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Review

Architecture of tight junctions and principles of molecular composition

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ABSTRACT

The tight junction creates an intercellular barrier limiting paracellular movement of solutes and material across epithelia. Currently many proteins have been identified as components of the tight junction and understanding their architectural organization and interactions is critical to understanding the biology of the barrier. In general the architecture can be conceptualized into compartments with the transmembrane barrier proteins (claudins, occludin, JAM-A, etc.), linked to peripheral scaffolding proteins (such as ZO-1, afadin, MAGI1, etc.) which are in turned linked to actin and microtubules through numerous linkers (cingulin, myosins, protein 4.1, etc.). Within this complex network are associated many signaling proteins that affect the barrier and broader cell functions. The PDZ domain is a commonly used motif to specifically link individual junction protein pairs. Here we review some of the key proteins defining the tight junction and general themes of their organization with the perspective that much will be learned about function by characterizing the detailed architecture and subcompartments within the junction.

Contents

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1. Introduction to tight junctions

Transporting epithelia require a paracellular seal to allow the directional transport of ions and solutes across cell layers. This seal is formed by the tight junction, the apical-most junction of a series of cell contacts that form lateral connections between adjacent cells. The ultrastructure of epithelial junctions was first described in a seminal paper by Farquhar and Palade over 50 years ago [1]. A few years later, freeze fracture electron microscopy (FFEM) was used to visualize the tight junction-containing plasma membranes, Fig. 1; using this method, tight junctions were observed to consist of rows of membrane contacts [2] that varied in number and morphology among different tissues [3]. In parallel, there was a gradual recognition among physiologists studying epithelial transport that the tight junction, which had been considered to be an impermeable structure, was actually variably permeable to ions and solutes [4]. These observations together led to a remarkable period of investigation in which tight junctions in different tissues were compared and characterized by electron microscopic and physiologic methods and led to the conclusion that the barrier varied widely among epithelia in its physiology. It was assumed that tissue-specific barrier differences were the result of the variations in protein composition and architecture.

However, it was not until 1986 that ZO-1, the first protein component of the tight junction was identified and localized to the tight junction by immuno-electron microscopy (immuno EM), Fig. 2A [5]. This discovery was soon followed by the identification of two ZO-1-related proteins that could co-immunoprecipitate with ZO-1, termed ZO-2 [6] and ZO-3 [7] and by the discovery of an unrelated protein termed cingulin [8]. However, since all four of these were found to be peripheral membrane proteins, none could directly create the intercellular barrier. In a tour de force, the Tsukita Laboratory, using a similar biochemical fractionation method as had Stevenson and Goodenough, identified the first tight junction transmembrane protein, occludin [9]. When mouse knock-out studies demonstrated occludin was dispensable for barrier formation [10], this group went on to identify several members of the claudin family of proteins [11]. Morphologic and functional studies subsequently demonstrated that claudins were the critical barrier forming proteins [12].

The identification of these proteins was just the beginning of an extensive enumeration of tight junction components that continues today. Identification of proteins has occurred though both systematic efforts to enumerate the junction proteome [13–15] and from serendipitous discovery of single proteins. Some cataloging represents isolated reports of an antigen that co-localized with ZO-1 or another known tight junction protein. Others represent recognition that previously characterized proteins are also at the junction, for example, many well-defined junctional signaling or cytoskeletal proteins. The current list of proteins is likely incomplete and the 3-dimensional architecture and functional interactions of these proteins are not well defined.

The goal of this review is to develop a more complete and nuanced model of tight junction functional and structural compartments based on the variety of techniques that have been used to probe protein interactions and localization. To do this, we will consider interactions among and spatial compartmentalization of the core tight junction proteins and some relevant cytoskeletal proteins. Mixed within this network are dozens of signaling proteins that control junction function and provide differentiation signals to the cell. We will not only highlight the relationships between these different groups of proteins but also pose important unanswered questions in tight junction structure and function.

2. Core components of the tight junction: integral membrane and scaffolding proteins

2.1. Integral membrane proteins: claudins, TAMPs and JAMs

2.1.1. Claudins

There is overwhelming evidence that the main freeze-fracture fibril forming proteins are the 25-plus members of the claudin family [16]. When expressed in fibroblasts which do not normally form tight junctions and the transfected cells are examined by FFEM, these small, 20–25 kDa, integral membrane proteins can recapitulate fibrils similar to those of epithelial cell tight junctions [11]. In addition, much physiologic evidence supports the idea that claudins form the paracellular seal (reviewed in [17]).

2.1.2. Claudin architecture

Although tight junction strands that can be visualized by FFEM are a hallmark of epithelial tissues, the appearance of these strands differs in different tissue in terms of their number and the degree of crosslinking between strands [18]. Within most tight junctions, there exists a polarity to strand organization. FFEM images typically reveal one continuous apical-most strand, variably cross-linked medial strands and looser, less well-organized and sometimes discontinuous basal strands [19,20], Fig. 1. How these strands are organized and the basis for their structural gradients is not well understood, but the strand organization may reflect a maturation process from basolateral to apical. As early as 1973, this gradient of organization led Staehelin to suggest that the seal formed by a "zippering up" of cell–cell contacts in the lateral to apical direction [19]. All claudins (except claudin-12) end in a carboxyl terminal PDZ binding motif; these motifs interact with the first of the three PDZ domains of the tight junction scaffolding proteins ZO-1, -2 and -3 [21] and this interaction contributes to strand organization. Binding to other PDZ domain-containing proteins has also been reported, including MPDZ (MUPP1, Multi PDZ domain protein 1) [22] and PatJ (protein associated with tight junctions) [23]. In addition, preferential interactions between distinct claudins and different PDZ domain-containing proteins have been reported [23,24], although this remains to be more fully investigated. There does appear to be a requirement for PDZ-dependent interactions in setting up a tight junction, since for example in the mouse breast epithelial cell line, Eph4 cells, the scaffolding proteins ZO-1 and ZO-2 are required to create the claudin-based tight junction strands [25]. It is notable however, that depletion (as opposed to knockout) of greater than 90% of ZO-1 and ZO-2 does not result in major tight junction fibril disruption [26], suggesting that only a small amount of ZO protein is required for nucleating claudin strand assembly. It is possible that an apical/basal gradient in localization of ZO (or other) scaffolding proteins within the junction or the fact that different claudins may have variable affinities for their scaffolds may contribute to a non-homogeneous strand organization, but there is no direct evidence for this. Additionally, it is also possible that posttranslational modification of either claudins or the scaffolding proteins may factor into their relative affinities and thus organization [27].

Although scaffolding proteins may be required to set up tight junctions, it is also clear that claudins have the capacity to self-organize into different strand architectures [28], an ability that has not only structural but physiologic and pathologic consequences [29]. Claudins lacking PDZ binding motifs form patches of tight junction fibrils when expressed in fibroblasts and, in epithelial cells, claudins lacking this motif will still accumulate at tight junctions, suggesting claudin oligomerization is not dependent on interaction with scaffolding proteins [30]. Differing affinities for both cis and trans homo and hetero-oligomerization among different claudins have been well documented [31–33], so that stand maturation might result in claudin "sorting". This could result in different

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