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Channeling Diversity: Gap Junction Expression in the Heart

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Introduction

Gap junctions (GJ) are comprised of clusters of intercellular channels that electrotonically and metabolically couple apposing cells. Each channel is formed from the pairing of two connexons (or hemi-channels), which in turn are formed by the assembly of six connexin monomers. The mammalian genome comprises almost two 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 five different connexins expressed in the adult mammalian heart (Table I). These include members of the alpha (Cx43 and Cx40), beta (Cx37, Cx30.2 in mouse or its human orthologue Cx31.9) and gamma (Cx45) families. All encode proteins with high degrees of structural homology, including four membrane-spanning helices and two extracellular domains. There is 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 four-chamber heart and the specialized nodal cells and 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.

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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 SA 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 SA 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[1]. In contrast, as one moves toward the periphery of the SA node into the so-called “paranodal area”, there is a heterogeneous mixture of myocytes, some of which express Cx43[1]. In the atrium proper, both Cx43 and Cx40 are found. Within the AV node, Cx45 and Cx30.2 (at least in the murine heart) are arranged to create low-conductance GJ[2]. 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 upon 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 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 (CCS) across the Purkinje-ventricular (PV) junction into the ventricular myocardium, while at the same time providing a degree of protection against retrograde conduction and reentrant arrhythmias[3]. The difficulty associated with this balancing act may explain why the PV junction is thought to be a common element in various arrhythmic syndromes[4].

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 that found in the embryonic human heart[5]. Cx45 is the earliest expressed connexin (E8.5 in mice), initially found in all cardiac compartments but ultimately restricted to primarily cells of the specialized CCS 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[5]. Cx43, while present throughout early cardiac development, increases in abundance during late gestation in both atrial and especially in working ventricular cardiomyocytes[5].

Targeted mutagenesis studies in the mouse have revealed that each of these connexins are required for normal heart formation and function. $Cx45^{-/-}$ mice have endocardial cushion defects[6]. 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[7]. Germline Cx43 knockout mice die perinatally with heart defects involving the right ventricular outflow tract[8]. While cardiac-specific Cx43 knockout mice circumvent this congenital defect, they develop spontaneous lethal ventricular arrhythmias beginning around one month after birth[9]. The mechanisms supporting conduction in the absence of Cx43 are uncertain. It is conceivable that low level expression of alternative isoforms such as Cx45 provides adequate electrotonic coupling[10]. Alternatively, computational modeling and recent studies of sub-cellular localization of voltage-gated sodium channels suggest the intriguing possibility of a role for ephaptic propagation[11–13]. Mice lacking Cx30.2 do not display structural heart defects, but do have a shortened PR interval on the surface EKG and therefore lack the physiologic delay between atrial and ventricular contraction[14]. While these knockout studies demonstrate the requirement for multiple connexins during cardiac development, they do not provide much insight into underlying mechanism. Conceivably, channels comprised of specific connexins regulate the flow of critical morphogens within or between different compartments of the developing heart. In addition, non-channel properties of connexins, i.e., interaction with β-catenin or other binding partners may regulate key developmental processes such as cellular proliferation, apoptosis or differentiation[15].

Regulatory Diversity

While each of the cardiac connexins form channels with distinct biophysical properties, the multitude of connexin genes provides for several additional levels of control, including transcriptional and post-translational regulation. For example, by virtue of sequence variation, individual connexins differ in their sensitivity to various kinases, acetylases, or other post-translational modifying enzymes, facilitating cell-type specific regulation by various signaling pathways. This form of regulation may provide a mechanism to rapidly and potently modulate coupling of populations of cells in response to physiologic cues or pathologic stimuli.

The surprisingly larger number of connexin genes may also provide a mechanism for fine-tuning connexin expression in stage- and lineage-specific manners through transcriptional control. The different connexin genes vary in their *cis*-acting regulatory regions, and consequently each can respond individually to the cell autonomous transcriptional pathways. For example, the T-box family transcription factor Tbx5 has been shown to activate the mouse Cx40 promoter, either alone or in concert with the homeoprotein Nkx2-5[16]. Conversely, mouse hearts that overexpress Nkx2-5 have reduced expression of Cx43[17]. Further, Tbx2 (similar to Tbx5) has been shown to be a negative regulator of Cx43 expression[18]. Recently, the Iroquois homeobox protein Irx3 has been shown to repress Cx43 expression and conversely activate Cx40. Irx3 mutant mice display QRS widening and ectopic Cx43 expression in the proximal bundle branches[19]. Such findings offer a glimpse into the complex mechanisms responsible for the heterogeneity of connexin expression.

Connexins and Disease

Connexin diversity may also provide some protective advantage for the heart when challenged by pathologic stressors, although evidence supporting this hypothesis is currently lacking. Certainly “modern” diseases such as coronary artery disease that present after reproductive age would not impart evolutionary pressure, however it is conceivable that differential gating of connexins in response to adrenergic activation (i.e., as part of the fight-or-flight response or with acute hemorrhage), might provide some benefit. For example, expression of multiple connexins with differential sensitivity to protein kinase A dependent phosphorylation might allow for preservation of electrotonic coupling and successful

impulse propagation while at the same time minimize diffusion of toxic metabolites from ischemic regions of the heart.

Looking to the future, it is conceivable that “designer” connexins with potenti anti-arrhythmic activity could be expressed in diseased hearts. Our laboratory has already demonstrated that a Cx43 mutant resistant to dephosphorylation in the carboxy terminus diminishes pathologic gap junction remodeling[20]. One can imagine fine tuning the structure of connexins to optimize their anti-arrhythmic efficacy.

Conclusion

The large number of connexin genes actively transcribed in the mammalian genome suggests that significant evolutionary pressure exists to maintain this high degree of complexity. Connexin diversity in the heart appears well designed to coordinate the initiation and propagation of the action potential from the sinus node to the ventricular myocardium. Moreover, the multitude of connexins expressed in the developing heart may facilitate the remarkable structural and functional plasticity that occurs as the heart matures from a simple linear peristaltic tube to a highly complex four-chambered pump. Finally, connexin diversity may provide some protection against pathologic stimuli.

What does the future hold for gap junction biologists? Despite significant progress, our understanding of the molecular determinants of the cardiac connexin life-cycle are still relatively immature. Further studies in this area will be essential to identify novel targets to manipulate connexin expression and function in the heart for therapeutic effect. In addition, the list of connexin-interacting proteins is expanding, but the biological significance of this interactome is in its infancy[21]. A multi-disciplinary strategy, channeling the efforts of a diverse assembly of investigators, with expertise in molecular, cellular and systems biology will be essential to unravel the nature of these interactions.

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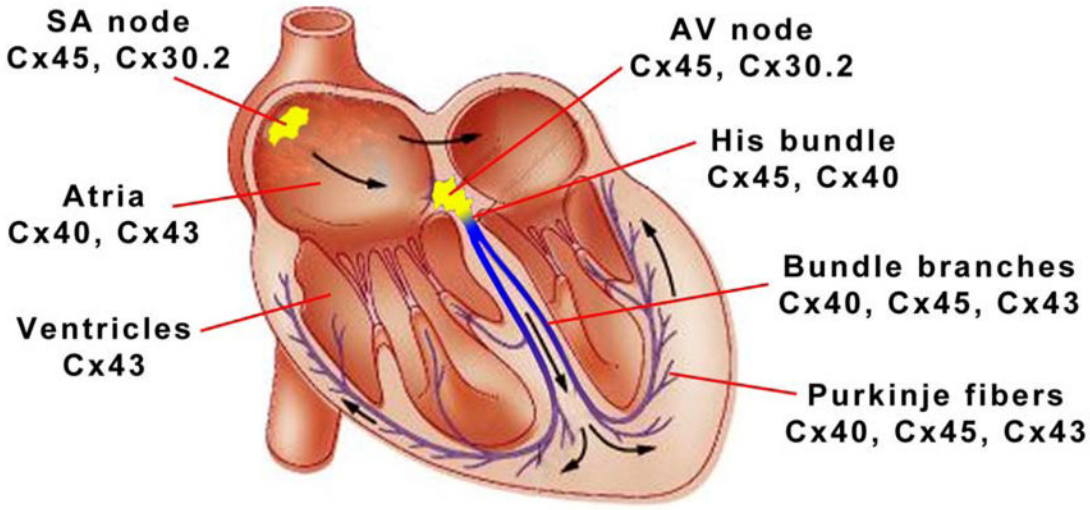

Figure 1.
Connexin expression profiles in the adult murine heart.

Table 1

The murine connexin gene family

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

Connexin	Gene	Chr	AA	Expression	g_j (pS)	Knockout phenotype
Cx30.2	Gjd3	11	278	SAN, AVN	9[2]	impaired delay in AV nodal conduction[14]
Cx37	Gja4	4	333	EC	300[22]	female sterility[23]
Cx40	Gja5	3	358	A, VCS, EC	162[24]	arrhythmias, BBB, congenital defects[7]
Cx43	Gja1	10	382	A, V	60–100[25]	RV outflow tract malformations[26]
Cx45	Gja7	11	395	SAN, AVN, VCS	32[27]	endocardial cushion defects[6]

Chr, chromosome; AA, amino acid residues; A, atrial cardiomyocytes; V, ventricular cardiomyocytes; SAN, SA nodal cells; AVN, AV nodal cells; VCS, ventricular conduction system; EC, vascular endothelial cells; g_j, unitary channel conductance in picosiemens; BBB, bundle branch block; RV, right ventricle.