Original of Cardiomyocytes in the Adult Heart
Annarosa Leri, Marcello Rota, Francesco S. Pasqualini, Polina Goichberg, Piero Anversa

Abstract: This review article discusses the mechanisms of cardiomyogenesis in the adult heart. They include the re-entry of cardiomyocytes into the cell cycle; dedifferentiation of pre-existing cardiomyocytes, which assume an immature replicating cell phenotype; transdifferentiation of hematopoietic stem cells into cardiomyocytes; and cardiomyocytes derived from activation and lineage specification of resident cardiac stem cells. The recognition of the origin of cardiomyocytes is of critical importance for the development of strategies capable of enhancing the growth response of the myocardium; in fact, cell therapy for the decompensated heart has to be based on the acquisition of this fundamental biological knowledge. (Circ Res. 2015;116:150-166. DOI: 10.1161/CIRCRESAHA.116.303595.)

Key Words: cell dedifferentiation  ■  cell transdifferentiation  ■  stem cells

Several organs are characterized by dividing and nondividing cells. Nondividing cells can rest in G0 and re-enter the cell cycle on stimulation or become terminally differentiated and die at the end of their lifespan without further division. This latter cell population is not dormant in G0; it has lost permanently the ability to replicate and generate cells with identical properties. For several decades, cardiomyocytes have been considered to belong to this cell category. The notion that myocytes cannot divide originated from the difficulty of identifying mitotic nuclei and from the negligible level of DNA synthesis measured by $^3$H-thymidine incorporation. The lack of DNA replication and the failure to recognize mitotic cells has led to the conclusion that myocyte renewal is absent in the adult heart. The dogma was introduced that adult cardiomyocytes are terminally differentiated cells, which are irreversibly withdrawn from the cell cycle. These cells are unable to proliferate but can perform their physiological functions, undergo cellular hypertrophy, and ultimately die by apoptosis or necrosis.

The widely accepted paradigm is that the heart is an organ characterized primarily by a fixed number of myocytes, which is maintained throughout life until death of the organism. The turnover of structural proteins has been considered the mechanism involved in the preservation of myocyte performance and the youth of the cell. Differences with this philosophy have been rejected as scientifically incorrect or the product of technical errors. The most common argument brought forward against the regenerative potential of the myocardium is that the heart does not repair itself after infarction. The statement must age at the same pace and, at any given time, the heart should be composed largely of a homogeneous population of cells of identical age. Because of this assumption, the principle of cellular senescence has never been applied to the heart. This process reflects the close relationship between number of cell divisions, telomeric shortening, oxidative stress, and replicative senescence in vivo. A mitotic clock regulates the lifespan of cells, which is independent from organ and organism age and lifespan. The heterogeneity in the properties of myocytes, together with evidence in favor of the regeneration of the young, adult, and aged myocardium, has questioned the conventional concept of myocardial biology and offered a novel perspective of the growth dynamics of the heart and its myocyte compartment.

The recognition that myocyte apoptosis and myocyte necrosis are natural components of the wear and tear of the organ, and increase dramatically with age and cardiac pathologies, has raised the challenging question concerning the origin of the newly formed cardiomyocytes needed for the preservation of the structure and function of the myocardium. There are 5 possibilities that have to be considered as potential sources of cardiomyocytes in the adult heart (Figure 1; Table): (1) cardiomyocytes are not postmitotic terminally differentiated cells parenchymal cells proliferate, the outcome of infarction is identical in all organs including the bone marrow, the testis, the skin, the kidney, the brain, and the intestine. In these self-renewing organs, stem cells do not normally migrate and home to the damaged area replacing the infarcted tissue.

According to the traditional view, the age of myocytes corresponds to the age of the organ and organism. All cardiomyocytes must age at the same pace and, at any given time, the heart should be composed largely of a homogeneous population of cells of identical age. Because of this assumption, the principle of cellular senescence has never been applied to the heart. This process reflects the close relationship between number of cell divisions, telomeric shortening, oxidative stress, and replicative senescence in vivo. A mitotic clock regulates the lifespan of cells, which is independent from organ and organism age and lifespan. The heterogeneity in the properties of myocytes, together with evidence in favor of the regeneration of the young, adult, and aged myocardium, has questioned the conventional concept of myocardial biology and offered a novel perspective of the growth dynamics of the heart and its myocyte compartment.

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and can re-enter the cell cycle and divide; (2) cardiomyocytes dedifferentiate in vivo reacquiring a primitive cell phenotype and then multiply; (3) cardiomyocytes derive from the engraftment and commitment of circulating hematopoietic stem cells (HSCs); (4) cardiomyocytes constitutes the progeny of resident cardiac stem cells (CSCs), which control cell turnover physiologically and cardiac repair after injury; and (5) cardiomyocytes are generated by a combination of these 4 cellular mechanisms. The recognition of the origin of cardiomyocytes is of critical importance for the development of strategies capable of enhancing the growth response of the myocardium; in fact, cell therapy for the decompensated heart has to be based on the acquisition of this fundamental biological knowledge.

This article discusses information obtained in humans and animals in favor or against each of these potential origins of myocytes.

**Myocyte Regeneration in Humans: Complexity of the Problem**

The possibility of taking advantage of the growth reserve of the myocardium to improve the management of human heart failure (HF) clinically requires understanding of the level of cardiomyocyte regeneration occurring in the intact and damaged organ.

Difficulties exist in the evaluation of myocyte proliferation by various methodologies. Ideally, in the absence of cell death, the measurement of the number of ventricular myocytes would seem to be the only approach that can demonstrate unequivocally the degree of myocyte cellular hyperplasia. However, the concomitant presence of widespread myocyte loss, together with multiple foci of myocardial injury and tissue scarring, complicates this type of analysis resulting in an underestimation of the extent of cell replacement in the decompensated heart.

Several reports have suggested that ventricular myocytes in humans re-enter the cell cycle and synthesize DNA. But whether DNA replication leads to the formation of ploidy, karyokinesis in the absence of cytokinesis, or to karyokinesis followed by cytokinesis has been a matter of intense debate. Although morphometric studies have shown that myocyte regeneration occurs in the severely hypertrophied heart with no changes in the proportion of mononucleated and binucleated cells, mitotic figures in myocytes have been difficult to identify. The lack of a direct documentation of dividing myocytes has been raised frequently as a critical argument questioning the validity of these morphometric results. The first demonstration of the localization of the proliferating cell nuclear antigen in human fetal and adult cardiomyocytes and the identification of myocyte mitotic images in routine histology sections was obtained in the mid-1990s (Figure 2); the mitotic index of myocytes and interstitial cells was also measured, providing an estimate of this phenomenon.

**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>α-MHC</td>
<td>α-myosin heavy chain</td>
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<tr>
<td>BMPCs</td>
<td>bone marrow progenitor cells</td>
</tr>
<tr>
<td>CSCs</td>
<td>cardiac stem cells</td>
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<tr>
<td>HF</td>
<td>heart failure</td>
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<tr>
<td>HSCs</td>
<td>hematopoietic stem cells</td>
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<td>OCM</td>
<td>oncostatin M</td>
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Figure 1. A to D. Potential mechanisms of cardiomyogenesis. See text. α-SA indicates α-sarcomeric actin; ANP, atrial natriuretic peptide; HSC, hematopoietic stem cells; and SM, smooth muscle.
Subsequent studies with more sophisticated techniques have acquired evidence of replicating cardiomyocytes and myocyte karyokinesis and cytokinesis. The implementation of immunolabeling and confocal microscopy has improved significantly image resolution and the accuracy of the quantitative data. Although routine histology was unable to detect with certainty mitotic images in cardiomyocytes of normal hearts, the expression of cell cycle proteins, nuclear mitotic division, and myocyte cell cycle proteins were identified by immunolabeling and confocal microscopy.28–31

Whether the fraction of replicating myocytes or the myocyte mitotic index is evaluated, these snap-shot results can be combined with the number of cardiomyocytes present in the ventricle and an approximate length of the cell cycle and mitosis to appreciate the actual magnitude of the process.28–31 When this type of analysis is introduced, cardiomycyte regeneration seems to be relatively high, suggesting that potentiation of this mechanism of cardiac recovery may have significant effects on the restoration of cardiac function after myocardial injury. However, the measured values are relatively small and it is difficult to extrapolate from these data the degree of myocyte formation occurring over time (Table). Although these factors have to be acknowledged, evidence accumulated in the past 15 years is consistent with the view that myocyte renewal is a relevant component of cardiac homeostasis and repair of the human heart.16,19

Myocyte Regeneration in Animals: the Controversy

The general opinion is that myocardial hypertrophy in the adult rodent heart is accomplished by enlargement of pre-existing cardiomyocytes with no cell proliferation. Studies aiming at the identification and quantification of DNA replication in the overloaded myocardium have documented none, little DNA synthesis in myocyte nuclei possibly representing polyploidy2,32 or significant DNA synthesis consistent with de novo formation of cardiomycocytes.1,33–36

Mitotic figures in myocytes have also been shown.37–39 Myocyte restoration occurs under a variety of experimental conditions characterized by a large and prolonged stress on the heart; they include myocardial infarction, nonocclusive coronary artery constriction, pressure overload, and aging.1,34–41 Similar findings have been found in larger animals.1,16,19

The impairment in cardiac performance seems to be critical for the activation of myocyte proliferation. Abnormal increases in diastolic wall stress promote myocyte proliferation, whereas changes in systolic stress have a lesser impact on this cellular process.37 The mechanism by which diastolic load triggers the formation of myocytes is currently unknown; however, alterations in ventricular compliance and sarcomere stretching have multiple effects on the synthesis and secretion of growth factors and cytokines implicated in cell viability, growth, and contractile behavior.42 The activation of

### Table. Levels of Myocyte Regeneration and Cell of Origin

<table>
<thead>
<tr>
<th>Myocyte Source</th>
<th>Extent of Myocardial Regeneration In Vivo</th>
<th>Assessed By</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Mitotic myocytes</td>
<td>0.12% of total myocytes</td>
<td>Presence of dividing myocytes in wild-type control groups</td>
<td>48; Figure 5</td>
</tr>
<tr>
<td></td>
<td>Negligible</td>
<td>Qualitative observation of Azan staining</td>
<td>49; Figure 1</td>
</tr>
<tr>
<td></td>
<td>Negligible</td>
<td>Presence of dividing myocytes in untreated control groups</td>
<td>50; Figure 3</td>
</tr>
<tr>
<td></td>
<td>0.01% of total myocytes</td>
<td>Presence of dividing myocytes in untreated control groups</td>
<td>51; Figure 3</td>
</tr>
<tr>
<td>Dedifferentiated myocytes</td>
<td>Undocumented. 43% of myocytes dedifferentiated</td>
<td>Presence of cells exhibiting characteristic of hibernating myocardium</td>
<td>68; Figure 2</td>
</tr>
<tr>
<td></td>
<td>Undocumented</td>
<td>Presence of markers of fetal-like gene expression program</td>
<td>69; Figure 4</td>
</tr>
<tr>
<td>Bone marrow cells</td>
<td>68% of infarcted area at day 9</td>
<td>Presence of sex-mismatched myocytes</td>
<td>70; Figure 1</td>
</tr>
<tr>
<td></td>
<td>Consistent with ref 66</td>
<td>Presence of sex-mismatched myocytes</td>
<td>88; Figure 4</td>
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<tr>
<td></td>
<td>Negligible</td>
<td>Absence of myocytes carrying ubiquitous fluorescent reporter</td>
<td>97; Table 1</td>
</tr>
<tr>
<td></td>
<td>Negligible</td>
<td>Absence of myocytes carrying ubiquitous or myocyte-restricted fluorescent reporter</td>
<td>98; Table 1</td>
</tr>
<tr>
<td>Cardiac stem cells</td>
<td>10% and 40% of total myocytes in young and old, respectively</td>
<td>Detection of dividing myocytes after 12-wk chase period</td>
<td>34; Figure 1</td>
</tr>
<tr>
<td></td>
<td>15% of total myocytes</td>
<td>Detection of dividing myocytes after 10-wk chase period</td>
<td>112; Figure 5</td>
</tr>
<tr>
<td></td>
<td>6% of total myocytes in old hearts untreated</td>
<td>Detection of dividing myocytes after 8-wk chase period</td>
<td>40; Figure 8</td>
</tr>
<tr>
<td></td>
<td>13%–22% of infarcted area after 10–20 days</td>
<td>Detection of differentiated cardiac progenitor cells</td>
<td>107; Figure 1</td>
</tr>
<tr>
<td></td>
<td>25% of infarcted area when using engineered cells</td>
<td>Detection of virally tagged myocytes</td>
<td>123; Figure 4</td>
</tr>
<tr>
<td></td>
<td>33% of infarcted area when using engineered cells</td>
<td>Detection of virally tagged myocytes</td>
<td>132; Figure 4</td>
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<tr>
<td></td>
<td>4% and 8.5% of total myocytes after isoproterenol damage from endogenous and injected progenitors, respectively</td>
<td>Detection of lineage-tagged myocytes</td>
<td>47; Figure 2</td>
</tr>
<tr>
<td></td>
<td>0.0055%–0.016% of total myocytes</td>
<td>Detection of lineage-tagged myocytes</td>
<td>161; Figures 2 and 3</td>
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Examples of the most updated studies on myocyte regeneration in normal homeostasis, after injury and with cell therapy. For a complete and critical overview of the older work on the subject, see Refs. 1 and 2.
autocrine/paracrine signals may influence dramatically the fate of cardiomyocytes.

Lineage tracing studies in mice have been performed in an attempt to measure the rate of myocyte renewal in the adult and old heart. By this genetic approach, myocyte formation was found to be minimal at baseline increasing modestly with pathological overloads. These results, however, are inconsistent with the magnitude of myocyte death occurring in the adult and aging mouse heart. The identification of cardiac troponin in the circulation has to occur for the heart to perform its function. If myocyte turnover is inconsequential, cardiomyocytes should share common characteristics. But this is not the case. Telomere attrition and the nuclear localization of p16, indicative of cellular aging, are not distributed uniformly in cardiomyocytes. The intrinsic mechanisms regulating myocyte regeneration have to occur for the heart to perform its function. If myocyte turnover is inconsequential, cardiomyocytes should share common characteristics. But this is not the case. Telomere attrition and the nuclear localization of p16, indicative of cellular aging, are not distributed uniformly in cardiomyocytes.

Similarly, apoptosis and the level of myocyte regeneration are conditioned by telomere length (Figure 3). Chronological age and cellular age do not coincide, but cellular age and cell death do coincide, although not all p16-positive myocytes undergo apoptosis or necrosis. Cardiac-restricted overexpression of insulin-like growth factor-1 (IGF-1) positively interferes with these negative variables of myocyte aging (Figure 4A–4C).

Thus, the heart is composed of a heterogeneous myocyte population undergoing constant death and renewal, a possibility in harmony with the maintenance of a dynamic steady state of the myocardium. Myocyte formation is substantial and plays a role in tissue homeostasis and in the response to aging and hemodynamic stress. The intrinsic mechanisms regulating myocyte regeneration are necessary and sufficient to preserve the integrity of the adult organ. Myocyte growth, however, fails to meet the demands of the old heart and, as in humans, an aging myocardium develops. Similarly, the partial restoration of myocytes after ischemia or increases in pressure and volume loads cannot prevent cardiac dysfunction and HF in rodents and patients.

**Cardiomyocytes Are Not Terminally Differentiated Cells**

Based on the assumption that a pool of mature ventricular myocytes synthesizes DNA and divides, mitogenic stimulation has been attempted by interfering with inhibitors of the cell cycle or overexpressing components of the replicating machinery. The retinoblastoma protein regulates the postmitotic myocyte phenotype, but cardiac-specific conditional deletion of retinoblastoma protein in transgenic mice does not increase the number of proliferating cells. Double deletion of retinoblastoma protein and p130 are required to synergistically upregulate Myc, E2F-1, and G1 cyclin-dependent kinases and expand the compartment of myocytes in S-phase and mitosis (Table). Early findings suggesting that cyclin D downregulation is implicated in the acquisition of the permanent dormant state of cardiomyocytes have prompted the development of transgenic mice overexpressing cyclin D1, D2, or D3 under the control of the myocyte-restricted α-myosin heavy chain (α-MHC) promoter. However, only mice overexpressing cyclin D2 show an increase in 3H-thymidine labeling in myocytes after infarction, leading to a significant reduction in scar size. These findings led to the interpretation that cell proliferation can be induced in mature myocytes by overexpression of cyclin D2.

Along the same principle, the growth factor neuregulin has been claimed to trigger myocyte regeneration physiologically and in the infarcted heart. The interaction of neuregulin with its tyrosine kinase receptor, ErbB4, may change the phenotypic properties of mature cardiomyocytes reactivating the cell cycle. This growth reserve, however, is confined to mononucleated cells, which comprise <10% of the myocyte population. Similarly, peristin, which stimulates integrins located on the plasma membrane of cardiomyocytes and the phosphatidylinositol-3-OH kinase pathway, promotes re-entry of mononucleated myocytes into the cell cycle. The relevance of peristin for cardiac hypertrophy has also been reported. Inhibition of p38 MAP kinase enhances myocyte renewal by reversing the postmitotic state. A defect in anillin localization at the midbody of dividing cells may be responsible for myocyte binucleation, ploidy, and lack of cytokinesis. Blockade of p38 MAP kinase reverses the process and reconstitutes cell division. Similarly, the delivery of cyclin A2 seems to trigger myocyte proliferation.
The conclusions reached in these mouse models are problematic. The examples of replicating myocytes given in these reports do not reflect the criteria dictated by the biology of the cell cycle. Adult myocytes measure \( \approx 20,000 \) to \( 25,000 \) \( \mu \text{m}^3 \); if these cells re-enter the cell cycle and divide, they have to acquire in mitosis a volume of \( \approx 40,000 \) to \( 50,000 \) \( \mu \text{m}^3 \) before 2 daughter cells of \( \approx 20,000 \) to \( 25,000 \) \( \mu \text{m}^3 \) each are formed.

Replicating myocytes in rodents are small, rarely binucleated, and vary in volume from 1500 to 7000 \( \mu \text{m}^3 \) (unpublished data); when binucleated myocytes enter the cell cycle, both nuclei are in mitosis and in a rather synchronized manner.38 In humans, dividing myocytes are predominantly mononucleated; thus far, replicating binucleated myocytes were not detected in the female or male human heart. In both animals and humans, myocyte cytokinesis has been shown by the localization of aurora B kinase at the duplicated sets of telo- phase chromosomes and cleavage furrow of the dividing cells. The forced re-entry of postmitotic myocytes into the cell cycle results in abortive mitosis and apoptosis.56 Overexpression of cell cycle genes favors the progression of cardiomyocytes from G\(_1\) to G\(_2\) and subsequently to the S-phase. But cells do not traverse the G\(_2\)/M checkpoint and divide; the arrest in G\(_2\)/M leads to cellular enlargement, senescence, and death.56

Hypertrophied, senescent myocytes attempt to proliferate but cannot complete successfully cell replication. These myocytes may generate chromosomal bridges (Figure 4D; courtesy of Dr Shao-Min Yan and Prof Alberto C. Beltrami, University of Udine, Italy). Accumulation of G-rich single-stranded DNA fragments, together with downregulation of the telomeric binding proteins TRF1 and TRF2, results in telomere erosion and formation of intercellular anaphase bridges. These cells possess properties that support the illegitimate rejoining of DNA broken ends: critical shortening of telomeres, telomere uncapping, and foci of phosphorylated histone H2AX.57 Chromosomal instability precludes completion of mitosis.

Death of adult cardiomyocytes in response to mitogenic signals occurs after the intramyocardial delivery of adenoviruses carrying the cell cycle regulator E2F-1.56 Expression of E2F-1 induces G\(_1\) exit and massive apoptosis, a process viewed as a repressive control against the inappropriate cell cycle re-entry of quiescent postmitotic myocytes. The intramyocardial injection of E2F-1 in p53 null mice stimulates DNA synthesis and the accumulation of myocytes in G\(_2\)/M; however, E2F-1 triggers apoptosis and rapid mortality of the animals. These findings are consistent with a cooperative role of p53 and the pocket protein family in maintaining myocyte quiescence in the adult heart.56

**Cardiomyocyte Dedifferentiation and Replication**

Changes in the phenotypic characteristics of cells contribute to the regenerative reserve of plants, invertebrates, teleost fishes, and amphibians. Cell dedifferentiation is a common phenomenon in plants.58 The main function of mesophyll cells in a leaf is to facilitate and perform photosynthesis. By the process of dedifferentiation, mesophyll cells can reacquire a primitive state, and the plasticity of plant protoplasts is reminiscent of the totipotency of embryonic stem cells.59 Plant protoplasts form calli, a mass of unorganized parenchyma cells, from which roots grow and generate entire plants.

In vertebrates, mostly in amphibians and fishes, quiescent specialized cells can revert their phenotype and assume the properties of replicating progenitors.70 These progenitors multiply and differentiate restoring the lost anatomic structure. When the morphological and functional integrity is reinstated,
progenitor cells lose their function. The repair mechanism originates from the formation of a mass of multipotent progenitors or different progenitors with restricted regeneration potential, that is, the blastema, from which the entire structure is regrown. The process that governs the replacement of anatomic parts is termed epimorphic regeneration. Conversely, stably committed mammalian cells are thought to be unable to dedifferentiate into stem cells. However, there are some exceptions; hepatocytes may revert their specific function, enter the cell cycle, and proliferate following partial hepatectomy. Similarly, airway epithelial cells can dedifferentiate into basal primitive cells, and exocrine pancreatic cells may change their intrinsic regulatory properties and form \(\beta\)-cells synthesizing insulin.

An interesting model of spontaneous myocyte proliferation with reconstitution of the injured heart is found in lower vertebrates such as zebrafish. In this organism, the myocardium retains an extraordinary capacity for regeneration throughout life, a process that results in minimal residual tissue scarring of the organ. The replicating cardiomyocytes, however, have an immature phenotype raising the question whether they constitute a pool of cells with the inherent ability to replicate or dedifferentiated cells, which have reacquired the propensity to multiply. In addition, whether the zebrafish heart contains a compartment of resident progenitors that acquire the cardiomyocyte lineage and contribute to cardiac repair remains to be determined. A similar phenomenon is observed in the neonatal mouse heart where the resection of the apical region can be efficiently restored by the ability that the spared cardiomyocytes have to re-enter the cell cycle and divide, reconstituting the integrity of the organ, a characteristic which is lost rapidly with maturation. In addition, activation of the IGF-1-IGF-1 receptor system at a later time point promotes myocyte formation.

The definition of cardiomyocyte dedifferentiation in the adult mammalian heart has been based on the interpretation of electron micrographs; dedifferentiated cardiomyocytes are characterized by partial loss of contractile material and abnormal Z lines. The sarcoplasmic reticulum and T-tubules are decreased, whereas the number of mitochondria is increased, in combination with areas of glycogen accumulation (Table). Recently, the distribution of the actin depolymerizing protein destrin was analyzed. Destrin was not detected in control hearts but was strongly visible in the perinuclear...
region of myocytes bordering the infarcted human myocardium; these myocytes also expressed the stem cell marker Runx1. Runx1 is a transcription factor that regulates the differentiation of HSCs into mature blood cells. The mRNA level of the surface antigen c-kit, which is shared by HSCs, CSCs, and lung stem cells, was upregulated only in culture preparations of dedifferentiated myocytes.89

Although these are interesting findings supporting the notion that adult cardiomyocytes retain a degree of plasticity, which was not anticipated previously, there are several caveats that need to be discussed. Similar ultrastructural alterations have been described with myocyte degeneration.73 In fact, dedifferentiated myocytes were interpreted initially as damaged cells in areas of hibernating myocardium with a phenotype consistent with a prelude to cell death.91

The electron microscopic characterization of dedifferentiated cardiomyocytes mimics closely the changes observed after muscle unloading and cellular atrophy.74 Dedifferentiation has been claimed to result in the acquisition of a fetal neonatal program, but no quantitative data have been obtained to demonstrate that the alteration in the proportion of myofibrils and mitochondria with cell dedifferentiation reflects the cytoplasmic composition of fetal–neonatal myocytes.75 The notion that neonatal myocytes are immature cells lacking sarcomere striation is incorrect (Figure 5A). Well-oriented myofibrils with intact sarcomeres are present in the fetal heart and the undifferentiated cytoplasm is rapidly replaced with myofibrillar structures and mitochondria shortly after birth in rodents.75 If the re-expression of the fetal program, postulated but not proven, pertains to dedifferentiated cardiomyocytes, protein synthesis needs to be shown by light and electron microscopic autoradiography (Figure 5B and 5C).75–77 Cells undergoing degenerative changes can be distinguished from dedifferentiating myocytes experiencing active protein synthesis.

Braun and colleagues have extended this original hypothesis and prospected that myocyte dedifferentiation constitutes a fundamental mechanism able to reprogram the replicating process in postmitotic myocytes. After this molecular reprogramming, cardiomyocytes can divide and contribute to the homeostatic control of the organ and be responsible for the restoration of the structural and functional integrity of the myocardium after injury. Oncostatin M (OCM), through the activation of the OCM receptors in cardiomyocytes, converts adult myocytes into fetal–neonatal cells.

Myocytes with the dedifferentiated cell phenotype were detected in idiopathic dilated cardiomyopathy and in the border zone of infarcted human hearts. The expression of OCM was increased in these pathological hearts and in a mouse model of dilated cardiomyopathy. However, these findings were not associated with indication of myocyte multiplication. Similarly, adult myocytes in culture exposed to OCM for 6 days acquired dedifferentiated properties but cell division was not shown, although adult myocytes have been reported to divide in vitro.73 Cell cycle progression was illustrated only in cultures of neonatal myocytes after exposure to OCM and stimulation with FGF-2. But neonatal myocytes have high telomerase activity and divide spontaneously in vitro.79 Moreover, the in vivo administration of OCM produced cellular changes consistent with myocyte dedifferentiation but myocyte replication was not apparent.

Myocyte dedifferentiation was not appreciated in human studies of myocardial aging, idiopathic dilated cardiomyopathy, myocardial infarction, and aortic stenosis.72–74 Myocyte mitotic images were identified, together with a significant increase in number of replicating cardiomyocytes expressing the cell cycle proteins Ki67 and MCM5. Large foci of newly formed small cardiomyocytes were found in the absence of morphological changes of cell dedifferentiation.90 Clusters of immature cardiomyocytes were also occasionally seen within the necrotic infarcted myocardium.31 Analogous observations have been made experimentally after myocardial infarction, coronary artery constriction, myocardial aging, and pacing-induced HF.34,36–38,80

Whether embryonic and fetal myocytes reverse to an immature phenotype assuming stem cell characteristics has been tested. Mice with enhanced green fluorescent protein (EGFP)-tagged c-kit–positive cells were used. Myocytes collected from fetal hearts at E16–E18 were cultured for a period of 2 to 3 days and the expression of EGFP in dividing myocytes was determined; 102 myocytes in mitosis were examined and none showed EGFP labeling. Similarly, nondividing fetal myocytes expressing different levels of sarcomeric proteins were all EGFP negative. In addition, c-kit–positive cells had a volume of ≈115 μm³, whereas the size of cardiomyocytes varied from 600 to 1100 μm³. These findings suggest that myocytes failed to reacquire a stem/progenitor cell fate.

In summary, the question whether the evidence provided supports the notion that postmitotic cardiomyocytes have the ability to reprogram themselves, alter the complex, tightly organized composition of the cytoplasm and obtain a replicative cell phenotype cannot be positively answered. Similarly, whether upregulation of the inflammatory cytokine OCM has to be considered critical in the initiation and regulation of myocyte division is questionable. Adult myocytes exposed to OCM increase significantly in size and lose sarcomeric structures,89 a phenomenon that is inconsistent with the acquisition of fetal–neonatal characteristics. Fetal–neonatal myocytes have a volume of ≈1000 to 1500 μm³, whereas the volume of adult cardiomyocytes is ≈20,000 to 25,000 μm³. Cell volume should decrease dramatically for myocytes to acquire the properties of proliferating fetal–neonatal cells. There are no examples of replicating myocytes in rodents with a volume of ≈20,000 μm³ or larger.88 Thus, the possibility that c-kit–positive CSCs represent dedifferentiated multiplying myocytes re-expressing the stem cell antigen c-kit is not supported by the majority of published observations.

Cardiomyogenesis and Bone Marrow Progenitor Cells

Despite the controversy that permeates the field, it is now generally accepted that myocyte renewal occurs in the adult heart and the debate is confined to the magnitude of the process rather than to the process itself. The recognition that myocytes are replaced continuously has put forward the challenging question concerning the cell(s) responsible for myocyte renewal. During the past decade, contrasting results have been
planted in male recipients. In these cases of sex-mismatched transplantation, female hearts transplanted to male recipients have been characterized by a significant number of Y-chromosome–positive myocytes and coronary vessels. Although discrepancies exist among laboratories in terms of the degree of cardiac chimerism, these results suggested that male cells can colonize the female heart and differentiate into myocytes and vascular structures (Figure 6A). The presence of male cells in the female heart was consistent with the contention that stem-like cells migrate to the cardiac allograft and give rise to the 3 main cardiac cell lineages: cardiomyocytes, endothelial cells, and smooth muscle cells.

To date, the c-kit–positive HSC-BMPC is the most versatile stem cell capable of breaking the law of tissue fidelity. Obtained on the contribution of HSCs and, more in general, bone marrow progenitor cells (BMPCs) to myocyte regeneration. If HSCs-BMPCs are directly implicated in the replacement of cardiomyocytes, this process requires stem cell transdifferentiation.

This biological process belongs to the class of cell transformations defined as metaplasia; metaplasia includes cases in which stem cells of 1 tissue acquire the cell phenotype of another tissue. Prenatally, undifferentiated cells undergo a progressive restriction of developmental options and this mechanism of embryonic specification was thought to be irreversible in adult life. This notion, however, has been challenged by several examples of transition from 1 cell type to another and from 1 cell lineage to another cell lineage. The ability of adult stem cells to generate cells beyond their own tissue boundary has been defined as cellular plasticity. Currently, the terms plasticity and transdifferentiation are used as synonyms.

The concept of HSC-BMPC transdifferentiation was based on the identification of male cells in female human hearts transplanted in male recipients. In these cases of sex-mismatched cardiac transplant, the female heart in a male host showed a significant number of Y-chromosome–positive myocytes and coronary vessels. Although discrepancies exist among laboratories in terms of the degree of cardiac chimerism, these results suggested that male cells can colonize the female heart and differentiate into myocytes and vascular structures (Figure 6A). The presence of male cells in the female heart was consistent with the contention that stem-like cells migrate to the cardiac allograft and give rise to the 3 main cardiac cell lineages: cardiomyocytes, endothelial cells, and smooth muscle cells.

To date, the c-kit–positive HSC-BMPC is the most versatile stem cell capable of breaking the law of tissue fidelity. The documentation in 2001 that c-kit–positive HSCs-BMPCs commit to cardiac cell lineages, replace the infarcted myocardium, and improve ventricular function has divided the scientific community initiating a debate that continues inexorably today (Table). These early results argued in favor of the differentiation of the injected cells into the myogenic and vascular cell phenotypes as the mechanism of the myocardial recovery after infarction. Subsequent studies have confirmed the original observations.

C-kit–positive HSCs-BMPCs constitute the class of hematopoietic progenitors that has been at the center of the controversy of stem cell plasticity. However, CD34-positive cells, CD133-positive cells, endothelial progenitor cells, and bone marrow side population cells have all been shown to transdifferentiate to a certain degree. Similarly, bone marrow mesenchymal stromal cells can acquire the cardiomyocyte lineage, although their principal function seems to be mediated by the release of growth factors and cytokines with subsequent activation of resident CSCs. Recently, c-kit–positive cortical bone–derived stem cells have been shown to generate myocytes and coronary vessels. Cortical bone–derived stem cells differentiate into myocytes and activate indirectly the formation of coronary arterioles and capillaries by secreting proangiogenic factors that stimulate endogenous neovascularization. Thus, CD34-positive cells, CD133-positive cells, endothelial progenitor cells, bone marrow side population cells, mesenchymal stromal cells, and cortical bone–derived stem cells can create in variable proportions new myocardium; however, paracrine signals originated by the delivered cells may be as relevant as transdifferentiation.

It is difficult to recognize the variables that condition a negative versus a positive result and provide the reader with an explanation for the discrepancy among laboratories.

Figure 5. Protein synthesis in developing or hypertrophying cardiomyocytes. A, Phase contrast micrographs of fetal atrial (upper left) and ventricular (upper right) myocardium. Myofibrils are located predominantly at the subsarcolemmal region. The lower 2 panels illustrate by light microscopic autoradiography 3H-leucine incorporation (silver grains) indicative of ongoing protein synthesis within the atrial (left) and ventricular (right) fetal myocardium. Scale bars, 10 μm. B, Electron microscopic autoradiographs of developing fetal ventricular cardiomyocytes. Silver grains (3H-leucine) are located over contractile filaments and mitochondria and are frequently associated with the periphery of the cells. Scale bars, 100 nm. C, Autoradiographs of transverse sections of left ventricular myocardium after sham-operation (upper) or after constriction of the abdominal aorta (lower). The number of silver grains (3H-leucine) per high power field is 43% higher in the hypertrophied heart. Scale bars, 10 μm. Adapted from Anversa et al and with permission of the publisher.
Initially, the data in favor of the plasticity of c-kit–positive HSCs-BMPCs were challenged based on the protocol used for the recognition of the injected cells and characterization of the differentiated progeny. The identification of cardiomyocytes derived from HSC-BMPC transdifferentiation was criticized as being the product of unspecific staining and autofluorescence artifacts. This comment was triggered by the erroneous interpretation of poor fixation of skeletal muscle, the uneven distribution of green fluorescence in sections of skeletal muscle was considered equivalent to the fluorescence resulting from immunolabeling of EGFP-positive cells. The autofluorescence generated by the cross-linking of skeletal muscle proteins during aldehyde fixation has nothing in common with the detection of EGFP with anti-GFP; the intensity of the fluorescent signal is 20- to 30-fold stronger than background fluorescence. More importantly, a methodology was introduced in which immunolabeling of proteins was obtained in the absence of autofluorescence inherent in tissue sections of formalin-fixed myocardium. Myocardial regeneration promoted by c-kit–positive HSCs-BMPCs was confirmed strengthening the original findings.

In a comparable manner, methodological artifacts were raised as the source of negative results. The use of frozen myocardial sections for light and confocal microscopy was criticized as having serious problems related to the poor quality of the preparation, inaccuracy of immunolabeling, and inadequate microscopic resolution. Major difficulties exist in the recognition of small newly formed structures, and cardiomyocytes derived from bone marrow cell transdifferentiation have often a diameter of 3 to 5 μm and a volume of 500 μm³. The infarct is rarely preserved in frozen sections and the regenerated cells are 100% restricted to the injured myocardium. Technical limitations were combined with unrealistic statements, which fueled the controversy. To justify some unusual results, a 100% degree of success in the injection of cells in the mouse heart was claimed. Moreover, a mortality rate of 8% with infarcts affecting 60% of the mouse left ventricle was reported. In spite of the perfect care that patients have in the most sophisticated medical centers, a 46% infarct results in intractable cardiogenic shock. Rodents are not different, although they can survive slightly larger infarcts. The hearts analyzed for the presence of cardiac regeneration were not the same studied functionally or with routine histology. Whether coronary ligation was unsuccessful or a small or large infarct was obtained was not determined and negative claims were based on only 2 animals that were supposedly properly infarcted and injected with cells comparable with those used in the earlier positive study.

A double transgenic mouse model for genetic lineage mapping was introduced to determine whether c-kit–positive BMPCs transdifferentiate and generate cardiomyocytes after infarction. In this model, all cardiomyocytes express β-galactosidase but, after a pulse of 4-OH-tamoxifen, myocytes convert the expression of β-galactosidase to EGFP as a result of Cre-mediated DNA recombination. The changes in the proportion of β-galactosidase and EGFP-positive cardiomyocytes were used to monitor the origin of myocytes from (1) BMPCs (myocytes negative for β-galactosidase and EGFP), (2) endogenous progenitors (myocytes positive for β-galactosidase only), or (3) pre-existing cardiomyocytes (myocytes positive for EGFP only).

In this study, a 40% to 50% infarct size resulted in no increase in end-diastolic pressure, and systolic pressure was higher in infarcted than in sham-operated animals. Moreover, the measurements of chamber volume were highly problematic and inconsistent with stroke volume and cardiac output in this model. Apparently, the injected BMPCs activated an unknown pool of resident cells, which led to myocyte formation in the infarct border zone; these myocytes were β-galactosidase–positive only. A major effort was then made to exclude that the administered c-kit–positive BMPCs, or the resident c-kit–positive CSCs had any role in the stimulation of cardiomyogenesis. The injected BMPCs disappeared rapidly, being undetectable by 4 weeks; myocyte formation was not seen at 2 weeks but was evident at 8 weeks.

Myocardial infarction and the delivery of c-kit–positive BMPCs led to a 2.7-fold increase in the number of regenerated cardiomyocytes in the infarct border zone, but this remarkable

![Figure 6. Stem cell homing and myocardial regeneration.](image-url)

A Detection of Male Chromosome

B c-kit BMPCs

C CD45-Positive Cells

- β-actin-EGFP
- α-MHC-EGFP
- α-MHC-αmyc-nuc-Akt

- <0.05 vs 12 hours; **P<0.05 vs 24 to 36 hours. Adapted from Rota et al and with permission of the publisher. Copyright © 2007, National Academy of Sciences. Authorization for this adaptation has been obtained both from the owner of copyright in the original work and from the owner of copyright in the translation or adaptation. α-MHC indicates α-myosin heavy chain; and EGFP, enhanced green fluorescent protein.
growth response had no impact on infarct dimension or cardiac performance. The high level of myocyte formation was consistent with the rather striking increase in the percentage of bromodeoxyuridine (BrdU)-labeled cardiomyocytes during the first week after myocardial infarction. However, there was no increase in myocyte regeneration at 2 weeks; both sets of data cannot be correct. Moreover, the dramatic increase in Nkx2.5, GATA4, and Nkx2.5-GATA4-positive cells in the infarct border zone at 1 week can hardly be reconciled with the lack of newly formed myocytes at 2 weeks.100

Another confusing factor is the absence of information concerning whether the injected c-kit–positive BMPCs engrafted within the infarcted myocardium. Although an extravagant number of BMPCs (6×105) was administered, it can be assumed that only a low fraction homed to the intact myocardium bordering the infarct. The large majority of cells would be expected to accumulate at the interface between the infarct and the spared tissue where the regenerative response commonly occurs. Importantly, newly formed cardiomyocytes in the infarct border zone and within the necrotic myocardium are small, often in mitosis, and are easily distinguishable from pre-existing cells.70,85,87 Unexpectedly, the new myocytes were all fully differentiated and showed morphology identical to that of postmitotic surviving cells. In the absence of β-galactosidase staining, it would be impossible to recognize pre-existing EGFP-positive cardiomyocytes from the regenerated cells. It is difficult to reconcile biologically that only EGFP-negative cardiomyocytes in which somehow DNA recombination did not occur re-entered the cell cycle and divided, whereas the EGFP-positive cells hypertrophied only.

The implementation of genetically engineered mice enabling lineage mapping studies is unquestionably valuable but, in this case, there are problems with either the mouse model or the protocols used for the detection of the engraftment and lineage specification of the delivered cells. It is unfortunate that the authors did not attempt to reconcile their data with the results obtained in all previous studies whether in favor or against BMPC transdifferentiation. In all cases, rare myocytes, inflammatory cells, macrophages, or vascular profiles derived from the c-kit–positive cells were found; the claim that no cells of bone marrow origin were present in the infarcted or surrounding myocardium100 is in contrast with all published reports.70,83,87,88,96,97,102-104

To address the question concerning homing, engraftment, and differentiation of c-kit–positive BMPCs, and demonstrate reproducibility of results, 4 laboratories with complementary expertise undertook a series of joined experiments.87 BMPCs for myocardial regeneration were obtained from 3 transgenic mice. In the first, EGFP was driven by the ubiquitous β-actin promoter; in the second, EGFP was driven by the cardiac-specific α-MHC promoter; and in the third, a c-myc–tagged nuclear-targeted Akt transgene was driven by the α-MHC promoter. With the first category of BMPCs (β-actin-EGFP), all cardiac cells formed by BMPC differentiation were expected to express EGFP; with the second category of BMPCs (α-MHC-EGFP), only myocytes formed by BMC differentiation were expected to express EGFP; and with the third category of BMPCs (α-MHC-c-myc–tagged nuc-Akt), only myocytes formed by BMC differentiation were expected to express c-myc in their nuclei. In all cases, male BMPCs were injected in female infarcted mice so that cell genotyping would allow the distinction between resident female cardiac cells and newly generated male cells.

By this approach, it was demonstrated that c-kit–positive BMPCs engraft in proximity to the infarcted myocardium and differentiate into cells of the cardiogenic lineage, forming functionally competent cardiomyocytes and vascular structures; this process is associated with the loss of bone marrow cell epitopes (Figure 6B and 6C). Cell fusion was also excluded by documenting a diploid DNA content together with 1 set only of sex chromosomes in nuclei of newly formed myocytes and vascular cells. Importantly, cardiac repair attenuated ventricular remodeling and the deterioration in cardiac function that occurs after infarction. Thus, adult c-kit–positive BMPCs implanted in the infarcted heart lose the hematopoietic fate and form de novo myocardium. These genetic studies97 argue strongly against the negative results recently reported.100

Why myocardial regeneration mediated by HSC-BMPC transdifferentiation has been caught in the controversy remains unexplained. The discrepancy may be dictated by differences in the methodology used and the complexity related to the identification of small developing myocytes, often searched for with tools that lack the necessary resolution required for this analysis. The localization of the Y-chromosome in newly formed cells is more sensitive than the detection of the fluorescent tag, which leads to underestimation of cell plasticity.105 There is no evidence that the entire transdifferentiated progeny retains EGFP or that the level of EGFP in the regenerated cells is higher than background autofluorescence. The expansion of the small pool of engrafted donor cells carrying EGFP may affect transgene expression even when driven by a ubiquitously active promoter.105 The contrasting results, however, raise the possibility that distinct classes of c-kit–positive HSCs-BMPCs may have been tested and only a selective subset of bone marrow cells retains the ability to transdifferentiate and acquire the cardiomyocyte lineage. Moreover, the molecular pathway that controls the change in the phenotypic properties of HSCs-BMPCs remains to be defined. The identification of this determinant of HSC-BMPC fate is currently under intense investigation in view of its biological significance and important clinical implications.

Cardiomyogenesis and Resident Cardiac Progenitor Cells

The new century has observed a dramatic change in our understanding of myocardial biology. Toward the end of the last century and the beginning of the new century, several reports have suggested that a pool of cardiomyocytes is cycling in the normal and pathological human heart,26-31 and similar observations were made in small and large animals after myocardial infarction, pressure overload, physiological aging, or pacing-induced HF.35,34,36,40,41,45,80 This body of work has provided the fundamental information that has led to the identification of a compartment of resident CSCs in 2003.106 Using the surface antigen c-kit, CSCs were first recognized and characterized in rats106 and subsequently in mice,35,107 dogs,108 and, ultimately,
in human beings. The c-kit–positive CSCs are self-renewing clonogenic and multipotent in vitro and in vivo meeting the criteria of bona fide tissue-specific adult stem cells. Serial transplantation assays in vivo, together with different models of myocardial damage and HF, have shown that this primitive cell class is necessary and sufficient for the regeneration and repair of the damaged myocardium. The c-kit–positive CSCs are present in the left and right ventricle but tend to accumulate in the atria and apex, which correspond to anatomic areas exposed to low levels of hemodynamic stress.

Several laboratories have made a major effort to define the distribution of this class of cardiac progenitors in the different anatomic regions of the human heart and in various pathological conditions (Table). CSCs are present in the outflow tract of the right ventricle in infants with tetralogy of Fallot and can be isolated and propagated in vitro from explanted hearts of pediatric patients with end-stage HF. These progenitors are most abundant in the neonatal period and decrease rapidly in infants and children, losing in part their potency to reconstitute the injured myocardium. In the adult human heart, CSCs tend to accumulate in the subepicardium and, in the presence of severe ventricular dysfunction, may lose some of their differentiation properties and ability to reach functional competence. The right atrium is the most appropriate source for c-kit–positive CSCs, although no detectable differences in the growth behavior of human CSCs obtained from the 4 cardiac chambers have also been reported. The number of intact CSCs decreases with age, diabetes mellitus, and chronic coronary artery disease, emphasizing the relevance that resident progenitors may have in the manifestations of the aging and diabetic myopathy and ischemic HF. In a clever manner, adoptive transfer has been introduced to rejuvenate human CSCs and restore their impaired regenerative capacity. In this regard, multiple protocols have been developed to optimize the acquisition and expansion of human c-kit–positive CSCs for clinical use.

In a comparable manner, several independent studies have identified, collected, and expanded c-kit–positive CSCs in small and large animals and developed strategies aiming at the enhancement of their growth reserve and effectiveness in promoting cardiac repair after ischemic myocardial damage. In addition, activation and commitment of endogenous c-kit–positive CSCs occur with pregnancy and dynamic exercise and can be isolated and propagated in vitro from explanted hearts of pregnant and dynamic exercise. An increase in maternal cortisol during pregnancy stimulates activation and commitment of endogenous c-kit–positive CSCs and results in an increase in heart weight.

Cardiac niches create the necessary, permissive milieu for the activation and repair of the damaged myocardium. The recognition that CSCs reside in the adult heart has raised questions about the origin of CSCs. If the CSCs are not a subpopulation of HSCs, they may be present early during development and be responsible for cardiomyogenesis in the embryonic and fetal heart, a role that they may continue to have postnatally and in adulthood. Conversely, if these primitive cells represent a HSC subset, they are located in the hematopoietic system, and continuously translocate to the developing heart, where they assume specific functions, leading to the formation of the mature cardiac phenotype. To address this issue, the entire live embryo, including the yolk sac, has been cultured using a mouse model with genetically tagged CSCs and analyzed by 2-photon microscopy. EGFP-positive cells were present in the yolk sac but did not translocate to the heart. CSCs were detected in the heart tube where they showed morphogenetic movements only, supporting the notion that prenat al cardiac development is controlled by growth and differentiation of CSCs, which are responsible for the formation of the myocyte progeny present at birth. Although homing of noncardiac EGFP-positive cells to the myocardium was not identified with certainty, it cannot be excluded that the bone marrow contributes partly to the growth of the embryonic and fetal heart, a mechanism consistent with the ability of HSCs to acquire the cardiomyocyte fate. With maturation, CSCs progressively lose their primitive state, contractile proteins accumulate, and when the adult cell phenotype is acquired cell division is suppressed.

Stem cells are stored in niches that constitute the microenvironment in which stem cells are maintained in a quiescent state. After activation, stem cells replicate and migrate out of the niches to sites of cell replacement where they differentiate and acquire the adult phenotype. Niche homeostasis is controlled by stem cell division, which preserves the proportion of primitive and committed cells within the parenchyma. In the niches, stem cells are connected to the supporting cells which anchor stem cells to the niche and modulate growth signals from the surrounding tissue. Stem cells divide rarely and their growth kinetics and localization can be assessed by the label-retaining assay; the long-term label-retaining property of a cell documents its stemness, whereas the progressive dilution of the label identifies the formed progeny.

Based on this premise, long-term BrdU-positive CSCs have been identified mostly in the atria and apex of the adult mouse heart. Clusters of uncommitted CSCs are surrounded by fibronectin and are connected by junctional complexes, made by connexin 43 and N-cadherin, to postmitotic cardiomyocytes and fibroblasts. Connexins are gap junction channel proteins that mediate passage of small molecules involved in cell-to-cell communication. Cadherins are calcium-dependent transmembrane adhesion molecules, which have a dual function: they anchor stem cells to the microenvironment and promote a cross-talk between stem cells and between stem cells and the supporting cells. In addition, CSCs divide symmetrically and asymmetrically in vitro and in vivo and their fate is dictated by the distribution of Numb and α-adaptin and the activity of the Notch receptor. Cardiac niches create the necessary, permissive milieu for the long-term residence, survival, and growth of CSCs.

The identification of the mechanisms responsible for the activation and lineage specification of CSCs has been a difficult challenge. Ca2+ has 2 fundamental functions in the heart: it activates growth processes and modulates the mechanical behavior of cardiomyocytes. Spontaneous Ca2+ oscillations occur in adult human CSCs and in mouse embryonic-fetal and adult CSCs, and this process promotes their entry into the cell cycle (Figure 7). Similarly, Ca2+ oscillations control the progression into the cell cycle of embryonic stem cells and favor proliferation and differentiation of mesenchymal stromal cells.
Ca²⁺ oscillations of c-kit–positive fetal mouse cardiac progenitor cells (CPCs) at baseline (Ctrl) and after downregulation of IP3 receptor type-2 (sh-RNA-IP3-R2). *,†

CPCs displaying Ca²⁺ oscillations in tyrode or after exposure to ATP is attenuated in sh-RNA-IP3-R2 (sh-RNA). *,†

Ca²⁺ oscillations of c-kit–positive fetal mouse cardiac progenitor cells (CPCs) at baseline (tyrode solution) and after exposure to IGF-1. *,†

Cytosolic Ca²⁺ plays a crucial role in CSC growth, and induction of Ca²⁺ oscillations in human CSCs, before their intramyocardial delivery in vivo, is coupled with enhanced cell engraftment within the infarcted myocardium, increased cell expansion, and potentiation of myocyte regeneration.150

The growth-promoting effects of c-kit–positive CSCs and embryonic stem cells are both initiated by spontaneous oscillations in intracellular Ca²⁺ through the activation of inositol-1,4,5-triphosphate receptors, which condition CSC asymmetrical division and myocyte lineage specification.79,150 The differentiation of CSCs into mature myocytes may involve the loss of T-type Ca²⁺ channels, which is associated with withdrawal of amplifying myocytes from the cell cycle and binucleation, Ca²⁺ influx via L-type Ca²⁺ channels, and transient receptor potential channels. Increases in L-type Ca²⁺ channels and transient receptor potential current activate NFAT (nuclear factor of activated T-cells) signaling and myocyte hypertrophy.153–155

Collectively, significant information has been acquired as a result of the effort made by multiple groups in defining the properties of c-kit–positive human and nonhuman CSCs. In vivo protocols have provided strong evidence concerning the ability of these cells to form functionally competent cardiomyocytes and coronary vessels integrated with the recipient myocardium.71,106,108–110,156,157 Human CSCs can divide by asymmetrical and symmetrical chromatid segregation110,158 and CSCs inheriting only the mother DNA have an unprecedented capacity to repair the infarcted myocardium experimentally, emphasizing the clinical relevance of carefully defined biological processes. Gain- and loss-of-function assays have shown that scattered myocyte loss activates the endogenous regenerative process by which c-kit–positive CSCs restore the structural and functional integrity of the injured heart.150 Based on high-throughput transcriptional profiling, c-kit–positive CSCs represent the most primitive cell population in the myocardium and their molecular signature is distinct from c-kit–positive HSCs.159

Conclusions

In summary, cardiomyocytes derive from transdifferentiation of HSCs-BMPCs and activation and lineage specification of resident CSCs. The data in favor of myocyte dedifferentiation are inconclusive and far from proving the validity of this mechanism of cell replication. Similarly, the possibility that postmitotic myocytes can re-enter the cell cycle and divide...
has little basis. As described previously in several review articles, in addition to c-kit–positive CSCs, other progenitor classes have been identified in the adult heart and their ability to form myocytes in vitro and in vivo has been shown carefully. Despite the importance of these other categories of primitive cells, the c-kit–positive CSC has been chosen here for discussion in view of the current controversy and the attention it has received in the scientific community worldwide. Laboratories in different continents have been able to isolate and propagate c-kit–positive CSCs from animals and humans and document their relevance in preclinical studies. Although the translation of this significant body of work to patients is in its infancy, it is reasonable to prospect clinical trials aiming at the recognition whether the delivery of autologous CSCs interferes with the unfavorable evolution of advanced HF.

The phase during which the existence and significance of c-kit–positive CSCs were questioned is ending and the collaborative effort of several laboratories will unquestionably promote the implementation of this discovery in human beings. Complex animal models cannot be taken and interpreted at face value ignoring the extraordinary progress that has been made concerning our understanding of myocardial biology. There is no organ in our organism in which stem cells have been identified and shown to have no fundamental importance in regulating cell homeostasis and regeneration. Problematic, often uninterpretable experimental results cannot condition the inclusion of CSCs into the management of human HF.

Recently, a lineage tracing study in the mouse has disputed the implications that c-kit–positive CSCs have in the regulation of myocyte renewal in the adult heart. The knock in strategy used has led to the loss of 1 allele of the c-kit gene and this may have affected the function of the c-kit receptor and the ability of CSCs to proliferate and form a myocyte progeny. Defects in c-kit receptor function may be organ specific and the myocardium could have been more severely influenced than the bone marrow and the lung. The discrepancy between the number of cardiomyocytes and the number of alveolar epithelial cells derived from c-kit–positive cells in this transgenic mouse model clearly indicates that this may be actually the case. In addition, given the substantial evidence supporting a role of c-kit–positive CSCs in myocardial homeostasis, repair after injury and aging, the new results are surprising. Rectifying the bases of the differences in findings with this new mouse model versus those of our group and others is clearly important to the field.

During the past 25 years, a large number of transgenic mice have been generated and this remarkable effort has advanced our knowledge of the multiple signaling pathways of myocyte growth. Disappointing advances, however, have been made in the elucidation of the pathogenesis of human HF and its treatment. The translation of basic information to patient care has been an occasional event created predominantly by the ingenuity and curiosity of isolated investigators. This is true for the implications that myocyte death, myocyte regeneration, and exogenous and endogenous stem cells have in the progression and future treatment of HF. However, there is still a strong debate in the field on the robustness of the regenerative response of the adult myocardium (Table) and the mechanisms by which new myocytes are formed. It is difficult to predict at present when a general consensus on the extent and origin of myocytes will be achieved in the scientific community, but an optimistic view can be prospected because several laboratories are engaged in resolving this biological problem, which has critical implications in the pathogenesis and severity of human HF.

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Disclosures

None.

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Origin of Cardiomyocytes in the Adult Heart
Annarosa Leri, Marcello Rota, Francesco S. Pasqualini, Polina Goichberg and Piero Anversa

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In the article by Leri et al, “Origin of Cardiomyocytes in the Adult Heart,” which appeared in the January 2, 2015, issue of the journal (Circ Res. 2015;116:150-166. DOI: 10.1161/CIRCSAHA.116.303595), a correction was needed.

Piero Anversa, MD, discloses that he is a member of Analogous, LLC.

The author regrets this omission.

This correction has been made to the current online version of the article, which is available at http://circres.ahajournals.org/content/116/1/150.full