Published in final edited form as:

Nat Biotechnol. 2013 January ; 31(1): 31–32. doi:10.1038/nbt.2480.

Reprogramming paces the heart

Edward G. Lakatta and

The Laboratory of Cardiovascular Science, NIA (IRP), NIH, Baltimore, Maryland, USA

Victor Maltsev

The Laboratory of Cardiovascular Science, NIA (IRP), NIH, Baltimore, Maryland, USA

Edward G. Lakatta: LakattaE@grc.nia.nih.gov; Victor Maltsev: maltsevvi@mail.nih.gov

Abstract

Rodent cardiomyocytes are converted into pacemaker cells by viral delivery of a single transcription-factor gene.

The heart beat is initiated by electrical impulses from specialized pacemaker cells in the sinoatrial node. If the pacemaker fails, the heart rate becomes abnormally low or irregular, leading to possible syncope and life-threatening circulatory collapse. In this issue, Kapoor *et al.*¹ demonstrate a strategy for restoring pacemaker activity by reprogramming working cardiomyocytes. They show that viral delivery of a gene important in pacemaker development, *Tbx18*, converts rodent cardiomyocytes into functional pacemaker cells within the heart, which the authors call induced sinoatrial node (iSAN) cells. This new biotechnology has a potential to revolutionize treatment in millions of people suffering pacemaker failure.

Currently, pacemaker dysfunction is treatable only by implantation of an artificial pacemaker—a small electronic-pulse generator with leads attached to heart. These devices are often associated with complications and limitations. Each year in the US, ~200,000 artificial pacemakers are implanted and ~175,000 require replacement, removal or repair. In pediatric patients, pacemakers have to be adjusted in additional operations as the heart and body grow. To overcome the shortcomings of pacemaker therapy, in the past decade researchers have investigated the possibility of creating ‘biological pacemakers’ by deriving pacemaker cells from non-pacemaker cells through genetic engineering². So far, the success of these approaches has been limited. To understand why, and to appreciate the future prospects of this emerging biotechnology, we need to look more closely at how the heartbeat is initiated.

At first glance, pacemaker activity appears to be wholly electrical in nature. During diastole—the period between heartbeats—the plasma membrane of pacemaker cells undergoes a slow spontaneous depolarization. When this depolarization reaches a critical level (excitation threshold), an all-or-none electric impulse (action potential) is generated within and between pacemaker cells. The action potential propagates across the atrium and enters conduction pathways that lead to the ventricle, where it stimulates the contraction of ventricular myocytes that expulses blood from the heart. Understanding of this pathway led the field of cardiac-pacemaker research to believe until recently that pacemaker activity is

fully controlled by electrogenic molecules, namely ion channels. This idea was bolstered by the work of Hodgkin and Huxley (awarded the Nobel Prize in Physiology and Medicine in 1963) on membrane ion currents that generate nerve impulses, and it inspired many extensive studies aimed at discovering the specific ion currents that drive spontaneous depolarization of the pacemaker-cell membrane.

This research showed that pacemaker cells lack the inward rectifier potassium current I_{K1} that keeps the membrane of ventricular myocytes strongly polarized to prevent spontaneous depolarization. Pacemaker cells were found to exhibit a non-selective ‘funny’ current, I_f , which is activated when the membrane repolarizes and diastolic depolarization begins. I_f seemed to be the long-sought ion current that controls diastolic depolarization and was often referred as ‘the pacemaker current’. Early efforts to create biological pacemakers were focused on increasing I_f (ref. 3) or suppressing I_{K1} (ref. 4). Surprisingly, this straightforward approach was insufficient to transform non-pacemaker cardiac cells into robust, responsive pacemaker cells.

More recent studies have revealed a greater complexity in cardiac pacemaker function. As shown in Figure 1, a ‘symphony’ of locally distributed molecules contributes to electrical and chemical oscillations in pacemaker cells⁵. Specifically, the sarcoplasmic reticulum—a network of intracellular Ca^{2+} stores—spontaneously generates rhythmic local Ca^{2+} oscillations, a phenomenon sometimes referred to as a ‘calcium clock’. The ensemble of these rhythmic local Ca^{2+} releases beneath the plasma membrane accelerates the rate of diastolic depolarization indirectly, but substantially, by activating an inward current of the Na^+/Ca^{2+} exchanger (I_{NCX}). The membrane depolarization reaches a threshold of activation of voltage sensitive Ca^{2+} channels. They open and generate Ca^{2+} current that generates the action potential upstroke. Then potassium currents become activated during the action potential and they timely repolarize the membrane. Although the resultant action potential is an important output of the pacemaker cell function, it simultaneously ‘feeds’ (via Ca^{2+} current) the intracellular local Ca^{2+} oscillators with Ca^{2+} , their oscillatory substrate, and resets their periods to prepare for the next duty cycle. The funny current acts in concert with I_{NCX} to drive diastolic depolarization. Thus, like all biological systems, the pacemaker system exhibits functional redundancy to guarantee robust operation.

Both electrical and Ca^{2+} cycling events are regulated by common chemical signaling pathways. These include Ca^{2+} activation of calmodulin-dependent kinase II and adenylyl cyclases to generate cAMP, which activates protein kinase-A. The resulting enhanced protein phosphorylation coordinates functions (e.g., activation kinetics) of intracellular and cell-surface proteins, resulting in crosstalk that is required for robust generation of rhythmic action potentials. Thus, Ca^{2+} and these regulatory molecules are critical nodes within the system that link to redundant pacemaker mechanisms to insure fail-safe pacemaker operation. Importantly, these nodes remain active to keep the heart rate near the middle of its full range even in the absence of autonomic receptor stimulation. In response to signals that call for a change in heart rate, the system’s nodes are adjusted by neurotransmitter activation of β -adrenergic and cholinergic receptors to effect functional changes in the different molecular components, shifting the tempo of the pacemaker-cell symphony.

In addition to adding or removing ion channels, efforts to create bio-pacemakers have also manipulated cells at a systems level, for example, by overexpressing β -adrenergic receptors⁶ (which receive and transfer neurotransmitter signals) or adenylyl cyclases (which generate cAMP). Most recently, overexpression of a Ca^{2+} -activated adenylyl cyclase was shown to be sufficient, in the absence of funny-current activation, to produce biological pacemaking for seven days in experimental dogs⁷.

It is likely that the most successful approach to creating biological pacemakers will recapitulate embryonic development, borrowing the transcription factors used by nature, such as members of Tbx family. A recent study showed that Tbx3 reprograms cardiomyocytes into pacemaker-like cells *in vitro*⁸. In the present report, Kapoor *et al.*¹ used rodents to test five transcription factors known to be expressed in developing cardiac and pacemaker cells and found that Tbx18 was most effective. Transduction of bicistronic adenoviral vectors expressing Tbx18 generated persistent biological pacemakers (for up to 8 weeks) not only in cell culture but, importantly, in intact myocardium (in a small area of the ventricle near the injection site).

iSAN cells exhibited the morphology and the major functional properties of sinoatrial node cells. These include downregulation of I_{K1} channels, expression of funny channels and increased levels of cAMP to activate them and cAMP-dependent PKA-mediated phosphorylation of phospholamban, which accelerates Ca^{2+} cycling by sarcoplasmic reticulum. Several other features of the pacemaker molecular symphony (Fig. 1) were documented, including generation of rhythmic diastolic local Ca^{2+} releases that cause rhythmic spontaneous diastolic depolarizations leading to rhythmic action potentials. The cells also responded to various stimulations that mimic neurotransmitter signals with an appropriate increase or decrease of action potential firing rate, and their rate and rhythm depended on protein phosphorylation.

Future investigations of this approach could explore several questions left unanswered by Kapoor *et al.*¹. Many experiments were performed in neonatal rodent myocytes (*in vitro*) and in rodents (*in vivo*), and these should be extended to adult cells and larger mammals. Long-term evaluation beyond 8 weeks is important because persistent higher cAMP levels, which would affect expression of numerous genes regulated by cAMP-dependent phosphorylation, may have undesirable effects, such as promoting cell death or some types of neoplasm⁹. In fact, cAMP levels in SA node cells are higher than in ventricular myocytes. The higher cAMP level are maintained in about their middle range of possible changes by a relatively higher rate of turnover by adenylyl cyclases and phosphodiesterases. This allows effective regulatory changes in cAMP signaling in response to changes in Ca^{2+} levels. While cAMP levels are naturally higher in SA node cells, these cells have some protecting mechanisms against cell death. Thus, the future studies will clarify whether the reprogrammed cells also have a similar protection.

It would be instructive to learn more about the mechanisms involved in generating high cAMP levels and about the nature of the electrical coupling between iSAN cells and the host heart. Finally, overexpression or co-expression of transcription factors other than Tbx18 and

Tbx3, such as Tbx5 and Shox2 and factors involved in earlier stages of cardiac cell catecholamine synthesis¹⁰, may also prove useful.

References

1. Kapoor N, Liang W, Marbán E, Cho HC. Transcription factor-driven conversion of quiescent cardiomyocytes to pacemaker cells. *Nature Biotechnology*. 2012 (in print).
2. Rosen MR, Robinson RB, Brink PR, Cohen IS. The road to biological pacing. *Nature reviews*. 2011; 8:656–666.
3. Qu J, et al. Expression and function of a biological pacemaker in canine heart. *Circulation*. 2003; 107:1106–1109. [PubMed: 12615786]
4. Miake J, Marban E, Nuss HB. Biological pacemaker created by gene transfer. *Nature*. 2002; 419:132–133. [PubMed: 12226654]
5. Lakatta EG, Maltsev VA, Vinogradova TM. A coupled SYSTEM of intracellular Ca^{2+} clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ Res*. 2010; 106:659–673. [PubMed: 20203315]
6. Edelberg JM, Aird WC, Rosenberg RD. Enhancement of murine cardiac chronotropy by the molecular transfer of the human beta2 adrenergic receptor cDNA. *J Clin Invest*. 1998; 101:337–343. [PubMed: 9435305]
7. Boink GJ, et al. Ca^{2+} -Stimulated Adenylyl Cyclase AC1 Generates Efficient Biological Pacing as Single Gene Therapy and in Combination With HCN2. *Circulation*. 2012; 126:528–536. [PubMed: 22753192]
8. Bakker ML, et al. T-box transcription factor TBX3 reprogrammes mature cardiac myocytes into pacemaker-like cells. *Cardiovascular research*. 2012; 94:439–449. [PubMed: 22419669]
9. Conkright MD, Montminy M. CREB: the unindicted cancer co-conspirator. *Trends in cell biology*. 2005; 15:457–459. [PubMed: 16084096]
10. Ebert SN, Rong Q, Boe S, Pfeifer K. Catecholamine-synthesizing cells in the embryonic mouse heart. *Annals of the New York Academy of Sciences*. 2008; 1148:317–324. [PubMed: 19120124]

Figure 1.

The coupled-clock pacemaker cell system. The same regulatory factors or nodes (red lettering) couple intracellular Ca^{2+} to surface-membrane proteins to generate rhythmic spontaneous action potentials at rest. β -adrenergic and cholinergic receptor activation change action potential firing rate via signaling through the same nodes. Musical notes at the ion channels and also same notes during action potential schematically illustrate the sequence of their activation underlying rhythmic and robust pacemaker function.