

Genetic studies define MAGUK proteins as regulators of epithelial cell polarity

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ABSTRACT Polarized epithelial cells play critical roles during early embryonic development and organogenesis. Multi-domain scaffolding proteins belonging to the membrane associated guanylate kinase (MAGUK) family are commonly found at the plasma membrane of polarized epithelial cells. Genetic studies in *Drosophila melanogaster* and *Caenorhabditis elegans* have revealed that MAGUK proteins regulate various aspects of the polarized epithelial phenotype, including cell junction assembly, targeting of proteins to the plasma membrane and the organisation of polarized signalling complexes. This review will focus on the genetic studies that have contributed to our understanding of the MAGUK family members, Dlg and Lin-2/CASK, in controlling these processes. In addition, our recent genetic analysis of mouse Dlg, in combination with genetic and biochemical studies of Lin-2/CASK by others suggests a model placing Dlg and Lin-2/CASK within the same developmental pathway.

KEY WORDS: *MAGUK, epithelial polarity, Dlg/SAP 97, CASK, PDZ*

Introduction

One of the earliest differentiation events to occur during embryonic development is the formation of a polarized epithelial cell sheet. As morphogenesis proceeds, polarized epithelial cells play a fundamental role in the co-ordinated rearrangement and differentiation of cells to create the structural architecture of the embryo and individual organs. Genetic studies using classical developmental model systems in various organisms have demonstrated the importance of the cell biology of polarized epithelial cells in the development of the embryo. These models include, among others, the development of embryonic ectoderm, the midgut and imaginal disc epithelia in *Drosophila*; the development of the epidermis, vulva, pharynx and intestine in *C. elegans*; and the development of the pre-implantation embryo, gut and kidney in mammals (Bryant, 1997; Müller, 2000; 2001; Michaux *et al.*, 2001; Horster *et al.*, 1999).

The establishment and function of polarized epithelial cells requires extracellular cues, changes in cell shape and the orientation of the cytoskeleton, and the asymmetric distribution of cytoplasmic organelles and plasma membrane proteins, to create two functionally distinct apical and basolateral membrane domains. These two domains are separated by apico-lateral tight junctions in vertebrates or septate junctions in invertebrates. These junctions form a transepithelial barrier and restrict membrane proteins to either the apical or basolateral membranes (Fig. 1). The apical

domain is often covered with microvilli and faces the exterior or the lumen, whereas the basolateral membrane communicates with neighbouring cells and the extracellular matrix of the basal lamina through cell adhesion molecules, growth factors and receptors (Yeaman *et al.*, 1999).

Along the basolateral membrane protein complexes are targeted to spatially and functionally distinct sites (cell junctions). Many of these complexes are assembled upon scaffolding proteins which, by means of multiple protein binding domains, serve to bring together and organise the various components of a protein complex. Multi-domain scaffolding proteins can assemble a combination of cell adhesion molecules, cytoskeletal proteins, receptors, ion channels and their associate signalling components at specific membrane sites. Genetic and biochemical studies have recently demonstrated that a family of scaffolding proteins known as the membrane associated guanylate kinase (MAGUK) family are important regulators of epithelial polarity

Abbreviations used in this paper: CAMK, calcium/calmodulin-dependent protein kinase; *C. elegans*, *Caenorhabditis elegans*; Dlg, Discs Large; EGFR, Epidermal growth factor receptor; GKAP, guanylate kinase associated protein PDZ-PSD-95, Dlg and ZO-1; GUK, guanylate kinase; L27, Lin-2-Lin-7 binding domain; Lgl, Lethal giant larvae; MAGUK, Membrane associated guanylate kinase; MEE, medial edge epithelial; Pals, proteins that associate with Lin-7; PSD-95, postsynaptic protein-95; SAP, synapse associated protein; Scrib, Scribble; SH3, src homology 3; ZO-1, zonula occludens.

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during development. This review will focus on genetic studies performed in *Drosophila*, *C. elegans* and mouse developmental model systems which have contributed to our current understanding of the role of two MAGUK scaffolding proteins, Dlg and Lin-2/CASK, in targeting and organising protein complexes to the plasma membranes of epithelial cells. The zonula occludens (ZO) group of MAGUKs which also play an important role in polarized epithelial cells at the tight junction will not be addressed in this review (for review see Gonzalez-Mariscal *et al.*, 2000). Our recent mouse genetic studies in combination with biochemical and cell biology studies allows a model to be presented in which Dlg and Lin-2/CASK lie within a common developmental pathway controlling craniofacial morphogenesis.

The MAGUK Family Members

All members of the MAGUK family have a basic protein domain structure in common that includes: one to three PDZ domains (named after the MAGUK family members PSD-95, Dlg and ZO-1), a src homology 3 (SH3) domain, and a guanylate kinase (GUK) domain (Anderson, 1996; Dimitratos *et al.*, 1999). PDZ domains are found in a variety of proteins in single or multiple copies. They can dimerise or bind the C-termini of integral membrane and intracellular proteins recognising either a S/TXV/I/L motif (Class I PDZ), or a $\psi\chi\psi$ motif (Class II PDZ), in which ψ represents a hydrophobic residue (Sonyang *et al.*, 1997). PDZ containing proteins are generally restricted to specific subcellular domains, such as regions of cell-cell contact in epithelial cells, the plasma membrane of red blood cells and lymphocytes, and synaptic and neuromuscular junctions (Woods and Bryant, 1991; Marfatia *et al.*, 1997; Lue *et al.*, 1994; Woods and Bryant 1993; Rafael *et al.*, 1998). MAGUK PDZ domains bind and mediate the clustering of membrane receptors and channel proteins (Kim *et al.*, 1995). The SH3 domain is a well characterised protein-binding domain also found in a variety of proteins and binds to proline-rich motifs (Pawson, 1994). Due to sequence divergence the GUK domain of MAGUKs shows low or no kinase activity and appears to also act as a protein binding domain (Kim *et al.*, 1997; Satoh *et al.*, 1997; Takeuchi *et al.*, 1997; Hanada *et al.*, 2000). MAGUK proteins containing the basic core PDZ, SH3, GUK domain structure can be further subdivided based on the number of PDZ domains they contain, the presence of additional domains and sequence similarity. The subfamilies include those resembling Dlg, Lin-2/CASK, ZO-1 and p55 (Dimitratos *et al.*, 1999) (Fig. 2).

The Dlg subfamily of proteins have a domain structure in common with the founding member of the MAGUK family, the product of the *Drosophila* tumor suppressor gene *dlg* (*lethal (1) discs-large*). Members include mammalian Dlg (also known as synapse associated protein 97 (SAP 97)), postsynaptic density protein 95 (PSD-95)/ SAP 90, PSD-93/Chapsyn-110, neuroendocrine Dlg/ SAP 102 and P-Dlg. Each of these subfamily members contain three PDZ domains at their NH₂-termini belonging to the Class I type.

The Lin-2/CASK subfamily are homologues of the proteins encoded by *C. elegans lin-2* and *Drosophila Camguk*. They contain a NH₂-terminal region which has homology to the calcium/

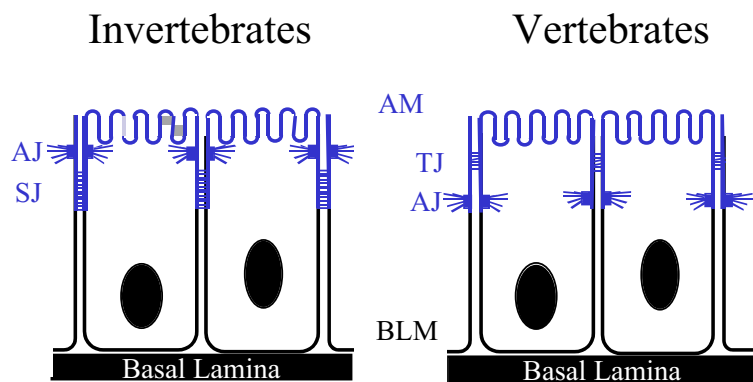


Fig. 1. Schematic diagram of the membrane domains and junctions of polarized epithelial cells in invertebrates and vertebrates. The apical membrane (AM) domain of the cell is depicted in blue and contains microvilli facing the lumen or exterior of the cell. The apico-lateral membrane contains specialized cell junctions, including the adherens junction (AJ) and septate junction (SJ) in invertebrates and the tight junction (TJ) and adherens junction in vertebrates. MAGUK proteins are found at various membrane domains of polarized epithelial cells. In invertebrates Dlg localizes to the SJ in *Drosophila* and at the adherens junction in *C. elegans*. ZO-1 localizes to the TJ and Dlg and CASK localize to the basolateral membrane (BLM; depicted in black) in vertebrates. Diagram adapted from Bryant and Huwe, 2000.

calmodulin-dependent protein kinase (CAMK), a repeated L27 (lin2-lin7 binding region) domain (Kamberov *et al.*, 2000; Doerks *et al.*, 2000) followed by the basic core domain structure (PDZ, SH3, GUK). The PDZ domain is of the Class II type. The CAMK domain as with the GUK domain also contains mutations rendering it kinase inactive.

The ZO-1 subfamily members (ZO-1, 2, 3) are homologous to *Drosophila Tamou*. The ZO-1 subfamily derives its name from the cell junction at which these members are localized, the zonula occludens or tight junctions of epithelial cells. These members contain three PDZ domains of which the first domain binds the C-terminal YV sequence of claudins and ZO-1 can interact with ZO-2 and-3 via their second PDZ domain. The ZO members also contain C-terminal proline-rich regions and a divergent C-terminus that can be alternatively spliced (Gonzalez-Mariscal *et al.*, 2000).

The p55 subfamily consists of p55, Dlg2, Dlg3, Pals1 (proteins that associate with Lin-7), and Pals2/p55T/VAM-1 and contain a single PDZ domain. The PDZ domain of p55 binds the C-terminal EYYV motif of glycophorin C (Marfatia *et al.*, 1997). As with the Lin-2/CASK subfamily this family, except for p55, also contains L27 repeat domains (Doerks *et al.*, 2000; Kamberov *et al.*, 2000; Tseng *et al.*, 2001).

Several MAGUK members also contain a region (HOOK or protein 4.1 binding domain) that binds certain actin-associated proteins of the Band 4.1 protein/ERM (Ezrin, Radixin, Moesin) superfamily and hence links MAGUKs to the cortical cytoskeleton (Lue *et al.*, 1994; Marfatia *et al.*, 1995; Cohen *et al.*, 1998; Kamberov *et al.*, 2000).

Drosophila Genetics: Function of Dlg in Imaginal Disc Epithelial Cells

The *dlg* gene was originally identified as a tumor suppressor in *Drosophila* as recessive lethal mutations resulted in neoplastic overgrowth of imaginal disc epithelium. The epithelial cells lost

their columnar structure becoming more cuboidal in shape, demonstrated irregular apico-basal polarity, a lack of differentiation, disruption to septate junction structure and developed wide intercellular spaces, suggesting a failure of cell adhesion (Stewart *et al.*, 1972; Woods and Bryant, 1989). The *dlg* protein product is normally located at the cytoplasmic face of septate junctions and was one of the earliest molecular markers of this structure (Woods and Bryant, 1991).

Identification of the mutations underlying the severity of the imaginal disc phenotypes in *Drosophila dlg* loss-of-function mutants, in conjunction with transgenic analysis have defined the roles of the various domains of Dlg (Woods *et al.*, 1996; Hough *et al.*, 1997). Three mutants in particular *dlg^{m52}*, *dlg^{m30}* and *dlg^{X1-2}* shed light on the role of the various domains of Dlg. Based on genetic complementation studies *dlg^{m52}* has been classified as a functional null allele although a truncated protein product retaining the PDZ1 and PDZ2 domains is still produced. This mutant demonstrated that one or all of the deleted domains (PDZ3, SH3, protein 4.1 and GUK) are important for *dlg* function within the imaginal disc epithelium. *Dlg^{m30}* contains a single point mutation in the SH3 domain resulting in the change of a conserved leucine to a proline. This causes the loss of septate junctions and overproliferation of imaginal disc epithelial cells. *Dlg^{X1-2}* results in the deletion of the GUK domains and C-terminus and causes epithelial overgrowth without affecting septate junction structure or cell polarity. Dlg was also shown to play a role in the organization of the cytoskeleton and localization of transmembrane proteins, including fasciclin III and neuroglian, which are involved in cell adhesion. Interestingly, even though septate junction structure was severely disrupted in *dlg^{m52}* mutants, the adherens junction which lies apical to the septate junction was still intact (Woods *et al.*, 1996) (Fig. 1).

The functions of each of the Dlg domains were further characterised *in vivo* by first constructing transgenic lines expressing altered Dlg proteins and then analysing their localization to the septate junction. These studies revealed that the HOOK domain was essential for targeting Dlg to the plasma membrane, possibly due to its association with the cytoskeleton. The PDZ2 domain specified the location of Dlg to the septate junction, possibly through the interaction with membrane binding proteins. The PDZ2 and PDZ3 domains were shown to be required for growth regulation but not epithelial structure. The SH3 and HOOK domains were required for septate junction formation and the control of cell

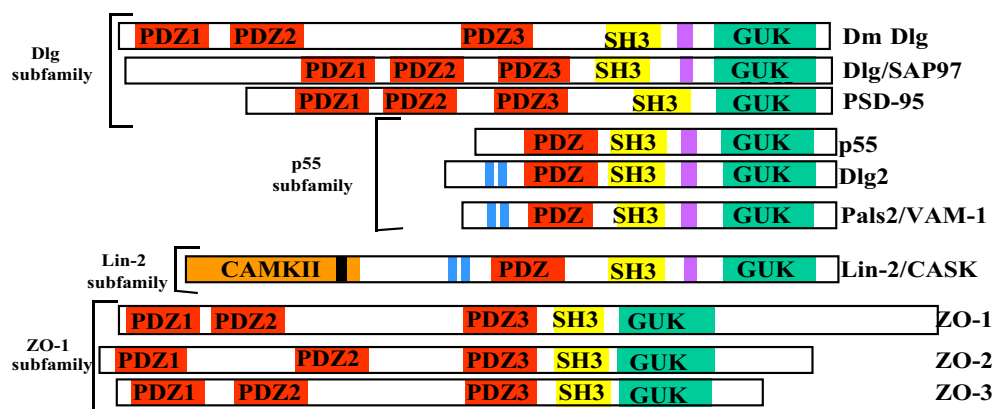
proliferation. The GUK domain acted as a negative regulator, inhibiting the function of Dlg in controlling proliferation (Hough *et al.*, 1997). These results demonstrated that the functions of Dlg in regulating cell proliferation and maintaining septate junction structure and apico-basal polarity could be assigned to an individual or a combination of domains.

Similar studies have been performed in mammalian systems to determine the domain(s) required for the lateral membrane targeting of mammalian Dlg. In contrast to *Drosophila* Dlg, the targeting function of mammalian Dlg lies within the first 65 amino acids, which are absent in the *Drosophila* protein. In mammalian epithelia, the PDZ1-2 and protein 4.1/HOOK domains provide stabilization of the protein at the membrane (Wu *et al.*, 1998). It has recently been shown that the same amino-terminal domain found in mammalian Dlg is used by *C. elegans* Dlg-1 for protein targeting to adherens junctions (Firestein and Rongo, 2001). In contrast to *Drosophila* Dlg and *C. elegans* Dlg-1, which are located to the apico-lateral membrane, mammalian Dlg is more broadly expressed along the basolateral membrane (Fig. 1).

Genetic Linkage of *dlg* to Epithelial Polarity Genes

Recently, a number of *Drosophila* tumor suppressor genes in which mutations also cause defects in epithelial polarity, proliferation and differentiation have been genetically linked to *dlg*. These include *Scribble* (*Scrib*) and the *lethal giant larvae* (*lgl*) genes (Bilder *et al.*, 2000; Mechler *et al.*, 1985). *Scrib* encodes a LAP protein containing four PDZ domains and sixteen leucine-rich repeats localised to the septate junctions of imaginal disc epithelial cells. *Lgl* encodes a WD-40 repeat containing protein, which is distributed along the membrane but is not exclusively membrane associated. Co-localization studies suggest that Dlg and *Scrib* ensure that *Lgl* is correctly localized and, that *Lgl* is essential for the localization of *Scrib* and Dlg to the septate junction (Bilder *et al.*, 2000). How these three proteins are associated has not been determined. Given that *Scrib* and Dlg possess PDZ domains it is likely that they interact with transmembrane and cytoskeletal proteins forming a protein complex that localizes to the plasma membrane. *Lgl* may have a role in protein trafficking in epithelia through its binding with tSNARE proteins which promote the fusion of cargo-carrying vesicles with target membranes in a Dlg- and *Scrib*-dependent fashion (Bilder *et al.*, 2000). Disruption in the normal localization of these proteins can lead to defects in epithelial

Fig. 2. Domain structure of some of the members of the MAGUK family. MAGUK proteins contain a basic domain structure comprised of a PDZ, SH3 and GUK domain. Subfamilies of MAGUKs (*Dlg*, *p55*, *Lin-2* and *ZO-1*) contain combinations of additional domains, including a HOOK or protein 4.1 binding domain (purple); L27 (*lin-2* binding domain (blue); and a calcium/calmodulin dependent kinase (CAMKII) domain (orange), which contains a calmodulin-binding region (black box). *Dm* = *Drosophila*.



lial structure and polarized signalling pathways, resulting in loss of proliferative control.

In addition, Lgl and Dlg are required for the localization of apical proteins, such as Bazooka, which is homologous to the *C.elegans* Par3, and is found in a complex with Par 6 and PKC in epithelial cells (Wodarz *et al.*, 2000; Kim, 2000; Lin *et al.*, 2000; Bilder, 2001). The above mentioned proteins also play a role in the polarization of neuroblasts (Wodarz *et al.*, 2000; Peng *et al.*, 2000; Bilder 2001). Understanding the mechanisms that interconnect these proteins to maintain a polarized epithelial structure and to control proliferation awaits the identification of additional interacting binding partners for these multi-domain proteins.

C.elegans Genetics: Function of *lin-2* in Vulval Epithelial Cells

The *lin-2* gene was originally identified in a genetic screen for vulvaless *C.elegans* mutants (Horvitz and Sulston, 1980; Ferguson and Horvitz, 1985). Vulval development requires the activation of LET-23, the homologue of the epidermal growth factor receptor (EGFR) in *C.elegans*. LET-23 is localized to the basolateral surface of polarized vulval epithelial precursor cells. The six vulval precursor cells, P3.p to P8.p, which are derived from the ectoderm, are aligned in an anterior-posterior fashion along the ventral midline of the hermaphrodite worm. Lin-3, an EGF-like growth factor, is secreted from the gonadal anchor cell in the mesoderm positioned adjacent and dorsal (basal) to P6.p. Thus, Lin-3 is secreted into the basal extracellular space surrounding P6.p where it activates the basolaterally positioned LET-23 on this precursor cell and induces vulva formation (Kornfeld, 1997) (Fig. 3).

Mutations in *lin-2*, and the genetically linked genes *lin-7* and *lin-10*, decrease Lin-3 mediated vulval signalling. This is due to the mislocalization of LET-23 to the apical membrane of the vulval epithelial cells resulting in a vulvaless phenotype. Similarly, a *let-23* mutant (*sy1*) which encodes a C-terminally truncated LET-23 also results in the localization of the receptor to the apical membrane. Interestingly, the truncated C-terminal region of LET-23 in the *sy1* mutant contains a PDZ type 1 binding motif (T-C-L). A combination of elegant genetic and biochemical studies led to the discovery that Lin-7/Lin-2 and Lin-10 form a ternary protein complex. These studies demonstrated that the PDZ domain of Lin-7 binds the C-terminus of Let-23 and restricts the receptor to the basolateral membrane (Hoskins *et al.*, 1996; Simske *et al.*, 1996; Kaech *et al.*, 1998; Whitfield *et al.*, 1999). In turn, a region N-terminal to the PDZ domain in Lin-7, interacts with a region between the CAMK and PDZ domains of Lin-2. The CAMK domain of Lin-2 interacts with a region N-terminal to the PTB/PDZ/PDZ motifs of Lin-10 (Fig. 3).

In contrast to *dlg* loss-of-function mutants in which the integrity of the septate junctions and epithelial polarity are destroyed, *lin-2*, *lin-7* and *lin-10* loss of function mutants retain distinct functional apical and basal domains. The mutations appear specifically targeted to the mislocalization of LET-23, thus disrupting polarized signalling and the trigger for vulval differentiation. It is interesting to note that LET-23 was mislocalized to the apical membrane rather than being diffusely distributed at all membranes. It has been

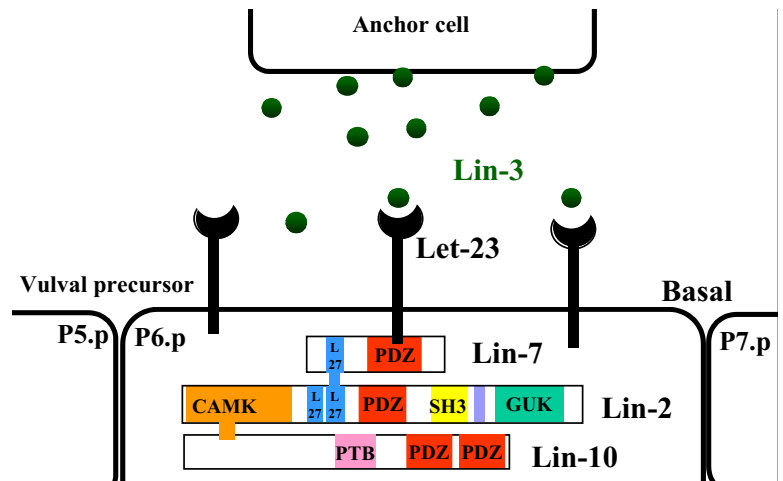


Fig. 3. Localization of the LET-23 receptor to the basolateral membrane of the epithelial vulval precursor cell (P6.p) in *C.elegans* via the *lin-7/lin-2/lin-10* protein complex. The C-terminus of LET-23 binds the PDZ domain of Lin-7. Lin-7 heterodimerizes with the L27 domain of Lin-2 and the CAMK domain of Lin-2 interacts with Lin-10. Basolateral localization of LET-23 allows it to interact with the Lin-3 growth factor produced by the gonadal anchor cell positioned on the basal side the P6.p vulval cell. The protein 4.1 binding domain is depicted in purple.

suggested that LET-23 may have apical as well as basal sorting signals and in the absence of functional Lin-2/Lin-7/Lin-10 basal mediated sorting, apical sorting occurs instead (Kaech *et al.*, 1998). The direct roles of these proteins in basolateral membrane targeting have been further investigated in mammalian systems.

Lin-2/CASK Protein Complexes at the Basolateral Membranes of Mammalian Epithelial Cells

The mammalian homologues of Lin-7, Lin-2 and Lin-10 have been identified and are known as mLin-7 or Veli, mLin-2 or CASK, and mLin10/ Mint or X11, respectively. Although the interaction of mammalian CASK, mLin-7 and X11 has been demonstrated in the brain (Borg *et al.*, 1998; Butz *et al.*, 1998), interactions between CASK and X11 do not occur in epithelial cells. Several isoforms of X11 exist and it may be that the CASK interacting isoform of X11 has not been identified as yet (Straight *et al.*, 2000). In contrast to the interaction of Lin-7 and LET-23 in *C.elegans*, an equivalent interaction between mLin-7 and any of the mammalian EGFRs does not occur. This is due to divergence from the PDZ Class 1 binding consensus sequence at their C-termini (Straight *et al.*, 2000). However, the PDZ domain of mLin-7 has been shown to interact with BGT, the γ -aminobutyric (GABA) transporter, in a kidney epithelial cell line (MDCK; Perego *et al.*, 1999; Straight *et al.*, 2000). The role of mLin-7 in this interaction is not to target BGT to the membrane but rather to retain BGT at the basolateral surface, preventing it from internalization (Perego *et al.*, 1999). Retention of BGT is dependent on the association of mLin-7 with the E-cadherin/ β -catenin-cell adhesion complex. The association of mLin-7 with this complex occurs via its PDZ domain binding to the C-terminal PDZ target motif of β -catenin and is Ca^{2+} - dependent (Perego *et al.*, 2000). Dlg/SAP97 has also been shown to associate with an E-cadherin/ β -catenin complex. However, direct binding to the components of this complex were not detected. Association

with this complex is believed to occur through the attachment of Dlg/SAP97 to the cortical cytoskeleton which is assembled by E-cadherin mediated cell-cell adhesion (Reuver and Garner, 1998).

The interaction between CASK and mLin-7 has been further characterised in mammalian systems and involves heterodimerization via the recently identified L27 domains (Fig. 3) (Doerks *et al.*, 2000). The L27 region in mLin-7 is also required for its proper targeting to the basolateral membrane and also involves CASK (Straight *et al.*, 2000). However, what targets CASK to the basolateral membranes of mammalian epithelial cells has not been identified. Neither a NH₂-terminal CASK fragment consisting of the CAMK, L27 and PDZ domains nor a C-terminal fragment consisting of the SH3, protein 4.1 binding and GUK domains could properly target to the membrane (Straight *et al.*, 2000; Kamberov *et al.*, 2000). This suggests that a combination of C-terminal and NH₂-terminal domains and their interacting binding proteins are required for CASK targeting.

One possible scenario that fits this model is that CASK is able to bind the C-terminal tail of syndecan (EFYA) in epithelial cells via its PDZ domain (Cohen *et al.*, 1998). Syndecans are heparan sulphate proteoglycans which are able to bind to the extracellular matrix and growth factors such as FGF, forming co-receptor complexes. CASK and syndecan-1/-2 are co-expressed at the basolateral membranes of epithelial cells. CASK can also interact with the actin-binding protein 4.1 which in turn attaches CASK to the cortical cytoskeleton (Cohen *et al.*, 1998). Thus, this protein complex involves an NH₂-terminal domain (PDZ) and a C-terminal domain (protein 4.1) of CASK. The interaction with syndecan may target CASK to the basolateral membrane enabling the other domains of CASK to interact with their binding partners at this membrane site, such as Lin 7 (Fig. 4).

Another important CASK interaction which may be involved in its targeting involves the C-terminal GUK domain. It has been shown that several of the MAGUKs can undergo intramolecular and intermolecular interactions involving the SH3 and GUK domains (McGee & Bredt, 1999; Nix *et al.*, 2000; Shin *et al.*, 2000; Wu *et al.*, 2000).

SH3 and GUK Intramolecular and Intermolecular Interactions

The SH3 domain can engage in MAGUK intermolecular and intramolecular interactions with the GUK domain via a mechanism that does not involve the usual proline rich recognition site of SH3 domains. The SH3-GUK intramolecular association, which predominates over the intermolecular association, has been shown to regulate intermolecular binding of MAGUKs, the binding of the guanylate kinase associating protein (GKAP) to the GUK domain, and the clustering of PDZ binding proteins (Nix *et al.*, 2000; Wu *et al.*, 2000; Shin *et al.*, 2000). This mechanism may explain the phenotypes seen in the *Drosophila* mutants where the GUK or SH3 domains are altered. Indeed, the equivalent point mutation in the SH3 domain of *dlg^{m30}* prevents the interaction with the GUK domain (McGee & Bredt, 1999).

One such intermolecular MAGUK interaction involves the GUK domain of CASK with the SH3 domain of Dlg. This interaction was identified via a yeast 2-hybrid screen using the C-terminal domain of CASK. The interaction was subsequently demonstrated *in vivo* in the gut epithelial cell line, CACO, by co-immunoprecipitation (Nix *et al.*, 2000). Both proteins are also co-expressed at basal and lateral membranes of gut epithelial cells (Nix *et al.*, 2000) and have been independently shown to be expressed at the basal and lateral membranes of ureteric and metanephric mesenchyme derived kidney epithelial cells as well as a kidney cell line (Straight *et al.*, 2000; Ide *et al.*, 1999; Caruana & Bernstein, 2001; Caruana & Bertram, unpublished).

Given the various functions already discussed in this review for CASK and Dlg, the interaction of these two MAGUK proteins has important implications in regulating protein targeting, signalling, cell proliferation and the structure and maintenance of polarized epithelial cells. The interaction between Dlg and CASK may result in the formation of large multi-scaffolding protein complexes involving the various binding partners of these two MAGUKs. Since Dlg and CASK interact with PDZ-binding proteins of the Class I and Class II type

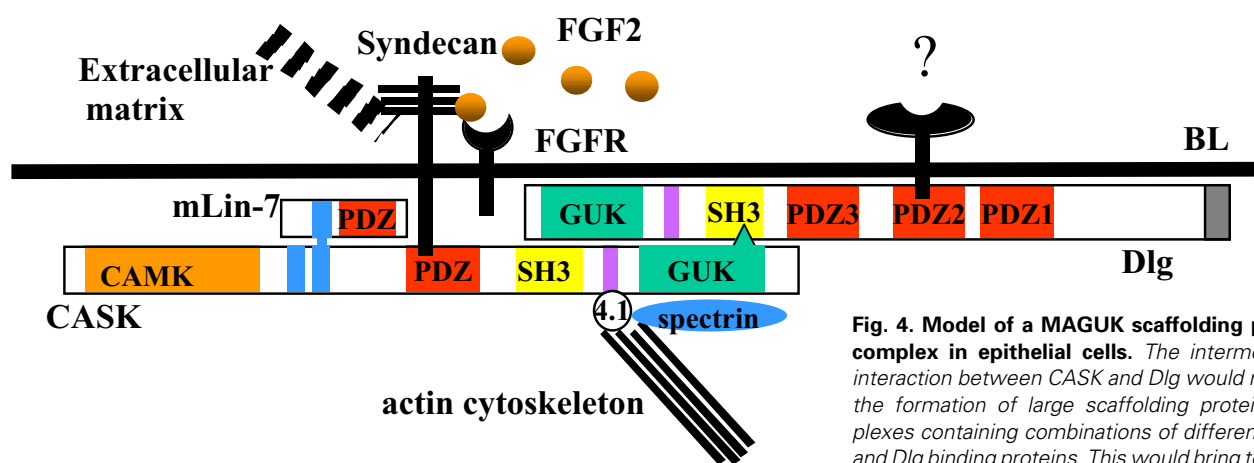


Fig. 4. Model of a MAGUK scaffolding protein complex in epithelial cells. The intermolecular interaction between CASK and Dlg would result in the formation of large scaffolding protein complexes containing combinations of different CASK and Dlg binding proteins. This would bring together a variety of transmembrane receptors at the

basolateral (BL) surface and their associated cytoplasmic proteins. Epithelial expressed binding partners known thus far for CASK and Dlg have been depicted. The PDZ domain of CASK can bind the C-terminus of syndecan. Syndecan acts as a co-receptor for growth factors within the extracellular matrix (eg. FGF2). Syndecans can also attach to extracellular matrix components. The interaction of CASK and Dlg to protein 4.1 provides a link for this scaffolding complex to the actin cytoskeleton and may be involved in maintaining these proteins at the basolateral membrane. Similarly, Dlg could also attach to protein 4.1 (not depicted). CASK could also heterodimerize with the L27 domain (blue) of Lin-7. The membrane targeting domain of Dlg is represented in grey and the protein 4.1 domain in purple. (Diagram adapted from Cohen *et al.*, 1998).

respectively, this also increases the repertoire of proteins within the envisaged scaffolding protein complex. Dlg may play a role in the mLin-7/CASK and/or CASK/syndecan protein complexes (Fig. 4). An intriguing question that remains to be answered is that if both Dlg and CASK have domains that are necessary for targeting, do they independently target to the basolateral plasma membrane and then interact with one another? Or does one MAGUK require the other for targeting to the membrane? The localisation of CASK and Dlg proteins needs to be analysed in systems where altered Dlg and CASK proteins have been expressed, respectively. In addition new binding partners for CASK and Dlg need to be identified in epithelial cells. In particular, transmembrane PDZ-binding proteins (receptors) need to be identified, which will provide insight into the signalling pathways these MAGUKs are involved in organising. To date, no epithelial expressed receptors have been shown to bind directly to mammalian Dlg (Fig. 4).

Mouse Genetics: Dlg and CASK function in the Same Developmental Pathway

Our recent analysis of mice containing a gene trap insertion in *dlg* (*dlg^{gt}*) has demonstrated that *dlg* plays an important role in normal mammalian development, in particular craniofacial morphogenesis (Caruana & Bernstein, 2001). The gene trap insertion results in a truncated protein product that contains the NH₂-terminal three PDZ domains of Dlg fused to the LacZ reporter and subsequently lacks the SH3, protein 4.1 and GUK domains. Mice homozygous for the *dlg* mutation (*dlg^{gt}/dlg^{gt}*) exhibit growth retardation *in utero*, have hypoplasia of the premaxilla and mandible, have a cleft secondary palate, and die perinatally. Consistent with this phenotype, the Dlg-LacZ fusion protein was expressed in mesenchymal and epithelial cells throughout craniofacial and palatal development. These results suggested that protein-protein interactions involving the SH3, protein 4.1 and/or the GUK domain of Dlg were necessary for normal craniofacial development.

Interestingly, the phenotype exhibited by the *dlg^{gt}/dlg^{gt}* pups is similar to that resulting from a transgene insertion in *CASK*. Insertion of the transgene occurs within the CAMK domain but the resulting protein product is not well characterised. The gene encoding *CASK* is X-linked, and insertional mutagenesis results in hypoplasia of the mandible and a cleft secondary palate in male mice (Wilson *et al.*, 1993; Laverty *et al.*, 1998).

Given the similarity in the phenotypes of the Dlg and CASK mutant mice and the ability of these two proteins to form intermolecular interactions, the following model can be proposed. In *dlg^{gt}/dlg^{gt}* mutants, the truncation of Dlg results in the loss of the SH3 domain and therefore the interaction with CASK. This could result in the disruption of a potential Dlg-CASK scaffolding protein complex (similar to that depicted in Fig. 4). In addition, the truncation of Dlg may disrupt targeting of Dlg and/or CASK as has been discussed above and may prevent the interaction with, as yet to be identified, transmembrane proteins (receptors) involved in craniofacial development.

Development of the palate is initiated by the bilateral outgrowths of the palatal shelves from the maxillary process of the first branchial arch. The shelves grow vertically down the sides of the tongue, elevate into a horizontal position above the tongue, adhere at the midline and then fuse (Ferguson, 1988). Analysis of mouse mutants has demonstrated that disruption to any one of these

processes or the development of other craniofacial structures can lead to a cleft palate. Which of these events contributes to the cleft palate phenotype in *dlg^{gt}/dlg^{gt}* and CASK mutant mice has yet to be determined. It is possible that hypoplasia of the mandible in both *dlg^{gt}/dlg^{gt}* and CASK mutants results in mis-positioning of the tongue leading to either a delay or complete obstruction in palatal shelf growth and elevation. However, in both CASK and *dlg^{gt}/dlg^{gt}* mutants elevated palatal shelves were never found to be fused (Caruana & Bernstein, 2001; Wilson *et al.*, 1992).

During palatal shelf fusion, adhesion occurs between the polarized medial edge epithelial (MEE) cells on opposing palatal shelves. Transformation of the MEE cells into mesenchymal cells then occurs to produce a continuous palate. Lack of adhesion and/or epithelial-to-mesenchyme transformation can cause a cleft palate as has been demonstrated in the TGF- β 3 and EGFR knockout mice (Proetzel *et al.*, 1995; Miettinen *et al.*, 1999). Dlg shows a temporal and spatial expression pattern in mesenchymal and epithelial cells during palatal development and is expressed in the MEE cells (Caruana & Bernstein, 2001). Analysis of the expression pattern of CASK in craniofacial tissue has not been reported. However, it is interesting to note that one of the binding partners of CASK, syndecan-1, which acts as a co-receptor for growth factors, is expressed at the basolateral surfaces of MEE cells and is lost as cells transform into mesenchymal cells (Sun *et al.*, 1998). It is possible that Dlg and CASK together play a role in the localization of receptors, growth factors, adhesion molecules and cytoskeletal components in the polarized MEE cells at the tips of the palatal shelves. Disruption in the localization or association of these components, due to mutations in CASK or Dlg, may result in altered adhesion and/or signalling events. This would inhibit epithelial-to-mesenchyme transformation, thus preventing palatal fusion.

This review has demonstrated how *Drosophila* geneticists have used imaginal discs and *C.elegans* geneticists have used vulval precursor cells as developmental systems to study the roles of Dlg and Lin-2/CASK in regulating epithelial polarity. We are currently using several mammalian systems in addition to the palatal model described above to further explore the roles of Dlg and CASK in epithelial cells. We have demonstrated that truncation of Dlg results in its mislocalization in the gut epithelial cells of *dlg^{gt}/dlg^{gt}* embryos (Caruana and Bernstein, 2001) even though it retains its N-terminal lateral membrane targeting sequence (Wu *et al.*, 1998). It will be interesting to determine whether the mislocalization of Dlg affects the localization of the CASK protein complexes. Similar studies are underway using the developing kidney (metanephros) as a model system (Caruana & Bertram, unpublished). During the development of the kidney, branching of the ureteric epithelial bud occurs within the surrounding mesenchyme. The tips of the branches induce the surrounding mesenchyme to condense and undergo a mesenchyme-to-epithelial transformation. These epithelial cells take on a polarized phenotype and continue to differentiate and specialize to produce the excretory unit of the kidney, known as the nephron (Clark & Bertram, 1999). One advantage of the kidney as a model organ is that these epithelial polarization events can be studied during their progression in whole metanephric organ cultures.

Summary

Members of the MAGUK family, in particular Dlg and CASK, play important regulatory roles in polarized epithelial cells. Many of

these functions, through a combination of genetic, cell biology and biochemical studies, have been shown to be attributed to either a specific or a combination of MAGUK domains (PDZ, SH3, GUK, Protein 4.1, L27). The protein complexes organised on these MAGUK scaffolds (many of which still need to be identified) are involved in maintaining cell junction structure, apico-basal polarity, cell adhesion, targeting of proteins to the plasma membrane, retention and clustering of receptors and polarizing signalling cascades. Disruption in these processes resulting in an alteration in the polarized epithelial phenotype can result in developmental defects such as lack of differentiation of the wing imaginal disc in flies and loss of vulva formation in the worm. Similarly, disruption to CASK or Dlg function in the mouse results in craniofacial dysmorphogenesis including a cleft palate. The model put forth attributes this to the loss of the intermolecular interaction between Dlg and CASK, suggesting that an important signalling cascade is being disrupted due to the lack of this interaction that is important to craniofacial development. This model needs to be tested by examining the localization of these proteins and their binding partners not only in the context of palatal development but also in other tissues that depend on polarized epithelial cells for their structural organization and function, such as the gut and kidney. Given the number of domains contained in both Dlg and CASK, additional binding partners, in particular transmembrane proteins which are likely to bind to the PDZ domains, still need to be identified to further our understanding of how MAGUKs regulate epithelial polarity.

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During the preparation of this review, Lee et al (*Mol. Cell. Biol.* 22: 1778-1791, 2002) reported a novel interaction between Dlg and CASK involving the N-terminus of Dlg and the L27N domain of CASK. They report that the association of Dlg to CASK is crucial for lateral localization of Dlg in MDCK cells.

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