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Review

Polarity complex proteins ☆

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Abstract

The formation of functional epithelial tissues involves the coordinated action of several protein complexes, which together produce a cell polarity axis and develop cell–cell junctions. During the last decade, the notion of polarity complexes emerged as the result of genetic studies in which a set of genes was discovered first in *Caenorhabditis elegans* and then in *Drosophila melanogaster*. In epithelial cells, these complexes are responsible for the development of the apico-basal axis and for the construction and maintenance of apical junctions. In this review, we focus on apical polarity complexes, namely the PAR3/PAR6/aPKC complex and the CRUMBS/PALS1/PATJ complex, which are conserved between species and along with a lateral complex, the SCRIBBLE/DLG/LGL complex, are crucial to the formation of apical junctions such as tight junctions in mammalian epithelial cells. The exact mechanisms underlying their tight junction construction and maintenance activities are poorly understood, and it is proposed to focus in this review on establishing how these apical polarity complexes might regulate epithelial cell morphogenesis and functions. In particular, we will present the latest findings on how these complexes regulate epithelial homeostasis.

Keywords: Tight junction; Epithelium; PAR complex; CRUMBS complex; SCRIBBLE complex

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Abbreviations: 5-HTR, serotonin receptor; Amot, angiomotin; APC, adenomatous polyposis coli; aPKC, atypical protein kinase C; CAR, Coxsackievirus and adenovirus receptor; CASK, calmodulin associated ser/thr kinase; C-kit, proto-oncogene (receptor for stem cell factor); CRB, crumbs; CRIB, Cdc42 Rac interaction binding; DLG, discs large; EMP55, 55-kDa erythrocyte membrane protein (p55); EPB41L5, erythrocyte protein band 4.1-like 5; EST, expressed sequence tag; FERM, band 4.1 ezrin radixin moesin; Has, heart and soul; HURP, hepatoma upregulated protein; InaD-like, inactivation no after-potential D; JAM, junctional adhesion molecule; K, chromosome; L27, Lin2 lin7; LAP, LRR and PDZ; LGL, lethal giant larvae; LRR, leucine-rich repeats; MAGUK, membrane-associated guanylate kinase; MOE, mosaic eyes; MPP, membrane protein palmitoylated; MUPP1, multi PDZ domain protein; MW, molecular weight; NG2, membrane-spanning proteoglycan; NOK, Nagie oko protein; Ome, oko meduzy; PALS, proteins associated with Lin seven; PAR, partition defective; PATJ, protein-associated with tight junction; PDZ, PSD-95, discs large, ZO-1; PI3K, phoshoinositide 3-kinase; PTEN, protein tyrosin phosphatase and tensin homologue; SAP102, synapse-associated protein 102; SCRIB, scribble; Sdt, stardust; SH3, Src homology domain 3; TAPP, tandem-PH (pleckstrin-homology)-domain-containing protein; TARPs, transmembrane AMPA receptor regulated proteins; TSC, tuberous sclerosis complex; TSHR, thyroid stimulating hormone receptor; VAM, veli-associated MAGUK; YMO, yurt mosaic eyes like; ZO, zonula occludens

☆ *D. melanogaster* and *C. elegans* gene and protein names are written in minuscule whereas mammal GENE and PROTEIN names are in capital letters. All gene names are in italic.

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1. Introduction

Cell polarity, which is fundamental to many aspects of cell and developmental biology, is involved in the processes of differentiation, proliferation and morphogenesis in both unicellular and multicellular organisms. In a wide range of elementary cellular processes, many constituents of the cell, such as plasma membrane proteins, organelles, and cytoskeletal components are organized asymmetrically within the cell. This asymmetrical pattern of organization is enhanced by cell differentiation processes resulting in dynamic cell compartments specialized in complex vectorial functions. Cell polarity is essential for processes such as the growth of budding yeast [1], cell division [2], the development of a fertilized egg into an organism [3], the transmission of nerve impulses [4], the transport of molecules across an epithelial cell layer [5], cell crawling [6] and lymphocyte homing [7], etc. Transient or stable cell polarization therefore constitutes a universal cellular trait in most multicellular organisms. One of the main challenges arising in this field during the last 15 years has been finding common molecular denominators between all these cellular events and processes, which may look very different in some respects but are all based on the development of cell polarity. Studies on these lines have led to the discovery of three polarity protein complexes that are all essential to epithelial polarity. In this review, it is proposed to report on how these complexes were discovered and to describe the structure of their components, focusing on the mammalian epithelial cells and tight junctions. The polarity of epithelial cells results in the presence of at least two plasma membrane domains: the apical surface facing the external medium and the basolateral surface connected to adjacent cells and connective tissue. The protein and lipid composition of these domains differs, reflecting their specific functions. Tight junctions provide a physical border between apical and lateral domains but their exact role in establishing and/or in maintaining this epithelial cell membrane asymmetry is still under debate. The newly identified partners at work in polarity protein complexes will be reviewed here, as well as the connections between them.

2. Genetic analysis of polarity complexes

Model organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster* provide useful means of describing basic biological processes. They lend themselves particularly well to addressing some of the questions arising in this field because of their structural simplicity and stereotyped features. In addition, their genomes have been completely sequenced and large-scale genetic screens are easy to perform. Genetic studies have shown the existence of evolutionarily conserved and ubiquitously expressed proteins that regulate cell polarization in many different cellular contexts (Table 1).

2.1. Par complex discovery

The first genes involved in cell polarization were identified by Kemphues et al. in 1988 [8], based on the asymmetric divisions occurring in the *C. elegans* zygote. After the fertilization step, zygotic asymmetric divisions leading to the formation of the germline contribute importantly to embryonic patterns of organization [9]. Each of these divisions is asymmetric, producing daughter cells differing in their size, cell division timing, cleavage pattern, and ultimate fate. In addition, the germline-specific P granules (ribonucleoprotein particles) are located at one pole of the cell prior to each division partition into the smaller daughter cells [10,11]. By performing genetic screens to detect maternal-effect lethal mutations disrupting these asymmetries, Kemphues et al. identified six genes called *par* (for partition defective) [8]. These mutations lead to abnormalities in the cleavage pattern, its timing and the distribution of P granules. Par proteins are therefore essential to the partitioning of early determinants and the development of polarity. Based on sequence analysis Par1 and Par4 were predicted to be protein kinases [12,13], while Par3 and Par6 contain PDZ (PSD-95, discs large, ZO-1) domains [14,15], Par5 is a 14.3.3 protein [16], and Par2 is a protein containing a zinc-binding domain of the ring finger class [17]. Par2 has no homologues in other species as far as we know. In line with their role in polarity, many of the Par proteins are asymmetrically distributed in the *C. elegans* one-cell embryo: Par1 and Par2 are

Table 1
Polarity complex proteins in *Drosophila melanogaster*, *Caenorhabditis elegans* and mammals

	Drosophila melanogaster	*Caenorhabditis elegans (1)*	Mammals				
			Gene symbols (2)	Human (2,3)			
				Aliases and previous symbols	K location	Isoforms	MW (kDa) unprocessed (4)
Par6/Par3/aPkc complex	DmPar6	–	*PARD6A*	PAR6; PAR6A; TIP40; PAR6α; PAR6C	16q22	2	37
		–	*PARD6B*	PAR6B	20q13	1	41
		Par-6	*PARD6G*	PAR6G; Par6γ; PAR6D	18q23	1	41
	Bazooka	–	*PARD3*	PAR3A; PAR3; PAR3α; ASIP; CTCL Tumor antigen se 2–5	10p11	10	180
		Par-3	*PARD3B*	PAR3β; PAR3L; ALS 2CR 19	2q33	5	140
	DmaPkc	PKC-3	*PRKCi*	PKCI; nPKCι; aPKCι/λ; PRKCι/λ	3q26	1	67
		–	*PRKCz*	PKC2; nPKCζ	1p36	1	68
Crb/Pals/Patj complex	Crb	Crb1	*CRB1*	RP12; LCA8	1q31–q32	4	154
		Eat-20	*CRB2*		9q33	3	134
		–	*CRB3*		19p13	2	13
		–	*MPP1*	EMP55	Xq28	1	52
		–	*MPP2*	DLG2	17q12–q21	3	65
		–	*MPP3*	DLG3	17q12–q21	1	66
		–	*MPP4*	DLG6	2q33	5	73
	Sdt	TAG-117	*MPP5*	Pals1	14q23	2	77
		C50F2.8	*MPP6*	Pals2; VAM-1; p55T	7p15	1	61
		Y55B1BR.4	*MPP7*		10p12	1	65
	Dpatj*	–	*INADL*	PATJ	1p31	5	196
		MPZ-1	*MPDZ*	MUPP1	9p24–p22	3	219
Scrib/Dlg/Lgl complex	Scrib	LET-413	*SCRIB*	SCRB1; CRIB1; Vartul; LAP4	8q24	4	175
	Dlg	dlg-1	*DLG1*	SAP97; hDlg	3q29	7	100
		–	*DLG2*	PSD-93; Chapsyn110	11q21	4	97
		–	*DLG3*	SAP102; NE-DLG	Xq13	1	90
		–	*DLG4*	PSD95; SAP90	17p13	2	80
		F44D12.1	*DLG5*	P-DLG	10q23	2	202
	Dlgl	– (5)	*LLGL1*	L2GL1; DLG4; HUGL1; LGL1	17p11	1	115
		–	*LLGL2*	L2GL2; HGL; LGL2	17q24–q25	3	113

(1) Source: http://www.wormbase.org/, *C. elegans* orthologs obtained after a blast search with human protein sequences in the worm base. (2) Source: http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl. (3) Source: http://www.expasy.org/. (4) Molecular weight of the amino acid sequence of the longest isoform in kiloDaltons (kDa). (5) Only one ortholog of the LGL-related protein family, Tomosyn (Tom-1), has been found to exist so far in *C. elegans*. (–) No orthologs were found in the database. *old name: Dlt.

located in the posterior cortex, Par3 and Par6 in the anterior cortex, while Par4 and Par5 are diffused throughout the cytoplasm. The *par* genes provide the polarity information required during the first cell cycle for cell-fate regulators to be properly asymmetrically distributed (reviewed in [18]). The proper distribution of Par1 requires the presence of the actin cytoskeleton and the non-muscle myosin, NMY-2, which interacts directly with a C-terminal region of Par1 [19]. Depletion of NMY-2 by RNA interference leads to *par*-like phenotypes in *C. elegans* embryos [19]. Par3 contributes to determine the restricted patterns of distribution of Par1 and Par2, because these proteins are uniformly present in *par3* mutants at the periphery of the embryo [15,20]. In 1996, Watts et al. [21] suggested that Par6 may act via Par3 by targeting or maintaining the Par3 protein at the cell periphery in *C. elegans* early embryos. These genetic analyses show that the localizations of Par proteins result from interdependent processes, although these proteins act together in a common process, which still remains to be identified. Two years later, Tabuse et al. [22] discovered a seventh *par* gene coding for an atypical protein kinase C (PKC3). Embryos lacking PKC3 as the result of RNA interference die, showing *par3*-like phenotypes [22]. Par3 and PKC3 proteins, which are colocalized in early *C. elegans* embryos, interact physically in vitro and are mutually dependent in terms of their asymmetric peripheral localization [22]. The concept of the first polarity protein complex, Par6/Par3/PKC3, was born.

2.2. Crb complex discovery

In 1990, Tepass et al. [23] established the existence of a link between epithelial polarity and *crumbs* (*crb*), a gene previously identified by Jürgens et al. [24] in *D. melanogaster*. The structure of the embryonic cuticle reflects the organization of the underlying epithelial epidermis from which it is secreted. *Crb* mutations are characterized by severe disruption of the cuticle (hence the name crumbs) and the epithelia originating from the ectoderm and extensive cell death in the epidermal primordium [23]. In the *Drosophila* embryo, the first cleavages occur in the absence of cytokinesis, giving rise to the syncytial blastoderm. Cellularization results from the invagination of plasma membranes to form the membrane furrow (reviewed in [25]). Cellularization of *Drosophila* embryo results in the formation of a cell monolayer showing many of the characteristics of polarized epithelia. In epithelial cells of *Drosophila* embryos at

later stages, the lateral plasma membrane consists of three distinct domains. The most apical domain comprises the subapical complex. It is followed by the zonula adherens, and further basally, by the septate junction. Embryos with *crumbs* mutations fail to assemble or stabilize a zonula adherens from spot adherens junctions [23]. This leads to the loss of cell polarity and adhesion, followed by breakdown of the epithelial structure and extensive cell death.

In epithelial cells, Crumbs (Crb) is found exclusively in the apical membranes and at the border between cells [23]. The *Drosophila crb* gene encodes an integral membrane protein (200 kDa) with a large extracellular part composed of 29 EGF-like and 3 laminin A/G domain-like repeats and a short cytoplasmic region of 37 amino acids [23]. This small domain is crucial for Crb function, since a stop mutation (crb^{8F105}) in this domain leads to a complete loss of function [26]. Conversely, overexpression of Crb results in the expansion of the apical plasma membrane and a concomitant decrease in the basolateral domain [27], and transforms the single-layered epidermis into a multilayered tissue [28]. In parallel there is a redistribution of β-Heavy-spectrin, a component of the membrane cytoskeleton [27]. Indeed Medina et al. have shown that the cytoplasmic domain of Crb links directly the β-Heavy-spectrin in *Drosophila* ectodermal cells [29]. Crb therefore plays a key role in specifying the apical plasma membrane domain of ectodermal epithelial cells of *Drosophila*. In 1993, Tepass and Knust [30] observed that the developmental defects caused by *stardust* (*sdt*) mutations were very similar to those associated with *crb* mutations. Studies on double mutant combinations of *crb* and *sdt* suggested that these genes may be part of a common genetic pathway (the *crb*/*sdt* pathway) in which *sdt* acts downstream of *crb* and is activated by the latter [30,31]. *Sdt* encodes a MAGUK (membrane-associated guanylate kinase) protein containing a single PDZ domain, a SH3 (Src homology region 3) domain and a GUK (homologous to the known guanylate kinases without enzymatic activity) domain [32], and as the result of alternative splicing, at least two isoforms of Sdt exist in *Drosophila*. Sdt binds to the four C-terminal amino acids of Crb via its PDZ domain [32,33].

In 1999, Bhat et al. [34] characterized a new protein containing four PDZ domains (called Disc Lost) that interacts with Crb and is essential for epithelial cell polarity. In fact, subsequent studies have shown that *dlt* locus encoded several genes. Mutations corresponding to the *dlt* phenotype disrupt *Drosophila* Codanin-1 homologue, a cytoplasmic protein, and not the formerly identified PDZ protein [35], which is now known as Dpatj. While Pielage et al. [35] reported that *Dpatj* mutations had no polarity phenotype, a recent study has shown that a truncated Dpatj protein containing the first two PDZ domains was still expressed and that a null allele indeed showed polarity defects in the early embryo [36]. These data confirmed those obtained upon removing Dpatj (using RNA interference methods) prior to the process of cellularization, which induced the complete loss of polarity, as shown by the pattern of Crb distribution [34]. Dpatj is a component of the Crb complex in *Drosophila* embryos [28,37] as well as adult flies [38]. By analogy with vertebrates, it has been suggested that the L27 (Lin2 Lin7 binding) domain of Dpatj may bind to the N-terminal L27 domain of Sdt. It has therefore emerged that Crb, Sdt and Dpatj form the second polarity complex.

2.3. Scrib complex discovery

The *scribble* (*scrib*) gene was identified in *Drosophila* using a screen for maternal mutations disrupting aspects of epithelial morphogenesis such as cell adhesion, shape and polarity [39]. The wild-type embryonic cuticle was found to have smooth, continuous sheet cuticles, whereas the *scrib* mutant embryos produce a corrugated cuticular surface that is riddled with holes (hence the name scribble) [39]. *Scrib* mutations cause broad defects in the epithelial organization leading to cells that are rounded, irregularly shaped and piled upon each other. The *scrib* gene encodes for a protein of 195 kDa with 16 leucine-rich repeats (LRR) and four PDZ domains, belonging to the LAP (LRR and PDZ) protein family [40].

The pattern of distribution of Scrib evolves from basal membranes to the zonula adherens at early embryonic stages, and it later targets the septate junction in mature epithelial cells. In *scrib* mutants, the polarity defects of epithelial cells do not result from the total loss of cell polarity but from a specific misdistribution of apical proteins. In *scrib* mutant, for example, apico-lateral markers such as Crb, Dpatj, E-cadherin and Armadillo are distributed throughout the plasma membrane of epithelial cells [39]. By contrast, basolateral markers such as β spectrin keep their restrictive localization. These findings indicate that one of the main functions of Scrib in epithelial polarity consists in excluding apical proteins (like the Crb complex) from the basolateral domain [39]. Bilder et al. [41] established the existence of correlations between two other genes, *lethal giant larvae* (*lgl*) and *lethal discs large* (*dlg*), which give the same embryonic phenotype as *scrib* mutant when they are mutated. These two genes were initially identified as tumor suppressors, because mutations resulted in tissue-specific tumors in larvae and their death, hence their names [42,43].

Mutated *lgl* affects cell growth and the cell adhesion properties of neural and imaginal disc cells during embryonic and larval development [44]. Lgl is a 130-kDa protein showing short motifs with sequence similarities with proteins having cell adhesion properties [45]. These short motifs are WD repeats consisting of approximately 40 amino acids and often ending in a Trp-Asp (W-D) dipeptide.

Mutations in the *dlg* tumor suppressor gene of *Drosophila* lead to neoplastic overgrowth of the imaginal discs [46]. Dlg is a 102-kDa protein and its amino acid sequence includes a core arrangement of several domains which are conserved in other MAGUK proteins: a L27 domain, a GUK domain, a SH3 motif, and three PDZ domains [46]. PDZ sequences [47] have previously been described as GLGF repeats [48] and Dlg Homology region domains [49].

The Lgl and Dlg proteins are located in an apical belt in the lateral cell membrane, at the septate junction. Scrib and Dlg overlap with Lgl in epithelial cells [41]. It has been suggested that these three proteins, Dlg, Lgl, and Scrib, which show common patterns of distribution and “loss of function”

phenotypes during the development of *Drosophila*, may form a biochemical complex that controls and refines the segregation of apical and basolateral membrane domains, although no physical interactions occur between them [39,41].

3. Polarity complex composition and interactions

3.1. Mammalian PAR complex

The PAR complex initially described in the nematode *C. elegans* and later in the fruit fly *D. melanogaster* and vertebrates is composed of two scaffold proteins, PAR6 and PAR3 and an atypical protein kinase C, aPKC (Table 1). This tripartite complex named PAR6/PAR3/aPKC is conserved from worms to vertebrates. PAR6, PAR3 and aPKC were first described as essential proteins for asymmetric division of the *C. elegans* zygote [21,50].

3.1.1. PAR6

Three PAR6 proteins encoded by three different genes have been identified in mammals: PAR6A/C, PAR6B and PAR6D/G (Table 1). All three have a similar molecular weight (37 kDa), function as part of a protein complex and contain three conserved domains mediating their interactions with the other members of the complex (Fig. 1). A Phox/Bem 1 (PB1) domain that binds to other PB1-domain-containing proteins such as aPKC is located at the N-terminal. The adjacent Cdc42/Rac interaction binding (CRIB) motif binds to the Cdc42 or Rac GTPases only in their activated GTP-bound state (reviewed in [51]). Lastly, all three have a PDZ domain that binds to other proteins such as PAR3 and CRB3 [52–55].

Although the three PAR6 proteins are structurally similar, they have different patterns of tissue and subcellular distribution. PAR6G is widely expressed, showing higher levels of expression in the kidney than elsewhere, and PAR6A and B show more restricted patterns with higher levels of expression (for details, see [52]; the authors used different nomenclature for the PAR6 compared to the database search genes: PAR6A is PAR6G, PAR6B is PAR6B and PAR6C is PAR6A). PAR6A and B are both present in the pancreas, kidney and placenta, but show different patterns of lung and liver expression, and PAR6A alone is also found in the skeletal muscle, brain and heart. A difference was recently reported in mammals between the patterns of expression of PAR6A and B in the oocytes during mouse embryonic development [56]. Louvet-Vallee et al. [57] have reported that PAR6A is only detected at the 2-cell stage, whereas PAR6B is expressed from the 2-cell to blastocyst stage [58]. In addition, before cell compaction occurs, PAR6B is located only in nuclei, and after the compaction PAR6B is targeted the apical pole of the 8-cell stage blastomere [58]. This is also the case in epithelial MDCK cells, where PAR6 proteins show different patterns of distribution [59]. PAR6A is present in both at tight junctions and in the cytosol, whereas PAR6B is localized to the cytosol; whereas PAR6G predominantly colocalizes with tight junction markers [59]. The specific spatio-temporal localizations for each PAR6 proteins strongly suggest different functions.

We will focus on the function of PAR6 in epithelial cells. Since PAR6 proteins do not include enzymatic domains, they function by bringing together several proteins at a specific localization (tight junctions, the leading edge of a migrating cell, the tip of the growing axon, etc.) (reviewed in [60]). It has been reported that in mammalian epithelial cells, overexpression of PAR6B delays the formation of tight junctions without affecting the adherens junctions [61], and that when PAR6B is overexpressed in cells already polarized, the tight junctions are disrupted [52,62]. Interestingly, Joberty et al. [52] reported that the PDZ domain of PAR6B is sufficient to disrupt tight junctions. Surprisingly, PAR6G, which is known to be associated with tight junctions, does not affect these junctions when it is overexpressed [59]. Although the PDZ domains are very similar between PAR6B and G, they do not bind with the same affinity to PALS1 [59,63], a protein known to be tightly associated with tight junctions. When PAR6B is overexpressed, it may take PALS1 away from the junctions; PAR6B binding to PALS1 can in fact interfere with PATJ binding to PALS1, and these interactions do not work synergistically [63].

The exact function of PAR6 has not yet been elucidated, but since Garrard et al. [64] established that Cdc42-GTP can induce a conformational change in PAR6B, Gao and Macara [59] developed a functional model for PAR6, in which the N-terminal folds back and interacts with the CRIB-PDZ domain. Cdc42-GTP binding to PAR6 [65] results in the unfolding of PAR6B and A, thus exposing the PALS1 binding site (PDZ domain in PAR6), whereas in PAR6G, this domain is too stable to be released.

The key function of PAR6 is to allow the interaction between aPKC and its downstream effectors such as PAR3 and LGL (described in (Sections 3.1.2, 3.1.3, and 3.3.3)) [66]. Phosphorylation of LGL will result in its detachment from the aPKC/PAR6 dimer, leading to the formation of another functional complex: PAR6/PAR3/aPKC [66].

3.1.2. PAR3

Two *PAR3* genes, *PAR3A* and *PAR3B*, have been identified in mammals (Table 1). *PAR3A* encodes three proteins with different molecular weights (180 kDa, 150 kDa, 100 kDa). All the PAR3A protein isoforms contain three PDZ domains (Fig. 1) and only the shortest isoform lacks the aPKC binding region. The first PDZ of PAR3A interacts with PAR6 [54]. PAR3B proteins (140 kDa) include a region which is homologous to the aPKC-binding domain, as well as the three PDZ domains. Strikingly, PAR3B does not seem to interact with aPKC [67,68], and its interaction with PAR6 is still not clearly understood. PAR3A is widely expressed in various tissues, showing higher levels of expression in the heart, kidney (where all three isoforms are present), and brain (where the 180-kDa and 150-kDa isoforms are present) [54]. During mouse embryonic development, PAR3A 180-kDa and 100-kDa isoforms are expressed earlier than the 150-kDa isoforms. The last one can be detected from day 14.5 of development onwards. PAR3B is strongly expressed in the kidney, lung and skeletal muscle and in lower levels in the brain heart, liver and pancreas. PAR3A and PAR3B are partially

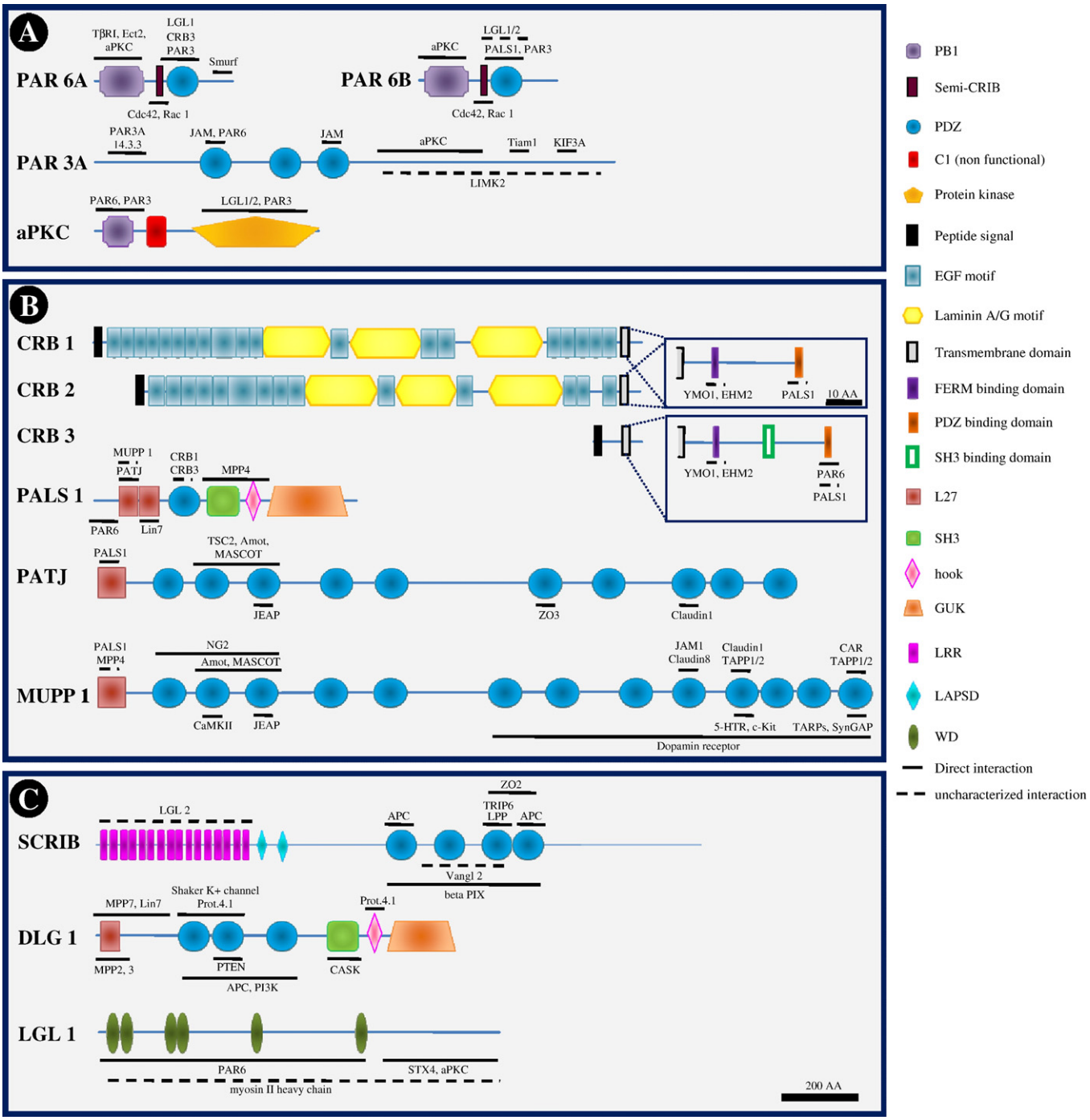

Fig. 1. Structure and interactions of mammalian polarity complex proteins. (Part A) PAR complex, (part B) CRB complex and (part C) SCRIB complex. Domains are shown according to the SMART (Simple Modular Architecture Research Tool) database (http://smart.embl-heidelberg.de.). All interactors are mentioned in the text except for TβR1 (TGFβ receptor 1) [182], Smurf [182], Ect2 [183], KIF3A [184], MASCOT [166], JEAP [166], CamKII [185], TAPP1/2 [186], TARPs [187], synGAP [185], dopamine receptor [188], Vangl2 [128], Shaker K^+ channel [189].

located in cell–cell contact regions and colocalized with ZO1 at tight junctions [65,67–69].

PAR3A has been extensively studied in epithelial cells. It seems likely that the starting point required for PAR3A to target the tight junctions is its ability to form self-associations and to bind to the junctional adhesion molecules (JAMs). PAR3A forms a homodimer via its N-terminal region; this association seems to be required for the correct association of PAR3A at the apical side of the cell–cell contact region during the process of polarization [70]. This mechanism may also be adopted by PAR3B [52]. PAR3A may then be stabilized upon binding directly to JAM via its first PDZ domain, and these two proteins may then be co-distributed to the sites of cell–cell contact [71]. As JAMs are present at newly formed cell–cell contacts prior to

PAR3A, they can serve as anchors for the recruitment of PAR3A to the junctional complex at an early stage in the junction formation process [72]. Once PAR3A occupies this site, it can play the role of a scaffold in the recruitment of proteins involved in the formation of the junctions, such as PAR6 [52] or aPKC [73]. Many studies have in fact shown that overexpression or depletion of PAR3A in epithelial cells leads to the disruption of tight junctions, along with the mislocalization of PAR6, aPKC and tight junction markers [52,69,70,74].

PAR3A interacts with members of the PAR complex and with other proteins such as 14.3.3 [75], LIMK2 [76,77], Tiam1 (reviewed in [78]) and certainly with other unknown partners. These interactions seem to be dependent on the state of phosphorylation of PAR3 [75,76], which is partially regulated by aPKC. The mechanisms underlying these different interactions suggest that PAR3A may be involved in the regulation of the dynamics of the cytoskeleton (reviewed in [78]).

3.1.3. aPKC

In mammals, two *aPKC* genes, *aPKCλ/ι* and *aPKCζ* encoding two different proteins have been identified (Table 1) [79,80]. They have similar molecular weights (75 kDa) and function as part of the polarity complex PAR (reviewed in [51]). Unlike conventional PKCs, aPKCλ/ι and aPKCζ are unique in having a PB1 domain in the N-terminal, which is known to interact with PAR6. The lack of a C2 domain and the incomplete C1 domain prevent aPKCλ/ι and aPKCζ from being activated by Ca^{++}, diacyl-glycerol and phorbol esters in the same way as classical PKCs [81]. The only domain conserved among PKC proteins is the catalytic domain present within the C-terminal region [82,83] (Fig. 1). This domain is known to phosphorylate several proteins such as PAR3 [84] and LGL [85]. In addition, Yamanaka et al. [66] have demonstrated that both LGL and PAR3 can form independent complexes with aPKC/PAR6 to regulate epithelial cell polarity.

aPKCλ/ι and aPKCζ are strongly expressed in the lung and brain, and the latter protein is also present in the kidney and testis. Many studies have been performed on epithelial cells, where both isoforms are expressed, but the data available so far do not point to the existence of any specific function for one or other isoform. We will therefore not distinguish here between the two isoforms.

In MDCK epithelial cells, aPKCs localize with the other members of the PAR complex at tight junctions [69]; and it is worth noting that aPKCs are the only members of the complex showing catalytic activity. This kinase activity is required for the formation of tight junctions to occur, as overexpression of kinase-dead aPKC mutant blocks their formation, and leads to mislocalization of PAR6 and PAR3 [73]. The results of several studies have led to the conclusion that the interaction between the N-terminal regions of PAR6 and aPKC is constitutive in epithelial cells [86]. As Cdc42-GTP binds to PAR6, it seems likely that Cdc42-GTP may form a complex with aPKC via the adaptor PAR6. Noda et al. [86] have established that the expression of Cdc42-GTP leads to the translocation of aPKC from the nucleus to the cytoplasm and cell periphery, where the complex will be involved in tight junction formation. Cdc42 is activated upon E-cadherin mediated cell–cell adhesion [87], resulting in phosphorylation and thus activation of aPKC, and this chain of events is crucial to tight junction formation [88,89].

3.2. Mammalian CRB complex

The Crumbs complex was identified in the epithelia of *Drosophila* and subsequently in vertebrates (Table 1). Mammalian CRB are transmembrane proteins, whereas the other proteins present in this complex, PALS1 and PATJ, are cytoplasmic scaffolding proteins.

3.2.1. CRB

Although there is only one *crumbs* gene in *D. melanogaster*, three genes denoted *CRB1*, *2*, and *3* have been identified in mammals, and their products have been detected in expressed sequence tag (EST) databases [90]. CRB1 and CRB2 are mainly expressed in the retina and brain [91] and CRB2 is also present in the kidney [92]. CRB3 is mainly expressed in skeletal muscles and in all epithelial tissues [53,93,94]. These localizations suggest the existence of a tissue-related functional specialization of CRB proteins in mammals.

In their extracellular domains, CRB1 (154 kDa) and CRB2 (134 kDa) consist of 3 Laminin A/G domains and 19 and 14 EGF-like domains, respectively [95,96] (Fig. 1). CRB3 (13 kDa) on the contrary has a very short extracellular domain but no recognizable protein domain and includes O and N glycosylation sites [90] (Fig. 1). These 3 proteins have a transmembrane domain and a cytoplasmic domain very well conserved, although different splice variants of *CRB*1 and *CRB*2 encoding secreted proteins lacking the transmembrane and intracellular domains have been described [92,95–97].

CRB1 mutations are responsible for retinal diseases, Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP) [91,95]. It has been suggested that CRB1 may contribute to these disorders by impairing cell adhesion properties or the formation of the apical surface [91,95]. Despite the high degree of similarity existing between the structure and patterns of expression of CRB1 and CRB2, CRB2 has not been found to be involved in these pathologies [92]. The function of CRB2 is not clearly understood yet.

CRB1, CRB2 and CRB3 have a very similar cytoplasmic domain showing 63%, 68% and 54% identity with *Drosophila* Crumbs, respectively [98,99]. In their cytoplasmic tails, CRB proteins contain two motives: a FERM (band 4.1-ezrin-radixin-moesin) protein-binding domain consisting of 12 amino acids containing a GTY motif [28,100] and a PDZ-binding domain consisting of ERLI residues [53,55]. The FERM domain is a protein–protein interaction domain which exists in various proteins, many of which serve as adapters linking transmembrane proteins to the cortical actin cytoskeleton [101]. The FERM domain is involved in recruiting a Dmoesin and β-Heavy-spectrin network in *Drosophila* ectodermal cells [29]. CRB3 has an additional SH3 binding site (consisting of PxxP residues), which has not been found to exist in any other CRB [53,102], and may mediate the involvement of CRB3 in specific pathways.

Crb contributes to stabilizing apical cell junctions in *Drosophila*, and many studies have shown during the last few years that it plays a similar role in mammals. Fogg et al. [93] have shown that loss of the ERLI sequence or point mutations in the FERM binding motif severely affects the ability of CRB3 to induce tight junctions in human epithelial MCF10A cells, while the SH3 binding motif is not involved in this process. Overexpression of CRB3 in MDCK cells delays the formation of tight junctions, and this effect depends on the last four ERLI residues of CRB3 [53,103].

In addition to playing a role in the formation of tight junctions, CRB3 is involved in the differentiation of the apical membrane. Fan et al. [104] have shown, for example, that in addition to its previously observed presence at apical and tight junctions, CRB3 is also present in discreet puncta in the primary cilia of MDCK. The authors have established that CRB3 contributes to cilium formation, since downregulation of CRB3 expression resulted in the inability of MDCK cells to form cilia [104].

3.2.2. PALS1

PALS1, also known as membrane-associated palmitoylated protein 5 (MPP5), is the mammalian homologue of the *Drosophila* Sdt protein. PALS1 was first cloned as a new binding partner for Lin7 [105]. Roh et al. [106] have established that PALS1 is an adaptor protein mediating indirect interactions between CRB and PATJ. High levels of PALS1 mRNA expression have been detected in the placenta and kidney, and moderate levels in the brain, heart and skeletal muscle [105].

PALS1 (77 kDa) is a scaffold protein that has multiple protein–protein interaction domains and belongs to the MAGUK (membrane-associated guanylate kinase) family [105] (Fig. 1). PALS1 consists of two L27 domains, a PDZ domain, an SH3 domain, a hook domain and a GUK domain [32,33,105]. The function of the SH3 domain and GUK domain of PALS1 still remains to be established. However, extensive intramolecular interactions have been found to occur between SH3 domain and GUK domain in MAGUK proteins [107,108].

In mammals, PALS1 interacts directly via its PDZ domain with the ERLI motif of the cytoplasmic domain of CRB1 [106]. PALS1 was the only interactor identified in a yeast two-hybrid screen performed on a retinal cDNA library using the intracellular domain of CRB1 as bait [106]. CRB3 is also linked to the PDZ domain of PALS1 [94]. In addition, the first L27 domain interacts with PATJ and the second with Lin7. Knock down of PALS1 in MDCK cells leads to tight junction and polarity defects [109] and to the mis-targeting of E-cadherin to the cell membrane [110]. Furthermore, loss of PALS1 resulted in concomitant loss of PATJ expression, but had apparently no effect on CRB3 expression or distribution [109,110]. It is not yet known whether the absence of PALS1 affects PATJ expression via the transcription, translation or stability of the protein. Since the presence of PALS1 in mammalian epithelia depends on interactions with PATJ [111], it seems possible that the stability of these two proteins may depend on interactions between them [109].

3.2.3. PATJ/MUPP1

There exist two mammalian homologues of the *Drosophila* Dpatj: PATJ (Pals-associated tight junction protein) [37], which is also known as human InaD-like (inactivation no after-potential D) [112], and MUPP1 (multi-PDZ domain protein) [113] (Table 1).

3.2.3.1. PATJ. PALS1 can bind to PATJ, a multiple PDZ domain containing protein localized at tight junctions. PATJ (196 kDa) expression has been found to occur mainly in epithelial tissues: bladder, testis, ovary, small intestine, colon, heart, pancreas, kidney, and lung, as well as the brain and skeletal muscle [99,112].

The interactions between PALS1 and PATJ involve the binding of the first L27 domain of PALS1 to L27 domain of PATJ (domain also called Maguk Recruitment: MRE) [106]. The PATJ L27 domain present at the N-terminal is followed by up to ten PDZ domains [37,99] (Fig. 1). The 6th and 8th PDZ modules of PATJ interact directly with ZO3 and Claudin1, respectively, via the PDZ-binding domain present at the C-terminal ends of these proteins [37]. The ZO3 cytoplasmic protein is linked to tight junctions via its association with Occludin [114]. It is worth noting that Occludin and Claudin1 are transmembrane proteins that constitute the basic architecture of tight junctions. The fact that overexpression or downregulation of PATJ in epithelial cells disrupts the tight junction-specific localization of ZO1, ZO3 and Occludin, suggests that PATJ might be involved in stabilizing tight junctions [99,111,115]. The downregulation of PATJ also affects the distribution of the other CRB complex proteins, CRB3 and PALS1, which were no longer accumulated at the apical membrane and at tight junctions [111]. These data strongly suggest that PATJ provides a link between the lateral (Occludin and ZO3) and apical (PALS1 and CRB3) components of tight junctions and stabilizes the CRB3 complex [37,111].

3.2.3.2. MUPP1. MUPP1 (219 kDa) was originally identified as a protein that interacts with the C-terminal of the serotonin 5-hydroxytryptamine type 2 receptor [113]. MUPP1 has a L27 domain in its N-terminal and 13 PDZ domains (Fig. 1). MUPP1 and PATJ PDZ domains show high levels of homology and bind to some identical proteins. For example, PDZ 10 of MUPP1 and PDZ 8 of PATJ show 78% homology and both bind to Claudin1. MUPP1 is concentrated at tight junctions [116,117] and has been detected in the heart, brain, placenta, liver, skeletal muscle, kidney and pancreas [113].

MUPP1 interacts with several molecules via its PDZ domains. Although its functional characteristics in epithelial cells have not yet been addressed, MUPP1 may act as a scaffold for several tight junction components, since a number of tight junction proteins have been found to bind to MUPP1, including JAM and CAR (coxsackievirus and adenovirus receptor), as well as Claudins [116–119]. MUPP1 may serve as a cross-linker in tight junctions between Claudin-based tight junction strands and JAM oligomers. However, the occurrence of 13 PDZ domains in tandem within single MUPP1 molecules might

indicate that many proteins other than Claudins and JAM may be tethered to the tight junction via MUPP1 molecules.

MUPP1 is linked to the CRB complex, since its L27 domain binds to PALS1. Roh et al. [103,106] have established that PALS1 binds to the L27-like domain of PATJ and MUPP1, and that PALS1 binds to CRB1. Studies based on the immunoprecipitation of MUPP1 from retinal lysates recently showed that endogenous MUPP1 interacted in photoreceptor cells in a protein complex with CRB1, PALS1 and MPP4, in agreement with the finding that CRB1 and MUPP1 are colocalized in the retina [120]. In *CRB1*$^{-/-}$ retinas before the onset of retinal degeneration, the complex consisting of MUPP1, PALS1 and MPP4 was formed in the absence of CRB1, which indicates that CRB1 is not essential to this interaction. MAGUK proteins may form heterodimers or homodimers [121], and indeed it has been reported that MPP4 interacts with PALS1 [122]. All these data suggest that in photoreceptor cells CRB1/PALS1-MPP4/MUPP1 complexes are probably present. Since PATJ is also colocalized with CRB1 and MUPP1 at the subapical region, it is conceivable that CRB1/PALS1-MPP4/PATJ complexes may also exist.

3.3. Mammalian SCRIB complex

Mammalian SCRIB, LGL and DLG show similar sequences and functions to those of their *Drosophila* homologues (Table 1). SCRIB, LGL and DLG are localized in the basolateral domain of epithelial cells [123–125]. The exact nature of the physical interactions between SCRIB, LGL and DLG has not yet been clearly defined. In *Drosophila* neuronal synapses, Scrib associates physically with Dlg via a protein termed GUK Holder [126], which was recently characterized in humans [127], and physical interactions between SCRIB and LGL2 were recently found to occur in polarized mammalian epithelial cells [128].

3.3.1. SCRIB

The only homologue of *Drosophila* Scrib described so far in mammals [129,130], which is also known as VARTUL, was first isolated in a biochemical screen designed to identify the proteins targeted by the E6 oncoprotein of the human papilloma tumor virus (HPV), the main agent responsible for cervical cancer [129]. The expression levels of SCRIB protein are low in the kidney, skeletal muscles, liver and lung and high in the breast, intestine, placenta and skin [123]. In these tissues, positive staining for SCRIB was mostly detected in epithelial cells [123]. SCRIB is also present in mouse eye and human colon epithelia, along with DLG1 [131,132].

SCRIB (175 kDa) is a large cytoplasmic multidomain protein that plays many roles in flies and mammals [39,41,129,133–135]. SCRIB is a member of the LAP protein family, which also includes Erbin [136], Densin180 [137] and Lano [138]. SCRIB has 16 LRR at its N-terminal, followed by 2 LAP-specific domains (LAPSD), a linker region, 4 PDZ domains and a C-terminal lacking any identifiable motives [130] (Fig. 1). Navarro et al. [123] have established that the LRR repeats occurring in SCRIB determine its ability to target the basolateral epithelial membranes.

SCRIB binds directly to the C-terminal motif of ZO2 via its PDZ domains 3 and 4 [139]. ZO2 interacts with several components of the tight junctions, including ZO1, Occludin, Cingulin and Claudins [140–142]. Metais et al. [139] detected a partial pattern of colocalization between GFP-SCRIB and ZO2 at the cell junctions of unpolarized cells. SCRIB was not colocalized with tight junction markers in polarized cells, whereas it was colocalized with β-catenin, an adherens junction marker [123]. The SCRIB/ZO2 interaction therefore probably takes place at the cell junctions before ZO2 is segregated in the tight junctions [139]. SCRIB and E-cadherin are mutually dependent for their proper targeting to adherens junctions [123,143]. Suppression of SCRIB expression in MDCK cells causes a delay in tight junction assembly and affects the epithelial morphology [143].

3.3.2. DLG

There exist five mammalian DLG proteins with molecular weights ranging from 80 to 200 kDa. Mammalian family DLG members show the characteristic MAGUK structural domains, including three PDZ domains, a SH3 domain, a hook domain (also known as 4.1 binding domain) and a GUK domain (Fig. 1). In addition to this basic structure, DLG1 has a L27 domain at the N-terminal (as occurs in the case of *Drosophila* Dlg), and DLG5 has a fourth PDZ domain and an extension forming a coiled-coil structure at its N-terminal. We will focus here on DLG1 because it is the most closely related to *Drosophila* Dlg and the most frequently studied DLG in epithelial cells [144]. The authors detected *DLG1* transcripts in the human brain, skeletal muscle, kidney, liver, cardiac muscle, and lung tissue. In addition, DLG1 targets the basal membrane in intestinal cells [145].

The L27 domain of DLG1 is able to bind to MPP2, MPP3 [146], MPP7, Lin7 [147,148] and Lin2/hCask [149]. The interaction between DLG1 and MPP7 facilitates epithelial tight junction formation, and this complex combined with Lin7 regulates the stability of DLG1 and its distribution to cell junctions [148]. Lin2/hCask is a calmodulin-associated Ser/Thr kinase (Cask), another member of the MAGUK protein family, and its association with DLG1 (via Cask L27N and DLG1 L27domains) is crucial to the lateral distribution of DLG1 in MDCK cells [149]. In addition, the GUK domain of Cask is also able to interact with the SH3 domain of DLG1 [121]. The lateral localization of DLG1 may also depend on interaction with the cytoskeleton protein 4.1, via PDZ 1 and 2 on DLG1 and a hook domain [150]. The hook domains provide a link with the cortical actin cytoskeleton after binding to members of the FERM superfamily [151].

Matsumine et al. [152] described the occurrence of interaction between PDZ domains of DLG1 and the C-terminal region of adenomatous polyposis coli protein (APC) associated with β-catenin. APC is mutated in familial adenomatous polyposis and in sporadic colorectal tumors. In addition, APC and DLG1 are colocalized in the basolateral membrane in rat colon epithelial cells. The second PDZ of DLG1 interacts with PTEN (protein tyrosine phosphatase and tensin homologue) [153], and this link is disrupted by phosphorylation of the PTEN

PDZ-binding domain present at its C-terminal (ITKV). Tyrosine-phosphorylated DLG1 recruits PI3K (phosphoinositide 3-kinase) to the lateral membrane by interacting with the SH2 domain of p85/PI3K in post-confluent differentiating Caco2 cells [145]. Cells containing low levels of DLG1 (using RNA interference method) fail to recruit PI3K to E-cadherin-mediated cell–cell contacts and their cortical actin cytoskeleton remains disorganized [154].

DLG1-deficient mice expressing a truncated DLG1 protein lacking SH3, protein 4.1 and GUK domains show delayed growth in utero and die perinatally [155,156]. The basolateral localization of truncated DLG1 was altered and restricted to the adherens junction, disrupting the epithelial polarity and the process of nephrogenesis, due to either ureteral branching morphogenesis and/or delayed mesenchyme-to-epithelial transition [156]. DLG1 is involved in regulating the structural organization of the epithelial ducts during ontogeny [156]. Identification and characterization of GUK domain partners will help to further elucidate the functional role of DLG1.

3.3.3. LGL

Two LGL proteins encoded by two different genes have been identified in mammals, namely LGL1 (115 kDa) and LGL2 (113 kDa), which are homologous to the Lgl *Drosophila* protein (Table 1). Two other related LGL proteins have been described in mammals: syntaxin-binding protein 5 (LGL3, 120–130 kDa) and syntaxin-binding protein-5-like (LGL4, 130 kDa) [157], which are homologous to the Tomosyn *Drosophila* protein. They all contain repeated WD40 domains: six in the case of LGL1, LGL3 and LGL4, and five in the case of LGL2 (Fig. 1). These WD40 repeats are known to form β-propellers that act as protein interacting modules for SCRIB, but this point remains to be further investigated, as Kallay et al. [128] have only demonstrated it for LGL2. Among these four human *LGL* genes, *LGL1* and *LGL2* have been the most thoroughly studied to date [66,125], and we will therefore use LGL1/2 here to denote both of these isoforms. LGL1 is expressed in the kidney and brain, and LGL2 is expressed in the lung, brain and testis. In the epithelia of vertebrates, LGL1/2 are localized to the lateral membrane [66,125,128], the region below the adherens junctions.

In MDCK epithelial cells, it has been reported that overexpression of LGL1/2 during the polarity establishment phase disrupts the formation of junctional complexes; whereas its overexpression in confluent polarized cells has no such effect [66]. Previously data indicated that LGL1/2 have to be phosphorylated to be able to adopt its restricted basolateral localization, as non-phosphorylatable LGL1/2 leaked into the apical domain of MDCK cells [125]. This phosphorylation is mediated by aPKC during the epithelial polarity establishment phase [66,125], leading to the detachment of LGL1/2 from the PAR6/aPKC dimer [66].

As LGL3 was found to exist in complex with the plasma membrane protein t-SNARE syntaxin 1 in neuronal cells, and since antibodies raised against LGL3 inhibit the exocytosis of dense core vesicle, Musch et al. [125] suggested that LGL1 may contribute to cell polarity by regulating polarized exocytosis in MDCK epithelial cells. LGL1 has in fact been found to interact specifically with syntaxin4 (STX4) (a t-SNARE specific for the lateral plasma membrane), which suggests that LGL may contribute to apico-basal polarity by regulating basolateral exocytosis [125].

4. Functional interactions between complexes and activation

The formation of a polarized epithelial cell layer with functional tight junctions requires spatio-temporal coordination of the activity of the polarity complexes. The three complexes previously described in this review regulate the establishment and maintenance of the apical polarity in the cell. However, their localizations are distinct: CRB and PAR complexes are restricted to the apical region of the lateral membrane whereas SCRIB complex is concentrated along the lateral membrane.

In this part, we will review how the different polarity complexes cooperate or interact together. It has by now been clearly established that the three complexes are connected through several protein–protein interactions. Genetic studies on flies have shown that mutants for Scrib complex show opposite phenotypes to those observed for mutants of members of the Crb and Par complexes [39], which suggests that the basolateral SCRIB complex and the apical CRB and PAR complexes have antagonistic activities. A connection between these different complexes was shown using genetic studies on flies and biochemical studies on mammalian cells [39,158] (Fig. 2).

E-cadherin/E-cadherin interactions in the cell–cell adhesion region trigger Cdc42-GTP activation [87] and the phosphorylation of aPKC, which in turn phosphorylates LGL. Phosphorylated LGL dissociates from PAR6/aPKC dimer and distributes to the lateral membrane, where it could interact with DLG and SCRIB [85]. aPKC is then able to interact with and phosphorylate PAR3, allowing the formation of the active PAR complex at the apical junctions [69,159]. A direct connection therefore exists between the activity of the basolateral complex containing LGL and the active apical PAR complex.

aPKC is required for the stable localization of PAR3, and PAR3 phosphorylated at S827 residue accumulates at tight junctions [84,160]. Once it has been phosphorylated at the S827 residue, PAR3 therefore dissociates from aPKC [84]. The existence of this mechanism is supported by the fact that in *Drosophila* Par1 phosphorylates Par3 inducing the fixation/binding of the adaptor 14.3.3 protein, preventing the association between Par3 and DmPar6/DaPkc dimer [161]. The kinase domain of aPKC is then released and is free to phosphorylate other proteins.

aPKC is able to bind directly to the CRB cytoplasmic tail that contains two threonine residues (T6 and T9) in an evolutionarily conserved region, which are potential targets for aPKC phosphorylation. Interestingly, Dpatj is able to modulate the phosphorylation of Crb by aPkc that is required for the proper localization of aPkc and Dpatj to the apical membrane and that of Scrib to the basolateral domain [162].

CRB3 binds to PAR6 directly [53] or via PALS1 [62], to promote the differentiation of the premature junctional structure into mature epithelial structures. In addition, overexpression of

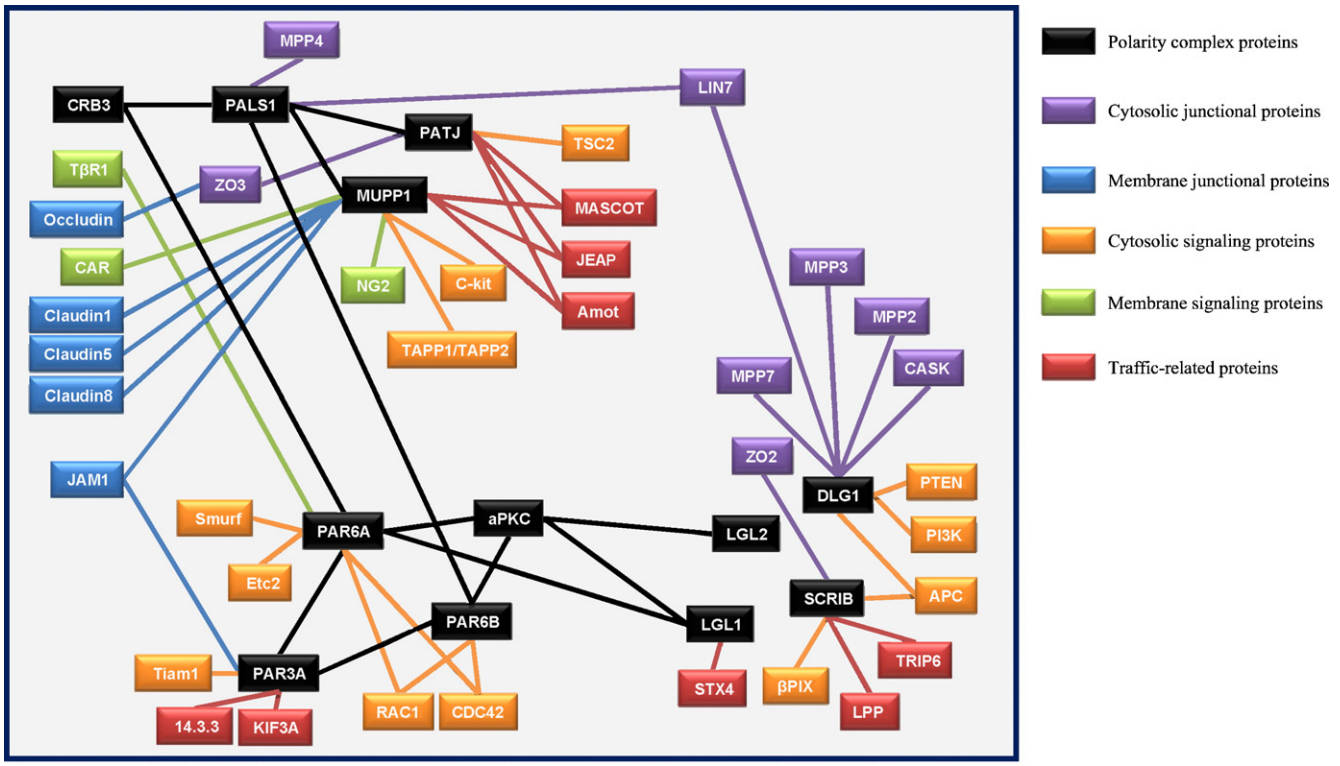

Fig. 2. Polarity complex protein network in mammalian epithelial cells. This figure illustrates some of the many interactions that occur in epithelial cells. Colors show the specific localization (cytosolic or membrane-associated) and function of the proteins.

both PAR6 and CRB3 in MDCK cells delays the assembly of tight junctions [53,61], which strongly suggests that PAR6 and CRB3 are involved in the same pathway, leading to the formation of tight junctions. The existence of a relationship between CRB complex and PAR complex has been further supported by the finding that localization of aPKCζ to tight junctions was inhibited in PALS1 siRNA expressing epithelial cells [109], probably via the scaffold protein PAR6 that binds to aPKC and PALS1. Interestingly, PAR3A was not mislocalized in the PALS1 knock down cells, which suggests that PAR3A is not recruited at the apical domain epithelial cells via PAR6, but that it is rather anchored to junctional complexes by JAM proteins present just below the CRB complex [71].

Furthermore, a study in *Drosophila* has reported that Dpatj can interact directly with Dmpar6 providing another potential mechanism linking the Crb to the Par complex [163]. In contrast, Wang et al. in 2004 [63] have shown that at least in MDCK epithelial cells, PAR6 binding to PALS1 interferes with PATJ binding to PALS1, and that these interactions do not work synergistically. The CRB-conserved ERLI motif may therefore link CRB to the PAR complex via multiple mechanisms.

In addition to interact with each others, these three complexes have been found to interact with cytoskeleton-related proteins, such as 14.3.3 protein for PAR complex [75], YMO1 (yurt/mosaic eyes like 1) protein for CRB complex [164] and Myosin II for SCRIB complex [165]. These interactions modulate the dynamic of actin cytoskeleton, one of the key events controlling cell shape and polarity.

All these data suggest that the SCRIB complex restricts the localization of CRB and PAR complexes to the apical region of epithelial cells, where they may act together to regulate tight junction formation.

5. Conclusion and perspectives

Although polarity complexes are involved in the formation and maintenance of tight junctions in mammalian epithelial cells, the exact mechanisms underlying their role are not yet understood. In addition, these polarity complexes have been found to be involved in other cellular events such as gene expression, differentiation, motility and growth by the identification of new molecular interactions (Fig. 2).

Recently, new interactors have been identified like EHM2 (expressed in high-metastatic cell) and YMO1, which is also known as EPB41L5 (erythrocyte protein band 4.1-like 5), which interact in a FERM domain-dependent manner with CRB1, CRB2 and CRB3 [164]. Their *Drosophila* homologue (Yurt) acts as a negative regulator of Crb activity both in epithelial polarity processes and at the apical membrane surface via a mechanism that is not yet fully understood [164].

Wang et al. [110] recently reported that PALS1 is involved in the regulation of E-cadherin trafficking. E-cadherin accumulates in intracellular structures inside PALS1 knock down cells, which indicates that the CRB complex might regulate the recycling of adhesion molecules. The existence of link between the CRB complex and vesicular trafficking agrees with the finding that PATJ and MUPP1 bind to Angiomotin (Amot)

[166], which is required (along with Rich1, a Cdc42-GAP) for the targeting of PALS1 and PAR3 to the plasma membrane [167]. Moreover in *Drosophila*, proteins involved in membrane recycling such as Avalanche and Rab5 also contribute importantly to the proper localization and functional integrity of the Crb complex [168].

The occurrence of 10 and 13 PDZ domains in PATJ and MUPP1, respectively, might indicate that many proteins other than Claudins and JAM can be tethered to the tight junction via MUPP1 and PATJ molecules. MUPP1 certainly interacts via these domains with several molecules (Figs. 1 and 2), including the proto-oncogene c-Kit [169], the transmembrane proteoglycan NG2 [170], an adenovirus E4-ORF1 oncoprotein [171], and Amot [166], which suggests the possible involvement of MUPP1 and PATJ in growth, proliferation and cell movement.

This hypothesis is in line with a recent interaction found to occur between PATJ and the tuberous sclerosis complex 2 (TSC2 or Tuberin) protein [172]. TSC2 associated to TSC1 (Hamartin) forms a complex that regulates the mTOR cascade through the GAP activity of TSC2 on Rheb, a small GTP-binding protein [173]. PATJ binds to the last amino acids of TSC2 via its PDZ domains 2 and 3, and depletion of PATJ from human epithelial intestinal cells induced an upregulation of the mTOR pathway, indicating that the CRB3 complex is able to regulate the activity of TSC on the mTOR cascade [172]. This finding opens new paths for understanding the role of the CRB complex in the regulation of cell metabolism, size and survival.

SCRIB has been found to be involved in the recycling and signaling of transmembrane receptor TSHR (thyroid stimulating hormone receptor) via the βPIX/GIT1/ARF6 pathway [174]. The interactions occurring between SCRIB and members of the Zyxin family (TRIP6 and LPP) have also suggested the existence of a new communication pathway between cell contact and the nucleus [175,176].

The PDZ domains of DLG interact with tumor suppressor proteins, APC and PTEN, as well as with several viral oncoproteins such as the E6 protein present in oncogenic human papillomavirus (reviewed in [177]). It has been reported that overexpression of DLG in fibroblasts impairs the events in the G0/G1 to S phase and inhibits cell proliferation [178]. DLG, like SCRIB, is therefore likely to be involved in the negative regulation of cell proliferation [177]. The interaction between APC and DLG regulated by PAR complex is involved in polarized cell migration [179].

Mammalian LGL proteins are linked to the exocytic machinery by direct interactions with SNARE proteins such as Syntaxin [125,180,181]. Zhang et al. [181] proposed that interactions between LGL proteins and the exocyst are important for the establishment and reinforcement of cell polarity. LGL1 is also associated with non-muscle heavy chain myosin II in a cytoskeletal network [165].

In conclusion, epithelial polarity complexes act at several cellular levels. PAR complex is required for defining the proper apico-lateral axis via mechanisms that are probably conserved between asymmetric cell division and establishment of epithelial polarity. CRB and SCRIB complexes are essential to setting up and maintaining apical and lateral identities acting as opposite forces. These effects may be mediated via the exo/endocytosis pathways. All these complexes contribute to the formation of apico-lateral junctions via mechanisms that are not yet understood. One might speculate that the actin cortical cytoskeleton plays a fundamental role in this process. It is quite certain, however, that polarity complexes also control cell metabolism and proliferation, and research in this area will predictably lead to some exciting new discoveries during the next decade.

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