Tight Junction, a Platform for Trafficking and Signaling Protein Complexes

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Epithelial cells are associated laterally in a sheet, with the apical surface facing the lumina, often covered by microvilli, and the basolateral side underlying connective tissue. Development and maintenance of epithelial polarity require cell–substrate and cell–cell adhesions that promote localized assembly of submembranous cytoskeleton networks and specialized membrane intracellular transport pathways. The assembly of tight junctions (TJs) plays a key role in the formation of structurally and functionally distinct basolateral and apical plasma membrane (PM) domains. How are the PM proteins delivered to specialized and polarized cell surface domains? Even though the involvement of v- and t-soluble N-ethylmaleimide–sensitive factor attachment protein receptors (SNAREs) in apical and basolateral PM delivery is now well established, are these proteins sufficient to specify vesicle docking and fusion at sites in the appropriate membrane domains? Recent data highlight TJs as major sites for localization of proteins involved in vesicle docking/targeting and signaling. This review will focus on how TJ constituents contribute to these processes.

Molecular Architecture of TJ, Plasma Membrane Barriers

TJs are specialized PM microdomains that form continuous branching strands around each epithelial cell, separating apical from basolateral sides. By freeze–fracture electron microscopy, they appear as a network of continuous anastomosing fibrils. These fibrils are tightly connected laterally to those in apposing membranes (kisses) to seal the paracellular space between adjacent cells. This architectural organization confers two important properties to TJs: they act as (a) a selective intercellular barrier regulating diffusion of molecules and ions across the paracellular route, and (b) a fence within the lateral PM, thus impairing the intermixing of apical and basolateral membrane proteins and lipids. Significant progress in understanding the functional architecture of TJs was achieved by identifying key components of TJs.

Occludin was the first integral membrane protein found concentrated within TJ fibrils. Several pieces of evidence suggest that occludin is involved in the barrier and fence functions of TJs (Tsukita and Furuse, 1999). Overexpression of the chicken occludin in MDCK cells increases transepithelial electrical resistance, whereas COOH-terminal truncated occludin increases paracellular flux of small tracers and induces leakage of N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl) sphingosyl phosphocholine (BODIPY)–sphingomyelin from the apical domain (Balda et al., 1996). Introduction of a peptide containing the second extracellular loop of occludin into Xenopus A6 epithelial cells causes a drop in transepithelial electrical resistance and mislocalization of occludin at TJs (Wong and Gumbiner, 1997). However, data from several experiments indicate that occludin is not sufficient to generate bona fide TJ strands. For example, differentiated embryonic bodies that were isolated from embryonic stem cells in which the occludin gene was knocked out still developed a normal network of TJ fibrils between adjacent epithelial cells (Saitou et al., 1998). In fact, establishment of TJ strands depends on claudins, which is another recently identified protein family that has at least 18 members. Claudins possess four transmembrane domains and are also localized at the site of close membrane–membrane apposition (kisses) within TJs. Expression of claudins1 and 2 into fibroblasts lacking TJs induce the formation of TJ strands that are morphologically similar to the epithelial TJ strands (Tsukita and Furuse, 2000). Analysis of oligodendrocyte–specific protein (OSP/claudin11) KO mice reveals the absence of TJ strands in myelin sheets of oligodendrocytes and sertoli cells (Gow et al., 1999), and paracellin1/claudin16 KO mice show an abnormal paracellular passage of Mg ions (Simon et al., 1999).
Table I. Tight Junction Proteins

| TJ proteins | Structural features | Function | Known partners |
|-------------|--------------------|----------|----------------|
| Claudins   | 4 TM, COOH-YV     | TJ barrier | ZO-1\(^1\) |
| Occludin   | 4 TM               | TJ barrier | ZO-1, ZO-2, Vap33, actin\(^{1,3}\) |
| JAM        | 1 TM, Ig-like      | Monocyte transmigration\(^4\) | |
| ZO-1       | 3 PDZ, 1SH3, GUK  | Scaffold protein, signaling molecule | Occludin, claudins, ZO-2, -3, cingulin, actin, ZONAB\(^{1,2,5,6}\) |
| ZO-2       | 3 PDZ, 1SH3, GUK  | Scaffold protein | ZO-1, actin\(^2\) |
| ZO-3       | 3 PDZ, 1SH3, GUK  | Scaffold protein | ZO-1, occludin\(^2\) |
| AF-6       | PDZ                | Scaffold protein | Ras, ZO-1\(^7\) |
| Dlg (Drosophila) | 3 PDZ, 1SH3, GUK | Scaffold protein, signaling molecule\(^8\) | ZO-1, -2, -3, occludin, myosin\(^7\) |
| Scribble (Drosophila) | 4 PDZ | Fence/membrane traffic\(^9\) | PKC \(^{11,12,13}\) |
| Cingulin   | coiled coil       | Signaling molecule\(^10\) | 5-PDE\(^{15,16}\) |
| Symplekin  |                    |           | G/C kinase\(^{17,18}\), exocyst subunits\(^{19}\) |
| ASIP/Par3  | 3 PDZ              |           | |
| Rab3b      | GDP/GTP binding   | Membrane traffic\(^{14}\) | |
| Rab13      | GDP/GTP binding   | Membrane traffic | |
| Rab8       | GDP/GTP binding   | Membrane traffic | |
| Sec6, Sec8 |                    | Vesicle docking | |

Exponent numbers indicate references: 1, Itoh et al., 1999; 2, Wittchen et al., 1999; 3, Lapierre et al., 1999; 4, Martin-Padura et al., 1998; 5, Cordenonsi et al., 1999; 6, Balda and Matter, 2000; 7, Yamamoto et al., 1997; 8, Woods and Bryant, 1991; 9, Bilder and Perrimon, 2000a; 10, Keon et al., 1996; 11, Joberty et al., 2000; 12, Lin et al., 2000; 13, Izumi et al., 1998; 14, Weber et al., 1994; 15, Zahraoui et al., 1994; 16, Marzesco et al., 1998; 17, Huber et al., 1993; 18, Ren et al., 1996; and 19, Grindstaff et al., 1998.

This finding leads to the proposal that Claudins and occludin generate a series of regulated channels within TJ membranes for the passage of ions and small molecules.

The cytoplasmic face of TJs is enriched in many peripheral membrane proteins (Table I). ZO-1, a 220-kD TJ phosphoprotein, is a member of a membrane-associated guanylate kinase domain (GUK) localized at cell–cell contacts (Mitic and Anderson, 1998). It contains three PDZ (PSD95, Dlg, and ZO-1), an SH3 domain, and an inactive GUK. PDZ domains are protein–protein interaction modules that recognize motifs of three amino acids at the COOH terminus of transmembrane proteins. ZO-1 may act as a molecular scaffold bringing together many proteins of TJs. ZO-1 binds Claudins, occludin, ZO-2, ZO-3, cingulin, and actin (Cordenonsi et al., 1999; Wittchen et al., 1999). It has three PDZ modules that could bind many different protein partners to control the dynamics of TJ assembly. Thus, it is tempting to speculate that the ZO-1/2/3 proteins are required for the clustering of Claudins and occludin to generate TJ fibrils and, presumably, the pores within these fibrils.

The complexity of TJ organization is reflected by the growing number of protein partners (Table I). A ZO1-associated nucleic acid–binding protein (ZONAB) associates with the SH3 domain of ZO-1 (Balda and Matter, 2000). In contrast, the partners for PDZ and SH3 domains of ZO-2/3 have not yet been identified. It is possible that these latter domains, and those contained in the other TJ proteins, also participate in targeting the proteins mentioned above, or different proteins, to TJs. Therefore, the presence of multiple PDZ modules in several TJ proteins points to a high degree of complexity of protein–protein associations. It remains to be determined how these proteins are spatially and dynamically arranged to form TJ complexes, and how the interaction at the junctional complex, with the underlying actin cytoskeleton, may regulate TJ functions. Exactly how actin is coupled to TJs is still an open question. A small number of actin filaments anchors at sites of kisses within TJs (Madara, 1998), presumably in close contact with occludin/claudins.

**Polarized Membrane Traffic in Epithelial Cells**

The occurrence of TJs implies that epithelial cells have specific routes to deliver and/or internalize proteins from the apical and basolateral PMs. These routes have been recently reviewed by several authors (Keller and Simons, 1997; Yeaman et al., 1999; Mostov et al., 2000) and can be classified into five groups (Fig. 1): (a) A1, the biosynthetic route to the apical PM; (b) A2, the apical recycling; (c) B1, the biosynthetic route to the basolateral PM; (d) B2, the basolateral recycling; and (e) B/A and A/B, the transcytosis from the basolateral to the apical PMs and vice versa, respectively.

Route A1 and B/A coexist in the human adenocarcinoma of the colon (CaCo-2) cell line and in hepatocytes, whereas route A1 is dominant in MDCK cells. There are also crossroads between routes B2, A2, and B/A, which meet in recycling and sorting endosomes in several types of epithelial cells. Endocytic signals play a role in routes B1 and B2, whereas N- and O-glycosylation and association with raft microdomains have been implicated in route A1 (Keller and Simons, 1997; Yeaman et al., 1999; Mostov et al., 2000). Interestingly, ZO-1 and occludin are associated with raft microdomains in T84 cells (Nusrat et al., 2000). Nevertheless, none of these signals seem to establish absolute rules for apical and/or basolateral sorting. Specific adaptors may play crucial roles in the sorting of apical and basolateral proteins. Hence, β1B, a subunit of an AP-1 coat, is required for the B1 route, and possibly B2 (Folsch et al., 1999).

**Two PM Domains—Two SNARE Machineries?**

Overwhelming evidence show that SNAREs are key proteins of membrane traffic. It is now clearly established that they provide the core machinery for lipid bilayer fusion (Weber et al., 1998), and that they are involved in all of the trafficking pathways (Bock and Scheller, 1999). SNAREs of the exocytic pathways include the syntaxin (1, 2, 3, and 4), synaptosome-associated protein (SNAP23/25), and brevin/Vesicle-Associated Membrane Protein...
Sec6/8 are concentrated in TJ areas (red rings).

Rab3b, Rab13, and Proteins internalized from each PM domain (yellow filling) recycling are delivered either to the apical or the basolateral PM. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical membrane and vice versa. Two distinct populations of postGolgi vesicles (purple transcytosis from the basolateral to the apical mem...
subsets of basolateral, apical, and/or junctional membrane proteins are preferentially delivered. As a consequence, the restriction of Rabs and Sec6/8 complex to TJs may enhance the specificity and efficiency of the targeting/docking of transport vesicles to their appropriate surface microdomains.

A crucial question is what maintains the correct distribution of targeted proteins to one PM domain? A possible answer is suggested by recent studies on Scrib, Dlg, and Lgl, three Drosophila proteins localized to the epithelial septate junctions (the analogue of vertebrate TJs). Loss of function of any of these genes leads to the disruption of cell polarity. These genes show strong genetic interactions, suggesting they are involved in a common pathway to control both cell growth and polarity. Furthermore, Scrib, Dlg, and Lgl are mutually dependent for proper localization, raising the possibility that they physically interact (Bilder et al., 2000). In the Scrib mutants, adherens junction proteins, including armadillo (a β-catenin homologue), are mislocated and found around the cell periphery, and apical transmembrane proteins exhibit unrestricted distribution to both apical and basolateral domains (Bilder and Perrimon, 2000). Thus, Scrib is required for maintaining apical membrane proteins at the apical domain, and it may play a role in polarized targeting of vesicles charged with apical proteins. In agreement with this hypothesis, the Lgl homologues in yeast (Sro7) and mammals (tomosyn) bind to PM t-SNAREs Sec9p and syntaxin 1, respectively, which directly promote fusion of transport vesicles with the PM (Fujita et al., 1998; Lehman et al., 1999). An attractive model for the function of Scrib, Dlg, and Lgl could be that the PDZ domains of Scrib and Dlg bind to transmembrane proteins and organize cell surface asymmetry, whereas Lgl locally promotes the assembly of SNAPE complexes. The localized assembly of SNAPEs at specific sites of the PM would restrict vesicle docking and fusion at these sites.

Scrib, like other leucine-rich repeats and PDZ domain (LAP) proteins, could also bind through its leucine-rich repeats to small GTPases of the Ras family known to play an important role in intracellular transport. Accordingly, PDZ-containing proteins may interact (directly or indirectly) with the Rab/exocyst and contribute to the specificity and accuracy of vesicle-targeting events. We speculate also that the interactions of Rab/exocyst/Lgl/Scrib/Dlg define a checkpoint site at TJs where misrouted proteins could be identified and rerouted to their correct destination.

**TJ, a Signal Transduction Site**

Various signaling molecules, such as protein kinase C (PKC), heterotrimeric G-protein, and phospholipase C (PLC), that affect the organization of the actin cytoskeleton and regulate membrane traffic are implicated in TJ functions. Recent findings show that Cdc42 and Rac, involved in the dynamics of actin cytoskeleton and cell polarity, bind to a protein complex containing Par6, Par3/ASIP, and atypical PKCζ isofrom. In the nematode Caenorhabditis elegans, Par-3 and Par-6 are implicated in asymmetric cell division. For example, Par-3 mutations cause mislocalization of several asymmetrically distributed proteins, disruption of mitotic spindle orientation and disruption of asymmetric cell division. In epithelial cells, Par3/ASIP localizes to TJs. Overexpression of either Par6, the NH2-terminal part of Par3, or the activated form of Cdc42 leads to mislocalization of ZO-1, indicating that the Cdc42/Par6/Par3/aPKCζ complex may be involved in maintaining cell–cell contact (Joberty et al., 2000; Lin et al., 2000). The specific binding of activated Cdc42/Rac1 to Par6 links Par6/Par3/aPKCζ complex to signaling pathways regulating actin cytoskeleton and vesicle targeting. Interestingly, microinjection of Cdc42T22N, a dominant-negative form of Cdc42, into MDCK cells leads to mislocalization of the basolateral membrane protein, gp58 (Kroschewski et al., 1999). Given the ability of Cdc42 to control actin cytoskeleton, it is possible that the Cdc42 mutation causes localized alterations in cortical actin filaments, leading to the loss of gp58's basolateral polarity. This result also suggests that the actin cytoskeleton may help to immobilize a Cdc42-interacting protein required to target basolateral membrane proteins. Control of the dynamics of F-actin rearrangement by the small GTPases Rho, Rac, and Cdc42 points to a role for these GTPases in TJ structure. Indeed, overexpression of activated forms RhoV14 and RacV12 induces the disorganization of TJ strands and a chaotic distribution of ZO-1 and occludin. ZO-1, as well as occludin staining, extends to the base of the cells expressing RhoV14 and RacV12 (Jou et al., 1998). One explanation is that RhoV14 and RacV12 cause dramatic changes in actin–myosin dynamics, leading to an alteration in TJ protein–protein interaction. Hence, the Rho-protein family, by regulating actin organization, could participate in targeting vesicles to the appropriate sites on the PM. Signal transduction at TJs could regulate membrane traffic by phosphorylation of Rab effectors, SNARE proteins, or their partners. For instance, these signaling pathways could play a major role in allowing exocytotic vesicles to reach the apical domain.

Several membrane-associated GUKs are involved in organizing signal transduction at TJs. In Drosophila, the Dlg mutation interferes with proliferation control, apical–basal polarity, development of septate junctions, and the ability of cells to differentiate (Woods and Bryant, 1991). In subconfluent epithelial cells, ZO-1, as well as symplekin, can be found in the nucleus, which raises the possibility that both proteins may have a role in regulating transcription (Gottardi et al., 1996; Keon et al., 1996). Recently, Balda and Matter (2000) have identified ZONAB, a protein homologous to Y-box transcription factors, which localizes to the nucleus and TJs. ZO-1 and ZONAB control endogenous ErbB-2 expression and regulate the paracellular permeability. These results indicate that ZO-1 is implicated in a signal transduction pathway that leads to the activation of specific gene expression. Expression of two NH2-terminal mutants of ZO-1 (ZO-11-422 and ZO-11-784) cause a dramatic change in cell morphology, reminiscent of an epithelium to mesenchyme transition (Ryeom et al., 2000), suggesting that ZO-1 is involved in regulating epithelial cell differentiation. In addition, it was recently reported that introduction of occludin into Raf-1–transformed Pa-4 epithelial cells results in reacquisition of epithelial phenotype, with formation of functionally intact TJs (Li and Mrsny, 2000). These data also suggest that occludin is a key molecule in Raf-1–transformation signaling. Taken together, the results imply that the TJ is a site of signaling.

Based on the studies reviewed here, TJs emerge as a platform used to coordinate multiple cellular processes. TJs function at crossroads of the secretory and signaling routes. They may serve both as a docking domain and
probably as a switch station for subsets of proteins and lipid domains destined to the basolateral and/or the apical PM. At least some of the basolateral and/or apical membrane proteins could be delivered to TJs via the Rab/exocyst/Scrib/Lgl complex. A major challenge for future TJ studies will be to investigate its potential role in organizing and orienting polarized membrane trafficking.

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|-------------|---------------------|----------|----------------|
| Claudins    | 4 TM, COOH-YV       | TJ barrier | ZO-1           |
| Occludin    | 4 TM                | TJ barrier | ZO-1, ZO-2, Vap33, actin1-3 |
| JAM         | 1 TM, Ig-like       | Monocyte transmigration4 | Occludin, claudins, ZO-2, -3, cingulin, actin, ZONAB1,2,5,6 |
| ZO-1        | 3 PDZ, 1SH3, GUK    | Scaffold protein, signaling molecule | Occludin, claudins, ZO-2, -3, cingulin, actin, ZONAB1,2,5,6 |
| ZO-2        | 3 PDZ, 1SH3, GUK    | Scaffold protein | ZO-1, actin2 |
| ZO-3        | 3 PDZ, 1SH3, GUK    | Scaffold protein | ZO-1, occludin2 |
| AF-6        | PDZ                 | Scaffold protein | Ras, ZO-17 |
| Dlg (Drosophila) | 3 PDZ, 1SH3, GUK | Scaffold protein, signaling molecule8 | |
| Scribble (Drosophila) | 4 PDZ | Fence/membrane traffic9 | ZO-1, -2, -3, occludin, myosin5 |
| Cingulin    | coiled coil         | Signaling molecule10 | PKC ε11,12,13 |
| Symplekin   | 3 PDZ               | Signaling molecule | Rab8 GDP/GTP binding |
| ASIP/Par3   | 3 PDZ               | Signaling molecule | Rab8 GDP/GTP binding |
| Rab3b       | GDP/GTP binding     | Membrane traffic14 | Sec6, Sec8 GDP/GTP binding |
| Rab13       | GDP/GTP binding     | Membrane traffic | Sec6, Sec8 GDP/GTP binding |
| Rab8        | GDP/GTP binding     | Membrane traffic | Sec6, Sec8 GDP/GTP binding |
| Exponent numbers indicate references: 1, Itoh et al., 1999; 2, Wittchen et al., 1999; 3, Lapierre et al., 1999; 4, Martin-Padura et al., 1998; 5, Cordenonsi et al., 1999; 6, Balda and Matter, 2000; 7, Yamamoto et al., 1997; 8, Woods and Bryant, 1991; 9, Bilder and Perrimon, 2000a; 10, Keon et al., 1996; 11, Joberty et al., 2000; 12, Lin et al., 2000; 13, Izumi et al., 1998; 14, Weber et al., 1994; 15, Zahraoui et al., 1994; 16, Marzesco et al., 1998; 17, Huber et al., 1993; 18, Ren et al., 1996; and 19, Grindstaff et al., 1998. TM, transmembrane.