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Review

Evolution of gap junction proteins – the pannexin alternative

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Summary

Gap junctions provide one of the most common forms of intercellular communication. They are composed of membrane proteins that form a channel that is permeable to ions and small molecules, connecting the cytoplasm of adjacent cells. Gap junctions serve similar functions in all multicellular animals (Metazoa). Two unrelated protein families are involved in this function; connexins, which are found only in chordates, and pannexins, which are ubiquitous and present in both chordate and invertebrate genomes. The involvement of mammalian pannexins to gap junction formation was recently confirmed. Now it is necessary to consider the role of pannexins as an alternative to connexins in vertebrate intercellular communication.

Key words: connexin, pannexin, gap junction, innexin, OPU.

Introduction

There are two fundamentally different ways for intercellular communication: the release of secreted molecules (hormones, neurotransmitters) into the extracellular space followed by binding to an adjacent cell, and by the formation of continuous channels that directly bridge the cytoplasm of the two cells. Such channels allow direct exchange of ions, metabolites and other messenger molecules, and mediate electrical coupling between neighbouring cells (Bruzzone et al., 1996; Chailakhyan, 1990; White and Paul, 1999). To enable essential intercellular communication, multicellular organisms have evolved distinct types of intercellular channels. Intercellular communication in plants occurs *via* elongated cytoplasmic bridges, called plasmodesmata, which traverse the thick cell walls that surround plant cells. Plasmodesmata allow translocation of big molecules (proteins and nucleic acids) and viruses between cells. The ultrastructure of plasmodesmata is known from cytological studies, but their molecular composition remains elusive (Heinlein, 2002; Zambryski and Crawford, 2000). Fungi have similar structures called septal pores (Potapova et al., 1988; Shepherd et al., 1993). Intercellular channels in plant and fungi are membrane-lined channels and are fundamentally different from intercellular channels in multicellular animals.

Animal specific intercellular channels are formed of proteins and are called gap junctions (GJ). Physiological and morphological studies have identified GJ in different tissues of various metazoan species. They all appear to have similar physiological properties. Surprisingly it was found that two unrelated protein families are involved in this function. Connexins are found only in chordates. Pannexins (innexins) are present both in invertebrate and chordate genomes (Baranova et al., 2004; Bruzzone et al., 1996; Kumar and Gilula, 1996; Levin, 2002; Panchin et al., 2000; Phelan et al., 1998a; Phelan and Starich, 2001).

This article is a brief overview of current knowledge of the two families of gap junction proteins, with an emphasis on the pannexin family and the evolution of gap junction function.

Initial gap junction studies

Two methods contributed greatly to GJ experimental studies: electrophysiological measurements of cell coupling, and dye injection experiments showing that small fluorescent dye molecules injected into one cell can pass directly into adjacent cells (Dermietzel et al., 1990; Furshpan and Potter, 1959; Levin, 2002; Loewenstein, 1981). Using these two approaches, experimental data for GJ function was collected from numerous biological models both in vertebrate and invertebrate species and in various tissue and cell types. For instance, in the human body GJ are present in nearly all tissues, being absent only in adult skeletal muscle cells (which are fused to form functional syncytia) and some circulating blood cells (Dermietzel et al., 1990). Vertebrate and invertebrate GJ share similar calculated pore size, voltage-gating properties and sensitivity to the same classes of pharmacological agents (Bruzzone et al., 1996; Levin, 2002; Phelan and Starich, 2001).

Before any GJ genes were identified and sequenced the GJ molecular structure was predicted from X-ray diffraction and

electron microscopy (Caspar et al., 1977; Makowski et al., 1977). A model was proposed in which a gap junction hemichannel is formed as six subunits oligomerize to form a hexameric torus. The unit gap junction channel is a pair of hemichannels, one from each cell, apposed in the narrow intercellular gap between neighbouring cell membranes.

Although GJ are the most common intercellular channels in animals, and only membrane-lined intercellular channels are known in plants and fungi, membrane-coated pores can nevertheless be observed in certain animal cell types (Huckins, 1978; Rustom et al., 2004; Shestopalov and Bassnett, 2000).

Connexins

Connexins were identified as the molecular components of vertebrate GJ about 20 years ago. The first connexin cDNA was cloned in 1986 (Paul, 1986), followed by the isolation of several related molecules of this multi-gene family (Willecke et al., 2002). This work allowed prediction of the protein structure. Each connexin contains four membrane-spanning α helices and intracellular C and N termini (Fig. 1A,C). The six subunits are thought to associate to form a connection with a central aqueous pore (Paul, 1986; Yeager and Nicholson, 1996). Both cysteine scanning and crystallography data suggest that the pore is lined by two transmembrane segments from each subunit, one of them tilted (Unger et al., 1999; Zhou et al., 1997). Following the development of the oocyte expression system, it was shown that connexin encoding mRNA injected into frog oocyte induced cell–cell channels between paired oocytes. These experiments presented direct proof of connexins' GJ function and provided a powerful tool for its study (Dahl et al., 1987; Swenson et al., 1989; Werner et al., 1989). At present a number of connexins have been cloned from cDNA collections and predicted from genomic sequences of various vertebrate species (Bruzzone et al., 1996; Sasakura et al., 2003; White and Paul, 1999; Willecke et al., 2002).

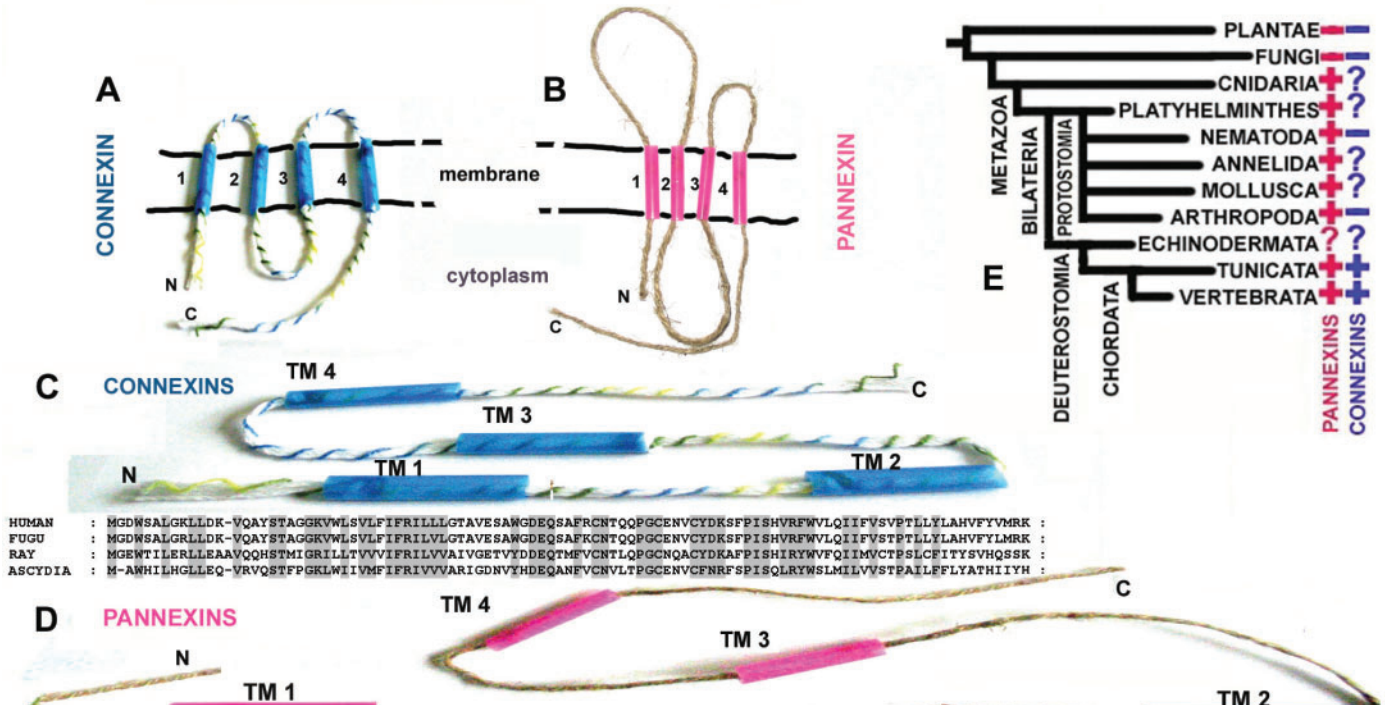

Fig. 1. The topology of (A) connexins and (B) pannexins (formerly innexins) with four transmembrane (TM) domains and intracellular N and C termini is the same, yet their sequences are not related (C,D). Alignments in C and D are limited to the most conserve regions that include the first two transmembrane domains TM1 and TM2 and the first extracellular loop; for connexins, mammals: human; bony fish, *Fugu*, ray; tunicate *Ascidia* (GeneBank accession numbers: P17302, AAL89668, Q92107 and AAQ90187); for pannexins, cnidarian: *Hydra*; flatworm: *Planaria*; annelid: leech, nematode; mollusc: *Clione*; insect, fly; tunicate *Ascidia*; mammals: human; ichnovirus (accession numbers: BK005478, AF207819, CAD55801, CAA79529, AAF75839, AAA28745, BK005483, AAK91714 and AAO45829). (E) Presence (+) or absence (–) of connexins (blue) and pannexins (red) in the main taxonomic groups of multicellular organisms are indicated in simplified phylogenetic tree.

Pannexins

Numerous attempts to clone connexins from invertebrates have failed, so alternatives to connexin gap junction protein candidates were investigated. At some point ductins (the major component of the proton channel of the vacuolar H^+-ATPase) were proposed to be the missing GJ proteins (Finbow and Pitts, 1993), yet this hypothesis has not found broad support (Bruzzone and Goodenough, 1995). Finally, it was suggested that invertebrate GJs are assembled from proteins unrelated to the connexin gene family. This protein family was originally designated OPUS, an acronym derived from the founding members, ogre, passover, unc-7 and shaking-B (Barnes, 1994; Krishnan et al., 1993; Phelan et al., 1998b; Starich et al., 1993, 1996; Watanabe and Kankel, 1992). It was suggested that these are specific invertebrate gap junction proteins, and they were later renamed innexins (invertebrate analog of connexins; Phelan et al., 1998a; Phelan and Starich, 2001). Although connexins and innexins have very different primary structures they nonetheless have some similar features (Fig. 1A–D). Proteins of both unrelated families have similar topology with four transmembrane domains (Bruzzone et al., 1996; Phelan and Starich, 2001). Fly and nematode innexin mRNA injection induced cell–cell channels between paired frog oocytes (Landesman et al., 1999; Phelan et al., 1998b; Stebbings et al., 2000). The presence of innexin homologs in different taxonomic groups, including vertebrates, was then demonstrated (Baranova et al., 2004; Bruzzone et al., 2003; Dykes et al., 2004; Panchin et al., 2000; Potenza et al., 2003). Innexins were reclassified with their vertebrate homologes in a bigger family. Given the ubiquitous distribution of this protein family in the animal kingdom these proteins were termed, pannexins (from the Latin pan – all, throughout and nexus – connection, bond) (Panchin et al., 2000; PROSITE: PS51013, www.expasy.org/prosite/).

Both the human and the mouse genomes contain three pannexin-encoding genes. The mammalian PANX1 (pannexin-1) mRNA is ubiquitously, although disproportionately, present in different tissues; in the embryonic central nervous system it is expressed noticeably more strongly than in other tissues. PANX2 is a brain-specific gene. A low level of PANX3 was detected in the brain and EST data suggest that PANX3 is expressed in osteoblasts and synovial fibroblasts (Baranova et al., 2004; Bruzzone et al., 2003; Panchin et al., 2000).

Direct proof of the vertebrate pannexins GJ function was provided by Bruzzone and coworkers (Bruzzone et al., 2003). They demonstrated that in paired oocytes, rodent PANX1, alone and in combination with PANX2, induced the formation of intercellular channels. However, it is not clear if pannexins duplicate GJ functions of connexins in vertebrates or play some special physiological role (Bao et al., 2004).

Recently vinnexins (viral homologs of pannexins/innexins) were identified in Polydnaviruses that occur in obligate symbiotic associations with parasitoid wasps. It was suggested that virally encoded vinnexin proteins may alter gap junctions in infected host cells, possibly affecting encapsulation responses in parasitized insects (Kroemer and Webb, 2004; Turnbull and Webb, 2002).

Gap junction protein evolution

Comparison of genomes from model organisms suggested that similar functions are supported by related molecules derived from a common ancestor. In this respect GJ proteins present an extremely interesting case for evolutionary and comparative analysis.

The growing number of cDNA and genomic sequences from different organisms provide evidence that connexins are present in all vertebrates and also in animals of the chordate branch, tunicates, ascidians and appendicularians, (see Sasakura et al., 2003; GenBank accession numbers AY380580, AY386312 and AY386311).

As recently as a few years ago there were no means of checking reliably whether some genes are really absent in given genomes. This uncertainty has changed with the availability of complete genome sequences from many model organisms and allows us to assert that connexin and pannexin homologs are absent in prokaryotes, plants and fungi (Fig. 1E). This fact is consistent with the hypothesis that multicellularity in plants, fungi and animals emerged independently (Baldauf, 2003).

The most intriguing outcome of the survey of complete genomes for GJs was the absence of the connexin homologs in non-chordate metazoan genomes such as those nematode worms and fruit fly (The *C. elegans* Sequencing Consortium, 1998; Adams et al., 2000).

The analysis of gene loss and acquisition is a powerful tool for evolutionary studies (Koonin et al., 2004). Specific genes for multicellularity are of particular interest. Connexins appear to be chordate-specific genes (Fig. 1E), but can this assertion be substantiated? If connexins are present in the genome of other deuterostomes, like echinoderms, we may expect connexins to arise from an earlier deuterostome ancestor, and if they are found in basal radial metazoans, such as Cnidaria, the plausible scenario will support the hypothesis that the connexin gene(s) was lost in the non-chordate (protostome?) common ancestor.

Unfortunately no complete genomes from non-chordate deuterostomes or radial animals are available so this question cannot be resolved at present. We can only state that in current databases no connexins are present outside the Chordata. The pannexin story appears to be clearer. Pannexins are present in all major bilaterian groups (Fig. 1E). Recently they were found in chordate branch tunicates (Sasakura et al., 2003; GenBank accession number TPA: BK005483). Apparent pannexins are also present in hydra (Cnidaria) database sequences. From hydra ESTs in the GeneBank we were able to reconstruct two complete coding sequences (CDS) of pannexin and three more partial CDS of obvious pannexin orthologs (GenBank accession numbers TPA: BK005478-BK005482). This finding strongly supports our postulate that pannexins are ubiquitous metazoan proteins and further justifies their name.

Gap junction specificity and brain function

The discovery of pannexins and the demonstration of their role in GJ formation, both in vertebrates and invertebrates, strengthens the role of model animals for the fundamental gap junction study. These model animals include genetically tractable invertebrate organisms such as fruitfly and nematode, and animals with large identifiable neurons such as molluscs, which are favorable for single cell physiological studies. The use of the model animals allows one to study the specificity of GJ formation. The importance of GJ specificity is particularly evident in the nervous system, where they are common and form electrical synapses. GJs are very simple in the sense that just a single type of molecule expressed in two adjacent cells appears to be sufficient for junction formation. The experiments on heterologous expression of connexins and pannexins in paired frog oocytes support this view. So what accounts for the specificity of GJs? It is possible that, unlike chemical connections, whose assembly depends on numerous types of molecules expressed in two cells on both sides of the synaptic cleft, GJ formation depends entirely on GJ proteins providing both conduction and recognition functions.

For both connexins and pannexins it was suggested that hemichannels in opposing cells may have different subunit compositions and that this difference can affect the assembly of the functional cell–cell channel (Ebihara et al., 1999; He et al., 1999; Kelmanson et al., 2002; Starich et al., 1996; Stebbings et al., 2000; White et al., 1995). The diversity of gap junction molecules is high. Chordates encode about 20 different connexins and three pannexins in their genomes, whereas invertebrates have about 20 different pannexins. Even the simple metazoan, *Hydra*, has at least five pannexins and probably more. The number of hexameric structures that can be produced by combinations of 20 monomers is vast. There is growing evidence that hemichannel properties really depend on the subunit composition. For the vertebrate GJ molecules (connexins), it was shown that differential expression of two different connexins by two distinct types of cells in mammalian heart is responsible for selective coupling (White et al., 1995). In nematode and fly pannexins (innexins), mutations revealed defects in specific GJ connections in the pharyngeal muscles and nervous system. For example, *eat-5* mutants lose detectable dye coupling, a reliable indicator of GJ communication, between anterior and posterior pharyngeal muscle groups (Starich et al., 1996).

In molluscs the key role of pannexins in the process of GJ selection was proved by intracellular injections of synthetic mRNA coding cPanx1, which led to specific changes in the electrical connection patterns formed by injected neurons (Kelmanson et al., 2002).

Conclusions

The discovery of the two unrelated GJ protein families responsible for the same fundamental function in multicellular animals has many implications for intercellular communication studies. Here we have briefly discussed the evolutionary aspects of this finding, its impact on the study of vertebrate GJs and the possible role of pannexins and connexins in the specificity of electrical connections formed by GJs.

We conclude that pannexins are ubiquitous metazoan proteins, whereas connexins appear to be chordate specific. As chordates apparently possess two distinct types of gap junction molecules it is important to understand what is the balance between them. Do pannexins duplicate GJ functions of connexins or does each play its own physiological role? Very little data are available on this subject, especially data describing pannexin function.

GJ intercellular communication is peculiar because it is likely to require only one type of molecule to build a functional communication channel between two cells. It is suggested that the same GJ molecules that are responsible for channel formation are also mediating cell–cell recognition, and that diversity of GJ proteins, together with the capability of forming heteromeric channels, provides the molecular basis for specificity of intercellular connections.

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