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Review

Functional redundancy and compensation among members of gap junction protein families? ☆,☆☆

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ABSTRACT

Gap junctions are intercellular conduits for small molecules made up by protein subunits called connexins. A large number of connexin genes were found in mouse and man, and most cell types express several connexins, lending support to the view that redundancy and compensation among family members exist. This review gives an overview of the current knowledge on redundancy and functional compensation — or lack thereof. It takes into account the different properties of connexin subunits which comprise gap junctional intercellular channels, but also the compatibility of connexins in gap junctions. Most insight has been gained by the investigation of mice deficient for one or more connexins and transgenic mice with functional replacement of one connexin gene by another. Most single deficient mice show phenotypical alterations limited to critical developmental time points or to specific organs and tissues, while mice doubly deficient for connexins expressed in the same cell type usually show more severe phenotypical alterations. Replacement of a connexin by another connexin in some cases gave rise to rescue of phenotypical alterations of connexin deficiencies, which were restricted to specific tissues. In many tissues, connexin substitution did not restore phenotypical alterations of connexin deficiencies, indicating that connexins are specialized in function. In some cases, fatal consequences arose from the replacement. The current consensus gained from such studies is that redundancy and compensation among connexins exists at least to a limited extent. This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.

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1. Introduction

Cells need to communicate with each other, and they do so by direct cell–cell communication via cell-adhesion molecules [1,2] notch-delta [3] or ephrin receptor-ephrin signaling [4] or by so-called gap junctions which are conduits for the free exchange of small molecules between the cytoplasm of neighboring cells [5]. Gap Junctions are dodecameric structures built up by two hexamers or hemichannels of connexin subunits (20 different subunits in mouse and 21 in man) which are located in the apposed plasma membranes of two individual cells [5,6]. Invertebrates express innexins, and more than 25 distinct innexins have been identified which mediate similar gap junctional coupling [7–9]. Innexins are evolutionary distinct from connexins, and innexin junctions show a morphology in electron microscopy which is distinct from gap junctions made up by connexins [10]. This distinct morphology together with the fact that chimeric gap junctions made up by connexins in one cell and innexins in another cell do not function as intercellular conduits indicated incompatibility of innexins and connexins [11]. Recently, 3 mammalian homologs of innexins have been identified which were called pannexins, showing a sequence similarity of about 20% to innexins [8,9,12–14]. Even though first reports indicated that pannexins can form gap junctional channels when expressed in Xenopus oocytes [15], current studies indicate that glycosylation of pannexins at the extracellular loops prevents the docking of hemichannels from the apposed plasma membranes of neighbouring cells [16,17]. All three gene families encode proteins with similar topology: A cytoplasmic N-terminus, four transmembrane domains with an embedded cytoplasmic loop of variable size and a cytoplasmic C-terminus. Innexins and pannexins have two conserved cysteine residues in both extracellular loops while connexins have three conserved cysteines in each loop instead. The conserved cysteine residues are required for the assembly of multimeric hemichannels [8].

Paracrine signaling is achieved by soluble ligands secreted from cells which travel short distances and bind to receptors from neighbouring cells [18,19] and via release of intracellular signaling molecules through hemichannels into the extracellular space [8,20]. Both functional connexin and innexin/pannexin hemichannels have been described. Innexins have both gap junctional channel (GJC) and hemichannel function, while pannexins and connexins show some form of specialization of channel function. For many connexins, hemichannel showed a conductance which was twice as high compared to a full gap junctional channel built by the same connexin. Since pannexin channels do not seem to form functional gap junctions in the first place, quotation marks are usually added to the term 'hemichannel' when referring to pannexins. The current consensus is that pannexin 'hemichannels' show many features which allow a 'hemichannel' function under physiological conditions [8]. By contrast, connexin hemichannels are mostly closed under normal conditions, but can be open in pathophysiological states, such as metabolic inhibition and hypoxia [20–23].

2. The connexin and pannexin gene families in mouse and man

A search for 'GJ' at http://www.informatics.jax.org/ and consultation of the web site http://www.genenames.org/genefamily/gj.php showed that twenty different connexin genes were described so far in mouse and 21 connexin genes have been identified in humans (see also [6,8,24,25]. Two nomenclatures exist: One uses the prefix Cx and is based on the calculated molecular weight of the corresponding connexin; Cx43 is connexin43 with a predicted molecular weight of 43 kDa. Note that the molecular weight sometimes differs between orthologous connexins of the species and that mouse Cx29 corresponds to human Cx30.2, mouse 30.2 to human 31.9, mouse Cx39 to human Cx40.1 and mouse Cx57 to human Cx62. Cx25 and Cx59 in humans have no mouse orthologue, while mouse Cx33 has no human orthologue (Table 1). The other nomenclature is based on the evolutionary relationship of connexins which forms alpha, beta, gamma, delta and epsilon subfamilies. Here, the subfamily members are numbered historically according to their order of detection. This nomenclature provides important information regarding evolutionary conservation (Table 1), but has quite a number of inconsistencies due to reassignment of connexins to newly emerged subfamily classes.

Why are there so many members of the connexin family? The different cell types of the body require distinct, tailored properties of gap junctions, which indeed differ depending on their connexin composition with respect to voltage gating, pH dependence, pore size, open probability and size and charge preferences for transported molecules [6,26,27]. In addition, variable amounts of connexin proteins are required for efficient gap junctional coupling depending on the stereology of cell–cell contact. Estimates indicate that only few gap junctions are required between pancreatic beta cells for control of insulin secretion [28] and a reduction of Cx43 between cardiomyocytes by 95% is tolerated for proper impulse propagation in the heart [29,30]. By contrast, gap junctions between hippocampal astrocytes which have only weakly overlapping territories, require more than one type of connexins in their gap junctions for proper function, as loss of either connexin in astrocytes already led to decreased tracer coupling [31–34].

Three pannexin genes have been described so far in humans and in rats and mice [12,15,35], numbered Panx1 to Panx3. Panx1 is ubiquitously expressed, while Panx2 is restricted to the central nervous system and Panx3 has been found in osteoblasts and in the skin [12,16]. The specialization of functional properties and large differences in the amount of junctional proteins required in various cell types probably furthered the expansion of the connexin and pannexin gene families throughout evolution.

3. Composition of gap junctions

A GJC is formed by the docking of two connexons (a synonym for hemichannels) from the apposed plasma membranes of two neighbouring cells. Connexons can be homomeric hexamers, i.e. made up by the same connexin subunits, or they can be heteromeric, i. e. made up by different connexins (Fig. 1). Provided connexins can form heteromeric connexons, a multitude of combinations is possible. If two hemichannels dock to each other which contain identical connexin makeup, the GJCs are called homotypic. If the hemichannels are made by different connexins, the channels generated are called heterotypic (Fig. 1). Recently, so-called bi-homotypic channels made up of Cx36 and Cx45 containing channels were described in retinal neurons, which are associated in common gap junctional plaques

Table 1
Mouse and human connexins known and compatibility of heterotypic and heteromeric channels. The table uses the current nomenclature of the gap junction/connexin gene family following discussions at the Gap Junction Conference (2007) in Elsinore, Denmark. M: mouse; h: human; a, A: α family; b, B: β family; g, G: γ family; d, D: δ family; e, E: ε family; n.d.: not determined; n.a.: not applicable. Bold letters show the compatibility of Cxs to form in addition to heterotypic channels also heteromeric hemichannels. # inconsistent data regarding the ability to form heterotypic channels. * Functional channels between myotubes of the diaphragm but non-functional channels between transfected HeLa cells.

Mouse Cx	Human Cx	Functional channels	Nonfunctional channels
Gje1, mCx23	GJE1, hCx23	No channel activity detected[18]	n.a.
Gjc3, mCx29	GJC3, hCx30.2	No channel activity detected[19]	n.a.
Gjb2, mCx26	GJB2, hCx26	Cx26[1,4], **Cx30**[2,24], **Cx32**[1,4,8,25], Cx46[1,4], Cx50[1,4]	Cx30.3[2], Cx31[1], Cx31.1[2,12], Cx36[9], Cx37[1], Cx40[1,4], **Cx43**[1,30], Cx45[1], Cx47[5], Cx57[2]
Gjb6, mCx30	GJB6, hCx30	**Cx26**[2,24], Cx30[2], Cx30.3[2], **Cx32**[2,24], Cx40[2], Cx43[2], Cx45[2], Cx46[2], Cx47[3], Cx50[2]	Cx31[2], Cx31.1[2], Cx36[9], Cx37[2], Cx57[2]
–	GjJB7, hCx25	n.d.	n.d.
Gjd3, mCx30.2	GJD3, hCx31.9	Cx30.2[7], **Cx40**[7,26], **Cx43**[7,26], **Cx45**[7,26]	n.d.
Gjb4, mCx30.3	GJB4, hCx30.3	Cx30[2], Cx30.3[13], Cx37[23], Cx40[23], Cx43[23], Cx57[3]	Cx26[2], Cx31[23], Cx31.1[23], Cx32[23], Cx36[23], Cx45[23]
Gjb3, mCx31	GJB3, hCx31	Cx31[1]; *hCx26*[6], *hCx30*[6], *hCx32*[6], *hCx45*[6]	Cx26[1], Cx30[2], Cx32[1], Cx36[9], Cx37[1], Cx40[1], Cx43[1], Cx45[1], Cx57[3]
Gjb5, mCx31.1	GJB5, hCx31.1	No channel activity detected[12,13]	Cx26[2, 12,13], Cx30[2], Cx31.1[12], Cx32[12], Cx37[23], Cx40[23], Cx43[12], Cx45[23], Cx57[3]
Gjb1, mCx32	GJB1, hCx32	**Cx26**[1,4,8,25], **Cx30**[2,24], Cx32[1,4,16], Cx46[4], Cx50[4]	Cx31[1], Cx31.1[8], Cx33[16], Cx36[9], Cx37[1], Cx40[1,4], **Cx43**[1,31], Cx45[1], Cx47[5], Cx57[3]
Gja6, mCx33	–	No channel activity detected[12]	Cx32[16], Cx33[12], Cx37[12,16], Cx43[12,16]
Gjd2, mCx36	GJD2, hCx36	Cx36[9]	Cx26[9], Cx30[9], Cx31[9], Cx32[9], Cx37[9], Cx40[9], Cx43[9], Cx45[9], Cx50[9]
Gja4, mCx37	GJA4, hCx37	Cx37[1,14], Cx40[1,4,11,14,17], **Cx43**[1,11,32], Cx45[1], Cx57[3]	Cx26[1], Cx30[2], Cx31[1], Cx32[1], Cx33[12,16], Cx36[9]
Gjd4, mCx39	GJD4, mCx40.1	Cx39[10] *	n.d.
Gja5, mCx40	GJA5, hCx40	Cx30[2], **Cx30.2**[7,26], Cx37[1,4,11,14,17], Cx40[1,14,17], **Cx43**[21,22]#, Cx45[1,17]	Cx26[1,4], Cx31[1,4], Cx32[1], Cx36[9], Cx43[1,4,11,17] #, Cx46[4], Cx50[4], Cx57[3]
Gja1, mCx43	GJA1, hCx43	Cx30[2], **Cx30.2**[7,26], **Cx37**[1,11,32], **Cx40**[21,22]#, Cx43[1,15,17,20], **Cx45**[1,17,20,29], **Cx46**[4,15,27], Cx47[5], Cx57[3]	**Cx26**[1,30], Cx31[1], Cx31.1[12], **Cx32**[1,31], Cx33[12,16], Cx36[9], Cx40[1,4,11,17] #, Cx50[15]
Gjc1, mCx45	GJC1, hCx45	Cx30[2], **Cx30.2**[7,26], Cx37[1], Cx40[1,17], **Cx43**[1,17,20,29], Cx45[1,17,20]	Cx26[1], Cx31[1], Cx32[1], Cx36[9], Cx57[3]
Gja3, mCx46	GJA3, hCx46	Cx26[4], Cx30[2], Cx32[4], **Cx43**[15,27], Cx46[4,15], **Cx50**[15,28]	Cx40[4], Cx57[3]
Gjc2, mCx47	GJC2, hCx47	Cx30[5], Cx43[5]	Cx26[5], Cx32[5]
Gja8, mCx50	GJA8, hCx50	Cx26[2,4], Cx30[2], Cx32[4], **Cx46**[15,28], Cx50[4,15]	Cx36[9], Cx40[4], Cx43[15], Cx57[3]
–	GJA9, hCx59	n.d.	n.d.
Gja10, mCx57	GJA10, hCx62	Cx30.3[3], Cx37[3], Cx43[3], Cx57[3]	Cx26[3], Cx30[3], Cx31[3], Cx31.1[3], Cx32[3], Cx40[3], Cx45[3], Cx46[3], Cx50[3]

[1] [41]; [2] [42]; [3] [43]; [4] [44]; [5] [45]; [6] [46]; [7] [47]; [8] [48]; [9] [37]; [10] [49]; [11] [50]; [12] [51]; [13] [52]; [14] [53]; [15] [54]; [16] [55]; [17] [56]; [18] [57]; [19] [58]; [20] [59]; [21] [60]; [22] [61]; [23] [62]; [24] [63]; [25] [64]; [26] [65]; [27] [66]; [28] [67]; [29] [68]; [30] [69]; [31] [70]; [32] [71].

where they form separate homotypic GJCs (Fig. 1; [36]). Cx45 and Cx36, when expressed in HeLa cells separately, do not allow dye coupling between these two transfected cell populations [37]. Likewise, HeLa cells expressing Cx36 do not form gap junctional plaques with other cells expressing Cx45 [36]. In fact, freeze fracture immunogold labeling indicated that in retinal neurons homotypic Cx36 GJCs stabilize plaque formation of homotoypic Cx45 containing gap junctions [36]. This is in line with the observation that mice lacking either Cx36 or Cx45 exhibit visual transmission deficits [38,39]. Intriguingly, Cx36 can functionally replace Cx45 in retinal neurons [40] in knock-in mice where the coding region of Cx45 is replaced by the coding region of Cx36 (Cx45KICx36 mice). This indicates that Cx36 can probably take over the function of Cx45. In these mice, Cx36 is expressed from the wildtype Cx36 gene locus and also expressed instead of Cx45 from the Cx45 gene locus. Since no visual transmission deficits were detected in these Cx45KICx36 mice, probably the amount of connexins expressed (Cx36 or Cx45) was most important, while possible differences between these two connexins were not relevant.

Not all connexins form compatible hemichannels which allow the formation of GJCs; an overview of heterotypic and heteromeric compatibility is given in Table 1, based on references [37,41–71]. Some connexin subunits form heterotypic GJICs with many different connexins, with a general tendency to form heterotypic channels among members of the same subfamily of connexins [72]. Others are restricted to a few other connexin family members or to themselves: Cx31 only couples homotypically in mouse [41]. This may be a species-specific phenomenon, since human Cx31 coupled to all connexins tested in a more recent study (Cx26, Cx30, Cx32, Cx45, [46]; Table 1). This finding indicates that

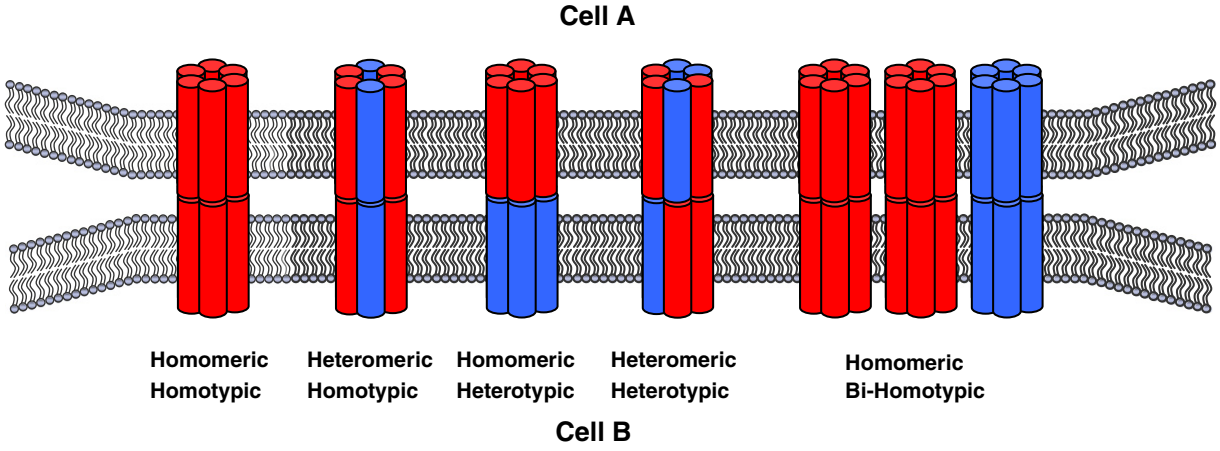

Fig. 1. Types of gap junction channels. For explanations, see text. Cylinders: connexin subunits; colours indicate different connexins.

compatibility of connexins can be species-specific. Some connexins lack GJIC activity altogether, for example Cx23 which has only two cysteine residues in each extracellular loop much like pannexins and innexins [57]. Cx33 actively inhibits functional expression of Cx43 channels in testes [55], most likely by increasing the rate of endocytosis of the resulting heteromeric channels [73]. Connexins do not only determine the properties of the channels they build up. Compatibility and incompatibility of connexins also increases the spectrum of intercellular communication, since it allows the generation of separate communication compartments for distinct molecules. Different channel properties and incompatibility of connexins are important determinants for redundancy and compensatory function. In fact, by replacing one connexin with another connexin which has different functional properties and a different compatibility pattern (Section 7), not only the transport characteristics but also the coupling compartments may be subject to change. Thus, several aspects of connexin function are changed by the replacement, which makes the interpretation of possible redundancy and compensation among connexins somewhat difficult.

The ability of Cxs to form heteromeric hemichannels seems to be restricted to members of the same phylogenetic subgroup (Table 1, [70]). For example, the member of the α-subgroup Cx43 has been shown to form heteromeric channels with Cx37 and Cx40, which both belong to the same group, but not with the β-subgroup members Cx26 and Cx32 (Table 1). To elucidate the molecular basis for connexin oligomerization compatibility, Lagree et al. compared α and β connexin sequences and identified two N-terminal amino acid residues (at positions 12 and 13) which seems to be crucial for heteromeric compatibility [74]. Replacement of these two residues in Cx43 with the corresponding amino acids of Cx32 resulted in mutant Cx43 proteins that were able to form heteromeric channels with Cx32 [74].

4. Non-channel functions of connexins

Connexins do not only exhibit GJC and hemichannel function [20], but also mediate adhesion between cells [75,76] and through their interaction with many other proteins, also affect intracellular signaling and gene expression, as also pannexins do [77]. Both the C-terminal tail of Cx43 and the full length protein have been found in the nucleus which resulted in growth inhibition [78]. Cx43 also interacts with N-cadherin and may indirectly regulate cell–cell adhesion [79]. In addition, Cx43 binds to β-catenin and suppresses β-catenin dependent gene regulation [80]. Such functions have to be taken into account when interpreting functional changes due to lack or replacement of connexins.

5. Composition of pannexin channels

Pannexins, when overexpressed in Xenopus oocytes, formed Panx1/Panx2 heteromeric GJCs [15]. The same study also demonstrated 'hemichannel' activity of pannexins [15]. Recent findings showed that pannexins are subject to glycosylation [12,16,17], which prevents the formation of GJCs. The possibility of communicating junctions made up by heteromeric pannexin channels was further questioned by a study which showed that Panx1 hemichannels are hexameric, while pure Panx2 hemichannels have an octameric structure and that heteromeric hemichannels made up by Panx1/Panx2 are unstable [81]. Panx1 which is expressed ubiquitously may be present in the same cell types as Panx2 in the CNS, while it may be co-expressed with Panx3 in osteoblasts and skin [12,16,82]. Three sources for Panx1 deficient mice are available: A Panx1 KO mouse [83], a gene-trapped ES cell line (www.genetrap.org; sequence tag: DD0964) in which the Panx1 gene has been disrupted, and a conditional Panx1 deficient mice generated by the KOMP consortium, which also prepared ES cells for Panx2 and Panx3 deficient mice (search for Panx at https://www.komp.org/). The investigation of compensation and redundancy among pannexins is in its infancy and awaits the further generation and characterization of the different pannexin deficient mice. In the following, we will therefore focus on connexins.

6. Most cell types contain more than one connexin

Mouse keratinocytes express 8 different connexins: in the basal layer, Cx43, Cx40, Cx37 and Cx31 were observed, while in the spinous layers Cx43, Cx37, Cx31.1, Cx31 and Cx30.3 were detected. The granulous layers express Cx37, Cx31.1, Cx31, Cx30.3, Cx30 and Cx26 (cf. [84]). Hepatocytes express Cx32 and Cx26 [85]. Retinal neurons coexpress Cx36 and Cx45 [36] and inhibitory interneurons express Cx36 and Cx30.2 [86–88]. While mice lacking Cx36 showed phenotypical aberrations due to a deficiency of intercellular coupling between interneurons [86,87], no significant alterations were observed in Cx30.2 deficient mice [88]. Astrocytes express Cx43 and Cx30 [89]. They may also express Cx26, even though the presence of Cx26 in astrocytes is under debate [90]. Oligodendrocytes express Cx47, Cx32 and Cx29 [91]. An interesting exception are pancreatic beta cells, which seem to exclusively express Cx36 [28], oocytes expressing Cx37 [92] and fibrocytes of the inner ear expressing Cx31. The fact that most cell types express more than one connexin subunit supports the possibility of redundancy and compensation (see Table 2 and references [88,93–105]). However, the large number of connexin genes might as well have allowed specialization of channel properties and channel regulation in order to optimize the channel properties for individual cell types and functions, giving rise to tailor-made GJCs. The next chapter deals with the suitability of human connexin mutations, connexin deficient mice and with knock-in mice in which one connexin was replaced by another connexin for the investigation of redundancy and compensation among connexins.

7. Tools to study functional compensation and redundancy among connexins

Mutations in connexin genes are associated with human genetic diseases: Mutations of Cx32 cause the X-linked form of Charcot-Marie-Tooth disease [106,107], mutations in Cx47 cause Pelizaeus-Merzbacher-like Disease [108,109], while another mutation led to hereditary spastic paraplegia [110]. A large number of mutations in Cx43 led to oculodentodigital dyplasia (ODDD), a syndrome characterized by ocular, dental, digital and craniofacial abnormalities [111]. Mutations in Cx31, Cx30.3, Cx30, Cx26 and Cx43 are associated with nonsyndromic deafness [112], and mutations in Cx26, Cx30, Cx30.3 and Cx31 are associated with skin diseases [112]. With few exceptions, most of these mutations are not loss of function mutations in the sense of knockout mutations but rather point mutations or truncations which may lead to changed connexin function. Therefore, dominant negative effects on other connexins interacting with the mutant forms either as heterotypic or heteromeric channels cannot be excluded. In addition, mice expressing an ODDD mutation of Cx43 exhibit not only loss of channel function but also increased hemichannel activity under hypoxia [25,113]. Even though mice expressing mutant forms of connexins have been generated [25,107,113,114], they are probably of limited value for the study of redundancy and compensation among connexins for the abovementioned reasons.

Connexin-deficient mice can be investigated for similarities in phenotypical aberrations and double deficient mice can be screened for aggravation of these phenotypical changes. Such studies could show that Cx43 and Cx30 probably have similar functions in astrocytes [31–34,115–118], and Cx32 and Cx47 may share similar functions in oligodendrocytes [118,119]. Especially compensatory changes in the expression of connexins with related functions can give a clue on redundancy and compensation [31,120].

Transgenic mice in which a connexin has been replaced by another connexin [121,122] provided insights into which endogenous

Table 2
Cell types expressing connexins.

Cell type	Expressed connexins	Ref.
CNS		[93]
Neurons	Cx36, Cx43, Cx45, Cx47, Cx30.2	[88]
Retinal Neurons	Cx36, Cx45, Cx57, Cx30.2	[94]
Astrocytes	Cx26, Cx30, Cx43	
Oligodendrocytes	Cx29, Cx32, Cx47	
Microglia	Cx36, Cx43	
Heart		[95]
Working myocytes	Cx40, Cx43	
Conductive myocytes	Cx30.2, Cx40, Cx45	
Lens		[96]
Epithelial cells	Cx43, Cx50	
Fibers cells	Cx46, Cx50	
Pancreas		[97]
Acinar cells	Cx26, Cx32	
Pancreatic beta-cells	Cx36	
Skin		[84]
Keratinocytes	Cx26, Cx30, Cx30.3, Cx31, Cx31.1, Cx37, Cx40, Cx43	
Vascular system		[98]
Smooth muscle cells	Cx43, Cx45	
Endothelial cells	Cx37, Cx40, Cx43	
Testis		[99]
Germ cells	Cx26, Cx31, Cx32, Cx33, Cx40, Cx43, Cx50	
Leydig cells	Cx30.2, Cx43	
Sertoli cells	Cx26, Cx32, Cx33, Cx43	
Endothelial cells	Cx30.2, Cx37, Cx43	
Mammary gland		[100]
Luminal epithelial cells	Cx26, Cx30, Cx32	
Myoepithelial cells	Cx43	
Placenta		[101]
Spongiotrophoblast cells	Cx31, Cx43	[102]
Trophoblastic giant cells	Cx43	
Syncytiotrophoblast cells	Cx26	
Glycogen cells	Cx31, Cx31.1	
Ovary		[92]
Oocytes	Cx37	
Granulosa cells	Cx32, Cx43, Cx45	
Liver		[103]
Hepatocytes	Cx32, Cx26	
Kidney		[104]
Endothelial cells	Cx37, Cx40, Cx43	
Epithelial cells	Cx26, Cx30, Cx30.3, Cx32	
Podocytes	Cx43, Cx45	
Inner ear		[105]
Supporting cells	Cx26, Cx30, Cx43	
Fibrocytes	Cx31	

connexin functions are unique and which are redundant. However, one caveat is the possible generation of new coupling compartments when connexins with different compatibility patterns are replacing an endogenous connexins. Both knockout mice and knock-in mice are artificial situations not found in nature, as opposed to connexin mutations. In the following chapter, we will deal with transgenic mice with lack of connexin function or connexin replacement.

8. Investigation of functional compensation and redundancy among connexins: Transgenic mouse studies

8.1. Developmental disturbances

Cx43 deficient mice develop to term but show morphological alterations of the heart vasculature which led to early postnatal death [123]. Mice lacking Cx26 died in early embryogenesis due to a placental deficit which is specific to mouse development and not observed in humans [124]. Cx45 KO embryos exhibited striking abnormalities in vascular development and die at early embryonic stages [125]. Premature death of mice lacking Cx43, Cx26 and Cx45 required the generation of cell-type restricted knockout mice for Cx43 [126–129] and Cx45 [39] in order to study later developmental stages and adult mice.

8.2. Heart

Four different Cxs, Cx43, Cx40, Cx45 and Cx30.2, are expressed in the mouse heart (Table 2) and each exhibits high regional and cell type specific expression patterns [95]. Cx43, the most abundant gap junction protein in the heart, is mainly expressed in the atrial and ventricular working myocytes; Cx40 is predominantly found in the atrial working myocytes, as well as in the His bundle and its two branches; Cx45 and Cx30.2 are mainly detected in the conduction system (sinoatrial (SA) and atrioventricular (AV) nodes, the His bundle and its brunches) [95]. Each of the cardiac Cxs forms channels with distinct unitary conductances, selectivity, sensitivity to transjunctional voltage as well as chemical and biochemical gating [27]. The unitary conductance, for instance, ranges from 10 pS (Cx30.2) to 200 pS (Cx40). In addition, cardiac Cxs can form heterotypic and heteromeric channels with unique gating and conductance properties (Table 1, [65,130,131]). For example, Cx43 and Cx45 have been shown to form homomeric–homotypic, homomeric–heterotypic, heteromeric–homotypic and heteromeric–heterotypic channels and each of these channel types possesses specific features [59].

Neonatal Cx43 knockout mice display ventricular arrhythmia and die at birth due to obstruction of the right ventricular outflow tract [123]. Cardiomyocyte-restricted conditional Cx43 knockout mice have normal heart structure but display slowed ventricular conduction velocity and die within 2 months after birth as a result of spontaneous ventricular arrhythmias [128]. To answer the question of whether another gap junction isoform can substitute Cx43 in the heart, four different knock-in mice have been generated. Plum et al. replaced the coding region of Cx43 by the coding region of Cx40 (Cx43KICx40) or Cx32 (Cx43KICx32) [121]. Since Cx40 belongs to the α- and Cx32 to the β-subgroup, these two knock-in mice lines offer the possibility to compare the substitutability within and between phylogenetic groups. Cx40 colocalizes with Cx43 in the atrial working myocytes, but is more abundantly expressed in the conductive myocytes. In contrast, Cx32 is a non-cardiac connexin with prominent expression in hepatocytes, oligodendrocytes and Schwann cells. The unitary conductance of Cx40 channels is about twice that of Cx43 channels (200 pS vs. 100 pS), whereas that of Cx32 is about two-thirds lower (35 pS) [95]. The voltage dependence as well as the pH sensitivity of the three channels is similar. Cx43 and Cx40 but not Cx32 are able to form functional heterotypic channels with Cx45 (Table 1). All three connexins are phosphoproteins [132]. The results obtained from Cx43KICx40 and Cx43KICx32 mice show that both Cx32 and Cx40 can rescue the lethality of Cx43-deficient mice. The cardiac morphology of Cx43KICx40 mice was relatively normal, whereas Cx43KICx32 mice exhibited mild morphological defects. Also the ECGs of both mouse lines were surprisingly normal, even though spontaneous ventricular arrhythmias were observed in most Cx43KICx40 and some Cx43KICx32 mice [121]. Even though such genetic replacements do not occur naturally, these findings demonstrate that Cx43, Cx32 and Cx40 replace each other with respect to some functions, but not all, and that each of these connexins also possess unique features (Table 3 and references [39,40,121–123,125,128,133–147].

In another study Cx43 was replaced by the β-subgroup member Cx26 (Cx43KICx26) [135]. Cx26 is normally expressed in hepatocytes, in the epidermis, the cochlea and many other cell types. The unitary conductance of Cx26 channels is somewhat higher than that of Cx43 (140 pS) [148]. Cx26 has slightly lower voltage sensitivity and a slightly higher sensitivity to acidification than Cx43. The gating properties of Cx43 are regulated by phosphorylation, whereas there are no reports on phosphorylation of Cx26 [132]. Cx26 hemichannels are not able to form functional heterotypic channels neither with Cx43 nor with Cx40 or Cx45 (Table 1). Cx43KICx26 mice were viable and showed no heart malformations. However, in ECG analysis, the

Table 3
Knock-in transgenic mice: phenotypical comparison of deficiency and replacement.

Functional replacement	Groups	KO phenotype of the replaced connexin	Phenotypic consequences of the replacement
Cx43 by Cx26	α by β	Cx43 General deletion: Neonatal ventricular arrhythmia. Lethal shortly after birth due obstruction of the right ventricular outflow tract [123].	Viable but decreased postnatal survival and growth due to impaired mammary gland development. Slowed ventricular conduction in the heart. Male and female mice infertile [135].
Cx43 by Cx32	α by β	Cardiac-restricted deletion: normal heart structure; slowed ventricular conduction velocity; postnatal lethal due to spontaneous ventricular arrhythmias [128]. Sertoli cells-restricted deletion: arrest of spermatogenesis at the level of spermatogonia or "Sertoli cell only" syndrome [133,134].	Viable but decreased postnatal survival and growth due to defective milk ejection. Male and female mice infertile. Spontaneous ventricular arrhythmias. Mild cardiac morphological defects. Cataracts [121].
Cx43 by Cx40	α by α		Viable but decreased postnatal growth and survival. Male and female mice infertile. Spontaneous ventricular arrhythmias [121].
Cx43 by Cx31	α by β		Neonatal ventricular arrhythmia. Lethal shortly after birth due obstruction of the right ventricular outflow tract [136].
Cx45 by Cx36	γ by δ	Cx45 Lethal around embryonic day 10.5 from cardiovascular defects and conduction failure in the heart [125,137]. Neuron-restricted deletion: defects in visual transmission [39].	General replacement: Embryonic lethal due to defects in cardiac morphogenesis and conduction. Neuron-directed replacement: animals viable, no defects in visual transmission [40].
Cx40 by Cx45	α by γ	Cx40 Slower conduction velocity in the conductive myocardium; right bundle branch block; spontaneous atrium-related arrhythmias [138–141]. Marked hypertension and irregular arteriolar vasomotion [142]. Increased renin synthesis and secretion [143].	Significant reduced conduction velocity in the left atrium; partial loss of function in the right bundle branch. Impaired conduction of endothelium-dependent dilations along arterioles. Moderate hypertension [145].
Cx50 by Cx46	α by α	Cx50 Reduced ocular growth and cataracts [144].	Impaired postnatal ocular growth [122].
Cx26 by Cx32	β by β	Cx26 Lethal between embryonic days 9.5–10.5 due to placental dysfunction [124]. Ectoderm-restricted deletion: lethal between embryonic days 16–18.5 due to severe lymphedemas [146]. Cochlea-restricted deletion: severe hearing impairments [129].	General heterozygous replacement: lethal between embryonic days 16.5–18.5 due to severe lymphedemas. Normal placental function [146]. Ectoderm-specific heterozygous replacement: embryonic lethal due to severe lymphedemas [146]. Cochlea-restricted replacement: almost normal hearing [147].

knock-in mice exhibited slowed ventricular conduction, similar to that found in conditional Cx43 KO mice even though the changes were somewhat milder [135]. These results show that Cx26 can partially substitute for Cx43 in the heart (Table 3).

Zheng-Fischhöfer et al. replaced the coding region of Cx43 with that of Cx31 (Cx43KICx31) [136], again a member the β-subgroup. Cx31 is like Cx32 and Cx26 a non-cardiac connexin with prominent expression in the skin, cochlea, uterus and placenta [132]. The unitary conductances of the two gap junction isoforms are similar, whereas Cx31 possesses a slightly higher voltage and pH sensitivity [46]. Cx31 like Cx43 can be regulated by phosphorylation. Mouse Cx31 hemichannels cannot form functional heterotypic channels with any other connexin (Table1; [41]), but coupling between human Cx31 and Cx45 hemichannels has been described [46]. Replacement of Cx43 by Cx31 could not rescue the lethal phenotype of Cx43-deficient mice. Similar to the Cx43 KO mice, Cx43KICx31 mice died shortly after birth due to obstruction of the right ventricular outflow tract. Furthermore, neonatal Cx43KICx31 mice showed electrophysiological abnormalities similar to those found in Cx43 KO mice [136]. These data suggest that Cx43 fulfils special functions in the heart that cannot be compensated by Cx31 (Table 3).

To further assess the functional equivalence of different gap junction isoforms, Frank et al. generated a knock-in mouse line in which Cx45 was replaced by Cx36 [40]. Hence, the substitutability of a γ-Cx by a δ-Cx was investigated in this case. Cx45 is the only connexin channel expressed in the early embryonic heart (E8.5, at the stage of the first contractions) and at that time point it can be found in all cardiac compartments [149]. In the adult heart Cx45 is expressed in the SA and AV nodes, as well as in the His bundle and its branches. General and cardiomyocyte-specific deletion of Cx45 in mice results in lethality around embryonic day 10.5 from cardiovascular defects and conduction failure in the heart [125,137]. Cx45 exhibits among all connexin isoforms the highest voltage sensitivity (half-maximal inactivation at ±13.4 mV) [150] and possesses a high cation selectivity (cation/anion selectivity ratio = 10:1; [151]). In addition the pH sensitivity of Cx45 is higher than for most other connexins [152]. Cx36 is not expressed in the heart but is mainly expressed in neurons of the CNS and retina [153] as well as in pancreatic beta-cells [97]. Common features of Cx45 and Cx36 are low unitary conductances and regulation by phosporylation. In contrast, Cx36 channels exhibit significantly lower voltage sensitivity and lack the ability to form heterotypic channels with any other connexin isoforms. The pH sensitivity of Cx36 channels represents a special case: while the activity of all other connexin channels decreases following acidosis, Cx36 channels are inhibited by alkalosis [154]. Replacement of Cx45 by Cx36 resulted in lethality on embryonic day 11.5 due to a defect in cardiac morphogenesis and conduction, similar to those found in Cx45 KO mice [40]. These findings show that Cx45 channels play a crucial role in the developing heart that cannot be taken over by Cx36 (Table 3).

Alcolea et al. replaced Cx40 by Cx45 and investigated the consequences on cardiac electrical conductance [145]. Cx40 and Cx45 are co-expressed in the conduction system, albeit Cx40 is also expressed in the atrial working myocytes [95]. Cx40 channels possess the largest

unitary conductance among the cardiac connexins, which has been suggested to be important for fast propagation of electrical excitation in the His-Purkinje system [155]. Consistent with this, Cx40-deficient mice display slower conduction velocity in the conductive myocardium, as shown by prolonged P-waves and QRS complex in the ECG [138,139]. Furthermore a right bundle branch block and spontaneous atrium-related arrhythmias have been found in Cx40 KO mice [139–141]. The knock-in of Cx45 into the Cx40 locus resulted in significantly reduced conduction velocity in the left atrium and normal conduction velocity in the right atrium. Whereas the conduction through the AV node was unaffected a partial loss of function was found in the right bundle branch [145]. These data demonstrate that, at least in the left atrium, Cx40 cannot be replaced by Cx45 (Table 3).

Taken together the knock-in studies on cardiac Cxs indicate that Cx43 possesses redundant as well as specialized functions in the heart which can partially be fulfilled by Cx26, Cx32 or Cx40 but not by Cx31. The function of Cx45 could not be taken over by Cx36 and that of Cx40 just to a very limited extent by Cx45, pointing to more specialized roles of these two gap junction isoforms in the heart.

8.3. Reproductive system

Several connexin isoforms are expressed in the testis, including Cx26, Cx30.2, Cx31, Cx32, Cx33, Cx37, Cx40, Cx43, Cx45, Cx46 and Cx50 (Table 2). The predominant connexin in testis is Cx43, which has been shown to be expressed in Sertoli cells, Leydig cells, spermatogonia and spermatocytes [99]. Cx43-mediated coupling has been demonstrated to occur between adjacent Sertoli cells, between adjacent Leydig cells as well as between Sertoli cells and germ cells [99]. Important information about the crucial role of Cx43 in spermatogenesis was obtained from Cx43 deficient mice. These mice show severely reduced germ cell numbers and, probably as a consequence, unusually small gonads [156]. Since Cx43 KO mice die shortly after birth, the role of Cx43 in spermatogenesis was examined on transplanted testis from KO fetuses. Under these conditions a complete lack of spermatogenesis ("Sertoli cell only" phenotype) has been observed [157]. A significant reduction of the spermatogonia number was also observed in conditional KO mice, in which Cx43 was deleted exclusively in Sertoli cells (Table 3; [133,134]).

Further insights into the role of Cx43 during testis development and in the control of spermatogenesis have been gained from knock-in mice. Replacement of Cx43 by Cx32 or Cx40, both also expressed in Sertoli cells, resulted in a phenotype strongly resembling that of Cx43 KO mice. In these mice primary spermatogonia were severely reduced and the secondary spermatogonia were completely absent. Moreover, the mice exhibited hypotrophic testis [121]. A comparable phenotype resulted from the replacement of Cx43 by Cx26. Homozygous Cx43KICx26 mice also displayed hypothrophic testis and the "Sertoli cell only" syndrome [135]. These findings show that Cx43 has unique functions in spermatogenesis, which cannot be fulfilled by Cx26, Cx32 or Cx40 (Table 3).

In the ovarian follicle the expression of Cx32, Cx37, Cx43 and Cx45 have been demonstrated (Table 2). The major gap junction protein expressed in cumulus granulosa cells is Cx43, whereas just low expression levels of Cx32, Cx45 and presumably also Cx37 were found in these cells [92,158]. In contrast, the only connexin channel expressed by oocytes seems to be Cx37. Using electron microscopy it could be shown that Cx43-containing gap junction plaques are detectable on the cumulus cell side close to the oocyte surface [92] indicating that Cx43/Cx37 heterotypic channels are involved in the coupling of cumulus cells with oocytes. The coupling between these cell types has been proven to mediate the transfer of nutrients, inhibitory nucleotides (cAMP) and anti-apoptotic signals [159]. In Cx43 KO mice just very few (about 10%) germ line cells were found. In organ cultures and in transplanted null mutant ovaries the oocyte growth was retarded and folliculogenesis arrested in most cases before the follicles had become multilaminar [156] demonstrating the pivotal role of Cx43 in folliculogenesis. Replacement of the coding region of Cx43 by that of Cx32 or Cx40 resulted in a nearly total infertility of homozygous females despite the fact that no morphological abnormalities in the germ-cell generation were found. In both mouse lines all stages of follicular maturation and ovulation could be found, but only few of the Cx43KICx32 females and none of the Cx43KICx40 females got pregnant after mating [121]. Hence, Cx32 and Cx40 can at least partially compensate for the loss of Cx43 in folliculogenesis and the reason for the female infertility of both knock-in mice remains to be determined. The fact that the replacement of Cx43 by Cx32 and Cx40 resulted in a similar phenotype is interesting, since Cx40 is able to form heterotypic channels with Cx37 whereas Cx32 cannot. The phenotypes of these knock-in lines therefore may indicate that Cx43/Cx37 heterotypic channels between cumulus cells and oocytes have unique properties which cannot be substituted by Cx40/Cx37 channels. In contrast to Cx32 and Cx40, no compensatory effect could be achieved by the knock-in of Cx26 into the Cx43 locus. These mice displayed a phenotype similar to that observed in Cx43 deficient mice. Like in Cx43 KO mice, the ovaries revealed only few follicles and the maturation of follicles was arrested at the early secondary stage (Table 3; [135]). In conclusion, unique features of Cx43 seem to be crucial for proper folliculogenesis, which cannot be realized by Cx26 and just to a limited extent by Cx32 and Cx40.

In mammary tissue, Cx32, Cx30 and Cx26 are colocalized in the secretory epithelium, whereas Cx43 is expressed in the myoepithelium of the mammary gland [63]. In epithelial cells the predominant Cx isoform during pregnancy is Cx26 and after parturition Cx32. Cx30 is transiently expressed during late pregnancy and early lactation [63]. From connexins expressed in the secretory epithelium only Cx30 hemichannels are able to form heterotypic channels with Cx43 hemichannels. Therefore intercellular coupling between luminal and myoepithelial cell compartments may occur only during the time of Cx30 expression. Heterozygous Cx43KICx32 and Cx43KICx26 mice, but not Cx43KICx40 mice, were unable to nourish their pups to weaning age. Histological analysis revealed that replacement of Cx43 by Cx32 led to a defect in milk ejection, whereas replacement of Cx43 by Cx26 resulted in a dysmorphogenesis of gland arborization [121,135]. These functional impairments of the mammary gland could be caused by aberrant coupling between luminal and myoepithelial cell compartments during early pregnancy and/or after parturition. This observation is supported by the fact that the replacement of Cx43 by Cx40, which cannot form heterotypic channels with Cx26 or Cx32, results in mice with normal mammary gland function (Table 3). These results indicate that Cx43 and Cx40 share unique properties which are crucial for proper mammary gland development and milk delivery to the pups and which Cx26 and Cx32 do not possess.

The expression of Cx26, Cx31, Cx31.1 and Cx43 has been documented in the mature rodent placenta (Table 2). Studies with knockout mice have revealed that Cx26 is critical for metabolic transport across the placental barrier, whereas Cx31 and Cx31.1 are crucial for the trophoblast cell lineage development [102]. Cx43 has been shown to be expressed in trophoblast giant cells (Table 2), but its function in the placenta remains unclear. Since Cx43-deficient mice display normally developed placentas [123] it is not surprising that replacement of Cx43 by Cx31 had no influence on placental development or function [136]. Hence, Cx43 seems to be replaceable by other Cxs in the placenta.

8.4. Vascular system

In the vascular tissue the expression Cx37, Cx40, Cx43 and Cx45 has been shown (Table 2). The expression levels of the four connexins

vary with vessel size, vascular regions and vascular beds as well as during embryogenesis and the progress of disease [160]. Vascular smooth muscle cells express mainly Cx43 and Cx45, whereas the expression of Cx37, Cx40 and Cx43 has been described in endothelial cells. Gap junctional coupling between vascular smooth muscle cells plays a central role in synchronization of vasomotor tone by coordinating changes in membrane potential and intracellular Ca^{2+} between adjacent cells [98]. Furthermore it is involved in the coordination of vessel constriction in resistance arteries and of cell proliferation and migration in the vasculature [98]. The predominant gap junction protein in endothelial cells is Cx40 which has been shown to play an essential role in the regulation of blood pressure by mediating the propagation of endothelium-dependent vasodilator responses and by controlling the renin synthesis and release. In addition, heterocellular communication between smooth muscles and endothelial cells play a crucial role in the Ca^{2+}-mediated responses induced by endothelium-dependent vasodilators [98]. Besides homotypic channels, heterotypic and heteromeric channels may play an important role for the intercellular coupling between vascular cells (Table 1; [161]). For example, the formation of heteromeric Cx43 and Cx40 channels has been demonstrated in vascular smooth muscle cells [162].

Most information about the role of Cx40 in vascular tissue has been obtained from the phenotype of Cx40 deficient mice, which display marked hypertension and irregular arteriolar vasomotion [142]. In kidney, were endothelial cells are coupled with juxtaglomerular cells via Cx40, deletion of Cx40 caused an increase of renin synthesis and secretion as well as increased number and altered distribution of renin-secreting cells. In addition, Cx40 KO mice exhibited a defect in the ability to recruit renin-expressing cells after stimulation [163]. To assess the functional equivalence of Cx40 and Cx45, vascular function was analyzed in Cx40KICx45 mice [145]. Cx40 channels exhibit much higher unitary conductance than Cx45 channels (200 vs. 30 pS) and are much less voltage sensitive [150]. Cx40KICx45 mice exhibited just moderate hypertension, indicating that Cx45 can at least partially replace the Cx40 function of blood pressure regulation. However, Wölfle et al. showed that the spread of dilations induced by endothelium-dependent stimuli was impaired to a similar degree in Cx40KICx45 mice than in Cx40 KO mice, demonstrating that this function of Cx40 cannot be rescued by Cx45 [164]. Since blood pressure is also regulated by renin secretion, a mechanism which is also disturbed in Cx40 KO mice, Schweda et al. assessed the ability of Cx45 to restore this function [165]. They showed that Cx40KICx45 animals possess normal plasma renin concentrations as well as normal number of renin producing cells, demonstrating that the control of renin secretion was normalized by Cx45 expression (Table 3). However, replacement of Cx40 by Cx45 could not restore the capability to recruit renin-expressing cells after stimulation [166]. These data indicate that the marked hypertension phenotype observed in Cx40 KO mice is mainly caused by the dysfunction in renin release and just to a minor extent by the impairment in the endothelial spread of dilations.

Cx43 is the predominant gap junction protein in smooth muscle cells, but is less abundant in endothelial cells. Its role in regulation of arterial tone as well as in the modulation of renin secretion has been investigated in Cx43KICx32 mice. Lopez et al. showed that the replacement of Cx43 by Cx32 attenuates aortic endothelium-derived hyperpolarisation factor (EDHF)-mediated relaxation, whereas aortic responses to endothelium-independent agonists were not modified [167]. They concluded that EDHF-mediated relaxations are at least partially dependent on Cx43 channels and that this function cannot be compensated by Cx32 expression. It is, however, unclear if the lack of response to endothelium-independent agonists indicates that Cx43 channels are not essential for these responses or that Cx32 can functionally replace Cx43 in this function [167]. Haefliger et al. used Cx43KICx32 mice to investigate the role of Cx43 during hypertension [168]. In an earlier study they reported that experimentally induced renin-dependent hypertension increases the expression of Cx43 in a tissue-specific manner. Furthermore, Liao et al. [127] reported that conditional endothelium-specific KO of Cx43 causes hypotension, albeit no changes of blood pressure have been found in a second study performed on other mice with endothelial Cx43 deletion [126]. Homozygous Cx43KICx32 mice displayed reduced renin levels and failed to show any increase in the blood pressure or renin levels after experimental induced hypertension.

Taken together, the knock-in studies demonstrate that in the vascular system Cx45 can replace Cx40 in the regulation of renin release but not in its function in endothelial signal conductance. In addition, unique functions of Cx43 seem to be necessary for the modulation of the renin secretion that cannot be fulfilled by Cx32 (Table 3; [168]).

8.5. Retina

In the retina at least four different connexin isoforms are expressed: Cx30.2, Cx36, Cx45 and Cx57 (Table 2; [94,153]). Cx36 is the predominant gap junction protein in the retina and is expressed in most subtypes of retinal neurons [153]. Cx45 expression has been described in subpopulations of bipolar and ganglion cells [39]. Initially, AII amacrine cells and ON cone bipolar cells were thought to be coupled by heterotypic channels composed of Cx36 and Cx45 [169], but recent findings indicated that Cx36 stabilizes Cx45 in the plaques [36]. This assumption is supported by the fact that both Cx36 KO and conditional Cx45-deficient mice display a similar reduction in the b-wave in the electroretinogramm (ERG) as well as severely impaired glycin transfer from AII amacrine cells to ON cone bipolar cells [153]. To investigate if Cx36 and Cx45 have similar functions in the retina, a knock-in mouse line with neuron-directed replacement of Cx45 by Cx36 has been generated [40]. As mentioned above, Cx45 channels possess the highest voltage sensitivity among all connexins and high cation selectivity and pH sensitivity [150–152]. In contrast, Cx36 channels exhibit a slightly lower unitary conductance and significant lower voltage sensitivity. Heterotypic or bi-homotypic channels composed of Cx45 and Cx36 connexons could have unique properties, which may play a crucial role in the retinal signal transmission. ERG and neurotransmitter coupling analysis performed on Cx45KICx36 mice revealed neither defects in transmission of visual signals nor in the metabolic coupling, providing strong evidence that Cx36 can functionally compensate for the loss of Cx45 in retinal neurons [40].

8.6. Lens

In the mammalian lens the occurrence of three different connexin isoforms has been described, which are expressed in distinct spatial and temporal patterns. Cx50 is present in all cells of the lens, whereas the expression of Cx43 seems to be restricted to the lens epithelium and that of Cx46 to the lens fibers (Table 2; [96]). Since the lens is an avascular structure, the gap junctional coupling presents a critical mechanism for providing cells located in the interior of the organ with metabolites and signal molecules [170]. In order to distinguish between unique and shared functions of Cx46 and Cx50 in the lens, White generated a knock-in mouse line, in which the coding region of Cx50 was replaced by that of Cx46 [122]. The two Cxs belong to the same phylogenetic group (α-group; Table 3). Cx50 channels exhibit higher unitary conductance than Cx46 channels (220 vs. 140 pS) and higher sensitivity to transjunctional voltage. The sensitivity to acidification of the two channels is similar [96]. Hemichannels composed of Cx46 are able to form functional heterotypic channels with both Cx43 and Cx50, whereas no functional heterotypic channels between Cx50 and Cx43 can form (Table 1). In addition, heteromeric Cx46/Cx50 hemichannels with unique electrophysiological properties have been described in mature lens fiber cells

[171,172]. Cx50-deficient mice display reduced ocular growth and cataracts [144]. Replacement of Cx50 by Cx46 prevented cataracts but the growth deficiency remained unchanged. In a subsequent work, Martinez-Wittinghan et al. showed that the coexpression of Cx46 and Cx50 at the Cx50 locus in heterozygous knock-in mice rescued ocular growth but produced dominant cataracts, independent of whether the native Cx46 locus was wild-type ($Cx46^{+/+}$ $Cx50^{+/Cx46}$), heterozygous ($Cx46^{+/-}$ $Cx50^{+/Cx46}$), or null ($Cx46^{-/-}$ $Cx50^{+/Cx46}$) [173]. The authors demonstrated that in heterozygous knock-in lenses the biochemical coupling and not the levels of ionic gap junctional coupling were reduced [173]. Interestingly, double heterozygous KO mice ($Cx46^{+/-}$ $Cx50^{+/-}$) displayed normal lens growth and clarity, whereas knock-over mice, in which native Cx46 was deleted and homozygously knocked into the Cx50 locus ($Cx46^{-/-}$ $Cx50^{Cx46/Cx46}$), showed lens growth deficiency but were free of cataracts [173]. Taken together, these findings show that Cx46 can substitute Cx50 in its function in the maintenance of lens transparency, but unique properties of Cx50 were required for proper lens growth and development [98]. In addition the data suggest that lens clarity and growth are not regulated by the magnitude of ionic coupling but rather by unique modes of biochemical gap junctional communication which in turn are modulated by the composition of the gap junction channels [173]. Newborn Cx43 KO mice displayed functional changes in the lenses similar to those observed at early stages in cataract development [174]. Consistent with this observation, cataracts have been found in about 10% of the Cx43KICx32 mice, but not in Cx43KICx40 or Cx43KICx26 mice [121,135]. These findings suggest that Cx40 and Cx26 can compensate for the loss of Cx43 in the lens, whereas Cx32 can just partially replace Cx43 function (Table 3).

8.7. Brain

Redundancy and compensation among connexins in the CNS has not been studied with knock-in mice, but rather by comparing single and double knockout mice of connexins expressed in neural cell types.

8.7.1. Astroglial connexins

Due to early postnatal death of Cx43 knockout mice [123], the function of astrocytic Cx43 was initially mainly studied in neonatal Cx43-deficient astrocyte cultures [175–177], where Cx43-deficient cultures showed impaired growth, decreased saturation density and strongly decreased tracer-coupling [45,175,177,178], the latter suggesting that indeed Cx43 was the major gap junction protein in cultured astrocytes. Yet other connexins such as Cx26, Cx30, Cx40, Cx45 and Cx46 were detected in astrocytes in vitro [177,179]. Interestingly, the expression of Cx30 seemed to be influenced and furthered by the presence of neurons co-cultured with astrocytes [180]. Possibly, compensation by other Cxs in Cx43-deficient astrocyte cultures accounted for the residual electrical and dye coupling observed [175–177]. A decreased dye uptake upon metabolic inhibition was also noted which is probably mediated by HC function either directly exerted by Cx43 or at least dependent on the presence of Cx43 [181].

The gross morphology of the brain was normal in Cx43-deficient neonatal mice [177]. Subsequently, the Cre/loxP system allowed the investigation of Cx43 function in the CNS of adult mice. Such mice also showed normal gross morphology [31]. Indeed, deficits in neuronal migration observed in Cx43 deficient mice seem to be compensated during development [182]. Another study described severe morphological changes in the brain of mice lacking Cx43 in astrocytes, which indicated a role for Cx43 during neurodevelopment, but the phenotype occurred only in a defined genetic background, i.e. 129SVEV [183]. Consistently, such dramatic morphological changes were not observed in mice lacking both Cx43 and Cx30 [32,33].

In mice lacking astrocytic Cx43, residual gap junction coupling of about 50% was observed probably due to the expression of Cx30 [31–33]. Mice lacking Cx43 in astrocytes exhibited a compensatory upregulation of Cx30 protein levels [31,120]. Consistently, in the same area (stratum radiatum of the hippocampal CA1 region) mice lacking Cx30 showed a 20% decrease in gap junctional coupling [34]. Interestingly, lack of Cx30 does not lead to a compensatory increase in Cx43 expression [34]. In mice lacking both Cx43 and Cx30, astrocytes were indeed devoid of tracer coupling [32,33]. Cx30 channels exhibit higher unitary conductance than channels composed of Cx43 (179 vs. 100 pS) but are more sensitive to transjunctional voltage [184]. In contrast to Cx43, Cx30 seems not to be regulated by phosphorylation. Cx43 and Cx30 can form functional heterotypic channels. In addition, astrocytes communicate with oligodendrocytes through heterotypic Cx43/Cx47, Cx30/Cx47 and Cx30/Cx32 but not through Cx43/Cx32 channels (Table 1; [45]).

Mice lacking Cx43 showed an increased velocity of spreading depression [31] indicating that astroglial Cxs in gray matter may be involved in spatial buffering of potassium ions released into the extracellular space (ECS) by neuronal activity, since critical potassium ion levels in the ECS required for spreading depression are achieved much faster. Astrocytic connexins are indeed involved in spatial buffering since DKO mice deficient for both Cx43 and Cx30 showed a prolonged increase and delayed recovery from stimulus-induced increases in extracellular potassium ions concentrations and a lowered threshold for generation of spontaneous and induced epileptiform discharges in acute hippocampal slices [32]. Recent observations in DKO mice indicate that Cx43 and Cx30 in astrocytes help to provide metabolites to neurons for their sustained activity, which is a function that would oppose the role of astrocytic connexins in spatial buffering [33]. Another recent study on DKO mice from the same lab showed that astrocytic connexins decrease neuronal activity by clearance of glutamate and potassium from the extracellular space [115], confirming earlier findings [32].

On the behavioral level, mice lacking either Cx43 in astrocytes or Cx30 have shown no or only mild behavioral impairments based on short and long term memory tests [185,186]. Mice lacking Cx43 in astrocytes showed increased locomotory activity in the open field and impaired motor capacities on the rotarod [31,185]. Cx30 deficient mice showed changes in the open field test that are opposed to those observed in mice lacking astroglial Cx43 and were linked to increased emotionality and decreased exploratory activity, while motor capacities were not impaired [186]. Cx43/Cx30 double KO mice showed a deficit in novel object recognition indicating impairment of spatial memory exceeding that of single deficiencies, while the motor impairment observed was similar to that found in Cx43 deficient mice [117].

Cxs expressed in radial glia have a strong impact on neurogenesis, and disruption of Cxs led to deficits in neuronal migration that were mainly attributed to adhesive functions of Cxs [75,76], even though the role of the C-terminal cytoplasmic tail of Cx43 in this process is under debate. Radial Glia-like cells in the adult dentate gyrus which are neural stem cells express Cx43 and Cx30 and are partially tracer-coupled. Both Cxs are required for proliferation of RG-like cells which give rise to neurons. Mice lacking Cx43 and Cx30 in astrocytes show an almost complete inhibition of proliferation in the subgranular zone, the neurogenic niche of the adult hippocampus, and a strong decrease in the number of new granule cells in the dentate gyrus. These findings suggest the requirement for gap junction coupling of RG-like cells for intact neurogenesis in the adult brain [116].

Altogether, Cx43 and Cx30 seem to cooperate in the interastrocytic coupling [31–34], and the upregulation of Cx30 protein in Cx43 deficient mice [31,120] may be one of the rare examples of compensatory expression changes observed in the connexin family. The finding of an increased velocity of spreading depression in the astrocyte-directed Cx43 deficient mice which needs elevated extracellular potassium concentration for its propagation [31] together with clear deficits in spatial buffering of potassium ions in Cx43/Cx30 DKO mice [32] indicated that both connexins serve the same

function and that Cx30 may have exerted residual spatial buffering function in the astrocytic Cx43 KO mice. If Cx43 and Cx30 share similar functions in RG-like cells remains to be determined.

8.7.2. Oligodendrocytic connexins

Three oligodendrocytic Cxs are known (Table 2) and knockout mice have been generated: Cx29 deficient mice did not show any signs of myelin abnormalities, gross anatomical changes or motor deficits [91,187]. Aged mice lacking Cx32 showed signs of a progressively demyelinating peripheral neuropathy starting around 3 months of age [188,189]. The phenotypical changes in the CNS were rather mild [190]. By contrast, lack of Cx47 led to pronounced myelin vacuolation [118,119]. Cx32 and Cx47 display similar unitary conductance and sensitivity to transjunctional voltage [148,191]. Cx32 has been shown to be regulated by phosphorylation, but there are no reports of Cx47 phosphorylation. Cx32 and Cx47 hemichannels cannot form functional heterotypic channels (Table 1; [45]). Mice lacking both Cx47 and Cx32 developed an action tremor before death around postnatal week 6. In addition, the vacuolation of the central white matter was much more severe when compared to that evoked by Cx47-deficiency alone [118,119]. This indicated that Cx32 and Cx47 may have a similar function.

8.7.3. Connexins in inhibitory interneurons

Cx36 was unambiguously described in inhibitory interneurons with double stainings for embedded reporter genes and cell type-sprecific markers [86,192,193]. Cx36 deficiency caused impairments in sharp waves, high-frequency ripple oscillations [194] and in 30–80 Hz gamma rhythms [87]. Also, weakened and spatially more restricted rhythmic inhibitory potentials were observed [86], which may be related to short-term memory impairments observed in these Cx36 deficient mice [195,196]. More recently, Cx30.2 was described in inhibitory interneurons, again using embedded reporter genes in Cx30.2 deficient mice [88] and showed frequent colocalization with Cx36, but no phenotypical alterations compared to controls. Common features of Cx30.2 and Cx36 channels are low unitary conductances and weak gating sensitivities to transjunctional voltage [37,47]. The two connexins differ in their sensitivity to pH: while the activity of Cx30.2 channels decreases at low pH, Cx36 channels are inhibited by high pH. Cx36 cannot form functional heterotypic channels with any other connexin isoform, whereas Cx30.2 has been shown to form heterotypic channels with Cx40, Cx43 and Cx45 (Table 1; [47]). An additional difference between Cx30.2 and Cx36 might be the regulation by phosphorylation: Cx36 but not Cx30.2 has been shown to be a phosphoprotein. Compensation among connexins expressed in inhibitory interneurons remains to be determined in doubly deficient mice.

8.7.4. Connexins in microglia

Cx43 was described in microglial culture following stimulation with interferon-γ, and LPS or TNF-α [197] and in microglial cultures treated with *Staphylococcus aureus* toxin [198], whereas different stimulation paradigms did not induce Cx43 expression in microglia [199–202]. In addition, Cx36 has been described in microglial cells [203,204]. Indirect evidence indicated expression of Cx32 in microglia in one report [205].

9. Conclusion and outlook

So far, the great majority of connexins which have been targeted by homologous recombination showed at least limited phenotypical alterations, indicating that the large number of connexins has specialized functions and is required for tailor-made intercellular communication in various tissues and organs. The use of doubly deficient mice allows one to examine aggravation of phenotypical alterations, i.e. whether two connexins share a similar function. Compensatory upregulation of connexins sharing similar functions gives further insight into limited redundancy. Functional replacement among connexins in knock-in mice was limited to a set of phenotypical parameters. For example, Cx36 could functionally replace Cx45 only in retina but not in the heart [40] and Cx32 or Cx40 could replace Cx43 during heart morphogenesis and in heart function, but not in the reproductive system [121]. Nevertheless, the generation of new coupling compartments in knock-in mice may have been responsible for some of the phenotypical alterations observed. So far, the results obtained from deficient mice and from knock-in mice indicate a limited extent of functional redundancy and compensation.

9.1. Conditional knock-ins to differentiate redundancy and compensation in various tissues

Conditional replacement of one connexin by another will allow us to separately investigate the impact of replacement, i.e. rescue of phenotypical alterations or complications due to aberrant trafficking of molecules. For example, conditional replacement of Cx26 by Cx32 rescued the hearing deficit of Cx26 deficient mice [147]. By contrast, deletion of Cx26 or heterozygous replacement of Cx26 by Cx32 in the embryonic skin or in all cells of the body led to severe lymphedemas due to the absence of the dermal lymphatic capillary system [146]. Cx26 KO mice died at 10,5 dpc due to a placental deficit in nutrient transport which led to a severe growth retardation [124]. Heterozygous expression of Cx32 in Cx26 expressing cells led to a 90% reduction in Cx26 expression in the placental labyrinth, but obviously Cx32 still allowed nutrient transport across the placenta [146]. Thus, replacement led to a rescue in the inner ear and in the placenta, while in the lymphatic system replacement led to a fatal complication.

9.2. Forced expression of connexins

Cx43 is strongly expressed in astrocytes. What would happen, when we induced ectopic expression of Cx43 in neurons via the tetOff system of conditional mutagenesis [206,207]? Would this allow coupling between neurons and astrocytes, or would other factors, for example the extracellular matrix or simple physical separation of neurons and astrocytes preclude such coupling? NG2 glial cells are not coupled by gap junctions [32,208]. Would forced expression of Cx43 in NG2 glial cells induce them to become part of the astrocytic coupling compartment?

9.3. Transgenic mice to differentiate the mechanisms by which connexins act

A number of functions can be exerted by connexins such as intercellular coupling, hemichannel activity, adhesion, or control of gene expression (see also Section 4). It remains to be determined which of those modes of connexin action are most relevant and which are actually compensated. Many connexin mutants have been generated and described for Cx43, which is abundantly expressed in astrocytes and in neural stem cells of the CNS. For this reason, we focus on Cx43 mutants and the CNS in the following: Astrocyte-directed Cx43-deficient mice [31] lack a major astrocytic Cx and thus none of the known connexin functions can be exerted. A number of Cx43 mutant proteins have been generated to differentiate the various modes of Cx43 action. The Cx43K258Stop mouse lacks the C-terminal tail (implicated in adhesion and control of gene expression) but the mutant protein allows, at least in transfected Hela cells, robust intercellular coupling [209], even though a decreased coupling rate has been described in primary astrocyte cultures [210]. If interastrocytic coupling is mediated by this mutant also in the hippocampus *in situ*, where the astrocytes have a much smaller contact area compared to cultured cells, remains to be determined. Another mutant Cx43 (Cx43C61S) has been described, which lacks both adhesion and

intercellular coupling [211]. Both mutations have been used to study the role of Cx43 mediated adhesion in early neurogenesis [75,76]. Another group of Cx43 mutants lacks channel activity due to point mutations but constitutes a full length protein to mediate the functions attributed to the C-terminus, such as adhesion and control of gene expression. One mutation, called Cx43G138R was found in some patients with oculodentodigital dysplasia [111]. Such a conditional point mutation of Cx43 was generated in mice [25,113]. This particular mutant exhibited lack of intercellular coupling but preserved HC activity. Another channel-dead mutant (called Cx43T154A) was found to form robust gap junction plaques at the EM level [212]. This arsenal of constructs and mice may help to decipher the most relevant mode of connexin action.

Future work with conditional gene replacements, forced expression of connexins and the investigation of various connexins mutants in which one specific aspect of connexin function is altered (channel function, hemichannel function, adhesion or control of gene expression) are likely to gain new insights into functional compensation and redundancy among connexins.

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