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Role of Lgl/Dlg/Scribble in the regulation of epithelial junction, polarity and growth

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1. ABSTRACT

Genetic studies in *Drosophila* have revealed that three tumor suppressors, Discs large (Dlg), Scribble (Scrib) and Lethal giant larvae (Lgl), which localize to the basolateral region of epithelial cells, cooperatively regulate cell polarity, junction formation and cell growth in epithelial cells. Subsequent studies in *Drosophila*, vertebrates and *C. elegans* have shown the evolutionary conservation of some of their functions in epithelial cells. Also, these studies revealed the importance of antagonistic interactions between these tumor suppressors and apical polarity regulators such as Crumbs and aPKC for the establishment of apical-basal polarity with organized cell-cell junctions and regulation of cell growth in epithelial cells.

2. INTRODUCTION

Epithelial cells are polarized along the apical-basal axis, having functionally separated membrane domains, apical and basolateral domains and organized cell-cell junctions in the lateral region. Establishment of epithelial cell polarity is critical for cell morphology, tissue physiology and cell signaling. Loss of cell polarity and tissue overgrowth are characteristics of cancers derived from epithelial cells. In *Drosophila*, mutations in the tumor suppressor genes *dlg*, *lgl* and *scrib* disrupt cell polarity and cellular junctions and induce extensive overproliferation of epithelial cells. Subsequent studies have identified an evolutionally conserved molecular system involved in the regulation of cell polarity, junctions and growth of epithelial

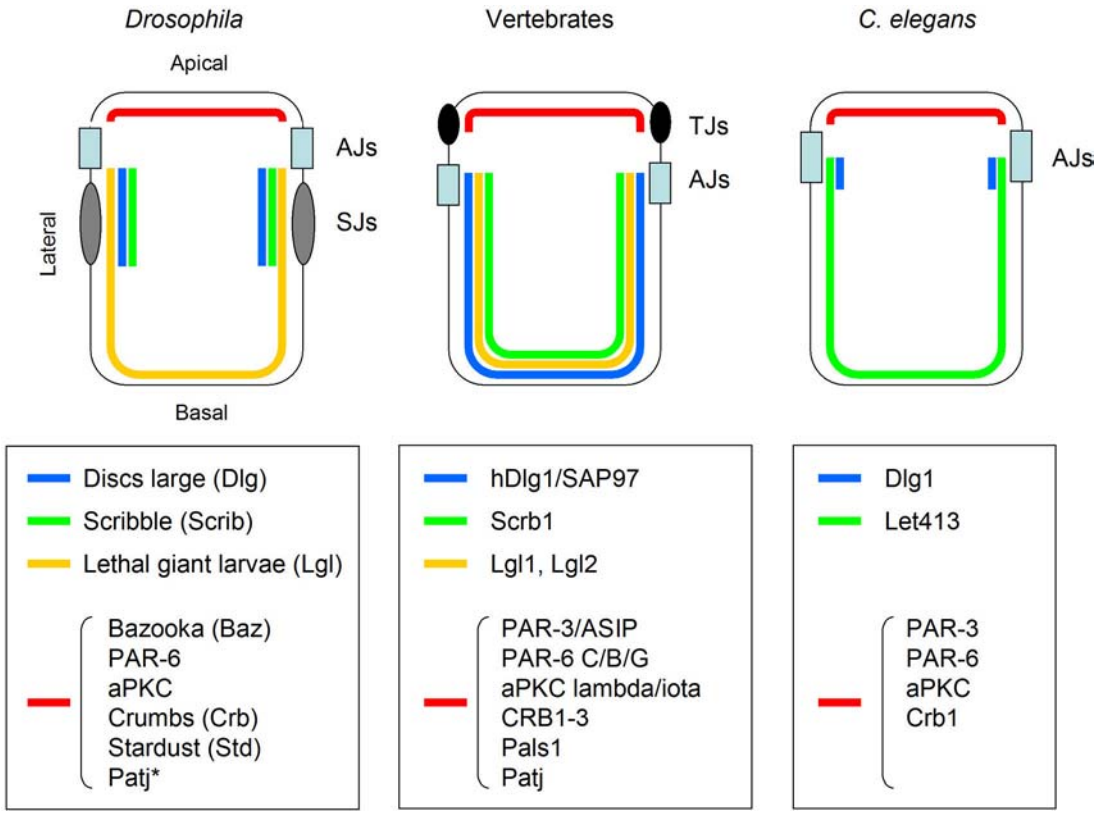

Figure 1. Cell-cell junctions and protein localization in epithelial cells. In *Drosophila* epithelial cells, Dlg and Scrib localize to septate junctions (SJs) below adherens junctions (AJs), whereas Lgl diffusely localizes in the basolateral region. In vertebrate epithelial cells, homologues of Dlg, Scrib and Lgl localize to the basolateral region, including AJs, but not to tight junctions (TJs). In *C. elegans* epithelial cells, Dlg homologue (Dlg1) localizes to AJs, whereas the Scrib homologue (Let413) localizes to the basolateral region. Localizations of apical polarity regulators are also shown (*previously called Discs lost).

cells in *Drosophila*, vertebrates and *C. elegans*. In this review, we first focus on *Drosophila* Lgl, Dlg and Scrib because the importance of these proteins for epithelial cell junctions, polarity and growth was first shown by genetic studies in *Drosophila*. We then describe the roles of these proteins in vertebrates and discuss the molecular mechanisms underlying the roles of Lgl, Dlg and Scrib in the regulation of epithelial cell junctions, polarity and growth as well as their functional conservation and divergence among organisms.

3. LGL/DLG/SCRIBBLE IN *DROSOPHILA*

In *Drosophila*, epithelial cells have two characteristic types of cell-cell junctions: adherens junctions (AJs) in the apical region and septate junctions (SJs) below them (1-3) (Figure 1). AJs form the zonula adherens, an ultrastructurally distinct belt that surrounds the apexes of epithelial cells. The core components of AJs are Shotgun (the *Drosophila* E-cadherin homologue), alpha-catenin and Armadillo (the *Drosophila* beta-catenin homologue). Although *Drosophila* epithelial cells do not have tight junctions (TJs) like vertebrate epithelial cells, SJs are thought to compensate for the functions of TJs, such as control of paracellular transport. The tumor suppressors Dlg and Scrib localize to SJs, whereas Lgl localizes to the entire basolateral region (Figure 1).

3.1. Identification of the tumor suppressors Lgl, Dlg and Scribble and their roles in the establishment of epithelial cell polarity and junctions in *Drosophila*

lgl was identified in 1985 as a gene whose homozygous mutation causes the formation of neoplastic tumors in brain and imaginal discs in larva (4). Its gene product, Lgl, has at least four WD repeats, each of which comprises a small four-stranded antiparallel beta sheet (Figure 2). Subsequent studies have revealed the localization of Lgl to the basolateral membranes of epithelial cells in a variety of tissues, including epidermis and digestive tract (Figure 1) (5-7). Biochemical analysis has revealed that a fraction of membrane-associated Lgl is resistant to solubilization by non-ionic detergents (7). Furthermore, Lgl forms homo-oligomers and associates with non-muscle myosin II (8). In addition, phosphorylation of Lgl releases it from the plasma membrane (9).These observations suggest that Lgl is associated with the cortical cytoskeleton, including myosin, in the basolateral region, and that this is regulated by phosphorylation of Lgl. Analysis using a temperature-sensitive mutant of *lgl* that behaves as a hypomorphic allele at a restrictive temperature revealed the requirement of Lgl for changes in epithelial cell shape during embryonic development (10).

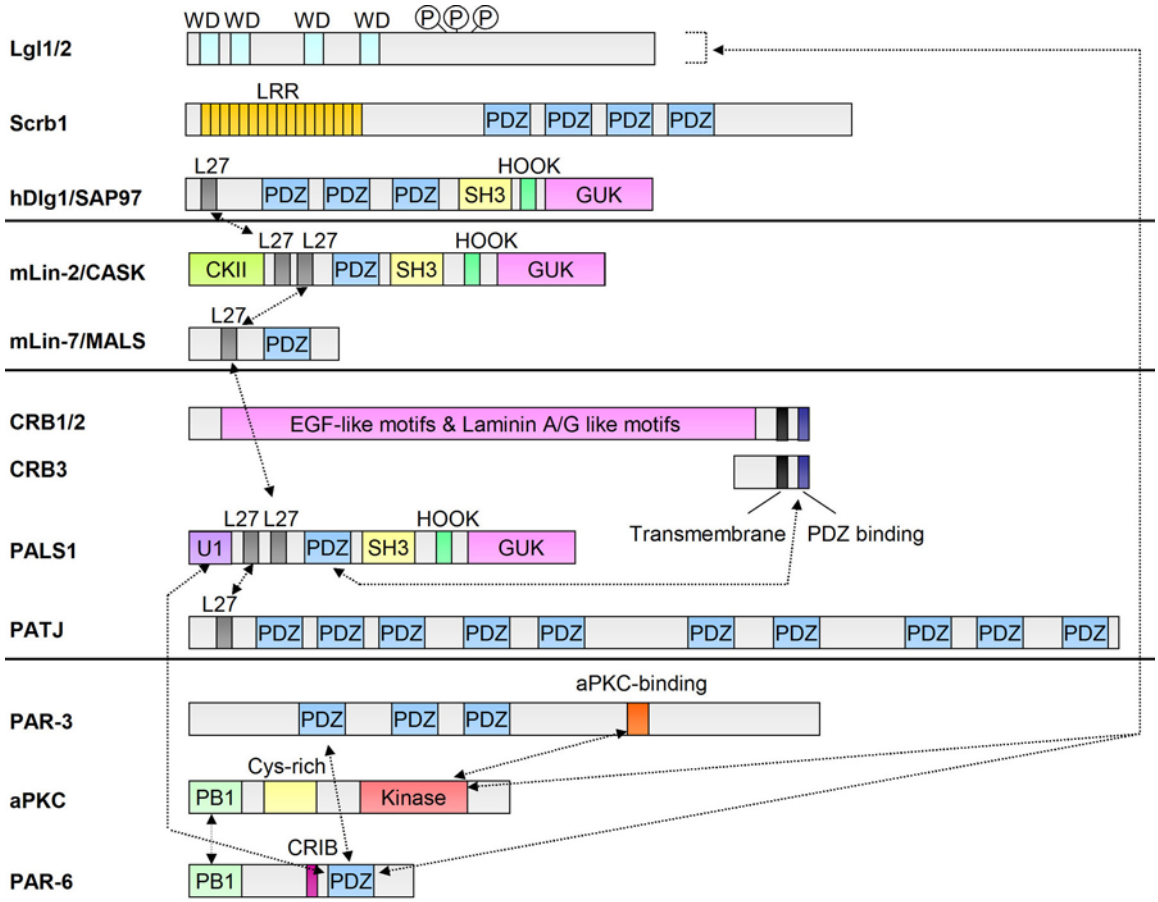

Figure 2. Domain structures of mammalian Dlg, Scrib and Lgl and their interacting proteins. P in Lgl-1/2 indicates the conserved Ser residues phosphorylated by aPKC. WD, WD repeat, GUK, guanylate kinase-like domain; CKII, CaM kinase-like domain, LRR, leucine-rich-repeats. The overall structures of these proteins are also conserved in *Drosophila* and *C. elegans* with the exceptions of *C. elegans* Scrib (Let-413), which has only one PDZ domain, and *Drosophila* Patj, which has four PDZ domains. Protein interactions are shown by dotted lines with arrows.

dlg was identified in 1991 as a gene whose mutation causes neoplastic tumor formation in the imaginal discs in larva (11). Its gene product, Dlg, is a MAGUK (membrane- associated guanylate kinase) protein that contains three PDZ domains, an SH3 domain and a guanylate kinase (GUK) domain (Figure 2) (11). Dlg also contains another evolutionally conserved region, HOOK or I3, between the SH3 and GUK domains (Figure 2) (12). Dlg localizes to SJs in epithelial cells (Figure 1) (11). Zygotic mutation of *dlg* results in loss of SJs, mis-positioning of AJs, and mis-localizations of apical or lateral membrane proteins in larval epithelial cells, suggesting that, in addition to cell growth control, Dlg is required for the establishment of cell-cell junctional structures and apical-basal polarity in epithelial cells (13).

Recently, *scrib* was identified as a gene whose maternal and zygotic mutation causes disorganization of epithelia in embryos (14). Its gene product, Scrib, is a LAP (leucine-rich repeats and PDZ domain) protein containing 16 leucine-rich-repeats (LRRs) and 4 PDZ domains (Figure 2), and localizes to SJs in epidermis (Figure 1). Immunofluorescence analysis revealed that loss of *scrib* results in the misdistribution of apical proteins and AJs to the basolateral cell surface, whereas basolateral protein localization remains intact (14). Thus, Scrib in the lateral domain functions in restricting apical membrane identity and correctly placing AJs.

In 2000, Bilder *et al.* showed that Scrib acts in concert with Dlg and Lgl to regulate both epithelial cell structure and cell proliferation (15). They showed that *scrib* zygotic mutation causes disorganization and overproliferation of epithelial cells in larval imaginal discs, corresponding to the phenotype of *lgl* or *dlg* zygotic mutant discs. In addition, maternal and zygotic mutations in *lgl* or *dlg* result in misdistribution of apical protein and disruption of AJs in the embryonic epidermis similarly to maternal and zygotic *scrib* mutations. Further, *scrib*, *dlg* and *lgl* mutants show very similar polarity defects in follicle cells: a monolayered epithelium of somatic cells that encases the germ line. In the embryonic epidermis, cortical Scrib or Lgl localization depends on Dlg, whereas Dlg localization to SJs requires both Scrib and Lgl. Finally, theses three genes showed strong genetic interactions. These observations revealed that Scrib, Dlg and Lgl act together in a common

pathway to regulate cell polarity, AJs and growth control.

Drosophila neuroblasts, which develop from an apicobasally polarized epithelium through delamination, undergo a series of asymmetric cell divisions, each of which produces a large mitotically active apical cell (neuroblast) and smaller basal cell (ganglion mother cell) that differentiates into two neurons or glial cell (16-18). Basal targeting of cell fate determinants including Numb during cell division is important for the development of ganglion mother cells (16, 17). Studies of tumor suppressor gene mutants in neuroblasts have identified critical roles for Lgl, Dlg and Scrib in the asymmetric distribution of basal proteins as well as in the asymmetry of cell size and mitotic spindle during division of neuroblasts (19-21). As in the embryonic epidermis, the cortical localizations of Lgl and Scrib are dependent on Dlg. Thus, similarly to the cell polarization of epidermis, these proteins also function in the polarization of neuroblasts.

3.2. Antagonistic interaction between laterally localized Lgl/Dlg/Scribble and apical polarity proteins

In the embryonic epidermis, mutation in *scrib*, *dlg* or *lgl* causes leakage of the apical protein Crumb (Crb) to the lateral region (14, 15). Crb is an apically localized transmembrane protein, and is necessary to establish the apical membrane domain and AJs in cooperation with its binding partners Stardust (Std) and Patj (previously called Discs lost) (22-27). Interestingly, Crb overexpression induces expansion of the apical domain and affects the formation of AJs (26, 27), which corresponds to the *scrib* mutant phenotype (14), suggesting that lateral tumor suppressors regulate apical membrane polarity by restricting localization of Crb to the apical region. Consistent with this, genetic analysis revealed antagonistic interactions between apical *crb/stardus*t and lateral tumor suppressor genes; that is, *crb* and *std* mutant phenotypes were rescued by mutations in *lgl*, *dlg* or *scrib* (28, 29). These observations support the notion that apical Crb complex and lateral tumor suppressors function competitively to define apical and basolateral membrane domains.

3.3. Molecular mechanism of the regulation of epithelial cell junctions, polarity and proliferation by Lgl

Another apical protein complex composed of a Ser/Thr kinase, atypical PKC (aPKC), and two PDZ proteins, Bazooka (Baz) and PAR-6, also has important roles in the assembly of AJs and apical-basal polarization in *Drosophila* epithelial cells (30-32). In neuroblasts, the aPKC-Baz-PAR-6 protein complex localizes to the apical cortex during asymmetric cell division, and also has an important role in polarization; mutation of each gene causes mis-localization of cell fate determinants and mis-orientation of the mitotic spindle (30, 31, 33-35). In 2002, Betschinger *et al.* showed a direct interaction between apical aPKC-PAR-6 and Lgl in neuroblasts (36). Interestingly, aPKC phosphorylates Lgl on three conserved Ser residues, which releases Lgl from the membrane cortex. They proposed that aPKC phosphorylates Lgl at the apical cortex to restrict Lgl activity and localization of cell fate determinants to the opposite, basal side of neuroblasts. Betschinger *et al.* further suggested that this phosphorylation induces autoinhibition of Lgl (37). In their model, the C-terminal region of Lgl mediates the interaction of Lgl with the cytoskeleton, and, upon phosphorylation, the N-terminal domain of Lgl binds to the C-terminal region to compete for binding to the cytoskeleton.

Hutterer *et al.* showed that, in *par-6* mutants, Lgl is not excluded from the apical region where Lgl is localized in the initial stage of epidermal development (38). Importantly, exogenously expressed mutant Lgl, in which phosphorylatable serine residues are substituted with alanines, mis-localized to the apical region and could not rescue the polarity defects in *lgl* mutants, indicating that the phosphorylation of Lgl by aPKC is required for exclusion of Lgl from the apical region and cell polarization. They further showed that *lgl* mutation causes mis-localization of PAR-6 to the lateral region in the embryonic epidermis (38). Thus, the localizations of PAR-6 and Lgl are mutually exclusive in these cells, although the mechanism of restriction of apical localization of PAR-6 by Lgl remains to be clarified. The antagonistic interaction between Lgl and aPKC is also supported by genetic analysis: Rolls *et al.* showed that mutation in *apkc* strongly suppress most cell polarity phenotypes of epithelial cells and neuroblasts in *lgl* mutants (39).

Rolls *et al.* further identified the importance of the antagonistic interaction between aPKC and Lgl in the regulation of cell proliferation in addition to cell polarity; they showed that *apkc* mutation reduced cell proliferation in both neuroblasts and epithelia, which is the opposite of the *lgl* phenotype, and that reduced aPKC levels strongly suppress the overproliferation phenotype in *lgl* mutants (39). Interestingly, exogenous expression of a membrane-bound form of aPKC in the epithelial cells of imaginal discs induces the cytoplasmic release of Lgl and causes massive overgrowth of the imaginal discs (40). In neuroblasts, *lgl* mutation in combination with a mutation in *pins*, a regulator of the asymmetric cell division of neuroblasts, or overexpression of a membrane-bound form of aPKC, induces symmetric cell division to self-renew the neuroblasts, and finally fills the brain with neuroblasts (41). These observations support the notion that the antagonistic interaction between Lgl and aPKC is important for the regulation of cell proliferation in addition to cell polarity, and suggest that a decrease in the level of cortical Lgl by gene mutation or phosphorylation by aPKC may be crucial step for uncontrolled cell growth in these cells.

3.4. Molecular mechanism of the regulation of epithelial cell junctions, polarity and proliferation by Dlg and Scrib

Although genetic analyses have clearly revealed the antagonistic interaction between *dlg* or *scrib* and apical polarity genes, the molecular mechanism underlying the regulation of cell polarity, AJ formation and cell proliferation by Dlg or Scrib remains obscure. Because Dlg and Scrib contain several separable domains, the roles of these domains in their localizations or functions have been analyzed. Analysis using transgenic *Drosophila* expressing Dlg deletion mutant identified two essential domains for the localization of Dlg to SJs in epithelial cells: the HOOK

Table 1. Polarity regulating proteins in *Drosophila*, *C. elegans* and vertebrates

Drosophila	C. elegans	Mammals (vertebrates)	
		Designations	(Gene symbols)
Lethal giant larvae (Lgl)	-[1]	Lgl1	(LLGL1)
		Lgl2	(LLGL2)
Discs large (Dlg)	Dlg-1	hDlg1 / SAP97	(DLG1)
		PSD-93	(DLG2)
		NE-Dlg / SAP102	(DLG3)
		PSD95 / SAP90	(DLG4)
		LP-Dlg	(DLG5)
Scribble (Scrib)	LET-413	Scrb1 / Vartul	(SCRB1)
DLin-2 / CAKI	Lin-2	mLin-2 / CASK	(CASK)
Dlin-7 / VELI	Lin-7	mLin-7A / MALS-1	(LIN7A)
		mLin-7B / MALS-2	(LIN7B)
		mLin-7C / MALS-3	(LIN7C)
Crumbs (Crb)	Crb1	CRB1	(CRB1)
		CRB2	(CRB2)
		CRB3	(CRB3)
Stardust (Std)	-	Pals1	(MPP5)
Patj	-	Patj	(INADL)
Bazooka (Baz)	PAR-3	PAR-3 / ASIP	(PARD3)
		PAR-3 beta / PAR-3L	(PARD3B)
PAR-6	PAR-6	PAR-6C/alpha	(PARD6A)
		PAR-6B/beta	(PARD6B)
		PAR-6G/gamma	(PARD6G)
aPKC	PKC-3	aPKC lambda/iota	(PRKCi)
		aPKC zeta	(PRKCz)

[1] (-) No homologues have been reported.

domain, which is required for the cortical localization of Dlg, and the second PDZ domain, which is required for accumulation of Dlg to SJs (42). Interestingly, rescue experiments using *dlg* mutants revealed that the second and third PDZ domains are required for growth regulation, but not for regulation of the epithelial structure, and that SH3 and HOOK are essential for both aspects of function (42). Thus, it seems that localization of Dlg to SJs through a PDZ domain is not essential for the establishment of epithelial cell polarity and junctions, but is required for the regulation of cell proliferation.

In the case of Scrib, experiments using Scrib transgenes revealed that N-terminal leucine-rich repeat (LRR) is important for cortical localization, whereas the first and second PDZ domains (PDZ1-2) are required for accumulation to SJs (43). Rescue experiments using *scrib* mutants have shown that LRR is necessary for both cell polarity and control of cell proliferation, and that the PDZ domains enhance the ability of the LRR to provide full proliferation control (43). Similar analysis in neuroblasts revealed that the LRR domain is necessary and sufficient for cortical localization in mitotic neuroblasts, that the PDZ2 domain is required for the apical localization of Scrib, and that both are required for basal localization of cell fate determinants in neuroblasts (44).

These observations suggest that, at least in epithelial cells, localization of Dlg or Scrib to the cortical region is important for the regulation of epithelial cell architecture, whereas localization of each protein to SJs through their PDZ domains is important for growth control. It should be noted that Dlg forms a complex with Scrib through a protein named GUK-holder in the synapses of neuronal tissues, and that within this complex, the GUK of Dlg interacts with GUK-holder, which interacts with PDZ1-2 of Scrib (45). However, these interactions may not be important for Dlg functions in epithelial cells, because experiments using Dlg mutants have shown that GUK is dispensable for localization to SJs and for regulation of epithelial architecture and proliferation (42).

4. LGL/DLG/SCRIBBLE IN *C. ELEGANS*

In *C. elegans*, embryonic epithelial cells have only AJs at the apical tip of cell-cell contact regions (Figure 1) (2). AJs contain three proteins, Hmr-1, Hmp-1 and Hmp-2, *C. elegans* homologues of mammalian cadherin, alpha-catenin and beta-catenin, respectively. These three genes are essential for anchoring the actin cytoskeleton to the plasma membrane, but not for cell adhesion or polarity in *C. elegans* epithelial cells (46). PAR-3, aPKC and PAR-6 localize to the apical region of epithelial cells (47, 48). The *C. elegans* homologue of Dlg (Dlg1) localizes to AJs, whereas the *C. elegans* homologue of Scrib (Let-413) localizes to AJs and the basolateral region of epithelial cells (Table 1, Figure 1) (47-50).

4.1. Essential roles of Dlg1 and Let-413 in the establishment of AJs in *C. elegans*

In 2001, three papers were published describing the importance of Dlg1 for the establishment of AJs in epithelial cells (47-49). Dlg1 knockdown affects the establishment of mature, belt-like AJs, and accumulation of AJM-1 (AJs marker stained by MH27 monoclonal antibody) to apical junctions in epidermis and intestinal epithelial cells. In contrast, the localizations of PAR-6 and aPKC to the apical surface were not affected, suggesting that the effects of Dlg1 knockdown specifically affected AJs, but not cell polarity. Dlg1 physically binds to and co-localizes with AJM-1 at apical junctions just below the regions where Hmr-1/Hmp-1/Hmp-2 is localized; knockdown of Dlg1 mis-localizes AJM-1, whereas Dlg1 localization is not affected by loss of AJM-1 (51). AJM-1 mutation results in a very similar phenotype to Dlg1 knockdown; that is, loss of mature AJs but not mis-localization of apical proteins (51). These observations suggest that Dlg1 functions to recruit AJM-1 to the apical end of the lateral region to establish mature, fully differentiated AJs at this position, whereas it is dispensable for epithelial cell polarization.

Mutation in *let-413* (*scrib* homologue) also affects AJ formation in epithelial cells, although the phenotypes are different from those resulting from Dlg1 knockdown (48, 50). Mutation in *let-413* mislocalizes AJs as well as Dlg1 and AJM-1 along the lateral region of epithelial cells (48, 50). Apical PAR-3 is also mis-distributed to lateral regions, suggesting that apical-basal polarity is affected by loss of Let-413 (48, 50). Let-413 localizes to the basolateral region of epithelial cells in contrast to Dlg1, which localizes to apical junctions with AJM-1 (48, 50). Thus, basolateral Let-413 is important for the restriction of junctional proteins as well as apical proteins to the apical region, to establish mature AJs and apical-basal polarity in epithelial cells. Overexpression experiments using deletion mutants of Let-413 revealed that LRR is necessary and sufficient for

rescue of the *let-413* mutant phenotype in *C. elegans* (52).

Thus, in *C. elegans* epithelial cells, Dlg1 and Let-413 cooperate with each other to establish mature AJs. Because the localization of Let-413 is not affected by Dlg1 knockdown (48), Let-413 acts upstream of Dlg1 in AJ formation. This is in contrast to data from *Drosophila*, in which the cortical localization of Scrib is dependent on Dlg (15). Currently, a functional homologue of Lgl has not been identified in *C. elegans*.

5. LGL/DLG/SCRIBBLE IN VERTEBRATES

In the mammalian epithelium, cell-cell junctions are composed of different types of junctions, tight junctions (TJs) and adherens junctions (AJs), which are distributed asymmetrically in the lateral region (53, 54). Tight junctions, the most apical structure of the cell-cell junctions demarcating the border between apical and basolateral membrane domains, are composed of three types of transmembrane proteins, claudins, occludin and JAM, and their scaffolding proteins including ZO-1-3. AJs, which are positioned immediately below tight junctions and characterized by two apposing membranes, are composed of two types of cell-cell adhesion complexes, the nectin-afadin complex and the classical complex composed of E-cadherin and alpha/beta-catenin.

Similarly to *Drosophila* epithelial cells, mammalian aPKC, PAR-3/ASIP (a Baz homologue) and PAR-6 form a protein complex, localize to TJs and the apical region, and regulate TJ formation and apical-basal polarity in epithelial cells (Figure 1,2) (55-66). A complex containing Crb, protein associated with Lin-7 (Pals1: Stardust homologue) and Patj (Table 1) physically interacts with the aPKC-PAR-3-PAR-6 complex, localizes to TJs and the apical domain, and regulates TJ formation and apical-basal polarity (Figure 1,2) (67-78). Cdc42, a Rho family GTPase, seems to be an activator of these protein complexes, because it has been shown that Cdc42 binds to the CRIB region of PAR-6 in a GTP-dependent manner and activates aPKC through PAR-6 (66). Cdc42 binding to PAR-6 also has been shown to facilitate the interaction of PAR-6 with Pals1 (74).

In contrast, mammalian homologues of Dlg (hDlg1/SAP97), Lgl (Lgl1, Lgl2) and Scrib (Scrb1) (Table 1) localize to the basolateral region of epithelial cells and do not co-distribute with TJs proteins in polarized epithelial cells (52, 79-83) (Figure 1). These homologues could rescue polarity defect and tumorous overgrowth of the respective *Drosophila* mutants (84-86). Thus, these proteins are functional homologues of the *Drosophila* tumor suppressors. Analysis using cultured epithelial cells, such as canine epithelial MDCK cells and human colon epithelial Caco-2 cells, has revealed that the cortical localizations of Lgl1/2, hDlg1/SAP97, and Scrb1 are dependent on cell-cell adhesion because removal of calcium from the culture medium, which dissociates E-cadherin-mediated cell-cell adhesion, localizes these proteins to the cytosol, and re-addition of calcium (calcium switch) re-localizes these proteins to the basolateral cortical region (81-83, 87). The direct requirement of E-cadherin for lateral localization of hDlg1/SAP97 and Scrb1 has been also shown (83, 87).

Although the functional significances of Lgl/Dlg/Scrib on epithelial cell polarity, junctions and growth were first shown in *Drosophila*, studies of Lgl/Dlg/Scrib homologues in vertebrates epithelial cells provided us deeper insights into molecular mechanisms underlying the regulation of epithelial cell polarity, junctions and growth by these proteins.

5.1. Molecular mechanism and functional significance of antagonistic interaction between Lgl1/2 and apical polarity proteins in vertebrate epithelial cells

Concurrently with the finding of the interaction of Lgl and aPKC-PAR-6 in *Drosophila* described above (36), mammalian Lgl1/2 was also shown to interact with PAR-6 and aPKC, and be phosphorylated by aPKC in epithelial cells (82) and fibroblasts (88). In epithelial cells, a calcium switch induces transient co-localization of Lgl2 with aPKC and PAR-6 to the cell-cell contact region and increases the amount of phosphorylated Lgl2. In addition, overexpression of kinase-negative aPKC induces abnormal co-distribution of PAR-6 and Lgl2 in the entire cortex (82). Further, in addition to the basolateral region, a phosphorylation-resistant mutant of Lgl is abnormally distributed to the apical region where aPKC and PAR-6 exist in epithelial cells (81). These observations are consistent with those observed in *Drosophila* studies, and suggest that the phosphorylation of Lgl2 by aPKC is an important step to segregate Lgl2 from the apical PAR-6-aPKC complex during epithelial cell polarization. Importantly, studies using mammalian cells further clarified the molecular mechanism by which Lgl antagonizes apical protein complex; that is, Lgl2 competes for PAR-3 in forming a protein complex with PAR-6 and aPKC, and overexpression of Lgl2 suppresses calcium switch-induced TJ formation (82). Thus, lateral Lgl2 functions as a negative regulator of the apical aPKC-PAR-3-PAR-6 complex by restricting its localization and/or function to the apical region during polarization of epithelial cells, and aPKC may modulate this function of Lgl by phosphorylating it (Figure 3).

Analysis of Lgl1/2 double-knockdown MDCK cells revealed the important role of mammalian Lgl in the establishment of the apical lumen (89). Lgl1/2 knockdown MDCK cells could not generate epithelial cysts when cultured in collagen-I gel, resulting in the formation of cell aggregates. Detailed analysis has shown that Lgl knockdown increases the amount of PAR-3 and Cdc42 that interact with the aPKC-PAR-6 complex in MDCK cells, and impairs apical domain disassembly induced by collagen-I, whereas PAR-3 knockdown accelerated this process. These data suggests that, upon cell-matrix interactions, Lgl mediates the apical domain disassembly by suppressing PAR-3-aPKC-PAR-6 complex activity, leading to the orientation of epithelial cell polarity (Figure 3). p32, a novel Lgl-binding protein, forms a protein complex with Lgl and aPKC and enhances aPKC-mediated phosphorylation of Lgl (90). Overexpression of p32 also resulted in a similar phenotype to Lgl knockdown in terms of cyst formation, suggesting that p32 negatively regulates Lgl function by enhancing its phosphorylation by aPKC to regulate the orientation of apical membrane polarity (Figure 3).

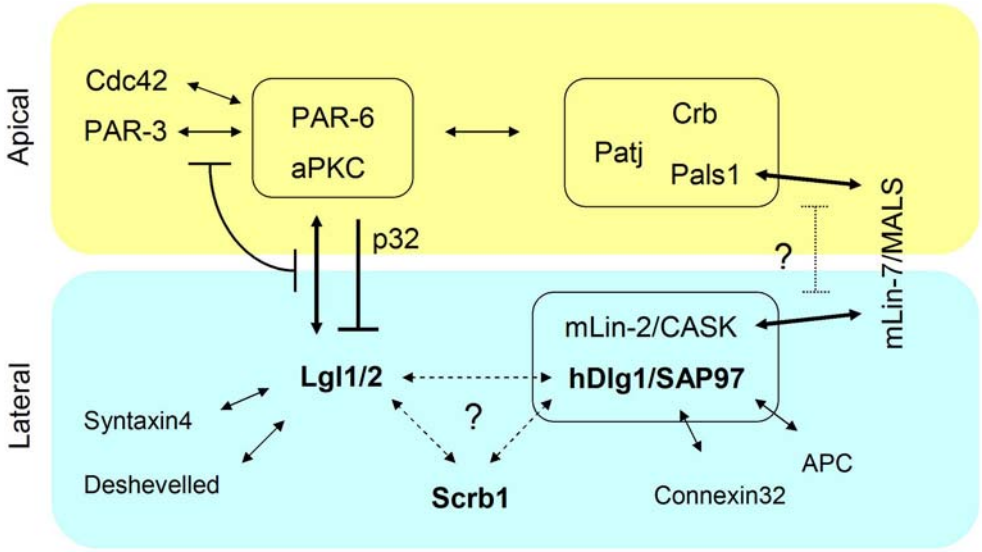

Figure 3. Molecular interactions in mammalian epithelial cells. Lgl1/2 interacts with a PAR-6-aPKC complex and antagonizes the interaction of PAR-3 (and Cdc42) with this complex. This suppressive effect of Lgl1/2 is relieved or lost following phosphorylation of Lgl by aPKC in cooperation with p32. In contrast, one of the Crb complex components, Pals1, interacts with the L27 region of mLin-7/MALS. hDlg1/SAP97 also interacts with the same L27 region of mLin-7/MALS through mLin-2/CASK. Thus, mLin-7/MALS may exclusively interact with either Pals1 or mLin-2/CASK in epithelial cells. Other interacting proteins for Lgl1/2 or hDlg1/SAP97 are also shown. Although a weak interaction between Scrb1 and Lgl2 has been reported, the molecular interactions among Lgl-1/2, Scrb1 and hDlg/SAP97 remain unclear in epithelial cells.

Mutual inhibition between aPKC and Lgl is also important for the establishment of epithelial cell polarity during early embryonic development in *Xenopus* (91). Overexpression of aPKC in the blastomere epithelium results in the expansion of the apical domain at the expense of basolateral and repositions tight junctions in the new apical-basolateral interface. In contrast, overexpression of Lgl2 or dominant-negative aPKC disrupts the apical domain and tight junctions and results in the expansion of the basolateral domain into the apical side. Importantly, overexpression of Lgl2 rescues the apicalization caused by aPKC overexpression, supporting the possibility that an antagonistic interaction between aPKC and Lgl2 is required to define apical-basolateral membrane domain and TJ organization. In zebrafish, *lgl2* mutant *penner* shows an overgrowth in the epidermis and rounded up cells, with detachment of the epidermis from the underlying tissues (92). In this mutant, hemidesmosomes are lost in the basal epidermis, whereas desmosomes and tight junctions were present in the lateral region and showed a normal ultra-structural appearance. Thus, in zebrafish, Lgl2 is required for hemidesmosome formation in the developing basal epidermis, although the involvement of aPKC in this process remains to be clarified.

5.2. Functional analysis of hDlg1/SAP97 and Scrb1 in mammalian epithelial cells

It has been shown that knockdown of hDlg1/SAP97 suppresses accumulation of E-cadherin and F-actin to the cell-cell contact region in human epithelial Caco-2 cells, suggesting that hDlg1/SAP97 is required for AJ assembly in these cells (93). In contrast, another group reported that knockdown of hDlg1/SAP97 does not affect the localization of the AJs markers E-cadherin and beta-catenin, or TJ markers, whereas it partly delays functional TJ formation after a calcium switch (94). In addition, hDlg1/SAP97 knockout mice developed various abnormalities in their renal and urogenital organs, whereas cell-cell junctional complexes were not affected in the ureter epithelia (95, 96). Thus, the role of the hDlg1/SAP97 in the polarity and junctions of mammalian epithelial cell remains obscure.

Knockdown of Scrb1 in MDCK cells disrupts E-cadherin-mediated cell-cell adhesion, with the result that these cells acquire a mesenchymal appearance, migrate more rapidly and lose directionality (97). Scrb1 knockdown in the mammary cell line MCF10A affects directional migration of cells during wound closure (98). A wound closure defect is also observed in the epidermis of the Scrb1 mutant mouse (*rumpelschtilzchen*) which has a missense mutation in LRR of Scrb1 and shows very low level expression of Scrb1 protein (98). Thus, it seems that Scrb1 has important roles in the controlled migration of epithelial cells, although knockdown of Scrb1 in MDCK or MCF10A cells did not result in clear defects in epithelial cell polarity and TJs (97, 98).

Although the roles of Scrb1 in apical-basal polarity of mammalian epithelial cell remains obscure, its involvement in the regulation of planar cell polarity has been shown by analysis of another mutant mouse (*circletail*), which has a premature termination codon between the second and third PDZ domains. This mouse shows a defect in neural tube closure during embryonic development (99). A similar neural tube closure defect is induced by mutation in Vangl2, a mammalian homologue of *Drosophila*

Strabismus involved in the establishment of planar cell polarity (100-102). Subsequent studies revealed that Scrb1 and Vangl2 interact with each other through the third and fourth PDZ domains of Scrb1 and the C-terminus of Vangl2, and are required for the establishment of planar cell polarity in mouse cochlea epithelial cells (103, 104).

5.3. Molecular interactions of Lgl1/2, Scrb1 and hDlg1/SAP97 at lateral region of vertebrate epithelial cells

Dollar *et al.* identified *Xenopus* Lgl as a binding protein of Dishevelled, an essential mediator of Wnt signaling (105). Lgl co-localizes with Dishevelled in the basolateral region of *Xenopus* ectoderm and *Drosophila* follicular epithelium, and reduced expression of Dishevelled results in a loss of basolateral Lgl. Importantly, reduced expression of either Dishevelled or Lgl results in apical-basal polarity defects; that is, a reduction in the level of aPKC in the apical region and ectopic localization of occludin to this region. Thus, Dishevelled is important for the basolateral localization of Lgl to maintain epithelial cell polarity and junctions. In mammalian epithelial cells, Lgl1 has been also shown to interact with syntaxin-4, a component of the exocytic machinery, at the basolateral membrane of epithelial cells (81). This observation suggests the possible involvement of Lgl1 in the basolateral exocytosis to contribute to epithelial cell polarization. It should be noted that the yeast homologue of Lgl (Sro7p or Sro77p) is involved in polarized exocytosis (106).

Immunoprecipiration studies using transfected MDCK cells have suggested an existence of week interaction of Lgl2 with LRR of Scrb1 (107). Importantly, overexpression experiments using Scrb1 deletion mutants have revealed that the LRR of Scrb1 is necessary and sufficient for its localization to cell-cell contact regions (52, 83), which is mostly corresponding to the observations in *Drosophila* and *C. elegans* (43, 52). These observations suggest a possible involvement of Scrb1-Lgl2 interaction in lateral localization of these proteins in epithelial cells.

The lateral localization of hDlg1/SAP97 has been shown to be mediated through the HOOK region (alternative spliced domain I3) between the SH3 and GUK domains of hDlg1/SAP97 (Figure 2) (12, 108, 109), which is mostly corresponding to the observations in *Drosophila* (42). A cytoskeletal protein, protein 4.1., interacts with this region through the FREM (Four.1-Ezrin-Radixin-Moesin) domain of protein 4.1. (12, 108, 109). Connexin32, a gap junction protein, also interacts with the region containing HOOK of hDlg1/SPA97 and is required for hDlg1/SPA97 localization to the cell-cell contact region (110). Studies of mammalian hDlg1/SAP97 have identified another important region for cell-cell targeting; that is, the L27 domain located in the N-terminal region of hDlg1/SAP97 (originally called the MRE domain) (111, 112). This L27 domain of hDlg1/SAP97 interacts with mLin-7 through CASK, forming an hDlg1/SAP97-CASK-mLin-7 ternary complex (Figure 2,3) (113). CASK and mLin-7 are mammalian homologues of *C. elegans* lin2 and lin7, respectively (Table 1), which are important for basolateral trafficking of Let-23 growth factor receptor in the body wall epithelium in *C. elegans* (114). Overexpression studies using dominant-negative CASK suggest that lateral localization of hDlg1/SAP97 is dependent on CASK (113). In addition to CASK, MPP2, MPP3 and MPP7, which are structurally similar to CASK, also form protein complexes with hDlg1/SAP97 and mLin-7 (94, 115, 116), suggesting that these MPP proteins may also have a role in the targeting of hDlg1/SAP97 to the lateral region (MPP2 and MPP3 are also referred ambiguously to as Dlg2 and Dlg3, respectively, in two referenced papers (115, 117), but these are not same as PSD-93 and NE-Dlg/SAP102 listed in Table 1).

Pals1 was originally identified as a binding protein of mLin-7 (78). Because Pals1 is structurally similar to CASK and both interact with the same region (L27) of mLin-7 (Figure 2), mLin-7 may form a protein complex with apical Crb3-Pals1-Patj in epithelial cells independently of lateral hDlg1/SAP97-CASK. Indeed, mLin-7 immunoprecipitates contain both Crb3-Pals1-Patj and hDlg1/SAP97-CASK complexes, and mLin-7 localizes to TJs in addition to the lateral region in epithelial cells (Figure 3) (118). In cultured epithelial cells, knockdown of mLin-7 suppresses TJ formation during epithelial cell polarization (117). mLin-7C knockout mice have hypomorphic kidneys characterized by numerous cysts and fibrosis, and show polarity defects together with mislocalization and downregulation of Crb, Pals1, Patj and hDlg1/SAP97 in renal tube epithelial cells (118). Thus, mLin-7 could be a scaffold for both the lateral hDlg1/SAP97 complex and the apical Crb complex, and regulates their localizations and stabilities to establish epithelial apical-basal polarity and tight junctions (Figure 3). An interaction between Lin-7 and Std or Dlg has been shown in *Drosophila* (119), indicating the evolutionary conservation of these interactions among organisms.

5.4. Possible roles of Lgl1/2, hDlg1/SAP97 and Scrb1 in epithelial tumorigenesis in mammals

Although the roles of hDlg1/SAP97 and Scrb1 in apical-basal cell polarity remain obscure in mammals, there have been several reports describing the interaction of these proteins with tumor-related proteins. hDlg1/SAP97 has been shown to interact with two famous tumor suppressor proteins, APC (adenomatous polyposis coli) and PTEN through the PDZ domains of hDlg1/SAP97 (120, 121). Negative regulation of cell cycle progression by the hDlg1/SAP97-APC complex has been also shown in NIH3T3 fibroblasts and human colon cancer cell lines (122). The PDZ domains of hDlg1/SAP97 also interact with several transforming proteins, including Net1 (a nuclear RhoA exchange factor) (123), and viral proteins such as high risk human papilloma virus E6 (124), human T-cell lymphotrophic virus-1 Tax (125), and adenovirus 9 E4ORF1 (126). These interactions are suggested to suppress hDlg1/SAP97 function by inducing degradation (127, 128) or translocation (123, 126), or suppressing hDlg1/SAP97 interaction with APC (125). Considering the requirement of PDZ domains of Dlg for cell proliferation control in *Drosophila* epithelial cells (42), these observations imply that the interactions of hDlg1/SAP97 with other protein such as APC through its PDZ domains are important for cell growth control and disruption of these interactions by

transforming proteins may induce cell transformation and/or uncontrolled cell growth in epithelial cells. High risk human papilloma virus E6 also interacts with the PDZ domain of Scrb1 to degrade it (129), suggesting the possible involvement of Scrb1 in cell transformation induced by the E6 protein.

An important role of mammalian Lgl in the regulation of cell proliferation and asymmetric cell division was revealed by analyses of its knockout mice; Lgl1 knockout mice showed severe brain dysplasia, including formation of neuroepithelial rosette-like structures, similar to the neuroblastic rosette in human primitive neuroectodermal tumors (130). Detailed analysis revealed that a large proportion of neural progenitor cells fail to exit the cell cycle and differentiate, but continue to proliferate, through a failure of asymmetric divisions. This is very similar to the phenomenon observed in *lgl* mutant *Drosophila*, in the brains of which the population of neuroblasts is increased by symmetric cell division (41).

There are a growing number of papers describing the reduction or delocalization of Lgl1/2, hDlg1/SAP97 or Scrb1 in human cancer tissues (40, 83, 131-137). Interestingly, overexpression of aPKC is observed in several cancer tissues (138, 139), and in some cancer tissues, an increase in cortical aPKC is accompanied by a decrease in cortical Lgl1 (40). Thus, controlled expression/localization of Lgl1/2, hDlg1/SAP97, Scrb1 and aPKC seems to be important for epithelial cell proliferation and tissue integrity.

6. SUMMARY AND PERSPECTIVES

Studies in *Drosophila* have established the critical roles of the lateral tumor suppressors Lgl, Dlg and Scrib in the establishment of apical-basal polarity with organized cell-cell junctions and regulation of cell proliferation in epithelial cells. The vertebrate Lgl homologue and *C. elegans* Dlg and Scrib homologues have also been shown to be important for the establishment of epithelial cell polarity and junctions. Genetic analysis using *Drosophila* has further identified antagonistic interactions between these tumor suppressors and apical polarity regulators during epithelial cell polarization. Subsequent studies of Lgl in *Drosophila* and vertebrates have identified a novel molecular mechanism involving mutual inhibition between Lgl and an aPKC-PAR-6 complex (Figure 3), which is required for the correct positioning of apical and basolateral membrane domains and cell-cell junctions in epithelial cells.

Recently, mutation in *Drosophila* syntaxin *avalanche* or *rab5* was shown to suppress endocytosis-mediated degradation of Crb, resulting in the accumulation of Crb and causing polarity defects and tumor formation in imaginal discs, corresponding to Crb overexpresion phenotypes (140). In contrast, mutation in *Drosophila* exocyst, exo84, suppresses apical localization of Crb, leading to a loss of apical membrane polarity and AJs, corresponding to *crb* mutant phenotypes (141). Importantly, reductions in the levels of Dlg or Lgl rescue apical polarity defects in exo84 mutants (141). Thus, the controlled localization of Crb by intracellular trafficking is important for the regulation of cell polarity, AJs and cell proliferation in epithelial cells, and Lgl and Dlg may be involved in this process. In mammals, overexpression of Crb in MDCK cells cultured in collagen gel suppresses lumen formation, resulting in the formation of cell aggregates without a lumen (72), corresponding to the phenotypes of Lgl1/2 double knockdown MDCK cells (89). It should be noted that Lgl1/2 knockdown suppresses internalization of apical proteins during depolarization of MDCK cells induced by cell-cell dissociation (89). Thus, Lgl may regulate cortical localizations of apical proteins, including Crb, by modulating the intracellular trafficking of these proteins in epithelial cells.

Although clear genetic and functional interactions among Lgl, Dlg and Scrib have been shown in *Drosophila*, how these proteins cooperate to regulate cell polarity, junction formation and cell proliferation remains obscure. One possibility is that Dlg and Scrib regulate these phenomena by regulating Lgl localization, because cortical Lgl is lost in *dlg* and *scrib* mutants in the epidermis and neuroblasts (15, 19-21). Alternatively, loss of Dlg or Scrib itself induces polarity defects and cell overgrowth through an unidentified pathway. In contrast to *Drosophila*, *C. elegans* Dlg1 specifically acts in AJ formation, but not in cell polarization, whereas *C. elegans* Scrib, Let-413, functions in both processes, suggesting the divergence of Dlg function among organisms. On the other hand, in mammalian epithelial cells, knockdown of hDlg1/SAP97 or Scrb1 does not result in clear defects in apical-basal cell polarity or cell-cell junctions in cultured epithelial cells. One possibility is that other proteins structurally similar to hDlg1/SAP97 or Scrb1 compensate for the loss of function of hDlg1/SAP97 or Scrb1 (Table 1), because the hDlg1/SAP97-related proteins NE-dlg, PSD-95/SAP90 and lp-dlg, and the Scrb1-related protein Erbin, have been shown to interact with junctional proteins and localize to cell-cell contact regions in epithelial cells (142-147). Combinational suppression of these proteins related to hDlg1/SAP97 and Scrb1 would be required to fully understand the roles of mammalian Dlg or Scrib in the formation of epithelial cell polarity and junctions. At least in the *Drosophila* synapse, an interaction between Dlg and Scrib, through GUK-holder, has been reported (45). In addition, during synaptic formation, Dlg is phosphorylated and regulated by PAR-1 Ser/Thr kinase, a downstream target of aPKC in mammalian epithelial cells (148-150). Analysis of these interactions in epithelial cells will help us to understand the molecular mechanism underlying the regulation of cell polarity by Dlg and Scrib.

In summary, studies of Dlg, Lgl and Scrib have identified novel molecular machinery regulating cell polarization, junction formation and growth control in epithelial cells. Further investigations of these molecules and their interacting partners will help us to understand the molecular mechanisms involved in both the establishment of epithelial tissue architecture during development and its disruption by pathological processes such as cancer.

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Abbreviations: Dlg: Discs large, Scrib: Scribble, Lgl: Lethal giant larvae, Crb: Crumb, Std: Stardust, aPKC: atypical PKC, Baz: Bazooka, APC: adenomatous polyposis coli, AJs: adherens junctions, SJs: septate junctions, TJs: tight junctions

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