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Cardiac regeneration strategies: Staying young at heart

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Abstract

The human heart is continually operating as a muscular pump, contracting on average 80 times per minute to propel 9 tons of blood through body tissues each day. Whereas damaged skeletal muscle has a profound capacity to regenerate, heart muscle, at least in mammals, has poor regenerative potential. This deficiency is attributable to the lack of resident cardiac stem cells, combined with roadblocks that limit adult cardiomyocytes from entering the cell cycle and completing division. Insights for regeneration have recently emerged from studies of animals with an elevated innate capacity for regeneration, the innovation of stem cell and reprogramming technologies, and a clearer understanding of the cardiomyocyte genetic program and key extrinsic signals. Methods to augment heart regeneration now have potential to counteract the high morbidity and mortality of cardiovascular disease.

Through normal wear and tear, our tissues experience regular cell loss that is countered by replenishment mechanisms. Aging itself is argued to be a cumulative outcome of the gradual decline in our bodies' natural capacity to balance these events. Each tissue displays specific rates and mechanisms of cell turnover. Human intestinal epithelial cells last about a week, whereas erythrocytes persist for 4 months before elimination. At the other end of the spectrum, cardiomyocytes, the contractile cells of the heart, display an estimated turnover rate of 0.3–1% per year, with most renewal events reported to occur in the first decade of life.

Due to this low turnover rate, each of us will die with many or most of the cardiomyocytes we had at birth (1). Besides longevity, cardiomyocytes possess brute strength and resilience. Their kryptonite is coronary artery occlusion and tissue ischemia, which can locally devastate the cardiomyocyte population and cause myocardial infarction (MI) with resultant scarring, along with non-ischemic causes of muscle loss. Without an evolved mechanism for rapid cardiomyocyte renewal, the spared myocardial constituents adapt and compensate to preserve pump function. Unfortunately, a scarred heart is much more likely to fail over time, and an estimated 38 million patients worldwide currently suffer from heart failure.

Several lower vertebrates have been found to display robust heart regeneration at the adult stage (2). Fetal and neonatal mice also respond constructively to cardiac injury with regeneration of new cardiomyocytes (3). However, this response fades by juvenile and adult stages in mammals. Understanding the heart's capacity for regeneration, and how to control

it, are key objectives of cardiovascular research. The past two decades of discoveries have unearthed promising avenues. Cardiac reconstitution, using stem cells and laboratory-grown cardiomyocytes, is one strategic front, as is manipulation of the cardiomyocyte genetic program or the signaling environment to awaken innate regenerative programs. Here we consider what we have learned and have yet to learn about heart regeneration and how these approaches might be applied in the clinic.

Challenges to heart regeneration

Cardiac and skeletal muscle are functionally and anatomically similar: each cell type is large and enriched with sarcomeres and mitochondria. However, the tissues differ markedly in their injury responses. Skeletal muscle is composed of post-mitotic, multinucleated muscle fibers, and it is spiked with satellite cells, an archetypical adult stem cell population that occupies the space under the basement membrane ensheathing the fiber. Although normally quiescent, these cells activate myogenic programs and differentiate into myoblasts after injury. Myoblasts proliferate and fuse with each other and remaining muscle fibers to quickly regenerate lost muscle mass. In his initial report in 1961 on the identification of skeletal muscle cells (4), Alexander Mauro wrote a single footnote, "It is exciting to speculate whether the apparent inability of cardiac muscle cells to regenerate is related to the absence of satellite cells." As discussed below, the identification of a resident cardiac stem cell population has been elusive with reports shrouded in controversy; however, it is indisputable that new heart muscle regeneration is extremely limited after MI.

Unlike differentiated skeletal myofibers, cardiomyocytes are capable of cell division. In fact, the heart grows by, and chamber features are patterned primarily by, regulated cardiomyocyte division. However, in mammals this mode of cardiomyogenesis has a limited, secondary role after birth. In mice, a fundamental shift in cardiomyocyte cell cycle dynamics occurs during the first week of postnatal life when cardiac growth becomes driven by karyokinesis in the absence of cytokinesis, and hypertrophy, expanding cardiomyocyte cell volume without division (5). This transition generates a population of binuclear murine cardiomyocytes that, by the adult stage, dwarfs the mononuclear population several-fold (6). Massive increases in ventricular pressure, a general shift from glycolytic to oxidative metabolism, and changes in sarcomere protein composition all occur in this life stage. A second, pre-adolescent wave of cardiomyocyte division associated with changes in thyroid hormone levels has been reported to occur in mice (7), although this finding is controversial (8, 9). Humans are likely to experience an analogous developmental timeline of cardiomyocyte proliferative capacity. The timing and affiliated hallmarks of the transitions are not as clear in humans, but more than half of all adult human cardiomyocytes become polyploid while remaining mononucleate (10). Generally, as mammals mature and the cardiac workload increases, a mechanistic shift occurs, from boosting contractile machinery with added cells and their genomes to harvesting sarcomere components from added genomes alone.

Whether the low reported rates of cardiomyocyte turnover in adult mammals is attributable to rare cardiomyocyte division events is unclear. Polyploid cells like hepatocytes can divide; thus, polyploidy should not by definition prohibit injury-induced proliferation. Yet,

cardiomyocyte division is exceedingly rare in adult mammals, either healthy or injured, and the ability to unambiguously monitor division is clouded by the preponderance of endoreplication events. There are many studies that have probed the molecular basis of this deficit. For instance, G1/S and G2/M cyclins and cyclin-dependent kinases are typically downregulated in maturing cardiomyocytes, whereas levels of cell cycle inhibitors increase. Centrosomes have been reported to lose functionality in mature mammalian cardiomyocytes (11). Forced transgenic cyclin overexpression or deletion of tumor suppressor genes can stimulate cardiomyocyte DNA synthesis or mitosis in adult mice; however, new cardiomyocyte production appears to be inefficient in these contexts (12, 13).

Additionally, the milieu of the injured mammalian heart is not optimized for regeneration. Vascular supply is limited after ischemic injury, and cardiac fibroblasts are present in equal or greater numbers than myocytes. These fibroblasts infiltrate inflamed injuries, and within several days create mature collagen-rich scars thought to be irreversible. Matrix stiffening that occurs after injury can further suppress the already limited potential for cardiomyocyte proliferation (14). Injuries often progress to chamber dysfunction and heart failure. Thus, there are numerous challenges to heart regeneration, including restrictions on the cardiomyocyte genetic program and the ischemic, pro-fibrotic injury environment.

Rise (and fall?) of cardiac stem cells

One of the most controversial topics from the past two decades of cardiac regeneration research is the existence of endogenous stem cells. Several types of resident cardiac progenitor (or stem) cells (CPCs) have been reported to reside in the adult heart, identifiable by surface markers c-Kit, Sca1, or PDGFR α , expression of the transcription factor Isl1, efflux of Hoechst dye, or the ability to form cardiospheres in culture (13–16).

Viewed collectively, CPCs are rare and heterogeneous in origin and apparent behavior, which in part explains the surrounding technical challenges and controversies associated with virtually all reports of their regenerative potential (15). Whereas cultured CPCs display a degree of lineage plasticity, it is unclear whether this accurately models *in vivo* behavior.

The most trumpeted, and also most contested, of the proposed resident CPCs are blood lineage-negative, c-Kit⁺ cells, reported in 2003 to give rise *in vitro* and *in vivo* to major cardiac cell types, including cardiomyocytes (16). This study and others like it generated extensive further investigation and popular excitement about prospects for human heart regeneration. In the years since, conflicting results have been obtained with respect to the potential of these cells (17). The cardiogenic potential of injected cells varied among reports from absent to robust, and comprehensive fate mapping of c-Kit-expressing cells by three independent groups found some vascular endothelial potential but minimal cardiomyogenic potential in injured adult mouse hearts (first reported in (18)). The current prevailing view is that c-Kit⁺ CPCs are rare, and their ability to replace lost cardiomyocytes after cardiac injuries is functionally insignificant.

Intriguingly, injection of a variety of purified cells into a MI have yielded small, short-term benefits to cardiac function in rodents, even if those cells and any progeny die within days of

introduction (15). Bone marrow-derived stem cells have been infused into patients for over a decade, although no clear conclusions can be made of the effects on functional recovery in these trials. Long-term engraftment of transplanted CPCs and any differentiation to cardiomyocytes in the setting of a mammalian MI are very limited, and the reported improvement in heart function has been attributed to paracrine release of soluble factors that mediate cardiac tissue survival and neovascularization (12, 15). In summary, most scientists in the field now recognize that CPCs are rare and may overlap with other characterized cell types, and that transplanted CPCs appear more likely to have pro-survival or –angiogenic effects than cardiomyogenic potential.

Stem cell-derived cardiomyocytes and their transplantation

Activation or addition of cardiomyogenic stem cells would be a regenerative solution to lost or damaged myocardium, but why not simply add back the contractile tissue that was lost? The cardiac regeneration field was launched with this in mind. Early attempts flooded MI injuries with contractile precursor cells in the form of skeletal myoblasts, which generated physiologically distinct muscle that ultimately did not electromechanically couple with spared myocardium (13). For many years, a cardiac reconstitution approach was limited by source availability; that is, the ability to procure hundreds of millions, even billions of cardiomyocytes, needed for transplantation into small and large mammalian hearts (Fig. 1).

Protocols for directed differentiation of embryonic stem cells and induced pluripotent stem cells (iPSCs) using the appropriate growth factors (e.g. BMP4, FGFs) or small molecules have enabled large-scale production of relatively pure cardiomyocytes. Stem cell-derived cardiomyocytes mature to a limited extent in culture. When transplanted they lack the anatomy and physiology of adult ventricular cells, and thus they must grow and mature quickly in vivo upon leaving the culture dish. A many-fold excess of cardiomyocytes must be transplanted, as the vast majority of these cells die or are washed out immediately after injection (13, 19). Human cardiomyocytes have been transplanted into rodents given ischemic injuries, including guinea pigs whose cardiomyocytes exhibit a similar slow rate of contraction, as well as non-human primate (macaque) models (Fig. 1). Results have shown promise; in particular, long-term survival of muscle grafts in the settings of host scar tissue has been observed. Electrical coupling occurs in these transplant models, and cardiac function can improve (20).

Among several remaining challenges is production scale, to generate several hundred million *surviving* transplanted cardiomyocytes per patient. Cardiomyocytes can be frozen and thawed for transplantation, meaning that an infarct's worth of replacement cardiomyocytes made from a patient's reprogrammed cells can be stored for future use. This process, however, requires formidable time and cost. Allogeneic transplants would have practical advantages over autologous transplants, yet prolonged use of immunosuppression is associated with significant morbidity and mortality. A final major hurdle to overcome is the prevalence of transient or sustained arrhythmias (20, 21). Long-term electromechanical coupling of donor and recipient cells is necessary to avoid events like ventricular tachycardia that can cause cardiac arrest. These arrhythmogenic risks might be alleviated with implantation of expensive internal cardioverter-defibrillator devices.

Tissue scaffolds, either synthetic or from natural materials like collagen, fibrin, gelatin, hyaluronic acid, chitosan, alginate, or decellularized tissues, can assist engraftment of transplanted cardiomyocytes (Fig. 1). Scaffolds, applied alone or with molecular factors, supporting vascular cells, and/or cardiomyocytes to create engineered contractile cardiac patches, can better replicate cardiac complexity, improving survival and maturation of donor cells. In these cases, the interplay between donor cells, scaffold, and host is crucial, and potential issues of host-donor coupling and arrhythmias remain. Bioengineering recipes for scaffold materials, cells and growth factors that comprise cardiac patches, and how to pre-condition these constructs, are regularly evolving (22).

Reprogramming approaches for heart regeneration

iPSCs, the key source for cardiomyocyte production in vitro, were initially derived from mouse fibroblasts infected with viruses encoding the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 (23). Methodologies have since evolved to employ other gene combinations, small molecule effectors, and non-viral vector delivery. Introduction of the transcription factors Gata4, Mef2c, Tbx5 (with or without Hand2), studied for years as regulators of the cardiomyocyte genetic program during heart development, was reported in 2010 to be sufficient to directly reprogram cardiac fibroblasts into induced cardiomyocyte-like cells (iCMs) that express contractile genes (24) (Fig. 2). This result is in line with contemporary direct reprogramming experiments deriving other tissue lineages, and with experiments from decades ago revealing that the expression of a single transcription factor MyoD can reprogram fibroblasts into skeletal myoblasts (25).

Combinations of specific miRNAs or pharmacological inhibitors of signaling pathways (e.g. JAK, Bmi1) with epigenetic modulators can improve the conversion of fibroblasts to iCMs (Fig. 2). Similarly, chemical regimens were reported to reprogram mouse fibroblasts to multipotent cardiac progenitors, which could be expanded and maintained in culture and developed into cardiomyocytes, endothelial cells, and smooth muscle cells in vitro and in vivo (reviewed in (26)). Although converting human fibroblasts into iCMs has proved more difficult, several recent studies identified cocktails that efficiently reprogram human fibroblasts to beating cardiomyocytes or multipotent cardiac progenitors (27, 28).

A MI injury presents a vernal pool for cardiac fibroblasts, and the idea of converting a portion of these cells in situ to contractile cells is a transformative concept. Initial experiments employed viral vectors loaded with cardiogenic transcription factors and injected directly into the infarcted tissue. In these studies, a modest proportion of cardiomyocytes in the MI border zone was traced as progeny of infected fibroblasts, concomitant with reduced scar area and improved left ventricular function (26). Although the robustness of the in vivo reprogramming process as well as the use of viral infection delivery are under debate, this technique provides a novel, cell-free platform for cardiac repair (Fig. 2).

Models for innate cardiac regenerative capacity

Regeneration has been studied in a menagerie of laboratory model systems over more than two-and-a-half centuries. Among vertebrates, fish and salamanders have elevated regenerative capacity, displayed impressively during regeneration of transected spinal cords, amputated limbs or fins, and resected portions of brain, intestine or jaw. Heart regeneration, too, is possible, and among lower vertebrate model systems has been demonstrated most capably by zebrafish, which regenerate muscle lost after resection of 20% of their single ventricle, a similar-sized cryoinjury, or a genetic ablation injury that depletes 60% or more of their cardiomyocytes (2) (Fig. 3). One week after genetic ablation of cardiomyocytes, over 40% of the spared ventricular cardiomyocytes show indicators of cell cycle entry. Although transient collagen deposition occurs and long-lasting cardiac scarring is possible in certain contexts, the innate cardiac injury response in zebrafish involves little or no fibrosis (2, 29). It is thought that this natural capacity for a meaningful regenerative response, which has also been reported to certain extents in other fish species and some amphibians (2), might serve as a beacon for regenerative methodologies in adult mammals.

The central question in innate heart regeneration for several years was whether a stem cell pool regenerated new cardiomyocytes. Years of research have yielded no definitive evidence of CPCs in adult zebrafish. On the contrary, genetic fate mapping techniques that permanently label cardiomyocytes before injury definitively trace the label into newly created cardiac muscle (30, 31). Thus, the vast majority if not all new cardiomyocytes regenerate from the division of spared cardiomyocytes.

Given the hurdles for adult mammalian cardiomyocyte division, how are zebrafish and other lower vertebrates able to stimulate cardiomyocyte proliferation upon injury? Possibly lowering the threshold is that adult zebrafish cardiomyocytes are smaller than mammalian cardiomyocytes, lack certain structural elements like T-tubules, and are predominantly mononuclear. Upon injury, zebrafish cardiomyocytes adjacent to the wound show features of a reduced contractile program, alter expression of many cardiogenic factors, and enter the cell cycle. This generally reflects dedifferentiation - a transient developmental reversal from a fully functional state. The ventricular pressure necessary for circulation in zebrafish is lower than that in mammals, possibly facilitating this transition. Transcriptional programs that could mediate cardiomyocyte dedifferentiation are under investigation. Expression analysis and functional tests have identified Stat3, Gata4, and Nf-kB transcription factors as required for zebrafish heart regeneration, the key downstream targets of which are of interest (2). Interestingly, multiple lines of evidence, including multicolor clonal analysis, indicate that most or all ventricular cardiomyocytes are capable of participating in regeneration to a similar extent, as compared to a hierarchical structure involving elite, regenerative cells (2). Mammals might differ by, for instance, possessing only a rare minority of cardiomyocytes that can divide upon injury. If so, studies in non-mammalian models have the potential to pinpoint molecular markers for this subpopulation of responsive cells. Additional reports demonstrate that innate heart regeneration requires not only proliferation-competent cardiomyocytes, but also a pro-regenerative injury environment orchestrated by diverse cell types (Box 1- Non-muscle cells and heart regeneration) .

BOX 1**Non-muscle cells and heart regeneration**

Pro-regenerative factors induced by injury could originate from heart muscle itself, or from non-muscle sources like circulating cells or resident cardiac cell types. Candidate cells include cardiac fibroblasts, which are less prominent in zebrafish than in mammals; the endocardium and epicardium, cell layers lining the inside and outside, respectively, of cardiac chambers; nerves; vasculature; and inflammatory cells like macrophages. Genetic ablation of the epicardium revealed a necessity for injury-induced cardiomyocyte proliferation (47), implicating this tissue as a source of mitogens like NRG1, retinoic acid, and Bmp ligands, in addition to its proposed roles in vascularization (2). The mammalian epicardium also has roles in myocardial survival and vascularization, and has been implicated as a target for regeneration strategies (36). Macrophages have been implicated as a pro-regenerative influence in many contexts of tissue repair (48), and chemical ablation experiments indicate they are critical for heart regeneration in neonatal mice (49).

Recently, an additional model for heart regeneration was established, taking advantage of an oft-cited principle that tissue regenerative capacity declines with age. Regenerative capacity of the mouse heart, and presumably other mammals, is present at the fetal stage (32). More strikingly, it persists in the early neonatal period concomitant with massive hyperplastic cardiac growth, and then precipitously declines (3). Neonatal mouse heart regeneration is similar to zebrafish heart regeneration, involving cardiomyocyte dedifferentiation and proliferation. A notable difference is that injury-induced cardiogenesis occurs throughout the injured, growing organ in mice, whereas in hearts of mature zebrafish this response is focused at the injury site (2). Neonatal mice provide an informative model that can be dissected with advanced genetic tools, and investigations to date have bolstered the idea of a conserved heart regeneration program (Fig. 3).

Eliciting heart regeneration through cardiomyocyte proliferation

The accepted source of new heart muscle for the innate regenerative response is direct division of cardiomyocytes; yet, adult mammalian cardiomyocytes are famously non-proliferative. Several additional lines of evidence contribute a glass-half-full scenario to revive the idea of induced cardiomyocyte proliferation for therapeutic benefits. First, as indicated earlier, there is evidence for production of new cardiomyocytes in adult humans, and cell labeling experiments have suggested a low renewal rate and probable low-grade increase in division after injury in adult mice (33). Second, some reports support the idea of a rare proliferative subpopulation of murine cardiomyocytes, also referred to above. These cells might be mononucleate and diploid, and could have gene expression signatures or features like hypoxia that tip the balance toward division versus hypertrophy (34, 35). Third, several manipulations have been shown to boost the proliferation of adult cardiomyocytes and initiate an apparent regenerative response in the adult rodent heart (Fig. 4; reviewed in (2, 36)) (Box 2- To regenerate, or not to regenerate?).

BOX 2**To regenerate, or not to regenerate?**

Making an accurate determination of heart regeneration in mammals is challenging. MI injuries in mice typically involve a permanent left anterior descending (LAD) artery ligation, which reduces variability compared to a more clinically relevant ischemia/reperfusion model; yet, injury size can still range widely. Quantifiable recovery of ventricular function is often dependent on multiple factors and not necessarily related to regeneration versus, for instance, remodeling. Histological snapshots also can err in distinguishing regenerated cardiac muscle from pre-existing muscle, and scar quantification is indirect. Surrogates for regeneration include assays for markers of cardiomyocyte cell cycle phases or exceedingly rare markers of cytokinesis. However, estimating rates of cardiomyocyte cycling is difficult without the use of precise cell type-specific nuclear markers, plus cycling mammalian cardiomyocytes are much more likely to be endoreplicating rather than initiating division (13, 36). When injury-induced cardiomyocyte proliferation is so rare, even one or two misclassified events can alter an estimation of regeneration. Live tracking of dividing cardiomyocytes in the process of heart regeneration is technologically daunting, but use of retrospective multicolor clonal analysis tools and discovery of new markers tightly predictive of cardiomyocyte division should bolster what is currently available.

Neuregulin1 (NRG1), an EGF-like growth factor that binds ErbB4 and ErbB2 receptors, is critical for CM proliferation, maturation, and polarizing cell behaviors during development, and also for adult mammalian cardiac homeostasis (2, 36). Soluble recombinant NRG1 can stimulate a low level of cardiomyocyte proliferation in adult murine hearts after MI (35), although this finding is contested (37). NRG1 also stimulates overt hyperplasia in uninjured adult zebrafish hearts when expressed from a transgene (38), and increases cycling when applied to human neonatal and juvenile cardiomyocytes in vitro (39). In mice, the NRG1 co-receptor ErbB2 is limiting after day 7; however, transgenic expression of an activated ErbB2 receptor in cardiomyocytes extends the postnatal regenerative window after MI and promotes repair in adults (40). NRG1 isoforms have been explored as therapeutic agents for heart failure patients with some benefits reported several years ago (41), and again recently (42). Systemic NRG1 delivery might have limited or undesirable effects compared with combinatorial or more targeted delivery approaches.

Hippo signaling is a conserved pathway that restrains cellular proliferation to regulate organ size by phosphorylation of transcriptional co-activators YAP and TAZ. Cardiac YAP activity decreases during murine development, and genetic YAP augmentation in mouse cardiomyocytes stimulates their proliferation and causes cardiomegaly (43). Moreover, mice mutant for the Hippo pathway protein Salvador homolog1 (Sav1) or genetically overexpressing YAP in cardiomyocytes show evidence of enhanced cardiomyocyte proliferation and reduced scar size following adult MI, indicating this pathway as a viable target for regeneration (36).

Extracellular biomechanical cues like matrix rigidity that affect cytoskeletal integrity and sarcomere organization in cardiomyocytes might act upstream or downstream of the Hippo signaling cascade to influence proliferation (14, 44) (Fig. 4). The adult cardiac fibroblast population has the potential to regulate ECM deposition, which in turn may impact the survival or proliferative capacity of cardiomyocytes. Cardiomyocyte division might also be modulated by emergence at birth from relatively hypoxic conditions in utero to atmospheric oxygen. This transition has been described to involve numerous metabolic changes, including the accumulation of reactive oxygen species and DNA damage (Fig. 4). Recent studies have reported proliferative effects of experimental hypoxia on cardiomyocytes in vivo, making regulated hypoxia an intriguing idea to prod the regenerative response (45).

Conclusions and prospects

“The heart has its reasons that reason does not know,” mused Blaise Pascal in the 17th century. Why heart regeneration occurs naturally in certain contexts but not others defies reasoning at some level, and investigations to date have not yielded ingredients and methods to effect perfect cardiac repair. Yet, the field of cardiac regeneration has experienced a watershed moment - many or most now believe that latent regeneration machinery can be awakened even in adult mammals. Developmental biologists are defining mechanisms of innate and coerced regeneration, and stem cell biologists along with genetic and tissue engineers are putting into action ideas for novel therapeutic approaches to counter heart failure.

From the discovery side, a deeper understanding of the upstream and downstream regulators, as well as the gene regulatory elements that activate regeneration programs (46), will provide context and ever more starting materials. Stem cell-based methods for in vitro cardiomyocyte generation and maturation are progressing. Concurrently, just as newly formed cardiomyocyte products of cell division must couple with existing muscle during heart development and regeneration, methods to coax maturation and electromechanical incorporation of transplanted cardiomyocytes must also progress. Also required from the applied side are precise delivery materials and methodologies to trigger cardiomyogenesis, by activating accelerators or disrupting brakes with reprogramming factors or mitogens. If an old heart can be taught new tricks, the impact will resound.

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Figure 1. Implanting stem cell-derived cardiomyocytes for regeneration

ESC- or iPSC-derived cardiomyocytes are produced and expanded in vitro for delivery into the injured heart. Synthetic or natural scaffolds can assist engraftment of transplanted cardiomyocytes or used to stimulate endogenous repair mechanisms when transplanted alone.

Figure 2. Direct reprogramming: from scar to muscle

Methodology for direct cardiac programming uses combinations of known factors or small molecules to reprogram fibroblasts into cardiomyocyte-like cells. For genes or compounds to be effective in vivo, the appropriate vectors, factors, and drugs must be injected directly into the infarct in attempts to reprogram resident cardiac fibroblasts.

Figure 3. Current models for heart regeneration

(Top) Adult zebrafish regenerate cardiac muscle lost from resection of the ventricular apex (shown) or other injuries through cardiomyocyte proliferation. (Middle) Neonatal mice possess a regenerative response to cardiac injury (an MI model is shown including a suture), with compensatory proliferation that minimizes effects of the injury during a period of cardiac growth. (Bottom) Adult mice show minimal hyperplasia in response to an MI injury, which instead results in scarring.

Figure 4. Pathways to regeneration by cardiomyocyte proliferation

Pathways that promote division of cardiomyocytes can be targets for improving heart regeneration. Recent studies indicate that modulation of NRG1-ERBB signaling, Hippo-YAP signaling, hypoxia and reactive oxygen species, or influences on the dystrophin glycoprotein complex (DGC) and/or sarcomere organization can alter the limited proliferative response of cardiomyocytes (CM) in vivo. (Call out each feature here or in the text above or eliminate that detail. Now done)