# REVIEWS

## Diversity and properties of connexin gap junction channels

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***Key words:*** *connexins; connexons; gap junction channels; structure; function.*

***Summary.*** *Gap junction channels are composed of two apposing hemichannels (connexons) in the contiguous cells and provide a direct pathway for electrical and metabolic signaling between adjacent cells. The family of connexin genes comprises 20 members in the mouse and 21 genes in the human genome. Connexins are expressed in all tissues except differentiated skeletal muscle, erythrocytes, and mature sperm cells. Various tissues express more than one type of connexins; therefore, homotypic, heterotypic, and heteromeric gap junction channels may form between cells. In this article, we briefly review basic gating and permeability properties of homotypic and heterotypic gap junction channels as well as recent achievements in the research of their regulation by transjunctional voltage, intracellular calcium, pH, and phosphorylation.*

### Families of gap junction proteins

Gap junction (GJ) channel proteins are subdivided into three families: innexins, pannexins, and connexins (1–3). Innexins are expressed in protostomes, while connexins and pannexins have been identified in deuterostomes. Pannexins have no homology with connexins and share 20% homology with innexins. All these proteins have four alpha helical transmembrane domains (M1–M4), intracellular N- and C-termini, two extracellular loops (E1, E2), and a cytoplasmic loop (I1) (4–6) (Fig. 1).

Six innexin/pannexin/connexin subunits form a hemichannel (innexon/pannexon/connexon). The presence of cysteine residues in the extracellular loops is critical for gap junction formation by two apposing hemichannels in contiguous cells. Connexins and innexins possess 3 or 2 cysteines in each extracellular loop, respectively, and form hemichannels and gap junction channels. The pannexins contain 2 cysteines in each extracellular loop; however, glycosylation of extracellular loops and protein in general precludes formation of functional gap junction channels (7). In that way, pannexins most likely form only nonjunctional channels and play paracrine role releasing ATP or glutamate into extracellular space and uptaking certain membrane-impermeant molecules into cells (8, 9). In contrast to connexin-based gap junctions, innexin-based channels are sensitive to membrane potential, closing with depolarization (10).

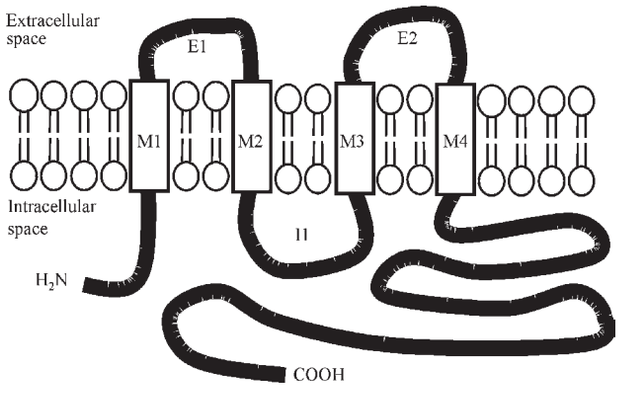


*Fig. 1.* Topological model of a connexin
Connexins, as well as pannexins and innexins, possess four transmembrane domains (M1–M4), intracellular N- and C-termini, two extracellular loops (E1–E2) and one intracellular loop (I1)

### Structure of connexin gap junction channels

GJ channels provide a direct pathway for electrical and metabolic signaling between adjacent cells (11–14). The family of connexin (Cx) genes consists of 20 members in the mouse and 21 genes in the human genome. hCx25, hCx59 occur only in the human genome and mCx33 only in the mouse genome. Also, unusual Cx23 with four instead of six cysteine residues in its two extracellular loops was identified in the mouse (15, 16). All other genes are orthologous pairs. Connexins are named by their molecular mass within the range of 23–62 kDa. For example, Cx30 molecular mass is 30366 Da, Cx43 – 43036 Da. A gap junction channel pore is approximately 100–150 Å in length and 12.5 Å in width with a 20 Å gap between contiguous cells. A single GJ channel is formed by stable, noncovalent interactions of two hemichannels located

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in the plasma membranes of adjacent cells via H-bonds between extracellular loops of their connexins. The specialized domains in the intracellular loop and the carboxyl terminus are responsible for specific channel properties of different connexins, including unitary conductance, pH dependence, voltage dependence, and selective permeability to small molecules up to 1 kDa. Molecular mass does not determine the single channel conductance. For example, Cx40 forms channels with larger conductances (150–160 pS) than Cx43 channels (90–115 pS) (17–20). In contrast, Cx45 channels exhibit a much lower main state conductance of ~30 pS. The process of docking of apposed connexons is poorly understood. Ultrastructurally, in freeze-cleaved replicas, GJ plaques can be seen to consist of tightly clustered GJ channels (~10 000/$\mu m^2$) (21, 22). Recently, crystal structure of human Cx26 GJ channel was demonstrated at 3.5 Å resolution (23). In the absence of plaques, there is no electrical coupling between contiguous cells. Plaques exceeding several hundred channels always confer coupling, but only a small fraction of channels is functional at any given time (24). Recently, a fusion protein consisting of Cx43 and green fluorescent protein (GFP) attached to its carboxyl terminus (Cx43-GFP) was transfected into mammalian cells and was shown to be transported to the cell surface and assembled into functional GJs (25). GJ plaques and unapposed hemichannels imaged in our laboratory are shown in Fig. 2.

Microfilaments and microtubules may be involved in turnover mechanisms and trafficking of Cxs to, within, and from the cell membrane (see (26) for more details). Formation of gap junctions requires appropriate cell adhesion, especially that mediated by $Ca^{2+}$-dependent molecules, cadherins (27). Most connexins are cotranslationally integrated into the endoplasmic reticulum membrane. The oligomerization of six connexins into a hemichannel starts in the endoplasmic reticulum and ends in the trans-Golgi network (28–30). Then vesicles containing connexons are transported along microtubules and actin filaments to the cell membrane and recruited to the outside of existing plaques (31). Moreover, recently it has been shown that tethering of the microtubule plus ends at the adherens junction promotes delivery of connexin hemichannels directly to the cell-cell border (32). Internalization of GJ channels starts from the middle of the plaque via vesicular structures called “annular junctions” (33) that are rapidly degraded by lysosomal and proteosomal pathways (34–36). Gap junction biosynthesis and assembly are strictly regulated, and intercellular junctions have a short half-life time of only a few hours (35, 37). The continuous synthesis and degradation of connexins through these mechanisms may provide for the quick adaptation of tissues to changing environmental conditions.

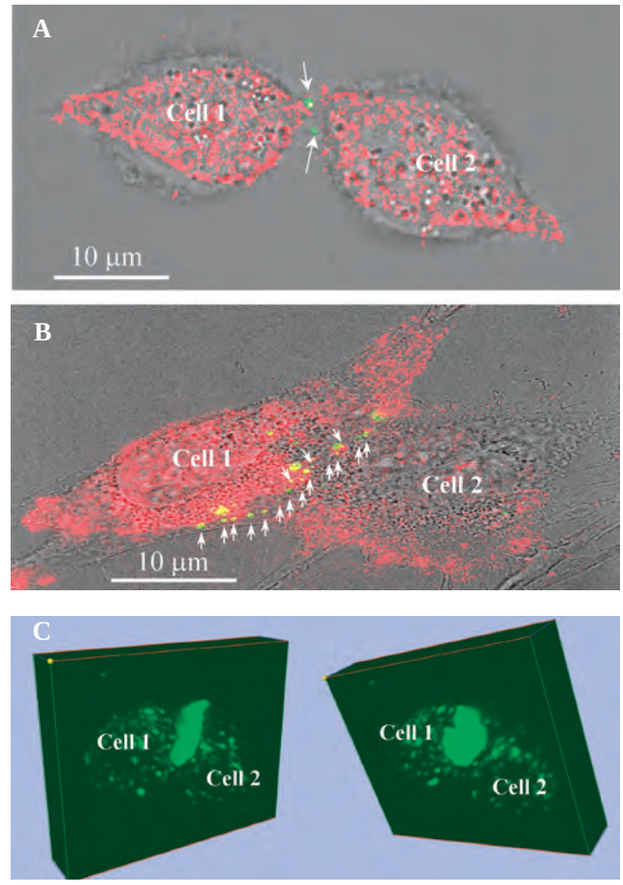


*Fig. 2.* Combined representation of Cx43-GFP gap junction plaques, imaged by conventional fluorescent microscopy (visible in green pseudocolor and indicated by arrows), clusters of unapposed Cx43-GFP hemichannels in the cell membranes, imaged by TIRF microscopy (visible in red pseudocolor), and phase contrast (gray scale) of adjacent HeLa cells (A) and rabbit’s skeletal myoblasts (B). 3D-image of two adjacent HeLa cells, containing huge gap junction plaque and numerous clusters of hemichannels (C).

**Expression patterns of connexins**

Connexins are expressed in all tissues except differentiated skeletal muscle, erythrocytes, and mature sperm cells (see Table 1). Big variety of connexin isoforms has been reported in the nervous system, where different cell types often express different sets of connexins (38–40). Major connexins in the neurons of CNS are Cx36, Cx30.2, and Cx45 (41). Astrocytes most abundantly express Cx30 and Cx43; endothelial cells of blood-brain barrier express Cx40 and Cx43 (42). Ten isoforms of connexins have been identified in different layers of the skin. Their physiological role

is not well determined; however, mutations of Cx26, Cx30, Cx30.3, Cx31, and Cx43 have been shown to be related with congenital diseases of the skin. Main connexins in the liver are Cx32 and Cx43. Moreover, Cx26 is expressed in the periportal acinar area, while liver vascular cells express Cx37 and Cx40. Also, small amounts of Cx39 and Cx30.2 (in the mouse liver) and Cx31.9 (in human liver) have been identified (43). In the heart, mCx30.2, Cx40, Cx43, and Cx45 can form GJs between myocytes of the conduction system and working myocardium of atria and ventricles. Cx45 and recently identified mCx30.2 are most abundantly expressed in the sinoatrial and atrioventricular nodes (44). Cx40 is expressed in the atria and the conduction system of ventricles, while Cx43 is a major connexin forming GJs between working cardiomyocytes (45). These distinct expression patterns are important for synchronous excitation of the atria and ventricles and for imparting a substantial AV delay that ensures the effective blood circulation (46). Expression of Cx40, Cx43, Cx45 together with Cx37 has also been reported in blood vessels, with most abundant expression of Cx40 in endothelial cells and Cx43 in smooth muscle cells (47).

*Table 1*. Expression patterns and single channel conductances ($g_j$) of human and mouse connexins

| Human Connexins | Mouse Connexins | $g_j$ (pS) | Expression patterns of connexins in different tissues |
|---|---|---|---|
| Cx23 | Cx23 | ND* | *Human and mouse genomes*. Transcription and translation have not been demonstrated in humans (48) |
| Cx25 | | ND | *Human genome* |
| Cx26 | Cx26 | 115–150 | Breast (49); skin (50); cochlea (51); liver (52); endometrium (53); glial cells (54); airway epithelium (55); somniferous tubules (56); pancreas (57) |
| Cx30 | Cx30 | 160 | Skin (58); brain (59); cochlea (60); airway epithelium (61); exocrine gland (62) |
| 31.3 | Cx29 | ND | Oligodendrocytes (63, 64), skeletal muscle, liver, pancreas, kidney (65) |
| Cx30.3 | Cx30.3 | ND | Skin (58) |
| Cx31 | Cx31 | 85/15 | Skin (58); airway epithelium (61); cochlea (66); placenta (67) |
| Cx31.1 | Cx31.1 | ND | Skin (58) |
| Cx31.9 | Cx30.2 | 15 | Mouse heart (68); mouse brain (69) |
| Cx32 | Cx32 | 58–70 | Liver (70); skin (58); Schwann cells (71); oligodendrocytes (72); endometrium (53); gland cells (62) |
| | Cx33 | | Testes (73) |
| Cx36 | Cx36 | 5–15 | Retina (74); pancreatic beta cells (75); neurons throughout the central nervous system (76) |
| Cx37 | Cx37 | 219–300 | Vascular smooth muscle (77); endothelium (78); ovaries (79); skin (58) |
| Cx40 | Cx40 | 158–198 | Skin (58); nervous system (80); endothelium (81); heart (82) |
| Cx40.1 | Cx39 | ND | *Human genome;* developing muscle of mouse (83) |
| Cx43 | Cx43 | 90–110 | Most widely expressed connexin, present in at least 34 tissues and 46 cell types (84) |
| Cx45 | Cx45 | 30 | Human pancreatic ductal epithelial cells (85); SA and AV nodes of the heart (82); neurons (86); oligodendrocytes (87), astrocytes (88), vascular system (89), skin (58); osteoblasts (90); retina (74); uterus (91) |
| Cx46 | Cx46 | 140–152 | Lens (92); alveolar epithelium (93) |
| Cx47 | Cx47 | 55 | Brain, spinal cord (94), oligodendrocytes (64) |
| Cx50 | Cx50 | 212 | Lens (92) |
| Cx58 | | ND | *Human genome* |
| Cx62 | Cx57 | 57 | Mouse oocytes (95); horizontal cells of the retina (24, 96) |

*ND, not determined.

**Regulation of connexin gap junction channels**

***Transjunctional voltage.*** Voltage sensitivity is particularly important in regulating the intercellular coupling between excitable cells. Cx43 channels are relatively insensitive to changes in transjunctional voltage compared with channels composed of Cx45 (17, 97–99) (Fig. 3). Each GJ composing hemichannel contains two $V_j$ sensitive gates (100). The fast gate is located at the cytoplasmic entrance of hemichannels and operates from open to residual state. The slow, or "loop," gate is located toward extracellular ends of hemichannels and exhibits slow gating transition to the fully closed state. Functional and structural studies conducted mainly on Cx26 and Cx32 channels indicate that the first several positions of the cytoplasmic NT-domain contain charged residues that determine the magnitude and also polarity of fast $V_j$ gating. For instance, Cx26, Cx30, Cx50 close at positive voltages, and Cx31, Cx32, Cx37, Cx40, Cx43, Cx45, Cx57 at negative. Interestingly, Cx46 hemichannels close at both, positive and negative, voltages; however, gating mechanisms are different. At inside positive voltages, fast gate, located on N-terminal domain, closes unapposed Cx46 hemichannels to the residual state, while at inside negative voltages, slow or "loop" gate, likely located on the extracellular domains, closes to the fully closed state (101, 102). Moreover, $g_j$ of some connexins, like of innexins in invertebrates, appears to be sensitive to membrane potential, $V_m$, and connexins can be classified in two groups according to their polarity of closure, e.g., Cx45 and C57 channels close upon hyperpolarization, whereas Cx43, Cx26, Cx30 channels close upon depolarization (103). Interestingly, $V_m$ sensitivity of Cx43 depends on species, i.e. in HeLa cells Cx43 shows no sensitivity to $V_m$ (100), while in *Xenopus laevis* oocytes $V_m$ sensitivity is obvious (104), and $V_m$ sensors probably are located in the CT region between 242 and 257 residues (104).

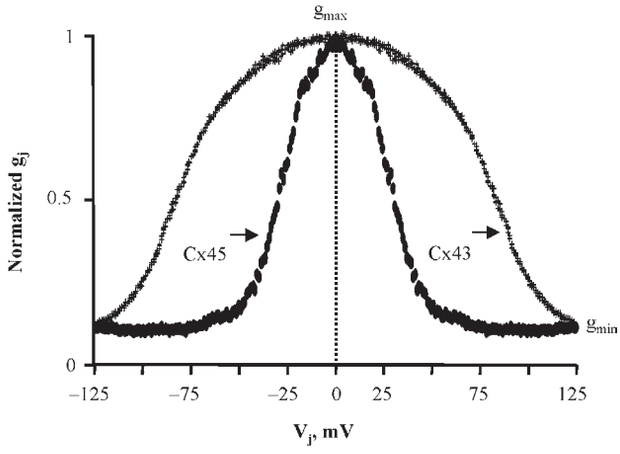


*Fig. 3.* Voltage dependence of Cx43-GFP and Cx45-CFP gap junction channels expressed in HeLa cells (arrows) $V_j$ sensitivity of the channel is characterized by voltage corresponding half conductance between $g_{min}$ and $g_{max}$.

Behavior of GJs may be predicted by mathematical modeling. Earlier, gating properties of homotypic and heterotypic GJ channels were described by two-state Boltzmann function (105, 106), assuming that each hemichannel gates independently (to open and fully closed states), which may be accurate only when both hemichannels have the same gating polarity and single channel conductance and are relatively insensitive to $V_j$. Recently the stochastic four-state model was proposed that accounts for voltage distribution inside the pore, i. e., takes into account contingent gating. It also takes into consideration residual and open-state conductances, gating polarities, voltage sensitivity, steepness of $g_j$ decay, and $V_j$-dependent rectification of each hemichannel (107).

***Intracellular $Ca^{2+}$.*** The closure of the channel by intracellular $Ca^{2+}$ plays a vital role in protecting intact cells from membrane depolarization and leakage of metabolites through gap junctions by disconnecting them from damaged cells. This process is called healing-over (Deleze, 1970) and occurs not only during different pathological conditions but also after incisions performed during surgery. It is still not determined if $g_j$ is affected by the physiological $Ca^{2+}$ transients during the process of the excitation-contraction coupling. The GJ sensitivity to $Ca^{2+}$ ranges from nanomolar to micromolar concentrations and depends on connexin and cell types (108–113). However, it is not completely understood whether $Ca^{2+}$ acts on GJ channel gating directly or through some intracellular intermediates. High $Ca^{2+}$ medium does not alter the permeability of Cx32 hemichannels incorporated into liposomes (114). In contrast, the D178Y mutant of Cx32 that destroyed the divalent cation-binding site caused a complete loss of the blocking actions exerted by $Ca^{2+}$ on hemichannel activity in *Xenopus laevis* oocytes (115). On the other hand, many experimental studies suggest that $Ca^{2+}$-dependent GJ gating may be mediated by calmodulin (see (116) for more details). It contains specialized domains in the N- and C-lobes that follow $NH_2$ terminus (117). $Ca^{2+}$ binding to these domains induces conformational changes enabling calmodulin to interact with receptors. Such interaction was demonstrated with Cx38 (118), Cx32 (119), Cx37 and Cx43 (120), Cx44 (121), and Cx50 (122).

***Intracellular pH.*** In virtually all cells, acidification of intracellular milieu decreases $g_j$; however, sensitivity to intracellular pH depends on connexin type. Cx32 and Cx43 are less sensitive to $pH_i$ than Cx38, Cx50, and Cx57 (123–125). Delmar and coworkers tested $g_j$-$pH_i$ dependence in oocyte pairs expressing different connexins and showed the following order of decreasing sensitivity to $pH_i$: Cx50>Cx46>Cx45>>Cx26>Cx37>Cx43>Cx40>Cx32 (126). Protonation of histidine residues in carboxyl tail and cytoplasmic loop of connexins modulates GJ channel permeability. The latest study provided evidences that $pH_i$-dependent increase in $g_j$ of Cx57 GJ channels was caused by an increase of channel open probability and number of functional channels (125). Interestingly, Cx36 GJ channels demonstrate opposite $g_j$ dependence on $pH_i$, uncoupling upon alkalosis rather than acidosis (127). However, these data contradict earlier report demonstrating uncoupling of Cx36 GJs under acidification with $CO_2$ (128). By now, it is not completely clear, whether $H^+$ acts directly on GJ channels. Heteromeric Cx26/Cx32 hemichannels incorporated into liposomes were insensitive to low pH when $H^+$ was buffered with maleate, bicarbonate, or Tris, but showed some pH sensitivity in the presence of aminosulfonate buffers (114). Therefore, it was concluded that $H^+$ affected GJ gating indirectly via protonation of endogenous aminosulfonate taurine (129). However, sensitivity of Cx46 hemichannels in excised patches to cytoplasmic pH suggests that gating is affected by direct protonation (130).

***Phosphorylation.*** Cytoplasmic C-tail of connexins contains multiple serine, threonine, and tyrosine residues that may be phosphorylated by various protein kinases. Cx36 and Cx56 also can be phosphorylated within cytoplasmic loop (131). Many connexins (Cx31, Cx32, Cx36, Cx37, Cx40, Cx43, Cx45, Cx46, Cx50, and Cx56) have been shown to be phosphoproteins (132, 133). Activation of protein kinases (134–136) or phosphatases (137) may cause changes in cell-to-cell communication and rapid turnover of channels (133, 138, 139). Phosphorylation modifies electrical and metabolic communication between contiguous cells by changing channel molecular structure that affects channel unitary conductance (99), mean open time (134), or open probability (140). Moreover, phosphorylation alters the net charge of C-terminus that in turn may modulate voltage or pH sensitivity of the connexins.

Cx43 is present in at least 34 tissues and 46 cell types, and has been the most intensively studied connexin (see (141) for more details). Cx43 does not contain serine residues in cytoplasmic loop, and there are no reports on phosphorylation of Cx43 N-terminus; however, activation of PKA, PKC, cyclin B kinase, casein kinase CK1, MAPK, and Src tyrosine kinase can cause, respectively, increased phosphorylation of S262, S265, S279, S282, S325, S328, S330, S364, S365, and I382 residues in C-terminus. Consequently, activation of PKA increases Cx43 insertion into plasma membrane (142, 143), PKC, MAPK, and epidermal growth factor activation accelerates the internalization of Cx43 (144–146), CK1 regulates assembly of Cx43 hemichannels to GJ plaques (147). The examination of the role of single kinase (e.g. PKC) in regulation of connexin properties and expression is quite sophisticated because it often exerts not only direct effects but causes the activation of other kinases (e.g. MAPK, Src) with successive phosphorylation of multiple residues and overlapping consequences (141).

**Heterotypic gap junction channels**

Since various tissues express more than one type of connexins, homotypic, heterotypic, and heteromeric GJ channels may form between cells. The number of combinations of heteromeric connexons and GJ channels is very large, and little is known about their biological significance in the heart, CNS, and other tissues. Some of connexins are incompatible to form heterotypic junctions, and this property may affect not only electrical and metabolic communication, but also cell differentiation during development. Typically, most embryonic cells express one or several Cx isoforms and strong connexin-mediated cell-cell coupling tend to eliminate intercellular gradients of permeants, such as ions, metabolites, small peptides, oligonucleotides, and small interfering RNA (siRNA) (129, 148, 149). Thus, in order for neighboring cell populations to develop independently, it may be important to express connexin isoforms that are incompatible to form heterotypic junctions, thereby preventing electrical synchronization, transfer of signaling molecules, or metabolic communication. Several studies reported formation of functional heterotypic junctions between cells expressing Cx45 with those expressing Cx40 and Cx43 (150–154). Recent evidences also suggest that Cx43 and Cx45 can form both heteromeric connexons and homomeric, heterotypic channels (155, 156). In general, among all “cardiac” connexins (Cx30.2, Cx40, Cx43, and Cx45), only Cx40 and Cx43 are not compatible to form heterotypic gap junction channels (157, 158).

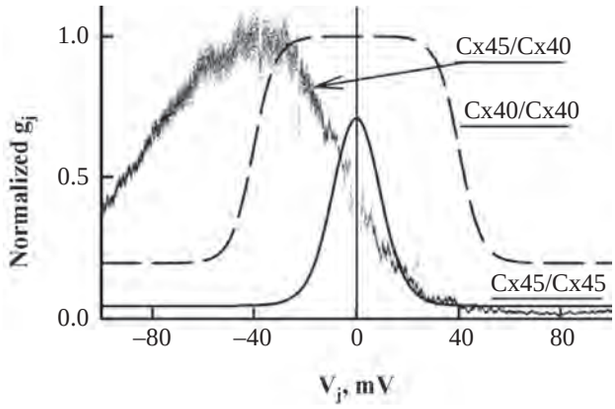


*Fig. 4.* Voltage gating of heterotypic Cx40/Cx45 GJs
Superposition of a $g_j$-$V_j$ plot of a Cx40/Cx45 heterotypic junction with $g_j$-$V_j$ plots of Cx40 (dashed line) and Cx45 (solid line) homotypic GJs (157).

Gating of heterotypic junctions is typically asymmetric with respect to $V_j$=0 and the degree of asymmetry depends on the intrinsic gating properties of the component hemichannels. Cx45 homotypic junctions exhibit the highest $V_j$-gating sensitivity among all members of the connexin family and this property contributes to the high degree of $V_j$-gating asymmetry in all heterotypic junctions containing Cx45 on one side, such as mCx30.2/Cx45 (44), Cx31/Cx45 (159), Cx43/Cx45 (154), and Cx40/Cx45 (157) (see Fig. 4).

In all cases, there is higher $V_j$-gating sensitivity when the Cx45 side is made relatively negative, which has been shown to result predominantly from closure of the slow gate of the Cx45 hemichannel (154). The fast gate of Cx45 also closes on this polarity, but its voltage sensitivity is shifted to higher $V_j$s. The fast and slow $V_j$-sensitive gates of Cx43 GJs also close on relative negativity. Differences in the unitary conductances of component hemichannels resulted to higher $V_j$-gating asymmetry in Cx43/Cx45 junctions than that predicted by simple connection of two hemichannels exhibiting equal unitary conductances. Most of the $V_j$ applied across a Cx43/Cx45 junction falls across the Cx45 hemichannel, which has ~3.5-fold smaller conductance than the Cx43 hemichannel, resulting in increased and decreased $V_j$-gating sensitivities of Cx45 and Cx43 hemichannels, respectively (154).

The strong $g_j$-$V_j$ gating asymmetry of Cx43/Cx45 (154) and Cx31/Cx45 (159) heterotypic junctions produces signal transfer asymmetry that can be increased or decreased by making the cell expressing Cx45 relatively more negative or positive, respectively. Therefore, this cell-to-cell signaling asymmetry seems to be a common feature of heterotypic junctions containing a Cx45 hemichannel on one side. Such signaling asymmetry may be functionally relevant in the CNS where signal propagation in one direction is preferred and both Cx43 and Cx45 are expressed. It has been shown in heterotypic Cx43/Cx45 GJs that dye transfer can be enhanced or reduced depending to which cell action potential arrived first, expressing Cx43 or Cx45 (160).

Cx30.2 was recently characterized as the fourth cardiac Cx, which is expressed preferentially in the SA- and AV-nodal regions of the mouse heart (44). mCx30.2-EGFP/Cx40 junctions are functional and exhibit an asymmetric steady-state $g_j$-$V_j$ relation with higher Vj-sensitivity at voltages relatively negative on the mCx30.2 side. In cocultures of HeLaCx30.2-EGFP and HeLaCx43-CFP, the steady-state $g_j$-$V_j$ relation of this heterotypic junction is strongly asymmetric and exhibits an increase in $g_j$ when the cell expressing mCx30.2 is made more positive. mCx30.2/Cx45 junctions are characterized by a markedly asymmetric $g_j$-$V_j$ relation. Steep and sensitive gating occurs at $V_j$s relatively negative on the Cx45 side (44, 158).

These findings have potential implications for intercellular coupling in specific regions of the heart, such as the interface between the sinus node and atrial myocardium or Purkinje fibers and ventricular myocardium.

**Permeability of gap junction channels**

GJs are permeable to second messengers and metabolites, such as $Ca^{2+}$, $PI_3$, glutamate, glutathione, ADP, and ATP. To study permeability of homotypic and heterotypic GJ channels formed of different connexin isoforms, fluorescent dyes of different net charge and size are being used. Techniques to evaluate dye permeability include the monitoring of dye transfer after scrape loading in the cell monolayer; the injection of dye in a single cell through a microelectrode and monitoring fluorescence recovery after photo bleaching; the measurement of single channel permeability by correlating cell-to-cell transfer of fluorescent dyes with GJ numbers estimated by electron microscopy (152, 161–164).

A few studies have examined single-channel permeability of homotypic and heterotypic GJ channels using simultaneous double whole-cell patch-clamp electrophysiology and fluorescence imaging recordings, when fluorescent dye was loaded into one cell of a cell pair through a patch pipette, and dye transfer to the neighboring cell was measured. Valiunas with coworkers examined single channel permeabilities of homotypic and heterotypic Cx40 and Cx43 GJ channels

*Table 2.* Single channel permeability ($\times 10^{-15}$ $cm^3/s$) of cardiac homotypic and heterotypic gap junctions for Alexa Fluor ($AF^{350}$) and Lucifer yellow (LY) (158)

| Dye\Cx | Cx30.2 | Cx40 | Cx43 | Cx45 | Cx30.2/Cx40 | Cx30.2/Cx43 | Cx30.2/Cx45 | Cx40/Cx45 | Cx43/Cx45 |
|---|---|---|---|---|---|---|---|---|---|
| $AF^{350}$ | 0.04 | 33.1 | 86 | 5.5 | 0.22 | 0.09 | 0.09 | 14.5 | 15.9 |
| LY | n.p. | 6.9 | 24.6 | 1.1 | n.p. | n.p. | n.p. | 2 | 2.3 |

n.p., non permeable.

to fluorescent dye Lucifer yellow (LY) in HeLa cells and showed that heterotypic channels demonstrated intermediate permeability (152). Another study has evaluated single channel permeabilities of homotypic Cx26, Cx32, Cx37, Cx40, Cx43, Cx45 and heterotypic Cx26/Cx32, Cx37/Cx43 GJ channels for series of Alexa Fluor (AF) dyes in *Xenopus laevis* ovocytes (162) and in contrast to the first one, shown that permeability of heterotypic channels was determined by permeability of more restrictive connexin. A recent study (158) has examined single-channel permeabilities of homotypic and heterotypic GJ channels formed of all known cardiac connexins, mCx30.2, Cx40, Cx43, and Cx45, to fluorescent dyes LY and AF. Single channel permeabilities calculated for homotypic and heterotypic GJs are presented in Table 2. The ratio of single channel conductance to permeability for $AF^{350}$ was 40- to 170-fold higher for mCx30.2 GJs than for Cx40, Cx43, and Cx45, suggesting that recently identified in the conductive system of the heart Cx30.2 GJs are more adapted to perform electrical rather than metabolic cell-to-cell communication.

**Concluding remarks**

In the last two decades, a huge number of studies have improved our knowledge about cell-to-cell communication through connexin gap junction channels. However, despite relatively well-examined properties of the channels as the single entities, very little is known about organization of spatio-temporal signaling cascades, nexuses, involved in connexin trafficking, docking, removal, phosphorylation/dephosphorylation, protein-protein interactions. Therefore, the major future challenges are to identify and quantify these proteins forming complexes, to picture their geometry, the hierarchy of organization and dynamic regulation by microscopic, structural and molecular biology approaches, and to understand the functional significance of these protein interactions in intercellular signaling and pathophysiology.

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## Plyšinių jungčių įvairovė ir savybės

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**Raktažodžiai:** koneksinai, koneksonai, plyšinės jungtys, struktūra, funkcija.

**Santrauka.** Plyšinės jungtys užtikrina elektrinį ir metabolinį ryšį tarp ląstelių. Jos yra sudarytos iš dviejų puskanalių (koneksonų), esančių besiliečiančiose ląstelėse. Puskanaliai sudaryti iš šešių subvienetų – koneksinų. Pelės genome koneksinų genų šeimą sudaro 20 narių, žmogaus genome – 21 narys. Koneksinų yra visuose audiniuose, išskyrus diferencijuotas skeleto raumenų ląsteles, eritrocitus ir subrendusias spermos ląsteles. Daugelyje audinių gali būti daugiau nei vieno tipo koneksinų, todėl tarp ląstelių gali formuotis ne tik homotipinės, bet ir heterotipinės, heteromerinės plyšinės jungtys. Šiame straipsnyje trumpai aptariamos pagrindinės homotipinių ir heterotipinių plyšinių jungčių elektrinės ir pralaidumo savybės, taip pat naujausi pasiekimai tiriant jų priklausomybės nuo jungties įtampos, viduląstelinio $Ca^{2+}$, pH ir fosforilinimo mechanizmus.

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