Myocardial Isl$^+$land
A Place With Lots of Rhythm, but No Beat
Mark A. Sussman

“Life is about rhythm. We vibrate, our hearts are pumping blood, we are a rhythm machine, that’s what we are.”

—Mickey Hart

As you read these words, an intrinsic rhythm of life ripples through your heart. Your personal “metronome” is required to begin at the earliest stages of embryonic development and continues unceasingly as life unfolds. Understanding the genesis of our rhythm continues to be refined by intense investigation from developmental biologists who are unraveling the intertwined orchestration of myocardial organization, timing, and cardiac precursor cell incorporation. Almost a decade ago, a remarkable publication identified an embryonic stem cell subpopulation essential for cardiac development and continues expressing a LIM homeodomain transcription factor called Isl1.$^1$ Isl1-null mice fail to develop major cardiac formation resulting in embryonic lethality, convincingly demonstrating an essential role of Isl1$^+$ stem cells in the initial stages of cardiogenesis. Potential significance of these cellular developmental biology observations did not go unnoticed by those committed to the comparatively nascent field of myocardial regeneration who long for a specific cardiac stem cell marker. Following closely on the heels of initial Isl1-related revelations, a spate of high-impact publications touted precursors capable of generating all cardiovascular lineages.$^{2-5}$ Thereafter, as the proverbial saying goes, “then all hell broke loose” and researchers became embroiled in yet another controversy about cardiac stem cell biology.$^6-^9$ Fortunately, time discovers truth and a clearer vision emerges, as presented in this issue of Circulation Research by Weinberger et al$^{10}$ that helps reconcile speculation with reality and dispels some of the hyperbole about Isl1 in the adult heart.

At the core of the controversy are the questions of whether Isl1$^+$ cells persist in the adult heart, what types of cells maintain Isl1 expression in the mature myocardium, and whether these cells contribute to myocardial regeneration. It stands to reason that if Isl1 is a marker of embryonic stem cells essential for cardiac development, then finding such cells in an adult heart would likely point toward a population capable of producing new myocardium. A variety of genetic manipulations in murine lines have produced clear evidence that Isl1$^+$ cells do persist beyond postnatal development and do contribute to myocardial structure. The group led by Sylvia Evans$^{11}$ responsible for the seminal studies in cardiac development related to Isl1 concluded that this marker is predominantly associated with a subset of pacemaker cells, endothelium, and vascular smooth muscle rather than myocytes (although “myocardialized” cells in the outflow tract coincident with cardiac myosin were noted). Also, this study used an inducible Cre/lacZ lineage tracking assessment to demonstrate that by embryonic day 9, most cells that were Isl1$^+$ have migrated to the heart and have ceased expressing the marker altogether. However, the key role of Isl1$^+$ cells in adult cardiomyogenesis remains debatable and has been confounded by observations of “highly efficient conversion to mature cardiac phenotype” in vitro.$^{2,3}$ Presumably, deletion of Isl1$^+$ cells in early embryonic development is likely to produce widespread collateral effects for inductive signaling responsible for recruitment, organization, and specification of other cell types, including those giving rise to myocytes. Thus, substantial loss of myocardial tissue resulting from Isl1 deletion is not necessarily tantamount to proof that myocardium is a primary derivative of the Isl1 cell lineage. Moreover, if Isl1$^+$ cells were present in adult myocardium and had robust cardiomyogenic potential, then even the most cynical skeptics would be persuaded of a role for these cells in regeneration if they re-emerged after pathological injury in the ventricular myocardium, as is common for many other markers of fetal and embryonic development; put in layman’s terminology, you cannot participate if you do not show up.

This brings us to the current study by Weinberger et al$^{10}$ that localizes Isl1$^+$ cells in adult myocardium—both in normal hearts as well as in those challenged by infarction injury. The study uses a heterozygous Isl1 “knock-in” mouse line in which one allele of Isl1 has been replaced with a nuclear-localized β-galactosidase gene to tag the cells. Thus, any cell that is expressing Isl1 will be identified by the presence of the transgene that can be readily detected by X-Gal enzymatic labeling; this is a helpful method because the expression or detection of the Isl1 signal may be relatively low or difficult to ferret out with immunohistochemistry. This genetically engineered mouse is one of the same lines used by the Evans group$^{11}$ in their study of prenatal and early postnatal development. In contrast to the previous work, Weinberger et al$^{10}$ chose to focus on an extended longitudinal time course of heart samples ranging in age from 10 weeks to 18 months to cover most of an adult mouse lifespan. Literally
thousands of myocardial sections were analyzed for presence of X-Gal reactivity. Reassuringly, the findings reinforced earlier reports from the Evans study: a few subregions showed concentrated labeling indicative ofIsl1+ expressing cells. These corresponded to cardiac ganglia, roots of the aorta and pulmonary artery proximal to the valves, a few isolated cells in the ventricular outflow tract, and sino-atrial node. Of these four groups, the sino-atrial node cells appeared both consistent and well-defined, prompting confirmation of their findings with mRNA-based polymerase chain reaction in human tissue samples in which Isl1 message was also robustly expressed. Having validated the fidelity of their detection system, the final experiments in the study assessed the involvement of Isl1+ cell-mediated response to infarction injury at multiple time points ranging from days to weeks after challenge. In >4000 sections of tissue analyzed for X-Gal reactivity indicative of Isl1, there were no cells identified in the damaged region, almost. In the best spirit of full disclosure, the authors admit that in one heart out of the 13 examined, “10 questionable cells (were) showing a rather atypical but clearly nuclear LacZ staining.” And that is literally the last line of the results section, leaving the reader to wonder whether we will ever be able to unambiguously close the door on this debate. Perhaps we are not meant to.

Researchers like to have clear and incontrovertible results, because they allow us to build our paradigms and move the field ahead by establishing universally held truths. Unfortunately, nature does not always cooperate and the cardiac stem cell field is rife with ambiguity. The present study by Weinberger et al demonstrates that cements the presence of Isl1 throughout adult lifespan in the myocardium, more or less by extending into adulthood the same places identified by the Evans group in 2007 in prenatal/postnatal development. Isl1 marks a subset of sino-atrial node, parasympathetic neurons, some smooth muscle cells at the roots of the great arteries, and a few myocardialized cells in the outflow tract. As the authors point out, all of these Isl1+ cells are coincident with other markers characteristic of lineage-committed cells; none of the Isl1+ cells was observed to exist as undifferentiated cells, as might be anticipated for a multipotent cardiac precursor cell. Also, aside from the 10 “atypical” cells found at 7 days after infarction in one mouse, the other 25 million cells at this time point were all negative for Isl1, and this is true for all other time points studied after myocardial infarction. The complete absence of Isl1+ cells in the adult working ventricular myocardial tissue, either in healthy samples or after infarction challenge, stands as compelling evidence against the likelihood of Isl1+ cells playing a biologically relevant role in myocardial regeneration. Of course, one could argue that the detection of Isl1 in the myocardium depends on ongoing expression from the promoter driving the reporter β-galactosidase protein, and that expression may be short-lived as the cells pass through an Isl1+ phase on their way to eventual commitment. However, the aforementioned Isl1+ committed cells do not appear to have a problem maintaining concurrent expression of the reporter protein and their lineage-characteristic proteins, and the myocardialized cells even coexpress myogenic proteins.

More plausibly, arrival of Isl1 cells in the embryonic cardiac field would certainly be consistent with a primary role in the organizational events surrounding establishment of rhythm by E8.5 that are essential for progression of cardiac development and survival. Collectively, the possibility of Isl1+ cells as mediators of adult myocardial regeneration appears to be fading away with the combined weight of observations from Weinberger et al that demonstrate Isl1+ cells do not exist in adult contractile ventricular tissue and are not recruited by injury.

I have managed to get through this Editorial without naming any other stem cell types in comparison to cardiac Isl1+ precursors (until now). I offer a closing reconciliation that hopefully will not be the literary equivalent of touching the “third rail.” Perhaps the reason why we have trouble reaching closure is because we long for “either/or” resolutions for our stem cell markers. The evidence that Isl1+ cells are essential to cardiogenesis is irrefutable, as is the growing recent evidence in support of c-kit+ cells for the same role in cardiac development. Rather than choose a side, what if the ultimate answer lies in a population of Isl1+/c-kit+ cardiac progenitor cells as reported last year for human embryonic and fetal hearts? However, existence of a dual-positive c-kit+Isl1+ stem cell does not necessitate c-kit and Isl1 stem cells sharing a common precursor, nor am I aware of any objective evidence that c-kit cells derive from Isl1 precursors (or vice versa). In the end, the biology of myocardial regeneration may settle for the reassurance that nature does not respect our self-imposed boundaries or territorial markers of cell type-specificity. Such a shared population of precursors concurrently expressing both Isl1 and c-kit simultaneously could explain the requirement of both cell types for cardiogenesis and point toward a continuum of stem cell marker expression that ebbs, flows, and becomes restricted to distinct cell populations as the heart progresses from genesis to maturation. The heart cannot function without rhythm originating in the sino-atrial node, where Isl1+ cells reside, but imposing rhythm without the corresponding beat from ventricular tissue is equally pointless. Cells in a dish can adopt strange intermediate phenotypes and are comfortable showing off their ambivalence by accommodating our desires to push cell fate decisions on them. As scientists, we should learn from nature and accept that ambivalence may be part and parcel of the impressionable personality of the stem cell. We might even be happier in the long run, because, as Erica Jong said, “ambivalence is a wonderful tune to dance to. It has a rhythm all its own.”

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References


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