment specifically [25].

A more recent finding is that SAP97 directs Kv4.2 to spines in hippocampal neurons [55]. Kv4.2 is a key component of the A-type potassium channel, which is involved in dendritic excitability and plasticity. Firstly, subcellular fractionation in hippocampal cultured neurons has shown that the Kv4.2 channel is enriched in the PSD fraction, the total homogenate and in the crude membrane fraction but has low expression levels in the synaptosomal membrane and cytosolic fraction [55]. This pattern is similar to that of SAP97 and SAP102. As shown in the COS cells, co-immunoprecipitation in hippocampal neurons shows that Kv4.2 co-precipitates with SAP97 and this occurs in all subcellular fractions [55]. Similar results are obtained in cultured organotypic slices virally infected with SAP97 and Kv4.2 [55]. In cultured hippocampal neurons SAP97 is responsible for the trafficking of Kv4.2 channels to synaptic sites as SAP97 knockdown (using RNAi) decreases Kv4.2 localisation in the PSD enriched fraction [55].

It is also known that both Kv4.2 and SAP97 are substrates for CAMKII in hippocampal neurons [45,56]. Specifically, CAMKII dependent phosphorylation of SAP97 Ser39 regulates the localisation of SAP97 as well as SAP97 interacting proteins [30,45]. Interestingly, CAMKII phosphorylation of SAP97 (Ser39) does not affect SAP97 clustering with Kv4.2 but causes a redistribution of Kv4.2 channels from internal compartments towards the cell surface in both hippocampal neurons and COS cells [55]. In the hippocampal neurons Ser39 phosphorylated SAP97 increases Kv4.2 channels in spine-like structures and in dendrites [55]. Therefore, CAMKII phosphorylation of SAP97 Ser39 is involved in the synaptic trafficking of Kv4.2.

Similarly the interaction between SAP97 and Kv4.2 channels has also been demonstrated in the heart. The voltage-dependent Kv4.2 and Kv4.3 channels are one of the major regulators of the transient outward potassium current in the heart, which regulates cardiac

cycle length [57,58]. In the heart SAP97 has been shown to interact with Kv4 channels in pull-down assays and to colocalise with these channels in immunocytochemistry experiments [59]. In Chinese hamster ovary cell lines, SAP97 was specifically necessary for the CaMKII regulation of Kv4.3 channels as well as for the clustering of Kv4.3 channels [59]. Therefore SAP97 regulates the transient outward potassium current in the heart by interacting with Kv4 channels.

Furthermore, in rat cerebellum and heart, it has been shown that SAP97 forms interactions with Kir2.1, Kir2.2 and Kir2.3 and that the second PDZ domain of SAP97 is sufficient for the interaction with Kir2.2 [60,61]. These Kir channels as well as Kir4.1 bind to SAP97 and CASK with different affinities as shown in affinity pull down experiments [60]. Furthermore, co-immunoprecipitation has shown that Kir2.2 is associated with SAP97 in both rat cerebellum and heart and that phosphorylation of the Kir2.2 C-terminus by PKA inhibits this interaction with SAP97 [61]. Altogether, SAP97 associates with both Kv and Kir channels to regulate their trafficking and localisation, ultimately affecting cell function, excitability and cell–cell communications.

7. Conclusions and future directions

The complex roles of SAP97 in targeting and regulating ion channels in neurons is far from completely understood, and future studies that decipher the differential roles that each isoform plays will be important in our understanding of neuronal excitability, synapse function and plasticity. Whether SAP97 isoforms work in tandem or alone remains to be determined. Moreover, a role for SAP97 has been raised in numerous brain diseases including schizophrenia [62], Alzheimer's Disease [63], Parkinson's disease [64] and epilepsy [65]. Although glutamate receptor targets are attractive candidates for therapeutic intervention, the critical roles of these receptors in many brain processes produced serious side effects. Should each SAP97 isoform prove to display independent functional roles, each could serve as individual targets in the development of pathophysiology or treatments of a number of these diseases in which neuronal excitability and/or glutamatergic synaptic transmission are altered.

References

[1] A. Bayes, L.N. van de Lagemaat, M.O. Collins, M.D.R. Croning, I.R. Whittle, J.S. Choudhary, S.G.N. Grant, Characterization of the proteome, diseases and evolution of the human postsynaptic density, Nat. Neurosci. 14 (2011) 19–21.

[2] M. Sheng, C.C. Hoogenraad, The postsynaptic architecture of excitatory synapses: a more quantitative view, Annu. Rev. Biochem. 76 (2007) 823–847.

[3] C. Verpelli, M. Schmeisser, C. Sala, T. Boeckers, Scaffold proteins at the postsynaptic density, in: M.R. Kreutz, C. Sala (Eds.), Synaptic Plasticity, 970, Springer Vienna, 2012, pp. 29–61.

[4] W. Xu, PSD-95-like membrane associated guanylate kinases (PSD-MAGUKs) and synaptic plasticity, Curr. Opin. Neurobiol. 21 (2011) 306–312.

[5] M. Sheng, E. Kim, The postsynaptic organization of synapses, Cold Spring Harb. Perspect. Biol. 3 (2011).

[6] M.K.A. Howard, G.M. Elias, L.A.B. Elias, W. Swat, R.A. Nicoll, The role of SAP97 in synaptic glutamate receptor dynamics, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 3805–3810.

[7] D. Li, C.G. Specht, C.L. Waites, C. Butler-Munro, S. Leal-Ortiz, J.W. Foote, D. Genoux, C.C. Garner, J.M. Montgomery, SAP97 directs NMDA receptor spine targeting and synaptic plasticity, J. Physiol. 589 (2011) 4491–4510.

[8] J. Montgomery, P. Zamorano, C. Garner, MAGUKs in synapse assembly and function: an emerging view, Cell. Mol. Life Sci. 61 (2004) 911–929.

[9] G.M. Elias, L. Funke, V. Stein, S.G. Grant, D.S. Bredt, R.A. Nicoll, Synapse-specific and developmentally regulated targeting of AMPA receptors by a family of MAGUK scaffolding proteins, Neuron 52 (2006) 307–320.

[10] B. Muller, U. Kistner, R.W. Veh, C. Cases-Langhoff, B. Becker, E.D. Gundelfinger, C. Garner, Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the *Drosophila* discs-large tumor suppressor protein, J. Neurosci. 15 (1995) 2354–2366.

[11] R.A. Lue, S.M. Marfatia, D. Branton, A.H. Chishti, Cloning and characterization of hdlg: the human homologue of the *Drosophila* discs large tumor suppressor binds to protein 4.1, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 9818–9822.

[12] J.G. Valtschanoff, A. Burette, M.A. Davare, A.S. Leonard, J.W. Hell, R.J. Weinberg, SAP97 concentrates at the postsynaptic density in cerebral cortex, Eur. J. Neurosci. 12 (2001) 3605–3614.

[13] N. Klöcker, R.C. Bunn, E. Schnell, G. Caruana, A. Bernstein, R.A. Nicoll, D.S. Bredt, Synaptic glutamate receptor clustering in mice lacking the SH3 and GK domains of SAP97, Eur. J. Neurosci. 16 (2002) 1517–1522.

[14] M. McLaughlin, R. Hale, D. Ellston, S. Gaudet, R.A. Lue, A. Viel, The distribution and function of alternatively spliced insertions in hDlg, J. Biol. Chem. 277 (2002) 6406–6412.

[15] C.L. Waites, C.G. Specht, K. Härtel, S. Leal-Ortiz, D. Genoux, D. Li, R.C. Drisdel, O. Jeyifous, J.E. Cheyne, W.N. Green, Synaptic SAP97 isoforms regulate AMPA receptor dynamics and access to presynaptic glutamate, J. Neurosci. 29 (2009) 4332–4345.

[16] O.M. Schlüter, W. Xu, R.C. Malenka, Alternative N-terminal domains of PSD-95 and SAP97 govern activity-dependent regulation of synaptic AMPA receptor function, Neuron 51 (2006) 99–111.

[17] G. Rumbaugh, G.M. Sia, C.C. Garner, R.L. Huganir, Synapse-associated protein-97 isoform-specific regulation of surface AMPA receptors and synaptic function in cultured neurons, J. Neurosci. 23 (2003) 4567–4576.

[18] T. Hanada, A. Takeuchi, G. Sondarva, A.H. Chishti, Protein 4.1-mediated membrane targeting of human discs large in epithelial cells, J. Biol. Chem. 278 (2003) 34445–34450.

[19] C.Y. Zheng, G.K. Seabold, M. Horak, R.S. Petralia, MAGUKs, synaptic development, and synaptic plasticity, Neuroscientist 17 (2011) 493–512.

[20] S. Roberts, E. Calautti, S. Vanderweil, H. Nguyen, A. Foley, H. Baden, A. Viel, Changes in localization of human discs large (hDlg) during keratinocyte differentiation is associated with expression of alternatively spliced hDlg variants, Exp. Cell Res. 313 (2007) 2521–2530.

[21] K.H. Yamada, T. Hanada, A.H. Chishti, The effector domain of human Dlg tumor suppressor acts as a switch that relieves autoinhibition of kinesin-3 motor GAKIN/KIF13B, Biochemistry (Mosc). 46 (2007) 10039–10045.

[22] H. Wu, J.E. Nash, P. Zamorano, C.C. Garner, Interaction of SAP97 with minus-end-directed actin motor myosin VI, J. Biol. Chem. 277 (2002) 30928–30934.

[23] H.J. Wu, S.M. Reuver, S. Kuhlendahl, W.J. Chung, C.C. Garner, Subcellular targeting and cytoskeletal attachment of SAP97 to the epithelial lateral membrane, J. Cell Sci. 111 (1998) 2365–2376.

[24] E. Kim, M. Sheng, Differential K+ channel clustering activity of PSD-95 and SAP97, two related membrane-associated putative guanylate kinases, Neuropharmacology 35 (1996) 993–1000.

[25] A.M. Tiffany, L.N. Manganas, E. Kim, Y.-P. Hsueh, M. Sheng, J.S. Trimmer, PSD-95 and SAP97 exhibit distinct mechanisms for regulating K+ channel surface expression and clustering, J. Cell Biol. 148 (2000) 147–157.

[26] H. Mok, H. Shin, S. Kim, J.R. Lee, J. Yoon, E. Kim, Association of the kinesin superfamily motor protein KIF1Bα with postsynaptic density-95 (PSD-95), synapse-associated protein-97, and synaptic scaffolding molecule PSD-95/discs large/zona occludens-1 proteins, J. Neurosci. 22 (2002) 5253–5258.

[27] N. Asaba, T. Hanada, A. Takeuchi, A.H. Chishti, Direct interaction with a kinesin-related motor mediates transport of mammalian discs large tumor suppressor homologue in epithelial cells, J. Biol. Chem. 278 (2003) 8395–8400.

[28] H. Wu, C. Reissner, S. Kuhlendahl, B. Coblentz, S. Reuver, S. Kindler, E.D. Gundelfinger, C.C. Garner, Intramolecular interactions regulate SAP97 binding to GKAP, EMBO J. 19 (2000) 5740–5751.

[29] M. Colledge, R.A. Dean, G.K. Scott, L.K. Langeberg, R.L. Huganir, J.D. Scott, Targeting of PKA to glutamate receptors through a MAGUK–AKAP complex, Neuron 27 (2000) 107–119.

[30] D. Mauceri, F. Cattabeni, M. Di Luca, F. Gardoni, Calcium/calmodulin-dependent protein kinase II phosphorylation drives synapse-associated protein 97 into spines, J. Biol. Chem. 279 (2004) 23813–23821.

[31] S. Lee, S. Fan, O. Makarova, S. Straight, B. Margolis, A novel and conserved protein–protein interaction domain of mammalian Lin-2/CASK binds and recruits SAP97 to the lateral surface of epithelia, Mol. Cell. Biol. 22 (2002) 1778–1791.

[32] O. Jeyifous, C.L. Waites, C.G. Specht, S. Fujisawa, M. Schubert, E.I. Lin, J. Marshall, C. Aoki, T. De Silva, J.M. Montgomery, SAP97 and CASK mediate sorting of NMDA receptors through a previously unknown secretory pathway, Nat. Neurosci. 12 (2009) 1011–1019.

[33] K.Y. Petrosky, H.D. Ou, F. Löhr, V. Dötsch, W.A. Lim, A general model for preferential hetero-oligomerization of LIN-2/7 domains, J. Biol. Chem. 280 (2005) 38528–38536.

[34] L. Chen, D.M. Chetkovich, R.S. Petralia, N.T. Sweeney, Y. Kawasaki, R.J. Wenthold, D.S. Bredt, R.A. Nicoll, Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms, Nature 408 (2000) 936–943.

[35] A.S. Leonard, M.A. Davare, M. Horne, C.C. Garner, J.W. Hell, SAP97 is associated with the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit, J. Biol. Chem. 273 (1998) 19518–19524.

[36] N. Sans, C. Racca, R.S. Petralia, Y.X. Wang, J. McCallum, R.J. Wenthold, Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway, J. Neurosci. 21 (2001) 7506–7516.

[37] T. Nakagawa, K. Futai, H.A. Lashuel, I. Lo, K. Okamoto, T. Walz, Y. Hayashi, M. Sheng, Quaternary structure, protein dynamics, and synaptic function of SAP97 controlled by L27 domain interactions, Neuron 44 (2004) 453–467.

[38] I. Ehrlich, R. Malinow, Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity, J. Neurosci. 24 (2004) 916–927.

[39] E. Schnell, M. Sizemore, S. Karimzadegan, L. Chen, D.S. Bredt, R.A. Nicoll, Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 13902–13907.

[40] M.P. Regalado, R.T. Terry-Lorenzo, C.L. Waites, C.C. Garner, R.C. Malenka, Transsynaptic signaling by postsynaptic synapse-associated protein 97, J. Neurosci. 26 (2006) 2343–2357.

[41] G. Caruana, A. Bernstein, Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene, Mol. Cell. Biol. 21 (2001) 1475–1483.
[42] M. Sheng, C. Sala, PDZ domains and the organization of supramolecular complexes, Annu. Rev. Neurosci. 24 (2001) 1–29.
[43] D. Mauceri, F. Gardoni, E. Marcello, M. Di Luca, Dual role of CaMKII-dependent SAP97 phosphorylation in mediating trafficking and insertion of NMDA receptor subunit NR2A, J. Neurochem. 100 (2006) 1032–1046.
[44] Y.A. Nikandrova, Y. Jiao, A.J. Baucum, S.J. Tavalin, R.J. Colbran, Ca2+/calmodulin-dependent protein kinase II binds to and phosphorylates a specific SAP97 splice variant to disrupt association with AKAP79/150 and modulate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor (AMPAR) activity, J. Biol. Chem. 285 (2010) 923–934.
[45] F. Gardoni, D. Mauceri, C. Fiorentini, C. Bellone, C. Missale, F. Cattabeni, M.D. Luca, CaMKII-dependent phosphorylation regulates SAP97/NR2A interaction, J. Biol. Chem. 278 (2003) 44745–44752.
[46] S. Nattel, L. Yue, Z. Wang, Cardiac ultrarapid delayed rectifiers, Cell. Physiol. Biochem. 9 (1999) 217–226.
[47] D. Godreau, R. Vranckx, A. Maguy, C. Goyenvalle, S.N. Hatem, Different isoforms of synapse-associated protein, SAP97, are expressed in the heart and have distinct effects on the voltage-gated K+ channel Kv1.5, J. Biol. Chem. 278 (2003) 47046–47052.
[48] S. Petitprez, A.F. Zmoos, J. Ogrodnik, E. Balse, N. Raad, S. El-Haou, M. Albesa, P. Bittihn, S. Luther, S.E. Lehnart, SAP97 and dystrophin macromolecular complexes determine two pools of cardiac sodium channels Nav1.5 in cardiomyocytes novelty and significance, Circ. Res. 108 (2011) 294–304.
[49] A.L. Hodgkin, A.F. Huxley, A quantitative description of membrane current and its application to conduction and excitation in nerve, J. Physiol. 117 (1952) 500.
[50] Y. Jan, L.Y. Jan, M. Dennis, Y. Jan, L.Y. Jan, M. Dennis, Two mutations of synaptic transmission in *Drosophila*, Proc. R. Soc. Lond. B. Biol. Sci. 198 (1977) 87–108.
[51] B.L. Tempel, D.M. Papazian, T.L. Schwarz, Y.N. Jan, L.Y. Jan, Sequence of a probable potassium channel component encoded at Shaker locus of *Drosophila*, Science (New York, N.Y.) 237 (1987) 770.
[52] G.A. Gutman, K.G. Chandy, S. Grissmer, M. Lazdunski, D. Mckinnon, L.A. Pardo, G.A. Robertson, B. Rudy, M.C. Sanguinetti, W. Stühmer, International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels, Pharmacol. Rev. 57 (2005) 473–508.
[53] Y. Kubo, J.P. Adelman, D.E. Clapham, L.Y. Jan, A. Karschin, Y. Kurachi, M. Lazdunski, C.G. Nichols, S. Seino, C.A. Vandenberg, International Union of Pharmacology. LIV. Nomenclature and molecular relationships of inwardly rectifying potassium channels, Pharmacol. Rev. 57 (2005) 509–526.
[54] E. Kim, M. Niethammer, A. Rothschild, Y.N. Jan, M. Sheng, Clustering of Shaker-type K+ channels by interaction with a family of membrane-associated guanylate kinases, Nature 378 (1995) 85–88.
[55] F. Gardoni, D. Mauceri, E. Marcello, C. Sala, M. Di Luca, A. Jeromin, SAP97 directs the localization of Kv4.2 to spines in hippocampal neurons: regulation by CaMKII, J. Biol. Chem. 282 (2007) 28691–28699.
[56] A.W. Varga, L.L. Yuan, A.E. Anderson, L.A. Schrader, G.Y. Wu, J.R. Gatchel, D. Johnston, J.D. Sweatt, Calcium–calmodulin-dependent kinase II modulates Kv4.2 channel expression and upregulates neuronal A-type potassium currents, J. Neurosci. 24 (2004) 3643–3654.
[57] E. Bou-Abboud, J.M. Nerbonne, Molecular correlates of the calcium-independent, depolarization-activated K+ currents in rat atrial myocytes, J. Physiol. 517 (1999) 407–420.
[58] J.L. Greenstein, R. Wu, S. Po, G.F. Tomaselli, R.L. Winslow, Role of the calcium-independent transient outward current Ito1 in shaping action potential morphology and duration, Circ. Res. 87 (2000) 1026–1033.
[59] S. El-Haou, E. Balse, N. Neyroud, G. Dilanian, B. Gavillet, H. Abriel, A. Coulombe, A. Jeromin, S.N. Hatem, Kv4 potassium channels form a tripartite complex with the anchoring protein SAP97 and CaMKII in cardiac myocytes, Circ. Res. 104 (2009) 758–769.
[60] D. Leonoudakis, L.R. Conti, S. Anderson, C.M. Radeke, L.M.M. McGuire, M.E. Adams, S.C. Froehner, J.R. Yates, C.A. Vandenberg, Protein trafficking and anchoring complexes revealed by proteomic analysis of inward rectifier potassium channel (Kir2.x)-associated proteins, J. Biol. Chem. 279 (2004) 22331–22346.
[61] D. Leonoudakis, W. Mailliard, K. Wingerd, D. Clegg, C. Vandenberg, Inward rectifier potassium channel Kir2.2 is associated with synapse-associated protein SAP97, J. Cell Sci. 114 (2001) 987–998.
[62] J. Sato, D. Shimazu, N. Yamamoto, T. Nishikawa, An association analysis of synapse-associated protein 97 (SAP97) gene in schizophrenia, J. Neural Transm. 115 (2008) 1355–1365.
[63] E. Marcello, R. Epis, C. Saraceno, F. Gardoni, B. Borroni, F. Cattabeni, A. Padovani, M. Di Luca, SAP97-mediated local trafficking is altered in Alzheimer disease patients' hippocampus, Neurobiol. Aging 33 (2012) 422, (e421-422. e410.).
[64] J. Nash, T. Johnston, G. Collingridge, C. Garner, J. Brotchie, Subcellular redistribution of the synapse-associated proteins PSD-95 and SAP97 in animal models of Parkinson's disease and L-DOPA-induced dyskinesia, FASEB J. 19 (2005) 583–585.
[65] A. Finardi, F. Gardoni, S. Bassanini, G. Lasio, M. Cossu, L. Tassi, C. Caccia, F. Taroni, G. LoRusso, M. Di Luca, NMDA receptor composition differs among anatomically diverse malformations of cortical development, J. Neuropathol. Exp. Neurol. 65 (2006) 883–893.