The explosion of stem cell research in cardiology has yielded an increasing recognition that understanding developmental cell fate decisions is critical for everything from disease models to cellular therapeutics. However, the biology is particularly rich and complex and has not yielded easily to traditional investigative methods. One area of intense focus is the study of the cues responsible for the development of specialized conduction tissues in the heart. Direction of native cells or exogenous cells into the conduction system lineage might offer therapeutic insights into degenerative conduction disease.

The most distal subendocardial fibers of the cardiac conduction system were first described by Jan Purkinje in 1839. Over 100 years ago, in painstakingly delicate dissections, Sunao Tawara characterized the cardiac conduction system further, describing the atrioventricular node, the penetrating bundle of His, the bundle branches, and the Purkinje fiber network. During the past 20 years, however, the pace of discovery of this highly specialized tissue has accelerated. Beginning with the observations that peripheral and central conduction tissue derives from cardiomyocyte progenitors in the chick, investigators have now identified key roles for many important molecular signals and transcription factors in conduction system development. Signaling molecules implicated in the formation of the conduction system include endothelin, neuregulin, Wnts, and bone morphogenetic proteins, and complex transcriptional roles have been identified for NKx2.5, members the T-box transcription factor family, and hop.

In this issue of Circulation, Rentschler et al take a significant step toward understanding the determinants of conduction cell fate. In a series of elegant experiments, they demonstrate that activation of the Notch signaling pathway can directly develop cardiomyocytes to become specialized conduction cells. To do this, the investigators used a conditional transgenic mouse, one that turns on the Notch signal only in developing cardiomyocytes, to demonstrate the upregulation of transcript levels of many proteins typically expressed in specialized conduction tissue. In the atrioventricular node, which had been shown previously to become expanded in size after Notch activation, the increase in size was shown to be the result of increased developmental recruitment of cells into the conduction system, and not because the cells were simply dividing more rapidly.

The timing of these effects is particularly interesting. At birth, conduction system markers were not increased in the ventricles of the Notch-activated hearts. However, by 3 weeks of age, these markers were observed outside the regions where conduction tissues are normally found. These results suggest that ongoing Notch activation not only influenced the cell fate decisions during early development, but also appeared to do so even after birth.

These intriguing findings raise a number of questions that are central to cardiac cell and developmental biology. For example, are these cells truly conduction cells, or could they represent ventricular cardiomyocytes that have been altered experimentally to express conduction cell proteins ectopically? To address this question, the investigators characterized these Notch-activated cells functionally using patch clamp techniques. They demonstrate that the Notch-activated cells not only express markers of conduction cells, but they also exhibit action potential profiles with a characteristic plateau found uniquely in Purkinje cells. Of interest, the degree of phenotypic conversion was heterogeneous because there were also some cells that were caught in transition with only a partial plateau, and some that were complete nonresponders. These observations about the relative efficiency of the conversion process are important and raise interesting questions regarding the determinants of the response to the Notch signal.

Although the in vivo data on Notch activation in developing cardiomyocytes address predominantly the question of cardiac cell lineage decision, the question of cardiac cell phenotype conversion (ie, reprogramming) by Notch activation remained open. To examine this, the investigators went on to demonstrate further that Notch signaling in committed neonatal cardiomyocytes also leads to increased conduction tissue marker expression and prolonged action potentials, supporting the notion that Notch signaling may be able to reprogram working chamber cardiomyocytes toward a conduction cell fate. These results add to a growing body of evidence that Notch signaling is a key determinant for cardiac conduction system development, and that Notch activation can influence cell fate decisions of the developing cardiomyocytes and may potentially convert mature postnatal cardiomyocytes into conduction-like cells.

These findings are particularly relevant given the intense interest in direct cell lineage reprogramming in recent years.
Inspired by the groundbreaking work of Takahashi et al.\(^{31,32}\) to generate embryonic stem cell-like cells from the enforced expression of pluripotency-associated transcription factors, investigators have now shown the ability of lineage-specific transcription factors to induce neuronal, hematopoietic, pancreatic, and cardiomyogenic phenotypes in somatic cells.\(^{23-36}\) Although the primary intention of activating Notch in cardiomyocytes in this study was to examine the role of this developmentally critical signaling pathway on the proper formation of the cardiac conduction system, the finding of an induced conduction cell phenotype in an otherwise committed cardiomyocyte places the results of this study in an entirely different context. Namely, the triggering of a signaling pathway in committed cells may also lead to phenotypic reprogramming.

To take this one step further, one could envision the possibility that by manipulating signaling pathways alone, we may be able to generate pluripotent stem cells, cardiomyocytes, neurons, pancreatic β-cells, and hematopoietic stem cells without the need for viral or transfection-mediated overexpression of transcription factors. Indeed, the requirement for bone morphogenetic proteins and fibroblast growth factor 2 supplementation to maintain human pluripotent stem cells, and the enhancement of pluripotent phenotype in the presence of chemical inhibitors of transforming growth factor-β, mitogen activated protein kinase kinase, and oxygen synthase kinase 3-β all support the notion that signaling pathway modulation plays an important role in direct cell lineage reprogramming.\(^{35}\) In this regard, the dramatic difference between the efficiency of fibroblast-to-cardiomyocyte reprogramming in vitro (1%–5%) and in vivo (10%–30%) by the overexpression of Gata4, myocyte-specific enhancer factor 2C, and T-box 5 may be the result of the presence of critical growth factors that are present only in vivo.\(^{36,38}\)

However, to examine the findings from Rentschler et al.\(^{29}\) in a reprogramming context, a number of key issues need to be addressed. For example, are the Notch-activated cells truly reprogrammed epigenetically or do they manifest a conduction system phenotype only under the continuous stimulus of Notch signaling? A demonstration of the maintenance of conduction system gene expression and functional phenotype in the absence of continuous Notch signaling would be important. Furthermore, the presence of epigenetic silencing at sarcomeric gene loci and epigenetic activation on ion channel gene loci would be essential. Also, are the cardiomyocytes that are induced in this study truly mature or are they still immature and somewhat plastic for conduction cell development? It has been reported that the cardiac conduction system in mice has not finished developing at birth, based on elegant experiments that have demonstrated postnatal requirements for NKX2.5\(^{19}\) and T-box 5\(^{18}\) in conduction system maturation. Hence, it is possible that results from the current study support the role of Notch signaling on lineage commitment and not reprogramming per se. In addition, what are the barriers to conversion of cardiomyocytes to conduction cells given that only 2 out of 11 patched cells were found to exhibit the full conduction cell phenotype? Because multiple other signals are involved in conduction system development, it is likely that modulation of these other pathways will be necessary to improve the efficiency of the reprogramming process. Also, it would be important to investigate the ability of Notch signaling to induce a conduction cell phenotype in adult cardiomyocytes or even cardiac fibroblasts given the potential utility of this strategy to create replacement cells for the degenerative conduction system in the elderly.

The reprogramming biology of cardiovascular cells is in its infancy. Although the pioneering studies from Takahashi et al.\(^{31,32}\) have shown the power and possibilities of what can be achieved with transcription factor overexpression, how much these findings can be adapted to generate mature cardiomyocytes and conduction cells remains to be seen. We envision a day in the future when electrophysiologists will discuss with their patients the reprogramming of their cardiomyocytes instead of their permanent pacemaker. The work of Rentschler et al.\(^{29}\) reported here provides a new dimension to this arena by showing that modulation of signaling pathways can lead to direct phenotype conversion of cardiomyocytes toward conduction cells.

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Disclosures
None.

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