REVIEWS

Diversity and properties of connexin gap junction channels

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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).

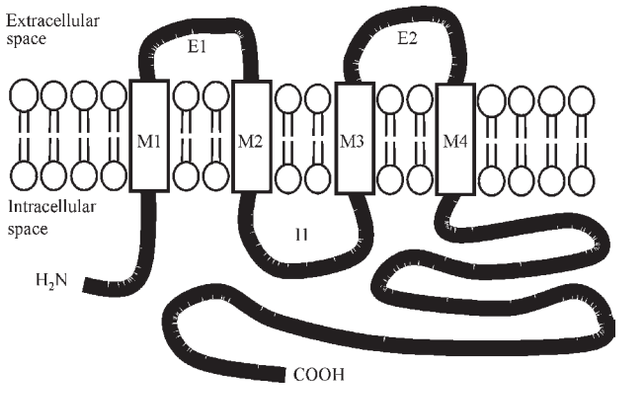

Fig. 1. Topological model of a connexin
Connexins, as well as pannexins and innexins, possess four transmembrane domains (M1–M4), intracellular N- and C-termini, two extracellular loops (E1–E2) and one intracellular loop (I1)

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/μm^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.

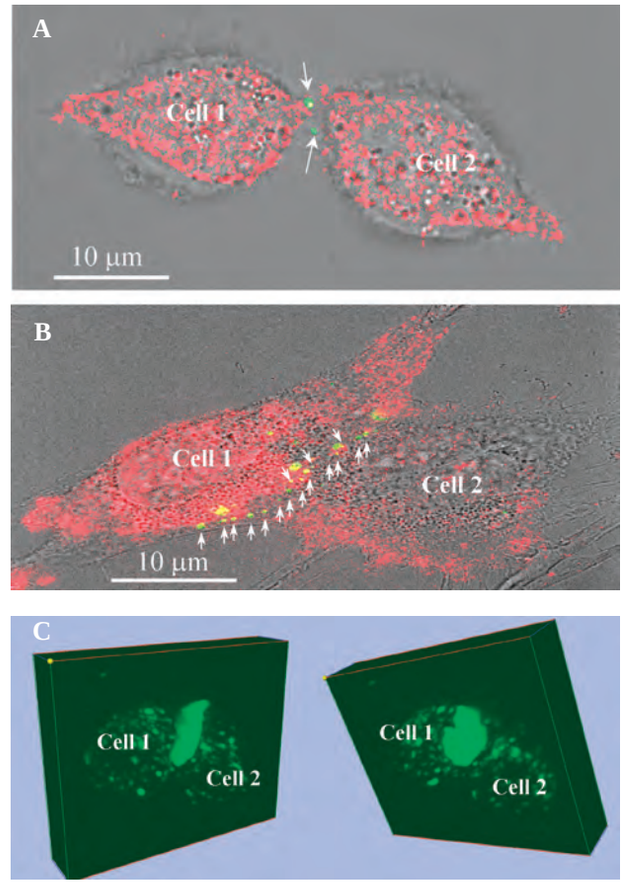

Fig. 2. Combined representation of Cx43-GFP gap junction plaques, imaged by conventional fluorescent microscopy (visible in green pseudocolor and indicated by arrows), clusters of unapposed Cx43-GFP hemichannels in the cell membranes, imaged by TIRF microscopy (visible in red pseudocolor), and phase 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).

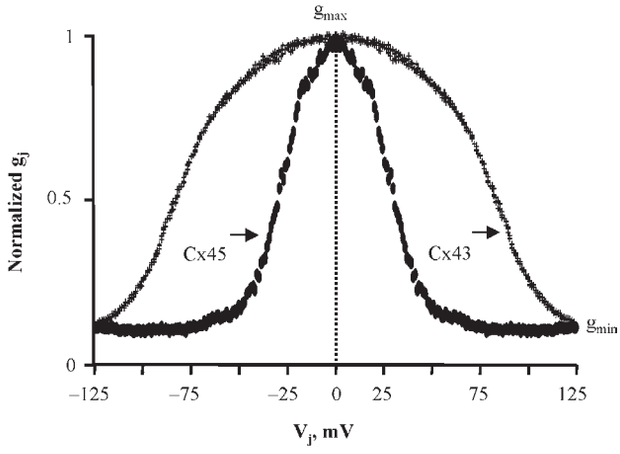

Fig. 3. Voltage dependence of Cx43-GFP and Cx45-CFP gap junction channels expressed in HeLa cells (arrows) V_j sensitivity of the channel is characterized by voltage corresponding half conductance between g_{min} and g_{max}.

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).

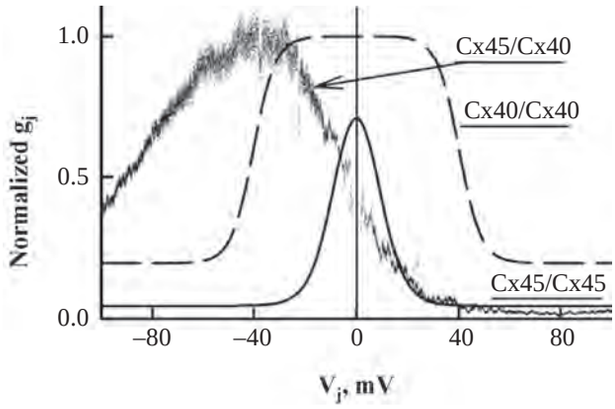

Fig. 4. Voltage gating of heterotypic Cx40/Cx45 GJs
Superposition of a g_j-V_j plot of a Cx40/Cx45 heterotypic junction with g_j-V_j plots of Cx40 (dashed line) and Cx45 (solid line) homotypic GJs (157).

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_js. 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_js 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.

References

1. Yen MR, Saier MH Jr. Gap junctional proteins of animals: the innexin/pannexin superfamily. Prog Biophys Mol Biol 2007;94(1-2):5-14.
2. Willecke K, Eiberger J, Degen J, Eckardt D, Romualdi A, Guldenagel M, et al. Structural and functional diversity of connexin genes in the mouse and human genome. Biol Chem 2002;383(5):725-37.
3. Scemes E, Spray DC, Meda P. Connexins, pannexins, innexins: novel roles of "hemi-channels". Pflugers Arch 2009;457(6):1207-26.
4. Hertzberg EL, Disher RM, Tiller AA, Zhou Y, Cook RG. Topology of the Mr 27,000 liver gap junction protein. Cytoplasmic localization of amino- and carboxyl termini and a hydrophilic domain which is protease-hypersensitive. J Biol Chem 1988;263:19105-11.
5. Yancey SB, John SA, Lal R, Austin BJ, Revel JP. The 43-kD polypeptide of heart gap junctions: immunolocalization, topology, and functional domains. J Cell Biol 1989;108: 2241-54.
6. Yeager M, Unger VM, Falk MM. Synthesis, assembly and structure of gap junction intercellular channels. Curr Opin Struct Biol 1998;8(4):517-24.
7. Boassa D, Qiu F, Dahl G, Sosinsky G. Trafficking dynamics of glycosylated pannexin 1 proteins. Cell Commun Adhes 2008;15(1):119-32.
8. Stout C, Goodenough DA, Paul DL. Connexins: functions without junctions. Curr Opin Cell Biol 2004;16(5):507-12.
9. Locovei S, Bao L, Dahl G. Pannexin 1 in erythrocytes: function without a gap. Proc Natl Acad Sci U S A 2006;103(20): 7655-9.
10. Verselis VK, Bennett MV, Bargiello TA. A voltage-dependent gap junction in Drosophila melanogaster. Biophys J 1991; 59:114-26.
11. Bennett MV. Connexins in disease (news). Nature 1994; 368:18-9.
12. Elfgang C, Eckert R, Lichtenberg-Frate H, Butterweck A, Traub O, Klein RA, et al. Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. J Cell Biol 1995;129:805-17.
13. Goodenough DA, Goliger JA, Paul DL. Connexins, connexons, and intercellular communication. Annu Rev Biochem 1996;65:475-502.
14. Paul DL. Molecular cloning of cDNA for rat liver gap junction protein. J Cell Biol 1986;103:123-34.
15. Gustincich S, Batalov S, Beisel KW, Bono H, Carninci P, Fletcher CF, et al. Analysis of the mouse transcriptome for genes involved in the function of the nervous system. Genome Res 2003;13(6B):1395-401.
16. Sohl G, Willecke K. Gap junctions and the connexin protein family. Cardiovasc Res 2004;62(2):228-32.
17. Brink PR, Ramanan SV, Christ GJ. Human connexin 43 gap junction channel gating: evidence for mode shifts and/or heterogeneity. Am J Physiol 1996;271(1 Pt 1):C321-31.
18. Beblo DA, Wang HZ, Beyer EC, Westphale EM, Veenstra RD. Unique conductance, gating, and selective permeability properties of gap junction channels formed by connexin40. Circ Res 1995;77:813-22.
19. Bukauskas FF, Elfgang C, Willecke K, Weingart R. Biophysical properties of gap junction channels formed by mouse connexin40 in induced pairs of transfected human HeLa cells. Biophysical J 1995;68:2289-98.
20. Beblo DA, Veenstra RD. Monovalent cation permeation through the connexin40 gap junction channel. Cs, Rb, K, Na, Li, TEA, TMA, TBA, and effects of anions Br, Cl, F, acetate, aspartate, glutamate, and NO3. J Gen Physiol 1997;109(4): 509-22.
21. McNutt N, Weinstein RS. The ultrastructure of the nexus. A correlated thin section and freeze-cleave study. J Cell Biol 1970;47:666-88.
22. Goodenough DA, Revel JP. A fine structural analysis of intercellular junctions in the mouse liver. J Cell Biol 1970; 45:272-90.
23. Maeda S, Nakagawa S, Suga M, Yamashita E, Oshima A, Fujiyoshi Y, et al. Structure of the connexin 26 gap junction channel at 3.5 A resolution. Nature 2009;458(7238):597-602.
24. Palacios-Prado N, Sonntag S, Skeberdis VA, Willecke K, Bukauskas FF. Gating, permselectivity and pH-dependent modulation of channels formed by connexin57, a major connexin of horizontal cells in the mouse retina. J Physiol 2009;587(Pt 13):3251-69.
25. Jordan K, Solan JL, Dominguez M, Sia M, Hand A, Lampe PD, et al. Trafficking, assembly, and function of a connexin43-green fluorescent protein chimera in live mammalian cells. Mol Biol Cell 1999;10(6):2033-50.
26. Olk S, Zoidl G, Dermietzel R. Connexins, cell motility, and the cytoskeleton. Cell Motil Cytoskeleton 2009;66(11):1000-16.
27. Jongen WM, Fitzgerald DJ, Asamoto M, Piccoli C, Slaga TJ, Gros D, et al. Regulation of connexin 43-mediated gap junctional intercellular communication by Ca2+ in mouse epidermal cells is controlled by E-cadherin. J Cell Biol 1991; 114:545-55.
28. Musil LS, Goodenough DA. Multisubunit assembly of an integral plasma membrane channel protein, gap junction connexin43, occurs after exit from the ER. Cell 1993;74:1065-77.
29. Sarma JD, Wang F, Koval M. Targeted gap junction protein constructs reveal connexin-specific differences in oligomerization. J Biol Chem 2002;23:20911-8.
30. Laird DW. Life cycle of connexins in health and disease. Biochem J 2006;394:527-43.
31. Gaietta G, Deerinck TJ, Adams SR, Bouwer J, Tour O, Laird DW, et al. Multicolor and electron microscopic imaging of connexin trafficking. Science 2002;296:503-7.
32. Shaw RM, Fay AJ, Puthenveedu MA, von Zastrow M, Jan YN, Jan LY. Microtubule plus-end-tracking proteins target gap junctions directly from the cell interior to adherens junctions. Cell 2007;128(3):547-60.
33. Jordan K, Chodock R, Hand AR, Laird DW. The origin of annular junctions: a mechanism of gap junction internalization. J Cell Sci 2001;114(Pt 4):763-73.
34. Laing JG, Beyer EC. The gap junction protein connexin43 is degraded via the ubiquitin proteasome pathway. J Biol Chem 1995;270:26399-403.
35. Musil LS, Le AC, VanSlyke JK, Roberts LM. Regulation of connexin degradation as a mechanism to increase gap junction assembly and function. J Biol Chem 2000;275(33):25207-15.
36. Qin H, Shao Q, Igdoura SA, Alaoui-Jamali MA, DW. L. Lysosomal and proteasomal degradation play distinct roles in the life cycle of Cx43 in gap junctional intercellular communication-deficient and -competent breast tumor cells. J Biol Chem 2003;32:30005-14.
37. Saffitz JE, Laing JG, Yamada KA. Connexin expression and turnover: implications for cardiac excitability. Circ Res 2000; 86(7):723-8.
38. Dermietzel R, Hertzberg EL, Kessler JA, Spray DC. Gap junctions between cultured astrocytes: immunocytochemical, molecular, and electrophysiological analysis. J Neurosci

1991;11:1421-32.
39. Nagy JI, Rash JE. Astrocyte and oligodendrocyte connexins of the glial syncytium in relation to astrocyte anatomical domains and spatial buffering. Cell Commun Adhes 2003; 10(4-6):401-6.
40. Li J, Habbes HW, Eiberger J, Willecke K, Dermietzel R, Meier C. Analysis of connexin expression during mouse Schwann cell development identifies connexin29 as a novel marker for the transition of neural crest to precursor cells. Glia 2007; 55(1):93-103.
41. Van Der Giessen RS, Maxeiner S, French PJ, Willecke K, De Zeeuw CI. Spatiotemporal distribution of Connexin45 in the olivocerebellar system. J Comp Neurol 2006;495:173-84.
42. Nagasawa K, Chiba H, Fujita H, Kojima T, Saito T, Endo T, et al. Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells. J Cell Physiol 2006;208:123-32.
43. Vinken M, Henkens T, De Rop E, Fraczek J, Vanhaecke T, Rogiers V. Biology and pathobiology of gap junctional channels in hepatocytes. Hepatology 2008;47(3):1077-88.
44. Kreuzberg MM, Söhl G, Kim J, Verselis VK, Willecke K, Bukauskas FF. Functional properties of mouse connexin30.2 expressed in the conduction system of the heart. Circ Res 2005;96:1169-77.
45. Lo CW. Role of gap junctions in cardiac conduction and development: insights from the connexin knockout mice. Circ Res 2000;87(5):346-8.
46. Kreuzberg MM, Willecke K, Bukauskas F. Connexin-mediated cardiac impulse propagation: connexin 30.2 slows atrioventricular conduction in mouse heart. Trends Cardiovasc Med 2006;16:266-72.
47. Bruzzone R, Haefliger JA, Gimlich RL, Paul DL. Connexin40, a component of gap junctions in vascular endothelium, is restricted in its ability to interact with other connexins. Mol Biol Cell 1993;4:7-20.
48. Sohl G, Willecke K. An update on connexin genes and their nomenclature in mouse and man. Cell Commun Adhes 2003; 10(4-6):173-80.
49. Kalra J, Shao Q, Qin H, Thomas T, Alaoui-Jamali MA, Laird DW. Cx26 inhibits breast MDA-MB-435 cell tumorigenic properties by a gap junctional intercellular communication-independent mechanism. Carcinogenesis 2006;27(12):2528-37.
50. Di WL, Common JE, Kelsell DP. Connexin 26 expression and mutation analysis in epidermal disease. Cell Commun Adhes 2001;8(4-6):415-8.
51. Sun J, Ahmad S, Chen S, Tang W, Zhang Y, Chen P, et al. Cochlear gap junctions coassembled from Cx26 and 30 show faster intercellular Ca2+ signaling than homomeric counterparts. Am J Physiol Cell Physiol 2005;288:C613-23.
52. Zhang JT, Nicholson BJ. Sequence and tissue distribution of a second protein of hepatic gap junctions, Cx26, as deduced from its cDNA. J Cell Biol 1989;109:3391-401.
53. Jahn E, Classen-Linke I, Kusche M, Beier HM, Traub O, Grummer R, et al. Expression of gap junction connexins in the human endometrium throughout the menstrual cycle. Hum Reprod 1995;10:2666-70.
54. Altevogt BM, Paul DL. Four classes of intercellular channels between glial cells in the CNS. J Neurosci 2004;24(18):4313-23.
55. Carson JL, Reed W, Moats-Staats BM, Brighton LE, Gambling TM, Hu SC, et al. Connexin 26 expression in human and ferret airways and lung during development. Am J Respir Cell Mol Biol 1998;18(1):111-9.
56. Brehm R, Marks A, Rey R, Kliesch S, Bergmann M, Steger K. Altered expression of connexins 26 and 43 in Sertoli cells in seminiferous tubules infiltrated with carcinoma-in-situ or seminoma. J Pathol 2002;197(5):647-53.
57. Serre-Beinier V, Mas C, Calabrese A, Caton D, Bauquis J, Caille D, et al. Connexins and secretion. Biol Cell 2002;94 (7-8):477-92.
58. Di WL, Rugg EL, Leigh IM, Kelsell DP. Multiple epidermal connexins are expressed in different keratinocyte subpopulations including connexin 31. J Invest Dermatol 2001; 117(4): 958-64.
59. Nagy JI, Patel D, Ochalski PA, Stelmack GL. Connexin30 in rodent, cat and human brain: selective expression in gray matter astrocytes, co-localization with connexin43 at gap junctions and late developmental appearance. Neuroscience 1999;88(2):447-68.
60. Ahmad S, Tang W, Chang Q, Qu Y, Hibshman J, Li Y, et al. Restoration of connexin26 protein level in the cochlea completely rescues hearing in a mouse model of human connexin30-linked deafness. Proc Natl Acad Sci U S A 2007; 104(4):1337-41.
61. Wiszniewski L, Sanz J, Scerri I, Gasparotto E, Dudez T, Lacroix JS, et al. Functional expression of connexin30 and connexin31 in the polarized human airway epithelium. Differentiation 2007;75(5):382-92.
62. Michon L, Nlend Nlend R, Bavamian S, Bischoff L, Boucard N, Caille D, et al. Involvement of gap junctional communication in secretion. Biochim Biophys Acta 2005;1719(1-2): 82-101.
63. Sargiannidou I, Ahn M, Enriquez AD, Peinado A, Reynolds R, Abrams C, et al. Human oligodendrocytes express Cx31.3: function and interactions with Cx32 mutants. Neurobiol Dis 2008;30(2):221-33.
64. Kleopa KA, Orthmann JL, Enriquez A, Paul DL, Scherer SS. Unique distributions of the gap junction proteins connexin29, connexin32, and connexin47 in oligodendrocytes. Glia 2004; 47(4):346-57.
65. Sohl G, Nielsen PA, Eiberger J, Willecke K. Expression profiles of the novel human connexin genes hCx30.2, hCx40.1, and hCx62 differ from their putative mouse orthologues. Cell Commun Adhes 2003;10(1):27-36.
66. Forge A, Becker D, Casalotti S, Edwards J, Marziano N, Nevill G. Gap junctions in the inner ear: comparison of distribution patterns in different vertebrates and assessement of connexin composition in mammals. J Comp Neurol 2003;467(2):207-31.
67. Malassine A, Cronier L. Involvement of gap junctions in placental functions and development. Biochim Biophys Acta 2005;1719(1-2):117-24.
68. Kreuzberg M, Schrickel J, Ghanem A, Kim J, Degen J, Janssen-Bienhold U, et al. Connexin30.2 containing gap junction channels decelerate impulse propagation through the atrioventricular node. Proc Natl Acad Sci U S A 2006;108:5959-64.
69. Kreuzberg MM, Deuchars J, Weiss E, Schober A, Sonntag S, Wellershaus K, et al. Expression of connexin30.2 in interneurons of the central nervous system in the mouse. Mol Cell Neurosci 2008;37(1):119-34.
70. Wilgenbus KK, Kirkpatrick CJ, Knuechel R, Willecke K, Traub O. Expression of Cx26, Cx32 and Cx43 gap junction proteins in normal and neoplastic human tissues. Int J Cancer 1992;51:522-9.
71. Bergoffen J, Scherer SS, Wang S, Scott MO, Bone LJ, Paul DL, et al. Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 1993;262:2039-42.
72. Menichella DM, Goodenough DA, Sirkowski E, Scherer SS,

Paul DL. Connexins are critical for normal myelination in the CNS. J Neurosci 2003;23(13):5963-73.
73. Fischer P, Brehm R, Konrad L, Hartmann S, Kliesch S, Bohle RM, et al. Connexin 33: a rodent-specific member of the gap junction protein family? J Androl 2005;26(1):75-84.
74. Massey SC, O'Brien JJ, Trexler EB, Li W, Keung JW, Mills SL, et al. Multiple neuronal connexins in the mammalian retina. Cell Commun Adhes 2003;10(4-6):425-30.
75. Serre-Beinier V, Bosco D, Zulianello L, Charollais A, Caille D, Charpantier E, et al. Cx36 makes channels coupling human pancreatic beta-cells, and correlates with insulin expression. Hum Mol Genet 2009;18(3):428-39.
76. Condorelli DF, Belluardo N, Trovato-Salinaro A, Mudo G. Expression of Cx36 in mammalian neurons. Brain Res Brain Res Rev 2000;32(1):72-85.
77. Isakson BE, Duling BR. Heterocellular contact at the myoendothelial junction influences gap junction organization. Circ Res 2005;97(1):44-51.
78. Kwak BR, Mulhaupt F, Veillard N, Gros DB, Mach F. Altered pattern of vascular connexin expression in atherosclerotic plaques. Arterioscler Thromb Vasc Biol 2002;22(2):225-30.
79. Gershon E, Plaks V, Dekel N. Gap junctions in the ovary: expression, localization and function. Mol Cell Endocrinol 2008;282(1-2):18-25.
80. Chang Q, Gonzalez M, Pinter MJ, Balice-Gordon RJ. Gap junctional coupling and patterns of connexin expression among neonatal rat lumbar spinal motor neurons. J Neurosci 1999;19(24):10813-28.
81. Yeh HI, Rothery S, Dupont E, Coppen SR, Severs NJ. Individual gap junction plaques contain multiple connexins in arterial endothelium. Circ Res 1998;83(12):1248-63.
82. Severs NJ, Rothery S, Dupont E, Coppen SR, Yeh HI, Ko YS, et al. Immunocytochemical analysis of connexin expression in the healthy and diseased cardiovascular system. Microsc Res Tech 2001;52(3):301-22.
83. von Maltzahn J, Wulf V, Willecke K. Spatiotemporal expression of connexin 39 and -43 during myoblast differentiation in cultured cells and in the mouse embryo. Cell Commun Adhes 2006;13(1-2):55-60.
84. Laird DW. Life cycle of connexins in health and disease. Biochem J 2006;394(Pt 3):527-43.
85. Chanson M, Scerri I, Suter S. Defective regulation of gap junctional coupling in cystic fibrosis pancreatic duct cells. J Clin Invest 1999;103(12):1677-84.
86. Maxeiner S, Kruger O, Schilling K, Traub O, Urschel S, Willecke K. Spatiotemporal transcription of connexin45 during brain development results in neuronal expression in adult mice. Neuroscience 2003;119(3):689-700.
87. Pastor A, Kremer M, Moller T, Kettenmann H, Dermietzel R. Dye coupling between spinal cord oligodendrocytes: differences in coupling efficiency between gray and white matter. Glia 1998;24(1):108-20.
88. Dermietzel R, Gao Y, Scemes E, Vieira D, Urban M, Kremer M, et al. Connexin43 null mice reveal that astrocytes express multiple connexins. Brain Res Brain Res Rev 2000;32(1):45-56.
89. Figueroa XF, Duling BR. Gap junctions in the control of vascular function. Antioxid Redox Signal 2009;11(2):251-66.
90. Steinberg TH, Civitelli R, Geist ST, Robertson AJ, Hick E, Veenstra RD, et al. Connexin43 and connexin45 form gap junctions with different molecular permeabilities in osteoblastic cells. EMBO J 1994;13:744-50.
91. Albrecht JL, Atal NS, Tadros PN, Orsino A, Lye SJ, Sadovsky Y, et al. Rat uterine myometrium contains the gap junction protein connexin45, which has a differing temporal expression pattern from connexin43. Am J Obstet Gynecol 1996;175 (4 Pt 1):853-8.
92. Gerido DA, White TW. Connexin disorders of the ear, skin, and lens. Biochim Biophys Acta 2004;1662(1-2):159-70.
93. Abraham V, Chou ML, George P, Pooler P, Zaman A, Savani RC, et al. Heterocellular gap junctional communication between alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 2001;280(6):L1085-93.
94. Teubner B, Odermatt B, Guldenagel M, Sohl G, Degen J, Bukauskas F, et al. Functional expression of the new gap junction gene connexin47 transcribed in mouse brain and spinal cord neurons. J Neurosci 2001;21(4): 1117-26.
95. Ratchford AM, Esguerra CR, Moley KH. Decreased oocyte-granulosa cell gap junction communication and connexin expression in a type 1 diabetic mouse model. Mol Endocrinol 2008;22(12):2643-54.
96. Hombach S, Janssen-Bienhold U, Söhl G, Schubert T, Büssow H, Ott T, et al. Functional expression of connexin57 in horizontal cells of the mouse retina. Eur J Neurosci 2004;19:2633-40.
97. Moreno AP, Sáez JC, Fishman GI, Spray DC. Human connexin43 gap junction channels. Regulation of unitary conductances by phosphorylation. Circ Res 1994; 74:1050-7.
98. Veenstra RD. Size and selectivity of gap junction channels formed from different connexins. J Bioenerg Biomembr 1996;28(4):327-37.
99. Moreno AP, Fishman GI, Spray DC. Phosphorylation shifts unitary conductance and modifies voltage dependent kinetics of human connexin43 gap junction channels. Biophys J 1992;62:51-3.
100. Bukauskas FF, Verselis VK. Gap junction channel gating. Biochim Biophys Acta 2004;1662:42-60.
101. Verselis VK, Ginter CS, Bargiello TA. Opposite voltage gating polarities of two closely related connexins. Nature 1994;368:348-51.
102. Srinivas M, Kronengold J, Bukauskas FF, Bargiello TA, Verselis VK. Correlative studies of gating in Cx46 and Cx50 hemichannels and gap junction channels. Biophys J 2005; 88(3):1725-39.
103. González D, Gómez-Hernández JM, Barrio LC. Molecular basis of voltage dependence of connexin channels: an integrative appraisal. Prog Biophys Mol Biol 2007;94:66-106.
104. Revilla A, Bennett MV, Barrio LC. Molecular determinants of membrane potential dependence in vertebrate gap junction channels. Proc Natl Acad Sci U S A 2000;97(26):14760-5.
105. Spray DC, Harris AL, Bennett MV. Equilibrium properties of a voltage-dependent junctional conductance. J Gen Physiol 1981;77:77-93.
106. Harris AL, Spray DC, Bennett MVL. Kinetic properties of a voltage-dependent junctional conductance. J Gen Physiol 1981;77:95-117.
107. Paulauskas N, Pranevicius M, Pranevicius H, Bukauskas FF. A stochastic four-state model of contingent gating of gap junction channels containing two "fast" gates sensitive to transjunctional voltage. Biophys J 2009;96(10):3936-48.
108. Rose B, Loewenstein WR. Permeability of a cell junction and the local cytoplasmic free ionized calcium concentration: a study with aequorin. J Membr Biol 1976;28:87-119.
109. Dahl G, Isenberg G. Decoupling of heart muscle cells: correlation with increased cytoplasmic calcium activity and with changes in nexus ultrastructure. J Membr Biol 1980; 53:63-75.

110. Noma A, Tsuboi N. Dependence of junctional conductance on proton, calcium and magnesium ions in cardiac paired cells of guinea-pig. J Physiol (Lond) 1987;382:193-211.
111. Neyton J, Trautmann A. Single-channel currents of an intercellular junction. Nature 1985;317:331-5.
112. Peracchia C. Increase in gap junction resistance with acidification in crayfish septate axons is closely related to changes in intracellular calcium but not hydrogen ion concentration. J Membr Biol 1990;113:75-92.
113. Lazrak A, Peracchia C. Gap junction gating sensitivity to physiological internal calcium regardless of pH in Novikoff hepatoma cells. Biophys J 1993;65:2002-12.
114. Bevans CG, Harris AL. Regulation of connexin channels by pH. Direct action of the protonated form of taurine and other aminosulfonates. J Biol Chem 1999;274(6):3711-9.
115. Gómez-Hernández JM, de Miguel M, Larrosa B, González D, Barrio LC. Molecular basis of calcium regulation in connexin-32 hemichannels. Proc Natl Acad Sci U S A 2003; 100(26):16030-5.
116. Peracchia C. Chemical gating of gap junction channels; roles of calcium, pH and calmodulin. Biochim Biophys Acta 2004;1662(1-2):61-80.
117. Kretsinger RH. Hypothesis: calcium modulated proteins contain EF-hands. In: E. Carafoli FC, W. Drabinowski, Margreth A., editors. Calcium transport in contraction and secretion. Amsterdam: Elsevier; 1975. p. 469-78.
118. Peracchia C, Wang X, Li L, Peracchia LL. Inhibition of calmodulin expression prevents low-pH-induced gap junction uncoupling in Xenopus oocytes. Pflugers Arch 1996;431: 379-87.
119. Peracchia C, Sotkis A, Wang XG, Peracchia LL, Persechini A. Calmodulin directly gates gap junction channels. J Biol Chem 2000;275(34):26220-4.
120. Sotkis A, Wang XG, Yasumura T, Peracchia LL, Persechini A, Rash JE, et al. Calmodulin colocalizes with connexins and plays a direct role in gap junction channel gating. Cell Commun Adhes 2001;8(4-6):277-81.
121. Zhou Y, Yang W, Lurtz MM, Chen Y, Jiang J, Huang Y, et al. Calmodulin mediates the Ca2+-dependent regulation of Cx44 gap junctions. Biophys J 2009;96(7):2832-48.
122. Zhang X, Zou T, Liu Y, Qi Y. The gating effect of calmodulin and calcium on the connexin50 hemichannel. Biol Chem 2006;387(5):595-601.
123. Wang X, Li L, Peracchia LL, Peracchia C. Chimeric evidence for a role of the connexin cytoplasmic loop in gap junction channel gating. Pflugers Arch 1996;431(6):844-52.
124. Liu S, Taffet S, Stoner L, Delmar M, Vallano ML, Jalife J. A structural basis for the unequal sensitivity of the major cardiac and liver gap junctions to intracellular acidification: the carboxyl tail length. Biophys J 1993;64:1422-33.
125. Palacios-Prado N, Sonntag S, Skeberdis VA, Willecke K, Bukauskas F. Gating, permselectivity and pH-dependent modulation of channels formed by connexin57, a major connexin of horizontal cells in the mouse retina. J Physiol 2009;587(Pt 13):3251-69.
126. Stergiopoulos K, Alvarado JL, Mastroianni M, Ek-Vitorin JF, Taffet SM, Delmar M. Hetero-domain interactions as a mechanism for the regulation of connexin channels. Circ Res 1999;84(10):1144-55.
127. González-Nieto D, Gómez-Hernández JM, Larrosa B, Gutiérrez C, Muñoz MD, Fasciani I, et al. Regulation of neuronal connexin-36 channels by pH. Proc Natl Acad Sci U S A 2008;105(44):17169-74.
128. Teubner B, Degen J, Sohl G, Guldenagel M, Bukauskas FF, Trexler EB, et al. Functional expression of the murine connexin 36 gene coding for a neuron-specific gap junctional protein. J Membr Biol 2000;176(3):249-62.
129. Harris AL. Emerging issues of connexin channels: biophysics fills the gap. Q Rev Biophys 2001;34:325-427.
130. Trexler EB, Bukauskas FF, Bennett MVL, Bargiello TA, Verselis VK. Rapid and direct effects of pH on connexins revealed by the connexin46 hemichannel preparation. J Gen Physiol 1999;113(5):721-42.
131. Urschel S, Hoher T, Schubert T, Alev C, Sohl G, Worsdorfer P, et al. Protein kinase A-mediated phosphorylation of connexin36 in mouse retina results in decreased gap junctional communication between AII amacrine cells. J Biol Chem 2006;281(44):33163-71.
132. Saez JC, Martinez AD, Branes MC, González HE. Regulation of gap junctions by protein phosphorylation. Braz J Med Biol Res 1998;31(5):593-600.
133. Solan JL, Lampe PD. Connexin phosphorylation as a regulatory event linked to gap junction channel assembly. Biochim Biophys Acta 2005;1711(2):154-63.
134. Cottrell GT, Lin R, Warn-Cramer BJ, Lau AF, Burt JM. Mechanism of v-Src- and mitogen-activated protein kinase-induced reduction of gap junction communication. Am J Physiol Cell Physiol 2003;284(2):C511-20.
135. Duncan JC, Fletcher WH. Alpha 1 Connexin (connexin43) gap junctions and activities of cAMP-dependent protein kinase and protein kinase C in developing mouse heart. Dev Dyn 2002;223(1):96-107.
136. Sirnes S, Kjenseth A, Leithe E, Rivedal E. Interplay between PKC and the MAP kinase pathway in Connexin43 phosphorylation and inhibition of gap junction intercellular communication. Biochem Biophys Res Commun 2009;382(1): 41-5.
137. John S, Cesario D, Weiss JN. Gap junctional hemichannels in the heart. Acta Physiol Scand 2003;179(1):23-31.
138. Laird DW. Connexin phosphorylation as a regulatory event linked to gap junction internalization and degradation. Biochim Biophys Acta 2005;1711(2):172-82.
139. Lampe PD, Lau AF. The effects of connexin phosphorylation on gap junctional communication. Int J Biochem Cell Biol 2004;36(7):1171-86.
140. van Veen TA, van Rijen HV, Jongsma HJ. Electrical conductance of mouse connexin45 gap junction channels is modulated by phosphorylation. Cardiovasc Res 2000;46(3): 496-510.
141. Solan JL, Lampe PD. Connexin43 phosphorylation: structural changes and biological effects. Biochem J 2009;419(2):261-72.
142. Paulson AF, Lampe PD, Meyer RA, TenBroek E, Atkinson MM, Walseth TF, et al. Cyclic AMP and LDL trigger a rapid enhancement in gap junction assembly through a stimulation of connexin trafficking. J Cell Sci 2000;113 (Pt 17):3037-49.
143. TenBroek EM, Lampe PD, Solan JL, Reynhout JK, Johnson RG. Ser364 of connexin43 and the upregulation of gap junction assembly by cAMP. J Cell Biol 2001;155(7):1307-18.
144. Leithe E, Rivedal E. Ubiquitination and down-regulation of gap junction protein connexin-43 in response to 12-O-tetradecanoylphorbol 13-acetate treatment. J Biol Chem 2004;279(48):50089-96.
145. Leithe E, Rivedal E. Epidermal growth factor regulates ubiquitination, internalization and proteasome-dependent degradation of connexin43. J Cell Sci 2004;117(Pt 7):1211-20.
146. Solan JL, Lampe PD. Connexin 43 in LA-25 cells with active

v-src is phosphorylated on Y247, Y265, S262, S279/282, and S368 via multiple signaling pathways. Cell Commun Adhes 2008;15(1):75-84.
147. Cooper CD, Lampe PD. Casein kinase 1 regulates connexin-43 gap junction assembly. J Biol Chem 2002;277(47):44962-8.
148. Neijssen J, Herberts C, Drijfhout JW, Reits E, Janssen L, Neefjes J. Cross-presentation by intercellular peptide transfer through gap junctions. Nature 2005;434:83-8.
149. Valiunas V, Polosina YY, Miller H, Potapova IA, Valiuniene L, Doronin S, et al. Connexin-specific cell-to-cell transfer of short interfering RNA by gap junctions. J Physiol 2005; 568:459-68.
150. Valiunas V, Weingart R, Brink PR. Formation of heterotypic gap junction channels by connexins 40 and 43. Circ Res 2000; 86(2):E42-9.
151. Cottrell GT, Burt JM. Heterotypic gap junction channel formation between heteromeric and homomeric Cx40 and Cx43 connexons. Am J Physiol Cell Physiol 2001;281(5): C1559-67.
152. Valiunas V, Beyer EC, Brink PR. Cardiac gap junction channels show quantitative differences in selectivity. Circ Res 2002;91(2):104-11.
153. Elenes S, Martinez AD, Delmar M, Beyer EC, Moreno AP. Heterotypic docking of Cx43 and Cx45 connexons blocks fast voltage gating of Cx43. Biophys J 2001;81(3):1406-18.
154. Bukauskas FF, Bukauskiene A, Verselis VK, Bennett MVL. Coupling asymmetry of Heterotypic connexin 45/connexin 43-EGFP gap junctions: properties of fast and slow gating mechanisms. Proc Natl Acad Sci U S A 2002;99:7113-8.
155. Moreno AP, Fishman GI, Beyer EC, Spray DC. Voltage dependent gating and single channel analysis of heterotypic channels formed by Cx45 and Cx43. Prog Cell Res 1995; 4:405-8.
156. Koval M, Geist ST, Westphale EM, Kemendy AE, Civitelli R, Beyer EC, et al. Transfected connexin45 alters gap junction permeability in cells expressing endogenous connexin43. J Cell Biol 1995;130:987-95.
157. Rackauskas M, Kreuzberg MM, Pranevicius M, Willecke K, Verselis VK, Bukauskas FF. Gating properties of heterotypic gap junction channels formed of connexins 40, 43 and 45. Biophys J 2007;92(6):1952-65.
158. Rackauskas M, Verselis VK, Bukauskas FF. Permeability of homotypic and heterotypic gap junction channels formed of cardiac connexins mCx30.2, Cx40, Cx43, and Cx45. Am J Physiol Heart Circ Physiol 2007;293(3):H1729-36.
159. Abrams CK, Freidin MM, Verselis VK, Bargiello TA, Kelsell DP, Richard G, et al. Properties of human connexin 31, which is implicated in hereditary dermatological disease and deafness. Proc Natl Acad Sci U S A 2006;103:5213-18.
160. Palacios-Prado N, Bukauskas FF. Heterotypic gap junction channels as voltage-sensitive valves for intercellular signaling. Proc Natl Acad Sci U S A 2009;106(35):14855-60.
161. Verselis VK, Veenstra RD. Gap junction channels. Permeability and voltage gating. In: Hertzberg E, editor. Advances in molecular and cell biology. JAI Press Inc; 2000. p. 129-92.
162. Weber PA, Chang HC, Spaeth KE, Nitsche JM, Nicholson BJ. The permeability of gap junction channels to probes of different size is dependent on connexin composition and permeant-pore affinities. Biophys J 2004;87(2):958-73.
163. Valiunas V. Biophysical properties of connexin-45 gap junction hemichannels studied in vertebrate cells. J Gen Physiol 2002;119:147-64.
164. Eckert R. Gap-junctional single-channel permeability for fluorescent tracers in mammalian cell cultures. Biophys J 2006;91(2):565-79.

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