

Survey

Disparate cell types use a shared complex of PDZ proteins for polarized protein localization

Christopher Rongo *

Waksman Institute/Rutgers University, 190 Frelinghuysen Rd., Piscataway, NJ 08854, USA

Abstract

Based on their morphology and function, epithelial cells and neurons appear to have very little in common; however, growing evidence indicates that these two disparate cell types share an underlying polarization pathway responsible for sorting proteins to specific subcellular sites. An evolutionarily conserved complex of PDZ domain-containing proteins thought to be responsible for polarized protein localization has been identified from both brain and epithelial tissue, both from mammals and from the nematode *C. elegans*. Some of the most recent data on PDZ proteins and the proteins with which they interact are summarized. In particular, some of the more recently proposed models for their function in cells, and the in vivo and in vitro data that support these models are focussed upon. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: PDZ proteins; Synapse formation; Cell polarity; MAGUK proteins

Contents

1. Introduction	349
2. Polarized protein localization: a tripartite complex identified in <i>C. elegans</i>	351
3. A corresponding mammalian tripartite complex that might assemble synaptic junctions between neurons	353
4. Models for tripartite complex function: polarized secretion versus selective retention	355
5. Conclusion	356
Acknowledgements	357
References	357

1. Introduction

The polarity of a cell is one of the fundamental aspects of multicellularity. Within a tissue, a single cell can take on a polarized shape so that it can separately interact with different groups of adjacent cells by using

* Tel.: +1-732-445-0955; fax: +1-732-445-5735.
E-mail address: rongo@waksman.rutgers.edu (C. Rongo).

different cell surfaces. By simply targeting separate sets of membrane proteins and signal transduction machinery to each cell surface, a polarized cell can thereby specifically determine how and what it communicates to each of its neighbors.

Polarization also allows the cell to specialize particular cell surfaces for particular functions. Epithelial cells of the intestine are a prime example since they are polarized into an apical surface that faces the lumen of the gut, and a basolateral surface that is situated on the

basement basal lamina (Fig. 1). The uptake of nutrients from the intestinal lumen occurs through an apical surface in which membranes are organized into microvilli to maximize surface area for absorption. The apical surface also contains membrane transport proteins to move nutrients from the intestinal lumen into the cell. In contrast, the basolateral surface has a simpler morphology and contains its own unique proteins, like Na^+/K^+ ATPases, that must be strictly localized to this cell surface in order to move nutrients

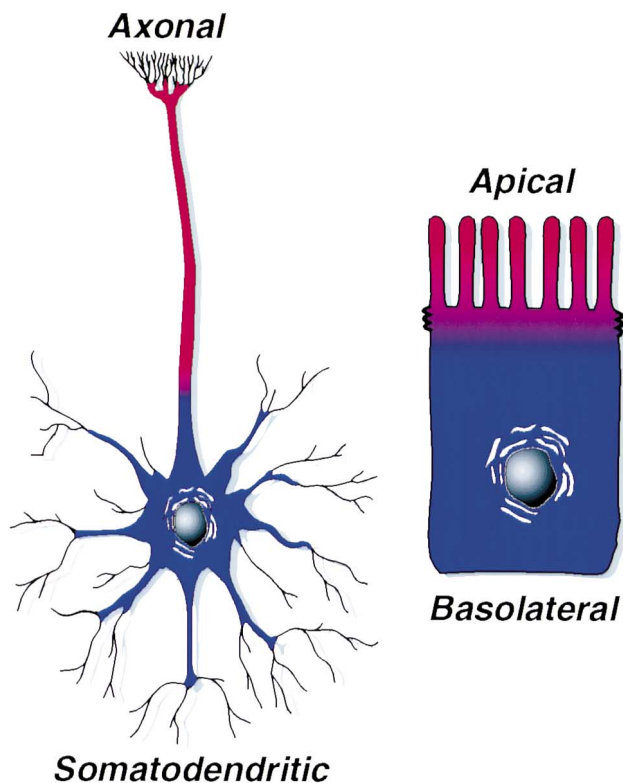


Fig. 1

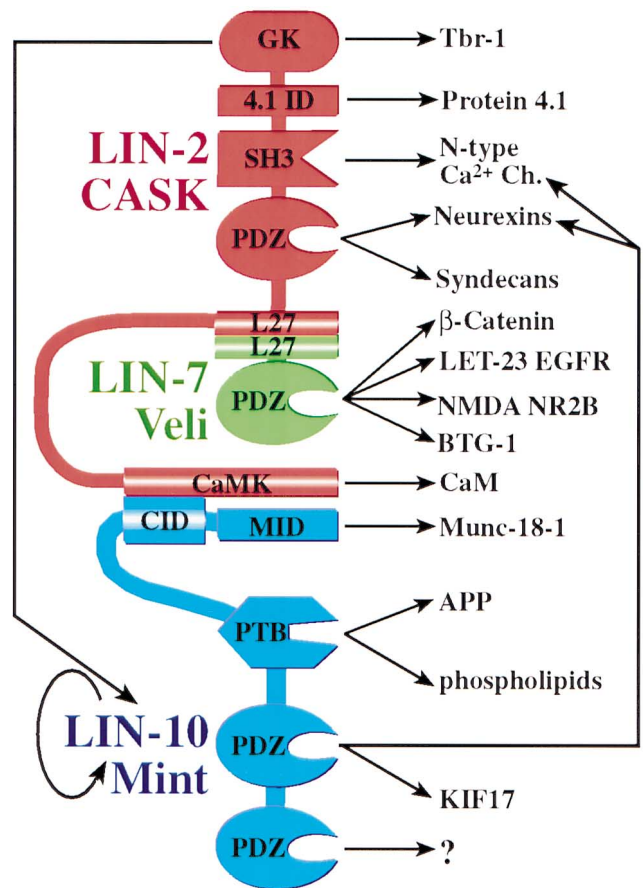


Fig. 2

Fig. 1. Neurons and epithelia differ in polarized morphology but have a common polarized protein localization system. Neurons are morphologically polarized into axons, which extend away from the neuron cell body to reach and innervate their targets, and dendrites, which arborize to provide postsynaptic sites to receive input from other neurons. Whereas epithelial cells appear to be simpler in overall morphology, they are no less polarized. Apical surfaces often form microvilli, long projections of membrane, to increase their surface area, thereby maximizing the amount of nutrients and other small molecules that they can exchange with the lumen. In contrast, the basolateral surface is simpler yet and contains the adhesive cell surface molecules necessary to attach the cell to the basal lamina. The trafficking system that localizes proteins to the somatodendritic and basolateral compartments (both labeled in blue) seems to be shared by both neurons and epithelial. Similarly, the system that localizes proteins to axons and apical surfaces (both labeled in red) seems to be partly shared.

Fig. 2. Schematic representation of LIN-2/CASK (red), LIN-7/Veli (green), and LIN-10/Mint (blue) proteins assembled into the tripartite complex. Each individual domain of these multidomain proteins is labeled, including the guanylate kinase domain (GK), Protein 4.1 interaction domain (4.1 ID), Src-homology 3 domain (SH3), PDZ domain, LIN-2/LIN-7 heterodimerizing domain (L27), CaMKII-like domain (CaMK), CASK interaction domain (CID), Munc18-1 interaction domain (MID), and phosphotyrosine binding domain (PTB). Arrows point to proteins that have been shown to interact with the indicated domain. The GK domain of LIN-2/CASK has been shown to provide a second binding site for LIN-10/Mint, and to interact with the CASK SH3 domain [50,92]. LIN-10 also has been shown to interact with itself, although the exact domains involved are unknown [93]. Proteins that bind to the second PDZ domain of LIN-10/Mint have not been identified, although spinophilin has been incorrectly reported in the literature [94]. Spinophilin binds to the rat homolog of the *C. elegans* protein originally but incorrectly identified as LIN-10 [30,95].

from the lumen to the extracellular fluid underneath the cell basement membranes.

Neurons of the brain are dramatically different in shape and size from epithelial cells, yet also require polarization to signal over long distances and to many different targets (Fig. 1). In its simplest form, a neuron sends out cylindrical membrane-bound processes called axons that can travel long distances (from centimeters to meters) to contact target cells and form synaptic connections. Channels must be localized to the axon, and vesicle release machinery must be localized to presynaptic sites at axonal endings in order to propagate electrochemical signals along the axon length and cause the release of neurotransmitters into synapses to signal to target cells. Neurons also send out arborized processes called dendrites to provide postsynaptic sites of contact for other neurons to innervate. Neurotransmitter receptors and signal transduction components must be localized to postsynaptic sites on these dendrites so that signals released by innervating neurons can be received and interpreted. In a simple sense, neuron polarity controls the flow of information in the nervous system.

One can think about cell polarity as having two components: polarized morphology and polarized protein localization. Upon differentiation, a cell must subdivide its membranes into specific surfaces and organize underlying cytoskeletal elements to drive each membrane surface into a particular membrane shape, thereby giving the cell a polarized morphology. Once each surface has been specialized, specific proteins are targeted to one membrane or another by polarized protein localization. Until the last few years, the pathways for polarized protein localization remained a mystery.

One of the first major clues to understanding polarized protein localization came from a hypothesis pioneered by Dotti and Simons that suggested that the underlying machinery for protein localization might be shared between many different cell types [1]. In particular, neurons and epithelial cells seem to share some aspects of polarized protein localization even though the polarized morphology of these cells appears quite distinct (Fig. 1). Support for this idea came from experiments in which glycoproteins that are normally localized to either basolateral or apical surfaces of epithelial cells were found to be localized to somatodendritic and axonal surfaces, respectively, when ectopically expressed in neurons [1–3]. There have been exceptions to these observations, particularly with regard to axonal trafficking [3]. However, it generally seems that proteins that are destined for apical surfaces in epithelia are sorted based on their interactions with lipid rafts, whereas proteins that are destined for basolateral surfaces in epithelia and somatodendritic surfaces in neurons share a localization pathway and are sorted by this

pathway based on several different *cis*-acting sequences found in these proteins [3–7]. Because many of these *cis*-acting sequences are utilized for localization in both epithelia and neurons, it has been presumed that the sorting machinery that recognizes these sequences would be present in both cell types.

2. Polarized protein localization: a tripartite complex identified in *C. elegans*

Evidence for a model in which different cell types share the same polarized localization came from the work of many labs studying the role of PDZ proteins. The PDZ domain was originally identified as a 90 amino acid motif found to be repeated three times in three different membrane-associated guanylate kinases (MAGUKs): PSD-95, a synaptic protein; DLG-1, a *Drosophila* protein found at cell and neuromuscular junctions; and ZO-1, a tight junction protein [8–11]. The PDZ domain is a protein–protein interaction domain that binds to specific amino acid sequences found at the carboxy-terminus of its binding partners [12,13]. Because PDZ proteins bind to proteins that are often found to be localized to either epithelial junctions or synapses, they are thought to act as molecular scaffolds that assemble transmembrane proteins and signaling molecules into clusters at these synapses and tight junctions. Genetic evidence to support this hypothesis has been found in only a few cases, two of which come from research on *Drosophila*. One case is the PDZ protein InaD, which is expressed in the photoreceptors of the *Drosophila* compound eye [14,15]. It has been shown to assemble the TRP channel and IP₃ signal transduction components into a ‘transducisome’ complex at a specialized plasma membrane, the rhabdomere, to facilitate the rapid signaling required for vision [16]. Another case is *lethal (1) discs large (dlg)*, which is required to localize Shaker potassium channels and the adhesion molecule FasII to neuromuscular junctions [17–19].

A third case that demonstrated that PDZ proteins mediate protein localization was demonstrated in the nematode *C. elegans* by examining the localization of the LET-23 EGF receptor (EGFR) [20–22]. In *C. elegans*, the vulval opening of the animal is induced by an anchor cell to form from a group of epithelial cells called vulval precursor cells. During early larval stages of the animal, the anchor cell secretes an EGF-like protein, LIN-3, into the extracellular space beneath the basement membrane of the vulval precursor cells [23]. The LET-23 EGFR is localized to the basolateral surface of the vulval precursor cells where it faces the anchor cell and can receive the LIN-3 signal [20–22,24]. The cells closest to the anchor cell are thought to be in range for the LIN-3 ligand to bind LET-23 and thereby

activate the receptor. LET-23 is a receptor tyrosine kinase, and activation of the receptor results in a signal cascade through the Ras/MAP kinase pathway that results in the cell division and eventual morphogenesis of the activated cells into a functioning vulva [25–27]. Mutations that inactivate LIN-3, LET-23, or the Ras/MAP kinase pathway result in nematodes that lack vulval openings. Based on this easily scorable phenotype, several vulval induction gene were isolated, and three of them (*lin-2*, *lin-7*, and *lin-10*) were particularly interesting because they could be placed genetically between the *lin-3* ligand and *let-60* Ras [28,29]. Kim and colleagues cloned all three genes and found that all three encoded PDZ proteins (Fig. 2, a novel protein was incorrectly identified as LIN-10; a PDZ protein was subsequently shown to be the correct LIN-10) [20,22,29,30].

The PDZ proteins LIN-2, LIN-7, and LIN-10 were the first proteins identified that showed that PDZ proteins not only cluster other molecules but were important for polarized protein localization within epithelial cells. Mutations in either *lin-2*, *lin-7*, or *lin-10* cause the normally basolateral LET-23 EGFR to become apically localized in the vulval precursor epithelia, whose overall polarized morphology remains intact (Fig. 3) [20–22]. Thus, rather than being required for signaling from the LET-23 EGFR to the Ras/MAP kinase pathway, the three PDZ proteins were required to properly localize the EGFR to its appropriate site of action, the basolateral surface, where it could receive the LIN-3 ligand.

How do these three PDZ proteins regulate polarized localization in epithelial cells? The sequence of these proteins provides some clues (Fig. 2). LIN-7 contains a single PDZ domain [20]. LIN-10 contains a phosphotyrosine binding domain (PTB) and two PDZ domains [22]. LIN-2 exists as two splice variants and resembles MAGUK proteins like PSD-95 because of its guanylate kinase and SH3 domains [29]. Unlike other MAGUKs, LIN-2 contains a single PDZ domain and a domain that resembles CaMKII. Surprisingly, neither the CaMKII domain nor the guanylate kinase domain have kinase activity [29]. Using yeast two-hybrid, *in vitro* binding, and coimmunoprecipitation experiments from cultured Schneider S2 cells, Kim and colleagues were able to show that these three proteins can form a complex, that LIN-10 can bind to LIN-2, LIN-2 can bind to LIN-7, and that none of these interactions were mediated through the protein–protein interaction domains (e.g. PDZ, PTB, SH3) known at the time [21]. Moreover, they found that the PDZ domain of LIN-7 could bind directly to the carboxy-terminal sequences of LET-23, suggesting that LET-23 EGFR is recognized through its carboxy-terminal tail by a complex of LIN-2, LIN-7, and LIN-10, and that this recognition targets the receptor to basolateral rather than apical membranes (Fig. 3A, C, E).

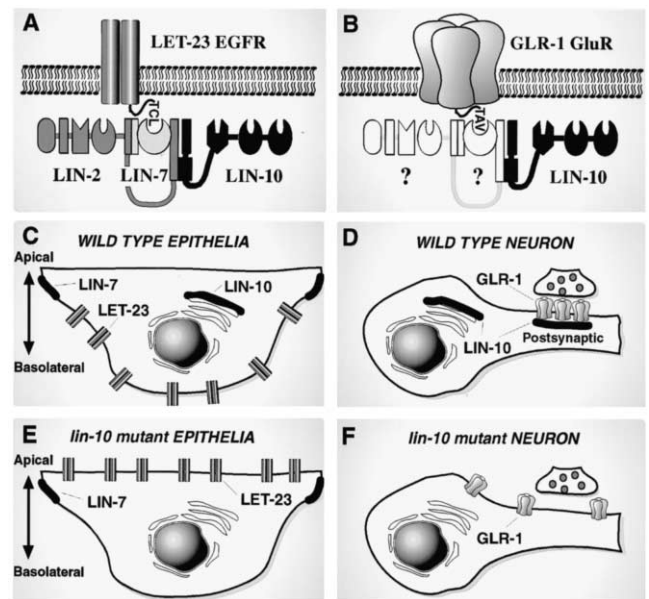


Fig. 3. LIN-2, LIN-7, and LIN-10 are required for protein localization in *C. elegans*. (A) LIN-2, LIN-7, and LIN-10 are thought to form a tripartite complex in vulval precursor epithelial cells. The complex binds the LET-23 EGFR directly through a PDZ interaction between LIN-7 and the LET-23 carboxy terminus. (B) LIN-10 is required to localize GLR-1 to postsynaptic specializations. The GLR-1 carboxy terminus matches a PDZ consensus; however, LIN-10 does not directly bind GLR-1 and additional proteins involved in the localization process have not yet been identified. (C) Wild-type epithelia localize LET-23 EGFR to their basolateral surfaces and (D) wild-type neurons localize GLR-1 to postsynaptic specializations. (E) Epithelia that lack LIN-10 localize LET-23 EGFR to their apical rather than their basolateral surfaces. (F) Neurons that lack LIN-10 fail to localize GLR-1 to synapses, resulting in diffuse GLR-1 channels throughout the neural process.

To further solidify their model, Kim and colleagues provided an elegant *in vivo* experiment as evidence. PDZ domains fall into classes depending upon the target sequence to which they bind [13,31]. The PDZ domain of LIN-7 is a type I PDZ protein, and the LET-23 carboxy-terminal sequence (-TCL) matches the type I consensus. Kim and colleagues changed either the carboxy-terminal sequence of LET-23 to a type II consensus (-YFI) or the PDZ domain of LIN-7 to a type II PDZ domain [21]. Either change alone abrogated LIN-7/LET-23 binding *in vitro* and resulted in apically-localized LET-23 *in vivo*. Combining the two compensatory changes restored both the binding and the basolateral localization of LET-23 *in vivo*, providing very strong evidence for a direct and functionally important interaction of LIN-7 with LET-23.

The demonstration in a genetic system that LIN-2, LIN-7, and LIN-10 facilitate polarized protein localization in epithelial cells prompted the question of whether these proteins were functioning in the same capacity in neurons. Are these PDZ proteins the polarization machinery shared between two disparate cell types?

Kaplan and colleagues had been studying the localization of an AMPA-type glutamate receptor, GLR-1, within *C. elegans* neurons [32]. GLR-1 was identified in screens for mechanosensory-defective mutants, and its expression is required in a group of interneurons, termed the command interneurons because of their governance over nematode locomotion activity [33,34]. Through double label analysis, GLR-1 was shown to be localized to postsynaptic specializations at mechanosensory neuron to interneuron synapses (Fig. 3D) [32]. It is thought that in response to touch, the mechanosensory neurons release glutamate into the synaptic cleft where it binds GLR-1-containing channels on the postsynaptic membrane. These channels presumably open in response to glutamate, depolarize the membrane, and activate the interneurons, resulting in the animal moving away from the mechanosensory stimulus.

One strong possibility was that PDZ proteins regulate GLR-1 localization since the PDZ proteins PSD-95 is enriched at synapses in mammalian brain [9,10,35]. PSD-95 can directly bind to Shaker-type K⁺ channels and NMDA-type glutamate receptors, and can cluster them when co-expressed with them in COS-7 cells, a non-neural cultured cell [36,37]. To identify *trans*-acting factors involved in GLR-1 localization, Kaplan and colleagues examined GLR-1 localization in the background of various mutants, including *lin-2*, *lin-7*, and *lin-10* mutants [32]. They found that nematodes that lack LIN-10 fail to localize GLR-1 to synapses (Fig. 3F). Instead, GLR-1 accumulates diffusely throughout much of the neuron. Polarized morphology and axon guidance in *lin-10* mutant neurons is unaffected, and presynaptic markers seem to be properly localized, indicating that LIN-10 is required specifically for the proper localization of GLR-1 to postsynaptic membranes. Interestingly, like *glr-1* mutants, *lin-10* mutants are defective for the same mechanosensory behaviors, even when localized to synapses by a LIN-10-independent pathway, indicating that some other aspect of LIN-10 function is required to transduce the glutamate signal from the mechanosensory neuron to the interneurons. Taken together, these experiments identified LIN-10 as a shared component of the polarized protein localization pathways in epithelia and neurons, and provided the first genetic evidence that PDZ proteins are required for the localization of synaptic proteins to central nervous system synapses *in vivo*.

Is the entire tripartite complex required for GLR-1 localization? Surprisingly, neither LIN-2 nor LIN-7 are required for GLR-1 localization (Fig. 3B) [32]. One explanation is that LIN-2, LIN-7, and LIN-10 function at separate steps along the pathway of LET-23 EGFR localization in epithelia, whereas only the LIN-10 step is shared between neurons and epithelia. Alternatively, LIN-10 might interact with a different set of proteins in neurons.

3. A corresponding mammalian tripartite complex that might assemble synaptic junctions between neurons

Is this a facet of biology unique to *C. elegans* alone, or is the tripartite complex conserved? At the same time that LIN-2 was cloned, Südhof and colleagues had identified the rat protein CASK based on its interaction with the neurexins, a family of neuronal cell surface molecules [38,39]. CASK is similar in sequence and domain organization to LIN-2, and together they define a subfamily of MAGUK proteins that now includes p55, Dlg2, Dlg3, Pal1, and Pal2 (Fig. 2 and Table 1) [40–44]. CASK is expressed in both neural and non-neural tissues throughout most of development.

Putative mammalian homologs of LIN-10 have also been found by several different groups [45–47]. One sequence, called X11, was originally identified as a candidate for the Friedreich ataxia locus but was later found to be a PDZ and PTB domain-containing protein capable of binding the amyloid precursor protein (APP) [47,48]. LIN-10-like proteins were also identified independently by their interaction with the synaptic protein Munc18-1, and were named Munc interacting proteins or Mints [45]. For simplicity, the mammalian homologs of LIN-10 are referred to as Mint1, Mint2, and Mint3 (Table 1). The Mints, like CASK, are broadly expressed in both neural and non-neural tissues throughout most of development [46,49,50].

Several different groups have identified putative mammalian LIN-7 homologs from mouse ESTs based on sequence identity to LIN-7 (Table 1) [21,51–54]. The Kim and Margolis labs have named them mammalian LIN-7 (mLin7a, mLin7b, and mLin7c), the Südhof lab has named them vertebrate LIN-7 (Veli1, Veli2, and Veli3), and the Brecht lab has named them mammalian LIN-7 (MALS-1, MALS-2, and MALS-3). For simplicity, I will refer to them as Veli1, Veli2, and Veli3. The Velis appear to have a more restrictive expression pattern than CASK or the Mints, with Veli1 and Veli2 found in separate but overlapping regions of the brain, whereas Veli3 is less abundant in the brain but more abundant in non-neural tissues [51,55].

The Kim, Margolis, and Südhof labs independently discovered that the mammalian homologs of LIN-2, LIN-7, and LIN-10, like their nematode counterparts, interact with each other and can be found in a tripartite complex from brain lysates (Fig. 4A) [21,51,52]. These results demonstrated that the PDZ tripartite complex is conserved from nematodes to mammals, but left open the question of whether the role of the complex in polarized localization was also conserved. The CASK-neurexins and Mint1-Munc18-1 interactions suggested that the complex might link cell adhesion (via neurexins) with synaptic vesicle release machinery (via Munc18-1).

As proposed by Südhof and colleagues, the Veli/CASK/Mint complex could potentially bring together many different proteins, which through protein–protein interaction, might drive synapse formation (Fig. 4A) [51]. Their model is centered on the interaction of neurexin with neuroligins, a family of cell adhesion molecules found at postsynaptic specializations [56–59]. The intracellular domain of neuroligin binds to PSD-95, whereas the extracellular neuroligin domain can reach across the synaptic cleft and bind to certain splice variants of the neurexins on the presynaptic side. The intracellular domain of the neurexins can bind to CASK and recruit the tripartite complex [38,51]. Mint1 can then recruit Munc18-1, which can bring along its own binding partner, syntaxin, a key SNARE needed for synaptic vesicle release [45,60]. Moreover, Mint1

and CASK both are able to bind N-type Ca^{2+} channels, thereby bringing the voltage-activated Ca^{2+} channel next to the Ca^{2+} triggered synaptic vesicle release machinery [61].

This model suggests that neuroligin, when presented to neurons, should be sufficient to induce the organization of presynaptic specializations in axons. Serafini and colleagues demonstrated that such induction can occur through a remarkable set of experiments [62]. Neuroligin, when expressed in non-neuronal HEK293 cells and cocultured with either pontine explants or cerebellar granule cells, induced the accumulation of presynaptic structures in axons. Synapsin, synaptotagmin, synaptophysin, CASK, and most importantly synaptic vesicles accumulated at the sites of neuroligin contact, and synaptic vesicle exocytosis could be ob-

Table 1
Members of the LIN-2, LIN-7, and LIN-10 families of proteins

Protein	Other names	Primary site of expression	Subcellular localization	Known binding partners	Ref.
<i>Veli-like proteins</i>					
LIN-7		Epithelia	Cell junctions	LET-23, LIN-2	[20,21]
Veli1	MALS-1,	Brain	Axonal and somatodendritic in neurons, postsynaptic in cultured hippocampal neurons	NMDA NR2B, DLG2, DLG3, CASK	[21,51–55]
Veli2	MALS-2	Brain	Somatodendritic in neurons	NMDA NR2B, DLG2, DLG3, CASK	[51,53–55]
Veli3	MALS-3,	Brain, kidney, liver	Diffuse in neurons, basolateral in epithelia	BTG-1, DLG2, CASK, Pals1, Pals2	[44,51,53–55] [77,96]
<i>CASK-like MAGUKS</i>					
LIN-2		Epithelia, neurons	Cell junctions in epithelia	LIN-7, LIN-10	[21,29]
CASK	mLin-2	Brain, lung, liver, kidney	Axonal and somatodendritic (pre and postsynaptic) in neurons, basolateral in epithelia	Mint1, Veli1, Veli2, Neurexins, Protein 4.1, Syndecans, N-type Ca^{2+} Ch., Thr-1, hDlg	[21,38,50–52], [67,68,91,92], [96,97]
Dlg3		Brain, skeletal muscle, testis, kidney, lung	ND	Veli1, Veli2	[42,43,51]
Pals 1		Heart, brain, skeletal muscle, kidney	Basolateral in epithelia	Veli3	[44]
Pals2		Heart, skeletal muscle, liver, testis, kidney, brain, spleen, lung	Basolateral in epithelia	Veli3	[44]
<i>Mint-like proteins</i>					
LIN-10		Epithelia, neurons	Postsynaptic in neurons, perinuclear (Golgi) in neurons and epithelia	LIN-2	[21,22,32,52], [93]
Mint1	X11 α , Mlin10	Brain	Somatodendritic, perinuclear	Munc-18-1, CASK, APP, Neurexins, KIF17, N-type Ca^{2+} Ch.	[45,48–50,52], [61,69,98,99]
Mint2	X11 β	Brain	ND	Munc-18-1, APP, Neurexins	[45,49,50,98]
Mint3	X11 γ	Most tissues	Perinuclear	APP	[46,49,50,96]

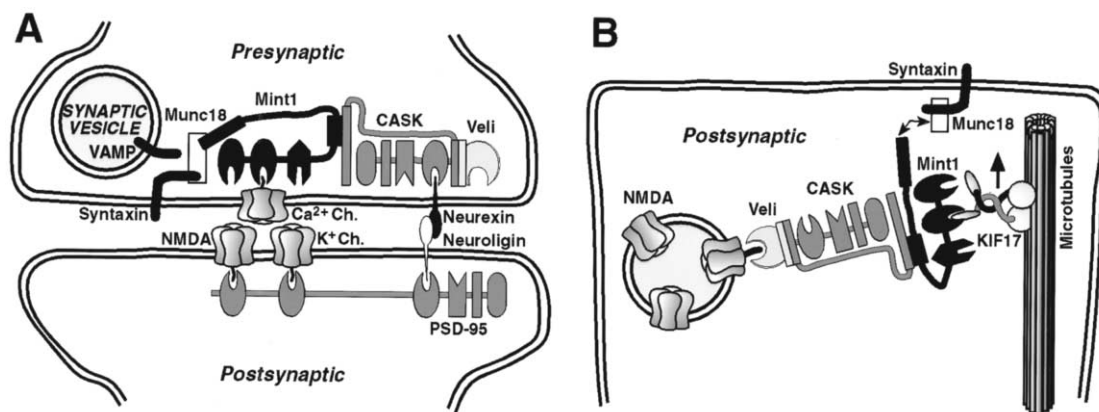


Fig. 4. Possible functions of the mammalian tripartite complex. (A) One tantalizing model of synapse formation suggests that a transsynaptic complex of proteins might sow the synapse together. In this model, postsynaptic cells would present neuroigin to the presynaptic cell, where it would bind and recruit neurexin. Neurexin would recruit the tripartite complex, which through its interaction with Munc18-1 and Ca^{2+} channels, could assemble the synaptic vesicles and their release machinery at the nascent presynaptic terminal. PSD-95, which can bind neuroigin at postsynaptic specializations, would assemble NMDA receptors and K^{+} channels at the postsynaptic membrane. (B) A postsynaptic model for the function of the tripartite complex suggests cargo vesicles containing NMDA receptor are delivered to postsynaptic specializations. In this model, the tripartite complex recognizes the receptors on the vesicles by a direct interaction through Veli. The vesicle complex could then be transported along polarized microtubules using the motor protein KIF17, which can bind to Mint1. Upon arrival at synaptic membranes, cargo vesicles might be fused to the membrane through an interaction between Mint1 and Munc18-1/syntaxin.

served at these sites. This phenomena could be blocked by the addition of soluble neurexin to the media, supporting the idea that neuroigin induces presynaptic development by binding to neurexin on presynaptic membranes.

This mammalian model stands in contrast to the function of the tripartite complex observed *in vivo* in *C. elegans*, where the complex is found to localize proteins to basolateral membranes of epithelia, and where LIN-10 localizes receptors to the postsynaptic side of synapses in neurons (the mammalian model predicts a presynaptic role) [21,32]. Is there genetic support for the mammalian model? So far, mouse knock-outs of the various genes in the complex have been less than informative. There are three neurexin genes and three neuroigin genes, all of which are alternatively spliced [39,63–65]. Neuroigin-1 knock-out mice have no obvious phenotype; however, neuroigin-2 can also induce presynaptic differentiation and thus cannot be discounted [59,62]. Similarly, double knock-outs of Veli1 and Veli2, which are abundant in brain, also do not yield a detectable phenotype, although Veli3 expression is upregulated in these mice, perhaps compensating for the lack of Veli1 and Veli2 [55]. On the postsynaptic side, knock-outs of PSD-95 properly localize NMDA receptors and have normal synaptic morphology, although other related MAGUK proteins could be compensating [66]. It seems that we might have to wait until all the molecules within a family are knocked-out before we have an answer as to what the tripartite complex is doing *in vivo* in mammals.

4. Models for tripartite complex function: polarized secretion versus selective retention

In contrast to the presynaptic mammalian model mentioned above, recent data from several groups points to a postsynaptic function for members of the tripartite complex. Veli1, Veli2, and Mint1 appear to be localized to the somatodendritic compartment of neurons, and CASK has been found on both pre and postsynaptic membranes [50,55,67,68]. The Bredt lab has recently shown that Veli1 and Veli2 can interact the NR2B subunit of the NMDA receptor [54]: Veli proteins coimmunoprecipitate with NR2B both from solubilized brain and when coexpressed in COS-7 cells. Moreover, Veli proteins cocluster with PSD-95 and NMDA receptors in cultured hippocampal neurons. Taken together, these results suggest that Veli proteins could participate in the recruitment of NMDA receptors to postsynaptic specializations.

How might the proteins in the tripartite complex localize proteins like the NMDA receptor to synaptic sites? Receptors might be clustered to specific synaptic sites through selective retention by an anchored tripartite complex. Alternatively, receptors might utilize the polarized secretion machinery of the cell, thereby being delivered as cargo on vesicles destined for synaptic sites. Recent data suggest that neurons probably use both of these strategies to deliver and cluster synaptic proteins.

In the case of the NMDA receptor, the Hirokawa lab has shown that the kinesin motor protein KIF17 can bind to Mint1 through a PDZ interaction (Fig. 4B) [69].

KIF17 is a dendrite-specific motor that associates with 50-nm membranous cargo vesicles, and KIF17, Veli, CASK, Mint1, and NR2B cofractionate with these vesicles and colocalize within dendrites of cerebral cortex neurons. Interestingly, vesicles purified from brain by anti-KIF17 antibodies contain NR2B and move on microtubules when provided with wild-type KIF17 protein but not KIF17 lacking the Mint1 interaction domain, even though this mutant form of KIF17 still has motor activity. Taken together with the result that Veli1 and Veli2 can directly bind to NR2B, these data suggest that the tripartite PDZ complex can couple NMDA receptor-containing vesicles to a kinesin motor protein, which could be used to selectively transport these cargo vesicles to synaptic sites. Because Mint1 can interact with Munc18-1, and Munc18-1 can bind to syntaxin on synaptic membranes, the cargo vesicle could conclude its journey by fusing with the synaptic membrane after a Mint1/Munc18-1/syntaxin interaction [45,60]. The tripartite complex could act like a SNARE protein in this model, taking the place of VAMP/synaptobrevin as the vSNARE. Such a model has been proposed for AMPA-receptor trafficking based on the interaction of GluR1 with NSF and SNAP proteins [70,71].

Once transported to the postsynaptic membrane, NR2B might switch its association from Veli proteins to PSD-95, thereby firmly anchoring it in the membrane and preventing its lateral diffusion [36,37]. PSD-95 and its associated proteins could then assemble the receptor into a complex with other signaling molecules like nNOS and synGAP [72,73]. Interestingly, overexpression of PSD-95 has been shown to enhance the maturation of glutamatergic synapses [74]. Thus, the association of the NMDA receptor with PSD-95 at the mature synapse could contribute to synaptic remodeling in the adult brain and activity-dependent synaptic plasticity. This postsynaptic model describing the function of the tripartite complex suffers from some of the same downfalls as the mammalian presynaptic model described earlier: lack of support from knockout studies. However, there is some *in vivo* data to support this model since in *C. elegans* LIN-10 is required to localize glutamate receptors to postsynaptic specializations [32].

In addition to their potential role in transporting proteins to basolateral and synaptic membranes, and anchoring those proteins at those membranes to prevent lateral fusion, there is some evidence to suggest that the LIN-2/LIN-7/LIN-10 complex of proteins might also play a role in selectively retaining proteins at the membrane by preventing their internalization. The epithelial GABA transporter BGT-1 contains a type I PDZ consensus at its carboxy-terminal end, and is sorted to basolateral surfaces of hypertonically cultured MDCK cells [75,76]. BGT-1 can bind to LIN-7 and Veli proteins through its carboxy-terminal sequences. Surprisingly, a deleted version of BGT-1 that lacks the last five amino

acids (BGT-1 Δ 5) is still localized to basolateral surfaces, in contrast to LET-23 EGF receptors deleted for the last six amino acids, which are mislocalized to apical surfaces [21,77]. BGT-1 Δ 5 transporters also accumulate in intracellular vesicles, and microinjection of a 17-mer BGT-1 carboxy-terminal peptide can cause wild-type BGT-1 transporters to accumulate intracellularly as well [77]. The maturation of BGT-1 Δ 5 is no different from wild-type BGT-1 transporters, and BGT-1 Δ 5 does not colocalize with the ER or Golgi, indicating that the intracellular accumulations are not an early intermediate in BGT-1 trafficking. When surface glycoproteins are pulse labeled with wheat germ agglutinin and chased into the endocytic pathway, they colocalize with BGT-1 Δ 5 transporters in intracellular vesicles, raising the possibility that a PDZ interaction with BGT-1 is required to retain it at the surface and prevent internalization. While it is clear that the last five amino acids of BGT-1 are important for BGT-1 surface retention, this result should be interpreted with caution since a requirement for a PDZ domain protein in this process has not yet been demonstrated.

Similar to BGT-1 endocytosis in epithelia, AMPA-type glutamate receptor endocytosis in neurons appears to be an important means of regulating synaptic efficacy [78–81]. AMPA receptor internalization at synapses requires clathrin-mediated endocytosis and depends upon the carboxy-terminal sequences of the receptor, which contain a PDZ consensus motif and protein kinase C (PKC) phosphorylation sites. The PDZ proteins GRIP/ABP and PICK1 can bind to AMPA receptor carboxy-terminal sequences, and these PDZ interactions can be regulated when AMPA receptors become phosphorylated by PKC or interact with NSF, which in both cases results in the internalization of the receptor by endocytosis [70,71,82–90]. Thus, like in the case of BGT-1, cell surface retention of AMPA receptors is probably one more aspect of their localization and function that is regulated by PDZ-proteins.

5. Conclusion

One disconcerting aspect of studying the tripartite complex proteins is the promiscuity of their interactions with other proteins (Fig. 2). For example, the PDZ domain of CASK can bind neurexins, but it can also bind members of the syndecan family of heparin sulfate proteoglycans, which could potentially link the tripartite complex to the extracellular matrix [38,67,68]. Most surprising of all, CASK has been shown to bind to a T-box transcription factor, Tbr-1, via the CASK guanylate kinase domain [91]. Sheng and colleagues have shown that CASK, along with Tbr-1, can enter the nucleus of COS-7 cells and embryonic neurons, and act as a coactivator of transcription. Such experimental

evidence lends support to the idea that many PDZ proteins are multifunctional interaction cassettes whose function can be suitably tailored depending on the context, including tissue of expression, subcellular location, and stage of development. Hence, CASK might be signaling during embryogenesis by shuttling from the cell junction to the nucleus to regulate genes involved neural development. Upon maturation of the nervous system, it switches its functions, moving to both the pre and postsynaptic membranes where it can assemble synaptic proteins and maintain neural polarity. Thus, the function and subcellular distribution of CASK is dictated by development and growth.

Of course, the promiscuity of these proteins is probably a blessing for researchers as much as it is a curse. New and interesting models for their function are proposed based on the identity of factors identified by various protein–protein interactions assays with members of the complex. Such experiments will no doubt provide fuel for this field for years to come. How will researchers sort through the plethora of binding partners to identify the *in vivo* role of these proteins? Hopefully simpler genetic systems like *C. elegans* and *Drosophila*, which are likely to have fewer redundant proteins than mice, will provide a framework for understanding the function of these proteins in cell polarity.

Acknowledgements

The author thanks Bonnie Firestein for many animated discussions and critical comments on this manuscript.

References

- [1] Dotti CG, Simons K. Polarized sorting of viral glycoproteins to the axon and dendrites of hippocampal neurons in culture. *Cell* 1990;62(1):63–72.
- [2] Cameron PL, Sudhof TC, Jahn R, De Camilli P. Colocalization of synaptophysin with transferrin receptors: implications for synaptic vesicle biogenesis. *J Cell Biol* 1991;115(1):151–64.
- [3] Jareb M, Banker G. The polarized sorting of membrane proteins expressed in cultured hippocampal neurons using viral vectors. *Neuron* 1998;20(5):855–67.
- [4] Keller P, Simons K. Post-Golgi biosynthetic trafficking. *J Cell Sci* 1997;110(Pt 24):3001–9.
- [5] Keller P, Simons K. Cholesterol is required for surface transport of influenza virus hemagglutinin. *J Cell Biol* 1998;140(6):1357–67.
- [6] Matter K, Yamamoto EM, Mellman I. Structural requirements and sequence motifs for polarized sorting and endocytosis of LDL and Fc receptors in MDCK cells. *J Cell Biol* 1994;126(4):991–1004.
- [7] Trowbridge IS, Collawn JF, Hopkins CR. Signal-dependent membrane protein trafficking in the endocytic pathway. *Annu Rev Cell Biol* 1993;9:129–61.
- [8] Woods DF, Bryant PJ. The discs-large tumor suppressor gene of *Drosophila* encodes a guanylate kinase homolog localized at septate junctions. *Cell* 1991;66(3):451–64.
- [9] Cho K-O, Hunt CA, Kennedy MB. The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* 1992;9:929–42.
- [10] Kistner U, Wenzel BM, Veh RW, Cases-Langhoff C, Garner AM, Appeltauer U, et al. SAP90, a rat presynaptic protein related to the product of the *Drosophila* tumor suppressor gene *dlg-A*. *J Biol Chem* 1993;268(7):4580–3.
- [11] Willott E, Balda MS, Fanning AS, Jameson B, Van Itallie C, Anderson JM. The tight junction protein ZO-1 is homologous to the *Drosophila* discs-large tumor suppressor protein of septate junctions. *Proc Natl Acad Sci USA* 1993;90(16):7834–8.
- [12] Doyle DA, Lee A, Lewis J, Kim E, Sheng M, MacKinnon R. Crystal structure of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. *Cell* 1996;85:1067–76.
- [13] Songyang Z, Lu K, Kwon Y, Tsai LH, Filhol O, Cochet C, et al. A structural basis for substrate specificities of protein Ser/Thr kinases: primary sequence preference of casein kinases I and II, NIMA, phosphorylase kinase, calmodulin-dependent kinase II, CDK5, and Erk1. *Mol Cell Biol* 1996;16:6486–93.
- [14] Shieh BH, Zhu MY. Regulation of the TRP Ca²⁺ channel by INAD in *Drosophila* photoreceptors. *Neuron* 1996;16(5):991–8.
- [15] Chevesich J, Kreuz AJ, Montell C. Requirement for the PDZ domain protein, INAD, for localization of the TRP store-operated channel to a signaling complex. *Neuron* 1997;18(1):95–105.
- [16] Tsunoda S, Sierralta J, Sun Y, Bodner R, Suzuki E, Becker A, et al. A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. *Nature* 1997;388(6639):243–9.
- [17] Tejedor FJ, Bokhari A, Rogero O, Gorczyca M, Zhang J, Kim E, et al. Essential role for *dlg* in synaptic clustering of Shaker K⁺ channels *in vivo*. *J Neurosci* 1997;17(1):152–9.
- [18] Thomas U, Kim E, Kuhlendahl S, et al. Synaptic clustering of the cell adhesion molecule fasciclin II by discs-large and its role in the regulation of presynaptic structure. *Neuron* 1997;19(4):787–99.
- [19] Zito K, Fetter RD, Goodman CS, Isacoff EY. Synaptic clustering of Fasciclin II and Shaker: essential targeting sequences and role of *Dlg*. *Neuron* 1997;19(5):1007–16.
- [20] Simske JS, Kaech SM, Harp SA, Kim SK. LET-23 receptor localization by the cell junction protein LIN-7 during *C. elegans* vulval induction. *Cell* 1996;85(2):195–204.
- [21] Kaech SM, Whitfield CW, Kim SK. The LIN-2/LIN-7/LIN-10 complex mediates basolateral membrane localization of the *C. elegans* EGF receptor LET-23 in vulval epithelial cells. *Cell* 1998;94(6):761–71.
- [22] Whitfield CW, Benard C, Barnes T, Hekimi S, Kim SK. Basolateral localization of the *Caenorhabditis elegans* epidermal growth factor receptor in epithelial cells by the PDZ protein LIN-10. *Mol Biol Cell* 1999;10(6):2087–100.
- [23] Hill RJ, Sternberg PW. The gene *lin-3* encodes an inductive signal for vulval development in *C. elegans*. *Nature* 1992;358(6386):470–6.
- [24] Aroian RV, Koga M, Mendel JE, Ohshima Y, Sternberg PW. The *let-23* gene necessary for *Caenorhabditis elegans* vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. *Nature* 1990;348:693–9.
- [25] Beitel GJ, Clark SG, Horvitz HR. *Caenorhabditis elegans* *ras* gene *let-60* acts as a switch in the pathway of vulval induction. *Nature* 1990;348(6301):503–9.
- [26] Han M, Sternberg PW. *let-60*, a gene that specifies cell fates during *C. elegans* vulval induction, encodes a *ras* protein. *Cell* 1990;63:921–31.

- [27] Lackner MR, Kornfeld K, Miller LM, Horvitz HR, Kim SK. A MAP kinase homolog, mpk-1, is involved in ras-mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev* 1994;8(2):160–73.
- [28] Ferguson EL, Horvitz HR. Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. *Genetics* 1985;110(1):17–72.
- [29] Hoskins R, Hajnal AF, Harp SA, Kim SK. The *C. elegans* vulval induction gene lin-2 encodes a member of the MAGUK family of cell junction proteins. *Development* 1996;122(1):97–111.
- [30] Kim SK, Horvitz HR. The *Caenorhabditis elegans* gene lin-10 is broadly expressed while required specifically for the determination of vulval cell fates. *Genes Dev* 1990;4(3):357–71.
- [31] Stricker NL, Christopherson KS, Yi BA, Schatz PJ, Raab RW, Dawes G, et al. PDZ domain of neuronal nitric oxide synthase recognizes novel C-terminal peptide sequences. *Nat Biotechnol* 1997;15(4):336–42.
- [32] Rongo C, Whitfield CW, Rodal A, Kim SK, Kaplan JM. LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. *Cell* 1998;94(6):751–9.
- [33] Hart AC, Sims S, Kaplan JM. Synaptic code for sensory modalities revealed by *C. elegans* GLR-1 glutamate receptor. *Nature* 1995;378:82–4.
- [34] Maricq AV, Peckol E, Driscoll M, Bargmann CI. Mechanosensory signalling in *C. elegans* mediated by the GLR-1 glutamate receptor [published erratum appears in *Nature* 1996 Feb 22;379(6567):749]. *Nature* 1995;378(6552):78–81.
- [35] Muller BM, Kistner U, Veh RW, Cases-Langhoff C, Becker B, Gundelfinger ED, et al. Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the Drosophila discs-large tumor suppressor protein. *J Neurosci* 1995;15(3 Pt. 2):2354–66.
- [36] Kornau H-C, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* 1995;269:1737–40.
- [37] Niethammer M, Kim E, Sheng M. Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *J Neurosci* 1996;16(7):2157–63.
- [38] Hata Y, Butz S, Sudhof TC. CASK: a novel dlg/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. *J Neurosci* 1996;16(8):2488–94.
- [39] Ushkaryov YA, Petrenko AG, Geppert M, Sudhof TC. Neurexins: synaptic cell surface proteins related to the alpha-latrotoxin receptor and laminin. *Science* 1992;257(5066):50–6.
- [40] Ruff P, Speicher DW, Husain-Chishti A. Molecular identification of a major palmitoylated erythrocyte membrane protein containing the src homology 3 motif. *Proc Natl Acad Sci USA* 1991;88(15):6595–9.
- [41] Mazoyer S, Gayther SA, Nagai MA, Smith SA, Dunning A, van Rensburg EJ, et al. A gene (DLG2) located at 17q12-q21 encodes a new homologue of the Drosophila tumor suppressor dlG-A. *Genomics* 1995;28(1):25–31.
- [42] Lin L, Peters LL, Ciciotte SL, Chishti AH. cDNA sequence and chromosomal localization of mouse Dlg3 gene adjacent to the BRCA1 tumor suppressor locus. *Biochim Biophys Acta* 1998;1443(1-2):211–6.
- [43] Smith SA, Holik P, Stevens J, et al. Isolation of a gene (DLG3) encoding a second member of the discs-large family on chromosome 17q12-q21. *Genomics* 1996;31(2):145–50.
- [44] Kamberov E, Makarova O, Roh M, Liu A, Karnak D, Straight S, et al. Molecular cloning and characterization of Pals, proteins associated with mLin-7. *J Biol Chem* 2000;275(15):11425–31.
- [45] Okamoto M, Sudhof TC. Mints, Munc18-interacting proteins in synaptic vesicle exocytosis. *J Biol Chem* 1997;272(50):31459–64.
- [46] Okamoto M, Sudhof TC. Mint 3: a ubiquitous mint isoform that does not bind to munc18-1 or -2. *Eur J Cell Biol* 1998;77(3):161–5.
- [47] Duclos F, Boschert U, Sirugo G, Mandel JL, Hen R, Koenig M. Gene in the region of the Friedreich ataxia locus encodes a putative transmembrane protein expressed in the nervous system. *Proc Natl Acad Sci USA* 1993;90(1):109–13.
- [48] Borg JP, Ooi J, Levy E, Margolis B. The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. *Mol Cell Biol* 1996;16(11):6229–41.
- [49] Borg JP, Yang Y, De Taddeo-Borg M, Margolis B, Turner RS. The X11alpha protein slows cellular amyloid precursor protein processing and reduces Abeta40 and Abeta42 secretion. *J Biol Chem* 1998;273(24):14761–6.
- [50] Borg JP, Lopez-Figueroa MO, de Taddeo-Borg M, Kroon DE, Turner RS, Watson SJ, et al. Molecular analysis of the X11-mLin-2/CASK complex in brain. *J Neurosci* 1999;19(4):1307–16.
- [51] Butz S, Okamoto M, Sudhof TC. A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. *Cell* 1998;94(6):773–82.
- [52] Borg JP, Straight SW, Kaech SM, de Taddeo-Borg M, Kroon DE, Karnak D, et al. Identification of an evolutionarily conserved heterotrimeric protein complex involved in protein targeting. *J Biol Chem* 1998;273(48):31633–6.
- [53] Irie M, Hata Y, Deguchi M, Ide N, Hirao K, Yao I, et al. Isolation and characterization of mammalian homologues of *Caenorhabditis elegans* lin-7: localization at cell–cell junctions. *Oncogene* 1999;18(18):2811–7.
- [54] Jo K, Derin R, Li M, Brecht DS. Characterization of MALS/Velis-1, -2, and -3: a family of mammalian LIN-7 homologs enriched at brain synapses in association with the postsynaptic density-95/NMDA receptor postsynaptic complex. *J Neurosci* 1999;19(11):4189–99.
- [55] Misawa H, Kawasaki Y, Mellor J, Sweeney N, Jo K, Nicoll RA, et al. Contrasting localizations of MALS/LIN-7 PDZ proteins in brain and molecular compensation in knockout mice. *J Biol Chem* 2001;276(12):9264–72.
- [56] Ichtchenko K, Nguyen T, Sudhof TC. Structures, alternative splicing, and neurexin binding of multiple neuroligins. *J Biol Chem* 1996;271(5):2676–82.
- [57] Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, Moomaw C, et al. Neuroligin 1: a splice site-specific ligand for beta-neurexins. *Cell* 1995;81(3):435–43.
- [58] Irie M, Hata Y, Takeuchi M, Ichtchenko K, Toyoda A, Hirao K, et al. Binding of neuroligins to PSD-95. *Science* 1997;277(5331):1511–5.
- [59] Song JY, Ichtchenko K, Sudhof TC, Brose N. Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proc Natl Acad Sci USA* 1999;96(3):1100–5.
- [60] Hata Y, Slaughter CA, Sudhof TC. Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. *Nature* 1993;366(6453):347–51.
- [61] Maximov A, Sudhof TC, Bezprozvanny I. Association of neuronal calcium channels with modular adaptor proteins. *J Biol Chem* 1999;274(35):24453–6.
- [62] Scheiffele P, Fan J, Choi J, Fetter R, Serafini T. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell* 2000;101(6):657–69.
- [63] Ushkaryov YA, Hata Y, Ichtchenko K, Moomaw C, Afendis S, Slaughter CA, et al. Conserved domain structure of beta-neurexins. Unusual cleaved signal sequences in receptor-like neuronal cell-surface proteins. *J Biol Chem* 1994;269(16):11987–92.
- [64] Ushkaryov YA, Sudhof TC. Neurexin III alpha: extensive alternative splicing generates membrane-bound and soluble forms. *Proc Natl Acad Sci USA* 1993;90(14):6410–4.

- [65] Ullrich B, Ushkaryov YA, Sudhof TC. Cartography of neuexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. *Neuron* 1995;14(3):497–507.
- [66] Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, et al. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 1998;396(6710):433–9.
- [67] Hsueh YP, Yang FC, Kharazia V, Naisbitt S, Cohen AR, Weinberg RJ, et al. Direct interaction of CASK/LIN-2 and syndecan heparan sulfate proteoglycan and their overlapping distribution in neuronal synapses. *J Cell Biol* 1998;142(1):139–51.
- [68] Hsueh YP, Sheng M. Regulated expression and subcellular localization of syndecan heparan sulfate proteoglycans and the syndecan-binding protein CASK/LIN-2 during rat brain development. *J Neurosci* 1999;19(17):7415–25.
- [69] Setou M, Nakagawa T, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* 2000;288(5472):1796–802.
- [70] Nishimune A, Isaac JT, Molnar E, Noel J, Nash SR, Tagaya M, et al. NSF binding to GluR2 regulates synaptic transmission. *Neuron* 1998;21(1):87–97.
- [71] Osten P, Srivastava S, Inman GJ, Vilim FS, Khatri L, Lee LM, et al. The AMPA receptor GluR2 C terminus can mediate a reversible, ATP-dependent interaction with NSF and alpha- and beta-SNAPs. *Neuron* 1998;21(1):99–110.
- [72] Brenman JE, Chao DS, Gee SH, McGee AW, Craven SE, Santillano DR, et al. Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha-1-syntrophin mediated by PDZ domains. *Cell* 1996;84(5):757–67.
- [73] Kim JH, Liao D, Lau LF, Haganir RL. SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron* 1998;20(4):683–91.
- [74] El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, Brecht DS. PSD-95 involvement in maturation of excitatory synapses. *Science* 2000;290(5495):1364–8.
- [75] Yamauchi A, Kwon HM, Uchida S, Preston AS, Handler JS. Myo-inositol and betaine transporters regulated by tonicity are basolateral in MDCK cells. *Am J Physiol* 1991;261(1 Pt. 2):F197–202.
- [76] Perego C, Bulbarelli A, Longhi R, Caimi M, Villa A, Caplan MJ, et al. Sorting of two polytopic proteins, the gamma-aminobutyric acid and betaine transporters, in polarized epithelial cells. *J Biol Chem* 1997;272(10):6584–92.
- [77] Perego C, Vanoni C, Villa A, Longhi R, Kaeck SM, Frohli E, et al. PDZ-mediated interactions retain the epithelial GABA transporter on the basolateral surface of polarized epithelial cells. *EMBO J* 1999;18(9):2384–93.
- [78] Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, et al. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat Neurosci* 2000;3(12):1291–300.
- [79] Man YH, Lin JW, Ju WH, Ahmadian G, Liu L, Becker LE, et al. Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. *Neuron* 2000;25(3):649–62.
- [80] Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, Wyszynski M, et al. Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. *Nat Neurosci* 2000;3(12):1282–90.
- [81] Ehlers MD. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 2000;28(2):511–25.
- [82] Matsuda S, Mikawa S, Hirai H. Phosphorylation of serine-880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor-interacting protein. *J Neurochem* 1999;73(4):1765–8.
- [83] Noel J, Ralph GS, Pickard L, Williams J, Molnar E, Uney JB, et al. Surface expression of AMPA receptors in hippocampal neurons is regulated by an NSF-dependent mechanism. *Neuron* 1999;23(2):365–76.
- [84] Luthi A, Chittajallu R, Duprat F, Palmer MJ, Benke TA, Kidd FL, et al. Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction. *Neuron* 1999;24(2):389–99.
- [85] Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Haganir RL. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature* 1997;386(6622):279–84.
- [86] Srivastava S, Osten P, Vilim FS, Khatri L, Inman G, States B, et al. Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. *Neuron* 1998;21(3):581–91.
- [87] Xia J, Zhang X, Staudinger J, Haganir RL. Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. *Neuron* 1999;22(1):179–87.
- [88] Song I, Kamboj S, Xia J, Dong H, Liao D, Haganir RL. Interaction of the *N*-ethylmaleimide-sensitive factor with AMPA receptors. *Neuron* 1998;21(2):393–400.
- [89] Xia J, Chung HJ, Wihler C, Haganir RL, Linden DJ. Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins [In Process Citation]. *Neuron* 2000;28(2):499–510.
- [90] Chung HJ, Xia J, Scannevin RH, Zhang X, Haganir RL. Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *J Neurosci* 2000;20(19):7258–67.
- [91] Hsueh YP, Wang TF, Yang FC, Sheng M. Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2. *Nature* 2000;404(6775):298–302.
- [92] Nix SL, Chishti AH, Anderson JM, Walther Z. hCASK and hDlg associate in epithelia, and their src homology 3 and guanylate kinase domains participate in both intramolecular and intermolecular interactions. *J Biol Chem* 2000;275(52):41192–200.
- [93] Walhout AJ, Sordella R, Lu X, Hartley JL, Temple GF, Brasch MA, et al. Protein interaction mapping in *C. elegans* using proteins involved in vulval development. *Science* 2000;287(5450):116–22.
- [94] Ide N, Hata Y, Hirao K, Irie M, Deguchi M, Yao I, et al. Interaction of rat lin-10 with brain-enriched F-actin-binding protein, neurabin-II/spinophilin. *Biochem Biophys Res Commun* 1998;244(1):258–62.
- [95] Ide N, Hirao K, Hata Y, Takeuchi M, Irie M, Yao I, et al. Molecular cloning and characterization of rat lin-10. *Biochem Biophys Res Commun* 1998;243(2):634–8.
- [96] Straight SW, Karnak D, Borg JP, Kamberov E, Dare H, Margolis B, et al. mLin-7 is localized to the basolateral surface of renal epithelia via its NH(2) terminus. *Am J Physiol Renal Physiol* 2000;278(3):F464–75.
- [97] Cohen AR, Woods DF, Marfatia SM, Walther Z, Chishti AH, Anderson JM, et al. Human CASK/LIN-2 binds syndecan-2 and protein 4.1 and localizes to the basolateral membrane of epithelial cells. *J Cell Biol* 1998;142(1):129–38.
- [98] Biederer T, Sudhof TC. Mints as adaptors. direct binding to neuroligins and recruitment of munc18. *J Biol Chem* 2000;275(51):39803–6.
- [99] Okamoto M, Matsuyama T, Sugita M. Ultrastructural localization of Mint1 at synapses in mouse hippocampus. *Eur J Neurosci* 2000;12(8):3067–72.