the cytoarchitecture may be competing processes. Cellular stress induces the buildup of massive amounts of misfolded proteins; chaperones help to rescue and refold them. This may impair their anchorage to the cytoarchitecture, and an increased diffusion may develop. Similarly, an accelerated protein synthesis, such as that of malignant or virally infected cells, may also impair cellular rigidity by shifting the chaperone aid toward protein folding instead of the regular support of the cytoarchitecture (Fig. 4).

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References
cellular space. In all tissues, the particles are ~10 nm across and yet spaced at a center-to-center distance of 18 nm. This raises the unanswered question of whether the extracellular portions of the particles are interdigitated like the two halves of a zipper or meet head to head, as shown in Fig. 3. In either case, the manner in which these proteins contact across, and fill, the intercellular space must determine the properties of the barrier. As a general rule, the mean number of strands encountered along the apical-basal axis is proportional to the junction’s electrical resistance (Table 1). Resistance varies by 100,000-fold between “tight” and “leaky” epithelia. The morphological pattern of the strands also varies among tissues, e.g., how parallel or branched they are; however, as yet there is no physiological correlate of these differences.

Aqueous “pores” in the tight junction exhibit size and charge selectivity

Several lines of experimental evidence support the existence of aqueous permeation routes, or pores, through the tight junction. First, the permeability for hydrophilic nonelectrolytes is graded up to a cutoff of ~20-Å radius, consistent with transport through a hydrated pathway that discriminates on the basis of size (16). Second, in leaky epithelia, in which paracellular conductance significantly exceeds transcellular conductance (15), tight junctions typically have permeability ratios for alkali-metal cations that differ by only a few fold (Table 1). This low degree of discrimination for similarly charged but different-sized ions again suggests that the permeation pathway contains fairly large aqueous spaces. This is in contrast to the behavior of transmembrane ion channels, which show a very high degree of discrimination. At physiological pH, most tight junctions are slightly cation selective, an observation that has been interpreted to result from anionic charges lining the aqueous pore. Such a model is supported by the observation that changing the solution pH (15), and presumably titrating the charged groups lining the pore, can reverse the charge selectivity. This “isoelectric point” (pK) differs among tissues, suggesting that the chemistry of their pores, i.e., the proteins creating the contacts, also differs (Table 1). Finally, morphological characteristics of the tight junction strands have also been invoked in support of a pore model. Phillipa Claude (4) is credited with first drawing attention to the positive correlation between strand number and the electrical resistance (Table 1). She noted that this relationship was not linear but logarithmic and interpreted this mathematically by modeling the junction as a series of branched, interconnected barriers containing pores that fluctuate between open and closed conformations (4).

**Table 1. Paracellular resistance, ionic permeability ratios, isoelectric point, and mean strand numbers of some epithelia**

<table>
<thead>
<tr>
<th>Epithelium</th>
<th>Resistance (Ω·cm²)</th>
<th>PNa/PK</th>
<th>PNa/PCl</th>
<th>pK</th>
<th>Strands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal tubule (dog)</td>
<td>6–7</td>
<td>0.91</td>
<td>1.4</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>Gallbladder (rabbit)</td>
<td>21</td>
<td>0.43</td>
<td>3.3</td>
<td>3.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Ileum (rabbit)</td>
<td>67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum (rat)</td>
<td></td>
<td>0.71</td>
<td>7.5</td>
<td>2.7</td>
<td>5.3</td>
</tr>
<tr>
<td>MDCK (dog)</td>
<td>60–4,000</td>
<td>0.83</td>
<td>10.0</td>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>Colon (rabbit)</td>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric fundus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necturus</td>
<td>10,573</td>
<td></td>
<td></td>
<td>11.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td></td>
<td></td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder (toad)</td>
<td>6–300,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Resistance is expressed in Ω·cm², and nos. of strands are expressed as means. Free solution mobility ratios are PNa/PK = 0.68 and PNa/PCl = 0.66. For references, see Refs. 4, 15, and 16.
actin microfilaments. Second are numerous “signaling” proteins proposed to be involved in junction assembly, barrier regulation, gene transcription, and perhaps other, presently undefined pathways. Third are proteins likely to regulate membrane vesicle targeting, such as sec6/8 and the occludin-binding protein VAMP-associated protein (VAP-33). Last, there are three known transmembrane proteins, junction adhesion molecule (JAM), occludin, and claudin. These are obviously the most likely candidates for creating the paracellular barrier. JAM is a member of the immunoglobulin superfamily and may be involved in immune cell transmigration or cell adhesion (3) but has not been localized to the junction strands. Claudin and occludin were both discovered and characterized by Tsukita and associates (5). This work has had a profound impact on our understanding of the molecular basis of the paracellular barrier and the direction of future research.

Do claudins create the barrier?

Both circumstantial and limited direct experimental evidence support the notion that claudins create the variable properties of the barrier. There exist at least 20 claudin genes encoding proteins that range from ~20 to 27 kDa, with primary sequence identity as low as 12% (5, 13). They are predicted to have four transmembrane-spanning helices with cytoplasmic NH₂ and COOH terminals (Fig. 3). A few reports have described that some claudins have very restricted expression patterns. For example, claudin-5 is predominately expressed in tight junctions of endothelial cells (14), and claudin-11 is the only claudin expressed in tight junctions that show linear noncross-linked fibrils [e.g., Sertoli cells, oligodendrocytes, and epithelial cells of the organ of Corti (9)]. Claudin-16 (a.k.a. paracellin-1) is limited in the adult to tight junctions of the thick ascending loop of Henle (TALH) in the kidney (18). Thus, consistent with a role in providing diversity, there are many different claudins and they show distinct expression patterns.

There is excellent evidence that claudins are both sufficient and necessary to form the junction strands. When individual claudins are expressed by transfection in junction-null fibroblasts, they assemble into long strands of 10-nM particles and the strands adhere between cells (7). According to a study of a limited number of claudins (8), they all are capable of homophilic adhesion between cells, and some but not all pairs are capable of heterophilic adhesion. This raises the interesting question of how many claudins compose a tight junction particle and whether a single particle contains more than one type of claudin (Fig. 3). The particle size is similar to that observed for gap junctions. Each gap junction particle represents a complex of six connexin proteins with a total mass of ~200 kDa. By comparison, this strongly suggests that particles within the tight junction are also multimeric complexes of claudins. With 20 claudins, the combinatorial possibilities for particle structure and function are very large. It is of note that different claudins create slightly different fibril morphology when expressed in fibroblasts, suggesting that the specific combination of claudins expressed in each epithelial cell type creates the variable morphology observed in vivo (8). The necessity of claudins for strand formation in vivo is supported by the phenotype of claudin-11 knockout mice. As mentioned above, claudin-11 is found in junctions with parallel, nonbranched strands, such as in oligodendrocytes in the central nervous system and in Sertoli cells in the testis. When claudin-11 is deleted in mice through homologous recombination, tight junctions are absent in these specific cell types. As a functional correlate, the mice show both central nervous system nerve conduction delays and male sterility (9). Together these results provide strong evidence that claudins are the principal structural element of the fibrils, define fibril morphology, and show selectivity in formation of adhesive contacts in the extracellular space.

Only a few studies have directly addressed claudin(s) function. Two groups have reported (10, 11) that overexpression of claudin-1 in MDCK cell monolayers increases the transepithel-
occludin is a facultative component of the barrier since it is absent from some junctions in vivo and its deletion from embryonic stem cells through homologous recombination interferes neither with formation of strands nor of a functional paracellular barrier (17).

Conclusions

Provocative but currently incomplete evidence supports speculation that the claudin family is responsible for creating the tight junction barrier and the variable properties of paracellular transport. Proof will require more detailed characterization and correlation of the structure and physiology of individual claudins. This will likely come from both genetic manipulation of claudins in mice and physiological study of claudin in various expression systems. It will be important to determine whether, and how, the 20 different claudins confer different solute and charge selectivity. Ultimately, the answer to this question will require determination of the three-dimensional structure of the extracellular sequences to visualize how they form pores.

It remains unclear how cells regulate paracellular permeability in the short and long term. Now we can begin to ask whether some of these changes are based in posttranslational modifications of claudins or alteration of their protein levels through transcriptional mechanisms.

It is also unknown whether claudins, or related proteins, are expressed in Drosophila or Caenorhabditis elegans; if they are, then genetic methods will greatly facilitate their study. The phenotype of mutations in human claudin-16 provided a striking insight into the physiological role of claudins and raises the possibility that there are additional claudin mutations or polymorphisms that present as epithelial diseases.

Finally, we should keep in mind the possibility that additional proteins that control paracellular permeability remain to be discovered. Despite these many questions, recent insights into the molecular structure of tight junctions are beginning to explain their important physiological differences and contribution to paracellular transport.

References

Small is Mighty: EmrE, a Multidrug Transporter as an Experimental Paradigm

Shimon Schuldiner, Dorit Granot, Sonia Steiner Mordoch, Shira Ninio, Dvir Rotem, Michael Soskin, Christopher G. Tate, and Hagit Yerushalmi

EmrE is a multidrug transporter from Escherichia coli that functions as a homooligomer and is unique in its small size. In each monomer there are four tightly packed transmembrane segments and one membrane-embedded charged residue. This residue provides the basis for the coupling mechanism as part of a binding site “time shared” by substrates and protons.

Multidrug transporters (MDTs) recognize a broad range of substrates with relatively high affinity and actively move them away from the cytoplasm. Since at times the substrates are toxic, these transporters have been associated with resistance to the effects of multiple drugs, antibiotics, and antineoplastic agents (4, 8). Multidrug resistance is a major concern in medical and agricultural diseases. In medicine, the emergence of resistance to multiple drugs is a significant obstacle in the treatment of several tumors as well as many infectious diseases. In agriculture, controlling the resistance of plant pathogens is of major economic importance. Because of the clinical relevance of these proteins and because of their apparently paradoxical ability of high affinity multidrug recognition, MDTs have been the topic of extensive studies.

A family comprising the smallest multidrug transporters (SMRs) has been identified on the basis of their primary amino acid sequence similarity (9). In those cases in which they have been studied (5, 15), they were shown to extrude various drugs in exchange with protons, thereby rendering bacteria resistant to these compounds. All of the genes identified thus far as coding for SMR proteins are restricted to the eubacterial kingdom, both in Gram-negative and -positive organisms. SMRs have been identified in many pathogens (Fig. 1).

One of the SMR genes, emrE, is an Escherichia coli gene coding for a highly hydrophobic 12-kDa protein. A hydrophatic analysis of the sequence reveals the presence of four putative transmembrane segments. This model is experimentally supported by a variety of techniques (see Ref. 18). Only one charged residue (Glu) is found in the putative transmembrane domain, and there is a total of eight charged amino acids throughout the protein (Fig. 1).

The EmrE protein has been characterized, purified, and reconstituted in a functional form (15, 18). EmrE-mediated transport is driven by a proton electrochemical gradient both in intact cells and in proteoliposomes (15). It behaves as an MDT capable of recognizing a wide range of substrates and inhibitors, including methyl viologen, ethidium, acriflavine, benzalkonium, and others (11, 15, 18).

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