Desmosomes: New Perspectives on a Classic

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Desmosomes are highly specialized anchoring junctions that link intermediate filaments to sites of intercellular adhesion, thus facilitating the formation of a supracellular scaffolding that distributes mechanical forces throughout a tissue. These junctions are thus particularly important for maintaining the integrity of tissues that endure physical stress, such as the epidermis and myocardium. The importance of the classic mechanical functions of desmosomal constituents is underscored by pathologies reported in animal models and an ever-expanding list of human mutations that target both desmosomal cadherins and their associated cytoskeletal anchoring proteins. However, the notion that desmosomes are static structures that exist simply to glue cells together belies their susceptibility to remodeling in response to environmental cues and their important tissue-specific roles in cell behavior and signaling. Here, we review the molecular blueprint of the desmosome and models for assembling its protein components to form an adhesive interface and the desmosomal plaque. We also discuss emerging evidence of supra-adhesive roles for desmosomal proteins in regulating tissue morphogenesis and homeostasis. Finally, we highlight the dynamic nature of these adhesive organelles, examining mechanisms in health and disease for modulating adhesive strength and stability of desmosomes.

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Introduction

Desmosomes, named after the Greek ''desmo'', meaning bond or ligament, are intercellular adhesive organelles that have been characterized as cellular spot welds by classical histologists. As the most prominent cell surface specializations in epidermal keratinocytes, desmosomes have fascinated cutaneous morphologists for almost 150 years (Getsios *et al.*, 2004b; Calkins and Setzer, 2007). These highly organized, disc-shaped junctions are formed by mirror image, tri-partite electron-dense plaques, each closely opposing the plasma membranes contributed by neighboring cells. The junctions serve as tethers for cytoplasmic intermediate filaments (IFs), pliant 10 nm cytoskeletal fibers designed to withstand a high degree of mechanical strain without breaking (Coulombe and Omary, 2002). Together with their attached IF network, desmosomes form

Editor's Note

Ever since the discovery that skin is composed of multiple separate cells that are able to hold themselves together, scientists have been interested in keratinocyte junctions and adhesion. With the development of microscopes, the basic structure of the desmosome was seen initially with light microscopes and then with the electron microscope. Over the last 3 decades, however, there has been an explosion of knowledge regarding the molecular mechanisms that hold keratinocytes together (See Milestones in Cutaneous Biology Desmosomes, http://www.nature.com/milestones/skinbio1/full/intro.html). The next two issues of JID Perspectives will explore keratinocyte adhesion and cell junctions in-depth. In this issue Dr. Kathy Green and co-authors detail the basic structure and function of desmosomes; Dr. Gabriele Richard and colleagues describe Gap junctions; and Dr. Carien Niessen and co-authors discuss Tight Junctions and Adherens Junctions. Next month the series will continue with Dr. John McGrath describing the genetic diseases of cell junctions.

These Perspectives will update the cutaneous biologist on our current knowledge of these critical epithelial structures that allow keratinocytes to stand together. As Aesop stated in his fable The Four Oxen and the Lion, ''United we stand, divided we fall''.

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Abbreviations: DP, desmoplakin; DP-GFP, DP fused with green fluorescent protein; Dsc, desmocollin; Dsg, desmoglein; IF, intermediate filament; PG, plakoglobin; PKC, protein kinase C; PF, pemphigus foliaceus; PKP, plakophilin; PV, pemphigus vulgaris

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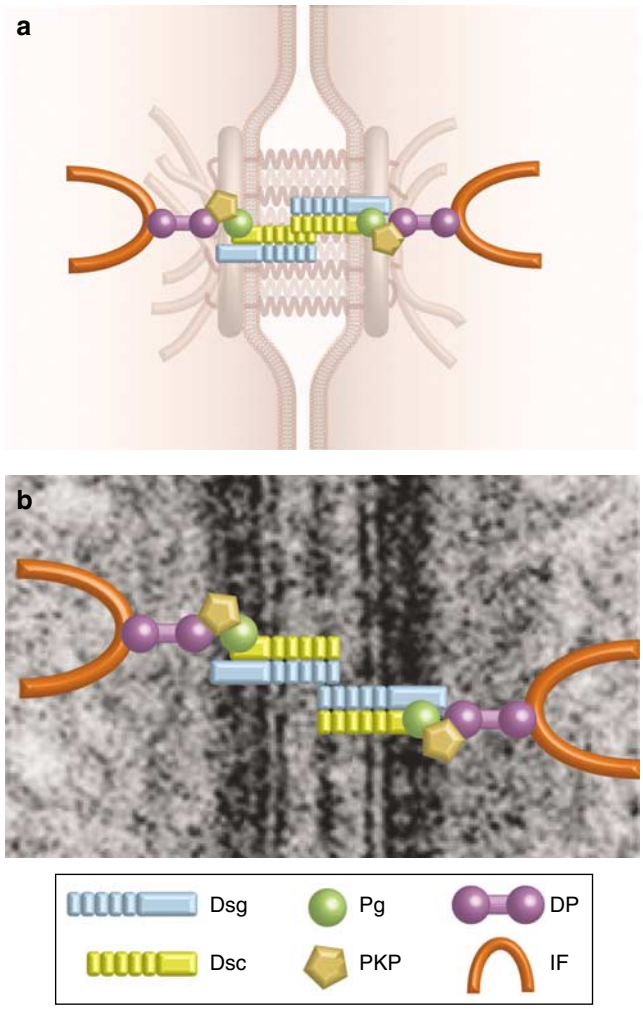

Figure 1. Molecular blueprint of the desmosome. (**a**) Diagram of a 'spot-weld' desmosome inspired by (Staehelin and Hull, 1978) and (**b**) an electron micrograph onto which are super-imposed the major desmosomal protein constituents from three families. Transmembrane desmosomal cadherins, Dsg and Dsc, bind the armadillo family protein PG, which in turn anchors the plakin family member DP and PKP. The cytoplasmic plaque, which is further stabilized by lateral interactions among these proteins, anchors the IF cytoskeleton to the desmosome.

a supracellular scaffolding that functions to distribute the forces of physical stress throughout the tissues. Their important contribution to cell and tissue architecture is revealed in patients with diseases of the skin and heart whose desmosomes are dysfunctional owing to inherited mutations or attack by autoimmune antibodies or bacterial toxins (for recent reviews, see Cheng and Koch, 2004; Getsios *et al.*, 2004b).

Although a majority of studies to date have focused on identifying the structure and function of the molecular building blocks that are the basis of the desmosomes' physical properties, recent evidence has emerged to suggest that these junctions are more structurally and functionally complex than once thought. In this review, we describe how recent findings have reshaped our perspective of the classic textbook model, allowing us to gain a new appreciation of the structural and functional variability that exists among desmosomes. We challenge the concept that this intercellular organelle is simply a mechanical weld that tethers IF to the membrane, providing evidence that it is a more central participant in the processes of differentiation and tissue morphogenesis. Finally, we discuss recent advances in our understanding of the mechanisms regulating junction homeostasis and remodeling, and how these mechanisms are co-opted during pathogenic processes, leading to human heart and skin disease.

Molecular blueprint of desmosomes and variations on a theme

Desmosome proteins come from three major gene families: cadherins, armadillo proteins, and plakins (Figure 1). Transmembrane members of the cadherin family, the desmogleins (Dsgs) and desmocollins (Dscs), cooperate to form the adhesive interface (reviewed in Koch and Franke, 1994; Angst *et al.*, 2001; Garrod *et al.*, 2002; Getsios *et al.*, 2004b). The cytoplasmic tails of the cadherins provide a binding platform for the armadillo family members plakoglobin (PG), plakophilins (PKPs) 1–3, and p0071 (reviewed in Cowin and Mechanic, 1994; Hatzfeld, 1999; Anastasiadis and Reynolds, 2000; Schmidt and Jager, 2005). The plakin family member, desmoplakin (DP), in turn, links the stress-bearing IF cytoskeleton to this specialized region of the plasma membrane (Hatsell and Cowin, 2001) (Figure 1). Lateral interactions among proteins in the junctional plaque reinforce its stability (reviewed in Getsios *et al.*, 2004b).

Variations on this molecular blueprint exist in the desmosomes of different cell types. These variations correlate with structural differences, revealed by electron microscopy, showing that desmosomes in different cells and distinct layers of the epidermis vary in appearance and size. For instance, during epidermal differentiation, smaller, less-organized desmosomes in cells of the basal layer are replaced by larger, more electron-dense desmosomes suprabasally. These compositional and structural differences are thought to tailor junctions to suit specialized functions in different organs and stages of differentiation.

In the case of the adhesive ''core'', four Dsg (Dsg1–4) and three Dsc genes (Dsc1–3) have been identified. The Dsc genes give rise to two differentially spliced isoforms, Dsc ''a'' and ''b'', the latter of which is a shorter version lacking the PG-binding site. Simple

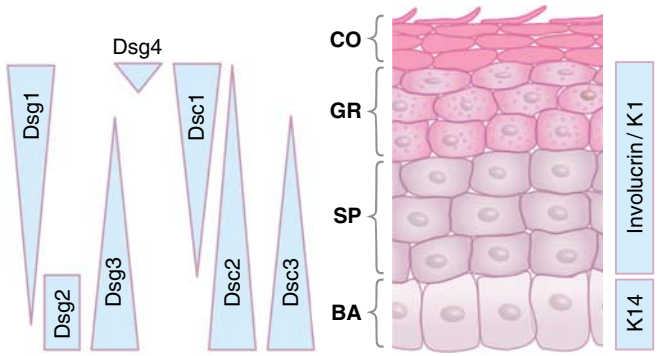

Figure 2. Isoform-specific expression pattern of desmosomal cadherins in the epidermis. A schematic of the epidermis (right) demonstrates the basal (BA), spinous (SP), granular (GR), and cornified (CO) layers. Tightly controlled expression levels and localization of the Dsgs and Dscs (left) within the epidermal layers establish a complex, isoform-specific patterning of desmosomal cadherins that likely contributes to tissue morphogenesis and differentiation. Mouse models (see Table 1) have been utilized to examine cutaneous functions of desmosomal cadherins by targeted ablation or misexpression utilizing epidermal-specific promoters (far right) that specify basal (e.g., keratin 14) or suprabasal (e.g., keratin 1, involucrin) expression.

epithelia express only the Dsg2/Dsc2 pair, whereas stratified complex epithelia, such as the epidermis, express primarily Dsc1/3 and Dsg1/3 with low levels of Dsg2/Dsc2 in the basal layers and Dsg4 concentrated in the granular and cornified layers (Figure 2) (Garrod *et al.*, 2002; Mahoney *et al.*, 2006; Dusek *et al.*, 2007). The special attributes and developmental contributions of the differentially expressed desmosomal cadherins are not fully understood, but the highly patterned distribution of different adhesion molecules may ensure maintenance of cell relationships during morphogenesis of multilayered tissues. Consistent with this idea, cell adhesion recognition peptides that interfere with the adhesive functions of Dsg2/Dsc2 and Dsg3/Dsc3 prevent proper sorting of lumenal and myoepithelial cells, which express different desmosomal cadherins within three-dimensional mammary cultures (Runswick *et al.*, 2001). Further, it has been proposed that the differentiation-specific pattern and ratios of desmosomal cadherins may regulate epidermal development and differentiation (Garrod, 1996; Chidgey *et al.*, 1997; Ishii and Green, 2001).

While the desmosomal cadherins have been thought to be the primary transmembrane molecules within desmosomes, others have been reported, including Perp, a tetraspanin molecule that is a transcriptional target of p63 (Ihrie *et al.*, 2005). Perp is not expressed in all tissues, but it is tempting to speculate that other Perp-like membrane proteins are widely present in desmosomes, where they may play a more general role in regulating junctional assembly or function. The modified desmosomes of suprabasal cornified layers, sometimes referred to as corneodesmosomes, contain a secreted glycoprotein called corneodesmosin, which has been reported to have homophilic adhesive properties (Jonca *et al.*, 2002). During the final stages of differentiation, the desmosome membrane complex contributes to the construction of the crosslinked cornified cell envelope by serving as a substrate for transglutaminases, likely contributing to some of the unique morphological features of corneodesmosomes revealed by ultrastructural studies (Steinert and Marekov, 1999).

Desmosomal plaque proteins, such as the armadillo family members, PKPs and PG, and plakin family members, including plectin, envoplakin, and periplakin, could also be used to customize desmosomes during differentiation in ways that likely translate into differences in desmosome function in different tissues or histological layers (Hatzfeld, 1999; DiColandrea *et al.*, 2000; Getsios *et al.*, 2004b). For instance, PKP1, one of the three desmosomal PKPs, is concentrated in the desmosomes of suprabasal cells, and its loss due to human mutations results in a skin fragility syndrome that is accompanied by a loss of DP from junctions and retraction of IF (McGrath *et al.*, 1999). Other specialized molecules such as pinin (Ouyang and Sugrue, 1996; Shi and Sugrue, 2000) and the periplakin-binding protein kazrin (Groot *et al.*, 2004) have been implicated in strengthening adhesive interactions by tailoring the IF cytoskeleton deep in the plaque or contributing to cornified envelope structure, respectively. Clearly, coordination of the various functions of desmosome membrane and plaque components is a necessary part of orchestrating proper development and homeostasis of complex tissues such as the epidermis.

Components originally defined as ''desmosomal'' can also be found in non-classical desmosome-related junctions in other tissues, where they differ considerably in ultrastructure from epithelial desmosomes. For instance, DP is a component of specialized vascular and lymphatic endothelial cell junctions called complexus adhaerentes, which contain vascular endothelial cadherin along with PG and DP (Borrmann *et al.*, 2006; Franke *et al.*, 2006; Hammerling *et al.*, 2006). In addition, recent immunoelectron microscopical analysis of cardiac muscle tissue has provided a new perspective on the composition of the intercalated disc, a specialized region of the cardiomyocyte membrane classically thought to contain both desmosomes and adherens junctions (fascia adherens), in addition to gap junctions. Franke *et al.*, (2006) made the surprising observation that desmosome molecules intermingle intimately with classic cadherin components in both junction types, independent of ultrastructural appearance. For this reason, intercalated discs have been reclassified as the ''area composita'' of adhering junctions. Loss of DP from these junction types may explain the observed defects in heart and microvasculature development in tetraploid rescued DP-null embryos (Gallicano *et al.*, 2001).

Mechanical functions of desmosomes revealed: breaking them down and building them up

Experimental and spontaneous animal models reveal desmosomal functions in adhesion and tissue integrity. The crucial importance of desmosomes during embryogenesis and in the adult is highlighted by observations from engineered and spontaneous mutations in animal models and further supported by evidence from human diseases of skin, heart, and hair (Cheng and Koch, 2004; McGrath, 2005). Mutations in both the transmembrane adhesive molecules and the plaque components that anchor the IF cytoskeleton lead to a spectrum of phenotypes that have been largely attributed to mechanical defects, but, as will be discussed later, the resulting pathologies appear to encompass defects that go beyond the structural roles of this organelle.

DP-null embryos do not survive beyond embryonic day 6.5, the time during when the egg cylinder normally expands (Gallicano *et al.*, 1998). Not only did keratin 8/18 IF appear disorganized in these developing embryos, but also the number and structure of desmosomes were impaired. These observations led the authors to suggest that desmosomes are not simply uncoupled from IF in DP-deficient animals, but their assembly is also altered. This same group followed up their initial studies by performing a tetraploid rescue experiment in which extraembryonic functions were rescued, allowing an analysis of specific embryonic defects owing to loss of DP (Gallicano *et al.*, 2001). The resulting animals were unable to survive much past gastrulation owing to dysfunctions of the heart, neuroepithelium, and skin. As mentioned above, defects in microvasculature, in which the endothelial cells are joined by complexus adhaerentes instead of desmosomes, were also observed.

Conditional ablation of DP in the heart (Garcia-Gras *et al.*, 2006) or null mutations of the armadillo proteins PG or PKP2 (Bierkamp *et al.*, 1996; Ruiz *et al.*, 1996; Grossmann *et al.*, 2004) yield animals with cardiac abnormalities and associated lethality beginning at approximately embryonic day 11 (for review, see Cheng *et al.*, 2005). In PG-null embryos surviving until birth, skin fragility was also observed (Bierkamp *et al.*, 1996). Similarly, a conditional knockout of DP in skin yielded animals with severe epidermal fragility and loss of barrier function leading to perinatal lethality (Vasioukhin *et al.*, 2001). While DP-null animals were previously reported not to assemble desmosomes, intercellular junctions were still present in the epidermis of the keratin 14-Cre mice with targeted ablation of DP, but lacked an inner plaque and could not anchor IF properly.

More recent reports are revealing a similar range of phenotypes owing to ablation of or mutations in desmosomal cadherins. Engineered and spontaneous mutations in desmosomal cadherins that are expressed in a more limited distribution in stratified epithelia or epidermal appendages have restricted phenotypes, including skin fragility and barrier defects (reviewed in Cheng and Koch, 2004) (Figure 2 and Table 1). In contrast, ablation of Dsg2, which is expressed in very early embryogenesis and throughout epithelial tissues, exhibited an embryonic lethal phenotype, dying shortly after implantation (Eshkind *et al.*, 2002). Moreover, the authors suggested that this cadherin is necessary for normal embryonic stem cell proliferation before the appearance of cell junctions. Surprisingly, loss of Dsc3, which was originally reported as being present specifically in stratified epithelia (Chidgey *et al.*, 1997; King *et al.*, 1997), leads to an even earlier, pre-implantation lethal phenotype in mice (Den *et al.*, 2006). The authors reported that embryos die within the first 2 days of development, before the appearance of mature desmosomes, and suggested that this cadherin may operate independently of desmosomes in early embryonic adhesion or other functions. Interestingly, a truncated form of Dsc1 lacking the PG- and PKP-binding tail was shown to be sufficient to support a normal epidermal phenotype *in vivo* (Cheng *et al.*, 2004). The possibility that desmosome molecules play desmosome-independent functions will be developed further in the sections that follow.

Human diseases provide support for mechanical functions. While investigators in the lab probed the function of desmosome genes by engineering their deletion and mutation over the past decade, representatives of each of the three major gene families as well as accessory molecules found in the desmosome have been revealed as targets for mutation in human diseases of skin and heart. In addition to DP mutations causing skin, hair, and/or heart defects, human mutations have also been identified in PG (skin, hair, heart), PKP1 (skin), PKP2 (heart), Dsg1 (skin), Dsg4 (hair), Dsg2 (heart), and Dsc2 (heart) (reviewed in McGrath, 2005; Pilichou *et al.*, 2006; Syrris *et al.*, 2006). These observations suggest that mutational attack on any individual component of the complex is sufficient to unravel or compromise junctional structures, resulting in defects in tissue integrity, development, and differentiation. Desmosomal component mutations can be inherited as both dominant and recessive alleles, and the defects range from relatively minor skin conditions, resulting from haploinsufficiencies and point mutations, to severe fragility and morphogenetic defects. In some cases, other phenotypic characteristics are observed, including arrhythmogenic right ventricular cardiomyopathy and woolly hair.

The spectrum of mutations and disease phenotypes supports a general conclusion that desmosomes and their molecular building blocks are important for the proper function of the heart and skin, but leaves us with some puzzling unanswered questions. There does not seem to be a particularly strong correlation between the location of a mutation and phenotypic characteristics that arise in families in the case of DP. In some cases, haploinsufficiency of DP leads to skin disease alone (Armstrong *et al.*, 1999) and in others to heart defects alone (Norman *et al.*, 2005; Yang *et al.*, 2006). It is also unclear why defects in internal epithelial tissues have not been reported in patients. For instance, one might speculate that alterations in the intestinal epithelium, which is another constantly renewing tissue like epidermis that is exposed to its own set of

Table 1. Mouse models demonstrating desmosomal cadherin function

Gene	Author	Targeting/mis-expression	Observed phenotype
Dsc1	Chidgey *et al.* (2001)	Targeted Dsc1-null mutation	Epidermal flaking, defective barrier function, hyperproliferation, and granular layer acantholysis
Dsc1	Henkler *et al.* (2001)	Dsc1 transgene driven by keratin 14 promoter (basal)	Normal epidermis
Dsc1	Cheng *et al.* (2004)	Targeted C-terminal truncation of Dsc1a/b	Normal epidermis
Dsc3	Hardman *et al.* (2002)	Dsc3 transgene driven by keratin 1 promoter (suprabasal)	Ventral alopecia, dermal cysts, epidermal hyperproliferation and hyperkeratosis, and altered β-catenin stability/signaling
Dsc3	Den *et al.* (2006)	Targeted Dsc3-null mutation	Lethal before implantation
Dsg2	Eshkind *et al.* (2002)	Targeted Dsg2-null mutation	Lethal shortly after implantation; decreased proliferation of embryonic stem cells
Dsg2	Brennan *et al.* (2007)	Dsg2 transgene driven by involucrin promoter (suprabasal)	Epidermal hyperkeratosis, hyperplasia, and hyperproliferation, with increased resistance to apoptosis
Dsg3	Allen *et al.* (1996)	N-terminally truncated Dsg3 transgene driven by keratin 14 promoter (basal)	Epidermal flaking, hyper- and para-keratosis, and autoamputation of tail
Dsg3	Koch *et al.* (1997, 1998)	Targeted Dsg3-null mutation	Runting, erosions of oral mucosa, supra-basilar epidermal acantholysis, and hair loss
Dsg3	Elias *et al.* (2001)	Dsg3 transgene driven by involucrin promoter (suprabasal)	Post-natal death with increased trans-epidermal water loss and scaling of stratum corneum with lamellar structure as in mucous membranes
Dsg3	Merritt *et al.* (2002)	Dsg3 transgene driven by keratin 1 promoter (suprabasal)	Epidermal flaking and pustules, hyper-proliferation, altered differentiation with hyper- and para-keratosis, and hair loss
Dsg4	Kljuic *et al.* (2003)	Spontaneous Dsg4 mutations (lanceolate hair mouse)	Defective hair keratinization and premature differentiation of keratinocytes with hyperproliferation

environmental stresses, would be observed in patients with DP or desmosomal cadherin mutations. Possible defects in the processes of wound healing and neovascularization, the latter requiring remodeling of DP-expressing endothelia, might also be predicted. Thus it is clear that additional studies into the molecular consequences of specific desmosomal mutations will be necessary to provide further functional insight into these phenotypic differences.

Desmosome membrane glycoproteins are not only subject to defects due to gene mutation, but also to inactivation by autoimmune antibodies and toxins produced by infectious bacteria (Payne *et al.*, 2004; Stanley and Amagai, 2006). The targets in this case are primarily members of the Dsg subfamily of desmosomal cadherins. The presence of Dsg autoantibodies in patients with pemphigus vulgaris (PV) and pemphigus foliaceus (PF) is associated with blistering in distinct epidermal layers and different body sites (Stanley, 1995; Amagai, 1999), which correlate with the expression patterns of Dsg3 and Dsg1, respectively (Shimizu *et al.*, 1995; Amagai *et al.*, 1996). Although there has been controversy regarding their role in pathogenesis (Nguyen *et al.*, 2000; Amagai *et al.*, 2006), compelling evidence supports the idea that Dsg autoantibodies contribute to disease (Amagai *et al.*, 1995, 1994a). Whether acantholysis is caused by direct interference with Dsg-dependent adhesion or antibody-triggered cellular signaling events required for blister development is a matter of current debate (Kottke *et al.*, 2006). Recent reports have also shown that the pathogenic agent produced by *Staphylococcus aureus* in Staphylococcal scalded-skin syndrome and bullous impetigo, exfoliative toxin A, is a serine protease that specifically cleaves the ectodomain of Dsg1. This protease results in epidermal blisters that are virtually identical to those seen in PF (Amagai *et al.*, 2000). Again, exactly how Dsg1 proteolysis leads to generation of subcorneal blisters, and how the effects of exfoliative toxin A compare functionally with those of anti-Dsg1 antibodies in PF to produce identical epidermal pathologies is unknown. Collectively, however, these autoimmune and infectious diseases support the idea that desmosomes make a key contribution to keratinocyte adhesion required for epidermal integrity.

Reconstituting desmosomes in a test tube

The adhesive interface. Previous studies have demonstrated that classic cadherins, which make up the core of adherens junctions, can impart Ca^{2+}-dependent adhesion onto normally non-adherent fibroblasts. Thus, it was logical to ask whether desmosomal cadherins are similarly sufficient to confer adhesive properties on cells. In spite of the fact that homophilic adhesive or ''trans'' interactions between

desmosomal cadherins can occur *in vitro* using recombinant molecules or peptides (Syed *et al.*, 2002; Waschke *et al.*, 2005), a series of studies demonstrated that individual desmosomal cadherins are unable to confer adhesive properties comparable to those of epithelial cells on non-adherent fibroblasts (Amagai *et al.*, 1994b; Chidgey *et al.*, 1996; Kowalczyk *et al.*, 1996; Marcozzi *et al.*, 1998). Collectively, these studies suggest not only that both Dsgs and Dscs are required to reconstitute intercellular adhesion (Kowalczyk *et al.*, 1996; Marcozzi *et al.*, 1998; Tselepis *et al.*, 1998), but also that the ratio of these cadherins must be carefully regulated or else adhesion will not occur (Getsios *et al.*, 2004a). In addition, co-immunoprecipitation experiments demonstrated that ectopically expressed Dsc and Dsg from neighboring cells can interact heterophilically (Chitaev and Troyanovsky, 1997). These experiments strongly support the idea that both Dsgs and Dscs are needed for normal adhesive function, but stop short of detailing how they are organized *in situ*, and whether interactions at the molecular level are homophilic, heterophilic, or both.

Recent tomographic studies have attempted to address how desmosomal cadherins are organized *in situ*. Based on the analysis of cryosections of desmosomes from mouse epidermal tissue, the authors interpreted their data to suggest that the organization of desmosomal cadherin ectodomains is consistent with a previously proposed model for classic cadherin ectodomain interaction. However, the identity of contributing partners was not addressed in this study. Advances in biochemical crosslinking approaches, similar to those employed to analyze classic cadherins (Troyanovsky, 2005), may provide a strategy for sorting out how these cadherins are organized in cells.

The plaque. A combination of *in vitro* protein–protein interaction studies and reconstitution strategies has also been used to try to understand the minimal requirements for assembling a desmosomal plaque and for establishing principles governing IF anchorage. Protein interaction studies have provided a picture of desmosomes that establishes a linear chain in which the Dsc and Dsg tails all associate with PG via an intracellular cadherin-type segment domain resembling the classic cadherin-binding site for β-catenin. In turn, PG binds via its central armadillo repeat domain to the N-terminus of DP. Finally, through its C-terminus, DP completes the link to the IF cytoskeleton. In general, PKPs exhibit a much more complex repertoire of interactions, associating with desmosomal cadherins, other armadillo proteins (both classic and desmosomal), the N-terminus of DP, and IF directly, thus likely contributing to a complex web of lateral stabilizing interactions in the plaque (Hatzfeld, 2006). While high-resolution structural data, such as those recently obtained for the arm repeats of PKP1, are likely to aid in defining how these family members engage in interactions, so far all the identified partnerships occur through the PKP head domain (Choi and Weis, 2005; Hatzfeld, 2006).

The anchorage of IF to the desmosomal plaque is thought to occur largely through interactions of the C-terminus of DP with keratin, desmin, or vimentin IF (Stappenbeck and Green, 1992; Stappenbeck *et al.*, 1993b; Kouklis *et al.*, 1994; Meng *et al.*, 1997; Fontao *et al.*, 2003). This IF-binding domain comprises three subdomains, each consisting of 4.5 copies of a 38-residue plakin repeat domain (Green *et al.*, 1990; Choi *et al.*, 2002). The subdomains are separated by flexible linking regions and the final ''C'' plakin repeat domain is followed by a terminal 68-residue tail. Based on the pattern of charged residues in the C-terminal domain, we previously hypothesized that the C-terminus of DP interacts with IF (Green *et al.*, 1990). Molecular modeling based on more recent structural analysis suggested that approximately four turns of an α-helix, such as those present in the rod domains of IF, might be able to interact with a shallow positively charged groove present within each plakin repeat domain (Choi *et al.*, 2002).

While cell culture, *in vitro* overlay, and yeast two/three hybrid experiments all support the idea that DP associates with IF, there is still uncertainty regarding the relative contributions of the plakin repeat domains themselves and the flexible linking regions that divide and terminate these domains. Co-sedimentation analyses are consistent with the idea that plakin repeat domains are sufficient for binding to vimentin. These studies also suggest that the more the plakin repeat domains, the better the binding: B plus C, or a protein containing all three plakin repeat domains, co-sediment more vimentin than individual domains (Choi *et al.*, 2002). Other studies suggest that the plakin repeat domains cannot bind in the absence of associated flexible regions, and also underscore the important contribution of the terminal tail (Fontao *et al.*, 2003). Collectively, however, the studies agree that different sequences within the linkers and tail fine-tune and specify interactions with different IF types (Green *et al.*, 1990; Stappenbeck *et al.*, 1993a; Kouklis *et al.*, 1994; Meng *et al.*, 1997; Choi *et al.*, 2002; Fontao *et al.*, 2003; Lapouge *et al.*, 2006). Further, interaction of the C-terminus of DP with IF is modulated from strong to weak by serine phosphorylation at residue 2849 (Stappenbeck *et al.*, 1994), and a point mutation at this site (S2849G) impairs DP assembly into desmosomes (Godsel *et al.*, 2005). The recent identification of additional serine sites located in key linking regions surrounding the plakin repeat domain and tail suggests further opportunities for regulating the conformation and IF-binding properties of this domain (Beausoleil *et al.*, 2004).

Our understanding of DP–intermediate filament interactions is limited by a lack of knowledge about the IF end of the partnership. Type I and II keratin IF, which are most frequently associated with DP in desmosomes of epithelial cells, are known to form obligate heteropolymers *in vivo* (Coulombe and Omary, 2002). Early work suggested that formation of the heterodimer is not required for IF binding, at least not in the case of epidermal keratins (Kouklis *et al.*, 1994;

Meng *et al.*, 1997). However, more recent *in vitro* studies suggest that both type III IF and keratins, including epidermal keratins, require the presence of the α-helical rod domain for DP association (Fontao *et al.*, 2003), although head and/or tail sequences may be required for optimal binding.

How all of these individual interactions are harnessed by the cell to construct the plaque is not well understood, and efforts to deconstruct the plaque have been hampered by the highly insoluble nature of the desmosome. In an extension of the approaches used to reconstitute adhesion in a test tube, investigators endeavored to construct plaques *in vitro* by expressing a combination of armadillo proteins and DP in concert with full-length desmosomal cadherins or chimeric membrane molecules containing either Dsc or Dsg tail domains. While these experiments are subject to a number of interpretations, some common themes have emerged. These include the following: (1) the complexity and insolubility of the desmosomal plaque increases with introduction of each class of components (Bornslaeger *et al.*, 2001); (2) in concert with PG and the DP N-terminus, the desmosomal cadherin tails are sufficient to nucleate the formation of an electron-dense plaque (Kowalczyk *et al.*, 1997); but (3) the proper clustering and/or segregation of plaque components into punctate plaque structures requires the coexpression of a PKP and PG (Bornslaeger *et al.*, 2001; Koeser *et al.*, 2003); and (4) the extent of plaque length is regulated by PG end domains (Palka and Green, 1997).

While these biochemical studies have laid a foundation for our understanding of how desmosomes are built from the ground up, understanding assembly in cells and tissues will require different strategies, such as live cell imaging, which will be discussed below.

Non-traditional roles of desmosome proteins in differentiation and disease

The studies described so far underscore the importance of desmosomes as intercellular adhesive organelles that are required for the integrity of epithelial and cardiovascular tissues. However, desmosome molecule functions are not limited to their traditional roles in desmosomes or in providing mechanical integrity to tissues, but extend to supra-adhesive functions *in vivo*. Consistent with a role for desmosomal cadherins in morphogenesis and tissue homeostasis, misexpression or targeted deletion of desmosomal cadherins in mice affects keratinocyte homeostasis and differentiation (Allen *et al.*, 1996; Chidgey *et al.*, 2001; Elias *et al.*, 2001; Merritt *et al.*, 2002; Cheng and Koch, 2004; Hardman *et al.*, 2005) (Table 1). Forced suprabasal expression (via the involucrin promoter) of Dsg2, which is normally only present at low levels deep in the epidermis, led to epidermal hyperplasia and the development of pre-cancerous papillomas (Brennan *et al.*, 2007). These changes were accompanied by an increase in the activity of multiple cell growth and survival pathways. These findings are particularly interesting in light of the previous observation that Dsg2 is important for proliferation of embryonic stem cells (Eshkind *et al.*, 2002). However, the underlying molecular mechanisms directly linking Dsg2 to cell growth and survival have not been determined. Suprabasal expression of Dsg3 and Dsc3 driven by the keratin 1 promoter similarly resulted in epidermal hyperproliferation, but a different outcome was observed when Dsg3 was misexpressed suprabasally via the involucrin promoter. In this case, Dsg3 expression resulted in a Dsg profile similar to that seen in normal mucous membranes and conversion to an epithelium with histological and functional attributes of a non-keratinized mucosal epithelium (Elias *et al.*, 2001). This observation supported the idea initially proposed by Garrod that the tightly regulated expression profile of desmosomal cadherins may be required for proper patterning of stratified tissues.

In hair follicles from mice with Dsg4 mutations, Kljuic *et al.* (2003) observed an abrupt, rather than gradual, transition from the proliferative to differentiating zones of follicular cells, a defect resulting in a lanceolate hair phenotype. Although this observation suggests a role for Dsg4 in morphogenesis of the hair follicle, it was not possible in this initial study to differentiate between adhesion-dependent and adhesion-independent defects. A somewhat similar impairment in the transition from the basal proliferative to suprabasal differentiating keratinocyte compartments was observed in organotypic epidermal ''rafts'' in which Dsg1 expression was limited using shRNA-mediated knockdown (Getsios S, Simpson CL, Green KJ, unpublished data). In Dsg1-deficient raft cultures, the intermediate layers and markers of spinous differentiation were under-represented, suggesting that this cadherin is required for normal epidermal morphogenesis. Interestingly, specific removal of the Dsg1 adhesive ectodomain by chronic exposure to exfoliative toxin A did not inhibit stratification or intermediate layer morphogenesis, suggesting that Dsg1's role in this process is adhesion-independent.

How might desmosomes be involved in adhesion-independent signaling? One potential mechanism is through PG, which is an armadillo family member and the closest relative of β-catenin, a well-known regulator of the canonical Wnt/wingless signaling pathway (Zhurinsky *et al.*, 2000; Huelsken and Birchmeier, 2001; Yin and Green, 2004). Because PG is capable of associating with, and perhaps competing for, many of the same partners as β-catenin, its ability to modulate the Wnt pathway has been thought by some to be indirect, by interfering with β-catenin's turnover and/or transcriptional activity. This idea is supported by one study in which a stabilized form of PG (ΔN122-PG) led to the formation of additional hair germs and hyperplastic hair follicles (Teuliere *et al.*, 2004), similar to those seen in mice with elevated β-catenin (Gat *et al.*, 1998); however, this effect is seen only in a β-catenin wild-type, not null, background. The notion that altering desmosome function could impact on β-catenin signaling is supported by the observed ability of the β-catenin binding partner PKP2 to modulate β-catenin-dependent TOP-FLASH reporter activity *in vitro* (Chen *et al.*, 2002); in addition, forced suprabasal expression

of Dsc3 enhanced β-catenin signaling activity and altered epidermal differentiation in transgenic mice (Hardman *et al.*, 2005).

On the other hand, recent reports have demonstrated PG's ability to signal in a β-catenin-deficient background, providing support for β-catenin-independent functions in signaling (Maeda *et al.*, 2004; Teuliere *et al.*, 2004; Williamson *et al.*, 2006). These included a regulatory role in differentiation and proliferation of keratinocytes within the interfollicular epidermis of mice expressing stabilized PG in a β-catenin-null background (Teuliere *et al.*, 2004). Another study demonstrated that ectopically expressed PG suppressed hair growth and follicle development even in a wild-type β-catenin background (Charpentier *et al.*, 2000). The notion that PG has functions distinct from β-catenin is also supported by cell culture studies showing that PG-null keratinocytes display reduced motility, even of single cells, and reduced sensitivity to UV-induced apoptosis (Yin *et al.*, 2005b; Dusek *et al.*, 2007).

Post-translational modification of PG has been shown to regulate its protein partnerships and subcellular distribution, and this ultimately has an impact on the available signaling pool. Tyrosine phosphorylation has been reported to promote PG's translocation into the nucleus in keratinocytes, resulting in its increased association with LEF/TCF transcription factors and inhibition of β-catenin-dependent reporter activity (Hu *et al.*, 2003). PG can be phosphorylated by multiple protein tyrosine kinases with different effects depending on the site of phosphorylation, which modulates both its association with intercellular junctions and availability for regulation of LEF/TCF signaling (Miravet *et al.*, 2003). For instance, Y549 phosphorylation increases PG binding to adherens junction components, resulting in an increase in the transcriptional activity of the β-catenin–Tcf4 complex (Miravet *et al.*, 2003). Whereas EGF receptor-dependent tyrosine phosphorylation of PG can impair DP recruitment to junctions, weakening desmosomal adhesion (Yin *et al.*, 2005a), a recent study has shown that loss of DP from junctions can result in PG translocation into the nucleus of cardiac myocytes (Garcia-Gras *et al.*, 2006). This resulted in a reduction in canonical Wnt signaling through Tcf/Lef1, decreases in β-catenin target genes c-Myc and cyclin D1, and an increase in adipogenic gene expression. In animals, these alterations were accompanied by excess adipocytes and fibrosis in the heart, increased myocyte apoptosis, cardiac dysfunction, and ventricular arrhythmias, similar to the pathologies observed in patients with arrhythmogenic right ventricular cardiomyopathy. These data shed new light on the previous proposal that the pathogenesis in arrhythmogenic right ventricular cardiomyopathy is due to transdifferentiation of cardiac myocytes to adipocytes in response to stress (d'Amati *et al.*, 2000). In fact, this transdifferentiation may be due more directly to alterations in PG signaling. These data also shed light on the ''woolly hair'' phenotype observed in humans with certain mutations in either PG or DP, raising the possibility that similar to β-catenin, PG may be important in fate determination during hair follicle morphogenesis, and DP mutations alter this function (MacRae *et al.*, 2006).

Signaling pathways linked to desmosomes have also been revealed by cellular responses to pemphigus antibodies directed against Dsgs, including increases in inositol triphosphate, mobilization of intracellular calcium with concomitant activation of protein kinase C (PKC) (Esaki *et al.*, 1995; Seishima *et al.*, 1995; Osada *et al.*, 1997), and phosphorylation of the Dsg3 cytoplasmic domain accompanied by loss of PG binding (Aoyama *et al.*, 1999). Further, antibody-induced interference with RhoA and activation of p38 mitogen-activated protein kinase have been reported to be required for loss of keratinocyte adhesion mediated by pathogenic antibodies (Berkowitz *et al.*, 2005, 2006; Waschke et al., 2005, 2006). p38 mitogen-activated protein kinase activation has recently been proposed to occur late in a signaling cascade involving the upstream activation of EGF receptor and Src in response to PV antisera (Chernyavsky *et al.*, 2007). These authors suggest further that Dsgs are not the sole or even primary mediators of this cascade, based on its continued stimulation in cells with siRNA-mediated knockdown of Dsg1 or 3. Associated armadillo proteins may be important contributors to cellular responses leading to acantholysis, as pathogenic PV antibodies are unable to induce keratin IF retraction in PG-null keratinocytes (Caldelari *et al.*, 2001). Further, PV antibodies appear to reduce nuclear accumulation of PG, thus alleviating PG-dependent inhibition of c-Myc transcription, which was proposed to contribute to an increase in proliferation and loss of intercellular adhesion in PV patients (Williamson *et al.*, 2006).

While PG has been implicated in several ''non-traditional'' roles, non-desmosomal functions of the PKP subfamily of armadillo proteins are just beginning to emerge. In addition to its roles in junctions, PKP2 may play a more general role in regulation of transcriptional machinery as part of the RNA polymerase III holoenzyme complex (Mertens *et al.*, 2001). Its access to the transcriptional machinery is regulated by C-TAK1-dependent phosphorylation and 14-3-3 binding, which determine whether it can gain entrance to the nucleus (Muller *et al.*, 2003). The family members PKP1 and 3 have also been found in association with RNA-binding proteins, and may likewise participate in processes of translation and RNA metabolism (Hofmann *et al.*, 2006). The PKP-related protein p0071 (sometimes referred to as PKP4) was recently shown to be required for spatially localizing RhoA during cytokinesis through its association with the Rho GTPase exchange factor Ect2 (Wolf *et al.*, 2006). Finally, the periplakin-binding protein, kazrin, associates with the nuclear matrix in a cell cycle-dependent manner before blastocyst formation during embryogenesis (Gallicano *et al.*, 2005). Although kazrin is not a PKP protein, it shares plakin-binding properties with PKP family members. Collectively, these studies raise the possibility that plakin-binding proteins have diverse nuclear functions outside of junctions.

While we have focused so far on non-desmosomal signaling and nuclear functions in this section, recently an unexpected role for DP in regulating the distribution of the microtubule cytoskeleton during epidermal differentiation was observed. Microtubules were shown to shift away from a radially arrayed, centrosome-associated organization in basal cells to accumulation in the cortical region near cell–cell junctions within suprabasal keratinocytes (Lechler and Fuchs, 2007). Along with this, there is a redistribution of the centrosomal protein called ninein to desmosomes. This switch in ninein distribution requires DP. Although desmosomal localization of another microtubule-associated protein, CLIP170, was reported years ago (Wacker *et al.*, 1992), until now a functional association between these junctions and microtubule organization had not been revealed. This finding opens up new avenues for examining the role of desmosomes in regulating cell polarity and cytoarchitecture.

Desmosome dynamics and regulation in health and disease

Much has been done to catalog the protein–protein interactions of desmosome molecules, but how these interactions are coordinated temporally and spatially in living cells to regulate assembly, disassembly, and adhesive strength is less well understood. Characterizing these key determinants of junctional stability will be important not only for understanding desmosome homeostasis and remodeling, but also for discovering how mechanisms that regulate assembly state are co-opted in human disease.

Assembly. A series of papers in the 1980s mapped out alterations in the localization, biosynthesis, and fate of the desmosomal plaque and membrane components following the elevation of extracellular calcium levels to trigger junction assembly (e.g., Watt *et al.*, 1984; Jones and Goldman, 1985; Mattey and Garrod, 1986; Penn *et al.*, 1987; Pasdar and Nelson, 1988a,b). Collectively, these studies demonstrated that desmosomal components are synthesized and initially recruited into a soluble pool, followed by acquisition of insolubility correlating with junction assembly. The evidence suggested that the plaque and membrane pools exist in spatially and biochemically separate compartments until the final assembly steps at the plasma membrane, and that DP is associated with the IF cytoskeleton whereas membrane proteins are associated with the microtubule cytoskeleton (Figure 3). Early ultrastructural studies also suggested that the DP–IF scaffold is closely associated with the cortical actin cytoskeleton (Green *et al.*, 1987). However, further analysis has been hampered by the insoluble nature of desmosomes and desmosome precursors (Pasdar *et al.*, 1991). Recent developments in optical microscopy have helped overcome these limitations. Using fluorescently tagged DP and desmosomal cadherins, several studies have revealed previously unappreciated dynamic behaviors of both plaque and membrane proteins (Windoffer *et al.*, 2002; Gloushankova *et al.*, 2003; Godsel *et al.*, 2005).

Assembly/regulation of the desmosomal plaque. Using DP fused with green fluorescent protein (DP-GFP), we recently mapped the behavior of DP in three dimensions during cell contact-initiated desmosome assembly (Godsel *et al.*, 2005). These studies suggested that assembly occurs in three temporally overlapping phases, beginning with the rapid accumulation of DP at contacting borders during the first few minutes following contact. Contact produces a signal that triggers the formation of fluorescent bright non-membrane-bound DP-containing particles in the cortical region of the cell (phase II) that subsequently translocate at a very slow rate (slower than most conventional microtubule-based motors) to cell–cell borders, where they join the progressively brightening clusters that formed during the earlier phase. This observation is consistent with earlier work, suggesting that at least some endogenous DP particles observed in the cytoplasm are desmosome precursors (Jones and Goldman, 1985), rather than all being remnants of previously internalized junctions (Mattey and Garrod, 1986).

Based on electron microscopy analysis, the cytoplasmic particles range in size, the largest frequently exceeding the diameter of a large keratin IF bundle (Jones and Goldman, 1985; Godsel *et al.*, 2005). While their biochemical nature is poorly understood, immunogold electron microscopy and ''retrospective'' immunofluorescence microscopy of cells first imaged live and then counterstained for other junctional molecules revealed that the DP-associated protein PKP2 is uniquely associated with assembly-competent cytoplasmic particles in the cells used in this study. Membrane components such as Dsc and Dsg were not associated with anterograde moving particles, only retrogradely moving particles, which may have represented endocytosed or engulfed desmosomes.

The mechanism of translocation of the precursor particles is unclear. However, the observation that the microfilament poison, cytochalasin D, significantly impairs DP particle movement (Godsel *et al.*, 2005) suggests that microfilaments contribute to the translocation process. As previous studies have suggested that the formation of adherens junctions is functionally linked to desmosome assembly (Lewis *et al.*, 1997), it is tempting to speculate that events associated with maturation of adherens junction-associated cortical actin may be required for proper formation of desmosomes upon cell—cell contact. Interestingly, PKPs can associate with actin, providing a potential link between the DP–IF network and actin-dependent desmosome assembly (Hatzfeld *et al.*, 2000). Although previous studies have suggested that IF are not required for DP accumulation and desmosome assembly, the behavior of DP-GFP mutants with compromised or enhanced binding to IF suggests that they play a regulatory role in the biphasic enhancement of DP at cell–cell borders revealed by live cell imaging (Godsel *et al.*, 2005). A C-terminal phosphorylation-deficient serine-to-glycine mutant, DP-S2849G, exhibits delayed outward trafficking, in part owing to its aberrant retention on IF, to which it binds with higher affinity (Stappenbeck

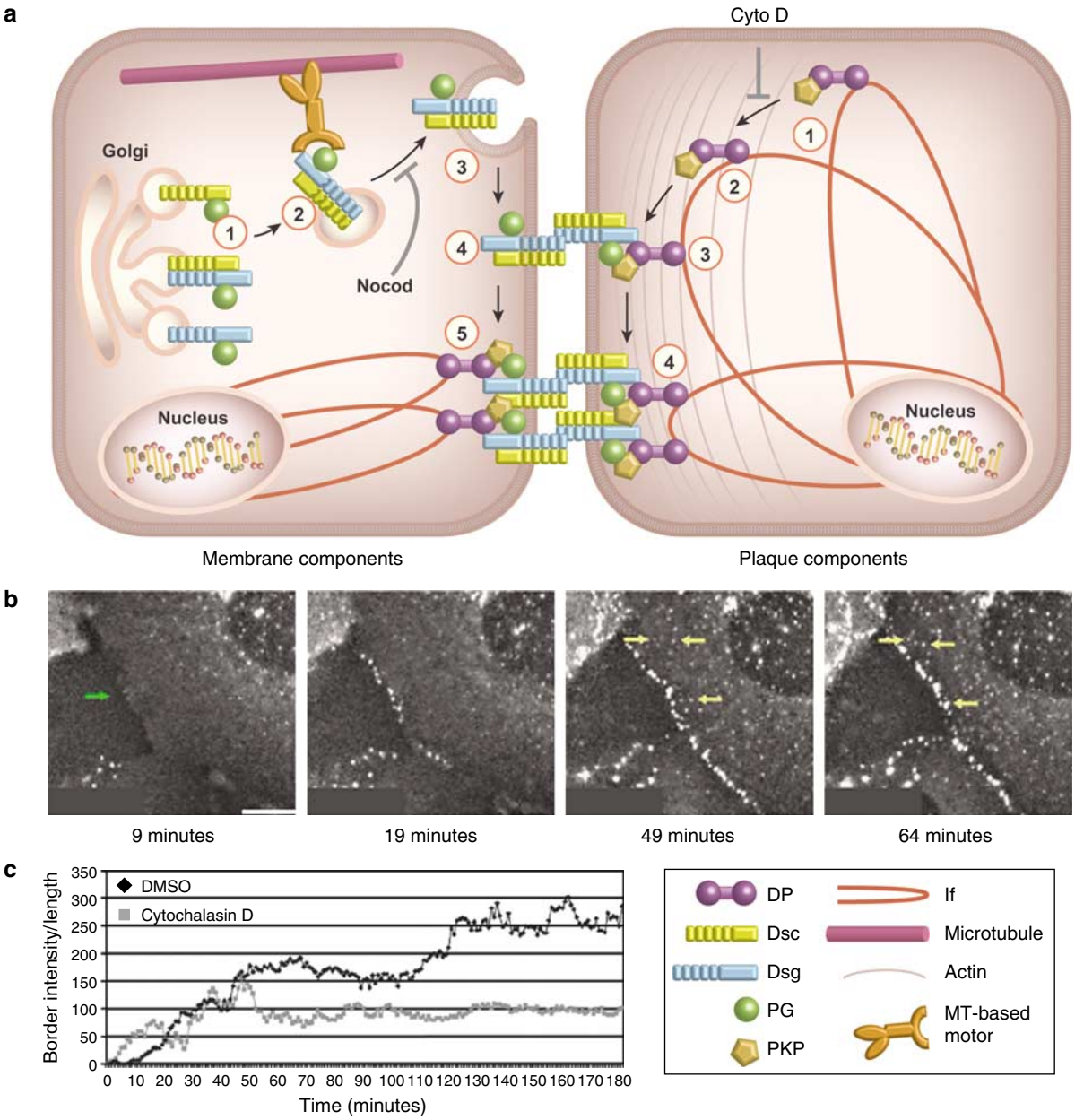

Figure 3. Model of desmosome assembly from distinct membrane and plaque compartments. (**a**) On the left, Dsg- and/or Dsc-containing vesicles bud from the Golgi (1) and appear to be transported along microtubules (MT) (nocodazole-sensitive) by unknown motors (2), which deliver them to the cell surface (3), allowing formation of cadherin trans-dimers (4) and later incorporation into mature desmosomes (5). It is not completely clear whether Dsgs and Dscs traffic together or in separate vesicles. On the right, non-membranous DP- and PKP-containing particles appear in the cytoplasm (1), then move toward the periphery, where actin-based motility (cytochalasin D-sensitive) seems to facilitate delivery to the cell surface (2), allowing formation of immature junctions (3) and eventual incorporation into mature desmosomes (4). (**b**) Live imaging of wounded epithelial cells shows appearance of cytoplasmic DP-GFP particles (arrows), which move toward the cell periphery and incorporate into nascent intercellular junctions. (**c**) Quantification of cell–cell border fluorescence reflects a two-stage increase in DP-GFP accumulation over time, the second of which is completely inhibited by application of cytochalasin D to destabilize actin filaments (adapted in part from Godsel *et al.*, 2005).

et al., 1994; Meng *et al.*, 1997; Fontao *et al.*, 2003). Achieving maximum intercellular adhesive strength requires proper attachments of junctions to both IF and cortical actin, as intercellular adhesion is synergistically strengthened by the presence of IF and actin attachments (Huen *et al.*, 2002). Further, adherens junctions are also unable to mature properly in cells lacking DP and normal IF attachments (Vasioukhin *et al.*, 2001).

Interestingly, knockdown of the α-isoform of PKC impairs calcium-dependent desmosome formation (Hobbs RP, Hsieh SN, *et al.*, personal communication) and results in retention of DP along IF in a manner similar to that observed for the S2849G DP mutant. Activation of PKC had previously been reported to trigger desmosome

formation in low-calcium conditions, or in cells lacking desmosomes owing to mutation of adherens junction proteins (Sheu *et al.*, 1989; Hengel *et al.*, 1997). Thus, PKC may act as a rheostat to control both the availability of DP for junction assembly and the rate at which DP reinforcements are assembled into particles and traffic to join their counterparts already building up the desmosomal plaque. Recently, a mutation located within the regulatory terminal tail of DP, which also contains two other serine phosphorylation sites (Beausoleil *et al.*, 2004), was discovered in patients with arrhythmogenic right ventricular cardiomyopathy (Yang *et al.*, 2006). It seems plausible that this mutation could affect phosphorylation-dependent regulation of DP either by altering the phosphorylation state of the tail or by interfering with the conformation of this region and thus the ability of the tail to properly fine-tune interactions with IF. Future studies to examine the role of DP phosphorylation regulation in skin wound healing and cardiac function will be important to understand the physiological importance of these assembly regulatory pathways in tissue function.

Interfering with intracellular calcium homeostasis also results in the accumulation of cytoplasmic DP in cells derived from patients with mutations in the gene encoding the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 pump (Sakuntabhai *et al.*, 1999; Dhitavat *et al.*, 2003). These patients present with a disorder called Darier's disease, characterized by skin abnormalities owing to impairment of intercellular adhesion and desmosome structure in epidermal keratinocytes (Dhitavat *et al.*, 2004). The authors of the report suggested that DP retention and aggregation in the cytoplasm may be due to improper protein folding, but it also seems possible that calcium-dependent alterations in other regulatory pathways, such as those upstream of calcium-dependent kinases like PKC, could contribute to the observed defects in desmosome assembly.

Assembly of the desmosomal cadherins. Understanding desmosomal cadherin assembly behavior has also benefited from the use of fluorescently labeled probes. Using Dsg2 fused to GFP (Dsg2-GFP), the continual assembly of nascent desmosomes has been observed within stable contacts located at the middle of an epithelial sheet (Gloushankova *et al.*, 2003). New GFP-labeled structures appear as a group of fine puncta, which after a few minutes coalesce into a single structure. This observation is in line with the rapid appearance of Dsg2-GFP puncta in newly contacting cells at the edge of a wound (Amargo EV and Green KJ, unpublished data). Desmosomal cadherins can continue to exchange into stable junctions once they are formed, as demonstrated by fluorescence recovery after photobleaching, showing that the desmosomal cadherin Dsc2 exchanges into existing junctions with a half-life of 30 minutes (Windoffer *et al.*, 2002). Although the extent of desmosome dynamics *in vivo* during epidermal differentiation is unknown, clearly new desmosomes with different compositions reflecting differentiation-dependent expression patterns have to be made either *de novo* during this process or remodeled or both (North *et al.*, 1996).

Previous studies suggested that Dsg in Madin–Darby canine kidney cells was trafficked toward the plasma membrane on microtubules (Pasdar and Nelson, 1989). Our recent data support this idea, as dual-label live cell imaging shows Dsg-GFP-containing particles moving rapidly toward the periphery on microtubules labeled with mCherry-tubulin, probing the plasma membrane region before appearing to fuse with existing fluorescent puncta that appeared shortly after cell contact. This rapid transport occurs at a rate consistent with conventional kinesin motors, and is dependent on microtubules, as the motile behavior is inhibited by nocodazole.

The spatial relationship between Dscs and Dsgs as they traverse the secretory apparatus on their way to newly forming desmosomes is not well understood. Electron microscopic level analysis has suggested that desmosome formation in MDCK cells occurs in two stages. The first involves the early transport of 60 nm vesicles containing mostly Dsc2 and lower amounts of Dsg and E-cadherin to the cell surface. The second stage involves transport of Dsg, E-cadherin, PG, and β-catenin in 200 nm vesicles that were specifically targeted to the basolateral plasma membrane, rather than diffusely all over the cell surface, as was the case with the smaller vesicles. Plaque proteins such as DP I/II were added subsequently to these sites of junction nucleation (Burdett and Sullivan, 2002). Consistent with this, electron microscopy pulse–chase experiments demonstrated that Dsg3 first forms small clusters on the surface lacking IF attachment, chasing into larger IF-attached half desmosomes, before presumably incorporating into desmosomal junctions (Sato *et al.*, 2000). These data suggest a multistage mechanism for regulating the distribution and rate of junction assembly. Further studies will be necessary to address whether Dscs and Dsgs are functionally interdependent at any stage during the secretory process, or whether their proper sorting and delivery require association of specific armadillo proteins. In addition, the potential involvement of specific Rab GTPases and/or a unique desmosomal exocyst complex, similar to that emerging for classic cadherins, is yet to be tested (Langevin et al., 2005; Lock and Stow, 2005).

Modulation of desmosome adhesive status and desmosome disassembly. Desmosome assembly is typically followed by a maturation phase characterized by increasing stability and resistance to environmental influences (Figure 4). Although this process is poorly understood, it is likely to involve both remodeling of the associated cortical cytoskeleton and other undefined post-translational modifications. In tissues or cells that have been cultured for long periods of time, desmosomes ultimately attain a hyperadhesive state known as ''calcium independence''. In this state, desmosomes are stable even in the absence of extracellular calcium (Watt *et al.*, 1984; Garrod *et al.*, 2005; Kimura *et al.*, 2007), which would normally disrupt junctions and cause their rapid

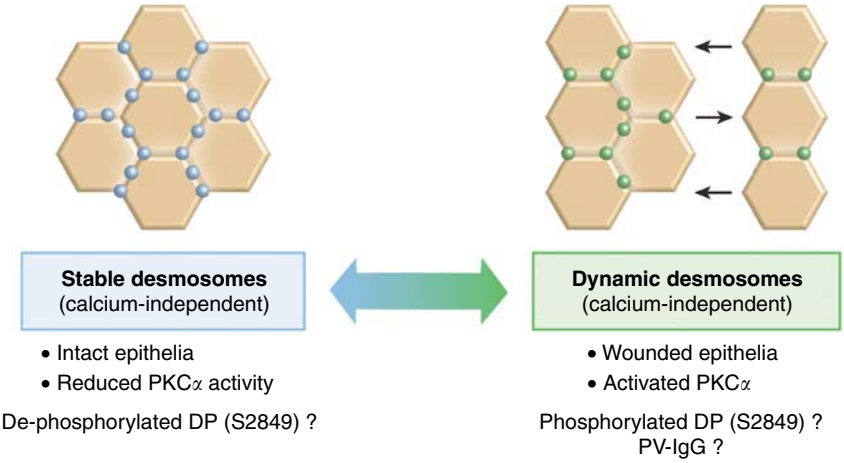

Figure 4. Desmosomal stability and remodeling. (Left) In confluent sheets of cells or tissues subjected to high levels of calcium, desmosomes are stable and insensitive to low-calcium conditions. This hyper-adhesive state is promoted by de-activation of PKCα (Wallis *et al.*, 2000). (Right) On the other hand, in more sparse cultures or after wounding of an epithelial sheet, PKCα activation appears to promote more dynamic, less adhesive desmosomes, permitting cell mobility as required for re-epithelialization during wound healing. Moreover, desmosome stability and adhesive strength are compromised by treatment with PV-IgG, which may also work through PKC-dependent pathways to de-stabilize desmosomes (Kitajima *et al.*, 1999). It seems plausible that PKCα activity may alter the phosphorylation state of desmosomal proteins, such as DP, affecting their interactions and association with IF, which could modulate adhesive strength and/or stability. In support of this, a PKC phosphorylation consensus site mutation in DP (S2849G) alters its affinity for keratin IF and impairs its assembly into desmosomes.

internalization (Mattey and Garrod, 1986; Kartenbeck *et al.*, 1991; Windoffer *et al.*, 2002). Activation of PKCα, which has been shown to occur at wound edges, causes a reversion to a calcium-dependent state and has been proposed to facilitate epithelial remodeling (Wallis *et al.*, 2000). Although a complete understanding of how PKCα contributes to desmosome stability is lacking, altered phosphorylation of desmosome and/or associated cytoskeletal components may underlie the shift to a less adhesive phenotype. In light of the reported role for PKC in internalization of classic cadherins (Le *et al.*, 2002), it is tempting to speculate that PKC similarly regulates the balance of desmosomal cadherin trafficking. Finally, the fact that PKCα also plays a positive role in desmosome assembly supports the idea that this kinase is important for maintaining a biologically ''metastable'' state, which would allow rapid desmosome remodeling (either assembly or disassembly) in response to environmental cues (Figure 4).

Tyrosine phosphorylation of the cadherin tail complex is another potential mechanism for modulating cadherin-based junctions (Gumbiner, 2005). Such a pathway is readily revealed in squamous cell carcinoma cells overexpressing EGF receptor, in which tyrosine phosphorylation of the Dsg2 tail and PG is accompanied by reduced levels of both Dsg2 and Dsc2 as well as accumulation of an internalized pool of desmosomal cadherins. Treatment of these cells with an EGF receptor inhibitor (PKI) not only resulted in stabilization and retention of desmosomal cadherins on the cell surface, but also enhanced desmosome assembly and dramatically increased adhesive strength of cell monolayers (Lorch *et al.*, 2004). The effect of EGF receptor inhibition was potent, as these increases in intercellular adhesive strength occur even under conditions of reduced extracellular calcium. These findings provide mechanistic insight into the therapeutic value of EGF receptor inhibitors for patients with invasive lung and head and neck cancers, many of which over express EGF receptor.

A number of tyrosine phosphorylation sites on the armadillo protein PG have been identified (Miravet *et al.*, 2003; Yin and Green, 2004). EGF receptor-dependent phosphorylation of PG on Y693, Y724, and/or Y729 impairs its association with DP and results in decreased intercellular adhesive strength, presumably by impairing association with the IF cytoskeleton (Gaudry *et al.*, 2001; Miravet *et al.*, 2003; Yin *et al.*, 2005a). Phosphorylation of PG at Y549 also decreases association with DP. Src phosphorylation of PG on Y643, on the other hand, increases its binding to DP, and at the same time reduces PG's ability to associate with components of the classic cadherin complex, to which both β-catenin and PG can bind. Collectively, these studies put PG at a critical juncture in the adhesive axis of adherens junctions and desmosomes. These phosphorylation events may also indirectly regulate PG's distribution in nuclear and cytosolic pools, where it is subject to regulation by the proteasomal degradation machinery. Proteasome-dependent degradation of PG is inhibited by its O-glycosylation, which in turn enhances keratinocyte intercellular adhesion (Hatsell *et al.*, 2003; Hu *et al.*, 2006).

Emerging data suggest that cadherin stability and internalization are key components of pathogenesis in the autoimmune disease pemphigus, as has recently been reviewed elsewhere (Kottke *et al.*, 2006). Dsg3 is degraded in response to PV autoantibody (PV-IgG) binding of DJM cells, resulting in Dsg3-depleted desmosomes (Aoyama and Kitajima, 1999). Recent work tracking the fate of antibody- or biotin-labeled cell surface protein is consistent with the idea that rapid

internalization of the soluble pool of PV-IgG-bound Dsg3 occurs upon antibody ligation (Calkins *et al.*, 2006), consistent with previous electron microscopy pulse–chase analysis, suggesting that PV-IgG-mediated internalization targets peri-junctional ''free'' Dsg3, which may be more accessible to pathogenic antibodies (Sato *et al.*, 2000). Data regarding the fate of associated PG differ, some suggesting that PG dissociates from phosphorylated Dsg3, and others suggesting that PG, but not DP, remains associated with the cadherin (Calkins *et al.*, 2006); more recently, it has been suggested that PV-IgG binding to Dsg3 results in a reduction in nuclear PG with downstream transcriptional effects (Williamson *et al.*, 2006). Following internalization, Dsg3 associates with endosomes and lysosomes, where it is presumably degraded (Calkins *et al.*, 2006). As mentioned above, several signaling pathways, including PKC activation, are initiated in response to antibody binding. In light of PKC's reported ability to revert desmosomes to a calcium-dependent state and to promote classic cadherin internalization (Le *et al.*, 2002), a potential contribution of PKC to Dsg3 internalization in PV seems possible (Kitajima, 2002). PV-IgG-induced alterations in both IF attachment (Caldelari *et al.*, 2001; Calkins *et al.*, 2006) and cortical actin organization (Waschke *et al.*, 2006) may also regulate this process.

Desmosomes are also subject to regulation by proteolysis. Dsg2 is processed by matrix metalloproteinases in tumor cells, and was recently identified as a substrate for members of the ADAM family of proteases (Bech-Serra *et al.*, 2006). EGF receptor and matrix metalloproteinase inhibitors blocked Dsg shedding and strengthened cell–cell adhesion in squamous cell carcinoma cells (Lorch *et al.*, 2004). Both matrix metalloproteinase-dependent ectodomain shedding and caspase-dependent processing of the intracellular domains of desmosomal cadherins occur during apoptosis (Weiske *et al.*, 2001; Dusek *et al.*, 2006a). These events are coordinated with cleavage of plaque components, and ultimately lead to the dissolution of desmosomes (Aho, 2004). In contrast to classic cadherins, which are reported to be pro-survival factors, the presence of Dsg1 sensitizes keratinocytes to programmed cell death (Dusek *et al.*, 2006a), as does the associated protein PG (Dusek *et al.*, 2007). Finally, regulated proteolysis of Dsg1 by tryptic and chymotryptic enzymes of the stratum corneum is thought to be important for normal granular layer function, as deficiency in the protease inhibitor LEKTI leads to elevated degradation of Dsg1 accompanied by altered desquamation and impaired barrier function in a mouse model of Netherton syndrome (Descargues *et al.*, 2005).

Perspectives and prospects for the future

The last 20 years have seen tremendous progress in understanding the protein–protein interactions in desmosomes responsible for anchoring intermediate filaments to intercellular junctions as well as the importance of this tethering function for adhesive strength and tissue integrity. The accelerated pace of discovery of human mutations in desmosome proteins during the last 5 years has intensified interest in intercellular junction assembly and function in human heart and skin. These studies have revealed that desmosome molecules serve not only as structural ties that bind intermediate filaments to the plasma membrane, but also as regulators of cell signaling and differentiation. Recent work on cellular responses triggered by autoimmune antibodies against desmosomes is also prompting investigators to reconcile the diversity of signaling responses and cellular pathways affected. Further work to expose these interdependent adhesive networks will be important in determining the best targets for therapeutic strategies to interfere with the pathogenesis of diseases targeting the desmosome.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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