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# Intercellular Junction Assembly, Dynamics, and Homeostasis

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Intercellular anchoring junctions are highly specialized regions of the plasma membrane where members of the cadherin family of transmembrane adhesion molecules on opposing cells interact through their extracellular domains, and through their cytoplasmic domains serve as a platform for organizing cytoskeletal anchors and remodelers. Here we focus on assembly of so-called “anchoring” or “adhering” junctions—adherens junctions (AJs) and desmosomes (DSMs), which associate with actin and intermediate filaments, respectively. We will examine how the assembly and function of AJs and DSMs are intimately connected during embryogenesis and in adult cells and tissues, and in some cases even form specialized “mixed” junctions. We will explore signaling and trafficking machineries that drive assembly and remodeling and how these mechanisms are co-opted in human disease.

In multicellular organisms intercellular junctions serve to maintain cell and tissue polarity and integrity. In vertebrates these organelles include: gap junctions, which chemically and electrically couple neighboring cells; tight junctions, which are essential for establishing the epithelial barrier; and “adhering junctions,” which organize the cortical cytoskeleton beneath the plasma membrane to modulate cell and tissue behavior. Together, these organelles integrate intra- and intercellular signaling, including regulation of nuclear functions and transcriptional pathways.

Calcium-dependent, cadherin-based anchoring junctions known as adherens junctions (AJs) and desmosomes (DSMs) organize and tether microfilaments and intermediate filaments (IF) to the plasma membrane, respectively. In polarized cells, AJs appear as continuous zonula, subjacent to the tight junctions, and are uniformly distributed along the plasma membrane in a belt that is closely opposed to an actin-rich mat underneath the plasma membrane (Niessen and Gottardi 2008). DSMs appear as “spot welds” distributed subjacent to the zonula and scattered throughout the

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K.J. Green et al.

lateral border, where they distribute forces of mechanical stress via interactions with the IF cytoskeleton (Holthofer et al. 2007; Garrod and Chidgey 2008). These junction types appeared at different times during the evolution of multicellular organisms, but share common blueprints, linked assembly mechanisms and synergistic functions.

### Building Blocks for Assembly of Intercellular Adhering Junctions

The structure of AJs and DSMs is roughly parallel in blueprint and can be broken down into three major components: the (1) transmembrane cadherins, the tails of which provide a scaffold for (2) armadillo family members, which help build a cortical platform for additional (3) cytoskeletal adapter proteins (see also Meng and Takeichi 2009). Together these components associate with and organize actin and IF, respectively, at the plasma membrane.

**Cadherins and Nectins:** Classical cadherins (e.g., E- and N- and P-cadherin) are single-pass transmembrane proteins (Hulpiau and van Roy 2009). Their extracellular cadherin domains contain a series of five conserved cadherin repeats, which extend from the cell surfaces and bind to cadherins present on adjacent cells (see Shapiro and Weis 2009). The cytoplasmic domains contain binding sites for associated catenins (see the following discussion) and other regulatory proteins (Perez-Moreno and Fuchs 2006). The desmosomal cadherins, desmogleins and desmocollins, have a similar ectodomain structure but more divergent cytoplasmic domains (Getsios et al. 2004b). In particular, the desmogleins have extended cytoplasmic tails containing variable numbers of RUD (repeating unit domain) motifs, whose function is unknown.

Nectins are more recently identified calcium-independent adhesion molecules in the IgG superfamily (Irie et al. 2004). They are thought to prime adhesive interactions for cadherins, thus initiating AJ assembly. Notably, the nectin cytoplasmic domain interacts with an actin binding protein called afadin/AF6, which has been shown to bind to

$\alpha$ -catenin and has thus been implicated in recruiting/clustering cadherin complexes in nascent junctions, and as a possible alternative way to link actin to the plasma membrane, as described below (Tachibana et al. 2000).

**Armadillo Proteins:** Both desmosomal and classic cadherins contain conserved intracellular domains that bind members of the armadillo family (Hatzfeld 2007). Junctional armadillo proteins fall into two subclasses that are each characterized by a central domain built from a series of  $\sim 42$  amino acid armadillo repeats. Armadillo drives the transcriptional program responsible for segment polarity in the fly, in addition to being a key component of fly AJs (Brembeck et al. 2006). Intercellular adhering junctions contain members from two armadillo subfamilies: One family contains armadillo and its close relative plakoglobin; the other group contains p120catenin,  $\delta$ -catenin/NPRAP (neural plakophilin related arm protein, NPRAP), ARVCF (armadillo repeat gene deleted in Velo-Cardio-Facial syndrome), and plakophilins (PKPs) 1–3 and p0071/PKP4 (Hatzfeld 2007).

**Cytoskeletal Adaptor Proteins:** The cytoplasmic cadherin tails and associated armadillo proteins are embedded within a cortical meshwork containing cytoskeletal adaptor proteins and associated cytoskeletal fibers that make up the electron dense junctional “plaque.” Contained within this plaque region are the  $\beta$ -catenin binding protein  $\alpha$ -catenin, and the plakoglobin- and PKP-binding protein desmoplakin (Green and Simpson 2007; Niessen and Gottardi 2008). In previous textbook models, these adaptors are final links in a chain that connect cadherins to their respective cytoskeletons, actin and IF. However, cadherin- $\beta$ - $\alpha$ -catenin and  $\alpha$ -catenin-actin interactions have recently been shown to be mutually exclusive in vitro (Yamada et al. 2005). Thus, the identity or identities of the real actin anchors are uncertain.  $\alpha$ -Catenin may still contribute to the effort indirectly via associated proteins such as afadin,  $\alpha$ -actinin, vinculin, ZO-1, or EPLIN (Abe and Takeichi 2008; Stemmler 2008). Data from AJ studies also prompt new consideration of the model proposed for

DSMs, whereby desmoplakin directly links IF to the desmosomal cadherin complex.

These core junctional components are assisted by additional cell-type specific and accessory molecules including actin remodeling proteins, IF binding and cornified envelope proteins, tyrosine kinases and phosphatases, growth factor receptors, and their associated proteins, which cooperate to tailor junctions structurally and functionally (Perez-Moreno and Fuchs 2006; Holthofer et al. 2007).

## EMBRYOGENESIS AND JUNCTION ASSEMBLY

### An Evolutionary Perspective

The cadherin superfamily comprises over one hundred members (Hulpiau and van Roy 2009). Cadherins first appeared in single-celled organisms, such as the choanoflagellate *Monosiga brevicollis*, where they are speculated to have served a role in binding bacterial prey (Abedin and King 2008; King et al. 2008). With the acquisition of multicellularity, cadherins recruited other structural and signaling proteins into primitive AJs. This ancient “assembly” step advanced AJ functions in regulating tissue morphogenesis through junctional and nonjunctional pathways, the latter through armadillo proteins that moved between the surface and the nucleus (Grimson et al. 2000; Schneider et al. 2003). With the appearance of vertebrates came the desmosomal cadherins. Their expanded diversity allowed tailoring of adhesive junctions for new structural and morphogenetic requirements, providing complex tissues with mechanical integrity whose importance is underscored by human diseases of the desmosome and corresponding animal models (reviewed by Kowalczyk and colleagues [Delva et al. 2009]) (Cheng and Koch 2004; Lai-Cheong et al. 2007). Consistent with the mantra “ontogeny recapitulates phylogeny,” AJs appear first during embryonic development. A rich body of literature regards AJs as master regulators of TJ and DSM assembly, suggesting that their assembly routes may have

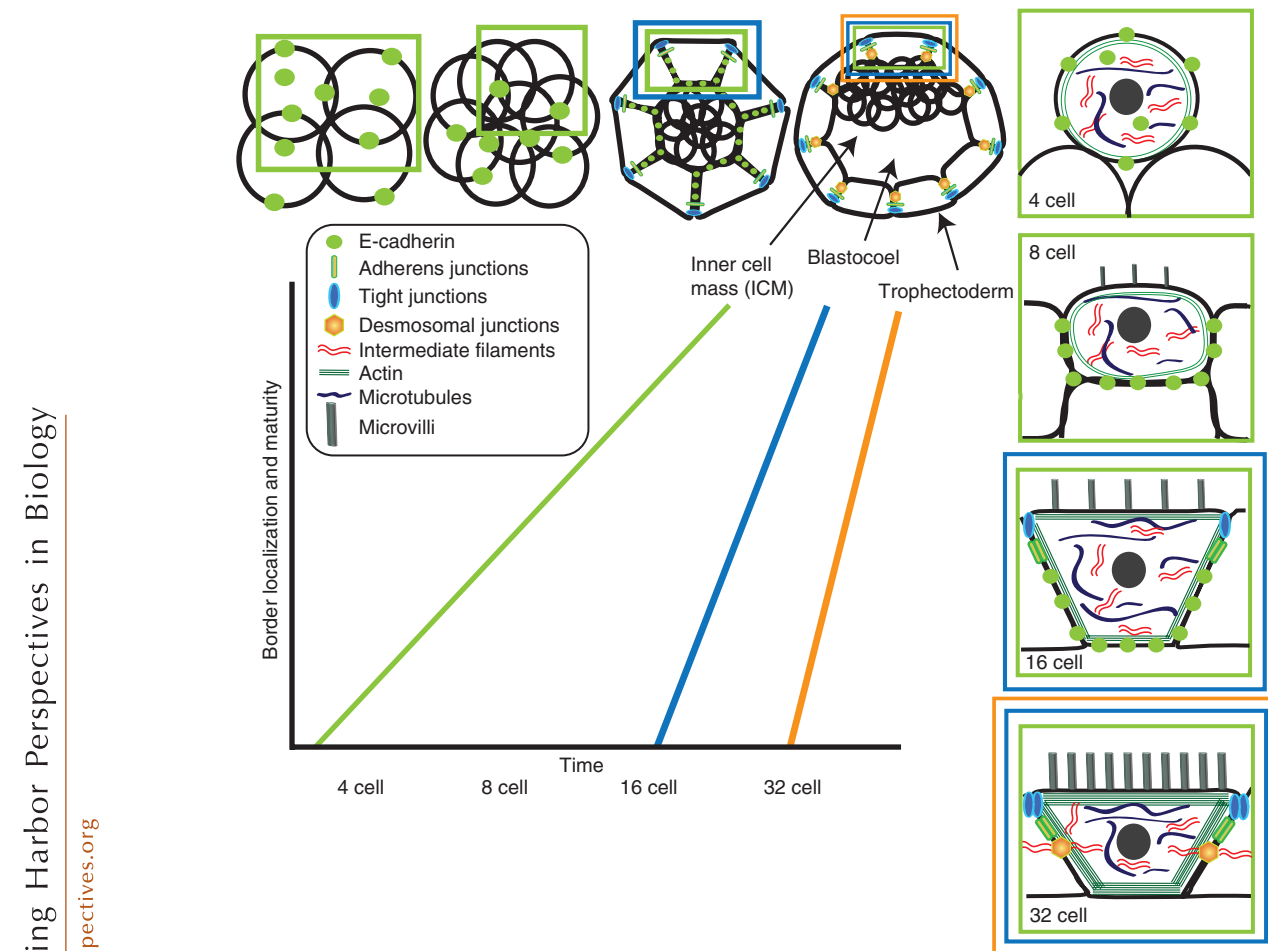
been overlaid on pre-existing cytoskeletal and signaling pathways.

### De Novo Junction Assembly in the Preimplantation Embryo

Intercellular junctions are initially formed during compaction of the 8-cell stage embryo in a process that culminates with the differentiation of the first epithelial layer, the trophoctoderm (Fig. 1) (Vestweber et al. 1987; Fleming et al. 1991; Fleming et al. 1993; Barcroft et al. 1998). Up until the early 8-cell stage, the embryo consists of loosely adherent blastomeres. On compaction, these relatively spherical blastomeres are converted into highly polarized cells that flatten to maximize contact points and begin to elaborate AJs along with focal tight and gap junctions (Fleming et al. 1993; Eckert and Fleming 2008). In contrast, DSMs anchoring intermediate filaments to cell–cell contacts are only found in the mature trophoctodermal epithelium at the 32-cell stage when a blastocoel cavity has formed and tight junctions have adopted a more zonular distribution. This sequence of developmental events places E-cadherin upstream in a pathway that allows for the formation and organization of other anchoring junctions.

E-cadherin-based adhesion complexes are required for embryo compaction and the subsequent formation of a trophoctodermal epithelium (Larue et al. 1994; Riethmacher et al. 1995; Torres et al. 1997; De Vries et al. 2004). The mere presence of E-cadherin or its cytoplasmic partners ( $\alpha$ -,  $\beta$ -,  $\gamma$ -catenin) is not sufficient to trigger compaction because all of these proteins are diffusely expressed along the cell surface in blastomeres of 4-cell embryos (Kidder and McLachlin 1985; Vestweber et al. 1987; Fleming et al. 1991; Fleming et al. 1993; Collins et al. 1995; Ohsugi et al. 1996; Barcroft et al. 1998). Although the process that activates the cadherin complex during compaction is not known, PKC $\alpha$  is redistributed to cell–cell contacts in 8-cell blastomeres and its activation can trigger compaction and  $\beta$ -catenin phosphorylation (Sefton et al. 1992; Pauken and Capco 1999). Because depletion of  $\beta$ -catenin

K.J. Green et al.



**Figure 1.** Junction biogenesis in preimplantation mammalian embryos. Following the initial cleavage event of the zygote and throughout the entire 4-cell stage, blastomeres are loosely connected to one another and exhibit a diffuse distribution of E-cadherin. Embryo compaction at the 8-cell stage stabilizes E-cadherin along increasing areas of cell–cell contact and initiates a process of junction assembly, cytoskeletal (actin microfilaments and microtubules) reorganization, and increased polarization of apico-basolateral membrane domains (apical microvilli). Asymmetric cell divisions that give rise to a 16-cell embryo allocate inner daughter cells lacking epithelial characteristics and an outer epithelial cell layer that gradually elaborates actin cytoskeleton-associated adherens and tight junctions (reviewed in Eckert and Fleming 2008). A functional tight junction barrier triggers cavitation and the formation of a fluid-filled blastocoel that contacts the inner cell mass; this process is coordinated with the abrupt assembly of intermediate filament-associated desmosomes in the outer trophectodermal epithelium.

results in postimplantation loss of embryos (Haegel et al. 1995), this armadillo family member is not the only target for PKC-mediated compaction events. Vezatin, which bridges the unconventional myosin VIIa motor to the cadherin–catenin complex, is a potential candidate because it maintains the levels of E-cadherin and  $\beta$ -catenin to allow for

compaction (Hyenne et al. 2007). Accordingly, compaction also requires active actin remodeling and contraction (Kabir et al. 1996; Clayton et al. 1999).

Desmosomes form fairly abruptly in the trophectodermal epithelium at the 32-cell stage, in contrast to the more gradual maturation and redistribution of tight junctions

into a zonular pattern at the apical interface. Although plakoglobin and Dsc3 are present at the time of compaction, most other desmosome proteins, including membrane (Dsg2, Dsc2) and plaque (desmoplakin) components, are first detected in the early blastocyst upon formation of an outer trophectoderm layer that surrounds the inner cell mass (Jackson et al. 1980; Fleming et al. 1991; Den et al. 2006). The induction of Dsc2 mRNA transcripts is tightly coordinated with the appearance of desmosomes and subsequent blastocoel formation (Fleming et al. 1991; Collins et al. 1995). The developmental signal that triggers Dsc2 expression appears to be E-cadherin-independent because Dsc2 and desmoplakin can be induced in the presence of function blocking antibodies for this classical cadherin. Moreover, desmosomal cadherins are normally distributed at the cell surface of E-cadherin-null blastocysts that have undergone compaction owing to the persistence of maternally derived E-cadherin or its ectopic replacement by transgenic N-cadherin expression (Ohsugi et al. 1997; Kan et al. 2007). Although Dsc2 induction appears to be a key initiator of desmosome assembly, E-cadherin is uniquely required for trophectoderm maturation leading to the death of these embryos during the preimplantation stages.

Embryos completely lacking zygotic and maternal E-cadherin cannot form DSMs and never reach the 32-cell stage because of a failure in compaction (De Vries et al. 2004; Kan et al. 2007). These results are mirrored by the observation that depletion of classic cadherins in keratinocytes results in a failure to form DSMs in vitro (Tinkle et al. 2008; Michels et al. 2009). These findings suggest that the temporal pattern of junction assembly is linked to their functional interdependence, a concept that will be further explored in the following section.

#### IN VITRO ANALYSIS OF CELL JUNCTION ASSEMBLY AND DYNAMICS

As during evolution and embryogenesis, AJ formation precedes DSM formation in vitro;

however, rather than being drawn out over days of embryogenesis, events occur within minutes of de novo cell contact or elevation of extracellular calcium (Figs. 2 and 3). AJs and DSMs exhibit interdependence from the initiation of nascent complexes through segregation of components into specific domains. Functionally, mature junctions synergize to maintain epithelial polarity and integrity. Further, junction homeostasis is a balance of biosynthetic and endocytic pathways, each step subject to post-translational modifications and pathogenic input.

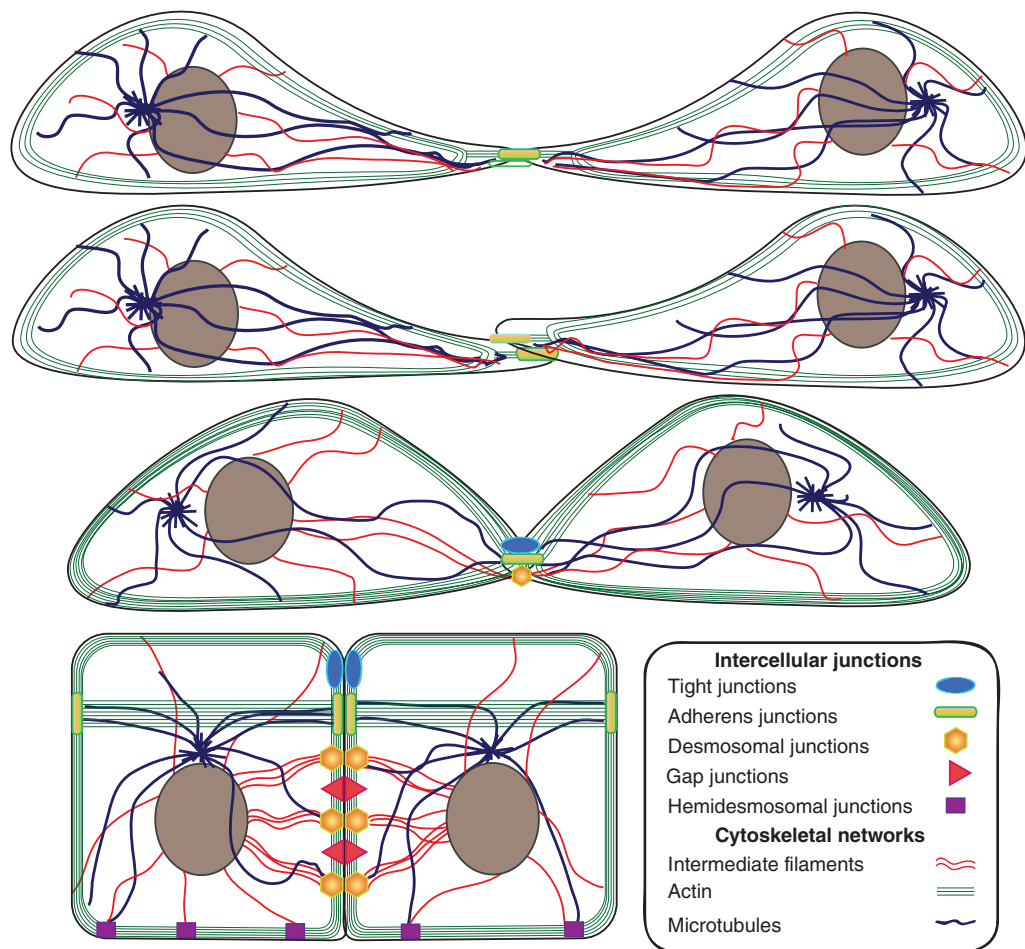
#### Assembly of the Adherens Junction and Desmosome Adhesive Interface

*Trafficking behavior of cadherins and associated armadillo proteins:* Desmosomal and classic cadherins are constantly synthesized and delivered to the plasma membrane (Fig. 4) (Penn et al. 1987; Pasdar and Nelson 1989; Shore and Nelson 1991). In response to de novo contact or elevation of extracellular calcium, cadherins cluster and become stabilized, and, in most cell types, segregate into distinct domains.

Cadherins follow a classic secretory route beginning with synthesis in the endoplasmic reticulum (ER), followed by transport to the Golgi for processing, sorting into post-Golgi carriers, and long range trafficking via MT-dependent motors (reviewed in Bryant and Stow 2004). Transport of tubulovesicular carriers containing E-cadherin out of the trans-Golgi network is dependent on the tethering protein Golgin-97 (Lock et al. 2005) and association with ankyrin-G and  $\beta$ -2-spectrin (Kizhatil et al. 2007). The ankyrin-G-spectrin complex later stabilizes E-cadherin by directly connecting it to the spectrin/actin cytoskeleton (Kizhatil et al. 2007), and these functions are both critical for coordinating cadherin trafficking with formation of cell-cell contacts in both cultured cells and developing embryos (Kizhatil et al. 2007). Studies linking desmosomal PKPs to vesicle trafficking have begun to emerge, with the discovery that PKP3 associates with the dynamin-like protein DNM1L, which is involved in vesicle trafficking within the



K.J. Green et al.



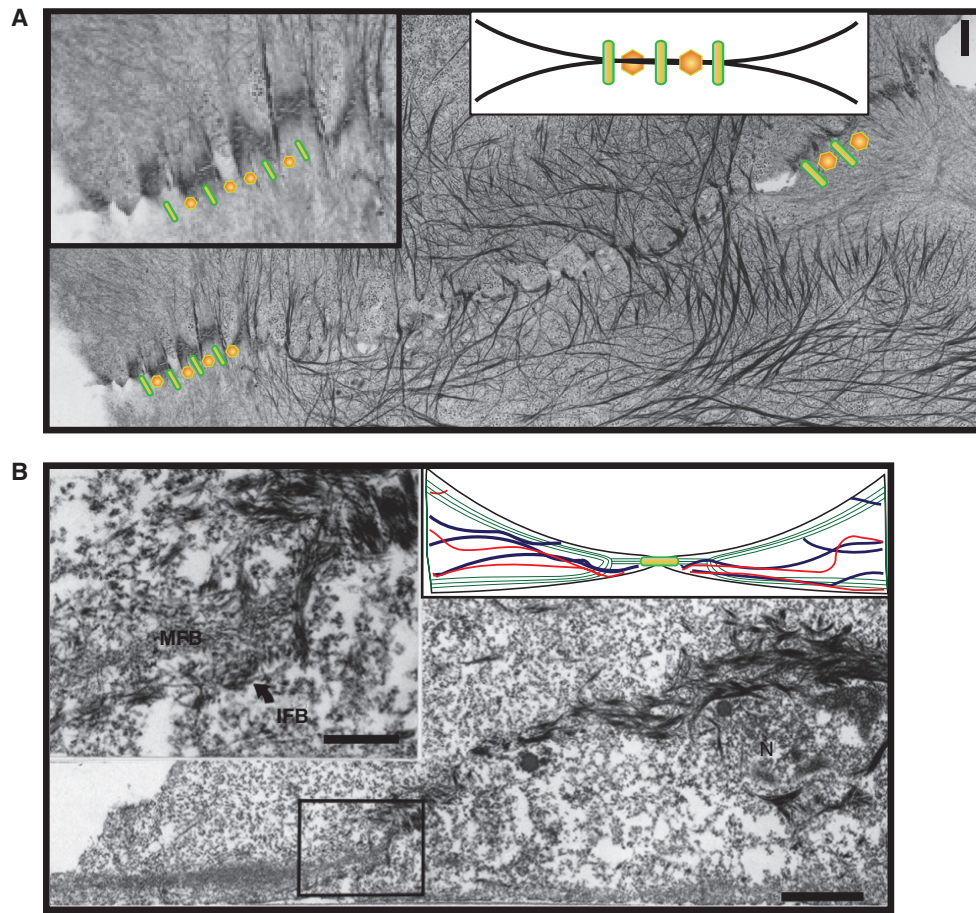
**Figure 2.** Junction biogenesis in vitro and associated cytoskeletal arrangements. Following cell contact in vitro, initial cadherin engagement triggers reorganization of the cytoskeleton, coordinated with downstream assembly of tight junctions and desmosomes. In polarized simple epithelial cells, the maturation of junctional contacts is accompanied by the formation of a circumferential zone of parallel microfilament bundles near the apical region of the cell, below the tight junction barrier. Desmosomes are subjacent to this complex and also appear as disclike structures that extend along the entire lateral border, where they anchor IF to the plasma membrane. MTs also anchor at AJs and DSMs.

secretory pathway in lung carcinoma cells (Furukawa et al. 2005).

Both classic and desmosomal counterparts are synthesized as precursor propeptides and processed via a furin-dependent mechanism before reaching the cell surface (Muller et al. 2004; Yokouchi et al. 2009). The presence of the propeptide is thought to prevent premature adhesion inside the cell (Ozawa and Kemler 1990). Glycosylation does not appear to be required for efficient processing of the

precursor or transport of E-cadherin to the cell surface, but in the case of desmosomal cadherins, may contribute to their notable insolubility (Shore and Nelson 1991).

The E-cadherin tail is unstructured in the absence of its binding partners  $\beta$ -catenin and p120catenin, which protect it from degradation (Huber et al. 2001; Davis et al. 2003) and shepherd the cadherin from the ER to the plasma membrane (Chen et al. 1999). A soluble pool of plakoglobin, which is capable of associating



**Figure 3.** Ultrastructural relationships between microfilaments, IFs, and associated adhering junctions at keratinocyte cell–cell contacts and during contact formation. (A) Transverse section through cell contact zone between two mouse keratinocytes showing alternating arrangement of AJs and DSMs at the cell margins with their associated actin and IF networks. (B) Cross section of mouse keratinocyte during contact initiation shows the intimate connection between keratin IF bundles, which emanate from the nuclear surface and dive down towards and integrate within actin bundles near the substrate (Reprinted, with permission, from Green et al. 1987).

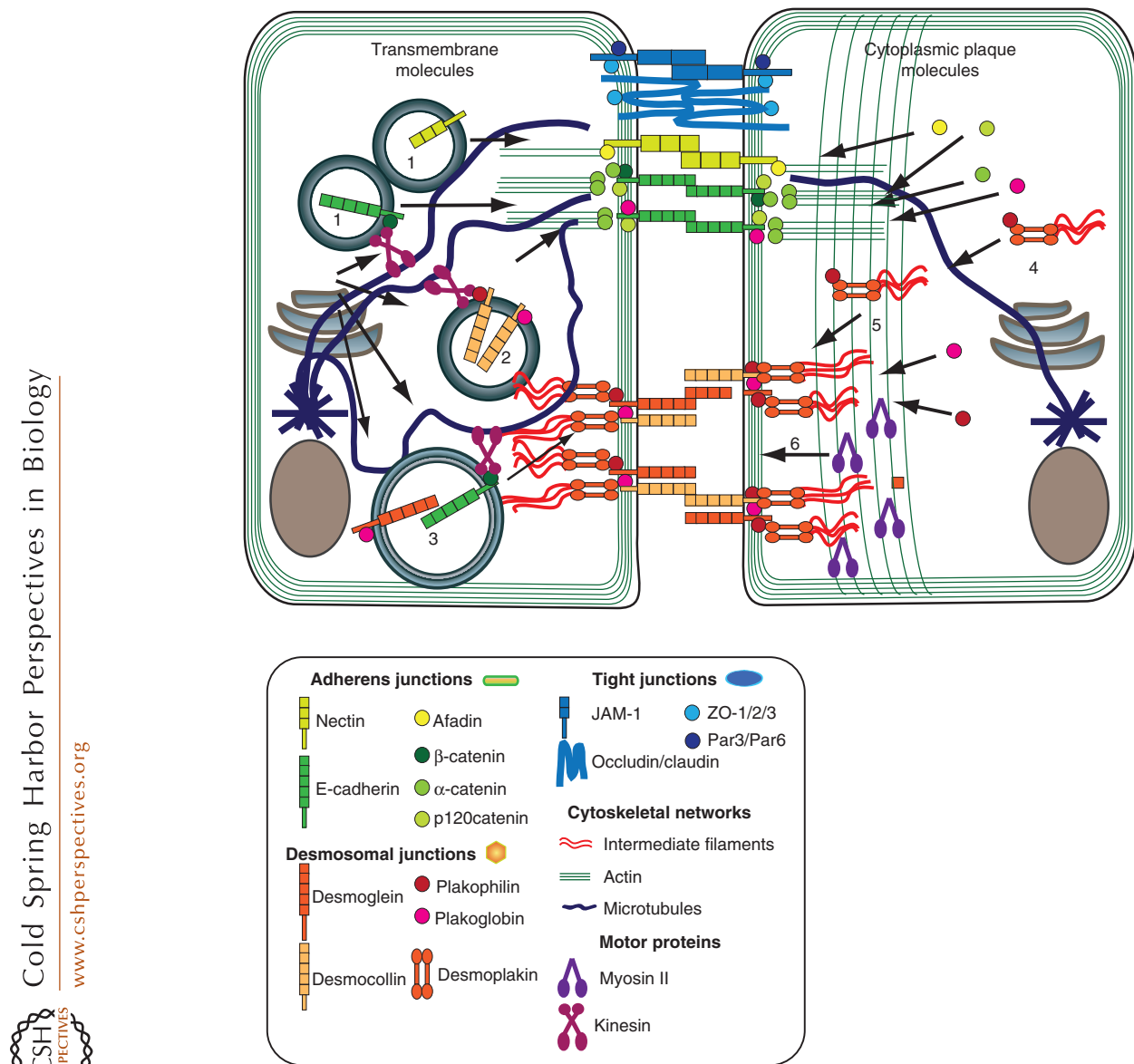
with both classic and desmosomal cadherins, initially associates primarily with E-cadherin and may serve a similar role (Hinck et al. 1994). An insoluble pool of newly synthesized plakoglobin associated with desmoglein is required for its stabilization and incorporation into DSMs (Pasdar et al. 1995). The functional implications of a potential temporal switch in partnership between classic and desmosomal cadherins was indicated by experiments suggesting that desmosome assembly in A431 cells depends on the presence of plakoglobin

associated with E-cadherin (Lewis et al. 1997). Whether plakoglobin serves a chaperone role for desmosomal cadherins has not been directly tested, although desmogleins are also unstructured in the absence of plakoglobin binding (Kami et al. 2009), and truncated desmogleins deficient in plakoglobin binding fail to properly localize to plasma membranes (Andl and Stanley 2001).

In polarized epithelial cells,  $\alpha$ -catenin and p120 associate with E-cadherin as it reaches the plasma membrane (Hinck et al. 1994;



K.J. Green et al.



**Figure 4.** Model depicting possible molecular dynamics of intercellular junction assembly. Molecular players in assembly of the adhesive core (*left cell*) and junctional plaques (*right cell*) are depicted, with emphasis on AJs and DSMs assembly. Transmembrane molecules are transported in a MT-dependent fashion toward the plasma membrane, possibly via molecular motors in the kinesin family. Cadherins and nectins (1) initiate the process of assembly of the AJ adhesive interface, whereas initial recruitment of desmocollins (2) is followed by stabilization at sites of junction assembly by desmogleins (3). Cargo loading may be facilitated through armadillo family members in some cases. Junctional plaque assembly occurs through dynamic remodeling of the cortical cytoskeleton, initiated by the cadherin-dependent concentration of actin remodeling proteins at sites of junction assembly to drive actin polymerization and reorganization from branched to bundled actin. In coordination with these changes, desmosomal precursors containing desmoplakin and plakophilin are formed in the cytoplasm in close association with IF. Desmosome precursors (4) subsequently translocate to sites of desmosome assembly in an actomyosin-dependent manner to reinforce the plaque (5), in conjunction with actin rearrangement depending on myosin II (6).

Miranda et al. 2003). Here p120 promotes junction formation by stabilizing cadherins through interfering with endocytic internalization (Davis et al. 2003; Kowalczyk and Reynolds 2004) and association with the actin-associated protein afadin in a Rap1 GTPase-dependent manner (Hoshino et al. 2005).

### *Cadherin Dynamics and Role of Microtubules*

Intercellular adhesions depend on the presence of an intact microtubule (MT) cytoskeleton (Grindstaff et al. 1998; Waterman-Storer et al. 2000; Stehbens et al. 2006; Kizhatil et al. 2007; Stehbens et al. 2009). MTs facilitate long-range trafficking of classic and desmosomal cadherins including their anterograde transport to the plasma membrane (Pasdar et al. 1991; Mary et al. 2002; Chen et al. 2003). Evidence is consistent with transport via kinesins, possibly through associated armadillo proteins (Mary et al. 2002; Chen et al. 2003; Teng et al. 2005) (Fig. 4).

Established junctions interact with both plus- and minus-end-directed MTs and associated motor proteins. These interactions regulate junction assembly state and provide transport routes to sites of cell adhesion (Ligon and Holzbaaur 2007; Shaw et al. 2007).  $\beta$ -catenin-dynein (Ligon et al. 2001),  $\alpha$ -E-catenin-dynactin (Lien et al. 2008) and Nezha/PLEKHA7-KIFC3 (Meng et al. 2008) associations have been revealed as critical for MT interaction and AJ stability. Further, dynamic MTs at adhesion sites promotes active myosin II recruitment, which in turn increases junctional E-cadherin (Stehbens et al. 2006). Intercellular junctions also control the spatial distribution of MTs. Notably, DSMS are required for reorganization of MTs to cell–cell contacts in differentiating keratinocytes through interactions with the centrosomal protein ninein, whose redistribution to cell–cell contacts along with MTs requires desmoplakin (Lechler and Fuchs 2007). Collectively, these data paint a picture of cooperative interactions between cadherin-based junctions and MTs in junction assembly and regulation of MT dynamics and organization.

### *Role of Rabs and the Exocyst*

Cadherin trafficking and junction assembly is tailored through small GTPases called Rabs which recruit specific effectors in the secretion process (Caviston and Holzbaaur 2006). E-cadherin has been reported to associate with an intermediate Rab11-positive recycling compartment in MDCK cells (Lock and Stow 2005). Further, Rab11 is associated with the exocyst, a multisubunit complex responsible for correct targeting of proteins to the plasma membrane. Loss of function of the exocyst components sec 5, 6, and 15 results in accumulation of *Drosophila* E-cadherin in a Rab11 compartment, inhibiting its delivery to the membrane (Langevin et al. 2005). Interfering with the tight junction scaffold protein associated with Lin seven 1 (PALS1) results in mislocalization of the exocyst and impairs E-cadherin delivery to the plasma membrane (Wang et al. 2007), and coordination between these junctions is facilitated by the balance of activities of Rab13 and Rab8 and their associated proteins (Yamamura et al. 2008).

How desmosomal and classic cadherins are coordinated during their egress out to the cell surface is not well understood. Based on the existence of “mixed” junctions that occur naturally or because of genetic interference, it seems plausible that cotrafficking of classic and desmosomal cadherins may be coupled with mechanisms to remodel or segregate components into distinct junctions in epithelial tissues (Bornslaeger et al. 1995; Ruiz et al. 1996; Borrmann et al. 2006). Temporal analysis of vesicular trafficking following a calcium switch suggests that 60-nm vesicles containing mostly Dsc2 are first observed to transport to the entire cell surface (Burdett 1998), consistent with the possibility that desmocollins serve as initiators of junction assembly in vitro (Hanakawa et al. 2000) and during embryogenesis. Larger vesicles containing desmoglein and E-cadherin were later observed to target basolaterally where they presumably associate with desmocollins and stabilize at sites of junction assembly (Wollner et al. 1992; Burdett 1998). Electron microscope pulse-chase

K.J. Green et al.

experiments demonstrated that in keratinocytes, desmogleins first form small clusters on the surface lacking IF attachment, and later found associated with IF-attached desmosomal plaques (Sato et al. 2000). Collectively, these data suggest both a temporal and spatial staging of cadherin targeting and incorporation into junctions.

### **Establishing Functional Adhesion**

Two models have been proposed to explain how intercellular adhesion is established once cadherins have arrived at the plasma membrane. The first depends on the initial formation of lateral or “cis” cadherin dimers, which then come together on adjacent cells to interact in *trans*, followed by a zippering of the adhesive interface (Shapiro et al. 1995; Pokutta and Weis 2007). The second suggests that cadherin monomers interact in *trans* to form a “strand dimer” (Boggon et al. 2002), an arrangement supported by site-specific cross-linking experiments (Trojanovsky et al. 2003) and visualized by tomographic analysis of DSMs (He et al. 2003). Additional support for this idea comes from recent single-molecule structural (fluorescence resonance energy transfer) and functional (atomic force microscopy) assays that identified *trans* but not *cis* interactions (Zhang et al. 2009).

In contrast to classic cadherins, robust desmosomal cadherin-mediated adhesion requires the presence of both desmogleins and desmocollins in the correct ratio (Amagai et al. 1994; Chidgey et al. 1996; Kowalczyk et al. 1996; Marozzi et al. 1998; Tselepis et al. 1998; Getsios et al. 2004a). Biochemical and atomic force microscopy (AFM) suggest that desmosomal cadherins are capable of interacting in both homophilic and heterophilic configurations (Syed et al. 2002; Waschke et al. 2005; Heupel et al. 2008) and desmocollin and desmoglein from neighboring cells can be co-precipitated (Chitaev and Trojanovsky 1997). However, the organization of desmosomal cadherins in situ is still unknown.

It has been suggested that weak *trans* interactions (Chen et al. 2005) are subsequently

strengthened by lateral cadherin clustering and cytoskeleton attachment (Gumbiner 1996; Kusumi et al. 1999). But the possible existence of higher affinity interactions is supported by the isolation of cadherin dimers from cells (Chitaev and Trojanovsky 1997) and the observation that cadherin mutants lacking catenin-binding sites can support cell–cell adhesion (Ozawa and Kemler 1998). The discrepancy between in vivo and in vitro data could arise from incomplete unfolding of the cadherin EC1 domain in vitro that makes strand dimer formation impossible (Trojanovsky et al. 2007). The observed range of mechanical strengths between *trans*-bonded E-cadherin pairs is also consistent with the possibility that longer lived associations can exist (Perret et al. 2004).

### **Assembly of the Junctional Plaque: Actin Remodeling and Desmosome Assembly are Linked**

Although one can debate the existence of weak versus strong cadherin–cadherin dimers, the cortical actin cytoskeleton and myosin II activity are clearly required for adhesion strengthening, junctional plaque assembly and maintenance (Chu et al. 2004; Mege et al. 2006; Miyake et al. 2006). Classic cadherin ligation triggers rearrangements and polymerization of cortical actin that drive formation of the AJ plaque (Yonemura et al. 1995; Perez-Moreno and Fuchs 2006; Pokutta et al. 2008). During this time, the process of DSM assembly and establishment of IF attachments is set into motion, the latter stages occurring in an actin-dependent manner (Figs. 3 and 4) (Green et al. 1987; Godsel et al. 2005). A functional link between actin rearrangements and desmosome assembly may help explain why interfering with classic cadherins or  $\alpha$ -catenin suppresses desmosome formation (Gumbiner et al. 1988; Watabe et al. 1994; Amagai et al. 1995; Taniguchi et al. 2005). Here, we will discuss actin remodeling pathways triggered by cadherin-dependent cell contact and then explore how these events may couple with downstream assembly of DSMs.

### *Classic Cadherins Trigger Rho GTPase-Dependent Actin Polymerization and Contractile Signaling*

Cell contact occurs through the extension of lamellopodia driven by the polymerization of branched actin filaments at the leading edge of simple polarized epithelial cells (Drees et al. 2005), or filopodial projections driven by polymerization of linear actin bundles, as has been described in primary keratinocytes and developing *Drosophila/Caenorhabditis elegans* during tissue morphogenesis (Raich et al. 1999; Vasioukhin et al. 2000; Bloor and Kiehart 2002). The extent to which cells use one or both of these actin-dependent processes to drive the initial stages of adhesion in specific cell types is not known. It seems likely that a combination of mechanisms and actin modulators tailor remodeling of the cell cortex to contextual and cell type differences (Gates and Peifer 2005).

In the contacting lamellopodia of MDCK cells, a highly mobile fraction of E-cadherin (Adams et al. 1998) converts into immobile puncta and ultimately into larger plaques whose formation and stability requires an intact actin cytoskeleton (Adams et al. 1996). Actin rearrangements occur in concert with alterations in Rac1, Cdc42, and RhoA signaling triggered by nectin and cadherin-mediated contacts (Noren et al. 2001; Irie et al. 2004). Rac-dependent recruitment of arp2/3 (Kovacs et al. 2002), which promotes polymerization of branched actin filaments, collaborates with actin binding proteins such as cortactin, to facilitate expansion of the contact zone (Helwani et al. 2004). Actin remodeling progresses through the local accumulation and dimerization of  $\alpha$ -catenin near contact sites. In its dimerized form,  $\alpha$ -catenin inhibits Arp2/3, converting actin from branched to linear, bundled arrays typical of maturing AJs (Yamada et al. 2005).

Rho activity facilitates contact expansion by activating actomyosin contractility in the flanking regions of the junctional contact zone (Yamada and Nelson 2007). Rho also regulates formins, actin-associated proteins that

mediate polymerization of linear bundles. The Rho effector Dia is required for junction stability and attenuation of protrusive activity in vertebrates and *Drosophila* (Sahai and Marshall 2002; Homem and Peifer 2008), depending on myosin II activity for reinforcement of cell–cell junctions (Carramusa et al. 2007). Formins are also recruited by  $\alpha$ -catenin to E-cadherin-tipped filopodia that project into neighboring keratinocytes to draw them together and initiate AJ assembly (Kobielak et al. 2004). Rho activity can also destabilize junctions through alternate pathways (Sahai and Marshall 2002; Zandy et al. 2007; Fang et al. 2008). The ability of the cadherin-associated protein p120catenin to inhibit Rho through p190RhoGAP may ensure that Rho activity is properly titrated at cell–cell junctions (Wildenberg et al. 2006). The continued activities of Rho and its downstream effector ROCK help to transform keratinocyte sheets to stratifying tissues (Vaezi et al. 2002) and increase the height of polarized epithelial cells (Zhang et al. 2005).

### *AJ Maturation and Assembly of the Desmosomal Plaque*

Shortly after calcium-induced cell contact in keratinocytes, electron-dense desmosome precursors associated with keratin IF align along perpendicular actin bundles that appear to emanate from newly formed AJ plaques (Green et al. 1987; Godsel et al. 2005). These precursors eventually disappear from the cytoplasm as DSMs mature (Jones and Goldman 1985; Pasdar and Nelson 1988), frequently alternating with AJs along contacting cell borders (Green et al. 1987) (Fig. 3). Live cell imaging of desmoplakin-GFP suggests that these precursors represent part of a three phase process that occurs in response to cell–cell contact in cultured keratinocytes at the leading edge of a wound. Desmosomal plaque assembly begins with the rapid accumulation of desmoplakin at contacting borders followed by a second phase (within  $\sim 20$ – $30$  min of cell contact) in which fluorescent bright nonmembrane bound desmoplakin-containing particles form

K.J. Green et al.

in the cortical region of the cell. The subsequent translocation of particles to nascent junctions to reinforce the plaque, is coordinated with actin rearrangements and dependent on myosin II activity (Godsel et al. 2005 and unpublished results). Once formed, desmosomes continue to exhibit dynamic fusion events as well as internalization and retrograde transport (Windoffer et al. 2002).

How is the desmoplakin-IF system coupled to AJ assembly and the actin-based machinery? Although plakoglobin has been the focus of early studies as important for junctional cross talk (Lewis et al. 1997), inducible expression of the p120-related protein PKP1 has also been reported to initiate DSM assembly in a classic cadherin-dependent fashion (Wahl 2005). In addition to interacting with desmoplakin and IF, PKPs can associate with actin and cause p120-like rearrangements of the actin cytoskeleton (Hatzfeld et al. 2000) (Chen, Bass-Zubek, and Green, unpublished). PKP2 deficiency also results in major alterations in the actin cytoskeleton and rhoA signaling, likely interfering with AJ-dependent actin remodeling driving DSM assembly (Bass-Zubek, Godsel and Green, unpublished). Thus, PKPs may provide a link between the desmoplakin-IF network and an actin-dependent phase of desmosome assembly.

Loss of PKP2 also results in retention of desmoplakin on IF, preventing its incorporation into DSMs. This “beads on a string” appearance of desmoplakin is reminiscent of the distribution of desmoplakin in PKC $\alpha$  knockdown cells (Bass-Zubek et al. 2008) and a phosphorylation deficient desmoplakin mutant (S2849G) that exhibits delayed outward trafficking (Godsel et al. 2005) because of its higher affinity for, and aberrant retention on, IF (Stappenbeck et al. 1994; Meng et al. 1997; Fontao et al. 2003). Our data suggest a model whereby PKP2 scaffolding of PKC $\alpha$  promotes desmoplakin phosphorylation and assembly competence (Bass-Zubek et al. 2008). Consistent with this, PKC restores DSM assembly in  $\alpha$ -catenin-null colon carcinoma cells and can promote DSM assembly even in reduced calcium (Sheu et al. 1989; Hengel et al. 1997). PKPs also collaborate

with plakoglobin to mediate desmosomal cadherin clustering into ultrastructurally identifiable plaques (Bornslaeger et al. 2001; Koeser et al. 2003).

Like AJs, DSMs undergo a period of maturation, ultimately attaining a hyper-adhesive state which can be reversed by the calcium-dependent kinase PKC $\alpha$  (Watt et al. 1984; Wallis et al. 2000; Garrod et al. 2005; Kimura et al. 2006). The fact that PKC $\alpha$  also promotes desmosome assembly supports the idea that this kinase is important for maintaining a dynamic state, which would allow rapid desmosome remodeling in response to environmental cues. Desmosomes are also defective in patients with Darier’s disease who have impaired calcium homeostasis because of mutations in the sarco(endo)plasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA) isoform 2 pump (Sakuntabhai et al. 1999; Dhitavat et al. 2003). Although these abnormalities were suggested to arise from improper folding of desmoplakin, it also seems possible that alterations in calcium-dependent kinase activity could contribute to the observed defects.

Achieving maximum mechanical integrity of epithelial cell sheets requires proper attachments of junctions to both IF and cortical actin, as intercellular adhesion is synergistically strengthened by the presence of IF and actin attachments (Huen et al. 2002). Further, AJs are unable to mature properly in cells lacking desmoplakin and normal IF attachments suggesting bi-directional regulation between AJs and DSMs (Vasioukhin et al. 2001b).

## JUNCTION HOMEOSTASIS: ROLE OF ENDOCYTOSIS

AJs and DSMs can be modified posttranslationally by physiological stimuli resulting in changes in phosphorylation state and proteolytic processing (Gumbiner 2005; Green and Simpson 2007). These processes have traditionally been proposed to alter interactions with the cytoskeleton, thus weakening adhesive strength. However, modification of the cadherins and their associated proteins can also regulate endocytic internalization and recycling (Bryant and



Stow 2004; Ivanov et al. 2005; Yap et al. 2007; Delva and Kowalczyk 2009). Derailed endocytosis of cadherins may contribute to tumor progression (Mosesson et al. 2008) and blister formation in the autoimmune disease pemphigus (Kottke et al. 2006).

Like other receptors, cadherins can be constitutively endocytosed by a clathrin-dependent pathway (Le et al. 1999; Palacios et al. 2002; Xiao et al. 2005), possibly via binding of the adaptor complex AP2 to the dileucine motif in the juxtamembrane region (Miyashita and Ozawa 2007). This motif may be exposed on dissociation of p120, consistent with earlier work demonstrating a role for p120 in E-cadherin stability (Davis et al. 2003; Xiao et al. 2003). However, other determinants must be responsible for clathrin-dependent endocytosis of VE-cadherin, because even though it is similarly regulated by p120 (Xiao et al. 2005), it does not possess such a motif. Further, N-cadherin intracellular regions lacking the dileucine motif facilitate endocytosis (Tai et al. 2007). Basolateral targeting of E-cadherin may also be regulated by the dileucine motif (Lock and Stow 2005), and assembly and recycling facilitated by the adapter mu1B subunit through a variant of PIPK $\gamma$  (Ling et al. 2007).

Growth factors such as TGF $\beta$  (Janda et al. 2006), EGF (Lu et al. 2003; Hirao et al. 2006; Bryant et al. 2007), HGF (Palacios et al. 2001), and, FGF (Bryant et al. 2005) can promote internalization and loss of junctions. Further, cadherin molecules can physically complex with growth factor receptors including EGF receptor, ErbB-1 (Dumstrei et al. 2002; Fedor-Chaiken et al. 2003) HGF receptor, c-Met (Davies et al. 2001) and FGFR (Suyama et al. 2002). These associations can either promote internalization or stabilize cell surface retention, impacting both junction integrity and growth factor-dependent signaling.

Small molecule inhibitors of EGFR/ErB2 have been shown to block internalization of E-cadherin and desmoglein 2 in tumor cells, resulting in increased adhesive strength even under conditions of reduced calcium, highlighting the therapeutic value of EGFR inhibitors for patients with cancers that overexpress EGFR

(Lorch et al. 2004; Klessner et al. 2009). The ADAM family of sheddases cooperates with EGFR in increasing the internalized pool of desmoglein 2, thus reducing adhesive strength (Klessner et al. 2009). Different endocytic pathways have been suggested to mediate growth factor-regulated endocytosis, including rac-dependent macropinocytosis (Akhtar and Hotchin 2001; Bryant et al. 2007) and caveolin-dependent endocytosis (Lu et al. 2003).

Growth factor stimulation has also been linked to activation of nonreceptor tyrosine kinases such as Src. Src-dependent phosphorylation of two tyrosines of E-cadherin (Y755 and Y756) produces a binding site for E3 ubiquitin-ligase Hakai, and ubiquitination of the E-cadherin–catenin complex facilitates clathrin-dependent internalization (Fujita et al. 2002) and targeting for lysosomal degradation (Palacios et al. 2005). This pathway likely explains cadherin internalization and dissolution of AJs shown to accompany the activation of ts-v-Src in MDCK cells (Warren and Nelson 1987; Palovuori et al. 2003) or reduced extracellular calcium via activation of Cdc42 (Shen et al. 2008).

Desmosomal cadherin internalization is a contributing factor in the autoimmune disease pemphigus (Kottke et al. 2006). Dsg3 is degraded in response to PV autoantibody binding resulting in Dsg3-depleted DSMs (Aoyama and Kitajima 1999), shown to be because of rapid clathrin- and dynamin-independent internalization of a soluble pool of PV-IgG-bound Dsg3 (Calkins et al. 2006; Delva et al. 2008). PV antibodies may impair assembly of new DSMs by targeting internalization of newly synthesized Dsg3 (Sato et al. 2000; Mao et al. 2009). Although it has been reported that plakoglobin, but not desmoplakin, remains associated with the cadherin (Calkins et al. 2006), others suggest that plakoglobin dissociates from phosphorylated Dsg3 after which it is degraded, depleting a nuclear pool required for attenuating c-myc transcription (Muller et al. 2006). PV induces alterations in both IF attachment (Caldelari et al. 2001; Calkins et al. 2006) and cortical actin (Waschke et al. 2006). The latter has been

K.J. Green et al.

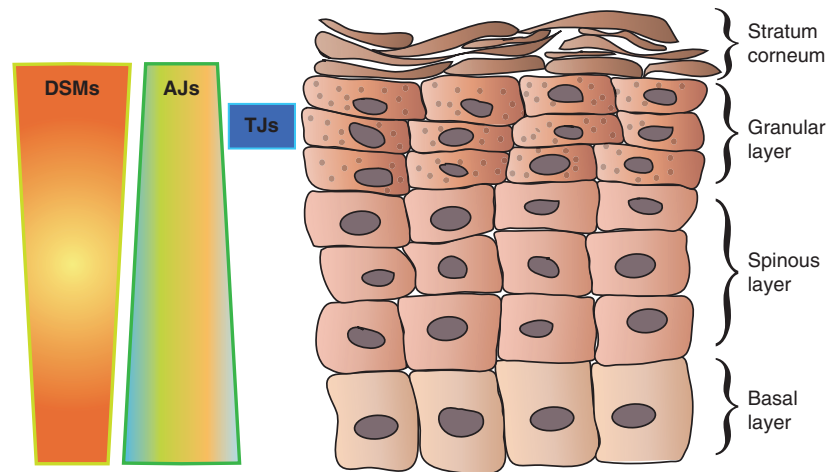
linked with a reduction in Rho A activity, suggesting another link between pathways regulating AJs and DSMs (Waschke et al. 2006).

Endocytosis can be envisioned to target two pools of cadherin molecules—engaged or unengaged in adhesion. In isolated epithelial MCF-7 cells, a pool of free cadherin is subjected to macropinocytosis (Paterson et al. 2003; Bryant et al. 2007) and clathrin-dependent endocytosis was suggested to target extrajunctional cadherin (Izumi et al. 2004; Hoshino et al. 2005). However, it is also plausible that cadherins are internalized directly from the intrajunctional region, forcefully breaking apart cadherin adhesive bonds. This would be consistent with the observed association of endocytic vesicles with AJs and supported by the protective effect of endocytosis inhibitors, which lead to the rapid expansion of the junctional interface (Trojanovsky et al. 2006). Thus, cadherin endocytosis may be essential to counterbalance a continuous assembly of cadherin dimers in AJs. Supporting this idea, defects in endocytosis induced by the lack of dynamin or proteins involved in Arp2/3-dependent actin polymerization prevented

completion of endocytosis in *Drosophila* epithelial cells (Georgiou et al. 2008; Leibfried et al. 2008). Together the data suggest that different endocytic routes collaborate to regulate the assembly state of adhering junctions in normal tissues and pathogenic states.

### JUNCTION ASSEMBLY IN COMPLEX TISSUES: CHALLENGES FOR THE FUTURE

Onto these basic modes of junction assembly and remodeling are overlaid additional complexities in the case of tissues such as the epidermis. This stratified squamous epithelium does not exhibit the typical apico-basolateral polarity found in simple epithelial cells. Yet, junctional components are integrated into specialized membrane complexes in a polarized fashion as basal layer keratinocytes stratify and differentiate (Green and Simpson 2007; Niessen 2007; Fuchs 2008). For example, adherens junction-enriched basal keratinocytes are accompanied by increasing numbers of DSMs, which change in molecular composition in the spinous layers and granular layers (Fig. 5 and Fig. 3 of Delva et al. 2009). Although many



**Figure 5.** Organization of intercellular junctions in the epidermis. Desmosomes and AJs are found throughout the epidermis but AJs enriched in E- and P-cadherin are found in the basal layers whereas the number and surface area occupied by DSMs increases during differentiation, during which time P-cadherin is lost. See Kowalczyk and colleagues (Delva et al. 2009, Fig. 3) for the expression patterns of DSM components during differentiation in epidermis. The TJ barrier is in the granular layer of the epidermis, with structurally definable junctions restricted to the second cell layer.

tight junction components (e.g., claudin-1, occludin, and ZO-1) are expressed throughout the epidermis, a tight junction barrier is sealed only in granular cells before these eventually give rise to a cornified dead cell layer (Furuse et al. 2002). Thus, junction assembly in the epidermis is coordinated throughout multiple cell layers instead of within distinct basolateral membrane domains of a simple epithelial cell.

As in the case of compacting embryos, keratinocytes devoid of classical cadherins (E- and P-cadherin) fail to elaborate DSMs and tight junctions in vitro (Tinkle et al. 2008; Michels et al. 2009). Although depletion of classical cadherins interferes with epidermal cohesion and the tight junction barrier in vivo, desmosomes still form in epidermis lacking classical cadherins or  $\alpha$ -catenin (Vasioukhin et al. 2001a; Tunggal et al. 2005; Tinkle et al. 2008).  $\alpha$ -catenin also plays an important role in orienting the mitotic spindle in the basal layer for asymmetric cell divisions that promote stratification into the differentiated layers (Lechler and Fuchs 2005). The lack of desmoplakin has little influence on the place of cell division in basal keratinocytes, but this IF binding protein regulates MT organization in the suprabasal epidermis via interactions with ninein (Lechler and Fuchs 2007). These studies emphasize that elaboration of AJs and DSMs into a stratified epithelium not only holds the tissue together but also provides positional cues for targeting cytoskeletal, trafficking, and endocytic machineries.

How junction assembly and remodeling occurs on the molecular level in vertebrate tissues in vivo is a challenge for future study. During stratification, DSMs with different compositions reflecting differentiation-dependent expression patterns must either be made de novo or remodeled or both (North et al. 1996). In part this remodeling may be driven by transcriptional programs, such as that regulated by grainyhead transcription factors that drive expression of desmoglein 1 in differentiated layers of the epidermis (Wilanowski et al. 2008). Once expressed, whether newly synthesized cadherins are immediately incorporated into junctions or require additional

regulatory cues or post-translational modifications, similar to those that occur during embryogenesis, is unknown. We also do not yet know whether, once assembly competent, molecules are inserted into pre-existing junctions or used to create new junctions. In the future, studies at the molecular level that address fundamental properties of junction dynamics, coupled with genetic analysis of in vivo models, should begin to reveal the complexity of mechanisms used in vivo.

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K.J. Green et al.

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## Intercellular Junction Assembly, Dynamics, and Homeostasis

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K.J. Green et al.

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