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Review

Connexin disorders of the ear, skin, and lens

Dwan A. Gerido, Thomas W. White*

Department of Physiology and Biophysics, State University of New York, T5-147, Basic Science Tower, Stony Brook, NY 11794-8661, USA

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Abstract

Gap junctions provide coupled cells with a direct pathway for sharing ions, nutrients, and small metabolites, thus helping to maintain homeostasis in various tissues. Abnormal function and/or expression of specific connexin genes has been linked to several diseases, including genetic deafness, skin disease, peripheral neuropathies, and cataracts. Research has provided significant insight into the function of gap junction proteins in both in vitro and in vivo models; however, questions regarding the exact mechanisms by which connexin related diseases occur in mammalian systems remain. Here, we discuss the disease states that are related to three human connexin genes, Cx26 (GJB2), Cx46 (GJA3) and Cx50 (GJA8), and recent scientific evidence characterizing those diseases in various experimental models.

Keywords: Connexin; Ear; Skin; Lens

1. Introduction

Multicellular organisms require various means of cellular communication to respond to internal and environmental stimuli. One method of communication is through the use of gap junctions, which allow the exchange of ions, nutrients and small metabolites from one cell to neighboring cells. Gap junctions are composed of oligomeric structures known as connexons, which are in turn formed from a large family of protein subunits called connexins. A connexon forms when six connexins oligomerize within the plasma membrane to complete one half of a gap junction channel. A fully functional gap junction channel is produced when two connexons from adjacent cells align in the extracellular space to allow direct communication between the two cells. Gap junction channels vary in complexity depending on the composition of connexin subunits within the channel. Homomeric connexons are formed when the hemichannel is composed of a single type of connexin, whereas heteromeric connexons contain different connexins. The arrangement of the complete gap junction channel amplifies this structural diversity, as it may be composed of connexons made from different connexins. Homotypic channels are composed of identical connexons, whereas heterotypic channels are composed of different connexons. The variations in the structural alignment of gap junction proteins allow for a vast array of possibilities for sharing metabolites and ions between coupled cells. Connexins are found throughout the vertebrate phyla, but nematodes and arthropods lack connexins and use an unrelated family of gap junction proteins known as innexins [66]. Although these gene families are different, and innexins are beyond the scope of this review, the invertebrate channels show obvious similarities in molecular and functional diversity as the vertebrate connexins [67,93].

2. Human connexin genes

In humans there are at least 20 connexin genes that exhibit complex and overlapping patterns of expression [102]. Comparison of connexin sequences has provided some insights into the evolution of connexin genes and suggests that they may have originated from a common ancestor and subsequently divided into several subgroups (Fig. 1). To root our phylogenetic tree, we have included a recently identified invertebrate chordate connexin, *Ciona intestinalis* Cx36.9 (T.W. White and P.R. Brink unpublished data, GenBank accession number BK001247; see also Ref. [82]). This tunicate connexin does not cluster

* Corresponding author. Tel.: +1-631-444-9683; fax: +1-631-444-3432.
E-mail address: thomas.white@sunysb.edu (T.W. White).

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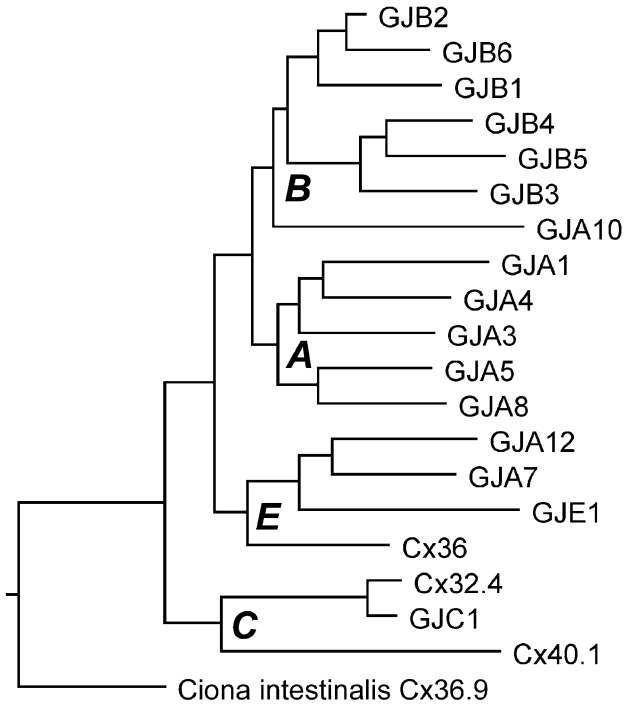

Fig. 1. Phylogenetic tree of chordate connexin genes. Human connexin genes were aligned according to their nucleotide sequence and compared to a more primitive tunicate gene that encodes for a gap junction protein. The human genes were segregated into three groups,β, α, and a third unnamed group. Connexin genes in the β group encoded gap junctions no more than 32 kDa in molecular weight, whereas the α group encoded connexins greater than or equal to 37 kDa. Older human connexin genes were aligned with the more primitive connexin from *C. intestinalis* (Cx36.9), suggesting a later evolutionary split between the α and β groups.

with the human genes and provides a unique opportunity to reexamine the sequence relationships between vertebrate connexins. The human connexin genes are commonly divided into α, β, and an unnamed third group, all of which encode for gap junction channels of different molecular weights [101]. Examination of these groups shows an apparent separation of channels that is partially dependant on the molecular weight of each individual gap junction protein. In general, connexin genes within the β group encode proteins smaller than 32 kDa, whereas the α genes encode connexins larger than 37 kDa. While these divisions are true for many of the connexin genes, there are at least seven connexins of intermediate molecular weight (31–47 kDa) that violate this dichotomy. Interestingly, many of these outliers show a closer sequence relationship with Ciona Cx36.9 than the α or β branches. Consistent with this view, two connexins from this third group also have introns within their coding regions (Cx36 and Cx40.1), a feature shared by the more ancient tunicate connexin. Taken together, these sequence relationships suggest that the α–β split was a more recent evolutionary event within the vertebrate connexin family and that the third outlying group may contain the more primitive genes.

3. Connexin mutations in human disease

Cellular communication is an important aspect in maintaining homeostasis within multicellular organisms. Processes such as hormone signaling, neural transmission, and cell-to-cell adhesion have provided animals with a wide variety of communication mechanisms that operate with very high specificity. In addition to these mechanisms of cellular communication, individual cells also contain numerous protein channels that allow communication with the extracellular environment. In fact, membrane proteins have the ability to pass a large number of ions and metabolites across the cell membrane in very short time periods. With such numerous possibilities of cellular communication, it may appear that the direct connection between the intracellular environments of two adjacent cells provided by gap junctions would be redundant. In contrast to this view, recent evidence has shown that mutations within several connexins give rise to various hereditary diseases in humans [22,99,102]. Currently, at least eight connexin genes have been implicated in hereditary human diseases (Table 1). For example, Charcot–Marie–Tooth disease is caused by mutations in the Cx32 gene [4], whereas erythrokeratodermia variabilis can result from mutations in either Cx31 or Cx30.3 [51,74]. In addition to these disorders, connexin mutations are also involved in deafness, cataracts, and numerous skin diseases. While research has provided some insight as to how mutations within these genes affect humans, the generation of suitable animal models for studying connexin related diseases has proven to be challenging in some cases. Here we discuss several diseases that are known to arise from mutations in connexins 26, 46, and 50, and research that further characterizes these mutations/deletions and their relation to connexin function. Although the focus of this review is limited to these three genes, the general principles discussed apply to other connexin disorders. One such principle is the apparent lack of redundancy that is evident in connexin disorders where more than one connexin gene is expressed in a particular tissue. The inability of other connexins to compensate for loss of function in a mutant connexin is a prevalent feature in connexin-related disorders, and may represent strict requirements between tissue homeostasis and gap junction-mediated cellular communication.

4. Mutations in Cx26 cause deafness

Genetic deafness has been extensively studied by a number of laboratories, and the data concerning both syndromic and nonsyndromic forms of genetic deafness clearly point to mutations within the Cx26 gene as a causative factor in the onset of hearing loss. Initially, the Cx26 gene was implicated in autosomal recessive forms of deafness, but was later found to be involved in both

Table 1
Summary of Cx genes, human diseases, and knockout mouse phenotypes

Gene	Expression patterns	Human hereditary diseases	KO mouse phenotypes	OMIM reference
GJB7 (Cx25)	unknown	unknown	unknown	no reference
GJB2 (Cx26)	cochlea, skin, liver, placenta, breast	Deafness, DFNB1	Embryonic lethal	220290
		Deafness, DFNA3		601544
		Deafness with keratopachydermia	Conditional KO	124500
		Keratitis-ichthyosis-deafness syndrome	hearing impairment	148210
		Ichthyosis, with deafness		602540
GJB6 (Cx30)	cochlea, skin, brain	Deafness, DFNB1	hearing impairment	220290
		Deafness, DFNA3		601544
GJB4 (Cx30.3)	skin	erythrokeratodermia variabilis	unknown	133200
GJB3 (Cx31)	skin, placenta	Deafness, DFNA2	placental	600101
		erythrokeratodermia variabilis	dysmorphogenesis	133200
GJB5 (Cx31.1)	skin	unknown	unknown	no reference
GJE1 (Cx31.3)	unknown	unknown	unknown	no reference
GJC1 (Cx31.9)	unknown	unknown	unknown	no reference
GJB1 (Cx32)	liver, oligodendrocytes, chwann cells	Charot–Marie–Tooth disease	liver carcinogenesis	302800
Cx32.4	unknown	unknown	unknown	no reference
Cx36	neurons	unknown	visual defects	no reference
GJA4 (Cx37)	endothelium	unknown	female sterility	no reference
GJA5 (Cx40)	heart, endothelium	unknown	atrial arrhythmias	no reference
Cx40.1	unknown	unknown	unknown	
GJA1 (Cx43)	heart, lens, brain, adrenal gland, etc	oculodentodigital dysplasia, ODDD	heart malformation	164200
GJA7 (Cx45)	heart, smooth muscle, neurons	unknown	lethal	no reference
GJA3 (Cx46)	lens	congenital cataracts, CZP3	nuclear-cataract	601885
GJA12 (Cx47)	Spinal cord, brain	unknown	unknown	no reference
GJA8 (Cx50)	lens	congenital cataracts, CZP1	microphthalmia, pulverulent cataract	116200
GJA10 (Cx59)	unknown	unknown	unknown	no reference
Cx62	ovaries	unknown	unknown	no reference

recessive and dominant forms of genetic deafness [11,60,65] (OMIM 220290, 601544). It was also found that pathological changes in the Cx26 sequence were polymorphic and those patients with both syndromic and nonsyndromic forms of deafness presented with a broad spectrum of mutations within Cx26. In fact, several nonsense and missense mutations within Cx26 have been found in families with genetic deafness. Although there is indisputable evidence linking Cx26 to genetic deafness, there remain questions as to how mutations in the Cx26 gene generate the wide variety of pathological phenotypes observed in patients with syndromic or nonsyndromic forms of this disease.

5. Cx26 function in the inner ear

Currently, the exact role of Cx26 in the onset of nonsyndromic deafness remains unclear; however, it appears that gap junctional communication influences the ionic environment of the inner ear sensory epithelia. Of particular interest is the fact that Cx26 is not expressed in vestibular hair cells, therefore mutant forms of Cx26 exert their effects independent of the mechanical processes of sound transduction. This observation is unique compared to other forms of deafness that are brought on by abnormalities in genes that directly participate in the mechanics of hearing, such as α-tectorin or myosin VIIA. [43,48,91] (OMIM602574). Immunohistological studies involving cochlear-supporting cells that are coupled by gap junctions have revealed a ubiquitous expression pattern for Cx26 [39,40]. Included within these cell populations are the nonsensory epithelial cells that anchor the vestibular hair cells, and the type II fibrocytes that form a layer of connective tissue that lies distally to the epithelial cells [41]. While the exact function of Cx26 within the cells of the inner ear is unknown, it has been proposed that Cx26 plays a major role in the cycling of K^+ ions during auditory transduction. The mammalian cochlea is organized in a manner that segregates the extracellular environment presented to the sensory fibers within this organ. The basolateral surfaces of hair cells are surrounded by perilymph, which has an ionic composition similar to that of other extracellular fluid compartments. The apices of

hair cells, however, are bathed in endolymph, which resembles intracellular fluids due to the high K^+ and low Na^+ concentrations present in both environments. Auditory signals (sound) stimulate the uptake of K^+ ions from the endolymph by hair cells, which in turn expel the K^+ ions into the interstitial space of the organ of Corti [34]. The expelled K^+ ions then pass into cochlear supporting cells through channels specific for K^+ ions [64]. It has been hypothesized that gap junctional communication is responsible for the recirculation of K^+ ions from the cochlear supporting cells back into the endolymph with gap junctions providing an intercellular pathway for this cycling mechanism.

Data supporting this hypothesis have emerged through the generation of mice lacking cochlear connexins 26 and 30. Complete removal of the Cx26 gene results in neonatal lethality, thereby preventing analysis of its function in hearing [23]. This limitation was overcome through the generation of a cochlear specific knockout of Cx26 using the Cre–LoxP recombination system [14] successfully deleting Cx26 in a subset of cochlear cells without effecting Cx26 expression in other organs. Animals with this deletion are a model of recessive deafness and displayed normal patterns of cochlear development, but showed an increase in postnatal cell death within the cochlea along with significant hearing loss. The initiation of cell death was found to occur near the inner hair cells, and coincided with the onset of audition. It was hypothesized that loss of Cx26 prevented recycling of K^+ ions after sound stimulation, and that elevated K^+ in the extracellular perilymph inhibited uptake of the neurotransmitter glutamate, which ultimately resulted in cell death within the hair cell population. While the evidence concerning Cx26 is compelling, this hypothesis becomes more complex when considering the activity of Cx30 in the inner ear. Lautermann et al. [46] have shown that Cx26 and Cx30 normally co-localize within the cochlea, and tissue-specific deletion of the Cx26 gene did not alter expression patterns of Cx30 [14]. This observation complicates the role of gap junctional communication in the recycling of K^+ ions in the onset of deafness due to the fact that Cx30 passes K^+ ions in an efficient manner [90] but could not prevent hearing loss in the absence of Cx26; thus, the presence of a single type of connexin with similar ionic selectivity was unable to rescue the phenotype observed in these mice.

A second mouse model has examined the pathology of dominant deafness mutations by transgenically expressing a dominant negative Cx26 mutant, R75W [44]. This missense mutation was originally characterized as causing syndromic deafness associated with skin disease and its dominant-negative effects were ascertained by using the paired oocyte expression system, in which channel activity was inhibited when expressed alone or when co-expressed with wild-type Cx26 [75]. Expression of the R75W mutation in mice resulted in profound deafness that was evident as early as 2 weeks of age. Furthermore, mice carrying the R75W mutation presented with significant histological abnormalities within the inner ear, which became more severe over time. At 2 weeks of age, R75W mutant animals displayed deformities of both the tunnel of Corti and the supporting hair cells, whereas by week 7, both structures had completely degenerated [44]. These findings suggest that the R75W mutation in Cx26 exerts its effects within the epithelial transport system of the inner ear by altering the circulation of K^+ ions into the cortilymph, instead of the extracellular perilymph, as was reported in the recessive Cx26 model [14]. This animal model also suggests that diverse cochlear pathologies may arise depending on the pattern of inheritance and/or the nature of the Cx26 mutation.

Similar to the tissue-specific loss of Cx26, deletion of Cx30 in mice resulted in hearing loss but did not alter development of the inner ear [88]. Although development was normal, Cx30 knockouts lacked the endocochlear potential that normally results from the asymmetric Na^+ and K^+ concentrations in the endolymph and perilymph. In addition, Cx30 knockouts also presented with increased apoptosis within the cochlear sensory epithelium. Deletion of Cx30 did not alter the cochlear expression of Cx26, again raising the question of why the continued expression of Cx26 was not able to compensate for the loss of Cx30 [88]. One possible explanation for these phenotypes is that gap junctions may have other roles in addition to recycling K^+ ions. This idea is supported by data from functional studies showing that while the K^+ conductances of Cx26 and Cx30 are similar, the channels display significant differences regarding the permeability of fluorescent dyes that are similar to cyclic nucleotides and second messengers in both size and charge [7,54,90]. These findings indicate that specific loss of either Cx26 or Cx30 within cochlear epithelial cells would not simply reduce the intercellular passage of K^+ ions, but would also significantly alter the availability of larger solutes that could be exchanged between the coupled cells.

6. Cx26 and autosomal recessive deafness

Many studies have confirmed that mutations in Cx26 result in recessive deafness [10,19,21,36,47,103]. The most common mutation associated with recessive deafness is a single-base deletion (35delG) within the Cx26 gene. The presence of the 35delG mutation in Cx26 raises several interesting questions regarding its function in hearing loss. First, it should be noted that the precise guanine residue deleted cannot be determined as there are normally six consecutive guanine residues from positions 30 to 35, and an equivalent mutation has also been described as 30delG in some studies [19,61]. Because this mutation appears to be common in several ethnic backgrounds, researchers have hypothesized that the six repeating guanine residues may represent a ‘‘mutational hotspot’’ within the Cx26

locus, which may explain the prevalence of this mutation in persons with recessive deafness. Second, there appears to be some diversity in the clinical phenotype of patients that were homozygous for the 35delG mutation. In one study, patients homozygous for the 35delG mutation were described as having moderate hearing loss, whereas a separate study involving compound heterozygotes and patients homozygous for the 35delG presented with complete deafness [19,30]. This variable phenotype may result from genetic modifiers that act in concert with the Cx26 mutation to affect the deafness phenotype.

In addition to the 35delG mutation, there are other mutations such as nonsense mutations, deletions and insertions within the Cx26 gene that result in hearing loss. One such mutation, M34T, has remained enigmatic in spite of numerous studies of this allele. Initially, the M34T mutant was characterized as an autosomal dominant mutation that resulted in deafness [38]. This finding was supported by functional data in which the M34T mutant exerted a dominant-negative effect on wild-type Cx26 when co-expressed [17,97]. However, these results were called into question when it was found that many individuals heterozygous for the M34T mutation retained normal hearing, challenging the notion that M34T was a dominant mutation [31,84]. Other in vitro studies detected defects in the assembly and trafficking of the M34T mutant, as well as reduced channel activity [55,89], which were incompatible with the assertion that M34T may represent a mere polymorphism [84]. Further analysis of deaf patients revealed that individuals homozygous for the M34T mutation presented with mild to high frequency hearing loss, and compound heterozygotes of M34T with other mutant Cx26 alleles such as 35delG were also deaf, suggesting that M34T may be a recessive allele [33,36,104], although some studies have hypothesized that the pathological effect of the M34T allele was highly dependant on the nature of the other Cx26 allele with which it was paired [16]. Early attempts to explain these discrepancies between dominant and recessive modes of action hypothesized that a second site mutation may silence some of the M34T alleles [97,99]. Subsequently, it was found that majority of the M34T alleles coincided with a 10-bp deletion in the 5′ UTR of Cx26 [33,104]. Whether this deletion is of any significance remains unclear, but its association with the M34T mutation may help to explain variability in the phenotypes of humans carrying the M34T allele. Currently, the only point on which there is apparent agreement is that the M34T mutation is present at a high frequency [30,104], and further research is needed to clarify its role in nonsyndromic deafness.

7. Cx26 and autosomal dominant deafness

While the importance of mutations in Cx26 is evident in autosomal recessive nonsyndromic deafness, dysfunction in the Cx26 gene also plays a role in the onset of dominant forms of deafness (DFNA3). In one well-characterized example, dominant nonsyndromic deafness was attributed to a G-to-C transition that resulted in a tryptophan-to-cysteine substitution at position 44 (W44C) in the Cx26 gene [18]. This mutation was detected in the 20 individuals that exhibited hearing loss, but none of the subjects with normal hearing. It was hypothesized that the additional cysteine residue interfered with disulfide bond formation, which in turn generated a dominant-negative effect on neighboring connexins [18]. Functional analysis of this mutant revealed the inability of W44C channels to allow passage of the gap junction permeable dye Lucifer yellow when expressed in HeLa cells [6]. When co-expressed with wild-type Cx26, W44C mutants further altered channel activity by reducing both the magnitude of the junctional current, and the voltage dependence of wild-type Cx26 channels [6]. In contrast to the W44C data, a separate recessive mutation, W77R, did not alter the activity or the voltage dependence of wild-type Cx26 channels in this study. Additional mutations within the Cx26 gene are also responsible for the onset of dominant nonsyndromic deafness. One such mutation is a G-to-A transition, which resulted in an arginine-to-glutamine substitution (R143Q). This mutation was found to affect a highly conserved residue within the third transmembrane domain of Cx26 [49]. Another Cx26 mutation involved in dominant forms of deafness was found in the fourth transmembrane domain of the Cx26 protein. This mutation resulted from a G-to-T transition at nucleotide 605, which in turn generated a cysteine-to-phenylalanine substitution (C202F). Subjects with this mutation exhibited a later onset of hearing loss, with effects becoming evident between 10 and 20 years of age [62]. Taken together, these studies have established a clear role for Cx26 mutations in dominant forms of nonsyndromic deafness.

8. Connexin 26 and skin disease

Dominant mutations in Cx26 have also been described in syndromic deafness associated with skin disease. These hereditary skin diseases can be described as a number of disorders with varying phenotypes, but similar genetic abnormalities. These disorders include keratitis-ichthyosis-deafness syndrome (KID), palmoplantar keratoderma with deafness (PPK), Vohwinkel syndrome (VS), and hystrix-like ichthyosis-deafness syndrome (HID) [37,73]. In spite of this range of hereditary skin disorders, there are important genetic and phenotypic characteristics that are common to each disease. Genetically, all of the skin disorders mentioned can result from mutations in the Cx26 gene, with the nature of each disease depending on the particular mutation. With regards to phenotype, skin disorders related to mutant forms of Cx26 show great variability, but generally involve an increased thick-

ness of the outer layers of the skin. This would indicate a critical role for connexins in maintaining the balance between proliferation and differentiation of the epidermis, with severe consequences when this balance is offset by mutant alleles of the Cx26 gene.

Although several different mutations have been identified, the exact role of Cx26 in skin disease cannot be attributed to loss of Cx26 channel activity alone due to the fact that many Cx26 mutations result in nonsyndromic deafness. This would indicate that mutant forms of Cx26 associated with skin disorders must impact additional genes in a manner that upsets tissue homeostasis and results in disease. Since the skin expresses several other connexins besides Cx26, these genes are good candidates for the trans-pathological actions of mutant forms of Cx26. Although this view has yet to be conclusively proven, it is clear that that connexins can form heteromeric structures [94]; therefore, mixing of normal connexin proteins with mutant forms of Cx26 may be the underlying cause of skin disease associated with mutations in the Cx26 gene. Consistent with this view, it was shown that mutant forms of Cx26 acted as a trans-dominant inhibitor of other connexin genes expressed in the skin [79]. In this study, it was found that wild-type Cx26 was able to form functional channels when co-expressed with wild-type Cx43, but that mutant forms of Cx26 that were associated with palmoplantar keratoderma significantly reduced channel activity of wild-type Cx43, thereby suggesting a trans-dominant effect of mutant Cx26. As an elegant control, expression of a Cx26 allele associated with nonsyndromic dominant deafness (W44C) did not inhibit the activity of Cx43, showing that trans-dominant inhibition of Cx43 was restricted to the syndromic deafness mutants. Most importantly, it was shown that one syndromic mutant allele of Cx26 (delE42) was co-localized with Cx43 in suprabasal keratinocytes in lesional skin from an affected patient, supporting the hypothesis that these proteins interact in vivo at the site of skin pathology [79]. Presumably, loss of function in these two gap junction proteins impacts homeostasis by preventing sharing of important nutrients and signaling molecules that help maintain the proper proliferative balance in the epidermis. These data support the hypothesis that skin disease-associated Cx26 mutants alter the function of other genes in addition to Cx26.

9. Connexin function in the skin

Adult skin consists of two distinct tissues, the mesodermally derived dermis and the ectodermally derived epidermis. The epidermis is primarily composed of keratinocytes that are subdivided into four layers: the inner most basal layer (stratum basale), the spinous layer (stratum spinosum), the granular layer (stratum granulosum), and the outermost cornified layer (stratum corneum). Cells in the basal layer continuously proliferate and terminally differentiate as they push upward into the spinous, granular and cornified layers, where they are eventually shed. Maintaining a uniform epidermal thickness requires tight regulation of keratinocyte growth and differentiation, and it has been suggested that gap junction-mediated cell communication may play a role in this regulation [8]. Ultrastructural analyses of human [9] and rodent [76] epidermis have detected gap junctions between keratinocytes residing in the basal, spinous, and granular layers, but not between cells in the cornified layers. These results are consistent with Lucifer yellow dye-transfer experiments that showed junctional communication between keratinocytes populating the three inner epidermal layers of newborn mouse [35], adult mouse [1] and adult human [81] skin. However, dye transfer experiments suggested that the epidermal keratinocytes were organized into small ‘‘communication compartments’’ consisting of 20–25 cells; dye transfer occurred between basal and suprabasal keratinocytes within a compartment, but was reduced between keratinocytes in neighboring compartments. The epidermal communication compartment was postulated to correlate with an epidermal proliferative unit [70], consistent with a role for gap junction-mediated cell communication in controlling keratinocyte growth [35,68].

The molecular basis underlying the development and maintenance of communication compartments in the epidermis is not known, but marked changes in connexin expression have been observed in human epidermis following pathological or experimentally induced changes in keratinocyte proliferation. In normal human skin, retinoic acid treatment resulted in a thickening of the epidermis and an increase in the number of keratinocytes, which were both correlated with a massive induction of Cx26 expression in all epidermal layers, and a somewhat smaller increase in Cx43 levels. [57]. In human psoriatic lesions, a similar large induction of Cx26 has been reported in the hyperproliferative psoriatic areas but not in the surrounding control epidermis [45,77]. In experimentally wounded human skin, up-regulation of Cx26 expression has also been observed, and it temporally preceded increases in cell proliferation [50]. In normal human interfollicular epidermis, Cx43 is broadly expressed while Cx26 is not detected, except at low levels in plantar epidermis [50,80]. Taken together, these studies establish an intriguing correlation between increased Cx26 expression and human keratinocyte proliferation and differentiation.

Similar changes in connexin expression have been reported in rodent epidermis undergoing changes in proliferation. In one study examining connexin changes after wounding, Cx26 expression rapidly increased in keratinocytes proximal to the wound, but was decreased in cells at the wound edge proper. The increased expression of Cx26 persisted in the hyperproliferative epidermis up to 6 days after wounding [25]. In another study of mouse skin

papillomas, expression of both Cx26 and Cx43 was elevated in the proliferating neoplasms, but not in surrounding normal skin [83]. Thus, mouse epidermis behaves very much like human epidermis with regard to changes in connexin expression associated with hyperproliferation; namely, a large induction in Cx26 expression is consistently correlated with an increase in keratinocyte numbers. This congruence between changes in epidermal connexin expression and keratinocyte proliferation in mice and humans supports the use of mice as experimental models to study human skin pathologies that arise from mutations in the Cx26 gene.

To date, only one mouse model of a human connexin skin disorder has been described. Vohwinkel syndrome was the first known skin disease to be attributed to mutations in the Cx26 gene [53,75]. In one large pedigree with classic Vohwinkel syndrome, affected patients were heterozygous for the D66H mutation in the Cx26 gene [53]. Based on the strong genetic evidence linking it to Vohwinkel syndrome, D66H was subsequently used to generate transgenic mice expressing the mutant under the control of a keratin promoter in suprabasal epidermal keratinocytes [2]. Instead of localizing at intercellular gap junctions, the transgenic D66H accumulated in the keratinocyte cytoplasm. Consistent with the model of trans-dominant effects of Cx26 skin disease mutants described above, Cx30 was also redistributed into the cytoplasm from intercellular junctions in the D66H transgenic animals. Early postnatal skin in the transgenic mice showed epidermal scaling with zonal hyperkeratosis of the tail and digits producing constriction bands, a hallmark feature of Vohwinkel syndrome. Microscopic analysis revealed areas of hyperkeratosis and a thickened stratum corneum associated with an increase in keratinocyte apoptosis as well as increased proliferation in the lesional areas. These results were consistent with the skin pathology seen in human patients and suggested that the D66H mutation caused premature keratinocyte death in the upper epidermis, with compensatory hyperproliferation in the basal layer, leading to a thickened stratum corneum.

10. Connexins in the mammalian lens

Mutations within human connexin genes have also been shown to produce cataracts [22,95], and research in this genetic disorder has been greatly facilitated by the development of numerous animal models where gap junction proteins expressed in the mammalian lens have been genetically manipulated [27,56,78,92,98,100]. Because of its unique function and anatomy, the mammalian lens is critically dependant on the proper functioning of gap junction proteins. The anterior surface of the lens consists of a simple cuboidal epithelium, while the rest of the lenticular mass is composed of highly differentiated and elongated fiber cells. The fiber cells are unusual in that they lose their intracellular organelles while accumulating high concentrations of soluble crystallin proteins that increase their refractive index. The loss of intracellular organelles presents the differentiated lens fiber cells with a unique homeostatic challenge, as these cells cannot independently support their own metabolism. Gap junctions contribute to lens function by helping maintain an environment that favors crystallin solubility and fiber transparency by coupling the metabolically active epithelium and the organelle lacking lens fibers into a syncytium [20,28,58,59]. Thus, connexin-mediated communication allows the fibers to maintain transparency and prevent light scattering so that images are properly focused onto the retina. In support of this hypothesis, both Cx46 and Cx50 knockout mice develop nuclear cataracts [27,98], but the nature of the defects is different depending on which connexin protein is deleted.

11. Genetic knockout of lens connexins

The lens expresses three different connexins, Cx43, Cx46, and Cx50, all of which appear to provide various functions that contribute to maintaining lens homeostasis [42,63,96]. Deletion of Cx43 results in neonatal lethality, which prevents investigators from determining the long-term effects of the loss of this protein in the lens; however, prenatal ocular development was found to proceed normally in the Cx43 knockout mice [100]. In contrast, deletion of either Cx46 or Cx50 does not compromise viability and the ocular phenotypes of these two animal models have been well documented [27,78,98].

Deletion of Cx50 in mice results in two distinct lens phenotypes, a mild nuclear cataract coupled with a significant ocular growth defect that includes both the eye and the lens [78,98]. Although both phenotypes are present in Cx50 knockouts, it appears that Cx50 plays a major role in the normal growth of the lens, with a relatively small role in maintaining lens clarity. It is believed that the ocular growth defect observed in Cx50 knock out mice results from a reduction of lens growth, an idea that is supported by previous studies showing a direct link between eye and lens growth [15]. Recent data concerning the growth defect in Cx50 knockouts show a significant retardation in lens cell division during the first postnatal week [85]. While the underlying factors governing the reduction in cell division remain unclear, it is obvious that the reduced rate of cell division contributes to the growth defect observed in these mice. The onset of the cataract phenotype in Cx50 knock out mice also occurs within the first postnatal week, and results in a mild nuclear cataract that is characterized by the precipitation of crystallin proteins [98] [78]. While the precise mechanisms by which loss of Cx50 generates crystallin precipitation are not known, it has recently been reported that the severity of crystallin precipitation, but not the growth defect, in Cx50 knockouts is under the influence of an unidentified genetic modifier(s) [24]. Thus, the two

phenotypes are independent of each other, and the growth defect is prominent in all mice with the Cx50 deletion, whereas the cataract phenotype can be influenced by secondary mechanisms which, to date, have not been identified. Additional data also revealed that the abnormalities observed in these mice did not simply result from the loss of intercellular communication. Data showing that expression patterns of Cx46 were normal in Cx50 knockouts, and that lenses from Cx50 knockouts were able pass gap junction permeable ions and dyes, supported this finding [3,98].

Disruption of the Cx46 gene also produced cataracts; however, the cataract phenotype was very different compared to the Cx50 knockout animals. Deletion of Cx46 resulted in severe cataracts associated with precipitation of several crystallin proteins and a specific proteolysis of γ-crystallins [27]. The severity of cataracts in these animals was also found to be influenced by genetic modifiers and cataract severity correlated well with the amount of cleaved γ-crystallin [26]. While both the Cx50 and the Cx46 knockouts display cataracts that are caused by crystallin precipitation, specific cleavage of γ-crystallins was unique to Cx46 knockout animals. Interestingly, Cx46 knockouts did not display the growth defects observed in the Cx50 knockouts. These data would argue that Cx46 has a central role in maintaining lens clarity, but has no effect on postnatal ocular growth. This hypothesis was supported by data showing that targeted replacement of the Cx50-coding region with the Cx46-coding region generated clear lenses with a growth defect similar to that observed in Cx50 knockout animals [92]. Thus, diversity of connexin channels was required for normal lens functioning, whereas the total channel number was much less important.

Recently, double knockout mice lacking both the Cx46 and Cx50 genes have been generated (Xiahuoa Gong, personal communication, Fig. 2). These animals presented with a dense opacity that was far more extensive than that observed in either the Cx46 or Cx50 single knockout mice. Presumably, the increased density of the opacities in these mice resulted from the total absence of gap junction proteins in the lens fiber cells. Double knockout mice also displayed a significant reduction in lens growth that was comparable to that observed in single Cx50 knockout animals. The presence of both the cataract and growth defect phenotypes in the double knockout mice further confirms the impor-

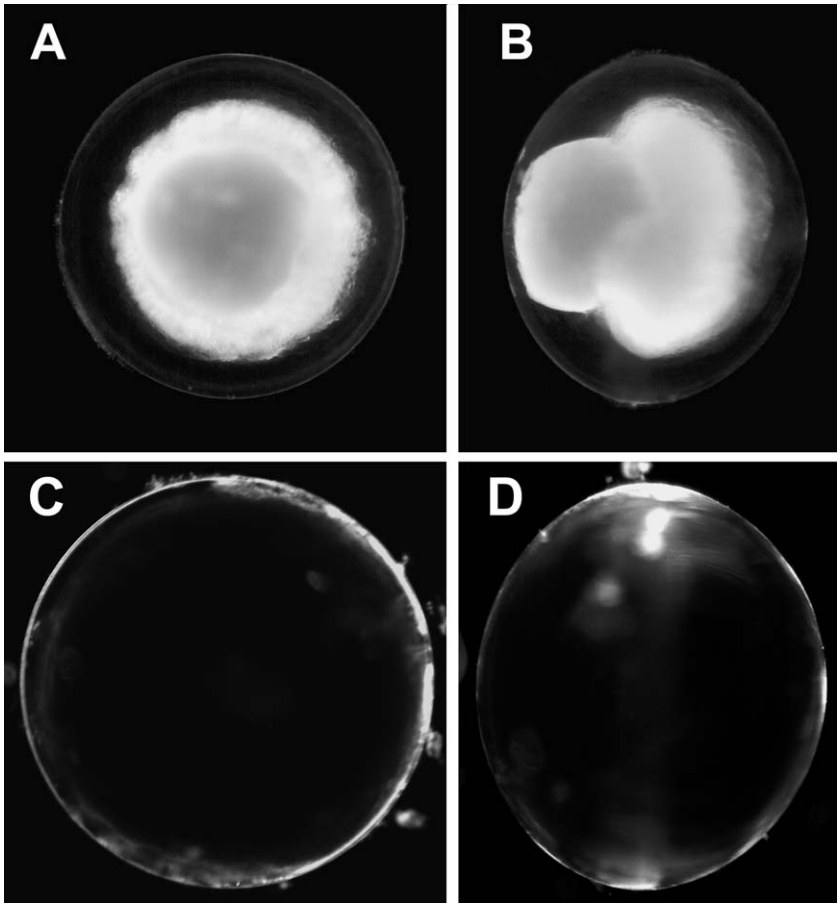

Fig. 2. Comparison of wild-type and double knockout mice. Lenses from mice lacking both Cx50 and Cx46 were photographed looking down on the anterior surface (A), or on the equatorial edge (B). Disruption of both connexin genes resulted in severe cataracts. Wild-type mice were also photographed on the anterior surface (C) and the equatorial edge (D). Opacities observed in double knockouts are far more severe than those seen in knockout of Cx50 or Cx46 alone, indicating a cumulative impact of connexins on lens transparency.

tance of connexins in maintaining lens homeostasis, especially with regards to the maintaining crystallin solubility.

12. Connexin mutations cause dominant cataracts in humans

Cataracts are one of the leading causes of visual impairment in humans. Early evidence described a congenital zonular pulverulent human cataract (CZP1, OMIM116200) that was later mapped to the long arm of human chromosome 1 which contains the Cx50 gene [13,72]. Further analysis of congenital cataracts revealed that mutations within the Cx50 gene were responsible for the observed cataracts. One such mutation is a C-to-T transition that resulted in the substitution of a serine residue at position 262 for a proline residue [86]. This mutation was only detected in individuals with cataracts in a large pedigree spanning several generations. Subsequently, additional mutations within the Cx50 gene were found to cause congenital cataracts. Like the P88S mutation, the variants R23T, E48K, and I247M all encoded single-amino-acid substitutions [5,32,69].

Mutations in the Cx46 gene have also been linked to zonular pulverulent cataracts in humans (CZP 3 OMIM 601885). To date, three separate mutations have been identified in the Cx46 gene that result in congenital cataracts. Two of these mutations are substitutions resulting in asn63ser and pro187leu Cx46 mutants [52,71]. The third mutation was identified as an insertion of a cytosine at nucleotide position 1137 within the Cx46 gene [52]. While there is a strong correlation between data linking cataracts to connexins, it should be noted that there are distinct differences in the inheritance patterns seen in humans and the knockout mice. Congenital cataracts in humans typically follow an autosomal dominant pattern of inheritance, whereas the phenotypes studied in knockout mice are recessive. To date, recessive cataracts caused by connexin mutation have not been described in humans, but dominant cataracts have been identified in mice which carry point mutations in Cx50 that resulted from treatment with chemical mutagens [12] [87] [29]. The finding of dominant cataracts in mice may provide researchers with additional animal models that more closely resemble the cataract phenotypes seen in humans.

13. Conclusions

The importance of gap junctions has become increasingly evident through the study of human disorders caused by mutations in connexin genes. While studying gap junction-mediated intercellular communication presents unique challenges, the advent of suitable animal models appears to aid the understanding of several connexin-mediated disorders. There appears to be two important aspects of gap junctional communication that are evident in most connexin related disorders. One is the inability of other gap junctions expressed in the same tissue to account for loss of function in one particular gene. This lack of redundancy increases the importance of each connexin, and restricts the ability of cells to compensate for mutant forms of these proteins. The second characteristic of connexin-mediated diseases is the ability of mutant genes to alter the activity of other genes expressed in the same tissue. This may result from simply mixing the subunits from different connexin genes, or may represent some other critical processes that are influenced by gap junctions. This leaves new and exciting challenges for future work in this field. Perhaps the most daunting challenge remaining for researchers will be understanding the downstream molecular events that ultimately bring about pathologies in persons expressing mutant connexin isoforms.

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