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Review

Mix and match: Investigating heteromeric and heterotypic gap junction channels in model systems and native tissues

Michael Koval [a,b,*], Samuel A. Molina [a], Janis M. Burt [c]

[a] Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Emory University, Atlanta, GA, United States
[b] Department of Cell Biology, Emory University, Atlanta, GA, United States
[c] Department of Physiology, University of Arizona, Tucson, AZ, United States

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ABSTRACT

This review is based in part on a roundtable discussion session: "Physiological roles for heterotypic/heteromeric channels" at the 2013 International Gap Junction Conference (IGJC 2013) in Charleston, South Carolina. It is well recognized that multiple connexins can specifically co-assemble to form mixed gap junction channels with unique properties as a means to regulate intercellular communication. Compatibility determinants for both heteromeric and heterotypic gap junction channel formation have been identified and associated with specific connexin amino acid motifs. Hetero-oligomerization is also a regulated process; differences in connexin quality control and monomer stability are likely to play integral roles to control interactions between compatible connexins. Gap junctions in oligodendrocyte:astrocyte communication and in the cardiovascular system have emerged as key systems where heterotypic and heteromeric channels have unique physiologic roles. There are several methodologies to study heteromeric and heterotypic channels that are best applied to either heterologous expression systems, native tissues or both. There remains a need to use and develop different experimental approaches in order to understand the prevalence and roles for mixed gap junction channels in human physiology.

1. Introduction

Proteins known as connexins form gap junction channels that provide a direct connection enabling the exchange of small molecules between adjacent cells. Different connexins form channels with different permeability and gating characteristics that dictate the type of intercellular communication they mediate. Moreover, different connexins are subject to different classes of posttranslational modification, such as phosphorylation, which further regulate gap junctional communication.

As an added level of complexity, gap junction channels can be formed containing more than one connexin isoform [1,2]. This allows formation of channels with unique gating and permeability that would not be otherwise attainable with channels composed of a single connexin isoform. Not all connexins are compatible to interact, which enables specific networks of interconnected cells to be formed and independently regulated.

There are now considerable data demonstrating which connexins are compatible to form a mixed gap junction channels and which cannot. Most of the evidence in support of the potential for connexins to interact has come from using transfected connexin-null cell models expressing one or more exogenous connexins. While this does provide useful information, observations obtained using expressed transgenes need to be interpreted in the context of native tissue systems. This requires taking into account tissue specific connexin expression, tissue architecture, molecular composition of cell–cell interfaces, and regulation via signal transduction pathways. In this review we summarize the current state of the art of how connexins interact and discuss implications for this in regulating tissue function.

2. Molecular basis for connexin compatibility

Connexins are multipass transmembrane proteins with both the N- and C-termini oriented towards the cytosol (Fig. 1). There are 21 human connexin genes that are translated into functional proteins.

* Corresponding author at: Emory University School of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, 615 Michael St., Suite 205, Atlanta, GA 30322, United States.
E-mail address: mhkoval@emory.edu (M. Koval).

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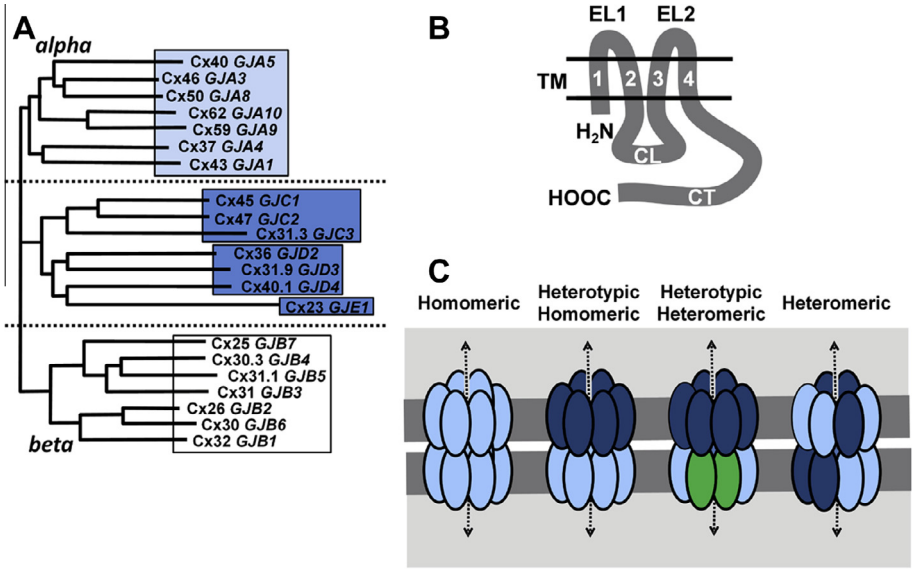

Fig. 1. Structure and interactions between human connexins. (A) Shown is a dendrogram arranged using amino acid homology [5], where human connexin protein names were used (connexin; Cx) and gene names are in italics. (B) Line diagram of a generic connexin, showing both the N-terminus (NH_2) and C-terminus (CT) oriented towards the cytoplasm. Other protein elements include the two Extracellular Loop (EL) domains, four Transmembrane (TM) domains, and Cytoplasmic Loop (CL) domain. (C) Diagram of different classes of channels, including homomeric, heterotypic and heteromeric.

By amino acid sequence homology connexins form three clusters, alpha connexins, beta connexins and a third cluster with intermediate homology composed of gamma, delta and epsilon connexins [3–5].

Twelve connexins interact in order to form a complete gap junction channel; six connexins in the plasma membrane of one cell oligomerize and dock with compatible hexamers on an adjacent cell [2,6]. Hexamers that act as *bona fide* plasma membrane channels without docking are called hemichannels. Gap junction channels composed of a single type of connexin protein are homomeric; heteromeric channels contain two or more different types of connexins (Fig. 1). Heterotypic channels are formed by a hexamer on one cell docked to a hexamer with different connexin composition on the other. Heterotypic channels are most typically formed from two homomeric hexamers (Fig. 1), however, they can also consist of a homomeric and heteromeric hexamer or two heteromeric hexamers. Based largely on sequence homology, connexin structure determination and the analysis of connexin interactions in model systems, there is a considerable amount known about the molecular determinants that regulate connexin compatibility.

Table 1
Motifs which regulate heteromeric compatibility.

R type			
Cx43	151	LLRTY	155
Cx46	145	LLRTY	149
Cx50	147	LLRTY	151
Cx45	173	LMKIY	177
Cx47	209	LMRVY	213
Cx36	195	ISRFY	199
Cx31.9	132	ARRCY	136
W type			
Cx26	132	LWWTY	136
Cx30	132	LWWTY	136
Cx30.3	127	LWWTY	131
Cx31	127	LWWTY	131
Cx32	131	LWWTY	135
Other			
Cx37	151	LMGTY	155
Cx40	149	LLNTY	153

Shown are motifs in the transition between the cytoplasmic loop (CL) and third transmembrane (TM3) domains that help confer heteromeric specificity. R type, and W type designation is from [7,8]. See also Figs. 2 and 3.

2.1. Heteromeric compatibility

The amino acid homology dendrogram in Fig. 1 provides a reasonable guide to heteromeric compatibility among connexins [5,7]. Heteromeric compatibility of alpha vs. beta connexins correlates well with a signature amino acid motif localized at the interface region where the cytosolic intracellular loop (CL) domain transitions into the third transmembrane domain (TM3) (Table 1; Fig. 2). For most alpha connexins, this motif contains a conserved arginine or lysine residue (which we have referred to as R type connexins) [7]. By contrast, beta connexins contain a di-tryptophan (“WW”) motif (W type connexins) [7,8].

Control of hetero-oligomerization by R and W motifs is most likely indirect. Based on the high resolution structure of Cx26, the WW motif is localized to the cytosol-membrane interface of the cytoplasmic leaflet and does not directly mediate interprotein connexin–connexin interactions [7,9]. Instead, several broadly conserved amino acids in TM2 and TM4 near the extracellular aspect of these domains form salt bridges or hydrogen bonds to stabilize hexamers [9]. Moreover, mutations in amino acids directly involved in connexin–connexin interactions are associated with human disease [10,11]. Thus, roles for R and W type motifs in regulating hetero-oligomerization are indirect and most likely due to control of the initiation of oligomerization as opposed to hexamer stabilization.

In fact, R type and W type connexins oligomerize via different pathways, which, in turn, plays a key role in preventing heteromer formation between them. The most thoroughly studied R type connexin is Cx43. In contrast to most oligomeric transmembrane channel proteins, Cx43 is stabilized as monomers in the endoplasmic reticulum (ER) and only oligomerizes after transport to the trans Golgi network (TGN) (Fig. 3) [7,12–14]. Part of this pathway is regulated by a quality control protein, ERp29, which binds to the second extracellular loop domain and stabilizes a conformation

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