Mini-Review

Breaking through the Tight Junction Barrier

Barry M. Gumbiner

Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York 10021

The tight junction, or zonula occludens, is an organelle of crucial importance to the development and function of most organ systems in vertebrates because it enables epithelia and endothelia to create compositionally distinct fluid compartments. The tight junction forms a continuous permeability barrier between adjacent cells that regulates the flux of molecules through the paracellular space (2). It also creates a boundary in the plasma membrane bilayer that separates the cell surface into biochemically and functionally distinct apical and basolateral membrane domains, enabling the cells to carry out polarized transport (8).

The tight junction is a fascinating structure. It forms the closest contact between adjacent cells known in nature, so close, in fact, that it was once believed to be a fusion between outer leaflets on the adjacent bilayers (11, 23). We now know that it does not make an absolute seal, but instead contains discrete ion selective pores through the extracellular portion of the junction (Fig. 1). The permeability properties of the tight junction are quite variable in different epithelia, depending on the specific physiological requirements for transepithelial (or transendothelial) solute transport (6). Moreover, tight junction permeability can be regulated physiologically within a given epithelium (13). Thus, the extracellular portion of the tight junction has to accomplish two main tasks; to form extremely narrow intercellular adhesions that occlude a region of extracellular space, and to create regulatable pores for selective molecular sieving.

The tight junction also has characteristics of a biological polymer. By freeze-fracture electron microscopy, which reveals intramembrane structure, the tight junction appears as a set of long, parallel, linear fibrils that circumscribe the cell, with short fibril fragments interconnecting the main parallel array (19). The assembly of these fibrils seems to be regulated in a way that is crucial to tight junction physiology. Various epithelial cell types differ in their number of parallel fibrils, and generally, a greater number of fibrils correlates with decreased junctional permeability (5, 15). Fibril assembly also seems to be quite dynamic in some cell types (Fig. 2). In the most impressive examples, a rapid, massive stimulation of fibril assembly occurs when epithelial cells are exposed to certain noxious stimuli (10, 20). More subtle alterations in fibril number also occur during less dramatic instances of junctional regulation (7, 12). Rapid fragmentation of the fibrils is also observed, for example, when leukocytes migrate across epithelia or when intestinal junctions open in response to ingestion of a meal rich in glucose (14, 18). Fragmentation may be due either to localized disassembly or to physical disruption by changes in the tension of the underlying actin cytoskeleton.

When considering these structural and functional properties of the tight junction, it is evident that an integral membrane protein is probably the most interesting and important tight junction component. Such a protein would be expected to have a remarkable number of features. In the simplest of models, it could be imagined to function simultaneously as a homophilic adhesion molecule, as a selective pore forming protein, and as a subunit of an integral membrane polymer. Indeed, the identification of such a protein has been the Holy Grail of the field. Despite significant progress on the identification and characterization of tight junction peripheral membrane proteins (1, 3), the integral membrane component has remained elusive. At long last, a major breakthrough has been made by Furuse et al. in the Tsukita laboratory, who report the identification, cloning, and sequence of a tight junction integral protein, named "occludin" (in this issue [7a]).

Furuse et al. (7a) took advantage of an earlier observation that an isolated rat liver adherens junction fraction was enriched in the tight junction peripheral protein ZO-1 (9). They prepared this membrane fraction from chick liver for use as an antigen to raise monoclonal antibodies, reasoning that the evolutionary distance between chicken and mouse would help overcome problems of immunogenicity. They obtained three mAbs to an ~65-kD integral membrane polypeptide that, by criterion of immunoelectron microscopy, is highly localized at the membrane of the zonula occludens. Because of its localization and integral membrane character, they named the protein "occludin".

The amino acid sequence of occludin, determined from a cloned cDNA, revealed no obvious sequence homology with other known sequences in the databases. However, analysis of the sequence predicts that the polypeptide will have four hydrophilic transmembrane passes. Based on the cytoplasmic localization of an epitope within the long hydrophilic COOH-terminal domain, the authors derive a model for the transmembrane topology of occludin having two short (~40 amino acids) extracellular loops between the two pairs of transmembrane segments (see Fig. 9 in Furuse et al. [7a]). The authors point out that this overall membrane topology is similar to the topology of other membrane proteins, such as the connexins of gap junctions, proteolipoprotein of myelin, and synaptophysin of synaptic vesicles. They speculate that this general structure may be functionally important for...
Figure 1. Schematic diagram of how ion selective pores might form in the extracellular occlusion of the tight junction. Presumably, subunits of a tight junction membrane protein pack very closely, but leave specific openings for the passage of ions.

proteins that form adhesions between membrane surfaces and locally restrict permeability.

This exciting discovery leads immediately to a great number of tantalizing questions about the properties of the protein with respect to the structure of the tight junction. Does occludin behave like a homophilic adhesion molecule when expressed at the cell surface? Does it have the capacity for self-assembly and polymerization in the membrane? To what extent does occludin assembly depend on the presence of traditional cell–cell adhesion molecules such as the cadherins? Does occludin interact directly with the known cytoplasmic plaque proteins of the tight junction, ZO-1 and ZO-2 and others, and are these proteins required for occludin polymerization or the formation of occludin-mediated cell contacts? A theoretical analysis of the occludin amino acid sequence may also prove to be fruitful. What is the significance of the high content of tyrosine and glycine residues in the putative extracellular loops? How might subunits having this structure pack to form ion selective pores in the extracellular space?

The identification of occludin also will provide powerful opportunities to re-examine several outstanding problems about the cell biology of the tight junction. One of the most pressing questions concerns the relationship between tight junction structure and paracellular permeability. A long standing hypothesis is that the number of tight junction fibrils, as observed by freeze-fracture EM, determines the electrical resistance of the paracellular pathway (4). However, there seem to be exceptions to this rule, and it has been proposed that the permeability of individual pores within the junctional fibril can vary (16, 21). It will be very informative to determine whether the levels of expression of occludin (or perhaps of still to be discovered occludin isoforms) or the extent of occludin polymerization into fibrils can account for variations in junctional permeability. Alternatively, are there observable biochemical changes in occludin that might regulate the permeability of individual pores in the assembled fibril?

A related problem concerns the mechanisms that control the number of junctional fibrils in various cell types. Is the extent of junction assembly determined by the level of occludin expression or by regulating occludin polymerization? In this regard it will be important to analyze the dynamics of the tight junction; are the dynamics of occludin polymerization/assembly comparable to the dynamics of the cytoskeletal filaments? Do the dynamics of polymerization/assembly play a role in the regulation of the tight junction during physiological and pathophysiological events? Furthermore, how do the junctional fibrils in most epithelial cells come to be assembled into the narrow junctional zone at the apical-most region of the lateral surface?

Another outstanding issue concerns the role of the tight junction in the development and maintenance of epithelial cell surface polarity. While it is clear that the zonula occludens forms a localized barrier in the plasma membrane at the boundary between apical and basolateral domains that prevents mixing of proteins and lipids (22), there is also evidence that the underlying membrane–cytoskeleton can restrict the distribution of proteins within membrane domains (17). It should now be possible to directly determine the relationship between the assembly of occludin fibrils within the bilayer (which could be independent of the formation of the actual junctional contact) with the development of cell surface polarity in developing tissues and cultured cell lines. Moreover, it's not hard to imagine designing specific probes for perturbing occludin assembly or function in order to determine the consequences of inhibiting junctional assembly on the establishment of surface polarity.

Many of us who have been intrigued by the structure and function of the tight junction have longed to be able to address these kinds of questions for years. Thus, it is apparent why the identification of occludin is such an important and exciting breakthrough for the field. Given the remarkable properties that might be expected for a tight junction integral membrane protein, it is likely that investigations of occludin...
structure and function will be of considerable interest to
scientists in other research areas, such as cell adhesion,
membrane structure and dynamics, and ion channel struc-
ture and regulation. Beginning with the publication of the oc-
ccludin sequence in this issue of the Journal of Cell Biology,
we can expect to experience a profusion of new discoveries
in this interesting and important field.

Received for publication 16 November 1993.

References
1. Anderson, J. M., M. S. Balda, and A. S. Fanning. 1993. The structure and
regulation of tight junctions. Curr. Opin. Cell Biol. 5:772-778.
2. Cereijido, M., L. Gonzalez-Mariscal, G. Avila, and R. G. Contreras.
1988. Tight junctions. CRC Crit. Rev. Anat. Sci. 1:171-192.
3. Citi, S. 1993. The molecular organization of tight junctions. J. Cell Biol.
121:485-489.
4. Claude, P. 1978. Morphological factors influencing transepithelial perme-
ability: a model for the resistance of the zonula occludens. J. Membr.
Biol. 39:219-232.
5. Claude, P., and D. A. Goodenough. 1973. Fracture faces of zonulae oc-
ccludentes from “tight” and “leaky” epithelia. J. Cell Biol. 58:390-400.
6. Diamond, J. 1977. The epithelial junction: bridge, gate, and fence. Physiol-
ologist. 20:10-18.
7. Easter, D. W., J. B. Wade, and J. L. Boyer. 1983. Structural integrity of
hepatocyte tight junctions. J. Cell Biol. 95:745-749.
7a. Furuse, M., T. Hirase, M. Itoh, A. Nagafuchi, S. Yonemura, S. Tsukita,
and S. Tsukita. Occludin: a novel integral membrane protein localizing
at tight junctions. J. Cell Biol. 123:1777-1788.
8. Gumbiner, B. 1987. Structure, biochemistry, and assembly of epithelial
tight junctions. Am. J. Physiol. (Cell Physiol.). 253:C749-C758.
9. Itoh, M., A. Nagafuchi, S. Yonemura, T. Kitani-Yasuda, S. Tsukita, and
S. Tsukita. 1993. The 220-kD protein colocalizing with cadherins in non-
epithelial cells is identical to ZO-1, a tight junction-associated protein in
epithelial cells: cDNA cloning and immunoelectron microscopy. J. Cell
Biol. 121:491-502.
10. Kachar, B., and P. Pinto da Silva. 1981. Rapid massive assembly of tight
junction strands. Science (Wash. DC). 213:541-543.
11. Kachar, B., and T. S. Reese. 1982. Evidence for the lipidic nature of tight
junction strands. Nature (Lond.). 296:464-466.
12. Madara, J. L. 1983. Increases in guinea pig small intestinal transepithelial
resistance induced by osmotic loads are accompanied by rapid alterations
in absorptive-cell tight-junction structure. J. Cell Biol. 97:125-136.
13. Madara, J. L. 1988. Tight junction dynamics: Is paracellular transport
regulated? Cell. 53:497-498.
14. Madara, J. L. 1989. Loosening tight junctions. J. Clin. Invest. 83:1089-
1094.
15. Madara, J. L. and K. Dharmasathaphorn. 1985. Occluding junction
structure-function relationships in a cultured epithelial monolayer. J. Cell
Biol. 101:2124-2133.
16. Martinez-Palomo, A., and D. Erlij. 1975. Structure of tight junctions in
epithelia with different permeability. Proc Natl. Acad. Sci. USA. 72:
4487-4491.
17. Nelson, W. J. 1992. Regulation of cell surface polarity from bacteria to
mammals. Science (Wash. DC). 258:948-955.
18. Parkos, C. A., S. P. Colgan, C. Delp, M. A. Arnaout, and J. L. Madara.
1992. Neutrophil migration across a cultured epithelial monolayer elicits
a biphasic resistance response representing sequential effects on transepi-
thelial and paracellular pathways. J. Cell Biol. 117:527-764.
19. Pinto da Silva, P., and B. Kachar. 1982. On tight-junction structure. Cell.
28:441-450.
20. Schnabel, E., J. M. Anderson, and M. G. Farquhar. 1990. The tight junc-
tion protein ZO-1 is concentrated along slit diaphragms of the glomerular
epithelium. J. Cell Biol. 111:1225-1263.
21. Stevenson, B. R., J. M. Anderson, D. A. Goodenough, and M. S.
Mooschke. 1988. Tight junction structure and ZO-1 content are identical
in two strains of Madin-Darby canine kidney ceils which differ in trans-
epithelial resistance. J. Cell Biol. 107:2401-2408.
22. van Meer, G., and K. Simons. 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 (Eur. Mol. Biol.
Organ.) J. 5:1455-1464.
23. van Meer, G., B. Gumbiner, and K. Simons. 1986. The tight junction does
not allow lipid molecules to diffuse from one epithelial cell to the next.
Nature (Lond.). 322:639-641.