The marker called chicken GATA-6 (cGATA-6) for conduction system

In 2001

The evolutionarily conserved GATA-6 transcription factor is an early marker of the cardiogenic lineage in vertebrate species.[1] In the early study [1]people showed that a cGATA-6 gene enhancer can be used to selectively express transgenes in the atrioventricular (AV) conduction system as it becomes manifest in the developing multichambered mouse heart. cGATA-6 gene is an early and persistent marker of the cardiogenic lineage. There are two enhancers upstream of the cGATA-6 gene that direct (spatially and temporally) distinct facets of heart-specific expression in transgenic mice. one enhancer showed in the study [1] functions in a heart-region-specific manner from very early stages of heart development and continues to function in the mature AVCS. The other one is inactivated when the heart further develops. At least one point is certain, A cGATA-6 gene heart-region-specific enhancer marks the AVCS in transgenic mouse embryos. As shown in Fig. 3, a superficial examination of a truncal section of an embryo (Fig. 3A) or an isolated heart (Fig. 3B) reveals that transgene expression is confined to the developing AVCS. In the isolated heart, robust expression is seen in the AV node (blue arrow), right AV ring (white arrow) whereas some weak expression still persists in the left AV junction (red arrow) at this stage.

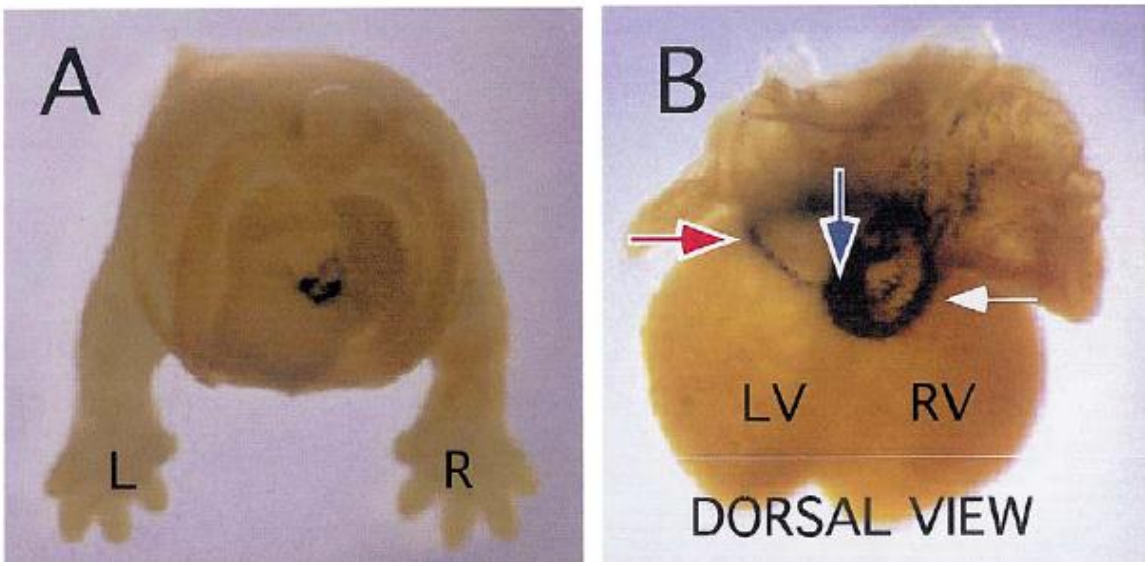

Figure 3

The 1.5 kb DNA fragment that drives the aforementioned transgene expression patterns must contain binding sites for a particular combination of active transcription factors that, in the mature heart, is restricted to the AVCS.

In 2005

Another study in 2005 further study the specific expression of c GATA-6. A functional pacemaking-conduction system is essential for maintaining normal cardiac function. However, the ability to accurately identify cells that function as cardiac pacemaking cells is crucial for being able to study their molecular phenotype.[2] In differentiating murine embryonic stem cells,

we demonstrate the development of an organized cardiac pacemakingconduction system in vitro using the coexpression of the minK-lacZ transgene and the chicken GATA6 (cGATA6) enhancer. These markers identify clusters of pacemaking “nodes” that are functionally coupled with adjacent contracting regions. cGATA6-positive cell clusters spontaneously depolarize, emitting calcium signals to surrounding contracting regions. Physically separating cGATA6-positive cells from nearby contracting regions reduces the rate of spontaneous contraction or abolishes them altogether.
In the previous study, its expression in the adult mouse becomes restricted to regions of the heart that contain the SA and atrioventricular (AV) nodes as well as the AV bundle (bundle of His) [3][4].
Because the cGATA6 enhancer identifies cells that organize and function as pacemaking cells, we used this marker to isolate a population of cells that resemble nodal cardiac myocytes with regard to gene expression and electrophysiological properties.

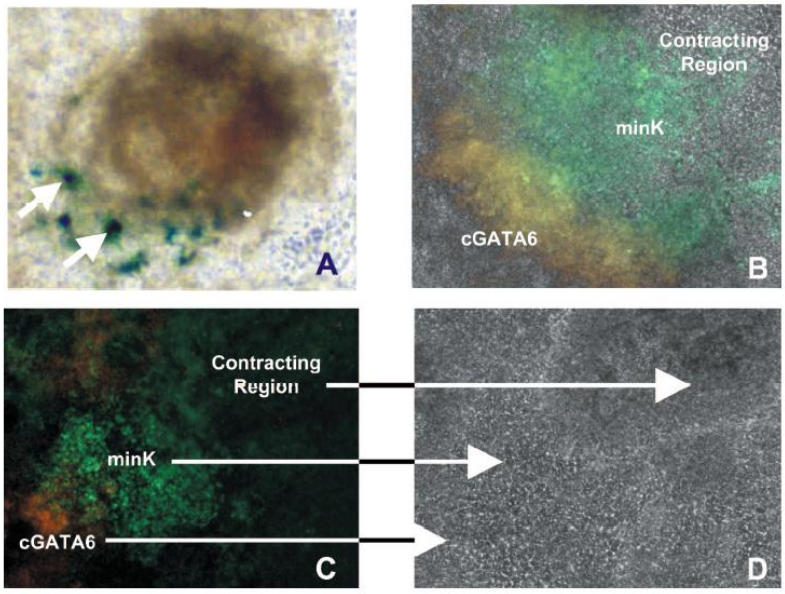

Fig. 1. Coexpression of chicken GATA6 (cGATA6) and *minK* depicts a cardiac conduction system in embryoid bodies (EBs). *A*: staining for β-galactosidase expression reveals distinct clusters of *lacZ*-positive cell clusters in a representative EB generated from embryonic stem (ES) cells expressing the *minK-lacZ* targeting vector. *B*: coexpression of cGATA6 (red) and *min*K (green) are shown overlaid on the corresponding phase-contrast image in a representative EB. *C*: another representative EB region showing a distinct cGATA6-positive “nodal” region (red) with bridging *minK* (green) expression is shown. *D*: phase-contrast image for *C* is shown, which corresponds to supplementary *movie 1* (http://ajpheart.physiology.org/cgi/content/full/00841.2004/DC1), demonstrating the location of the spontaneously contracting region.

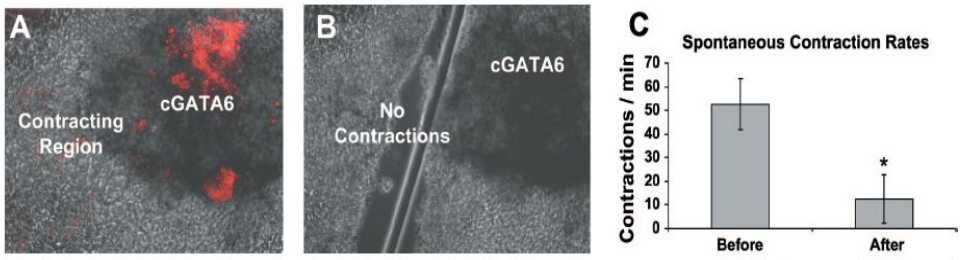

Fig. 3. Separating spontaneously contracting regions from cGATA6-positive cell clusters reduces spontaneous contraction frequency. *A*: representative region of an EB shows the spatial relationship between a cGATA6-positive cell cluster (red) and an adjacent spontaneously contracting region. *B*: an image of the same region (*A*) following physical separation of the cGATA6-positive cluster and the spontaneously contracting region, which resulted in the cessation of contractions. *C*: separating the cGATA6-positive cells from adjacent spontaneously contracting regions caused either a marked reduction or cessation of spontaneous contractions ($*P < 0.05$, $n = 11$).

In 2004
One cardiac troponin I (cTnI) gene promoter, displays AVCS specificity in a subset of the transgenic lines that harbor a particular cTnI/lacZ construct (Di Lisi et al., 2000). However, in other cases, this construct is expressed more broadly within the heart, in some cases recapitulating the pan-cardiac expression of the endogenous cTnI gene (Di Lisi et al., 1998; Habets et al., 2002). The other control region, a GATA-6 gene enhancer, consistently drives robust AVCS expression

in transgenic mice (Davis et al., 2001; Edwards et al., 2003). GATA factors have similarly been shown to regulate the cTnI promoter (Di Lisi et al., 1998), although it remains to be determined if the latter transfection results pertain to the AVCS-specific, or more general heart-specific, activity of the cTnI promoter in transgenic mice.

In 2011
In September this year a lab reported further study about the GATA 6 reporter[5]. 00635.2011.—This study examined transgenic mice whose expression of a-galactosidase (lacZ) reporter is driven by a GATA6 gene enhancer. Morphometric analysis determined that 1% of myocytes, often found in small clusters, express this GATA6-associated reporter in the adult heart. the expression patterns of cardiac genes, such as Nkx2.5 and GATA6, which are uniformly exhibited throughout the developing myocardium, were actually composites of multiple distinct transcriptional programs.

enhancer sequences in the GATA6 locus that drive that gene's expression in conduction tissue differ from those responsible for expression within working myocardium.

The transgenic tgG6/lacZ mice used in this study were generated by genomic insertion of a lacZ transgene, whose expression is driven by a 1,300-bp enhancer-containing region from the GATA6 gene (15, 29). This genetic region contains consensus binding sites for several heart-associated factors, including Nkx2.5, MEF2C, SRF, GATA-4/5/6, HAND1, and Hand2[6]. Optical clearing of the tissue disclosed that lacZ-positive cells were distributed throughout the entire myocardial wall.

In summary, these data show that the tgG6/lacZ reporter gene identifies a subpopulation of cardiomyocytes with a variant phenotype that correlates to decreased gap junctional coupling with the neighboring myocardium. The only differences we discerned were a correlation between the expression of the reporter molecule driven by a GATA6 gene enhancer and deficient expression of Cx43, which is the major gap junctional protein of working myocytes. In this regard, the combination of our injury and heptanol results may suggest a pathway whereby Cx43 disruption (due to injury or disease) or inhibition (heptanol) leads to downstream activation of a particular GATA6 enhancer.

Conclusion: for GATA6 enhancer, it is a good marker for conduction system in the development of embryonic cells; in the mature heart, it is amazing that it marks those who decrease the Cx43 connections, which implies that it could not be marked by conduction system.

In 2007 The comparison of 4 markers used in the study of conduction system

For deeper study, researchers studies more transgenetic markers in adult mice in 2007[7].
The specialized cardiac conduction system (CCS) consists of the sinoatrial node (SAN) and the atrioventricular (AV) conduction system (AVCS), which includes proximal (AV node, bundle of His and bundle branches) and distal (Purkinje fibers) components. The SAN is the impulse generating tissue or primary pacemaker of the heart.(Fig 4)
Four CCS marker mice [two transgenic (cGATA6|lacZ, CCS|lacZ) and two targeted gene knock-in (minK|lacZ, Hop|lacZ)] the expression of the lacZ gene (β -gal) has been used to compare. The conclusion is that none of the four CCS marker mice we studied specifically mark the SAN.

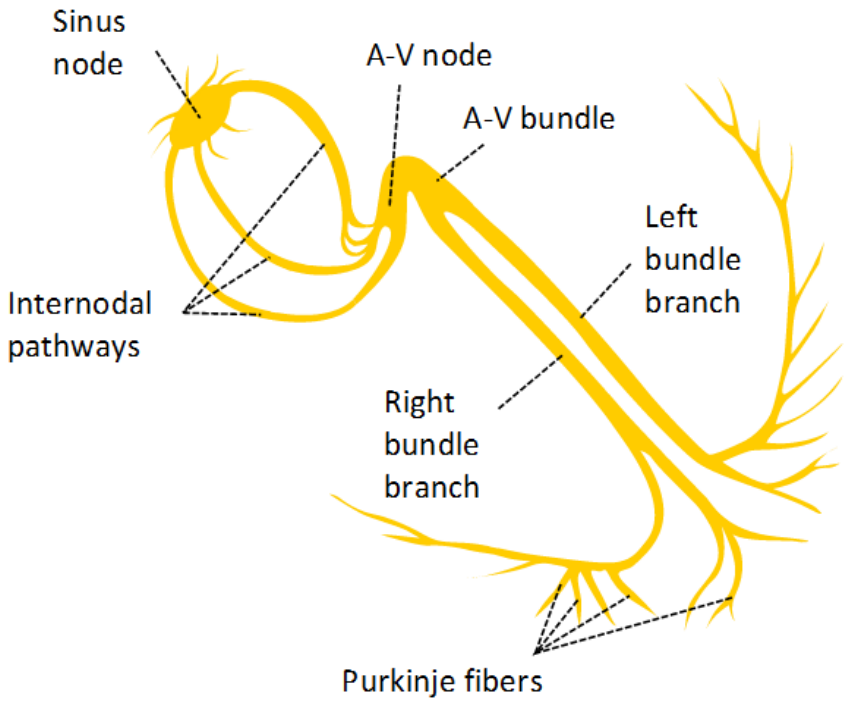

Figure 5a. The conduction system

However, this paper provides additional information.
For cGATA6, LacZ gene was strongly expressed over the entire intercaval region;
For CCS, X-gal staining of the hearts of these mice showed consistent proximal and distal AVCS and patchy and variable expression of the reporter within the genotype in theintercaval region, left and right atria including the appendages;
For mink, X-gal staining was consistent, strong and specific in the AVN, bundle of His and Purkinje, indicating expression in the proximal and distal AVCS. [8]
For Hop, it is generally expressed in all the heart. (Figure 5b)

β-galactosidase expression in various tissues, among the four CCS-marker mice

Mouse Genotype	SAN	Peri-SAN Atria	Atria	AVN
cGATA6|LacZ	−	+	++	+
CCS|LacZ	−	+	++	++
minK|LacZ	−	−	−	++
HOP|LacZ	+	+	++	++

Figure 5b

Conclusion: In adult mouse, none of these four marker could be expressed in SAN, which functions primarily in conduction system. However, mink could be specific in conduction system other than SAN.

The marker called minK for conduction system

In 1999, an early research found the mink gene's specific expression in conduction system. [9] The minK gene encodes a 129-amino acid peptide the expression of which modulates function of cardiac delayed rectifier currents (IKr and IKs). They generated mink-deficient mice in which LacZ gene take place of the mink coding region. Figure 4 shows the specific expression in the mice conduction system.

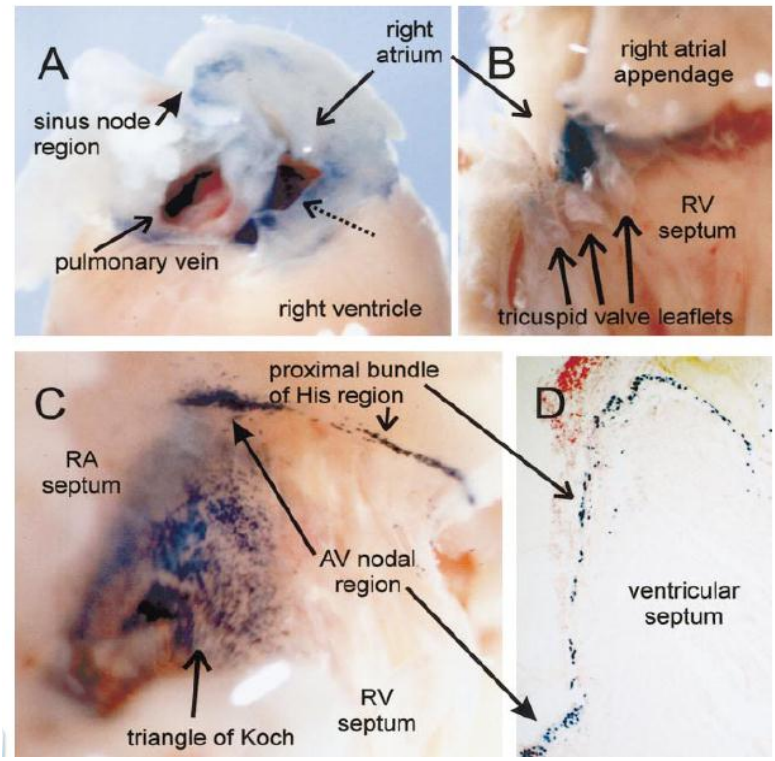

Figure 4. β-Galactosidase staining in adult *minK* (–/–) mice. A, Posterior view of the right atrium, showing intense staining in the region of the sinus node. The dashed arrow shows an area of intense staining visible on the atrial septum. No staining is visible on the ventricular epicardium. B, View of the right ventricular septum after removal of the atrial and ventricular free walls (in a different heart from that shown in panel A). As in panel A, there is an intense area of staining on the caudal right atrial septum, just beyond the tricuspid valve leaflets. C, Close-up of the area of intense staining shown in panels A and B. The staining is confined to the triangle of Koch and to the region of the AV node and proximal bundle of His. D, Microscopic section of the staining shown in panel C. The pattern of staining is virtually identical to that reported with Cx40 immunostaining.[31] E, Higher-power view of panel D showing nuclear β-galactosidase staining in 3 endocardial cells (magnification, ×40 oil; zoom 1.7). F, Cx40 immunostaining of the same section

In 2011

A new study ensure the specificity of mink gene. The study uses inducible Cre recombination, a powerful technology that allows for spatial and temporal modulation of gene expression in vivo. To enable inducible recombination in the murine CCS, the created a minK:CreERT2 bacterial artificial chromosome (BAC) transgenic mouse line. Cre activity is present after tamoxifen administration in the atrioventricular (AV) node, AV bundle, and bundle branches of adult transgenic mice. [10](Figure 2)

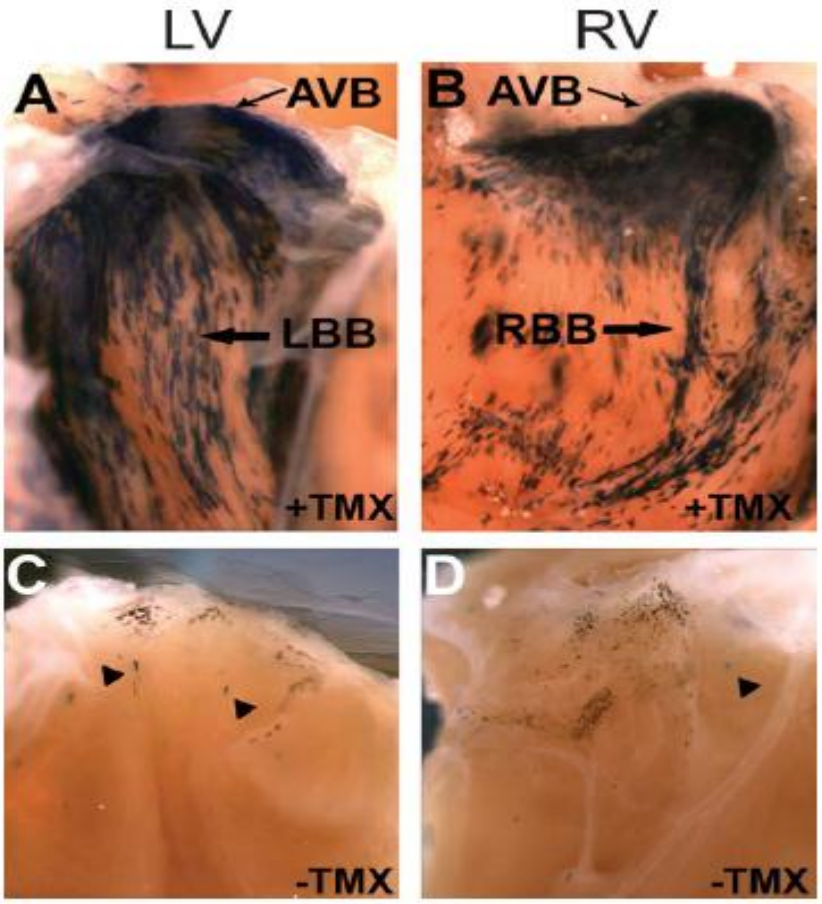

FIG. 2. Tamoxifen-inducible cre activity in the AV bundle and bundle branches of minK:CreER^{T2};ROSA26R double transgenic mice. Hearts from mice receiving (**a**-**b**) or not receiving (**c**-**d**) tamoxifen (TMX) were dissected to reveal the left (LV; a, c) and right (RV; b, d) ventricular septal surfaces and subsequently were stained with X-Gal to detect β-galactosidase activity (blue). β-galactosidase activity was detected in the AV bundle (a, b) and bundle branches (a,b) of mice that received tamoxifen, whereas only scattered blue cells (arrowheads) were found in the absence of tamoxifen (c,d). AVB: AV bundle; LBB: left bundle branch; RBB: right bundle branch.

This paper introduced another two markers that could be used in SAN: HCN4:CreERT2 (Hoesl et al., 2008) and Cx40:CreERT2-IRESmRFP (Beyer et al., 2010). Tamoxifen-inducible Cre activity in HCN4:CreERT2 mice has been reported in the SA node and AV node, but not the AV bundle or bundle branches (Hoesl et al., 2008). Within the CCS, tamoxifen-inducible Cre activity in Cx40:CreERT2 mice has been reported in the AV bundle, bundle branches, and Purkinje fibers, but not the AV node (Beyer et al., 2010).

Conclusion: mink gene is a good marker in the atrioventricular (AV) node, AV bundle, and bundle branches of adult transgenic mice.

The marker called HCN4 for conduction system

In 2008

The group uses temporally controlled gene deletion tech to exam gene function in vivo.[11] The tamoxifen-inducible CreERT2 construct was 'knocked in' into the pacemaker channel HCN4 locus. After injection of tamoxifen, highly selective and efficient recombination was observed in the sinoatrial and atrioventricular node. Expression of Cre and tamoxifen per se did not affect cardiac rhythm, basal heart rate and heart rate modulation. By crossing these animals with floxed HCN4 mice, complete deletion of this gene in the sinoatrial node could be achieved (Figure 2, Figure 3).

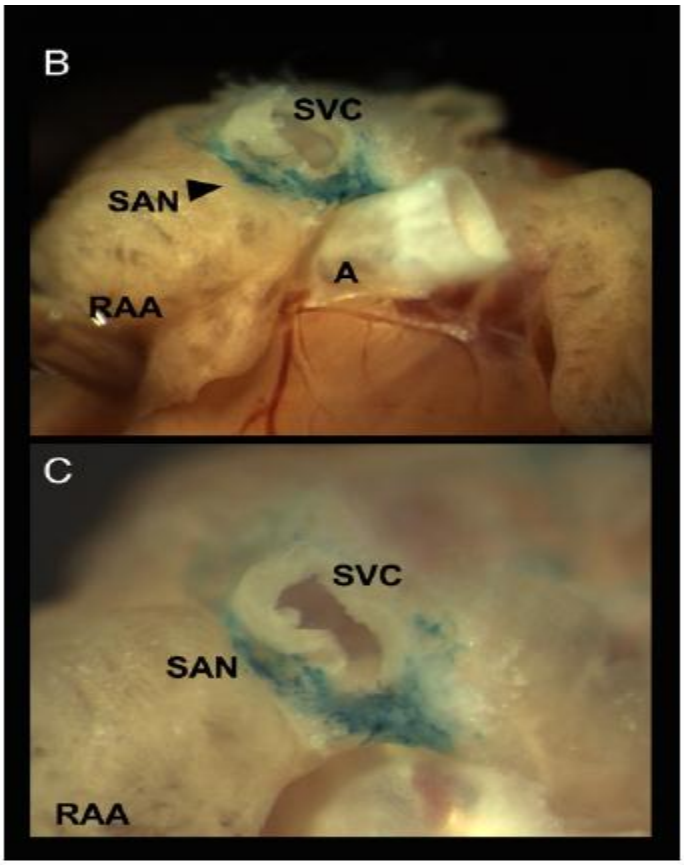

Fig. 2. CreER^{T2} activity examined by whole-mount X-Gal staining from tamoxifen-injected HCN4-KiT, ACZL tg/0 mice. (A) Midsagittal brain slice. (B) X-Gal staining (arrowhead) is found in the sinoatrial region close to the vena cava superior in a ventral top view of the heart. (C) Higher magnification shows staining around the superior vena cava in proximity to the right atrial appendage and the aorta. SVC, superior vena cava; A, aorta; RAA, right atrium appendage.

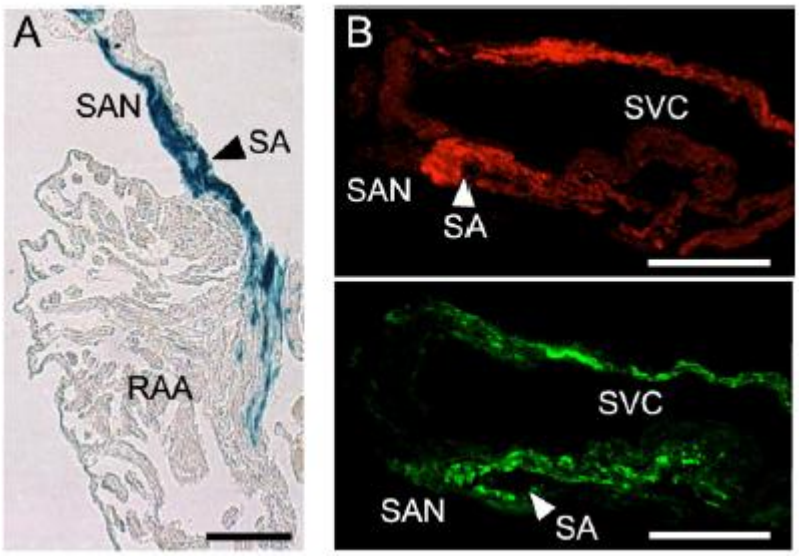

Figure 3. CreERT2 mediated recombination in the SAN and AVN. (A) X-Gal stained section from a tamoxifen-injected HCN4-KiT, ACZL tg/0 mouse shows strong recombination in the SAN. (B) Expression of HCN4 (red) and β-gal (green) in the SAN. Arrows highlight the SAN artery. SA, sinoatrial node artery; SV, sinus venosus.

In addition, two available heart-specific and inducible Cre lines MerCreMer and αMHC–CrePR1 do not lead to highly efficient recombination in the sinoatrial node (SAN).

Conclusion: the study successfully established inducible Cre-mediated recombination confined to cardiac pacemaker cells (SAN). The endogenous HCN4 promoter was used to direct expression of the recombinase to these cells.

Whether the HCN4 is specific in the conduction system?

"All four HCN isoforms have been expressed in heterologous cells and shown to form homomeric, hyperpolarization-activated, non-selective cation currents that are directly modulated by cAMP" " HCN4 is also the predominant HCN transcript in cardiac Purkinje fibers, specialized conducting tissue that can exhibit subsidiary pacemaker activity"[12]

1. Dorene L. Davis, A.V.E., Amy L. Juraszek, Aimee Phelps, and J.B.E.B. Andy Wessels, *A GATA-6 gene heart region-specific enhancer provides a novel means to mark and probe a discrete component of the mouse cardiac conduction system.* Mechanisms of Development, 2001. **108**: p. 105-119.
2. Steven M. White, W.C.C., *Embryonic stem cells form an organized, functional cardiac conduction system in vitro.* Am J Physiol Heart Circ Physiol, 2005. **288**: p. 670-679.
3. Adamo, R.F., et al., *GATA-6 gene enhancer contains nested regulatory modules for primary myocardium and the embedded nascent atrioventricular conduction system.* Anat Rec A Discov Mol Cell Evol Biol, 2004. **280**(2): p. 1062-71.

4. Edwards AV, Davis DL, Juraszek AL, Wessels A, and Burch JB. Transcriptional regulation in the mouse atrioventricular conduction system. Novartis Found Symp 250: 177–189; discussion 189–193, 276–179, 2003.
5. Mathieu C. Rémond, G.I., Michael P. O'Quinn, Nadejda V. Mezentseva, and B.S.H. Victor Garcia, Robert G. Gourdie, Carol A. Eisenberg, and Leonard M. Eisenberg, *GATA6 reporter gene reveals myocardial phenotypic heterogeneity that is related to variations in gap junction coupling.* Am J Physiol Heart Circ Physiol, 2011. **301**: p. 1952-1964.
6. Davis DL, Wessels A, Burch JBE. An Nkx-dependent enhancer regulates cGATA-6 gene expression during early stages of heart development. Dev Biol 217: 310–322, 2000.
7. S Viswanathan, J.B., GI Fishman, IP Moskowitz, and DW Benson, *Characterization of Sinoatrial Node in Four Conduction System Marker Mice.* J Mol Cell Cardiol, 2007. **42**(5): p. 946-953.
8. Kupershmidt S, Yang T, Anderson ME, Wessels A, Niswender KD, Magnuson MA, et al. Replacement by homologous recombination of the minK gene with lacZ reveals restriction of minK expression to the mouse cardiac conduction system. Circ Res Feb 5;1999 84(2):146–52.
9. Sabina Kupershmidt, T.Y., Mark E. Anderson, Andy Wessels, Kevin D.Niswender, Mark A. Magnuson and Dan M. Roden, *Replacement by Homologous Recombination of the minK Gene With lacZ Reveals Restriction of minK Expression to the Mouse Cardiac Conduction System.* Circulation Research, 1999. **84**: p. 146-152.
10. Arnolds, D.E. and I.P. Moskowitz, *Inducible recombination in the cardiac conduction system of minK: CreERT(2) BAC transgenic mice.* Genesis, 2011. **49**(11): p. 878-84.
11. Hoesl, E., et al., *Tamoxifen-inducible gene deletion in the cardiac conduction system.* J Mol Cell Cardiol, 2008. **45**(1): p. 62-9.
12. Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 2003;65: 453–80.