Cell Tissue Res (2013) 352:21–31
DOI 10.1007/s00441-012-1485-6

REVIEW

Gap-junction-mediated cell-to-cell communication

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Received: 29 March 2012 / Accepted: 1 August 2012 / Published online: 1 September 2012

Abstract Cells of multicellular organisms need to communicate with each other and have evolved various mechanisms for this purpose, the most direct and quickest of which is through channels that directly connect the cytoplasms of adjacent cells. Such intercellular channels span the two plasma membranes and the intercellular space and result from the docking of two hemichannels. These channels are densely packed into plasma-membrane spatial microdomains termed "gap junctions" and allow cells to exchange ions and small molecules directly. A hemichannel is a hexameric torus of junctional proteins around an aqueous pore. Vertebrates express two families of gap-junction proteins: the well-characterized connexins and the more recently discovered pannexins, the latter being related to invertebrate innexins ("invertebrate connexins"). Some gap-junctional hemichannels also appear to mediate cell-extracellular communication. Communicating junctions play crucial roles in the maintenance of homeostasis, morphogenesis, cell differentiation and growth control in metazoans. Gap-junctional channels are not passive conduits, as previously long regarded, but use "gating" mechanisms to open and close the central pore in response to biological stimuli (e.g. a change in the transjunctional voltage). Their permeability is finely tuned by complex mechanisms that have just begun to be identified. Given their ubiquity and diversity, gap junctions play crucial roles in a plethora of functions and their dysfunctions are involved in a wide range of diseases. However, the exact mechanisms involved remain poorly understood.

Keywords Connexin · Pannexin · Innexin · Electrical synapse · Gap junction

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Introduction

Gap-junction channels provide one of the most common forms of intercellular communication and fulfil similar functions in all multicellular animals (Metazoa). They are composed of membrane proteins that form a channel allowing the diffusion of ions and small molecules between the cytoplasm of adjacent cells (for a general presentation, see Harris 2001; Saez et al. 2003). Channels, clustered in plasma membrane spatial microdomains termed gap junctions, result from the docking of two hemichannels, which are hexameric torus of junctional proteins around an aqueous pore. All junctional channels have a similar overall structure but, unlike many other membrane channels, different gene families encode the membrane proteins that form them in different animal phyla. The permeability and gating characteristics of gap-junction channels depend on the junctional protein isoform and on the post-translational modifications present on them. In most cases, one junctional protein cannot fully substitute for another. Although many junctional channels are relatively nonselective in their permeability to ions and small molecules, some differences in permeability to cytoplasmic solutes (with potential biological consequences) have now been described (for references, see Properties of gap junctional channels).

Rather than being fixed passive conduits, as long regarded, gap-junction channels are regulated by complex mechanisms that are only now being identified. The level of cell-to-cell

communication might indeed be actively adjusted by multiple mechanisms including (1) changes in junctional protein expression, (2) regulation of their trafficking and turnover and (3) modulation of channel properties. The degree of intercellular communication is sensitive to a variety of stimuli, including changes in the level of intracellular Ca^{2+}, in pH, in transjunctional applied voltage, in phosphorylation/dephosphorylation processes and in response to the activation of various kinds of membrane receptors (see Dynamic modulation of gap-junctional communication). Alterations in the phosphorylation status of proteins, resulting from the dynamic interplay of protein kinases (PKs) and protein phosphatases (PPs), are thought, for example, to be involved in a broad variety of junctional protein processes (such as the trafficking, assembly/disassembly, degradation as well as the gating of junctional channels) but the underlying mechanisms still remain poorly understood.

Because of their mediation of the cell-to-cell diffusion of ions, metabolites and small cell-signalling molecules, junctional channels play pivotal roles in a wide range of physiological processes, for example, in regulating events not only in development, cell differentiation, growth and proliferation, electrical activation of the heart and of smooth muscles or neuronal signalling, but also in hormone secretion, auditory function, wound healing, lens transparency or immune functions. In addition to their actions as mediators of the transfer of small molecules between neighbouring cells, some junctional proteins also appear to also have direct gap-junction-independent effects, for example, on cell growth (see Properties of gap junctional channels).

Consequently, defects in junctional proteins and therefore in gap-junctional communication are associated with a wide variety of pathologies in humans and experimental animals because of alterations in protein expression and in the modulation of channel properties. On the other hand, the neuro- and cardioprotective effects of gap-junction blocking agents demonstrate that the closure of these channels can be beneficial in certain pathological situations. Gap-junction proteins have also been shown to act as tumour suppressors but their mechanism(s) of action remains unclear (see Properties of gap junctional channels).

Molecular structure of gap-junctional intercellular channels

Gap junctions are observed in electron microscopy as the close apposition of plasma membranes of adjacent cells, with the persistence of a remarkably uniform 2 to 3 nm intercellular space ("gap"). When membranes are cleaved by freeze-fracture, a characteristic clustering of intramembranous protein particles is observed (Fig. 1). All gap-junctional channels share a similar overall structure but, unlike many other membrane channels, different gene families encode the membrane proteins that form them in different animal phyla. For a long time, gap-junction structure and functions were mainly investigated in vertebrates, in which they were thought to be composed only of connexins (Cxs). Then, in *Caenorhabditis elegans* (a nematode) and *Drosophila* (an arthropod), neither of which has Cx genes, gap junctions were found to be formed of products of another gene family, termed the innexins (Inxs, invertebrate analogues of Cxs), which have no sequence homology to Cxs (Phelan et al. 1998). The list of animal phyla with identified Inx family members progressively extended to the Annelida, Platyhelminthes, Mollusca and Coelenterata. Subsequently, sequences with low similarity to the invertebrate Inxs were identified in vertebrate chordates, leading some authors to suggest that the protein family be re-named

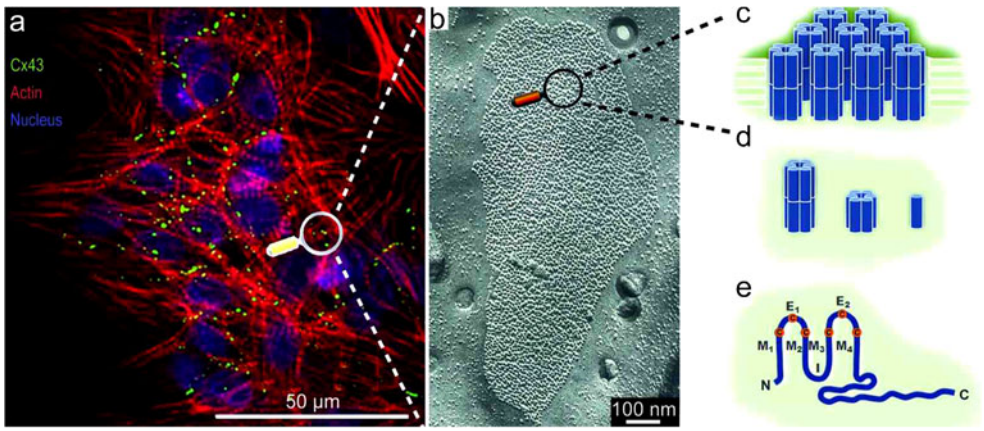

Fig. 1 Gap junctions in cell membranes. **a** Immunostaining of cultured neonatal rat cardiomyocytes. **b** Freeze-fracture replica of a gap junction in the plasmatic leaflet of the membrane of a cardiac Purkinje fibre of a sheep. **c** Diagram of clustered intercellular channels. **d** Each junctional channel is made by the docking of two connexons (*left*), with each connexon (*middle*) consisting of six connexins (*right*). **e** Transmembrane topology of the Cx43 polypeptide (*M* transmembrane domain, *E* extracellular loop, *I* cytoplasmic loop, *C in red dot* conserved cysteine residue, *N* N-terminal, *black C* C-terminal)

the pannexins, from the Greek "pan", meaning all/entire, and nexus, meaning connection (Panchin et al. 2000; Panchin 2005), abbreviated to Panx. It also emerged that Cx genes were not restricted to vertebrates but were also present in invertebrate chordates (for example, in tunicates, ascidians and appendicularians; for an analysis of their relationship to vertebrate Cxs, see White et al. 2004).

Cx, Inx and Panx proteins share the same topology (Fig. 2), with four alpha-helical transmembrane (TM) domains connected by two extracellular (EC) loops and a single cytoplasmic loop; both N- and C-terminals are intracellular. Inxs display only about 16% overall identity when their full-length amino-acid sequences are compared with either Panxs or Cxs, which might simply reflect the fact that these are all four transmembrane domain proteins. There is somewhat greater identity between Inxs and Panxs when only the first halves of the molecules (the first two TM domains and the intervening EC loop) are compared. A pair of cysteine residues in EC1 is absolutely conserved in all Inxs and Panxs as is a proline motif in TM2. A proline is found in the same relative position in all Cxs suggesting that this residue is strictly conserved in gap-junction proteins. Notably, Panxs do not possess a YYXWX motif in TM2, a motif regarded as a signature sequence of Inxs. Invertebrate Cxs share 25%–40% sequence identity with human Cxs. Twenty and twenty-one members of the Cx gene family appear to be expressed in the mouse and human genome, respectively (19 of which can be grouped into sequence-orthologous pairs) and orthologues are increasingly being characterized in other vertebrates; in invertebrate chordates, a comparable number (e.g. 17 Cx-like sequences in a basal marine chordate, the tunicate *Ciona intestinalis*) have been found. Far fewer Panx genes have been detected; as yet, only three have been described in mouse and human. The Inx family appears large, since well over 50 sequences have already been reported (e.g. eight in *Drosophila* and 25 in *C. elegans*) but functional studies of cell–cell communication have only been accomplished for some of them (see Phelan 2005). Cxs have been classified according to their predicted molecular weight and named accordingly (for example, Cx43 for the 43-kDa Cx, etc.).

Individual gap-junctional proteins assemble into an annular torus structure around an aqueous pore, forming a "hemichannel" called a connexon, innexon or pannexon, made of single (homomeric) or multiple (heteromeric) protein isotypes. Cxs, Inxs and Panx1 assemble into hexamers and Panx2 into octamers (the oligomeric number of a Panx3 membrane channel has not yet been determined; see Sosinsky et al. 2011). These proteins have conserved cysteine residues in their extracellular loops, namely six in all Cxs except for Cx23 (Iovine et al. 2008) and four in Inxs, Panx1 and Cx23; these residues form intra-protein disulfide bonds (for a review, see Kovacs et al. 2007). The end-to-end docking of a hemichannel in the membrane of one cell with a corresponding hemichannel in an adjacent cell membrane forms an intercellular channel that spans the two plasma membranes. Although Panxs have been found to be able to form cell-to-cell channels in paired oocytes (Bruzzone et al. 2003), uncertainty remains with regard to this capability in native tissues. Intercellular dye or electrical coupling are indeed generally absent in a variety of Panx-expressing host cells (see Dahl and Harris 2008) but Ca^{2+} wave propagation

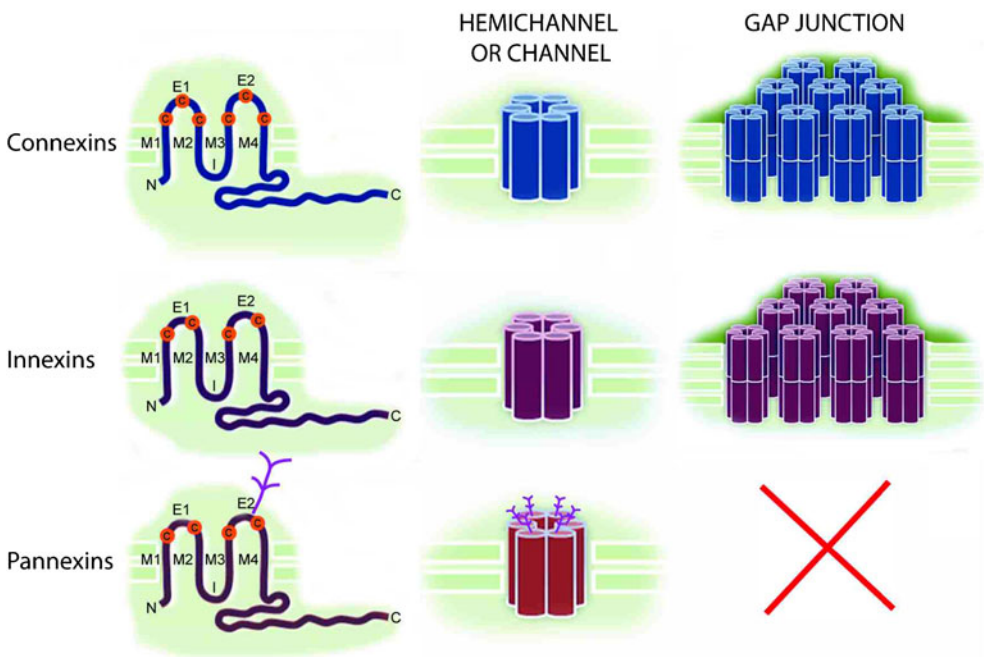

Fig. 2 Families of gap-junction proteins. Vertebrates express two protein families: the well-characterized connexins and the more recently discovered pannexins, the latter being related to invertebrate innexins ("invertebrate connexins"). The oligomeric assembly of junctional proteins around an aqueous pore cross the plasma membrane, the end-to-end docking of hemichannels form a cell-to-cell channel but is usually prevented in pannexins by glycosylation in their extracellular loops

between osteoblasts has recently been ascribed to Panx3 gap junctions (Ishikawa et al. 2011). The expression of multiple protein isotypes in the same cell type, the multiplicity of isoforms and their different structural combinations probably provide exquisite "functional tuning" of this unique family of membrane channels.

Moreover, as is now obvious, gap-junction proteins do not operate as free-floating entities in the plasma membrane but interact with specific cytoplasmic proteins that link them to the cytoskeleton and to intracellular signal transduction pathways. Such co-assembly into multiprotein complexes is likely to be important for immobilization and clustering of the cell-to-cell channels, for correct targeting of channels to specific subcellular sites, for the ability of channels to funnel ions and small molecules and for modulation of the channel functions by PKs, PPS, and other regulatory proteins. Conversely, gap-junction proteins might also, through their diverse partners, be involved in cell functions markedly different from their classical cell-to-cell tunnel-forming functions (for a recent review, see Hervé et al. 2012).

Experimental approaches for investigating gap-junctional communication

Macroscopic junctional conductance (Gj) is determined by using the simultaneous voltage-clamping of two coupled cells. Both cells are at first clamped at a common holding potential (Vh) and then a pulse is applied (e.g. for 125 ms every 30 s) to one cell while the second cell is maintained at Vh to generate a transjunctional voltage difference (Vj). Therefore, when contacting cells are connected by open junctional channels, this voltage gradient induced a junctional current (Ij) flowing through them from one cell to its neighbour and Gj can be calculated by dividing Ij by the amplitude of the Vj pulse.

Two main recording configurations are commonly used, either the conventional whole-cell or the perforated-patch configuration. In the latter, after the gigaseal formation, the membrane patch under the pipette tip is permeabilized with a pore-forming antibiotic (e.g. amphotericin B) instead of being ruptured. Antibiotic-made membrane pores are not voltage dependent, are permeable to monovalent ions (somewhat selective for monovalent cations over anions) and impermeable to multivalent ions and molecules of >0.8 nm in diameter. Therefore, this configuration allows the prevention of both the dilution of cytosolic components and the disruption of the normal intracellular Ca^{2+}-buffering mechanisms.

Currents through single intercellular channels can be recorded by a dual voltage-clamp in cases in which the number of functioning channels is sufficiently small or reduced by superfusion with a gap-junctional blocker (e.g. 1-heptanol or halothane) that is able to reduce considerably the open probability of channels, thereby lowering the junctional conductance to a sufficiently low level for the detection of single channel activity. Historically, the unpaired hemichannels scattered in nonjunctional membranes were considered as remaining permanently closed to avoid cell death but several sets of data reported in the past decade revealed that at least some of them mediated paracrine signalling by providing a flux pathway for ions such as Ca^{2+}, for ATP, for glutamate and plausibly for other compounds in response to physiological and pathological stimuli. Analysis of unopposed hemichannels in functional expression systems provides a unique opportunity to compare hemichannel properties with those of intact gap-junction channels between coupled cells. Each isoform of gap-junction protein indeed forms intercellular channels with unique properties of size selectivity, ion permeability, conductance and gating.

The intercellular diffusion of fluorescent dyes is the most common approach to the quantification of gap-junction selectivity. The first method for introducing the fluorescent dye into the cells was via microinjection by means of a sharp microelectrode that was filled with the respective dye and impaled into the cells, the dye (non-toxic, not lipophilic and not metabolized by the cells) being released into the cells by either pressure or iontophoresis. The diffusion of the injected dye to neighbouring cells is generally observed for compounds up to 1,000 Daltons. The addition of fluorescent probes of various charges and sizes to the filling solution of one of the patch pipettes is a useful tool for dissecting biophysical properties of gap-junction channels by the simultaneous measurement of junctional conductance and intercellular dye transfer. In living sliced preparations, the presence of a fluorescent dye in the patch pipette solution enables the delineation both cell morphology and connections.

The scrape loading method consists of exposing a monolayer of cultured cells to a calcium-free medium containing two fluorescent dyes of different molecular weights. A scrape line made on the monolayer by a surgical blade allows the dyes to penetrate into the injured cells. The dye of high molecular weight stains the injured cells but only the dye of low molecular weight (e.g. Lucifer yellow) can diffuse into the neighbouring cells. The pre-loading method is a non-invasive assay that allows the extent of dye spread and the effective diffusivities through gap junctions to be probed. Isolated cells are pre-loaded with two fluorescent dyes, namely calcein (which is able to pass through gap-junction channels) and DiI (1,1'-dioctadecyl 3,3,3',3'-tetramethylindocarbocyanine perchlorate, which is unable to pass) and plated with unlabeled cells (e.g. a cell monolayer). The existence of calcein transfer from each pre-loaded cell can then be ascertained.

These methods allow us to test whether a dye can diffuse between the cells but do not permit any precise quantification. This is made possible by analysing the kinetics of fluorescence recovery after photobleaching (FRAP): cultured cells are exposed to the ester form of a fluorescent dye (e.g. carboxyfluorescein), which is able to cross junctional channels. Inside the cell, the esterases release the dye, which is trapped inside the cells. After removal of the extracellular dye, the light emission is recorded. The dye inside one of the cells is then bleached by means of strong illumination (e.g. a laser beam). If the cells are interconnected, the diffusion of intact dye occurs and the light emission of the bleached cell increases until equilibrium is reached between the cells. The kinetics of the fluorescence emission in the bleached cell follows, at least initially, a mono-exponential time course, allowing the determination of its time constant. An important advantage of this method is that the measurement can be repeated, up to 4 times, on the same cell group (see Délèze et al. 2001).

Gap-junction channels have long been considered as non-specific or at least as poorly specific channels. However, they show some specificity, e.g. towards cyclic nucleotides, and specific techniques have been developed to investigate this property, for example, by Bedner et al. (2003). In their study, HeLa cells were transfected with DNAs coding for cyclic nucleotide-gated (CNG) and for Cx45 channels. After the cells had been incubated with a membrane-permeable caged cAMP, the nucleotide was released inside one of the cells; its diffusion through the intercellular channels activated, in turn, Ca^{2+}-conducting CNG channels in the neighbouring cells. The calcium transient, detected by means of a Ca^{2+}-indicator dye, was proportional to the extent of cAMP intercellular diffusion. Subsequent double whole-cell recordings allowed the number of gap-junction channels that were involved to be calculated and a comparison of the selective permeabilities of gap-junctional channels composed of different Cxs for cAMP or for cGMP.

Properties of gap-junctional channels

Permeability and selectivity of channels Generically, the pores of gap-junction channels are wide enough (approximately 1.2 nm) to be permeable to a wide variety of cytoplasmic molecules, i.e. to virtually (at least for some types of Cx channels) all soluble second messengers, amino acids, nucleotides, ions and glucose and its metabolites. Until a few years ago, any cytoplasmic molecule of appropriate size (up to about 1 kDa in molecular mass) was thought to be able to diffuse through gap-junction channels, with the pore diameter being the primary limit to cell-to-cell diffusion. The relatively large pore diameter was also widely presumed to lead to a lack of significant charge selectivity between permeants. Both expectations now appear incorrect since pore diameter, while obviously important, does not account for the observed molecular selectivities and since many Cx channels exhibit substantial charge selectivity, even between monovalent ions (for reviews, see Harris 2007; Ek-Vitorin and Burt 2012).

Voltage sensitivity Gap-junction channels, regardless of type (homomeric or heteromeric, homotypic or not), display voltage-dependent gating but the kinetics and sensitivity vary over a wide range. Most channels are sensitive to the voltage difference between the cell interiors (or transjunctional voltage, Vj), whereas other channels are also sensitive to absolute inside-outside voltage (i.e. the membrane potential, Vm). This dual voltage regulation can be explained either by a single gate capable of responding to both Vm and Vj or by the existence of two different gates each of which specifically senses one type of voltage (for a review, see González et al. 2007). The hypothesis of two separate gates is supported by the striking differences of the gating properties of Vj and Vm in a given Cx channel with respect to the polarity of closing, voltage sensitivity and kinetics. The mechanisms of Vj and Vm gating also diverge in terms of the class of gating events including their probabilities, residual conductances and intervening states. Furthermore, each of the hemichannels forming an intercellular channel has its own sets of Vj and Vm gates, which can interact internally within the hemichannel and also with those of the associated hemichannel (see Palacios-Prado and Bukauskas 2012). Finally, instantaneous/initial macroscopic and unitary junctional currents have been described as having rectification, which might be a voltage-dependent property of the conductive pore.

Dynamic modulation of gap-junctional communication

Direct chemical modulation Ca^{2+} was the first identified agent able to cause a "decoupling of the highly permeable intercellular bridges" (Délèze 1970), proposed to act via calmodulin (CaM), through an interaction between Ca^{2+}-bound CaM and one or more intracellular domains of Cxs (Peracchia et al. 1983). The way that CaM controls the chemical gating of hemichannels or intercellular channels is not yet understood, although Dodd et al. (2008) have proposed a model of CaM-Cx32 interaction, whereas a "cork-type" gating (a CaM "lobe" physically obstructing the cytoplasmic mouth of the channel pore) has been suggested for the Ca^{2+}-CaM-induced inhibition of gap-junctional intercellular communication (GJIC) mediated by the lens Cx43 (Zhou et al. 2007), Cx44 (Zhou et al. 2009) and Cx50 (Chen et al. 2011).

Gap-junction channels (particularly Cx43 channels) are able to close in response to acidification of the intracellular

medium. The proposed mechanism involves a Cx intramolecular interaction between the channel pore and a discrete C-terminal domain of the Cx, serving as gating particle, i.e. a "ball and chain" model (Liu et al. 1993), analogous to the inactivation of Shaker-type K^+ channels (Hoshi et al. 1990). Several classes of hydrophobic compounds, including some long-chain alkanols, aldehydes, fatty acids, steroids, fenamates, triphenylethylenes, phenols, cardiac glycosides and volatile anaesthetics (for a review, see Hervé and Sarrouilhe 2005) are able to reduce Cx channel activity. The most frequently proposed mechanism of action of lipophilic compounds is an insertion into the membrane lipid bilayer, with resulting modification in the microenvironment of the membrane proteins, including membrane channels. Perturbations of lipid-lipid and lipid-protein interactions might, in turn, alter the function of membrane proteins.

The absence of specific affinity reagents or toxins (compared with those that have been vitally important in elucidating the molecular mechanisms of several other channels) has hindered the biophysical study of Cx channels. Promising perspectives for the development of targeted drugs directed against Cx channels have been provided by both synthetic Cx-mimetic peptides of the extracellular loops of Cxs (small peptides that correspond to parts of their sequences and that are expected to inhibit the docking of the hemichannels of two adjacent cells) and peptides acting on gap junctions via membrane receptors, as natural antiarrhythmic peptides or endothelins (for a recent review, see Hervé and Dhein 2010) have been used to influence GJIC.

Protein phosphorylation Protein phosphorylation, a widespread mechanism of functional modulation, is regarded as a major mechanism of the regulation of gap-junctional communication and influences the extent of GJIC at several steps, by governing Cx trafficking from the Golgi complex to the plasma membrane, the aggregation of channels into selected areas and the prevention of their free diffusion throughout the lipid bilayer, their removal from the plasma membrane, their degradation as well as the gating of gap-junction channels. Most of the Cxs are indeed phosphoproteins, being phosphorylated on serine and, in some cases, on tyrosine residues. Among the investigated PKs, cAMP-activated PK (PKA), protein kinase C (PKC), protein kinase G (PKG), p34cdc2, casein kinase 1 (CK1), mitogen-activated protein (MAP) kinase and pp60Src have been shown to target Cxs (for a review, see Moreno and Lau 2007). The importance of PPs in phenomena regulated by protein phosphorylation was for many years underestimated; they were originally supposed to act mainly to reverse the action of regulated PKs, before it became obvious that PP activities were also modulated in a sophisticated manner by regulatory and targeting subunits. Phosphoserine/threonine PPs (PP1, PP2A and PP2B) and phosphotyrosine PPs have been reported to modify Cx phosphorylation status and/or GJIC (see Hervé and Sarrouilhe 2006).

This GJIC modulation appears to be much more complex than direct Cx phosphorylation and increasing evidence indicates that protein partners are involved. This view is supported by a lack of correlation between the degree of junctional coupling and the pattern of Cx phosphorylation (for example, of Cx43 or Cx45), suggesting that the participation of additional regulatory component is required (see Hervé et al. 2004). On the other hand, several associations of Cxs with protein partners have been found to depend on the phosphorylation status of Cx43 (for a review, see Hervé et al. 2012).

Humoral modulation GJIC is controlled by a range of biomolecules, including neurotransmitters (e.g. adrenaline and noradrenaline, dopamine or serotonin), growth factors and various bioactive compounds (such as lysophosphatidic acid).

The activation of adrenoceptors by transmitters, adrenaline (epinephrine) or noradrenaline (norepinephrine), is a potent modulator of the propagation of action potentials within interconnected cells, particularly in cardiac tissues and neuronal networks. In rat hippocampus, for example, noradrenaline reduces electrical coupling predominantly via β-adrenergic receptors via a cAMP/PKA-dependent pathway (Zsiros and Maccaferri 2008). In the heart, acute β-adrenergic activation enhances Cx40 conductance but reduces Cx45 conductance. In cultured rat neonatal cardiomyocytes, acute β-adrenergic stimulation enhances GJIC and induces a phosphorylation of Cx43 via PKA, Epac and PKC (Duquesnes et al. 2010). Chronic β-adrenergic stimulation enhances the expression of Cx43 mRNA and Cx43 protein together with Cx43 phosphorylation but does not affect Cx40 (Salameh and Dhein 2011). α-Adrenergic stimulation acutely uncouples the cells, whereas chronic stimulation enhances Cx43 expression without altering Cx40 expression (Salameh and Dhein 2011).

The dynamic regulation of GJIC by the light-activated neuromodulator dopamine has been particularly well investigated in the vertebrate retina (for a review, see Bloomfield and Völgyi 2009). Among the proposed mechanisms, the light-induced liberation of dopamine might both activate (via dopaminergic receptors D1) or reduce (via dopaminergic receptors D2/4) the activity of PKA, with resulting effects on the conductance of gap junctions (Bloomfield and Völgyi 2009).

Serotonin mediates a wide range of modulatory effects on neural circuit development and function (see Rörig and Sutor 1996). Serotonin also influences cardiac activity with the activation of several serotoninergic subtypes receptors (particularly 5-HT_{4b}, 5-HT_{2A} and 5-HT_{2B}) being seen to affect the intercellular coupling of cardiac myocytes markedly (Derangeon et al. 2010).

Thyroid hormones influence the electrical and contractile functioning of the heart by affecting the activities of diverse

channels (e.g. K^+ or Ca^{2+} channels). Thyroid hormone up-regulates Cx40 mRNA expression in the mouse atrium and concomitantly influences electrocardiographic parameters (Almeida et al. 2009).

Functional roles of gap-junctional channels

Gap-junction intercellular channels enable the formation of communication compartments that are essential for the maintenance of tissue function and homeostasis, with GJIC being actively involved in virtually all aspects of cellular life cycle, ranging from cell growth to cell death. Panxs seem mainly to form single plasma membranes channels (see Molecular structure of gap-junctional intercellular channels). Cxs might also be active as functional "hemichannels" in nonjunctional membranes, linking the cytoplasm of a cell with its extracellular microenvironment (see Goodenough and Paul 2003). Such hemichannels are thought to rest in a predominantly closed state in vivo, with transient openings in response to a wide range of stimuli (for a recent review, see Saez et al. 2010).

The cell-cell transfer of current or electrical coupling of cells is of fundamental importance in excitable tissues, e.g. the heart, in which current transfer occurs only through gap-junction channels. Hence, the spatial distribution and biophysical properties of gap-junction channels are important determinants of the conduction properties of cardiac muscle. In the nervous system, electrical synapses, without the need for receptors recognizing chemical messengers, allow a faster (by about 10 times) propagation of the signal than that which occurs across chemical synapses, the predominant kind of junctions between neurons. This higher speed of propagation also enables many neurons to fire synchronously and so electrical synapses are abundant in escape mechanisms and other processes that require quick responses, such as the response to danger. Moreover, currents carried by ions frequently travel in both directions through this type of synapse.

Gap junctions also mediate cell-to-cell propagation of some signalling molecules that might regulate cell growth, differentiation, functions and death. Junctional channel pores are indeed sufficiently wide to be permeable to a wide variety of cytoplasmic molecules; evidence exists for permeability through at least some types of Cx channels of virtually all soluble second messengers, amino acids, nucleotides and glucose and its metabolites.

In a wide diversity of cell types, coordinated long-distance responses are propagated by calcium waves, which are coordinated oscillations of the intracellular calcium concentration propagated from cell to cell as waves. Cell-to-cell propagation through gap-junction channels is one of the pathways mediating this response, as initiated by a focal mechanical, electrical or hormonal stimulus, and might serve to coordinate a global cellular response by being propagated to numerous adjacent cells. Although Ca^{2+} can move through gap junctions, its intracellular Ca^{2+} concentration is normally extremely low. A great deal of experimental evidence has however accumulated supporting the hypothesis that intercellular calcium waves result from the diffusion of inositol 1,4,5-trisphosphate (IP3) through gap junctions. According to this hypothesis, IP3 initially produced in a single cell binds to its receptors on the endoplasmic reticulum, triggering the release of Ca^{2+} into the cytoplasm. The diffusion of IP3 passing between cells via gap junctions thus progressively causes a calcium wave (for a review, see Dupont et al. 2007).

Membrane connexons (hemichannels) scattered in the non-junctional plasma membrane were originally regarded as precursors of cell-to-cell channels but evidence now exists showing that they can also play distinct roles in intercellular signalling. Hemichannels are normally kept closed in the presence of normal extracellular calcium concentrations but various cells can however tolerate some hemichannel opening, which might exert physiological or deleterious effects, depending on the situation. This pathway would allow the release, into the extracellular medium, of signalling molecules (e.g. ATP, glutamate, NAD^+, prostaglandin E_2, ions) and might even provide pathways for foreign and possibly infectious agents to enter cells.

Although Panxs (particularly Panx1 and Panx3) have occasionally been suggested to form intercellular gap-junction channels, the vast majority of the literature supports the view that their primary role is to form single membrane channels (see Penuela et al. 2012). These channel-forming glycoproteins are all capable of dye uptake and Panx1 has been extensively reported as an ATP/UTP release channel, even under conditions of physiological levels of calcium.

Gap-junction channels and other membrane channels and receptors mutually influence their behaviour

The various channels, receptors and transporters present in membranes frequently influence each other either directly (e.g. through direct protein–protein interactions or via an intermediate partner protein) or indirectly (one of them might, for example, mediate the transport of a molecule regulating the activity of the second or influence the membrane insertion of the second). Such mutual interactions might have important functional consequences on cellular homeostasis and functions.

Channels In cardiac ventricular tissue, the fast inward sodium current (I_{Na}) is the main depolarizing current and Cx43 is the major junctional channel-forming protein permitting current flow between adjacent cells. A decrease of I_{Na} or a

decrease in GJIC both lead to the slowing of conduction, thereby increasing the risk of life-threatening re-entrant arrhythmias. In fetal atrial myocytes, the genetic ablation of Cx43 decreases immunofluorescence signals of Cx40 and Cx45 and a marked decrease in Na^+ inward current (Desplantez et al. 2012). In mouse heart, a reduced heterogeneous expression of Cx43 results in decreased Nav1.5 expression (Jansen et al. 2012). Immunohistochemical studies have revealed that Nav1.5 channels are preferentially located in intercalated disks, closely associated with both N-cadherin and Cx43 (Malhotra et al. 2004). In cardiac intercalated discs, according to Sato et al. (2011), desmosomes, gap junctions and Na^+ channels do not act independently but rather as a "functional triad" in which changes in the composition of one can affect the function and integrity of the other, with significant consequences to electric cardiac synchrony.

Cystic fibrosis transmembrane conductance regulator (CFTR), an ion channel that transports chloride ions across epithelial cell membranes, influences the characteristics of gap-junction channels, e.g. Cx45 voltage-sensitivity and the gating or inhibition of Cx43-mediated GJIC by proinflammatory mediators (for a review, see Chanson et al. 2007).

Aquaporins are water channel proteins belonging to the major intrinsic protein superfamily of membrane proteins; aquaporin-0 (AQP0), the most abundant membrane protein in lens fibres, has been found to interact with two binding sites within the intracellular loop region of Cx45.6 (Yu et al. 2005) or of its human ortholog Cx50 (Liu et al. 2011). In mouse astrocytes, decreases in the expression of AQP4, the main water channel present in the brain, specifically reduces GJIC mediated by Cx43 (Nicchia et al. 2005).

In double-transfected Neuro2A cells, Panx1 has been identified as a binding partner of $K_v\beta3$, a protein belonging to the family of regulatory β-subunits of the voltage-dependent K^+ channels (Bunse et al. 2005).

Receptors Combining confocal microscopy and freeze-fracture replica immunogold labelling, Rash et al. (2004) observed, in "mixed" (chemical plus electrical) synapses of goldfish and rat brain, a close association between Cx35 and an N-methyl-D-aspartate glutamate receptor subunit (NR1). In rat myocardium, Cx43 has been seen to interact (possibly indirectly) with M2/M3 subtypes of acetylcholine seven transmembrane-domain muscarinic receptors (mAChR); ischaemia specifically impairs this M3-mAChR/Cx43 association (Yue et al. 2006).

Extracellular ATP binds to a plethora of ionotropic (P2X) and metabotropic (P2Y) receptors, which mediate its action; this plays particularly important roles in paracrine signalling, allowing multiple cells within a tissue to respond actively to environmental stresses (such as metabolic inhibition, mechanical shear or microbial invasion) sensed by only a few cells at the site of environmental insult or stimulation. Panx1 appears to be the molecular substrate of a large pore activated by either metabotropic P2Y or ionotropic $P2X_7$ receptors (for a review, see Hervé et al. 2012), allowing the diffusion of molecules of up to 900 Da in size.

Consequences of GJIC alterations

Because of their ubiquity and diversity, gap junctions play crucial roles in multicellular organisms, particularly in the maintenance of homeostasis, morphogenesis, cell differentiation and growth control. Their dysfunctions are involved in a wide range of diseases but the exact mechanisms remain relatively poorly understood. Naturally occurring functional mutations of Cx genes are associated with several human inherited diseases. Charcot-Marie-Tooth disease, which comprises a group of genetically heterogeneous disorders of the peripheral nervous system, is the most common inherited peripheral neuropathy, with an estimated frequency of about 1 in 2500 births. The X-linked form of Charcot-Marie-Tooth (CMTX) is associated with mutations in the gene encoding Cx32, which is expressed in Schwann cells; to date, more than 400 different mutations have been found in CMTX patients (see Nualart-Marti et al. 2012). From the clinical standpoint, the disease is characterized by progressive weakness of the distal leg and intrinsic hand muscles, absent or diminished deep tendon reflexes and variable sensory loss.

Cx26 mutations are responsible for genetic deafness (both syndromic and nonsyndromic forms), with an estimated frequency of about 1 in 1000 births, but the exact role of Cx26 in the onset of nonsyndromic deafness remains unclear; apparently gap-junctional communication influences the ionic environment of the inner ear sensory epithelia. The loss of Cx26 might disrupt the recycling of potassium from the synapses at the base of hair cells through the supporting cells and fibroblasts and therefore would result in a local poisoning of Corti's organ by potassium, leading to hearing loss (for reviews, see Apps et al. 2007; Levit et al. 2011).

Mutations in Cx26 are also responsible for skin disorders; with regard to phenotype, these skin disorders show great variability but generally involve an increased thickness of the outer layers of the skin. This indicates a critical role for Cxs in maintaining the balance between the proliferation and differentiation of the epidermis (for s recent review, see Scott et al. 2012).

Point mutations in Cx50 and Cx46 have been identified in patients with inherited cataracts. Perhaps indicative of the unique requirement for gap-junction communication in maintaining lens tissue homeostasis, human lens Cx mutations are largely restricted to the extracellular loop or transmembrane domains.

In addition to these hereditary human diseases consecutive to Cx dysfunctions, a variety of developed human diseases exist, e.g. various cardiac diseases in which cardiac Cxs are reduced in number or redistributed from the intercalated disks (the ends of the cardiac myocytes) to lateral cell borders, a phenomenon called "gap-junction remodelling", which is considered to be arrhythmogenic (see Severs et al. 2008).

Given their key roles in homeostatic control, Cxs and their channels are frequently targeted upon impairment of this critical balance and this notion has been extensively described in carcinogenesis (see Cronier et al. 2009). Despite the many exceptions that have been reported, tumour cells generally display reduced GJIC. Numerous mechanisms appear to be involved, including the relatively rare occurrence of mutations in Cx genes, epigenetic modifications able to trigger the silencing of Cx gene expression, inappropriate phosphorylation and aberrant cytosolic localization of Cxs. The loss of cell-to-cell junctional communication might allow tumour cells to escape from normal growth regulation by the surrounding cells, thereby representing their growth independence. Cx genes are therefore considered as class II tumour suppressor genes and their overexpression in tumour cells is known to result in decreased cell proliferation and increased cell death activities.

GJIC can also be altered, for example, during injury and two opposing theories have been presented. Such a decrease in gap-junctional communication attenuates the spread of toxic metabolites from the injured area to healthy organ regions (see Patel et al. 2012). Moreover, the decrease in cellular communication also reduces the loss of important cellular metabolites, such as ATP and glucose.

Conclusions and perspectives

Gap-junctional channels, which are unique structures found in most animal cell types, span the two cell membranes and the intercellular space, thereby creating a conduit between the intracellular media of apposed cells and allowing the passive transfer on ions and small molecular weight molecules, and are involved in a variety of cell functions. Gap-junctional coupling was long regarded as a "static" way of communication through stable intercellular tunnels before being shown to be finely regulated. The importance of the rapid dynamics of channel turnover, of the plasticity of Cx expression and of the fine modulation of channel permeability in response to various stimuli, thus offering possibilities for rapid remodelling of intercellular circuits, is now becoming apparent. Mutational alterations in the Cx genes are associated with the occurrence of multiple diseases, including peripheral neuropathy, cardiovascular disease, dermatological disease, hereditary deafness and cataract. Consequently, the modulation of GJIC is a potential pharmacological target. Future research, based, for example, on recent developments in genetics, might clarify gap-junction physiology. This will, in turn, provide promising perspectives for the development of targeted drugs.

References

Almeida NA, Cordeiro A, Machado DS, Souza LL, Ortiga-Carvalho TM, Campos-de-Carvalho AC, Wondisford FE, Pazos-Moura CC (2009) Connexin40 messenger ribonucleic acid is positively regulated by thyroid hormone (TH) acting in cardiac atria via the TH receptor. Endocrinology 150:546–554

Apps SA, Rankin WA, Kurmis AP (2007) Connexin 26 mutations in autosomal recessive deafness disorders: a review. Int J Audiol 46:75–81

Bedner P, Niessen H, Odermatt B, Willecke K, Harz H (2003) A method to determine the relative cAMP permeability of connexin channels. Exp Cell Res 291:25–35

Bloomfield SA, Völgyi B (2009) The diverse functional roles and regulation of neuronal gap junctions in the retina. Nat Rev Neurosci 10:495–506

Bruzzone R, Hormuzdi SG, Barbe MT, Herb A, Monyer H (2003) Pannexins, a family of gap junction proteins expressed in brain. Proc Natl Acad Sci USA 100:13644–13649

Bunse S, Haghika A, Zoidl G, Dermietzel R (2005) Identification of a potential regulator of the gap junction protein pannexin1. Cell Comm Adhes 12:231–236

Chanson M, Kotsias BA, Peracchia C, O'Grady SM (2007) Interactions of connexins with other membrane channels and transporters. Prog Biophys Mol Biol 94:233–244

Chen Y, Zhou Y, Lin X, Wong HC, Xu Q, Jiang J, Wang S, Lurtz MM, Louis CF, Veenstra RD, Yang JJ (2011) Molecular interaction and functional regulation of connexin50 gap junctions by calmodulin. Biochem J 435:711–722

Cronier L, Crespin S, Strale PO, Defamie N, Mesnil M (2009) Gap junctions and cancer: new functions for an old story. Antioxid Redox Signal 11:323–338

Dahl G, Harris AL (2008) Pannexins or connexins? In: Harris AL, Locke D (eds) Connexins, a guide. Humana, New York, pp 287–302

Délèze J (1970) The recovery of resting potential and input resistance in sheep heart injured by knife or laser. J Physiol (Lond) 208:547–562

Délèze J, Delage B, Hentati-Ksibi O, Verrecchia F, Hervé JC (2001) Fluorescence recovery after photobleaching. Meth Mol Biol 154:313–327

Derangeon M, Bozon V, Defamie N, Peineau N, Bourmeyster N, Sarrouilhe D, Argibay JA, Hervé JC (2010) 5-HT4 and 5-HT2 receptors antagonistically influence gap junctional coupling between rat auricular myocytes. J Mol Cell Cardiol 48:220–229

Desplantez T, McCain M, Beauchamp P, Rigoli G, Rothen-Rutishauser B, Parker KK, Kléber AG (2012) Connexin 43 ablation in fetal atrial myocytes decreases electrical coupling, partner connexins and sodium current. Cardiovasc Res 94:58–65

Dodd R, Peracchia C, Stolady D, Török K (2008) Calmodulin association with connexin32-derived peptides suggests trans-domain interaction in chemical gating of gap junction channels. J Biol Chem 283:26911–26920

Dupont G, Combettes L, Leybaert L (2007) Calcium dynamics: spatio-temporal organization from the subcellular to the organ level. Int Rev Cytol 261:193–245

Duquesnes N, Derangeon M, Métrich M, Lucas A, Mateo P, Li L, Morel E, Lezoualc'h F, Crozatier B (2010) Epac stimulation

induces rapid increases in connexin43 phosphorylation and function without preconditioning effect. Pflügers Arch 460:731–741
Ek-Vitorin JF, Burt JM (2012) Structural basis for the selective permeability of channels made of communicating junction proteins. Biochim Biophys Acta (in press)
González D, Gómez-Hernández JM, Barrio LC (2007) Molecular basis of voltage dependence of connexin channels: an integrative appraisal. Prog Biophys Mol Biol 94:66–106
Goodenough DA, Paul DL (2003) Beyond the gap: functions of unpaired connexon channels. Nat Rev Mol Cell Biol 4:285–294
Harris AL (2001) Emerging issues of connexin channels: biophysics fills the gap. Q Rev Biophys 34:325–472
Harris AL (2007) Connexin channel permeability to cytoplasmic molecules. Prog Biophys Mol Biol 94:120–143
Hervé JC, Dhein S (2010) Peptides targeting gap junctional structures. Curr Pharmaceut Des 16:3056–3070
Hervé JC, Sarrouilhe D (2005) Connexin-made channels as pharmacological targets. Curr Pharm Des 11:1941–1958
Hervé JC, Sarrouilhe D (2006) Protein phosphatase modulation of the intercellular junctional communication: importance in cardiac myocytes. Prog Biophys Mol Biol 90:225–248
Hervé JC, Plaisance I, Loncarek J, Duthe F, Sarrouilhe D (2004) Is the junctional uncoupling elicited in rat ventricular myocytes by some dephosphorylation treatments due to changes in the phosphorylation status of Cx43? Eur Biophys J 33:201–210
Hervé JC, Derangeon M, Sarrouilhe D, Giepmans BNG, Bourmeyster N (2012) Gap junctional channels are parts of multiprotein complexes. Biochim Biophys Acta 1818:1844–1865
Hoshi T, Zagotta WN, Aldrich RW (1990) Biophysical and molecular mechanisms of Shaker potassium channel inactivation. Science 250:533–538
Iovine MK, Gumpert AM, Falk MM, Mendelson TC (2008) Cx23, a connexin with only four extracellular-loop cysteines, forms functional gap junction channels and hemichannels. FEBS Lett 582:165–170
Ishikawa M, Iwamoto T, Nakamura T, Doyle A, Fukumoto S, Yamada Y (2011) Pannexin 3 functions as an ER Ca^{2+} channel, hemichannel, and gap junction to promote osteoblast differentiation. J Cell Biol 193:1257–1274
Jansen JA, Noorman M, Musa H, Stein M, Jong S de, Nagel R van der, Hund TJ, Mohler PJ, Vos MA, Veen TA van, Bakker JM de, Delmar M, Rijen HV van (2012) Reduced heterogeneous expression of Cx43 results in decreased Nav1.5 expression and reduced sodium current that accounts for arrhythmia vulnerability in conditional Cx43 knockout mice. Heart Rhythm 9:600–607
Kovacs JA, Baker KA, Altenberg G, Abagyan R, Yeager M (2007) Molecular modeling and mutagenesis of gap junction channels. Prog Biophys Mol Biol 94:15–28
Levit NA, Mese G, Basaly MG, White TW (2011) Pathological hemichannels associated with human Cx26 mutations causing keratitis-ichthyosis-deafness syndrome. Biochim Biophys Acta 1818:2014–2019
Liu J, Xu J, Gu S, Nicholson BJ, Jiang JX (2011) Aquaporin 0 enhances gap junction coupling via its cell adhesion function and interaction with connexin 50. J Cell Sci 124:198–206
Liu S, Taffet S, Stoner L, Delmar M, Vallano ML, Jalife J (1993) A structural basis for the unequal sensitivity of the major cardiac and liver gap junctions to intracellular acidification: the carboxyl tail length. Biophys J 64:1422–1433
Malhotra JD, Thyagarajan V, Chen C, Isom LL (2004) Tyrosine-phosphorylated and nonphosphorylated sodium channel beta1 subunits are differentially localized in cardiac myocytes. J Biol Chem 279:40748–40754
Moreno AP, Lau AF (2007) Gap junction channel gating modulated through protein phosphorylation. Prog Biophys Mol Biol 94:107–119
Nicchia GP, Srinivas M, Li W, Brosnan CF, Frigeri A, Spray DC (2005) New possible roles for aquaporin-4 in astrocytes: cell cytoskeleton and functional relationship with connexin43. FASEB J 19:1674–1676
Nualart-Marti A, Solsona C, Fields RD (2012) Gap junction communication in myelinating glia. Biochim Biophys Acta (in press)
Palacios-Prado N, Bukauskas FF (2012) Modulation of metabolic communication through gap junction channels by transjunctional voltage; synergistic and antagonistic effects of gating and ionophoresis. Biochim Biophys Acta 1818:1884–1894
Panchin YV (2005) Evolution of gap junction proteins—the pannexin alternative. J Exp Biol 208:1415–1419
Panchin Y, Kelmanson I, Matz M, Lukyanov K, Usman N, Lukyanov S (2000) A ubiquitous family of putative gap junction molecules. Curr Biol 10:R479–R480
Patel SJ, Milwid JM, King KR, Bohr S, Iracheta-Velle A, Li M, Vitalo A, Parekkadan B, Jindal R, Yarmush ML (2012) Gap junction inhibition prevents drug-induced liver toxicity and fulminant hepatic failure. Nat Biotechnol 30:179–183
Penuela S, Gehi R, Laird DW (2012) The biochemistry and function of pannexin channels. Biochim Biophys Acta (in press)
Peracchia C, Bernardini G, Peracchia LL (1983) Is calmodulin involved in the regulation of gap junction permeability? Pflügers Arch 399:152–154
Phelan P (2005) Innexins: members of an evolutionarily conserved family of gap-junction proteins. Biochim Biophys Acta 1711:225–245
Phelan P, Bacon JP, Davies JA, Stebbings LA, Todman MG, Avery L, Baines RA, Barnes TM, Ford C, Hekimi S, Lee R, Shaw JE, Starich TA, Curtin KD, Sun Y-A, Wyman RJ (1998) Innexins: a family of invertebrate gap-junction proteins. Trends Genet 14:348–349
Rash JE, Pereda A, Kamasawa N, Furman CS, Yasumura T, Davidson KG, Dudek FE, Olson C, Li X, Nagy JI (2004) High-resolution proteomic mapping in the vertebrate central nervous system: close proximity of connexin35 to NMDA glutamate receptor clusters and co-localization of connexin36 with immunoreactivity for zonula occludens protein-1 (ZO-1). J Neurocytol 33:131–151
Rörig B, Sutor B (1996) Regulation of gap junction coupling in the developing neocortex. Mol Neurobiol 12:225–249
Saez JC, Berthoud VM, Branes MC, Martinez AD, Beyer EC (2003) Plasma membrane channels formed by connexins: their regulation and functions. Physiol Rev 83:1359–1400
Saez JC, Schalper KA, Retamal MA, Orellana JA, Shoji KF, Bennett MV (2010) Cell membrane permeabilization via connexin hemichannels in living and dying cells. Exp Cell Res 316:2377–2389
Salameh A, Dhein S (2011) Adrenergic control of cardiac gap junction function and expression. N Schmied Arch Pharmacol 383:331–346
Sato PY, Coombs W, Lin W, Nekrasova O, Green KJ, Isom LL, Taffet SM, Delmar M (2011) Interactions between ankyrin-g, plakophilin-2, and connexin43 at the cardiac intercalated disc. Circ Res 109:193–201
Scott CA, Tattersall D, O'Toole EA, Kelsell DP (2012) Connexins in epidermal homeostasis and skin disease. Biochim Biophys Acta 1818:1952–1961
Severs NJ, Bruce AF, Dupont E, Rothery S (2008) Remodelling of gap junctions and connexin expression in diseased myocardium. Cardiovasc Res 80:9–19
Sosinsky GE, Boassa D, Dermietzel R, Duffy HS, Laird DW, MacVicar B, Naus CC, Penuela S, Scemes E, Spray DC, Thompson RJ, Zhao HB, Dahl G (2011) Pannexin channels are not gap junction hemichannels. Channels (Austin) 5:193–197
White TW, Wang H, Mui R, Litteral J, Brink PR (2004) Cloning and functional expression of invertebrate connexins from *Halocynthia pyriformis*. FEBS Lett 577:42–48

Yu XS, Yin X, Lafer EM, Jiang JX (2005) Developmental regulation of the direct interaction between the intracellular loop of connexin 45.6 and the C terminus of major intrinsic protein (aquaporin-0). J Biol Chem 280:22081–22090

Yue P, Zhang Y, Du Z, Xiao J, Pan Z, Wang N, Yu H, Ma W, Qin H, Wang WH, Lin DH, Yang B (2006) Ischemia impairs the association between connexin 43 and M3 subtype of acetylcholine muscarinic receptor (M3-mAChR) in ventricular myocytes. Cell Physiol Biochem 17:129–136

Zhou Y, Yang W, Lurtz MM, Ye Y, Huang Y, Lee HW, Chen Y, Louis CF, Yang JJ (2007) Identification of the calmodulin binding domain of connexin 43. J Biol Chem 282:35005–35017

Zhou Y, Yang W, Lurtz MM, Chen Y, Jiang J, Huang Y, Louis CF, Yang JJ (2009) Calmodulin mediates the Ca^{2+}-dependent regulation of Cx44 gap junctions. Biophys J96:2832–2848

Zsiros V, Maccaferri G (2008) Noradrenergic modulation of electrical coupling in GABAergic networks of the hippocampus. J Neurosci 28:1804–1815