European Journal of Cell Biology 90 (2011) 787–796

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Review

A look at tricellulin and its role in tight junction formation and maintenance

Cibelle Mariano[a], Hiroyuki Sasaki[b,c], Dora Brites[a], Maria Alexandra Brito[a,*]

[a] Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), and Department of Biochemistry and Cell Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal
[b] Division of Fine Morphology, Core Research Facilities, The Jikei University School of Medicine, Tokyo, Japan
[c] The Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka, Japan

ARTICLE INFO

Article history:
Received 21 January 2011
Received in revised form 10 June 2011
Accepted 11 June 2011

Keywords:
Barrier
Central tube
Deafness
Intercellular junctions
Lipolysis-stimulated lipoprotein
MARVEL
Occludin
Tight junctions
Tricellulin
Zonula occludens

ABSTRACT

Tight junctions are elaborate networks of transmembrane and cytosolic proteins that regulate epithelial permeability. Tricellulin was the first tight junction protein found at tricellular tight junctions, the specialized structures occurring where three cells meet together. Here, we summarize the current knowledge about tricellulin (marvelD2), a MARVEL domain protein. We address tricellulin location at tricellular junctions, and establish the comparison with the other members of the MARVEL family, occludin (marvelD1) and marvelD3. The structure of tricellulin and its membrane folding, as well as the proposed molecular interactions of tricellulin with other tight junction proteins, together with the interplay between those proteins are also discussed. In addition, we address the role of tricellulin in barrier properties, discriminating the involvement of the protein in paracellular permeability at bicellular and at tricellular tight junctions. Moreover, the key importance of the protein for hearing is highlighted based on the fact that mutations in *TRIC*, the human tricellulin gene, lead to deafness. Furthermore, this review points to some of the aspects that still deserve clarification for a better understanding of the biology of tight junctions in general and of tricellulin in particular.

Introduction

An important property of epithelial and endothelial cells is their assembly into a physical and ion- and size-selective barrier separating tissues, known as intercellular junctions. Intercellular junctions consist of tight junctions (TJs), adherens junctions (AJs) and desmosomes, which are elaborate structures formed by integral membrane proteins connected to the actin cytoskeleton by linker or adaptor proteins. Whereas TJs are located on the apical region of epithelial cells, AJs are usually situated in basolateral direction from TJs, and desmosomes are positioned below (Farquhar and Palade, 1963). Generally, TJs are responsible for intercellular sealing, whereas AJs and desmosomes mechanically link adjacent cells, but this organization may be more complex in specialized epithelia as in the blood–brain barrier (BBB) where TJs and AJs are intermingled. Collectively, intercellular junctions provide structural integrity and function as landmarks, spatially confining signaling molecules and polarity cues, in addition to serving as docking sites for vesicles and as permeability restraints. These issues have been extensively reviewed (Cardoso et al., 2010; Chiba et al., 2008; Nelson, 2003; Niessen, 2007; Paris et al., 2008; Steed et al., 2010). Here, we will focus on TJs proteins and particularly on tricellulin, a TJ protein mainly found at tricellular contacts that has attracted the attention of several research groups, as revealed by the number of papers published since its first report in 2005 by Ikenouchi et al. (2005).

Tight junctions

TJs function as a seal that regulates lateral diffusion between the apical and basolateral plasma membrane domains and form a semi-permeable barrier to paracellular flux, thus contributing to the establishment of distinct fluid compartments within the body (Schneeberger and Lynch, 2004; Tsukita et al., 2001). They are constituted by networks of transmembrane and cytosolic proteins, though the participation of lipids has also been proposed and is still under debate. In fact, models where lipids are organized in inverted cylindrical micelles (Kachar and Reese, 1982) and the existence of

Abbreviations: AJ, adherens junction; BBB, blood–brain barrier; bTJ, bicellular tight junction; CK, casein kinase; GK, guanylate kinase; JAM, junction adhesion molecule; LSR, lipolysis-stimulated lipoprotein receptor; MAGUK, membrane-associated guanylate kinase; MAPK, mitogen-activated protein kinase; PDZ, Post-synaptic density 95, Disk-large and ZO-1 proteins; PI3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; PKC, protein kinase C; PPAR, peroxisome proliferator activated; SH3, Src homology 3; TAMP, tight junction-associated MARVEL protein; TJ, tight junction; tTJ, tricellular tight junction; ZO, zonula occludens.

* Corresponding author at: Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal. Tel.: +351 217946400; fax: +351 217946491.
E-mail address: abrito@ff.ul.pt (M.A. Brito).

0171-9335/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2011.06.005

a lipid-protein hybrid complex in which proteins function within a lipid infrastructure similar to the plasma membrane (Lee et al., 2008) were proposed.

Analysis of TJs in ultrathin section electron microscopy showed focal attachments of adjacent cell membranes that exclude the intercellular gap (Farquhar and Palade, 1963). Freeze-fracture electron microscopy revealed a set of intramembranous anastomosing chains of particles forming continuous linear fibrils on one fracture face, with complementary vacant grooves on the other, known as TJ strands (Staehelin, 1973; Wade and Karnovsky, 1974). These elaborate structures appear as a series of apparent fusions of adjacent cells, named as kissing points, where the intercellular space is completely obliterated (Fig. 1). This contributed to our understanding of the three-dimensional organization of TJs, where each strand within the plasma membrane associates laterally with another strand in the apposing membrane of an adjacent cell to form paired TJ strands, responsible for the occlusion of intercellular space that accounts for zonula occludens (Tsukita et al., 2001).

Bicellular and tricellular tight junctions

TJs are usually perceived as structures circumscribing individual cells and sealing the intercellular space between two adjacent cells, as shown in Fig. 1. However, the narrow extracellular space at tricellular contacts, where there are three epithelial cells meeting together, should also be considered as contributing to the sealing of the intercellular space throughout the cellular sheet. These junctions should be referred as tricellular TJs (tTJs) to more precisely distinguish them from those between two adjacent cells, known as bicellular TJs (bTJs) (Ikenouchi et al., 2005).

tTJ present a structurally specialized organization that was first observed between three plasma membranes by freeze-fracture electron microscopy (Staehelin, 1973; Staehelin et al., 1969; Wade and Karnovsky, 1974). Analysis of freeze-fracture replicas revealed that the belt of bTJs from adjacent cells is not continuous at tricellular contacts, where the most apical elements of the TJ strands in the bTJ turn to extend in basal direction, attaching to one another (Fig. 2). These TJ strands form the central sealing elements, which have an orientation perpendicular to the bicellular tight-junctional belt, where bTJ and tTJ strands are interconnected and form a continuous complex (Staehelin, 1973, 1974). The vertically orientated triple pair strand structure, constituting the central sealing elements, attach to form a very narrow tube in the extracellular space at the center of each tricellular contact. This structure with ~10 nm diameter and known as the "central tube" (Staehelin, 1973; Wade and Karnovsky, 1974; Walker et al., 1994), initially thought to impede the diffusion of solutes (Staehelin, 1973), was recently demonstrated to control the paracellular flux to macromolecules (Krug et al., 2009).

Tight junction proteins

Since the finding of occludin as a TJ protein in 1993 (Furuse et al., 1993), a multitude of proteins have been identified, including claudins (Furuse et al., 1998; Morita et al., 1999a) and the junction adhesion molecules (JAMs). Among the TJ proteins are the transmembrane proteins and the cytoplasmic proteins, as schematically represented in Fig. 3. Claudins, occludin and JAMs are integral membrane proteins of the TJ that interact with those of neighboring plasma membrane, as well as with the cytosolic proteins, and contribute to the barrier properties.

Claudins comprise a multigene family with already known 27 members (Mineta et al., 2011). These proteins, with 20–27 kDa, bear four transmembrane domains, two extracellular loops, one intracellular turn, a short N-terminal and a longer C-terminal cytoplasmic domains (Mineta et al., 2011; Morita et al., 1999a; Tsukita et al., 2001). Claudins are thought to constitute the backbone of TJ strands (Tsukita et al., 2001) and are considered as the major barrier-forming proteins of TJs (Tsukita and Furuse, 1999). In most cell types, multiple claudin types are coexpressed in individual cells and the combination and proportions of different claudins vary among cell types, which provide functional diversity to the barrier properties of TJs depending on the organ characteristics (Furuse, 2009). As an example, claudin-5 is particularly abundant in TJs of brain microvasculature (Morita et al., 1999b) and the barrier properties of the BBB are severely affected in claudin-5 knockout mice (Nitta et al., 2003). Another example is claudin-11, also known as oligodendrocyte-specific protein, which appears to be involved in the formation of TJs in oligodendrocytes and in the tight electric sealing of myelin in the central nervous system (Gow et al., 1999). Consequently, knockout animals for this protein lack TJ strands in myelin and show slowed nerve conduction and hind limb weakness. Interestingly, such animals suffer from deafness (Gow et al., 2004; Kitajiri et al., 2004), which is also observed in patients with mutations in the claudin-14 gene (Wilcox et al., 2001), pointing to the role of TJs in the generation/maintenance of the endocochlear potential and establishment of the stria vascularis compartment, indispensable for hearing. Recent studies using loss of function of claudins have demonstrated that TJs have crucial roles in morphogenesis by their control of fluid accumulation, which is thought to be an important factor determining the shape of epithelial structures (Furuse and Moriwaki, 2009), thus unraveling novel and interesting roles for TJs.

Occludin is a protein with approximately 65 kDa, also bearing four transmembrane domains and not showing any sequence similarity with claudins (Furuse et al., 1993, 1998). As for claudin-5, brain endothelial cells exhibit a strong junctional staining for occludin, indicating that the expression levels of occludin also differ considerably between different types of epithelial cells (Hirase et al., 1997). Occludin appears to be incorporated into claudin-based TJ strands (Saitou et al., 1997; Tsukita et al., 2001), but TJ strands without occludin were reported in non-brain endothelial cells and in Sertoli cells in some species (Hirase et al., 1997; Moroi et al., 1998). Accordingly, occludin-deficient mice possess morphologically normal TJs (Saitou et al., 2000), despite the various complex phenotypes including chronic inflammation and hyperplasia of gastric epithelium, calcification in the brain, testicular atrophy, loss of cytoplasmic granules in striated duct cells of the salivary gland and thinning of the compact bone. These phenotypes cannot be explained in terms of barrier dysfunction of TJs and suggest that occludin may be involved in epithelial differentiation as well (Furuse, 2009; Saitou et al., 2000; Schulzke et al., 2005). Knockdown of occludin expression in epithelial cells also leads to diverse phenotypic alterations, and revealed the involvement of Rho signaling pathway, through which occludin elicits reorganization of the actin cytoskeleton and alters the dynamic behavior of TJ strands (Yu et al., 2005). These findings therefore reveal that the functions of the TJ occludin are more complex than previously supposed.

JAMs are a group of three proteins, named JAM-1, -2 and -3, or JAM-A, -B and -C, respectively, with approximately 40 kDa (Arrate et al., 2001; Cunningham et al., 2000; Martin-Padura et al., 1998). In contrast to claudins and occludin, JAMs are single-pass transmembrane proteins, with a transmembrane domain and an extracellular portion folded into two immunoglobulin-like domains (Arrate et al., 2001; Martin-Padura et al., 1998). JAMs are concentrated at TJs, where they associate laterally with the claudin-based backbone of TJ strands in epithelial cells (Tsukita et al., 2001). They appear to be clustered at intercellular contacts, playing a role in TJs formation and in endothelial cells polarity, as well as in paracellular permeability (Ebnet et al., 2003; Mandell et al., 2005). JAMs also belong to the immunoglobulin superfamily and are involved in cell-cell adhesion/junctional assembly of epithelial/endothelial cells