JCB Comment

Keratinocyte junctions and the epidermal barrier: how to make a skin-tight dress

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Although intercellular junctions are known to be the major regulators of permeability of simple epithelia, they had not been thought to be important in regulating the permeability of stratified mammalian epithelia. Furuse et al. (2002, this issue) demonstrate that functional tight junctions may indeed be a necessary part of the permeability barrier of the skin.

A key function of endothelial, epithelial, and mesothelial cells is to form physical barriers that define body compartments. For instance, vascular endothelial cells separate blood from perivascular tissues, thus restricting leakage of plasma proteins and outflow of circulating cells. In addition, intestinal epithelial cells create the boundary between visceral lumen and interstitial space, which is instrumental in the protection from pathogens and in the adsorption of nutrients. Finally, epidermal keratinocytes, besides protecting the body interior from the external environment, limit the dispersion of fluids through the wide surface area of the skin.

In single-layer epithelia (e.g. in the small intestine), intercellular junctions represent the major barrier restricting paracellular permeability, i.e., the diffusion of water and solutes across intercellular spaces (Madara, 1998). In comparison, in multilayer epithelia (e.g. in the epidermis), junctions are somewhat less organized, and their role in barrier function has not been clearly defined. Among stratified epithelia of the skin, keratinocytes in the stratum corneum assemble an insoluble complex of cross-linked proteins and lipids, i.e., the cornified cell envelope (CCE),* building up an efficient barrier against physical trauma and fluid dispersion (Roop, 1995). Until now, the prevailing view has been that stratified epithelium, such as the epidermis, lacked continuous and functional tight junctions. A paper in this issue draws our attention to a possible role of keratinocyte tight junctions (TJs) in the permeability barrier of the skin (Furuse et al., 2002).

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*Abbreviations used in this paper: CCE, cornified cell envelope; TJ, tight junction.

TJs are the most apical component of the junctional complex, which also comprises desmosomes, adherens junctions, and gap junctions. In general, the complex mediates cell-to-cell adhesion and communication (Bazzoni et al., 1999). In particular, TJ control paracellular permeability and maintain cell polarity, which are often referred to as barrier and fence function, respectively (Tsukita et al., 2001). Like other junction organelles, TJs are composed of transmembrane and intracellular molecules. At the TJ, integral membrane proteins are represented by occludin, junction adhesion molecule, and claudins (Bazzoni and Dejana, 2001; Stevenson and Keon, 1998). Claudins are members of a family that comprises more than twenty proteins with four membrane-spanning regions, two extracellular loops, and two cytoplasmic termini (Furuse et al., 1998).

In this issue, Furuse et al. (2002) report that genetic ablation of *claudin-1* in mice induces neonatal death, which is associated with rapid appearance of wrinkles in the skin. *Claudin-1*–deficient pups die within 24 h after birth, probably because of significant body dehydration. Surprisingly, however, the morphology of keratinocyte TJs (which are concentrated, as in normal animals, in the stratum granulosum) is unaffected in mutant mice. Similarly, the distribution of other TJ components, such as occludin and claudin-4, is unchanged. Finally, the organization of the epidermis in layers is normal, the only exception being a more compact and thicker stratum corneum. Nonetheless, in the stratum corneum, the content of lipids, the presence of the CCE components involucrin and loricrin, and the expression of the transcription factor Klf4 are all unchanged, thus ruling out possible indirect effects of *claudin-1* deletion on the structural organization of the stratum corneum.

Overall, this paper is highly significant. In particular, it compels us to reconsider the actual mechanisms whereby the epidermis acts as a permeability barrier. As to junction-independent systems, genetic studies have recently reported that Klf4 and transglutaminase-1 are essential for expressing and cross-linking CCE molecules, respectively (Steinert, 2000). Interestingly, ablation of either *Klf4* (Segre et al., 1999) or *transglutaminase-1* (Matsuki et al., 1998) induces a phenotype that is comparable to that of *claudin-1* absence. Hence, both mechanisms, i.e., correct organization of TJs in the stratum granulosum and of CCE in the stratum

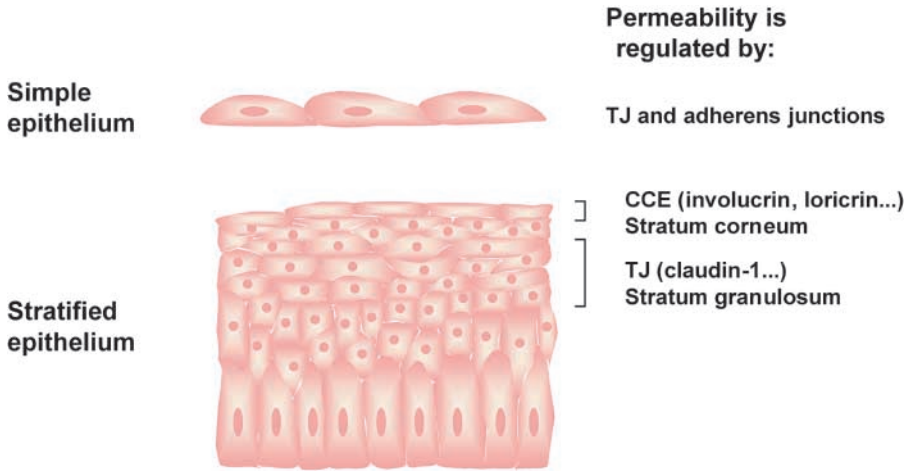

Figure 1. **The two major mechanisms, whereby epithelial cells control permeability, are schematically depicted.** In simple (i.e., single-layer) epithelia, permeability predominantly follows the paracellular route and is mostly dependent on intercellular junctions. In multilayer epithelia, the permeability barrier depends on junctions and other cell structures, such as CCEs in the uppermost stratum corneum of the skin.

corneum, are required for full control of skin permeability (Fig. 1).

As is often the case for interesting and thought-provoking reports, the recent study by Furuse et al. (2002) raises a series of questions that calls for further research. First, the seemingly normal morphology of TJs in *claudin-1*–deficient animals is puzzling. Expression of claudin-1 in fibroblasts induces formation of TJ strands and restricts permeability (Furuse et al., 1998). However, in the light of the findings reported herein, lack of *claudin-1* only affects the function, and not the structure, of TJs (Furuse et al., 2002). A possible explanation for the discrepancy is that other TJ proteins, such as claudin-4 and occludin, may vicariously promote TJ organization, even if the resulting TJs are perhaps unable to function efficiently in the control of permeability. In this respect, it might be useful to analyze TJ function in other types of epithelial cells, such as the stratified and columnar epithelial cells of esophagus and intestine, respectively. Finally, as a practical corollary, it appears that the observation of normal TJ morphology not always allows the prediction of normal TJ behavior. Clearly, in these and similar conditions, further morphological analysis using freeze-fracture electron microscopy would help confirm that TJ structure is unquestionably normal.

Second, the mechanism of enhanced permeability remains elusive. Although evidence presented in the manuscript points to a primary defect in permeability to water, further studies are warranted to examine permeability to cations. Notably, recent studies suggest that individual claudins might act as selective cation channels. For instance, expression of exogenous claudin-4 specifically affects the permeability to sodium (Van Itallie et al., 2001). In addition, mutations of *claudin-16* cause a hereditary form of hypomagnesaemia associated with renal wasting of magnesium (Simon et al., 1999). By analogy, absence of *claudin-1* might result in enhanced permeability to cations with consequent loss of isosmotic fluids through the skin.

Finally, other open questions concern the actual mechanism of the skin defect. Clearly, a physical imperfection of TJs strands in the keratinocytes is the most obvious hypothesis. However, an alternative possibility is that claudin-1 might play a more indirect and complex role. For instance, it is known that some TJ molecules (e.g., ZO-1) interact with transcription factors (Balda and Matter, 2000). Hence, one might speculate that claudin-1 is required for expression of genes, which in turn are indispensable for controlling permeability in the stratum corneum. In addition, it is possible that the observed abnormalities of the skin indirectly reflect general homeostatic alterations. Authors show that grafting *claudin-1*–deficient skin onto a nude mouse fails to rescue the gross morphological alterations of the donor tissue. However, albeit suggestive, these data are not conclusive. For instance, if irreversible changes occur in the skin during development, they might not revert to normality in the allograft. Along the same line, another important point for future research regards the functional role of claudin-1 in organs, such as kidney and liver, in which it is also expressed.

Analysis of TJ function is still in its infancy. The long list of individual TJ molecules and the bewildering complexity of their molecular interactions already prefigure a lengthy and demanding task. However, in spite of all the unanswered questions, the approach taken by Furuse et al. (2002) is likely to pave our way toward deeper understanding of TJ function.

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