Tight Junctions/Adherens Junctions: Basic Structure and Function

Carien M. Niessen[1,2]

Adherens and tight junctions are intercellular junctions crucial for epithelial adhesion and barrier function in a wide variety of tissues and organisms. In stratifying epithelia, such as the epidermis, the role of adherens and tight junctions was considered less important owing to the abundance of desmosomes, mediating firm mechanical stability between the cells, and to the barrier function of the stratum corneum, respectively. This view has changed in recent years because of different studies that showed the importance of these structures for proper skin physiology and barrier function. The current review provides an overview of the crucial molecular constituents of these structures and highlights some recent results on their regulation. In particular, I will discuss their importance in skin biology.

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Introduction

An important property of epithelial and endothelial cells is their assembly into a physical and ion- and size-selective barrier separating tissues. Intercellular junctions, such as adherens and tight junctions, play a crucial role in the formation and maintenance of epithelial and endothelial barriers. Adherens and tight junctions were first identified on the ultrastructural level as part of the terminal bar, a tripartite junctional complex bordering the apico-basolateral membrane in a variety of polarized simple epithelia and implicated in barrier function (Farquhar and Palade, 1963). Desmosomes form the third structure of this complex but will be discussed in detail in another part of this series. Tight junctions are the most apical structure of the apical complex demarcating the border between apical and basolateral membrane domains. The intercellular membrane space of tight junctions is almost completely obliterated, hence their alternative name zonulae occludens. Adherens junctions are positioned immediately below tight junctions and characterized by two apposing membranes, which are separated by ~20 nM, that run parallel over a distance of 0.2–0.5 μm. Adherens junctions are also found outside of the tripartite complex in both epithelial and non-epithelial cells, often showing a more discontinuous or punctate pattern. Both adherens and tight junctions are closely associated with a circumferential belt of actin.

Remarkably, without any knowledge at the time on the molecular composition of the junctional complex, scientists were able to make accurate functional predictions based on this ultrastructure. Tight junctions do provide epithelia with a semipermeable size- and ion-specific barrier, which varies depending on their exact molecular composition (reviewed in Anderson *et al.*, 2004). They also restrict the diffusion of apical and basolateral membrane components, the so-called ''fence function.'' Moreover, as predicted, adherens junctions are crucial for the initiation and maintenance of intercellular adhesion in a wide variety of tissues and cell populations (reviewed in Irie *et al.*, 2004; Gumbiner, 2005). Although their ultrastructure suggests that adherens and tight junctions form stable structures, it is now obvious that they are highly dynamic complexes even in fully polarized epithelia.

A more recently identified function of intercellular junctions is that they provide the cell not only with structural integrity but also function as landmarks, spatially confining signaling molecules and polarity cues as well as serving as docking sites for vesicles (reviewed in Nelson, 2003). This is reflected in many components known to be associated with adherens and tight junctions. Whereas some of these molecules are structural and form an integral part of such junctions, others are either transiently associated with these junctions or found guilty through association with one of the structural junctional components. In addition, for several structural components, novel functions outside of the junctions have been identified. It is often not clear if such functions are directly coupled to their junctional localization.

[1]*Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany and* [2]*Department of Dermatology, University of Cologne, Cologne, Germany*

Correspondence: Dr Carien M. Niessen, Center for Molecular Medicine, University of Cologne, LFI, 05, room 59, Joseph Stelzmannstrasse 9, Cologne D-50931, Germany. E-mail: carien.niessen@uni-koeln.de

Abbreviations: EC, extracellular; p120cat, p120 catenin; JAMs, junctional adhesion molecules; GEFs, GTPase-specific nucleotide exchange factors

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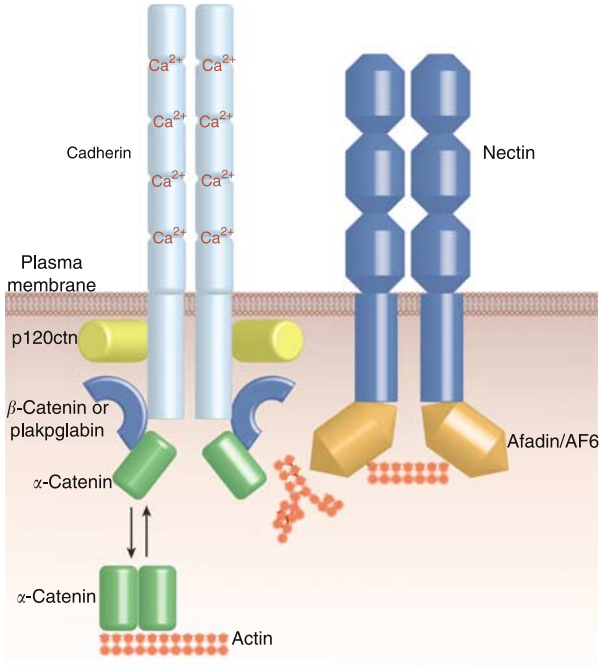

Figure 1. Schematic representation of the basic structural components of the adherens junctions. Shown are the cadherin–catenin complex and the nectin–afadin complex and their potential interactions with actin (see text).

Broadly speaking, we can define the junctional components in three general categories: (1) structural proteins necessary for initiation of the junctions, (2) plaque proteins associated with the cytoskeleton, and (3) signaling/polarity proteins. Although the division between the groups is actually a rather gray area, it serves the purpose to distinguish between protein complexes that are required for the basic structure of the junctions and, those that are more likely involved in regulation of junctional integrity or in intercellular communication. This review discusses the basic structural components of adherens and tight junctions and their importance in skin and will provide some key examples of regulation. Several excellent recent reviews exist for a more comprehensive overview of regulation of and signaling by junctions (Matter and Balda, 2003; Irie *et al.*, 2004; Gumbiner, 2005; Halbleib and Nelson, 2006; Perez-Moreno *et al.*, 2006).

Molecular composition of adherens junctions

The adherens junction consists of two basic adhesive units: the nectin–afadin complex and the classical cadherin–catenin complex (Figure 1). Both nectins and cadherins are multimember families and the cell-specific expression of cadherins and nectins ultimately determine the strength and adhesive specificity of the adherens junctions.

The nectin–afadin complex. The nectin family of IgG-like adhesion receptors consists of four members, Nectin-1 to-4. For each nectin, multiple splice variants have been described. Nectins form lateral homodimers that can engage in both homophilic and heterophilic adhesion with other nectins or nectin-like receptors, although with variable specificity and affinity. Nectins consist of an extracellular (EC) domain comprising three IgG-like loops, a single transmembrane region and a cytoplasmic domain with a C-terminal PDZ binding motif present in most splice variants (Irie *et al.*, 2004).

Nectins forms a structural adhesive unit with the actin-binding protein afadin, also known as ''AF-6,'' providing these adhesion molecules with a direct link to the cytoskeleton. Afadin was initially identified as a fusion partner of ALL-1, a translocation found in a subset of acute myeloid leukemias (Prasad *et al.*, 1993). This protein also consists of a PDZ domain (to which nectins bind), two Ras/Rap binding domains, a dilute domain, a forkhead-associated domain, and three proline rich regions (in humans two), sugges ting the potential to function as a signal integrator at adherens junctions. A smaller variant, S-Afadin, lacks the actin-binding region. Unlike the longer variant, knockdown of this variant did not affect intercellular adhesion (Lorger and Moelling, 2006).

Nectins may provide the first scaffold for adherens and tight junction formation. Utilizing many different *in vitro* assays Takai and co-workers (reviewed in Irie *et al.*, 2004) showed that cadherin mediated cell-cell adhesion and tight junction formation was dependent on nectins. Nevertheless, single knockout mice of different nectins did not reveal an essential role for nectins in embryogenesis. This is likely owing to compensation and/or redundancy by other nectins because inactivation of afadin did result in early embryonic lethality due to perturbation of intercellular junctions and polarity (Ikeda *et al.*, 1999; Zhadanov *et al.*, 1999). Moreover, phenotypes related to junctional alterations have been observed in specific organs in the different existing nectin knockouts (Irie *et al.*, 2004).

The cadherin–catenin complex. The type I classical cadherins belong to a large super family of proteins, the characteristic of which is the cadherin EC repeat. The desmosomal cadherins are also part of the cadherin super family. Cadherins are considered homophilic adhesion molecules although recent data indicate that binding can be more promiscuous (Niessen and Gumbiner, 2002; Duguay et al., 2003). If cadherins can mediate heterophilic adhesion and what the exact molecular requirements of the EC domain for adhesion are, are both subjects of an ongoing debate (reviewed in Gumbiner, 2005). Inactivation of different cadherins in a variety of organisms have shown their importance in tissue morphogenesis (Gumbiner, 2005; Halbleib and Nelson, 2006).

Classical cadherins form a basic complex with the catenins, α-, β-, and p120 catenin (p120ctn). Both p120ctn and β-catenin bind directly to the cadherin via their armadillo repeats, whereas α-catenin connects via β-catenin. Plakoglobin, also known as γ-catenin, is a close relative of β-catenin. Although primarily associated with desmosomal cadherins, it can substitute for β-catenin in the classical cadherin/catenin complex. Binding of β-catenin to cadherin is crucial for full adhesive function and this is largely dependent on its ability to bridge the cadherin with the actin-binding protein, α-catenin (reviewed in Aberle *et al.*, 1996). This makes β-catenin an excellent candidate to mediate signal-induced changes in cadherin adhesive contacts. β-catenin can directly bind to several signaling proteins, for example, the EGF receptor or tyrosine phosphatases. Moreover, growth factor-induced tyrosine phosphorylation of the cadherin–catenin complex is associated with changes in intercellular adhesion concomitant with changes in complex composition (reviewed in Nelson and Nusse, 2004). *In vitro* studies using cadherin–α-catenin fusion mutants indeed suggested a role for β-catenin in regulation of intercellular motility (Nagafuchi *et al.*, 1994). However, both *in vitro* and *in vivo* studies using similar constructs showed no β-catenin-dependent impairment of adhesion regulation (Takeda *et al.*, 1995; Pacquelet and Rorth, 2005). The significance of β-catenin in regulation of adhesion is therefore still under debate. As β-catenin is the central player in Wnt signaling, a pathway that regulates cell fate determination, it is tempting to speculate that its function in adherens junctions relates to the coordination of morphogenetic movements with cell fate determination. This is corroborated by a multitude of studies showing a close reciprocal relationship between intercellular adhesion and Wnt signaling (Nelson and Nusse, 2004; Brembeck *et al.*, 2006).

P120ctn is the poster child of a subfamily of Armadillo repeat-containing proteins, which also includes δ-catenin, Armadillo-Repeat gene deleted in Velo-Cardio Facial syndrome (ARVCF), and plakophilins. The first three members appear to be functionally redundant with respect to classical cadherin binding. Plakophilins are predominantly found at desmosomes. The cadherin interaction with p120ctn is crucial for cell-surface stability by regulating endocytosis (Xiao *et al.*, 2007). In the absence of p120ctn cadherin, cell-surface expression is strongly diminished. Furthermore, p120ctn has emerged as a major regulator and integrator of signaling by the Rho family of small GTPases (Anastasiadis, 2007), and this is at least partially dependent on its interaction with the cadherin (Wildenberg *et al.*, 2006).

Actin binding at the adherens junction. Adherens junctions are closely connected to the actin cytoskeleton as their disturbance perturbs the actin cytoskeleton. Because α-catenin can directly bind either β-catenin or actin, it was considered textbook knowledge that α-catenin provided the connection of cadherins to actin. Indeed, many other *in vitro* and *in vivo* studies corroborated such a direct link. However, recent studies from the Nelson and Weis groups found no *in vitro* evidence for the existence of a ternary cadherin-β–α-catenin–actin complex (Yamada *et al.*, 2005). Instead, binding of α-catenin to actin or β-catenin is mutually exclusive (Drees *et al.*, 2005). In addition, actin dynamics at intercellular adhesive contact sites were very different to those of the cadherin complex, suggesting the absence of a stable interaction. Regardless, both genetic and cell biological data strongly indicate that regulation of actin polymerization does take place at or in close vicinity of the adherens junctions. This is at least partially dependent on α-catenin (reviewed in Gates and Peifer, 2005; Scott and Yap, 2006). The actin-binding protein afadin is another candidate to connect adherens junctions directly to actin.

The actin nucleating proteins formin and Arp2/3 are associated with the cadherin complex (Kobielak *et al.*, 2004; Verma *et al.*, 2004). Thus it is possible that both Arp2/3-dependent actin branching activity, important for lammelipodia formation, and formin-dependent linear actin filament polymerization actin cables, important for fillapodia formation, occur at or near adherens junctions. How and which factors regulate local activity is less clear. A wide variety of actin-binding and regulatory proteins, such as ZO-1, vinculin, spectrin, cortactin, moesin, α-actinin, Ena/Vasp, Wave, and Wasp, can associate with and affect adherens junctions and may thus be responsible (reviewed in Gates and Peifer, 2005; Scott and Yap, 2006). Overall, the results suggest a dynamic interaction of adherens junctions with the cytoskeleton (Figure 1).

Cooperation of nectins and cadherins in adherens junction formation. Many potential physical links between the nectin–cadherin adhesion systems have been identified, the most direct one being those between afadin and α-catenin or p120ctn (Hoshino *et al.*, 2005). However, thus far, it has not been possible to show the existence of a cadherin/nectin-containing complex *in vivo*. Nevertheless, both systems appear to be required for formation and function of adherens junctions. For example, both adhesion complexes cooperate in the formation and plasticity of synaptic junctions, a specialized form of adherens junctions (Togashi *et al.*, 2006).

Either cadherin or nectin engagement can control the activity of the Rho family of small GTPases, which is a crucial regulator of actin dynamics. Activated forms of these GTPases affect cadherin activity and junction stability (Braga and Yap, 2005), suggesting a close reciprocal relationship. One model suggests that initial engagement of cells occurs via nectins that activate Rac and Cdc42, which then stimulate the formation of lammelapodial protrusions and cadherin binding (Irie *et al.*, 2004). In addition, Rho GTPases may regulate actomyosin contractions, which is important for junctional rearrangements during morphogenetic movements (Bertet *et al.*, 2004). Local regulation of small GTPase activity is most likely mediated by the recruitment of Rho family GTPase-specific nucleotide exchange factors (GEFs) and

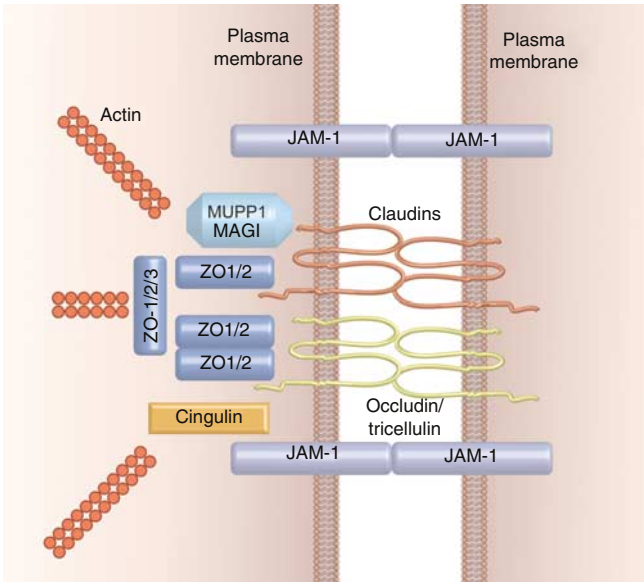

Figure 2. Schematic representation of the basic structural transmembrane components of tight junctions. ZO-1 or ZO-2 is important for clustering of claudins and occludin, resulting in the formation of tight junctional strands. The role of the other scaffolding proteins (ZO-3/MAGI/MUP1) is less clear. The ZOs and cingulin can provide a direct link to the actin cytoskeleton.

GTPase-activating proteins to adherens junctions, several of which interact with core components. Adherens junctions may regulate Rho small GTPases via their upstream regulators, the Rap small GTPases. RapGEFs directly interact with E-cadherin and engagement of E-cadherin activates Rap. Afadin is an effector of Rap activity, suggesting the possibility of a positive loop reinforcing intercellular adhesion (reviewed in Kooistra *et al.*, 2007).

Molecular composition of tight junctions

Transmembrane components. Three types of structural transmembrane components that are enriched at tight junctions have the potential to mediate cell–cell adhesion (Figure 2): the IgG-like family of junctional adhesion molecules (JAMs), the claudin, and occludin families of four transmembrane spanning molecules (Schneeberger and Lynch, 2004; Furuse and Tsukita, 2006).

Occludin was the first identified transmembrane component of tight junctions. Although tight junctions without occludin are rare, its physiological function in tight junctions is still unclear. Tight junctional strands and barrier function were still present in cells and epithelial tissues deficient for occludin. Nevertheless, the mice exhibit several different phenotypes, such as growth retardation, mineral deposits in the brain, male sterility, and gastritis, suggesting barrier impairment (Saitou *et al.*, 2000). Alternatively, occludin serves an as yet unidentified function independent of the classical tight junctional barrier and fence function. Indeed, studies done using cells in which occludin expression was knocked down by RNAi indicate a crucial role for this protein in the communication of apoptosis to surrounding cells (Yu *et al.*, 2005). Only recently a protein, tricellulin, was identified with structural similarity to occludin. Unlike other tight junctional proteins, tricellulin is enriched only at tricellular tight junctions, where it enforces the barrier function of epithelial cell sheets (Ikenouchi *et al.*, 2005). Its mutation contributes to deafness in humans (Riazuddin *et al.*, 2006). This finding further illustrates the growing molecular complexity of tight junctions.

The presence of functional tight junctions in the absence of occludin leads the group of Tsukita to the identification of two other tight junctional transmembrane proteins, named claudin-1 and -2. Around the same time other groups independently found that mutation or inactivation of certain proteins, now recognized as claudins, resulted in diseases such as hypomagnesaemia (claudin-16), deafness (claudin-14), and absence of central nervous system myelin and sertoli cell tight junction strands (claudin-11) (reviewed in Furuse and Tsukita, 2006).

The claudin family consists of at least 24 members, with each showing a specific organ and tissue distribution. Exogenous expression of claudins in fibroblasts not only induced Ca^{2+}-independent cell–cell adhesion but also resulted in the formation of tight junction fibers, indicating its crucial role in tight junction formation. More importantly, experimentally manipulating the type of claudin expression directly affected paracellular ion and/or size selectivity (Van Itallie *et al.*, 2001; Nitta *et al.*, 2003). As the EC loops of occludin contain very few charged amino acids, these loops are charged in claudins and their isoelectric point varies widely between the different claudins. Changing the charge in the first EC loop of claudin-15 altered barrier ion specificity (Colegio *et al.*, 2002). It is now widely recognized that the large variety in strength, size, and ion specificity of tight junctional barriers in different epithelia and endothelia is largely due to the type of claudin(s) found at specific tight junctions (Anderson *et al.*, 2004; Furuse and Tsukita, 2006).

The IgG-like family of JAMs is the third group of transmembrane receptors found at tight junctions. The family consist of the closely related molecules JAM-A, -B and, -C and the more distantly related Coxsackie and adenovirus receptor, endothelial cell-selective adhesion molecule, and JAM-4 (Ebnet *et al.*, 2004). JAMs can be engaged in homophilic and heterophilic adhesion but do not induce the formation of tight junctional strands when expressed in fibroblasts. JAMs are not exclusively found on cells that form tight junctions but also on cells such as leukocytes, thereby contributing to their transendothelial migration (Ebnet *et al.*, 2004). In addition, Jam-C regulates polarization and differentiation of spermatids by recruiting polarity protein complexes (Gliki *et al.*, 2004).

Scaffolding proteins. The incorporation and association of occludin, claudins, and JAMs in tight junctional strands require local clustering of these proteins. As no direct interactions have been found between occludins, claudins, and JAMs, cytoplasmic binding partners must fulfill this scaffolding function (Figure 2). An important group of tight junctional scaffolding molecules are the zonula occludens proteins ZO-1, ZO-2, and ZO-3. These proteins belong to the membrane-associated guanylate kinase-like homologs family and are characterized by three N-terminal PDZ domains, an SH3 domain followed by the GUK domain. These proteins can interact directly with occludin and claudins via their PDZ domains, whereas their C-terminus can associate with actin, thus providing a direct link with the cytoskeleton (Schneeberger and Lynch, 2004). Localization of ZO-1 to tight junctions requires its actin-binding domain (Fanning *et al.*, 2002). In addition, ZO-1 can also directly interact with JAMs and form homodimers or heterodimers with either ZO-2 or ZO-3. Only very recently, it was shown that either ZO-1 or ZO-2, but not ZO-3, is crucial for clustering of claudins, strand formation, and barrier function (Umeda *et al.*, 2006). Surprisingly, apico-basolateral polarity was not obviously disturbed in the absence of tight junctional strands, suggesting that tight junctions are not involved in the separation of the apical and basolateral membrane domains (the fence function). One should keep in mind that these studies were done under 2-D culture conditions that provide external polarity cues. More stringent tests, such as 3-D culture conditions or animal models, will thus be necessary to rule out a function for tight junctions in apico-basolateral polarity and fence function (Shin and Margolis, 2006).

Several other PDZ-containing scaffolding proteins, such MUPP1 and MAGI proteins are associated with the tight junctional cytosolic plaque and can directly interact with one or more of the tight junctional transmembrane components (Schneeberger and Lynch, 2004). It is at present unclear if these molecules are directly involved in the formation of the tight junctions or serve a more regulatory function.

Cingulin, a non-PDZ tight junctional plaque protein, interacts with ZOs, JAMs, and actin via its head domain, whereas its central rod domain is required for homodimerization and interacts with myosin. As such, this protein may be an important regulator of tight junctional dynamics during actomyosin contraction (Clayburgh *et al.*, 2005). However, cells with a deletion of the cingulin head domain showed no obvious disturbance in tight junctional strands (Guillemot *et al.*, 2004). Instead, cingulin may couple junctional integrity to cytoskeletal regulation and proliferation by binding to the Rho-specific exchange factor GEF-H1. Not only does GEF-H1 affects paracellular permeability but it also regulates Rho activity and G1/S transition (Aijaz *et al.*, 2005).

Regulation of intercellular junctions

A large group of molecules can either directly interact with components of adherens or tight junctions or are localized at these junctions. Generally, they are either involved in the dynamic regulation of intercellular adhesion and junction assembly/disassembly or in communicating signals from the junctions. The mechanisms that regulate the formation and dynamic maintenance of adherens junctions can occur on multiple levels varying from transcriptional regulation to more local regulation, such as cytoskeletal dynamics, proteolytic cleavage by proteases, phosphorylation of key components by growth factors or endocytosis.

Formation of adherens junctions facilitates the assembly of tight junctions. This is reflected by several interactions between core adherens and tight junctional components, such as those between ZO-1 and α-catenin or afadin. ZO-1 is recruited to early cadherin-containing intercellular contacts (Itoh *et al.*, 1997), thus providing a first scaffold for the tight junctions. Interference with nectin or cadherin-based adhesion disturbed tight junctions (Irie *et al.*, 2004; Tunggal *et al.*, 2005) but absence of both ZO-1/ZO-2 had no effect on adherens junctions (Umeda *et al.*, 2006).

Studies in *Drosophila* made the important observation that junction formation is functionally coupled to the establishment of polarity (Nelson, 2003). Several different multiprotein complexes regulate the set-up of polarity by specifying membrane domains. In mammalian polarized simple epithelial cells, apical domain polarity complexes, such as Par3/Par6/aPKC and Crumbs/Pals/Patj, are localized at tight junctions. More importantly, functional interference with any of these proteins affects paracellular permeability, indicating their importance in the assembly of functional tight junctions (Anderson *et al.*, 2004; Shin *et al.*, 2006). Vice versa, loss of ZO1/ZO-2 did result in a more lateral distribution of Par3 but without obvious loss of apico-basolateral polarity (Umeda *et al.*, 2006). The polarity protein scribble, important for basolateral membrane domain identity in *Drosophila*, is recruited to E-cadherin junctions in mammalian cells and its downregulation affects cell–cell adhesion (Qin *et al.*, 2005). Many direct interactions exists between polarity proteins and core junctional components (reviewed in Shin *et al.*, 2006), but their exact contribution to the molecular mechanisms that underlie the interplay between intercellular junctions and polarity is far from clear. Interestingly, tissue growth factor-β-induced disassembly of tight junctions during epithelial to mesenchymal transition appears to require interactions of its receptor with the polarity protein Par6 and occludin (Barrios-Rodiles *et al.*, 2005).

The importance of adherens and tight junctions in the skin

The importance of adherens and tight junctions in skin physiology and pathology is best illustrated by studies from junctional component knockout mice, which revealed crucial roles for these structures in the epidermis. Although other cell compartments do form adherens and tight junctions, their presence has only been poorly studied in the context of skin function and disease and will thus not be discussed. The epidermis forms an important barrier that protects the organism from the outside while also preventing

unnecessary loss of water. Although cells are not polarized in an apicobasolateral sense, the tissue shows a polarized distribution of intercellular junction components, differentiation markers and polarity proteins. In addition, since this is a self-renewing tissue with a continuous upward movement of cells, intercellular junctions must dynamically rearrange without losing their adhesive strength or barrier properties.

The best indication that adherens junctions play a crucial role in the mechanical stability of keratinocytes comes from mice carrying an epidermal specific deletion of α-catenin (Vasioukhin *et al.*, 2001a). This caused detachment of the epidermis associated with impaired intercellular adhesion and loss of adherens junctions. In addition, epidermal loss of E-cadherin, expressed on all viable layers of the epidermis, resulted in hair loss owing to impaired intercellular adhesion (Young *et al.*, 2003; Tinkle *et al.*, 2004).

Several results suggest that adherens junctions and desmosomes cooperate to assure proper epidermal cohesion. P-cadherin loss alone has no obvious skin phenotype but enhances the blistering defects caused by the absence of the desmosomal cadherin desmoglein-3 (Lenox *et al.*, 2000). Lack of a desmosomal plaque protein, desmoplakin, in keratinocytes not only impaired desmosomes but also adherens junctions (Vasioukhin *et al.*, 2001b).

Adherens junctions may have other functions next to their structural intercellular adhesive role. Loss of α-catenin in the epidermis results in overgrowth and the formation of epithelial cysts, associated with alterations in growth factor signaling (Vasioukhin *et al.*, 2001a). Thus, α-catenin may be an important regulator for signaling receptors, and this may depend on its association with adherens junction. Alternatively, α-catenin-dependent overgrowth may result from aberrations in stem cell division. For example, α-catenin appears to be required for the proper positioning of aPKC in cells undergoing asymmetric cell division in the basal layer of the epidermis (Lechler and Fuchs, 2005), and this likely contributes to the balance between stem cells and differentiated cells.

Deletion of p120ctn in the epidermis activates an inflammatory response in mice, which is linked to increased NF-κB signaling in keratinocytes (Perez-Moreno *et al.*, 2006). Interestingly, expression analysis of α-catenin-deficient keratinocytes also revealed upregulation of this pathway (Kobielak and Fuchs, 2006). Together, these reports suggest that adherens junction components may couple regulation of inflammation to structural integrity of the epidermis, even though the molecular mechanisms are far from clear.

The stratum corneum physically separates the organism from its environment and protects it from unnecessary water loss as well as detrimental influences from the outside. Owing to the existence of this barrier, it was assumed that tight junctions would not contribute to the epidermal barrier, despite expression of its components in the epidermis (Brandner *et al.*, 2006). This perception changed when extensive epidermal water loss was observed in claudin-1-deficient mice (Furuse *et al.*, 2002). These mice had an apparently normal functioning stratum corneum but a dysfunctional occludin-positive barrier in the granular layer. The ultrastructural observation of continuous strands in the epidermal granular layer indeed showed the presence of an integral tight junctional barrier (Schluter *et al.*, 2004). The importance of tight junctions for physiological barrier function is further underscored by the observation that claudin-1 mutations are found in neonatal ichtyosis-sclerosing cholangitis syndrome (Hadj-Rabia *et al.*, 2004). Next to claudin-1, other claudins are expressed in skin and they may be important for the selective transport of small solutes through the skin.

The adherens junction protein E-cadherin is important for functional epidermal tight junctions since early epidermal deletion of this protein largely phenocopied the claudin-1-deficient mice. This was associated with inappropriate localization of polarity proteins like aPKC, which is localized not only at tight junctions but at cell–cell contacts in all viable epidermal layers (Tunggal *et al.*, 2005). Blocking aPKC function in keratinocytes interfered with *in vitro* barrier formation, suggesting that polarity proteins also contribute to epidermal barrier formation, similar to simple epithelia. Interestingly, tight junction assembly was not visible disturbed, suggesting that aPKC regulates a late step in the formation of functional tight junctions (Suzuki *et al.*, 2002; Helfrich *et al.*, 2007).

Several papers indicate that the stratum corneum and tight junctions cooperate in the formation of a functional skin barrier. Overexpression of claudin-6 under the involucrin promotor in mice results in epidermal barrier defects associated with changes in both the tight junctional and stratum corneum barrier (Turksen and Troy, 2002). Inactivation of the membrane-anchored channel-activating serine protease 1/Prss8 disturbed both barriers, although the molecular mechanism is unclear (Leyvraz *et al.*, 2005). Because of the importance of the lipid composition in the stratum corneum, and the presumed fence function of tight junctions in simple epithelia, it is tempting to speculate that tight junctions in the stratum granulosum regulate ''apical'' protein and lipid vesicle targeting toward the stratum corneum. However, recent data suggest that that the fence function is independent of tight junctions (Umeda *et al.*, 2006). Addressing the relationship between the two barriers may thus also contribute to solving the question if tight junctions do or do not contribute to epithelial fence function (Shin and Margolis, 2006).

A concept potentially important for skin pathology is the observation that junctions and their associated proteins are hijacked by a variety of viruses and bacteria to obtain entry into cells and/or replicate. This happens on at least two different levels. First, certain viruses or bacteria use transmembrane components of cellular junctions as receptors. For example, nectin-1 is a receptor for herpes simplex virus. Second, bacteria and viruses modulate junctional structures resulting in at least a partial disruption of such structures. This can happen either by inserting effectors into the cell or activating signals that locally regulate the actin cytoskeleton or by direct binding to

junctional components (Sousa *et al.*, 2005).

Concluding remarks

The last 10 years have brought dramatic new insights into the function of adherens and tight junctions in skin physiology and pathology. It is now obvious that these structures are crucial components of skin barrier function, and perhaps link structural integrity to proliferation and inflammatory responses of the skin. It will be important to dissect how tight junctions only seem to form a structural barrier in the granular layer of the epidermis, even though many of its components are already at sites of intercellular contacts in the spinous and/or basal layer. Another exciting direction is the dissection of the role of intercellular junctions in inflammatory diseases associated with impaired barrier function, such as psoriasis, and if mutations in junctional components underlie uncharacterized barrier diseases.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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