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Review

Conceptual barriers to understanding physical barriers☆

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ABSTRACT

The members of the large family of claudin proteins regulate ion and water flux across the tight junction. Many claudins, e.g. claudins 2 and 15, accomplish this by forming size- and charge-selective paracellular channels. Claudins also appear to be essential for genesis of tight junction strands and recruitment of other proteins to these sites. What is less clear is whether claudins form the paracellular seal. While this seal is defective when claudins are disrupted, some results, including ultrastructural and biochemical data, suggest that lipid structures are an important component of tight junction strands and may be responsible for the paracellular seal. This review highlights current understanding of claudin contributions to barrier function and tight junction structure and suggests a model by which claudins and other tight junction proteins can drive assembly and stabilization of a lipid-based strand structure.

Contents

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1. Introduction

Since their discovery in 1998 [1], claudin proteins have become a central focus of tight junction research. It has become clear that expression of members of this large family of tetra-membrane spanning proteins modulates paracellular, i.e. tight junction, permeability to ions and water in a size- and charge-selective manner [2–8]. Increases in paracellular conductance induced by specific claudins can be defined as either anion- or cation-selective [6,9–13]. The conductance pathways that are enhanced by increased expression of pore-forming claudins are size-selective, and appear to only admit solutes and solvents with radii up to ~3.5 Å [7,8,14–16]. These claudins are often referred to as "pore-forming" claudins. Other claudins have been described as "sealing" claudins [17,18]. There is some evidence to support the idea that these claudins form paracellular seals, including the severe epidermal barrier defects in claudin-1-deficient mice [19] and the observation that expression of specific claudins reduces transepithelial ion conductance in cultured monolayers [20,21]. However, while this is a convenient model, it may well be an oversimplification of a far more complex biology. In this review, we will explore the mechanisms by which claudins, other proteins, and lipids form and regulate the tight junction barrier, both at steady-state and in response to exogenous stimuli.

2. Claudins: tight junction components, organizers, or both?

The initial report that identified claudins showed that claudin-1 and claudin-2 co-localized with occludin by fluorescence microscopy and were present within tight junction strands seen by freeze-fracture electron microscopy [1]. This was rapidly followed by the observation that, when expressed in fibroblasts, which lack tight junctions, claudin proteins concentrated at cell contact sites and induced formation of tight junction like strands [22]. This, along with the beaded appearance of tight junction strands was taken as evidence that the strands are composed primarily of claudins. However, it is important not to forget previous work concluding that tight junction strands are lipid-based [23–26] as well as more recent studies showing that tight junctions can be defined as low density, cholesterol- and glycolipid-rich, detergent-resistant membrane domains [27–31]. It may, therefore, be more accurate to think of claudins as essential organizers of tight junction strands. This view is supported by the observation that occludin and other members of the tight junction associated MARVEL protein (TAMP) family are recruited to strands by claudins [22,32,33].

3. Claudins as paracellular ion channels

Abundant data are available to support the conclusion that claudins form paracellular ion channels. Initial work demonstrated, for example, that the differences between MDCK cell lines characterized by high (MDCK I) and low (MDCK II) transepithelial electrical resistance (TER) were almost entirely explained by expression of claudin-2 in the latter, but not the former [16]. Specifically, claudin-2 expression in high resistance MDCK monolayers resulted in increased paracellular Na^+ and K^+ conductance without any effect on Cl^- conductance or paracellular flux of larger solutes, including mannitol, lactulose, and 4 kD dextran [2,16]. This high capacity, size- and charge selective conductance route has been termed the pore pathway (Fig. 1). Further study showed that treatment of cultured monolayers with the TH2 cytokine IL-13 induced claudin-2 expression as well as similar size- and charge-selective increases in paracellular permeability that could largely be prevented by inhibition of claudin-2 upregulation [8,34]. Thus, while

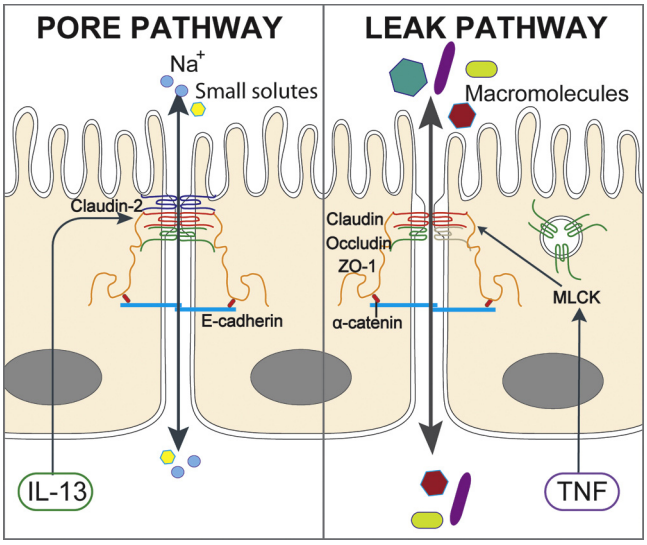

Fig. 1. Distinct routes and regulatory mechanisms are involved in trans-tight junction flux. Paracellular flux across the epithelial tight junction can defined as two distinct pathways, pore and leak. In intestinal epithelia, the cytokine IL-13 induces claudin-2 expression that, in turn, enhances water and small solute, e.g. ion, flux across the high capacity, size- and charge-selective pore pathway. Conversely, TNF activates myosin light chain kinase (MLCK) that triggers caveolin-mediated occludin removal from the tight junction. This results in increased macromolecular flux across the low capacity, relatively nonselective leak pathway.

claudin-2 expression can regulate tight junction permeability to cations, it cannot explain differences in paracellular flux of larger molecules [2].

The ability to form charge- and size-selective channels has been linked to residues within the first extracellular loop of claudin proteins [3–5]. However, it is important to recognize that many, if not all, studies exploring these issues in living cells and tissues are complicated by expression of claudins other than those mutated within the same cells. Despite this technical obstacle, mutagenesis studies have identified essential residues that define aspects of the conductance pore and are necessary for size-selectivity of claudin-2-based channels [14,35,36]. These studies have also mapped the sequences that form the interior of and entry to the claudin-2 channel [14,15,35–37].

The recently solved crystal structure of claudin-15 has provided new insight and allowed the generation of hypotheses that may define the protein domains that form the channel [38–40]. These models support the observation that claudin-2-based channels are size-selective, with a maximal radius of ~3.5 Å, and can accommodate some cations that are larger than Na^+, including methylamine and ethylamine (methylamine is only slight less permeable than Na^+ [15]). Notably, tight junction channels traverse intercellular junctions and are, therefore, oriented parallel to the plasma membrane, i.e. orthogonal to transmembrane ion channels. The precise manner in which claudin monomers interact to form these channels and, potentially, the paracellular seal, remain areas of active investigation.

To date, most measures of tight junction conductance have relied on measurements across relatively large multicellular sheets that include many paracellular channels [41]. Higher resolution approaches, including measurements using scanning electrodes [42,43], have been unable to detect single channel events. As a result, many have concluded that tight junction channels are static, i.e. are either open or closed, but do not regularly transition between these states. Recent work demonstrating that protein interactions at the tight junction are highly dynamic and are modulated during barrier regulation [33,41,44–49] has caused some to question this view. One recent report describes a modified