Gap junctions (Gj) form conduits between adjacent cells that are composed of connexin (Cx) protein subunits and allow direct intercellular communication. To date, the connexin gene family comprises 20 members in the mouse and 21 members in the human genome, 19 of which can be grouped as sequence-orthologous pairs. The structure of connexin genes is relatively simple. An untranslated exon 1 is separated by an intron of different length from exon 2, containing the uninterrupted coding region and the 3′-untranslated region (3′-UTR). However, in some connexin genes, the untranslated regions and the reading frame are spliced. Among the known “cardiovascular” connexins, Cx37 and Cx40 were demonstrated to be functionally expressed in mouse and human endothelial cells and Cx40, Cx43 as well as Cx45 in cardiomyocytes of both species. Functional properties, like permeabilities, charge selectivity and unitary conductivity were investigated after directed expression of these connexins in cultured cell lines or paired Xenopus oocytes. Targeted deletion of their coding sequence in the mouse genome allowed study of the biological relevance of Cx37, Cx40, Cx43 and Cx45 with regard to cardiovascular morphology and function. After ablation of Cx37 or Cx40, mice were viable and could be used to study defects in the adult cardiovascular system but loss of Cx43 or Cx45 led to neonatal or embryonic lethality, respectively. Conditional and cell-type specific deletion of both connexins in the heart or blood vessels can help to overcome this obstacle. As yet only little is known about mutations in the human genes for Cx37, Cx40, Cx43 and Cx45. Thus, a profound comparison between human and mouse phenotypes is not yet possible.

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### 1. Structure of gap junctions and connexin genes

Among the various cell contact mediating protein complexes like tight junctions, desmosomes and cell adhesion molecules, gap junctions (Gj) allow direct communication between adjacent cells. Various compounds up to a molecular mass of 1000 Da can be exchanged by passive diffusion through gap junctional conduits, i.e. metabolites, ions, second messengers, water and electrical impulses [1,2]. Recent reports suggest that hemi-channels can also function under certain conditions, allowing cellular release and uptake of diffusible molecules as summarized by Refs. [3,4].

Six connexin (Cx) subunits can form a hemi-channel (connexon) in the plasma membrane that can dock to another hemi-channel in the plasma membrane of an adjacent cell, in order to assemble a complete gap junctional channel (Fig. 1A). Cell adhesion proteins may contribute to the docking and assembling process. The connexin subunit is a four-transmembrane spanning protein (Fig. 1B), harboring two extracellular loops, one cytoplasmic loop and one cytoplasmatic N- as well as C-terminal region. Within both extracellular loops, the order of three cysteine residues is highly conserved (except for Cx31): first loop: [C–X6–C–X3–C] and second loop [C–X5–C–X3–C]. Opposing cysteines in both loops were suggested to form disulfide-bridges stabilizing the loops during the docking of two connexons (Fig. 1A and see Ref. [1]). Innexins form another family of gap junctional proteins that are only expressed in invertebrates (D. melanogaster or C. elegans) and do not show any sequence similarity to connexins [5]. Recently, genes that show some sequence identity to innexins were also discovered in the genome of higher vertebrates therefore designated as pannexins [6].
mammalian cell types, it has often been found that more than one type of connexin is expressed. For example, Cx36, Cx45 and Cx57 have been detected in mouse neurons, whereas Cx26 and Cx32 occur in hepatocytes (cf. Ref. [7]). Apparently, different compositions of connexin isoforms can contribute to heteromeric and heterotypic gap junction channels (cf. Ref. [1]), which may lead to regulatory variety. Two different nomenclatures are in use: (i) Cx are designated according to the species from which they were derived and their theoretical molecular mass (in kDa). For example, mCx32 means mouse connexin 32, a protein of about 32 kDa (cf. Ref. [8]). (ii) On the other hand, connexins can be divided into subgroups (α, β or γ) with respect to their extent of sequence identity and length of the cytoplasmic loop [9]. Connexins are then abbreviated with “Gj” for gap junction and numbered according to the order of discovery. For example, mCx43 was the first connixin of the α-group (Gja1) and mCx32 was the first connixin of the β-group (Gjb1). There are, however, still some discrepancies between both nomenclatures, which should be clarified when all connexin genes, at least in mouse and man, have been unequivocally identified (see Table 1).

To date, the family of connexin genes comprises 20 members in mouse and 21 genes in the human genome, 19 of which can be grouped as orthologues pairs (Table 1). Assuming that both genomes are completely sequenced and annotated, there are, however, connexin genes that occur only in the mouse (mCx33) or the human genome (hCx25 and hCx59). The biological reason for this is not known. Furthermore, orthologous connexins may not necessarily be expressed in the same tissue or cell type, since hCx30.2, hCx31.9, hCx40.1 and hCx62 were recently found to be additionally expressed in the human heart, whereas their mouse orthologues mCx29, mCx30.2, mCx39 and mCx57 are not, as deduced from Northern blot results [10]. Even the transcript sizes of these orthologous connexin pairs are different [10]. The chromosomal location of mouse and human connexin genes is different in man and mouse but does not seem to be randomized. Connexin genes tend to occur in clusters on certain chromosomes, which were maintained during evolution of mouse and man for the syntenic Cx30.3, Cx31, Cx31.1 and Cx37 genes and the syntenic Cx26, Cx30 and Cx46 genes (Table 1). The general genomic structure of connexin genes is relatively simple. A 5′-untranslated region (5′-UTR) designated as exon 1 is separated from the complete connexin coding region and the subsequent 3′-UTR located on exon2 (Fig. 2A). But many exceptions from this simple scheme have already been identified, which can be divided into two categories: First, different 5′-UTRs can be spliced in a consecutive or alternate manner. This might reflect variations of the transcription pattern due to alternate promoter usage, altered mRNA stability and/or translational efficacy (Fig. 2B). Second, the coding region can be interrupted by introns. This affects the following connexin protein sequences of mCx36, hCx36, mCx39, hCx40.1 and mCx57, but it needs to be clarified yet whether these connexin genes indeed express different transcript isoforms (Fig. 2C).

Concerning mouse and human heart, there are many studies characterizing the expression (focusing on either
transcription or translation) of the four α-group connexins Cx37, Cx40, Cx43 and Cx45 in various cell types. Cx40, Cx43 and Cx45 were found to be expressed between cardiomyocytes, whereas Cx37 and Cx40 are present between endothelial cells (cf. Ref. [11]). These connexin genes are highly conserved in both species with regard to their nucleotide- and protein sequence similarity, chromosomal location, transcript size and transcriptional pattern (Table 2). This relatively high sequence similarity usually allows comparison of functional aspects between pairs of these connexins in mouse and man. But in the case of hCx30.2, hCx31.9, hCx40.1 and hCx62, it will become much more difficult to study their functional impact, since no counterparts are known that are expressed in mouse heart.

2. Strategies to analyze the function of connexins

Different approaches have been established to study functional properties of connexins. An often used method is the transfection of cultured cell lines (i.e. HeLa or N2A cells) with connexin coding DNAs or the injection of *Xenopus* oocytes with connexin coding RNAs. After verification of stable or transient expression, dye and tracer injections revealed specific permeability and charge selectivity of the corresponding gap junction channels. HeLa cells, stably transfected with mouse Cx37, Cx40, Cx43 and Cx45 coding DNAs were demonstrated to be permeable to either the negatively charged Lucifer yellow (443 kDa; 2-) and the positively charged neurobiotin (287 kDa; 1+) [12]. Furthermore, it was corroborated that Cx40 hemi-channels were not able to couple with Cx43 hemi-channels in a heterotypic manner [12]. The unitary conductances, as determined in connexin transfectants of cultured mammalian cells, are listed in Table 2. Interestingly, Cx45 channels exhibit unusual high voltage sensitivity after application of various transjunctional voltages [13]. This might reflect to the dual expression and function of Cx45 on the one hand in cardiomyocytes/endothelial cells of the cardiovascular system and, on the other hand, in neurons of the central nervous system [14,15]. The biophysical aspects of connexin channels will be discussed elsewhere in this Spotlight Issue.

Targeted deletion of different connexin genes in the mouse genome yielded evidence for their biological relevance. Today, 13 out of the 20 identified mouse connexin genes have been “knocked out”, in several cases by replacement of the corresponding coding DNA with a reporter gene (cf. Ref. [7]). For example, ablation of Cx26 or Cx30 protein in the cochlea both leads to hearing loss [16,17], which corresponds to nonsyndromic deafness in patients carrying mutations in their Cx26 or Cx30 coding regions (cf. Ref. [18]; http://www.crg.es/deafness). A similar correlation exists for Cx46 and Cx50, which are expressed in lens fiber cells of human and mouse eyes. Null mutation in the mouse [19] as well as single base mutations in the human orthologues [20,21] both lead to connexin specific cataract formations. However, these nearly perfect matches of mouse and human phenotypes appear to be exceptions, since point mutations can have transdominant negative effects, not necessarily similar to a complete loss of function in connexins.

Table 1

<table>
<thead>
<tr>
<th>Mouse connexin</th>
<th>GJ</th>
<th>chr</th>
<th>Human connexin</th>
<th>Chr</th>
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<th>Chr</th>
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<td>1</td>
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<td>hCx30</td>
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<td>hCx30.3</td>
<td>17</td>
<td>Gjb4</td>
<td>hCx30.3</td>
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<td>Gjb5</td>
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</tr>
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<td>Gja9</td>
<td>hCx36</td>
</tr>
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<td>6</td>
<td>Gja10</td>
<td>hCx62</td>
</tr>
</tbody>
</table>

This table reflects the current state of the sequence information available from the NCBI database (http://www.ncbi.nlm.nih.gov/genomes/static/euk_g.html). Note: mCx23 [33] and hCx23 are gene and protein predictions from the database, which show only two cysteine residues in their presumed cytoplasmic loops. Cx31.3 is the only transcript isoform known of the hCx30.2 gene [34]. chr, chromosomal assignment.

Fig. 2. Structures of mouse connexin genes. (A) Common gene structure. The 5′-untranslated exon 1 (5′-UTR) is separated by an intron of variable length from exon 2 harboring the complete coding region (shaded box) and the subsequent 3′-UTR. (B) Different 5′-UTRs can be spliced alternatively and/or consecutively to that exon carrying the coding region. (C) In mCx36, hCx36, mCx39, hCx40.1 and mCx57, the coding region is spliced (cf. Ref. [7]).
from female infertility [26]. Cx40(−/−) and fertile except for the Cx37(−/−) females, which suffer from female infertility [26]. Cx40(−/−)-deficient mice showed a tendency towards arrhythmias [27,28], whereas the Cx37(−/−)-deficient mice were unaffected with regard to heart function [26] (see Table 2). Interestingly, the Cx37(−/−)/Cx40(−/−) double deficient mice also died around birth due to vascular abnormalities with pronounced dilatation of blood vessels [29], since both connexins are co-expressed in endothelial cells. In the meantime, other studies have been started to investigate whether downregulation of either Cx43 or Cx45 in the hearts of Cx40-deficient animals aggravated the already existing arrhythmias (Table 2). Therefore, Cx40(−/−) mice were crossed with mice heterozygous for Cx43 or Cx45. These Cx40(−/−)/Cx43(+/−) and Cx40(−/−)/Cx45(+/−) mice (Krüger et al., unpublished experiments) both showed abnormal cardiac conduction and morphogenesis leading to neonatal death in most of these animals. Thus, the 50% reduction of Cx43 or Cx45 expression (in heterozygous mice) crossed into the corresponding Cx40(+/+)/Cx43(+/−) and Cx40(+/+)/Cx45(+/−) background aggravated the Cx40(−/−)-background. This dose-dependent effect is not obvious in the corresponding Cx40(+/+)Cx43(+/−) and Cx40(+/+)Cx45(+/−) controls [30] (Krüger et al., unpublished experiments). To determine the redundancy between different members of the connexin gene family, we replaced Cx43 with either Cx40 or Cx32 in corresponding “knock-in” mice (Cx43KICx40 and Cx43KICx32) [31]. Both connexins are able to largely restore heart malformations observed in Cx43(−/−) neonates [23] and thus sufficient to circumvent neonatal lethality but the resulting mice suffer from infertility. Thus, the different members of the connexin gene family apparently fulfill unique functions in certain cell types but can largely compensate each other in other cell types.

Table 2

<table>
<thead>
<tr>
<th>Protein sequence identity mouse vs. human*</th>
<th>Cx37</th>
<th>Cx40</th>
<th>Cx43</th>
<th>Cx45</th>
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<tr>
<td>Transcript sizesb</td>
<td>1.7 kb</td>
<td>3.5 kb</td>
<td>3.0 kb</td>
<td>2.2 kb</td>
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<tr>
<td>Cell types with major expressionb</td>
<td>endothelial cells</td>
<td>cardiomyocytes</td>
<td>ubiquitous</td>
<td>endothelial cells, neuronsc, smooth musclec, cardiomyocytes</td>
</tr>
<tr>
<td>Cell-type specific expression in heartf</td>
<td>endothelial cells</td>
<td>cardiomyocytes</td>
<td>cardiomyocytes</td>
<td>cardiomyocytes</td>
</tr>
<tr>
<td>Unitary conductance</td>
<td>300 pSf</td>
<td>200 psf</td>
<td>60–100 pSb</td>
<td>20–40 pSf</td>
</tr>
<tr>
<td>Phenotype(s) of Cx-deficient mice</td>
<td>female sterilityj</td>
<td>atrial arrhythmiasa</td>
<td>heart malformationsl</td>
<td>defective vascular developmentm</td>
</tr>
<tr>
<td>Human hereditary disease(s)</td>
<td>association with atherosclerosisa</td>
<td>n.a.</td>
<td>oculodentodigital dysplasia (ODDD) syndactyly type IIIn</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

6: Elfgang et al. [12], n.a., not analyzed.

91% 85% 98% 98%
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References