Tight Junctions and Cell Polarity

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Key Words

tight junction proteins, epithelial polarity, CRB complex, Par complex

Abstract

The tight junction is an intracellular junctional structure that mediates adhesion between epithelial cells and is required for epithelial cell function. Tight junctions control paracellular permeability across epithelial cell sheets and also serve as a barrier to intramembrane diffusion of components between a cell's apical and basolateral membrane domains. Recent genetic and biochemical studies in invertebrates and vertebrates indicate that tight junction proteins play an important role in the establishment and maintenance of apico-basal polarity. Proteins involved in epithelial cell polarization form evolutionarily conserved multiprotein complexes at the tight junction, and these protein complexes regulate the architecture of epithelia throughout the polarization process. Accumulating information regarding the regulation of these polarity proteins will lead to a better understanding of the molecular mechanisms whereby cell polarity is established.

Contents

INTRODUCTION

Tight junction: a cell-cell junction between epithelial cells that seals adjacent cells together to prevent free diffusion across the epithelial cell sheet

Basolateral membrane: membrane residing opposite the apical membrane, functioning in cell-cell or cell–extracellular matrix adhesion

Cell polarity: asymmetric distribution of macromolecules such as proteins, lipids, and carbohydrates within a cell

The formation of adhesive contacts between cells is essential for the function of many tissues. This is particularly true for epithelial cells, which adhere tightly to one another to form epithelial sheets that line organ cavities and act as barriers between body compartments. The tight junction is one type of specialized intercellular junctional complex that mediates adhesion between epithelial cells. Tight junctions serve to form tight seals between epithelial cells, creating a selectively permeable barrier to diffusion through the intercellular space. Tight junctions also demarcate the boundary between the apical and basolateral membrane domains of a cell and serve as a barrier to intramembrane diffusion of proteins and macromolecules between the apical and basolateral membrane domains (also termed the fence function). In conventional electron micrographs, tight junctions appear as regions of tight apposition of neighboring cells, where the plasma membranes of adjacent cells actually appear to fuse together (so-called kissing points). In freeze-fracture electron micrographs, the tight junctions appear as a continuous, branching network of particle strands encircling the apical end of the lateral membrane of epithelial cells. Numerous studies have now shown that these particle strands are composed of transmembrane proteins that bind directly to protein partners on neighboring cells, thus occluding and sealing the intercellular space.

The barrier and fence functions of tight junctions have been well appreciated. It is only recently, however, that the tight junction has come to be recognized as a complex, multiprotein structure that also plays a role in other diverse cellular processes, including the regulation of cell polarity, proliferation, and differentiation. In addition to integral membrane proteins that mediate direct contact between cells, the tight junction also contains a large number of cytoplasmic proteins associated with the transmembrane proteins in a dense, cytoplasmic plaque. Some of these cytoplasmic proteins serve as adaptors that link the integral membrane proteins to the cell's actin cytoskeleton, thus stabilizing the tight junction structure. Other cytoplasmic proteins play roles in transcription, cell polarity, or other signaling functions. A great many of these tight junction–associated proteins contain postsynaptic density/discs large/zonula occludens (PDZ) domains and act as scaffolds to recruit other proteins into the tight junction plaque. Thus, the tight junction can be seen as a multiprotein complex that functions as a signaling platform. The list of proteins that have been identified as tight junction components is long and continues to grow. In the first half of this review, we give an overview of the major structural protein components of the epithelial tight junction, with a special emphasis on the signaling roles of these proteins. In the second half of this review, we focus on the role of tight junctions in the control of epithelial cell polarity. We concentrate in detail on two tight junction–associated protein complexes that have been shown to

play critical roles in the development of cell polarity: the Crumbs (CRB) complex and the partitioning defective (Par) complex. Finally, we discuss the interaction between the polarity complexes and the structural proteins of the tight junction.

COMPOSITION OF THE TIGHT JUNCTION

Tight junctions consist of transmembrane proteins and peripheral membrane proteins, and these proteins interact with each other to form a complex protein network (**Figure 1**). Scaffolding proteins bind to the transmembrane proteins, linking these proteins to the cytoskeleton. Some proteins are involved in signal transduction pathways to regulate polarity and proliferation. In this section, we discuss the structural details of the major proteins of the tight junction as well as their function and interactions.

Transmembrane Proteins

Transmembrane proteins are important components of the tight junction that reach across the junction and connect the membranes of adjacent cells to make a tight seal. The three main families of transmembrane proteins found in tight junctions—occludin, claudins and junctional adhesion molecules—are discussed below.

Occludin. Occludin was identified in chicken liver as the first transmembrane protein at the tight junction and is one of the constituents of the tight junction intermembrane strands (Furuse et al. 1993). Occludin contains four transmembrane domains, two extracellular loops, and two intracellular domains (reviewed in Feldman et al. 2005). Occludin directly interacts with the proteins zonula occludens (ZO)-1 and ZO-2 as well as ZO-3 at the tight junction, and these interactions are required for the tight junction localization of occludin. Studies have shown that occludin's C-terminal half, consisting of ~250 amino acids, is responsible for the interaction with ZO-1 and ZO-2 (**Figure 1**). Also, occludin interacts with the actin cytoskeleton and junctional adhesion molecules (JAMs) indirectly via its interaction with ZO proteins (**Figure 1**) (reviewed in Gonzalez-Mariscal et al. 2000, Mandell & Parkos 2005).

Expression of a C-terminally truncated occludin mutant in MDCKII cells resulted in increased paracellular permeability, showing the functional importance of this region (Balda et al. 1996). Furthermore, Chen et al. (1997) showed that the C-terminal region played an important role in tight junction assembly in *Xenopus* embryo development. The extracellular domain of occludin has also been implicated in tight junction development. Expression of occludin in occludin-null fibroblasts resulted in increased intercellular adhesion; synthetic peptides representing the extracellular loops prevented this occludin-induced adhesion, suggesting that the extracellular domains of occludin are involved in cell-cell adhesion (Van Itallie & Anderson 1997). Synthetic peptides corresponding to the extracellular domains have also been shown to disrupt the tight junction permeability barrier of epithelial cells, the resealing of tight junctions following a calcium-switch assay, and the localization of endogenous occludin to the tight junction (Wong & Gumbiner 1997). However, studies of occludin knockout mice show that the morphology of tight junctions is not affected and the barrier function of intestinal epithelium appears normal, although there are histological abnormalities in gastric epithelium (Saitou et al. 2000).

Another possible role for occludin is in the regulation of various signaling events emanating from the tight junction (**Figure 2**). Raf-1 disrupts the tight junction through the downregulation of occludin, and overexpression of occludin suppresses Raf-1-induced tumor growth via the second loop of occludin's extracellular domain (Li & Mrsny 2000, Wang et al. 2005). Also, occludin may be involved in RhoA activation via GEF-H1/Lfc, a tight

PDZ: postsynaptic density/discs large/zonula occludens

Crumbs (CRB) complex: evolutionarily conserved multiprotein complex consisting of CRB, PALS1/Sdt, and PATJ/DmPATJ; plays a crucial role in apico-basal polarity of epithelia cells

Partitioning defective (Par) complex: evolutionarily conserved multiprotein complex consisting of Par3/Baz, Par6, and aPKC; required for proper polarization of many cell types

ZO: zonula occludens

JAM: junctional adhesion molecule

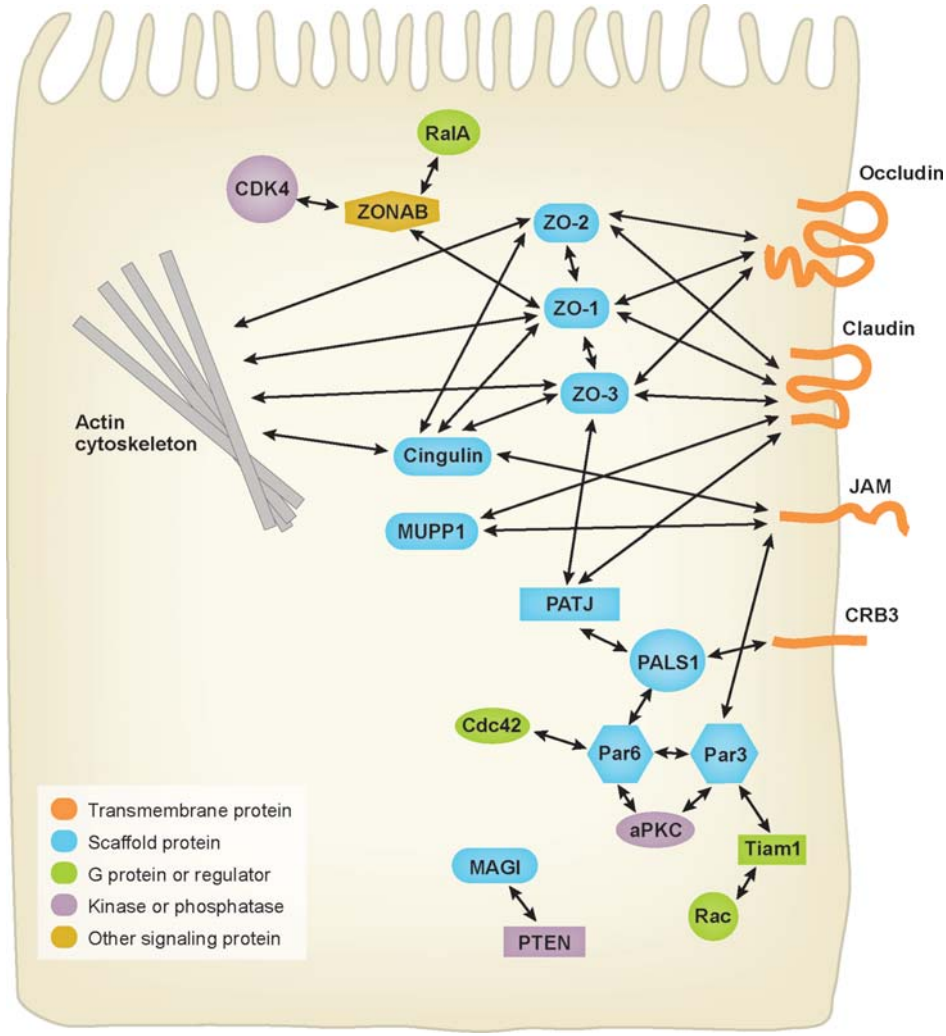

Figure 1

Complexity of protein-protein interactions at the tight junction. This figure illustrates some of the many interactions that occur at the tight junction. Some of these interactions, such as those between claudins and ZO proteins, are necessary for the structural integrity of the tight junction. Others, such as those between ZO proteins and the transcription factor ZONAB, regulate signaling pathways that emanate from the junction (see text for details). aPKC, atypical protein kinase C; CDK4, cell division kinase 4; CRB3, Crumbs 3; JAMs, junctional adhesion molecules; MAGI, membrane-associated guanylate kinase with inverted domain structure; MUPP1, multi-PDZ domain protein 1; PALS1, protein associated with Lin seven 1; Par, partitioning defective; PATJ, PALS1-associated tight junction protein; RalA, Ras-like GTPase; Tiam1, T-lymphoma invasion and metastasis; ZO, zonula occludens; ZONAB, ZO-1-associated nucleic acid–binding protein.

junction–associated guanine nucleotide exchange factor (Aijaz et al. 2005, Benais-Pont et al. 2003). Recent studies have suggested that occludin has an important role in targeting TGF-β receptors to the tight junction. This may be important for the TGF-β-mediated epithelial-to-mesenchymal transition, which requires loss of polarity and dissolution of junctions (Barrios-Rodiles et al. 2005). Thus, although the role of occludin

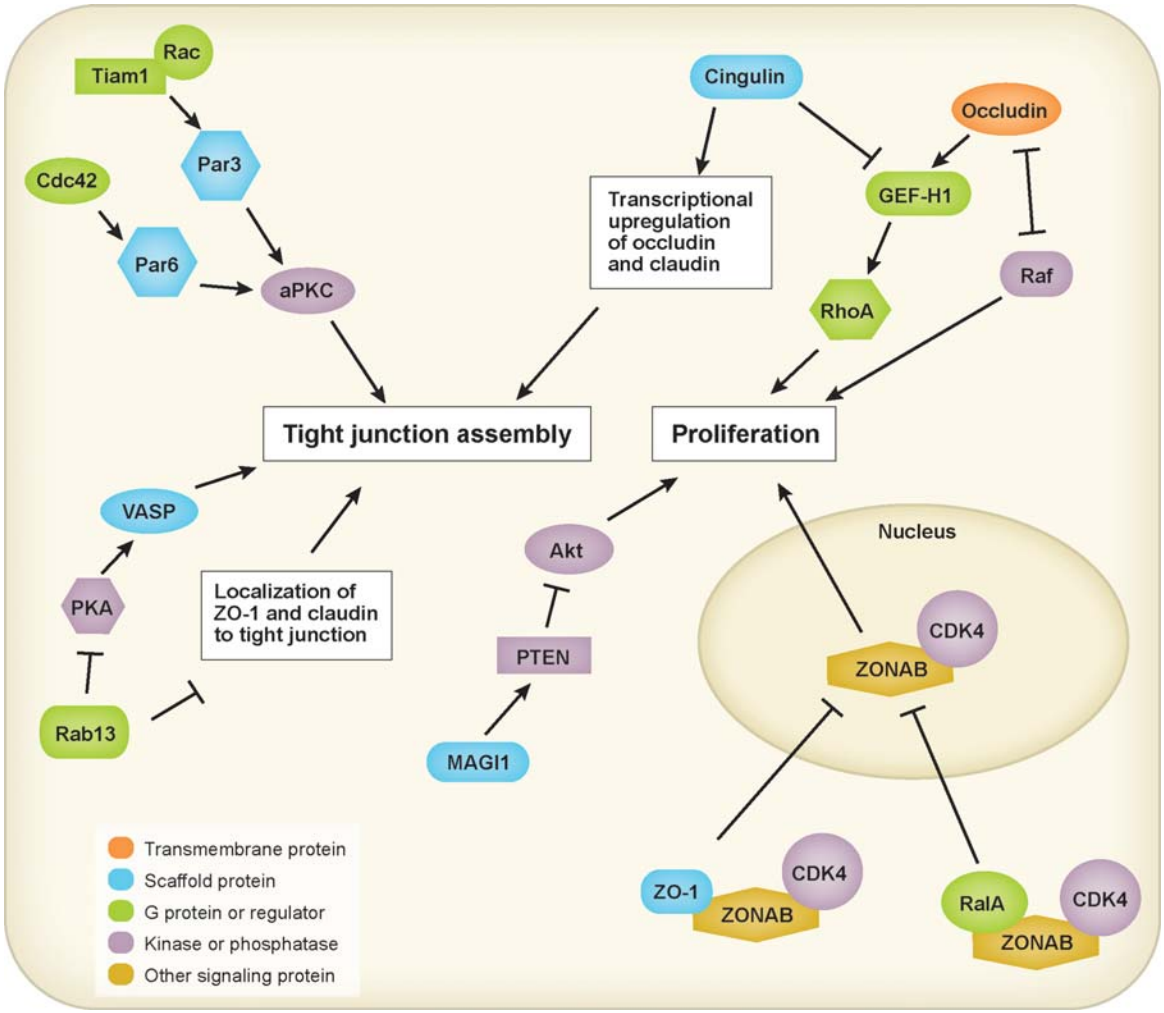

Figure 2

Signaling events at tight junctions. Tight junction proteins regulate the assembly of tight junctions by controlling transcription and localization of tight junction proteins. In addition, the proteins at tight junctions are involved in proliferation and differentiation control (see text for details). aPKC, atypical protein kinase C; CDK4, cell division kinase 4; MAGI1, membrane-associated guanylate kinase with inverted domain structure; PKA, protein kinase A; RalA, Ras-like GTPase; Tiam1, T-lymphoma invasion and metastasis; VASP, vasodilator-stimulated phosphoprotein; ZO, zonula occludens; ZONAB, ZO-1-associated nucleic acid–binding protein.

in tight junction formation is unclear, studies have revealed an emerging role for this protein in the regulation of signaling events at this junction.

Claudins. Claudins are a family of transmembrane proteins that are constituents of the tight junction intercellular strands. Similar to occludin, claudins contain four transmembrane domains, two extracellular loops, and two intracellular domains. Despite this topological resemblance, claudins have no sequence similarity to occludin. Tsukita and coworkers (Furuse et al. 1998a) first identified

PALS1: protein associated with Lin seven 1

PATJ: PALS1-associated tight junction protein

Transepithelial resistance: electrical resistance across an epithelial monolayer owing in part to the presence of tight junctions

Paracellular permeability: ability of molecules to penetrate the barrier between cells

claudin-1 and -2 from a chicken liver fraction. Subsequent database searching and genomic cloning identified more claudin family members, expanding the number to 24 as of this writing. Most cells express at least two claudin family members; some claudins, such as claudin-5 and claudin-11, have tissue-specific expression patterns (reviewed in Tsukita et al. 2001).

Claudin proteins mediate calcium-independent cell-cell adhesion. Expression of claudin-1 and claudin-2 in L fibroblasts lacking tight junctions induces cell-cell aggregation and the formation of cell-cell contact planes where claudins are concentrated (Furuse et al. 1998b). Freeze-fracture electron microscopy revealed that these cell contact planes are composed of a well-developed network of particle strands, similar in structure to tight junctions. When occludin and claudin were coexpressed in L fibroblasts, occludin was recruited along with claudin into these tight junction–like strands. These results suggest claudins are the main proteins important for tight junction strand formation. Claudins interact with each other between different tight junction strands or within individual strands in a homotypic as well as heterotypic manner (Furuse et al. 1999). The C-terminal amino acids of claudins encode PDZ-binding motifs, and these motifs are highly conserved throughout the claudin family. Through these PDZ-binding motifs, claudins directly interact with peripheral PDZ-domain-containing proteins, including ZO-1, ZO-2, ZO-3, and protein associated with Lin seven 1 (PALS1)-associated tight junction protein (PATJ) (**Figure 1**) (Roh et al. 2002a; reviewed in Tsukita et al. 2001).

Cell-type-specific differences in tight junctions appear to be determined by different combinations of claudin isoforms. MDCK type II cells with low transepithelial resistance (TER) have a high level of claudin-2 expression, but MDCK type I cells with high TER express low levels of claudin-2 (Furuse et al. 2001). Moreover, mutations in claudin-16 are associated with human disease syndromes in which normal paracellular permeability to calcium and magnesium is impaired (Hou et al. 2005, Simon et al. 1999). In addition, claudin-4 expression in epithelia induces a decrease in paracellular conductance by reducing sodium permeability (Van Itallie et al. 2001). Furthermore, claudin-1 knockout mice die from dehydration within 1 day of birth owing to defects in epidermal barrier function (Furuse et al. 2002). These studies indicate that claudins are crucial for the barrier function of tight junctions.

Studies have identified several potential mechanisms for the regulation of claudin function. Some studies indicate that WNK4, a serine/threonine kinase, hyperphosphorylates claudin-1–4 and decreases TER by increasing the permeability of chloride (Yamauchi et al. 2004). D'Souza et al. (2005) showed claudin-3 is phosphorylated in ovarian cancer cells by protein kinase A (PKA) and this phosphorylation event increases paracellular permeability and disrupts the tight junction barrier function. Studies using an intestinal epithelial monolayer showed interferon gamma (IFN-γ) increases the paracellular permeability and disrupts the epithelial barrier by inducing endocytosis of tight junction proteins such as claudin-1 and occludin (Madara & Stafford 1989, Utech et al. 2005). The protein Snail regulates claudin function at the transcriptional level. Snail is a transcription factor expressed during epithelial-to-mesenchymal transition, and it downregulates the expression of junctional proteins, including E-cadherins, claudins, and occludin. Snail decreases claudin expression, resulting in an increase in tight junction paracellular permeability and disruption of barrier function (Ikenouchi et al. 2003).

Junctional adhesion molecules. JAMs are members of the immunoglobulin superfamily of proteins and are expressed in epithelial cells. JAMs are also found in many other cell types, including leukocytes, endothelia, and platelets. Four members of the JAM protein family have been identified: JAM-A, JAM-B,

JAM-C, and JAM4/JAML (JAM4 refers to the mouse protein, whereas JAML refers to the human homolog of JAM4) (Mandell & Parkos 2005). In epithelia, JAM-A and JAM-C localize to the tight junction, whereas JAM-B exists along the lateral membrane (Aurrand-Lions et al. 2001).

Unlike occludin and claudin, JAMs have a single transmembrane domain, an extracellular domain containing two Ig-like motifs, and a cytoplasmic tail (Kostrewa et al. 2001). The extracellular domains of JAM-A, JAM-B, and JAM-C contain dimerization motifs that play a role in their interactions (Kostrewa et al. 2001, Mandell et al. 2004). In Chinese hamster ovary cells, exogenously expressed JAM-A localizes to the cell-cell contact site formed by two transfected cells, not to cell-cell junctions formed by one transfected cell and one untransfected cell; this suggests that JAM-A may participate in cell adhesion through homophilic interactions (Bazzoni et al. 2000a). Other studies have reported heterophilic interactions of JAM-B with JAM-C as well as with integrins (Cunningham et al. 2002, Liang et al. 2002).

Recent studies have implicated JAM proteins in the formation of intercellular junctions and in epithelial barrier function. Inhibition of JAM protein function with a monoclonal antibody results in decreases in TER and defects in tight junction assembly (Liu et al. 2000). JAM-A homodimer formation is implicated in JAM-A regulation of tight junctions (Mandell et al. 2004). Despite this evidence, little is known of the detailed mechanisms whereby JAM proteins regulate tight junction formation. Unlike the case with occludin and claudins, evidence is lacking for a direct role of JAM proteins in the formation of tight junctions and regulation of the paracellular barrier. JAMs are known to interact with many other proteins, and JAMs may indirectly regulate tight junction formation by targeting other proteins to the tight junction. Except for JAML, all JAM proteins have a PDZ-binding motif at their C termini that appears to be involved in the interaction with tight junction scaffolding proteins (**Figure 1**). These scaffolding proteins include ZO-1, calcium/calmodulin-dependent serine protein kinase (CASK/Lin2), membrane-associated guanylate kinase with inverted domain structure 1 (MAGI-1), multi-PDZ domain protein 1 (MUPP1), and partitioning defective 3 (Par3) (Ebnet et al. 2001, Itoh et al. 2001). The interaction of JAMs with many of these proteins appears to be important for proper function of the tight junction (reviewed in Mandell & Parkos 2005). However, further work is necessary to understand the detailed role of JAM proteins in tight junction formation.

Par3: partitioning defective 3

MAGUK: membrane-associated guanylate kinase

Peripheral Membrane Proteins

Transmembrane proteins of the tight junctions bind via their intracellular domains to peripheral membrane proteins. These peripheral membrane proteins, reviewed below, allow the transmembrane proteins to organize in the membrane, attach to the cytoskeleton, and initiate cell signaling.

Zonula occludens. ZOs are tight junction proteins that belong to the membrane-associated guanylate kinase (MAGUK) family of proteins. MAGUKs contain a core structure consisting of one or more PDZ domains, an Src homology 3 (SH3) domain, and a guanylate kinase (GUK) domain (reviewed in Gonzalez-Mariscal et al. 2000). The majority of MAGUK proteins localize to specific regions of cells such as junctions or synapses and act as peripheral membrane scaffolding proteins. The GUK domains of MAGUK proteins can bind to GMP but have no catalytic activity, consistent with their role in protein interactions rather than catalysis (Kistner et al. 1995, Masuko et al. 1999, Olsen & Bredt 2003). Indeed, the GUK domain of PSD-95 can interact with proteins such as guanylate kinase–associated protein (Kim et al. 1997). In PSD-95, an intramolecular interaction between the SH3 and GUK domains that did not require a conventional

ZONAB: ZO-1-associated nucleic acid–binding protein

SH3-binding polyproline motif was identified; this suggests that the SH3 domain of MAGUKs may play a unique role in the regulated assembly of these proteins (McGee et al. 2001).

There are three isoforms of ZO proteins: ZO-1, ZO-2, and ZO-3. ZO-1 localizes to the tight junction of epithelial and endothelial cells and is also present in nonepithelial cells such as fibroblasts, astrocytes, and Schwann cells (reviewed in Gonzalez-Mariscal et al. 2000). In addition to the MAGUK core structure of PDZ, SH3, and GUK domains, ZO-1 also contains two nuclear localization signals and a carboxyl region with several proline-rich regions. Studies show that ZO-1 localizes to the nucleus during the maturation of the epithelial monolayer and regulates proliferation by interacting with a Y-box transcription factor, ZO-1-associated nucleic acid–binding protein (ZONAB) (Balda et al. 2003, Balda & Matter 2000). Moreover, ZO-1 plays an important role in the assembly and function of tight junctions. ZO-1 has been implicated in the development of tight junctions during mouse blastocyst formation, and epithelial cells derived from ZO-1 knockout mice show delayed tight junction formation following a calcium-switch assay (Sheth et al. 1997, Umeda et al. 2004). In T84 epithelial cells, reduced barrier function induced by IFN-γ is mediated, in part, by a reduction of ZO-1 expression in T84 epithelia (Youakim & Ahdieh 1999).

ZO-2 and ZO-3 were identified as binding partners of ZO-1 (reviewed in Gonzalez-Mariscal et al. 2000). ZO-2 also has two nuclear localization signals in its first PDZ domain and in its GUK domain; studies indicate that ZO-2 localizes to the nuclei in subconfluent epithelial cells and directly interacts with various nuclear proteins such as Fos, Jun, CCAAT/enhancer binding protein, and DNA-binding protein scaffold attachment factor-B (Betanzos et al. 2004, Jaramillo et al. 2004, Traweger et al. 2003). Studies showing that ZO-1 expression is lost in breast cancer cells suggests the involvement of ZO proteins in tumorigenesis; additionally, ZO-2 is a cellular target for tumorigenic adenovirus type 9 E4-ORF1 (Glaunsinger et al. 2001, Hoover et al. 1998).

As mentioned above, ZO proteins act as scaffolding proteins and interact with many binding partners at the tight junction, including transmembrane proteins and cytoskeletal proteins (**Figure 1**). ZO-1 is able to bind to ZO-2 and ZO-3 via its second PDZ domain. All ZO proteins interact directly with the C-terminal domain of occludin (reviewed in Feldman et al. 2005, Gonzalez-Mariscal et al. 2000). The C-termini of claudins can bind to the first PDZ domain of ZO proteins (Itoh et al. 1999). Moreover, cingulin, a cytoplasmic protein of the tight junction, interacts with all ZO proteins (Cordenonsi et al. 1999). ZO-1 binds to ZONAB through its SH3 domain (Balda & Matter 2000, Balda et al. 2003). Furthermore, ZO proteins can interact with F-actin and the actin-binding protein Band 4.1 to link tight junction transmembrane proteins to the actin cytoskeleton (reviewed in Gonzalez-Mariscal et al. 2000). In addition to interactions with tight junction proteins, ZO-1 can interact with adherens junction proteins such as E-cadherin and catenin in nonepithelial cells and nonpolarized epithelial cells (Itoh et al. 1993, Rajasekaran et al. 1996). Also, ZO-1 is recruited to the nectin-based cell-cell adhesion complex through its interaction with afadin, an F-actin-binding protein (Yokoyama et al. 2001). Moreover, ZO-2 can bind to the lateral membrane protein, hScrib, in epithelia (Metais et al. 2005). These observations indicate that ZO proteins form a complex with adherens junction proteins in nonpolarized cells in which tight junctions have not formed, but upon polarization, ZO proteins can separate from the adherens junction and concentrate in the tight junction, where they interact with tight junction proteins such as claudins and occludin (Muller et al. 2005).

Membrane-associated guanylate kinase inverted. MAGI proteins belong to the MAGUK family of proteins but have three

features that distinguish them from all other members of the family: (*a*) The GUK domain is located on the N terminus rather than the C terminus; (*b*) the SH3 domain is replaced by two WW domains, another type of protein-protein interaction module that binds proline-rich peptides; and (*c*) MAGI proteins contain five PDZ domains rather than the usual one or three (Dobrosotskaya et al. 1997). MAGI-1 and MAGI-3 are expressed widely in various adult tissues, whereas MAGI-2 is primarily but not exclusively expressed in the brain.

Although MAGI-1 localizes to the tight junction (Ide et al. 1999), it also binds to β-catenin and is associated with β-catenin in E-cadherin-based adherens junctions. This association becomes stronger in calcium-depleted nonpolarized epithelial cells. These observations imply that, similar to the ZO proteins, MAGI-1 is involved in the formation of both adherens junctions and tight junctions and that interactions with junctional proteins are regulated during the polarization process (Dobrosotskaya & James 2000, Rajasekaran et al. 1996). Furthermore, two actin-binding proteins, synaptopodin and α-actinin-4, are also capable of binding to MAGI-1, and this interaction is mediated by the second WW and PDZ5 domain of MAGI-1, respectively. This suggests that MAGI-1 plays a role in actin-cytoskeleton dynamics within polarized epithelial cells (Patrie et al. 2002).

MAGI proteins are involved in various signal transduction events (**Figure 2**). MAGI proteins interact with the tumor suppressor protein PTEN via their second PDZ domain and recruit PTEN to cell-cell junctions. The interaction of MAGI-2 with PTEN is regulated through the phosphorylation of the C terminus of PTEN and stabilized by the cytoskeletal protein, vinculin. MAGI-mediated recruitment of PTEN to E-cadherin-based adherens junctions appears to stabilize these junctions and suppress Akt-induced cell invasiveness, suggesting that MAGI plays a role in tumorigenesis (Subauste et al. 2005, Tolkacheva et al. 2001). The involvement of MAGI proteins in tumorigenesis is further suggested by studies showing that oncogenic viral proteins, such as E4-ORF and E6 of adenovirus type 9 as well as high-risk human papillomaviruses, interact with MAGI proteins and target them for degradation (Glaunsinger et al. 2000, Thomas et al. 2002). In a recent study, Ohashi et al. (2004) showed that MAGI3 binds to the Tax1 protein of human T cell leukemia virus type 1 (HTLV-1) and that this interaction may play a role in the pathogenesis of HTLV-1-associated diseases.

Cingulin. Cingulin is a 140–160-kD protein localized to the cytoplasmic surface of epithelial tight junctions. This protein was originally identified as a peripheral membrane protein at the tight junction in avian brush border cells (Citi et al. 1988). It is expressed during early mouse embryo development by both maternal and embryonic genomes, suggesting a potential role in embryogenesis and epithelial maturation (Fleming et al. 1993, Javed et al. 1993). Cingulin has globular head and tail domains as well as a central α-helical rod domain that is responsible for the formation of coiled-coil parallel dimers (Citi et al. 2000, Cordenonsi et al. 1999). Cingulin interacts with other tight junction proteins, including ZO-1, ZO-2, ZO-3, JAM-A, and actin, suggesting that cingulin is an important component of tight junctions and links tight junction proteins to the actin cytoskeleton (**Figure 1**) (Bazzoni et al. 2000b, Cordenonsi et al. 1999, D'Atri & Citi 2001, D'Atri et al. 2002).

Recent evidence suggests that cingulin plays an important role in transcriptional regulation and cell proliferation (**Figure 2**). Embryoid bodies derived from cingulin knockout mice showed alterations in the mRNA expression levels of several tight junction proteins, including claudins and occludin (Guillemot et al. 2004). Also cingulin may inhibit the activity of RhoA, which is required for cell-cycle progression, by interacting with the RhoA GEF protein, GEF-H1/Lfc (Aijaz et al. 2005). On the basis of these studies, one can propose that cingulin plays a role in tight junction formation via the transcriptional

regulation of tight junction protein expression and by the downregulation of RhoA activity blocking G1/S phase progression.

ZO-1-associated nucleic acid–binding protein. ZONAB was identified as a binding partner of ZO-1 and localizes to both the tight junction and nucleus (Balda & Matter 2000). It is a Y-box transcription factor protein that binds to inverted CCAAT box-containing sequences. ZONAB binds the promoter of the epidermal growth factor receptor 2 (ErbB-2) and regulates ErbB-2 expression. ZONAB also interacts with cell division kinase 4 (CDK4), a protein involved in S phase entry that regulates cell proliferation (Balda et al. 2003). ZONAB activity is regulated by its interaction with ZO-1 and by cell density (**Figure 2**). Under conditions of high cell density, ZONAB interacts with ZO-1 at the tight junction and is sequestered from the nucleus, and thus it is unable to regulate gene expression. ZONAB also serves to sequester CDK4 away from the nucleus under these conditions, resulting in a block of cell entry into S phase. Under conditions of low cell density, however, the formation of tight junctions is not complete, and the expression of ZO-1 is low. ZONAB is thus released from the tight junction and able to localize to the nucleus, where it can regulate gene expression. It also translocates CDK4 into the nucleus, stimulating cell entry into S phase. Thus, ZONAB appears to play a unique role as a tight junction–associated protein that serves as a sensor of cell density, regulating transcription and cell proliferation in a density-dependent manner.

More recently, Frankel et al. (2005) demonstrated that ZONAB binds to Ras-like GTPase (RalA) proteins, which are members of the Ras superfamily and are involved in a variety of signaling events such as vesicle transport, cellular transformation, actin-cytoskeleton remodeling, and cell-cycle control. As with ZO-1, interaction with RalA appears to regulate the transcriptional activity of ZONAB in a cell density–dependent manner (**Figure 2**). Increased amounts of RalA-ZONAB complex form under conditions of high cell density, and transcriptional repression by ZONAB is relieved. When cells grow under low-density conditions, the amount of RalA-ZONAB complex formed is decreased, and ZONAB is transcriptionally active. The exact mechanisms by which RalA regulates ZONAB transcriptional activity are still unclear; it is also unclear whether RalA and ZO-1 act together in the regulation of ZONAB activity.

Rab13. Rab13 is a member of the small GTPase Rab family of proteins that is involved in the regulation of exocytic and endocytic pathways, including vesicle movement and fusion. Zahraoui et al. (1994) identified it as a mammalian homolog of the yeast secretory protein, Sec4. Rab13 localizes to the tight junction in polarized epithelial cells and is recruited to cell-cell contacts from a cytosolic pool at an early stage of junctional complex assembly, suggesting a role in early junctional formation (Sheth et al. 2000). Expression of a constitutively active form of Rab13 inhibits trafficking of claudin-1 and ZO-1 to the plasma membrane (Marzesco et al. 2002). Furthermore, dominant active Rab13 was also shown to inhibit the post-endocytic recycling of occludin to cell junctions. These results are consistent with studies showing a negative effect of activated Rab13 on tight junction assembly (Marzesco et al. 2002, Morimoto et al. 2005). In a recent study Kohler et al. (2004) demonstrated that activated Rab13 directly interacts with PKA and inhibits the PKA-dependent phosphorylation at tight junctions (**Figure 2**). As a result, the PKA-dependent phosphorylation of vasodilator-stimulated phosphoprotein, an actin-remodeling protein whose phosphorylation is required for tight junction barrier function, is inhibited, and the localization to the junction of vasodilator-stimulated phosphoprotein, as well as of tight junction proteins including claudin-1 and ZO-1, is disrupted (Kohler et al. 2004, Lawrence et al.

2002). Thus, Rab13 plays an important role in the dynamics of tight junction assembly by regulating PKA signaling and protein trafficking.

CELL POLARITY AND JUNCTIONAL ASSEMBLY

Recent studies suggest that many tight junction proteins form evolutionarily conserved multiprotein complexes and function as molecular cassettes. In this section, we discuss the detailed function of two of these evolutionarily conserved protein complexes, the CRB3(CRB)/PALS1(Stardust)/PATJ(DmPATJ) and the Par3(Bazooka)/Par6/aPKC complexes. These protein complexes not only guide tight junction formation but also play an important role in the establishment and maintenance of apico-basal polarity.

Function of the Crumbs Complexes in Epithelial Cell Polarization

Drosophila CRB is a transmembrane protein with 30 EGF-like and 4 laminin A G-domain-like repeats in its extracellular domain. It localizes to the apical membrane and concentrates at the subapical region (also called the marginal zone), a region above the zonula adherens (ZA), a belt-like junctional structure encircling the apex of *Drosophila* epithelia. The loss-of-function CRB mutants show severe disorganization and degeneration of ectodermally derived embryonic epithelia (reviewed in Tepass et al. 2001). CRB is an important apical membrane determinant, as the insertion of CRB is sufficient to confer apical characteristics to the plasma membrane, and overexpression of CRB induces an expansion of the apical domain and a reduction of the basolateral domain (Wodarz et al. 1995). CRB overexpression also leads to the redistribution of beta heavy spectrin, one of the membrane cytoskeleton proteins, which is consistent with a study suggesting that CRB stabilizes the apical spectrin–based membrane skeleton by interacting with beta heavy spectrin as well as with Dmoesin, a 4.1/ezrin/radixin/moesin (FERM)-domain containing protein (Medina et al. 2002). Furthermore, a recent study showed that CRB accumulates at the apical membrane in syntaxin Avalanche or Rab5 mutants, genes that encode core components of the vesicle trafficking machinery. This accumulation of CRB also leads to an expansion of the apical membrane in *Drosophila* epithelia (Lu & Bilder 2005). These results suggest that endocytic traffic is critical for the maintenance of apico-basal polarity and confirm that CRB plays an important role in the determination of the apical domain.

In addition to apical determination, CRB in concert with Stardust (Sdt) is required for the biogenesis of the ZA, which plays an important role in the establishment of epithelial integrity (Grawe et al. 1996, Tepass 1996). Sdt is a MAGUK protein and the *Drosophila* homolog of PALS1. Mutations in Sdt produce a phenotype nearly identical to that associated with CRB mutations in the development of epithelial polarity (Tepass & Knust 1993). The ZA is formed during and after gastrulation, and the reorganization of spot adherens junctions into ZA during cellularization is a critical step of ZA formation (Tepass et al. 2001). During the polarization of epithelia, CRB seems to delimit the apical border where the ZA will be formed, whereas Sdt appears to be required for the assembly of spot adherens junctions during gastrulation (Grawe et al. 1996). CRB and Sdt are mutually dependent for their stability and localization; expression of CRB is decreased in Sdt mutants and vice versa (Bachmann et al. 2001, Hong et al. 2001). Indeed, CRB physically interacts with Sdt, just as mammalian CRB3 interacts with PALS1 (see below) (Bachmann et al. 2001, Hong et al. 2001, Roh et al. 2002b). CRB also controls polarity and ZA formation in *Drosophila* photoreceptor cells (Izaddoost et al. 2002, Pellikka et al. 2002).

The mammalian CRB proteins were identified as homologs of the *Drosophila* CRB protein. They are integral membrane

CRB3: Crumbs3

Apical membrane: membrane of epithelial cells that faces the free surface, opposite to the basal membrane, which contacts the basement membrane

Sdt: Stardust

proteins consisting of a transmembrane domain, an extracellular domain, and an intracellular domain with conserved PDZ-binding and FERM-binding motifs. Although three isoforms of mammalian CRB have been identified, only two, CRB1 and CRB3, have been functionally characterized. CRB1 is the human ortholog of *Drosophila* CRB, and it is expressed primarily in the eye and brain; mutations in CRB1 cause Leber congenital amaurosis and retinitis pigmentosa (den Hollander et al. 1999, 2001). A recent study with CRB1 knockout mice suggests that CRB1 is required for the maintenance of photoreceptor cell polarization and adhesion during light exposure (van de Pavert et al. 2004). The CRB3 isoform is expressed mainly in epithelial tissues. Unlike CRB1 and *Drosophila* CRB, which have large extracellular regions containing laminin G and epidermal growth factor–like domains, CRB3 has a very short extracellular domain. CRB3 localizes to the apical membrane of epithelial cells and is concentrated at tight junctions, where it interacts with PALS1, the mammalian homolog of the *Drosophila* Sdt protein (**Figure 1**). Interaction of CRB3 with the PDZ domain of PALS1 is mediated by an evolutionarily conserved C-terminal PDZ-binding motif, the amino acid sequence ERLI (Makarova et al. 2003).

Several studies have now established an important role for CRB3 in epithelial cell polarization and tight junction formation. Overexpression of CRB3 in MDCKII cells leads to the delayed formation of tight junctions and a disruption of cell polarity; these phenotypes require the C-terminal ERLI sequence of CRB3, demonstrating the functional importance of the PDZ-binding motif (Lemmers et al. 2004, Roh et al. 2003). Moreover, a recent study using mammary epithelial MCF10A cells indicated a critical role for CRB3 in tight junction formation. MCF10A cells express little endogenous CRB3 and do not form tight junctions in vitro. It was found that the introduction of exogenous CRB3 into MCF10A cells was sufficient to induce the formation of functional tight junctions. Mutations in either the PDZ-binding motif or FERM-binding motif led to defects in the ability of CRB3 to promote tight junction development. Thus, both conserved intracellular motifs appear important for CRB3-mediated tight junction formation (Fogg et al. 2005). In addition to having a role in tight junction formation, CRB3 has also been implicated in the formation of primary cilia. CRB3 has been localized to cell cilia, and small interfering RNA experiments show that CRB3 expression is required for ciliogenesis in epithelial cells. (Fan et al. 2004).

PALS1 is a MAGUK protein consisting of two L27 domains, one PDZ domain, an SH3 domain, a Band 4.1–binding domain, and a GUK domain (Kamberov et al. 2000). RNA interference (RNAi)-mediated inhibition of PALS1 expression in mammalian epithelial cells causes defects in tight junction formation and cell polarity. These defects appear to result from a failure of recruitment of the Par6/Par3/aPKC complex to the tight junction (see below) (Straight et al. 2004). The *Drosophila* ortholog of PALS1, Sdt, was also shown to control the polarity of epithelia in flies as mentioned above. The zebrafish ortholog of PALS1, Nagie Oko (Nok), is essential for morphogenesis of photoreceptor cells in the retina (Bachmann et al. 2001, Hong et al. 2001, Straight et al. 2004, Wei & Malicki 2002). In a recent study Rohr et al. (2006) demonstrated that Nok, in concert with Heart and Soul/aPKCι, is required for myocardial coherence and remodeling during cardiac morphogenesis.

PALS1 has two L27 domains. One of the L27 domains interacts with mLin-7, whereas the other interacts with PATJ. PATJ is a multiple PDZ-domain-containing protein that localizes to the tight junction. Of its ten PDZ domains, binding partners have been identified for only two. PATJ interacts with ZO-3 via its sixth PDZ domain and with claudin-1 via its eighth PDZ domain. (Lemmers et al. 2002, Roh et al. 2002a,b). MUPP1, a paralog of PATJ, contains 13 PDZ domains and also interacts with tight junction proteins,

including claudins and JAM (Hamazaki et al. 2002, Poliak et al. 2002). However, the function of MUPP1 in epithelial polarity and junction formation is not clear, although its role as a tumor suppressor has been suggested by studies showing that MUPP1 is a target protein of oncogenic viruses such as human papillomaviruses (Lee & Laimins 2004, Massimi et al. 2004).

Recent studies indicate that PATJ plays an important role in the development of mammalian epithelial cell polarity. Using the MDCK cell cyst model, Shin et al. (2005) demonstrated that RNAi-mediated reduction of PATJ expression leads to defects in cell polarity. Studies in MDCK cells demonstrate that when cells are nonpolarized, PATJ localizes to an intracellular compartment related to the vacuolar apical compartment (Low et al. 2000, O'Brien et al. 2002, Rodriguez-Boulan et al. 2005, Shin et al. 2005, Utech et al. 2005, Vega-Salas et al. 1988). During cell polarization, PATJ moves from this compartment to the apical region and then to the tight junction. Suppression of PATJ expression in Caco-2 cells resulted in decreased stability of the CRB3 complex and localization of CRB3 to the intracellular compartment (Michel et al. 2005). These results indicate a role for PATJ in apico-basal polarity and CRB3 trafficking during polarization of mammalian epithelia. Furthermore, PATJ is required for the formation of tight junctions. Adenovirus E4-ORF1 protein induces the disruption of tight junctions by interacting with PATJ and sequestering it from the junction, suggesting a critical role for PATJ in tight junction integrity (Latorre et al. 2005).

In *Drosophila*, DmPATJ, which has one L27 and four PDZ domains, is maternally expressed and localized to the leading edge of the furrow canal, the ingrowing plasma membranes during blastoderm cellularization. During gastrulation, DmPATJ is redistributed to an apical region, where CRB and Sdt localize. Although the localization of DmPATJ is disrupted during gastrulation, DmPATJ is correctly targeted to the furrow canal during cellularization in an Sdt mutant, suggesting CRB and Sdt are required for the maintenance of DmPATJ but not essential for the initial localization of DmPATJ (Bachmann et al. 2001, Bhat et al. 1999, Hong et al. 2001). DmPATJ has only four PDZ domains. Bhat et al. (1999) incorrectly reported that mutation of the gene for DmPATJ led to the discs' lost phenotype; however, this study was the first to suggest an indirect interaction between PATJ and CRB. Subsequent studies showed that the defects in the formation of imaginal discs were caused by mutation in a *Drosophila* codanin1 homolog, not DmPATJ (Bhat et al. 2003, Pielage et al. 2003). More recent studies show that DmPATJ is required for planar cell polarity and retinal morphogenesis in the *Drosophila* eye (Djiane et al. 2005, Richard et al. 2006). DmPATJ stabilizes the CRB complex and is required for stalk membrane and rhabdomere maintenance during photoreceptor development (Richard et al. 2006). Moreover, DmPATJ interaction with Frizzled is required for the development of planar cell polarity. DmPATJ recruits atypical protein kinase C (aPKC) to Frizzled, resulting in phosphorylation and inhibition of Frizzled activity (Djiane et al. 2005). This study suggests that DmPATJ plays a critical role connecting planar cell and apico-basal polarity.

aPKC: atypical protein kinase C

Function of the Par Complexes in Polarization of Epithelial Cells

Par3 and Par6 were first identified as PDZ-domain-containing proteins affecting anterior-posterior partitioning in *Caenorhabditis elegans* embryo development. Subsequent studies identified mammalian homologs of Par3 and Par6 and their interaction with each other as well as with aPKC. In mammalian epithelial cells, the Par3/Par6/aPKC complex localizes at tight junctions (reviewed in Macara 2004, Ohno 2001). Conserved region 3 of Par3 directly binds to the kinase domain of aPKC, and the N terminus of Par6 interacts with that of aPKC. Also, Par6 is able to bind to the PDZ domain of Par3. Par6 also interacts

Baz: Bazooka

with the active form of Cdc42, a Rho GTPase, via its semi-Cdc42/Rac interacting binding (CRIB) domain, and with part of the PDZ domain (Joberty et al. 2000, Johansson et al. 2000, Lin et al. 2000, Qiu et al. 2000). A recent study revealing the crystal structure of this interaction showed that the semi-CRIB domain and adjacent PDZ domain form a continuous eight-stranded sheet for Cdc42 binding and that the Cdc42-Par6 interaction induces conformational changes in Par6 (Garrard et al. 2003). The Par3/Par6/aPKC complex can be pulled down with the active form of Cdc42 in MDCK cells, suggesting the existence of this multiprotein complex in vivo (Joberty et al. 2000).

Bazooka (Baz) is the *Drosophila* ortholog of mammalian Par3. In *Drosophila*, a Baz/Par6/aPKC complex is required for the correct development of epithelia (Petronczki & Knoblich 2001, Wodarz et al. 2000). This complex localizes to the subapical region along with the CRB complex. The components of the Baz/Par6/aPKC complex are mutually dependent upon one another for correct localization during epithelial morphogenesis. Baz directly binds to aPKC, and aPKC mutant embryos show mislocalization of Baz, resulting in the disruption of apico-basal polarity (Wodarz et al. 2000). Baz also interacts with Par6 and is required for the correct localization of Par6 (Rolls et al. 2003). Similarly, in Par6 *Drosophila* mutants, Baz fails to localize apically, and epithelial polarity is disrupted (Petronczki & Knoblich 2001). A recent study suggests the involvement of Par1 and 14-3-3 proteins in the regulation of the Baz/Par6/aPKC complex (Benton & Johnston 2003). Baz is phosphorylated by Par1, and this phosphorylation provides Baz with the binding sites for 14-3-3 proteins. Subsequent binding of 14-3-3 to Baz inhibits the formation of the Baz/Par6/aPKC complex by blocking the binding of aPKC to Baz. Par1 resides in the lateral membrane, and its activity there leads to lateral exclusion of the Baz/Par6/aPKC complex. This restricts the Baz/Par6/aPKC complex to the apical domain and maintains the integrity of apico-basal polarity (**Figure 3**).

Unlike the CRB complex, which appears to be involved in the establishment of polarity in a limited number of cells, the Baz/Par6/aPKC complex also plays a role in specifying the polarity of several other cell types, such as neuroblasts (Schober et al. 1999, Wodarz et al. 1999). The formation of neuroblasts requires delamination from the neuroectodermal epithelium and subsequent asymmetric cell division along the apico-basal axis to generate two different daughter cells, a neuroblast and a ganglion mother cell. Asymmetric localization of several cell-fate-determinant proteins is required for this process. The protein Inscuteable localizes to the apical cortex, and the proteins Numb and Miranda target to the basal cortex. In Baz mutants, Inscuteable fails to localize to the apical cortex and is distributed in the cytoplasm, resulting in the mislocalization of Numb and Miranda during metaphase of neuroblast division (Schober et al. 1999, Wodarz et al. 2000). Additionally, mitotic spindles in neuroblasts are misoriented in these Baz-mutant embryos (Cai et al. 2003). This suggests that Baz controls the asymmetric division of neuroblasts by regulating the localization of cell-fate-determinant proteins and the orientation of the mitotic spindle. In addition to regulating neuroblast polarity, the Baz/Par6/aPKC complex also regulates polarization and the development of *Drosophila* oocytes (Cox et al. 2001, Huynh et al. 2001).

Recent studies in *Drosophila* have attempted to elucidate the functional hierarchy of Baz, Par6, and aPKC during polarization and adherens junction formation in epithelia (Harris & Peifer 2004, 2005; Le Bivic 2005). These studies suggest that Baz acts upstream of adherens junction formation during polarization of *Drosophila* epithelia even though adherens junctions are known as key landmarks for establishing epithelial cell polarity (Nelson 2003). Adherens junction mutants show normal localization of Baz at the apical region, whereas the assembly of *Drosophila*

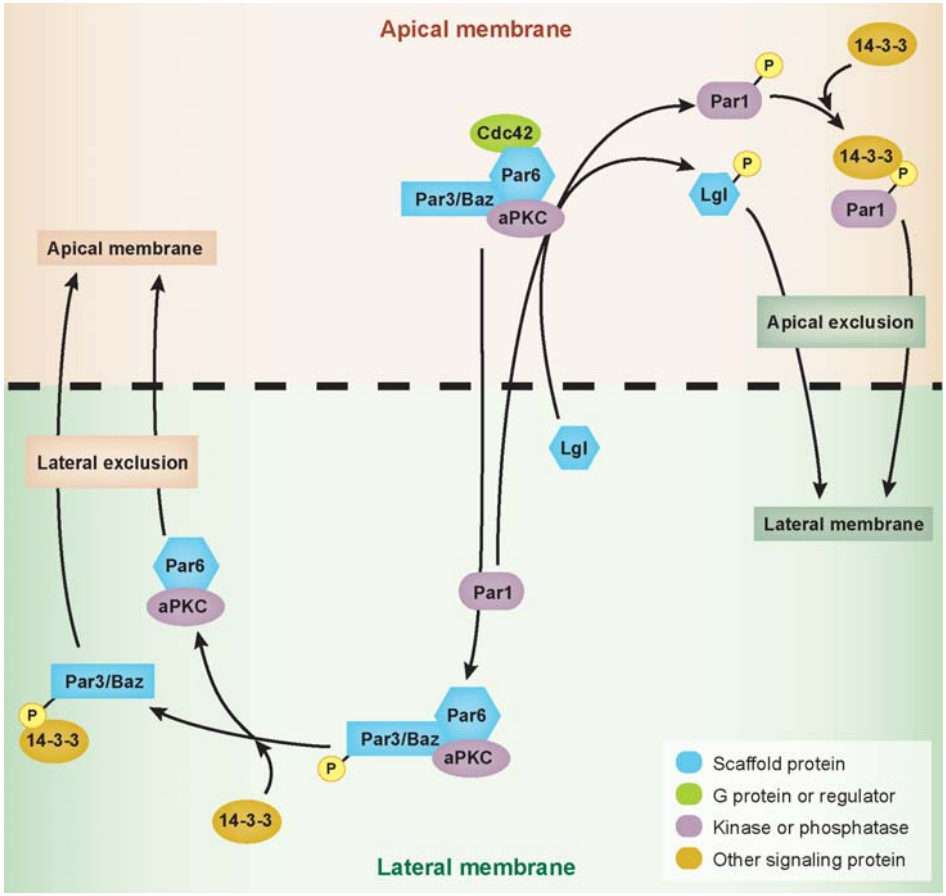

Figure 3

Mutual exclusion of polarity complexes in apico-basal polarity. Apical and lateral membranes are balanced by mutual exclusion of polarity proteins. The exclusion of proteins from either the apical or lateral domains is mediated by phosphorylation. For example, when Lethal Giant Larvae (Lgl) crosses to the apical domain, it is phosphorylated and sent back to the basolateral surface. Similarly the localization of partitioning defective 3 (Par3) and Par1 is controlled by phosphorylation (see text for additional details). aPKC, atypical protein kinase C; Baz, Bazooka.

E-cadherin in adherens junctions is disrupted in the absence of Baz (Harris & Peifer 2004). A subsequent study indicates that Baz targets to an apical region below aPKC and Par6 and that it colocalizes with the adherens junctions as epithelia first form (Harris & Peifer 2005). The study shows that Baz positioning is dependent on cytoskeletal cues and is independent of aPKC and Par6. The targeting of these polarity proteins results in the formation of a stratified apical domain, with Baz and adherens junctions localizing basally to aPKC and Par6. However, further studies are necessary to elucidate the exact hierarchy between the components of the Baz/Par6/aPKC complex and adherens junction during cellularization and gastrulation of the *Drosophila* embryo. In mammalian epithelia, there is still a strong belief that cell adhesion and adherens junction formation precede epithelial polarization (Nelson 2003).

On the basis of these biochemical studies of the Par complex, one can propose a model mechanism whereby the Par complex is

Lgl: Lethal Giant Larvae

regulated (**Figure 3**). The N-terminal region of Par6 is necessary to activate aPKC (Yamanaka et al. 2001). However, full-length Par6 is not able to stimulate aPKC owing to structural restrictions, although it is able to bind aPKC. Activated Cdc42 binds to Par6 and induces a conformational change in Par6 that results in increased affinity for aPKC (Garrard et al. 2003). Increased binding between the N terminus of Par6 and the regulatory domain of aPKC activates the kinase activity of aPKC. Par3 also participates in the regulation of this complex. Par3 promotes epithelial tight junction formation through interactions with aPKC (Hirose et al. 2002). Moreover, Par3 also regulates the polarity of epithelia by interacting in a phosphorylation-dependent manner with 14-3-3 (Hurd et al. 2003a). This interaction seems to regulate the targeting of the Par6-aPKC complex rather than aPKC activity. As mentioned above, Par3 also interacts with JAM, a transmembrane protein at the tight junction (Ebnet et al. 2001, Itoh et al. 2001). Par3 may be recruited by JAM to the tight junction and function there as a scaffolding protein to bring Par6-aPKC and Cdc42 into spatial proximity upon the formation of cell-cell contacts (Kim et al. 2000). Recently, an additional mode of activation of the Par complex has been proposed. T lymphoma invasion and metastasis (Tiam1), a Rac-specific guanine nucleotide exchange factor, directly interacts with and activates Par3 and aPKC through the activation of Rac and regulates the polarity of epidermal keratinocytes (Mertens et al. 2005). This regulation is independent of Cdc42 activation. In contrast, in another study Chen & Macara (2005) showed that Par3 binds to and inhibits Tiam1-mediated Rac activation, resulting in the promotion of tight junction assembly in epithelial cells. A third study in neuroblastoma cells suggests the Par complex mediates Rac activation by Cdc42, leading to cell migration (Nishimura et al. 2005). Further studies are necessary to understand these contrasting results.

Finally, there is the question of how exactly the Par complex regulates epithelial polarity. Studies have suggested that aPKC activity is required for the establishment of epithelial cell polarity (Suzuki et al. 2001, 2002). Overexpression of a kinase-dead mutant of aPKC leads to the disruption of the barrier function of tight junctions and the mislocalization of tight junction proteins, including ZO-1, occludin, and claudin-1. The localization of lateral and apical membrane proteins is also affected, suggesting that aPKC plays an important role in the formation and/or maintenance of epithelial polarity. Moreover, the zebrafish homolog of aPKC, Heart and Soul, is required for the formation and maintenance of the ZA during early epithelial development in zebrafish (Horne-Badovinac et al. 2001). One of the target proteins of aPKC is Lethal Green Larvae (Lgl), originally identified as a tumor suppressor gene and required for basal protein targeting and asymmetric cell division in *Drosophila* (Ohshiro et al. 2000, Peng et al. 2000). Studies show that aPKC directly interacts with and phosphorylates Lgl and that this phosphorylation event regulates the localization of Lgl, controlling polarity in *Drosophila* and mammalian cells (Betschinger et al. 2003, Plant et al. 2003, Yamanaka et al. 2003). Lgl localizes to the basolateral membrane in completely polarized mammalian epithelia. On the basis of the results from several studies, it is proposed that Lgl is bound to Par6-aPKC, preventing the localization of Par6-aPKC to the junctional complex. Once Cdc42 becomes activated, Lgl is phosphorylated by aPKC, dissociates from Par6-aPKC, and is restricted to the lateral membrane (Plant et al. 2003, Yamanaka et al. 2003). As a result, the balance between the apical domain and basolateral domain is determined by the activity of the Par complex at the apical domain and that of Lgl at the lateral domain (**Figure 3**). Par1 kinase, one of the evolutionarily conserved polarity determinants, is another target of aPKC. Recent studies show that aPKC contributes to epithelial polarity by phosphorylating Par1

and dissociating it from the membrane in a context similar to aPKC and Lgl (**Figure 3**) (Hurov et al. 2004, Suzuki et al. 2004).

Interplay Between Polarity Protein Complexes

Recent biochemical and genetic studies have shown physical and functional interactions between the CRB and Par polarity complexes. In mammalian epithelial cells, PALS1 physically interacts with Par6 through the N-terminal region of PALS1 and the PDZ domain of Par6, providing a direct link between the CRB3 and Par6 complexes (Hurd et al. 2003b). This interaction is implicated in the development of epithelial polarity and is regulated by the binding of active Cdc42 to Par6. Moreover, this interaction is evolutionarily conserved, as Sdt is able to interact with *Drosophila* Par6 through a conserved Par6-binding domain (Wang et al. 2004). The PDZ domain of Par6 also interacts directly with CRB3 (Lemmers et al. 2004). Interaction between the CRB and Par polarity complexes also exists in *Drosophila*. In *Drosophila* photoreceptors, the CRB and Par complexes are colocalized in the rhabdomeres stalk, and interaction between these complexes is mediated, in part, by the binding of DmPATJ to Par6 (Nam & Choi 2003). These polarity complexes are mutually dependent upon one another for proper localization in the *Drosophila* photoreceptor, as loss of the CRB complex leads to mislocalization of the Par complex and vice versa. Furthermore, aPKC binds to CRB and DmPATJ and phosphorylates CRB in *Drosophila*. This phosphorylation appears functionally significant. A nonphosphorylatable mutant of CRB behaves as a dominant negative and disrupts epithelial cell polarity. Overexpression of a kinase-dead aPKC also disrupts localization of apical and basolateral determinants to the appropriate membranes, supporting the idea that aPKC-mediated phosphorylation of CRB is important for cell polarity (Sotillos et al. 2004).

Scrib: Scribble

Epithelial polarization involves the establishment of distinct membrane domains represented by different polarity determinants, including the CRB and Par complexes. An important question is how these multiprotein complexes are coordinated and regulated to create the epithelial architecture of apicobasal polarity. Recent studies in *Drosophila* describe a functional hierarchy of interactions between polarity complexes during epithelial polarization. These studies give rise to a model in which polarity protein complexes act to antagonize one another and mutually exclude one another from the same region, thus setting up defined membrane domains with distinct compositions (Bilder et al. 2003, Tanentzapf & Tepass 2003). There are three major multiprotein complexes involved in epithelial polarization. As discussed above, the CRB and Par complexes act as apical membrane determinants. A third complex, the Scribble (Scrib) complex, localizes to the lateral domain and acts as a determinant of the lateral membrane domain. The Scrib complex consists of three proteins: Scrib, Discs Large, and Lgl. Although it is not clear if these proteins physically form a complex, studies in *Drosophila* demonstrate genetic interactions between these proteins (Bilder & Perrimon 2000, Bilder 2004).

Genetic studies in *Drosophila* suggest the following model: The CRB complex is recruited to the apical domain by the Par complex at an early stage of *Drosophila* epithelial development. Initial localization of the Par complex is not disrupted in CRB mutant embryo, but the CRB complex is required for apical maintenance of the Par complex at later steps of polarization. Activity of Cdc42 is required for the apical localization of Par6 (Hutterer et al. 2004). The Scrib complex determines the lateral domain and represses apical identity on the lateral region by antagonizing the Par complex. Also, the

apically localized CRB complex antagonizes the activity of the Scrib complex at apical domain. As mentioned above, this mutual exclusion of distinct domains is regulated through the phosphorylation of polarity protein complexes (**Figure 3**) (Plant et al. 2003, Yamanaka et al. 2003). The mutual antagonism of apical and lateral domains is also suggested in vertebrate development. Chalmers et al. (2005) demonstrated that loss of CRB3 or aPKC leads to the expansion of basolateral markers to the apical domain and that this is phenocopied by the overexpression of Lgl, suggesting that antagonistic regulation of protein complexes maintains the integrity of apico-basal polarity in mammalian cells. However, in mammalian epithelia, much work remains to understand the functional interaction of the basolateral and apical complexes, and the ordering of polarity protein targeting described in *Drosophila* may not be applicable.

RELATION OF POLARITY COMPLEXES AND TIGHT JUNCTION STRUCTURAL COMPONENTS

Above we highlight structural proteins of the tight junction and then review polarity complexes that target to the tight junction. The interaction between structural proteins of the tight junctions, such as claudins and ZO-1, and polarity complexes continues to be an area of active investigation. Structural proteins such as claudins play an important role as a fence preventing the mixing of apical and basolateral membranes. In this manner they act to reinforce polarity, but it appears unlikely that this fence function initiates polarity. This view is supported by studies in *Drosophila* in which epithelia polarize without a fence between apical and basolateral surfaces. The CRB and Par complexes localize to a region called the subapical or marginal zone that straddles the border between apical and basolateral surfaces. The seal between *Drosophila* epithelial cells is the septate junction, and that exists solely at the lateral side of the cell and not at the boundary between the apical and basolateral domains. In higher organisms this seal has moved to the tight junction and thus probably acts to reinforce polarity rather than establish it.

In our view, the polarity complexes function to determine the site of the tight junction in the process of cell polarization. In MDCK cells, RNAi-mediated reduction of PATJ expression results not only in polarity defects but also in a severe delay of tight junction formation, including mislocalization of occludin and ZO-3 to the lateral membrane (Michel et al. 2005, Shin et al. 2005). Mislocalization of tight junction proteins on the lateral membrane is similar to the results showing the failure of separation of the tight junction protein ZO-1 from adherens junctions in PATJ knockdown MDCKII cells (K. Shin, unpublished data). As mentioned above, some tight junction proteins, such as ZO proteins, initially localize to the cell-cell contact sites and then separate from the nascent adherens junction to form tight junctions (Itoh et al. 1993, Muller et al. 2005, Rajasekaran et al. 1996). In addition, a study shows that claudin-1 and occludin are correctly recruited to the functional tight junctions introduced by CRB3 expression in MCF10A cells (Fogg et al. 2005). These studies suggest that polarity proteins, including CRB3 and PATJ, play an important role in the correct localization of tight junction proteins. An emerging concept is that polarity complexes define the apico-basal polarity and create the landmark where the tight junction will form. This leads to a close interdependence between polarity complexes and tight junction structural components. Polarity complexes target proteins to the tight junction, and the formation of the tight junction then serves to reinforce polarity by preventing admixing of apical and basolateral membrane proteins.

SUMMARY POINTS

1. The tight junction is an important structure in mammalian epithelial cells that mediates a selective diffusion through the intercellular space and demarcates the boundary between the apical and basolateral plasma membrane domains.
2. Occludin, claudin, and JAM are transmembrane proteins that are important components of the tight junction. These proteins link to the actin cytoskeleton through interactions with scaffolding proteins such as ZO proteins. Transmembrane proteins play an important role in paracellular permeability.
3. Tight junction proteins are also involved in signal transduction to regulate cell proliferation and differentiation. These proteins include ZONAB, Rab13, and cingulin and control cell proliferation by participating directly in signaling cascades or by regulating gene expression in the nucleus.
4. Two evolutionarily conserved protein complexes, the CRB and Par complexes, are localized at the tight junction and are involved in the establishment and maintenance of epithelial cell polarity.
5. The CRB and Par complexes determine the apical domain of epithelial cells by excluding a lateral determinant, the Scrib complex, from the apical domain. Similarly, the Scrib complex antagonizes CRB and Par complexes at the lateral membrane. This mutual exclusion between polarity complexes leads to the establishment and maintenance of apico-basal polarity in epithelia.

FUTURE ISSUES

1. Although it is known that polarity proteins play an important role in apico-basal polarity and that interaction between these proteins is crucial for their function, the detailed molecular mechanisms whereby these proteins control and regulate epithelial polarity are still unclear. It is important to understand better how these polarity proteins interact with other signaling events during polarization as well as to find new binding partners for polarity proteins that may provide missing links between polarity proteins and other signaling pathways.
2. Although the hierarchy among polarity proteins during *Drosophila* epithelial polarization is well established, the temporal and spatial nature of polarity protein interactions during mammalian epithelial development is unclear. More information on the interactions and regulation of polarity proteins is required to elucidate the detailed molecular mechanisms of the polarization process.
3. Recent studies suggest that tight junction proteins such as ZONAB and Rab13 regulate epithelial proliferation and differentiation. Further work is necessary to understand the mechanisms whereby tight junctions regulate cell proliferation, how this role of tight junctions is functionally related to the polarization of epithelia, and how polarity proteins are involved in proliferation.

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