

WORKING OUT THE STRENGTH AND FLEXIBILITY OF DESMOSOMES

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Desmosomes have long been regarded as essential ‘spot welds’ that externally glue together cells within a tissue, and internally anchor the cytoskeletal network of intermediate filaments. Inactivation of desmosomal components by mutation, autoimmune antibodies and bacterial toxins breaches the structural integrity of embryos and adult tissues. But desmosomes are also functionally flexible organelles that recruit molecules capable of instructing cells within a tissue to undergo proper morphogenesis and patterning.

INTERMEDIATE FILAMENT

A cytoskeletal filament, of typically 10 nm in diameter, that occurs in higher eukaryotic cells.

ADHERENS JUNCTION

A cell–cell adhesion complex that contains classical cadherins and catenins that are attached to cytoplasmic actin filaments.

TIGHT JUNCTION

A belt-like region of adhesion between adjacent epithelial or endothelial cells. Tight junctions regulate paracellular flux, and contribute to the maintenance of cell polarity by stopping molecules from diffusing within the plane of the membrane.

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Histologists and cell biologists have presumed for years that desmosomes — derived from the Greek words for ‘bound’ (desmo) and ‘body’ (soma) — are structural ‘spot welds’ that hold cells together within tissues. This idea was based on their highly ordered appearance, which was revealed by ultrastructural analysis, and their prominence in tissues that experience mechanical stress. The recent identification of mutations in many desmosomal constituents that cause skin, hair and heart defects in humans, along with phenotypic information from engineered and spontaneous mutations in mice, underscores the essential functions of specific molecular components within these adhesive complexes. So, desmosomes are assured a place in biology textbooks as intercellular junctions that are essential in maintaining tissue integrity. They achieve this by forming robust extracellular bonds that are anchored at the cell surface to the resilient INTERMEDIATE-FILAMENT-based cytoskeleton.

The intercellular-junctional complexes of epithelial cells also comprise ADHERENS and TIGHT JUNCTIONS, but these structures associate with the dynamic actin-microfilament network. Desmosomes and adherens junctions work with each other to hold epithelial sheets together, whereas tight junctions are specialized structures that are adapted to establish a diffusion barrier between the APICAL- and BASOLATERAL-membrane domains of these polarized cells. Epithelial cells are not only physically anchored to one another but are also chemically coupled by intercellular channels, which are

known as GAP JUNCTIONS, that allow for the direct exchange of small molecules between the cytoplasm of adjacent cells.

Looking beyond a reductionist view of how a generic desmosome is engineered, it becomes apparent that the function of these membrane complexes extends beyond that of ‘intercellular velcro’ — in fact, desmosomes also encode instructions that drive tissue morphogenesis, and which regulate tissue homeostasis and responses to environmental signals. In the future, an important challenge will be to define the relative contributions of the mechanical (adhesive) and chemical (non-adhesive) mechanisms that govern desmosome-dependent functions. Achieving this goal will be key to understanding why desmosomes vary in their molecular composition from cell type to cell type and during tissue differentiation.

The structural complexity of desmosomes

Desmosomes bring together proteins from at least three separate families: the cadherins, the armadillo proteins and the plakins (FIG. 1). The human desmosomal cadherin genes are tightly clustered on chromosome 18 and include four desmogleins (*DSG1–4*) and three desmocollins (*DSCI–3*; FIG. 2)^{1–3}. The desmosomal cadherins interact directly with a subset of armadillo-family members that includes plakoglobin (also known as γ -catenin) and the p120^{CTN}-related proteins, plakophilin-1–3 and p0071 (also known as plakophilin-4; REFS 4–7). The integration of keratin-based

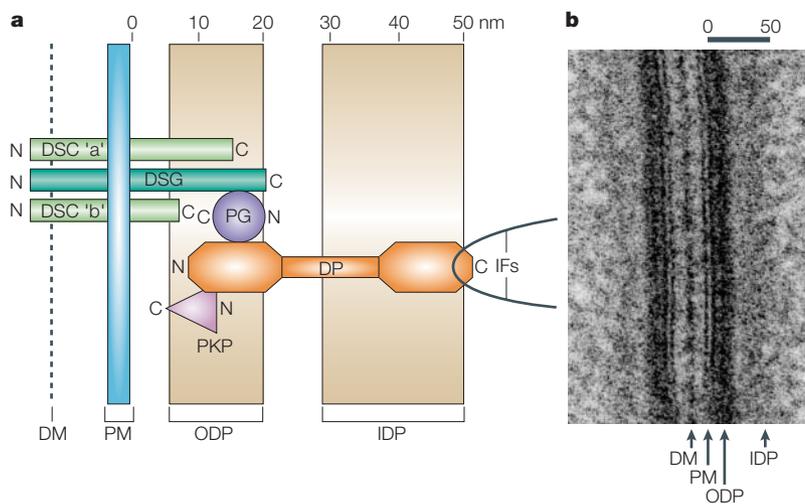


Figure 1 | A model for the structural organization of desmosomes. The relative distance (in nm) from the plasma membrane (PM) and orientation (N, amino terminus; C, carboxyl terminus) of the main desmosomal proteins (a) is compared to the ultrastructural domains of the desmosome, on the basis of immunogold localization studies (b)¹²⁹. The proteins include: the desmosomal cadherins (DSG, desmoglein; DSC 'a' and 'b', desmocollin 'a' and 'b' isoforms); the armadillo-family members plakoglobin (PG) and the plakophilins (PKP); and the intermediate-filament (IF)-binding protein desmoplakin (DP). An electron micrograph of a desmosome from bovine tongue highlights the single extracellular dense midline (DM) and the outer and inner dense plaques (ODP and IDP, respectively) in the cytoplasm of adjacent cells. Part b is reproduced with permission from REF. 130 © (1994) Elsevier.

APICAL-MEMBRANE DOMAIN
The surface of an epithelial cell that faces the lumen.

BASOLATERAL-MEMBRANE DOMAIN
The surface of an epithelial cell that adjoins underlying tissue.

GAP JUNCTION
A junction between two cells that consists of pores that allow passage of molecules (up to 1 kDa).

DESOMOSOMAL PLAQUE
An electron-dense region that is present beneath the plasma membrane of desmosomes, and which can be readily observed at the ultrastructural level.

MENINGES
Three connective-tissue layers (dura, arachnoid and pia mater) that line the outer surface of the brain and spinal cord.

TYPE I INTEGRAL MEMBRANE GLYCOPROTEIN
A single-pass transmembrane protein that contains an amino-terminal luminal domain with sugar moieties and a carboxy-terminal cytoplasmic domain.

intermediate filaments into the **DESOMOSOMAL PLAQUE** of epithelial cells is coordinated by the plakin-family member desmoplakin^{8,9}.

Desmoplakin is present in all desmosome-containing tissues and in specialized endothelial cell junctions that are known as complexus adhaerentes, but it can anchor different intermediate-filament subtypes at the cell surface¹⁰. For example, the ability of desmoplakin to link filaments that are composed of the **vimentin** protein to the complexus adhaerentes has a crucial role in the development of the microvasculature in mouse embryos, and also couples this intermediate-filament protein to the desmosomes that are present in the **MENINGES**¹¹. By contrast, the desmosomes that are present in the intercalated discs of myocardial cells, which have been shown by gene-targeting studies involving plakoglobin and desmoplakin to be essential for normal cardiac development, are connected to **desmin**^{11–13}.

The desmosomal canvas is coloured by the variable molecular components that can be found in different tissues. For instance, intestinal epithelial cells contain both plakophilin-2 and plakophilin-3, whereas hepatocytes express only plakophilin-2, which indicates that there are probably functional differences between the desmosomes of these two cell types¹⁰. The desmosomal cadherins and the plakophilins have differentiation-dependent expression patterns in the epidermis (FIG. 3), hair follicles and mammary glands^{1,2,10,14–16}. In addition, the plakin-family members envoplakin and periplakin have been found to be concentrated in the uppermost layers of the epidermis^{17,18}.

Desmosomal cadherins

Functional analyses of the desmosomal cadherins have been guided by our more advanced understanding of the closely related classical cadherins — calcium-dependent cell-adhesion molecules that are present in adherens junctions.

As adhesion molecules... The classical and desmosomal cadherins are both **TYPE I INTEGRAL MEMBRANE GLYCOPROTEINS** that contain five extracellular subdomains (ECs), the most membrane-proximal of which is sometimes referred to as an extracellular anchor domain¹ (EA; FIG. 2). A highly conserved, short amino-acid sequence that is present in the amino-terminal EC1 subdomain of the classical cadherins has been termed the cell adhesion recognition (CAR) sequence¹⁹. Garrod and colleagues designed DSG- and DSC-specific peptides that recognized the corresponding sequence in the desmosomal cadherins; these peptides were therefore designated 'CAR-site peptides'²⁰. A single CAR-site peptide was sufficient to interfere with classical-cadherin-based homophilic adhesion, but both DSG- and DSC-specific peptides were required to block adhesion in desmosome-forming cells, indicating that heterophilic interactions might be important in desmosomal-cadherin-based adhesion^{15,20}. These observations are consistent with the identification of homophilic and heterophilic DSG–DSC complexes in cells and in binding experiments *in vitro*^{21–23}. However, elucidating the relative disposition of these molecules at the cell surface continues to challenge researchers.

Cadherins are thought to interact on the surface of the same cell in a *cis* orientation and between adjacent cells in a *trans* orientation (FIG. 4). In general, classical and desmosomal cadherins form distinct dimers, although, under certain circumstances, lateral interactions between these two different cadherin-subfamily members have been described²². High-resolution structural studies of the classical cadherins provide some important clues to how these adhesive interactions are coordinated at the cell surface. For example, X-ray crystallographic analysis of the entire *Xenopus laevis* **C-cadherin ectodomain** showed that these molecules adopt a stable, curved conformation that engages in the symmetrical exchange of a highly conserved, amino-terminal tryptophan residue with another C-cadherin in a *trans* orientation; the tryptophan inserts into the hydrophobic pocket of the EC1 subdomain²⁴. The resulting structure presumably represents an adhesive dimer between adjacent cells. These and other structural studies also identified *cis* interactions between cadherin ectodomains — the molecular basis of which remains controversial — indicating that a highly ordered interdigitation of cadherins occurs along the plane of the membrane in these junctions^{24,25}.

By contrast, the use of **ELECTRON TOMOGRAPHY** to visualize the molecular organization of desmosomes from the neonatal mouse epidermis revealed a less-organized array of cadherins at the cell surface²⁶. Variable groups of between 10 and 20 desmosomal cadherins were identified that assembled into a series of discrete knots via their amino-terminal domains. The pairing of individual

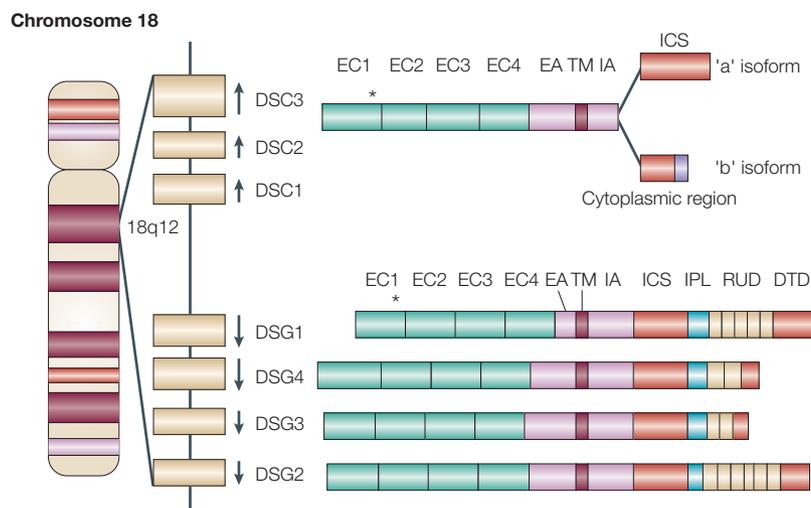


Figure 2 | The genomic and molecular organization of the human desmosomal cadherins. The four desmoglein (*DSG1–4*) and three desmocollin (*DSC1–3*) genes are clustered on the q arm of chromosome 18. These integral membrane glycoproteins are composed of four highly conserved extracellular subdomains (EC1–4), a more variable extracellular anchor (EA), a single transmembrane domain (TM), an intracellular anchor (IA) and additional cytoplasmic subdomains. EC1 contains a cell adhesion recognition (CAR) amino-acid sequence (indicated by asterisks) that contributes to the adhesive function of the desmosomal cadherins. The desmocollin 'a' and 'b' isoforms contain, respectively, an intracellular cadherin-typical sequence (ICS), which is also present in the desmogleins, and a shorter cytoplasmic region that contains unique sequences (shown in purple). The desmogleins possess an extended cytoplasmic region downstream of the ICS that comprises an intracellular proline-rich linker domain (IPL), a variable number of repeated-unit domains (RUD) and a desmoglein-specific terminal domain (DTD).

cadherins on adjacent cells resulted in three predominant geometrical shapes that resembled the letters W, S and λ . By modelling the crystal structure of the C-cadherin ectodomain onto these tomographic maps, the authors determined that a flexible intermolecular exchange of the amino-terminal tryptophan residue was consistent with the organization of the respective cadherins into these conformations and might accommodate both *cis* and *trans* interactions within desmosomes.

Calcium is also required to maintain the structural conformation and homodimerization of classical cadherin ectodomains and seems to be essential for the recognition of desmosomal cadherins by autoimmune pemphigus antibodies and a bacterial toxin, exfoliative toxin-A (BOX 1)^{27,28}. This probably contributes to the calcium-dependence of desmosomal-cadherin function that occurs during the initial assembly of these junctions in epithelial cells that have been induced to form stable cell–cell contacts.

...and regulators of morphogenesis. Recent studies have uncovered evidence that desmosomal cadherins have crucial roles in the formation and organization of complex tissues. For example, desmosomal CAR-site peptides are capable of disrupting the patterning of mammary epithelial cells in three-dimensional cultures¹⁵. Furthermore, alterations in the normal DSG and DSC expression patterns have a profound effect on morphogenesis in humans and mice. Mutations in the human

DSG1 gene cause striate palmoplantar keratoderma, an epidermal-thickening disease, whereas *DSG4* mutations result in defective hair-follicle differentiation^{2,29}. By contrast, the targeted deletion of the gene that encodes the more broadly expressed desmosomal cadherin Dsg2 results in early embryonic lethality in mice³⁰.

Desmosomal cadherins might function as molecular sensors that regulate cellular behaviour, an idea that is further supported by the various epidermal differentiation and barrier-formation defects that result from the targeted deletion of *Dsc1* or forced overexpression of *Dsg3* or *Dsc3* in the uppermost layers of the skin, where they are not normally expressed at high levels (C. Byrne, personal communication)^{31–33}. In the *Dsc3* transgenic mice, these morphogenetic changes correlated with alterations in the intracellular signalling pathways that involve β -catenin — an adherens-junction protein that is also capable of interacting with transcription factors of the lymphoid-enhancer binding factor/T-cell factor (LEF/TCF) family — altering transcriptional activity in the nucleus and regulating cell-fate decisions in the murine epidermis and its appendages^{34,35}.

The complex phenotypes that are seen in these transgenic models therefore raise the possibility that desmosomal cadherins participate in intracellular signalling processes. Culturing human KERATINOCYTES with anti-*DSG3*-containing sera from patients with the autoimmune blistering disease pemphigus vulgaris results in the phosphorylation of *DSG3*, its dissociation from plakoglobin and a transient increase in intracellular calcium concentrations and protein kinase C activity³⁶. This lends support to the idea that desmosomal cadherins are capable of transmitting extracellular information into intracellular responses.

The unique DSG and DSC cytoplasmic domains are obvious candidates for mediating intracellular signalling (FIG. 2). Each *DSC* gene can be expressed as two isoforms: an 'a' isoform with a cytoplasmic domain that is related to that of classical cadherins, and a shorter 'b' isoform with different cytoplasmic amino-acid sequences³⁷. DSG and the *DSC* 'a' isoforms all contain a common intracellular cadherin-typical sequence (ICS) that mediates binding to plakoglobin, and additional interactions of these proteins with plakophilins, p0071 and desmoplakin have been reported^{1,4–7,38,39}. However, the DSG isoforms also contain domains downstream of the ICS that are not found in any other members of the cadherin family, including an intracellular proline-rich linker domain, a variable number of repeated-unit domains and a terminal domain. The functions of these domains remain a complete mystery. So too does the role of the *DSC* 'b' tail, although, recently, plakophilin-3 was the first desmosomal component to be shown to interact with this region⁶. Identifying further transient interactions within desmosomal complexes is challenging because of their insoluble nature, but focusing on these unique cytoplasmic regions will probably yield important information about the potential non-adhesive functions of the different desmosomal cadherins.

ELECTRON TOMOGRAPHY
A technique for modelling three-dimensional (3D) objects using a series of two-dimensional electron-microscope images.

KERATINOCYTE
A specialized epithelial cell that is present in the skin.

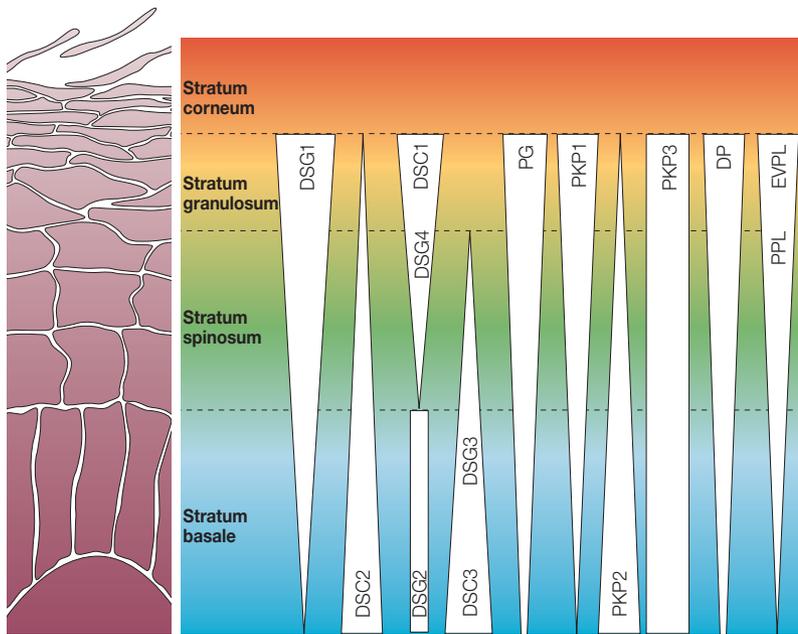


Figure 3 | Desmosomal proteins in the different epithelial cell layers of the skin. Several different desmosomal cadherin, armadillo and plaklin proteins are expressed in a differentiation-dependent manner in the mammalian epidermis. As the basal keratinocytes (in the stratum basale layer) exit the cell cycle and enter the strata spinosum and granulosum, the absolute number of desmosomes is increased (for simplicity, this is not depicted). The keratinocytes eventually enter the stratum corneum, which is composed of anucleate cells that contain chemically crosslinked desmosomal complexes, known as corneodesmosomes. DSG, desmoglein; DSC, desmocollin; PG, plakoglobin; PKP, plakophilin; DP, desmoplakin; EVPL, envoplakin; PPL, periplakin.

Armadillo proteins with dual roles

Plakoglobin in junctions... The armadillo protein plakoglobin is an adaptor that links desmosomes to the intermediate-filament cytoskeleton by directly binding the desmosomal cadherins, desmoplakin, plakophilin-2, plakophilin-3 and p0071 (REFS 4–7). Targeting the gene that encodes plakoglobin in humans and mice causes structural defects in the skin and heart and can result in embryonic lethality as a consequence of ruptured cardiac ventricles^{12,13,40}. Although desmosomes form in these null embryos, this might result from the redistribution of the highly related adherens-junction protein β -catenin to these intercellular complexes⁴¹. As β -catenin specifically binds to the tails of the classical cadherins, it is usually excluded from desmosomes. Plakoglobin, on the other hand, can bind to both desmosomal and classical cadherins, and has been proposed to be a mediator of crosstalk between adherens junctions and desmosomes (BOX 2).

Plakoglobin is also required for the retraction of keratin filaments in cultured keratinocytes in response to pemphigus serum, which thereby implicates this armadillo protein in intracellular signalling pathways downstream of the desmosomal cadherins⁴².

...and out of junctions. Armadillo, the *Drosophila melanogaster* homologue of β -catenin, was originally identified as a component of the Wnt signalling pathway and is directly implicated in transcriptional regulation³⁴.

In general, β -catenin expression is maintained at low levels in the cytoplasm by the PROTEASOME protein-degradation machinery, but its stabilization by Wnt signals allows this catenin subtype to translocate to the nucleus and bind LEF/TCF-family members, where it is thought to contribute a transcriptional-activation domain to this family of DNA-binding proteins. A corresponding non-junctional role for plakoglobin seems likely. Plakoglobin can activate LEF/TCF reporter activity in a human mesothelial cancer cell line with a homozygous deletion of the gene that encodes β -catenin⁴³. However, it has also been suggested that plakoglobin might more generally interfere with the ability of β -catenin to interact with the proteasome, thereby upregulating gene transcription through the LEF/TCF transcriptional complex by increasing β -catenin levels in the cytoplasm and nucleus^{43–45}. Alternatively, plakoglobin interactions with β -catenin–TCF4 might interfere with DNA binding and subsequent transcriptional activation, causing plakoglobin to function as a negative regulator of this signalling pathway^{44,46}. Although plakoglobin and β -catenin can compensate for some of each other's adhesive functions, the roles of these two armadillo proteins outside the context of junctions are clearly not equivalent. This was made apparent by striking differences in cellular differentiation when the two proteins were transgenically overexpressed in mouse epidermis. Whereas β -catenin induced transcriptional activity that resulted in hyperproliferation and hair-follicle differentiation in the skin, plakoglobin suppressed both of these cell-fate decisions *in vivo*^{47,48}.

Plakoglobin also interacts with tyrosine kinases, including the epidermal growth factor receptor (EGFR), and with the protein tyrosine phosphatases leukocyte-antigen-related phosphatase and protein tyrosine phosphatase- κ (REFS 49–51). The phosphorylation of plakoglobin by distinct protein tyrosine kinases differentially alters its ability to interact with desmosomal and adherens-junction proteins, but might also influence downstream intracellular signalling events that are mediated by this armadillo-family protein^{52–54}. Recent work in *X. laevis* embryos has also shown that plakoglobin regulates cell shape by maintaining the cortical-actin-, but not the cytoskeleton- or microtubule-based, cytoskeleton⁵⁵. The ability of this desmosomal armadillo protein to coordinate cytoskeletal dynamics might explain why mouse keratinocytes that are isolated from embryos that are null for the gene that encodes plakoglobin are more motile than their wild-type counterparts in single-cell-tracking experiments, where cell–cell junctions do not form (T. Yin, E. Müller and K.J.G., unpublished observations).

Plakophilins in and out of junctions. Other desmosome proteins that might function outside of desmosomes are members of the p120^{CTN}-related subfamily of armadillo proteins, the plakophilins. Plakophilins-1–3 can be found in the nucleus, as well as in junctions^{4,56}. In desmosomes, the plakophilins probably regulate the

PROTEASOME

A large multisubunit protease complex that selectively degrades intracellular proteins. Targeting to proteasomes most often occurs through the attachment of multi-ubiquitin tags.

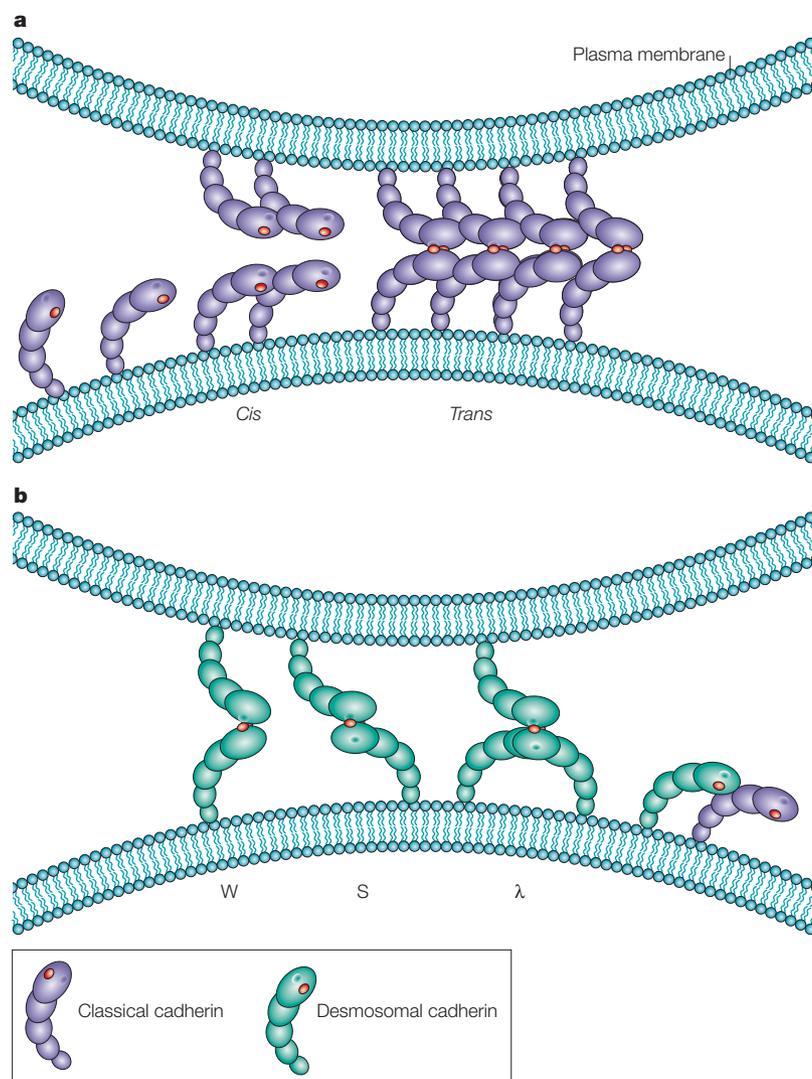


Figure 4 | Structural models for cadherin interactions at the cell surface. a | The five extracellular subdomains of classical cadherin monomers (purple) are represented as extending out from the plasma membrane and are believed to interact *trans* to form an adhesive bond with adjacent cells. *Cis* interactions, meanwhile, allow lateral dimer formation on the same cell. Judging from the crystallization of the entire C-cadherin ectodomain, it seems likely that an amino-terminal tryptophan (red sphere) inserts into the hydrophobic pocket (indentation) in the EC1 domain of an adjacent cadherin to form an adhesive dimer²⁴. Lateral extension of several dimers is thought to occur through *cis* interactions, which might be mediated by different amino acids²⁵. Lateral dimers might also form in the absence of any *trans* dimers. Interactions between the cadherin cytoplasmic domains and catenins (not shown) also probably contribute to lateral clustering. **b** | Electron-tomographic mapping of desmosomes has identified three recurring structures, resembling the letters W, S and λ, that form between desmosomal cadherins (green) at the cell surface²⁶. Modelling the C-cadherin structure onto these maps indicates that a flexible engagement of the conserved tryptophan with the hydrophobic pocket on EC1 can mediate both *cis* and *trans* interactions among desmosomal cadherins. Depletion of extracellular calcium alters the conformation of the cadherin ectodomain and results in lateral complexes forming between classical and desmosomal cadherins in cultured cells²². The molecular basis of these mixed dimers remains poorly characterized.

integrity of adhesive complexes. Patients with null mutations in the gene that encodes plakophilin-1 have skin-fragility defects that are accompanied by a redistribution of desmoplakin from the plasma membrane to the cytoplasm⁵⁷. Indeed, one of the common characteristics of all plakophilins seems to be their ability to

recruit desmoplakin to cell borders^{6,39,58,59}. Data emerging from the study of p120^{CTN} indicate that this adherens-junction-associated protein might regulate the trafficking, stabilization, clustering and recycling of classical cadherins at the cell surface^{60–62}. Similarly, the reintroduction of plakophilin-1 into keratinocytes that are isolated from humans with null mutations in the gene that encodes plakophilin-1 increased desmosomal-protein expression⁶³. Taken together, these observations point to a high degree of functional conservation between p120^{CTN} and the plakophilins.

The similarities between p120^{CTN} and the plakophilins might also be reflected in non-adhesive functions. In particular, p120^{CTN} causes dramatic changes in the shape of NIH 3T3 cells (a type of mouse fibroblast cell) by modulating the effects of RHO-FAMILY GTPASES on the actin-based cytoskeleton^{64,65}. Plakophilin-2, but not plakophilin-1 or plakophilin-3, also alters the actin cytoskeleton and shape of COS cells in a manner that is related to Cdc42 activity (X. Chen, M. Hatzfeld and K.J.G., unpublished observations). In addition, p120^{CTN} interacts with a transcriptional repressor that is known as Kaiso⁶⁶. Although plakophilins have yet to be found in a direct complex with known transcription factors, plakophilin-2 does associate with the RNA polymerase III holoenzyme and enhances LEF/TCF transcriptional activity^{5,67}.

p0071 is more closely related to p120^{CTN} than to the other plakophilins, and it localizes to both adherens junctions and desmosomes⁷. In endothelial cells, p0071 can displace p120^{CTN} from complexes that contain vascular endothelial cadherin (VE-cadherin) and binds desmoplakin, indicating that it has a unique role in regulating these specialized junctions⁶⁸. However, p0071 also contains a PDZ (Postsynaptic-density protein of 95 kDa, Discs large, Zona occludens-1)-binding motif that is not present in the other desmosomal armadillo proteins, and it interacts with the PDZ-motif-containing proteins *erbin* and *papin*^{69–71}. *Erbin* has also been detected in a complex with the receptor tyrosine kinase ERBB2 and can inhibit the mitogen-activated protein (MAP)-kinase pathway, providing another possible link between junctional complexes and intracellular signalling pathways^{72,73}. Less is known about *papin*, although it has been found to colocalize with *erbin* and *ErbB2* in MDCK (Madin–Darby canine kidney) cells⁷⁴. As PDZ DOMAINS are commonly found in proteins that are implicated in the establishment of epithelial polarity, p0071 might facilitate the organization and stability of newly established junctions.

Linking intermediate filaments to desmosomes

Molecular basis for desmosome–intermediate-filament connections. The organization of the intermediate-filament-based cytoskeleton with membrane complexes is regulated by a family of related proteins that are known as plakins⁷⁵. Ancestral members of this gene family are similar to the *D. melanogaster* protein *Shot*, which contains both actin- and microtubule-binding domains and has an essential role in a variety of morphogenetic processes^{76,77}. The inability of *shot* mutants to interact with these two cytoskeletal components at cell junctions

RHO-FAMILY GTPASE
A Ras-related GTPase that is involved in controlling the polymerization of actin.

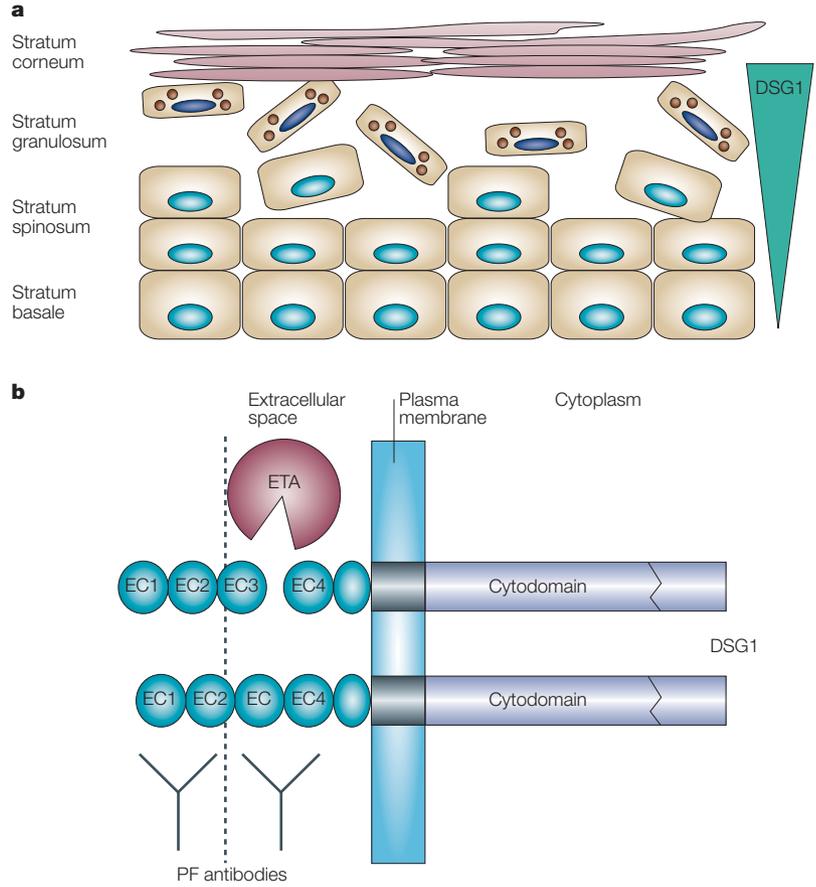
Box 1 | **DSG1 as a common target in diseases that lead to superficial skin blisters**

One of the first indications that DSG1 could mediate cell adhesion came from studies of sera that were taken from patients with a rare autoimmune disease known as pemphigus foliaceus. These sera were shown to contain anti-DSG1 antibodies that recognized the desmosomes of epidermal keratinocytes and were believed to interfere with the adhesive function of this desmosomal cadherin, resulting in cell–cell dissociation and subsequent skin erosions and blister formation in the upper layers where this molecule is most highly expressed (see part a of the figure)¹¹⁷.

A direct role for the DSG1 autoantibodies in the pathogenesis of this disease was subsequently shown by passively injecting affinity-purified anti-Dsg1 antibodies into neonatal mice¹¹⁸. The resulting superficial epidermal

blisters could be prevented by the forced overexpression of Dsg3, which is normally concentrated in the basal epidermal layers¹¹⁹. The fact that Dsg3 can compensate for the inactivation of Dsg1 might explain why the skin blisters that form in these patients occur primarily in the cellular layers of epidermis that lack high levels of Dsg3 expression, and why blisters do not form frequently in oral mucosa, where Dsg3 is abundantly expressed¹²⁰.

The exfoliative toxins (designated ETA in the figure) produced by *Staphylococcus aureus* were shown to cause superficial skin blisters in staphylococcal scalded-skin syndrome and bullous impetigo over 30 years ago¹²¹. It was not until recently, however, that the striking similarity between these diseases and pemphigus foliaceus was recognized and a common mechanism unravelled — the molecular targeting of DSG1 (REF. 122; see part b of the figure). The exfoliative toxins that are produced by these bacteria are serine proteases that are capable of cleaving within a presumptive calcium-binding site at the border between the extracellular EC3 and EC4 subdomains of DSG1, but not of cleaving the related DSG3 or E-cadherin¹²³. Consequently, this cleavage is believed to inactivate DSG1-mediated adhesion in the upper layers of the epidermis. PF, pemphigus foliaceus.



is responsible for a subset of the embryonic-lethal defects that have been described to date^{78,79}. More-recently evolved members of the plakin family might have adopted a specialized region — known as the plakin-repeat domain — to address the advent of intermediate filaments in organisms such as *Caenorhabditis elegans*⁸⁰.

Although several plakin-family members — including desmoplakin, **plectin**, **envoplakin** and **periplakin** — bind intermediate filaments and localize to desmosomes, genetic studies indicate that only desmoplakin is indispensable for these intercellular junctions. For example, plectin is present in both desmosomes and the hemidesmosomes, which connect epithelial cells to the basement membrane, but genetic defects in plectin did not alter intercellular adhesion in mice or humans^{81,82}.

Similarly, the deletion of envoplakin had little effect on murine epidermal junctions⁸³. By contrast, an autosomal dominant mutation in the gene that encodes desmoplakin has been identified in patients with striate palmoplantar keratoderma, and a recessive mutation has been linked to striate palmoplantar keratoderma, cardiomyopathy and woolly hair^{84,85}. Deleting the desmoplakin gene in mice causes lethal defects in extra-embryonic tissues⁹. Mixing wild-type tetraploid and desmoplakin-mutant diploid MORULAE to rescue these extra-embryonic phenotypes results in further problems with adhesion in the heart, brain and skin, although these embryos eventually perish as a consequence of abnormalities in the microvasculature system¹¹.

PDZ DOMAIN
(Postsynaptic-density protein of 95 kDa, Discs large, Zona occludens-1). A protein-interaction domain that often occurs in scaffolding proteins and is named after the founding members of this protein family.

MORULAE
A mulberry-like mass of early-stage embryonic cells.

Box 2 | **Molecular dialogue between desmosomes and adherens junctions**

Desmosomes are believed to have evolved after adherens junctions and form later during embryonic development, which indicates a potential hierarchical relationship in junction formation. Recent work in cells that are forming new contacts has added weight to the argument that adherens junctions form before desmosomes. Whereas adherens junctions localized to the tips of early membrane contacts, desmosomes formed in the flanking regions that were already in apposition¹²⁴. This early adhesive event coordinates junction assembly, as function-blocking antibodies that are specific for E-cadherin interfere with desmosome formation¹²⁵. The molecular sentinels that respond to the order for junction formation from E-cadherin are probably proteins that can associate with both adherens junctions and desmosomal components, which include plakoglobin, plakophilin-2 and p0071 (REFS 5,7,126). Of these, plakoglobin has been shown to trigger desmosome formation, but only on the platform of classical cadherin complexes¹²⁷.

The marriage between desmosomes and adherens junctions benefits from good communication on both sides. Keratinocytes that lack desmoplakin cannot progress beyond an early stage of adherens-junction formation⁸⁶. In some cases, however, desmosomes can even function without obvious adherens junctions. For example, fibroblastic cell adhesion following transfection with desmosomal components occurs without classical cadherins^{20,128}. Although adherens junctions and desmosomes independently promote adhesion in artificial reconstitution systems, both complexes are probably coordinated to enhance adhesive strength in epithelial cells.

Compelling evidence that the integration of intermediate filaments into desmosomes holds adult tissues together comes from studies in which desmoplakin has been knocked-out specifically in the epidermis⁸⁶. Not only were desmosome–intermediate-filament connections lost, but the structural integrity of this tissue was severely compromised. Somewhat surprisingly, a reduction in the number of adherens junctions was also found in the mutant skin and this corresponded with defects in adherens-junction maturation in keratinocytes that were isolated from these animals (BOX 2).

That actin- and keratin-based intercellular junctions are functionally interdependent was also shown using epithelial cells that can be induced to express a truncated amino-terminal polypeptide of desmoplakin. This dominant-negative mutant desmoplakin retains binding sites for plakoglobin and plakophilins but lacks the intermediate-filament-binding domain⁸. The amino-terminal mutant protein localized to, and caused clustering of, desmosomal-cadherin-containing complexes at the cell surface, but was unable to couple intermediate filaments to the plaque and so severely compromised intercellular adhesion^{8,87}. When attachments between cortical actin and the plasma membrane were also disrupted, a synergistic effect on decreased adhesion was observed, showing that these two cytoskeletal connections to junctions work together to regulate adhesive strength⁸⁷.

These studies point to a structural and functional polarity of desmoplakin (FIG. 5). The desmoplakin amino terminus contains a plakin domain that is responsible for targeting desmoplakin to junctions by mediating interactions with desmosomal armadillo proteins and possibly by directly binding the DSG1 and DSC1 cytoplasmic tails^{38,39,75}. The reintroduction into desmoplakin-deficient keratinocytes of a construct that contained the complete amino-terminal domain of desmoplakin — but which still lacked the proposed downstream, intermediate-filament-binding domains — not only allowed junction assembly to occur and restored cell adhesion, but also allowed for some intermediate-filament associations with the plasma membrane⁸⁶. This might be due to the ability of this desmoplakin subdomain to interact

with other intermediate-filament-associated proteins, including the plakophilins³⁸. Recently, a point mutation in the desmoplakin gene, which alters a putative phosphorylation site in a region that has been implicated in binding plakoglobin, was identified in patients with cardiac disease⁸⁸. This raised the possibility that defects in these protein–protein interactions might cause human disease.

Clues to the structural basis for desmosome–intermediate-filament connections. By analysing the amino-acid sequence of the carboxy-terminal domain of desmoplakin, an intrinsic code was identified that consisted of three plakin-repeat domains — called the A, B and C subdomains — with a specialized linker sequence between the B and C subdomains that is conserved in many plakin-family members (FIG. 5)⁷⁵. These sequences had previously been implicated in intermediate-filament binding by colocalization, biochemical and yeast two-hybrid studies, but we are now beginning to understand the structural basis for these interactions given the recent crystallization of the desmoplakin B and C subdomains⁸⁹. These plakin repeats adopt a globular structure that accommodates a groove lined with basic residues, and which has been proposed to contribute to intermediate filament binding. *In vitro* studies showed that individual plakin-repeat domains could interact with vimentin with low affinity, but that binding was enhanced when all three repeats were included. Although the specialized linker sequence had no effect on binding in this study, this and other carboxy-terminal sequences downstream of the plakin repeats have been shown to be sufficient for regulating the strength and specificity of intermediate-filament interactions in desmoplakin and other plakin-family members^{90,91}. So, although the relative importance of the plakin repeats and linker sequences is yet to be resolved, these studies agree that many interactions contribute to the binding of the desmoplakin tail to intermediate filaments, and it seems almost certain that different sequences within the carboxyl terminus accommodate distinct intermediate-filament proteins in various cellular contexts.

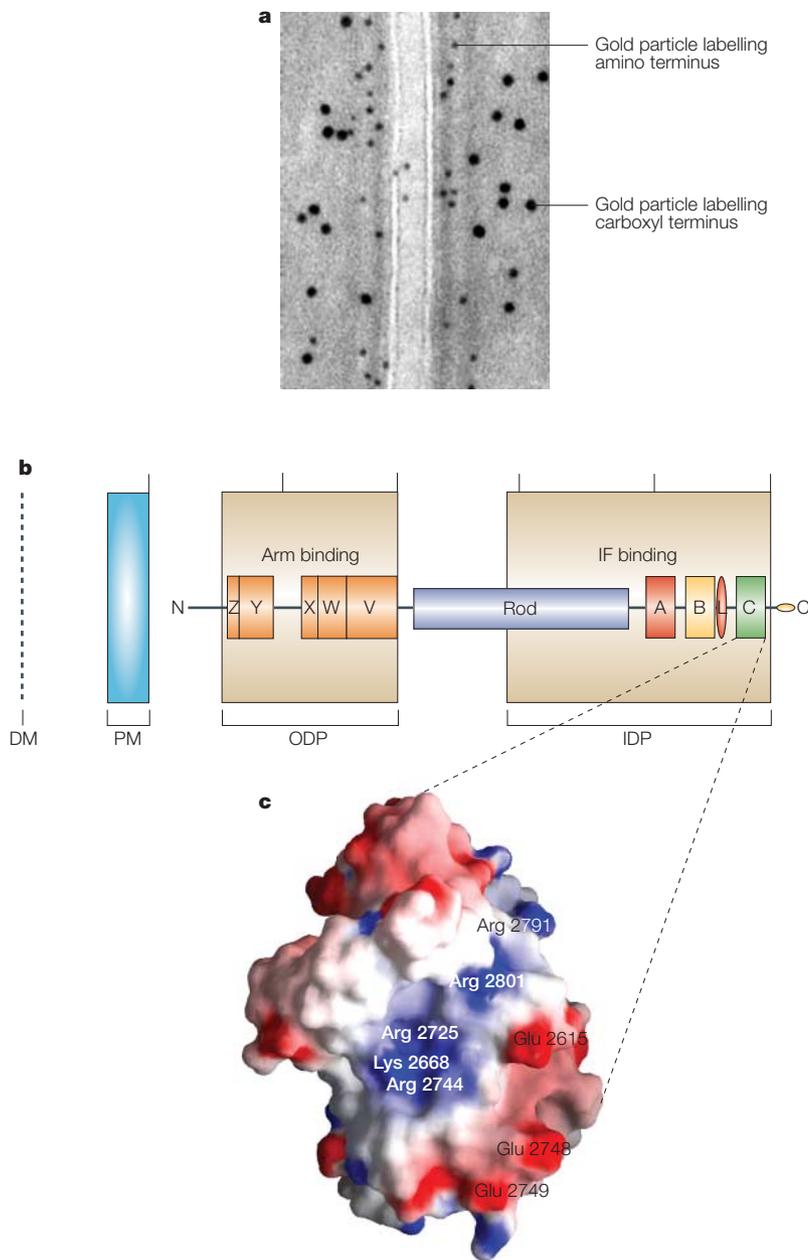


Figure 5 | Segregating the functions of desmoplakin according to its structural ends.
a | Double-labelling immunogold-localization studies of bovine epidermis, using antibodies specific for the desmoplakin amino- or carboxy-terminal ends (5 and 10 nm gold particles, respectively), showed that this protein adopts a perpendicular orientation with respect to the ultrastructural domains outlined in FIG. 1b. Reproduced with permission from REF. 129 © (1999) The Company of Biologists. **b** | The termini are bound by a central α -helical coiled-coil-rod domain that varies in length between the two alternatively spliced gene products of desmoplakin (for simplicity, only one of these products, desmoplakin I, is depicted). The amino-terminal plakin domain is predicted to form a series of α -helical bundles — termed Z, Y, X, W and V — and mediates junctional localization via the desmosomal armadillo proteins. By contrast, the plakin-repeat domains — A (red), B (yellow) and C (green) — at the carboxyl terminus of desmoplakin, along with a specialized linker subdomain (L), are involved in intermediate filament (IF) binding. **c** | A surface representation of the globular C subdomain from crystallized structures, made up of a short β -hairpin and two anti-parallel α -helices, revealed a conserved basic groove (blue) and acidic surfaces (red) in the plakin-repeat domains. Part **c** is reproduced with permission from REF. 89 © (2002) Macmillan Magazines Ltd. DM, dense midline; IDP, inner dense plaque; ODP, outer dense plaque; PM, plasma membrane; N, amino terminus; C, carboxyl terminus.

Putting it together and taking it apart

Early approaches to the study of desmosome dynamics were aimed at tracking components after junction formation was initiated, but we now know that desmosomal plaques can form even in the absence of cell–cell contacts⁹². More recently, cell biologists have attempted to reconstitute desmosomal plaques by transfecting desmosomal proteins into cultured cells^{39,59,93}. In this context, the desmosomal cadherins and desmoplakin are essential building blocks, but both plakoglobin and the plakophilins sculpt the finer structural details into these plaques. In particular, plakoglobin provides informational cues that limit desmosome size and composition, whereas the plakophilins mediate the lateral clustering of desmosomal cadherins at the cell surface; this lateral clustering, for classical-cadherin-containing complexes, is an important determinant of adhesive strength^{94,95}. The process of junction assembly in living cells is now being worked out by using fluorescently tagged desmosomal proteins. Although the desmosomal cadherins are relatively stable at the cell surface, DSC2a is rapidly redistributed to areas that undergo junctional reorganization⁹⁶.

Post-translational modification. Desmosomal proteins are remarkably stable in junctions and their extended half-life in cultured epithelial cells (>24 and >72 hours for DSG2 and desmoplakin, respectively) has focused research on the post-translational events that regulate desmosome assembly^{97,98}. For example, the DSG cytoplasmic domain is phosphorylated during trafficking to the cell surface and in response to growth factors, and the inhibition of phosphatase activity using okadaic acid could disrupt desmosome assembly^{52,99}. Desmoplakin–intermediate-filament interactions are modulated by the phosphorylation of a specific serine residue that is present in the carboxy-terminal domain and this can alter trafficking of cytoplasmic desmoplakin into newly formed cell contacts (L. M. Godsel and K.J.G., unpublished observations)¹⁰⁰. Although the phosphorylation of p120^{CTN} can influence classical-cadherin-based adhesion, similar processes that regulate the plakophilins and p0071 have not been thoroughly examined so far¹⁰¹.

Transcriptional regulation. The compositional differences of desmosomes in adult tissues and the remodeling of these junctions during embryonic development and in cancer indicate that there must be tight transcriptional controls for these junctions. A member of the zinc-finger family of transcription factors, known as Slug, disrupts epithelial desmosomes but can also bind and repress the E-cadherin promoter in certain cell lines; this might then influence desmosomal-protein expression (BOX 2)^{102–104}. Teasing apart the direct and indirect effects of transcription factors on desmosomal-protein expression will require more-thorough promoter analysis. Initial attempts to delineate the *Dsg1* promoter in transgenic mice have had only limited success, perhaps due to long-range genetic controls on expression¹⁰⁵.

14-3-3 PROTEIN

A protein that binds to two phosphoserine/ phosphothreonine-containing polypeptides to form crosslinks.

RNA INTERFERENCE

The process by which double-stranded RNA specifically silences the expression of genes by causing the degradation of their cognate mRNAs.

Conclusion and future directions

In conclusion, desmosomes are junctional complexes that coordinate with other adhesive junctions to maintain the integrity of epithelial sheets, and many of the molecular and structural details of desmosomes are beginning to reveal themselves. The desmosomal cadherins not only function as adhesion molecules at the cell surface, but probably provide the molecular basis for cell-sorting and -segregation events during development and in adult tissues. Several armadillo proteins have adopted structural roles by organizing desmosomal cadherins at the cell surface and also interact with signalling pathways in the cytoplasm and nucleus. In recent years, the requirement for desmoplakin and for its connections to intermediate filaments in maintaining tissue integrity has been confirmed, but it has become clear that there are also synergistic relationships between desmoplakins, adherens-junction maturation and actin-cytoskeleton dynamics in epithelial cells. The advent of live-cell-imaging techniques is now enabling these concepts to be refined into dynamic models for junction assembly in cultured cells.

The number of known desmosomal components continues to grow and includes two newly identified genes in mice that are highly related to human *DSG1* — the original mouse *Dsg1* has consequently been renamed *Dsg1 α* and the more recently characterized genes termed *Dsg1 β* and *Dsg1 γ* (REFS 106,107). Unravelling specific roles for the distinct Dsg isoforms in mice promises to expand our understanding of the multiplicity of desmosomal cadherin functions.

A definitive demonstration of 'outside-in' signalling through desmosomes has yet to be made and lags behind work in other intercellular junctions. The interaction between E-cadherin and the EGFR is coordinated to determine cell survival in *D. melanogaster* embryos and might explain how a loss of this classical cadherin directly regulates apoptosis *in vivo*^{49,108,109}. The desmosomal cadherins are also molecular targets during the apoptotic cascade, but their role in this cellular process remains unknown (R. L. Dusek and K.J.G., unpublished observations)¹¹⁰. The binding of cadherins to specific growth-factor receptors might also directly regulate downstream signalling events. In support of this, interactions between neuronal cadherin (N-cadherin) and the fibroblast growth factor receptor are required for cellular migration in breast cancer cell lines, and complexes

containing VE-cadherin and the vascular endothelial growth factor receptor inhibit cellular proliferation in endothelial cells^{111,112}. It seems likely that the distinct desmosomal cadherins interact with their own set of receptors to coordinate cellular-differentiation pathways during development.

In vitro binding studies have led to much progress in the area of desmoplakin-protein interactions, but how different intermediate filaments interact with the carboxyl terminus of desmoplakin remains to be discovered. The first crystals that were generated for this plak-in-family member provided the germ of an idea, but more elaborate high-resolution studies are required to truly illuminate the structural basis for these interactions. Similarly, plakoglobin and the plakophilins can be found near the cell surface, in association with the cytoskeleton and in the nucleus, but we have a poor understanding of what regulates the trafficking between these subcellular compartments. The discovery of previously unknown binding partners might provide some insight into this process, as the phosphorylation of plakophilin-2 by a CDC25C-associated kinase (C-TAK-1) has been shown to alter its ability to interact with 14-3-3 PROTEINS and to enter the nuclear compartment¹¹³. A similar set of molecular chaperones might exist for the other desmosomal armadillo proteins.

E-cadherin can interfere with the pools of β -catenin that are available for intracellular signalling and can inhibit cancer-cell invasion in an adhesion-independent manner¹¹⁴. The fact that the artificial introduction of the cytoplasmic domain of DSG1 can suppress plakoglobin-mediated axis duplication in *X. laevis* embryos indicates that desmosomal cadherins can also saturate plakoglobin and/or the plakophilins in epithelial junctions¹¹⁵. Signalling events downstream of E-cadherin can further regulate the expression levels of this classical cadherin during murine hair-follicle development and in human colon cancer cell lines — might an autoregulatory loop also exist for desmosomal molecules during tissue formation?^{104,116} In these cases, it will be important to better delineate how plakoglobin mediates intracellular signalling events independently of its effects on β -catenin. Sketching out the signalling capacity of these junctional proteins on a less redundant canvas by using RNA INTERFERENCE strategies will surely open our eyes to the multitude of pathways that emanate from desmosomes.

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The authors declare that they have no competing financial interests.

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