

## Review

# Desmosomes and disease: an update

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**Summary.** Desmosomes play a critical role in the maintenance of normal tissue architecture. Skin blistering can occur when desmosomal adhesion is compromised by antibodies in autoimmune diseases such as pemphigus. Inherited mutations in genes encoding desmosomal constituents can adversely affect the skin, and result in heart abnormalities. Desmosomes may have a tumour suppressor function: expression of desmosomal components is reduced in some human cancers, and desmosomal cadherins have the capacity to suppress the invasiveness of cells in culture. Transgenic animal research has provided important insights into the role of these junctions in normal epithelial morphogenesis and disease.

**Key words:** Desmosome, Cadherin, Armadillo, Plakin, Disease

### Introduction

Desmosomes are complex intercellular junctions that mediate cellular adhesion. They are highly organised structures of up to 0.5  $\mu\text{m}$  in diameter that appear to rivet cells together, and are particularly abundant in tissues such as epidermis that experience mechanical stress (Fig. 1). At the ultrastructural level, two identical electron-dense cytoplasmic plaques are separated by a central core region that bridges the gap between apposing cells. The intermediate filament (IF) cytoskeleton is associated with the cytoplasmic plaque regions. Desmosomes anchor keratin IFs to the membrane in epithelia, but they are also found in some non-epithelial cells such as the myocardial cells of heart, where they associate with desmin IFs, and follicular dendritic cells of lymph nodes, where they interact with vimentin IFs. By linking the IF networks of adjacent cells desmosomes provide structural continuity, and so confer mechanical strength on entire tissues. In some

human diseases desmosomal adhesion is disrupted, which can result in severe consequences for tissue integrity. This confirms the importance of desmosomes for cellular adhesion, but can lead to the misleading impression that desmosomes are simply inert 'spot welds'. The focus of this review is on the role of desmosomes in human disease and therefore by necessity I concentrate on the adhesive function of desmosomes. However, it should be borne in mind that desmosomes have an important regulatory role in epithelial morphogenesis (Runswick et al., 2001), can be rapidly modulated in situations such as wounding (Wallis et al., 2000) and may act as signalling centres (Green and Gaudry, 2000). This review is intended to update a previous article on the subject (Chidgey, 1997), and will concentrate on recent advances in the field. Other reviews that cover related topics are available (Burdett, 1998; Amagai, 1999; Garrod et al., 1999; Kowalczyk et al., 1999; McGrath, 1999).

### Molecular composition and related structures

The molecular composition of desmosomes varies in different cell types, and can sometimes depend on the location of a particular cell within a stratified tissue. The major constituents belong to three gene families: the cadherin, armadillo and plakin families. The desmosomal cadherins, of which there are six, three desmocollins (Dsc 1-3) and three desmogleins (Dsg 1-3), span the plasma membrane and are localised in the central core and membrane proximal region of the cytoplasmic plaque (Fig. 2). Armadillo family members are found in the cytoplasmic plaque and include plakoglobin (PG; also known as  $\gamma$ -catenin) and three plakophilins (PKP 1-3). In addition to their structural role, desmosomal armadillo family members may be involved in intracellular signalling: PKPs are found in nuclei as well as in the desmosomal plaque. Desmoplakin (DP), a member of the plakin family of cytoskeletal linker proteins, is located in the plaque region. DP is an indispensable component of the desmosome because it acts as a linker between other constituents of the junction and IFs. Other members of the plakin family such as envoplakin, periplakin and

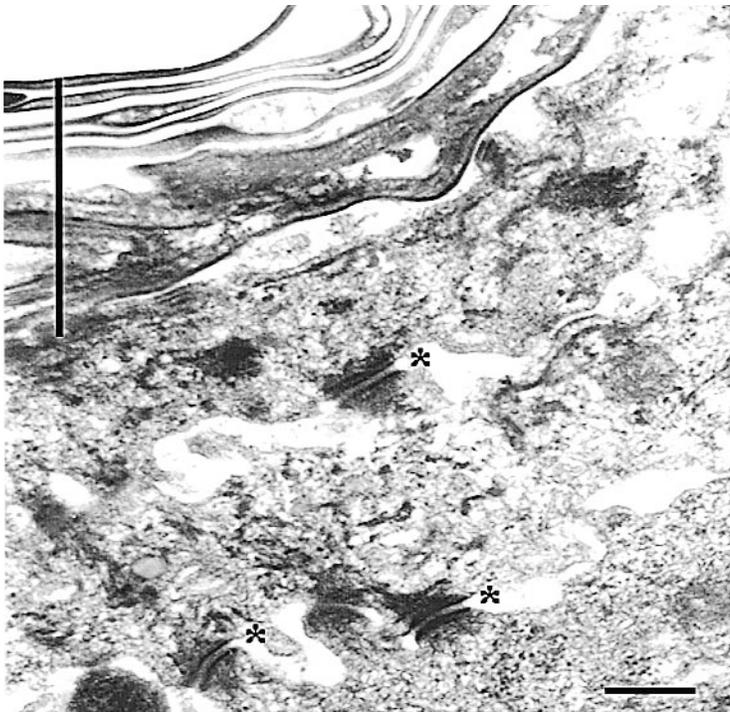
plectin are found at the periphery of the desmosomal plaque, and are likely to be involved in anchoring DP to IFs. Another protein, pinin, is recruited to mature desmosomes and is thought to play a role in stabilisation of the desmosome-IF complex. Pinin is also localised in the nucleus in various tissues and in some cultured cell lines.

Desmosome-related structures have been described. For example, in the late stages of epidermal differentiation keratinocytes are transformed into anucleated, flattened cells called corneocytes. These cells form the cornified layers, or stratum corneum (see Fig. 1). In the stratum corneum the desmosomal plaque is incorporated into the cornified envelope, a covalently cross-linked protein layer that is deposited at the cytoplasmic face of the plasma membrane, and the central core region is converted into an electron-dense plug. These structures are known as corneodesmosomes and they are retained until just below the surface of the skin where they are degraded, a process of major importance in cell shedding (desquamation). Corneodesmosin (Cdsn), a recently characterised protein, is expressed during the final stages of terminal differentiation in epidermis, and associates with the desmosomal core soon before the transformation of desmosomes to corneodesmosomes. Endothelial cells do not produce desmosomes but they do synthesise unique structures called complexus adherens junctions in which a classical cadherin, VE-cadherin, is linked to the vimentin IF network *via* the cytoplasmic desmosomal proteins PG and DP (Schmelz et al., 1994; Valiron et al., 1996; Kowalczyk et al., 1998).

### Desmosomal cadherins

Desmocollins 1-3 are the products of three distinct genes, each of which generates a pair of proteins of different sizes (the 'a' and 'b' forms) by alternative splicing. The 'a' and 'b' proteins differ only in the size of their cytoplasmic domains with the 'a' form possessing the longer of the two. Three distinct genes also encode Dsgs 1-3. All six desmosomal cadherin genes are clustered at chromosome 18q12.1 (Hunt et al., 1999). The Dscs and Dsgs are type 1 transmembrane proteins that show tissue-specific patterns of expression. In simple epithelia such as colon only Dsc2 and Dsg2 are produced whereas in epidermis, a stratified squamous epithelium, all six are expressed. In epidermis the situation is complicated by the fact that expression of desmosomal cadherins is differentiation-specific. Thus expression of the '1' proteins is largely confined to upper, terminally differentiating strata whilst expression of the '3' proteins is strongest in lower layers (Fig. 3). All desmosomes possess at least one Dsc and one Dsg but there appears to be no barrier to the presence of more than one of each (North et al., 1996). As a result the ultimate cadherin composition of a desmosome in epidermis may be very complex, particularly in intermediate layers where all 6 isoforms are expressed.

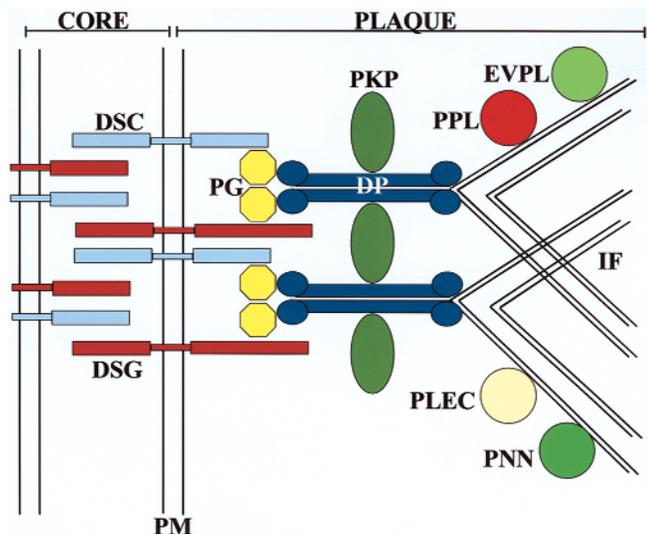
Little is known about the ways in which desmosomal cadherins interact with each other to generate adhesion. Transfection experiments in cultured mouse fibroblasts, which do not produce desmosomes, have shown that expression of one desmosomal cadherin alone, be it a Dsc (Chidgey et al., 1996) or Dsg (Amagai



**Fig. 1.** Ultrastructure of the upper layers of mouse epidermis. Desmosomes are indicated by asterisks and the vertical bar shows the position of the cornified layers. Bar: 0.5  $\mu\text{m}$ . Image courtesy of S. Kirk, University of Manchester.

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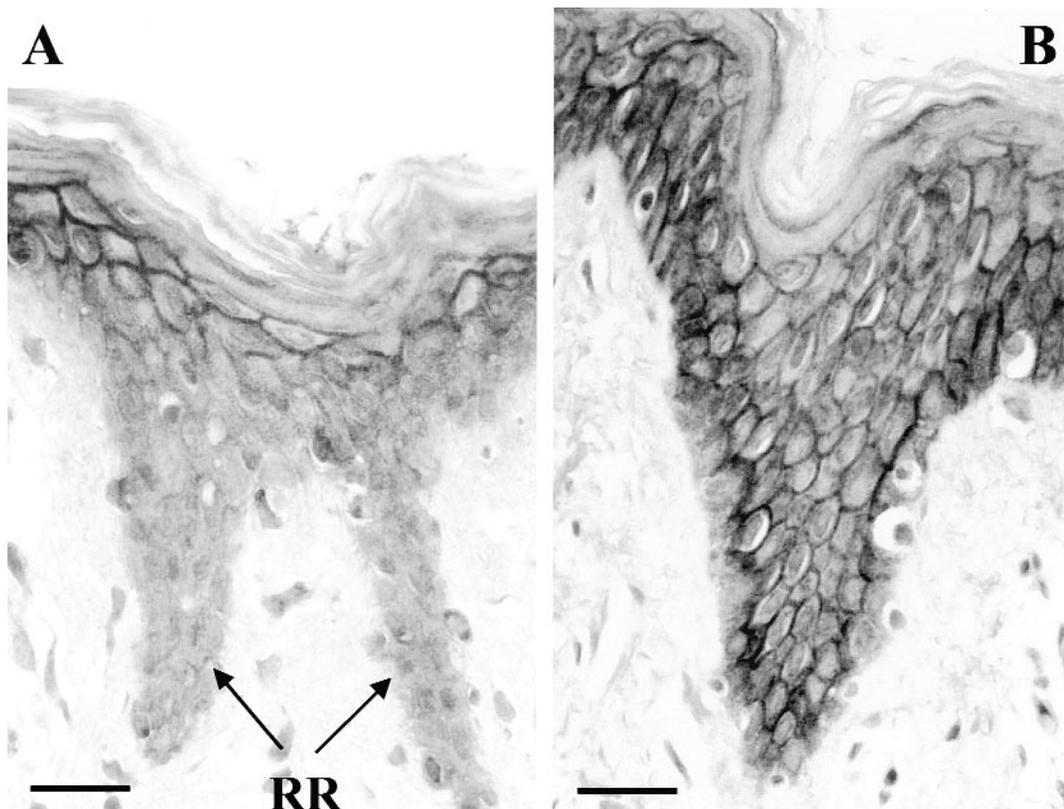
et al., 1994), is insufficient to generate strong cell-cell interactions. However, it is possible to generate adhesion in aggregation assays by transfecting cells with a Dsc,



**Fig. 2.** Molecular model of a desmosome. The major desmosomal constituents, their approximate location and some potential interactions are shown. DSC: desmocollin; DSG, desmoglein; PG, plakoglobin; DP, desmoplakin; PKP, plakophilin; PPL, periplakin; EVPL, envoplakin; IF, intermediate filament; PLEC, plectin; PNN, pinin; PM, plasma membrane.

Dsg and PG (Marcozzi et al., 1998; Tselepis et al., 1998), although full desmosome assembly does not occur and the transfected proteins do not interact with the cell cytoskeleton. These experiments, and those of Chitaev and Troyanovsky (1997), suggest that desmosomal adhesion is mediated by heterotypic (i.e. Dsc-Dsg) interactions between apposing cells. However, additional homotypic interactions cannot yet be fully discounted. In addition both hetero- and homotypic lateral contacts between desmosomal cadherins expressed by the same cell are possible. At present, it is not known whether desmosomal cadherins show inherent differences in their adhesive properties. For example, one might predict that the '3' isoforms are the least adhesive to facilitate cell proliferation and motility in lower layers of the epidermis, whilst the '1' isoforms are the most adhesive to generate strong resistance in upper layers to mechanical stress and abrasion. Unfortunately there is currently no model system that can easily be used to test such a prediction.

The adhesive function of the desmosomal cadherins has been confirmed by gene targeting experiments in mice. Targeted disruption of mouse *Dsg3* causes loss of keratinocyte cell adhesion with epidermal splitting (acantholysis) occurring just above the basal cell layer where *Dsg3* is most strongly expressed (Koch et al., 1997). Acantholysis also occurs in mice lacking *Dsc1* although in these animals loss of adhesion takes place in the upper, granular layers of the tissue (Fig. 4) where

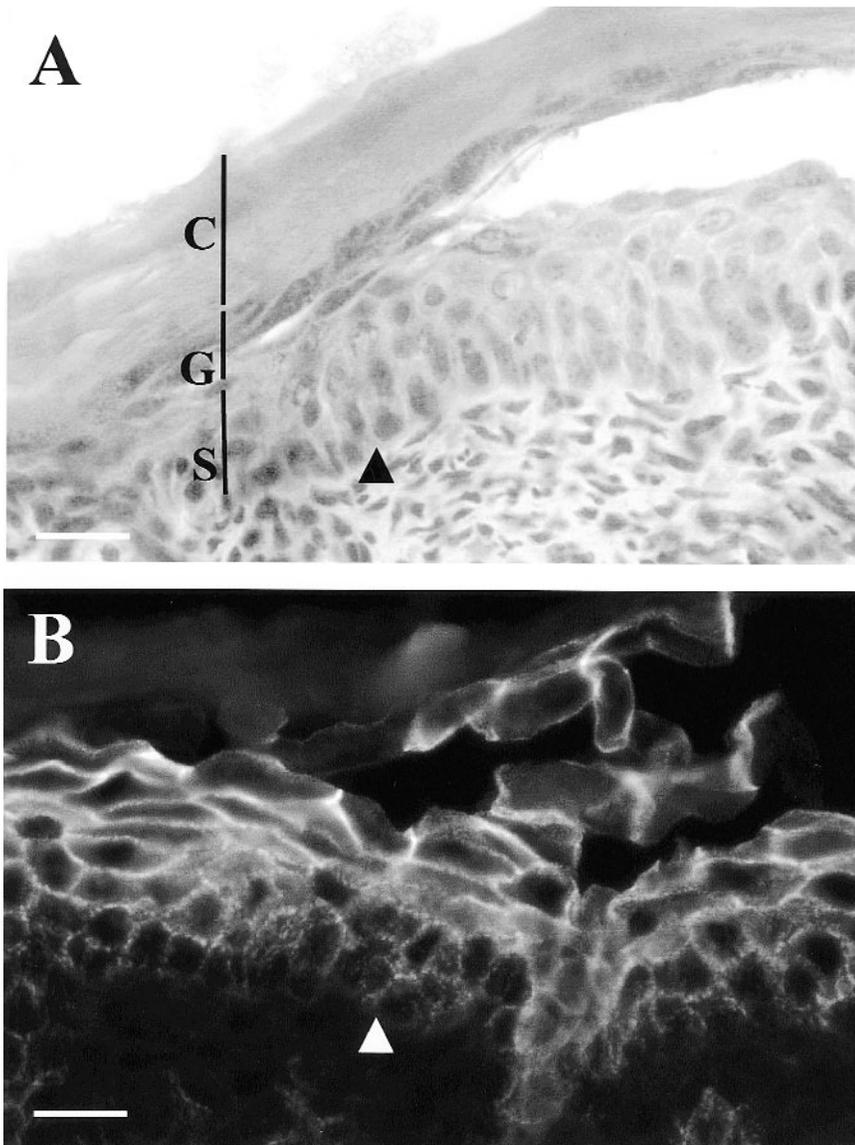


**Fig. 3.** Expression of desmocollins in human epidermis. **A** Dsc1. **B** Dsc3. Dsc1 is most strongly expressed in upper cell layers and is largely absent from rete ridges (RR) whilst Dsc3 expression is strongest in the lower layers of rete ridges. Note that individual desmosomes are not visible at the magnification shown. Bars: 25  $\mu$ m.

expression of Dsc1 is strongest, and in neonatal mice results in the formation of localised lesions that compromise skin barrier function (Chidgey et al., 2001). Perhaps surprisingly, the distribution of other desmosomal components, and the ultrastructure of desmosomes are apparently normal in both Dsg3 and Dsc1 null mice. Both Dsg3 and Dsc1 are expressed in the hair follicle (King et al., 1997). Absence of Dsg3 results in a loss of adhesion between the hair bulb and the cells of the outer root sheath and causes hair loss at a specific (telogen) phase of the hair cycle (Koch et al., 1998). In contrast absence of Dsc1 apparently has no effect on the hair cycle, although Dsc1 null mice often develop alopecia and chronic dermatitis in later life (Chidgey et al., 2001).

The importance of desmosomes for normal skin

barrier function is further illustrated by recent experiments in which full-length Dsg3 was expressed under the control of the involucrin promotor (i.e. throughout the tissue rather than only in the deep epidermis) in transgenic mice (Elias et al., 2001). In these animals the epidermal stratum corneum is abnormal with gross scaling. Mice die shortly after birth due to severe dehydration, with loss of barrier function caused by premature dissolution of corneodesmosomes and loss of adhesion between corneocytes. It is not clear why weakened corneocyte adhesion should result from the misexpression of Dsg3. One possibility is that Dsg3 interferes with the adhesive function of Dsg1 (which is normally expressed in upper epidermal layers) and is itself either non-functional in the upper epidermis or intrinsically less adhesive than Dsg1. In light of these



**Fig. 4.** Desmosomes are essential for normal epidermal adhesion. Epidermal splitting without cell lysis (acantholysis) in the upper epidermis of a desmocollin 1 knockout mouse as detected by: **(A)** H&E staining; **(B)** immunofluorescent staining, using an antibody specific for desmoplakin. Arrowheads indicate the basal cell layer. S, spinous layers; G: granular layers; C: cornified layers. Bars: A, 50  $\mu\text{m}$ ; B, 25  $\mu\text{m}$ .

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data it is perhaps surprising that misexpression of full-length Dsc1 in basal layers of transgenic mouse epidermis has no discernable effect on either desmosome ultrastructure or skin histology (Henkler et al., 2001).

Pemphigus is a human autoimmune blistering disease that has two main forms: pemphigus foliaceus (PF) and pemphigus vulgaris (PV). In PF patients possess antibodies directed against Dsg1 and develop blisters of the superficial epidermis. In early PV autoantibodies against Dsg3 result in mucous membrane lesions. In later forms of the disease patients develop additional autoantibodies against Dsg1 and develop the mucocutaneous form with blisters in both mucous membranes and deep epidermis. These differences in clinical profile can, at least partially, be explained by the distribution profile of Dsgs in target tissues (Shirakata et al., 1998; Mahoney et al., 1999a). In PF, antibodies against Dsg1 cause superficial blisters in epidermis because, of the Dsgs, only Dsg1 is expressed in significant amounts in upper layers. Similarly, in early PV autoantibodies against Dsg3 cause mucosal blistering because Dsg3 is the only Dsg that is expressed to any great extent in human mucous membranes. Epidermal blistering does not occur in early PV because Dsg1 is present in all layers of skin (although it is most strongly expressed in upper layers), and presumably is able to compensate for loss of Dsg3 function. In the mucocutaneous form of the disease epidermal blistering occurs as the result of the combined effects of autoantibodies directed against both Dsg3 and Dsg1. As anti-Dsg1 antibodies are required in late PV the question arises as to why the blisters are invariably located in immediately suprabasal cell layers. It may be that lower layers are more exposed to pathogenic antibodies that penetrate from the dermis or that cell adhesion is weaker in this part of the tissue (Mahoney et al., 1999a). Overall, it appears that the presence of one Dsg is sufficient to protect tissues from the pathogenic effects of autoantibodies directed against another. Experiments carried out using transgenic animals have provided evidence that supports this model. For example, expression of Dsg3 in the superficial layers of epidermis markedly diminishes the ability of PF IgG to induce superficial blister formation (Wu et al., 2000). Furthermore, in Dsg3 null mice PF IgG is sufficient to cause blister formation in epidermis (as expected) and mucous membranes (Mahoney et al., 1999a).

The mechanisms that allow sensitisation to self antigens and result in the generation of pathogenic autoantibodies in pemphigus are not understood, although both environmental and genetic factors are likely to be involved (Anhalt and Diaz, 2001). An animal model for the active form of PV has recently been developed. Recombinant mouse Dsg3 was injected into Dsg3 null mice, splenocytes isolated and transferred to immunodeficient mice that express Dsg3. The recipient mice produced anti-Dsg3 antibodies and developed a PV phenotype (Amagai et al., 2000b). This model does not help define the factors that lead to the

loss of self-tolerance in the initial stages of the disease, but may be useful for evaluating potential therapies.

Steric interference by autoantibodies that prevent desmoglein molecules on adjacent cells from interacting is the most likely mechanism for blister formation in pemphigus. The phenotype of Dsg3 knockout mice (Koch et al., 1997), which show many similarities to that of patients with PV, is consistent with this idea. However there are other possibilities. In cultured cells PV IgG induces the phosphorylation of Dsg3 and dissociation of PG (Aoyama et al., 1999). PV IgG has also been shown to cause the retraction of keratin filaments in cultured keratinocytes from normal, but not PG null, mice (Caldelari et al., 2001). Together these data suggest that PG may have a role in the aetiology of pemphigus. Other molecules may also be important. For example it has been suggested that blistering in PV is the result of synergism between anti-Dsg and anti-cholinergic receptor antibodies (Nuygen et al., 2000a,b). Plasminogen activator activation has long been suspected of playing a part in the disease process. However pemphigus autoantibodies are pathogenic in both urokinase plasminogen activator and tissue-type plasminogen activator knockout mice (Mahoney et al., 1999b) so it is unlikely that either of these enzymes has a primary role in the pathogenesis of the disease.

A number of other autoimmune blistering diseases that affect desmosomes and their constituents have been described. IgA pemphigus (sometimes known as intercellular IgA dermatosis) is characterised by the presence of blisters, neutrophilic infiltration and depositions of IgA autoantibodies at the epidermal cell surface (see Robinson et al., 1999). There are two forms of this disease: the intraepidermal neutrophilic form where pustules occur in the lower epidermis and the target antigen has yet to be fully characterised, and the subcorneal pustular dermatosis (SPD) form where the pustules occur in the upper epidermis and the target antigen is thought to be Dsc1 (Hashimoto et al., 1997). At present it is not known whether the anti-Dsc1 antibodies in serum from SPD patients are pathogenic. However it is of interest that Dsc1 null mice develop lesions that resemble those found in patients (Chidgey et al., 2001). Pemphigus herpeticiformis is a pemphigus variant that is also characterised by subcorneal pustules and neutrophilic infiltration. The autoantigen in this disease, at least in the majority of cases, is Dsg1 (Ishii et al., 1999). In this disease, as in IgA pemphigus, the mechanism of neutrophilic recruitment is not known. However, it has been suggested, at least in the case of pemphigus herpeticiformis, that the cytokine IL-8 may be important (O'Toole et al., 2000).

Exfoliative toxin A, produced by *Staphylococcus aureus*, causes epidermal blistering in a rare disease called staphylococcal scalded-skin syndrome (SSSS; also known as Ritter disease), a more extensive and severe form of bullous impetigo. In SSSS infection results in release of toxin into the circulation and widespread blistering, whilst the effects are restricted in

the milder bullous impetigo. In both diseases blisters occur in the superficial layers of the epidermis and resemble those found in PF. The explanation for these observations has recently been revealed: exfoliative toxin A specifically cleaves Dsg1 (Amagai et al., 2000a) and results in a loss of desmosomal adhesion and epidermal splitting.

The palmoplantar keratodermas (PPKs) are a diverse and heterogeneous group of genetic skin diseases that primarily affect the palm and sole. The striated form is characterised by longitudinal hyperkeratotic lesions on the palms and localised keratin masses on the soles. Inherited mutations in the gene encoding Dsg1 results in the striate PPK phenotype (Rickman et al., 1999; Hunt et al., 2001). The mutations segregate in an autosomal dominant fashion and the majority so far described occur in DNA encoding the extracellular domain and result in truncated proteins. One, which introduces a stop codon in DNA coding for the N-terminal pro-peptide, effectively results in a null allele, suggesting that the disorder is due to haploinsufficiency.

### Armadillo proteins

The armadillo family is characterised by the presence of a central domain, consisting of a variable number of imperfect, 42 amino acid armadillo (arm) repeats (see Hatzfeld, 1999). It includes  $\beta$ -catenin, a protein found in adherens junctions (AJs), which link classical cadherin adhesion molecules to the actin cytoskeleton via  $\alpha$ -catenin. The arm family also includes PG, which is found in both AJs and desmosomes, and PKPs. In desmosomes PG and PKPs are located in the portion of the intracellular plaque (Fig. 1) adjacent to the plasma membrane (North et al., 1999). PG apparently does not associate with Dsc 'b' proteins, but it does interact with the cytoplasmic domains of Dsc 'a' forms and Dsgs (Trojanovsky et al., 1993). It also interacts with DP suggesting a linear chain of desmosomal cadherin-PG-DP interactions (Bornslaeger et al., 2001), although PG independent desmosomal cadherin-DP associations have been reported in *in vitro* overlay assays (Smith and Fuchs, 1998).

The participation of desmosomal proteins in intracellular signalling has yet to be clearly defined. In contrast, the role of  $\beta$ -catenin in the Wnt signalling pathway, which is involved in the determination of cell fate during embryonic development, is well established. A pool of free  $\beta$ -catenin is found in the cytoplasm of cells: the size of this pool is tightly regulated by a complex including glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), the tumour suppressor protein adenomatous polyposis coli (APC) and a scaffolding protein axin. Phosphorylation by GSK3 $\beta$  targets  $\beta$ -catenin for degradation but in response to binding of the secreted morphogen Wnt to its receptor, the phosphoprotein dishevelled inhibits GSK3 $\beta$  leading to hypophosphorylation of  $\beta$ -catenin, its accumulation in the cytoplasm and subsequent translocation to the

nucleus. Nuclear  $\beta$ -catenin is involved in the transcriptional activation of Wnt-responsive genes in complex with HMG-type transcription factors such as lymphoid enhancer factor-1 (LEF-1) and T-cell enhancer factors (TCFs). PG can interact with many of the same proteins as  $\beta$ -catenin (see Zhurinsky et al., 2000), although there appear to be significant differences in their activities in signalling assays. Over-expression of either  $\beta$ -catenin or PG in the early *Xenopus* embryo results in the duplication of the embryonic body axis (Funayama et al., 1995; Karnovsky and Klymkowsky, 1995). However, depletion of  $\beta$ -catenin RNA in the early embryo prevents dorsal signalling (Heasman et al., 1994) whereas depletion of PG mRNA has no effect on signalling (Kofron et al., 1997). It is possible that PG acts indirectly by preventing the degradation of  $\beta$ -catenin (Miller and Moon, 1997), or it may have a distinct,  $\beta$ -catenin-independent, role in Wnt signalling (Hakimelahi et al., 2000; Kolligs et al., 2000).

Transgenic mouse experiments have provided further evidence that suggests that  $\beta$ -catenin and PG have distinct signalling activities. Over-expression of  $\beta$ -catenin in epidermis results in *de novo* hair follicle morphogenesis and hair tumours (Gat et al., 1998) whereas PG suppresses epithelial proliferation and hair growth (Charpentier et al., 2000). Furthermore, in null mice absence of  $\beta$ -catenin signalling results in defects in anterior-posterior axis formation at embryonic day of development 5.5 (Huelsenken et al., 2000), whereas PG signalling is clearly not critical for early embryonic development as PG null mice survive until at least day 12 and die as a result of a loss of intercalated disc integrity in the heart (Bierkamp et al., 1996; Ruiz et al., 1996). Indeed some PG null mice survive until birth and show an additional skin phenotype with blistering and subcorneal acantholysis. Interestingly,  $\beta$ -catenin is localised to desmosomes in the epidermis of these mice but clearly cannot fully substitute for PG as desmosomes are reduced in number and exhibit ultrastructural abnormalities (Bierkamp et al., 1999).

Heart and skin abnormalities are also seen in the human autosomal recessive Naxos disease. This disorder is characterised by arrhythmogenic right ventricular cardiomyopathy (ARVC), diffuse PPK and woolly hair. Diffuse PPK, which differs from the striate form in that it presents with a thick, even hyperkeratosis over palm and sole, and woolly hair are evident from birth or shortly after. ARVC causes arrhythmias, heart failure and sudden death but does not normally manifest until about 15 years of age. Naxos disease is caused by a homozygous 2 base pair deletion in the PG gene that results in a 56-residue truncation in the C-terminal end of the protein (McKoy et al., 2000). The mutant protein clearly retains some functional activity despite the absence of the C-terminal tail as the patients' phenotype is far less severe than that of PG null mice (Bierkamp et al., 1996; Ruiz et al., 1996).

Currently three plakophilins have been described, each representing the product of a distinct gene. In

contrast to the desmosomal cadherin genes, which are clustered, genes encoding PKPs 1-3 are found on human chromosomes 1q32, 12p11 and 11p15 respectively (Bonne et al., 1998, 1999). The PKPs show tissue- and cell type-specific patterns of expression (Bonne et al., 1999; Hatzfeld, 1999; Schmidt et al., 1999). Among epithelia PKP1 is largely restricted to the upper layers of stratified tissues. In contrast, PKP2 is ubiquitously expressed in both simple and stratified epithelia (where it is usually restricted to lower layers), and non-epithelial desmosome bearing tissues such as myocardium. PKP3 shows an intermediate pattern of distribution and is generally found only in simple and stratified epithelia. PKPs 1 and 2 are also produced in numerous cell types that do not synthesise desmosomes. In these cells they have an exclusively nuclear localisation whereas in tissues that produce desmosomes PKPs have a dual localisation, being found both at the cell membrane and in the nucleus.

RNA encoding both PKP 1 and 2 is alternatively spliced. In humans the PKP1 and PKP2 'b' forms are identical to the 'a' forms but for the addition of 21 and 44 amino acids respectively in the central arm portion of each molecule (Mertens et al., 1996; Schmidt et al., 1997). In the case of PKP1, the 'b' form is found exclusively in cell nuclei, whereas the shorter 'a' protein is located in both desmosomes and nuclei (Schmidt et al., 1997). The significance of the nuclear localisation is not known although PKP2 has been detected in association with components of the RNA polymerase III holoenzyme (Mertens et al., 2001).

The precise nature of the interactions between PKPs and other desmosomal constituents are not fully understood. In reconstitution experiments in transfected cells both PKP and PG are able to interact with desmosomal cadherins and DP and recruit the latter to cell-cell borders (Bornslaeger et al., 2001). However, formation of structures that resemble desmosome-like plaques at the ultrastructural level requires expression of both armadillo proteins (Bornslaeger et al., 2001). Direct interactions between PKP and IFs have been shown in yeast two-hybrid and *in vitro* overlay assays (Smith and Fuchs, 1998; Hofmann et al., 2000). Hence PKPs may be able to interact with IFs both directly and indirectly (through DP), and their main role may be to strengthen desmosomal adhesion by increasing the number of IF binding sites at the desmosomal plaque.

The crucial role of PKPs in desmosomal adhesion has been demonstrated in ectodermal dysplasia/skin fragility syndrome. This is an autosomal recessive disease that has resulted, in all cases so far described, from mutations causing premature chain termination in both alleles of PKP1 (McGrath et al., 1997, 1999; Whittock et al., 2000). Family members of patients who are heterozygotic for mutant alleles do not show symptoms, which suggests that haplosufficiency is not a significant factor in the disorder. The disease is characterised by skin blistering, dystrophic nails and sparse hair. In epidermis desmosomes are small and

poorly formed with reduced connections to keratin IFs. Keratins are condensed and compacted around the nucleus, so adding weight to the view that PKPs play an essential role in stabilising desmosome-cytoskeleton interactions.

## Plakins

Desmoplakin is a member of the plakin family of proteins that interact with IFs and localise to IF attachment sites at the cell membrane (Ruhrberg and Watt, 1997). DPI and DPII are two proteins that are derived from the same gene and generated by alternative splicing (Green et al., 1990). In humans DPI and DPII are 330 and 260KDa in size respectively and differ only in the size of the coiled-coil rod domain that separates the two globular ends. DPs are thought to exist as homodimers and both forms appear in all desmosome-bearing tissues except heart muscle tissue where DPII is absent (Angst et al., 1990). The N-terminal globular domain of DP interacts indirectly (via PG) with desmosomal cadherins in desmosomes, and with VE-cadherin in complexus adherens junctions, whilst the C-terminal end associates with IFs. As discussed above, DP also associates with PKPs and direct interactions with desmosomal cadherins may occur.

Gene targeting experiments in mice have shown the importance of DP for early embryonic development. DP null embryos do not survive beyond day of development 6.5 due to a loss of integrity of extra-embryonic tissues (Gallicano et al., 1998). In these tissues desmosomes are dramatically reduced in number and show loss of keratin filament attachment. DP null embryos supported by wild-type extra-embryonic tissues survive longer (until E10) and display marked abnormalities in both desmosome-containing tissues such as heart muscle and epidermis, and complexus adherens junction-containing tissues such as the microvasculature (Gallicano et al., 2001). Epidermal-specific DP knockout mice survive until birth, but upon mild mechanical stress show large areas of denuded skin due to epidermal peeling (Vasioukhin et al., 2001). Despite the fact that all desmosomes in null skin lack keratin filaments, epidermal separations are most severe in the basal and spinous layers, suggesting that desmosomal adhesive function is reinforced in upper layers, perhaps by formation of the cornified envelope.

The importance of DP is further emphasised from the study of human disease. Autosomal dominant palmoplantar keratoderma is caused by mutations in the DP gene that result in a null allele and haploinsufficiency (Armstrong et al., 1999; Whittock et al., 1999). Affected skin is characterised by absence of cell-cell contact and disruption of normal desmosome-IF interactions. Desmosomes are small and of abnormal appearance. Another genetic disease involving DP is caused by an autosomal recessive, homozygous mutation that produces a premature stop codon and results in a truncated protein lacking the C-terminal tail. The disease

is characterised by dilated left ventricular cardiomyopathy, striate PPK and woolly hair (Norgett et al., 2000), and affected individuals often die in adolescence from heart failure. Again, affected skin is characterised by a breakdown of normal cell-cell adhesion. The heart and skin phenotypes are clinically distinct from those seen in Naxos disease (see above). However the two diseases do bear some similarities. In both only heart, skin and hair are affected, in spite of the much more widespread expression of both DP and PG. It is likely that both mutant DP and PG retain some activity and are able to maintain desmosomal adhesion in organs that are not subject to high levels of mechanical stress.

Envoplakin and periplakin belong to the plakin family of cytoskeletal linker proteins. They are expressed in stratified and simple epithelia (Ruhrberg et al., 1996, 1997) and are up-regulated during terminal differentiation of epidermal keratinocytes. Both associate with the desmosomal plaque and with keratin filaments and may have an accessory role in coupling desmosomes to IFs. In terminally differentiating cells envoplakin and periplakin are cross-linked into the cornified envelope and it has been suggested that these proteins form the initial scaffold on which the cornified envelope is built. However gene targeting of envoplakin does not inhibit cornified envelope assembly and the mice have no pathological phenotype (Maatta et al., 2001).

Paraneoplastic pemphigus (PNP) is mucocutaneous blistering disease that occurs in association with neoplasia, particularly malignant lymphomas. One of the characteristic features of PNP is the presence of serum antibodies which recognise a number of proteins including envoplakin, periplakin, DPI and DPII, and the hemidesmosomal component BPAG1 (see Robinson et al., 1999). Autoantibodies against Dsg3 are also present in the sera from patients with PNP and these antibodies are pathogenic when injected into neonatal mice (Amagai et al., 1998). Antibodies against the desmosomal plakins probably arise as a result, rather than a cause, of the disease although it is possible that they enter damaged cells and amplify the autoimmune response.

Plectin, which is identical to IFAP300 (Clubb et al., 2000), is another member of the plakin family. It is abundantly expressed in a wide variety of mammalian tissues and cells. Plectin is thought to anchor desmosomes and hemidesmosomes to IFs (Skalli et al., 1994). In addition, it is a versatile cytoplasmic cross-linker that is able to form cross bridges between IFs and microfilaments and microtubules (Svitkina et al., 1996). Plectin is able to bind to DP *in vitro* (Eger et al., 1997), but is likely to have a more peripheral role in desmosomes than DP. Desmosomes are not affected in plectin knockout mice (Andra et al., 1997) and no abnormalities in desmosomes have been reported in the disease muscular dystrophy with epidermolysis bullosa simplex (MD-EBS), which is characterised by structural failure in both muscle and skin (at the level of the

hemidesmosome), and is caused by mutations in the plectin gene (McLean et al., 1996; Smith et al., 1996).

### Other desmosomal constituents

Pinin is a widely expressed protein that appears to localise to both desmosomes and nuclei (Brandner et al., 1997; Ouyang, 1999; Shi and Sugrue, 2000). It is absent from newly formed junctions but associates with the cytoplasmic plaque of mature desmosomes (Ouyang and Sugrue, 1992). Yeast two hybrid screens indicate that pinin is able to directly interact with IFs and transfection of the pinin cDNA into cultured cells results in enhanced cell-cell adhesion and increased IF organisation (Ouyang and Segrue, 1996). It is likely that pinin, like plectin, is not an integral part of the desmosome but acts at the periphery to stabilise the desmosome-IF complex. The nuclear role of pinin is unknown. Pinin may be important for tumour progression: down-regulation of pinin expression has been observed in transitional cell carcinomas and renal cell carcinomas (Shi et al., 2000).

Corneodesmosin (Csdn) is a secreted protein that is expressed during late keratinocyte differentiation, and is located in lamellar bodies (specialised secretory vesicles) in the cells of the upper spinous and granular layers. When migrating keratinocytes reach the upper, granular layers Csdn is transported to the cell surface and secreted into the extracellular space where it associates with the core of desmosomes, just before their transformation into corneodesmosomes (see Simon et al., 1997). It has been suggested that Cdsn may be important for corneocyte adhesion in lower layers of the stratum corneum, and its proteolytic degradation may be required for desquamation (Guerrin et al., 1998; Simon et al., 2001). It has a high glycine content, which implies a potential for adhesive loop structures at the cell surface, but experimental evidence for an adhesive role is not currently available. The gene encoding Csdn is located on chromosome 6p21.3, within a susceptibility region for psoriasis, a multifactorial skin disease characterised by keratinocyte proliferation and altered differentiation, including hyperkeratosis (thickening of stratum corneum) and increased shedding of epidermal scale, with vascular changes and accumulation of inflammatory cells. Because of its location in the genome and its putative role in corneocyte maturation, the Cdsn gene is a possible candidate gene for genetic susceptibility to psoriasis (Tazi Ahnini et al., 1999).

### Desmosomes and cancer

The role of desmosomes in human cancer is not yet clear, but some evidence is beginning to emerge that suggests that they may play a role in the progression of the disease. A recent report has described three PG mutations in two chemically induced invasive carcinomas of murine bladder (Shiina et al., 2001). Mutations that affect presumptive GSK3 $\beta$  phosphorylation sites of  $\beta$ -catenin are common in a

variety of cancers (see Polakis, 2000), and are thought to lead to elevated intracellular levels of the protein, and uncontrolled proliferation through the inappropriate transcriptional activation of  $\beta$ -catenin:TCF target genes such as c-myc and cyclin D1. The mutations described by Shiina et al. (2001) occurred down-stream of the GSK3 $\beta$  phosphorylation site, suggesting that stabilisation may not be required for the putative oncogenic activity of PG, although one mutation in the GSK3 $\beta$  phosphorylation site has been described in a gastric cancer cell line (Caca et al., 1999). In contrast to wild-type  $\beta$ -catenin, unmutated PG has the ability to transform cells in culture, through activation of c-myc (Kolligs et al., 2000) and induction of the anti-apoptotic protein Bcl-2 (Hakimelahi et al., 2000). These data, which suggest that PG may have oncogenic activity, are at odds with experiments that demonstrate that over-expression of PG in transformed cell lines, even those which do not express desmosomal or AJ proteins, suppresses their tumorigenicity in nude mice (Simcha et al., 1996). Furthermore, reduced expression of PG correlates with adverse outcome in patients with a number of different cancers. In some cases such as prostate (Morita et al., 1999), oesophageal (Nakanishi et al., 1997), and non-small cell lung cancer (Pirinen et al., 2001) this appears to be a part of a general loss of expression of AJ components, whilst in others such as neuroblastoma, one of the most common extracranial solid tumours in children, only PG is affected (Amitay et al., 2001). A tumour suppressor role for PG is also inferred from the loss of heterozygosity (LOH) that has been observed at the PG locus on chromosome 17q21 in some sporadic breast and ovarian cancers (Aberle et al., 1995).

LOH in the region of chromosome 18 containing the desmosomal cadherin gene cluster has been observed in squamous cell carcinoma of oesophagus (Karkera et al., 2000) and head and neck (Takebayashi et al., 2000). Immunohistochemical studies have shown a general reduction in expression of desmosomal components in a number of cancers, and correlated reduced staining with invasive and metastatic behaviour. For example in oral squamous cell carcinoma expression of Dscs, Dsgs and DP is inversely correlated with differentiation status and lymph node metastasis (Shinohara et al., 1998). Similar results have been obtained in studies of squamous cell carcinoma of the oesophagus (Natsugoe et al., 1997) and skin (Krunic et al., 1998). Melanocytes are specialised cells that produce the pigment melanin, and are dispersed among keratinocytes in skin. Dsg1, the only desmoglein expressed by melanocytes, and E-cadherin are down-regulated during development of melanoma, a process that may involve autocrine secretion of hepatocyte growth factor by melanoma cells (Li et al., 2001). Overall, it appears that desmosomes may have a tumour invasion and metastasis suppressor role. In support of this idea experiments have shown that expression of multiple desmosomal components (Dsc1, Dsg1 and PG) in cultured fibroblasts generates adhesion

in aggregation assays, and reduces the ability of transfected cells to invade collagen gels (Tselepis et al., 1998). Adhesion is inhibited, and invasion restored, by treating the cells with specific function-blocking peptides directed against the cell adhesion recognition sites of Dsc1 and Dsg1, indicating that desmosomal adhesion specifically inhibits invasion.

It remains to be established whether reduced expression of one or more desmosomal constituents is indicative of a loss of desmosomal function and aids the progression of malignant disease. Down-regulation of desmosomal components does not occur in all cancers. For example, in colorectal cancer expression levels of Dsc, Dsg and DP do not appear to correlate with differentiation status and metastasis (Collins et al., 1990). In some cancers switching of desmosomal cadherin isoforms has been observed. In oesophageal cancer Dsc2 and Dsc3 are down-regulated whilst Dsc1, which is not expressed in normal oesophagus, is up-regulated (Tselepis et al., unpublished). Inappropriate expression of Dsc1 also occurs in colorectal cancer (Hardy et al., unpublished). At present it is not clear whether the aberrant expression of Dsc1 is merely a by-product of neoplastic progression, or whether Dsc1 has a role in the tumorigenic phenotype. It is possible that expression of Dsc1 is initiated to compensate for the reduction in expression of other Dscs but is unable to fully do so.

#### Hailey-Hailey and Darier disease

Hailey-Hailey and Darier disease are non-immunological disorders that are inherited in an autosomal dominant fashion, and are characterised by persistent blistering and erosions of the skin. Desmosome ultrastructure is disrupted in lesional skin from patients, and until recently it was thought that these diseases could be caused by defects in desmosomes. However it is now known that the primary cause of Hailey-Hailey disease and Darier disease are mutations in *ATP2C1* (Hu et al., 2000) and *ATP2A2* (Sukunthabhai et al., 1999) respectively, genes which encode sarco/endoplasmic reticulum calcium pumps. Although the mechanisms by which mutant calcium pumps cause acantholysis is not known the findings do illustrate the importance of intracellular Ca<sup>2+</sup> homeostasis for normal epidermal function.

#### Conclusion

In the past five years an astonishing amount of progress has been made in our understanding of desmosomes and their role in human disease. A detailed picture of the role of desmosomes in autoimmune blistering diseases has emerged, and inherited mutations in the genes encoding a number of desmosomal constituents (Dsg1, PG, PKP1 and DP) have been identified. Targeted mutagenesis of the Dsc1, Dsg3, PG and DP genes has been carried out in mice. Overall,

these studies have confirmed the importance of desmosomes for cell adhesion and the maintenance of normal tissue architecture, and revealed new insights into the molecular makeup of the desmosome. In human cancer, altered expression of desmosomal constituents has been observed although it remains to be seen whether this is important for the aetiology of the disease. In the long term the realisation that desmosomes play a crucial role in both autoimmune and inherited disorders may pave the way to novel and more efficient therapies.

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