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Desmosomes from a structural perspective

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Summary

Desmosomes are cell-cell junctions responsible for maintaining the structural integrity of tissues by resisting shear forces. Defects result in diseases of mechanically challenged tissues such as skin and heart. The architectural design represents the key to understanding the strength and durability inherent to desmosomes. A number of different proteins contribute to this architecture and x-ray crystallography has made considerable progress in defining the atomic structure of various isolated domains. Electron tomography has been used to determine the three-dimensional structure of intact desmosomes *in situ*. By combining information from x-ray crystallography, cell and molecular biology and electron tomography, it should ultimately be possible to deduce the specific protein interactions that define the mechanical properties of this important adhesive junction.

Introduction

Desmosomes are intercellular junctions that confer mechanical stability to a wide range of tissues. Also, desmosomes are critical during embryogenesis for sorting cells and thus for the formation of organs and tissues [1]. In the mature organism, desmosomes are most abundant in areas subject to mechanical stress and, as a consequence, defects are often manifested as diseases of the skin and heart [2,3]. For example, pemphigus and epidermolysis bullosa are blistering skin diseases and arrhythmogenic right ventricular cardiomyopathy is a heart disease, both of which result from weakened desmosomal contacts that ultimately compromise the mechanical integrity of the corresponding tissue.

Functionally, desmosomes act like buttons that join the lateral edges of adjacent cells. The architectural principles, depicted in Fig. 1, are analogous to those of the adherens junction and the hemi-desmosome, though each of these junctions has a distinct set of protein components. The primary site of adhesion is in the extracellular domains of type I transmembrane proteins, which for both desmosomes and adherens junction belong to the cadherin family characterized by five tandem extracellular domains (EC1-5). On the intracellular side of the desmosome is a plaque, divided into regions closer to the membrane (outer dense plaque, ODP) and further from the membrane (inner dense plaque, IDP). As shown most graphically by immunolabeling and electron microscopy [4], the ODP comprises the intracellular domains of desmosomal cadherins as well as two proteins from the armadillo family, namely plakoglobin and plakophilin. The IDP is composed of desmoplakin, which serves to couple the sites of

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intercellular adhesion to the intermediate filament network, thus providing mechanical reinforcement to the primary, intercellular adhesion site.

Extracellular Interactions between Cadherins

Cadherin interactions in adherens junctions are known to be homotypic, meaning that a single type of “classical” cadherin [5] is expressed on the surface of two adjoining cells. In contrast, desmosomal adhesion requires expression of two complementary types of cadherins called desmocollin and desmoglein [6,7] and there is evidence that this involves heterotypic interaction between desmocollin and desmoglein emanating from opposing cell surfaces [8, 9]. Nevertheless, there remains uncertainty regarding the physical nature of these interactions and the determinants of cadherin binding specificity. These issues have been approached through a variety of biophysical and crystallographic studies of classical cadherins [10,11]. In particular, a series of x-ray crystallographic studies provide a compelling model for homotypic interactions in the form of a so-called trans dimer, shown in Fig. 1. This dimer is formed via a domain swap involving the conserved tryptophan 2 in the N-terminal, membrane-distal EC1 domain of the cadherin molecule [12,13]. Alternatively, molecular force microscopy has been used to study the physical interactions between cadherin molecules, either individually or in an ensemble [14-18]. These force measurements reveal multiple bound states and have been used to support a different model involving interdigitation of the finger-like cadherin molecules [19-21]. These competing models are fundamentally incompatible, because the strand dimer emphasizes symmetric interactions between the EC1 domain whereas interdigitation implies alternative interactions of EC1 with domains EC2-5. However, a recent implementation of intermolecular force microscopy brings some relief to this controversy [22 **]. As before, this study shows multiple binding states for cadherins, but further analysis of lifetimes and bond lengths supports an alternative to molecular interdigitation: namely an enhanced bending of the naturally curved cadherin molecule that would bring cadherin domains into a parallel alignment at the midpoint between the two cells. This model would allow for the domain swap between EC1 domains illustrated by Shapiro and colleagues, as well as interactions between other extracellular domains proposed by Leckband and colleagues. Furthermore, the proposed flexibility in the bending of cadherin would explain the marked difference in the intermembrane distance of desmosomes vs. adherens junctions, despite structural similarity in their respective cadherin molecules.

Desmosomal cadherins form a subfamily that is distinct from the classical cadherins [23]. Nevertheless, the domain architecture and characteristic sequence motifs, such as the N-terminal tryptophan and calcium binding residues, are conserved (Fig. 2). Sequence identities for the entire extracellular portion of desmosomal cadherins are 30-35% relative both to each other and to classical cadherins. By comparison, sequence identities between individual extracellular domains from either N-cadherin or C-cadherin are only 10-20% despite having a conserved fold [24]. These comparisons strongly suggest that extracellular portions of desmosomal cadherins will have the same basic architecture as classical cadherins, though important details such as binding specificity, flexibility and the angle between individual extracellular domains are likely to be different. An x-ray crystallographic analysis of type II classical cadherin provides an example of such differences [25 **]. These structures show an enhanced dimer interface between EC1 domains that are postulated to be a major determinant in the binding specificity of type II cadherins. In particular, a second tryptophan residue is involved in the domain swap and additional set of bonds extend the dimer interface along the entire length of the EC1 domain. As a result, the angle between dimeric EC1 domains is far more constrained in type II relative to type I cadherins, suggesting extra rigidity in the corresponding type II dimer interface. Sequence comparisons with desmosomal cadherins indicate that they lack the hydrophobic residues that produce this extended interface (Fig. 2), suggesting that both desmosomes and adherens junctions could depend on flexible cadherin

interactions in order to engage in the multiple binding states and thus confer additional mechanical strength to the respective junctions.

Intracellular Interactions: plakoglobin, plakophilin, desmoplakin

The intracellular region of desmosomes is compositionally heterogeneous and it has been challenging to untangle the interactions between the constituent proteins and to determine their elements of specificity. Plakoglobin (Pg) is found in both adherens junctions and desmosomes [26] and is highly homologous to β -catenin. Both are characterized by 12 armadillo (ARM) repeats as well as globular domains of unknown structure with ~100 residues at both N- and C-termini. The ARM repeats of β -catenin have been shown by x-ray crystallography [27] to form an α -helical solenoid that binds extended polypeptide chains along a groove formed by this superhelical structure. In particular, the cytoplasmic tail of E-cadherin [28], the transcription factor LEF/TCF [29] and components of the WNT signaling pathway [30] all bind in the groove of β -catenin. A similar interaction likely occurs between Pg and the conserved region of desmoglein and desmocollin, known as the intracellular catenin binding site [31,32]; this interaction undoubtedly represents an early step in desmosome assembly.

Plakophilins (Pp) are also ARM repeat proteins, but they belong to the p120^{ctn} subfamily, which is distinct from the subfamily containing Pg and β -catenin. Plakophilins consist of 9 ARM repeats and have a considerably larger N-terminal domain of 275-380 residues depending on the isoform, but no C-terminal domain to speak of. The N-terminal domain has been reported to bind to essentially every other component in the desmosome, using blot overlay, co-immunoprecipitation, yeast two-hybrid, or recruitment assays [reviewed in 33,34]. Ironically, no binding partner has been reported for the ARM repeats, which contain the characteristic LxNL motifs along the groove that has been shown in the case of β -catenin to bind a variety of extended peptides [35 **]. Nevertheless, a consensus has evolved according to which Pg is largely responsible for binding the cytoplasmic tails of cadherins and Pp provides lateral association of cadherins within the plane of the membrane [36-38]. Although this consensus is plausible, we lack the definitive structural and functional results specifying a specific set of interactions that would dictate the architecture of the molecular scaffold underlying the intracellular plaque of the desmosome.

Desmoplakin (Dp) is a critical component of this molecular scaffold, playing an important role both in lateral clustering of cadherins and in linking intermediate filaments to the junction. Dp is a huge molecule, with N-terminal and C-terminal domains of almost 1000 amino acids separated by a central, α -helical domain of almost equal size. Dp has been predicted to form dimers based on a coiled-coil interaction along this central domain, which indeed is consistent with images recorded by rotary-shadow electron microscopy [39]. These images also show that the molecule can span up to 180 nm, with a 130-nm long central rod connecting two globular heads. A shorter splice variant is about half this length, consistent with its loss of two-thirds of the central rod [40]. Many studies report an interaction between the N-terminal domains of Dp and Pp and assign this interaction a critical role in clustering the cadherins [reviewed in 33,41]. In particular, failure to form desmosomes in Dp knockout cells can be restored by expressing only the N-terminal domain of Dp [42], suggesting an important role for the Dp N-terminal domain in the intracellular scaffold. The C-terminal end of Dp interacts with intermediate filaments (IF) and features three plakin-repeat domains (PRD) denoted A, B and C, which are connected by variable length linker sequences [40]. The structures of PRD-B and PRD-C have been recently characterized by x-ray crystallography [43]. Each PRD consists of 4.5 tandem repeats of a 38-residue motif and the structures show that, unlike the regular, linear arrangement of most tandem repeats (e.g., Pg and Pp), the PRD's form an irregular structure with the end of the fifth repeat interacting with the beginning of the first repeat. Thus, these domains have been postulated to form beads on a string, rather than an extended structure with

inserted loops. However, this “string” is likely to be more than a flexible tether. The 46-residue linker between PRD-A and PRD-B is included the structure of PRD-B, where it forms a small globular sub-domain that packs against the main body of the PRD. The linker between PRD-B and PRD-C is longer (~150 residues), is conserved with other members of the plakin family, and has been suggested to play a definitive role in intermediate filaments binding [44,45]. Indeed, co-sedimentation studies of vimentin with PRD's from Dp showed that the presence of the PRD-BC linker greatly enhanced the binding of constructs to vimentin [43].

Structure of Intact Desmosomes

Electron microscopy has long been used to characterize the appearance of desmosomes *in situ*. These junctions are characterized by a prominent mid-line running halfway between opposing cell surfaces and a densely stained plaque on the intracellular face of the membrane. Recent advances have made it possible to evaluate the organization of the intact desmosome and to attempt to correlate the results from x-ray crystallography. In particular, we have used electron tomography to evaluate the three-dimensional structure of desmosomes from newborn mouse epidermis prepared by freeze-substitution and thin sectioning [24]. By using the x-ray structure of C-cadherin as a template for interpreting the organization of extracellular densities, we produced a model for the interactions between the desmosomal cadherins, though no attempt was made to distinguish desmoglein from desmocollin given the lack of necessary resolution. The result was a disordered series of molecular groups in which cadherins interacted via their N-terminal domains, consistent with the domain swap described for classical cadherins. However, unlike crystallographic structures, interactions within the intact desmosome were not constrained to symmetric dimers. We postulated that flexibility in the N-terminal strand allowed multimers with a variety of geometries to be assembled. The curved shape of cadherins placed EC1 and EC2 domains running approximately parallel to the membrane, thus defining the midline as a region of increased protein density and offering the potential for numerous secondary interactions that would add to the overall strength of the adhesive bond.

An alternative view of this intermembrane space has come from an image of frozen, unstained sections of epidermis from human forearm [46,47 *]. This image shows densities crossing the extracellular space between two cells with an apparent periodicity of ~5 nm. These densities run straight to the midline, which is a potential contradiction with the curved structure revealed by x-ray crystallography that can only be resolved by using electron tomography to determine the three-dimensional organization in this frozen tissue. A recent analysis of a lanthanum-infiltrated desmosome from the guinea pig heart also shows a regular spacing of intercellular densities, this time with a ~7.5 nm periodicity [48,49]. Although it is possible that the different preparative protocols are generating different appearances of these extracellular regions, it is also possible that different tissue environments influence packing of the cadherin molecules. In particular, desmosomes have been reported to undergo a transition from a calcium-dependent, low adhesion state to a calcium-independent high adhesion state. This transition is not accompanied by changes in protein composition and may instead involve differences in cadherin packing [48,50 **]. Also, the repeated and regular mechanical stress experienced in cardiac tissue could induce a particular arrangement of molecules, given that the individual intermolecular bonds are low affinity and transient [51 **].

A higher density and greater heterogeneity of protein interactions in the intracellular plaque make its structure more difficult to define. Nevertheless, zones can be distinguished based on changes in density and texture. In stained material from newborn mouse skin (Fig. 3), the ODP is densely stained and extends ~18.5 nm from the surface of the membrane; the IDP is more fibrous and extends a further ~37.5 nm to the border of the IF network. In the image of frozen-unstained desmosomes from human skin, the ODP appears as an 11-nm translucent band

bordered by a 7nm thick layer of material. These dimensions are consistent with immunolocalization of the various protein constituents [4], which indicate that the ODP contains the cytoplasmic tails of desmoglein and desmocollin as well as Pp, Pg, and the N-terminal domain of Dp. The C-terminal domain of Dp, however, was 50 nm away at the innermost boundary of the IDP. Based on these immunolabeling results and on the tomographic images such as Fig. 3, the total extent of Dp in an intact desmosome is ~40 nm, which is considerably less than the length of the extended Dp molecule (180 nm). The N- and C-terminal domain can be expected to be globular with a dimension of 5-10 nm each, suggesting that the central, α -helical domain must be folded up within the IDP. One might speculate that such folding would provide an extensibility to the IDP, which could be useful in maintaining cell adhesion when the tissue is under shear stress.

Concluding remarks

Although desmosomes have been studied for many decades, we still have a lot to learn about the physical interactions that produce one of the most stable structure in the cell. We need continued progress in revealing the structures of individual protein constituents and their various domains. However, a full understanding will require elucidation of the architecture of the intact desmosome. Electron tomography is well suited for this work, though challenges exist for sample preparation and for interpreting the resulting 3D maps. With the development of technologies for preparing and imaging frozen, unstained sections of cells, we can look forward continued progress in this regard. Once we establish a basic architecture, there are many questions to be asked. What is the assembly pathway during desmosome formation? What is the disassembly pathway during wound healing or cell transformation? What are the architectural consequences of genetic and autoimmune defects that lead to various diseases? Given the fundamental physical role that desmosomes play in holding tissues together, this structural approach is essential to understanding their function.

Acknowledgements

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Figure 1.

Protein components of the desmosome. Individual components are superimposed on the grey-scale electron micrograph at the center of this figure. The membranes of opposing cells are shown as red lines with the distinctive mid-line indicated by the white arrow. White strands correspond to cadherin molecules, which are type I membrane proteins that directly mediate the intercellular bond. The intracellular plaque is segregated into two regions. The outer dense plaque is immediately adjacent to the membrane and comprises the intracellular tails of cadherins (white), plakoglobin (orange), plakophilin (yellow) and the N-terminal domain of desmoplakin (blue). The inner dense plaque comprises the rod domain and C-terminal domain of desmoplakin (blue), the latter of which associates with a network of intermediate filaments (green). X-ray crystal structures related to these various components are shown around the periphery of this figure at approximately the same scale. The cadherin trans dimer corresponds to the two-fold symmetric interaction seen in the x-ray crystal structure of the five extracellular domains of C-cadherin [12]. The ARM repeats of plakoglobin have been shown to interact with the intracellular tails of desmosomal cadherins and are represented here by the structure of the highly homologous β -catenin [27]; the orange spheres represent N- and C-terminal domains of unknown structure (~ 100 residues each). The ARM repeats of plakophilin [35 **] are shown associated with an N-terminal domain of unknown structure (~ 300 residues), which has been shown to interact with desmoplakin and many other components of the outer dense plaque. The central domain of desmoplakin is predicted to form a long coiled coil consisting of 889 residues and is shown here as three discontinuous lengths derived from the x-ray structure of tropomyosin. The C-terminal domain is characterized by three plakophilin repeat domains that bind intermediate filaments; the corresponding structures [43] have been packed arbitrarily within a sphere with a volume comparable to the entire 926-residue C-terminal

domain. The N-terminal domain, which has been shown to interact with plakophilin and other components of the outer dense plaque, is depicted simply as a sphere of unknown structure. Scale bar corresponds to 3 nm.

Figure 2.

Alignment of cadherin N-terminal EC1 domains. The top three sequences are from type I classical cadherins, followed by four type II classical cadherin sequences. Desmocollin and desmoglein are desmosomal cadherins. Calcium binding residues are shaded yellow. Residues shown by x-ray crystallography of type I and type II cadherins to be involved in the dimer interface are shaded in grey. Additional residues, outlined in black, are involved in the more extensive type II interface that is postulated to confer extra rigidity to the corresponding dimer [25 **]. In this respect, desmosomal cadherins appear to be more similar to type I cadherins, suggesting that they maintain the flexible geometry seen in the corresponding dimeric structures [12].

Figure 3.

Architecture of the intracellular plaque. (a) A tomographic slice through a desmosome from new-born mouse epidermis preserved by freeze-substitution and thin sectioning. (b) 3D rendering of components based on segmentation of the tomographic volume. Red planes correspond to the plasma membrane from opposing cells. The outer dense plaque (ODP) is shown in gold, extends ~ 18.5 nm from the membrane and has very dense staining. The inner dense plaque (IDP) is shown in green, extends ~ 37.5 nm from the ODP and consists of strand-like connections to the intermediate filaments (IF). Several ribosomes are shown in purple at the periphery of the junction. Although densities corresponding to cadherins are visible in the intercellular space, they have not been rendered in the 3D model. (c) Another tomographic section through new-born mouse epidermis. A desmosome appears to be forming (black arrow) with a low density of individual cadherin molecules visible in the intercellular gap and a light accumulation of material in the intracellular plaque. Another junction is visible with a smaller intercellular spacing (white arrow), which could be an adherens junction. In fact, adherens junctions are believed to have a role in directing desmosome assembly [52]. Individual densities are also visible protruding from the plasma membrane outside of junctions (black arrowhead) and could correspond to free cadherins. White arrowhead indicates ribosomes. Scale bars in (a) and (c) correspond to 5 nm.