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Editorial

Membrane channels formed by gap junction proteins☆

The acquisition of multicellularity, a major step in animal evolution, allowed continuous increase in morphological complexity and opened numerous adaptive opportunities. In multicellular organisms, communication between neighboring cells and between cells and their extracellular space is essential for the proper functioning of basic cellular activities. Therefore, plants as animals evolved specific structures with selective permeability to ensure its fine control. Communication via transmembrane channels made of gap junction proteins (innexins, connexins, pannexins), very old in the evolutionary history of metazoans, allowed to provide speed, synchrony, switching, symbiosis, and stimulus/suppression to diverse functions of cell collectives. Whereas pannexin channels and innexin/connexin hemichannels serve as diffusional pathways for ions and small molecules between intra- and extra-cellular compartments, gap junction channels connect the cytoplasms of contacting cells and coordinate electric and metabolic activities thanks to direct exchanges of ions and small molecules (including second messengers like Ca^{2+}, IP3, cyclic nucleotides and oligonucleotides).

The present issue of Biochimica et Biophysica Acta-Biomembranes summarizes the current state of knowledge concerning the nature, characteristics and properties of the membrane channels formed by gap junction proteins.

1. Families of gap junction proteins

In deuterostomes (Echinodermata, Urochordata, Cephalochordata and Vertebrata), gap junctions are formed by connexins while in protostomes (Nematoda, Mollusca, Plathyhelmintes, Arthropoda, Annelida), they are formed by innexins. Interestingly, vertebrates and lower chordates contain innexin homologs, the pannexins, which also form channels, but rarely (if ever) make intercellular channels. If the connexin and the innexin/pannexin polypeptides do not share significant sequence similarity, the three protein families exhibit a similar membrane topology and some similarities in their quaternary structure and share some structural and functional commonalities. Eric C. Beyer and Viviana M. Berthoud [1] summarize the differences and similarities between the **gap junction gene and protein families connexins, innexins and pannexins**.

2. Membrane channel structure and properties

A gap junction channel that joins the cytoplasm of adjacent cells is formed by docking of the extracellular domains of Cx or Inx membrane-spanning hemichannels contributed by neighboring cells. The presence of conserved residues and motifs correctly aligned results in a proper arrangement of the docking interface. Non-covalent interactions at the docking interface, including hydrogen bonds, are predicted to form between interdocked extracellular domains. Protein sequence alignment analysis on the docking compatible/incompatible Cxs have revealed that the extracellular loop E1 domain is important for the formation of the gap junction channel and the E2 domain is important in the docking compatibility in heterotypic channels. Donglin Bai, Benny Yue and Hiroshi Aoyama [2] show as **crucial motifs and residues in the extracellular loops influence the formation and specificity of Cx docking**.

The oligomeric assembly of junctional proteins contains an aqueous nanopore which can be gated, i.e. is capable of controllable switching between closed and open states, by electrical, mechanical and chemical stimuli. Connexin channels display multiple forms of voltage dependence that have different sensitivities and time courses. Each hemichannel displays two distinct voltage-gating mechanisms that are primarily sensitive to a voltage gradient formed along the length of the channel pore (the transjunctional voltage) rather than to the absolute membrane potential. Two distinct voltage-gating mechanisms (respectively a fast-gating and a loop- or slow-gating) appear to be involved in conformational changes. T.A. Bargiello, S. Oh, Q. Tang, N.K. Bargiello, T.L. Dowd and T. Kwon [3] summarize the recent progress in the validation of **atomic models of the open and voltage-driven closed states** of undocked Cx hemichannels.

In living cells, Cxs are constantly made, transported, and degraded, these processes being rapidly and exquisitely regulated by intracellular as well as extracellular cues. Cx43 hexamers (hemichannels, connexons) are formed in the traditional secretory pathway. They then trafficked to the cell surface where they can act as stand-alone channels or dock with hemichannels in appositional membranes to form intercellular channels which insert at the edge or into gap junctional plaques. Thereafter, full plaques or segments of plaques can be internalized at any time. Both junctional plaques and surrounding regions are highly dynamic with complex behavior of targeted insertion and internalization. Irina Epifantseva and Robin M.

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Shaw [4] highlight recent mechanisms identified in the **intracellular trafficking of Cx43 gap junction channels**, particularly the involvement of other proteins contributing to the delivery of channels to the cell-cell border.

In contrast with the other parts of the molecules, the C-terminal domains of different Cxs and Panxs are not conserved but differ in length, and are considered to confer functional differences and regulatory mechanisms to their respective proteins. In Cx43, this long cytosolic C-terminus is not only a target of phosphorylation but also serves as a scaffolding platform that associates with structural and signaling molecules leading to regulation of intracellular signaling, independent of channel activity. Edward Leithe, Marc Mesnil and Trond Aasen [5] review the current understanding of the regulation and unique **functions of the Cx43 C-terminus**, both as an essential component of full-length Cx43 and as an independent signaling hub, and highlight the complex regulatory and signaling networks controlled by the Cx43 C-terminus.

3. Modulation of membrane channel activity

Pannexins were originally thought to represent, owing to their sequence homology to the invertebrate gap junction family (innexins), a second and redundant brand of junctional proteins in addition to the well characterized connexins. However, it is now evident that Panx function primarily as single membrane channels in autocrine and paracrine signaling. This 3-membered family of proteins indeed forms large pore ion and metabolite channels in Chordates. Panx1 is ubiquitously expressed in many mammalian tissues whereas Panx2 and Panx3 appear to be more restricted in their expression. Panx1 channels are activated through diverse mechanisms including stretch and mechanical membrane stimulation, increased concentration of intracellular calcium or extracellular potassium, receptor-induced signaling pathways as well as proteolytic truncation of the distal C terminus. Paige Whyte-Fagundes and Georg R. Zoidl [6] provide an overview of the **mechanisms of Panx1 channel gating and regulation**. A tight regulation of channel opening is necessary to modulate their function in vivo and occurs through post-translational modifications (including phosphorylation, glycosylation, proteolysis, *N*-acetylation, S-nitrosylation, ubiquitination, lipidation, hydroxylation, methylation and deamidation), channel intermixing, and sub-cellular expression profiles. Post-translational modifications have been postulated as some of the regulating mechanisms for Panx1, while Panx2 and Panx3 have not yet been as well characterized. Andrew K. Boyce, Anna L Epp, Archana Nagarajan and Leigh Anne Swayne [7] delve into the **role of post-translational modifications in the regulation of trafficking and channel properties**, highlighting particularly the importance of glycosylation, phosphorylation, S-nitrosylation and proteolytic cleavage.

Most Cx are known to be phosphorylated by protein kinases that lead to modifications in tyrosine, serine, and threonine residues which have been implicated in the regulation of gap junctional communication at several stages of the Cx 'life cycle', including hemichannel oligomerization, export of the protein to the plasma membrane, hemichannel activity, gap junction assembly, gap junction channel gating and Cx degradation. Phosphorylation is capable of directly modulating connexin channel function but the most dramatic effects on gap junction activity occur via the organization of the gap junction structures themselves. Phosphorylation of Cx43 for example at different sites is known to control the assembly, size and turnover of gap junctions. Joell L. Solan and Paul D. Lampe [8] present an overview of the **spatio-temporal regulation of Cx43 phosphorylation and gap junction dynamics**.

Reactive oxygen and nitrogen species are small, inorganic and highly reactive compounds with short half-lives in biological systems which, given their strong electrophilic nature, rapidly react with and modify organic molecules (i.e. nucleic acids, lipids, and proteins). They act as important second messengers to intimately control the function of many membrane channels. Both the Cx and Panx families appear to serve as downstream targets of changes of intracellular redox potential. Nitric oxide (NO) is a gaseous reactive oxygen species formed by the action of an enzyme family known as nitric oxide synthases. Once produced by the cells, NO can diffuse to neighboring cells and act via several direct or indirect signaling pathways. Isaac E. Garcia, Helmut A. Shanchez, Agustin D. Martinez and Mauricio A. Retamal [9] outline the **redox-mediated regulation of connexin proteins** and more specifically focus on nitric oxide.

4. Permeation profiles of membrane channels

Gap junction channels formed by different Cxs exhibit specific permeability to a variety of small ions and larger solutes including second messengers, metabolites, and small interfering RNAs. They are generally considered to be poorly selective (but can sometimes be surprisingly selective among molecules with similar size), possess open probabilities approximating unity, and exhibit mean open times ranging from milliseconds to seconds. Virginijus Valiunas, Ira S. Cohen and Peter Brink [10] examine the **factors that affect solute permeation of gap junction channels** and discuss the possible underlying molecular mechanisms. For the Cxs taken into consideration (Cx26, − 37, − 40, − 43, and − 50), their analysis of monovalent action flux portrays the pore as equivalent to an aqueous space where hydrogen bonding and weak interactions with binding sites dominate.

Rectifying electrical synapses are rare gap junctions that favor transmission of signals in one direction. The rectification of electrical transmission is generally associated with molecular differences between both innexin and connexin-based hemichannels assembled to form intercellular channels. However, electrical rectification may also be observed at homotypic channels, arising from transjunctional structural asymmetries in some components forming electrical synapses, differentially distributed at each side of the junction. James I. Nagy, Alberto Pereda and John E. Rash [11] explain how **electrical synapses in the mammalian CNS** represent key elements in synaptic circuitry, govern the collective activity of ensembles of electrically coupled neurons, and in part orchestrate the synchronized neuronal network activity and rhythmic oscillations that underlie fundamental integrative processes.

5. Tissue functions of gap junction proteins

Multicellular organisms have developed long-range signaling modes of communication via the nervous and endocrine systems. In turn, cells of these two systems have developed interaction mechanisms with adjacent cells via membrane channels built of Cxs and Panxs, widespread in most tissues, allowing individual endocrine and neuro-endocrine cells to sense the state of activity of their neighbors and, accordingly, to regulate their own level of functioning. Moreover, genetic studies have revealed that Cxs also control the function of human glands, which are central to the pathogenesis of major endocrine diseases. Paolo Meda [12] shows how **gap junction proteins are key drivers of endocrine function** and may represent molecular targets for future prevention and treatment of diseases such as diabetes and hypertension.

Blood-tissue barriers are highly selective permeability barriers that separate the circulating blood from the brain (blood-brain barrier), the intestine (gut barrier) or Sertoli cells (blood-testis barrier) extracellular fluid. In the latter, one of the tightest tissue barriers in the mammalian body,