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# Molecular Analyses of Tight Junction Physiology: Insights and Paradoxes

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## Introduction

Epithelia and endothelia demarcate and regulate transport between tissue compartments in the body. The tight junction contributes to two central aspects of epithelial and endothelial physiology. First, the tight junction constitutes the principal barrier to passive movement of fluid, electrolytes, macromolecules and cells through the paracellular pathway (the ''gate'' function, Diamond, 1977). This pathway is defined as the space between cells made up of the tight junction and lateral intercellular space in series (Fig. 1). It is now abundantly clear that the tight junction is not a simple gasketlike barrier. The tight junction demonstrates ion selectivity, varies significantly in permeability between different tissues, may be subject to physiological regulation, and undergoes dynamic modulation upon passage of cells of the immune system from one tissue compartment to another (reviewed in Madara et al., 1992). Second, the tight junction may contribute to epithelial transport by promoting epithelial cell surface polarity. By restricting free diffusion of lipids and proteins in the plane of the plasma membrane (the ''fence'' function, Diamond, 1977) the tight junction may help maintain the distinct apical and basolateral surface compositions necessary for vectorial transport across epithelia (Gumbiner, 1990).

Despite the physiological importance of tight junctions, relatively little is known about the molecular and cellular mechanisms that underlie tight junction physiology. Based on macroscopic conductance and sieving properties of epithelia and the ultrastructural appearance of the tight junction, it has been hypothesized that the tight junction contains size- and charge-selective aqueous channels or pores formed by integral membrane proteins that otherwise act to occlude the extracellular space (Cereijido et al., 1989; Madara et al., 1992; Reuss, 1992). Such channels would allow the selective passage of ions, but not macromolecules or cells. In addition, it has been proposed that continuous chains of these integral membrane proteins prevent passive diffusion of plasma membrane constituents (Pisam & Ripoche, 1976). Attractive as these models may be, direct proof of such channel-containing integral membrane proteins has yet to emerge. However, significant progress has been made in characterizing the molecular constituents of the tight junction in the last decade. Ultimately, the aim of such research is to define the molecular mechanisms responsible for all aspects of tight junction function. In particular, there has been an intense search for the integral membrane protein (or proteins) that mediate the gate and fence functions of the tight junction. Recent attempts to study one such candidate molecule, occludin, have provided intriguing insights into, and paradoxes concerning, the molecular physiology of the tight junction.

## Ultrastructural and Molecular Characterization of the Tight Junction

The tight junction appears in thin section electron microscopy as a series of focal contacts between plasma membranes of adjacent epithelial and endothelial cells that restrict the movement of extracellular tracer molecules (Farquhar & Palade, 1963; Friend & Gilula,

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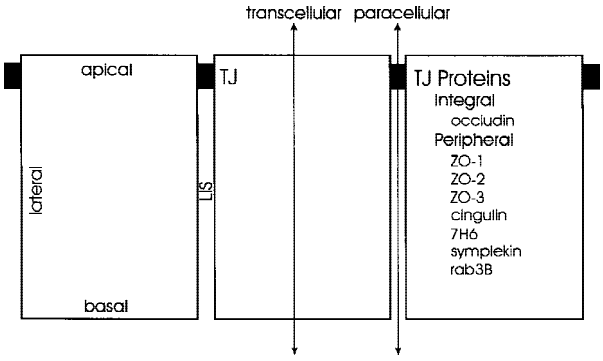


**Fig. 1.** Schematic representation of a transporting epithelium. Transepithelial permeation can occur through transcellular and paracellular pathways. The paracellular pathway consists of the tight junction (TJ) and lateral intercellular space (LIS). The tight junction lies at the interface of the apical and lateral plasma membrane domains. Integral and peripheral membrane proteins specifically localized to the tight junction are listed.

1972). In freeze-fracture, the contact sites are revealed as a variably complex network of intramembrane fibrils lying at the boundary of the apical and lateral cell surfaces (Staehelin, 1974; Bullivant, 1978). Fibrillar material is also visible on the cytoplasmic surface of the tight junction in thin section. This material is likely to be comprised, in part, of several tight junction-associated peripheral membrane proteins, including ZO-1 (Stevenson et al., 1986), cingulin (Citi et al., 1988), ZO-2 (Gumbiner, Lowenkopf & Apatira, 1991; Jesaitis & Goodenough, 1994), 7H6 (Zhong et al., 1993), symplekin (Keon et al., 1996), and rab3B (Weber et al., 1994), an epithelial-specific member of the rab family of membrane traffic regulators (Fig. 1). Actin filaments are also associated with the cytoplasmic surface of the tight junction membrane contact sites (Madara, 1987; Madara et al., 1988). The tight junction complex has also been hypothesized to include several other proteins, including p130, identified as an additional band in ZO-1 immunoprecipitations (Balda et al., 1993), another rab protein (rab13) (Zahraoui et al., 1994), and spectrin (Furuse et al., 1994). Tight junction localizations of rab13 and spectrin have yet, however, to be verified by immunoelectron microscopy. Further characterization of p130 has revealed that this protein is found at the tight junction and is, along with ZO-1 and ZO-2, a member of the membrane associated guanylate kinase (MAGUK) family of proteins. The name ''ZO-3'' has been proposed to replace p130 (Haskins et al., 1998). Members of the MAGUK family are found at sites of cell-cell interactions and may be involved in signal transduction and tumor suppression (Woods & Bryant, 1991; Willott et al, 1993).

To date, a single integral membrane protein, termed occludin, has been identified at the tight junction. First cloned in chicken (Furuse et al., 1993), occludin is a polypeptide of ~65 kDa whose amino acid sequence contains four predicted membrane-spanning regions, a short cytoplasmic amino terminus and a longer cytoplasmic carboxyl terminus. Subsequent cloning of marsupial (rat-kangaroo) and mammalian (human, mouse, canine) occludins demonstrated that the overall predicted topology of the protein was conserved despite significant differences in the deduced primary amino acid sequences between mammalian and chicken proteins (Ando-Akatusaka et al., 1996). It was noteworthy that the first predicted extracellular loop of all five occludins has an unusually high percentage of glycine and tyrosine residues (~65%), and the cytoplasmic tail was highly conserved between species. The evidence that occludin is an integral membrane protein is: (i) the conserved predicted transmembrane regions (Ando-Akatusaka et al., 1996); (ii) resistance to extraction under biochemical conditions that strip peripheral proteins from cell membranes (Furuse et al., 1993); (iii) accessibility of the first predicted extracellular loop, but not the cytoplasmic carboxyl-terminus, to domain-specific antibodies applied to non-permeabilized cells (van Itallie & Anderson, 1997); and (iv) disturbance of paracellular permeability by externally applied peptides corresponding to the extracellular loops of occludin (Wong & Gumbiner, 1997).

Information on the interactions among tight junction proteins is limited. Affinity columns displaying portions of occludin that include the predicted cytoplasmic tail specifically retain ZO-1, ZO-2 and spectrin from cell extracts and directly bind an isolated full length ZO-1 fusion protein (Furuse et al., 1994). Immunoprecipitation data indicate that ZO-1, ZO-2 and ZO-3 form a complex (Balda et al., 1993; Jesaitis & Goodenough, 1994) although specific interactions within this complex are not yet defined. Finally, a variety of evidence indicates a physical and functional association between actin and ZO-1 or a complex containing ZO-1. ZO-1 colocalizes with disrupted actin aggregates in cytochalasin D-treated Madin-Darby Canine Kidney (MDCK) and thyroid cells (Stevenson & Begg, 1994; Yap et al., 1995*b*), and activated rho proteins alter both actin organization and paracellular permeability (Nusrat et al., 1995). Recently, it has been demonstrated that ZO-1 can bind actin in cosedimentation assays (Itoh et al., 1997). Combined, these data suggest a tentative model in which occludin and possibly other integral membrane proteins are linked to the actin cytoskeleton by a complex of peripheral membrane proteins. It should also be noted that while our focus is on the molecular determinants of tight junction physiology, the observations that ZO-1 and symplekin may, under certain conditions, also be found outside the tight junction (Howarth, Hughes & Stevenson, 1992; Gottardi et al., 1996; Keon et al., 1996), suggest that some tight junction-associated elements play other roles in the cell.

## Assays of Paracellular Permeability

Abundant physiologic evidence indicates that the paracellular pathways of epithelia behave as aqueous passages restricting solute permeation on the basis of size and charge (reviewed in Madara et al., 1992; Reuss, 1992). Three commonly used approaches are available to investigate the permeability of the paracellular pathway to ions and uncharged hydrophilic macromolecules: passage of electron-dense dyes, transepithelial electrical resistance and other electrophysiological measures, and transepithelial flux of substances without affinity for membrane transporters. Barrier function can be assessed morphologically by the ability of tight junctions to block the passage of electron dense molecules, such as ruthenium red, lanthamum, and cationic ferritin (Mullin et al., 1997). A modification of this method, combining biotinylating compounds and fluorescence microscopy, has recently been described (Chen et al., 1997). This approach, which was responsible for the original identification of tight junctions as the primary barrier within the paracellular pathway (Farquhar & Palade, 1963), carries the advantage that heterogeneity in permeability across a population of cells may be detected. However, the large size of available electron dense tracers makes this a relatively insensitive technique.

Electrophysiological measurements provide, in contrast, the most sensitive assays of paracellular permeability to ions. Of the variety of techniques that are available (Kottra & Fromter, 1990; Reuss, 1992; Lewis, Berg & Kline, 1995), the simplest method involves electrodes placed on opposite sides of an epithelial sheet which are used to pass current pulses and measure resultant voltage deflections, permitting calculation of the total transepithelial electrical resistance. The common belief is that because the resistance of biological membranes is relatively high, transepithelial electrical resistance obtained for comparatively low resistance epithelia, such as that from the proximal convoluted kidney tubule, reflect largely the permeability of the tight junctions (Reuss, 1992). This apparent technical and analytic simplicity has ensured the appeal of this approach to many laboratories working on tight junction biology. As discussed below, however, additional factors, including variable transcellular ionic permeability and contributions of the lateral intercellular space, make this interpretation complex (Kottra & Fromter, 1990; Reuss, 1992; Lewis et al., 1995). Conditions have been identified in both high and low resistance epithelia where transepithelial electrical resistance does not equate directly with tight junction ionic permeability (Stoddard & Reuss, 1988; Jovov et al., 1994; Parkos et al., 1992; Reuss, 1992).

The third approach to determining paracellular permeability is to measure the passive movement or ''flux'' of hydrophilic uncharged molecules, commonly radioactively labeled, across an epithelial sheet over time. Molecules are selected in part for their lack of affinity for any transcellular transport systems (Madara et al., 1992; Reuss, 1992; Lewis et al., 1995). They include, for example, D-mannitol (MW182D), raffinose (MW594D), polyethylene glycol (MW 900D or 4000D) and various methylated dextrans with molecular weights as high as $2 \times 10^6$ D. Assuming that the lateral intercellular space provides a relatively minor impediment to the movement of these molecules, flux studies of this kind may measure different properties of the tight junction than electrical measurements, since even relatively small tracers, such as mannitol, differ from $Na^+$ and $Cl^-$ ions in size, shape, charge and hydration sphere. Tight junction permeability may change to one class of solutes but not to another. The tracer flux approach remains less sensitive to small changes in junctional permeability, and has inferior temporal resolution, compared with electrophysiological measurements (Reuss, 1992). Importantly, however, to the extent that these macromolecules are not actively transported through cells, flux assays permit an unambiguous determination of passage through the paracellular space and the tight junction. Electrophysiological and flux assays consequently provide complementary approaches to assessing paracellular permeability.

It should be noted, however, that cytotoxicity can result in changes to total transepithelial permeability that are not readily distinguishable from changes in transepithelial electrical resistance or flux measurements due solely to paracellular permeation events (Lewis et al., 1995; S. Lewis, *personal communication*). This interpretative problem is potentially most significant when dealing with low-resistance (''leaky'') epithelia, where >50% reductions in transepithelial electrical resistance may follow loss of as few as 0.3% of cells during the interval before neighboring cells can migrate to close the spaces left behind by dead cells. This level of cell loss may not be readily detected by common measurements of cytotoxicity, such as lactate dehydrogenase or $^{51}Cr$ release. Careful morphologic examination, assays for apoptosis, and use of electron dense dyes provide alternative methods to exclude cell death as a major cause of changes in total epithelial permeability.

## The Role of Occludin in Tight Junction Permeability

Several observations identify occludin as an attractive candidate to mediate the gate and fence functions of the tight junction. First, occludin expression and localization to the tight junction correlates with establishment of the paracellular permeability barrier in cultured epithelial monolayers (Wong & Gumbiner, 1997). The tissue distribution of occludin also correlates with tight junction-forming epithelia (Saitou et al., 1997). Second, immunoEM indicates that occludin localizes to the intramem-

brane fibrils visible in freeze-fractured tight junctions (Fujimoto, 1995; Furuse et al., 1996). The demonstration that occludin molecules can oligomerize (Chen et al., 1997) further suggests that polymers of occludin may form these junctional fibrils (Gumbiner, 1993). Finally, occludin increased cell-cell adhesion when expressed in fibroblasts, cells which do not normally contain occludin or display tight junctions (van Itallie & Anderson, 1997). This result, which apparently involves the first predicted extracellular loop of occludin, is consistent with the model of a tight junction transmembrane component acting as a homophilic cell adhesion molecule mediating the apposition of adjacent plasma membranes (Gumbiner, 1993). Although paracellular permeability properties and structural aspects of cell-cell contact were not examined in the fibroblasts expressing occludin, it may be unreasonable to expect that expression of occludin alone would suffice to reconstitute an intact tight junction, given that a host of other proteins are likely to be necessary for tight junction assembly and integrity.

Recently Wong and Gumbiner (1997) utilized peptides corresponding to the extracellular regions of the occludin molecule to assess occludin function in *Xenopus* epithelial cells (A6 cell line), a high-resistance epithelium. Addition of a 44 amino acid peptide corresponding to the second predicted extracellular loop of chicken occludin induced a progressive decrease in transepithelial electrical resistance and increased flux of hydrophilic markers. This apparent increase in paracellular permeability to ions and macromolecules was associated with loss of detectable junctional and total cellular occludin, likely due to increased turnover of the occludin protein. In contrast, there were no detectable changes in junctional localization or expression of other junction-associated proteins (ZO-1, ZO-2, cingulin) nor changes in the cell adhesion molecule E-cadherin. This study provides the strongest direct evidence to date that occludin plays a critical role in the tight junction permeability barrier, although it does not rule out the possibility that other as-yet-unidentified elements present at the extracellular face of the tight junction participate in the seal.

An alternative approach to studying protein function involves mutation or ablation of the polypeptide within cells. Attempts to ablate occludin expression by homologous gene recombination or antisense oligonucleotide technologies have yet to be reported. However, three groups have taken the approach of over-expressing wild-type or mutant occludin in junction-forming cells. In a recent report, chicken occludin molecules lacking regions of the cytoplasmic tail localized to the tight junction when expressed in early *Xenopus* embryos (Chen et al., 1997). This was associated with increased tight junction permeability to passage of biotinylating agents applied to the exterior of the embryo. These truncation mutants presumably perturbed the function of the endogenous occludin molecule in a dominant-negative fashion through altered protein-protein interactions (Herskowitz, 1987). Consistent with this, oligomerization between mutant chick and endogenous *Xenopus* occludin was demonstrated in co-immunoprecipitation experiments (Chen et al., 1997).

Two other groups expressed chicken occludin, either wild-type (Balda et al., 1996; McCarthy et al., 1996) or a truncated version lacking the cytoplasmic C-terminal tail (Balda et al., 1996), in the low transepithelial electrical resistance strain of MDCK cells. Both groups reported that wild-type occludin localized to the tight junction without apparent change in the expression or localization of endogenous occludin. This was associated with a variable increase in transepithelial electrical resistance, presumably consistent with a decrease in paracellular ionic permeability. Strikingly, however, expression of full-length occludin was also accompanied by an increase in the flux of hydrophilic macromolecules through the paracellular pathway. In addition, Balda et al. (1996) reported that the chicken occludin construct missing the cytoplasmic tail also localized to the tight junctions of MDCK cells. However, in contrast to the linear distribution of endogenous canine and wild-type chicken occludin, the truncated molecule localized in clumps along cell borders and induced the co-aggregation of endogenous occludin without affecting the distribution of the peripheral membrane protein ZO-1. Expression of truncated occludin again increased both transepithelial electrical resistance and flux of nontransported solutes with molecular masses between 4–40 kDa, but to a greater degree than wild-type occludin.

These findings raise several intriguing questions. First, how does expression of wild-type chicken occludin, which does not cause a redistribution of endogenous protein, produce physiological changes qualitatively similar to those obtained upon expression of a truncated construct which causes a complete relocalization of the endogenous molecules? This paradox may be resolved, in part, by the observation that over-expression of wild-type molecules can have a dominant negative effect rather than the enhancement of native function that might initially be expected (Herskowitz, 1987). This may be especially likely when the protein being expressed differs significantly in amino acid sequence from the endogenous protein, as is the case with chick and canine occludin, which show only approximately 50% identity (Ando-Akatusaka et al., 1996). Even more interestingly, how does transepithelial electrical resistance increase despite the clearly discontinuous distribution along cell borders of what is presumed to be a key transmembrane element of the tight junction? Assuming that the observed transepithelial electrical resistance increase reflects a decrease in the passage of ions through tight junctions, and taking into account that discontinuous oc-

cludin observed by immunofluorescence is not paralleled by a disturbance in the freeze-fracture appearance of tight junction fibrils, Balda et al. (1996) suggest that additional unidentified elements are involved in the paracellular permeability barrier. Although this is clearly a possibility, it emphasizes that interpretation of the results is dependent on the reliability of the functional assays used.

The most perplexing finding in both studies was the divergent effect of expression of exogenous occludin on transepithelial electrical resistance and flux determinations of paracellular permeability. Generally, maneuvers that increase paracellular permeability to nontransported macromolecules have been associated with a significant increase in ionic permeability and a consequent decrease in transepithelial electrical resistance. Or, put another way, paracellular passages large enough to accommodate increased movement of 40 kDa tracer molecules are usually associated with greater ionic permeability. Instead, both groups have hypothesized that the divergence in transepithelial electrical resistance and flux observed upon modulation of occludin expression may point to a more dynamic and complex junctional gating mechanism. In a reinterpretation of a model originally formulated by Claude (1978), they have proposed that a population of channels at the tight junction open and close in a temporal manner that results in decreased ionic permeability, as determined by instantaneous transepithelial electrical resistance measurements, while permitting increased passage of tracer molecules over the relatively longer time period of a flux assay. In addition, a channel modulating role of occludin-ZO-1 binding was proposed (Balda et al., 1996; McCarthy et al., 1996). More generally, these findings raise the important possibility that the dynamic behavior of the tight junction involves differential selectivity to various solutes. A critical question may be not just how permeable tight junctions are, but also what they are permeable to. The tight junction may become selectively permeable to one class of solutes, but not others, in response to cellular and environmental cues (Mullin et al., 1997).

It should be noted that the interpretation of the divergent transepithelial electrical resistance and flux findings rests upon the use of transepithelial electrical resistance as a specific index of tight junction ionic permeability alone. Formally, this is not the case. The total resistance across an epithelial sheet is comprised of both transcellular and paracellular ionic resistances, typically modeled as parallel resistors (Madara & Dharmsathaphorn, 1985; Kottra & Fromter, 1990; Reuss, 1992). Ionic resistance in the paracellular pathway is, in turn, determined by contributions from both tight junction and the lateral intercellular space acting in series. The exact contribution of each of these elements to total transepithelial electrical resistance is, however, difficult to determine both experimentally and analytically (Kottra & Fromter, 1990; Reuss, 1992). Channel-selective drugs (e.g., amiloride derivatives such as phenamil) have been used to inhibit the dominant apical ion channels of epithelia (Jovov et al., 1994; Yap et al., 1995*a*), thereby isolating the paracellular conductances, but this requires that the dominant transcellular conductances are known (Reuss, 1992). Scanning microelectrode techniques (Reuss, 1992) and optical measurements of ion flux in the lateral intercellular space (Kovbasnujuk et al., 1995) allow more direct assessment of ionic permeability in the paracellular pathway, but these are technically demanding procedures and not generally available outside specialized laboratories. Instead, in low resistance epithelia it is commonly assumed that the predominant ionic flux route is paracellular, based upon the relative resistances of biological membranes and epithelial monolayers. There are clear examples in high resistance epithelia where transcellular conductances contribute significantly to total transepithelial electrical resistance (Parkos et al., 1992). For example, addition of amiloride to inhibit apical sodium channels doubled transepithelial electrical resistance in *Xenopus* A6 epithelial cell monolayers (Jovov et al., 1994). However, in lower resistance epithelia such as the MDCK strain used in the occludin studies described above, where >90% of the current leak is paracellular, it is less likely that changes in transcellular conductance contribute significantly to changes in total transepithelial electrical resistance. In particular, it is unlikely that any decrease in transcellular ionic conductances could sufficiently counteract the dominant paracellular shunt to cause an increase in total transepithelial electrical resistance. Consequently, it seems probable that the changes in transepithelial electrical resistance observed by Balda et al. (1996) and McCarthy et al. (1996) reflect changes in paracellular ionic permeability.

Leaky epithelia, such as the MDCK cell lines used in these two studies present the additional analytic problem that changes in the properties of the lateral intercellular space may contribute significantly to changes in paracellular electrical resistance (Reuss, 1992; Lewis et al., 1995). The contribution of the lateral intercellular space to paracellular resistance has commonly been believed to be minor by comparison with that of the tight junction, based on measurements made under baseline conditions, particularly in high resistance epithelia. However, this does not necessarily hold under specific experimental conditions for low resistance epithelia. For example, although the lateral intercellular space contributed only 5% of the paracellular resistance of *Necturus* gall bladder epithelium under resting conditions, treatments which collapsed the lateral intercellular space increased its contribution to 30% of total paracellular resistance (Kottra & Fromter, 1993; Kottra, Hase & Fromter, 1993) and increased total transepithelial electrical resistance (Stod-

dard & Reuss, 1988). Furthermore, significant alterations in paracellular permeability, induced by cAMP and reflected in a 50% increase in total transepithelial electrical resistance, were attributable to changes in lateral intercellular space resistance rather than tight junction permeability (Kottra & Fromter, 1993; Kottra et al., 1993). Most recently, Bijlsma et al. (1997) presented evidence that in cAMP-stimulated intestinal epithelial cells an overall increase in transepithelial electrical resistance was attributable to collapse of the lateral intercellular space, overriding a concomitant increase in tight junction permeability to chloride.

Consequently, it seems reasonable to conclude that in low resistance epithelia, changes in transepithelial electrical resistance do not necessarily reflect changes in tight junction permeability with sufficient specificity to be used as a benchmark for assessing tight junction ionic permeability. This caveat implies that the divergent changes in transepithelial electrical resistance and flux observed by Balda et al. (1996) and McCarthy et al. (1996) may not solely reflect changes in tight junction behavior. In particular, it is possible that changes in lateral intercellular space properties, such as pathway length, volume and ionic composition, can cause significant shifts in paracellular permeability, and hence transepithelial electrical resistance (Stoddard & Reuss, 1988; Reuss, 1992; Kottra & Fromter, 1993; Kottra et al., 1993; Bijlsma et al., 1997). Moreover, these shifts can occur independently of changes in tight junction conductance. In particular, changes in lateral intercellular space ionic composition can potentially alter resistivity of fluid in this compartment that would produce concomitant changes in paracellular ionic permeability but not affect the permeability of uncharged tracer molecules. While it remains difficult to envision lateral intercellular space conditions under which there can be increased permeation of 40 kDa tracer molecules yet decreased transit of ions, this possibility can only be excluded by direct experimentation. This can be achieved using techniques such as impedance analysis and diffusion potential studies which better allow discrimination between tight junction and lateral intercellular space contributions to paracellular permeability (Kottra & Fromter, 1990; Reuss, 1992; Lewis et al., 1995).

A final possible explanation why both transepithelial electrical resistance and transepithelial mannitol flux increase in response to the transfection of mutant occludin involves the possibility that there are in effect two paracellular pathways in the epithelial cell sheet contained in the filter ring assembly (e.g., Costar Transwell) used for flux and resistance assays (Kottra & Fromter, 1990; Helman & Liu, 1997). The first is the tight junction/lateral intercellular space pathway described above. If looked on as tight junction density, or the length of junctions per unit area, the relative contribution of this paracellular pathway will increase as a function of the area of the cell sheet (and hence as the square of the radius of the filter). The second potential pathway is at the edge of the epithelial cell sheet where the epithelium contacts the plastic ring itself. Being the circumference of the filter ring, the contribution of this pathway to total paracellular flux will increase in arithmetic proportion to the radius of the cell sheet. If one then postulates that (i) the tight junction pathway has significant permeability to $Na^+$ but not to mannitol or other nonionic tracers, whereas the edge pathway has significant permeability to both; and (ii) transfection of mutant occludin affects the permeability of the two pathways differently such that resistance decreases and mannitol flux increases in the edge pathway, but resistance in the tight junction pathway increases (with mannitol flux remaining low), then the combination of an increase in both net transepithelial electrical resistance and mannitol flux is possible. As outlined by Helman and Liu (1997) this hypothesis is testable using epithelial cell sheets in filter rings of identical composition but different diameter.

## Role of Occludin in Epithelial Cell Polarity

In contrast to the generally accepted role of the tight junction in regulating paracellular permeability, the function of the tight junction in the maintenance of epithelial cell surface compositional asymmetry is less well understood. Although a large body of indirect evidence implies that the tight junction acts as a fence within the plane of the plasma membrane (van Meer & Simons, 1986; Stevenson et al., 1988), there are a number of alternate cellular mechanisms that influence the polarized distribution of proteins and lipids at the cell surface. These include polarized cellular targeting (Matter & Mellman, 1994) and selective retention of membrane proteins by association with the cytoskeleton (Nelson, 1992). A role for the tight junction in partitioning membrane lipids was inferred from the observation that the polarized distribution of lipids in the outer leaflet of the plasma membrane was disrupted when cells were exposed to calcium-free conditions that disrupt tight junctions (van Meer & Simons, 1986). These experimental conditions were not, however, specific to the tight junction, since cadherin-based adhesion, among other cellular processes, is also affected by removal of extracellular calcium (Yap, Brieher & Gumbiner, 1997). Manipulation of occludin provides an opportunity to specifically assess the role of an integral tight junction element in epithelial polarity.

Balda et al. (1996) reported that MDCK cells transfected with mutant chicken occludin lacking the cytoplasmic tail failed to retain fluorescent lipid markers added to the apical membrane compartment, in contrast to control cells or cells expressing wild-type chick occludin. The polarized distribution of protein markers was

not apparently affected. This suggests that the tight junction, and specifically occludin, participates in segregating membrane lipids in polarized epithelial cells. Inasmuch as lipid composition influences the function of many membrane proteins (Simons & Ikonen, 1997), it will be important to know the extent to which perturbation of occludin function alters the distribution of endogenous membrane lipids and consequently the activity of critical membrane elements, particularly transport proteins and ion channels which can affect the ionic composition and resistivity of the lateral intercellular space.

## Conclusions

Characterization of the molecular composition of the tight junction provides a powerful basis upon which to dissect tight junction physiology. As shown by recent advances, a number of techniques are now readily available with which to probe tight junction function using biochemical and molecular genetic tools. As critical first steps in this direction, the studies on occludin clearly seem to indicate that this protein plays an important role in determining tight junction permeability and also contributes to the fence role of this junction. We cannot yet say, however, whether occludin is solely responsible for these functions. Indeed, data presented to date suggest the presence of other transmembrane elements.

The divergent changes in transepithelial electrical resistance and tracer flux induced by over-expression of wild-type and truncated occludin are exciting observations with provocative implications for understanding the dynamic nature of tight junction permeability. But these findings are difficult to interpret unambiguously without further experimentation. In particular, an implication of these results is that one commonly used tool, the measurement of transepithelial electrical resistance, cannot always be assumed to reflect changes in tight junction permeability, even when low resistance epithelia are used as model systems. The assumptions that underlie the use of total resistance measurements as a first approximation to junctional permeability do not necessarily hold. Just as ''transepithelial'' does not always perfectly reflect ''paracellular,'' so too ''paracellular'' will not always exclusively indicate ''tight junctional.'' Instead, as methods to manipulate tight junction proteins increase in sophistication, clarification of the functional role of occludin and other possible transmembrane elements of the tight junction will require physiological techniques beyond simple transepithelial electrical resistance measurements, including impedance analysis, diffusion potential studies, and a wider range of paracellular flux probes, to measure and dissect changes in paracellular and tight junction permeability.

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