VIEWPOINT

Channeling diversity: Gap junction expression in the heart

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Introduction

Gap junctions are composed of clusters of intercellular channels that electrotonically and metabolically couple apposing cells. Each channel is formed from the pairing of 2 connexons (or hemichannels), which, in turn, are formed by the assembly of 6 connexin monomers. The mammalian genome comprises almost 2 dozen individual connexin genes, and a surprising number of these genes are actively transcribed in the developing or mature heart. The evolutionary advantage for cardiac gap junction diversity remains uncertain. Here, we consider the nature of this diversity and speculate on potential explanations.

Connexin diversity in the heart

There are at least 5 different connexins expressed in the adult mammalian heart (Table 1). These include members of the alpha (connexin 43 [Cx43] and connexin 40 [Cx40]), beta (connexin 37 [Cx37] and connexin 30.2 [Cx30.2] in mice or connexin 31.9 [Cx31.9] in humans), and gamma (connexin 45 [Cx45]) families. All encode proteins with high degrees of structural homology, including 4 membrane-spanning helices and 2 extracellular domains. There is a significant divergence in their intracellular segments, particularly in their carboxy terminal domains, which may contribute to the distinct biophysical properties of gap junction channels formed from each connexin.

Why such diversity? The primary function of the heart is to support the circulation as a dynamic pump—one that is dependable yet highly responsive to acute and chronic changes in metabolic demand. The 4-chamber heart, the specialized nodal cells and a rapidly conducting His-Purkinje network embedded within the heart have evolved to meet this challenge. Given the functional specialization of individual subsystems that are integrated into the organ as a whole (rate regulation by the nodes; blood collection in the atria; rapid myocardial activation through the His-Purkinje system; blood ejection in the ventricles), one might expect that each component would express connexins with appropriate gap junction channel properties.

Is the nature of connexin diversity consistent with these disparate functional requirements, indicative of strong evolutionary pressure? Indeed, connexin isotype expression in the heart appears well designed to support not only the unique functional attributes of each compartment but also the particular complexities that arise at interfaces between individual compartments (see Figure 1).

We begin with the sinoatrial node, where both theoretical and experimental studies have demonstrated that pacemaker function requires a population of weakly coupled intrinsically rhythmic cells apposed to a second population of well-coupled cardiomyocytes. Not unexpectedly, we find that the sinoatrial node includes a core of pacemaker cells expressing low levels of Cx45 and Cx30.2, each of which encode highly voltage-sensitive, small-conductance gap junctional channels.[11] In contrast, as one moves toward the periphery of the sinoatrial node into the so-called paranodal area, there is a heterogeneous mixture of myocytes, some of which express Cx43.[11] In the atrium proper, both Cx43 and Cx40 are found. Within the atrioventricular node, Cx45 and Cx30.2 (at least in the murine heart) are arranged to create low-conductance gap junction.[1] This expression pattern contributes to the slowing of conduction that is responsible for the delay between atrial and ventricular activation. In contrast, maximal pressure development within the pumping chambers of the heart is dependent on rapid and highly synchronized triggering of the ventricular myocardium by strongly coupled cells within the His-Purkinje network, and in fact, high-conductance Cx40 channels are robustly expressed in the specialized ventricular conduction system. Finally, the ventricular myocardium itself is highly coupled through abundant expression of Cx43. These channels provide an intermediate gating sensitivity and conductance that is best suited for widespread local propagation of the cardiac action potential.

The interface between the Purkinje fiber network and the ventricular myocardium presents a particularly intriguing example of structural and functional specialization. Here, it is believed that heterotypic channels are formed by Cx40 connexons in the Purkinje cells and Cx43 connexons in the

KEYWORDS Gap junctions; Connexins; Heart development; Arrhythmia

ABBREVIATIONS **Cx30.2** = connexin 30.2; **Cx31.9** = connexin 31.9; **Cx37** = connexin 37; **Cx40** = connexin 40; **Cx43** = connexin 43; **Cx45** = connexin 45 (Heart Rhythm 2012;9:1159–1162)

Research in the Fishman laboratory was supported by grants from the NIH (R01 HL82727, R01 HL105983, T32 HL098129) and the New York State Department of Health (NYSTEM N08G-132). **Address reprint requests and correspondence:** Dr Glenn I. Fishman, MD, FHRS, Leon H. Charney Division of Cardiology, New York University School of Medicine, 522 First Avenue, Smilow 801, New York, NY 10016. E-mail address: glenn.fishman@nyumc.org.

1547-5271/$ -see front matter doi:10.1016/j.hrthm.2011.11.040

Table 1 The murine connexin gene family

Connexin	Gene	Chr	AA	Expression	g_j (pS)	Knockout phenotype
Cx30.2	*Gjd3*	11	278	SAN, AVN	9[1]	Impaired delay in AVN conduction[2]
Cx37	*Gja4*	4	333	EC	300[3]	Female sterility[4]
Cx40	*Gja5*	3	358	A, VCS, EC	162[5]	Arrhythmias, BBB, congenital defects[6]
Cx43	*Gja1*	10	382	A, V	60–100[7]	RV outflow tract malformations[8]
Cx45	*Gja7*	11	395	SAN, AVN, VCS	32[9]	Endocardial cushion defects[10]

Displayed for each connexin is its chromosomal location, number of amino acids, cardiac expression pattern, channel characteristics, and cardiac phenotype of murine knockout.

A = atrial cardiomyocyte; AA = amino acid residue; AVN = atrioventricular nodal cell; BBB = bundle branch block; Chr = chromosome; Cx30.2 = connexin 30.2; Cx31.9 = connexin 31.9; Cx37 = connexin 37; Cx40 = connexin 40; Cx43 = connexin 43; Cx45 = connexin 45; EC = vascular endothelial cells; g_j = unitary channel conductance; RV = right ventricle; SAN = sinoatrial nodal cell; V = ventricular cardiomyocyte; VCS = ventricular conduction system.

ventricular cardiomyocytes. The differential voltage dependence of Cx40 and Cx43 connexons provides for rectification, supporting antegrade flow of current down the specialized cardiac conduction system across the Purkinje-ventricular junction into the ventricular myocardium while providing a degree of protection against retrograde conduction and reentrant arrhythmias.[12] The difficulty associated with this balancing act may explain why the Purkinje-ventricular junction is thought to be a common element in various arrhythmic syndromes.[13]

Connexin diversity during heart development

Expression of different connexin isotypes varies not only within distinct compartments of the adult heart but also as a function of cardiac developmental stage. The major spatiotemporal expression patterns appear relatively conserved across mammalian species, as the embryonic mouse heart closely parallels the embryonic human heart.[14] Cx45 is the earliest expressed connexin (E8.5 in mice), initially found in all cardiac compartments but ultimately restricted primarily to cells of the specialized cardiac conduction system by E15.5. Cx40 is also expressed throughout the early developing heart, most prominently in the trabecular myocardium, but is downregulated in late fetal stages.[14] Cx43, while present throughout early cardiac development, increases in abundance during late gestation both in atrial and especially in working ventricular cardiomyocytes.[14]

Targeted mutagenesis studies in the mouse have revealed that each of these connexins is required for normal heart formation and function. $Cx45^{-/-}$ mice have endocardial cushion defects.[10] Cx40 knockout mice have abnormalities in cardiac conduction and arrhythmias and an increased incidence of congenital defects, including pathologic hypertrophy, common atrioventricular junction, or ventricular septal defects.[6] Germ-line Cx43 knockout mice die perinatally with heart defects involving the right ventricular outflow tract.[15] While cardiac-specific Cx43 knockout mice circumvent this congenital defect, they develop spontaneous lethal ventricular arrhythmias beginning around 1 month after birth.[16] The mechanisms supporting conduction in the absence of Cx43 are uncertain. It is conceivable that the low-level expression of alternative isoforms, such as Cx45, provides adequate electrotonic coupling.[17] Alternatively, computational modeling and recent studies of subcellular localization of voltage-gated sodium channels suggest the intriguing possibility of a role for ephaptic propagation.[18–20] Mice lacking Cx30.2 do not display structural heart defects, but do have a shortened PR interval on the surface electrocardiogram and therefore lack the physio-

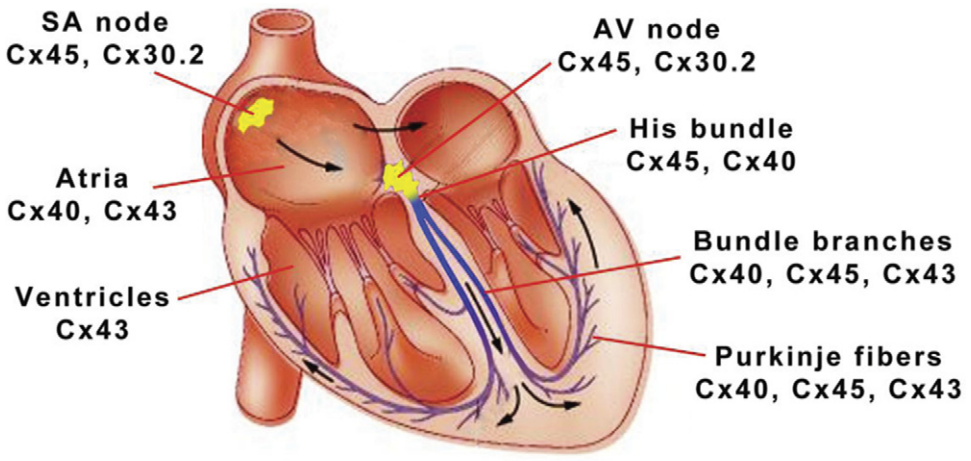

Figure 1 Connexin expression profiles in the adult murine heart.