Review TRENDS in Cell Biology Vol.16 No.3 March 2006



# Pathways and control of connexin oligomerization

**Michael Koval**

Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA

**Connexins form gap junction channels that link neighboring cells into an intercellular communication network. Many cells that express multiple connexins produce heteromeric channels containing at least two connexins, which provides a means to fine tune gap junctional communication. Formation of channels by multiple connexins is controlled at two levels: by inherent structural compatibilities that enable connexins to hetero-oligomerize and by cellular mechanisms that restrict the formation of heteromers by otherwise compatible connexins. Here, I discuss roles for secretory compartments beyond the endoplasmic reticulum in connexin oligomerization and evidence that suggests that membrane microdomains help regulate connexin trafficking and assembly.**

## Introduction

Gap junctions interconnect cells by forming a direct link to enable the diffusion of small aqueous molecules and ions from one cell to its nearest neighbor [1]. This enables the flow of specific intercellular signals and metabolic cooperation between communicating cells in a tissue. The importance of such communication is shown by the increasing number of human diseases that are directly attributable to mutants and deficiencies of the gap junction protein, connexin [2]. Mutations range from those that subtly change gap junctional permeability [3] to mutations that cause severe changes in connexin trafficking and that completely inhibit gap junctional coupling [4–7].

Connexins are a multigene family of transmembrane proteins [8] (Figure 1). Each connexin forms a channel with unique permeability characteristics, which is reflected in the types of metabolite and signaling molecule that flow through it [9]. A complete channel is formed from two hexameric hemichannels, one in each cell, that meet at the cell surface (Figure 2). Gap junction channels are organized into higher order semicrystalline arrays, known as plaques. In addition, connexin hemichannels function as bona fide plasma membrane channels that enable the diffusion of ATP and other aqueous molecules from the cytosol to the extracellular environment [10].

Connexins span the membrane bilayer four times with the N and C termini oriented towards the cytoplasm. The four connexin transmembrane domains are largely α helical [11]. Fleishman *et al.* [12] used a best-fit algorithm to assign the transmembrane domains of connexin 32 (Cx32) mathematically to the corresponding α helices in the structural model (Figure 2). On the basis of their model, the best fit was obtained by assigning the third transmembrane domain as the predominant pore-lining helix [12], although this is controversial [13].

Different types of cell express different connexins and cells frequently express two or more connexins [14–16]. Because an individual gap junction channel is composed of 12 connexins, cells that express multiple connexins can produce mixed channels, provided that the connexins are able to hetero-oligomerize. The formation of gap junction channels by two or more connexins enables cells to produce channels that have unique permeability and gating characteristics that could not be obtained using a single connexin [17]. However, the rules that govern connexin oligomerization and compatibility are complex.

## Determinants of connexin compatibility

### *Innate heteromeric compatibility*

Figure 2 shows the different classes of channel that can be formed when cells express two or more connexins. Connexins do not ubiquitously intermix; instead, compatibility is based on their protein structure. When two connexins expressed in the same cell form a mixed channel, this is referred to as heteromeric compatibility. Examples of heteromeric channels formed by endogenously expressed connexins include Cx32–Cx26 in the liver [15,18], Cx46–Cx50 in the lens [19] and Cx43–Cx46 in the lung [20]. Heteromeric connexin compatibility has been tested using transfected cell models, although there are many combinations that have not been examined. The heteromeric compatibility groups correspond loosely to α and β connexin subfamiles [15,17]. Connexins that do not belong in either of these subgroups have been found to form heteromers with either α connexins (e.g. Cx45–Cx43) [21] or β connexins (e.g. mCx29–Cx32) [22]. To date, no connexin has been identified that forms normal heteromeric channels with both α and β subfamily connexins.

Motifs that dictate innate heteromeric compatibility have been studied most extensively using two incompatible connexins, Cx32 and Cx43. A series of truncation and point mutants that alter connexin heteromeric specificity showed that the motifs that prevent hetero-oligomerization of Cx32 and Cx43 include pairs of amino acids in the N

*Corresponding author:* Koval, M. (mhkoval@emory.edu).

 0962-8924/$ - see front matter  doi:10.1016/j.tcb.2006.01.006

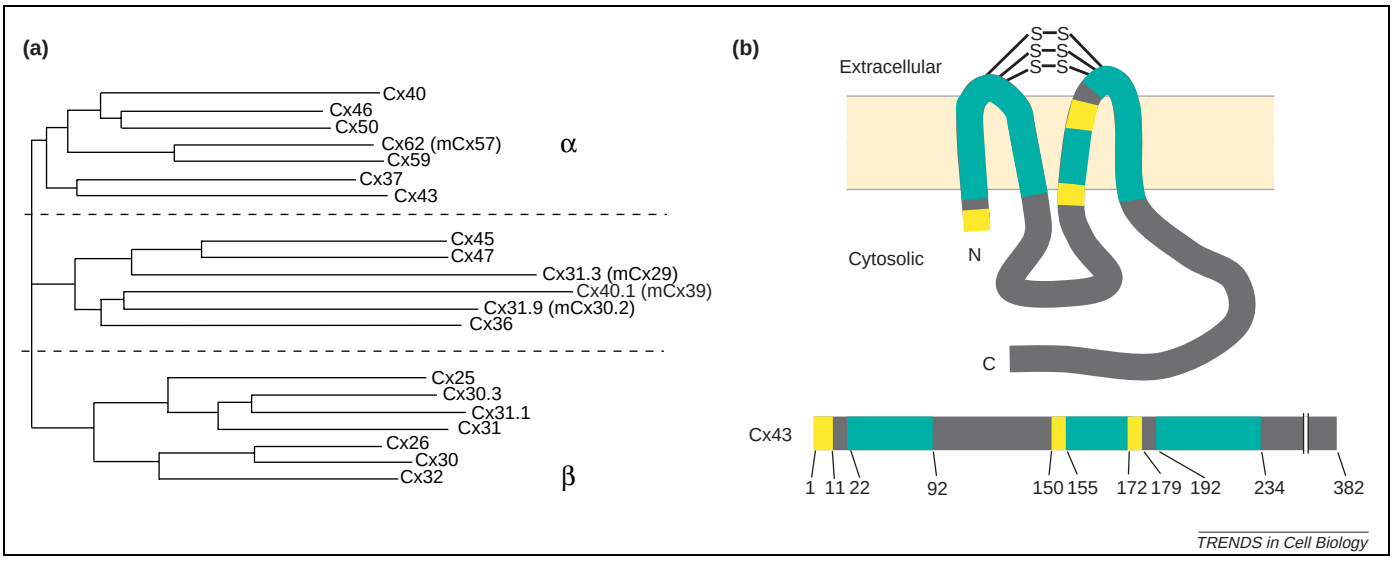


**Figure 1.** Connexin homology and structure. **(a)** Phylogram of 20 human connexins calculated using ClustalW and omitting C-terminal domains [85]. By protein homology, connexins form two major subgroups, α and β, with an additional group of connexins with intermediate homology [8]. Different connexins are denoted by Cx plus a number corresponding to the predicted molecular mass based on the amino acid sequence. Mouse connexin names that differ from their human orthologs are shown in parentheses*. **(b)** Line diagram corresponding to an individual connexin. The two extracellular loop domains are interconnected by three disulfide bridges. Green denotes regions with high amino acid sequence homology within the entire connexin protein family, yellow denotes regions in which amino acids are homologous within a subset of connexins and gray denotes divergent regions of connexins that vary in amino acid sequence and size. Numbers correspond to positions of Cx43 amino acids that define different classes of connexin homology domains.

terminus and at the cytosolic end of the third transmembrane domain [23].

An aberrant interaction between α and β connexins can have pathologic consequences. For example, although Cx26 and Cx43 are normally incompatible [24], some Cx26 mutants that are associated with the skin disorder palmoplantar keratoderma have a dominant-negative effect on Cx43, which is probably due to formation of an aberrant heteromer [25]. However, other Cx26 mutations that are associated with non-syndromic deafness are more specifically limited to the disruption of Cx26 and other β connexins, indicating that different mutations in the same connexin can cause different human diseases.

### *Innate heterotypic compatibility*

A gap junction channel formed by a head-to-head interaction between two different connexins is known as a heterotypic channel. Heterotypic channels have been identified in several settings *in vivo*, most notably in the central nervous system (CNS), in which astrocytes and oligodendrocytes are interconnected exclusively through heterotypic channels [26,27]. Although such gap junctions can contain channels with unique permeability characteristics, a more prominent role for specific heterotypic interactions is to form intercellular networks by either promoting or restricting cell interconnectivity. Disulfide bridges that interconnect the two extracellular domains are crucial for channel formation [28,29]. Two key amino acids in the second extracellular loop are the major determinants of heterotypic compatibility [15,30], although motifs in the first extracellular loop and other parts of the protein might also have a role [31]. In contrast to innate heteromeric compatibility, innate heterotypic compatibility does not correlate well with whether a given connexin is an α or β family member [15,32]. For example, Cx32 and Cx43 cannot form heterotypic channels; however, the α connexins Cx46 and Cx50 can form heterotypic channels with either Cx32 or Cx43 [30]. In addition, some connexins, including Cx31 and Cx36, appear to only form homotypic channels [32,33].

### *Regulated connexin compatibility*

Although connexins have structural determinants that define their ability to form heteromeric channels, there are several examples showing that this ability is also dependant on the context of cell expression. For example, two compatible connexins, Cx43 and Cx46, are expressed by type I alveolar epithelial cells and form heteromeric channels [20]. However, these same connexins, when expressed by type II alveolar epithelial cells or osteoblastic cells, do not hetero-oligomerize (Figure 3) [20,34,35]. Thus, formation of heteromeric Cx43–Cx46 channels depends on cell type and is a regulated process.

Another example of regulated connexin targeting is the myoendothelial junction between vascular endothelium and smooth muscle cells. These cells express different levels of Cx37, Cx40 and Cx43, all of which can hetero-oligomerize with one another; this has been demonstrated in transfected cell models [17]. Using a co-culture model, in which the cells maintained polarity and phenotype, Isakson and Duling [36] found that Cx37 was specifically excluded from myoendothelial junctions between

* At the 2005 International Gap Junction Conference, a proposal to develop a revised nomenclature to unify connexin naming between species was approved, but has not been finalized. Changes to the connexin nomenclature will be published elsewhere and posted to the Human Genome Organization website (http://www.gene.ucl.ac.uk/nomenclature/).