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Pacing the Heart with Genes: Recent Progress in Biological Pacing

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Abstract

The heartbeat originates within the sinoatrial node (SA node or SAN), a small highly specialized structure containing <10,000 genuine pacemaker cells. The ~5 billion working cardiomyocytes downstream of the SAN remain quiescent when it fails, leading to circulatory collapse and fueling a \$6B/year electronic pacemaker industry. The electronic pacemaker devices work quite well. But, device-related problems persist. These include lead failure/repositioning, finite battery life, and infection. For pediatric patients, the children outgrow the length of the leads, necessitating replacement with longer leads. These pitfalls have motivated creation of biological pacing, that are free from all hardware. Toward this goal, we and others have tested the concept of biological pacemakers. Combined with efforts to create clinically relevant, large animal models of biological pacing, the field is moving beyond a conceptual novelty toward a future with clinical reality.

Keywords

Bradycardia; Gene therapy; Biological pacemakers

Introduction

Electronic pacemaker devices can sustain heart rate or deliver shocks to terminate life-threatening tachycardias. However, the high cost of devices and complications such as pulmonary collapse, hemorrhage, bacterial infection, and lead/generator failure [1] represent limitations of the technology.

Biological pacing seeks to convert a focal, normally quiescent region of myocardium to one that beats on its own by creating a spontaneous, electrical activity in that focal region. Earlier approaches to creating biological pacing exploited the existing knowledge on the molecular correlates that give rise to the unique electrophysiology of the SA

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nodal pacemaker cells. Such effort demonstrated the astonishing simplicity with which automaticity could be elicited from ordinary cardiomyocytes. It has also exposed, however, limitations to this approach; the cardiomyocytes with nascent automaticity lacked many of the defining features of the native SA nodal pacemaker cells, which are important for impulse propagation as well as impulse generation.

A recent breakthrough presents a new paradigm that transcends the previous approaches, creating induced pacemaker cells with striking semblance to the native SA nodal counterparts. A parallel approach is to derive stand-alone, spontaneously beating cardiac tissues from pluripotent stem cells [2–4]. Beyond the natural formation of spontaneously beating embryoid bodies, however, the stem cell-based approach has gained rather incremental advances and is not discussed here. In the next sections, the gene-based approaches to creating biological pacing are discussed in detail, including the ongoing efforts to translate the basic findings to clinically relevant, large animal models.

Biological Pacing by Ion Channel Modulation

The first proof-of-concept study to trigger spontaneous action potentials (APs) in ventricular myocytes by modulating ionic currents was provided by Marbán and colleagues [5]. They posited that the cardiac myocytes in the chamber (ventricular and atrial) myocardium have the capacity to beat on their own but are suppressed from doing so. This is because the hyperpolarizing ion current, I_{K1} , conducted by Kir2 channels, establishes a stable and strong negative resting membrane potential, thereby functioning as an “electrical break” against spontaneous activity [6]. They sought to destabilize I_{K1} by adding a dominant-negative, mutant ion channel Kir2.1AAA. Dominant-negative Kir2 ion channels have been shown to indiscriminately co-assemble with all subfamilies of Kir2 channels (such as Kir2.1, 2.2, and 2.3) and downregulate I_{K1} selectively and effectively in ventricular cardiomyocytes [7, 8]. A complementary approach taken by us and others was to overexpress an ion channel, HCN4, known for its role for spontaneous phase 4 depolarization in the SA and AV nodal pacemaker cells [9]. HCN4-mediated ionic current, I_f , could also provide rate responsiveness to neurohumoral input owing to its gating dependence on cAMP [9]. The ion channel-based rationale led to multiple approaches to engineering biological pacemakers in both small and large animal models. The striking simplicity with which spontaneous APs could be generated is demonstrated by the self-oscillating APs created by exogenous expression of three sarcolemmal ionic currents in a non-excitable, kidney cell line [10]. These approaches may represent a fast method to generate automaticity since the heterologously expressed ion channels should reshape the AP profile instantly as the nascent proteins are delivered to the target cardiomyocytes’ sarcolemma. However, these approaches mimic only a single ionic profile of the native SA nodal cells and thus fall short of replicating the coupled mechanisms of intracellular Ca^{2+} cycling and sarcolemmal ionic flow, which give rise to the remarkable robustness of automaticity observed in native nodal pacemaker cells [11]. The readers are referred to the previous reviews for detailed description of the ion channel-based approaches [10].

Induced Pacemaker Cells by Transcription Factor-Mediated Reprogramming

Reconfiguring the ventricular myocytes' sarcolemmal ion channel repertoire elegantly demonstrates the minimal complement of ionic currents for ordinary cardiomyocytes to pace themselves. Still, the repurposed myocytes remain largely as chamber cardiomyocytes with the characteristically long AP duration [12]. And, the cell-to-cell electrical coupling in the ion channel-modified myocardium would continue to be mediated by Cx43, a predominant gap-junction protein in the chamber myocardium. In contrast, a low-conductance gap junction protein, Cx45, mediates the cell-to-cell electrical coupling in the SA node and is thought to be a major component for overcoming the source-to-sink mismatch in the nodal structure [13, 14]. The ion channel-based approach also lacks a design element for spontaneous intracellular Ca^{2+} oscillations that are one of the hallmarks of genuine SA nodal pacemaker cells [15].

To engineer faithful biological replicas of SAN cells as an alternative therapeutic strategy, we reasoned that a gene/genes critical for early SAN specification may be able to convert working cardiomyocytes to the nodal pacemaker cells. During cardiogenesis, cardiomyocytes become specialized to exhibit ventricular, atrial, or pacemaker properties [16]. Pacemaker cells are exceedingly rare, comprising <10,000 of the ~10 billion cells (including myocytes and non-myocytes) in the adult mammalian heart [17], and yet, the initiation of the heartbeat depends critically upon this diminutive subpopulation. Studies in mouse development suggest that embryonic SAN development is heavily dictated by transcriptional regulators such as *Shox2*, *Tbx3*, *Tbx5*, and *Tbx18* [18]. *Shox2* is a negative regulator of *Nkx2.5* in the sinus venosus, and *Shox2*-deficient mouse and zebrafish embryos display bradycardia [19, 20]. *Tbx3* is a potent regulator of SAN specialization, with developmental errors resulting from either deficiency or ectopic expression [21]. *Tbx5*, which shows an inverse correlation between its dosage and abnormal cardiac morphogenesis in Holt-Oram syndrome, is a positive regulator of *Shox2* and *Tbx3* [22]. Upstream of all these factors is *Tbx18*; mesenchymal progenitor cells expressing *Tbx18* define the sinus venosus and differentiate *de novo* into SA nodal cells. *Tbx18* is required for embryonic development of the SAN head area [23] but becomes undetectable by birth and in adulthood [24••]. We hypothesized that ectopic expression of one or more transcription factors may convert quiescent ventricular myocytes to pacemaker cells.

Tbx18 Reexpression Increases Automaticity in Neonatal Cardiomyocytes

Freshly isolated neonatal rat ventricular myocytes (NRVMs) were transduced individually with selected transcription factors in bicistronic adenoviral vectors. *Tbx18*-transduced NRVMs (*Tbx18*-NRVMs) exhibited an increased percentage of spontaneously beating monolayer cultures compared to control and to the other transcription factors (*Shox2*, *Tbx3*, *Tbx5*, and *Tbx20*). Multiple, spontaneously beating foci could often be observed in individual *Tbx18*-NRVM monolayers. This is likely due to the significant downregulation of *Cx43* (but not *Cx45* or *Cx40*) by *Tbx18* [25•].

NRVMs, being immature cardiomyocytes, often beat spontaneously when cultured as monolayers. The syncytial contractions are driven by a small number of autonomously beating cardiomyocytes in the monolayer [25•]. This is exemplified if the NRVMs are

plated sparsely so that the NRVMs cannot electrically couple with each other. Then, a majority of the control NRVMs (transduced singly with GFP) do not beat, eliciting action potentials only upon exogenous electrical stimulation. In contrast, most Tbx18-NRVMs beat autonomously and spontaneously. The AP parameters of Tbx18-NRVMs replicated the features of SA nodal pacemaker cells: relatively depolarized maximum diastolic potential (MDP), accompanied by reduced I_{K1} density but elevated I_f . Intracellular Ca^{2+} cycling events complement and couple with sarcolemmal ionic currents to generate automaticity in native pacemaker cells [26]. The number of Tbx18-NRVMs exhibiting spontaneous intracellular Ca^{2+} oscillations was ~6-fold higher relative to control.

In addition to the sarcolemmal ion channel-mediated pathways to pacemaking, subsarcolemmal, spontaneous local Ca^{2+} release events (LCRs) have been shown to generate automaticity in SA nodal pacemaker cells [26]. Referred to as a “ Ca^{2+} clock,” the LCRs activate Na^+ - Ca^{2+} exchanger currents (I_{NCX}), which is a critical component of phase 4 depolarization during late diastole [26]. Tbx18-NRVMs displayed LCRs preceding each whole-cell Ca^{2+} transient, and those LCRs were wider and longer lasting compared to the spontaneous Ca^{2+} release events in GFP-NRVMs. From first principle, larger Ca^{2+} stores in the sarcoplasmic reticulum (SR) would favor automaticity [26]. The SR Ca^{2+} content, reported by measuring the amplitude of caffeine-induced Ca^{2+} transients, was twice larger in Tbx18-NRVMs compared to the control group. Thus, Tbx18 expression in NRVMs produced distinctive changes in Ca^{2+} cycling which recapitulate key features of SAN pacemaker cells.

Tbx18-Reprogrammed Pacemaker Cells Function as Biological Pacemakers In Vivo

To examine conversion of adult ventricular myocytes into pacemaker cells in vivo, Tbx18 adenoviral vector was directly and focally injected into the apex of guinea pig hearts. Two to four days post-injection, ectopic pacemaker activity was analyzed by electrocardiography. Since the guinea pigs have relatively high resting heart rate at about 280 bpm, the animals' native sinus rhythm was suppressed with methacholine [27, 28]. All control animals (injected with an adenoviral vector expressing only GFP) exhibited a slow junctional rhythm with antegrade polarity and narrow QRS duration ($n=5$). This is expected since slow, ectopic ventricular activity arises from the atrioventricular junctional area when the sinus rhythm is substantially suppressed. Under the same conditions, five of seven Tbx18-injected animals demonstrated an ectopic ventricular rhythm with wide QRS complexes and retrograde polarity (Fig. 1). Tbx18 had been injected at the apex of the guinea pig heart, which is in line with the retrograde polarity of the QRS complexes. Furthermore, the wide QRS is likely the consequence of cardiac conduction through general myocardium without the use of the secondary conduction system.

To examine if the direct intramyocardial injection of Tbx18 led to spontaneously beating cells in situ, single Tbx18-positive ventricular myocytes (Tbx18-VMs) were isolated 4–6 days after the somatic gene transfer of Tbx18 (or GFP) in vivo. Freshly isolated, native SAN cells served as side-by-side comparison. GFP-VMs exhibit stable resting potentials, with APs elicited only upon electrical stimulation. In contrast, Tbx18-VMs fire spontaneous, rhythmic APs with prominent diastolic depolarization like that in native SAN cells. The

cellular electrophysiological parameters (MDP, APD₉₀, $V_{\max}$, beating rate) of Tbx18-VMs closely resembled those of native SAN pacemaker cells (Fig. 2). In addition to automaticity, native SAN cells are smaller and thinner than ordinary ventricular myocytes. Remarkably, some of the Tbx18-VMs faithfully reproduced the distinctive tapering morphology of SAN cells; in contrast, non-transduced and GFP-VMs retained the brick-like appearance typical of ventricular myocytes (Fig. 3).

Multiple lines of evidence from electrocardiographic recordings of the Langendorff-perfused, ex vivo heart indicate the site of transgene injection as the origin of biological pacing. Upon complete heart block, Tbx18-injected hearts illustrated ectopic ventricular beats with a wide QRS complex at a rate faster than the control hearts that exhibited slow junctional escape rhythm with a narrow QRS complex (154 ± 6 vs. 120 ± 7 bpm, respectively, $n=8$). Furthermore, the polarity and morphology of the QRS complexes were similar to those of electrode-paced QRS complexes at the site of transgene injection. A perceived advantage of biological pacemakers is their expected responsiveness to autonomic regulation; β -adrenergic stimulation accelerated the heart rate while cholinergic suppression decelerated it (235 ± 19 and then to 150 ± 23 bpm, respectively, $n=7$) in Tbx18-injected hearts. Collectively, the data demonstrate that the Tbx18-induced, ventricular pacemaker activity responds to autonomic regulation in vitro and in the intact heart.

The Induced Pacemaker Phenotype Is Not Dependent Upon Continued Expression of Tbx18 Transcript

Permanently reprogrammed cells are thought to remain altered without sustained transcription factor expression [29, 30]. In an effort to gauge durability of the conversion to the SA nodal pacemaker cell phenotype, the ventricular cardiomyocytes transduced with Tbx18 in vivo were freshly isolated at multiple time points and up to 2 months after the initial in vivo gene delivery. Then, the transcript levels of Tbx18 were quantified by single-cell, real-time PCR. When isolated just 3 days after in vivo gene transfer, the level of Tbx18 transcripts exhibited a wide dynamic range of expression in Tbx18-VMs. At 2 months after in vivo Tbx18 delivery, a few ventricular myocytes, freshly isolated from the injection site, remained weakly GFP-positive, and some of those GFP-positive myocytes beat spontaneously. The levels of Tbx18 transcript in most of these long-term spontaneously beating myocytes were negligible, close to the negative control level in ventricular myocytes expressing GFP alone. A significant proportion of Tbx18-VMs exhibited SAN-like, lean morphology as judged by cell length-to-width (L-to-W) ratio up to 6 weeks after initial gene transfer. At a similarly long-term time frame, Tbx18-injected hearts demonstrated ectopic idioventricular rhythm at 165 ± 14 bpm, with electrocardiograms consistent with biological pacing from the Tbx18 injection site. Furthermore, these long-term, Tbx18-injected hearts responded to autonomic regulation in a manner similar to the short-term, Tbx18-injected hearts. Thus, SAN-like cells maintain pacemaker function even after exogenous Tbx18 expression has waned.

This study provides the first evidence that Tbx18 converts adult ventricular myocytes to faithful replicas of SAN pacemaker cells in vitro and in vivo. The new SAN-like pacemaker cells exhibit automaticity and pacemaker cell morphology and create biological pacemakers

in vivo. Specific epigenetic changes in the bioengineered SAN-like pacemaker cells are consistent with direct and specific transformation rather than non-specific reversion to a primitive state [24••]. Newly created SAN-like pacemaker cells retain their phenotype even after the expression of exogenous *Tbx18* has waned, indicating durable conversion to a pacemaker phenotype. In another study, constitutive overexpression of *Tbx3* in transgenic mice led to spontaneous atrial rhythm but failed to create ectopic ventricular beats [31]. A logical next step would be to combine the two factors and examine for potential synergistic effects for better reprogramming.

Biological Pacemaker Created by Minimally Invasive Gene Transfer in Pigs with Complete Heart Block

Electronic pacemakers have been successfully used for more than five decades with continuous refinement [32]. But, they are limited in some instances by complications ranging from inadequate autonomic support to lead fracture, infections, and adverse cardiac remodeling [10, 33]. Proof-of-concept studies of biological pacemakers have been performed in large animals, using delivery methods that are transferrable to clinical procedures [5, 10, 33–36, 37•]. However, all previous large animal studies have used ion channel-based approaches to elicit spontaneously oscillating APs from normal myocardium [5, 34–36, 37•]. Motivated by in vitro and small animal in vivo reprogramming with *TBX18* [24••], we have employed the minimally invasive, porcine model of complete heart block [35] to achieve in vivo somatic reprogramming with *TBX18* in pigs [38••].

Twelve pigs were given percutaneous injections of either *TBX18* ($n=7$) or green fluorescent protein (*GFP*, control) ($n=5$) and monitored for 14 days. The baseline clinical, electrocardiographic (ECG), and laboratory data showed no significant differences between animals. The model involved first implanting an electronic pacemaker device (with the lead placed at the right ventricular apex) and then creating complete heart block by ablating the atrioventricular (AV) node with a radiofrequency catheter. The electronic device served as a backup pacing device for the duration between the AV node ablation and until the biological pacing is achieved after the transgene expression. Upon generating complete heart block, the transgene, *TBX18*, is then injected in the AV junctional area, downstream of the ablated site.

After gene delivery, mean heart rate was higher in *TBX18*-transduced animals compared to *GFP*-transduced control animals starting at day 2 and persisting for 2 weeks. *TBX18*-transduced pigs achieved a higher maximal HR and relied less on a backup electronic pacing device (<1 % from day 5 to day 11) compared to controls (8 to 40 % throughout the 2 weeks of follow-up). Thus, *TBX18* gene delivery successfully creates robust biological pacemaker activity, minimizing the need for backup electronic pacing.

The heart rhythm is known to correlate with the circadian rhythm [39]. Over the 14 days after the gene injection, both daytime and nighttime heart rates were higher in the *TBX18*-transduced group than in controls. The diurnal change (the difference between daytime and nighttime heart rate) was also greater in the *TBX18* group, resembling the behavior of the native SAN [39]. As a surrogate for the sinus node recovery time (SNRT) measurements [40], the pig's heart was paced electronically at a heart rate of 120 bpm for 1 min, and then,

electronic pacing was abruptly terminated to allow the biological pacemaker to emerge from overdrive suppression. Corrected recovery time after burst ventricular pacing was 10-fold shorter in the *TBX18* group at day 8 compared to control animals (whose pacing was presumably coming from the endogenous and slow junctional escape rhythm).

Autonomic Regulation Controls the Rate of the *TBX18* Biological Pacemaker in Pigs

Distinct, yet unproven, advantages of biological pacemakers are their potential capacity for heart rate variability (HRV) as observed under normal sinus rhythm [41, 42] and response to β -adrenergic stimulation. Normalized low-frequency component of the HRV tended to be higher, and normalized high frequency lower, in the *TBX18* group, which are compatible with sympathetic predominance of the SAN [41, 42] and in the *TBX18*-transduced group. Isoproterenol infusion increased heart rate by 71 % in *TBX18* animals, with less pronounced changes in control animals. The HRV analysis and the response to β -adrenergic agonist are indicative of the *TBX18* biological pacemaker's responsiveness to endogenous and pharmacologic autonomic stimuli.

The mean physical activity, measured by the implanted accelerometer, and the duration of bursts of activity were significantly greater in the *TBX18* group than in controls. The post-burst latency (time between bursts of activity) was shorter in *TBX18*-transduced animals than in controls. During maximal activity, animals in the *TBX18* group demonstrated a higher heart rate compared to controls, indicating superior accommodation to hemodynamic and metabolic demands (i.e., chronotropic adaptability). These data are highlights of another perceived advantage of biological pacing: Unlike the electronic backup pacing device, the *TBX18* biological pacemaker provides autonomically sensitive chronotropic adaptability appropriate to support physical activity.

***TBX18* Biological Pacing Originates at the Focal Injection Site and Does Not Increase Arrhythmic Risk**

In order to derive anatomical origin of ectopic ventricular pacing activity in vivo, electroanatomic mapping of the right ventricle (RV) and pace mapping from the injection site were performed. On the day of device implantation and gene injection, the earliest activation originated, in both groups, from the right ventricular apex at which the electronic device's lead had been positioned to pace the heart. At day 14 after gene delivery, activation arose in the high septal region, anatomically close to the transgene injection site in *TBX18*-transduced animals but not in control animals.

Transient pacing of the gene injection site with a stimulation catheter generated electrocardiographs that showed a 100 % match on all 12 of the 12 ECG leads with the *TBX18*-induced biological pacemaker rhythm. In contrast, there was no match between the catheter-paced ECGs with the non-specific escape rhythms observed in *GFP*-transduced controls. The QRS duration of the *TBX18*-induced rhythm was significantly shorter than that in controls (55.4 ± 3.1 vs. 67.4 ± 1.1 ms, $p=0.01$), consistent with fast conduction from the high septal region [43]. These findings suggest that *TBX18*-induced rhythms originated from the gene delivery site, whereas spontaneous rhythms in control animals rarely did.

During the 2-week follow-up period, the cumulative incidence of sustained ventricular arrhythmias was low and did not differ significantly between the two experimental groups. Corrected QT interval and dispersion from 12-lead ECG and APD₉₀ dispersion and duration did not differ in the two treatment groups. Programmed ventricular stimulation with and without isoproterenol infusion elicited no episodes of inducible ventricular tachycardia in either control or *TBX18*-transduced animals. These data suggest that *TBX18* biological pacemaker does not increase arrhythmic risk.

Prospects for Clinical Translation of Biological Pacemakers

This study demonstrates that in situ somatic reprogramming can stimulate proper chronotropic heart function in a large-animal model of complete heart block. The gene delivery techniques were consistent with routine clinical practice in which venous access is considered the safest route for electronic pacemaker implantation [44]. The *TBX18* biological pacemaker was superior to an electronic pacemaker in its responsiveness to the circadian rhythm and autonomic regulation, showing features of the native SAN.

A major concern of biologic-driven automaticity is the potential occurrence of ventricular arrhythmias [10, 45]. Here, no evidence of increased ventricular arrhythmias was seen during 2 weeks of continuous monitoring. Repolarization parameters (QT and APD) and gradients (QT and APD₉₀ dispersion) that can promote arrhythmogenesis [46, 47] were indistinguishable between *TBX18*-transduced animals and in the control animals. Moreover, no inducible ventricular tachycardia was seen by programmed ventricular stimulation 2 weeks after *TBX18* gene delivery. Together, these findings support the absence of pro-arrhythmic risk of the *TBX18*-induced biological pacemaker, at least in the short term. Biodistribution the *TBX18* adenoviral vector indicated that the viral vector was primarily localized at the injection site, with minimal systemic distribution. Nevertheless, the arrhythmic and systemic safety profiles should be rigorously sought out with a longer term study.

The gene delivery technique is readily transferrable to a clinical setting and exhibits many advantages: low invasiveness, minimal pain/distress, minimal blood loss, lower risk of stroke by advancing the catheter to the right-sided circulation, and the ability to deliver the transgene focally into the AV junction area, resulting in antegrade conduction through the specialized His-Purkinje system. In addition, the transvenous injection catheter (NOGA MyoStar) has been extensively used in multiple human biological therapy trials [48–50].

Conclusions

A distinct advantage of biological pacemakers to gene therapies for cardiac regeneration is that focal modifications of electrical properties suffice for effective treatment. Since the amount of exogenous biological material delivered can be correspondingly reduced, potential problems due to widespread dissemination may be more readily averted. A focal modification, in principle, is reversible: If needed, implanted biological pacemakers can be destroyed by conventional electrophysiological ablation, followed by electronic pacemaker implantation.

Gene delivery vectors could be tailored to different clinical needs. Adenoviral vectors, for example, achieve the peak expression earlier than other vectors albeit in a transient manner. This would be ideal for the temporary pacing needs to treat infected pacemaker devices [10, 35, 38••]. On the other hand, the high immunogenicity of these vectors could be circumvented by using helper-dependent adenovirus [51]. Accordingly, adeno-associated virus or lentivirus vectors would be more suitable for long-term applications [52].

Will biological therapies eventually replace drugs and devices? To answer this question, long-term, large animal studies must be performed to compare the efficacy of pacing by biologic vs. device. At the very least, current data justify testing of biological pacemakers for transient use in clinical conditions such as infected pacemaker devices [10, 35, 38••]. On the other hand, technological advances in devices, e.g., leadless pacemakers [53], will continue to improve the standard of care. Thus, it would be logical to predict a future in which a device and a biological therapy co-exist in a bradycardic patient, with the device functioning as a backup. Further work will be required to optimize the functional efficacies of biological therapies, to evaluate the safety and toxicology, and to establish the long-term stability of therapy. With the recent breakthroughs, biological pacing is now closer to clinical translation than to experimentation of concepts.

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Fig. 1.

Focal delivery of an adenoviral vector expressing Tbx18 and GFP (adeno-CMV-Tbx18-IRES-eGFP) into the apex of guinea pig hearts in vivo generates ectopic ventricular beats [24••]. Representative electrocardiograms of GFP- or Tbx18- injected guinea pig (**a** and **b**, respectively). Downward (negative) deflection in ECG recording on lead II after methacholine injection (0.05 mg/ml) revealed ectopic pacemaker activity originating from the ventricle with wide QRS complexes in Tbx18-injected guinea pigs (**b**). In contrast, GFP-injected guinea pigs ($n=7$) exhibited antegrade, junctional rhythm with narrow QRS complexes (**a**). No retrograde, ectopic beats with wide QRS complexes were observed in GFP-injected guinea pigs. (Adapted from: Kapoor N, Liang W, Marban E, Cho HC. Direct conversion of quiescent cardiomyocytes to pacemaker cells by expression of Tbx18. *Nature Biotechnology*. 2013;31(1):54–62. doi:[10.1038/nbt.2465](https://doi.org/10.1038/nbt.2465)) [24••]

Fig. 2.

Whole-cell action potentials (APs) recorded from freshly isolated single Tbx18-VMs illustrates rhythmic and spontaneous AP oscillations [24••]. The AP demonstrates slow diastolic depolarization, resembling the slow depolarization prominent in native SA node pacemaker cells (*left panel*). The *lower panels* show the same APs as in the *upper panels* in an expanded timescale. In contrast, GFP-VMs displayed no spontaneously firing AP, but rather a stable resting membrane potential. The GFP-VMs elicit single action potential only when they are provoked by an electrical stimulation. (Adapted from: Kapoor N, Liang W, Marban E, Cho HC. Direct conversion of quiescent cardiomyocytes to pacemaker cells by expression of Tbx18. *Nature Biotechnology*. 2013;31(1):54–62. doi:[10.1038/nbt.2465](https://doi.org/10.1038/nbt.2465)) [24••]

Fig. 3.

Exogenous expression of Tbx18 transforms adult ventricular myocytes into SA nodal pacemaker cells in vivo [24••]. **a** Representative images of freshly isolated SA nodal pacemaker cells (*left panel*), Tbx18-VMs (green myocytes, *middle panel*), and control GFP-VMs (*right panel*). **b** The freshly isolated cardiomyocytes in **a** were immunostained against α -SA. In contrast to the well-organized pattern of sarcomeres in the control myocytes (GFP-VMs), the native SA nodal pacemaker cells and the Tbx18-VMs display substantially disorganized myofibrillar structure. Scale bar=50 μ m. **c** Tbx18 expression makes the ventricular myocytes to become leaner and spindle-shaped compared to the control myocytes. Measurements of myocyte length-to-width ratio indicate that Tbx18-VMs are narrower compared to control. Whole-cell capacitance, as an indirect measure of cell size, demonstrates that Tbx18-VMs are significantly smaller compared to GFP-VMs. (Adapted from: Kapoor N, Liang W, Marban E, Cho HC. Direct conversion of quiescent cardiomyocytes to pacemaker cells by expression of Tbx18. *Nature Biotechnology*. 2013;31(1):54–62. doi:10.1038/nbt.2465) [24••]