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Myocardial Plasticity: Examples in Development, Regeneration and Disease

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Abstract

During a heart attack loss of blood flow and oxygen to regions of the heart results in the massive death of myocardial cells. Since, terminally differentiated adult myocardial cells proliferate at a very low rate the mammalian heart is unable to recover from the massive cell loss. This is in contrast to other animals such as adult zebrafish, axolotl, newts and mammalian neonates in which de-differentiation and proliferation facilitates regeneration after injury. Thus, a lack of myocardial plasticity and proliferation within adult mammalian myocardial cells results in insufficient repair after a myocardial infarction. Conversely, de-differentiation appears to be associated with the pathology of many chronic cardiomyopathies. Here we highlight studies from the developmental, regenerative and clinical cardiac fields which together provide insight into the molecular and conceptual underpinning of myocardial plasticity.

Introduction

Myocardial lineage and differentiation

Despite constituting only ~30% of heart, myocardial cells are the primary source of cardiac contractile function resulting in the efficient pumping of blood throughout the body [1,2]. The different myocardial sub-types are broadly divided into working myocardium responsible for blood flow (i.e. atrial, ventricular myocytes) and non-working myocardium responsible for the efficiency of blood flow (i.e. outflow tract, inflow tract and conduction myocytes) [3,4].

Myocardial cells are generated from a diverse set of lineages (for an in depth review see [3]). Two waves of mesodermal-derived cells during gastrulation, named first heart field (FHF) and second heart field (SHF) constitute the majority of the left ventricle (FHF), right ventricle (SHF), right and left atria (FHF/SHF) and outflow/inflow tracts (SHF)[5–8]. These contributions occur sequentially with myocardial cells derived from the FHF forming the outer layer of the primitive heart tube, which is then further expanded by the addition of late differentiating SHF cells [9,10]. A third wave closely associated with the SHF forms the Sino-atrial node (pacemaker) [11]. These cells are supplemented by a set of neural crest

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derived cells, which contribute to the septation of the heart [12]. Each lineage contributes to a specific anatomical set of myocardial cells. Yet, they contribute to an overlapping variety of myocardial sub-types, which are indistinguishable histologically[3,13].

Myocardial cells must maintain appropriate contractile function throughout the entire development and morphogenesis of the heart. As the embryo develops and grows into an adult, the requirements on the heart change dramatically requiring alterations in the number, size and efficiency of the myocardial muscle. As a result, myocardial cells dynamically differentiate during embryonic and fetal development to meet these functional demands, which include a metabolic switch from glycolysis to fatty acid metabolism, increases in cell size, diminished proliferative capacity, and changes in protein isoforms [14–23]

Many of these changes, such as loss of proliferative capacity in the adult mammalian heart, become detrimental during acute heart disease when there is a large loss of myocardial cells. In other animals such as the adult zebrafish, myocardial cells are able to respond to a similar injury by de-differentiating and proliferating [24]. De-differentiation has also been observed during chronic cardiac disease where it is associated with the progression of disease [25]. In this review, we bring together studies from cardiac development, regeneration and disease to summarize the role of myocardial plasticity in adaptive responses such as during regeneration as well as maladaptive responses such as during chronic cardiac disease.

(Each of these fields is extensive, thus we apologize in advance to our colleagues whose valuable work has been omitted due to space constraints. Further, we point the reader to more extensive reviews focused on each individual topic [3,25–29].)

Restriction of atrial and ventricular myocardial identity during development— Atrial and ventricular myocytes display dramatic biological differences; yet a series of genetic studies reveals that these differentiated myocytes are able to exchange their identities. Differences between atrial and ventricular myocytes can be observed in structural, physiological and molecular myocyte properties. Ventricular myocytes are rod-like in morphology with highly organized sacromeric structures, including specialized T-tubule sub-structures and also exhibit a flat action potential plateau. In contrast, atrial myocytes are more squamous with poorly developed, disorganized sacromeres and display a triangular shaped action potential with a shorter contraction and relaxation period [30–38].

These genetic studies have identified a set of counterbalancing transcription factors that maintain the identity of differentiated chamber myocytes by repressing the gene program of the opposing chamber (atrial or ventricular) and promoting their own chamber-specific gene program. For example, *COUP-TFII* is an ophraned nuclear hormone receptor that is expressed in atrial but not ventricular myocytes[39] where it functions to maintain atrial identity. Studies in mouse by Wu et al. show that conditional removal of *COUP-TFII* in the atrium using the *Myh6:cre* results in atria containing myocytes with ventricular identity. Conversely, forced ventricular expression of *COUP-TFII*, results in ectopic atrial fates [40**]. Thus, *COUP-TFII* is involved in restricting ventricular identity and promoting atrial identity. Microarray and ChiP-seq studies further revealed that *COUP-TFII* controls a large range of genes important for both atrial and ventricular identity including *Tbx5* (important

for promoting atrial identity[41–43]) and *hey2* (important for promoting ventricular identity [44]). Thus, *COUP-TFII* may act by repressing the ventricular transcriptional network while promoting the atrial expression program. Intriguingly, *COUP-TFII* restricts this atrial plasticity until e15.5, after which the *COUP-TFII* gene program is no longer needed to maintain chamber identity [40].

Conversely, the transcription factors *Nkx2.5*, *Hey2* and *Irx4* are important for maintaining ventricular chamber identity. *Nkx2.5* is expressed throughout the heart and is required for a range of functions during heart development including cardiac differentiation, conduction specification and septation [45–47] (reviewed-[48,49]). A recent set of studies in zebrafish have identified a new role for *Nkx2.5* in restricting the plasticity of ventricular cells. In the absence of *Nkx2.5* and its ortholog *Nkx2.7*, ventricular cells trans-differentiate into atrial cells leading to an enlarged atrium and a small ventricle without change in overall cardiac cell number [50,51]. These studies correspond with recent studies identifying plasticity in atrial lineages after *Amhc* expression [52] and with ventricular chamber defects in mammalian studies of *Nkx2.5* mutants [53–55].

Irx4 and *Hey2* are restricted to the ventricle, where loss and gain-of-function studies in the mouse reveal a similar exchange of ventricular identity [44,56–58]. However, unlike *Nkx2.5* mutants in zebrafish, distinct chambers are maintained despite ectopic expression of the atrial gene program within the ventricle of *Irx4* or *Hey2* mutants. The expression of *Irx4* and *Hey2* are lost in *Nkx2.5* mutants suggesting a hierarchy in which Nkx2.5 regulates Irx4 and Hey2 [50,59]. Furthermore, *Irx4* was shown to bind to *RxRa* and inhibit its binding to the *Myh6* (atrial specific myosin) promoter [60,61]. These results suggest that plasticity may be restricted by interacting with inductive pathways, such as Retinoic acid signaling. Atrial-ventricular antagonism also appears to occur at the level of myosin expression. As demonstrated by the ectopic ventricular expression of *MLC2a* in *MLC2v* mutants [62*]. Intriguingly, these studies of chamber plasticity are similar to Ebstein's anamoly a rare congenital heart defect in which a portion of the right ventricule is "atrialized" and to which mutations in *Nkx2.5* have been linked [63].

Separate from atrial-ventricular identity, another set of studies revealed that overexpression of the Notch-intercellular domain (NICD) or *Tbx18* in adult working myocardial cells can transform them into non-working conduction myocytes [64,65]. Whether there exists a regulatory network to suppress conduction identity in mature working myocytes or whether these experiments represent reprogramming events remains unclear.

Together these studies reveal a genetic network that regulates the ability of mature myocytes to exchange identities. Interestingly, these genes do not regulate all possible forms of plasticity but rather the transformation between atrial and ventricular states. The existence of a genetic network that regulates a specific identity transformation is similar to function of *Scl/Tal1* which inhibits endothelial cells from trans-differentiating to a myocardial lineage [66–68**]. The close proximity of these lineages (atrial/ventricular, endothelial/myocardial) and the existence of genetic programs that restrict their ability to trans-differentiate during development into each other suggests a possible fundamental property of proximal lineages. Indeed a system-level analysis of C. elegans lineages [69*], confirms the existence of

regulatory mechanisms that restrict the plasticity of proximal lineages to exchange identities. However, further experimentation is necessary to confirm this relationship and to elucidate the underlying mechanism of plasticity.

Myocardial plasticity during cardiac regeneration—While mammalian adult cardiac tissue does not undergo regeneration after injury[70,71*], the hearts of several amphibians and fish species have been shown to regenerate. Utilizing histological and SEM, classical studies in these animals observed cardiac cells near the site of injury which appear to undergo a process of de-differentiation (reduced sarcomere structure) and proliferation (increased incorporation of tritiated thymidine/BrdU and mitoses) [72–75*] [76*] similar to the phases of regeneration in other organs [77]. However, the question of whether new myocardial tissue is generated from an unknown stem cell population, the transdifferentiation of other cell types, or un-injured mature myocardial cells was unknown. Using cre-lox lineage tracing to mark and track myocardial cells, studies of adult zebrafish after ventricular injury revealed that new myocardial cells are generated almost exclusively from pre-existing differentiated myocardial cells[78,79**], which undergo a de-differentiation and proliferative response. During de-differentiation, myocardial cells display disorganized sarcomeric structures [80] as shown in earlier SEM studies and also express genes from embryonic development including fetal myosin genes and transcription factors such as *Gata4*, *Tbx20*, *Tbx5*, *Nkx2.5* and *Hand2* [79,81,82].

Many of these transcription factors are important for cardiogenesis during development. For example, the *Gata 4-6* and *Hand* transcription factors are essential for de-novo cardiac differentiation [83–91] and are part of the cocktail of factors sufficient to reprogram fibroblasts into cardiomyocytes [92–96]. Inhibition of *gata4* during cardiac injury in the adult zebrafish, reduces the generation of new myocardial cells leading to scar formation[97]. Similarly, over-expression of *Hand2* during cardiac injury increases myocardial proliferation after injury. [81]. However, their exact role in the processes of de-differentiation and re-differentiation remain to be elucidated.

A number of studies have begun to elucidate upstream signals responsible activating myocardial de-differentiation (reviewed here [28]). *NfKb*, BMP, RA, Shh, Pdgf, reactive oxygen, Paxillin have all been shown to be required for the activation of *gata4* and other embryonic genes [98–106]. Intriguingly, some signals (Notch, Igf) appear to be required for proliferative activity, but not the re-expression of this embryonic cardiac program [107,108], suggesting these signals may act in parallel or downstream. Of note are the studies focusing on Neuregulin signaling, which is upregulated in perivascular cells upon injury. Gemberling et al. show that Neuregulin signaling is not only necessary but also sufficient to trigger de-differentiation and proliferation, suggesting it provides an instructive signal to stimulate a regenerative response in myocardial cells upon injury [109**].

Intriguingly, studies of zebrafish larval hearts revealed a trans-differentiation response within atrial cells after ventricular injury. Larval hearts contain differentiated cardiomyocytes with highly organized sarcomeric structures [17,110]. When larval ventricular myocytes are ablated using nitroreductase and metrodinazole [111], a de-differentiation and proliferative response is induced in both ventricular and atrial myocytes. Genetic marking of

differentiated atrial cells revealed that atrial cells de-differentiate, migrate and then re-differentiate as ventricular myocyte, helping to repopulating the injured ventricle. Although, the molecular mechanisms underlying this atrial response still remain to be fully elucidated, initial studies indicate a requirement for Notch signaling within the endocardium [110**].

This trans-differentiation response of larval atrial cardiomyocytes to ventricular injury contrasts with adult injury, in which atrial cardiomyocytes have not been shown to contribute to the ventricle. Indeed the atrial trans-differentiation response diminishes with age [110]. However, adult atrial myocytes do retain a degree of plasticity; the adult zebrafish atrium regenerates after injury and adult newt and rat atrial cells re-enter the cell cycle upon ventricular injury [80,112–115]. Future investigations into the intrinsic and extrinsic differences between adult and larval atrial cells are likely to be informative in understanding the regulation of myocardial plasticity.

Neo-natal Mammalian Regeneration

Despite the intransgience of mammalian adult hearts to regenerate, observations from human case studies [116–122] and mouse studies of cardiac damage during development [123–125] have suggested that embryonic and neonatal mammalian hearts might possess regenerative potential. This hypothesis has been recently tested, with several researchers showing that neonatal hearts (P1) are able to regenerate upon injury but fetal (>P7) and adult hearts can not [123,126–129] (further reviewed here [24,29]). These studies reveal striking parallels between neonatal heart regeneration and adult zebrafish heart regeneration, including the generation of new myocytes from the de-differentiation and proliferation of pre-existing mature myocytes [129] and the critical role of the re-expression of embryonic transcription factors such as *Gata4* [130]. Additionally, Neuregullin signaling plays a pivotal role in both adult zebrafish and neonatal mouse regeneration. Addition of neuregulin stimulates proliferation in neonatal cardiomyocytes, but not after P7 due to the downregulation of the neuregulin receptor, *Erbb2*. However, a transient ectopic *Erbb2* signaling pulse in juvenile animals is sufficient to stimulate de-differentiation and proliferation [131**]. Further studies exploring whether changes to *Erbb2* expression is the only event that mediates the regenerative capacity of P1 compared to P7 animals will help to elucidate what portions of the regenerative program remain poised in adult cardiomyocytes.

Myocardial plasticity during pathological cardiac remodeling—De-differentiation has also been shown to occur during acute and chronic cardiac disease (reviewed here [25]). For example, de-differentiation is observed in the ventricle of human patients after acute myocardial injury [132,133] and in animal models of cardiac injury, such as pressure-overload in rats and rabbits [132,134]. Chronic cardiac diseases in humans such as dilated cardiomyopathies and mitral valve disease [135–137] as well as animal models such as MCP-1 also feature myocardial de-differentiation [138].

Myocardial de-differentiation during cardiac disease is characterized by changes from a highly organized striated sarcomeric structure to a less dense disorganized complex, [137] along with metabolic and molecular changes reminiscent of fetal cardiomyocytes. For example, myocardial cells in a diseased hearts switch from fatty acid metabolism, used

almost exclusively in adult myocytes, to the glycolytic pathway used in fetal myocytes [139]. Furthermore, fetal gene isoforms expressed during development are re-expressed during cardiac disease [137,140–142] as are transcription factors associated with early cardiac development, such as *Gata4*, *Nkx2.5* and *Mef2c* [143–147] (reviewed here [27,148]).

Several studies have begun to investigate the role embryonic cardiac transcription factors have on disease progression. For example, loss of Gata4 in adult cardiomyocytes attenuates TAC-induced hypertrophy, indicating an essential role for Gata4 in a pathological hypertrophic response[149]. Gata4 is expressed in adult cardiomyocytes where its activity is upregulated in response to pathological hypertrophy[150]. Loss of *Gata4* as well as gain of *Gata4* activity in adult cardiomyocytes leads to pathological hypertrophy indicating that a proper balance of *Gata4* activity is required to limit hypertrophy [149,151]. Similarly, *Mef2* activity is upregulated during pressure overload [147,152–154], where it is implicated in promoting hypertrophy [155,156]. These studies indicate an integral role for de-differentiation in the pathology of cardiac remodeling. Future studies investigating how de-differentiation is triggered and how it contributes to cardiac disease are likely to be highly informative for therapeutic interventions.

One of the mechanisms through which de-differentiation could be triggered is through inflammatory cytokines which are prevalent during cardiac disease. Expression of the inflammatory cytokine, oncostatin M (*OSM*) occurs in both patients who have suffered a myocardial infarct (MI) or who have dilated cardiomyopathy (DCM)[157**]. Studies by Kubin et al. show that exposure of adult rat myocardial cells to *OSM in vivo* and *in vitro* induces MAPK-dependent de-differentiation and EdU-incorporation. Interestingly, *OSM* was found to have opposing affects on models of MI and DCM. *OSM*-addition increased survival upon MI, but decreased survival in the chronic MCP-1 model of DCM [158][157]. Additionally, Neuregulin signaling has also been shown to stimulate de-differentiation and proliferation in adult cardiomyocytes [131,159], indicating that multiple signaling sources may stimulate de-differentiation during cardiac disease.

Future perspectives—Despite the identification of multiple factors that trigger or restrict myocardial plasticity, we still lack a complete understanding of how this plasticity is regulated or its role in cardiac disease. Epigenetic regulation such as chromatin remodeling represents a logical hypothesis for how myocardial plasticity is restricted and reactivated. Chromatin remodeling factors have been shown to be essential for cardiac differentiation [91,160], reprogramming [96,161] and cardiac disease[162,163]. Thus, identification of chromatin states and chromatin modulators essential for plasticity processes such as de-differentiation and trans-differentiation is likely to be essential for understanding these changes. These ongoing studies of myocardial plasticity represent the forefront of an exciting cross-disciplinary field important for a fundamental understanding of cell identity and for developing reparative therapies.

Acknowledgments

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Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

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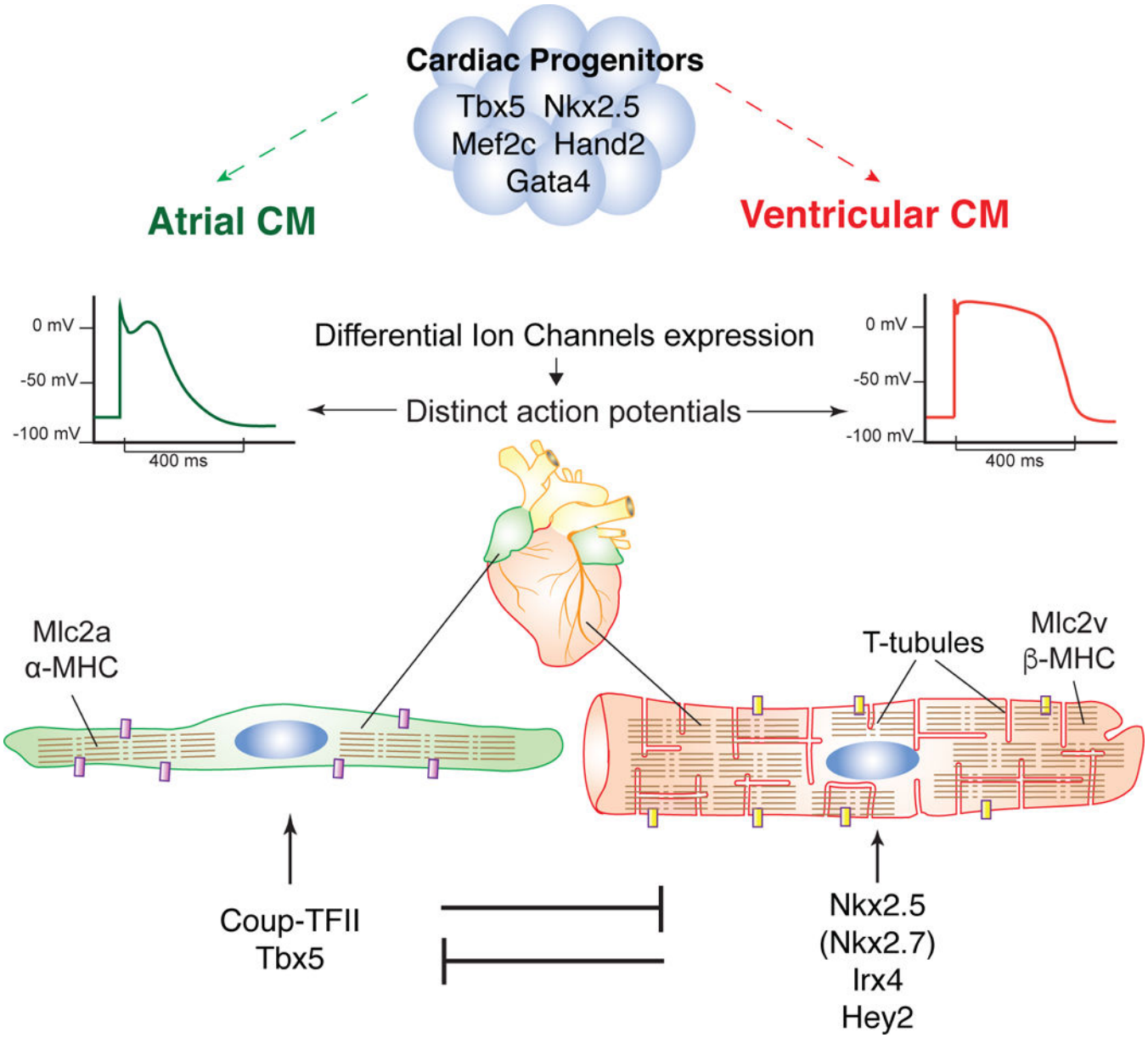

Figure 1. Restriction of atrial and ventricular identity

(A) Atrial (green) and ventricular (red) cells arise from distinct yet overlaping sets of cardiac progenitors, characterized by the expression of transcription factors such as *Tbx5, Nkx2.5, Mef2c, Hand2* and *Gata4.* (B) Atrial and ventricular myocytes display distinct physiological, structural and molecular differences. For example, ventricular cardiomyocytes display a flatter action potential plateau, have specialized T-tubule structures and express different gene variants e.g. *Mlc2v/Mlc2a*, *β-MHC/α-MHC.* (C) Atrial and ventricular myocytes each express a mutually exclusively transcriptional program that continues to promote their own identity and suppresses the other.

CM: Cardiomyocyte, Purple/Yellow block: different ion channels, Segmented lines represent sacromeres.

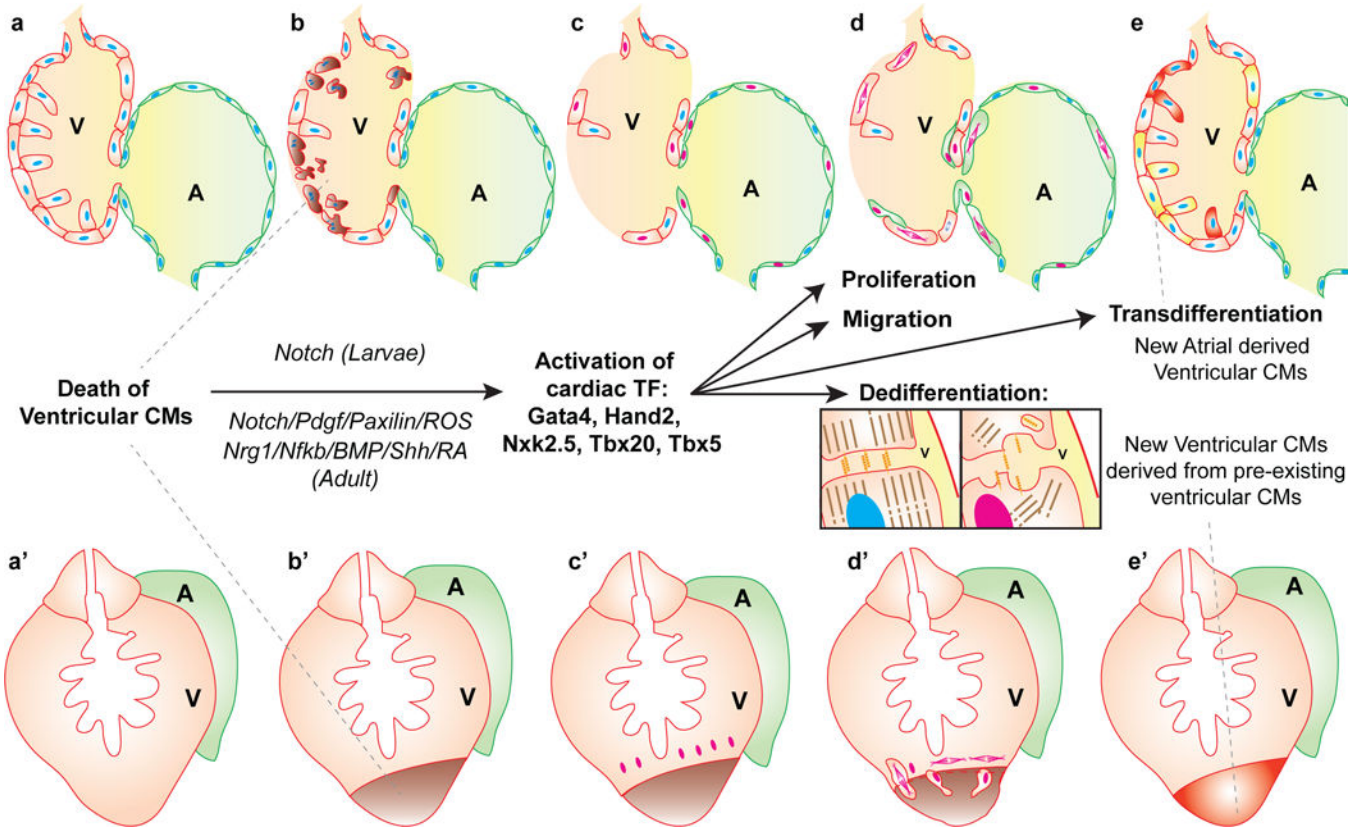

Figure 2. Myocardial changes after cardiac injury in larval and adult zebrafish

Larval (a-e) and adult (a′-e′) zebrafish hearts consist of a single ventricular (red) and atrial (green) chamber. After injury (b, b′) pre-existing mature cardiomyocytes de-differentiate, re-activate earlier cardiac transcription factors (red nuclei) (c, c′) and proliferate (d, d′) to contribute new cardiomyocytes to the injured area (e,e′). In larvae atrial cardiomyocytes also respond to injury by dedifferentiating, migrating and then transdifferentiating to a ventricular fate (Yellow cells, c-e). De-differentiation involves the disassembly of sacromeres and cell-cell contacts (inset).

V: Ventricle, A: Atrium, Blue and red nuclei: differentiated and de-differentiated nuclei, respectively.

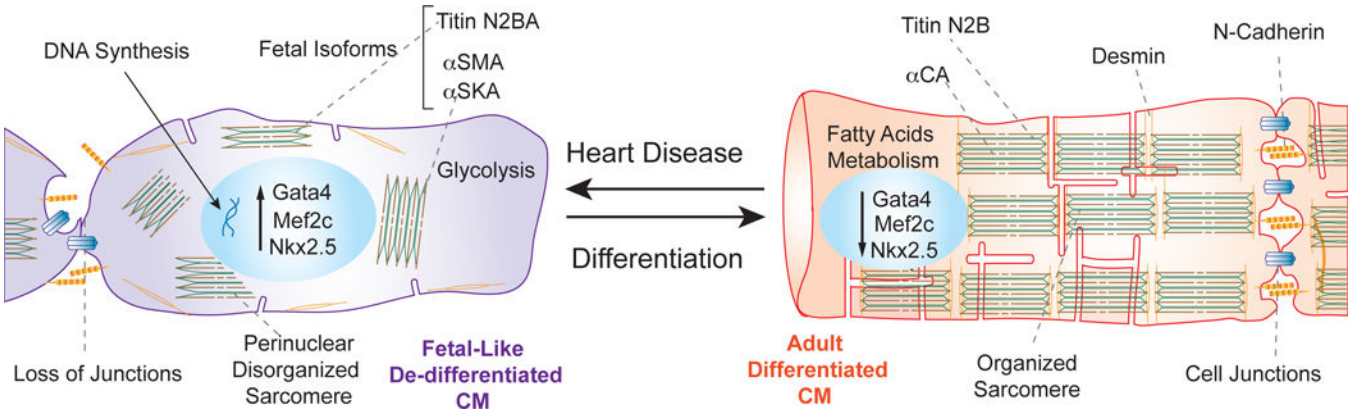

Figure 3. Schematic contrasting the different properties of de-differentiated and mature cardiomyocytes

De-differentiated cardiomyocytes (purple) associated with cardiac disease display fetal-like characteristics compared to a mature adult cardiomycoyte (red). These fetal-like characteristics include a metabolic change to glycolysis, the activation of early cardiac transcription factors such as *Gata4*, *Mef2c, Nkx2.5* and the activation of fetal specific isoforms such as Titin N2BA (green), αSMA, αSKA. Additionally, Desmin (orange) is re-localized in de-differentiated cardiomyocytes and sarcomeres are not as highly organized. CM: Cardiomyocytes,