Swapping Connexin Genes
How Big Is the Gap?

Steven M. Taffet, José Jalife

Manipulation of the expression of specific genes in transgenic or knockout (KO) mice has become an important strategy to study the biological and physiological roles of individual cardiac proteins. Several laboratories using transgenic and knockout methodologies have made substantial progress in generating mouse models with reduced connexin expression in the heart. This has provided the unique opportunity to characterize both qualitatively and quantitatively the role of cardiac gap junction channels in electrical wave propagation and has opened many interesting questions regarding the electrophysiological consequences and proarrhythmic effects of reduced intercellular coupling in the atria, ventricles, and His-Purkinje system of neonatal and/or adult mice lacking a given connexin.

The current count of 19 connexins in the mouse genome and 20 connexins in the human genome implies a highly intriguing diversity of connexin function. But the exact nature of that diversity is not clear. Connexins can no longer be looked at as passive pores that allow ions to pass from one cell to the next. At the least, these channels are dynamic pores that are regulated by a distinct pattern of cellular states and protein interactions. It is likely that these channels are more than that. Connexins are in many ways similar to scaffolding proteins that can bind cytoskeletal proteins, protein kinases, and cellular scaffolds. Recent evidence has even suggested that some of the biological properties of connexin channels are distinct from their pore ability to form gap junctions and pass electrical information from one cell to the next. Based on the complex regulation and interactions of connexins, it would be expected that each connexin may have a distinct role and that they may not be interchangeable. That some connexins have unique functions was demonstrated by the study of Plum et al, who generated two different knock-in (KI) mouse lines in which the coding region of the connexin43 (Cx43) gene was replaced either by the coding region of Cx32 or of Cx40. In Cx43KI32 or Cx43KI40 mice, it was possible to rescue the postnatal lethality of Cx43-deficient mice; however, there were significant functional and morphological differences between the wild-type and two knock-in mouse lines, supporting the need for diversity and specialization of connexins in various cell types.

In the study by Alcoléa et al, published in this issue of Circulation Research, Cx40 is replaced with Cx45. Both of these connexins are expressed in the cardiac conduction system and might be expected to have some functional equivalence. Mice were generated with the coding sequence of Cx45 replacing that of Cx40 (Cx40KO-Cx45KI). The mice were analyzed for proper expression of the KI gene and then examined for alterations in the conduction properties of the hearts. Interestingly the Cx40KO-Cx45KI mice had several distinct patterns. The initial finding was an alteration in the surface ECG. The changes were an increased duration of the P wave, implying a slowing of atrial activation and a prolongation of the QRS complex, reflecting delayed activation of the ventricles. These studies were followed by epicardial mapping, which indicated that the ventricles had an altered pattern of activation due to an increased conduction time to the right ventricle. Analysis of conduction velocity in the atria indicated that although conduction was unchanged in the right atrium, it was significantly slowed in the left. Finally, the Cx40KO-Cx45KI mice had a significant reduction of conduction velocity in the right bundle branch. These differences suggest that Cx40 and Cx45 are not functionally equivalent.

The lack of equivalence between two connexin channels is not surprising based on the distinct characteristics of these channels. Cx40 channels have more than four times the unitary conductance of Cx45 channels and a similar number of Cx40 channels could, in theory, pass much more current than Cx45 channels. The question is then whether that difference is significant enough to account for the slowing of conduction velocity. The answer is problematic. The results of Gutstein et al suggest that a reduction of Cx43 content in the ventricle equal to 90% only results in a 50% reduction in conduction velocity. Can the reduced ion flow through Cx45 channels account for the 23% reduction in conduction velocity in the left atria? Since there is no reduction in Cx43 in these mice, it would be assumed that the Cx43 channels in the left atria would still be capable of conduction, further minimizing the effect of reduced conduction through Cx45 channels. Might differences, other than ion transport, between Cx45 and Cx43 play some role in the slowing of impulse conduction?

Alternatively, it is not unreasonable to conclude that there is a lack of any effect of Cx45 in the atria. Verheule at al described a 30% decrease in atrial conduction in Cx40−/− mice. This figure was derived from the right atrium, and data were not presented from the left atria. Alcoléa et al did not measure conduction velocity from the Cx40−/− mice directly.
but relied on the values previously recorded. It is also 
intriguing that the conduction velocity for Cx40/−/− animals 
noted in the study of Alcoléa et al is substantially lower than 
that measured by Verheule and colleagues. As the value for 
atrial conduction velocity from control animals is substi-
tially different from the previous studies and there is no value 
determined from Cx40/−/− animals, it is not possible to 
accurately assess the effect of Cx45 substitution. It is possible 
that Cx45 is incapable of replacing Cx40 to maintain con-
duction in the atria.

Similar questions might be asked about the role of two 
distinct connexins in the specialized conduction system of the 
ventricles. The results presented by Alcoléa et al and by a 
previous study from the same group19 demonstrate that Cx45 
can act as the primary connexin in the His bundle and left 
bundle branch (LBB). In contrast, in Cx40KO mice, there is 
a right bundle branch (RBB) block, which apparently is only 
partially restored by Cx45 expression. In the Cx40KO- 
Cx45KI mice, there is a 46% reduction of conduction velocity 
compared with wild-type. Again, is this simply due to the 
lower single-channel conductance of Cx45 or some other 
mechanism?

At this juncture, it is important to stress the fact that the 
effect of Cx40 replacement by Cx45 on intraventricular 
conduction is not free of controversy. To begin with, while 
Cx40/−/− mice have normal heart rates and no arrhythmias, 
they have prolonged PR and QRS intervals. In the study of 
Verheule et al,2 the mean value of the QRS in the wild-type 
mouse was 11.0 versus 16.3 ms in the Cx40/−/− mouse. 
Similarly, Tamaddon et al20 reported a mean QRS of 
14.5 ms for the wild-type and 16.8 ms for the KO mouse. Using 
high-resolution optical mapping, Tamaddon et al20 
demonstrated that the prolonged atroventricular and intraventricular 
conduction times in the Cx40/−/− mouse were attributable 
in large measure to a reduction in the velocity of impulses 
propagating through the RBB. Interestingly, unlike Van Rijen 
et al,19 these authors did not observe complete RBB block in 
any of their experiments. The conduction velocity measured 
in Cx40/−/− mice (43.7 cm/s) was reduced from that recorded 
from the Cx40/−/− mice (74.5 cm/s). This 42% reduction is 
similar to the alteration in conduction velocity in the 
Cx40KO-Cx45KI mice relative to control. As such, the 
ability of Cx45 to have any effect in replacing Cx40 must be 
questioned. Moreover, the conduction velocity value present-
ed by Alcoléa is substantially lower in the control animals 
than that reported by Tamaddon et al.20 This raises a critical 
issue. In the Cx40KO-Cx45KI mice, the amount of Cx45 in 
the RBB is substantial, representing the endogenous expres-
sion of Cx45 plus the additional expression of Cx45 under 
the influence of the Cx40 promoter. Under these conditions, it is 
difficult to explain the blocking or even slowing of conduction. 
Computer models of impulse propagation in model 
cables indicate that there would need to be a substantial 
reduction in coupling to result in a lowering of conduction 
rate. It is not clear that the different unitary conductance of 
Cx45 and Cx40 would explain the present results. This 
interpretation of the results implies a much greater functional 
difference between Cx40 and Cx45 than just a difference in 
ion transfer.

An intriguing result in the study by Alcoléa et al,15 using 
the Cx40KO-Cx45KI, as well as in previous studies in the 
Cx40−/− mouse, is the demonstration that the lack of Cx40 in 
the two types of mice affects propagation through the RBB 
much more than the LBB. In the study of Tamaddon et al,20 
the absence of Cx40 in the RBB resulted in significant 
conduction slowing, which led downstream to delayed acti-
vation of the right ventricular free wall. While these authors 
were unable to optically map the LBB, they did notice that 
activation of the right ventricular septum occurred before the 
complete activation of the RBB in the Cx40−/− mice. Simul-
taneous optical mapping and ECG recording indicated that 
the activation of the RBB in those mice was delayed with 
respect to the start of the QRS complex. Using the Q time and 
right ventricular septal activation as substitute markers of 
LBB activation, it was concluded that the RBB was somehow 
more affected than the LBB. Alcoléa et al have shed substi-
tual light on the question of whether electrical and/or struc-
tural differences between the two major His bundle branches 
may explain the apparently selective consequences of the 
absence of Cx40 on RBB conduction. First, they carried out 
electrode mapping experiments in both bundle branches and 
demonstrated that conduction velocity was significantly re-
duced in the RBB but not in the LBB of the Cx40KO-Cx45KI 
mice with respect to the wild-type and heterozygote mice. 
Second, while not presented in the present study, they report 
having replaced one allele of the Cx40 gene by enhanced 
green fluorescent protein (eGFP) (Cx40KO−/−eGFP) and observed 
a significant difference in the anatomical complexity of the 
LBB with respect to the RBB, with the former emerging from 
the His bundle as a large group of separate parallel strands 
forming a layer on the left side of the ventricular septum, and 
the latter being constituted by a single thin strand. Based on 
the studies of Kucera et al,21 who have proposed a “pull-and-
push” mechanism of slow, but nevertheless, robust conduc-
tion in branching structures, it seems reasonable to speculate 
that, if Cx45 was upregulated similarly in both bundle 
branches, the safety factor for propagation was higher in 
the greatly branched LBB than in the unbranched RBB. How-
ever, improved technology and further studies will be neces-
sary to provide a definite explanation to this interesting result.

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