CHAPTER THREE

Structure and Function of Desmosomes

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Contents

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Abstract

Desmosomes are prominent adhesion sites that are tightly associated with the cytoplasmic intermediate filament cytoskeleton providing mechanical stability in epithelia and also in several nonepithelial tissues such as cardiac muscle and meninges. They are unique in terms of ultrastructural appearance and molecular composition with cell type–specific variations. The dynamic assembly properties of desmosomes are important prerequisites for the acquisition and maintenance of tissue homeostasis. Disturbance of this equilibrium therefore not only compromises mechanical resilience but also affects many other tissue functions as becomes evident in various experimental scenarios and multiple diseases.

Key Words: Desmoglein, Desmocollin, Plakoglobin, Plakophilin, Desmoplakin, Cadherin, Pemphigus, Cancer.

1. Introduction

Cell–cell adhesions are crucial for the function of multicellular organisms by providing mechanical stability and facilitating signal transmission between neighboring cells. Among the numerous cell–cell adhesion structures desmosomes are probably those that are most dedicated to mechanical coupling. Their high degree of adhesive strength is based on multiple and extremely strong noncovalent interactions between its molecular constituents. This architecture requires highly coordinated mechanisms of assembly to avoid premature association during synthesis and ectopic aggregation in various cell compartments during transport of its individual polypeptide components. Complex regulatory pathways exist to attenuate adhesive strength to specific requirements by coordinating the balance between assembly and disassembly and by controlling polypeptide turnover and degradation.

Desmosomes are especially abundant in skin and cardiac muscle, both of which have to withstand considerable mechanical stress. If these multiple "spot welds," referred to as maculae adhaerentes, are weakened either by mutation of their proteinaceous components or by environmental factors such as autoantibodies or specific microbial proteases, blisters form in the epidermis and rupture of cardiomyocytes occurs in the heart. The mechanical stability depends in large part on the tight association of desmosomes

with the intermediate filament cytoskeleton (Fig. 3.1A). While the biomechanical aspects of desmosomal function are clearly in the foreground, desmosomes are not only inert intercellular Velcro but are dynamically integrated into cell communication and signaling thereby determining properties of cell assemblies as distinct morphogenetic and functional entities that are capable of adapting to specific environments. These properties are becoming more and more apparent as *in vivo* experimentation using transgenic cell and animal systems increases in sophistication, revealing unexpected contributions of desmosomal components to diverse tissue functions.

Given the cornucopia of excellent reviews on cell–cell adhesion and, in particular, on desmosomes (Burdett, 1998; Cheng and Koch, 2004; Chidgey, 2002; Coulombe, 2002; Dusek *et al.*, 2007; Garrod *et al.*, 2002; Getsios *et al.*, 2004b; Green and Gaudry, 2000; Huber, 2003; Jamora and Fuchs, 2002; Kottke *et al.*, 2006; Kowalczyk *et al.*, 1999a; Yin and Green, 2004), we will especially focus on the complexity of desmosomal composition in different cell types, concentrate on the phenotypes observed in transgenic animals with defined molecular alterations of desmosomal components, and summarize the current knowledge on human diseases that are caused by specific desmosomal deficiencies. We intend to portray desmosomes as dynamic structures that are subject to modulation and are integrated into cellular-signaling cascades. Particular emphasis will be on the contribution of desmosomal cadherins to desmosomal function, since they are at the core of these adhesion sites not only participating directly in the adhesive process by transcellular interactions but also by providing the scaffolding onto which cytoplasmic polypeptides are recruited to mediate the anchorage of the intermediate filament cytoskeleton and to initiate manifold intracellular events.

2. Morphology

2.1. Ultrastructure of desmosomes

The Italian medical doctor Bizzozero detected desmosomes in the nineteenth century as tiny nodules by light microscopy (Bizzozero, 1864). He also recognized their bridging function between cells (Bizzozero, 1870), which had been postulated a few years before by Schrön (1865). The term desmosome, which is derived from the Greek words ὁ $\delta\varepsilon\sigma\mu\acute{o}\varsigma$ (tie) and τὸ $\sigma\tilde{\omega}\mu\alpha$ (body), was introduced by Schaffer in 1920. Upon the availability of transmission electron microscopes, the conspicuous ultrastructural morphology of desmosomes and their attached material in epidermis and other multilayered epithelia caught the attention of several morphologists (Horstmann and Knoop, 1958; Karper, 1959; Kelly, 1966; Odland, 1958; Porter, 1956). These early reports described quite accurately the insertion of densely packed "tonofibrils" into the electron-dense plaque region and the

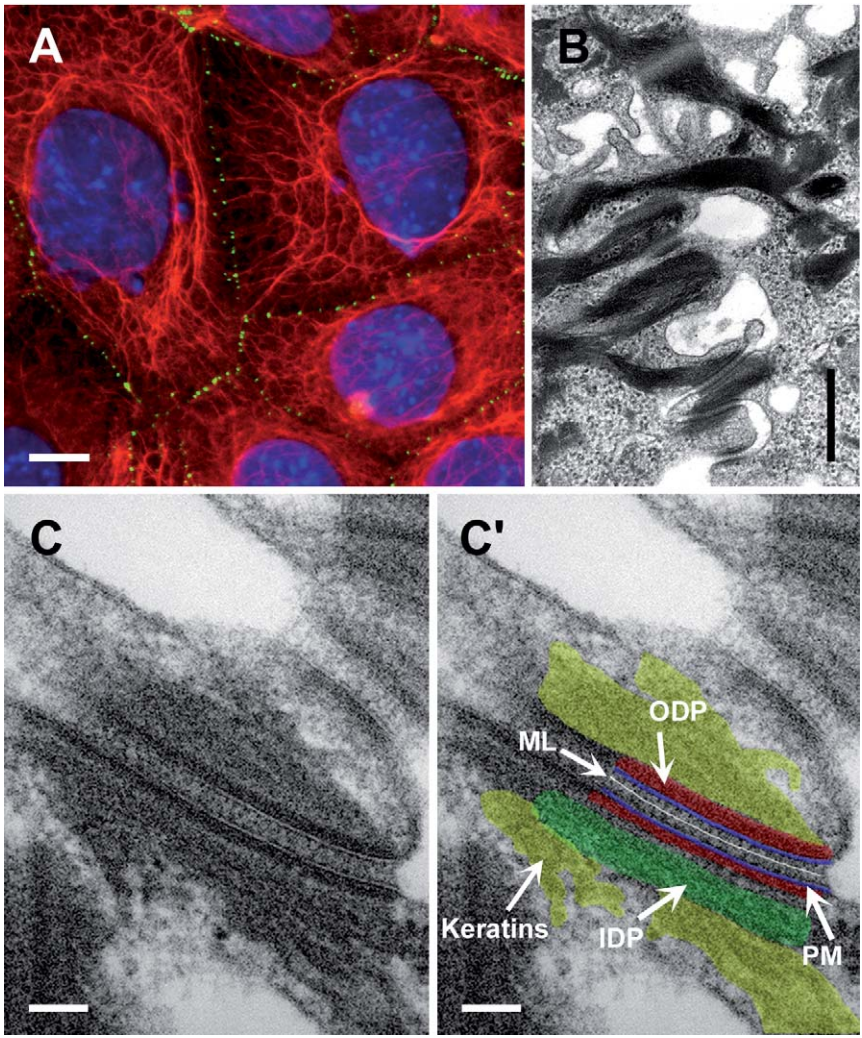

Figure 31 Detection of desmosomes by immunofluorescence microscopy (A) and electron microscopy (B, C). (A) Spontaneously immortalized murine mammary epithelial cells of line EpH4 (Fialka *et al.*, 1996) were reacted with monoclonal desmoplakin antibodies DP 2.15/2.17/2.20 (from Progen, Heidelberg, Germany) in combination with Cy2-conjugated secondary antibodies, with rat monoclonal antikeratin intermediate filament antibody TROMA1 (Developmental Studies Hybridoma Bank, University of Iowa) in combination with Cy3-labeled secondary antibodies, and with DAPI (nuclear stain). Note the anchorage of keratin intermediate filaments at desmosomal junctions thereby creating a transcellular cytoplasmic network. Bar: 10 μm. (B, C) Electron microscopy of human epidermis depicting multiple desmosomes cut at different angles in the survey (B) and at higher magnification (C). The position of the characteristic midline (ML) dividing the extracellular desmoglea in the desmosomal cleft between the adjacent plasma membranes (PM) with distinct filamentous substructures and the

presence of defined substructures in the intercellular space with a prominent midline. While suprabasal epidermal keratinocytes are decorated by multiple desmosomes throughout their entire surface, polarized epithelial cells produce desmosomes as part of a defined tripartite junctional complex (Farquhar and Palade, 1963). This complex consists of two circumferential belts, the zonula occludens, also referred to as the tight junction, and the zonula adhaerens or intermediary junction, in combination with the button-like maculae adhaerentes (i.e., the spot desmosomes) (Farquhar and Palade, 1963). The three different adhesion sites are arranged in an apicobasal order. This classical arrangement is typically found in the intestinal mucosa, but also occurs in the mucosal epithelium of the stomach, gallbladder, uterus, and oviduct, and was identified in other polarized glandular and duct epithelia of the liver, pancreas, salivary glands, stomach, and thyroid gland as well as in epithelial cells of the nephron (Farquhar and Palade, 1963; Kelly, 1966; Staehelin, 1974). It was soon realized that desmosomes are not restricted to epithelia but are also present in the junctional complexes of the disci intercalares of cardiomyocytes including cardiac Purkinje fiber cells (Fawcett and Selby, 1958; Sjostrand *et al.*, 1958), in meningeal cells (Gusek, 1962), and in follicular dendritic cells of lymph nodes (Muller-Hermelink and Caesar, 1969; Swartzendruber, 1965).

All desmosomes share distinct morphological hallmarks (Fig. 3.1B and C). The abutting plasma membranes are separated by a defined intercellular cleft of ~24 nm (range 22 to 50 nm in vertebrates), which is slightly more than that observed in the zonula adhaerens (Farquhar and Palade, 1963). The intercellular space, which is penetrable by water and ions, is filled with electron-dense material, the desmoglea. In mature desmosomes a distinct stratum can be discerned in the middle that is unique to desmosomes and is referred to as the midline (Odland, 1958). Often cross bridges between the midline and the plasma membrane are discernible with intercalated particles that are spaced 7 to 8 nm apart and are best seen after lanthanum infiltration (Kelly, 1966; Rayns *et al.*, 1969; Staehelin, 1974). High-resolution electron tomography of plastic sections revealed intertwined and presumably flexible cross bridges (He *et al.*, 2003), whereas micrographs of native vitrified material depicted straight staggered filamentous structures with a 5-nm periodicity (Al-Amoudi *et al.*, 2004, 2005). Interestingly, wounding leads to loss of the midline and reduction of the intercellular cleft in desmosomes of adjacent cells in epidermis and cultured monolayers (Garrod *et al.*, 2005; Wallis *et al.*, 2000).

location of the outer dense plaque (ODP) that is separated from the keratin filament-anchoring inner dense plaque (IDP) by a zone of reduced staining are all demarcated in C′. Bars: 250 nm (B); 50 nm (C). (The electron micrographs were kindly provided by Dr. Jastrow, Department of Anatomy and Cell Biology, Johannes Gutenberg University, Mainz.)

Plaque diameters range between 0.2 μm and 0.5 μm in most instances but may be as small as 0.1 μm or as large as several micrometers (Moll *et al.*, 1986; Staehelin, 1974). They are discoid or oval shaped. An inner, less dense plaque facing the cytoplasm and an outer, denser plaque that is adjacent to the plasma membrane, best seen in desmosome-rich tissues, are also distinguished (Burdett, 1998; Kowalczyk *et al.*, 1999a; North *et al.*, 1999). Both plaque partitions are 15 to 20 nm in size and are separated by a 10- to 20-nm gap. The desmosome-associated intermediate filament bundles converge on the inner plaque where they fray out into protofilamentous subunits. They loop in a more-or-less wide arc through the electron-dense material 40 to 70 nm away from the plasma membrane, but do not end there (Kelly, 1966; North *et al.*, 1999). Often, multiple desmosomes are linked by filament bundles (Lentz and Trinkaus, 1971; Tamarin and Sreebny, 1963; Troyanovsky *et al.*, 1993).

2.2. Morphological diversity of desmosomes and related junctions

All adhering junctions are characterized by prominent cytoplasmic plaques and attached cytoskeletal filaments. They can be subdivided into two major groups: desmosomes and adhaerens junctions. It is generally accepted that the term desmosome (macula adherens) should be used exclusively for the spot-like adhesion sites that anchor intermediate filaments and that are the topic of this chapter. In contrast, the other adhering junctions, which, on the basis of their morphology, have been referred to as belt desmosomes (zonulae adhaerentes), puncta adhaerentia, or fasciae adhaerentes, and anchor actin filaments are grouped as adherens or intermediate junctions.

Desmosomes vary in size despite their overall uniform appearance. Small desmosomes and those lacking a distinct midline and plaque morphology have been referred to as nascent, immature, or simplified desmosomes assuming that they represent primitive junctions. Accordingly, these small desmosomes have been detected in early embryonic stages and *in vitro* during desmosome assembly (Dembitzer *et al.*, 1980; Jackson *et al.*, 1980). Growth of desmosomes may occur by coalescence of such precursors (Gloushankova *et al.*, 2003; Windoffer *et al.*, 2002). Desmosomal size and morphology vary between tissues and different situations (for direct comparison see, e.g., Cowin *et al.*, 1985). Large desmosomes are generally present in tissues that are subject to intense mechanical stress. In addition, size differences have been noted between different epidermal body sites and strata (Wan *et al.*, 2003). Furthermore, desmosome morphology is subject to regulation during terminal differentiation in the epidermis leading to the formation of transition desmosomes, which further mature into

corneodesmosomes in the stratum corneum (Al-Amoudi *et al.*, 2005; Skerrow *et al.*, 1989). Conversely, desmosomes lacking a distinct midline were detected in wounded epidermis and cultured cells that may represent dedifferentiated desmosomes with reduced adhesive properties (Garrod *et al.*, 2005; Wallis *et al.*, 2000). Interestingly, it has been observed that carcinomas with a low degree of differentiation and poor prognosis appear to present smaller desmosomes (Oliveira Crema *et al.*, 2005).

It has long been known that removal of calcium in culture media or proteolytic treatment of cultured cells results in desmosomal dissociation and formation of half desmosomes in the cytoplasm that remain associated with intermediate filaments (Demlehner *et al.*, 1995; Duden and Franke, 1988; Kartenbeck *et al.*, 1982; Mattey and Garrod, 1986). Furthermore, complete desmosomal entities have been detected in the cytoplasm of skin keratinocytes upon wounding, in carcinomas, and, occasionally, in normal keratinocytes (Garrod *et al.*, 2005; Komura and Watanabe, 1975; Schenk, 1975). While the physiological function of these intracellular desmosomal fragments is not clear at present, such findings indicate that epithelial cells are able to endocytose large areas of their intercellular contacts including even entire adjoining desmosomal halves (see also below).

Hemidesmosomes—not to be confused with cytoplasmic desmosome halves—are morphologically but not compositionally related to desmosomes (Litjens *et al.*, 2006). They are adhesion sites occurring in basal cells of stratified and complex epithelia mediating the attachment of basal keratinocytes to the extracellular matrix. Similar to desmosomes they anchor keratin filaments that loop through an electron-dense plaque.

Recent observations suggest that the hitherto recognized adhering junctions do not fully represent the *in vivo* complexity of these adhesion sites. With the advent of molecular markers, multitudes of compositionally and structurally distinct entities have emerged. Examples include the complexus adhaerentes occurring in special vascular endothelia, notably the retothelial cells of lymph nodes (Schmelz and Franke, 1993; Schmelz *et al.*, 1994; Valiron *et al.*, 1996), the contactus adhaerentes detected in the cerebellar granule layer (Hollnagel *et al.*, 2002; Rose *et al.*, 1995), the area composita in the intercalated discs of cardiomyocytes (Borrmann *et al.*, 2006; Franke *et al.*, 2006), the junctions between photoreceptors and adjacent Müller glia cells (Paffenholz *et al.*, 1999), and the complex cortex adhaerens of lens fiber cells (Straub *et al.*, 2003).

The presence of desmosomes is not restricted to mammals. Desmosomes have been described in all vertebrates examined to date including the clawed frog *Xenopus laevis* and the zebrafish *Danio rerio,* both of which produce desmosomal components with high sequence similarities to their mammalian counterparts (Martin and Grealy, 2004; Ohga *et al.*, 2004). Desmosome-like structures have been reported in other nonvertebrate species as well, extending all the way to the nematode *Caenorhabditis elegans,*

whose continuous adhesion belts are part of the apical junction that shares structural and compositional features with the zonula adhaerens. These sites contain the E-cadherin ortholog HMR-1 and the α- and β-catenin orthologs HMP-1 and HMP-2, respectively, in their apical subunit (Knust and Bossinger, 2002). Furthermore, punctate adhesion sites are present in the hypodermis of *C. elegans* anchoring the intermediate filament cytoskeleton to the extracellular matrix in a hemidesmosome-type fashion, thereby providing mechanical continuity between muscle cells on one site and the cuticle on the other (Michaux *et al.*, 2001).

3. Molecular Architecture

Desmosomes are tightly packed assemblies of specific polypeptides that can be grouped into the membrane-spanning desmosomal cadherins and the cytoplasmic desmosomal plaque constituents. The transmembrane components comprising the desmogleins (Dsgs) and desmocollins (Dscs) bridge the extracellular space and are embedded in the cytoplasmic plaques. As such, they are dual-function molecules that are instrumental in the direct association with corresponding molecules in the neighboring cells and provide, at the same time, platforms for desmosomal plaque assembly in the adjacent cytoplasms. In addition, their strategic location may aid in signal transmission either outside-in or inside-out. The plaque components mediate the anchorage of the cytoplasmic intermediate filaments and promote clustering of the desmosomal cadherins. Two types of plaque molecule are distinguished. One type is characterized by multiple repeats of the so-called *arm*-motif and performs various functions in different cellular compartments. This group includes plakoglobin (PG) and several plakophilin isoforms (PPs1–3). The other plaque molecules are large cytoskeletal linker molecules that are referred to as plakins. Desmoplakin (DP) is the major desmosomal plakin. A schematic diagram of the main desmosomal polypeptides and their arrangement is presented in Fig. 3.2. Given that these interactions occur in three-dimensional space, that they are not static, that different binding partners may compete for the same or mutually exclusive binding domains, and, finally, that these interactions are subject to regulation, it is possible to obtain a glimpse of the true *in vivo* complexity of the resulting adhesion sites and their dynamics in health and disease.

3.1. Desmosomal cadherins

The desmosomal single-pass type I transmembrane glycoproteins are encoded by two multigene families, the desmocollins and desmogleins, that are synthesized in context-dependent combinations (Garrod *et al.*, 2002; Getsios *et al.*, 2004b). These proteins belong to the cadherin superfamily of

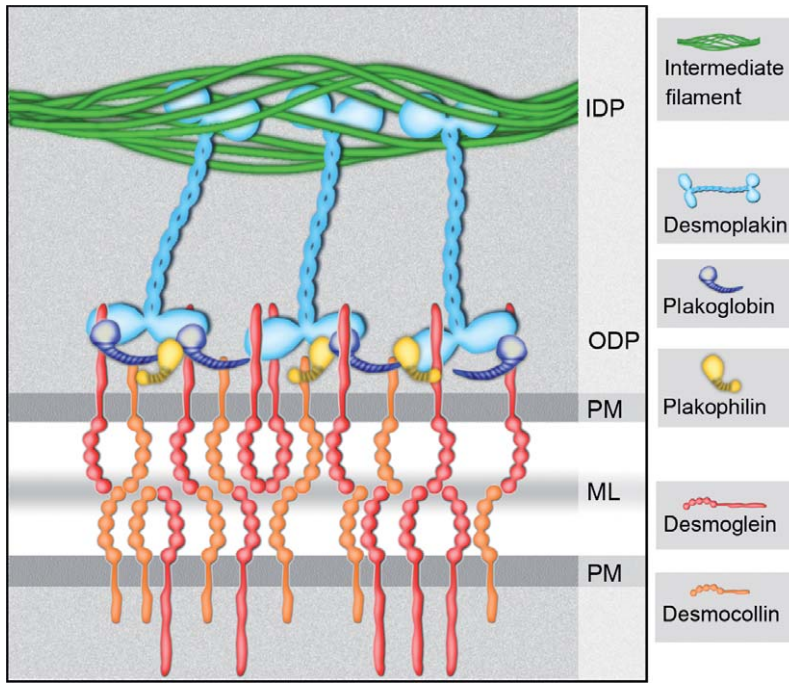

Figure 3.2 Scheme presenting the hypothetical arrangement of major desmosomal components. The scheme encompasses the symmetrical cytoplasmic plaques with inner and outer dense substructures, the plasma membranes (PM), and the intercellular cleft that is filled with the electron-dense material of the desmoglea and that is subdivided by the central midline (ML). Desmosomal cadherins of the desmoglein type (red) and desmocollin type (orange) are drawn as λ-, W-, and S-shaped multimers according to He *et al.* (2003). They bind to the globular plaque components plakoglobin (blue) and plakophilin (yellow), which, in turn, associate with the elongated desmoplakin dimers (blue). For simplicity, only the smaller desmoplakin variant II is integrated into the scheme, which connects the inner and outer desmosomal plaque (IDP, ODP) and probably binds to untwisted intermediate filaments (green).

calcium-dependent cell–cell adhesion molecules. In addition to the two groups of desmosomal cadherins, classical type I and atypical type II cadherins can be distinguished, all of which share characteristic extracellular cadherin (EC) domains (Nollet *et al.*, 2000). According to the consensus nomenclature (Buxton *et al.*, 1993) the desmosomal cadherin genes are referred to as DSC1, DSC2, and DSC3 coding for desmocollins (Dscs) and as DSG1, DSG2, DSG3, and DSG4 encoding the four desmogleins (Dsgs; scheme in Fig. 3.3). They are synthesized in a cell type– and development-dependent fashion. Furthermore, each of the DSC genes gives rise to variants a and b, which result from differential splicing of the last exons leading to Dscs differing only in their most carboxy-terminal domain.

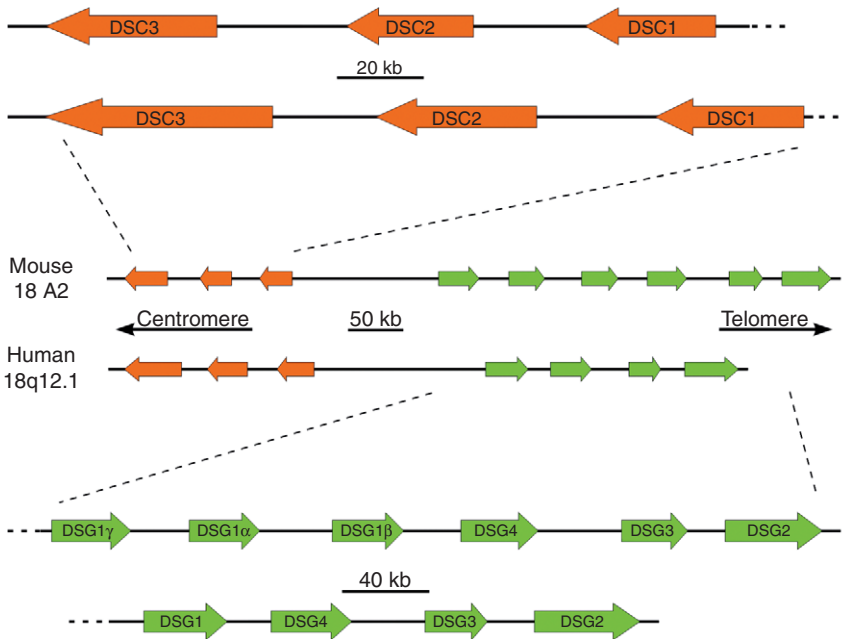

Figure 3.3 Genomic organization of desmosomal cadherins in human and mouse. The maps of the desmosomal cadherin gene clusters on the long arm of human chromosome 18 and the long arm of murine chromosome 18 are shown. Note that the direction of transcription for DSCs (top) and DSGs (bottom) is in an opposite orientation, while the various isoforms are transcribed in the same orientation. The murine gene cluster contains three DSG1-related gene loci that originated most likely from gene duplications. DSG1α is also occasionally referred to as DSG1, DSG1β as DSG5, and DSG1γ as DSG6.

All desmosomal cadherin genes are directly adjacent to each other on the long arm of chromosome 18 in humans with opposite directions of transcription for the DSC and DSG genes (see Fig. 3.3; Cowley *et al.*, 1997). A very similar arrangement is also found on mouse chromosome 18, which, however, presents two additional DSG genes that are most closely related to DSG1 (see Fig. 3.3). They are referred to as DSG1β (alternatively DSG5) and DSG1γ (alternatively DSG6) and are distinguished from the human DSG1 ortholog DSG1α/DSG1 (Kljuic and Christiano, 2003; Pulkkinen *et al.*, 2003; Whittock, 2003). Overall, it is safe to conclude that the desmosomal cadherin genes arose by divergent evolution of duplicated genes. Desmosomal cadherins have been observed in many vertebrate species including *X. laevis,* in which case cross-reactivity of antibodies against bovine Dsgs was reported (Ohga *et al.*, 2004).

The phylogenetic tree in Fig. 3.4 highlights features of desmosomal cadherin evolution presenting comparisons of isoforms identified in human, chimpanzee, mouse, rat, cow, and dog (see also Suzuki, 1996;

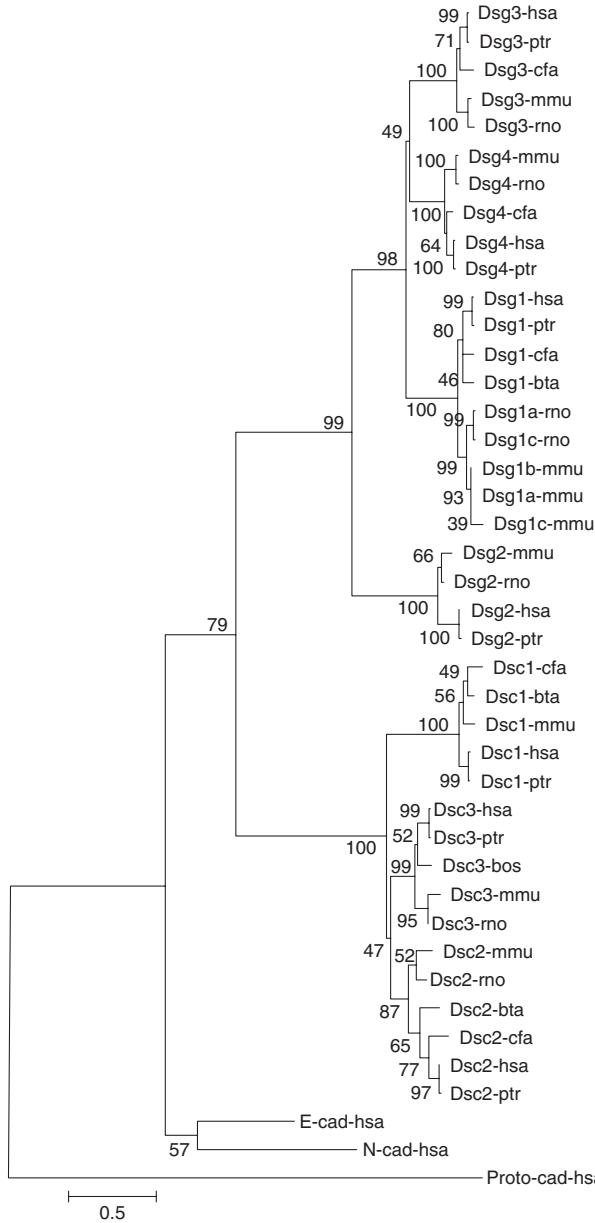

Figure 3.4 Phylogenetic tree of desmosomal cadherins from *Homo sapiens* (hsa), *Pan troglotydes* (ptr), *Mus musculus* (mmu), *Rattus norvegicus* (rno), *Bos taurus* (bta), and *Canis familiaris* (cfa). For comparison the two classical human cadherins E-cadherin (E-cad) and N-cadherin (N-cad) are included. The tree was rooted with the unrelated human protocadherin (Proto-cad). The phylogenetic tree underscores the common ancestry of the various Dsg and Dsc isoforms that are approximately equidistant to each other and the classical

Nollet *et al.*, 2000). The comparisons demonstrate the conserved nature of each desmosomal cadherin family and their divergence from classical and type II cadherins as well as the equidistant relationship of desmosomal and classical cadherins from protocadherins. It is interesting to note, however, that on the basis of comparing the first extracellular domain, desmosomal cadherins are less distant from classical cadherins than type II cadherins from classical cadherins (Nollet *et al.*, 2000). The specific isoform assignments of several DSG- and DSC-like gene sequences from chicken (predicted DSG2-like XM_42608, predicted DSG4-like XM_426082, and DSC2-like XM_426081) were not conclusive given the existing ambiguities in the proposed gene structure and questionable sequence fidelity. Yet, an overall sequence correlation was readily apparent (not shown).

The domain structures of desmosomal cadherins are exemplified in Fig. 3.5 for Dsg2 and Dsc2. Most prominent are the EC domains E1–E4 [the extracellular anchor region (EA) is also referred to as EC5]. EC domains have been crystallized in the case of the three different classical cadherins (Boggon *et al.*, 2002; Overduin *et al.*, 1995; Shapiro *et al.*, 1995) and also of the three type II cadherins (Patel *et al.*, 2006). The resulting structural data revealed that the EC domains of these two rather distant cadherin families with less than 50% sequence similarity share the same basic design: They are folded into seven-stranded β-barrels. The EC domains are connected by flexible linkers forming calcium-binding pockets each of which accommodates up to three calcium ions. Molecular modeling allows an easy fit of these experimentally determined structures onto the corresponding Dsc2 domains (Garrod *et al.*, 2005). Despite these apparent structural similarities, the extracellular domains of the desmosomal cadherins present several unique features. Most striking is the observation that structural changes induced by

cadherins. It is based on amino acid alignment of the extracellular domains by a neighbor-joining algorithm using the PAM matrix and Dayhoff's model of amino acid evolution with pairwise deletion and 1000 replications. The bar at the bottom equals 0.5 PAM distance and the numbers at the branch points represent the supporting bootstrap values. The following accession numbers were used for the calculations: Dsg1-hsa: NM.001942.1; Dsg1-ptr: XM.523899.2; Dsg1a-mmu: XM.484705.3; Dsg1b-mmu: NM.181682.1; Dsg1c-mmu: NM.181680.1; Dsg1a-rno: XM.001054208.1; Dsg1c-rno: XM.214616.4; Dsg1-bta: NM.174045.1; Dsg1-cfa: NM.001002939.1; Dsg2-hsa: NM.001943.1; Dsg2-ptr: XM.512079.2; Dsg2-mmu: NM.007883.1; Dsg2-rno: XM.001054396.1; Dsg3-hsa: NM.001944.1; Dsg3-ptr: XM.523900.2; Dsg3-mmu: NM.030596.2; Dsg3-rno: XM.001054333.1; Dsg3-cfa: NM.001002983.1; Dsg4-hsa: NM.177986.2; Dsg4-ptr: XR.021674.1; Dsg4-mmu: NM.181564.2; Dsg4-rno: NM.199490.1; Dsg4-cfa: XM.850325.1; Dsc1-hsa: NM.024421.1; Dsc1-ptr: XM.512078.2; Dsc1-mmu: X97986.1; Dsc1-bta: NM.174044.1; Dsc1-cfa: XM.547623.2; Dsc2-hsa: NM.024422.2; Dsc2-ptr: XM.512077.2; Dsc2-mmu: L33779.1; Dsc2-rno: BC101864.1; Dsc2-bta: XM.615164.2; Dsc2-cfa: XM.861837.1; Dsc3-hsa: NM.001941.2; Dsc3-ptr: XM.512076.2; Dsc3-mmu: NM.007882.2; Dsc3-rno: XM.001053804.1; Dsc3-bta: L33774.1; E-cad-hsa: NM.004360.2; N-cad-hsa: NM.001792.2; Proto-cad-hsa: NM.019120.2.

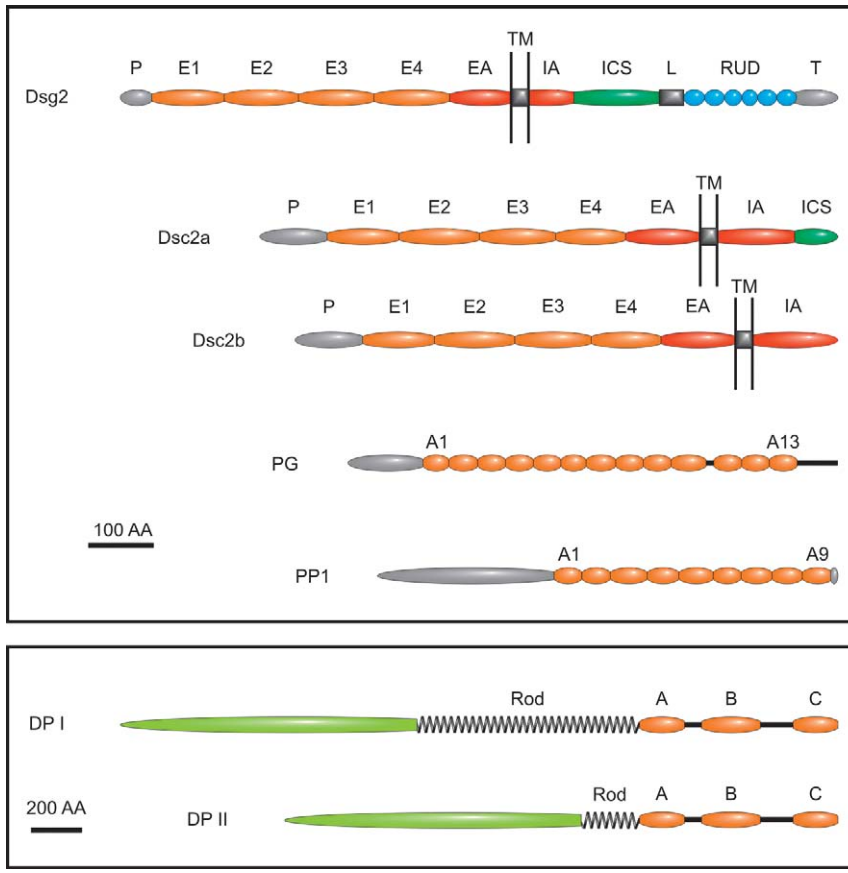

Figure 3.5 Domain structure of major desmosomal components (only one isoform is shown for each polypeptide family). The molecules are drawn as if they were linear unfolded polypeptides (amino acid scale bars are shown in the upper and lower parts of the figure). The desmosomal cadherins (Dsg2, Dsc2a, and Dsc2b) share the same extracellular features comprising an amino-terminal precursor-specific segment (P) that is cleaved off in the endoplasmic reticulum, four ~110 amino acid-long calcium-binding domains (E1–E4), and an extracellular anchor domain (EA) next to the membrane-spanning region (TM). The intracellular anchor domain (IA) is followed by the intracellular catenin-binding and cadherin-like sequence (ICS) in Dsc splice variants a and in Dsgs. The Dsc splice variants b lack this domain. Dsgs present additional segments including a short, proline-rich linker (L), the variable repeated unit domains (RUDs), and a carboxy-terminal domain (T). The plaque components PG and PPs differ in the number of their *arm*-repeats (A) and present divergent amino acid sequences in their terminal domains. Two alternatively spliced DP variants are shown at the bottom presenting globular amino-terminal and carboxy-terminal domains. The carboxy-terminal IF-binding regions are composed of homology units A, B, and C.

calcium depletion in Dsg1 cannot be reversed by calcium replenishment (Hanakawa *et al.*, 2003), pointing to differences in conformational stability in comparison to classical cadherins.

On the other hand, the structural similarities between classical and desmosomal cadherins can be taken as strong indications of shared functional properties such as the formation of strand dimers via the conserved tryptophane residue W2 in EC1, which was observed for C-cadherin (Boggon *et al.*, 2002). There is compelling evidence that such dimers play a pivotal role in classical cadherin-mediated adhesion (Troyanovsky, 2005). Much less is known, however, about intercadherin interactions in desmosomes. The complexity of desmosomal cadherin composition and the insolubility of desmosomes have been major impediments for standard biochemical approaches to study protein–protein interactions in desmosomes. Nevertheless, the formation of Dsg–Dsc heteromeric complexes in non-epithelial HT-1080 cells was detected by coimmunoprecipitation (Chitaev and Troyanovsky, 1997), demonstrating that desmosomal cadherins are capable of a stable dimeric association as is the case for classical cadherins. Furthermore, examination of homophilic and heterophilic interactions between Dsg and Dsc in solution showed that they are comparatively weak, which is also in accordance with observations on classical cadherins (Syed *et al.*, 2002). The determination of the exact structure of desmosomal cadherin adhesion, however, requires further studies.

The tripeptide HAV has been identified as a crucial element in the adhesion of classical cadherins (Blaschuk *et al.*, 1990). Administration of peptides containing this motif, which is referred to as the CAR (cell adhesion recognition) site, was shown to interfere with cadherin-based adhesion. The exact reason why such peptides are able to destroy cadherin-mediated adhesion is not understood, however. Evidence for the existence of a CAR site is even less clear in the case of the desmosomal cadherins, which contain only a rather divergent sequence of the candidate site (YAT or RAL; Tselepis *et al.*, 1998). It was reported, however, that blocking peptides corresponding to the CAR sites of desmosomal cadherins interfered with proper cell type–specific positioning of luminal and myoepithelial cells in a three-dimensional culture system of mammary epithelial cells (Runswick *et al.*, 2001).

High-resolution imaging of frozen samples by electron tomography has considerably enhanced our ideas about the arrangement of extracellular desmosomal cadherin domains *in vivo* (He *et al.*, 2003). Groups of desmosomal cadherins connected to each other by a series of discrete knots via their amino-terminal domains were observed. Three alternative configurations were detected: W, S, and λ shapes. It was demonstrated that the known X-ray structure of human C-cadherin could be superimposed on these images. By further molecular modeling a crucial role could also be assigned to W2-dependent strand–dimer interactions for desmosomal

cadherin multimerization in these specific spatial arrangements. Yet, much work needs to be done to understand the molecular details regulating homooligomerization versus heterooligomerization and *cis*- versus *trans*-multimerization. Even more, it is not known why the adhesive force differs among the different desmosomal cadherins. How do different relative levels of the two types of desmosomal cadherins and the presence of different isoforms affect desmosomal stability and adhesive strength? What happens to the extracellular domains when desmosomes become calcium independent (Garrod *et al.*, 2005; Kimura *et al.*, 2006)?

The extracellular desmosomal cadherin domains are connected to the membrane-spanning segment by a short and not very well conserved extracellular anchor domain (EA and TM in Fig. 3.5). The cytoplasmic domains also exhibit an overall high degree of sequence divergence except for a short element, the intracellular 72-amino acid-long cadherin-typical sequence (ICS). This element has been shown to bind to PG, one of the major linker molecules of desmosomes that is also present in other adhering junctions (Mathur *et al.*, 1994; Roh and Stanley, 1995; Troyanovsky *et al.*, 1994a,b). Interestingly, this sequence motif is present in all Dsgs and in all Dsc splice variants a but is absent in the alternatively spliced Dsc b isoforms that are not capable of associating with PG (Troyanovsky *et al.*, 1993, 1994a), but instead interact with the plaque protein PP3 (Bonne *et al.*, 2003).

Regardless of the striking similarities between the ICS of desmosomal and classical cadherins, their binding properties differ significantly, presenting unique features. In normal epithelial cells, classical cadherins bind to both PG and β-catenin, whereas desmosomal cadherins associate exclusively with PG. Even more, it was observed in cultured cells that Dsg2 is unable to interact with β-catenin even in the absence of PG (Chitaev and Troyanovsky, 1997), although such interactions have been observed in keratinocytes of PG knockout animals (Bierkamp *et al.*, 1999). The molecular details of the interfaces between desmosomal cadherins and desmosomal plaque proteins will be described below.

Further intracellular desmosomal cadherin domains are the juxtamembranous anchor region (IA), the proline-rich linker (L), the Dsg-specific repeated unit domains (RUDs), and the terminal domain (TD; see Fig. 3.5). The juxtamembranous region plays an important function in classical cadherins, providing a binding site for the *arm*-repeat protein p120 and bearing several other motifs such as the dileucine motif that may be responsible for correct trafficking (Miranda *et al.*, 2003). It is likely that the corresponding regions in desmosomal cadherins have similar functions. Therefore, the reported binding of Dsc splice variants b to PP3 (Bonne *et al.*, 2003) and probably also PP2 (Chen *et al.*, 2002) may be mediated by the membrane-proximal region and may extend to other members of the plakophilin family. Furthermore, we have shown that the IA region of Dsc1a contains a

DP-binding element (Troyanovsky *et al.*, 1994b). The functions of the L and RUD segments are not clear at present but probably determine isoform- and isotype-specific properties of desmosomal cadherins.

3.2. Desmosomal plaque components

3.2.1. *Arm*-repeat domain molecules

These molecules are characterized by multiple repeats of a 42 amino acid domain that was first identified in the segment polarity gene *armadillo* in the fruit fly *Drosophila melanogaster* (Peifer and Wieschaus, 1990; Riggleman *et al.*, 1989) and is hence referred to as the *arm*-repeat domain. The structure of this domain was solved for three members of this superfamily, namely for β-catenin (Huber *et al.*, 1997), PP1 (Choi and Weis, 2005), and importin-α (Kobe, 1999). It was found that each repeat is composed of three α-helices and that the repeats are packed together forming a superhelix (i.e., the *arm*-repeat domain). This superhelix is bent by nonhelical inserts to different degrees and has either a positively charged groove in the case of β-catenin and PP1 or a negatively charged groove in the case of importin-α, which spans the entire domain. These grooves serve as perfect surfaces for binding to either acidic or basic protein ligands in the form of extended peptides.

Desmosomes contain several *arm*-repeat proteins, namely the obligatory PG encoded by the JUP gene (Aberle *et al.*, 1995) and various plakophilins (PPs) that are encoded by corresponding single PKP genes in different chromosomal locations (Bonne *et al.*, 1998) and are synthesized in a cell type–specific pattern (Hatzfeld, 2006; Schmidt and Jager, 2005). Multiple binding partners have been identified for these molecules that reside in various junctions, in the cytoplasm, and in the nucleoplasm, thereby resulting in complex subcellular distribution patterns depending on cell type and cell function. Even within desmosomes, a plethora of binding sites has been characterized, contributing to an association with practically all other components. Thus, they are linking molecules par excellence, and it will be a continuing challenge to order the different binding reactions with respect to each other considering that they are governed by different affinities, that they are regulated by specific protein modification, and that they most likely influence each other. A particularly attractive scenario is that the various binding sites are sequentially exposed as the *arm*-domain molecules change their conformation within the context of developing desmosomes and thereby provide multiple connections between the various desmosomal components resulting in their subsequent compact dense clustering.

PG, also referred to as γ-catenin, is a typical *arm*-repeat domain molecule. Its central *arm*-repeat domain, which is very similar to that of β-catenin, is flanked by the amino- and carboxy-terminal tail domains. Some authors distinguish 12 repeats in analogy to β-catenin (Huber *et al.*, 1997), whereas

others distinguish 13 (Getsios *et al.*, 2004b; Wahl *et al.*, 1996; A1–A13 in Fig. 3.5). The positively charged groove of the PG *arm*-repeat domain provides binding sites for various ligands such as desmosomal cadherins (see above), classical cadherins (Aberle *et al.*, 1994; Sacco *et al.*, 1995), and components of the *wnt* signaling pathway such as the adenomatous polyposis coli (APC) protein (Ozawa *et al.*, 1995; Rubinfeld *et al.*, 1995), axin (Kodama *et al.*, 1999; Kolligs *et al.*, 2000), and TCF/LEF family transcription factors (Miravet *et al.*, 2002). Each ligand is characterized by specific binding features to the *arm*-repeat domain resulting in different binding affinities (Choi *et al.*, 2006). More importantly, different PG ligand complexes expose different secondary binding sites. Phosphorylation of PG and its ligands further increases the variability of complex formation (Hu *et al.*, 2001; Miravet *et al.*, 2003). Finally, intramolecular interactions of PG's amino- and/or carboxy-termini with the *arm*-repeat domain additionally enlarge the spectrum of PG-binding properties and interactions (Troyanovsky *et al.*, 1996).

Structural analyses of the E-cadherin–β-catenin complex showed that an extended stretch of 14 residues (region III) of E-cadherin is crucial for the interaction with the positively charged groove of the *arm*-repeat domain of β-catenin (Huber and Weis, 2001). Since the corresponding region III of the desmosomal cadherins share significant similarities with E-cadherin, it is not surprising that the *arm*-repeat domains of both β-catenin and PG are capable of interacting with Dsg. Yet, the amino- and carboxy-termini of β-catenin completely inhibit this interaction and may therefore explain the exclusive *in vitro* binding of Dsg and PG and the absence of Dsg–β-catenin association (Troyanovsky *et al.*, 1996; Wahl *et al.*, 2000).

Detailed molecular analyses of the PG-binding site of Dsg1 identified several hydrophobic amino acids that presumably interact with nine hydrophobic amino acids in the *arm*-repeats 1–3 of PG, which are located outside the positively charged groove (Chitaev *et al.*, 1998). This hydrophobic element of PG is also needed for the interaction with Dsc2a (Chitaev *et al.*, 1998). Interestingly, the hydrophobic portion of Dsg that was found to be involved in the interaction with PG corresponds to the so-called "hydrophobic cap" of E-cadherin, which is located in the carboxy-terminus downstream of region III (Huber and Weis, 2001). Taken together, these observations suggest that the assembly of the PG–Dsg complex is initiated by the interaction between the hydrophobic cap of Dsg and the hydrophobic PG element. In support of this, point mutations in both regions abolished the formation of the Dsg–PG complex (Chitaev *et al.*, 1998). Yet the same PG mutants still form a complex with E-cadherin. Furthermore, binding of Dsg to PG shields the α-catenin-binding site of PG located in the same region (Aberle *et al.*, 1996; Chitaev *et al.*, 1998; Miravet *et al.*, 2003; Sacco *et al.*, 1995), thereby preventing integration of α-catenin into the Dsg–PG complex.

In a consecutive step of desmosome assembly, the region adjacent to the hydrophobic portion of the Dsg tail, which corresponds to the E-cadherin region III, itself may interact with the positively charged groove of the PG *arm*-repeat domain, which may have been covered during the initial association step described in the previous paragraph. It was thus shown that an 11-amino acid-long stretch in the PG carboxy-terminus (K673-Q683) is important for preventing the interaction of the positively charged groove with Dsg (Troyanovsky *et al.*, 1996). Interestingly, in 19 patients suffering from the desmosomal Naxos disease, a premature termination was noted in the middle of this element at W679 (McKoy *et al.*, 2000). Furthermore, expression of carboxy-terminally deleted PG was shown to induce alterations in desmosome structure (Palka and Green, 1997). The transition of the Dsg–PG complex from one conformation to the next may open binding sites for other desmosomal plaque proteins such as DP or PPs. Such binding site alterations may be crucial for desmosomal assembly. Intramolecular binding of the positively charged groove to the acidic carboxy-terminal transactivation domain is interesting in light of the interdependency of the various binding domains and their different accessibility in certain situations and topologies (Zhurinsky *et al.*, 2000). The amino- and carboxy-termini may therefore have important regulatory functions for ligand specificities, which was also suggested to be the case for the related β-catenin (Solanas *et al.*, 2004).

Interactions of PG with other desmosomal components have been described including DP (Kowalczyk *et al.*, 1997), PP2 and PP3 (Bonne *et al.*, 2003; Chen *et al.*, 2002), p0071 (Hatzfeld *et al.*, 2003), and even keratins (Smith and Fuchs, 1998), although specificity and precise interfaces of these interactions have not been determined conclusively. In addition, an association of PG with DP has been shown to be influenced by Src-dependent tyrosine phosphorylation (Miravet *et al.*, 2003). Tyrosine phosphorylation, however, induced by association with the epidermal growth factor receptor, prevents interaction with DP and favors binding to Dsgs (Gaudry *et al.*, 2001; Hoschuetzky *et al.*, 1994). These effects may be counteracted by phosphatases such as the leukocyte common antigen-related (LAR) protein tyrosine phosphatase (Muller *et al.*, 1999) and protein tyrosine phosphatase kappa (Fuchs *et al.*, 1996). In addition, PG interacts with the DF3/MUC1 transmembrane oncoprotein (Li *et al.*, 2003; Yamamoto *et al.*, 1997). The latter association has been shown to be subject to regulation by heregulin and epidermal growth factor (EGF), leading to nucleolar localization of the MUC1/PG complex (Li *et al.*, 2003).

The PG-related *armadillo* gene product in *Drosophila* is involved in signal transduction in the canonical *wnt* pathway and serves a dual function as a signaling and a structural molecule that is implicated in human cancer as worked out in detail for the vertebrate ortholog β-catenin (Behrens, 2005; Brembeck *et al.*, 2006; Giles *et al.*, 2003). Similar to β-catenin and *armadillo,*

the amino-terminal domain of PG is subject to phosphorylation of S28 by glycogen synthase kinase-3β (GSK), a cytoplasmic mediator of the *wnt* signaling pathway (Kodama *et al.*, 1999). The carboxy-terminus of PG, on the other hand, contains a transcriptional activation domain that in a complex with LEF1/TCF transcription factors facilitates gene transcription (Hecht *et al.*, 1999; Huber *et al.*, 1996; Maeda *et al.*, 2004; Miravet *et al.*, 2002; Simcha *et al.*,1998). In addition, PG associates with the F-box protein β-TrCP of the SCF E3 ubiquitin ligase complex (Sadot *et al.*, 2000). While these interactions are possibly important in *wnt*-dependent signaling, the role of PG in this process continues to be a matter of debate (Ben-Ze'ev and Geiger, 1998). Despite the apparent similarities between PG and β-catenin, there are also significant differences (Ben-Ze'ev and Geiger, 1998) concerning desmosomal localization, which is reserved to PG, stability, transcriptional activation, proliferative effects, and binding properties to various partners (Solanas *et al.*, 2004). It is therefore not a surprise that mutation of either polypeptide leads to very different overall phenotypes (Bierkamp *et al.*, 1996; Giles *et al.*, 2003; Haegel *et al.*, 1995; Heasman *et al.*, 1994; Kofron *et al.*, 1997; Ruiz *et al.*, 1996), and caution should be taken not to extrapolate observations for one to the other.

PP1 was originally described in desmosomal preparations of bovine muzzle as the sixth largest Coomassie Blue-stained polypeptide band by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) ("band 6 protein" of M_r 75,000; Kapprell *et al.*, 1988). PPs are basic, positively charged desmosomal components whose main residence is in the nucleus. Based on structural analyses PPs contain probably only 9 and not 10 *arm*-repeats as previously suggested (cf. Hatzfeld, 2006; A1–A9 in Fig. 3.5). To date three PP isoforms are being distinguished (Hatzfeld, 1999, 2006; Schmidt and Jager, 2005). A more distantly related group of *arm*-proteins includes 120^{ctn}, ARVC, δ-catenin/NPRAP, and p0071 (also occasionally referred to as PP4), which are localized to adherens junctions. This group together with the PPs are referred to as the plakophilin-p120^{ctn} family. PPs 1–3 localize to desmosomes but also occur in nuclei of many different cell types. All major desmosomal components have been identified as potential PP-binding partners including the desmosomal cadherins Dsgs1–3, Dscs1–3 splice variants a, and Dsc3 splice variant b (Bonne *et al.*, 2003; Chen *et al.*, 2002; Hatzfeld *et al.*, 2000; Smith and Fuchs, 1998), DP (Hofmann *et al.*, 2000; Kowalczyk *et al.*, 1999a; Smith and Fuchs, 1998), PG (Bonne *et al.*, 2003), and keratins (Bonne *et al.*, 2003; Hatzfeld and Nachtsheim, 1996; Hatzfeld *et al.*, 1994, 2000; Hofmann *et al.*, 2000; Kapprell *et al.*, 1988; Kowalczyk *et al.*, 1999a; Smith and Fuchs, 1998). Therefore PPs are prime candidates to act as desmosomal crosslinkers. It has been reported that PPs are involved in the recruitment of DP to cell borders, and it was proposed that they provide lateral interactions between DPs (Kowalczyk *et al.*, 1999b). Accordingly, lack of PPs results in cytoplasmic

DP aggregate formation and reduction of size and number of desmosomes (McMillan *et al.*, 2003; South *et al.*, 2003). This is, in turn, associated with a reduction of the calcium stability of desmosomes and an increase in the migration of cultured keratinocyte cell sheets (South *et al.*, 2003). Binding to the nondesmosomal linker β-catenin was also reported (Chen *et al.*, 2002). One surprising feature of the PP subfamily is that the known molecular interactions of all members are mediated via their amino-termini (Hatzfeld, 2006). So far, the only known function of the *arm*-repeat domain is its influence on the dynamics of the actin cytoskeleton (Hatzfeld *et al.*, 2000). Future research is needed to identify ligands of the *arm*-repeat domain groove and to examine details of the complex relationship between PPs and the cytoskeleton.

Further associations were described between PPs and signaling molecules (Chen *et al.*, 2002; Muller *et al.*, 2003) and with factors affecting RNA metabolism (Hofmann *et al.*, 2006; Mertens *et al.*, 2001) pointing to multiple, nondesmosome-related functions of PPs.

3.2.2. Plakins

Plakins are humongous polypeptides that crosslink the different cytoskeletal filaments and attach them to membrane-associated complexes (Jefferson *et al.*, 2004). They were originally identified as tethers that attach the keratin intermediate filaments to cell–cell and cell–matrix junctions (Ruhrberg and Watt, 1997). Plakins share several basic architectural features (Jefferson *et al.*, 2004; Leung *et al.*, 2002): The conserved plakin domain, which is responsible for interactions with plasma membrane components, is the most characteristic feature of the plakin family. It is a globular region consisting of α-helical bundles that are located in the amino-terminal part of the respective molecules. The crystal structure of the plakin domain of the bullous pemphigoid antigen 1 (BPAG1) shows that it consists of two pairs of spectrin repeats that are interrupted by a putative Src-homology 3 (SH3) domain (Jefferson *et al.*, 2007). Next to the plakin domain is the central coiled-coil rod domain, which is important for dimerization and is present in most, though not all plakins. It is flanked at the carboxy-terminus by a variable number of plakin repeat domains (PRDs) that bind to intermediate filaments. A considerable variety of this basic scheme exists among the various plakin types and the multiple splice variants of individual isoforms. In addition, actin-binding calponin-homology regions, spectrin repeats, and microtubule-binding sites may be present. Taken together, plakins are modular molecules consisting of different combinations of various binding domains.

Interestingly, plakins have been identified in the fruit fly *D. melanogaster* (Gregory and Brown, 1998) and the nematode *C. elegans* (Bosher *et al.*, 2003). The *Drosophila* gene was originally termed *kakapo* and is now referred to as *shortstop* encoding the polypeptide Shot (short for Shortstop;

Leung *et al.*, 2002). Different splice variants participate in diverse processes such as neuronal process formation, attachment of the dorsal and ventral wing surfaces, and, most importantly, junctional attachment of epidermis and muscle (Gregory and Brown, 1998; Lee *et al.*, 2000; Prokop *et al.*, 1998). A major mechanical function concerning cytoskeletal anchorage has also been reported in *C. elegans* where the plakin VAB-10 occurs as two isoforms, VAB-10A and VAB-10B, that mediate the attachment of the hypodermis to the cuticle and muscle (Bosher *et al.*, 2003).

Several plakins have been localized to desmosomes depending on the tissue type and precise cellular localization. DP is certainly the most prominent desmosomal plakin and is an obligatory desmosomal component. It is well established that DP serves as a linker between the transmembrane complex and intermediate filaments of the keratin, vimentin, and desmin type, all of which may be anchored to desmosomes depending on the cell type (e.g., Kartenbeck *et al.*, 1983, 1984; Fig. 3.6 presents typical DP immunofluorescence patterns in liver and heart). DP, however, is not only present in desmosomes but has also been detected in related junctions such as the complexus adhaerentes of specialized endothelial cell junctions (Schmelz and Franke, 1993).

The two splice variants DPI and DPII differ only in the length of their central α-helical coil-coil domain but share amino- and carboxy-terminus (Fig. 3.5). Rotary shadowing electron microscopy revealed flexible dumbbell-like structures of up to 180 nm for purified DPI and up to 93 nm in the case of DPII (O'Keefe *et al.*, 1989). While the longer splice variant DPI has been detected in all desmosome-bearing tissues, DPII mRNA could not be detected in cardiac muscle (Angst *et al.*, 1990). This central coiled-coil domain separates the amino-terminal plakin domain from the carboxy-terminal tail. The latter contains three globular PRDs, termed A, B, and C, each of which consists of 4.5 copies of a unique 38-amino acid repeat motif. Crystal structure analyses of DP-PRDs (Choi *et al.*, 2002) revealed that this motif is composed of an 11-residue β-hairpin that is followed by two antiparallel α-helices that are typically 8 and 14 residues long, thereby forming a groove that is lined with basic residues contributing to intermediate filament binding in cooperation with the other PRDs. Additional sequence elements within or close to the multiple tripeptide GSR repeats that are located even further downstream also contribute to intermediate filament binding in a phosphorylation-dependent manner involving specifically S2849 (Fontao *et al.*, 2003; Stappenbeck *et al.*, 1994).

The amino-terminal plakin domain of DP, similar to domains of other plakins, interacts with the plasma membrane-attached structure (i.e., in this instance the desmosomal plaque). Almost all desmosomal plaque components, namely the *arm*-proteins PP1, PP2, and PG, and intracellular regions of the desmosomal cadherins exhibit binding activity in various assays (Bornslaeger *et al.*, 2001; Setzer *et al.*, 2004; Smith and Fuchs, 1998;

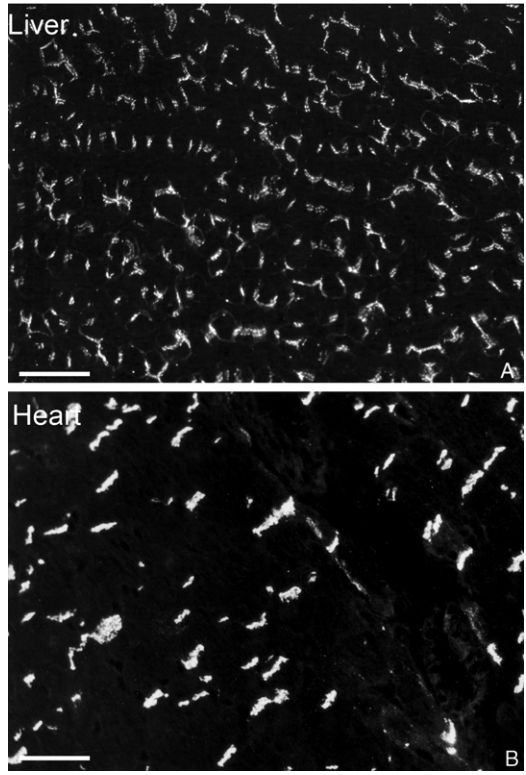

Figure 3.6 Indirect immunofluorescence microscopy of bovine liver (A) and rat heart (B) detecting desmosomes with murine monoclonal desmoplakin antibodies DP 2.15/2.17/2.20 (from Progen, Heidelberg, Germany). Note the decoration of bile canaliculi between hepatocytes (A) and the labeling of intercalating discs between cardiomyocytes (B). Bars: 100 μm in A; 50 μm in B. (The micrographs were kindly provided by Dr. Jürgen Kartenbeck, German Cancer Research Center, Heidelberg, Germany.)

Troyanovsky *et al.*, 1994b). In endothelial cells DP may link to the head domain of p0071, which is, in turn, coupled to VE-cadherin (Calkins *et al.*, 2003). Additional testing is needed to further substantiate the versatility of the plakin domain of DP in an *in vivo* context. Another important issue to be clarified is the coordination between the amino-terminal plakin domain and the carboxy-terminal intermediate filament-binding domains.

The large and ubiquitous cytoskeletal crosslinker plectin is another plakin that has been localized to desmosomes (Wiche *et al.*, 1983) but appears to occur primarily in hemidesmosomes (Litjens *et al.*, 2006). Intermediate filament-binding sites were also mapped to its PRDs (Nikolic *et al.*, 1996). In contrast to the three PRDs found in DP, however, plectin contains six PRDs (Leung *et al.*, 2002). Interactions of plectin with DP were also reported (Eger *et al.*, 1997).

Envoplakin (Ruhrberg *et al.*, 1996) and periplakin (Ruhrberg *et al.*, 1997) are other plakins that were localized to desmosomes by electron microscopy. They contain either one PRD (envoplakin) or none (periplakin). Both are constituents of desmosomes and the cornified envelope of suprabasal epidermal keratinocytes (see also Leung *et al.*, 2002).

3.2.3. Additional components

Further desmosomal polypeptides have been previously described, most of which have gained only limited recognition. Desmosomal specificity was demonstrated in most instances by immunolocalization of their epitopes to desmosomal junctions in suprabasal keratinocytes. It is necessary to keep in mind, however, that the surface of these cells is in large part occupied by closely spaced desmosomes and that desmosomal specificity is therefore difficult to assess conclusively. The following polypeptides have been described.

Desmocalmin: This ~240-kDa polypeptide was originally isolated from bovine muzzle epidermal desmosomes (Tsukita, 1985). It binds calmodulin in a calcium-dependent manner and interacts with keratin filaments (Tsukita, 1985). Further attempts to clone and identify it have not been successful to date.

Keratocalmin: This ~250-kDa polypeptide is also a calmodulin-binding polypeptide that has been localized to desmosomes in human epidermis (Fairley *et al.*, 1991).

Desmoyokin: This is a large, ~680-kDa peripheral desmosomal protein in the upper strata of stratified bovine epithelia (Hashimoto *et al.*, 1993; Hieda and Tsukita, 1989) whose human ortholog AHNAK was originally identified as a downregulated gene in neuroblastoma and was reported to be present in the cytoplasm and nucleoplasm of cells lacking desmosomes (Shtivelman *et al.*, 1992). Detailed localization studies of desmoyokin in keratinocytes further suggested that it may not be a true desmosomal component (Masunaga *et al.*, 1995).

Pinin: This ~140-kDa phosphoprotein was described as a facultative component of mature desmosomes (Ouyang and Sugrue, 1992, 1996) that may link keratin filaments to the desmosome (Shi and Sugrue, 2000). Its localization could not be confirmed by others who detected it primarily in the nucleus residing in nuclear "speckles" that are likely involved in RNA processing (Brandner *et al.*, 1997, 1998). In addition, the recently described pinin RNAi-induced reduction of corneal cell–cell adhesion is most likely due to transcriptional alterations (Alpatov *et al.*, 2004; Joo *et al.*, 2005).

Erbin: This ~180-kDa polypeptide binds via its PDZ domains to p0071, was partly colocalized with DP along the plasma membrane, and has been shown to be essential for epithelial integrity (Izawa *et al.*, 2002; Jaulin-Bastard *et al.*, 2002).

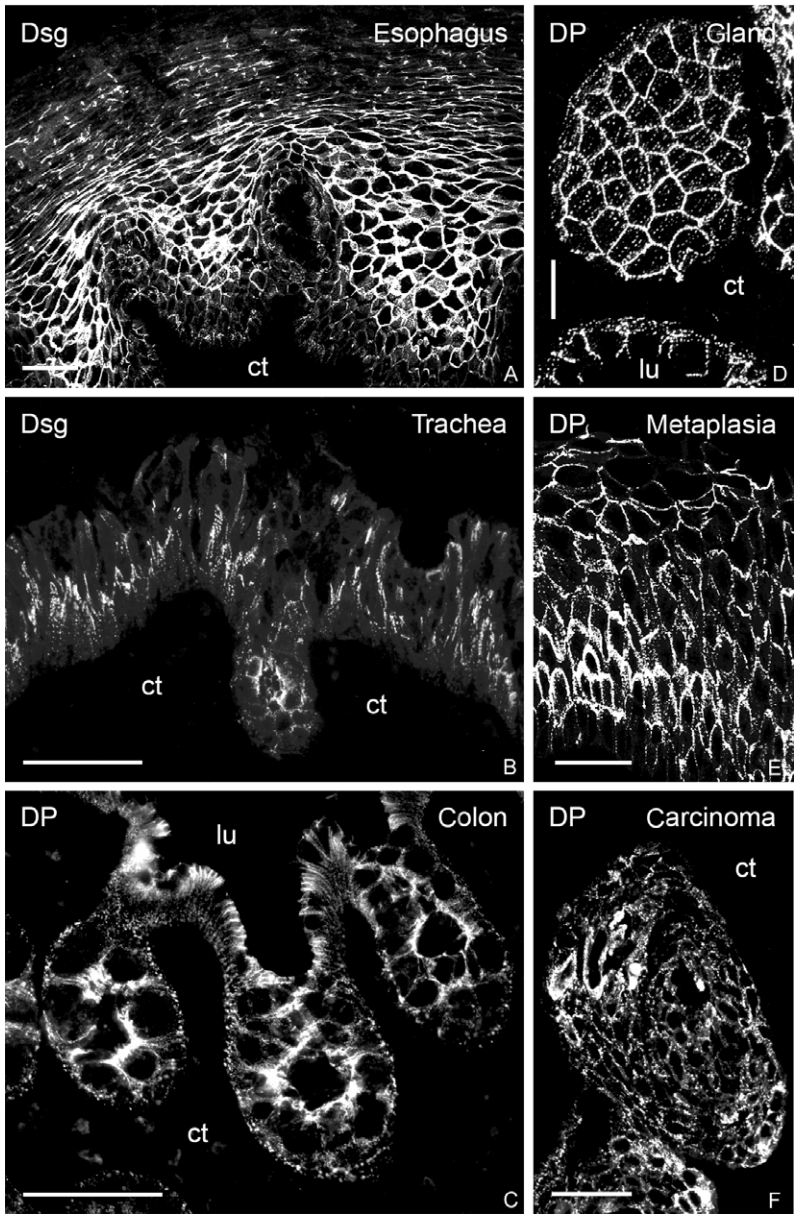

Figure 3.7 Indirect immunofluorescence microscopy detecting desmosomal components in human tissues including the multilayered squamous epithelium lining the esophagus (A), the complex pseudostratified tracheal epithelium (B), the simple mucosal epithelium of the colon (C), and simple glandular epithelium from bovine snout (D; tangential section on top, transverse section through acinus on bottom; lu, lumen). Note also the abundance of desmosomes in an extensive squamous cell metaplasia of the

Corneodesmosin: This 52- to 56-kDa phosphorylated glycoprotein was detected in upper layers of human epidermis and the inner root sheath of hair follicles (Levy-Nissenbaum *et al.*, 2003; Simon *et al.*, 2001). It is secreted and becomes integrated into the desmosomal gap in the upper granular and cornified layers of the epidermis (Simon *et al.*, 1997, 2001). It may stabilize desmosomes by acting as a homophilic adhesion molecule (Jonca *et al.*, 2002) and is covalently associated with the cornified envelope (Simon *et al.*, 1997). Its progressive proteolytic degradation further suggests a function in desquamation (Simon *et al.*, 1997, 2001).

3.3. Cell type specificity of desmosomal composition

Broad-reactive antibodies against the major desmosomal polypeptides have been used to determine the distribution of desmosomes in various normal tissues and also in metaplastic or cancerous lesions (examples are shown in Fig. 3.7). The polypeptide composition of desmosomes, however, varies among different tissue types and even within certain subcompartments of a given tissue. This variability is due to synthesis of different isoforms of obligatory desmosomal components and to the facultative presence of additional, nonessential constituents. Although the knowledge of the cell type–dependent composition of desmosomes has increased considerably during the past few years, its significance for specific desmosomal properties remains largely unknown.

Table 3.1 summarizes results for the tissue-specific synthesis of the desmosomal cadherins in human, cow, and mouse. Differences in the reported patterns of synthesis can be accounted for by various explanations. First and most important, the various detection methods used differ considerably in their respective sensitivity (e.g., reverse transcriptase polymerase chain reaction [RT-PCR] versus Northern blot hybridization) and their specific limitations (e.g., Northern blot versus immunoblot or immunoblot versus immunofluorescence). Second, tissues often present variable differentiation features depending on their precise location and functional requirements (e.g., foreskin epidermis versus trunk epidermis or palmar/plantar epidermis). Third, species differences have to be taken into account (e.g., the differing histology of the stomach of ruminating and nonruminating animals). Even when keeping these aspects in mind, it is very difficult to delineate distinct "expression rules" as has been suggested earlier (Getsios *et al.*, 2004b).

bronchial epithelium (E) and a tumor islet of a pulmonary squamous cell carcinoma (F). In each instance, surrounding connective tissue (ct) is negative. Primary antibodies used were murine monoclonal DP antibodies DP 2.15/2.17/2.20 and monoclonal Dsg antibodies DG 3.10 (from Progen). Bars: 25 μm in D; 50 μm in A–C, E, F. (Figure 3.7D was provided by Dr. Jürgen Kartenbeck.)

Table 3.1 Cell type–specific synthesis of desmosomal cadherins in human, cow, and mouse tissues (Holthoefer *et al.*, 2007)

	Dsg1/Dsg1α	Dsg1β/ Dsg5	Dsg1γ/ Dsg6	Dsg2	Dsg3	Dsg4	Dsc1	Dsc2	Dsc3
blastocyst				$+^{7}$		$-^{5}$		$+^{4,5}$	$+^{4}/-^{4}$
E7	$-^{22;27;32}$	$-^{27;32}$	$-^{32}$	$+^{22;32}$	$+^{22;32}$	$+^{32}$			
E11/E11.5	$-^{22;27;32}$	$-^{27;32}$	$-^{32}$	$+^{22;32}$	$+^{22;32}$	$+^{32}$			$(+)^{5}$
E12.5	$(+)^{10c,d,e}/-^{10g}$			$+^{10c,d,e,f,g}$	$(+)^{10c}/+^{10e}/-^{10d,g}$		$-^{10c,d,e,g}$	$+^{10c,d,e,f,g}$	$+^{5;10c,d,e}/-^{10g}$
E13.5	$+^{10e,f;11}/-^{4;10a,b}$			$+^{10a,b,e,f}$	$+^{10e,f}/-^{10a,b}$		$(+)^{10e}/+^{11}/-^{10a,b,f}$	$+^{10a,b,e,f}$	$+^{5;10b,e,f}/-^{8a}$
E14/E14.5	$(+)^{10c}_{g}/+^{3;10a,d,f}/-^{10b,}$	$+^{3}$	$+^{3}$	$+^{10a,b,c,d,f,g}$	$(+)^{10c,d}/+^{3;10a,f,g}/-^{10b}$		$+^{10f}/-^{10a,b,c,d,g}$	$+^{10a,b,c,d,f,g}$	$+^{5;10a,b,c,d,f}/-^{10g}$
E15/15.5	$+^{10a,b,c,d,e;11;22;27;32}$	$-^{27;32}$	$-^{32}$	$+^{10a,b,c,d,e;22;32}$	$(+)^{10c}/+^{10a,b,}_{d;22;32}/-^{10e}$	$+^{32}$	$+^{10e;11}/-^{10a,b,c,d}$	$+^{10a,b,c,d,e}$	$+^{5;10a,b,c,d,e}$
E16.5/17	$+^{10g,f;11;22;27;32}$	$+^{27;32}$	$+^{32}$	$+^{10g,f;22;32}$	$+^{10g,f;22;32}$	$+^{32}$	$+^{10f;11}/-^{10g}$	$+^{10f,g}$	$+^{5;8f,g}$
stomach	$-^{29}/-^{3}$	$-^{3}$	$+^{3}$	$+^{29;30}$	$-^{29}$	$-^{13}$	$-^{25;26}$	$+^{25}/+^{20}$	$-^{25;26}$
rumen							$-^{19}$	$+^{19}$	$+^{19}$
intestine							$+^{19}$	$+^{20}/-^{19}$	$-^{19}$
small	$-^{29}/-^{3}/-^{29}$	$-^{3}$	$+^{3}$	$+^{29}/+^{29}$	$-^{29}/-^{29}$	$-^{13}$	$-^{25;26}$	$+^{25}$	$-^{25;26}$
large	$-^{29}/-^{3}$	$-^{3}$	$+^{3}$	$+^{29}$	$-^{29}$		$-^{25;26}$	$+^{25}$	$-^{25;26}$
liver	$(+)^{29}/+^{22;32}/-^{3;13;14;27}/-^{29}$	$-^{3;27;32}$	$+^{13;14}/-^{3;32}$	$+^{29;30}/+^{22;32}$	$-^{29}/+^{22}/-^{32}/-^{29}$	$-^{33}/(+)^{13}/-^{13;32}$	$-^{25;26}/+^{19}$	$+^{25}/+^{20}/-^{19}$	$-^{25;26}/-^{19}$
pancreas	$-^{3}$	$-^{3}$	$+^{3}$			$+^{13}$			
gall bladder							$-^{19}$	$+^{19}$	$-^{19}$
uterus	$-^{3;13;14}$	$+^{3}$	$+^{3;13;14}$			$-^{33}$	$-^{19}$	$-^{19}$	$-^{19}$
kidney	$-^{29}/-^{3;13;14;27;32}/-^{29}(+)^{22}/-^{29}$	$-^{3;27;32}$	$-^{3;13;14;32}$	$+^{29}/+^{22;32}/+^{29}$	$-^{29}/(+)^{22}/-^{32}/-^{29}$	$+^{13}/-^{33}/-^{13;32}$	$-^{25}/-^{19}$	$+^{25}/+^{20}/+^{19}$	$-^{25}/-^{19}$
thyroid gland				$+^{29}$	$-^{29}$	$-^{33}$			
testis	$+^{22;27;32}/-^{3}$	$-^{27;32}/+^{3}$	$+^{3;32}$	$+^{22;32}$	$+^{22}/-^{32}$	$+^{33}/-^{13;32}$			
epididymis	$-^{3}$	$+^{3}$	$-^{3}$						
seminal vesicle	$-^{3}$	$-^{3}$	$+^{3}$						
prostate gland	$-^{29}$			$+^{29}$	$-^{29}$	$+^{33}$	$-^{26}$		$+^{26h}$
salivary gland	$-^{29}/-^{3}$	$-^{3}$	$-^{3}$	$+^{29;30}$	$-^{29}$	$(+)^{33}$	$-^{25}$	$+^{25}$	$+^{25}$
sebaceous gland	$-^{3}$	$+^{3}$	$+^{3}$						
sweat gland									
acinus							$-^{26}$		$-^{26}$
middle duct							$-^{26}$		$+^{26}$

upper duct							$-^{26}$		$-^{26}$
lung	$+^{22}/(+)^{27}/-^{3;32}$	$-^{3;27;32}$	$-^{3;32}$	$+^{22;32}$	$+^{22}/-^{32}$	$-^{13;33}/-^{13;32}$	$-^{19}$	$+^{20}/+^{19}$	$-^{19}$
trachea						$-^{33}$	$-^{26}/-^{19}$	$+^{19}$	$+^{26h}/-^{19}$
urothelium	$-^{29}/-^{3}/-^{29}$	$-^{3}$	$-^{3}$	$+^{29;30}/+^{29}$	$-^{29}/-^{29}$		$-^{26}/-^{19}$	$+^{20}/-^{19}$	$+^{26h}/-^{19}$
esophagus	$+^{29}/-^{3}/+^{29}$	$+^{3}$	$+^{3}$	$+^{29;30i}/+^{29}$	$+^{29}/+^{29}$		$-^{12;25;26;31}/(+)^{19}$	$+^{12;25;31h,j}/+^{20j}/+^{19j}$	$+^{12;25;26h,j}/+^{19h}$
exocervix							$-^{12;31}$	$+^{12;31h,j}$	$+^{12}$
vagina				$+^{30i}$			$-^{26}$		$+^{26h,j}$
gingival epithelium	$+^{28k}$			$+^{30i;28k}$	$+^{28k}$	$+^{28k}$	$+^{28k}$	$+^{28k}$	$+^{28k}$
junctional	$-^{9l}$			$-^{9l}$	$+^{9}$				
sulcular	$+^{9l}$			$+^{9l}$	$+^{9}$				
oral	$+^{9l}$			$+^{9l}$	$+^{9}$				
buccal mucosa									
tongue	$+^{29}/+^{3}/+^{29}$	$+^{3}$	$+^{3}$	$+^{29}/+^{29}$	$+^{29}/+^{29}$		$+^{16;19}$	$+^{12}$ $+^{20j}/+^{16;19}$	$+^{12}$ $+^{15;19}$
eye bulb									
cornea	$-^{23}$			$+^{23}$	$-^{23}$		$-^{23}$	$+^{23}$	$-^{23}$
limbus	$-^{23}$			$+^{23}$	$+^{23}$		$-^{23}$	$+^{23}$	$+^{23}$
conjunctiva	$-^{23}$			$+^{23}$	$+^{23}$		$-^{23}$	$+^{23}$	$+^{23}$
epidermis	$+^{2;16;21;29;34}/+^{3;13;14;32}/+^{29}$	$+^{3;32}$	$+^{3;13;14;32}$	$+^{2;21;29;30;34}/-^{16}/+^{32}/+^{29}$	$+^{2;21;29;34}/+^{32}/+^{29}$	$+^{2;13;21;33}/+^{13;32}$	$+^{12;25;26;31}/+^{16;19;24}$	$+^{12;25;31}/+^{20}/+^{16;19}$	$+^{12;25;26}/+^{15;19;24;35}$
basal	$+^{21}/+^{3}$	$-^{3}$	$-^{3}$	$+^{2;21;30;34}$	$+^{2;21;34}$	$-^{2;13;21}$	$-^{12;26;31}/-^{24}$	$+^{12;31}/-^{20}$	$+^{12;26}/+^{15;24;35}$
suprabasal	$+^{2;21;34}/+^{3}$	$+^{3}$	$+^{3}$	$-^{2;30;34}$	$-^{2;34}$	$+^{2;13;21}$	$+^{12;26;31}/+^{24}$	$+^{12;31}/+^{20}$	$+^{12;26}/+^{15;24;35}$
hair							$+^{26}$		$+^{26}$
irs	$+^{2;6;18;34}/+^{3}$	$+^{3}$	$+^{3}$	$-^{2;18;34}$	$-^{2;18;34}$	$+^{2;13}/+^{13}$	$+^{18;26}$	$-^{18}$	$+^{18;26}$
ors	$+^{34}/+^{3}$	$+^{3}$	$+^{3}$	$+^{2;34}$	$+^{2;34;17}/+^{17}$	$+^{13}/+^{13}$	$-^{26}$		$+^{26}$
upper:	$+^{2}$			$+^{2}$	$+^{2}$				
bc	$-^{18}$			$+^{18}$	$+^{18}$		$-^{18}$	$+^{18}$	$+^{18}$
cl	$+^{18}$			$-^{18}$	$+^{18}$		$-^{18}$	$+^{18}$	$+^{18}$
cc	$+^{18}$			$-^{18}$	$-^{18}$		$+^{18}$	$-^{18}$	$+^{18}$
lower:									
bc	$-^{18}$			$+^{18}$	$+^{18}$		$-^{18}$	$(+)^{18}$	$+^{18}$
cl	$+^{18}$			$+^{18}$	$+^{18}$		$-^{18}$	$+^{18}$	$+^{18}$
cc	$+^{18}$			$-^{18}$	$+^{18}$		$-^{18}$	$(+)^{18}$	$+^{18}$
precortex				$+^{2}$	$+^{2}$	$+^{2}$			
cortex	$-^{2}$			$-^{2}$	$-^{2}$	$+^{2;18}$			
matrix cells/ trichocytes	$(+)^{18}/-^{2;21}$			$(+)^{2;18;34}$	$+^{2;18;34}$	$+^{2;13}/+^{13}$	$-^{18}$	$+^{18}$	$+^{18}$

(*continued*)

Table 3.1 (*continued*)

	Dsg1/Dsg1α	Dsg1β/ Dsg5	Dsg1γ/ Dsg6	Dsg2	Dsg3	Dsg4	Dsc1	Dsc2	Dsc3
medulla	$+^{18}$			$-^{18;34}$	$+^{18;34}$		$-^{18}$	$+^{18}$	$+^{18}$
heart	$-^{29}/-^{3;13;14;27;32}/+^{22}/-^{29}$	$-^{3;27;32}$	$-^{3;13;14;32}$	$+^{8;29;30}/+^{22;32}/+^{8;29}$	$-^{29}/+^{22}/-^{32}/-^{29}$	$-^{13;33}/-^{13;32}$	$-^{25;26}/(+)/-^{16;19}$	$+^{8;25}/+^{20}/-^{16}/+^{19}$	$-^{26}/-^{19}$
skeletal muscle	$-^{3;13;14;22;32}/(+)^{27}$	$-^{3;27;32}$	$+^{13;14}/-^{3;32}$	$+^{22;32}$	$-^{22;32}$	$+^{13}/-^{33}/-^{13;32}$	$-^{19}$	$-^{19}$	$-^{19}$
lymph node				$+^{30m}$			$+^{25}$	$+^{25}$	$-^{25}$
spleen	$-^{29}/+^{22;27}/-^{3;32}$	$-^{3;27;32}$	$-^{3;32}$	$+^{29}/+^{22;32}/+^{29}$	$-^{29}/+^{22}/-^{32}/-^{29}$	$+^{13}/-^{33}/-^{13}$			
arachnoid mater	$-^{1}$			$+^{1}$	$-^{1}$		$-^{1}$	$+^{1}$	$+^{1o}$
thymus	$-^{3}/-^{29}$	$-^{3}$	$+^{3n}$	$+^{29}$	$+^{29}$	$+^{13}/-^{33}/-^{13}$	$+^{26n}$		$+^{26n}$
adrenal gland						$-^{33}$			
brain	$-^{3;13;14;27;32}/(+)^{22}$	$-^{3;27;32}$	$+^{13;14}/-^{3;32}$	$+^{22;32}$	$+^{22}/-^{32}$	$+^{13}/-^{33}/-^{13}$			
cerebellum	$-^{3}$	$-^{3}$	$-^{3}$			$-^{33}$			
fetal						$-^{33}$			
bone marrow						$-^{33}$			
ovary	$-^{3}$	$-^{3}$	$-^{3}$						$+^{5\star}$

[a] oral epithelium;
[b] tongue;
[c] dental epithelium;
[d] lip furrow;
[e] nasal epithelium;
[f] pad epidermis;
[g] gastric epithelium;
[h] basal;
[i] only basal;
[j] suprabasal;
[k] a mixture of monoclonal antibodies against Dsgs and Dscs was used;
[l] the antibody against Dsg1 showed crossreactivity with Dsg2;
[m] dendritic reticulum cells;
[n] Hassall bodies;
[o] dural border cells only
⋆ staining is limited to the oozyte
italic letters mark the earliest expression of the gene in question in the given tissue

[1] Akat *et al.*, 2003: immunohistology
[2] Bazzi *et al.*, 2006: immunohistology
[3] Brennan *et al.*, 2004: immunohistology
[4] Collins *et al.*, 1995: RT–PCR
[5] Den *et al.*, 2006: Northern Blot and immunohistology
[6] Donetti *et al.*, 2004: quantitative immunoelectron microscopy
[7] Eshkind *et al.*, 2002: immunohistology
[8] Franke *et al.*, 2006: quantitative immunoelectron microscopy
[9] Hatakeyama *et al.*, 2006: immunohistology
[10] King *et al.*, 1997: *in situ* hybridization
[11] King *et al.*, 1996: only the *in situ* hybridization results are included in the table
[12] King *et al.*, 1995: *in situ* hybridization and immunohistology
[13] Kljuic *et al.*, 2003a: RT–PCR, immunohistology, *in situ* hybridization
[14] Kljuic *et al.*, 2003b: RT–PCR
[15] Koch *et al.*, 1991: Northern Blot
[16] Koch *et al.*, 1992: Northern Blot
[17] Koch *et al.*, 1998: immunohistology
[18] Kurzen *et al.*, 1998: immunohistology
[19] Legan *et al.*, 1994: RT–PCR, Northern Blot and *in situ* hybridization
[20] Lorimer *et al.*, 1994: RT–PCR and *in situ* hybridization
[21] Mahoney *et al.*, 2006: immunohistology
[22] Mahoney *et al.*, 2002: RT–PCR
[23] Messent *et al.*, 2000: immunohistology and immunoblotting
[24] North *et al.*, 1996: quantitative immunoelectron microscopy
[25] Nuber *et al.*, 1995: Northern Blot and RNAse protection assay
[26] Nuber *et al.*, 1996: immunohistology
[27] Pulkkinen *et al.*, 2003: RT–PCR
[28] Sawa *et al.*, 2005: immunohistology
[29] Schafer *et al.*, 1994: RNAse protection assay
[30] Schafer *et al.*, 1996: immunohistology
[31] Theis *et al.*, 1993: *in situ* hybridization
[32] Whittock, 2003: RT–PCR
[33] Whittock and Bower, 2003: RT–PCR
[34] Wu *et al.*, 2003: immunohistology
[35] Yue *et al.*, 1995: immunohistology and immunoblotting

The methods used to detect the different Dsg and Dsc isoforms are given along with the appropriate citations. Signals were classified as either absent −, weak (+), or strong +. irs: inner root sheath; ors: outer root sheath; bc: basal cell layer; cl: central cell layer; cc: companion cell layer.

In particular, the generally assumed exclusive synthesis of Dsg2 and Dsc2 in simple epithelia (e.g., Nuber *et al.*, 1995; Schafer *et al.*, 1996) is not supported by all observations reported to date. Thus, Dsg1 (Dsg1γ/Dsg6) was shown to be present in the mucosal lining of the stomach, intestine, and uterus as well as in the glandular epithelia of pancreas and liver of mouse (Brennan *et al.*, 2004; Kljuic and Christiano, 2003; Kljuic *et al.*, 2003a) and also in human liver (Schafer *et al.*, 1994). In addition, Dsg4 is detectable in human liver and pancreas (Kljuic *et al.*, 2003a) and Dsc1 in bovine intestine and liver (Legan *et al.*, 1994). Most unexpected was the recent description of Dsc3 production during the earliest stages of embryogenesis also including cells lacking desmosomes (Den *et al.*, 2006), reminiscent of the detection of Dsg2 in such cells (Eshkind *et al.*, 2002). Similarly surprising is the early onset of Dsg3 and Dsg4 synthesis in E7 embryos preceding the formation of complex and stratified epithelia by several days (Mahoney *et al.*, 2002; Whittock, 2003).

It should be stressed that Dsg2 and Dsc2 are certainly the predominant isoforms during development. Both are detectable from the morula or the very early blastocyst stage (E3.5) onward (i.e., just prior to the formation of the desmosome-containing trophectoderm) (Collins *et al.*, 1995; Fleming *et al.*, 1991; Jackson *et al.*, 1980). An example of Dsg immunoreactivity in blastocysts is presented in Fig. 3.8A that coincides with DP at the plasma membrane (Fig. 3.8B), but appears to be somewhat more extensive including cytoplasmic staining that is most prominent in the inner cell mass. In addition, a presumably maternal pool of Dsc2 and of Dsc3 has been detected in unfertilized eggs and in cleavage stages up to the early eight-cell stage (Collins *et al.*, 1995; Den *et al.*, 2006). A detailed and quite comprehensive description

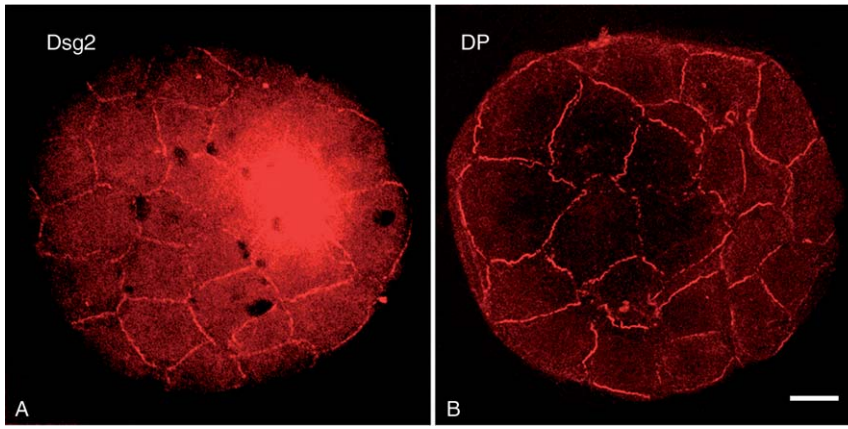

Figure 3.8 Whole-mount confocal immunofluorescence microscopy of murine blastocysts detecting desmosomal proteins Dsg2 (A) and DP (B; same antibodies as in Fig. 3.7). Bar: 10 μm. (The micrographs are taken from Fig. 2 of Eshkind *et al.*, 2002, with permission from Elsevier.)

of the temporospatially regulated occurrence of desmosomal cadherins in murine epithelia using *in situ* hybridization can be found in King *et al.* (1997) demonstrating individual patterns of regulation for each polypeptide and specific correlations to ongoing epithelial differentiation. Thus, unique and complex patterns of coexpression are generated. In general, the first desmosomal cadherin to be synthesized is Dsg2 and the latest, which is produced only in suprabasal layers of keratinizing epithelia, is Dsc1.

While the expression of Dsg1, 3, and 4 and of Dsc1 and 3 is somewhat variable in simple and complex epithelia (also including the urothelium), a consistent and strong synthesis of these polypeptides is noted in all stratified epithelia with the exception of the corneal and conjunctival epithelium lacking Dsg1 as well as Dsc1 (Table 3.1). Furthermore, Dsc3 and Dsg3 production is restricted to the limbus but is absent in the cornea proper, demonstrating that Dsc2 and Dsg2 suffice to support stratified epithelia on their own (Messent *et al.*, 2000). In the other stratified epithelia, isoforms 2 and 3 are most strongly expressed in the basal compartment with highest protein levels for isoforms 2, whereas isoforms 1 and Dsg4 show an inverse concentration gradient reaching highest levels in the suprabasal cell layers (Getsios *et al.*, 2004b). In general, the expression patterns of the respective isoforms 1 and 3 of each desmosomal cadherin type often coincide. Yet, minor differences are readily apparent (e.g., Dsc3 positivity in human urothelium in the absence of Dsg3 [Nuber *et al.*, 1996; Schafer *et al.*, 1994] or the inverse detectability of Dsg1 and Dsc1 in liver of human and cow [Legan *et al.*, 1994; Nuber *et al.*, 1996; Schafer *et al.*, 1994]), arguing against a strict transcriptional coregulation of DSG and DSC genes.

The distribution of desmosomal cadherins in different layers of the epidermis has been studied in detail: Dsc2 mRNA is localized primarily in the basal and lower levels of suprabasal cell layers whereas Dsc1 mRNA is restricted to suprabasal cells with the highest concentration in the spinous cell layer (Arnemann *et al.*, 1993; King *et al.*, 1995; Legan *et al.*, 1994; Theis *et al.*, 1993). Dsc3 synthesis is strongest in basal cells but extends into the first few suprabasal cell layers (Arnemann *et al.*, 1993; Legan *et al.*, 1994). These results could be corroborated by using isoform-specific antibodies directed at Dscs 1 and 3 (North *et al.*, 1996; Nuber *et al.*, 1996), especially emphasizing an inverse relationship between both isoforms, and further demonstrating that individual desmosomes may contain both isoforms. Similarly, the Dsg2 immunosignal was almost exclusively restricted to the basal compartment of epidermis (Arnemann *et al.*, 1993; Schafer *et al.*, 1996).

The most complex distribution patterns were observed in human hair, demonstrating unique profiles of desmosomal cadherins for individual layers and cell types (see Table 3.1). While Dsc3 was noted in all epithelial cell types, Dsc1 and Dsc2 exhibited an almost inverse and exclusive distribution (Kurzen *et al.*, 2003). In contrast, partial overlap was noted between the inversely distributed Dsg2 and Dsg1/3 (Kurzen *et al.*, 2003).

Desmosomes in nonepithelial tissues, most notably in cardiomyocytes, meningeal cells of the arachnoid mater, and dendritic reticulum cells of lymph nodes, all contain Dsg2 and Dsc2 (see Table 3.1). Other desmosomal cadherins are either detected only in trace amounts by RT-PCR or are restricted to certain cell layers such as DSC3 to dural border cells of the arachnoid mater (Akat *et al.*, 2003). The significance of spurious detection of various desmosomal cadherins in tissues lacking typical desmosomes is currently not clear. It remains to be shown whether these findings are due to contaminating desmosome-bearing tissue fragments or are an indication of the presence of alternative adhesion structures and/or cell compartments harboring desmosomal cadherins.

The information on Dsc splice variant expression is incomplete. Occasionally, double bands of equal intensity were resolved in Northern blots (Koch *et al.*, 1992). By RT-PCR both Dsc2 splice variants were detected in many different tissues at variable relative amounts (Nuber *et al.*, 1995). A further layer of desmosomal cadherin complexity is added by the coassembly of different desmosomal cadherin isoforms within single desmosomes (North *et al.*, 1996; Nuber *et al.*, 1996). The characterization of regulatory elements determining cell type–specific gene transcription is still in its infancy and few studies have addressed these issues in any depth (Marsden *et al.*, 1997).

So far, no strict correlations have been established for other desmosomal components that would suggest a strictly coregulated expression of specific combinations.

Differential synthesis of the *arm*-containing desmosomal linker proteins has been examined in case of the PPs revealing different levels of expressional complexity concerning cell type specificity and subcellular localization. In the absence of desmosomes these polypeptides are soluble and primarily nucleoplasmic, whereas in the presence of desmosomes a certain proportion is recruited into cell adhesion sites (Bonne *et al.*, 1999; Mertens *et al.*, 1996; Schmidt *et al.*, 1997). The signals that determine the subcellular localization are poorly understood at present. Interestingly, however, PP1b resides exclusively in the nucleus, whereas PP1a exhibits a nuclear and desmosomal distribution (Schmidt *et al.*, 1997). Both splice variants differ just by a 21 amino acid-encoding exon that is present only in splice variant b. A complete distribution map has not been established to date. But PP1a has been primarily detected in desmosomes of stratified and complex epithelia (Hatzfeld *et al.*, 1994; Heid *et al.*, 1994; Kapprell *et al.*, 1988; Schmidt *et al.*, 1997), whereas PP2 splice variants a and b have been identified predominantly in desmosomes of simple epithelia and nonepithelial tissues such as myocardium or dendritic reticular cells of lymph nodes, but were also found in certain complex and stratified epithelia (Mertens *et al.*, 1996, 1999). PP3 has been reported to be the most epithelium specific and has been localized in desmosomes and the nucleus of simple and stratified epithelia but not in hepatocytes and myocardium (Schmidt *et al.*, 1999). In contrast to

individual PPs, PG has been identified in all desmosomes and is also a universal component of other adhaerens junctions, thereby presenting a very broad distribution pattern (Cowin *et al.*, 1986; Kapprell *et al.*, 1987).

Both DP splice variants have been shown to be present in simple and stratified epithelia, although DPII could not be detected in cardiac muscle (Angst *et al.*, 1990), and there is some controversy on the presence/absence of DPII in certain epithelia (Cowin *et al.*, 1985). However, there is agreement that DPI is present in all desmosome-bearing cells/tissues and pathological alterations derived therefrom (examples are shown in Figs. 3.6 to 3.8). DP also localizes to the complexus adhaerens in vascular endothelia (Schmelz and Franke, 1993). In heart, it was recently shown to localize to both the desmosome-like and fascia adhaerens–type junctions as is the case for PP2 (Borrmann *et al.*, 2006; Franke *et al.*, 2006; Grossmann *et al.*, 2004). Since both types of junctions are molecularly and morphologically intermixed in the intercalated disc, it was recently suggested to be a junction type on its own, for which the name *area composita* was coined (Borrmann *et al.*, 2006; Franke *et al.*, 2006).

The precise expression patterns of the various accessory desmosomal proteins that are present only in cell type–specific contexts have not been examined systematically and have been cursorily mentioned above.

It should be noted that the production of desmosomal polypeptides is not restricted to normal tissues but is also detectable in tumors originating from desmosome-bearing tissues. As such, their detection has become a valuable additional criterion in the histodiagnosis of carcinomas and meningiomas (Akat *et al.*, 2003; Moll *et al.*, 1986).

4. Biogenesis

4.1. Desmosome formation during development

Desmosomes are first detected together with keratin filaments in the trophectoderm during the blastocyst stage of preimplantation mouse embryos (Collins and Fleming, 1995; Collins *et al.*, 1995; Ducibella *et al.*, 1975; Eshkind *et al.*, 2002; Fleming *et al.*, 1991; Jackson *et al.*, 1980), suggesting that desmosome formation strengthens adhesion to maintain tissue integrity in the presence of increasing mechanical stress imposed by the accumulating blastocoele fluid. Noticeably, adherens junctions and tight junctions are formed prior to desmosomes, indicative of a hierarchy of junctional complex formation (Fleming *et al.*, 1994). In addition to this temporal order, a spatial order is also evident early, as the different junctions become arranged in the characteristic junctional complex in which desmosomes are localized to the basolateral cell–cell borders. The typical midline structure and electron-dense intermediate filament-anchoring plaques as diagnostic

desmosomal hallmark features are already detectable at the blastocyst stage (Fleming *et al.*, 1991). By immunohistological examination desmosomal components can be detected as puncta at the lateral membrane contact sites between the trophectoderm cells from the 32-cell stage onward (Fleming *et al.*, 1991). Recently, a novel type of Dsg2-positive nondesmosomal punctate adhesion site was identified in embryonic stem cells (Eshkind *et al.*, 2002). It will be interesting to find out whether these adhesions represent specific desmosomal precursors. In addition, it will be informative to understand the significance of desmosomal cadherin synthesis noted in inner cell mass derivatives (Collins *et al.*, 1995; Eshkind *et al.*, 2002) and the lagging formation of desmosomes. Remarkably, desmosomes still assemble in the absence of keratin filaments in embryonal bodies (Baribault and Oshima, 1991) and in hepatocytes (Magin *et al.*, 1998), although alterations in DP distribution were noted in the latter instance (Loranger *et al.*, 2006).

In early postimplantation mouse embryos, desmosomes are present in the two major embryonic epithelia (i.e., the embryonic ectoderm and the visceral/proximal endoderm) (Jackson *et al.*, 1981). Molecular analyses corroborated the initial morphological studies and revealed that desmosomal protein synthesis precedes the morphogenesis of desmosomes (Fleming *et al.*, 1991). The advent of stratified epithelia during mouse embryogenesis is accompanied by changes in desmosomal cadherin synthesis (King *et al.*, 1997). The originally synthesized isoforms Dsg2 and Dscs2/3 are complemented by Dsg isoforms 1, 3, and 4 and by Dsc1 (see Table 3.1) probably furnishing additional desmosomal qualities.

4.2. Experimental analysis of desmosomal biogenesis

Studies of the synthesis of desmosomal components during embryogenesis suggest that desmosome formation is contingent on the presence of desmosomal cadherins, *arm*-repeat–containing polypeptides, and DP. Multiple attempts have been undertaken to put the essential components together in *in vitro* systems to examine the molecular requirements and the interactive surfaces participating in this process. Probably the most informative attempt has been presented by Koeser and colleagues (2003). They were able to reconstitute desmosome-like cell adhesion complexes together with anchored intermediate filaments in fibrosarcoma-derived HT-1080 cells. These desmosome-free cells synthesize the desmosomal cadherin Dsg2 in the absence of any Dsc and desmosomal plaque proteins. It was shown that all three major plaque proteins (i.e., DP, PG, and PP2) are necessary and probably sufficient to form structurally and functionally competent desmosomes, even in the absence of detectable amounts of Dsc. Interestingly, PG was essential for the segregation of desmosomal and adherens junction components whereas PP2 was able to efficiently recruit DP to cell–cell junctions. In the absence of PG, however, this recruitment was not specific to desmosomes and resulted in misdirection of DP into adherens junctions.

By using a tailless DP mutant lacking the intermediate filament binding sites it was also shown that desmosome assembly occurs independently of the intermediate filament system in accordance with various *in vitro* and *in vivo* situations in which desmosomes are present in the absence of an intact intermediate filament network (Baribault and Oshima, 1991; Bornslaeger *et al.*, 1996; Denk *et al.*, 1985; Loranger *et al.*, 2006; Magin *et al.*, 1998).

The aforementioned experiments extend earlier experiments in which reconstitution of desmosomal adhesion was attempted either in nonepithelial or in epithelial cells. The first approach was hampered by the fact that in contrast to the classical cadherins, adhesive properties cannot be efficiently conferred by either Dsg or Dsc alone in nonepithelial cells (Amagai *et al.*, 1994; Chidgey *et al.*, 1996), not even when combined individually with PG (Kowalczyk *et al.*, 1996). Instead, strong adhesion requires Dsgs, Dscs, and PG in a specific ratio (Dusek *et al.*, 2007; Getsios *et al.*, 2004a; Marcozzi *et al.*, 1998; Tselepis *et al.*, 1998). The second approach exploited the presence of desmosomal components, known and unknown, by employing desmosome-containing epithelial cells. An attempt was made to recruit and redirect desmosomal polypeptides to morphologically and immunologically distinct membrane sites in desmosome-rich vulvar carcinoma-derived A-431 cells (Chitaev *et al.*, 1996, Troyanovsky *et al.*, 1993, 1994a,b). To accomplish this, connexin transmembrane segments were fused to desmosomal cadherin tails thereby enriching them in ultrastructurally defined gap junctions. It was shown that connexin 32–Dsc1a cytoplasmic tail hybrids indeed induced the formation of large gap junctions that recruited DP- and PG-positive electron-dense plaque material and served as keratin filament anchorage sites (Troyanovsky *et al.*, 1993, 1994b). On the other hand, a comparable connexin 32–Dsg1 cytoplasmic tail hybrid was not only incapable of such an assembly but acted as a dominant-negative mutant interfering with connexin clustering and endogenous desmosome formation (Troyanovsky *et al.*, 1993, 1994a) possibly due to an altered balance between the different PG pools (Norvell and Green, 1998). Both paradigms, however, could be used to map the respective PG-binding sites (Troyanovsky *et al.*, 1994a,b). In addition, Dsc determinants for DP recruitment were identified (Troyanovsky *et al.*, 1994b). In a similar approach hybrids consisting of the four membrane-spanning domains of the synaptic vesicle protein synaptophysin were fused to PG to examine the molecular binding determinants of the latter to desmosomal cadherins and the desmosomal plaque region (Chitaev *et al.*, 1996; Troyanovsky *et al.*, 1996).

Another completely different approach to examine desmosomal biogenesis was taken by several laboratories using live cell imaging of fluorescently labeled desmosomal components (Gloushankova *et al.*, 2003; Godsel *et al.*, 2005; Windoffer *et al.*, 2002). The most comprehensive study was done by Godsel and coworkers (2005) using fluorescent DP hybrids and examining

desmosome formation after scratch wounding in cultured cell lines. Based on their observations a multistep process was proposed. Within a few minutes of cell–cell attachment DP-positive puncta are recruited to the newly formed contact zones presumably from a rapidly available cytoplasmic DP pool. This pool may correspond to the discrete and insoluble cytoplasmic particles that were previously identified by immunohistology in the absence of cell–cell contacts and was rapidly depleted upon cell–cell contact formation (Pasdar and Nelson, 1988b). Soon afterward, DP particles form in the cytoplasm that are associated with PP2 and translocate in an actin-dependent fashion to the plasma membrane, probably contributing to the enlargement of the existing DP-positive membrane sites. Much work needs to be done to further substantiate this rough working hypothesis, especially with regard to the other desmosomal components that appear to team up only at the plasma membrane (e.g., Pasdar *et al.*, 1991). It also should be noted, however, that desmosomal halves assemble even in the absence of direct cell–cell contact (i.e., in cells continuously grown in low calcium medium) (Demlehner *et al.*, 1995).

It is of interest that desmosome assembly may differ between different cell types and may even differ in the same cell type depending on the circumstance. Thus, it was reported that Dsg1 and Dsc1 are not recruited into desmosomes in cultured simple or squamous epithelial cells (Chitaev *et al.*, 1998; Ishii *et al.*, 2001) demonstrating desmosomal cadherin isotype specificity. Other striking examples of the context-dependent complexity of desmosome assembly are the apparent differences concerning the contribution of PG *in vitro* and *in vivo*. On the one hand, PG was shown to be absolutely required for desmosome formation in the *in vitro* HT-1080 culture system (Koeser *et al.*, 2003), and several studies presented evidence that the PG-binding site of Dsg is vital for normal function of this protein (Andl and Stanley, 2001; Palka and Green, 1997). On the other hand, ultrastructurally normal desmosomes are formed in epithelial cells of mice lacking PG (Ruiz *et al.*, 1996). These apparent contradictions imply that the pathways of desmosome assembly are much more limited in cultured cells than in cells that are embedded in a complex tissue context. It is likely that other cell–cell contact structures affect and coordinate desmosome assembly (see later), and their absence may result in the elimination of specific desmosomal assembly pathways that may be exclusively used in the *in vitro* situation.

5. Dynamics

5.1. Desmosome dynamics during interphase and mitosis

An important issue of desmosomal adhesion is how desmosomes can maintain their structural function while allowing dynamic cell behavior that requires cell–cell contact rearrangements as is the case (e.g., during

migration, differentiation, and tissue regeneration). Mechanisms must exist to attenuate desmosomal adhesive strength to local requirements. Regulation of desmosomal size may be one way to fulfill such a task. In support of this, immature ''nascent'' desmosomes are small, whereas desmosomes in tissues that are subjected to increased mechanical stress are rather large. Such growth of desmosomes from small precursor particles has been observed by live cell imaging (Gloushankova *et al.*, 2003; Godsel *et al.*, 2005).

Alteration in molecular composition is another way to modulate desmosomal adhesive properties by changing the isoforms present and/or the relative amounts of Dscs and Dsgs. The stratum-specific desmosomal composition in epidermal cell layers supports this notion and may contribute to the tight association of suprabasal cells contrasting with the capacity of basal cells to move to upper layers by asymmetric mitotic division.

Live cell imaging experiments revealed another aspect of desmosome dynamics (Windoffer *et al.*, 2002). Although individual desmosomes could be followed for many hours, a very rapid exchange of its constituents was observed including even the desmosomal cadherins (Fig. 3.9). In this way, minor modification of adhesive strength could be accomplished without complete disruption of adhesion sites. It is attractive to speculate that signal-dependent protein modification is involved in the regulation of turnover kinetics.

Interestingly, desmosomes are maintained during the entire cell cycle providing continuous anchorage to neighboring cells and cytoplasmic intermediate filaments even during cell division (Baker and Garrod, 1993; Shimizu *et al.*, 2000; Windoffer *et al.*, 2002). Yet, desmosomal stability was shown to be considerably altered in dividing cells exhibiting increased fusion of desmosomal particles into large plaque areas that were often concentrated around the cleavage furrow (Windoffer *et al.*, 2002). Simultaneously, increasing dispersion of desmosomal cadherins was noted during prophase. The characteristic finely punctate desmosomal fluorescence was reestablished after cytokinesis concomitant with loss of the diffuse distribution.

5.2. Calcium-dependent alterations of desmosomes

The classical method to examine desmosomal dynamics involves calcium-shift experiments. Reduction of extracellular calcium results in disassembly of desmosomes and uptake of desmosomal material into the cell interior (Fig. 3.10; Kartenbeck *et al.*, 1982, 1991; Mattey and Garrod, 1986). Cytoplasmic vesicles were identified containing complete desmosomal assemblies together with their adhering intermediate filaments. The uptake of desmosomal particles into the perinuclear area was shown to be dependent on both actin filaments (Fig. 3.11; Holm *et al.*, 1993; Windoffer *et al.*, 2002) and microtubules (see Fig. 3.11). It is, however, independent of clathrin (Holm *et al.*, 1993). Although the particles may initially reach a nonlysosomal compartment (Holm *et al.*, 1993) and may therefore

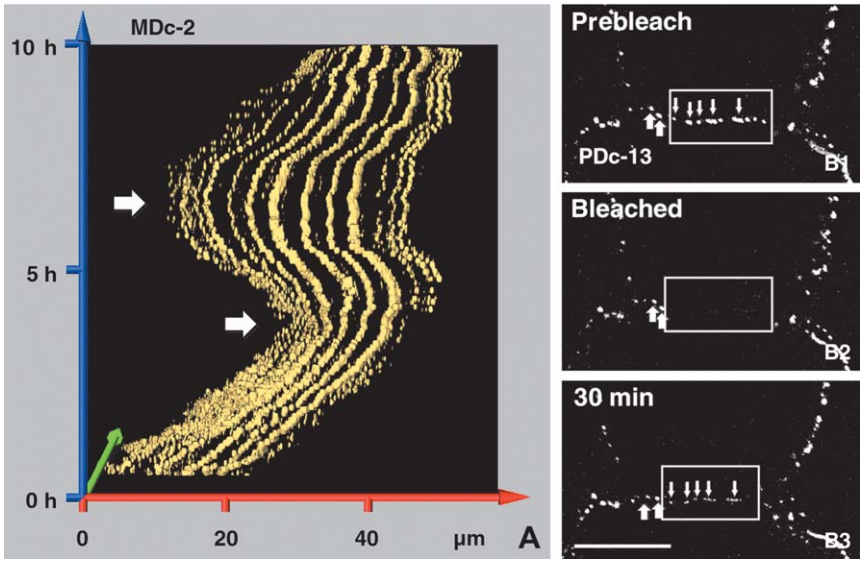

Figure 3.9 Time-space diagram of desmosomal motility (A) and image series from a fluorescence recovery after photobleaching (FRAP) experiment (B) of cell lines producing fluorescent Dsc2. (A) Derived from a 10 h epifluorescence recording of a desmosomal array in canine kidney MDCK-derived MDc-2 cells (recording intervals 5 min). The time is plotted along the *y*-axis in hours (h), the movement in the two-dimensional space dimension along the *x*- and *z*-axes in micrometers (μm). The trajectories of the time surface of desmosomal fluorescence highlight the coordinated movement of desmosomes whose overall arrangement, size, and shape remain mostly the same for the entire period. In contrast, the interdesmosomal distance varies considerably, albeit in a coordinated fashion (compare, e.g., the two time points that are marked by arrows) as if they are arranged on an elastic string. (B) The confocal fluorescence image series was taken from hepatocellular carcinoma PLC-derived PDc-13 cells that were subjected to intense photobleaching in the boxed area. The pictures depict the desmosomal fluorescence prior to bleaching (B1), immediately after bleaching (B2), and after a 30-min recovery period. Note the considerable recovery of fluorescence in the bleached desmosomes indicating that despite the overall longevity of single desmosomal entities (see A), a high turnover of integral desmosomal polypeptides occurs. Bar: 10 μm. (The figures are taken from Figs. 4 and 11 of Windoffer *et al.*, 2002.)

potentially retranslocate to the cell surface, experimental evidence rather suggests that they are not directly reused but either degraded (Burdett, 1993; Mattey and Garrod, 1986) and/or disassembled into nondesmosomal subunits/polypeptides (Windoffer *et al.*, 2002). A recent study examining the effect of pemphigus vulgaris serum also showed that desmosomal components are targeted first to an endosomal and subsequently to a lysosomal compartment (Calkins *et al.*, 2006). In this instance, however, the Dsg3–PG complex detached from DP and keratin filaments prior to uptake.

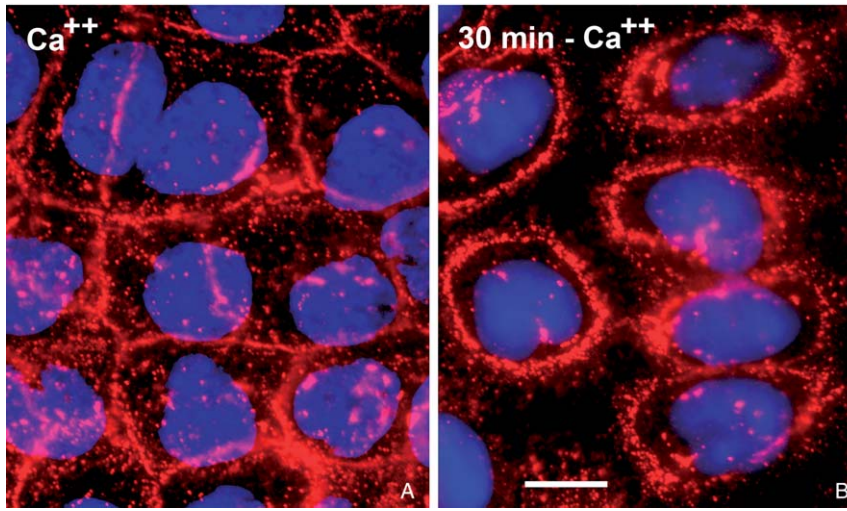

Figure 3.10 Immunofluorescence microscopy of colon carcinoma-derived CaCo-2 cells before (A) and 30 min after depletion of calcium (B, cf. Windoffer *et al.*, 2002) detecting the distribution of DP using monoclonal antibodies DP 2.15/2.17/2.20. Nuclei are stained with DAPI. Note the displacement of DP-positive puncta from the cell periphery to a cytoplasmic, perinuclear domain after calcium removal. Bar: 25 μm.

The uptake of desmosomal particles in low calcium medium depends on various parameters such as cell type, passage number, and time after plating (Mattey and Garrod, 1986; Wallis *et al.*, 2000; Watt *et al.*, 1984; Windoffer *et al.*, 2002). Interestingly, calcium sensitivity of desmosomal uptake can be transmitted to neighboring cells by protein kinase C-dependent signaling (Wallis *et al.*, 2000). It has been suggested that desmosomes reach a state of hyperadhesion whose signet feature is calcium independence and confers high stability (Garrod *et al.*, 2005). It is subject to regulation, however, and can transit to a calcium-dependent condition in situations that require reduced adhesion as is the case upon wounding (Wallis *et al.*, 2000).

Furthermore, endocytosis may not be the direct cause but rather a consequence of desmosomal destabilization in the absence of calcium. In support of this, live cell imaging revealed a rapid dissolution of desmosomal cadherin particles in the plane of the plasma membrane upon calcium depletion with only very little uptake of desmosomal particles into cytoplasmic carriers (Windoffer *et al.*, 2002). These observations could be interpreted as an overall reduction of desmosomal coherence resulting either in diffusion within the plasma membrane, in uptake of molecular assemblies that were below the detection limit, or in rapid degradation.

The calcium-switch system has also been used to examine desmosome formation by shifting cells from low to high calcium media. The resulting

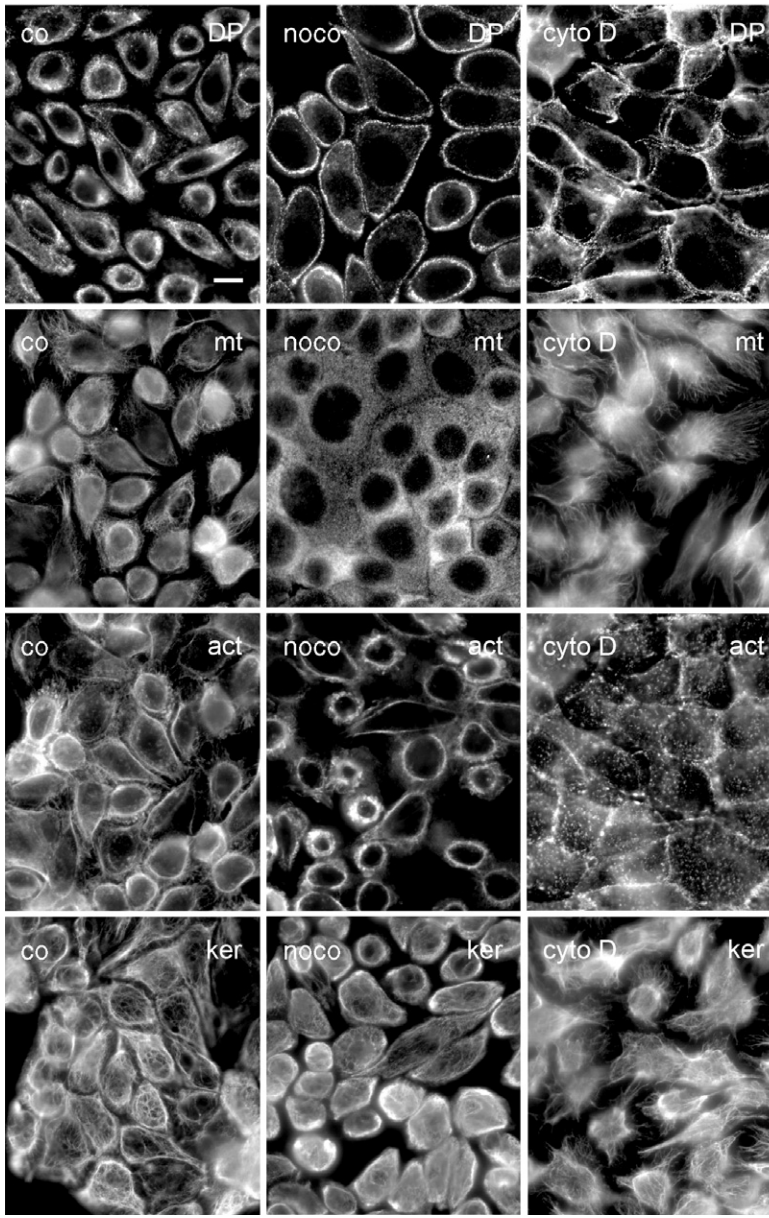

Figure 3.11 Indirect immunofluorescence microscopy of Madin–Darby canine kidney cells (MDCK) after transfer to a calcium-depleted medium (Windoffer *et al.*, 2002). In some instances, cells were preincubated for 15 min in standard medium in the presence of either 1 m*M* nocodazole (noco) or 5 μM cytochalasin D (cyto D). Subsequent incubation in calcium-depleted medium in the absence (co) or presence of drugs lasted 30 min prior to fixation in methanol/acetone. Desmosomal plaque protein DP was detected

experimental evidence suggests that desmosomes assemble at the plasma membrane, most likely by maturation of enlarging particles (Hennings and Holbrook, 1983; Pasdar and Nelson, 1989; Watt *et al.*, 1984; Windoffer *et al.*, 2002). It was found that desmosome assembly in MDCK cells upon calcium shift can be grouped into two phases (Burdett and Sullivan, 2002): During the first 30 min 60-nm vesicles containing mostly Dsc2 were found while at later time points larger vesicles of ~200 nm with Dsg, E-cadherin, PG, and β-catenin were directed to presumptive nucleation sites. The essential contribution of Dsc during early stages of desmosomal assembly was further underscored by observations in HaCaT cells producing amino-terminally deleted Dsc3. These cells presented impaired assembly of adherens junctions and desmosomes, whereas a comparable Dsg3 mutant affected only desmosome formation (Hanakawa *et al.*, 2000). The capacity of the Dsc mutant to bind to both β-catenin and PG may explain the observed differences. It will be interesting to find out how these processes relate to the delivery of DP to newly forming desmosomal adhesion sites (Godsel *et al.*, 2005). The current view is that DP and desmosomal cadherins are delivered separately to the plasma membrane based on their separate distribution in the insoluble pool (Pasdar *et al.*, 1991), their different distribution patterns in the cytoplasm (Pasdar and Nelson, 1988b; Pasdar *et al.*, 1991), and the actin-dependent delivery of DP (Godsel *et al.*, 2005) that appears to be associated with intermediate filaments (Pasdar *et al.*, 1991) while Dsg colocalized with microtubules (Pasdar *et al.*, 1991). It should be noted, however, that neither intermediate filaments nor microtubules are essential for desmosome assembly (Baribault and Oshima, 1991; Magin *et al.*, 1998; Pasdar *et al.*, 1992).

Some controversy exists with respect to the importance of vesicular half-desmosomes in cells grown for many passages in low calcium medium. It is unquestionable that such structures are formed under these conditions in different cell types (Demlehner *et al.*, 1995; Duden and Franke, 1988). However, vesicular localization may apply to only a small percentage of desmosomal proteins synthesized under such conditions (Burdett, 1993; Windoffer *et al.*, 2002), and biochemical analyses demonstrate an elevated solubility of desmosomal cadherins in this situation (Pasdar and Nelson, 1989; Penn *et al.*, 1987), suggesting that at least this biochemically defined fraction is not part of large assemblies.

with murine monoclonal antidesmoplakin 1/2 antibody mix DP-2.15/DP-2.17/DP-2.20, microtubules (mt) with monoclonal anti α-tubulin antibodies (Amersham Pharmacia Biotech, Freiburg, Germany), actin filaments (act) with Texas Red-conjugated phalloidin (Molecular Probes, Eugene, OR), and keratin filaments (ker) with murine monoclonal antibodies against keratin 8 (clone Ks8–17.2 from Progen). Note that disruption of microtubules and actin filaments interferes with desmosomal uptake. Bar: 10 μm.

An interesting aspect of calcium-dependent adhesion site dynamics was recently reported for E-cadherin that may also be of relevance for desmosomal adhesion (Troyanovsky *et al.*, 2006). Blocking endocytosis by ATP depletion or by hypertonic sucrose led to a rapid increase in adhesive E-cadherin dimers coinciding with acquisition of calcium resistance of intercellular contacts. It was suggested that endocytosis is needed for disassembly of the surprisingly stable cadherins' adhesive dimers.

5.3. Phosphorylation-dependent alterations of desmosomes

The importance of phosphorylation for desmosomal functions and dynamics has only been rudimentarily investigated, although most if not all desmosomal components are subject to phosphorylation (Amar *et al.*, 1999; Gaudry *et al.*, 2001; Miravet *et al.*, 2003; Pasdar *et al.*, 1995a; Shibamoto *et al.*, 1994; Stappenbeck *et al.*, 1994; Yin and Green, 2004). The relevance of phosphorylation is, once again, becoming apparent in disease. Thus, desmosomal cadherins become phosphorylated upon pemphigus vulgaris (PV) antibody ligation (Aoyama *et al.*, 1999). In this situation, Dsg3 is serine phosphorylated and dissociates from PG.

Multiple tyrosine phosphorylation sites have been identified in PG (Miravet *et al.*, 2003); however, these have different effects on its binding properties (Yin and Green, 2004). Therefore, tyrosine phosphorylation of PG was shown to allow association with Dsgs but to prevent interaction with DP (Gaudry *et al.*, 2001; Miravet *et al.*, 2003; Yin *et al.*, 2005). This may explain why EGFR inactivation promotes desmosomal assembly (Lorch *et al.*, 2004) and, conversely, why EGF as well as HGF/SF induce cell scattering (Shibamoto *et al.*, 1994). Other studies have revealed, however, that tyrosine phosphorylation of other PG sites may lead to increased binding to DP and simultaneous reduction of affinity to E-cadherin and α-catenin and vice versa (Miravet *et al.*, 2003). Furthermore, phosphorylation of PG may affect its solubility (Pasdar *et al.*, 1995b). Taken together, PG phosphorylation appears to be an important mechanism to regulate junctional localization and to determine desmosomal adhesive strength. In addition, PG phosphorylation modulates its transcriptional activation properties (Miravet *et al.*, 2003) and affects its targeting to the proteasomal degradative pathway. Interestingly, O-GlcNAc addition at the amino-terminal catenin-like "destruction box" counteracts phosphorylation, probably preventing consecutive degradation and, conversely, enhancing signal transduction, silencing gene transciption, and regulating multimolecular protein assembly (Hatsell *et al.*, 2003; Hu *et al.*, 2006). Therefore, alterations in the phosphorylation patterns finetune the balance of the multiple PG functions.

Phosphorylation of the other desmosomal *arm*-repeat proteins has been reported. Phosphorylation of PP2 by a CDC25C-associated kinase

(C-TAK-1) alters the interaction of PP with 14-3-3 proteins, thereby allowing entry of PP into the nuclear compartment (Muller *et al.*, 2003).

Phosphorylation of the DP carboxy-terminus, specifically of S2849, which is a potential protein kinase A target site, appears to affect keratin and desmin filament association and assembly into desmosomes (Fontao *et al.*, 2003; Godsel *et al.*, 2005; Lapouge *et al.*, 2006; Meng *et al.*, 1997; Stappenbeck *et al.*, 1994). Furthermore, an S299R mutation has been identified in the amino-terminal domain of DP in patients with arrhythmogenic right ventricular cardiomyopathy, which leads to altered PG binding (Rampazzo *et al.*, 2002).

In more general terms, the effects of nonspecific modulators of phosphorylation on overall desmosome dynamics have been examined. The serine–threonine phosphatase inhibitor okadaic acid was shown to prevent the formation of ultrastructurally recognizable desmosomal plaques in the calcium switch paradigm, although the trafficking of desmosomal components to the membrane was not affected (Pasdar *et al.*, 1995a). In contrast, the protein kinase inhibitor H-7 (and also staurosporine) did not inhibit desmosome assembly but rather stimulated it (Shabana *et al.*, 1998). In addition, an increased granular labeling was noted for major desmosomal proteins in the presence of a highly selective protein kinase C (PKC) inhibitor (Amar *et al.*, 1998; Shabana *et al.*, 1998). On the other hand, H-7 interfered with calcium-dependent disassembly and internalization after desmosomal splitting (Denisenko *et al.*, 1994; Pasdar *et al.*, 1995a). It was therefore suggested that the different sensitivities are mediated by calcium-dependent intracellular signal transduction pathways (Pasdar *et al.*, 1995a), most likely by PKC (Citi, 1992; Sheu *et al.*, 1989). Probably one of the most compelling examples of regulation of desmosomal properties and dynamics has been provided by Wallis *et al.* (2000). They presented evidence that wounding-induced, PKCα-mediated signaling alters the responsiveness of desmosomes to calcium reduction.

In addition to phosphorylation, other modifications have been shown to occur and to be of functional relevance for keratinocyte adhesion. Of note is the reported O-glycosylation of plakoglobin leading to posttranslational stabilization with a coincident increase in cadherin binding and adhesiveness (Hu *et al.*, 2006). The underlying mechanism might be competition with glycogen synthase kinase-3 phosphorylation for the amino-terminal threonine 14 (Hatsell *et al.*, 2003), which would result in protection against proteasomal degradation.

5.4. Regulators of desmosomal adhesion

Comparatively little information is available on the overall transcriptional regulation of desmosomal gene expression (Adams *et al.*, 1998; Marsden *et al.*, 1997; Potter *et al.*, 2001; Smith *et al.*, 2004). It has been shown that the

zinc-finger family of transcription factors (slug) disrupts desmosomes during epithelial–mesenchymal transition and during wound healing, possibly by direct effects on the promoters of desmosomal genes (Savagner *et al.*, 1997, 2005). Another report has identified members of the CCAAT/enhancer binding proteins (E/EBP) as important factors for DSC1 and DSC3 expression in keratinocytes (Smith *et al.*, 2004). It was further observed that a 525-bp DSC2 promoter fragment was active in kidney epithelial cells and early mouse embryos (Marsden *et al.*, 1997). Interestingly, the DSC3 promoter is aberrantly methylated in breast cancer cell lines and DSC3 transcription is enhanced by p53 (Oshiro *et al.*, 2003). Finally, methylprednisolone treatment increased Dsg1 and 3 as well as E-cadherin synthesis (Nguyen *et al.*, 2004). Simultaneously, methylprednisolone inhibited the PV antigen-induced increase in phosphorylation of PG and Dsg3 as well as of E-cadherin and β-catenin (Nguyen *et al.*, 2004).

In addition to desmosomal protein modifications that were discussed in the previous section, desmosome formation is regulated by protein stability. It has been known for a long time that cell contact formation alters the half-life of desmosomal constituents considerably (Pasdar and Nelson, 1988a, 1989). The shift in protein stability probably reflects the integration of these polypeptides into cell–cell contacts. On the other hand, proteolytic processing has been described for desmosomal proteins that may be active during differentiation, wound healing, or apoptosis. Thus, desmosomal cadherins and PG have been identified as targets of caspases in apoptotic cells (Brancolini *et al.*, 1998; Dusek *et al.*, 2006a; Weiske and Huber, 2005), and, even more importantly, desmosomal cadherins are cleaved on their extracellular domains by metalloproteinases resulting in shedding of these domains. Such a mechanism may be of relevance for metastatic tumor cells by contributing to loss of adhesion (Dusek *et al.*, 2006a; Weiske and Huber, 2005; Weiske *et al.*, 2001). The importance of proteolytic degradation is also observed in Netherton syndrome, a keratinizing disorder that is caused by a defect in the serine protease inhibitor Kazal-type 5 gene (SPINK5). The absence of this inhibitor leads to increased desmosomal cadherin degradation leading to fragility and a decrease of corneodesmosomes (Descargues *et al.*, 2006).

6. Imbalance of Desmosomal Protein Synthesis in Transgenic Mice

6.1. Reduced production of desmosomal proteins

The phenotypes of desmosomal gene knockouts are summarized in Table 3.2. Most unexpected were probably the observed consequences of DSC3 ablation (Den *et al.*, 2006). Embryonic lethality was observed prior to

Table 3.2 Summary of phenotypes observed in desmosomal transgenesis in mice (Holthoefer *et al.*, 2007)

Gene(s)/Genetic alteration	Phenotype	Reference
Knockouts		
DSG2: constitutive inactivation	– embryonic periimplantation lethality – defect in embryonal stem cell proliferation	Eshkind *et al.*, 2002
DSG3: constitutive inactivation	– intraepithelial lesions in mucous epithelia – crusted skin erosions upon trauma – suppurative conjunctivitis with suprabasilar blisters of eyelids and mucocutaneous conjunctiva – hair loss during telogen 3–4 weeks postpartum with separation of cells within outer root sheath, reduced number of desmosomes, and occurrence of separated half-desmosomes surrounding the hair bulb – additional inactivation of Dsg1 by exfoliative toxin A leading to loss of anagen hair with separation between outer and inner root sheath at plane of companion layer	Koch *et al.*, 1997 Koch *et al.*, 1998 Hanakawa *et al.*, 2004
DSG3 and **P-cadherin:** constitutive inactivation	– postnatal lethality – severe blister formation with ''row of tombstones'' in epidermis and oral mucosa together with desmosomal separation	Lenox *et al.*, 2000
DSC1: constitutive inactivation	skin: – localized acantholysis in stratum granulosum occasionally leading to ulcerating lesions and chronic dermatitis – flaky skin – neutrophil invasion – parakeratosis	Chidgey *et al.*, 2001

(*continued*)

Table 3.2 (*continued*)

Gene(s)/Genetic alteration	Phenotype	Reference
	– compromised barrier function – hyperproliferation (increased K6/16 and Ki67 reactivity) – localized and permanent hair loss with development of utriculi and dermal cysts – improved wound healing	
DSC1: constitutive inactivation resulting in carboxyterminally truncated Dsc1a and b isoforms lacking binding sites for PG and PP1	– normal skin! – Dsc1 incorporation into normal desmosomes – increased Dsc2 mRNA in suprabasal cell layers without up regulation of Dsc2 protein synthesis	Cheng *et al.*, 2004
DSC3: constitutive inactivation	– embryonic lethality before E2.5	Den *et al.*, 2006
DSP: constitutive inactivation	– embryonic postimplantation lethality (E6.5) – defect in elongation of egg cylinder elongation due to dissociation of extraembryonic tissues – reduced proliferation in all embryonic and extraembryonic tissues – reduced number and size of desmosomes – collapse of keratin filament network – no endothelial chord formation in ES cell-derived embryoid bodies – tetraploid rescue of extraembryonic tissues → postgastrulation lethality with defects in heart, neuroepithelium, skin, and blood vessels (reduced number of capillaries and disrupted capillaries)	Gallicano *et al.*, 1998, 2001
DSP: conditional inactivation in epidermis	– blistering – absence of inner desmosomal plaque	Vasioukhin *et al.*, 2001

	– disturbed keratin filament attachment to desmosomes and perinuclear keratin filament aggregates – reduced adherens junctions – immunohistology: reorganization of actin filaments, reduced PG, increased PP3 – immunoblotting: reduced PP2, DSC1; increased DSC1, PP3 – increased solubility of PG, PP1, DSC1, Dsg1, Dsg3	
plectin: constitutive inactivation	– postpartal lethality – skin blistering with reduced hemidesmosomes but normal desmosomes – focal disruption of sarcomeres in skeletal muscle – disintegration of intercalated discs in heart	Andra *et al.*, 1997
envoplakin: constitutive inactivation	– slight delay in epidermal barrier formation	Maatta *et al.*, 2001
periplakin: constitutive inactivation	– no apparent phenotype	Aho *et al.*, 2004
JUP: constitutive inactivation	– embryonic lethality from E10.5 onward until birth	Bierkamp *et al.*, 1996, 1999
	– heart rupture and absence of desmosomes in cardiomyocytes	Ruiz *et al.*, 1996
	– redistribution of desmoplakin to all plaque-bearing junctions in cardiac muscle – reduced compliance of heart fibers – skin blistering due to subcorneal acantholysis – reduced and abnormal desmosomes in skin and intestinal mucosa	Isac *et al.*, 1999

(*continued*)

Table 3.2 (*continued*)

Gene(s)/Genetic alteration	Phenotype	Reference
	– association of β-catenin with Dsg and localization of β-catenin to residual skin desmosomes – increase of apoptosis in cultured keratinocytes	
PKP2: constitutive inactivation	– embryonic lethality (E10.5 - E11) – heart defects: reduced trabeculation, atrial wall thinning, perforations of cardiac walls – DP mislocalisation in cytoplasmic granular aggregates – reduced Dsg2 immunofluorescence and immunoblot signals – increased Triton X-100 solubility of DP, Dsg2, PG	Grossmann *et al.*, 2004
desmoyokin/AHNAK: constitutive inactivation	– no apparent phenotype	Kouno *et al.*, 2004
Increased Synthesis		
JUP: full length, FLAG epitope-tagged cDNA and cDNA coding for N-terminally deleted (80 amino acids), myc epitope-tagged PG, keratin 14 promoter-driven transgenes	– reduced proliferation of epidermal and hair follicle keratinocytes – stunted hair growth: premature termination of growth phase	Charpentier *et al.*, 2000
Production of Mutants		
DSG3: cDNA coding for FLAG epitope-tagged Dsg3ΔN (deletion of extracellular domain), keratin 14 promoter-driven transgene	– swollen paws and digits – flaky skin (dorsolateral) – epidermal hyperproliferation – reduced and abnormal desmosomes – widening of intercellular spaces – altered integrin expression	Allen *et al.*, 1996

	– inflammation – progressive self-amputation of tail	
DSP: cDNA coding for FLAG epitope-tagged N-terminal mutants (v30M, Q90R), α-myosin heavy chain promoter-driven transgene	– embryonic lethality	Yang *et al.*, 2006
DSP: cDNA coding for FLAG epitope-tagged C-terminal mutant (R2834H), α-myosin heavy chain promoter-driven transgene	– cardiomyocyte apoptosis – cardiac fibrosis and lipid accumulation – ventricular enlargement and cardiac dysfunction – interrupted DP-desmin interactions	Yang *et al.*, 2006
Ectopic Synthesis		
DSG2: full-length FLAG epitope-tagged cDNA, involucrin promoter-driven transgene	– epidermal hyperkeratosis – epidermal hyperplasia – enhanced keratinocyte survival *in vitro* – spontaneous papillomatous skin lesions and increased susceptibility to chemically induced carcinogenesis	Brennan *et al.*, 2007
DSG3: full-length FLAG epitope-tagged cDNA, involucrin promoter-driven transgene	– perinatal lethality due to severe dehydration – skin barrier defect – no epidermal hyperproliferation – disturbance of epidermal stratum corneum with gross scaling and lamellar morphology typical of mucous epithelia – corneocyte separation with premature dissolution of desmosomes – abnormal skin differentiation: flaking, pustules, thinning of hair – protection against pemphigus foliaceus antibody–induced blistering	Elias *et al.*, 2001
DSG3: full-length cDNA, keratin 1 promoter-driven transgene	– no perinatal lethality – normal skin barrier	Merritt *et al.*, 2002

(*continued*)

Table 3.2 (*continued*)

Gene(s)/Genetic alteration	Phenotype	Reference
	– hyperproliferation: acanthosis, hypergranulosis, hyperkeratosis, localized parakeratosis, increased Ki67 staining, induction of K6/16 – late onset (>12 weeks) phenotype in skin and hair: flaky skin with pustules containing inflammatory cells and thinning of hair with abnormal hair follicles – altered terminal differentiation: suprabasal K14, increased filaggrin, loricrin, involucrin – widened intercellular spaces	
DSC3a and **DSC3b:** full-length cDNA, keratin 1 promoter-driven transgene	– localized progressive ventral alopecia with considerable variability: degenerated hair follicles (utriculi) filled with sebum and keratinized dermal cysts presenting ectopic K1 and loricrin expression indicative of interfollicular transdifferentiation – delayed ventral *and* dorsal hair growth after plucking – thickened epidermis in regions of alopecia: acanthosis, hypergranulosis, hyperkeratosis, increased basal and suprabsal keratinocyte proliferation with suprabasal K14 expression and strong K6 expression – altered keratinocyte differentiation – increased β-catenin–dependent transcriptional activity leading to increased cyclin D1 levels	Hardman *et al.*, 2005
DSC1a: full-length cDNA, keratin 14 promoter-driven transgene	– no apparent phenotype	Henkler *et al.*, 2001

implantation in this instance. Only 3% of homozygous mutants could be detected in E2.5 embryos at the 8 to 16 cell morula stage that were obtained from a heterozygous intercross. This demonstrates convincingly that Dsc3 fulfills crucial nondesmosomal functions, at least during this early developmental phase. One possibility is that its presence regulates compaction in some unknown way. Similarly, a nondesmosomal function is also likely for Dsg2, since its deletion prevented survival of embryonal stem cells that are derived from desmosome-free inner mass cells but did not induce defects in the desmosome-positive trophectoderm layer (Eshkind *et al.*, 2002). The provocative conclusion is that Dsg2 also fulfills, at least under certain circumstances, nondesmosomal functions that are essential for cell proliferation. The elucidation of the molecular mechanism that leads to the decrease in PP2 levels in $DSG2^{+/-}$ embryonal stem cells and its consequences on cytoskeletal organization and gene expression may help to unravel this mystery. It will be of interest to find out whether similar changes occur in the absence of Dsc3.

The slightly later embryonic lethality of DP-deficient mice at E6.5 appears to be caused by different defects (Gallicano *et al.*, 1998). A trophectoderm is formed, implantation takes place, but extraembryonic tissues do not develop properly and an overall defect in proliferation occurs. Remarkably, desmosomal-like structures were still detectable in embryonal endoderm and the ectoplacental cone, albeit at reduced number and size resulting in a collapse of the keratin filament network. Formally, it has not been excluded that partial transcripts of the mutant DSP gene are still generated giving rise to amino-terminally deleted polypeptide mutants and thereby explaining the surprising residual desmosome formation. Another bottleneck was identified for DP function by tetraploid rescue of the extraembryonic defects. In this case, lethal postgastrulation defects were noted around E10 affecting heart function, neuroepithelium, skin, and capillaries (Gallicano *et al.*, 2001). Knockdown experiments further showed that the presence of DP is important for tube formation by investigating capillary formation *in vitro* (Zhou *et al.*, 2004). To examine DP function in adult skin, epidermis-specific knockout animals were prepared (Vasioukhin *et al.*, 2001). Although the number of desmosomes was not significantly altered, they lacked keratin filaments, and mechanical stress led to intercellular blister formation. Cultivating keratinocytes from these animals further revealed defects in epidermal sheet formation demonstrating that DP is needed for functional desmosome assembly and, even more, reinforcement of stable intercellular adhesion.

Inactivation of the plakoblobin-encoding JUP gene and the plakophilin 2-encoding PKP2 gene induced embryonic lethality at E10.5 primarily due to heart defects (Bierkamp *et al.*, 1996; Grossmann *et al.*, 2004; Isac *et al.*, 1999; Ruiz *et al.*, 1996). The absence of these *arm*-repeat polypeptides led to redistribution of DP into granular aggregates and other junctions.

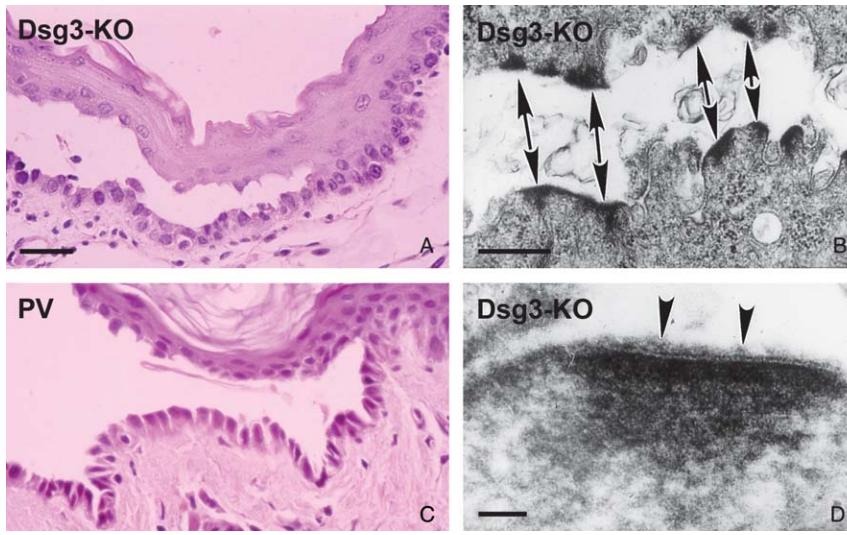

Figure 3.12 Microscopy of reduced desmosomal adhesion in DSG3 knockout mice (DSG3-KO; A, B, D) and pemphigus vulgaris patients (PV; C). Light microscopy of hematoxylin and eosin-stained sections of epidermis reveals intraepidermal blister formation (A, B). By electron microscopy half-desmosomes are readily apparent in DSG3 knockout mice (B, D) with adhering desmoglea (arrows in D) and large intercellular spaces (double arrows in C). Bars: A, 40 μm (same magnification in C); 0.5 μm in B; 50 nm in D. (The figures are taken from Figs. 4E and G and 5B and C of Koch *et al.*, 1997, by copyright permission of The Rockefeller University Press.)

In addition, skin blistering was noted in PG mutants with a reduced number of morphologically abnormal desmosomes and retracted intermediate filaments (Bierkamp *et al.*, 1996). It appears that in the absence of PG β-catenin can take over some of its functions, since it was localized to the residual desmosomes in this situation (Bierkamp *et al.*, 1999). On the other hand, PG cannot fully compensate for the loss of β-catenin, which induced ectoderm defects during the gastrulation stage and subsequent lethality (Haegel *et al.*, 1995; Huelsken *et al.*, 2000), although it is upregulated in heart upon β-catenin depletion to maintain apparently normal cardiac structure and function (Zhou *et al.*, 2007). Similarly, different phenotypes were elicited in *X. laevis*. Depletion of PG resulted in a partial loss of adhesion, and a loss of embryonic shape, but did not affect dorsal signaling (Kofron *et al.*, 1997), whereas downregulation of β-catenin inhibited dorsal mesoderm induction in early embryos (Heasman *et al.*, 1994). In addition, overexpression of PG in *X. laevis* induced anterior axis duplication upon nuclear accumulation (Karnovsky and Klymkowsky, 1995).

In contrast to the strong phenotypes induced by the absence of obligatory desmosomal components, gene ablation of other components has

generally led to less severe defects. In the case of the desmosomal cadherins, pathologies were mostly noted in skin and its appendages (DSC1, DSG3) as well as in mucous epithelia (DSG3) as detailed in Table 3.2. The phenotypes correlate well with the known expression patterns (Chidgey *et al.*, 2001; Koch *et al.*, 1997, 1998). They also highlight the important contribution of these adhesion molecules to mechanical stability of desmosomes, since split half-desmosomes were seen at the cell surface in blistered skin (Fig. 3.12). It is of note, however, that alterations result not only from reduced cell–cell adhesion but also include changes in cell proliferation (Chidgey *et al.*, 2001). Cooperative effects were observed between desmosomal and classical cadherins (Lenox *et al.*, 2000). Surprisingly little phenotypic changes were noted in a DSC1 deletion mutant lacking the Dsc1a- and Dsc1b-specific regions including the PG-binding site in Dsc1a (Cheng *et al.*, 2004). Finally, depletion of the facultative plakins resulted in variable deficiencies in desmosome-bearing tissues ranging from skin blistering and disintegration of the intercalated discs in myocardium in the case of plectin (Andra *et al.*, 1997), a slight defect in epidermal barrier formation for envoplakin (Maatta *et al.*, 2001), to the absence of detectable dysfunctions for periplakin (Aho *et al.*, 2004). Similarly, no defects were noted in desmoyokin$^{-/-}$ mice (Kouno *et al.*, 2004).

6.2. Overproduction and ectopic synthesis of wild-type and mutant desmosomal proteins

Orthotopic overexpression and ectopic production of desmosomal components as well as expression of mutant desmosomal components have been achieved by injecting specific gene constructs into the male pronuclei of murine zygotes and by examining the transgenic offspring. The resulting complex phenotypes are listed in Table 3.2. Although some of the reports contradict each other in part, several important conclusions can be drawn from these experiments. Dysbalance of desmosomal protein synthesis affects tissue homeostasis by altering tissue differentiation with coincident weakening of cell–cell adhesion and by altering proliferation. While the adhesive defects were expected, although they turned out to be rather mild-natured and affected primarily hair (Allen *et al.*, 1996; Charpentier *et al.*, 2000; Hardman *et al.*, 2005; Merritt *et al.*, 2002), changes in proliferation were much more difficult to understand. Reduced proliferation was noted in situations of PG overexpression (Charpentier *et al.*, 2000), whereas increased proliferation was observed in mice producing elevated levels of Dsg2 (Brennan *et al.*, 2007), or mice synthesizing either amino-terminally deleted Dsg3 or full length Dsg3 and Dsc3a/b in suprabasal epidermal cell layers (Allen *et al.*, 1996; Hardman *et al.*, 2005; Merritt *et al.*, 2002). The most plausible explanation is that the levels of available PG affect cell proliferation via its suppressor function on c-MYC gene expression

(Williamson *et al.*, 2006). Such a dysbalance may also be the underlying reason why reduced proliferation has been reported in Dsg2 and DP knockout mice (Eshkind *et al.*, 2002; Gallicano *et al.*, 1998) but does not readily explain the increased proliferation in Dsc1-deficient animals (Chidgey *et al.*, 2001). The complex crosstalk among the various plaque polypeptides of different junctions and their partially opposing effects on gene transcription in different cell types, all of which relies on specific stoichiometric ratios, remain to be elucidated to provide a molecular understanding of the underlying pathophysiological mechanisms. In addition, it is becoming clear that desmosomal cadherins present isotype-specific functions. In accordance, severe though variable defects were induced by increased suprabasal production of Dsg3 and Dsc3 that are normally restricted to the more basal compartment of the epidermis (Elias *et al.*, 2001; Hardman *et al.*, 2005; Merritt *et al.*, 2002), or ectopic production of Dsg2 in suprabasal keratinocytes (Brennan *et al.*, 2007). These situations also highlight the importance of the relative quantitative levels of desmosomal cadherins for epithelial proliferation and differentiation.

7. Interplay Between Desmosomes and Other Cell Components

7.1. Crosstalk with adherens junctions

Junction formation appears to be organized in a hierarchical fashion with respect to temporal and spatial coordination. Therefore, adherens junctions initiate cell–cell contacts that are later stabilized by desmosomes. During embryonic development, desmosomes are established only after adherens junctions are formed (see above). Similarly, the same order was observed in cultured MDCK cells employing the calcium switch system in combination with specific inhibitory antibodies (Gumbiner *et al.*, 1988). Also, cadherin function has been shown to be essential for desmosome assembly in keratinocytes (Amagai *et al.*, 1995a; Lewis *et al.*, 1994; Wheelock and Jensen, 1992). Detailed analysis of epithelial sheet formation revealed that this process starts with zippering of actin-containing junctions at the tips of early membrane contacts and that desmosome formation originates in flanking regions where cells were in apposition (Vasioukhin *et al.*, 2000).

Important molecular regulators of desmosome formation may be PG, p0071, and even β-catenin, which are, at least under certain circumstances, components of both types of adhaerens junctions (Hatzfeld *et al.*, 2003; Lewis *et al.*, 1997; Palka and Green, 1997; Setzer *et al.*, 2004). In addition, PPs may be of particular importance, because, in addition to their dual junctional localization, they affect actin organization (Chen *et al.*, 2002; Hatzfeld *et al.*, 2000). Furthermore, signaling mechanisms may contribute

to the crosstalk as suggested by the observation that α-catenin-mediated junction assembly can be bypassed by PKC to establish junctional complexes in colon carcinoma cell lines (van Hengel *et al.*, 1997).

Specifically, it has been shown that PG is required in a complex with E-cadherin to initiate desmosome assembly in A-431 cells (Lewis *et al.*, 1997). Similarly, introduction of PG into SCC9 cells lacking PG and E-cadherin led to an increase in expression and stability of N-cadherin and a decrease in the level and stability of β-catenin, which in turn induced desmosome formation (Parker *et al.*, 1998). An interesting example of crosstalk between desmosomes and adherens junctions in the context of overall cell behavior was provided by analyses of p0071/PP4 (Setzer *et al.*, 2004). Overexpression of this polypeptide in A-431 cells resulted in increased adherens junction assembly and reduced desmosome assembly that were accompanied by keratin filament retraction. These cells exhibited reduced migration in an *in vitro* wounding system without noticeable alterations in overall adhesive strength. It was suggested that PP4 regulates PG availability. Experimental evidence has also been accrued to suggest that PP2 mediates crosstalk between desmosome formation and β-catenin signaling. It was shown that PP2 binds to β-catenin *in vitro* and that upregulation of PP2 enhances β-catenin signaling (Chen *et al.*, 2002). On the other hand, PPs have been identified as Dsc-binding partners (Bonne *et al.*, 2003). Hence, the increased levels of β-catenin transcriptional activity observed in mice producing increased levels of Dsc3 under the K1 promoter suggest a link between both via a PP signaling activity (Hardman *et al.*, 2005).

Taken together, it appears that each *arm*-molecule contributes in a specific way to the delicate balance between adherens junctions and desmosomes: p120ctn together with p0071 increase the assembly and stability of adherens junctions, whereas p0071 negatively affects desmosome assembly and stability. On the other hand, PP1 increases desmosomal assembly and stability (Hatzfeld *et al.*, 2000; Sobolik-Delmaire *et al.*, 2006; Wahl, 2005). In out-of-balance situations *arm*-proteins may substitute for each other. Therefore, β-catenin has been localized to desmosomes of PG knockout mice (Bierkamp *et al.*, 1999).

Conversely, disruption of desmosomal functions also weakens adherens junctions. This has been noted in DP-deficient keratinocytes (Vasioukhin *et al.*, 2001). Here, DP was shown to be essential for the maturation of adhaerens junctions by clamping down the transient zippering ''courtship'' of classical cadherins and thereby promoting the maturation of puncta adhaerentia-type junctions and affecting cortical actin remodeling. Similarly, cells producing a DP mutant lacking its rod and IF-binding domains presented reduced mechanical resilience despite the continued presence of other adherens junctions (Huen *et al.*, 2002).

Crosstalk between desmosomes and adherens junctions also includes the transmembrane complexes. In support of this, the presence of Dsgs in

nondesmosomal junctions has been reported in embryonal stem cells (Eshkind *et al.*, 2002). In addition, Dsgs were found in association with β-catenin in PG-deficient keratinocytes (Bierkamp *et al.*, 1999). Desmosomal cadherins may even associate in *cis* with E-cadherin as suggested by *in vitro* observations in low calcium conditions (Troyanovsky *et al.*, 1999). Also, altered adherens junctions were found in transgenic mice synthesizing amino-terminally deleted Dsg3 (Allen *et al.*, 1996). Another recent report provided evidence for the presence of an N-cadherin–catenin–vimentin complex that appears to promote cell–cell adhesion in fibroblast L-cells (Kim *et al.*, 2005).

7.2. Crosstalk with cytoskeletal filaments

7.2.1. Intermediate filaments

Even though intermediate filaments and desmosomes are attached to each other, they are not essential for the morphogenesis of the other. Therefore, desmosomes are formed in the absence of an intact intermediate filament cytoskeleton (Baribault and Oshima, 1991; Magin *et al.*, 1998) and keratin filament networks exist in the absence of desmosomes, although they are usually collapsed around the nucleus and do not withstand mechanical stress (Gallicano *et al.*, 1998; Troyanovsky *et al.*, 1993, 1994a; Vasioukhin *et al.*, 2001). Interestingly, desmosome-anchored intermediate filaments are more resistant to phosphatase inhibitors and are more long lived than nonanchored filaments, suggesting that they acquired specific properties making them particularly stable and protecting them against disassembly (Strnad *et al.*, 2001, 2002; Windoffer *et al.*, 2004).

Desmosomes are capable of anchoring different types of intermediate filaments. Keratin filaments associate in epithelial cells, desmin filaments in cardiomyocytes, and vimentin in meningeal cells (see Fig. 3.6). The different filaments bind to the carboxy-terminus of the plakins desmoplakin, plectin, and periplakin (Fontao *et al.*, 2003; Karashima and Watt, 2002; Kazerounian *et al.*, 2002; Nikolic *et al.*, 1996; Stappenbeck and Green, 1992) but use different binding motifs (Stappenbeck *et al.*, 1993) and exhibit different binding affinities (Meng *et al.*, 1997).

7.2.2. Actin filaments and microtubules

The organization of the actin filament cytoskeleton has a major impact on desmosome formation as shown by drug-induced actin filament disassembly that resulted in compromised stability, assembly, and spatial organization of desmosomal components at the plasma membrane (Pasdar and Li, 1993). Furthermore, actin filaments have been implicated in the delivery of DP-PP2–containing particles to the plasma membrane (Godsel *et al.*, 2005). A connection between desmosome dynamics and actin filament organization may also be mediated by p0071, which is essential for Rho

A-mediated contractile ring formation during cytokinesis (Wolf *et al.*, 2006). A remarkable dual function was also described for periplakin, which binds to actin via its head-rod domain and to intermediate filaments by its carboxy-terminus, which can be separated *in vivo* by specific caspase 6-mediated cleavage (Kalinin *et al.*, 2005).

On the other hand, intact microtubules appear to be dispensable for desmosome formation (Pasdar *et al.*, 1992). A rather unexpected finding was, however, reported recently (Lechler and Fuchs, 2007). It was shown that DP is essential for the cell type–specific organization of the microtubule system in suprabasal keratinocytes. The effect was attributed to binding of DP to the microtubule-anchoring protein ninein that becomes relocalized in suprabasal cells from a centrosomal to a junctional position around which microtubules reorient. These findings extend much earlier observations in which the plus end microtubule-binding protein CLIP170 was localized to desmosomes in polarizing MDCK cells (Wacker *et al.*, 1992).

7.3. Crosstalk with the nucleus

7.3.1. Shuttling of desmosomal proteins

It has been established that the desmosomal *arm*-repeat–containing polypeptides also reside in the nucleus (Bonne *et al.*, 1999; Hu *et al.*, 2003; Klymkowsky, 1999; Schmidt *et al.*, 1997), where they can interact with the transcriptional apparatus to affect gene expression. In this way, direct regulatory mechanisms appear to exist that couple tight cellular adhesion to proliferation and may thus represent part of the molecular machinery that is responsible for the long-known contact inhibition, a basic property of nontransformed cells. One of the most interesting questions in this context concerns the exact origin of junctional proteins in the nucleus: Are they directly delivered from the endoplasmic reticulum to the nucleus or do they first pass through cell–cell junctions? Several observations suggest that the nuclear pool of β-catenin is fueled from the cadherin–catenin complex (Gottardi and Gumbiner, 2004; Klingelhofer *et al.*, 2003).

It is of note that PG exhibits weaker binding to the transcription factors LEF1 and TCF4 than β-catenin (Maeda *et al.*, 2004; Williams *et al.*, 2000) and that there are significant differences in the transactivation capacity of both (Hecht *et al.*, 1999; Simcha *et al.*, 1998). In contrast to β-catenin, PG decreases rather than increases the affinity of TCF4 and LEF1 for DNA (Miravet *et al.*, 2002; Zhurinsky *et al.*, 2000). It is therefore likely that PG antagonizes, at least in part, β-catenin, as also suggested by recent observations on the suppression of c-MYC gene expression by PG, which is in contrast to its activation by β-catenin (Williamson *et al.*, 2006). The situation becomes even more complicated when considering the binding of PP2 to β-catenin, which upregulates β-catenin/TCF signaling (Chen *et al.*, 2002).

The precise molecular signals and mechanisms that determine nuclear import and export are not known. In the case of PP2 it was shown that S28 phosphorylation by the serine kinase C-TAK-1 facilitates binding to 14-3-3, thereby preventing nuclear entry (Muller *et al.*, 2003).

7.3.2. Signal transduction

It should be kept in mind that the function of cell adhesion molecules extends beyond the mere mechanical coupling of cells engaging in processes of differentiation and proliferation and thereby facilitating tissue homeostasis. They are positioned at the extracellular to intercellular interface and might therefore be important mediators of signal transduction either outside-in or inside-out. Very little is known, however, about such sensing functions. Yet, the observation that anti-Dsg3–containing sera from patients with the autoimmune blistering disease pemphigus vulgaris leads to Dsg3 phosphorylation (Aoyama *et al.*, 1999) concomitant with a transient increase in intracellular calcium and PKC activity (Seishima *et al.*, 1995) supports the notion of information transmission from the outside to intracellular signaling. Autoantibodies directed against Dsg3 also resulted in p38 MAPK activation and HSP27 phosphorylation (Berkowitz *et al.*, 2005). Remarkably, all these changes take place prior to antibody-induced cell separation, suggesting that the specific antibodies do not simply interfere with cell–cell adhesion by steric hindrance (see also Waschke *et al.*, 2005) but elicit specific intracellular signaling events. Consequently, inhibition of p38 MAPK signaling prevented the antibody-dependent keratin filament retraction and actin reorganization (Berkowitz *et al.*, 2005). An important proof of principle was made by coinjecting PV antibodies together with specific p38 MAPK inhibitors intradermally to demonstrate that skin splitting can be prevented effectively in a living animal (Berkowitz *et al.*, 2006). Furthermore, one of the crucial upstream components in this cascade may be the plaque protein PG whose absence also prevented PV IgG-dependent keratinocyte splitting *in vitro* (Caldelari *et al.*, 2001). Another recent study implicated Rho A as a downstream target of the p38 MAPK-dependent cascade (Waschke *et al.*, 2006). It was shown that Rho A activation and p38 MAPK inactivation inhibited PV- and PF-IgG–dependent splitting as well as cytoskeletal reorganization in an *ex vivo* human skin model and between HaCaT keratinocytes. Much remains to be done to work out the precise sequence of events. Even more, it will be a major challenge to determine whether these events are directly or indirectly linked to the desmosomal adhesion-dependent regulation of cell survival and proliferation. In particular, the role of PG as an important suppressor of c-MYC transcription has been acknowledged recently (Williamson *et al.*, 2006), a mechanism that would also explain why its absence has an antiapoptotic effect in keratinocytes (Dusek *et al.*, 2006b). Similarly, the depletion of Dsg2 and/or of Dsc3

in knockout mice could exert its antiproliferative effects by setting PG free to suppress cell proliferation (Den *et al.*, 2006; Eshkind *et al.*, 2002).

Conversely, desmosomal dynamics are affected by signaling molecules. The best case so far is the wounding-induced destabilization of desmosomes. It has been shown that desmosomes transit from a calcium-insensitive to a calcium-sensitive state that appears to be mediated by PKC (Wallis *et al.*, 2000). Since the reversion to calcium independence does not rely on additional desmosomal components and occurs spontaneously in confluent cultures, it has been proposed by Garrod and colleagues (2005) that it is primarily associated with conformational changes possibly mediated by altered phosphorylation and resulting in hyperadhesion whose morphological correlate is the presence of a midline. Hyperadhesion is proposed to be the primary condition in healthy epithelia *in vivo*. Evidence for this model was provided by examining the mechanical stability of confluent HaCaT keratinocytes with hyperadhesive desmosomes presenting increased mechanical resilience (Kimura *et al.*, 2006).

8. Desmosomes and Disease

Much has been learned about desmosomal functions in human diseases that are associated with desmosomal malfunctions and are caused by different pathogenetic mechanisms due to either genetic defects, autoantibodies, bacterial toxins, or to malignant transformation. Table 3.3 lists the affected desmosomal polypeptides together with brief summaries of major symptoms. Since it is impossible to cover the entire literature on the topic, we will restrict the discussion to a few selected aspects.

8.1. Genetic diseases

In the past few years there has been an exponentially growing list of monogenic human diseases that are caused by mutations in genes coding for desmosomal proteins (Kottke *et al.*, 2006; McGrath, 2005; McGrath and Wessagowit, 2005). These include the desmosomal cell adhesion molecules as well as desmosomal plaque components and are associated with two major disease phenotypes: those that affect primarily the epidermis and its appendages and those whose clinical symptoms become manifest as cardiac dysfunction. Although the underlying genetic defects are known, much needs to be learned about the molecular pathogenetic mechanisms.

In skin, desmosomal deficiencies caused either by DSG1 or DSP mutations lead to hyperkeratosis that often occurs in the form of prominent bands on palms and soles. Hence, these diseases are referred to as striate palmoplantar keratoderma (SPPK; Alcalai *et al.*, 2003; Armstrong *et al.*,

Table 3.3 Summary of genetic diseases that are caused by distinct desmosomal gene mutations (Holthoefer *et al.*, 2007)

Type	Mutated gene – Inheritance	Clinical symptoms
Striate palmoplantar keratoderma (SPPK1)	**DSG1** – dominant	- hyperkeratotic bands on palms and soles
Striate palmoplantar keratoderma (SPPK2)	**DSP** – dominant and recessive	- hyperkeratotic bands on palms and soles (may be generalized) - varying degrees of ultrastructural alterations in desmosomes and intermediate filament organization - woolly hair and hair loss - nail dystrophy - arrhythmic right ventricular cardiomyopathy
Ectodermal dysplasia skin fragility syndrome	**PKP1** – recessive	- dysplasia of skin, hair, nails - skin fragility with thickening of epidermis - trauma-induced epidermal erosions and blistering - impaired wound healing due to reduced migration - ultrastructural and molecular alterations of epidermal desmosomes

Balding mouse (bal^{J}, bal^{pas})	**DSG3** – recessive	- epidermal acantholysis → cutaneous erosions - mucous membranes → runting at d 8–10 - patchy alopecia
Human: localized autosomal recessive hypotrichosis (LAH)	**DSG4** – recessive	- hypotrichosis of scalp, trunk, and extremities sparing facial, pubic, axillary hair - brittle, dystrophic hair: abnormal hair shaft with nodules and lance heads (trichorrhexia nodosa), impaired pigmentation, severe disturbance of inner root sheath
Mouse/rat: lanceolate hair phenotype (lah; Iffa Credo "hairless" rat)		- thickened interfollicular epidermis with hyperkeratosis and acanthosis
Arrhythmogenic right ventricular cardiomyopathy	**DSG2** – dominant	- arrhythmogenic right ventricular cardiomyopathy
	PKP2 – dominant	- atrophy of right ventricular myocytes and replacement with fatty or fibrofatty tissue
Naxos disease	**JUP** (plakoglobin) – recessive	- arrhythmogenic right ventricular cardiomyopathy - striate palmoplantar keratoderma - woolly hair

1999; Basso *et al.*, 2006; Hunt *et al.*, 2001; Kljuic *et al.*, 2003b; Norgett *et al.*, 2000; Pilichou *et al.*, 2006; Rickman *et al.*, 1999; Whittock *et al.*, 1999). The hyperkeratosis is paired with ultrastructural alterations in desmosomes and widening of intercellular spaces (Armstrong *et al.*, 1999; Milingou *et al.*, 2006; Whittock *et al.*, 1999). The severity of disease is generally more restricted in the case of DSG1 mutations corresponding to the type 1 SPPK, occasionally presenting only focal hyperkeratoses instead of the conspicuous striations (Milingou *et al.*, 2006). In contrast, the DSP-caused type 2 SPPK often affects the entire body surface and goes along with defects in hair, nails, and, most notably, the heart. Corresponding differences in severity were also noted by electron and immunofluorescence microscopy revealing that desmosome number, size, and morphology are severely altered and changes in keratin filament organization and composition take place (Wan *et al.*, 2004). The phenotypic differences between the two SPPK types are most likely accounted for by the partial compensation of Dsg1 deficiencies through Dsg3, whereas no such redundancy exists for DP. Interestingly, a pedigree was recently described with a mutation in the amino-terminal domain of DP (S299R) disrupting a putative phosphorylation site that may be implicated in PG binding and was exclusively associated with cardiac symptoms in the form of arrythmogenic right ventricular cardiomyopathy (ARVC; Rampazzo *et al.*, 2002). This finding suggests that DP functions can be separated into those that are essential in the heart and rely primarily on molecular interaction with PG and those that are needed for intermediate filament anchorage with an essential contribution to skin integrity. The latter conclusion is also supported by another DSP mutation leading to pathological manifestations in the skin in the form of lethal acantholysis (Jonkman *et al.*, 2005). The underlying molecular defects were compound heterozygous mutations of DSP in which both mutated alleles had an intact amino-terminus but lacked the carboxy-terminal keratin-binding sites, thereby completely abrogating intermediate filament anchorage although desmosomes were still formed.

The ectodermal dysplasia skin fragility syndrome is another type of epidermal disease that is caused by mutations in a gene encoding a desmosomal component. PKP1 has been identified as the major molecular target in this recessively transmitted affliction (Hamada *et al.*, 2002; McGrath *et al.*, 1997; South, 2004; Sprecher *et al.*, 2004; Whittock *et al.*, 2000). By immunohistology and electron microscopy desmosomal size and frequency were shown to be significantly reduced in the lower suprabasal layers (McMillan *et al.*, 2003). Although desmosome number was also reduced in the upper suprabasal layers, their size was considerably increased in comparison to control skin. In all suprabasal layers poorly developed inner and outer plaques were seen. In addition, a widening of intercellular spaces, reduced midlines, and perturbed intermediate filament organization with anchorage defects and prominent perinuclear aggregates were noted

(Hamada *et al.*, 2002; McGrath *et al.*, 1997; South, 2004; Sprecher *et al.*, 2004). The skin fragility was paired with thickening of the epidermis and, most notably, an increased sensitivity to trauma paired with compromised wound healing. It was proposed that reduced migration is responsible for the delayed wounding response (South *et al.*, 2003).

A skin phenotype with pronounced alterations in hair was described in the balding mouse strains that carry mutations in the DSG3 gene (Pulkkinen *et al.*, 2002). They present prominent acantholysis which, in contrast to the aforementioned diseases, is not accompanied by pronounced hyperkeratosis. These blisters are therefore considered to be a direct consequence of compromised adhesion, although we are not aware of any detailed ultrastructural analyses. As expected, the alterations are not restricted to the epidermis but extend to hair and oral mucosa, reflective of the broad distribution pattern of Dsg3. The adhesion defects, which result in impaired hair anchorage, are most notable in these mice, which are referred to as balding (*bal*) mice (Montagutelli *et al.*, 1997). Almost identical defects are seen in DSG3 knockout mice (Koch *et al.*, 1998). A different phenotype was described for DSG4 mutants that are the cause of localized autosomal recessive hypotrichosis (LAH) in humans (Kljuic *et al.*, 2003a; Moss *et al.*, 2004; Schaffer *et al.*, 2006; Zlotogorski *et al.*, 2006) and are observed in the lanceolate mouse (Kljuic *et al.*, 2003a) and rat (Bazzi *et al.*, 2004; Jahoda *et al.*, 2004; Meyer *et al.*, 2004). Most prominent are the distinct hair abnormalities that result in dystrophic alopecia and are accompanied by variable degrees of follicular hyperkeratosis. The hair is short, dysmorphic, and brittle with characteristic lance heads and alterations in the hair shaft as well as in inner and outer root sheath. The defects demonstrate that the adhesion dysfunction that most likely gives rise to the typical swelling of the hair shaft and hair loss is also coupled with impaired proliferation in the hair matrix and abnormal differentiation in the precortex zone (Bazzi *et al.*, 2004; Jahoda *et al.*, 2004; Kljuic *et al.*, 2003a). It was therefore proposed that Dsg4 acts as a regulator of the transition from proliferation to differentiation (Kljuic *et al.*, 2003a). In addition, mutation of the facultative desmosomal protein corneodesmosin that is synthesized in the upper suprabasal layers of the epidermis and in the inner root sheath of the hair follicle gives rise to the autosomal dominant disorder hypotrichosis simplex, which is characterized by reduced cell–cell adhesion in the inner root sheath and an accumulation of cytoplasmic aggregates (Levy-Nissenbaum *et al.*, 2003).

Naxos disease has long been known as a syndrome in which ARVC is coupled with palmoplantar keratoderma and woolly hair (Protonotarios *et al.*, 1986). Originally, defects in the PG-encoding JUP gene were described as the genetic cause of this recessive disease (McKoy *et al.*, 2000; Protonotarios *et al.*, 2001, 2002). Interestingly, reduced connexin 43 staining can be discerned early and a significant decrease of intercalated discs and ultrastructurally identifiable gap junctions becomes evident (Kaplan *et al.*,

2004) providing an explanation for the observed alterations in intracardiac conduction. Recently, it was found that DSP mutations lead to very similar disease phenotypes acting both in a dominant and recessive fashion (Alcalai *et al.*, 2003; Norgett *et al.*, 2000; Rampazzo *et al.*, 2002; Yang *et al.*, 2006). Quite unexpected was the identification of multiple mutations throughout the DSG2 gene in ARVC (Awad *et al.*, 2006; Basso *et al.*, 2006; Pilichou *et al.*, 2006; Syrris *et al.*, 2006; Tsatsopoulou *et al.*, 2006). In this instance, the dominantly inherited disorder exhibits partial penetrance and is also characterized by cardiac disease with ventricular tachyarrhythmias. Morphologically, an atrophy of ventricular cardiomyocytes and fibrofatty replacement are pathognomonic for all types of ARVC. This again shows that a desmosomal disease phenotype is not simply due to compromised adhesion but is instead the consequence of altered differentiation and cell survival. Given the similarities to the aforementioned cardiac disease phenotypes observed in DSP and JUP mutations, it may be postulated that these factors contribute to the same pathway. Furthermore, PKP2 mutations were reported with almost identical clinical symptoms (Antoniades *et al.*, 2006; Basso *et al.*, 2006; Gerull *et al.*, 2004; Tsatsopoulou *et al.*, 2006), indicating that all factors are intertwined. Therefore, a unique possibility exists to genetically sort out the hierarchy of the cascade of these various polypeptides in the pathogenesis of ARVC by examining their levels and localization in the different genetic backgrounds. Along this line, the observed downregulation of PP2 in DSG2$^{+/-}$ embryonal stem cells (Eshkind *et al.*, 2002) can be taken as an indication that Dsg2 is upstream of PP2. Another peculiarity is that DSC2 has not been implicated in ARVC so far, possibly indicating that it lacks the specific signaling function of Dsg2. The Dsg2-specific RUD region (see Fig. 3.5) would be a potential module to mediate such functions. Finally, it is puzzling why the DSG2 mutations manifest primarily in the heart and not in liver, colon, or other simple epithelia whose predominant, if not exclusive, Dsg isoform is Dsg2 (see Table 3.1). Maybe the long-lived nature of cardiomyocytes allows the full development of a clinically relevant phenotype whereas the high turnover in other tissues prevents it. Detailed examination of the various Dsg2-positive epithelia may reveal more subtle alterations.

Mutations in the plakin plectin do not lead to desmosomal defects, suggesting that this molecule does not fulfill essential desmosomal functions. Instead it is primarily involved in hemidesmosomal cell–extracellular matrix adhesion in the skin thus leading to junctional epidermolysis bullosa (McLean *et al.*, 1996; Pfendner *et al.*, 2005; Smith *et al.*, 1996).

The evidence for a direct contribution of desmosomal gene mutation to cancer is still surprisingly scarce despite the overwhelming evidence for such a role of the related β-catenin in several malignancies, most notably those that arise from the large intestine (Behrens, 2005; Giles *et al.*, 2003). The strongest case has been presented for PG: The presence of an allelic

variation of the JUP gene was shown to be associated with a predisposition to familial breast and ovarian cancer and loss of heterozygosity (Aberle *et al.*, 1995). In addition, a mutation of S28, a potential phosphorylation site, was identified in a gastric cancer (Caca *et al.*, 1999).

8.2. Autoimmune diseases

The examination of several autoimmune diseases that are caused by antibodies directed against desmosomal cadherins has contributed significantly to the understanding of the importance and the mechanisms of desmosomal adhesion. Since numerous reviews have been published on the topic (Bystryn and Rudolph, 2005; Kottke *et al.*, 2006; Payne *et al.*, 2004), we will only summarize a few well-established aspects of these diseases. A common feature of these life-threatening diseases is the formation of blisters that are prone to superinfection (Fig. 3.13). While Dsg3 autoantibodies result in acantholysis of lower suprabasal cell layers in the epidermis and in erosions of the oral mucosa (Figs. 3.12 and 3.13), only superficial and skin-restricted blister formation is elicited by Dsg1 autoantibodies. These differences have been known for a long time and led to the distinction of two major forms of pemphigus, namely the Dsg 3-related PV and Dsg 1-related pemphigus foliaceus (PF) that occurs also endemically as the Brazilian fogo selvagem. The desmoglein compensation theory states that both Dsgs can compensate for each other to maintain adhesion. Therefore, the lesions in PV arise in the deepest epidermal layers that lack Dsg1, leading to the notorious "tombstone-like" appearance (Fig. 3.13D and E). Conversely, blisters occur in PF in the most superficial layers where there is no Dsg3. This argument is further supported by experiments in which DSG3$^{-/-}$ animals were treated with anti-Dsg1 antibodies and suprabasilar blisters developed instead of subcorneal blisters (Mahoney *et al.*, 1999). Furthermore, regional differences in lesion formation in the skin and oral mucosa are most likely due to the different distribution of Dsg1 and Dsg3 (see Table 3.1). It is not clear, however, why Dsg2 is not able to make up for the loss of Dsg3 in PV.

The pathophysiology of PV and PF has been worked out in some detail. Specific autoantibodies that are directed against distinct domains of desmosomal cadherins have been isolated from affected patients where they occur bound to the surface of epidermal cells and in circulating body fluids (e.g., Allen *et al.*, 1993; Amagai *et al.*, 1991, 1994; Eyre and Stanley, 1987, 1988; Ishii *et al.*, 1997; Koulu *et al.*, 1984; Olague-Alcala *et al.*, 1994; Stanley *et al.*, 1986; Fig. 3.13C). Various transfer assays demonstrated that these isolated antibodies are sufficient to induce blister formation (Amagai *et al.*, 1992; Anhalt *et al.*, 1982; Mahoney *et al.*, 1999; Rock *et al.*, 1989). Most convincingly, passive transfer of affinity-purified anti-Dsg1-antibodies into neonatal mice led to subcorneal, epidermis-restricted acantholysis

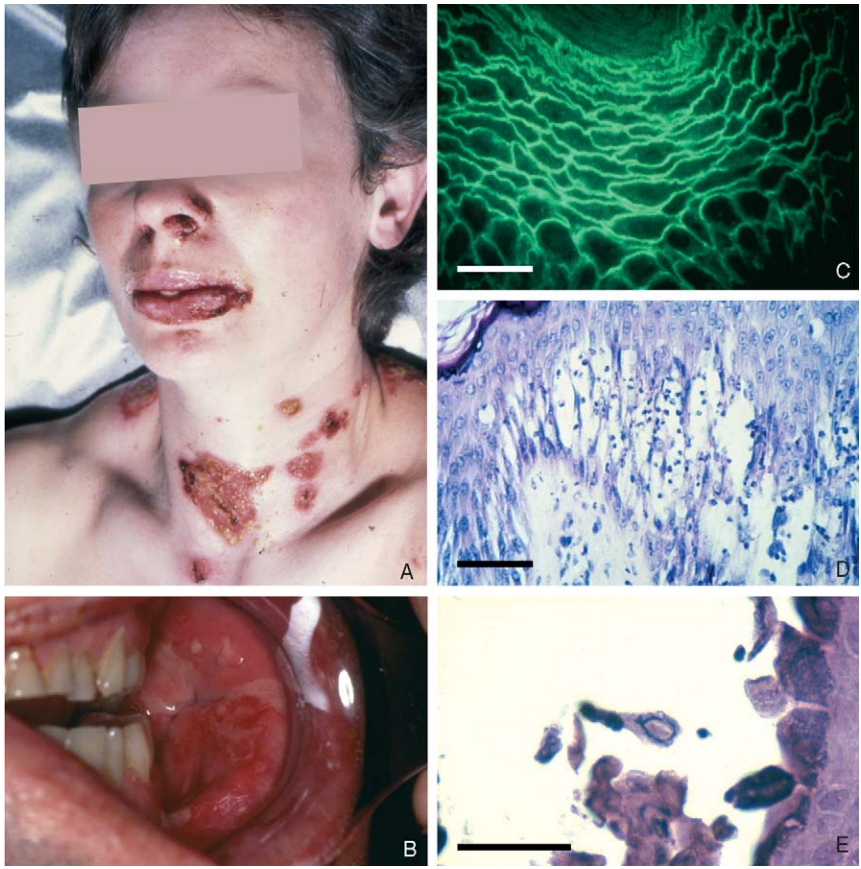

Figure 3.13 Alterations in patients suffering from pemphigus vulgaris (A, B, D, E) and pemphigus foliaceus (C). Note the blister formation in various parts of the skin and oral mucosa of the patient shown in A and B independent of mechanical stress. The affected areas have a tendency for bacterial superinfection. Patient sera contain autoantibodies that elicit a plasma membrane staining in the epidermis corresponding to desmosomes (C). In pemphigus foliaceus staining is strongest in suprabasal cell layers (C) and in pemphigus vulgaris in intermediate cell layers. The histology of affected areas reveals intraepithelial blister formation due to loss of cell–cell adhesion either in the upper cell layers in the case of pemphigus foliaceus (not shown) or in the lower cell layers along with formation of basal "tombstones" in the case of pemphigus vulgaris (D, E). Bars: 50 μm in C, E; 100 μm in D. (The original images were kindly provided by Dr. C. Sunderkötter and were prepared by the photolaboratory of the Clinic and Polyclinic of Dermatology, University Münster, thanks to Ms. J. Bückmann, P. Wissel, and Dr. T. Luger.)

without mucous membrane involvement (Amagai *et al.*, 1995b; Rock *et al.*, 1989). Conversely, antibodies against the extracellular domain of Dsg3 caused suprabasilar blisters as well as acantholysis in mucous membranes of neonatal mice (Amagai *et al.*, 1992). Furthermore, all pathogenic antibodies

could be depleted from either PV or PF sera by adsorption to the extracellular domains of Dsg3 and Dsg1, respectively (Amagai *et al.*, 1994, 1995b; Memar *et al.*, 1996). A highly sophisticated adoptive PV mouse transfer model was recently described: Splenocytes were isolated from $DSG3^{-/-}$ mice that had been immunized with recombinant Dsg3 and were introduced into $RAG2^{-/-}$ immunodeficient mice (Amagai *et al.*, 2000b). As expected, the recipient mice developed typical symptoms of PV. Thus, there is little doubt that anti-Dsg antibodies induce skin blistering. Two aspects are, however, the subject of intense discussion: (1) What is the exact molecular mechanism that leads to skin blistering? (2) How do other autoantibodies that are frequently observed in PV and PF patients contribute to the disease? For a detailed discussion of these topics, see Amagai *et al.* (2006).

Two major alternative pathomechanisms have been discussed in the past: (1) direct inhibition of adhesion by antibodies and/or (2) involvement of intracellular signaling processes. The first is supported by observations showing that the most potent pathogenic PV monoclonal antibodies interact with the functionally important N-terminal adhesive interface (Tsunoda *et al.*, 2003). Release of desmosomal cadherins into small clusters that are readily internalized and the formation of desmosomal halves at the cells surface are considered to be direct consequences of antibody binding (Calkins *et al.*, 2006; Sato *et al.*, 2000). Although pathogenic autoantibodies may not be able to penetrate inside desmosomes, it can be assumed that they are able to block turnover of desmosomal components, especially taking the high turnover rates noted in cultured systems into consideration (Gloushankova *et al.*, 2003; Windoffer *et al.*, 2002). The second pathogenetic mechanism is primarily supported by *in vitro* observations. Addition of IgG from PV sera to cultured keratinocytes caused a transient increase in intracellular calcium and inositol 1,4,5-triphosphate (IP_3) (Seishima *et al.*, 1995). This response was inhibited by an inhibitor of phospholipase C (PLC), suggesting that the latter is involved in PV-IgG-induced inositol bisphosphate hydrolysis to generate IP_3 (Esaki *et al.*, 1995). PLC also produces diacylglycerol, which activates PKC, of which certain isoforms were increased and translocated to a particulate cytoskeletal fraction within 30 sec of PV-IgG supplementation (Osada *et al.*, 1997). PV-IgG also induced phosphorylation of Dsg3 and dissociation of plakoglobin from Dsg3 in cultured keratinocytes (Aoyama *et al.*, 1999). Interestingly, keratin retraction from cell–cell contact sites was observed that required PG-mediated signaling (Caldelari *et al.*, 2001) and involves p38 MAPK and Rho A (Berkowitz *et al.*, 2005, 2006; Waschke *et al.*, 2006). It should be stressed that activation of intracellular pathways precedes cell splitting and desmosomal cadherin disengagement, which is not completely abolished by antibody binding (Waschke *et al.*, 2005). Eventually, signaling may lead to apoptosis (Arredondo *et al.*, 2005; Wang *et al.*, 2004). Clearly, antibody

binding itself does not instantaneously lead to splitting, which is temporally and spatially regulated (Shimizu *et al.*, 2004).

The ongoing controversy about the pathogenesis of PF and PV is even more complicated by the fact that patients develop, in addition to anti-Dsg1 and anti-Dsg3, a large spectrum of autoantibodies against cell surface molecules such as other Dsgs, all Dscs, E-cadherin, collagen XVIII, several subunits of the nicotinic acetylcholine receptors, and annexins, to name but a few (Amagai *et al.*, 2006). Obviously, anti-E-cadherin antibodies, for example, act synergistically enhancing the antiadhesion effect of anti-Dsg antibodies (Evangelista *et al.*, 2006). Also, these additional autoantibodies may be pathogenic on their own as shown for antiacetylcholine receptor antibodies both *in vitro* and *in vivo* (Nguyen *et al.*, 2000). Similarly, it was recently reported that activation of the EGF receptor and Src kinases can still be elicited in Dsg-depleted keratinocytes by PV-IgG (Chernyavsky *et al.*, 2007).

The involvement of Dscs in autoimmune diseases has been much less explored. It is likely linked to IgA-pemphigus, a vesiculopustular dermatosis in which Dsc antibodies were identified. Although initial observations suggested that different subtypes could be correlated with immunoreactivities directed either against Dsc3 or Dsc1 (Hashimoto *et al.*, 1996), more stringent assays could confirm the presence of only Dsc1-specific autoantibodies (Hashimoto *et al.*, 1997). Furthermore, Dsc antibodies were also observed in Hallopeau-type pemphigus vegetans (Hashimoto *et al.*, 1994), in pemphigus herpetiformis (Kozlowska *et al.*, 2003), and also in PF and PV (Dmochowski *et al.*, 1993, 1995), although these findings may not be related directly to the etiology of these diseases.

8.3. Bacterial toxins

The importance of Dsgs for epidermal integrity became even more apparent when the target of *Staphylococcus aureus* toxins was identified as Dsg1 (Amagai *et al.*, 2000a, 2002; Hanakawa *et al.*, 2002). *S. aureus* infections are among the most common bacterial infections in children. These patients usually present with bullous impetigo that may, especially in young children but also in immunocompromised adults, develop into a generalized and life-threatening form, the so-called staphylococcal scalded skin syndrome (SSSS). In this case, subcorneal epidermal blisters appear over the entire body surface. The disease-causing exfoliative toxins A, B, and D are serine proteases, all of which with very high specificity exclusively cleave Dsg1 after glutamic acid residue 381, which is positioned between extracellular domains 3 and 4 (see Fig. 3.5; Amagai *et al.*, 2002; Hanakawa *et al.*, 2002). The resulting symptoms phenocopy those observed in PF, namely cell–cell separation in the cell layers beneath the stratum corneum. In this way, bacteria open up a specific niche that is used for spreading in the otherwise hardly penetrable skin.

8.4. Cancer

The inverse interrelationship between cell–cell adhesion and malignant transformation is well recognized, and reduced cell–cell adhesion is considered to be one of the hallmarks of malignant tumors favoring formation of metastasis. The relevance of this concept for desmosomal adhesion is supported by observations of desmosome-free fibroblasts producing desmosomal components (Dsc1a and b, Dsg1, and PG) that not only exhibited increased adhesion but also reduced invasion in an *in vitro* assay (Tselepis *et al.*, 1998). On the other hand, components of certain adhesion structures, most notably of desmosomes, have served as reliable tumor markers in histodiagnosis, especially in the distinction of epithelial tumors and meningiomas (Akat *et al.*, 2003; Moll *et al.*, 1986).

Downregulation of desmosomal components has been reported for a number of different tumors such as squamous cell carcinoma of the head and neck (Bosch *et al.*, 2005), mouth (Depondt *et al.*, 1999; Harada *et al.*, 1992; Hiraki *et al.*, 1996; Shinohara *et al.*, 1998), pharynx (Depondt *et al.*, 1999), esophagus (Natsugoe *et al.*, 1997), and skin (Krunic *et al.*, 1998; Tada *et al.*, 2000), and was seen in urothelial carcinomas (Conn *et al.*, 1990). Reduced Dsg3 was also noted in breast cancer (Klus *et al.*, 2001; Oshiro *et al.*, 2003). In gastric cancer reduced and abnormally distributed Dsg2 was observed (Biedermann *et al.*, 2005; Yashiro *et al.*, 2006). Interestingly, a significant reduction in Dsgs was noted in comparisons of low- and high-grade intraepithelial lesions of the uterine cervix (Alazawi *et al.*, 2003; de Boer *et al.*, 1999). An absence of downregulation, however, was observed in colorectal carcinomas (Collins *et al.*, 1990), and even an upregulation of Dsg2 was described in squamous cell carcinomas with a positive correlation to high-risk tumors (Kurzen *et al.*, 2003). Dsc, Dsg, and DP expression was reported to be inversely correlated with differentiation status, invasiveness, and lymph node metastasis in oral squamous cell carcinomas (Hiraki *et al.*, 1996; Shinohara *et al.*, 1998). More detailed analyses suggested that the reduction of desmosomal components is most prominent in the respective invasion front (i.e., in a region where cells migrate into the surrounding connective tissue) (Depondt *et al.*, 1999; Hiraki *et al.*, 1996; see, however, Kurzen *et al.*, 2003) and is related to more aggressive types of tumors some of which undergo epithelial–mesenchymal transition (for desmosome dissociation during early stages of epithelial–mesenchymal transition, see Boyer *et al.*, 1989; Savagner *et al.*, 1997).

The direct molecular linkage between cell adhesion and altered gene expression in tumors has been elucidated, at least in part, for β-catenin (Behrens, 2005; Giles *et al.*, 2003). β-Catenin is a major structural protein of adherens junctions as part of the linker system coupling the transmembrane cadherin adhesion molecules to the actin cytoskeleton. Nonjunctional β-catenin is usually rapidly degraded by the ubiquitin–proteasome system

involving the serine/threonine kinase GSK 3β and the axin/APC multiprotein complex. The amino-terminally serine-phosphorylated β-catenin is recognized by the ubiquitin ligase β-TrCP. Stabilization of cytoplasmic β-catenin by *wnt* signaling or in tumors through a number of different mechanisms results in nuclear translocation where β-catenin interacts with LEF/TCF transcription factors leading to transactivation of LEF/TCF target genes, which include the dorsalizing genes *Siamois*, *Twin*, and *Xn3*, the protooncogene c-MYC, cyclin D1, fibronectin, and the matrix metalloprotease matrilysin. β-Catenin mutations occur frequently and are among the most common in colorectal carcinomas.

The situation is much more complicated for the related PG, which, as pointed out above, differs in several ways from β-catenin and is rarely mutated in tumors. Most observations suggest, however, that it may function as a tumor suppressor. In accordance, reduced PG synthesis has been noted in tumors and metastatic lesions of renal cells (Buchner *et al.*, 1998), oral and pharyngeal squamous cell carcinomas (Depondt *et al.*, 1999), esophageal carcinomas (Nakanishi *et al.*, 1997), prostate cancer (Shiina *et al.*, 2005), and skin carcinomas (Tada *et al.*, 2000). Furthermore, JUP mutations have been identified in gastric cancer (Caca *et al.*, 1999) and loss of PG has been observed during cancer progression (Aberle *et al.*, 1995; Amitay *et al.*, 2001). Loss of heterozygosity has been reported in some sporadic breast, ovarian, and prostate cancers (Aberle *et al.*, 1995; Shiina *et al.*, 2005). The tumor suppressor function is also supported by experiments in which PG was overexpressed in various cell lines leading to inhibited cell growth and reduced tumorigenicity (Simcha *et al.*, 1996; Winn *et al.*, 2002). Similarly, *de novo* expression of PG in SCC9 squamous cell carcinoma cells led not only to epidermoid differentiation with desmosome formation but also to a decreased growth rate and increased matrix adhesiveness (Parker *et al.*, 1998). Furthermore, overproduction of PG in skin of transgenic mice resulted in suppression of epithelial proliferation and hair growth (Charpentier *et al.*, 2000) in line with its proposed function as a key suppressor of c-MYC in skin (Williamson *et al.*, 2006).

On the other hand, PG protects keratinocytes from apoptosis possibly by an increase in the antiapoptotic molecule BCL-X_L in keratinocytes (Dusek *et al.*, 2006b). Furthermore and in contrast to the aforementioned studies, an oncogenic potential of PG was proposed by others. Thus a strong transforming capacity was described for PG in RK3E epithelial cells (Kolligs *et al.*, 2000) and overexpression of PG in squamous cell carcinoma cells of line SCC9 resulted in uncontrolled growth and foci formation with inhibition of apoptosis and induction of BCL-2 expression (Hakimelahi *et al.*, 2000). The discrepancies may be explained by cell type–specific differences and indirect effects of PG on β-catenin activity.

Several studies investigated the distribution of PPs in various types of carcinomas (Furukawa *et al.*, 2005; Mertens *et al.*, 1999; Moll *et al.*, 1997;

Papagerakis *et al.*, 2003; Schwarz *et al.*, 2006). In squamous cell carcinomas an inverse relationship between the degree of malignancy and synthesis of PP1 and PP3 was observed (Schwarz *et al.*, 2006). Interestingly, p0071 appeared to be associated with tumor growth, exhibiting an inverse relationship to tumor size (Papagerakis *et al.*, 2003). PP2 antibody staining was reported to be generally weak or absent in these tumors by some (Mertens *et al.*, 1999; Schwarz *et al.*, 2006) but not by others who found a positive correlation with metastasis formation (Papagerakis *et al.*, 2003). PP2 was, however, consistently detectable in adenocarcinomas (Mertens *et al.*, 1999; Schwarz *et al.*, 2006). In hepatocellular carcinoma PP2 was the only PP except for limited PP1-positive foci with nuclear reactivity (Schwarz *et al.*, 2006). In most other adenocarcinomas, with the exception of prostate cancer, PP2 was coexpressed with PP3, whereas PP1 was usually absent (Schwarz *et al.*, 2006). Interestingly and somewhat in contrast to other aforementioned reports, PP3 was shown to be elevated in all non–small-cell lung carcinomas including adenocarcinomas and squamous cell carcinomas, and it was proposed to be a useful prognostic marker (Furukawa *et al.*, 2005). Whether PPs themselves affect tumor development and progression in an isotype-specific fashion as suggested by some (Furukawa *et al.*, 2005; Schwarz *et al.*, 2006) and how this relates to their specific subcellular localization remain to be shown.

9. Concluding Remarks

The current review attempted to provide a broad overview of the molecular diversity of desmosomes and their functions as tissue stabilizers but also attempted to point out current developments that assign additional functions to desmosomes concerning tissue differentiation and proliferation. In the coming years it will be necessary to meet the challenge to work out the molecular details of the complex interactions that occur not only within desmosomes but that also take place with outside partners to identify upstream and downstream regulators whose intricate balance affects the basic cellular machinery resulting in finely tunable shifts between differentiating and proliferating activities.

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