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ROLE OF TIGHT JUNCTIONS IN ESTABLISHING AND MAINTAINING CELL POLARITY

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ABSTRACT

The tight junction (TJ) is not randomly located on the cell membrane, but occupies a precise position at the outermost edge of the intercellular space and, therefore, is itself considered a polarized structure. This article reviews the most common experimental approaches for studying this relationship. We then discuss three main topics. (*a*) The mechanisms of polarization that operate regardless of the presence of TJs: We explore a variety of polarization mechanisms that operate at stages of the cell cycle in which TJs may be already established. (*b*) TJs and polarity as partners in highly dynamic processes: Polarity and TJs are steady state situations that may be drastically changed by a variety of signaling events. (*c*) Polarized distribution of membrane molecules that depend on TJs: This refers to molecules (mainly lipids) whose polarized distribution, although not the direct result of TJs, depends on these structures to maintain such distribution.

INTRODUCTION

Polarity and tight junctions (TJs) are the two fundamental features of the transporting phenotype. Polarity provides the necessary vectoriality for substances to be transported across epithelia toward or away from the lumen. TJs ensure that those substances do not diffuse back through the intercellular space. In the past, TJs were regarded as hermetic, and vectorial movement of substances

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across epithelia was not considered to obey the laws of thermodynamics.[1] More recently, the use of Na-tracers has demonstrated that, in fact, the unidirectional fluxes of Na^+ may differ by a factor of 20 or more, even in the absence of an external driving force, and Koefoed-Johnsen & Ussing (55) proposed a model whose basic feature was the structural asymmetry of epithelial cells to account for such transport. In turn, it was found that the TJs of some epithelia may be highly leaky (8, 30, 100), but the TJ is still considered hermetic:[2] Its synthesis, structure, and physiological properties are not fully understood.

THE TIGHT JUNCTION

The TJ is a belt of anastomosing strands of proteins and lipids that surrounds the lateral membrane of epithelial cells and seals the outermost end of the intercellular space. For a further description, see the review by L Mitic and JM Anderson in this volume.

POLARITY

In a broad sense, polarity is not an exclusive feature of the cell membrane but is reflected in the position of the nucleus, the Golgi apparatus, microvilli, mitochondria, flagellae, dendrites, axons, microtubules, microfilaments, and the composition of the extracellular matrix, e.g. the basal lamina. However, this review is restricted to the polarity of the cell membrane. Actually, a certain degree of asymmetry, or at least regionality in the distribution of membrane components, is found in most cells, including some that do not have TJs. Thus neurons, spermatozoa, yeasts, skeletal muscle fibers, osteoclasts, and T cells have pumps, channels, carriers, and receptors, and some of them bud daughter cells and bind viruses in restricted domains of the membrane (6, 77, 92, 94, 111, 117).

EXPERIMENTAL APPROACHES TO STUDY THE RELATIONSHIP BETWEEN TJs AND POLARITY

Yeast budding is polarized, either in sites adjacent to the previous budding sites (axial budding pattern) or from the opposite end of the cell (bipolar budding pattern) in response to cues that are spatially and chronologically arranged in a hierarchy of mechanisms: (*a*) assembly of a signaling apparatus at the

[1]At the beginning of this century, when G Galeotti proposed that the electrical potential across the frog skin was due to a higher Na^+ permeability in the inward than in the outward direction, it was argued that such asymmetry would be in violation of the laws of thermodynamics (16, 18).

[2]The original meaning of hermetic, refers to Hermes Trimegistus, the Greek name for the Egyptian god Thoth, founder of alchemy and other occult sciences, and is used to allude to something that remains obscure.

budding site to decode the cue; (*b*) reinforcement of the cue and assembly of the cytoskeleton; and (*c*) propagation of signals controlling the secretory pathway, the actin cables, and microtubules that convey the information to the cytoplasm (34). One of the main advantages offered by yeasts is that they can be easily mutated to individualize genes, such as *Rho1p, cdc24,* and *cdc28,* that participate in the different stages of polarization, acting, e.g., through cyclins, GTP-binding proteins, and protein kinases (33, 40, 56, 113, 129).

Transitions from fertilized egg throughout embryogenesis have been used to study the moment and circumstances in which cells activate a given gene, express specific molecules (e.g. ZO-1, cingulin, E-cadherin), assemble the cytoskeleton, and depend on signals derived from the extracellular matrix, adjacent cells, or remote cells that send growth and differentiation factors (for an extensive review, see 37). Embryogenesis has been used also to understand the steps leading to the expression of TJs and apical/basolateral polarity. The retinal pigment epithelium (RPE), unlike most epithelia, expresses Na^+,K^+-ATPase on the apical rather than basolateral plasma membrane (7, 50, 102) and is a valuable tool for investigating the mechanisms of distribution of this enzyme (65, 82).

Epithelial cells that are harvested and allowed to reaggregate in suspension culture may form closed follicles in which microvilli extend into the lumen, or inverted follicles in which the microvilli are oriented outward, depending on the culture medium used. In both configurations, the TJs separate the apical from the basolateral domain (21, 72, 73, 74, 75, 86, 87).

Cultured monolayers of established cell lines that resemble native epithelia express TJs and polarity (19, 20, 78, 99). When dissociated with trypsin-EDTA, the epithelial cells lose these features, which are recovered in a few hours when seeding is made at confluence, so cells do not have to divide to fill the area available for growing. These preparations offer several advantages: use of a single cell type; can be synchronized; provide a sufficient amount of cells for biochemical studies; easily mutated by changing experimental conditions (media composition, growth factors, extracellular matrix); and large monolayers amenable to measurement of transepithelial fluxes and electrical parameters. To some extent, these methods can also be applied to primary cultures, which offer the advantage that observations can be readily extrapolated to the tissue of origin.

Formation of TJs can be followed through the development of a transepithelial electrical resistance (TER) and through restriction to permeation of extracellular markers (3, 45). Changes in the surface area of apical and basolateral membrane domains can be gauged through changes in the electrical capacity (29, 54, 79, 80, 108). Polarization, in turn, is studied by specifically marking the molecules in a given pole with biotin-avidin (58), antibodies (14), ^{3}H-ouabain (17, 24), and lipid probes (124).

Cramer and coworkers (27, 28) have developed a model system, using cultured monolayers, to study migration of leukocytes through the space between the epithelial cells that involves the opening and resealing of TJs.

Ca^{2+} is one of many agents involved in synthesis and maintenance of TJs and apical/basolateral polarity. Cells plated at confluence and transferred to a calcium-free medium (42, 43), or maintained in a stirred medium without calcium (1, 38, 41, 47, 104, 110, 112, 125), do not polarize or make TJs. However, with the addition of Ca^{2+}, TJs form. Thus calcium is used as a tool (Ca-switch) to trigger both polarization and assembly and sealing of TJs (39, 66, 93).

Ca^{2+} is needed primarily on the extracellular side of the membrane (26, 45), probably to activate E-cadherins (48, 49, 53). During a Ca-switch, La^{3+} inhibits the influx of Ca^{2+} and prevents the increase of its cytosolic concentration, without affecting the sealing of TJs (Figure 1). Cd^{2+} blocks both Ca^{2+} entry and junction formation; however, it cannot trigger junction formation (26). The contact between neighboring cells promoted by Ca^{2+} activates a cascade of intracellular reactions, including phospholipase C (PLC), protein kinase C (PKC), and calmodulin (CaM) (2). Although the participation of PKC in the development of TJs and polarity is well documented (35, 79, 114), some controversy remains about the identity of the protein(s) phosphorylated by PKC (3, 22, 57, 116, 118). TJ assembly and sealing also involve a receptor mediated by two G proteins (2). These reactions are not limited to the establishment of TJs and polarization of membrane molecules, as they also participate in the expression of a variety of other cell-cell contacts (3, 90) and in rearrangement of the cytoskeleton (2, 12, 23).

Inhibitors of transcription and translation block the development of TJs and polarity, depending on when they are added (Figure 2). In some instances, however, protein synthesis is not necessary for restoration of polarized expression of certain specific proteins. Contreras et al (24) have shown that the polarized expression of Na^+,K^+-ATPase in newly plated MDCK cells is prevented by cycloheximide; however, these cells have an intracellular pool of Na^+,K^+-ATPase whose transfer to the plasma membrane depends on the synthesis of other peptide(s).

One of the problems in separating TJ formation from apical/basolateral polarization is that during differentiation, TJ development and apical/basolateral polarization occur simultaneously, and agents that impair the development of one will also block the expression of the other. However, there are significant exceptions. Thus proteolytic enzymes elicit the expression of TJs in HT29 cells (95, 96), and mutations of the basolateral and transcytosis signals impair the polarized distribution or the transcytosis of pIgG receptors without noticeable effects on the TJ (14, 15, 71). [The pIgG receptor has a sequence of amino acids

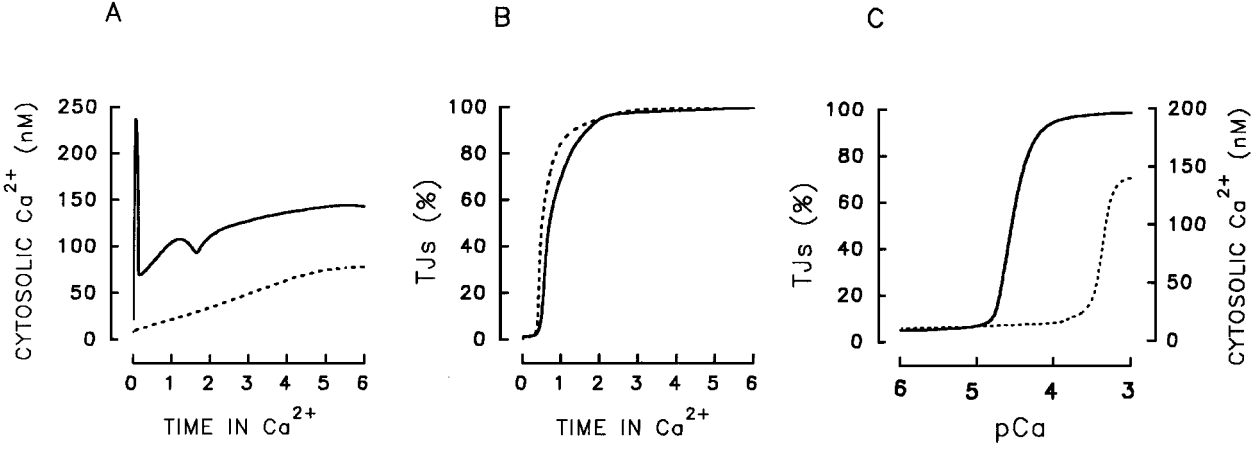

Figure 1 Ca^{2+} acts primarily at an extracellular site. (*A*) Before the Ca-switch, there is a low intracellular calcium concentration. With 1.8 mM Ca^{2+} the concentration rises to a peak, followed by a slow increase to control values (*full line*). The presence of La^{3+} suppresses the peak and markedly slows the increase of cytosolic Ca^{2+} (*dashed line*). (*B*) Time course of TJ sealing following a Ca-switch, in the absence and presence of La^{3+}. Junctions seal in spite of the low cytosolic Ca^{2+} concentration. (*C*) Junctional sealing (*full line*) and cytosolic Ca^{2+} (*dashed line*) following a switch to the concentration of Ca^{2+} in the abscissa. At 0.1 mM Ca^{2+}, the cytosolic Ca^{2+} concentration is essentially the same as before the switch, but TJs are 60% sealed (26, 45).

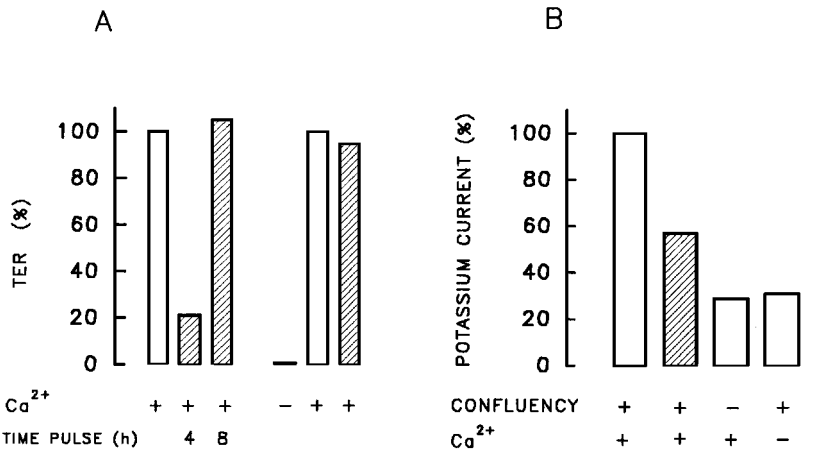

Figure 2 Dependence of tight junction formation and polarization on protein synthesis and Ca^{2+}. (*A*) Assembly and sealing of TJs is commonly gauged through the development of transepithelial electrical resistance (TER). MDCK cells plated at confluence ($t = 0$) and incubated for more than 15 h reach a maximum value of TER (first column). A pulse of cycloheximide between 2 and 4 h drastically reduces TER (second column). A similar pulse added between 6 and 8 h fails to inhibit the development of TER, suggesting that by this time, the necessary proteins are already synthesized (third column). Monolayers incubated for 20 h without Ca^{2+} do not develop TER (fourth column), but TER reaches a maximum value in 4 h of being transferred to 1.8 mM Ca^{2+} (fifth column) (Ca-switch). The increase in TER following the Ca-switch is not inhibited by cycloheximide (sixth column), which suggests that calcium acts mainly in the last steps of junction formation (17, 44). (*B*) MDCK cells express K^+ channels in a polarized manner: four types in the apical domain and a fifth in the basolateral domain. These channels are lost upon harvesting with trypsin-EGTA but recovered 8–15 h after plating at confluence (first column). Recovery is less in the absence of cell-cell contacts (second column) or Ca^{2+} (third column). Cycloheximide partially inhibits recovery (fourth column) (97, 120). In *A* and *B*, cross-hatching indicates the presence of inhibitor.

that addresses the protein to the basolateral domain and another that is required to send the receptor to the opposite side of the cell (transcytosis).]

MECHANISMS OF POLARIZATION THAT OPERATE REGARDLESS OF THE PRESENCE OF TJs

Early in the studies of polarization, it was assumed that it depended on a sorting signal and an addressing mechanism. Today, for every conceivable mechanism of polarization, there is a clear example of a molecule that depends on it. A thorough review of these mechanisms and signals would not be possible in this article, so we provide only a few examples of each type (Figure 3).

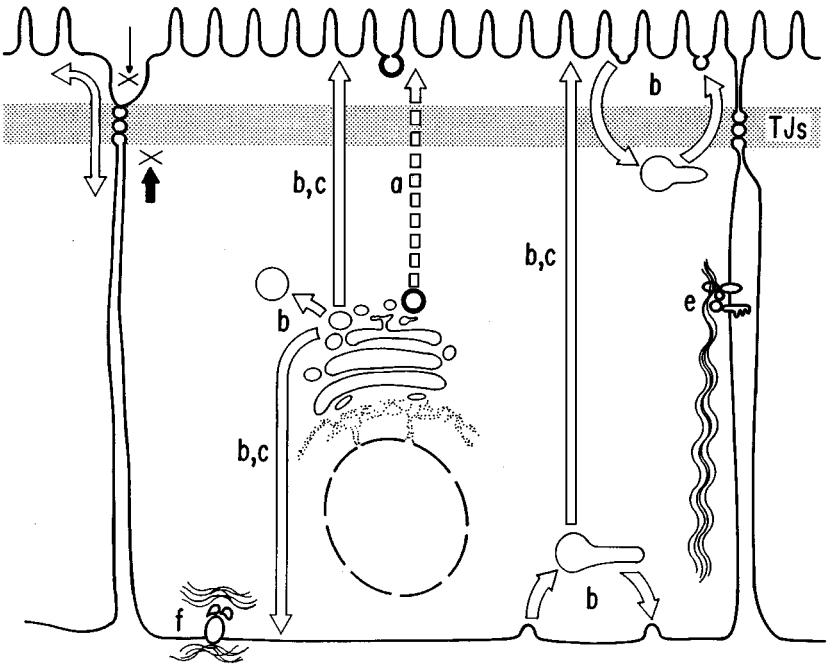

Figure 3 The relationship between TJs and polarity: epithelial cell in which the junctional belt (*dotted area*) separates the apical (*top*) from the basolateral (*bottom*) domain. Letters correspond to polarization mechanisms described in the text.

Anchorage to Glycosyl-Phosphatidylinositol (GPI)

GPI proteins are a class of glycoproteins whose extracellular domain is a polypeptide linked to a phosphatidic molecule of the membrane via an ethanolamine, a glycan, and an inositol. Lisanti et al (59, 60) have shown that GPI proteins owe their polarization to the fact that the lipid moiety is sorted in the *trans*-Golgi network (TGN) and addressed to the apical domain. Addition of a GPI signal to normally basolateral proteins, such as the G protein of the VSV virus, leads to its apical expression (10). GPI-anchored proteins appear to pack together to form lipid rafts in the TGN, which are then delivered to the apical membrane (reviewed in 105).

Other Addressing Signals

Basolateral delivery of numerous proteins depends on coated-pit related signals. These mechanisms are divided into two groups, depending on whether they are related to clathrin (69). For example, the proximal signal of the LDL receptor (68) and of the IgG Fc receptor are related to clathrin. These signals generally

rely on a tyrosine residue or on a dileucine motif. Typical examples of coated-pit mechanisms unrelated to clathrin are the distal determinant of the LDL receptor (52, 71) and of transferrin receptor (14). Clustering of other proteins in the membrane occurs through the recognition of carbohydrate moieties by a sorting receptor (36) or, as mentioned above, through a cytosolic protein coat structure.

Default Polarization

Deletion of the tetrapeptide Lys-Asp-Glu-Leu in some proteins normally expressed in intracellular organelles results in their insertion in the plasma membrane or secretion (62, 67, 81, 88, 106).

Hierarchical Signaling

There is now clear evidence of overlapping signals or differential interpretation of the same cue. Thus the LDL and polyimmunoglobulin receptors are expressed on the basolateral membrane by a dominant signal related to clathrin. However, once these receptors are exposed to the basolateral milieu and bind their respective ligands (LD, IgG, or IgM), they are readdressed and transcytosed to the apical domain (70, 15). HA antigen of the influenza virus is sorted to the apical domain, but when a cysteine 543 is mutated to tyrosine, the protein is readdressed to the basolateral domain (9). This suggests either that the tyrosine becomes a basolateral signal that now dominates over the apical signal, or that wild-type HA is being sorted to the apical domain by a default mechanism (until the basolateral signal, tyrosine, is incorporated) (69). The latter interpretation is supported by the fact that few apical determinants have been characterized (see above). Furthermore, Thy-1, a GPI-anchored protein that is addressed to the apical domain, is still delivered there upon removal of the GPI, implying that it must have a second apical signal or that apical delivery is through a default mechanism (98).

Anchoring to the Cytoskeleton

Disruption of microtubules and microfilaments with drugs such as colchicine, nocodazole, and cytochalasin results in missorting or reduction of the degree of polarization of HA proteins of influenza virus, but does not interfere with the delivery of G protein from stomatitis virus to the basolateral surface (101, 107). In most epithelial cells, Na^+,K^+-ATPase is delivered almost exclusively to the basolateral membrane (46) and is retained there through anchoring to ankyrin and fodrin (76, 83, 84, 85, 126).

Interaction with the Extracellular Matrix or with Neighboring Cells

TJs can be formed between different types of epithelial cells (44). Likewise, the junction-associated molecule ZO-1 can be expressed at junctions between cells

derived from dog Madin-Darby canine kidney (MDCK) or monkey (Ma104) epithelial cells (Figure 4 is available on the Annual Reviews site in the Supplementary Materials Section; http://www.AnnualReviews.org). On the contrary, MDCK cells express Na^+,K^+-ATPase and E-cadherin in the lateral membrane, provided these proteins are also expressed by the neighboring cell (Figure 4, see above). Intercalated cells (IC) from the collecting duct exist in two interconvertible forms: α-IC, which expresses H^+-ATPase in the apical domain and band 3 in the basolateral domain, and β-IC, which expresses the reversed polarity (109). Changes in pH induce the secretion of hensin, which becomes part of the extracellular matrix and can reverse the polarity of the α and β ICs (51, 119, 122).

Finally, there is clear evidence that some membrane proteins become polarized in the absence of TJs. Thus the newly plated human breast cancer cell line MCF-7, which expresses the antigen MAM-6 on the entire plasma membrane, reaches a 17:1 apical/basolateral polarization 2–6 h after plating, whereas TJs become evident only after 12–20 h (128).

DYNAMIC NATURE OF THE ROLE OF TJs IN EPITHELIAL CELL POLARITY

The cell membrane undergoes a continuous process of retrieval and restoration of molecules but does not randomize its components (91) nor perturb junctional sealing. In this respect, polarity and TJs should not be regarded as stable features, but as highly dynamic steady state configurations:

1. Binding of IgA or IgM to a membrane receptor (pIgR) located at the basolateral domain leads to receptor-ligand internalization followed by transcytosis to the apical membrane (15). In addition, the empty receptor is efficiently transcytosed, providing its serine-604 is phosphorylated (14).

2. Glucose, alanine, or leucine activate Na-cotransporters on the apical side of intestinal cells and elicit a decrease of the electrical resistance of the TJ, accompanied by the development of large dilatations between its strands (63; see review by JL Madara, this volume).

3. ADH triggers the incorporation of water channels in the apical membrane domain of renal collecting duct cells (11).

4. Intercalated cells retarget membrane domain markers in a pH/hensin-dependent manner (51, 119, 122; see above).

5. Leucine aminopeptidase is removed from the apical domain in MDCK cells and reinserted a few minutes later in the same domain (61).

6. The orientation of the apical and basolateral poles of cells in suspended follicles can be experimentally changed by adding serum, collagen, or hormones to the bathing medium. These changes are accompanied by large displacements of the TJ (21, 72, 73, 74, 86, 87).

7. TJs are retained during mitosis; thus cells in a mucosa proliferate, displace, and rearrange in the plane of the epithelium without breaking down its barrier function (5).

8. Additionally, TJs open and reseal to allow the passage of leukocytes during diapedesis (27, 28, 89).

9. TJs between Sertoli cells and between those cells and germ cells in seminiferous epithelium are sufficiently plastic to allow the transit of germ cells toward the lumen of seminiferous tubules without distorting the role of the blood-testis barrier (13).

10. Studies in the retinal pigment epithelium show that molecules such as Na^+,K^+-ATPase, spectrin, ankyrin, and antigen 5A11 polarize by moving at different times and even to opposite poles of the cell (103).

POLARIZED DISTRIBUTION OF MEMBRANE MOLECULES THAT DEPEND ON TJs

At the body temperature of mammals, the hydrophobic moiety of membrane lipids is in a liquid state, and in spite of strong hydrophilic bonds between polar groups and between the surrounding water, molecules can diffuse in the plane of the membrane. However, some lipid species are preferentially confined to a given membrane domain (32, 115, 121–124, 127). Lipid probes such as 5-[*N*-hexadecanoyl] amino fluorescein, 1-acyl-2-[*N*-4-nitrobenz-2-oxa-1, 3-diazole]aminocaproyl phosphatidylcholine, and gangliosides added to the apical membrane are unable to pass through the TJ. These molecules have a negligible flip-flopping rate. On the other hand, lipids that can readily flip-flop from the outer to the inner leaflet of the membrane (and back), such as 3, 3′-dihexacylin docarbocyanine iodide and 5-[*N*-dodecanoyl] aminofluorescein, are able to avoid the TJ and pass from the apical domain, where they were added, to the basolateral region. On this basis, the TJ is interpreted as a fence between the apical and the lateral domain of the outer leaflet only (31, 32).

As described by L Mitic and JM Anderson (this volume), occludin is probably the main protein constituent of the strands. Balda et al (4) found that MDCK cells expressing transfected chicken occludin, in addition to endogenous occludin, show a significant increase of transepithelial resistance (TER)

and prevent the apical-to-lateral diffusion of the fluorescent probe BODIPY-FL-sphingomyelin. Cells transfected with C-terminally truncated occludin do not form a continuous junctional belt of occludin or ZO-1, as revealed by immunofluorescence staining of these TJ proteins. Nevertheless, these monolayers exhibit an increased value of TER, indicating that a continuous junctional distribution of occludin is not required for the formation of electrically tight epithelia. Interestingly, in spite of this increase in TER, monolayers of cells transfected with C-terminally truncated occludin were no longer capable of preventing diffusion of the lipid probe from the apical to the basolateral domain (4).

CONCLUSIONS: INFLUENCE OF TJs ON POLARITY, AND OF POLARITY ON TJs

The relationship between TJs and polarity was once thought to be simple: Transport proteins were synthesized and somehow delivered to a given plasma membrane domain, and the TJ was regarded as a fence preventing lateral diffusion and randomization. As summarized above, the picture is now far more complex because membrane molecules can achieve polarization and redistribute in spite of the presence of the TJ. However, the TJ has been confirmed as a barrier that restricts the mixing of some freely diffusing membrane molecules (see, for instance, 64). Ironically, its precise position at the apical/basolateral interface is the result of a polarized insertion of its components. The polarized location of TJs, in principle, serves two functions: (*a*) The obvious one is to meet in register (contact at very specific site) a moiety in the neighboring cell; and (*b*) some of the molecules within the TJ have strong homology with proteins known to participate in signal-transducing cascades; thus signaling events are triggered by TJ components at specific points of the cell surface. TJs and polarity are not static features of cells; rather they represent highly dynamic arrangements of molecules in the plasma membrane that are sensitive to the composition of the extracellular matrix, contacts with neighboring cells, growth and differentiation factors, hormones, pharmacological agents, and cell cycle stages.

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Literature Cited

1. Anderson JM, Van Itallie CM, Peterson MD, Stevenson BR, Carew EA, Mooseker MS. 1989. ZO-1 mRNA and protein expression during tight junction assembly in Caco-2 cells. *J. Cell Biol.* 109:1047–56
2. Balda MS, González-Mariscal L, Contreras RG, Macias-Silva M, Torres-Marquez ME, Garcia Sains JA, Cereijido M. 1991. Participation of a G-protein modulation system in the assembly and sealing of tight junctions. *J. Membr. Biol.* 122:193–202
3. Balda MS, González-Mariscal L, Matter K, Cereijido M, Anderson JM. 1993. Assembly of the tight junctions: the role of diacylglycerol. *J. Cell Biol.* 123:293–302
4. Balda MS, Whitney JA, Flores C, González S, Cereijido M, Matter K. 1996. Functional dissociation of paracellular permeability and electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant membrane protein of tight junctions. *J. Cell. Biol.* 134:1031–49
5. Baker J, Garrod D. 1993. Epithelial cells retain junctions during mitosis. *J. Cell Sci.* 104:415–25
6. Baron R, Neff L, Louvard D, Courtoy PJ. 1985. Cell mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kDa lysosomal membrane protein at the osteoclast ruffled border. *J. Cell Biol.* 101:2210–22
7. Bok D. 1982. Autoradiographic studies on the polarity of plasma membrane receptors in retinal pigment epithelial cells. In *The Structure of the Eye*, ed. J Hollyfield, pp. 247–56. New York: Elsevier-North Holland
8. Boulpaep EL, Seely JL. 1971. Electrophysiology of proximal and distal tubules in the autoperfused dog kidney. *Am. J. Physiol.* 221:1084–96
9. Brewer CB, Roth MG. 1991. A single amino acid change in the cytoplasmic domain alters the polarized delivery of influenza virus hemagglutinin. *J. Cell Biol.* 114:413–21
10. Brown DA, Cride B, Rose JK. 1989. Mechanism of membrane anchoring affects polarized expression of two proteins in MDCK cells. *Science* 245:1499–501
11. Brown D, Katsura T, Kawashima N, Verknon AS, Sabolic I. 1995. Cellular distribution of the aquaporins: a family of the water channel proteins. *Histochem. Cell. Biol.* 104:1–9
12. Burgstahler AD, Nathanson MH. 1995. NO modulates the apicolateral cytoskeleton of isolated hepatocytes by a PKC-dependent, cGMP-independent mechanism. *Am. J. Physiol.* 269:G789–99
13. Byers S, Pelletier RM. 1991. Sertoli-Sertoli cell tight junctions and the blood-testis barrier. In *Tight Junctions*, ed. M Cereijido, pp. 279–304. Boca Raton: CRC Press
14. Casanova JE, Apodaca G, Mostov KE. 1991. An autonomous signal for basolateral sorting in the cytoplasmic domain of the polymeric immunoglobulin receptor. *Cell* 66:65–75
15. Casanova JE, Breitfeld PP, Ross SA, Mostov KE. 1990. Phosphorylation of the polymeric immunoglobulin receptor required for its efficient transcytosis. *Science* 248:742–45
16. Cereijido M, ed. 1992. Evolution on the ideas of the tight junction. In *Tight Junctions*, pp. 1–13. Boca Raton: CRC Press
17. Cereijido M, Ehrenfeld J, Fernández-Castelo S, Meza I. 1981. Fluxes, junctions and blisters in cultured monolayers of epithelial cells. *Ann. NY Acad. Sci.* 372:422–41
18. Cereijido M, Ponce A, González-Mariscal L. 1989. Tight junctions and apical/basolateral polarity. *J. Membr. Biol.* 110:1–9
19. Cereijido M, Robbins ES, Dolan WJ, Rotunno CA, Sabatini DD. 1978. Polarized monolayers formed by epithelial cells on a permeable and translucent support. *J. Cell Biol.* 77:853–80
20. Cereijido M, Rotunno CA, Robbins ES, Sabatini D. 1978. Polarized epithelial membranes produced in vitro. In *Membrane Transport Processes*, ed. J Hoffman, pp. 433–61. New York: Raven
21. Chambard M, Gabrion J, Mauchamp J. 1981. Influence of collagen gel on the orientation of epithelial cell polarity: follicle formation from isolated thyroid cells and from preformed monolayers. *J. Cell Biol.* 91:157–66
22. Citi S, Denisenko N. 1995. Phosphorylation of the tight junction protein cingulin and the effects of protein kinase inhibitors and activators in MDCK epithelial cells. *J. Cell Sci.* 108:2917–26
23. Citi S, Volberg T, Bershadsky AD,

Denisenko N, Geiger B. 1994. Cytoskeletal involvement in the modulation of cell-cell junctions by the protein kinase inhibitor H-7. *J. Cell Sci.* 107:683–92
24. Contreras RG, Avila G, Gutiérrez C, Bolívar JJ, González-Mariscal L, et al. 1989. Repolarization of Na-K-pumps during establishment of epithelial monolayers. *Am. J. Physiol.* 257:C896–905
25. Deleted in proof
26. Contreras RG, Miller J, Zamora M, González-Mariscal L, Cereijido M. 1992. The interaction of calcium with the plasma membrane of epithelial (MDCK) cells during junction formation. *Am. J. Physiol.* 263:C313–18
27. Cramer EB. 1992. The ability of leucocytes to cross tight junctions. In *Tight Junctions*, ed. M Cereijido, pp. 321–36. Boca Raton: CRC Press
28. Cramer EB, Milks LC, Ojakian GK. 1980. Transepithelial migration of human neutrophils: an in vitro model system. *Proc. Natl. Acad. Sci. USA* 77:4069–73
29. Crowe WE, Ehrenfeld J, Brochiero E, Wills NK. 1995. Apical membrane sodium and chloride entry during osmotic swelling of renal (A6) epithelial cells. *J. Membr. Biol.* 144:81–91
30. Diamond JM. 1974. Tight and leaky junctions of epithelia. *Fed. Proc.* 33:2220–24
31. Dragsten PR, Blumenthal R, Handler JS. 1981. Membrane asymmetry in epithelia: is the tight junction a barrier to diffusion in plasma membrane? *Nature* 94:718–22
32. Dragsten PR, Handler JS, Blumenthal R. 1982. Fluorescent membrane probes and the mechanism of maintenance of cellular asymmetry in epithelia. *Fed. Proc.* 41:48–53
33. Drgonová J, Drgon T, Tanaka K, Kollár R, Chen G-C, et al. 1996. Rho1p, a yeast protein at the interface between cell polarization and morphogenesis. *Science* 272:277–79
34. Drubin DG, Nelson WJ. 1996. Origins of cell polarity. *Cell* 84:335–44
35. Ellis B, Schneeberger E, Rabito CA. 1992. Cellular variability in the development of tight junctions after activation of protein kinase C. *Am. J. Physiol.* 263:F293–300
36. Fiedler K, Simons K. 1995. The role of N-glycans in the secretory pathway. *Cell* 81:309–12
37. Fleming TP, Johnson MH. 1988. From egg to epithelium. *Annu. Rev. Cell Biol.* 4:459–85
38. Forte JG, Naus AH. 1963. Effects of calcium removal on bullfrog gastric mucosa. *Am. J. Physiol.* 205:631–37
39. Franchi E, Camatini M. 1985. Evidence that a Ca^{2+} chelator and calmodulin blocker interfere with the structure of inter-sertoli junctions. *Tissue Cell* 17:13–25
40. Gimeno CJ, Fink GR. 1992. The logic of cell division in the life cycle of yeast. *Science* 257:626
41. González-Mariscal L. 1992. Biosynthesis of the tight junction. In *Tight Junctions*, ed. M Cereijido, pp. 243–55. Boca Raton: CRC Press
42. González-Mariscal L, Borboa L, López-Vancell R, Beaty G, Cereijido M. 1985. Electrical properties of MDCK cells. In *Tissue Cultured in Epithelial Cells*, ed. M Taub, pp. 25–36. New York: Plenum
43. González-Mariscal L, Chávez de Ramírez B, Cereijido M. 1985. Tight junction formation in cultured epithelial cells (MDCK). *J. Membr. Biol.* 86:113–25
44. González-Mariscal L, Chávez de Ramírez B, Lazaro A, Cereijido M. 1989. Establishment of tight junction between cells from different animal species and different sealing capacities. *J. Membr. Biol.* 107:43–56
45. González-Mariscal L, Contreras RG, Bolívar JJ, Ponce A, Chávez de Ramírez B, Cereijido M. 1990. Role of calcium in tight junction formation between epithelial cells. *Am. J. Physiol.* 259:C978–86
46. Gottardi CJ, Caplan MJ. 1993. Delivery of Na^+,K^+-ATPase in polarized epithelial cells. *Science* 260:552–54
47. Griepp EB, Dolan WJ, Robbins ES, Sabatini DD. 1983. Participation of plasma membrane proteins in the formation of tight junctions by cultured epithelial cells. *J. Cell Biol.* 96:693–702
48. Gumbiner B, Simons K. 1986. A functional assay for proteins involved in establishing an epithelial occluding barrier: identification of an uvomorulin-like peptide. *J. Cell Biol.* 102:457–68
49. Gumbiner B, Stevenson B, Grimaldi A. 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J. Cell Biol.* 107:1575–88
50. Gundersen D, Orlowski J, Rodríguez-Boulán E. 1991. Apical polarity of Na,K-ATPase in retinal pigment epithelium is linked to a reversal of the ankyrin-fodrin submembrane cytoskeleton. *J. Cell Biol.* 112:863–72
51. Hikita C, Takito J, Al-Awqati Q. 1995. Hensin, an ECM protein that reverses the polarity of band 3, is induced by cyclic AMP. *Mol. Biol. Cell* 65:401A

52. Hunziker W, Harter C, Matter K, Mellman I. 1991. Basolateral sorting in MDCK cells requires a distinct cytoplasmic determinant. *Cell* 66:907–20
53. Imhof BA, Vollmers HP, Goodman SL, Birchmeier W. 1983. Cell-cell interaction and polarity of epithelial cells: specific perturbation using a monoclonal antibody. *Cell* 35:667–75
54. Jovov B, Lewis SA, Crowe WE, Berg JR, Wills NK. 1994. Role of intracellular Ca^{2+} in modulation of tight junction resistance in A6 cells. *Am. J. Physiol.* 266:F775–84
55. Koefoed-Johnsen V, Ussing HH. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* 42:298–308
56. Kron SJ, Styles CA, Fink GR. 1994. Symmetric cell division in pseudohyphae of the yeast *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 5:1003–22
57. Kurihara H, Anderson JM, Farquhar MG. 1995. Increased tyr phosphorylation of ZO-1 during modification of tight junctions between glomerular processes. *Am. J. Physiol.* 268:F514–24
58. Le Bivic A, Real FX, Rodríguez-Boulán E. 1989. Vectorial targeting of apical and basolateral plasma membrane proteins in a human adenocarcinoma epithelial cell line. *Proc. Natl. Acad. Sci. USA* 86:9313–17
59. Lisanti MP, Caras IW, Davitz MA, Rodríguez-Boulán E. 1989. A glycophospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. *J. Cell Biol.* 109:2145–56
60. Lisanti MP, Sargiacomo M, Graeve L, Saltiel AR, Rodríguez-Boulán E. 1988. Polarized apical distribution of glycosyl-phosphatidylinositol-anchored proteins in a renal epithelial cell line. *Proc. Natl. Acad. Sci. USA* 88:9557–61
61. Louvard D. 1980. Apical membrane aminopeptidase appears at sites of cell-cell contact in cultured epithelial cells. *Proc. Natl. Acad. Sci. USA* 77:4132–36
62. Machamer CE, Rose JK. 1987. A specific transmembrane domain of a coronavirus E1 glycoprotein is required for its retention in the Golgi region. *J. Cell Biol.* 105:1205–14
63. Madara JL, Pappenheimer JR. 1987. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J. Membr. Biol.* 100:149–64
64. Mandel LJ, Bacallao R, Zampighi G. 1993. Uncoupling of the molecular "fence" and paracellular "gate" functions in epithelial tight junctions. *Nature* 361:552–55
65. Marrs JA, Anderson-Fisone C, Jeong MC, Cohen-Gould L, Zurzolo C, Nabi IR, et al. 1995. Plasticity in epithelial cell phenotype: modulation by the expression of different cell adhesion molecules. *J. Cell Biol.* 129:507–19
66. Martínez-Palomo A, Meza I, Beaty G, Cereijido M. 1980. Experimental modulation of occluding junctions in a cultured transporting epithelium. *J. Cell Biol.* 87:736–45
67. Matlin KS, Bainton D, Pesonen M, Genty N, Louvard D, Simons K. 1983. Transfer of a viral envelope glycoprotein from the apical to the basolateral plasma membrane of MDCK cells. I. Morphological evidence. *J. Cell Biol.* 97:627–37
68. Matter K, Hunzinker W, Mellman I. 1992. Basolateral sorting of LDL receptor in MDCK cells: The cytoplasmic domain contains two tyrosine-dependent targeting determinants. *Cell* 71:741–53
69. Matter K, Mellman I. 1994. Mechanisms of cell polarity: sorting and transport in epithelial cells. *Curr. Opin. Cell Biol.* 6:545–54
70. Matter K, Whitney JA, Yamamoto EM, Mellman I. 1993. Common signals control LDL receptor sorting in endosomes and the Golgi complex of MDCK cells. *Cell* 74:1053–64
71. Matter K, Yamamoto EM, Mellman I. 1994. Structural requirements and sequence motifs for polarized sorting and endocytosis of LDL and Fc receptors in MDCK cells. *J. Cell Biol.* 126:991–1006
72. Mauchamp J, Chambard M, Gabrion J, Verrier B. 1983. Polarized multicellular structures designed for the in vitro study of thyroid cell function and polarization. *Meth. Enzymol.* 98:477–86
73. Mauchamp J, Charrier B, Takasu N, Margotat A, Chambard M, Dumas D. 1979. Modulation by thyrotropin, prostaglandin E2 and catecholamines of sensitivity to acute stimulation in culture thyroid cells. In *Hormones and Cell Regulation*, ed. JE Dumont, J Nuñez, pp. 51–68. Amsterdam: North-Holland
74. Mauchamp J, Fayet G. 1974. Three-dimensional reorganization of thyroid cells in culture and iodide metabolism. *Endocrinol. Exp.* 8:170
75. Mauchamp J, Margotat A, Chambard M, Charrier B, Remy L, Michel-Bechet M. 1979. Polarity of three-dimensional structures derived from isolated hog thyroid cells in primary culture. *Cell Tissue Res.* 204:417–30

76. Mays RW, Beck KA, Nelson WJ. 1993. Organization and function of the cytoskeleton in polarized epithelial cells: a component of the protein sorting machinery. *Curr. Opin. Cell Biol.* 6:16–24
77. Miledi R. 1960. The acetylcholine sensitivity of frog muscle fibres after complete or partial denervation. *J. Physiol.* 151:1–23
78. Misfeldt DS, Hamamoto ST, Pitelka DR. 1976. Transepithelial transport in cell culture. *Proc. Natl. Acad. Sci. USA* 73:1212–16
79. Mullin JM, McGinn X. 1988. Effects of diacylglycerols on LLC-PK1 renal epithelia: similarity to phorbol ester tumor promoters. *J. Cell. Physiol.* 134:357–66
80. Mullin JM, Snock KV. 1990. Effect of tumor necrosis factor on epithelial tight junctions and transepithelial permeability. *Cancer Res.* 50:2172–76
81. Munro S, Pelham HR. 1987. A terminal signal prevents secretion of luminal ER proteins. *Cell* 48:899–907
82. Nabi IR, Mathews AP, Cohen-Gould L, Gundersen D, Rodríguez-Boulán E. 1993. Immortalization of polarized rat retinal pigment epithelium. *J. Cell Sci.* 104:37–49
83. Nelson WJ, Veshnock P. 1986. Dynamics of membrane-skeleton (fodrin) organization during the development of polarity in Madin-Darby canine kidney epithelial cells. *J. Cell Biol.* 103:1751–65
84. Nelson WJ, Veshnock P. 1987. Ankyrin binding to (Na^+,K^+)ATPase and implications for the organization of membrane domains in polarized cells. *Nature* 328:533–35
85. Nelson WJ, Veshnock P. 1987. Modulation of fodrin (membrane skeleton) stability by cell-cell contact in Madin-Darby canine kidney epithelial cells. *J. Cell Biol.* 104:1527–37
86. Nitsch L, Wollman SH. 1980. Thyrotropin preparations are mitogenic for thyroid epithelial cells in follicles in suspension culture. *Proc. Natl. Acad. Sci. USA* 77:2743–47
87. Nitsch L, Wollman SH. 1980. Ultrastructure of intermediate stages in polarity reversal of thyroid epithelium in follicles in suspension culture. *J. Cell Biol.* 86:875–80
88. Pääbo S, Bhat BM, Wold WSM, Peterson PA. 1987. A short sequence in the COOH-terminus makes an adenovirus membrane glycoprotein a resident of the endoplasmic reticulum. *Cell* 50:311–17
89. Parkos CA, Colgan SP, Madara JL. 1994. Interactions of neutrophils with epithelial cells: lessons from the intestine. *J. Am. Soc. Nephrol.* 5:138–52
90. Pasdar M, Li Z, Chan H. 1995. Desmosome assembly and disassembly are regulated by reversible protein phosphorylation in cultured epithelial cells. *Cell Motil. Cytoskelet.* 30:108–21
91. Patzac A, Winkler H. 1986. Exocytic exposure and recycling of membrane antigens of chromafin granules: ultrastructure evaluation after immunolabeling. *J. Cell Biol.* 102:510–15
92. Peng I, Dennis JE, Rodríguez-Boulán E, Fishman DA. 1987. Epithelial polarity in presumptive myocardial cells. *J. Cell Biol.* 105:2043–51
93. Pitelka DR, Taggart BN, Hamamoto ST. 1983. Effects of extracellular calcium depletion on membrane topography and occluding junctions of mammary epithelial cells in culture. *J. Cell Biol.* 96:613–24
94. Poindexter JS. 1964. Biological properties and classification of the Caulobacter group. *Bacteriol. Rev.* 28:231–95
95. Polack-Charcon S. 1992. Proteases and the tight junction. In *Tight Junctions*, ed. M Cereijido, pp. 257–77. Boca Raton: CRC Press
96. Polack-Charcon S, Shoham JJ, Ben-Shaul Y. 1978. Junction formation in trypsinized cells of human adenocarcinoma cell line. *Exp. Cell Res.* 116:1–13
97. Ponce A, Bolívar JJ, Vega J, Cereijido M. 1991. Synthesis of plasma membrane and potassium channels in epithelial (MDCK) cells. *Cell. Physiol. Biochem.* 1:195–204
98. Powell SK, Lisanti MP, Rodríguez-Boulán EJ. 1991. Thy-1 expresses two signals for apical localization in epithelial cells. *Am. J. Physiol.* 260:C715–20
99. Rabito CA. 1986. Reassembly of the occluding junctions in a renal cell line with characteristics of proximal tubular cells. *Am. J. Physiol.* 250:F734–43
100. Reuss L. 1992. Tight junction permeability to ions and water. In *Tight Junctions*, ed. M Cereijido, pp. 49–66. Boca Raton: CRC Press
101. Rindler MJ, Ivanov IE, Sabatini DD. 1987. Microtubule-acting drugs lead to the nonpolarized delivery of the influenza hemagglutinin to the cell surface of polarized Madin-Darby canine kidney cells. *J. Cell Biol.* 104:231–41
102. Rizzolo LJ. 1990. The distribution of Na^+,K^+-ATPase in the retinal pigmented

epithelium from chicken embryo is polarized in vivo but not in primary cell culture. *Exp. Eye Res.* 51:435–46
103. Rizzolo LJ, Zhou S. 1995. The distribution of Na^+,K^+-ATPase and 5A11 antigen in apical microvilli of the retinal pigment epithelium is unrelated to α-spectrin. *J. Cell Sci.* 108:3623–33
104. Rodríguez-Boulan E, Paskiet KT, Sabatini DD. 1983. Assembly of enveloped viruses in Madin-Darby canine kidney cells: polarized budding from single attached cells and from clusters of cells in suspension. *J. Cell Biol.* 96:866–74
105. Rodríguez-Boulán E, Powell SK. 1992. Polarity of epithelial and neuronal cells. *Annu. Rev. Cell Biol.* 8:395–427
106. Rothman JE. 1987. Protein sorting by selective retention in the endoplasmic reticulum and Golgi stack. *Cell* 50:521–22
107. Salas P, Misek I, Vega-Salas DE, Gundersen D, Cereijido M, Rodríguez-Boulán E. 1986. Microtubules and actin filaments are not critically involved in the biogenesis of epithelial cell surface polarity. *J. Cell Biol.* 102:1853–67
108. Schneeberger EE, Lynch RD. 1992. Structure, function, and regulation of cellular tight junctions. *Am. J. Physiol.* 262:L647–61
109. Schwartz GJ, Barasch J, Al-Awqati Q. 1985. Plasticity of functional epithelial polarity. *Nature* 318:368–71
110. Sedar AW, Forte JG. 1964. Effects of calcium depletion on the junctional complex between oxyntic cells of gastric glands. *J. Cell Biol.* 22:173–88
111. Shapiro L. 1985. Generation of polarity during Caulobacter cell differentiation. *Annu. Rev. Cell Biol.* 1:173–207
112. Siliciano JD, Goodenough DA. 1988. Localization of the tight junction protein, ZO-1, is modulated by extracellular calcium and cell-cell contact in Madin-Darby canine kidney epithelial cells. *J. Cell Biol.* 107:2389–99
113. Simon MN, De Virgilio C, Souza B, Pringle JR, Abo A, Reed SI. 1995. Role of the Rho-family GTPase Cdc42 in yeast mating-pheromone signal pathway. *Nature* 376:702–5
114. Singer KL, Stevenson BR, Woo PL, Firestone GL. 1994. Relationship of serine/threonine phosphorylation/dephosphorylation signaling to glucocorticoid regulation of tight junction permeability and ZO-1 distribution in nontransformed mammary epithelial cells. *J. Biol. Chem.* 269:16108–15
115. Spiegel S, Blumenthal R, Fishman PH, Handler JS. 1985. Gangliosides do not move from apical to basolateral plasma membrane in cultured epithelial cells. *Biochim. Biophys. Acta* 821:310–18
116. Staddon JM, Herrenknecht K, Smales C, Rubin LL. 1995. Evidence that tyrosine phosphorylation may increase tight junction permeability. *J. Cell Sci.* 108:609–19
117. Stowers L, Yelon D, Berg LJ, Chant J. 1995. Regulation of the polarization of T cells toward antigen-presenting cells by Ras-related GTPase cdcC42. *Proc. Natl. Acad. Sci. USA* 92:5027–31
118. Stuart RO, Nigam SK. 1995. Regulated assembly of tight junctions by protein kinase C. *Proc. Natl. Acad. Sci. USA* 92:6072–76
119. Takito J, Hikita C, Al-Awqati Q. 1996. Hensin, a new collecting duct protein involved in the in vitro plasticity of intercalated cell polarity. *J. Clin. Invest.* 98:2324–31
120. Talavera D, Ponce A, Fiorentino R, González-Mariscal L, Contreras RG, Sánchez SH, et al. 1995. Expression of potassium channels in epithelial cells depends on calcium-activated cell-cell contacts. *J. Membr. Biol.* 143:219–26
121. Tournier JF, Lopez A, Gas N, Tocanne JF. 1989. The lateral motion of lipid molecules in the apical plasma membrane of endothelial cells is reversibly affected by the presence of cell junctions. *Exp. Cell Res.* 181:375–84
122. van Adelsberg J, Edwards JC, Takito J, Kiss B, Al-Awqati Q. 1994. An induced extracellular matrix protein reverses the polarity of Band 3 in intercalated epithelial cells. *Cell* 76:1053–61
123. van Meer G, Gumbiner B, Simons K. 1986. The tight junction does not allow lipid molecules to diffuse from one epithelial cell to the next. *Nature* 322:639–41
124. van Meer G, Simons K. 1986. The function of tight junctions in maintaining differences in lipid composition between the apical and the basolateral cell surface domains of MDCK cells. *EMBO J.* 5:1455–64
125. Vega-Salas DE, Salas PJI, Rodríguez-Boulán E. 1987. Modulation of the expression of an apical membrane protein of Madin-Darby canine kidney epithelial cells: Cell-cell interactions control the appearance of a novel intracellular storage compartment. *J. Cell Biol.* 104:1249–59
126. Wang AZ, Ojakian GK, Nelson WJ. 1990. Steps in the morphogenesis of a polarized

epithelium. *J. Cell Sci.* 95:137–51
127. Wong V, Gumbiner B. 1997. A synthetic peptide corresponding to the extracellular domain of occludin perturbs the tight junction permeability barrier. *J. Cell Biol.* 136:399–409
128. Zhao ZS, Leung T, Manser E, Lim L. 1995. Pheromone signalling in *Saccharomyces cerevisiae* requires the small GTP-binding protein Cdc42 and its activator CDC24. *Mol. Cell. Biol.* 15:5246–57
129. Ziomek CA, Schulman S, Edidin M. 1980. Redistribution of membrane proteins in isolated mouse intestinal epithelial cells. *J. Cell Biol.* 86:849–57