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Toward an Understanding of the Genetics of Murine Cardiac Pacemaking and Conduction System Development

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ABSTRACT

We distinguish the cardiac pacemaking and conduction system (CPCS) from neighboring working cardiomyocytes by its function to generate and deliver electrical impulses within the heart. Yet the CPCS is a series of integrated but distinct components. The components must act in a coordinated fashion, but they are also functionally, molecularly, and electrophysiologically unique. Understanding the differentiation and function of this elegant and complex system is an exciting challenge. Knowledge of genes and signaling pathways that direct CPCS development is at present minimal, but the use of transgenic mice represents an enormous opportunity for elucidating the unknown. Transgenic marker lines have enabled us to image and manipulate the CPCS in new ways. These tools are now being used to examine the CPCS in mutants where its formation and function is altered, generating new information and directions for study of the genetics of CPCS development.

Key words: cardiac conduction; transgenic; genetic; development

Multiple genetic and signaling pathways must operate to regulate cardiac pacemaking and conduction system formation and function. Impulses originate from the sinoatrial (SA) node and are delivered to the atria and atrioventricular (AV) node. Following delay at the AV node, the impulse travels through the His bundle and is transmitted rapidly to each ventricle through the bundle branches and Purkinje fiber network. Just as the components functionally differ from the working cardiomyocytes, so they also differ from each other, rendering our understanding of how the system develops particularly complex (Moorman et al., 1998). In the mouse, the use of transgenic animals is being employed to explore this challenging biological problem.

TRANSGENIC TOOLS

Molecular markers of the murine conduction system are limited in number and relatively not specific for the CPCS at early stages of heart development (Myers and Fishman, 2003). In the last few years, however, the use of transgene reporters such as *lacZ* and green fluorescent protein (GFP) has allowed for enhanced imaging of the conduction system in the embryo and adult. As expected due to the heterogeneity of the CPCS, some markers are expressed only in a subset of cells. The cardiac-specific chicken GATA6 (cGATA6) enhancer has been used to drive *lacZ* expression in the atrioventricular canal during early embryogenesis and later in the AV junction, node, His bundle, and bundle branches (Davis et al., 2001). Transgene expression in the *Troponin I-lacZ* line is similar to the cGATA6 enhancer but even more restricted to the AV canal and node (Di Lisi et al., 2000). These promoter elements are powerful tools not only as CPCS markers but also to drive transcription of *Cre* recombinase, thus allowing for exquisitely specific elimination of gene function.

In the *minK-lacZ* line, β-galactosidase activity has been documented in the SA node, AV junction, and bundle branches and colocalizes with conduction system marker connexin40 (Cx40) in the subendocardium of the interventricular septum (Kupershmidt et al., 1999). *minK-lacZ* expression is similar to the line described by our laboratory, cardiac conduction system (CCS)-*lacZ* (Myers and Fishman, 2003). In this line, a random insertion of *Engrailed* promoter elements driving reporter gene expres-

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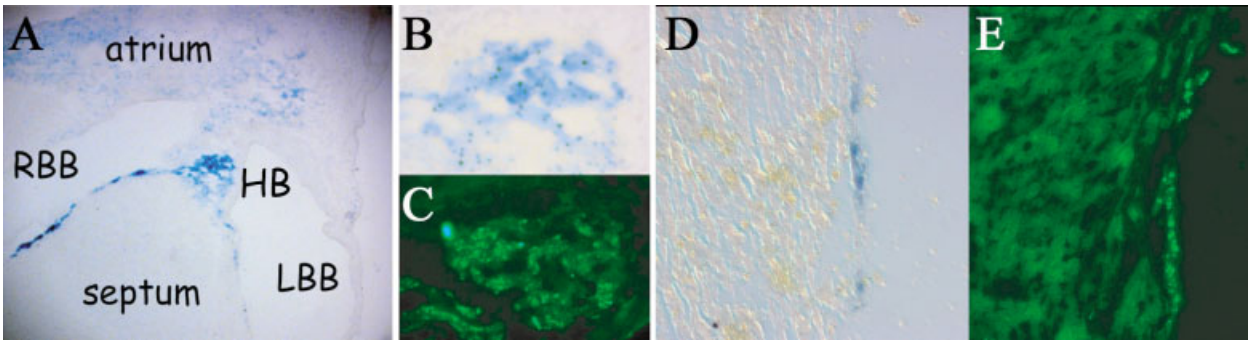

Fig. 1. **A:** *LacZ* expression in the His bundle (HB) and bundle branches (RBB, LBB) of the adult CCS-*lacZ* heart. Higher-power view of serial sections showing colocalization of *lacZ* (blue) and Cx40 (green) in the His bundle (**B** and **C**) and left bundle branch (**D** and **E**).

sion led to prominent CPCS labeling throughout heart development and in the adult (Rentschler et al., 2001). The unique aspect of the CCS-*lacZ* staining pattern is the visibility of the Purkinje fiber network during embryogenesis, not observed with any other markers. Full details of the developmental expression patterns in this line have been given elsewhere (Rentschler et al., 2001; Myers and Fishman, 2003). The adult expression pattern, however, has not been described and a comparison to ventricular conduction system labeling by GFP expressed under control of the *Cx40* gene is quite informative (Miquerol et al., 2004).

Late in embryogenesis and in adult mouse hearts, connexin40 expression as revealed by immunohistochemistry specifically labels the central AV node and ventricular conduction system tissues (Delorme et al., 1995; Coppen et al., 2003). Using serial sections of adult CCS-*lacZ* hearts, we colocalized Cx40 immunostaining with transgene expression in the node, His bundle, bundle branches, and subendocardial Purkinje fibers along the interventricular septum (Fig. 1). The expression pattern detailed by Cx40 immunostaining has been recapitulated in the *Cx40*-GFP line (Miquerol et al., 2004). In the Cx40-GFP hearts, the complete Purkinje fiber network is easily visible (Miquerol et al., 2004). Whole mount X-gal staining of adult CCS-*lacZ* hearts also shows transgene expression in the extensive fiber network as described for the *Cx40*-GFP (Fig. 2). The imaging of the CPCS by these transgenic lines is now being put to excellent use in analyzing mutants with conduction system defects. These tools hold considerable potential for unraveling the genes and signaling pathways that direct CPCS formation and function.

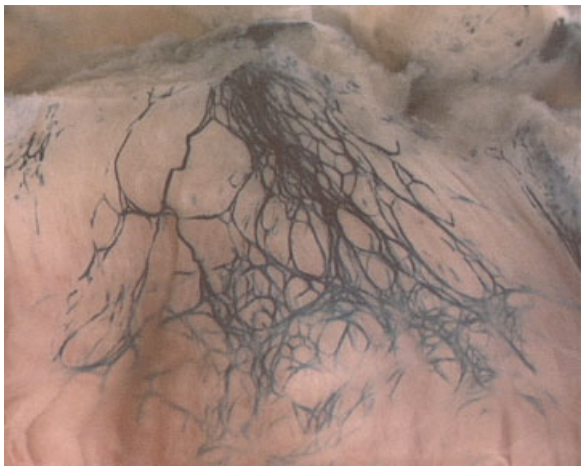

Fig. 2. Exposed septal surface of the left ventricle in an adult CCS-*lacZ* heart, whole mount-stained for *lacZ*. As described in the Cx40-GFP line, the left bundle forms a sheet and the dense Purkinje network covers the septal surface and the free walls.

CCS INDUCTION

We have employed the CCS-*lacZ* line to investigate factors that may influence CPCS development. In the avian heart, extensive work has revealed the role for endothelin in directing the formation of periarterial Purkinje fibers (Gourdie et al., 1998; Takebayashi-Suzuki et al., 2000; Hall et al., 2004). Therefore, we tested the ability of endothelin (ET) as well as other candidate factors, such as neuregulin (NRG), angiotensin II, and insulin-like growth factor I to influence the pattern of CPCS expression in cultured embryonic hearts (Rentschler et al., 2002). Only neuregulin resulted in a significant upregulation of *lacZ* expression, as well as a corresponding change in the functional activation sequence, as revealed by optical mapping (Rentschler et al., 2002).

This intriguing finding has led to many questions as to the in vivo action of neuregulin and endothelin in murine ventricular conduction system development. Unfortunately, answers to these questions remain elusive. The usefulness of NRG mutants is limited as these mice die very early in embryogenesis with extensive heart defects (Lee et al., 1995; Meyer and Birchmeier, 1995; Garratt et al., 2003). Conditional deletion of the NRG receptor ErbB2 leads to dilated cardiomyopathy but did not appear to affect CPCS function (Ozcelik et al., 2002). As there is redundancy in the ErbB receptors, this does not conclusively rule out involvement of NRG in ventricular conduction system development. Redundancy is also a problem in examining the function of endothelin signaling. Single ligand knockouts result in cardiac and craniofacial abnormalities, suggesting a role for endothelin in the neural crest (Baynash et al., 1994; Kurihara et al., 1994, 1995). Attempts to eliminate signaling by disrupting the endothelin-converting enzymes needed to generate active endothelin still did not completely block production of ma-

ture ET (Yanagisawa et al., 2000). As cardiac-specific deletion of the ET receptor endothelin A did not have an appreciable effect on ligand binding, the question of endothelin's action on the CPCS will await the generation of a double mutant line perturbing both receptor A and B expression in the heart (Kedzierski et al., 2003). Although ET showed little if any alteration of the *lacZ* pattern in our cultured embryonic hearts, its effect was not assayed by optical mapping. It is possible that functional changes arose from exposure to endothelin that were not identified by transgene expression. Clearly, further investigation is required to elucidate the issue of ET, NRG, and the murine conduction system.

FORWARD TO GENETICS

The revelation of conduction system hypoplasia in *Nkx2.5* mutant mice has generated increased interest in conduction system development (Jay et al., 2004). We are entering an exciting time when knowledge of the genes that direct specification and differentiation of the various cardiac pacemaking and conduction system components will be forthcoming. While the *Nkx2.5* mutants and CCS-*lacZ* mice suggest that the entire conduction system does share some level of transcriptional control, it is only logical to assume that the functional differences in the central (nodes, His bundle, proximal bundle branches) and the peripheral (distal bundle branches and Purkinje network) conduction system components should be reflected in their genetic differentiation pathways. Indeed, recent work demonstrates the expression pattern of the transcriptional regulator *Tbx3* is limited to the central conduction system as well as the internodal regions and the atrioventricular junction (Hoogaars et al., 2004). *Tbx3* appears to allow formation of the central conduction system by repressing transcription of chamber-specific genes (Hoogaars et al., 2004). One of these genes is *Cx40*, which until late in embryogenesis has broad chamber expression (Delorme et al., 1995). Therein lies the confusing and therefore particularly intriguing point, as Cx40 will come to be expressed in portions of the node and proximal bundle branches overlapping with *Tbx3* (Coppen et al., 2003; Miquerol et al., 2004). What other factors are at work to promote this critical transition? If anything, the *Tbx3* study highlights how much more there is to discover. While it provides clues regarding the formation of the central conduction system, genes that specifically influence Purkinje fiber formation remain unknown. To address these issues, genomic studies are needed to uncover more of the players in these processes.

CONCLUSION

The ability to manipulate the mouse genome makes it the optimal model system for dissecting the genetics of conduction system development. The combination of transgenic lines marking conduction system components and mutants with CPCS abnormalities and dysfunction is beginning to provide important insights into this intriguing biological issue, along with a plethora of new questions. The advances in imaging technology such as optical mapping and 3D reconstruction techniques are increasing the detail with which we can characterize CPCS defects. In dealing with the genetics of murine CPCS development, we are truly stepping in terra incognita. It is a slightly daunting, particularly exciting, and truly rewarding adventure.

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