New, emerging roles for cardiac connexins. Mitochondrial Cx43 raises new questions

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See also article by Boengler et al. [9] (pages 234–244) in this issue.

Connexins are protein subunits that constitute the gap junction channel, a dodecameric channel connecting two neighbouring cells formed by two hexameric hemichannels provided by either cell. The gap junction channel permits intercellular communication by allowing the propagation of the action potential and the transfer of small molecules such as cAMP. Six different gap junctional proteins (connexins; Cx) have been identified in the cardiovascular system: Cx31.9, Cx37, Cx40, Cx43, Cx45, Cx46, Cx50, and Cx57 (the molecular mass in kDa is given by the number) [1,2]. In the heart, there are mainly three isoforms that differ in their molecular weight: connexin 43 (a 43-kDa connexin; Cx43), which is found in most parts of the heart; connexin 40 (Cx40), which is mainly expressed in atria and in the conduction system; and connexin 45 (Cx45), which is expressed predominantly in embryonic stages and has been identified in parts of the ventricular conduction system of mice and rats [3]. Moreover, Cx45 was shown to be expressed in co-cultures of neonatal rat cardiomyocytes with fibroblasts [4] and is involved in myocyte/fibroblast coupling in the sinoatrial node [5]. Cx50 has been found in valvular tissue of the rat heart [6].

Connexins have four transmembrane regions, one intracellular and two extracellular loops, and intracellular N- and C-terminal tails. The length of the C-terminus differs among the various isoforms and is subject to phosphorylation by a number of kinases (for review, see Ref. [2]). Connexins are synthesized in the rough endoplasmic reticulum, folded, and transported to the Golgi apparatus within vesicles. In the trans-Golgi network, connexins are oligomerized to hexameric connexons, which are transferred thereafter to the plasma membrane along microtubular structures. They dock to the connexon of the neighbouring cell via the extracellular loops of the connexons, thereby forming the complete dodecameric gap junction channel. The single channel conductance is regulated by C-terminal phosphorylation. These gap junction channels are subject to high turnover and are degraded via lysosomal and (mainly) proteasomal degradation. The half-life of Cx43 was determined to be on the order of 90 min [7]. It has been assumed that phosphorylation and ubiquitinylation are involved in initiation of this connexin degradation. Finally, besides their function in intercellular communication, the connexins, at least Cx43, may have a role as gene regulators: thus, the C-terminus of Cx43 localizes to the cell nucleus and can inhibit cell growth [8].

In the article by Boengler et al. [9] (see this issue), the authors describe a new localization of connexins in cardiac tissue; they detected Cx43 in the mitochondria using a number of different, independent methods. Interestingly, >80% of Cx43 found in the mitochondria was phosphorylated (as detected indirectly by higher molecular weight in Western blots, see Fig. 5A of Boengler et al. [9]). The amount of mitochondrial Cx43 was enhanced after two periods of 5-minute ischemic preconditioning in rat hearts, which means a rapid translocation of the protein that – taking into consideration a half-life time of about 90 min – cannot be explained by inhibition of degradation. Similarly, they found this increase in mitochondrial Cx43 in pig hearts subjected to ischemic preconditioning and low-flow ischemia, while ischemia itself did not seem to influence translocation. Electron microscopy showed the location of immunogold-labelled anti-Cx43 antibody within the mitochondria (see Fig. 2B of Boengler et al. [9]), compatible with a localization near the inner mitochondrial membrane or the matrix.

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The existence of mitochondrial Cx43 (which was also found in homocysteine-treated endothelial cells [10]) raises a number of new questions. The first is related to the mechanism by which connexin 43 translocates to mitochondria, since this translocation normally requires a mitochondrial entry sequence in the form of N-terminal amino acids carrying a positive charge that are rich in serine and threonine. According to the method described by Emanuelsson et al. [11], such a pre-sequence is lacking in Cx43. To remain in the outer mitochondrial membrane, a membrane-anchoring sequence followed by a positively charged sequence would be required, which does not seem to be present in Cx43. For further movement into the inner parts of the mitochondria, the proton-motive force of the inner membrane is required while the pre-sequence will be removed. For import through the outer membrane, the mitochondrial TOM complex is required, which cooperates with the complexes TIM22 and TIM23 of the inner membrane for import of the protein into the inner compartments of the mitochondria. In some cases, mitochondrial proteins do not contain a pre-sequence but a targeting signal within the mature protein [12]. A protein being transported into the mitochondria is normally unfolded and elongated. Since Cx43 seems to lack a mitochondrial pre-sequence, it is present unclear how Cx43 reaches the inner membrane or matrix of the mitochondria as shown in the electron microscopic images of the article by Boengler et al. [9].

Next, taking the transport mechanism into account, Cx43 probably reaches the inner mitochondria as a single protein chain. Thus, to form connexons (=hemichannels), oligomerization would be required. At present, it is not known whether connexins can be oligomerized in mitochondria. This, on the other hand, raises the question about the function of this Cx43. If it is oligomerized, it may form hemichannels that could be important for volume control. Moreover, there is a proton gradient at the inner membrane between the matrix and the intermembrane space. Since gap junction channels close in response to high H+ concentrations, such hemichannels (if really present) could regulate volume as a function of the pH. However, the resulting question would be: how is the putative hemichannel oriented, with the carboxy tail in the matrix or in the intermembrane space? The present article does not show that hemichannels, i.e. oligomers, are present, it only gives evidence for the presence of connexin 43.

Another possibility is that the single protein chain of Cx43, or a part of it, has functions other than forming channels. Thus, the group of Elissavet Kardami showed elegantly that the carboxy tail of Cx43 can translocate into the nucleus, inhibiting cell growth and possibly regulating gene expression [8]. In a subsequent study, this group showed that serine-262 phosphorylation seems to be important for this translocation [13] and that S262-phosphorylation occurs in response to PKC stimulation with bFGF (via PKCe). Since it is known that ischemic preconditioning also activates certain isoforms of PKC, causing selective translocation of PKC\(D\) and PKCe [14], and that preconditioning seems to depend on PKCe activation [15,16], it is tempting to speculate that PKCe activation with subsequent Cx phosphorylation may give the signal for mitochondrial translocation, which also could explain the high amount of phosphorylated Cx43 in the mitochondria found by Boengler et al. [9]. Since mitochondria also contain DNA, one could also speculate that such translocated mitochondrial Cx43 could also exert gene-regulatory effects on mitochondrial DNA similar to those described for nuclear DNA (see above).

From these considerations and the work of Doble et al. mentioned above [13], the question arises whether ischemic preconditioning-dependent translocation of Cx43 also depends on PKC and which isoform of PKC may be involved. The issue of the isoform involved is important, since according to present knowledge, PKC\(D\) activation exerts different, gap junction conductance-enhancing effects [17] than PKCe, which reduces gap junction coupling [18]. In addition, it would then be interesting to know which sequence within Cx43 becomes phosphorylated. The most urgent question, however, is whether mitochondrial Cx43 is a monomeric protein or whether it forms hemichannels, which would provide information about a functional role. From the present literature, it seems that the positive effects of ischemic preconditioning are somehow related to Cx43, since in contrast to wild-type mice, hearts from heterozygous Cx43 knockout mice cannot be preconditioned [19]. Thus, the article by Boengler et al. gives rise to a number of interesting questions about cell biology and function and directs our view on connexins from their role in cell–cell communication to intracellular trafficking and regulatory functions.

References


