Gap Junctions: Basic Structure and Function

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Gap junctions allow the exchange of ions, second messengers, and small metabolites between adjacent cells and are formed by two unrelated protein families, the pannexins and connexins. Mutations in connexin genes cause a variety of genetic disorders, implicating a critical role in tissue homeostasis. Association of congenital skin disorders to mutations in different connexins has underscored the importance of gap junctional communication in the skin and its appendages. Here, we discuss the basic structure of gap junction channels and the function of connexin genes that have been associated with human disorders to explore the physiology of intercellular communication in skin.

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Introduction

Cellular communication is important for the maintenance of tissue/organ homeostasis in multicellular organisms. Using this communication, cells can review differences in environmental conditions and respond accordingly. This concept could involve either sending a signal to neighboring cells to generate a coordinated response or isolating groups of cells from the rest of the community to maintain tissue integrity. One type of communication between cells is mediated via intercellular channels that cluster in specialized regions of the plasma membrane to form gap junctions (Robertson, 1963; Revel and Karnovsky, 1967; Wei *et al.*, 2004). Gap junctional channels link the cytoplasm of two cells, and provide a means for the exchange of ions (K^+ and Ca^{2+}), second messengers (cAMP, cGMP, and inositol 1,4,5-triphosphate (IP_3)), and small metabolites (glucose), allowing electrical and biochemical coupling between cells (Kanno and Loewenstein, 1964; Lawrence *et al.*, 1978). Furthermore, Valiunas *et al.* (2005) recently showed that transfer of small interference RNAs between adjacent cells through gap junctions was possible, although it remains unclear if small interfering RNAs are normally exchanged *in vivo*. Gap junctional communication is essential for many physiological events, including cell synchronization, differentiation, cell growth, and metabolic coordination of avascular organs including epidermis and lens (White and Paul, 1999; Vinken *et al.*, 2006).

Gap junctions are present in both vertebrates and invertebrates from mesozoa to mammals, whereas higher plants use structures called ''plasmodesmata'' for direct intercellular communication. In chordate animals, gap junction channels are encoded by a family of genes called ''connexins'' (Goodenough, 1974), which can be categorized into three groups known as α, β, and γ according to their gene structure, overall gene homology, and specific sequence motifs (Harris, 2001). There are two conventions of nomenclature in the literature for connexins, one of which depends on molecular mass of the connexin (Cx26 represents the connexin protein of 26 kDa; Cx46, connexin isoform of 46 kDa, etc), whereas the other uses greek symbols based on evolutionary considerations (*GJB2* is gap junction beta 2 referring to Cx26, whereas *GJA3* stands for gap junction alpha 3, or Cx46). Gap junctional communication in nonchordate animals, however, is mediated via another family of integral membrane proteins called innexins (Inxs). Innexin proteins are not homologous to connexins in terms of primary sequence; nevertheless, gap junction channels formed from innexins share functional similarities with intercellular channels made of connexins. Recently, another group of proteins called pannexins (Panxs), which may be distantly related to innexins, were identified in vertebrates and have been shown to be expressed in various tissues including kidney, eye, and neurons (Panchin, 2005; Barbe *et al.*, 2006). So far, only connexin genes have been linked to human diseases, so we will limit our discussion to the structure and function of gap junctions that are formed by the connexins.

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Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; Cx, connexin; IP_3, inositol 1,4,5-triphosphate; MW, molecular weight; NAD^+, nicotinamide adenine dinucleotide; SNHL, sensorineural hearing loss

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Basic structural organization of connexins and gap junctions

Gap junctions are highly specialized membrane structures that contain clusters of channels. This organization requires the membranes of two neighboring cells to come close to each other leaving a 2–4 nm gap (Bruzzone *et al.*, 1996; White and Paul, 1999). Connexin family members share a similar structural topology. Each connexin has four transmembrane domains that constitute the wall/pore of the channels. These domains are connected by two extracellular loops that play roles in the cell–cell recognition and docking processes. There are three unchanged cysteine residues in each loop, which solely form intraconnexin disulfide bonds (Krutovskikh and Yamasaki, 2000). The transmembrane domains and the extracellular loops are highly conserved among the family members. Furthermore, connexin proteins have cytoplasmic N- and C-termini and a cytoplasmic loop linking the second and third transmembrane domains (Figure 1a). Although the N-terminus is conserved, the cytoplasmic loop and C-terminus show great variation in terms of sequence and length. For example, the Cx26 protein has the shortest C-terminus, whereas Cx50 has a long C-terminal tail. The cytoplasmic tail and loop are susceptible to various post-translational modifications (e.g. phosphorylation), which are believed to have regulatory roles (Cruciani and Mikalsen, 2002). Most connexins are phosphoproteins, and phosphorylation is considered to be important for the regulation of assembly and modulation of the physiological properties of the channels (Lampe and Lau, 2004; King and Lampe, 2005).

Gap junction biosynthesis and assembly are strictly regulated and intercellular junctions have a short half-life of only a few hours (Musil *et al.*, 2000). Most connexins are cotranslationally integrated into the endoplasmic reticulum membrane. The oligomerization of six connexins into a hemichannel is thought to occur in a progressive fashion starting in the endoplasmic reticulum and ending in the *trans*-Golgi network (Musil and Goodenough, 1993; Sarma *et al.*, 2002; Laird, 2006). Connexons (hemichannels) are then carried to the cell surface via vesicles transported through microtubules, which fuse to the plasma membrane. These hemichannels can either form nonjunctional channels in unopposed areas of the cell membrane (see below) or diffuse freely to regions of cell-to-cell contact to find a partner connexon from a neighboring cell to complete the formation of intercellular channels (Figure 1b) (Harris, 2001). Intercellular channels then cluster into gap junction plaques, a highly dynamic event involving removal of old channels from the center of the plaque, while adding new gap junction subunits to the periphery (Gaietta *et al.*, 2002). The intercellular channels from the middle of the plaque are internalized into vesicular structures called ''annular junctions'' (Jordan *et al.*, 2001), which either fuse with the lysosome for degradation by lysosomal enzymes or are targeted to the proteosomal pathway (Laing and Beyer, 1995; Musil *et al.*, 2000; Qin *et al.*, 2003). The continuous synthesis and degradation of connexins through these mechanisms may provide for the quick adaptation of tissues to changing environmental conditions. Unopposed hemichannels can also be functional under certain conditions, including mechanical and ischemic stress. Under these circumstances, open hemichannels are thought to facilitate the release of a variety of factors such as ATP, glutamate, and NAD^+ into the extracellular space, generating different physiological responses (Evans *et al.*, 2006). It is currently not known if active hemichannels become incorporated into gap junctions before degra-

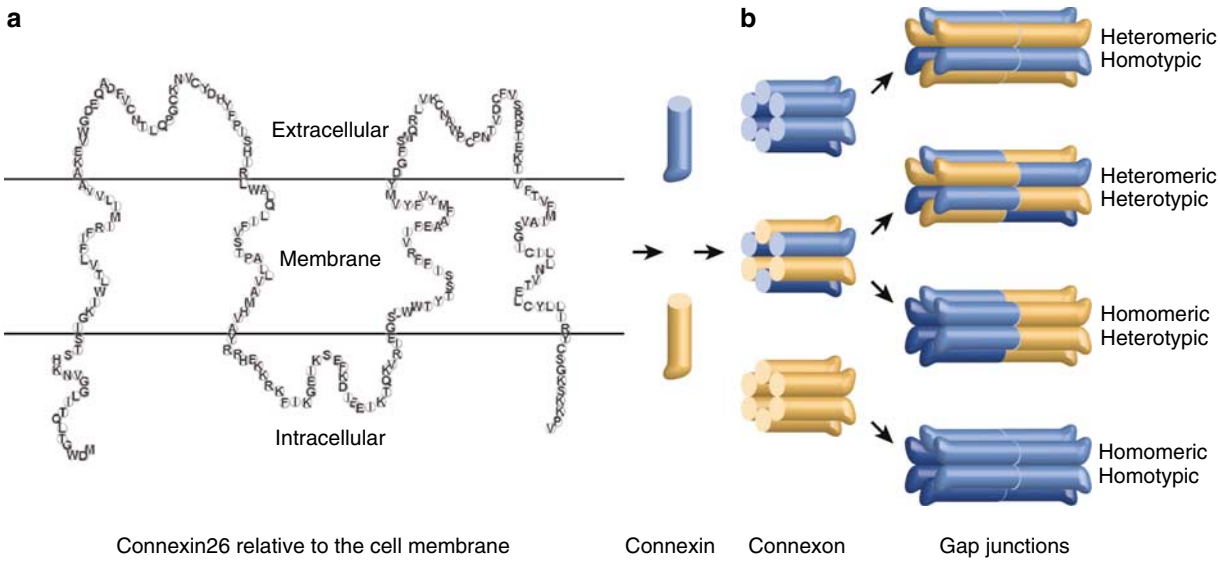

Figure 1. Schematic representation of connexins and gap junction channels. (**a**) Connexins have four transmembrane domains, two extracellular loops, a cytoplasmic loop, and cytoplasmic N- and C-termini. (**b**) Six connexins oligomerize to form hemichannels called ''connexons,'' which then align in the extracellular space to complete the formation of gap junction channels. Different connexins can selectively interact with each other to form homomeric, heteromeric, and heterotypic channels, which differ in their content and spatial arrangement of connexin subunits.

dation or follow a distinct recycling pathway.

There are at least 21 connexin isoforms in the human genome and nearly all cells in the body express at least one type of these genes at some point during development and in the adult life. For instance, Cx26 is highly expressed in cochlea, liver, skin, and placenta, while Cx46 and Cx50 are exclusively found in the eye. Moreover, connexins show overlapping expression patterns where an individual cell can use more than one type of isoform. Cx26, Cx30, Cx30.3, Cx31, Cx43, and others, for example, are found in keratinocytes (Wiszniewski *et al.*, 2000; Kretz *et al.*, 2003), whereas cardiomyocytes use Cx31.9, Cx40, Cx43, and Cx45 for intercellular communication (Beyer *et al.*, 1995; Bukauskas *et al.*, 2006). Coexpression of multiple genes within a single cell, consequently, can affect both the composition of connexons and intercellular channels formed and may provide a compensatory mechanism for the loss of one isoform. Connexons can be formed either from a single type of connexin or from more than one type, leading to the formation of either homomeric or heteromeric hemichannels, respectively. Another complexity is observed during the formation of the fully functional channels. Homotypic channels are formed from either the same homomeric or the same heteromeric connexons, whereas heterotypic channels contain different homomeric or heteromeric hemichannels (Figure 1b). The formation of these structures depends on the compatibility of connexins forming the channels, since not all connexins can interact with each other, such as Cx26 was shown to form heteromeric channels with Cx30 and Cx32, whereas it cannot form functional channels with Cx40 (Segretain and Falk, 2004). These complex interactions thus increase the structural and functional diversity, allowing a vast array of possibilities in the type of molecules shared between cells.

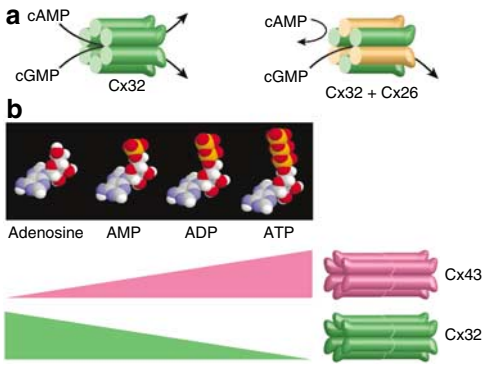

Figure 2. Selective permeability of gap junctions to second messengers. (**a**) Gap junctions are unique in terms of permeability. Cx32 hemichannels were permeable to both cAMP and cGMP whereas Cx26/Cx32 heteromeric hemichannels showed reduced transfer of cAMP but their permeability to cGMP was comparable to that of Cx32 hemichannels. (**b**) The addition of extra phosphate groups to adenosine changed its relative permeability through gap junctions. Cx32 intercellular channels were more permeable to adenosine than channels formed by Cx43. However, the Cx43 intercellular channels had progressively higher permeability to AMP, ADP, and ATP than those formed by Cx32.

Permselectivity of gap junctions

Early models of gap junctional communication described the channels as being nonspecific passive pores, which would freely allow the passage of any ions or metabolites smaller than 1.2 kDa (Simpson *et al.*, 1977). However, recent developments in the field highlighted the selective permeability (permselectivity) of gap junction channels, demonstrating that channels formed by different connexins are unique in terms of their conductance, gating, and permeability to specific molecules (Goldberg *et al.*, 2004) (Figure 2). Furthermore, the association of diseases to specific connexins has also emphasized the uniqueness of each protein, because the loss of one isoform cannot be compensated for by the presence of other connexins in the same tissue or a cell type and leads to pathophysiological defects. Gap junctions mediate the transfer of ions and metabolites/second messengers between cells, allowing ionic and biochemical coupling, respectively. In excitable cells such as neurons and heart, electrical coupling enables the generation of synchronized and rapid responses. In nonexcitable cells, the metabolic coupling may play role in the propagation of coordinated responses. To date, the ionic permeability of gap junctions made of different connexins has been shown to be similar to each other, presenting only minor alterations (Nicholson *et al.*, 2000; Harris, 2001). On the other hand, diverse connexins demonstrated differences in the exchange of larger metabolites and second messengers (Nicholson *et al.*, 2000; Goldberg *et al.*, 2004; Weber *et al.*, 2004). This characteristic of connexins could explain why different tissues need different types of intercellular channels.

Bevans *et al.* (1998) were one of the first to demonstrate variation in the permeability of connexins to distinct signaling molecules. They reconstituted liposomes with either Cx32 homomeric or Cx26/Cx32 heteromeric hemichannels and then loaded the liposomes with tritiated cAMP and cGMP. Using transport-specific fractionation, they determined the permeabilities of these second messengers through the reconstituted channels and observed that Cx32 homomeric hemichannels were equally permeable to both cAMP and cGMP, whereas the Cx26/Cx32 heteromeric channels favored the passage of cGMP over cAMP (Figure 2a).

In another study, Goldberg *et al.* (2002) compared the permeability of Cx32 and Cx43 to different cellular metabolites, including glutamate, glucose, adenosine, AMP, ADP, ATP, and glutathione. They developed a layered culture system using a porous membrane to separate donor and receiver cells from each other, while still allowing the formation of gap junctions between these cells. This study demonstrated that Cx32 gap junctions had a 10-fold higher relative permeability to adenosine compared with Cx43 channels. By contrast, the phosphorylation status of the adenosine shifted its

preferential selectivity from Cx32 channels toward those formed by Cx43. The Cx43 intercellular channels were eight times more permeable to AMP and ADP than Cx32 channels, and the permeability of ATP through Cx43 was more than 300-fold better than that through Cx32 channels (Figure 2b). These observations may suggest an effect of charge and size on the permselectivity of the molecule. However, Goldberg *et al.* (1999) had shown earlier that Cx32 intercellular channels were more permeable to calcein, a fluorescent dye with higher molecular weight and charge (MW: 623 and charge: −4) than ATP (MW: 507 and charge: −3), relative to the Cx43 channels. These data suggest the presence of determinants of gap junctional permselectivity, in addition to the size and the charge of the molecule.

The concept that each gap junction channel is unique in terms of permselectivity is supported by a large number of studies. This specificity is well demonstrated in connexin-associated diseases, where loss of one isoform cannot be compensated for by coexpressed connexins. Beltramello *et al.* (2005) compared the permeability of wild-type Cx26 and deafness-associated Cx26 mutant V84L channels to IP_3. Initially, they observed that both wild type and mutant channels were equally permeable to K^+ ions. Then, they extended their experiments to test if the passage of IP_3 between wild type or mutant channels was altered. Although mutant V84L channels were permeable to potassium ions, the IP_3 transfer was impaired between the cells expressing the mutant protein. The authors also analyzed the permeability of Cx30 to IP_3 and determined that the permeability coefficient of Cx30 was approximately half that of wild-type Cx26. Hence, this suggests that even though Cx30 is highly homologous to Cx26, they are not functionally redundant and Cx30 may not be able to compensate for the loss of IP_3 exchange between the cochlear supporting cells in the absence of Cx26 (Beltramello *et al.*, 2005). Furthermore, Zhang *et al.* (2005) examined the passage of IP_3 and Ca^{2+} waves in organotypic cochlear cultures to analyze if biochemical coupling through gap junctions was needed for normal cochlear function. When they injected IP_3 into the wild-type cells, they observed the generation of Ca^{2+} waves in the cells around the injected one. However, when they analyzed the deafness-causing mutants, V84L, V95M, and A88S, they observed a decrease in the intercellular transfer of propidium iodide and IP_3. These mutant gap junctions were still permeable to Na^+ and Ca^{2+} when they injected cells directly with these ions and detected their diffusion with fluorescent dyes (Zhang *et al.*, 2005). This suggested that although some of the deafness-causing Cx26 mutant channels still retained their ionic coupling, they had altered permeability to larger metabolites, which may play role in the etiology of the disease.

Gap junctional communication in skin

Connexins play a key role in tissue/organ homeostasis and the etiology of several human hereditary diseases was linked to mutations in connexin genes. The epidermis is highly coupled by intercellular channels and gap junctional communication plays a crucial role in keratinocyte growth and differentiation. At least nine connexin genes, including *Cx26*, *Cx30*, *Cx30.3*, *Cx31*, and *Cx43* were shown to be expressed during the keratinocyte differentiation process (Kelsell *et al.*, 2000; Di *et al.*, 2001). These genes show distinct spatial and temporal expression patterns as well as some overlapping tissue distribution during epidermal morphogenesis. *Cx43* is expressed throughout the interfollicular epidermis, whereas *Cx26* is only present in palmoplantar epidermis. *Cx26* and *Cx43* are also expressed in the hair follicles and sweat glands (Salomon *et al.*, 1994). Additionally, *Cx30*, *Cx30.3*, and *Cx31* have been found in the upper, differentiated epidermal layers. Although the exact role of gap junctional communication during keratinocyte differentiation is not exactly known, the association of mutations in the genes for *Cx26*, *Cx30*, *Cx30.3*, *Cx31*, and *Cx43* with different skin pathologies emphasized the importance of intercellular communication in the development and differentiation of epidermis (Richard, 2005). The first hereditary skin disease associated with connexin genes was erythrokeratodermia variabilis (EKV; OMIM# 133200), which is caused by mutations in the genes of either *Cx31* or *Cx30.3* (Richard *et al.*, 1998a; Macari *et al.*, 2000) In addition, mutations in *Cx26*, which are the leading cause of nonsyndromic deafness, were also associated with a variety of skin disorders including palmoplantar keratoderma associated with sensorineural hearing loss (PPK; OMIM 148350, Richard *et al.*, 1998b), keratitis–ichthyosis–deafness syndrome (KID syndrome; OMIM 148210; Richard *et al.*, 2002; van Steensel *et al.*, 2002), Bart–Pumphrey syndrome (OMIM 149200; Richard *et al.*, 2004), and Vohwinkel syndrome (OMIM 124500; Maestrini *et al.*, 1999). Mutations in the *Cx30* gene are the basis of Clouston syndrome, which is also known as ''hidrotic ectodermal dysplasia'' (OMIM 129500; Lamartine *et al.*, 2000). Finally, palmoplantar keratoderma, focal hyperkeratosis, and kinky hair have been described in oculo–dento–digital dysplasia, which is a rare, complex developmental disorder mainly affecting the face, eyes, teeth, hair, and limbs and is caused by mutations in the *Cx43* gene (Paznekas *et al.*, 2003; Gong *et al.*, 2006; Kelly *et al.*, 2006; Table 1). In this review, we will briefly summarize the known changes in functional activity of mutant connexin proteins that produce the various skin disorders.

Functional analyses of specific pathogenic connexin mutations associated with skin anomalies have been conducted to decipher the molecular mechanisms underlying these diseases and to highlight the normal role of intercellular communication in the epidermis. In contrast to connexin mutations causing skin disorders, autosomal recessive hearing loss without skin involvement (nonsyndromic) is often because of a simple loss of channel function altering cochlear intercellular communication (White, 2000; Bruzzone *et al.*, 2003), suggesting that loss of connexin function is not detrimental for development and function of the epidermis. Thus, mutant connexin proteins resulting in skin disorders must acquire novel functions

Table 1. Epidermal connexins and associated disorders

Gene	Hereditary disease	OMIM reference	Expression pattern
GJB4(Cx30.3)	Autosomal-dominant erythrokeratodermia variabilis	133200	Skin, kidney, and placenta
GJB3(Cx31)	Autosomal-dominant and -recessive erythrokeratodermia variabilis	133200	Skin, cochlea, placenta, kidney, testes, eye, and PNS
	Autosomal-dominant and -recessive nonsyndromic sensorineural hearing loss (DFNA3)	600101	
GJB2(Cx26)	Autosomal-recessive nonsyndromic sensorineural hearing loss (DFNB1)	220290	
	Autosomal-dominant nonsyndromic sensorineural hearing loss (DFNA3)	601544	Almost ubiquitous, including cochlea, skin, liver, placenta, breast, lung, and brain
	Vohwinkel syndrome	124500	
	Keratitis–ichthyosis–deafness syndrome	148210	
	Palmoplantar keratoderma associated with sensorineural hearing loss	148350	
	Bart–Pumphrey syndrome	149200	
GJB6(Cx30)	Autosomal-recessive nonsyndromic sensorineural hearing loss (DFNB1)	220290	
	Autosomal-dominant nonsyndromic sensorineural hearing loss (DFNA3)	601544	Skin, brain, cochlea, and cornea
	Clouston syndrome (Hidrotic ectodermal dysplasia)	129500	
GJA1(Cx43)	Oculo–dento–digital dysplasia	164200	Ubiquitous, including skin, heart, eye, and brain

not present in wild-type channels to generate epidermal abnormalities.

In *Xenopus* oocyte expression studies, three Cx26 mutants causing PPK and SNHL, R75W, delE42, and D66H, were found not only to lack gap junction channel function but also inhibited coexpressed wild-type Cx26 (Richard *et al.*, 1998b) in a dominant-negative fashion, thus illustrating a unique molecular mechanism for the contribution of these mutations to skin disease (Rouan *et al.*, 2001). When these mutants were coexpressed with another epidermal connexin, Cx43, they also produced a strong inhibition of Cx43 channel activity (Rouan *et al.*, 2001). This suggested that PPK-associated Cx26 mutations might perform their action through a common mechanism where they act as *trans*-dominant inhibitors of other epidermal connexins, such as Cx43.

Analysis of the Cx26 mutations G59A and D66H in Vohwinkel syndrome provided further insight about the generation of distinct skin phenotypes (Thomas *et al.*, 2004). Lucifer yellow dye transfer experiments demonstrated that these mutants failed to form functional channels. Coexpression with Cx26, Cx43, or Cx32 showed that both G59A and D66H exerted a dominant-negative action on Cx26, whereas having selective *trans*-dominant effects on the other two connexins. G59A reduced dye transfer between cells when coexpressed with either Cx32 or Cx43. In contrast, D66H mutants had a dominant-inhibitory effect only on Cx43 (Thomas *et al.*, 2004). The differential ability of each mutation to affect distinct connexins may explain variations in epidermal phenotypes, or disease severity of specific mutations. Similar *trans*-dominant interactions were observed for other epidermal disease-causing connexin genes. The EKV-associated Cx30.3 mutation F137L caused a reduction in the number and size of gap junctions formed, as well as decrease in coupling of HeLa cells when coexpressed with wild-type Cx31, suggesting a distinct *trans*-dominant inhibitory mechanism for this mutation (Plantard *et al.*, 2003). Thus, the spectrum of *trans*-dominant interactions produced by distinct connexin mutations could uniquely alter the magnitude of intercellular communication and/or the range of permeable molecules shared between epidermal keratinocytes, leading to specific skin disorders such as PPK and EKV.

More evidence for the importance of dominant-negative action on coexpressed connexins came from the only animal model of a connexin skin disorder to date, mimicking Vohwinkel syndrome due to the expression of Cx26-D66H in transgenic mice (Bakirtzis *et al.*, 2003). Cx26-D66H transgenic mice displayed premature keratinocyte cell death with a thickening of epidermal layers (hyperkeratosis), similar clinical features to human patients with heterozygous D66H mutations in *GJB2* (Cx26). Accumulation of Cx26-D66H protein was observed in the cytoplasm of keratinocytes, im-

plicating defective trafficking of the protein to the plasma membrane. Moreover, transgenic Cx26-D66H exerted a dominant-negative effect on both Cx26 and Cx30, also inhibiting the transport of these wild-type proteins to the cell membrane. Surprisingly, Cx26-D66H did not interfere with Cx43 membrane localization (Bakirtzis *et al.*, 2003), despite dominantly inhibiting Cx43 channel formation in two separate *in vitro* assays (Rouan *et al.*, 2001). These results underscore the importance of animal models in confirming molecular mechanisms and suggest that additional research will be required to fully understand the pathogenicity of the D66H mutation.

EKV-associated Cx31 mutations were also shown to impair protein trafficking. The recessive L34P mutation caused protein accumulation in the cytoplasm and prevented the formation of functional Cx31 intercellular channels (Gottfried *et al.*, 2002). Characterization of dominant Cx31 EKV mutations such as R42P, 66delD, G12R, G12D, and C86S further provided support for the role of impaired protein trafficking in this disease (Di *et al.*, 2002). Mutant proteins showed an aberrant cytoplasmic localization and cells expressing mutant proteins had a high incidence of cell death. The defective trafficking and cell death were also detected in cells expressing Cx26 keratitis–ichthyosis–deafness-associated mutants, G12R, S17F, and D50N (Common *et al.*, 2003). The reason for cell death has not been elucidated; however, the defective proteins may influence the functionality of other connexins or other cellular components or alter the hemichannel function, thus leading to the observed skin pathologies.

In addition to having an impact on the function or assembly of gap junction channels, some connexin mutations may promote the activity of hemichannels. Support for this idea came from studies of a Cx26 KID syndrome mutation, A40V. Injection of A40V complementary RNA into *Xenopus* oocytes resulted in a disorganization of cell pigmentation followed by cell death. Moreover, induction of large membrane currents not seen in wild-type Cx26 suggested the presence of aberrant A40V hemichannel activity (Montgomery *et al.*, 2004). The authors concluded that constitutively active hemichannels could contribute to the pathophysiology of this mutation. Functional evaluation of additional KID syndrome mutations in Cx26 will be needed to determine if this is a general mechanism for this disorder, as dominant inhibition seems to be for PPK. However, further support for disregulated hemichannels playing a role in pathology has emerged from studies of other skin disorders.

Similar to the A40V Cx26 mutation, the hidrotic ectodermal dysplasia-associated Cx30 mutants G11R and A88V-induced cell death in *Xenopus* oocytes, which could have resulted from the presence of functional hemichannels, an idea supported by the detection of large voltage-activated currents in single oocytes expressing mutant proteins that were not seen in cells injected with wild-type Cx30 (Essenfelder *et al.*, 2004). These currents could be suppressed by elevation of extracellular calcium, and under these conditions, G11R and A88V formed functional intercellular channels with altered voltage gating properties. Further support for active hemichannels came from the analysis of ATP leakage in HeLa cells transfected with G11R and A88V. Cells expressing mutant channels had an ATP leakage two to threefold higher than control cells, suggesting that ATP release through hemichannels may play a role in the hidrotic ectodermal dysplasia phenotype (Essenfelder *et al.*, 2004). Aberrant channel activity was also observed for the EKV-associated Cx31 mutant G12R (Diestel *et al.*, 2002). Cells expressing G12R started to die $\sim$48 hours after initiation of protein synthesis. The mutant channels displayed increased neurobiotin permeability 24 hours after protein expression relative to wild-type Cx31. This elevated junctional communication was proposed to be because of reduction or disruption of channel gating. However, these cells were not examined for functional hemichannels, which might have contributed to cell death. In addition to causing cell depolarization and death, hemichannels could induce the release of metabolites into the extracellular space in the epidermis and influence the regulation of keratinocyte growth and differentiation.

The etiology of several human hereditary skin diseases has been linked to mutations in a variety of connexin genes and different mutations within a single connexin gene can generate a range of functional defects, implicating that diverse channel activities contribute to the different disorders. Accumulating evidence shows that the mutations associated with distinct skin phenotypes give rise to novel functional activities. Three mechanisms could be proposed for the action of skin disease-associated mutations: (i) impaired protein trafficking and connexon assembly at the plasma membrane, (ii) *trans*-dominant inhibition of coexpressed connexins, and (iii) the production of constitutively active hemichannels, any or all of which could potentially alter cell survival (Figure 3). The *trans*-dominant inhibitory actions of mutant proteins change the range of channel types available in the epidermis, and therefore may alter the type of molecules exchanged and also the extent of intercellular communication. Leaky hemichannels might cause uncontrolled release of cell contents including metabolites and signaling molecules into the extracellular space, which could affect the behavior of surrounding cells in addition to initiating cell death.

In the adult human epidermis, connexins have distinct as well as overlapping expression patterns (Figure 4). Under pathological conditions, the normal distribution of connexins may be altered. For example, a PPK patient with a Cx26-E42del mutation showed ectopic expression of Cx26 in the spinous and granular layers, where Cx26 was found to colocalize with Cx43 (Rouan *et al.*, 2001). Colocalization of Cx26 with Cx43 in PPK lesions owing to upregulation of Cx26 could promote the interaction of Cx26 mutant protein with Cx43, altering its assembly or channel activity, consistent with *in vitro* studies showing a *trans*-dominant inhibition of Cx43 by Cx26 mutants (Rouan *et al.*, 2001). The resulting

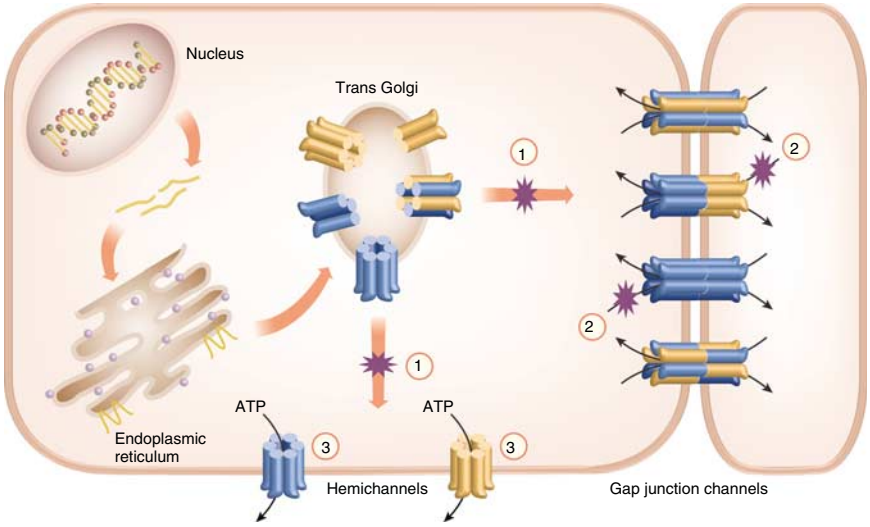

Figure 3. Consequences of pathogenic connexin mutations on gap junction biogenesis and function. Pathogenic mutations not only affect gap junction channel formation at different levels from protein translation to assembly of gap junctions but can also alter the channel function. Skin disease-associated connexin mutations have been shown to execute their action through three main mechanisms. They may inhibit protein trafficking to the plasma membrane, thereby impairing connexon/gap junction channel assembly (1). They may exert a dominant-negative action on coexpressed connexins, changing the types of molecules exchanged between the cells (2). Finally, they may result in the production of constitutively active hemichannels (3), leading to the unregulated release of cellular contents into the extracellular space.

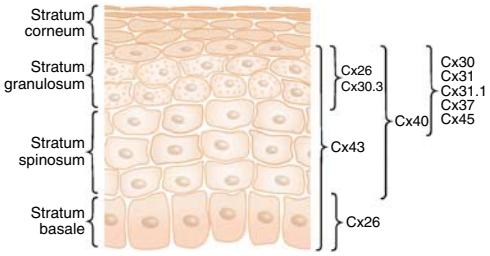

Figure 4. Expression pattern of connexins in the epidermis. At least nine connexin isoforms have been shown to be expressed during epidermal morphogenesis with distinct spatial and temporal expression pattern as well as some overlapping distribution. Cx43 is the most broadly expressed, whereas Cx26 is limited to basal keratinocytes in palms and soles, or occasionally cells in the granular layer. Cx40 is present throughout the spinous and granular layers, whereas the remaining six connexins are restricted to the upper spinous and granular layers.

alteration of gap junctional communication could modify the natural response pattern of keratinocytes during terminal differentiation and in response to stress or injury. It still remains unclear, however, how subtle changes in intercellular signal transmission within the upper layers of the epidermis result in epidermal thickening (acanthosis). Upregulation of Cx26 in keratinocytes preceding hyperproliferation has been observed during wound healing and inflammatory skin disorders (Labarthe *et al.*, 1998; Djalilian *et al.*, 2006). However, the relationship between the Cx26 upregulation and keratinocyte proliferation is not fully known, and the signaling cascades and/or molecules that could play role in this process remain to be identified. Examination of skin samples from patients with other connexin-based disorders, such as EKV or hypohidrotic ectodermal dysplasia, may further demonstrate if alterations in the connexin expression and distribution are a common feature of skin diseases. Additional characterization of disease-causing mutations and the generation and examination of transgenic animals will further improve our understanding of the function of gap junctional communication in keratinocyte growth and differentiation as well as the pathophysiology of diseases, where control of these processes has been corrupted.

CONFLICT OF INTEREST

G. Richard works for a private company involved in molecular diagnostic testing for rare genetic disorders. G. Meşe and T.W. White declare no conflict of interest.

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