The Scribble–Dlg–Lgl polarity module in development and cancer: from flies to man

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Abstract

The Scribble, Par and Crumbs modules were originally identified in the vinegar (fruit) fly, Drosophila melanogaster, as being critical regulators of apico–basal cell polarity. In the present chapter we focus on the Scribble polarity module, composed of Scribble, discs large and lethal giant larvae. Since the discovery of the role of the Scribble polarity module in apico–basal cell polarity, these proteins have also been recognized as having important roles in other forms of polarity,

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as well as regulation of the actin cytoskeleton, cell signalling and vesicular trafficking. In addition to these physiological roles, an important role for polarity proteins in cancer progression has also been uncovered, with loss of polarity and tissue architecture being strongly correlated with metastatic disease.

Introduction

Within a multicellular organism, cells exhibit different shapes, from the columnar epithelial cells that make up the skin and line the lung airways to the stellate fibroblast cells that make up the dermis of the skin and the migratory immune cells. Cell shape is fundamentally important during development for morphological movements and in tissue homoeostasis for cellular function and in defining tissue architecture. The deregulation of mechanisms regulating cell shape can lead to developmental disorders, tissue degeneration or cancer [1,2].

The shape of a cell depends on its cell polarity. Cell polarity can be loosely described as the asymmetric distribution of cellular constituents, including proteins, carbohydrates and lipids, to distinct cellular domains. Polarity is essential for many biological functions and plays a crucial role in processes as diverse as the growth of budding yeast, cell division, the transmission of nerve impulses, cell crawling and lymphocyte homing [3]. Several different types of cell polarity exist, including apico–basal, asymmetric cell division, planar and front–rear (migration) polarity [4–6].

ABCP (apico–basal cell polarity) defines the axis separating the apical and basal domains within a cell, and is established and maintained by the interplay between three evolutionarily conserved polarity modules, which define specialized domains along the apico–basal axis of a cell [7,8]. Establishing these cellular domains is important for the positioning of the adherens junction (composed predominantly of E-cadherin, α-catenin and β-catenin) and tight junctions [composed predominantly of ZOs (zona occludens), claudins and occludin] in epithelial cells, which are required for cell–cell contact and cell communication, thereby establishing a coherent epithelial tissue and regulating tissue growth. The ABCP regulators are the Scribble, Par and Crumbs polarity modules [3,9]. We define these as modules rather than complexes, since although well-defined physical interactions occur between proteins of the Par and Crumbs polarity modules, it is less clear for the Scribble polarity module. The Scribble polarity module is composed of Scrib (Scribble), Dlg (discs large) and Lgl (lethal giant larvae), the Par complex is composed of Par-3, Par-6 and aPKC (atypical protein kinase C), and the Crumbs complex is composed of the transmembrane protein Crumbs, Pals and Patj (Pals1-associated tight junction protein).

In addition, to ABCP there are other forms of cell polarity. PCP (planar cell polarity) is polarity across the plane of an epithelium and refers to the ability of cells or tissues to orient in a given direction, e.g. the organization of the hair cells within the cochlea [5]. Front–rear cell polarity is important for migration, which
occurs at both the level of individual cells, for example T-cell migration, and as sheets of cells, for example wound healing [4]. Finally, ACD (asymmetric cell division) refers to the ability of cells to produce two daughter cells with different cell fates and is a common process in development and in immune responses [6].

In the present chapter we focus on the Scribble polarity module. We take a historical perspective, describing the identification and biological functions of Scrib, Dlg and Lgl, as well as highlighting recent advances in our understanding of the function of the Scribble polarity module in development and cancer. We highlight the physiological function of the Scribble polarity module in different forms of cell polarity, as well as other cellular processes, including regulation of the actin cytoskeleton, cell signalling and vesicular trafficking. Furthermore, we describe how Scrib, Dlg or Lgl are altered in cancer.

The Scribble polarity module is highly conserved in evolution

Scrib, Dlg and Lgl were originally identified in the vinegar (fruit) fly *Drosophila melanogaster* by virtue of their tumorigenic mutant phenotype [10,11]. Scrib was so-called because of the disorganized epithelial phenotype observed by the mutant, Dlg was named due to the overgrowth of imaginal discs (the tissues that give rise to the *Drosophila* adult structures such as the eye and the wing) and Lgl was named because of the formation of overgrown larvae due to the inability of larval tissues to cease proliferation and differentiate. Homozygous mutants in any of the genes results in loss of ABCP and tissue overgrowth [12–15]. Since differentiation is blocked and tissue morphology is aberrant, these tumours are termed neoplastic. Indeed, genetic analysis in the *Drosophila* embryo revealed that Scrib, Dlg and Lgl function in a common pathway to regulate the establishment and maintenance of ABCP in epithelial cells [16]. Subsequently, homologues of Scrib, Dlg and Lgl have been identified in many multicellular organisms ranging from worms to man (see Table 1). There is a single mammalian homologue of Scrib (hScrib, Scrb1), four Dlgs, Dlg1 (hDlg, SAP97), Dlg2 (Chapsyn-110, PSD-93), Dlg3 (NE-Dlg, SAP102) and Dlg4 (PSD-95, SAP90), and two Lgls, Lgl1 and 2 (Hugl1 and 2) [3,9,17].

Scrib belongs to the LAP family, which describes proteins that contain either one or four PDZ [PSD (postsynaptic density)-95/Dlg/ZO (zona occludens)-1] domains and 16 LRRs (leucine-rich repeats) and function in controlling cell shape, size and subcellular protein localization [18]. Scrib contains four PDZ domains (see Figure 1), which are regions of 80–90 amino acids, found ubiquitously across the animal kingdom, and act through protein–protein interactions. LRR domains are believed to act in signalling and other LRR-domain-containing proteins, e.g. SUR-8, have been shown to interact with Ras members through their LRR domain [19]. The PDZ and LRR domains of Scrib are required for efficient activity, e.g. in correct localization and targeting to the membrane (see
the section on ABCP regulation). The LRR domain is critical for function, since *Drosophila* Scrib LRR domain mutants have similar phenotypes to the complete loss of Scrib protein; however, when overexpressed, a Scrib mutant lacking the PDZ domains can function similarly to the wild-type gene *in vivo* [20].

Dlg belongs to the MAGUK (membrane-associated guanylate kinase) superfamily, which are characterized by the presence of PDZ domains, an SH3 (Src homology 3) domain and a GUK (guanylate kinase-like) domain (see Figure 1). The MAGUK family act as scaffolding proteins and are important in tethering membrane structures, adhesion and in signalling [21]. The SH3 domain is so-named due to the discovery of a homologous region in the tyrosine product of the v-Src oncogene. Although SH3 domains are not catalytic, they have been shown to couple substrates to enzymes, thereby orchestrating their enzymatic activity [22]. The GUK domains in MAGUKs are catalytically inactive due to the absence of an ATP-binding site. The MAGUK GUK domain originated

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**Table 1. Polarity protein homologues across various species**

<table>
<thead>
<tr>
<th>Polarity complex</th>
<th><em>D. melanogaster</em> (vinegar fly)</th>
<th>Vertebrate (other aliases)</th>
<th><em>C. elegans</em> (worm)</th>
<th><em>D. rerio</em> (zebrafish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scribble complex</td>
<td>Scribble (hScrib, Scrib1, Vartul)</td>
<td>Scribble (hScrib, Scrib1, Vartul)</td>
<td>Let-413</td>
<td>Scrib (Scribble1)</td>
</tr>
<tr>
<td>Dlg</td>
<td>Dlg1 (SAP97, hDlg)</td>
<td>Dlg1 (SAP97A)</td>
<td>Dlg1 (SAP97A)</td>
<td>Dlg2 (PSD-93)</td>
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<tr>
<td></td>
<td>Dlg2 (PSD-93, Chapsyn-110)</td>
<td>Dlg2 (PSD-93)</td>
<td>Dlg2 (PSD-93)</td>
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<tr>
<td></td>
<td>Dlg3 (SAPI02, NE-Dlg)</td>
<td>Dlg3</td>
<td>Dlg3</td>
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</tr>
<tr>
<td></td>
<td>Dlg4 (PSD-95, SAP90)</td>
<td>Dlg4 (PSD/SAP90)</td>
<td>Dlg4 (PSD/SAP90)</td>
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<tr>
<td>Lgl</td>
<td>Lgl1 (HUGLI, LLGL1)</td>
<td>Lgl1</td>
<td>Lgl1</td>
<td>Lgl2 (Penner)</td>
</tr>
<tr>
<td></td>
<td>Lgl2 (HUGLI, LLGL2)</td>
<td>Lgl2</td>
<td>Lgl2 (Penner)</td>
<td></td>
</tr>
<tr>
<td>Par complex</td>
<td>Bazooka (ASIP, PARD3)</td>
<td>Par-3</td>
<td>Pard3</td>
<td></td>
</tr>
<tr>
<td>Par 6</td>
<td>Par6 α, β and γ (PARD6 A,B and G)</td>
<td>Par-6</td>
<td>Pard6 α, β γa and γb</td>
<td></td>
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<tr>
<td>aPKC</td>
<td>PKC1 and ζ (PKC1 and 2)</td>
<td>PKC-3</td>
<td>PKC-3</td>
<td>Prkci [heart and soul (has)]</td>
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<tr>
<td>Crumbs complex</td>
<td>Crb1</td>
<td>Crb-1</td>
<td>Crb-1</td>
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<tr>
<td>Crb</td>
<td>Crb2</td>
<td>Crb2 α and β</td>
<td>Crb2 α and β</td>
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<tr>
<td></td>
<td>Crb3</td>
<td>Crb3 α and β</td>
<td>Crb3 α and β</td>
<td></td>
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<tr>
<td>Stardust</td>
<td>PALS1 (MPP5)</td>
<td>Tag-117 (C01B7.4)</td>
<td>Mpp5 α and β</td>
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<tr>
<td>Patj</td>
<td>PALS2 (MPP6, VAM-1)</td>
<td>Mpp6 α and β</td>
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<td>PATJ (INADL)</td>
<td>Inadl</td>
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<td></td>
<td>MUPP1 (MPDZ)</td>
<td>Mpz-1</td>
<td>Mpdz (Mupp1)</td>
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<td>(C52A11.4)</td>
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from a catalytically active guanylate kinase domain and gradually lost enzymatic function during evolution [23]. It is now believed that the MAGUK GUK domain functions through interactions with the SH3 domain and by interacting with proteins associated with the actin cytoskeleton and/or microtubules [24,25].

Lgl contains several WD-40 repeats (also called WD or β-transduction repeats) and conserved phosphorylation sites (see Figure 1). WD-40 repeats are structural motifs composed of approximately 40 amino acids that are named because they usually terminate in a tryptophan–aspartic acid (WD) dipeptide. WD-40 repeats function in a wide variety of processes including signal transduction, vesicle trafficking, cytoskeleton assembly and cell division [26]. The specific function of particular proteins is determined by the sequences flanking the WD-40 repeats. The WD-40 motif in Lgl has similar characteristics to those of cell-adhesion proteins [27].

**Biological processes regulated by Scrib, Dlg and Lgl**

Mutations in *Drosophila scrib, dlg* or *lgl* result in tumours in epithelial tissues, characterized by a loss in ABCP, differentiation and proliferation control, indicating that these proteins regulate tissue architecture and act as tumour suppressors [1]. Similar phenotypes are exhibited by mutation of these genes in other organisms, although the effects vary and are tissue-specific, perhaps due to redundancy with other genes.

In *Drosophila* mutants of *scrib, dlg* or *lgl*, apical proteins are mislocalized basolaterally and the adherens junctions do not form a tight band to form the zonula adherens, which are necessary for forming tight connections between epithelial cells and epithelial tissue architecture [15,16]. *Drosophila* Scrib, Dlg
and Lgl also have roles in ACD and differentiation of the neural stem cells (neuroblasts) [6]. In addition, Drosophila Scrib and Lgl have been implicated in PCP [5], since they show genetic and physical interactions with core PCP regulators [28–30]. Finally, Lgl, Scrib and Dlg also have roles in cell migration of the Drosophila ovarian border cells; however, whereas lgl or dlg mutants increase border cell migration, scrib mutants inhibit it [31–33].

In the worm Caenorhabditis elegans, Dlg-1 or the Scrib homologue Let-413 are required for adherens junction formation [34,35]. However, in contrast with Drosophila, C. elegans Dlg-1 and Scrib appear to have distinct functions; Let-413-deficient embryos have defects in ABCP, whereas Dlg-1-deficient embryos are defective in recruitment of an adherens junction protein, AJM-1 [36,37]. The C. elegans Lgl homologue also has distinct functions to Dlg-1 and Let-413. Lgl functions redundantly with another cell polarity regulator, Par-2, in the maintenance of cell polarity in the early embryo [38,39].

In zebrafish, mutations in Lgl2 (Pannier) result in a failure to form hemidesmosomes (the connection between apical and basal surfaces of cells in multilayered epithelia, such as the skin) and tissue integrity of the basal epidermis [40]. By contrast, Scrib (Scribble1) has a role in PCP and cell migration [41,42].

In mammalian cells it appears that Dlg and Scrib have similar functions in cell polarity, but their requirement appears to be context-dependent. Knockdown of Dlg1 in the Caco-2 intestinal epithelial cells results in reduced accumulation of E-cadherin at the adherens junctions [43]. By contrast, Scrib knockdown does not perturb ABCP in MCF10A breast epithelial cells [44], but in SK-CO15 intestinal epithelial cells Scrib functions in the assembly of tight junctions [45]. However, Scrib has a role in directed epithelial cell migration in vitro and in the mouse epidermis [46]. Consistent with this, Scrib mutant mice have severe neural tube closure defects [47–49] and neural migration defects [42]. In the mouse Dlg1 also has a predominant role in epithelial migration, as evidenced by the Dlg1 mutant defects in craniofacial [50] and urogenital tract development [51]. Dlg1 is not essential for adherens junction formation, but is required for tight junction formation [52]. Lgl acts distinctly from Dlg and Scrib in mammalian cells. Lgl1-knockout mice show brain dysplasia, owing to defects in ACD and differentiation [53]. Lgl2-knockout mice exhibit branching morphogenesis defects in the placenta, which is considered to be a PCP and cell migration defect [54].

**The mechanism of Scrib/Dlg/Lgl function**

**Regulation of cell polarity**

ABCP regulation

In Drosophila epithelial cells, Scrib, Dlg and Lgl are localized to the cortex, basal to the adherens junction, at the septate (basolateral) junctions, and the plasma membrane localization of each protein depends on the function of the others [15]. Mammalian Scrib and Dlg are co-localized to the adherens junctions and extend basally [55,56]. In Drosophila the Scrib LRR region is important for...
plasma membrane localization, whereas the PDZ domains of Scrib are important for recruitment to the junctional complex in epithelial cells and neuroblasts [9,20,57]. Indeed, the role of the LRR domain of Scrib in localization is evolutionarily conserved, since point mutations in the LRR domain of mouse and the worm *C. elegans* Scrib (Let-413) result in abnormal protein localization [46,58,59]. Moreover, structure–function analysis of human Scrib has revealed that both LRR and PDZ domains are required for correct localization [56,60]. PDZ domains have also been shown to be required for the correct localization of *Drosophila* Dlg [61].

Key to the regulation of ABCP is the mutual antagonism between Lgl and aPKC. The localization of Lgl is regulated by phosphorylation by aPKC (see Figure 2). Phosphorylated Lgl is unable to localize to the cortex and is inactive [9,37,62]. Mutations from serine to alanine in the aPKC phosphorylation sites of *Drosophila* or mammalian Lgl prevent it from being inactivated by aPKC phosphorylation, and the protein accumulates at the cortex [63–66] (see Figure 2a). Through binding to the Par complex, Lgl can also inhibit aPKC activity, and the defects of *Drosophila* scrib, dlg or lgl mutants can be rescued by knockdown of aPKC [67–70]. This mutual antagonism between Lgl and aPKC is evolutionarily conserved, since it is also observed in the worm, *C. elegans* [38,39], and in the frog, *Xenopus* [71]. However, in hemidesmosome formation in the zebrafish epidermis, aPKC does not seem as important as the antagonistic interaction between Lgl2 and E-cadherin [72]. The Crumbs complex also acts antagonistically to the Scrib–Dlg–Lgl complex [70,71,73]; however, the precise manner by which this regulation occurs is not known. In *Drosophila*, the Yurt–Coracle–Neurexin IV complex, and Lkb1 (liver kinase B1)-AMP-regulated protein kinase module have also been shown to regulate ABCP in some types of epithelial cells or under metabolic stress [74] (see Chapter 10); however, it is currently unknown how well conserved these modes of regulation are in other organisms.

**Regulation of asymmetric cell division**

In ACD of *Drosophila* neural stem cells (neuroblasts), the antagonistic relationship between Lgl and aPKC is also key (see Figure 2b). The aPKC complex is localized apically, and through the phosphorylation of Lgl, restricts Lgl from the apical cortex, thereby enabling Par (PARtitioning defective)-3 to enter the aPKC complex and promote phosphorylation of the fate-determinant Numb [63,75]. This phosphorylation of Numb is required for its asymmetric localization to the basal part of the cell during cell division and its segregation into the daughter cell (the ganglion mother cell), which is essential for differentiation. The adaptor proteins, Mira (Miranda) and Pon (partner of Numb) are required for the asymmetric localization of Numb and two other fate-determinants, Pros (Prospero) and Brat [6]. aPKC via direct phosphorylation of Mira restricts Mira to the basal cortex [76]. ACD also involves the correct orientation of the mitotic spindle in mitosis, a process co-ordinated by Insc (Insutable), which is localized apically by binding to Par-3. Insc then recruits the NuMa [Pins (Lgn)–GαI–Mud]
Figure 2. Models of polarity: apico–basal and asymmetric cell division

(a) Epithelial cells are polarized along their apico–basal axis through the action of three core polarity complexes. The Par complex, composed of Bazooka (Par3 in mammalian cells), Par6 and aPKC, is localized to the sub-apical domain where it promotes activity of the Crumbs complex. The Crumbs complex composed of Crumbs, Stardust (PALS1 in mammalian cells) and Patj is also localized to the apical domain. Both complexes act through mutual antagonistic interactions to maintain basolateral localization of the Scribble complex, which is composed of Scribble, Dlg and Lgl. aPKC-mediated phosphorylation of Lgl excludes it from the apical cortex and ensures that the Scribble complex remains basally located at septate junctions (equivalent to the tight junction in mammalian cells). The model illustrated here has been described in *Drosophila* and although homologues for all proteins exist in vertebrates (see Table 1), not all interactions have been formally proven in mammalian settings. 

(b) *Drosophila* neuroblasts are commonly used as a model for ACD. The apically located aPKC phosphorylates Lgl which in turn phosphorylates Numb. This prevents cell determinants such as Mira, Pon, Brat and Pros moving apically and ensures their segregation to the basally derived GMC (ganglion mother cell) (inset and text for further details). Insc binds to the apical Par–aPKC complex which then recruits the Pins–Gαi–Mud complex. Mud binds microtubules and ensures correct spindle orientation (see text for further details).
complex, which is important for interacting with the mitotic spindle in order to correctly orientate the segregation of chromosomes during mitosis [6]. Dlg plays a different role to Lgl in ACD; it is required as a fail-safe mechanism, termed telophase rescue, to correctly align the mitotic spindle with the apical cortex via its binding to Pins [77]. Scrib shows a similar localization to Dlg in neuroblasts [78], although its precise role has not been defined. Mutations in Lgl, Dlg or Scrib show similar defects in neuroblast ACD; they exhibit defects in basal-determinant targeting, leading to symmetric divisions and an expansion of the neuroblast numbers and brain tumours [78–81]. Whether these mechanisms also occur in mammalian cells remains to be investigated; however, in the mouse skin and brain, the Pins homologue Lgn plays an important role in ACD [82,83].

Regulation of PCP
In addition to ABCP, it is becoming clear that PCP can control co-ordinated cell behaviour and is required for normal organ formation [84]. Extensive work has shown that the PCP pathway regulates the establishment of polarity within the plane of an epithelium, orthogonal to the axis of ABCP (see Figure 3a). In addition to regulating the patterning of external epidermal structures, such as wing and abdominal hair cells in Drosophila [85], the PCP pathway is more widely used for modifying cellular direction and movement of groups of cells, which is important for the formation of various tissues [86]. Formation of three-dimensional organs, such as the mammalian lung or kidney, requires co-ordinated behaviour across groups of cells to direct tissue morphogenesis. PCP is one such behaviour critical for organ formation, since uniform polarity across groups of cells underlies many basic cellular processes, including directional migration and orientated cell division. Disruption of PCP can lead to developmental defects, which in mammals include deafness, neural tube, heart and lung defects, and polycystic kidney disease [87,88].

The end result of the PCP pathway is polarization of the cytoskeleton, mediated by downstream effectors, which, in turn drives cellular morphogenesis and/or morphogenetic movement such as convergent extension, a process whereby cells intercalate between each other to drive tissue elongation along a particular axis. Convergent extension was originally observed in neurulation and gastrulation of the early embryo [89–91], but more recently is known to be required in various other tissues, including the mammalian cochlea [92] and kidney [93]. The downstream effector molecules of the PCP signalling pathway, including the small Rho family GTPases, RhoA, Rac1, Cdc42 (cell division cycle 42), the Rho effector ROCK (Rho-associated kinase), and the ROCK target myosin II, have all been described as PCP effectors during gastrulation of the early embryo. In addition, Rac1 mediates the polarization and morphogenesis of hair cells in the mouse inner ear, and myosin II is implicated in convergent extension of the cochlea [92].

Two parallel pathways, the ‘core’ PCP complex and the Fat/Dachsous system can influence planar polarity. We will focus only on the ‘core’ PCP pathway.
Figure 3. (Continued)
In flies, six proteins have been identified to operate within this ‘core’ pathway: Fz (Frizzled), Dsh (Dishevelled), Dgo (Diego), Vang (Vang Gogh, also known as Strabismus), PK (Prickle) and Fmi (Flamingo, also known as Starry-night). Complete loss of any of these proteins leads to a loss of PCP, a requirement for a protein to be considered part of the core cassette [5]. However, in vertebrates, the system is more complex due to the presence of homologues of a number of the core genes, including Frizzled, Dishevelled, Vangl and Celsr (the homologue of *Drosophila* Fmi). In addition, vertebrate genes do not always behave in the same manner as their *Drosophila* homologues. For example, overexpression of the *Drosophila* Otk (Off-track) results in defects in PCP [94], whereas loss of PTK7 (protein tyrosine kinase 7, the mammalian homologue of *Drosophila* Otk) results in identical phenotypes as core PCP mouse mutants. PTK7 has also been shown to genetically interact with core components of the PCP pathway, such as Vangl2 [95,96].

In mouse development, a clear role for Scrib has been revealed in the regulation of PCP, rather than in ABCP [47,97,98]. For example, Scrib mouse mutants exhibit classic PCP phenotypes, such as craniorachischisis, the most severe form of failed neural tube closure [47], as well as disruption to the polarity of inner ear hair cells [97]. Moreover, Vangl2, a core component of the PCP pathway, and Scrib show co-localized expression patterns and have strong genetic interactions [95,99]. In addition, Celsr1, Vangl2, Scrib and PTK7 are all required for the normal development of branched organs, including the lung [96,100]. Scrib along with Vangl2 and Celsr1 have been shown to operate via RhoA to modulate the actin–myosin cytoskeleton required for the formation and growth of new airway branches.

**Figure 3. Models of polarity: planar cell polarity and migration**

(a) This schematic diagram represents a simplified view of the PCP signalling pathway. The diagram shows the complex interactions between the core components of this signalling cascade. Interactions between the multiprotein complexes Celsr1–Vangl2–Prickle and Celsr1–Frizzled–Dishevelled establish planar polarity between neighbouring cells. The asymmetrical localization of these multiprotein complexes in an epithelial sheet directs cytoskeletal reorganization that allows a single hair to emerge solely from the distal edge of a cell. This same mechanism is used to direct cell movement and migration during convergent extension. During PCP signalling, Frizzled, Vangl2, Celsr1, PTK7, Prickle and Diego all signal via the cytoplasmic Dishevelled, activating downstream effector molecules, such as RhoA, ROCK and JNK (c-Jun N-terminal kinase), resulting in gene transcription, cytoskeletal remodelling and the establishment of polarity. Scribble interacts with Vangl2 and Celsr1, as well as modulating downstream effectors to regulate the establishment of PCP. (b) Cell migration requires the asymmetric localization of polarity molecules to opposite sides of the cell. Using T-cell migration as an example, this schematic diagram depicts how the Par complex is located to the front of the migrating cell and the Scribble complex towards the uropod at the rear of the cell. The precise location of the Crumbs complex is still to be determined, but current research suggests that it sits between the Par and Scribble complexes. The microtubule-organizing centre (MTOC) is required for the contractile motions that drive T-cell migration. Rho GTPases play key roles in uropod formation, through interactions with ERM (erzin/radixin/moesin) and by mediating the actomyosin cytoskeleton at the leading edge.

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While the majority of studies indicate the Scribble polarity module to have a role in ABCP in *Drosophila* and PCP in mammalian systems, more recently a role for Scrib in establishing PCP in *Drosophila* [28], and in ABCP in mammary and prostate epithelial cells [101,102] has been determined. Moreover, studies in zebrafish, revealed that Scrib is required for convergent extension of mesenchymal cells [41], which lack ABCP, suggesting additional functions for Scrib in PCP patterning. Indeed, *Drosophila* studies show that PCP and ABCP pathways are closely linked at the molecular level [28,103]. Furthermore, the PCP protein Dsh binds to Lgl and regulates its localization in *Drosophila* epithelium and frog (*Xenopus*) tissues [30]. Thus it is likely that many epithelial tissues require both ABCP and planar polarization for optimal organization and function, and the Scribble polarity module may be the link between these two polarity pathways.

**Regulation of directed cell migration**

Another role that has been attributed to the Scribble polarity module and components of the PCP pathway is the regulation of directed migration of cells and vertebrate wound healing. PCP signalling results in the reorganization and polarization of the actin cytoskeleton and subcellular organelles at the leading edge of tissues, such as that which occurs when the skin is wounded, so that keratinocytes (skin epithelial cells) can undergo co-ordinated cell movement from the wound edge to close the gap [104]. Scrib has been shown to be essential for proper epithelial cell movements in response to extracellular directional migration cues during wound healing [46]. Scrib co-localizes with Rac and Cdc42 at the leading edge of migrating cells and is required for the recruitment of these GTPases to this site. Furthermore, this function of Scrib in regulating cell migration is conserved between different epithelial cell types and species, as mutational disruption of Scrib function in the mouse results in defective wound healing of the epidermis *in vivo* [46]. A more recent study confirmed the role of Scrib in effective wound healing in the mouse and implicated both Celsr1 and the vertebrate PCP component PTK7 in this process [95,104]. Dlg1 is also required for epithelial cell migration during mouse embryogenesis, with loss-of-function mutants resulting in craniofacial defects [50], and epithelial duct formation and morphogenesis defects in urogenital development [51].

Scrib can also control the migration of non-epithelial cell types, such as zebrafish motor neurons, mouse astrocytes and T-lymphocytes, by regulating front–rear polarity [41,105,106]. Here we will focus on T-lymphocytes. T-cell shape is determined by the selective polarized recruitment of molecules to different regions of the cell and is crucial for T-cell function, from migration to cell killing. Scrib and other polarity proteins (Lgl, Par3 and Crumbs) are expressed in T-cells and are thought to be critical for their function (see Figure 3b). As with other migrating cells [4,107], the opposing action of the Scribble polarity module with the Par and Crumbs complexes is also thought to be important for T-cell front–rear polarity and migration. Indeed, knockdown of Scrib in murine...
T-lymphocytes results in a profound defect in cell polarity and loss of directed cell migration [105]. Whether Dlg or Lgl also function in this manner in T-cells is more difficult to discern due to the presence of multiple family members (see Table 1).

In summary, the Scribble polarity module plays a central role in different forms of cell polarity, ABCP, ACD, PCP and front–rear, in different cell types. An important theme to emerge from these studies is that Scrib, Dlg and Lgl often have distinct roles, and therefore cannot always be considered as working in a common pathway. Key questions still remaining are what are the precise mechanisms by which Scrib, Dlg and Lgl function to control these different types of polarity and how do other cellular processes that are regulated by these proteins [such as actin remodelling, cell signalling and vesicular trafficking (see below)] connect to cell polarity regulation?

**Regulation of the actin cytoskeleton by Scrib, Dlg and Lgl**

The regulation of cell polarity involves remodelling of the actin cytoskeleton; however, at present only sketchy details have been revealed on the molecular mechanism by which Scrib, Dlg or Lgl mediate this regulation. As described above, there are links via PCP proteins to the Rho-GTPase family of actin regulators, and in cell migration Scrib has been connected to Rac-GTPase regulation, via binding to and controlling its regulators, β-Pix and Git1 [108,109]. Recently another actin regulator connecting Scrib and Dlg to the actin cytoskeleton has emerged, Gukh. Gukh (NHS) is an evolutionarily conserved protein identified as tethering Dlg and Scrib together in *Drosophila* neuromuscular junctions [110,111]. NHS is mutated in the human developmental disorder Nance–Horan Syndrome, and is involved in remodelling the actin cytoskeleton and cell morphology in mammalian cells [112]. This role of NHS is likely to be important in linking Scrib to the regulation of PCP and cell migration [42].

Lgl appears to regulate the actin cytoskeleton differently. Lgl binds to and negatively regulates the non-muscle myosin protein, myosin II, a regulator of F-actin (filamentous actin) function in cell morphology regulation, in *Drosophila* and human cells [113–115]. Recently evidence has emerged that, in *Drosophila*, this regulation may play an important role in PCP [29], although the role of this interaction in *Drosophila* ABCP or ACD is unclear [29,76]. However, in *C. elegans*, Lgl-1 negatively regulates the accumulation of myosin II (NMY-2) on the posterior cortex of the early embryo in the establishment of ABCP [39]. In this system, the myosin II-mediated contraction of cortical F-actin, asymmetrically distributes PAR-3, PAR-6 and aPKC (PKC-3) to the anterior. Thus, in the worm embryo, Lgl seems to play an important role in ABCP through its regulation of myosin II as well as aPKC.

**Regulation of signalling pathways by Scrib, Dlg and Lgl**

The Scribble polarity module has been linked to the regulation of many signalling pathways that play critical roles in cell growth, proliferation and survival [1].
Here we highlight important new insights that have emerged for Scrib, Dlg and Lgl in regulating signalling pathways involved in tissue growth control.

In Drosophila, a role for Lgl in cell proliferation and survival has been uncovered, which is separable from its role in ABCP [116]. By making mutant patches of tissue in the developing Drosophila eye, the G1–S-phase cell-cycle regulator cyclin E and the cell-cycle transcription factor E2F1 were shown to be ectopically expressed, in a region of the developing eye where cells have normally exited from the cell cycle. Moreover, the cell death inhibitor Diap1 (Drosophila inhibitor of apoptosis) is up-regulated and developmental cell death blocked [68,116]. This up-regulation of cyclin E, E2F1 and Diap1 is due to the inhibition of the Hippo negative tissue growth control pathway.

The evolutionarily conserved Hippo signalling pathway consists of a kinase cascade involving the Hippo and Warts protein kinases that phosphorylate and inactivate the Yorkie (YAP (Yes-associated protein)/TAZ (transcriptional co-activator with PDZ-binding motif) in mammalian cells) transcriptional co-activator [117] (see Chapter 9). This finding that loss-of-function of Lgl (and up-regulation of aPKC) inactivates Hippo pathway signalling [thereby leading to increased expression of Yorkie targets, including tissue growth genes such as CCNE1 (cyclin E), E2F1 and Diap1], has provided a link between Lgl/aPKC balance and regulation of tissue growth via Hippo pathway regulation in Drosophila [68,118,119]. The mechanism by which Lgl/aPKC regulates the Hippo pathway is currently unclear; however, deregulation of Hippo signalling correlates with delocalization of the Hippo protein kinase from the apical membrane, where it normally functions [120,121]. Interestingly, depleting Scrib or Dlg does not lead to deregulation of Hippo pathway signalling unless ABCP is lost [68,122]. A possible mechanism by which Scrib may control the Hippo pathway has been revealed by a recent study showing that mammalian Scrib can bind to TAZ (the positive transcriptional co-activator that is negatively regulated by Hippo pathway signalling) and sequester it to the cell cortex in breast cancer stem cells [123]. Therefore, when Scrib or ABCP is lost, TAZ would be expected to be released from the cortex and function as a transcriptional co-activator. Whether this control extends to other cell types or other organisms remains to be determined. Another mechanism by which Scrib deregulation could result in Hippo pathway deregulation is via its effect on E-cadherin; in MDCK kidney epithelial cells, loss of Scribble destabilizes the coupling between E-cadherin and α-catenin and results in decreased adhesion [4]. Since α-catenin restricts Hippo pathway signalling by tethering YAP to the cortex [124,125], then knock down of Scrib would be expected to release YAP from the cell cortex enabling it to enter the nucleus to activate transcription of tissue growth genes. Furthermore, depletion of Scrib in Drosophila results in increased F-actin accumulation, which has been shown to reduce Hippo pathway signalling and increase tissue growth [126,127]. Loss of polarity induced by Scrib or Dlg depletion would also lead to expansion of the apical domain.
and higher levels of aPKC and Crumbs that could also contribute to Hippo pathway inactivation [68,118]. In development and tissue homeostasis, Lgl/aPKC balance, and perhaps also Scrib and Dlg, may relay cues from the surrounding cells and the microenvironment to regulate the Hippo tissue growth control pathway, thereby modulating cell proliferation, survival and differentiation. Indeed, in zebrafish, Scrib has been linked to Hippo pathway regulation in pronephros development [128].

Scrib has also been implicated in the EGFR (epidermal growth factor receptor)-Ras GTPase signalling pathway to control cell proliferation and survival in both mammalian cells and Drosophila [44,129]. The EGFR-Ras signalling pathway signals through a kinase cascade involving Raf, MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase] and MAPK (ERK), to promote proliferation and survival, and is one of the major pathways deregulated in human cancers [130]. In mammalian cells, Scrib functions as a scaffolding protein for Ras signalling; it binds to MAPK (ERK) through a conserved domain, KIM (kinase-interaction motif) [129]. However, it is likely that there are additional tiers of regulation of Ras-MAPK signalling by Scrib, for example Scrib can also interact with RSK2 (ribosomal S6 kinase 2), a negative regulator of the pathway [131], and GIT1 [G-protein-coupled receptor kinase-interacting Arf (ADP-ribosylation factor)-GAP (GTPase-activating protein) 1], an Arf-GAP that can act as a MEK-ERK scaffold [132,133]. Interestingly, in zebrafish, Lgl2 has also been linked to regulation of the Ras signalling pathway [134]; however, how direct this is and whether this regulation also occurs in other organisms remains to be determined.

The Scribble polarity module can also have an impact on the regulation of the PI3K (phosphoinositide 3-kinase) pathway. The PI3K pathway is regulated at adherens junctions and acts via the protein kinases, Akt and TOR (target of rapamycin), leading to up-regulation of translation and thereby cell growth and proliferation, as well as promoting cell survival [135,136]. In mammalian cells, Dlg1 has been shown to bind to PTEN (phosphatase and tensin homologue deleted on chromosome 10), a negative regulator of the PI3K pathway [137], and Dlg1 is required for the Adenovirus 9 E4-ORF1 (E4 region-encoded open reading frame 1) oncoprotein to promote the constitutive activation of PI3K [138]. Consistent with a role for Dlg in promoting PI3K signalling, a recent study in Drosophila showed that PI3K signalling is down-regulated in Dlg-depleted epithelial cells, and further knockdown of PI3K components resulted in synthetic lethality of Dlg-depleted tissue, even in the presence of oncogenic Ras [139]. Conversely, in mammalian cells, Scrib negatively regulates Akt activity via binding to Phlpp [PH (pleckstrin homology) domain and LRR protein phosphatase], a protein phosphatase that negatively regulates Akt, and localizes it to the plasma membrane [140]. Scrib forms a tripartite complex with Phlpp and Akt, thereby inhibiting Akt activity, but when Scrib is down-regulated, Phlpp is released, Akt activity is increased and cell growth, proliferation and survival is enhanced.
In summary, the common theme emerging from these studies is that the Scribble polarity module proteins may serve as signalling scaffolds controlling many signalling pathways, but which signalling pathways are regulated in specific cells or organisms may be context-dependent.

**Regulation of vesicular trafficking by Scrib, Dlg and Lgl**

In *Drosophila*, neoplastic tumour phenotypes are also observed in mutants affecting endocytosis, the process by which external and membrane-bound proteins are trafficked into a cell [141]. There is accumulating evidence for the role of vesicular trafficking in the localization of cell polarity proteins; however, the ABCP machinery can also control vesicular trafficking [142]. The Par complex has been implicated in endocytosis regulation from a genetic screen in *C. elegans* [143]. Furthermore, in *Drosophila* epithelial tissues, mutants in Par complex proteins exhibit defects in E-cadherin trafficking [144,145], and in endocytosis of apical proteins [146]. In mammalian cells, Scrib inhibits basal receptor endocytosis and promotes recycling of the TSHR (thyroid-stimulating hormone receptor) in PC12 cells [108].

Exocytosis, the trafficking of proteins after protein synthesis in the endoplasmic reticulum to the plasma membrane [147], has also been linked to the Scribble polarity module. In *Drosophila*, *scrib*, *dlg* and *lgl* genetically interact with mutants in a core component of the exocytic machinery, *exo84*, which is required for the apical delivery of proteins [148]. In MDCK (Madin–Darby canine kidney) mammalian epithelial cells, Lgl2 forms a complex with the t-SNARE [target SNARE (soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptor)] syntaxin 4, a component of the basolateral exocyst machinery [149], which parallels studies in yeast where the Lgl homologues Sro7 and Sro77 interact with the exocytic machinery [150,151]. Furthermore, in response to osmotic stress, syntaxin 4 forms a complex with Scrib, Dlg and Lgl and is required for their membrane localization [152]. Lgl1 has also been shown to interact with and regulate the small GTPase Rab10 in directional membrane insertion during mammalian axonal development [153]. Furthermore, in mammalian cells, Scrib, through its association with β-Pix and GIT1 (Rac GTPase regulators), has an important role in regulating exocytosis in neuroendocrine cells [133].

Collectively, these data suggest that members of the Scribble polarity module may be playing key roles in vesicle trafficking. Since many signalling pathways involve vesicle trafficking to promote signalling, or to down-regulate receptors and dampen signalling [154], this connection of Scrib–Dlg–Lgl to vesicle trafficking may be fundamental to their regulation of signalling pathways. Although this field is still in its infancy, distinct roles for Scrib, Dlg and Lgl in specific aspects of vesicular trafficking are being revealed. Future research will need to focus on discerning the universality of each of these mechanisms and how they relate to effects of Scrib, Dlg or Lgl on cell polarity, signalling pathways or actin cytoskeletal regulation.
Role of Scrib/Dlg/Lgl in cancer progression and metastasis

Since the original identification of Scrib, Dlg and Lgl as neoplastic tumour suppressors in *Drosophila*, much effort has focused on understanding their involvement in mammalian cancer progression and metastasis. We describe below evidence revealing an important role for the Scribble polarity module in mammalian cancer.

Scrib and Dlg are targets of viral oncoproteins

Over 80% of cervical cancers are caused by the high-risk human papilloma viruses HPV16 and HPV18, which target PDZ-domain-containing proteins for degradation via the E6 oncoprotein [155]. Several polarity proteins contain PDZ domains, including Scrib, Dlg1, MAGI-1 (membrane-associated guanylate kinase, WW and PDZ domain-containing protein 1) and MUPP1 (multi-PDZ-domain protein 1), and have been identified as targets of the E6 oncoprotein [155–159]. Importantly, the transforming capacity of the E6 oncoprotein is dependent on direct interaction with PDZ proteins, as mutants that lack the PDZ-binding motif no longer transform cells [160].

In addition to HPV16 and HPV18, several other human tumour viruses have been identified in which their oncogenic potential depends, in part, on their ability to inactivate polarity proteins. Adenovirus type 9 promotes transformation through E4-ORF1 via interactions with the polarity proteins Dlg1, MUPP1, PATJ and ZO (zona occludens)-2 [157,158,161,162]. HTLV-1 (human T-cell leukaemia virus type 1) Tax oncoproteins are causative agents for adult T-cell leukaemia and have been shown to target the polarity proteins Dlg and Scrib [162,163]. The role of polarity deregulation in tumorigenesis has primarily focused on epithelial tumours, however, the ability of Adenovirus 9 and HTLV-1 to promote sarcomas and leukaemias respectively, supports a wider role for polarity deregulation in the development of many cancer types [164].

Aberrant expression of Scrib, Dlg and Lgl in human cancer

Consolidated analysis from both primary tissue samples and cell lines suggests that altered expression of polarity proteins play a causal role in tumorigenesis [165]. All three members of the Scribble polarity module are mislocalized in various human cancers. A recent study found Scrib mislocalized in 50% of analysed DCIS (ductal carcinoma in situ) breast cancer lesions, and Scrib and Dlg has been shown to be mislocalized in colon and cervical cancer, whereas Lgl2 is mislocalized in gastric adenocarcinomas [101,166–168]. Members of the Scribble polarity module frequently show aberrant expression across a variety of tumour types, including breast, endometrial, cervical, colon, prostate and lung [101,102,166–172]. Altered expression patterns of other polarity complexes, for example the Par complex, are also seen in several tumour types, including breast, oesophageal and ovarian carcinomas [173–176]. Furthermore, enforced expression in transformed cell lines of polarity proteins, including
Scrib, Lgl and Crumbs3, results in an increase in cell adhesion and reversion to a less malignant phenotype, suggestive of a direct role for polarity regulators in tumour progression [44,177–179]. Taken together, these studies support the idea that aberrant expression of polarity proteins correlates with malignancy and invasion, although direct links to clinical outcome remain to be established. To bridge this gap, researchers are developing in vivo mouse models to investigate the consequences of polarity deregulation in tumorigenesis.

**In vivo models of Scrib, Dlg and Lgl knockdown in tumorigenesis**

Scrib, unlike other members of the complex, has a single homologue (see Table 1), enabling it to be studied without the complicating factors of redundancy. Mice that have lost both copies of Scrib die perinatally, however, analysis of heterozygous mice enables the study of the effects of reduced Scrib expression. Aged Scrib\(^{+/−}\) mice show prostate hyperplasia and have a high incidence of lung adenomas [102]. The effect of Scrib depletion has also been analysed by transplantation of epithelial cells in which Scrib was knocked down [using RNAi (RNA interference)] into the mouse mammary fat pad; Scrib-depleted tissues exhibit multilayering and eventually a small percentage develop tumours [101]. Similar to studies in *Drosophila*, where mutants in *scrib, dlg* and *lgl* co-operate in tumorigenesis with various oncogenes, such as Myc and Ras [180,181], the Myc oncogene also co-operates with Scrib depletion to promote tumorigenesis in mouse xenograft models [101]. Co-operation between Scrib and the Ras oncogene also occurs in other settings, such as the prostate [102], suggesting that such co-operation may be a conserved phenomenon.

**Conclusions and future perspectives**

As outlined in the present chapter, the Scribble polarity module has roles in a plethora of biological processes. In the years since the initial discovery of Scrib, Dlg and Lgl [12,14,15], we have learnt that their functions are far more extensive than the roles in ABCP, junctional integrity and cell proliferation for which they were originally identified. It is now clear that they play roles in the regulation of other types of polarity, the actin cytoskeleton, cell signalling and vesicle trafficking. The context-dependent roles that Scrib, Dlg and Lgl often exhibit have complicated studies investigating their physiological functions. For the most part, the structure and function of members of the Scribble polarity module are evolutionarily well conserved, which has enabled researchers to study the proteins in simpler organisms, such as the vinegar fly *Drosophila melanogaster*, the worm *C. elegans* and the zebrafish *Danio rerio*, before translating the findings into the more complex mammalian systems. The emergence of a crucial role for Scribble polarity module proteins in cancer progression and metastasis has spurred the development of many in vivo models to aid in the understanding of how their deregulation contributes to tumorigenesis. Although, much progress
has been made in this fascinating and ever-growing field, we still have a long way to go before fully understanding how Scrib, Dlg and Lgl function, and how they interact both among themselves and with other proteins physiologically and in tumorigenesis.

Summary

- The Scribble polarity module comprises three evolutionarily conserved proteins, Scrib, Dlg and Lgl.
- Scrib, Dlg and Lgl function in the same genetic pathway in ABCP regulation.
- Scrib, Dlg and Lgl also have specific roles in other forms of polarity: ACD, PCP and front–rear polarity in cell migration.
- The Scribble polarity module regulates the actin cytoskeleton via different mechanisms.
- Scrib, Dlg and Lgl have distinct regulatory effects on signalling pathways that control cell growth, proliferation and survival.
- The regulation of vesicular trafficking via the Scribble polarity module may be key to its function in regulating signalling pathways.
- Deregulation of the Scribble polarity module is commonly observed in human cancer and correlates with tumour progression.

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