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Biochimica et Biophysica Acta 1719 (2005) 3 – 5
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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Preface

Connexins, innexins and pannexins: Bridging the communication gap

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Multicellular organisms have evolved different mechanisms of intercellular communication, the most direct and quickest of which is through channels that link the cytoplasms of adjacent cells. In plants, a cytoplasmic continuity exists through elongated cytoplasmic bridges termed plasmodesmata, which cross the thick cell walls surrounding plant cells. In metazoans, cells are interconnected by channels which span the two plasma membranes and the intercellular space; these result from the docking of two half channels, which are hexameric torus of junctional proteins around an aqueous pore. These densely packed channels, localised in gap junctions, provide a direct route by which cells can exchange ions and small molecules, including oligonucleotides, siRNAs, and second messengers such as Ca^{2+}, inositol phosphates and cyclic nucleotides. Gap junctions are found in essentially all tissues at some stage of development hinting at an enormous diversity of functions beyond their traditional roles in coordinating electrical activity in excitable tissues.

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 (see Fig. 1). For a long time, gap junction structure and functions were mainly investigated in the vertebrates, where they were thought to be formed solely by connexins (Cxs). Then, in *Drosophila* (an arthropod) and *C. elegans* (a nematode), which have no Cx genes, gap junctions were found to be composed of another gene family, the innexins (Inxs, invertebrate analogues of Cxs), which have no sequence homology to Cxs [1]. The list of animal phyla with identified Inx family members progressively extended to annelida, platyhelminthes, mollusca and coelenterata. Inxs were also identified in polydnaviruses, symbiotic proviruses of parasitic wasps; these functional genes appear to have originated from, and co-evolved with, host insect innexins (see [2,3]). Sequences with low similarity to the invertebrate innexins were identified in vertebrate chordates, leading some authors to suggest that the protein family be re-named pannexins (from the Greek "pan", neuter of the adjective "pas", which means all, entire, and nexus, connection ; [4,5]. At present the vertebrate proteins and a few invertebrate innexins are referred to as pannexins (abbreviated Panx; see Fig. 1). For clarity, this term will be used here only to refer to the chordate innexin-like sequences. It also emerged that Cx genes were not restricted to vertebrate animals but were also present in invertebrate chordates (e.g. in tunicates, ascidians and appendicularians; see [6] for an analysis of their relationship to vertebrate connexins).

Cx, Inx and Panx proteins share the same topology, with four alpha-helical transmembrane (TM) domains connected by two extracellular (EC) loops and a single cytoplasmic loop; both N- and C-termini are intracellular. It is not yet clear whether Inxs and Panxs are members of the same superfamily. Inxs display only about 16% overall identity when their full-length amino acid sequences are compared to either Panxs or Cxs, which may 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 is all Cxs suggesting that this residue is strictly conserved in gap-junction proteins. Notably, Panxs do not possess a YYXWX motif in TM2, regarded as a signature sequence of innexins. Invertebrate Cxs share 25–40% sequence identity with human Cxs. Twenty and twenty-one members of the Cx gene family are likely to be expressed in the mouse and human genome,

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0005-2736/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.bbamem.2005.11.013

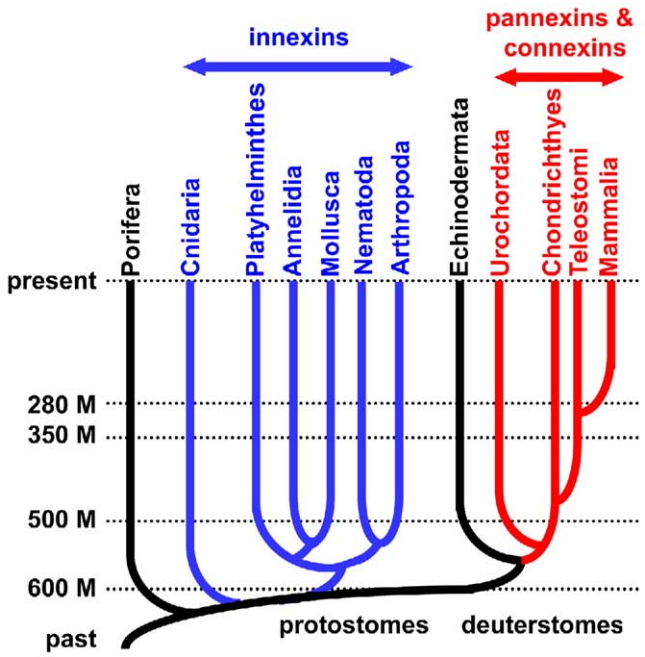

Fig. 1. Evolutionary distribution of connexins, innexins and pannexins. Among triploblast animals, deuterostomes have connexins and pannexins while protostomes use innexins instead. So far, only innexin sequences have been identified in diploblast animals.

respectively (19 of which can be grouped into sequence-orthologous pairs) and orthologues are increasingly characterised in other vertebrates; in invertebrate chordates, a comparable number (e.g. seventeen connexin-like sequences in a basal marine chordate, the tunicate Ciona intestinalis) have been found. There are far fewer Panx genes; as yet only 3 have been described in mouse and human. The Inx family appears large since well over 50 sequences have already been reported (for example they are 8 in *Drosophila* and 25 in *C. elegans*) but functional studies of cell-cell communication have only been realised for some of them (see [2]).

Most cell types express multiple Cx, Inx or Panx proteins, allowing for the construction of homo- and hetero-oligomeric hemichannels. These, in turn, can assemble into homotypic and heterotypic channels that consist of two identical or two different hemichannels, respectively. When expressed in the paired *Xenopus* oocyte system, the vast majority of Cxs form homotypic channels and several of these proteins interact selectively to form heteromeric or heterotypic channels. Only half of the investigated Inxs (6 vs. 12) were able to establish cell-to-cell channels independently (see [2]). A number of the proteins that do not appear to form homomeric channels (for example, *Drosophila* (Dm)-Inx3, Cx33 and Panx2) may influence the formation and properties of junctional channels built of other proteins expressed in the same cells (for discussion, see [7]). For example, Dm-Inx3 modifies the electrophysiological properties of channels made of Dm-Inx2, Cx33 specifically inhibits Cx37 channel formation and Panx2 reduces the amplitude and modifies the voltage gating kinetics of Panx1 hemichannels. The observed inhibitory effect of Cx33 raises the possibility that other proteins that do not form functional channels may similarly act as negative regulators of intercellular communication by inhibitory effects on the formation of gap junctions. The physiological significance of this is not known but it might, for example, provide a means of selectively coupling different cell types. In short, the wide variety of possible interactions adds great versatility to the functional modulation of gap junctions and provides a structural basis for the charge and size selectivity of the intercellular channels.

The strength of cell-to-cell communication through gap junctional channels may be actively adjusted by multiple mechanisms including changes in protein expression, regulation of protein trafficking and turnover as well as the modulation of channel properties via complex mechanisms that are only now being identified. The level of cell-to-cell communication is indeed influenced by a variety of stimuli, including changes in the level of intracellular Ca^{2+}, pH, transjunctional voltage and by phosphorylation/dephosphorylation balances. In spite of their similar overall structure, the influence of such factors on the physiological properties of gap junction channels is clearly dependent on their molecular composition.

The permeability and gating characteristics of the channels are indeed dependent on the protein isoform and on post-translational modifications present on them. For example, homotypic and heteromeric channels containing different Cxs (e.g. Cx40/Cx43), Inxs (e.g. Dm-Inx2/Dm-Inx3) or Panxs (e.g. Panx1/Panx2) display differential voltage sensitivity. Such voltage sensitivity is likely to be particularly important in regulating intercellular coupling between excitable cells; asymmetric voltage sensitivity of heterotypic channels might, for example, underlie rectification at some electrical synapses. Some data suggesting that membrane potential differences between neighbouring cells may also influence intercellular communication in non-excitable tissues have been reported. So far, the properties of Panx or Inx channels, such as size and charge selectivity, have not been examined; however, based on Cx studies, it is reasonable to assume that the molecular make-up of such channels will determine their permeability to ions and signalling molecules. Alterations in the phosphorylation status of proteins, resulting from the dynamic interplay of protein kinases and protein phosphatases, are also thought to be involved in a broad variety of processes including trafficking, assembly/disassembly and degradation of proteins, as well as the gating of junctional channels but the underlying mechanisms remain poorly understood. The effects of a variety of chemicals on cell-to-cell communication have been investigated in cells expressing Cxs [8], but data are still very rare in cell systems communicating through Panx or Inx channels. Some of the compounds which interrupt communication via Cx channels also abolish Panx-based intercellular communication (e.g. 18-glycyrrhetinic acid or its synthetic derivative carbenoxolone) whereas others have much more modest effects (e.g. flufenamic acid) [9].

Mutations in (or loss of) connexin genes have been linked to a variety of human and animal diseases, including deafness, peripheral neuropathy, skin disorders, infertility, cataracts,

etc.... Studies, to date, have revealed that the loss of Inx function in flies and worms also leads to a range of neural, muscle and epithelial anomalies, whereas the impact of pannexin knockouts is still unknown.

In addition to their roles in cell-cell coupling, several studies suggest that Cx proteins may also mediate other types of signalling; likewise, Panxs (e.g. Panx1) have been found at sites not previously recognized for gap junctional coupling, suggesting that junctional proteins might fulfil additional functions besides the formation of intercellular channels (see [10]). This could involve interactions with other protein partners (particularly with cytoskeleton and core components of adherens and tight/septate junctions) that may play a role not only in assembly, trafficking, gating and turnover of gap-junction proteins, but also in the coordinate regulation of cell-cell communication with cell adhesion and cell motility.

Alternatively, gap junction proteins may have a role as hemichannels. Although hemichannels present in the non-junctional regions of the plasma membrane are believed to be kept closed in the presence of normal extracellular Ca^{2+}, it now appears that different cells can tolerate some hemichannel (either Cx or Panx-made) openings, which might exert physiological or deleterious effects, depending on the situation [11].

In conclusion, while the structural basis for direct cell-cell communication (*viz*., the assembly of intercellular channels) has been conserved throughout evolution, gap junctional-forming proteins appear to be encoded by distinct gene families unequally distributed among the different animal phyla. Inx or Inx-like (Panx) genes have been identified in coelenterates, arthropods, nematodes, annelids, flatworms, molluscs and chordates. Their presence in coelenterates, such as *Hydra*, organisms that predated the protostome–deuterostome bifurcation suggests that Inxs were the primeval gap junction proteins. Invertebrate chordates (e.g. tunicates) are considered to share a common ancestor with modern vertebrates. Some of their representatives are present in the fossil record 550 million years ago, during the Cambrian explosion, and thus date to the same epoch when the protostomal invertebrates such as flies and nematodes were evolving. The presence of connexin-like sequences forming functional junctional channels in tunicates confirms that the Cxs were ancient genes that predated vertebrate evolution by hundreds of millions of years. In this scheme, Inxs evolved in diploblasts and provide the sole means of gap-junctional communication in these organisms and in all protostomes. Inxs have been largely superseded later in evolution by connexins that arose de novo in deuterostomes. It remains to be seen through functional studies whether Panxs can be regarded as vestigial Inxs that have survived in higher animals.

Given to space limitations, only a sampling of the available literature on this topic is given in the present review and we apologise to authors whose original works have not been quoted.

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