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Emerging role of the scaffolding protein Dlg1 in interfacing with the vesicle trafficking machinery

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Abbreviations: ADAM10, disintegrin and metalloproteinase 10; AMPARs, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-type glutamate receptors; AP-1, clathrin adaptator protein complex 1; Dlg1, Discs large 1; ER, endoplasmic reticulum; ESCRT, endosomal-sorting complex required for transport; GluR1, AMPA receptor subunit; Glut4, glucose transporter type 4; GUK, guanylate kinase; Hrs, hepatocyte growth factor receptor tyrosine kinase substrate; KIF, kinesin family member; L27, Lin-2, -7; NMDAR, N-methyl-D-aspartate receptor; NR2A, NMDA receptor subunit; PDZ, post-synaptic density-95/Discs large/zona occludens-1; PSD-93, post-synaptic density-93; SAP-102, synapse-associated protein-102; SH3, Src homology 3, SNARE, soluble N-ethyl-maleimide-sensitive fusion protein attachment-protein receptor; TGN, *trans*-Golgi network; VWF, von Willebrand Factor

Discs large 1 (Dlg1) is a modular scaffolding protein implicated in the control of cell polarity through assembly of specific multiprotein complexes, including receptors, ion channels and signaling proteins, at specialized zones of the plasma membrane. Recent data have shown that in addition to these well-known interaction partners, Dlg1 may also recruit components of the vesicle trafficking machinery either to the plasma membrane or to transport vesicles. Here, we discuss Dlg1 function in vesicle formation, targeting, tethering and fusion, in both the exocytotic and endocytotic pathways. These pathways contribute to cell functions as major and diverse as glutamatergic activity in the neurons, membrane homeostasis in Schwann cell myelination, insulin stimulation of glucose transport in adipocytes, or endothelial secretion of the hemostatic protein, von Willebrand factor (VWF).

Mammalian discs large 1 (Dlg1; also named synapse-associated protein-97) is a modular scaffolding protein, consisting of six protein-binding domains. These include a Lin-2, -7 (L27) domain, three post-synaptic density-95/Discs large/zona occludens-1 (PDZ) domains, a Src homology 3 (SH3) domain and a guanylate kinase (GUK) domain (Figure1B) (1, 2). The GUK domain is catalytically inactive, and so Dlg1 lacks intrinsic enzymatic activity (3). Dlg1 domains have various and sometimes intricate binding specificities. The PDZ domains contain a specific GLGF sequence that constitutes a hydrophobic cavity where the X-S/T-X-V/L C-terminal motif of their target protein binds (4, 5). This motif is present in a large number of proteins such as receptors, ions channels or signaling proteins (5). The L27 domain, which is exclusively found in scaffolding proteins, preferentially hetero- or homo-oligomerizes (6, 7). In solution, Dlg1 exists as an elongated tetramer (8).Therefore, L27 interactions between scaffolding proteins allow the formation of supramolecular complexes (6). This domain may also interact with other target sequences which are found in a number of proteins but have not been clearly defined (9, 10). Finally, the SH3 domain interacts conventionally with proline-rich sequences (11, 12). Alternatively, this domain also forms a specific binding module with the GUK domain, even though the GUK domain may also behave as an independent binding domain (13-15). No consensus target sequence has been reported in either case. However, the GUK domain is often involved in interactions with cytoskeleton-associated proteins (13, 14, 16). Consistent with its structure, Dlg1 is a scaffold that organizes multiprotein complexes by physical interaction (1). Dlg1 subcellular localization depends on its interaction with partners but this protein has been reported to be mostly present at the membrane-cytoskeleton interface of cell-cell junctions (1, 17, 18). Therefore Dlg1 specifically assembles a combination of cell adhesion molecules, receptors or ion channels with cytoskeletal proteins and/or associate signaling components at specific plasma membrane sites (1). Dlg1 has both structural and signaling roles. The function of this scaffolding protein in controlling cell polarity was first shown in *Drosophila*.

The *Drosophila dlg* gene encodes the sole orthologue of mammalian Dlg1 in this organism. Alternative splicing gives rise to two main isoforms of *Drosophila* Dlg, DlgS97, which contains an L27 domain, and DlgA, which lacks an L27 domain (Figure 1A) (19). DlgA is the only form expressed in the epithelial tissues of *Drosophila* and is localized at septate junctions (the *Drosophila* counterpart of vertebrate tight junctions) *via* its PDZ2 domain (19-21). Genetic analysis in the fruit fly has shown that *dlg* mutations cause embryonic lethality due to disruption of the epithelial cellular junctions, resulting in a defect in the polarization of apical proteins and in cell overgrowth (20, 22). The ectopic expression of mammalian Dlg1 reverses tumor formation in *dlg* mutant *Drosophila* (23, 24). However, whether

mammalian Dlg1 behaves like *Drosophila* Dlg as an oncosuppressor essential for establishing proper epithelial polarity is controversial. Some data support this notion: in humans, Dlg1 mislocalization and progressive loss of expression are frequently observed during epithelial cancer progression, and this protein is a target for several viral oncoproteins (25-30). In Dlg1$^{-/-}$ mice, which die as neonates because of craniofacial deformities, an increased proliferation of ocular lens cells was observed (31, 32). This phenomenon is accompanied by an alteration in the distribution of polarity and cell-cell adhesion markers (33). Finally, in epithelial cells, Dlg1 localizes laterally at the site of cell-cell junctions by an indirect interaction, probably *via* a cytoskeleton-associated protein, with E-cadherin and regulates adherens junction integrity by recruiting PI3K to the complex (34, 35). However, Dlg1 depletion does not appear to significantly alter epithelial apicobasal polarity since no obvious mislocalisation of tight junction markers has been reported (36). One possible explanation for these contradictory results is that the loss of Dlg1 has tissue specific effects on epithelial cell polarity. In addition, other Dlg family members (described below) have been shown to be expressed in mouse embryo epithelia and in human cultured epithelial cells that may have redundant functions with Dlg1 and/or may compensate for its loss (37, 38).

Dlg expression is not restricted to epithelial cells. In the *Drosophila* larva, Dlg is localized to the synaptic contacts that form the neuromuscular junction. DlgS97 was reported to be the more prevalent isoform in neurons and muscles during fruit fly development (19, 39). At the glutamatergic synapse, Dlg is required for the targeting and clustering of its PDZ1-2 domain partners, the adhesion protein, Fasciclin II, and the Shaker K^+ channel (40-42). Mammalian neuronal tissue has, in addition to Dlg1, three homologous proteins named post-synaptic density-95 (PSD-95), post-synaptic density-93 (PSD-93) and synapse-associated-protein-102 (SAP-102), all three lacking an L27 domain. These four proteins are thought to play a key role in synapse assembly and plasticity (1, 43). Considering the high degree of functional redundancy among these four proteins, the *in vivo* importance of Dlg1 for brain function is unclear. Nevertheless, PSD-95 and PSD-93 were proposed to be more associated with the clustering and function of glutamatergic receptors at the synapse, whereas Dlg1 and SAP-102 might be more involved in the trafficking of these receptors to the synapse (44).

Consistent with its evolutionarily conserved functions, Dlg1 has been shown to regulate polarity in a number of other cell types. Dlg1 function has been particularly well established in cultured astrocytes and T-cells. In migrating astrocytes, Dlg1 is involved in the establishment and maintenance of antero-posterior polarity in concert with microtubule-associated proteins: the tumor suppressor adenomatous polyposis coli and the motor protein dynein (45, 46). At the leading edge, by directly interacting with guanylate kinase-associated protein, Dlg1 recruits dynein which regulates microtubule dynamics (46). Dlg1 contribution to the establishment and stabilization of the immunological synapse by driving the formation of the T-cell receptor-associated functional signaling complex is also well documented. Dlg1 translocates to the T-cell-antigen-presenting cell junction in response to TRC engagement and brings together a number of TCR signaling molecules such as the kinases Lck, p38 and ZAP70, and the cytoskeleton-organizing proteins WASP and ezrin (47-51).

During the past decade, investigations on Dlg1 function in the formation and maintenance of specialized zones of the plasma membrane have contributed to the conclusion that conserved mechanisms govern the establishment of cell polarity. Among these mechanisms are protein trafficking and/or docking to the proper plasma membrane microdomains, protein clustering, and formation of dynamic multi-protein signaling complexes. More recently, Dlg1 function in the polarized trafficking of membrane components was shown to be dependent on its ability, directly or indirectly, to recruit a variety of vesicular trafficking proteins to vesicles or to the plasma membrane, and to bring them in close proximity to specific cargoes (Figure 1C). This review focuses on mammalian Dlg1 function in vesicle formation, targeting, tethering and fusion in exocytotic and endocytotic pathways.

Exocytosis

Newly synthesized membrane proteins are targeted to their final destination following their packaging into a series of specific vesicles (52-54). Briefly, after synthesis in the endoplasmic reticulum (ER), immature membrane proteins are delivered by vesicular intermediates to the Golgi network, where they undergo post-translational modifications. At the *trans*-Golgi network (TGN), proteins are sorted into vesicles according to their intrinsic sorting motif. In epithelial cells and neurons, the clathrin adaptator protein complex 1 (AP-1) has been clearly shown to recognize the cytoplasmic sorting motif (often Tyr-based or diLeu-based targeting motifs) of a number of proteins whose final destination is the basolateral or the somatodendritic membranes, respectively (55-57). In addition, AP-1 recruits clathrin, a structural protein, which contributes to the formation of vesicles by membrane deformation. Similarly, the sorting motifs that route proteins to the apical and the axonal membranes (such as N- or O-glycosylation or glycosyl phosphoinositol-anchor) are localized in the extracellular or the transmembrane domains of membrane proteins (58-63). However, how these latter sorting motifs are recognized and how transport vesicles are shaped is unclear. After their sorting at the TGN, newly formed vesicles are transported across lengthy distances along microtubules by microtubule plus-end-directed motors of the kinesin family, either directly to the proper plasma membrane domain or indirectly through an endosomal compartment (64-69). Finally, protein delivery requires vesicle tethering and fusion with the specific target membrane. The exocyst complex regulates tethering of vesicles to the basolateral membrane of epithelial cells and to

domains of neurite outgrowth and axonal synapse-assembly in neurons (70, 71). The soluble N-ethyl-maleimide-sensitive fusion protein attachment-protein receptor (SNARE) complexes, which comprise vesicle (v)-SNAREs and target (t)-SNAREs (the syntaxins) control the fusion of the vesicle to the target plasma membrane. Different t-SNAREs, syntaxin-3 and syntaxin-4, are localized at the apical and basolateral plasma membranes in epithelial cells (72-74).

The first evidence for the contribution of Dlg1 to the trafficking of newly synthesized membrane proteins was obtained in neurons. Dlg1 interaction with its PDZ neuronal partners, such as the GluR1 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-type glutamate receptors (AMPARs), the NR2A subunit of the N-methyl-D-aspartate receptor (NMDAR) or the α-subunits of the potassium channels Kv1 and Kv4.2, occurs very early in the partner biosynthetic pathway, while they are in the ER (75-78). These data suggest a role for Dlg1 in the immature partner processing and/or in their trafficking from the ER to the Golgi network. However, detailed data are still lacking concerning the exact functions of Dlg1in this early process.

More convincingly, Dlg1 has been shown to directly or indirectly recruit motor proteins to post-Golgi vesicles which contain newly synthesized neuronal Dlg1 partners. Among these motor proteins are the microtubule-dependent motor proteins of the kinesin family, KIF1Bα and KIF17 (79, 80). Although the functional consequence of Dlg1 and KIF1Bα interaction is unknown, it has been shown that the neuron-specific protein motor, KIF17 and Dlg1 are present, in rat hippocampal neurons, in a complex with the PDZ scaffolding proteins, CASK, velis/MALS and Mint, which contributes to the transport of NMDARs along microtubules (68, 79). Dlg1 interacts with the NMDAR subunits NR2A or NR2B *via* its PDZ1 domain, whereas KIF17 binds directly to a PDZ domain of Mint (68). After their synthesis in the somatic ER, NMDARs follow an unusual secretory route into a specialized dendritic ER subcompartment that bypasses the somatic Golgi network and directly enters into the denditric Golgi outposts (79). Dlg1 is necessary for sorting NMDARs into this alternative secretory pathway. Inward rectifier potassium Kir2 channels are also in complex with SAP97, CASK, Mint and Velis/MALS in neurons suggesting that this alternative pathway may not be specific for NMDARs (81). Dlg1 and NR2 subunits dissociate before the insertion of NMDAR in the postsynaptic membrane (78). This mechanism depends on Dlg1 phosphorylation by Calcium/calmodulin kinase II on Ser232, which is located in the Dlg1 PDZ1 domain. Together, these data suggest that Dlg1 participates in NMDAR-containing vesicle trafficking by bringing together NR2A/B and a multiprotein complex that includes KIF17. The interaction of Dlg1 and this complex may involve a direct association with CASK that was previously shown to bind Dlg1 *via* their respective L27 domains (82).

Dlg1 has also been shown to directly interact with the actin-dependent motor protein, myosin VI. This motor protein binds to the L27 domain of Dlg1 specifically in neurons, since an association between myosin VI and Dlg1 is not detectable in epithelial cells (10). In nerve growth factor-differentiated PC12 cells, Dlg1 and myosin VI partially colocalize with an overlapping punctate pattern, likely to represent vesicles. In addition, they form a tripartite complex with the AMPAR subunit GluR1 (which directly interacts with the PDZ2 domain of Dlg1) (10, 83). Nash and coworkers have recently shown that a dominant negative construct that disrupts Dlg1 and myosin VI interaction specifically blocks AMPAR but not NMDAR delivery at the hippocampal synapse (84). Therefore, Dlg1 participates in AMPAR trafficking to the plasma membrane by bringing together the receptor and myosin VI. It is noteworthy that myosin VI is a unique member of the myosin family that moves away from the plasma membrane towards the pointed-end of actin filaments (85). Nevertheless, this motor protein was shown to contribute to exocytotic pathways in different cell types (86). In MDCK cells, myosin VI was reported to be involved, in concert with the adaptor protein, optineurin, and the small GTPase, Rab8, in the polarized AP-1B-dependent sorting of secretory cargoes from endosomes to the basolateral membrane (87). Myosin VI and optineurin were also shown to be required for the polarized delivery of the epidermal growth factor receptor to the leading edge of motile cells of epithelial origin (88). Recent data have suggested that myosin VI and optineurin may regulate the fusion of transport vesicles with the plasma membrane (89). However, the exact role of myosin VI in Dlg1-dependent AMPAR trafficking remains to be established.

Besides its interactions with motor proteins in neurons, Dlg1 has also been shown to interact directly with the microtubule-dependent motor protein of the kinesin family, KIF13B (also named GAKIN), *via* its GUK domain. This interaction occurs in a variety of cells such as T-cells, epithelial and Schwann cells (13, 90, 91). Interaction with Dlg1 may regulate the microtubule-stimulated ATPase activity of KIF13B. Due to an intramolecular interaction within the full length protein that masks KIF13B motor domain to microtubules, the microtubule-stimulated ATPase activity of KIF13B was shown to be very low *in vitro* (92). The interaction of KIF13B with a recombinant protein corresponding to the SH3-GUK region of Dlg1 inhibits the closed state of KIF13B and activates its microtubule-stimulated ATPase activity. Analysis of the functional consequences of Dlg1 and KIF13B interaction has led to the suggestion that these partners may participate in the remodeling of plasma membranes. In spreading MDCK cells transfected with GFP-KIF13B, Dlg1 was shown to be transported to the tip of cell projections (90). Dlg1 is also transported to the plasma membrane of Schwann cells by KIF13B and, as further discussed below, has been recently shown to participate in myelin biogenesis, which depends on the regulated sorting and recruitment of specific lipids and proteins to specialized subdomains of the plasma membrane (91).

Together, these results suggest that KIF13B and Dlg1 are responsible for the transport of vesicles to the dynamic sites of membrane remodeling. However, the specific cargoes that are transported together with Dlg1 remain to be identified.

Although Dlg1 was first shown to be implicated in vesicle transport in neurons, this protein is also involved in protein sorting at the TGN in other cell types. In endothelial cells, Dlg1 was found to complex with clathrin and γ-adaptin, a subunit of AP-1 (93). Endothelial cells are responsible for the secretion by exocytosis of the hemostatic and proinflammatory adhesive protein, von Willebrand factor (VWF) (94). VWF is stored in specific granules called Weibel-Palade bodies. At the TGN, the biogenesis of these granules requires VWF aggregation and an external scaffolding complex that contains clathrin and AP-1 (95). Dlg1 was recently shown to be part of this complex and to control the formation of Weibel-Palade bodies (93). Whether the role of Dlg1 in clathrin-dependent protein sorting at the TGN is restricted to specific granules, such as Weibel-Palade bodies, or can be generalized to other secreted proteins remains to be established.

Dlg1 not only participates in vesicle transport and sorting, but this scaffold may also contribute to vesicle tethering and fusion with the plasma membrane by direct interaction with proteins of the exocyst complex and t-SNAREs, respectively. The exocyst complex protein, sec8, was first shown to directly interact with Dlg1 by performing a PDZ array using His-tagged sec8 protein as bait (96). In Schwann cells, Dlg1 interacts also with sec8 *via* its PDZ1-2 domains (91). The functional consequence of this interaction, in Schwann cells, was investigated by depletion of Dlg1 or sec8 expression leading to impairment of myelination. Dlg1 was proposed to behave like a scaffold platform that controls the membrane addition necessary for myelination by coordinating vesicle transport and adhesion to plasma membrane subdomains of Schwann cells by direct interaction with KIF13B and sec8, respectively. Similarly, Dlg1 and sec8 interaction was shown in adipocytes (96). In these cells, the glucose transporter type 4 (Glut4) is redistributed from intracellular vesicles into plasma membrane lipid rafts in response to insulin (97). Fractionation experiments on sucrose gradients have shown that Dlg1 knocked down by siRNA inhibits the insulin-induced assembly of the exocyst complex and Glut4 translocation to the lipid rafts (96). Dlg1 depletion also reduces glucose uptake. Data suggest that in response to insulin, Dlg1 interacts with sec8, to promote the anchoring of the exocyst complex to the lipid rafts, allowing tethering of Glut4 vesicles at the adipocyte plasma membrane (98). It is noteworthy that the tethering step which involves Dlg1 differs between Schwann cells and adipocytes. In Schwann cells, Dlg1 is present at the vesicle surface, while in adipocytes Dlg1 is located at the plasma membrane in lipid rafts. Therefore, in the first case Dlg1 recruits sec8 at the vesicle membrane and in the second case at the target compartment.

Dlg interaction with t-SNAREs was first shown in the *Drosophila* neuromuscular junction (99). The fly t-SNARE protein, Gtaxin, which shares sequence similarity with the vertebrate syntaxin-18, was shown to be a Dlg partner using a yeast two-hybrid screen with a late embryonic *Drosophila* cDNA library and the GUK domain of Dlg as bait (99, 100). Genetic analysis has shown that the localization of Gtaxin to the neuromuscular junction depends on Dlg expression and that both proteins participate in the expansion of the subsynaptic reticulum, a highly convoluted and multilayered specialized postsynaptic membrane. It was suggested that Dlg regulates the localization of membrane addition to the synapse *via* its interaction with Gtaxin. In HaCaT human keratinocytes, Dlg1 coimmunoprecipitates and partially colocalizes at the plasma membrane with syntaxin-4 (101). Dlg1 localization was suggested to be dependent on syntaxin-4. However, the functional role of this interaction remains to be established.

Endocytosis and endosomal trafficking pathways

Membrane proteins are targeted to endosomes and lysosomes for desensitization, regulated ligand uptake or degradation. Internalization may be clathrin- or caveolin-dependent (102, 103). The specific cargoes (mostly glycosylphosphatidylinositol-anchored proteins) that are internalized following caveolin-mediated endocytosis and the molecular details implicated in this pathway are not well identified (103). Clathrin-coated vesicle formation occurs in four major steps: 1/initiation or nucleation, 2/cargo selection that requires the clathrin adaptator protein complex 2 (AP-2) and other cargo-specific adaptator proteins, 3/coat assembly and 4/dynamin-dependent scission of the nascent vesicle from the plasma membrane (104-107). Vesicles are then uncoated and cargoes are sorted into early endosomes and are either recycled back to the plasma membrane in recycling endosomes or targeted to late endosomes for later degradation in lysosomes (102). A small number of studies have shown that Dlg1 participates in the vesicular trafficking implicated in endosomal trafficking pathways.

In hippocampal neurons, Dlg1 has been reported to contribute to AMPAR endocytosis. Indeed, Osterweil and coworkers have shown that Dlg1 recruits myosin VI to endocytotic vesicles containing AMPARs (108). Both proteins are likely to be involved in the early stages of clathrin-mediated AMPAR endocytosis, because they form a tripartite complex with AP-2. These data should not be extrapolated to all the receptors which interact with Dlg1. Indeed, the NR2B subunit of NMDARs contains an endocytotic motif that binds directly to the μ2 subunit of AP-2, and is in close proximity to the Dlg1 binding site (109). Both proteins are therefore in competition for NR2B binding (109). In addition, Dlg1, which directly interacts with the β1-adrenergic receptor, was shown to be required for its efficient recycling in a model of exogenous expression. However, Dlg1 function is upstream to vesicular trafficking (110, 111). In fact, Dlg1 recruits protein kinase A anchoring protein-79 to the complex that itself binds protein kinase A,

which phosphorylates the β1-adrenergic receptor. Receptor phosphorylation is the first step in the initiation of its internalization due to persistent activation.

It has also been suggested that Dlg1 may participate in endosomal routes. Using a yeast two-hybrid screen, Chetkovich and coworkers have shown that the L27 domain of Dlg1 binds to hepatocyte growth factor receptor tyrosine kinase substrate (Hrs), a multifunctional endosomal protein that plays a central role in the downregulation of receptors (9, 112). Hrs recruits clathrin to early ensosomes (113). In addition, this protein is a component of the endosomal-sorting complex required for transport-0 (ESCRT-0) that plays a crucial role in sequestering ubiquitinated cargoes in clathrin-coated microdomains of early endosomes and recruits other ESCRT complexes (114, 115). Hrs and ESCRT are thus implicated in the down regulation of membrane receptors by lysosomal sorting and in regulating cell signaling by more obscure pathways. Hrs was also shown to participate in ESCRT-0-independent pathways such as in the transport of low-density lipoprotein-derived cholesterol from endosomes to the ER (116). The function of the Dlg1-Hrs complex remains to be investigated.

Conclusion

Most hypotheses regarding the function of Dlg1 are based on its involvement in the establishment and maintenance of cell polarity by contributing to channel and receptor targeting and clustering at the plasma membrane, to the assembly of functional signaling complexes, or to the regulation of cytoskeletal dynamics. A growing number of recent reports have suggested that Dlg1 also participates in several exocytotic and endocytotic pathways by controlling specific vesicle trafficking steps which are summarized in Figure 2. In this context, Dlg1 behaves as a scaffolding platform that brings together at least one component of the vesicle trafficking machinery with a specific cargo. Dlg1-mediated coupling between vesicle components and cargoes may facilitate their specific delivery to microdomains of the plasma membrane or to endosomes (Figure 1C). Cargoes which depend on Dlg1 for their trafficking are listed in Table1. Data are however still lacking concerning the exact pathways which are regulated by Dlg1 in interaction with syntaxin-4 or Hrs.

Dlg1 function in vesicular trafficking may have some repercussion in human pathologies. A mutation in Dlg1 has been reported to be a risk factor for schizophrenia among male patients in the Japanese population (117, 118). However, the single-nucleotide polymorphism which was implicated was found in the fourth large intron in the Dlg1 sequence, suggesting that an indirect mechanism may be involved. In addition, an alteration of Dlg1 expression levels has been reported in the dorsolateral prefrontal cortex of postmortem brains from patients with schizophrenia (119, 120). In these patients, an increased content of GluR1 in early endosomes isolated from brain combined with an augmented Dlg1 expression in brain homogenates have recently been reported (119). These data are consistent with an increased Dlg1-dependent GluR1 endocytosis in schizophrenia. In addition, in the hippocampus of patients at initial stages of Alzheimer disease, the synaptic expression levels of disintegrin and metalloproteinase 10 (ADAM10) are reduced, whereas GluR1 levels are augmented (121). Dlg1 is not only involved in AMPAR trafficking to the glutamatergic synapse but also contributes to the exocytotic sorting of ADAM10, which has been shown to have an α-secretase activity that precludes amyloid-β formation (12). In the hippocampus of Alzheimer patients, the coimmunoprecipitation of Dlg1 and ADAM10 is reduced, whereas that of Dlg1 and GluR1 is augmented (121). These data suggest that an alteration in the ability of Dlg1 to bind and regulate the trafficking of ADAM10 and GluR1 may participate, as observed during the early stages of Alzheimer disease, in the shift of the amyloid precursor protein metabolism to amyloidogenesis and in impairing gutamatergic function of the synapse, respectively. Because a number of human diseases present associated membrane trafficking defects, our understanding of how scaffolding proteins such as Dlg1 may finely tune vesicular trafficking could be of help to design effective therapeutic strategies (122).

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Table1: Specific cargoes whose trafficking depends on Dlg1. Dlg1 localization depends on its direct or indirect interaction with integral membrane proteins. Cargoes are likely to recruit Dlg1 at nascent or preexisting post-Golgi or endocytotic vesicles. Dlg1 behaves like a platform that assembles specific complexes containing cargoes and proteins from the trafficking machinery. Therefore depending on the cell type, Dlg1 controls various cargo trafficking steps.

Cargo	Cell type	Trafficking steps in which Dlg1 participates	Reference
ADAM10	Neuron	Delivery at the hippocampal synapse	(12)
AMPAR	Neuron	Trafficking from the ER to the Golgi network?	(76)
		Myosin VI-dependent delivery at the hippocampal synapse	(10, 84)
		Myosin VI- and AP-2-dependent endocytosis	(108)
Glut4	Adipocyte	Sec8-dependent delivery to lipid rafts at the plasma membrane in response to insulin	(96)
Kir2 channel	Neuron	KIF17-dependent delivery to the post-synaptic membrane?	(81)
Kv1 and Kv4.2 channels	Neuron	Trafficking from the ER to the Golgi network?	(75, 77)
NMDAR	Neuron	Trafficking from the ER to the Golgi network?	(78)
		KIF17-dependent delivery to the post-synaptic membrane	(68, 79)
Membrane	Schwann cells	KIF13B- and sec8-dependent delivery to subdomains of the plasma membrane	(91)
VWF	Endothelial cells	Clathrin- and AP-1-dependent sorting at the Golgi network	(93)

Figure 1

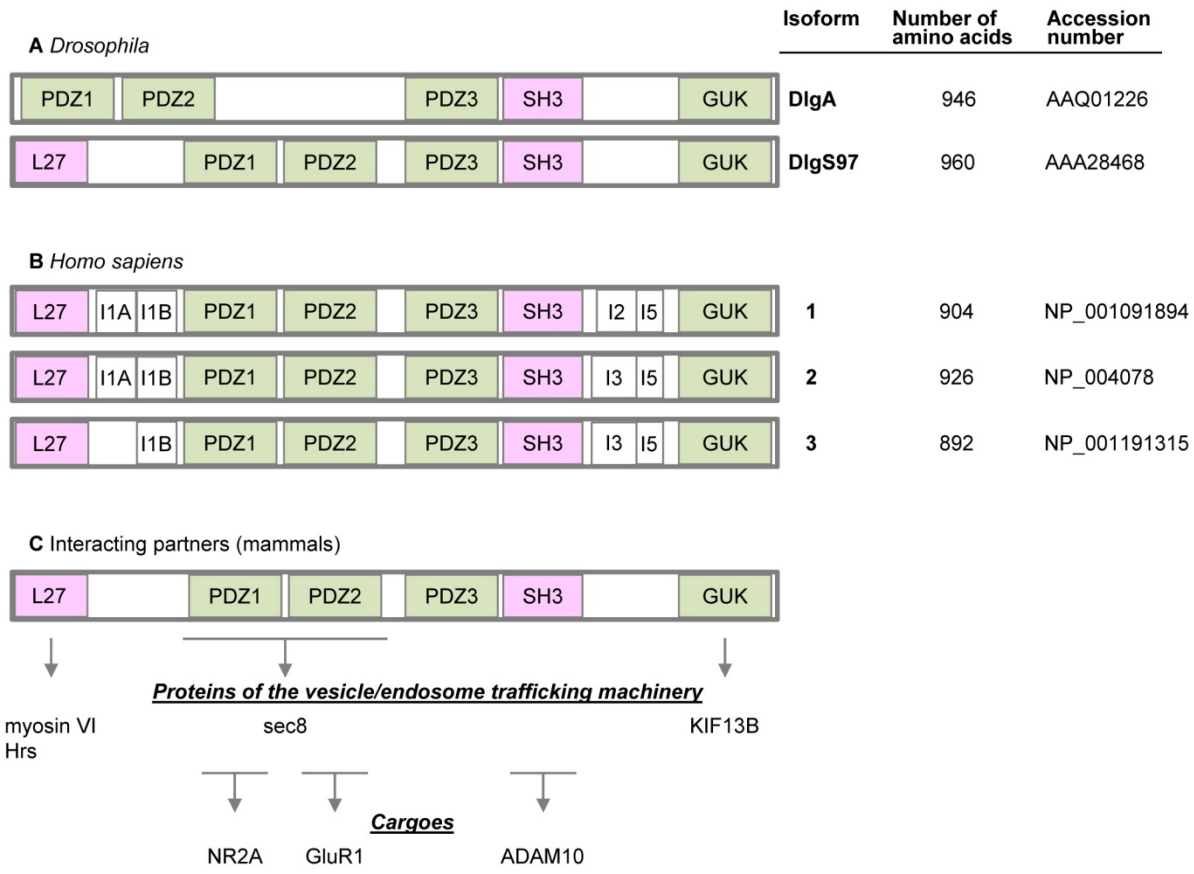
Isoform
Number of amino acids
Accession number
A Drosophila
PDZ1 PDZ2 PDZ3 SH3 GUK
DlgA 946 AAQ01226
L27 PDZ1 PDZ2 PDZ3 SH3 GUK
DlgS97 960 AAA28468
B Homo sapiens
L27 I1A I1B PDZ1 PDZ2 PDZ3 SH3 I2 I5 GUK
1 904 NP_001091894
L27 I1A I1B PDZ1 PDZ2 PDZ3 SH3 I3 I5 GUK
2 926 NP_004078
L27 I1B PDZ1 PDZ2 PDZ3 SH3 I3 I5 GUK
3 892 NP_001191315
C Interacting partners (mammals)
L27 PDZ1 PDZ2 PDZ3 SH3 GUK
Proteins of the vesicle/endosome trafficking machinery
myosin VI
Hrs
sec8
KIF13B
Cargoes
NR2A
GluR1
ADAM10

Figure 2

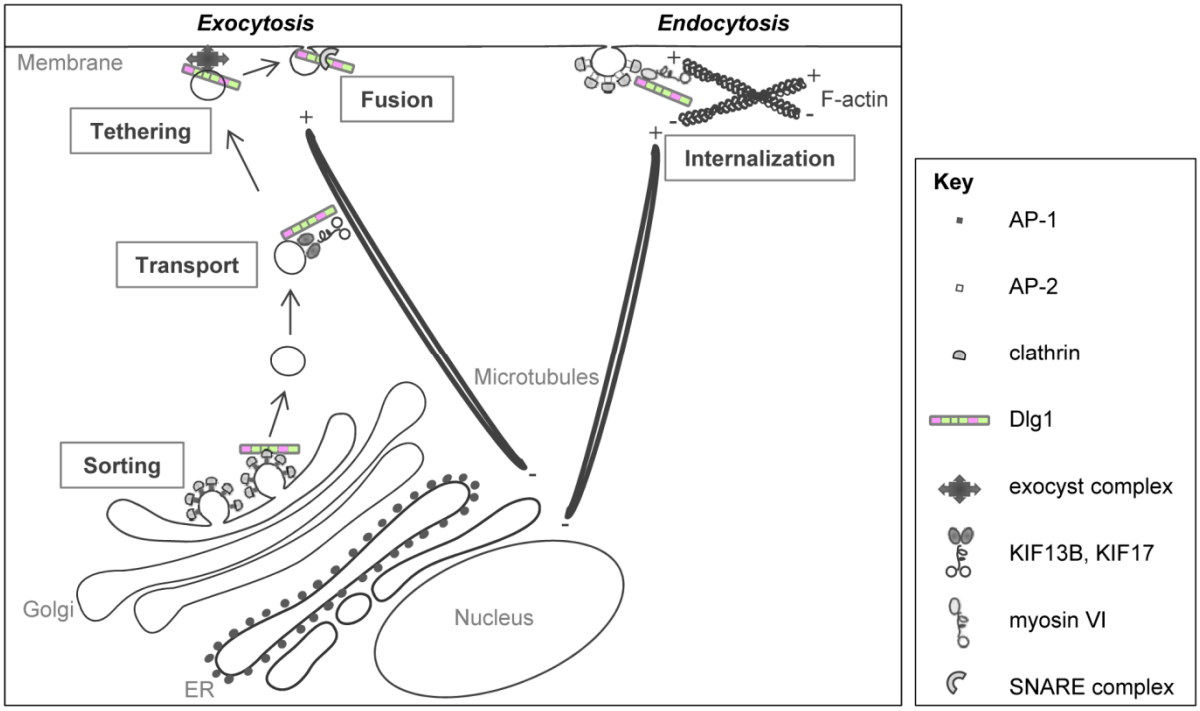
Exocytosis
Endocytosis
Membrane
Tethering
Fusion
F-actin
Internalization
Transport
Microtubules
Sorting
Golgi
Nucleus
ER
Key
AP-1
AP-2
clathrin
Dlg1
exocyst complex
KIF13B, KIF17
myosin VI
SNARE complex

Figure 1: Organization of the protein-protein binding domains of the *Drosophila* Dlg and the human Dlg1 and direct interacting partners of the vesicle trafficking machinery. The *Dlg(1)* gene encodes a multidomain scaffolding protein composed of six main protein-binding domains: L27, PDZ1-3, SH3 and GUK. (A)There are two major Dlg isoforms in *Drosophila* encoded by two alternative transcripts, one which lacks an L27 domain (DlgA) and one which contains an L27 domain (DlgS97). (B) In mammals, Dlg1 mRNAs are known to contain two regions that encompass alternatively spliced exons, leading to several isoforms (123, 124). The N-terminal region is located between the L27 and the PDZ1 domain. Two proline-rich insertions I1A and I1B may be alternatively present in this region with all possible combinations except I1A alone. The I1A+I1B variant binds to the SH3 domains of a number of tyrosine-kinases (123). The other alternatively spliced region is located between the SH3 and the GUK domain. This region generates variants containing a combination of four small insertions: I2, I3, I4 and I5 (123). Only the better described isoforms are represented here although at least seven different transcripts have been reported whose expression is tissue-specific (124). The I3 insertion may bind proteins associated with the actin cytoskeleton (2, 18). In addition, the I3 insertion is necessary for Dlg1-dependent activation of the microtubule-stimulated ATPase activity of KIF13B (92). The functionality of the other insertions is poorly understood. (C) In mammals, Dlg1 domains recruit proteins of the vesicle trafficking machinery and/or cargoes to form functional protein complexes and contribute to exocytosis and endocytosis pathways. Only direct interaction partners are represented.

Figure 2: Schematic representation, in a model cell, of the main vesicle trafficking steps in which Dlg1 was shown to be involved. By bringing together cargoes and proteins implicated in the trafficking machinery, Dlg1 participates in exocytic (left) and endocytic (right) pathways. Depending on cargoes and cell type, Dlg1 has been shown to participate in different trafficking steps. Sorting: in endothelial cells, Dlg1 is implicated in forming with clathrin and AP-1 the specific secretory granules that store VWF. Transport: in neurons and Schwann cells, Dlg1 recruits the motor proteins of the kinesin family, KIF17 and KIF13B, at transport vesicles for delivery of NMDARs or membrane components to specific zones of the plasma membrane. Tethering: in adipocytes and Schwann cells, Dlg1 recruits sec8, a member of the exocyst complex, to the plasma membrane or vesicle surface, respectively. Dlg1 therefore participates in Glut4 delivery to lipid rafts at the plasma membrane of adipocytes and to myelination in Schwann cells. Fusion: Dlg1 has been shown to interact with the t-SNARE, syntaxin-4, in keratinocytes, although the exact function of this interaction remains to be elucidated. Internalization: at the plasma membrane of hippocampal neurons, Dlg1 brings together myosin VI, AP-2 and AMPARs for receptor endocytosis.