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Structure and function of desmosomal transmembrane core and plaque molecules

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Abstract

Desmosomes are intercellular junctions that function in cell–cell adhesion and attachment of intermediate filaments (IF) to the cell surface. Desmogleins and desmocollins are the major components of the transmembrane adhesion complex, whereas desmoplakins (DPs) are the most prominent components of the cytoplasmic plaque. Based on sequence similarity, desmogleins and desmocollins are related to the calcium-dependent homophilic adhesion molecules known as cadherins. Like the classical cadherins, the desmosomal cadherins contain four homologous extracellular domains bearing putative calcium-binding sites, a single transmembrane spanning domain, and a C-terminal cytoplasmic tail. Molecules in the desmoglein subclass contain a unique C-terminal extension within which is found a repeating motif that is predicted to form two β-strands and two turns. Stable cell lines expressing desmoglein 1 have been generated from normally non-adherent L cell fibroblasts, to study the contribution of this cadherin to desmosomal adhesion. The predicted sequence of desmoplakin (DP) I suggests it will form homodimers comprising a central α-helical coiled-coil rod and two globular end domains. The C-terminus contains three regions with significant homology, each of which is made up of a 38-residue motif also found in two other molecules involved in organization of IF, bullous pemphigoid antigen and plectin. Ectopically expressed polypeptides including the C-terminus of DP I specifically align with keratin and vimentin IF in cultured cells, whereas those lacking this domain do not align with IF. The last 68 amino acids of DP are required for alignment along keratin but not vimentin IF, and residues 48–68 from the C-terminal end are critical for this interaction. These results suggest that the C-terminus of DP plays a role in the attachment of IF to the desmosome and that a specific site is necessary for interaction with keratin IF. A sequence at the most N-terminal end of DP appears to be required for efficient incorporation into the desmosomal plaque. Interestingly, this region has not been reported to be present in the homologous bullous pemphigoid antigen or plectin molecules and may represent a desmosomal targeting sequence.

Key words: Desmosomal transmembrane core; Intercellular junctions; Cell surface; Desmoplakin

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1. Introduction

Adhesive intercellular junctions play a critical role in the development and maintenance of epithelial tissue integrity. A prominent example of such a junction is the desmosome which not only functions in cell–cell adhesion but also serves as a specific attachment point for intermediate filaments (IF). In this way the IF cytoskeleton is linked to sites of adhesion between cells, creating a continuous network throughout a tissue that is thought to resist the forces of mechanical stress.

On the ultrastructural level, desmosomes appear as highly organized electron dense discs at sites of very close cell–cell contact (Fig. 1). Mirror image cytoplasmic plaques sandwich a membrane core region comprising transmembrane glycoprotein molecules which are thought to mediate cell adhesion. The plasma membranes of neighboring cells are separated by an ≈ 30 nm space filled with electron dense material that is often organized as a central dense midline with lateral projections extending to the membrane [1–4]. The tripartite plaque consists of an extremely electron dense region or outer plaque subjacent to the plasma membrane. A less dense layer separates this region from a fibrillar layer or inner plaque through which IF bundles appear to loop [5]. Finally, a set of fine "traversing filaments" appear to extend between the IF and the plasma membrane [6].

The highly ordered structure of the desmosome is presumably related to the requirement that desmosomes mediate strong adhesive interactions between cells. Even the harsh denaturing conditions that are used to isolate desmosomes fail to cause these adhesive structures to separate. However, it is likely that desmosomes in living cells are dynamic structures, as they appear to be modulated in response to growth factors and other environmental cues. For this reason, the regulation of desmosome assembly and disassembly is rapidly becoming an active area of investigation. In addition, the identification of cDNA clones encoding many of the desmosomal molecules now provides the opportunity to identify the function of individual desmosomal components. Here we summarize work in our laboratory in the context of the current literature, specifically addressing the mechanisms of desmosomal mediated cell–cell adhesion and attachment of the intermediate filament (IF) cytoskeleton to the cell membrane.

2. Results and discussion

2.1. The desmosomal cadherins are members of a diverse group of proteins comprising the cadherin gene family

The identification and analysis of cDNA clones encoding the transmembrane components of the desmosome has revealed that the desmosomal glycoproteins are members of the cadherin gene family of cell adhesion molecules [7]. Cadherins are transmembrane glycoproteins that mediate calcium-dependent cell–cell adhesion by binding homophilically to cadherins on adjacent cells [8–11]. Although a number of desmosomal glycoproteins have now been identified as cadherins, the mechanism of desmosomal cadherin-mediated adhesion remains to be defined. The desmosomal cadherins currently include two subclasses of molecules termed desmogleins and desmocollins (for a review on desmosomal cadherin nomenclature refer to ref. [12]). Three desmoglein isoforms have been identified to date: Dsg1 (pemphigus foliaceus antigen), Dsg2, and Dsg3 (pemphigus vulgarus antigen) [13–19]. Each of these desmogleins is the product of a separate gene. In addition, three desmocollin genes have been identified, Dsc1, Dsc2, Dsc3, although only the bovine homologue of Dsc2 has been cloned ([12,20–25]. Each desmocollin gene produces two alternatively spliced transcripts that encode molecules differing in the length of the cytoplasmic domain. The "b" form arises by the inclusion of a 46 base pair exon containing an in frame termination codon, resulting in a shortened version that includes 11 unique amino acids not found in the "a" form [23]. *In situ* hybridization data suggest that the desmosomal cadherins exhibit tissue and differentiation specific patterns of expression similar to those seen for the keratin

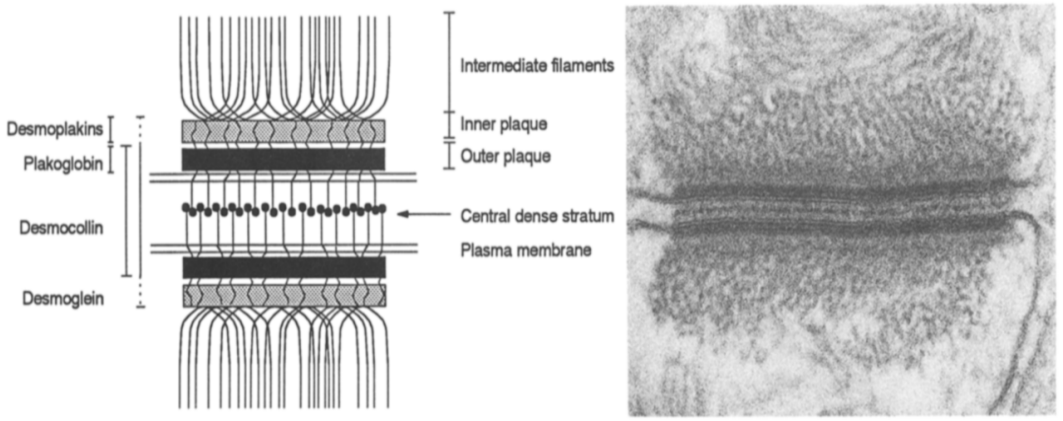


Fig. 1. Graphical depiction and ultrastructure of a desmosome from bovine tongue epithelium. The desmosome contains mirror image tripartite plaques consisting of a narrow electronlucent zone sandwiched between outer and inner plaques to which intermediate filaments are attached. The cytoplasmic plaques lie on either side of two opposed lipid bilayers, bisected by the central dense stratum of the intercellular space. The major molecular components found in each region are labeled at the left.

genes [24–27]. Although the functional significance of this observation is currently unknown, it is possible that the expression of different desmosomal cadherins may provide a mechanism for generating differences in adhesive potential.

Structurally, cadherins consist of an extracellu-

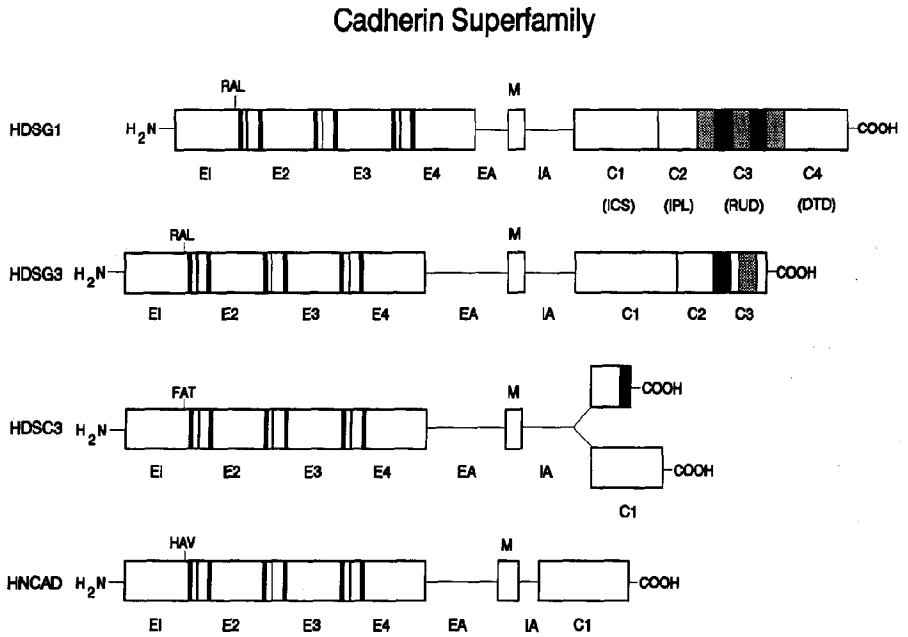


Fig. 2. Comparison between desmosomal cadherins and classical cadherins. The orientation of each molecule is shown with respect to the plasma membrane (M). The boundaries of the extracellular domains, E1 through E4 and EA (extracellular anchor), and of the cytoplasmic domains, IA (intracellular anchor) and C1 through C4 are shown for each molecule. Nomenclature proposed by Koch, et al are shown below Dsg1 in parentheses: ICS, intracellular cadherin-typical sequence; IPL, intracellular proline-rich linker; RUD, repeating unit domain; DTD, desmoglein-specific terminal domain. Putative calcium-binding domains in the amino terminus are shown as shaded rectangles. The two Dsc3 forms resulting from alternative splicing are shown; the black rectangle represents 11 unique amino acids at the COOH terminus of the shorter "a" form.

lar domain, a single pass transmembrane domain, and a cytoplasmic domain (Fig. 2). The extracellular domain of the originally described, or classical cadherins (E-cadherin (uvomorulin), P-cadherin, and N-cadherin), consists of four repeated homology units that are ≈110 amino acids in length and contain highly conserved calcium binding motifs [8,28]. Comparisons between human Dsg1 and N-cadherin [17] reveal that there is 31% sequence identity in the most N-terminal extracellular repeat (E1). The second repeat, E2, has 39% identity while E3 and E4 exhibit greater divergence in sequence identity with 27% and 24% respectively. The calcium binding domains appear to be crucial for the adhesive function of the classical cadherins, as a point mutation in one of these sites results in the loss of adhesive function of uvomorulin [29]. In addition to the calcium binding motifs, the amino acid sequence HAV in the first extracellular domain of uvomorulin has been identified as an important adhesive recognition site [30]. Binding specificity among the cadherins requires additional sequences outside the HAV motif [31]. HAV is replaced with RAL in the desmogleins (Dsg1 and Dsg3), with FAT in Dsc3, and YAT in Dsc1 (using nomenclature in ref. [12]) [7]. The functional significance of differences in this region are unknown.

The desmosomal cadherins contain sequences in the cytoplasmic C I domain that bear similarity to classical cadherins, and as discussed below, may comprise binding sites for related associated proteins. A striking difference between the two desmosomal cadherin subclasses is the presence of an extended cytoplasmic tail in the desmogleins [14,17]. This tail can be divided into several regions, the first of which is a proline rich stretch of 59 amino acids. This sequence is followed by a novel series of repeats each of which consists of 29 ± 1 residues predicted to form two β-turns and two β-strands. All desmogleins contain this distinguishing repeating motif, although the number of repeats varies among members of this subclass. The repeats are followed by a glycine rich stretch of amino acids that is apolar with the exception of a basic tail leading to the C-terminus of the molecule. The function(s) of the desmoglein-specific sequences in the cytoplasmic portion of the molecule are unknown.

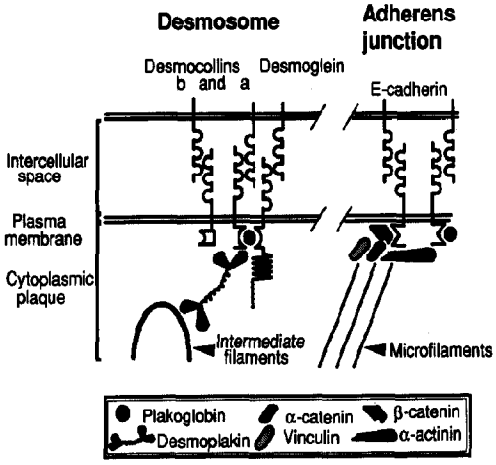


Fig. 3. Hypothetical model predicting the organization of desmosomes and adherens junctions. The adherens junction plaque contains catenins and other components (e.g. vinculin and α-actinin) that facilitate its interaction with microfilaments. In addition, this junction has also been reported to contain plakoglobin, which some investigators have suggested is identical to γ-catenin. Plaque components of desmosomes such as desmoplakin are thought to facilitate interaction with intermediate filaments. Please note that neither model includes every component that has been identified for a particular junction (e.g. plectin/300 kDa IFAP and desmocalmin in desmosomes). Note that the extracellular domains of both neighboring cells are shown here, but only a single cytoplasmic plaque (lower cell) is shown.

In differentiated tissues, the classical cadherins are often found in adherens-type junctions that associate with the actin cytoskeleton, whereas the desmosomal cadherins are located in junctions that interact with the IF network (Fig. 3). The C I domain contains a region that is highly conserved among the classical cadherins and serves as a binding site for three molecules called α-, β- and γ-catenins [32]. Of these, β-catenin seems to be the most tightly associated with the cadherin tail. β-catenin is thought to be the human homologue of the Drosophila *armadillo* gene product, an important link in the *wingless* signal transduction pathway that is required for the proper establishment of segment polarity [33]. Intriguingly, the obligate desmosomal molecule plakoglobin also bears a high degree of sequence

similarity to β-catenin/*armadillo*, and like β-catenin, plakoglobin co-immunoprecipitates with cadherin molecules [34]. Although plakoglobin associates more tightly with the desmosomal cadherins Dsg1 and Dsg3, it apparently also associates with E- and N-cadherin [35,36], consistent with its reported localization in adherens junctions (Fig. 3). Recent immunoprecipitation evidence suggests that E-cadherin forms complexes with either β-catenin or plakoglobin, but not both simultaneously [37]. β-catenin, on the other hand, has not been reported to interact with the desmosomal cadherins. Thus, it is likely that the most highly conserved region of the C I domain of cadherins serves as a binding site for plakoglobin and, in the case of classical cadherins, β-catenin.

In spite of the similarities between adherens junctions and desmosomes, the ability of the desmosomal cadherins to interact with cytoplasmic molecules that are unique to the desmosomal plaque is predicted to be an important mechanism to specifically recruit and attach IF to sites of desmosomal adhesion. As plakoglobin also associates with adherens junctions, it is unlikely that this molecule alone provides the specificity for IF attachment. Other molecules, such as desmoplakin, may perform such a function (see below).

2.2. *Mechanism of cadherin mediated cell adhesion*

The discovery that desmosomal glycoproteins are members of the cadherin superfamily suggests that these proteins are also cell–cell adhesion molecules. Indirect evidence supporting a role for the desmosomal cadherins in adhesion comes from studies in which antibodies recognizing desmocollin inhibit desmosome formation in cul-

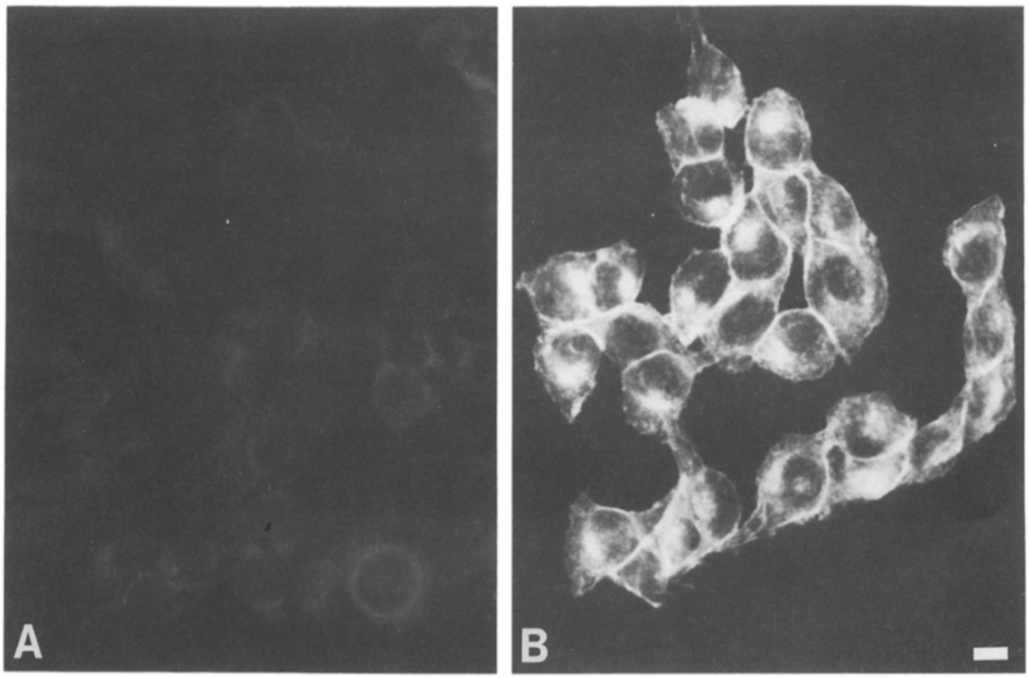


Fig. 4. Stable expression of Dsg1 in L-cells. L-cells were co-transfected with cDNA constructs encoding human Dsg1 and the pSV2neo antibiotic resistance marker. Clones were then isolated and analyzed by immunofluorescence microscopy for the expression of Dsg1 using human autoantibodies from pemphigus foliaceus patients (a generous gift from Dr. J. Stanley). Clones not expressing Dsg1 (A) and clones expressing Dsg1 were isolated (B). Note the concentration of Dsg1 staining at cell–cell borders (B). Bar = 10 μm.

tured cells [38]. Likewise, antibodies directed against Dsg1 and Dsg3 are present in the plasma of patients suffering from the autoimmune diseases pemphigus foliaceus and pemphigus vulgaris, respectively. These circulating antibodies are thought to disrupt cell–cell adhesion and thereby cause the skin blistering that characterizes these diseases (for a review, see ref. [39]).

In order to demonstrate directly that the desmosomal cadherin Dsg1 is a cell adhesion molecule, we have adopted the approach used to demonstrate the adhesive function of the classical cadherins [40]. In these studies, L-cell fibroblasts are transfected with cDNA constructs encoding putative adhesion molecules. Parental L-cells do not express cadherins and do not aggregate in suspension when released from the substrate. However, L-cells that express ectopic E-cadherin exhibit extensive aggregation in suspension which can be inhibited by incubation with antibodies directed against the extracellular domain of E-cadherin. In order to test the adhesive function of Dsg1, we have generated L-cell lines that stably express this molecule. These cell lines appear to properly synthesize and deliver Dsg1 to the plasma membrane where it localizes to cell-cell borders as assessed by immunofluorescence analysis (Fig. 4). However, preliminary studies using these L-cells suggest that Dsg1 alone is unable to mediate cell–cell aggregation in suspension using the approach discussed above (Kowalczyk and Green, unpublished observations).

One explanation for this result is that additional molecules may be required for adhesion mediated by desmoglein. In fact, the ability of the classical cadherins to mediate cell–cell adhesion is dependent on interactions between the cadherins and the cytoskeleton via the catenins, which are endogenous components of L-cells [32,41]. In contrast, the desmosomal molecules plakoglobin and desmoplakin are not expressed at detectable levels in the parental L-cells used in these experiments (data not shown). If desmosomal cadherins require interactions with the IF cytoskeleton in order to function in adhesion, then the expression of desmosomal cadherins alone in L-cells may not lead to cell–cell aggregation.

As discussed above, the desmosomal cadherins, Dsg1 and Dsg3, have been shown to interact with plakoglobin, a β-catenin-like protein. To determine if plakoglobin enhances the adhesive function of Dsg1, L-cell lines were generated that express both of these desmosomal components. Preliminary experiments indicate that L-cells expressing both Dsg1 and plakoglobin do not exhibit extensive aggregation compared to control cells expressing either a gene encoding an antibiotic resistance marker or Dsg1 alone. However, the ectopically expressed plakoglobin and desmoglein appear to interact, as these two proteins can be co-immunoprecipitated using antibodies directed against either Dsg1 or plakoglobin. This demonstrates that Dsg1 and plakoglobin expressed ectopically in L-cells form a complex in a manner similar to that in epithelial cells.

Interestingly, L-cell lines transfected with a cDNA construct encoding plakoglobin but not desmoglein express little or no plakoglobin protein. However, the mRNA for the exogenously expressed plakoglobin is present. Upon transient transfection of a plasmid encoding Dsg1 into cells expressing plakoglobin mRNA, plakoglobin protein levels increase dramatically. This postranscriptional regulation of plakoglobin by Dsg1 is similar to that observed with α-catenin and E-cadherin [42]. The mechanism leading to increased plakoglobin expression is currently unknown. One potential explanation for this observation is that plakoglobin is rapidly degraded unless it is bound to the cytoplasmic tail of Dsg1. The stabilization of plakoglobin by Dsg1 may represent an important event in the sequence of protein–protein interactions that occur during desmosome assembly.

The lack of significant aggregation in L-cells expressing both Dsg1 and plakoglobin may be due to the inability of plakoglobin to completely link Dsg1 to the cytoskeleton. Other desmosomal molecules such as desmoplakin may be required perform this function. To circumvent the need to transfect L-cells with numerous desmosomal molecules that would facilitate coupling of desmoglein with the cytoskeleton, we have undertaken an alternative approach that involves the creation of a chimeric cDNA molecule encoding

the cytoplasmic domain of E-cadherin and the extracellular domain of Dsg1. The chimera should facilitate interactions with the actin cytoskeleton via the E-cadherin cytoplasmic domain and the catenins endogenously present in L-cells, thus allowing examination of the function of the desmoglein extracellular domain. Although this approach did not result in a striking increase in the number of cell aggregates in adhesion assays, a small amount of weak adhesion could not be ruled out.

In similar studies from John Stanley's laboratory using a chimeric molecule consisting of the Dsg3 extracellular domain and the E-cadherin cytoplasmic domain, it was reported that the Dsg3 extracellular domain mediates weak adhesion when expressed in L-cells [43]. However, this adhesion is not comparable to the adhesion exhibited by L-cells expressing E-cadherin. One interpretation of these results, is that adhesion mediated by desmosomal cadherins is inherently weaker than that mediated by the classical cadherins. This seems unlikely, however, since desmosomes appear to be strong sites of attachment that are unusually resistant to chemical and mechanical stress.

Taken together, the results of these studies suggest that a single desmosomal cadherin is unable to mediate cell-cell adhesion comparable to that exhibited by E-cadherin. Several possible explanations may account for these observations. First, unlike classical cadherins, desmosomal cadherins may bind heterophilically, rather than homophilically. Heterophilic interactions between desmosomal cadherins might facilitate the localization of both types of cadherins to the desmosome and provide a mechanism to regulate the stoichiometry of these molecules. Second, the desmosomal cadherins may form heterodimers that function as cell adhesion molecules only if both molecules are present within the same cell. Third, adhesion through E-cadherin may be a prerequisite for desmosome assembly and desmosomal cadherin-mediated adhesion. Consistent with this latter possibility, are reports that antibodies directed against the extracellular domain of E-cadherin inhibit or delay the appearance of intercellular junctions, including desmosomes [44,45]. E-cadherin blocking antibodies also inhibit the aggregation of a keratinocyte cell line incubated in suspension [46]. Since these cells are fully capable of assembling desmosomes, this suggests that desmosomal adhesion can occur only after E-cadherin mediated adhesion has occurred.

One possible explanation for these results is that E-cadherin generates intracellular signals that are required for desmosomal adhesion and assembly. If this is the case, it is likely that desmosomal plaque components are targets of this signaling pathway. Thus, it will be important to elucidate the mechanism by which desmosomal cadherins interact with the plaque components of the desmosome and to determine how this assembly process is regulated. To this end, Franke and co-workers have generated chimeric molecules containing the cytoplasmic domain of the desmosomal cadherins coupled to the gap junction protein connexin 32 [47]. The connexin portion of the chimera functions as a membrane anchoring site for the cytoplasmic domain of the desmosomal cadherins. Expression of such a chimeric molecule containing the desmocollin cytoplasmic tail results in the recruitment of desmoplakin and IF to the chimeric gap junction/desmosome structure. However, a similar chimera containing the desmoglein cytoplasmic tail functions as a dominant negative mutant, leading to the disruption of desmosomes and accompanying attachment of IF to the cell membrane. One possible explanation for such a dominant negative effect could be that the availability of a limiting binding partner such as plakoglobin or DP might be reduced by association with ectopically expressed protein. However, control constructs in which the Dsg1 tail was fused with synaptophysin resulted in localization of protein in small cytoplasmic vesicles rather than at the plasma membrane. These vesicles were also able to bind plakoglobin, but formation of endogenous desmosomes was not inhibited in this case. The underlying basis for these observations is unknown; however, it is clear that desmosomal plaque assembly is a carefully regulated process that is sensitive to the stoichiometry and/or positioning of the cytoplasmic tails of the desmosomal cadherins.

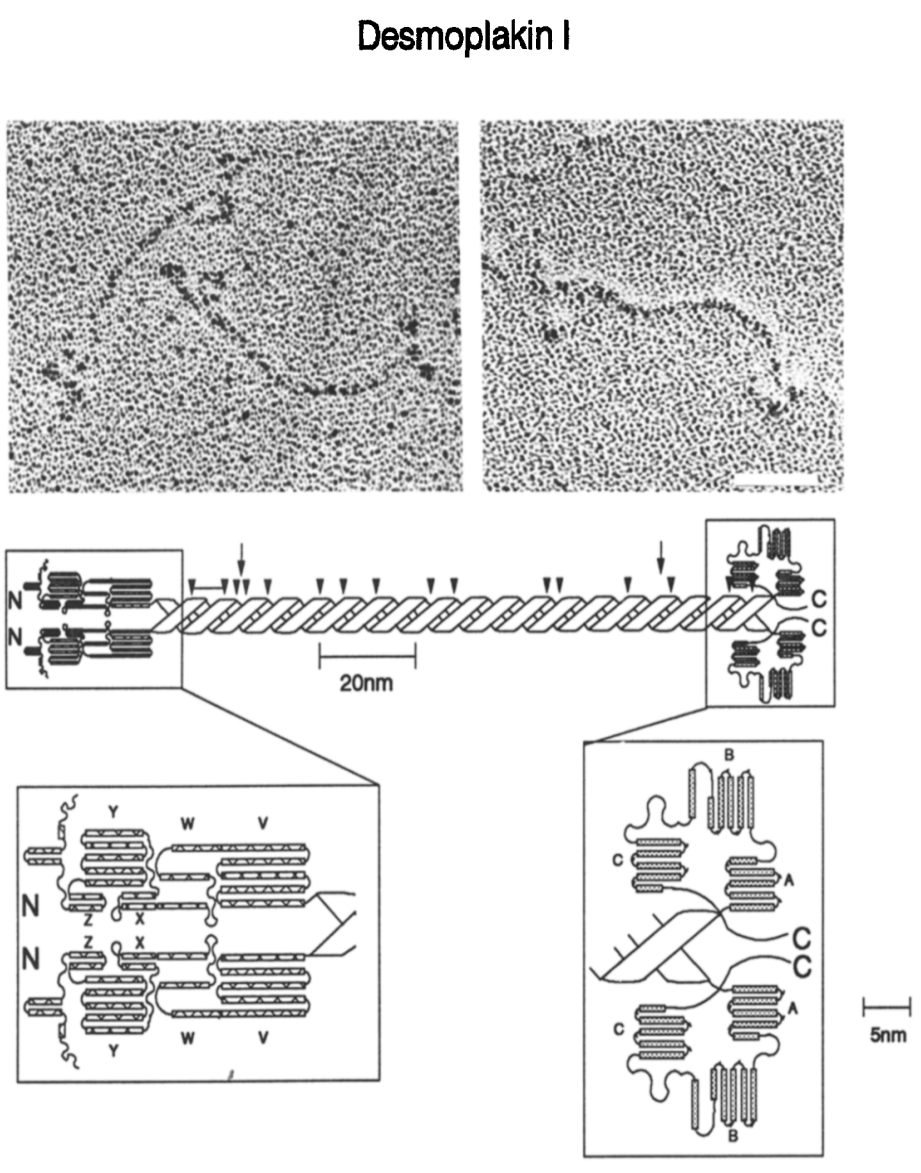


Fig. 5. Structure of a desmoplakin homodimer. The predicted model of DP structure is shown in comparison with rotary shadowed images of purified DPI. Note that the rod domain consists of a coiled-coil α-helix ≈ 130 nm in length. Possible break points and stutters predicted by the amino acid sequence are designated by small arrows above the rod domain, and an extended region of predicted flexibility is designated by a horizontal bar. Large arrows define the boundaries of the DPI specific region. Rectangles in the carboxyl terminus contain the 38 residue repeat motif and represent predicted α-helical regions that turn upon themselves and are stabilized by ionic interactions. Rectangles in the amino terminus have a heptad substructure and also represent predicted α-helical regions that turn upon themselves. Rotary shadowed images of DPI were prepared as described in ref. [55]. Note the existence of an extended, flexible central rod flanked by two more globular ends. Bar = 50 nm. (Rotary shadowed EM images kindly provided by Dr. Edward O'Keefe.)

2.3. The desmosomal plaque

In order to gain insights into the assembly and function of desmosomal plaque components we undertook an investigation of the structure and function of desmoplakins (DPs) I and II, the most abundant proteins in the plaque domain. The DPs are localized at a potentially critical interface between the IF cytoskeleton and the cytoplasmic domains of the core glycoproteins. Indeed, our studies described below suggest that DP is a bifunctional molecule with separate functions residing in the N- and C-terminal globular domains. It is likely that DPs thus play roles both in organization and/or regulation of the desmosomal core, as well as the IF cytoskeleton.

Analysis of cDNA and genomic clones indicates that DPI and II are likely to be encoded by a single gene that gives rise to predicted polypeptides of 332 and 260 kDa, respectively [48–52]. The smaller DPII molecule appears to be generated from an alternatively spliced message via an internal donor, and demonstrates a more restricted tissue distribution than the apparently obligate DPI molecule [53].

Based on sequence analysis, DP is predicted to form a homodimer consisting of a central heptad repeat-containing α-helical coiled-coil rod domain, flanked by two globular end domains [48–50,54] (Fig. 5). The predicted DPI rod length of ≈ 130 nm correlates well with measurements obtained from EM images of rotary shadowed molecules [55]. DNA sequence analysis reveals that DPII is lacking approximately two thirds of the central domain, thus containing a shorter rod of ≈ 43 nm. Based on the pattern of acidic and basic residues along the central coiled-coil helix, it is likely that DPI will aggregate with itself or similar molecules into higher order filamentous structures. Such a prediction is consistent with the possibility that DP may at least in part make up the thin 4–5 nm "traversing filaments" that appear to link IF with the plaque or the plaque with the plasma membrane [56].

The C-terminus of DP can be divided into three subdomains (A, B and C) each containing 176 residues that can be divided further into several repeats of a 38 residue motif [48,54]. It is predicted that these regions each fold into a compact globular conformation stabilized by intrachain ionic interactions (Fig. 5). Significantly, the periodicity in acidic and basic residues of the 38 residue motif is the same as that found in the 1B rod domain of IF proteins, suggesting the

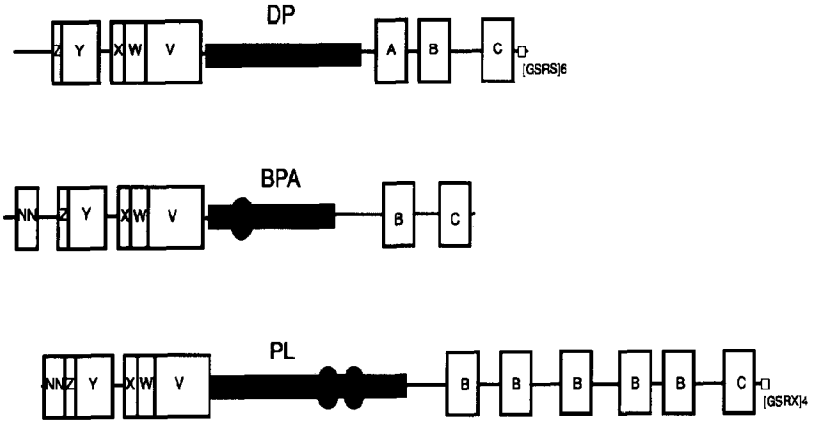


Fig. 6. Comparison of domain structure of desmoplakin (DP), the 230 kDa bullous pemphigoid antigen (BPA) and plectin (PL). Each molecule is predicted to contain a central rod domain flanked by two globular end domains. Homologous regions in the N-terminus are predicted to form several α-helical bundles labeled V, W, X, Y, Z and NN. The C-terminal domains are composed of subdomains each containing 176 residues that can be subdivided further into 38 residue repeating motifs. DP contains three of these domains (A, B, and C), whereas BPA contains two and PL contains six. These C-terminal repeats may play a role in interaction with IF networks.

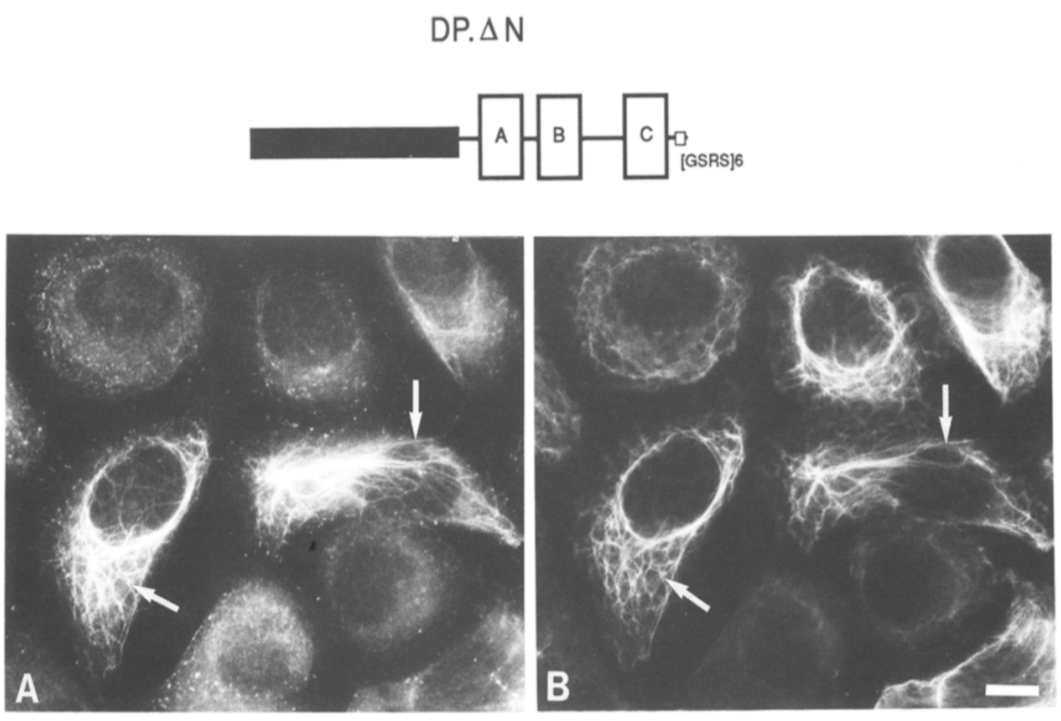
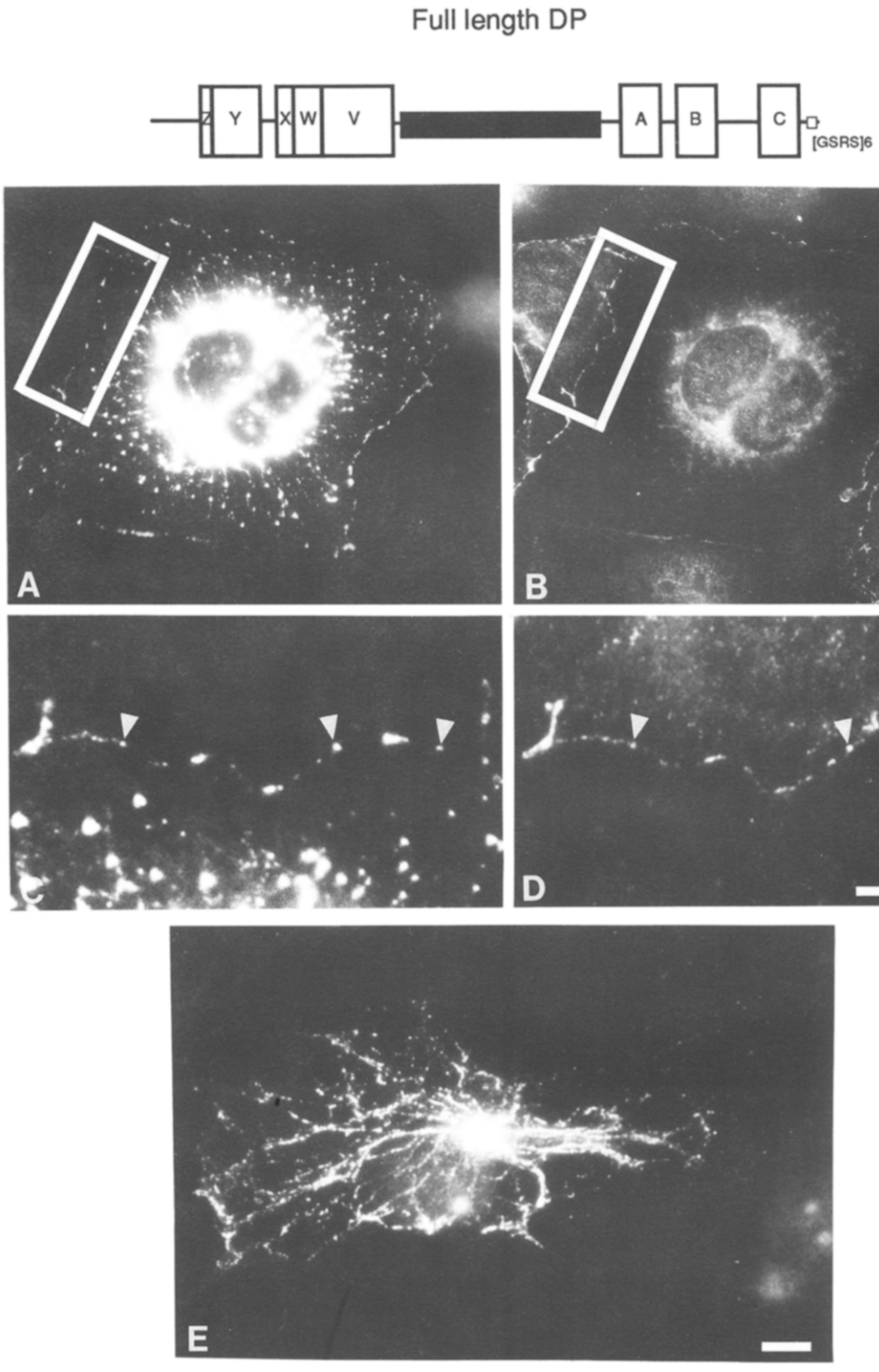


Fig. 7. Alignment of DP.ΔN with keratin IF in transfected HeLa cells. Cells were transfected with a construct encoding the rod plus C-terminus of DP. 24 h after glycerol shock, cells were fixed and reacted with a rabbit polyclonal to (A) DP (NW 38) and (B) a mouse monoclonal to keratin 18 (KSB17.2). Note coalignment of the DP polypeptide with keratin IF in transfected cells (arrows). Bar = 10 μm.

possibility that DP might interact with IF or IF-like proteins via their common periodicity of charged residues. The N-terminus is characterized by heptad repeat substructure over much of its length [50]. In contrast to the extensive series of heptads in the rod, the stretches of heptad are shorter and are likely to form a series of compact bundles rather than a coiled rope (Fig. 5).

Many of the structural characteristics displayed by DP can also be found in two related molecules, the hemidesmosomal 230 kDa bullous pemphigoid (BP230) antigen [15,57,58] and the intermediate filament associated protein (IFAP) plectin [59]. Each of these molecules has been implicated as playing a role in interacting with IF polypeptides or networks [60,61]. The three domain structural plan of all three members of this newly emerging gene family is similar to that described for DP, a central rod flanked by two globular ends (Fig. 6). Comparison of both the DP N- and C-termini among these molecules reveals extensive similarity that suggest an impor-

Fig. 8. COS-7 cells transfected with full length DPI. (A)–(D): 24 h after glycerol shock cells were fixed and reacted with the mouse monoclonal, 9E10.2, directed against the *c-myc* tag (A) and (C), and a rabbit polyclonal antibody directed against bovine desmoglein (Dsg) (B) and (D). The rectangle in (A) and (B) corresponds to (B) and (D), respectively (each rectangle is rotated 70° clockwise). Arrowheads in (C) and (D) indicate examples of 9E10.2 and Dsg spots that colocalize at cell–cell borders, suggesting that tagged DP can associate with desmosomes. (As modified from Stappenbeck et al., J. Cell Biol. 123, (1993) 691–705). (E): Indirect immunofluorescence of COS-7 cells transiently transfected with tagged full length DP, extracted with 0.5% triton for 15 min, fixed and reacted with 9E10.2. Note in this case the punctate, linear staining pattern, reflecting alignment with endogenous IF. Bars = 10 μm.


Full length DP
Y
X
W
V
A
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[GSRS]6
A
B
D
E

tant evolutionarily conserved function in protein–protein interactions possibly involving IF networks [54].

2.4. Experimental approaches for determining the function of DP domains

The position of DP in the desmosome suggests that this molecule might be an adapter that links the IF cytoskeleton to the transmembrane glycoprotein core. However, *in vitro* biochemical experiments have so far failed to identify direct interactions between DPs and any core glycoprotein or IF polypeptides [55,62]. In some cases, it is possible that the denaturing conditions used to extract DP from desmosomes during purification might affect its *in vitro* binding capabilities. It is also possible that DP may interact with IF only in the presence of accessory or linking proteins that stabilize or mediate the association. To avoid difficulties inherent in *in vitro* binding experiments, a molecular genetic approach was used to test the potential for interactions between various domains of DP and either IF or the desmosomal plaque in cultured cells [51,63].

Constructs encoding full length or individual domains of DPI were transiently expressed in cultured COS-7 or HeLa epithelial cells, and their association with the cytoskeleton and/or cell surface was investigated by immunolocaliza-

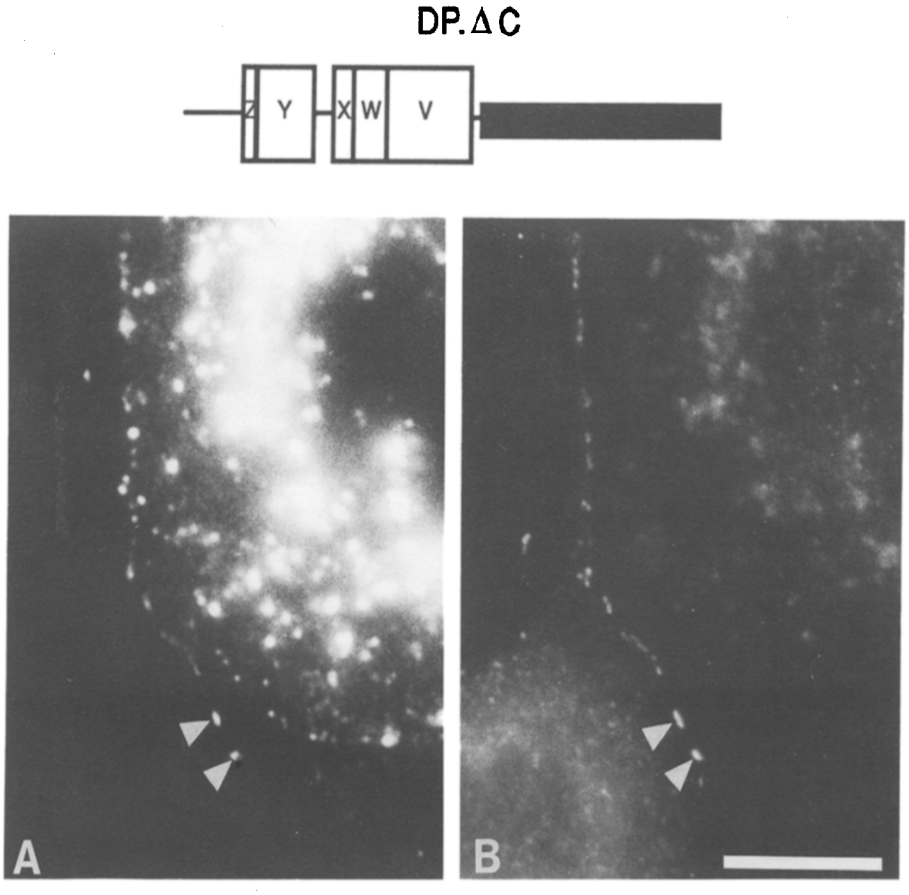

Fig. 9. Indirect double label immunofluorescence of COS-7 cells transfected with pDP.ΔC. 24 h after glycerol shock cells were reacted with (A) the mouse monoclonal, 9E10.2, directed against the c-*myc* tag and (B) a rabbit polyclonal antibody directed against bovine desmoglein (Dsg). Shown here is a high magnification of cell borders. Arrows indicate 9E10.2 and Dsg spots along cell–cell borders that colocalize, suggesting that DP.ΔC can associate with desmosomes. Bars = 10 μm.

tion and biochemical approaches. In order to distinguish between endogenous DP and ectopically expressed polypeptides, a peptide tag encoding a 10 amino acid epitope of *c-myc* [64] was introduced at the C-terminus of some constructs.

Immunolocalization studies revealed that ectopically expressed polypeptides containing the C-terminus but lacking the N-terminus are capable of aligning along IF networks (Fig. 7), consistent with the possibility that this domain is involved in attaching IF networks to the desmosome plaque. At the highest levels of expression, both vimentin and keratin IF networks are disrupted, although other cytoskeletal networks such as microfilaments and microtubules are unaffected.

In order to further define sequences in the C-terminus required for alignment with IF networks, constructs encoding various deletions of the C-terminus were introduced into several cell lines to test the ability of the truncated proteins to coalign with and disrupt vimentin (3T3 fibroblasts), keratin (HaCaT keratinocytes), or both types of IF in the same cell (HeLa cells). These studies revealed that the interaction with keratin, but not vimentin, IF networks is dependent on the presence of the 68 residues at the extreme C-terminus of the DP molecule, and that residues 48–68 from the C-terminus are critical for the interaction. Constructs missing these residues are unable to align with keratin as assessed by immunofluorescence analysis, and this lack of alignment correlates with a dramatic increase in triton solubility of the DP polypeptide [51].

The interaction with vimentin, on the other hand, requires the presence of at least two of the C-terminal repeat subdomains. Furthermore, extraction with non-ionic detergents indicates that DP associating with vimentin IF is significantly more extractable than DP associating with keratin IF, suggesting that the interaction of DP with keratin IF may be of a higher affinity. Although the rod domain alone does not associate with IF, addition of the rod to the C-terminus (DP.ΔN) is required for efficient association with vimentin IF, perhaps due to DP dimerization which would in effect increase the number of available C-terminal domains. In conjunction with IF polypeptides, overexpression of DP.ΔN results in the formation of higher order fine filamentous structures in transfected cells that resemble the 4–5 nm "traversing filaments" previously reported to be present in the desmosomal plaque [63].

The potential for differential interactions between DP and different IF types is consistent with reports that keratins preferentially interact with desmosomes in cells expressing both filament systems [65]. It is possible that the last 68 residues of DP may not only be necessary for specifying an interaction with DP, but may promote interaction with keratin at the expense of interactions with vimentin. The differential extractability of DP.ΔN in cells expressing keratin versus cells expressing vimentin only may also be a reflection of this fact. The physiological significance of possible differences in affinity between DP and different filament networks remains unclear, however.

Polypeptides containing the DP N-terminus localize to the desmosomal plaque, suggesting that sequences required for incorporation into desmosomes are present in this domain (Figs. 8, 9). Interestingly, ectopically expressed full length DP1 appears in some cells to associate with desmosomes (Figs. 8A–8D) and in others to interact with IF (Fig. 8E). Factors that allow the function of either the C- or the N-terminus to be manifested preferentially in individual cells are not yet understood. However, it is suspected that the incorporation-competent state may be dependent on the stage of cell cycle (e.g. transfected dividing daughter cells often appear to incorporate tagged DP at newly forming desmosomes at cell–cell interfaces) or other physiological states.

Although a membrane receptor for DP has not been identified, the cytoplasmic domains of one or more of the transmembrane desmosomal cadherins are excellent candidates. Sequences required for interaction with this putative "receptor" complex appear to reside in the N-terminal most 194 amino acids of DP, as molecules lacking this region are unable to associate with the desmosomal plaque [63]. It is interesting to note that this region may be unique to DP, as similar sequences have so far not been identified in

BP230 or plectin. It has not yet been determined whether this region is sufficient for targeting to the desmosomal plaque, only that it is necessary.

Deletion of the C-terminus from the full length DPI clone results in a protein still capable of associating with desmosomes but not of interacting with IF networks (Fig. 9). The inability of DP.ΔC to coalign with and disrupt IF provides additional support for the idea that the C-terminus of DP contains sites necessary and sufficient for interaction with IF. Because DP.ΔC can apparently incorporate into desmosomes, interaction with IF is probably not required for localization to desmosomes. While this result does not rule out the possibility that IF provide a route for DP to localize to desmosomes [66–68], it suggests that other mechanisms can be utilized. Our results are consistent with previous suggestions that IF are not required for delivery of DP to desmosomes [69–71]. The existence of an IF independent pathway for delivery of DP to the cell surface could explain the apparently normal assembly of the desmosomal plaque in IF deficient simple epithelia [72] or in epithelia with disrupted IF [73].

Thus, DP is a bifunctional molecule in which the N-terminus is apparently required for incorporation into the desmosomal plaque, while the C-terminus of DP is required for association with IF networks. With regard to the interactions of DP with IF networks, the possible involvement of additional accessory proteins has not been ruled out. And in fact, other molecules localized to desmosomes have been demonstrated to bind IF in vitro. Two of these proteins, band 6 (DP IV) and desmocalmin, have a restricted tissue distribution [74–76]. A third candidate, the 140 kDa lamin B related protein [77], is found in very small amounts and is restricted to desmosomes. In light of the fact that the DP C -terminus aligns along vimentin filaments in fibroblasts, cells which presumably are not expressing other desmosomal components that could act as accessory or linking molecules, it is unlikely that these candidate linkers are absolutely required for a DP-IF association. On the other hand, a protein with a widespread tissue distribution such as plectin [78] or 300 kDa IFAP [79] which are present in fibroblasts but have also been reported in some desmosomes cannot be ruled out in the interaction.

3. Prospects for the future

We now have a more clear understanding of desmosome composition, and know something about the structure and function of individual molecules such as desmoplakin. However, very little is known regarding the regulation of desmosome assembly, adhesion and association with the IF cytoskeleton. Desmosomes are modulated during processes such as wound healing and metastasis, during which protein markers disappear from the cell surface, and IF retract into a cap around the nucleus. Preliminary work indicates that phosphorylation of certain desmosomal components such as plakoglobin and desmoplakin may play a role in modulating protein–protein interactions and thus the assembly state and function of desmosomes. Future work will focus on defining how these molecules are regulated during assembly and disassembly, and in addition, will begin to address the function of desmosomes in higher levels of tissue organization by utilizing transgenic mouse models.

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