Cardiovascular Regeneration

Concepts

*Dogma
*Cardiac progenitors
*Hematopoietic stem cells
*Mesenchymal stem cells
*Cardiospheres
*Embryonic stem cells
*Induced pluripotent stem cells
*Induced cardiac myocytes via transdifferentiation
*Advantages and disadvantages

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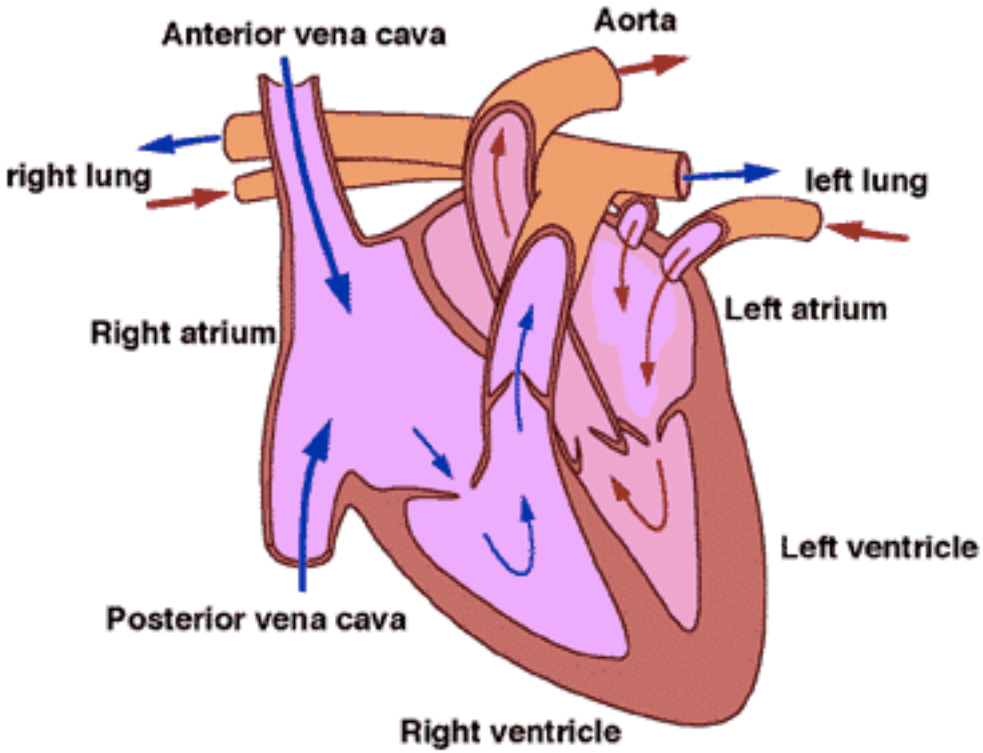
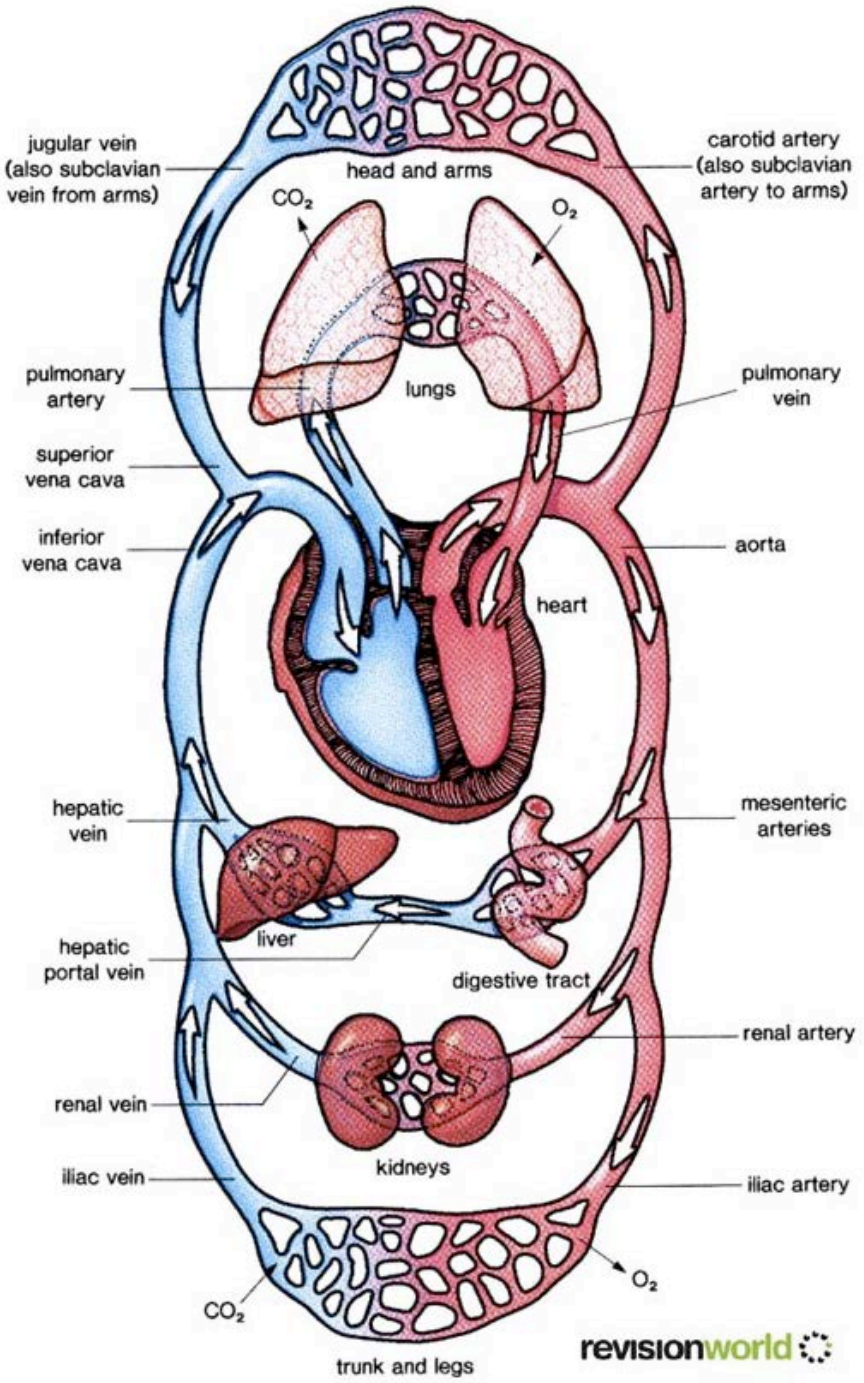
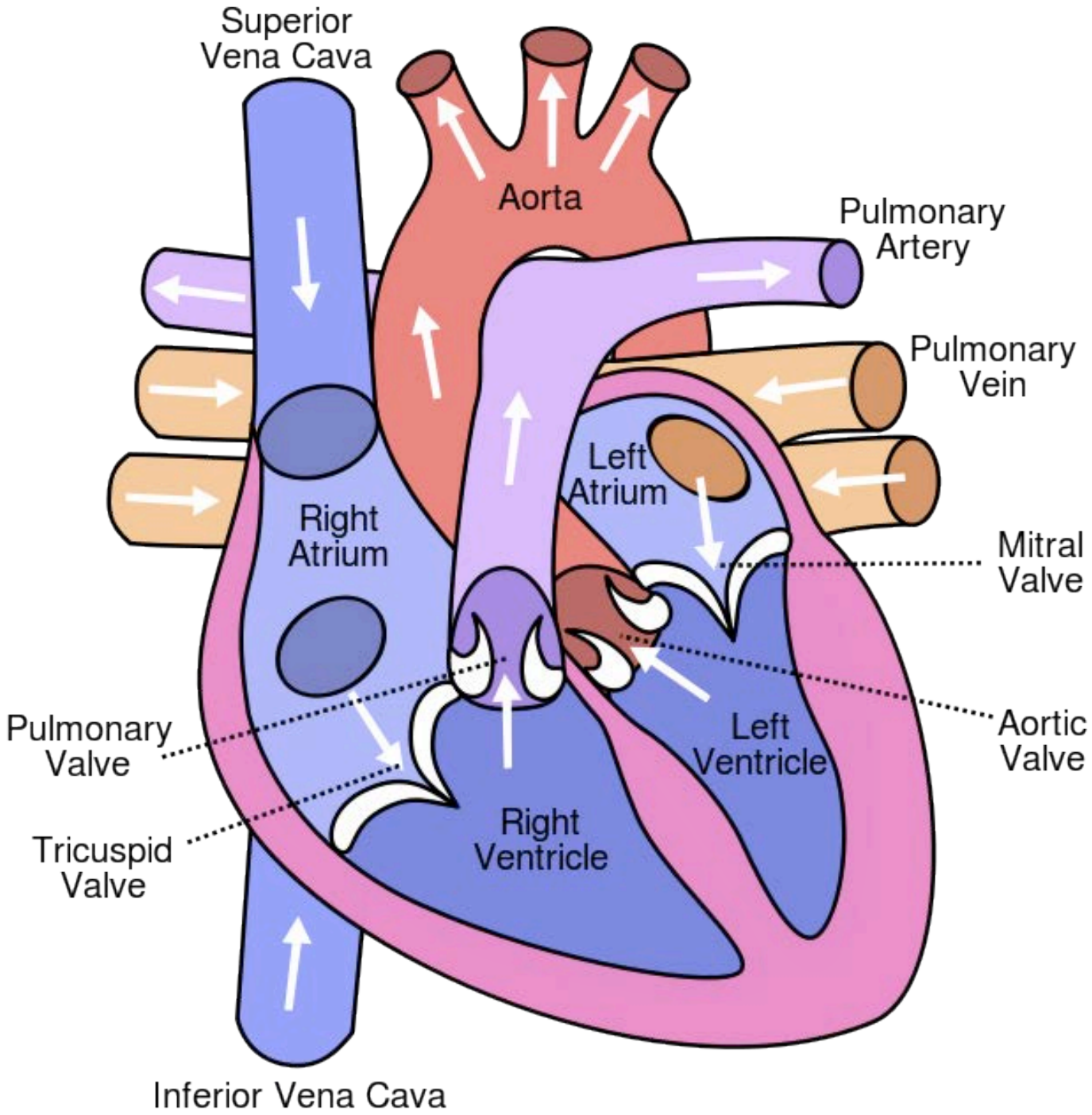
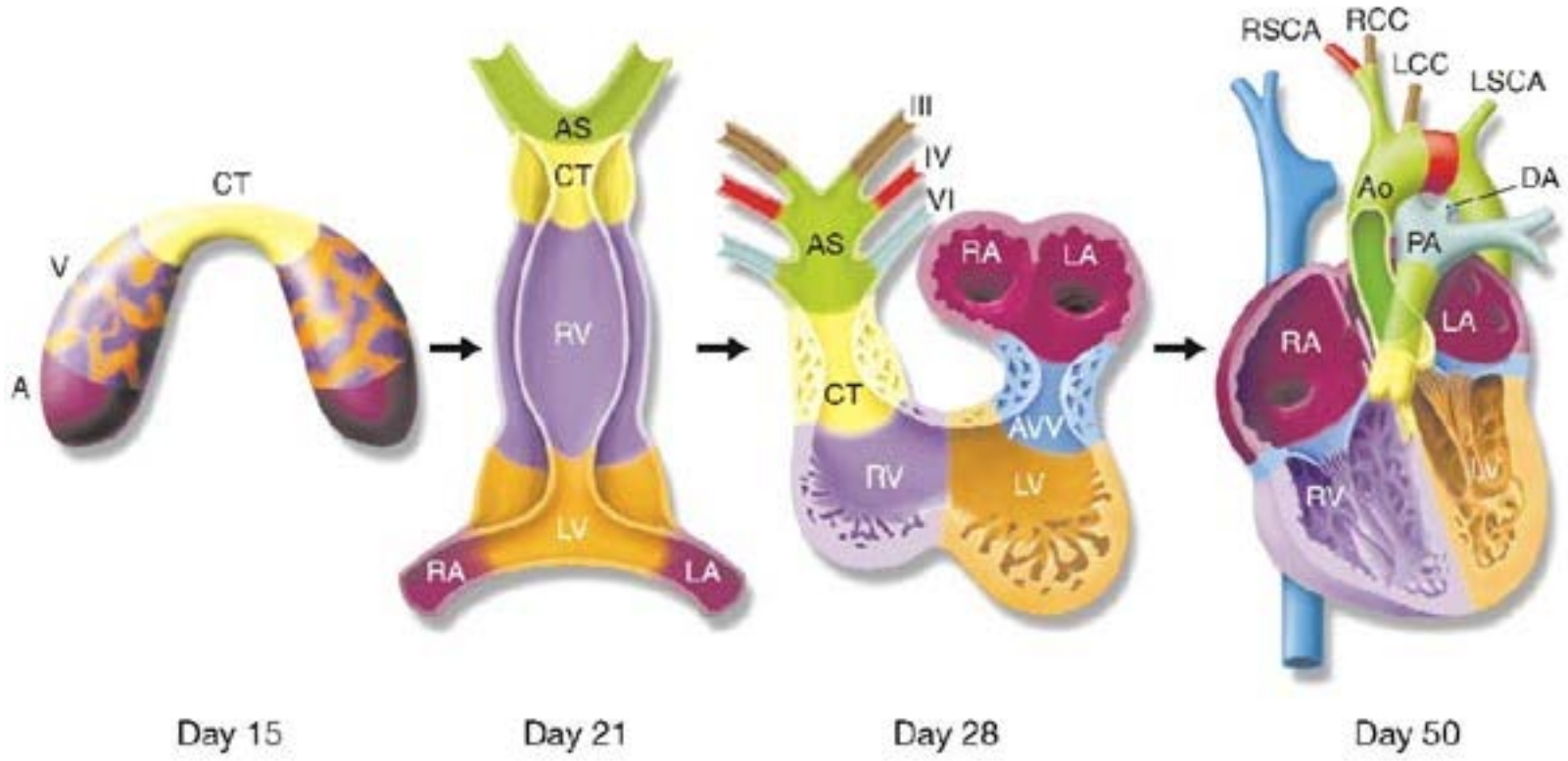
Cardiac Development

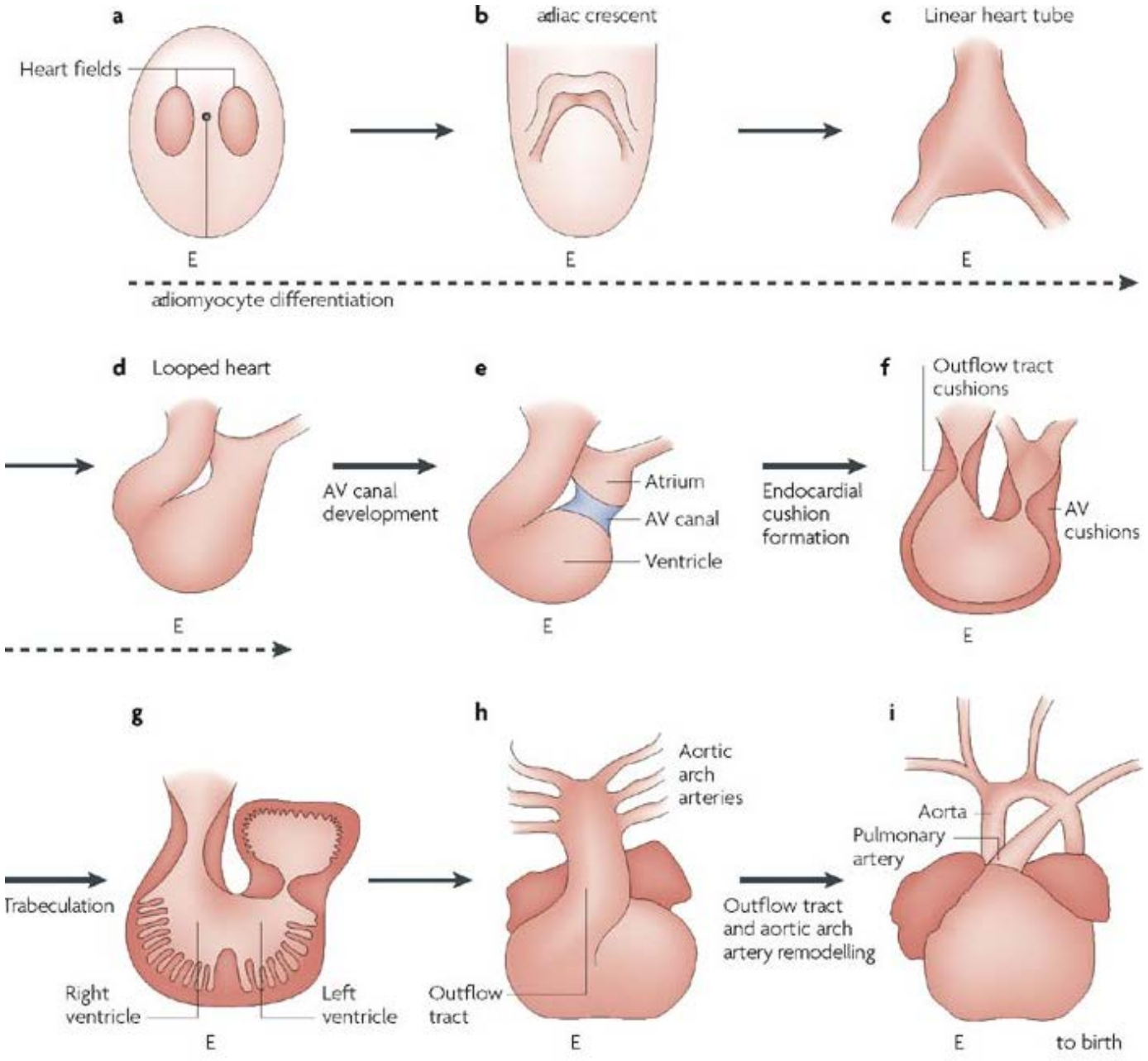

Anterior vena cava
Aorta
right lung
left lung
Left atrium
Right atrium
Left ventricle
Posterior vena cava
Right ventricle


jugular vein
(also subclavian
vein from arms)
head and arms
carotid artery
(also subclavian
artery to arms)
CO_2
O_2
pulmonary
artery
lungs
pulmonary
vein
superior
vena cava
inferior
vena cava
aorta
heart
hepatic
vein
mesenteric
arteries
liver
hepatic
portal vein
digestive tract
renal artery
renal vein
kidneys
iliac vein
iliac artery
O_2
CO_2
trunk and legs
revisionworld


Superior
Vena Cava
Aorta
Pulmonary
Artery
Pulmonary
Vein
Left
Atrium
Right
Atrium
Mitral
Valve
Pulmonary
Valve
Aortic
Valve
Left
Ventricle
Tricuspid
Valve
Right
Ventricle
Inferior Vena Cava

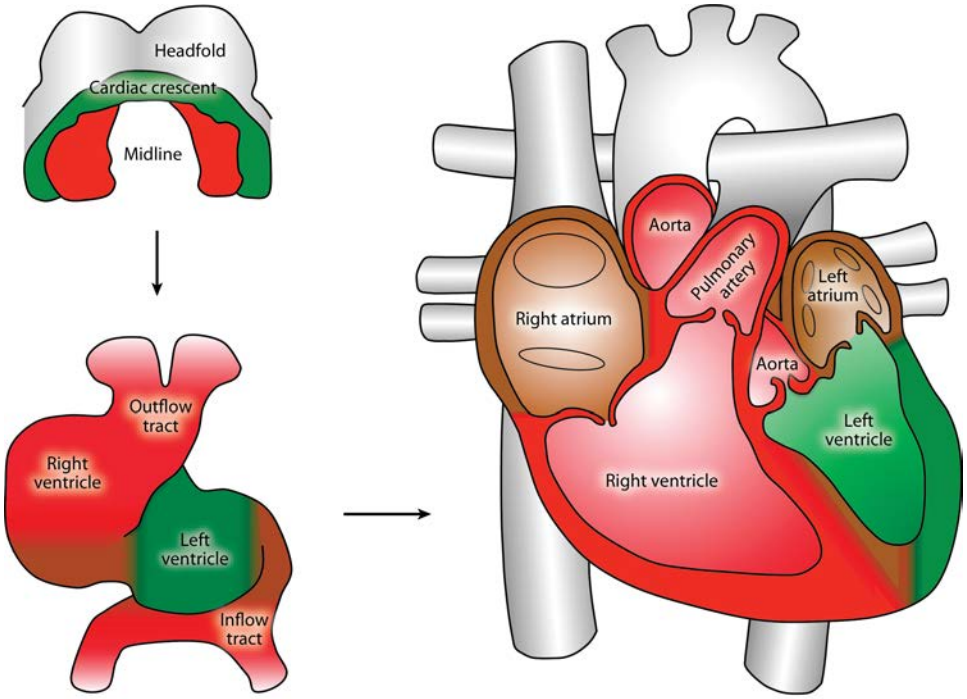
Heart field, linear tube, ballooning





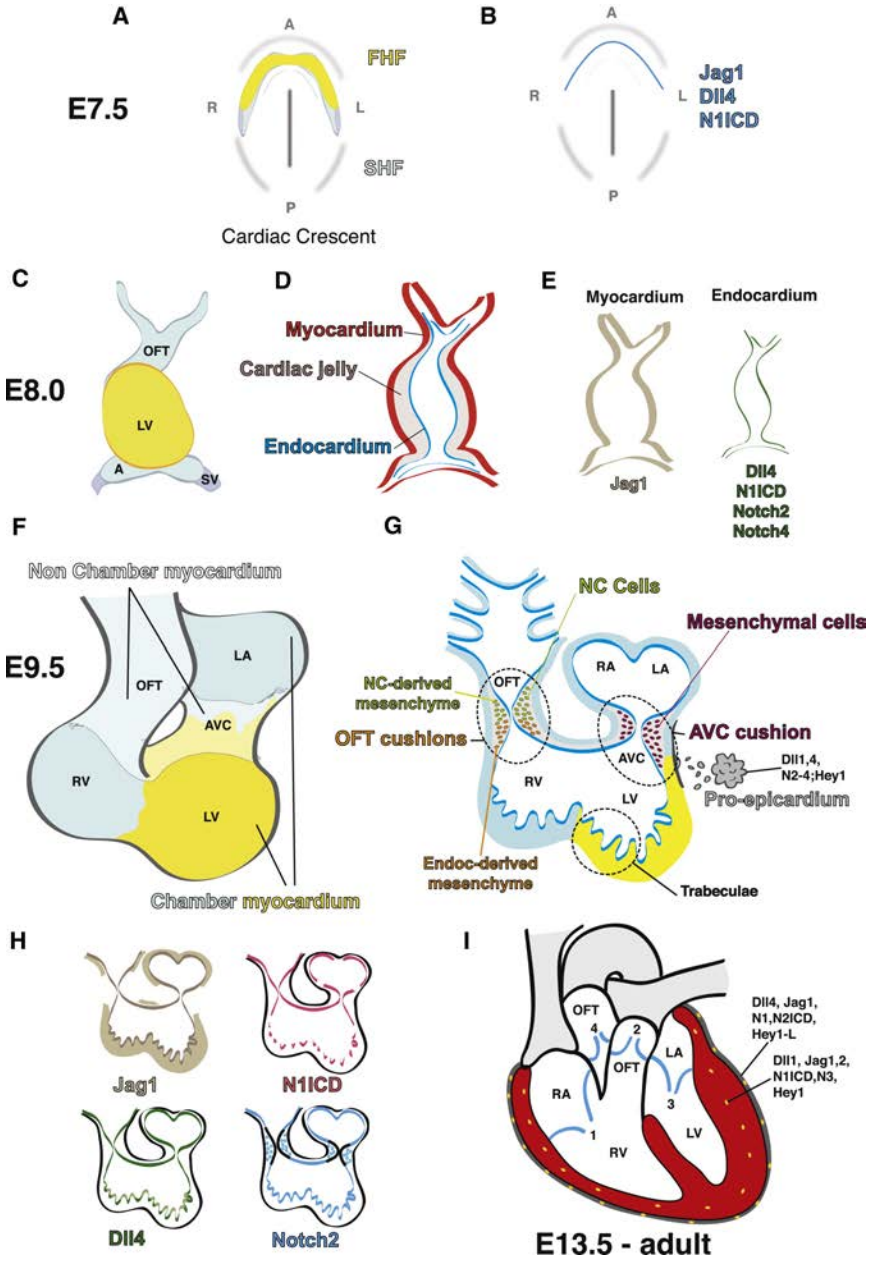
Nature Reviews | Genetics

Primary and secondary heart fields

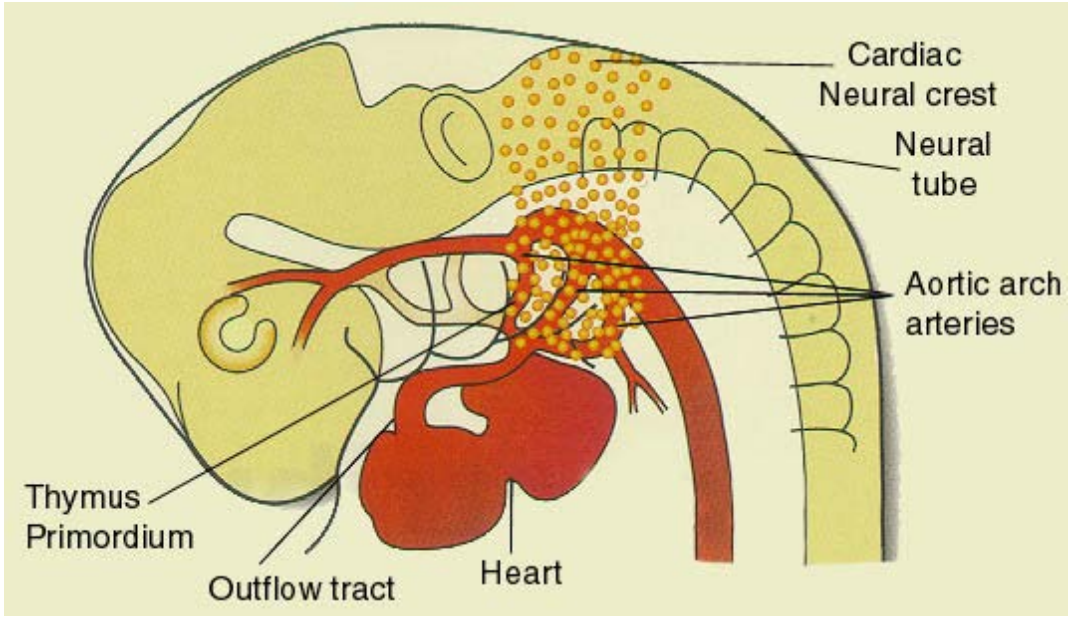


Musunuru K, et al. 2010.
Annu. Rev. Cell Dev. Biol. 26:667–87

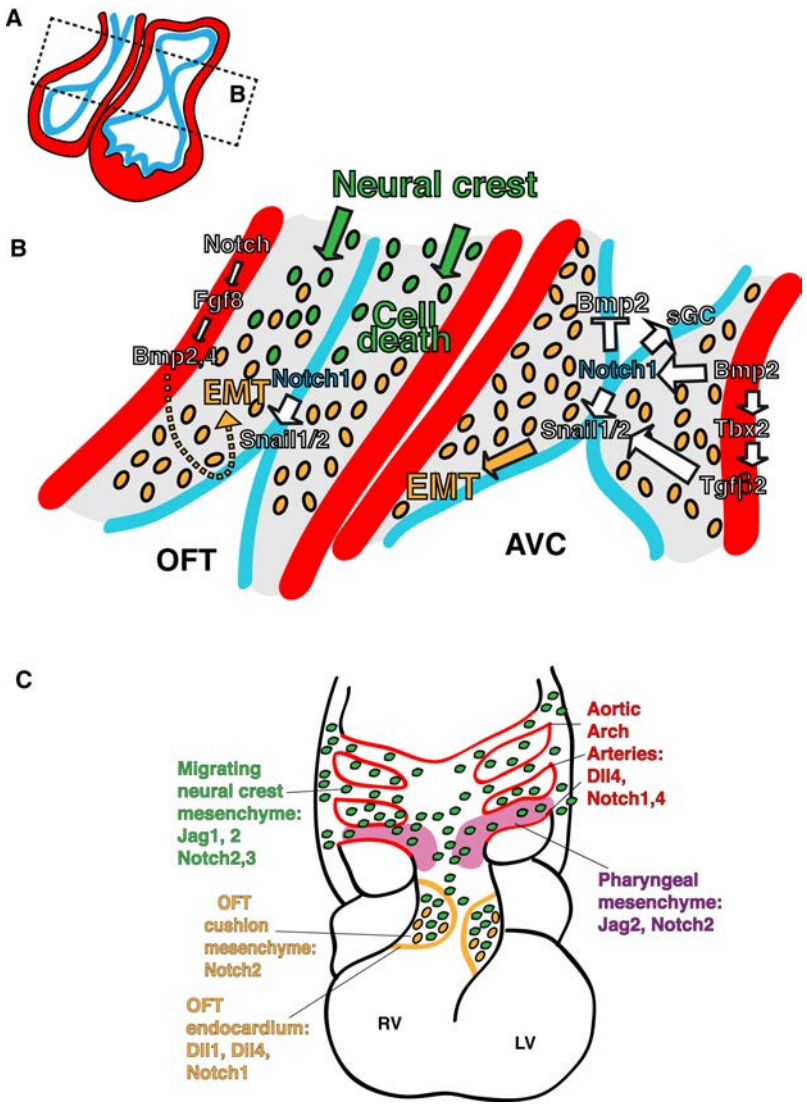
Formation of endocardial cushion (prospective cardiac valves)



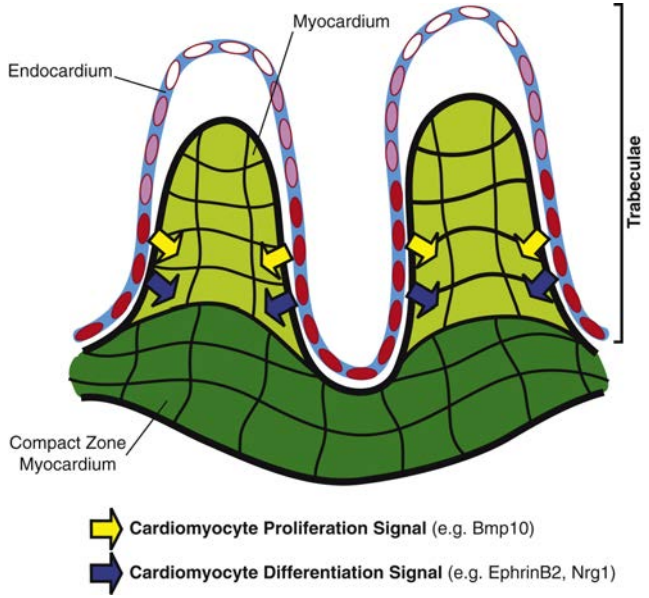
Participation of outside sources (neural crest) to OT endocardial cushion



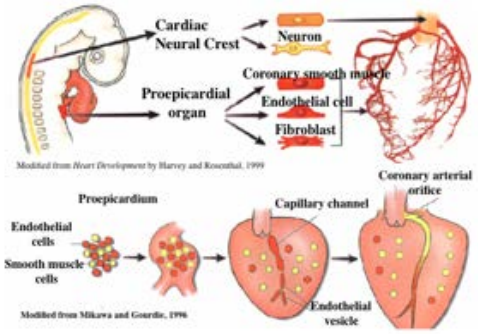
Participation of outside sources (neural crest) to OT endocardial cushion



Trabeculation and expansion of the compact myocardium



Contribution of the PEO (proepicardial organ) and epicardium to the coronary vasculature



Dogma

Cardiac myocytes are post-mitotic

CMs do not proliferate, or have limited proliferative capacity

Several experiments to attempt to address this important issue

The most conclusive and recent experiment consists of turnover measurements from individuals affected by radioactivity after the Chernobyl disaster

Conclusion:

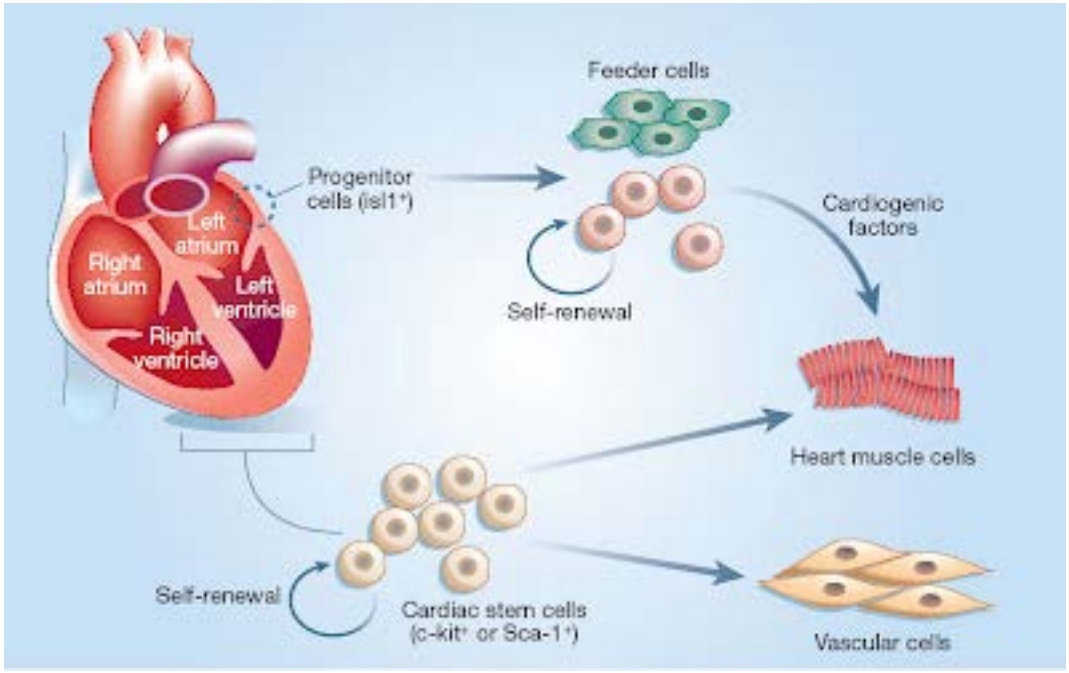
the heart has limited proliferative capacity, as it replenishes half of the total number of CMs in a lifespan. This limitation opens venues for further investigation of enhancement of internal sources of CM production or injection of external sources of CMs.

Internal sources:

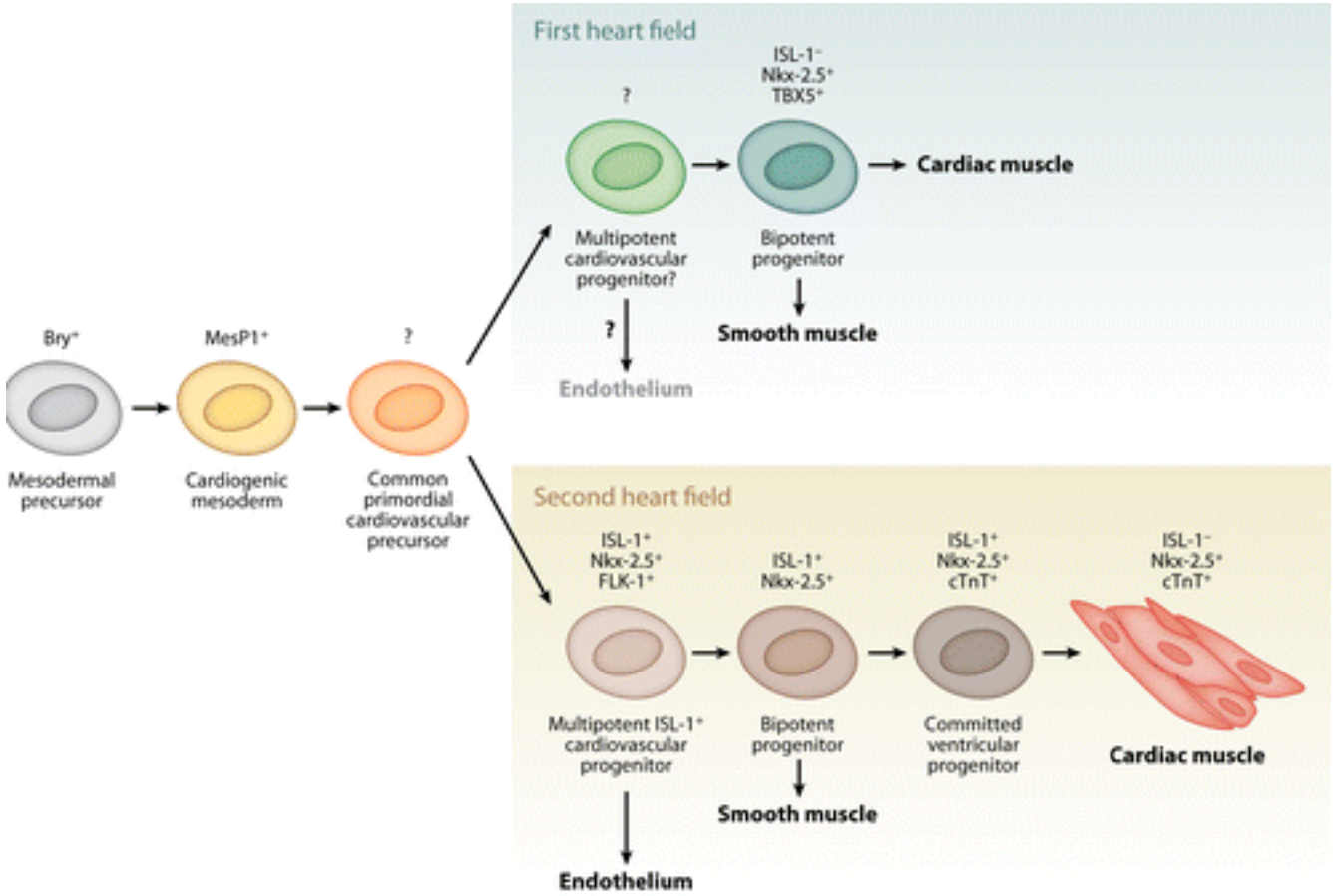
Cardiac stem cells (CSCs)
Small rounded rare cells, c-kit+ cells, respond to growth factors
Clonogenic (give rise to endothelial, smooth muscle and cardiac muscle)
Replenish infarcted area with new CMs

Cardiac progenitor cells (CPCs)
Islet1+ (Isl1+) cells seen in neonatal tissue, give rise mature CMs

CSCs/CPCs may not appear to be effective under a severe acute MI episode or under chronic heart failure.


Feeder cells
Progenitor cells (isl1+)
Left atrium
Right atrium
Left ventricle
Right ventricle
Self-renewal
Cardiogenic factors
Heart muscle cells
Self-renewal
Cardiac stem cells (c-kit+ or Sca-1+)
Vascular cells

Markers specific for first and second heart field



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Exogenous administration

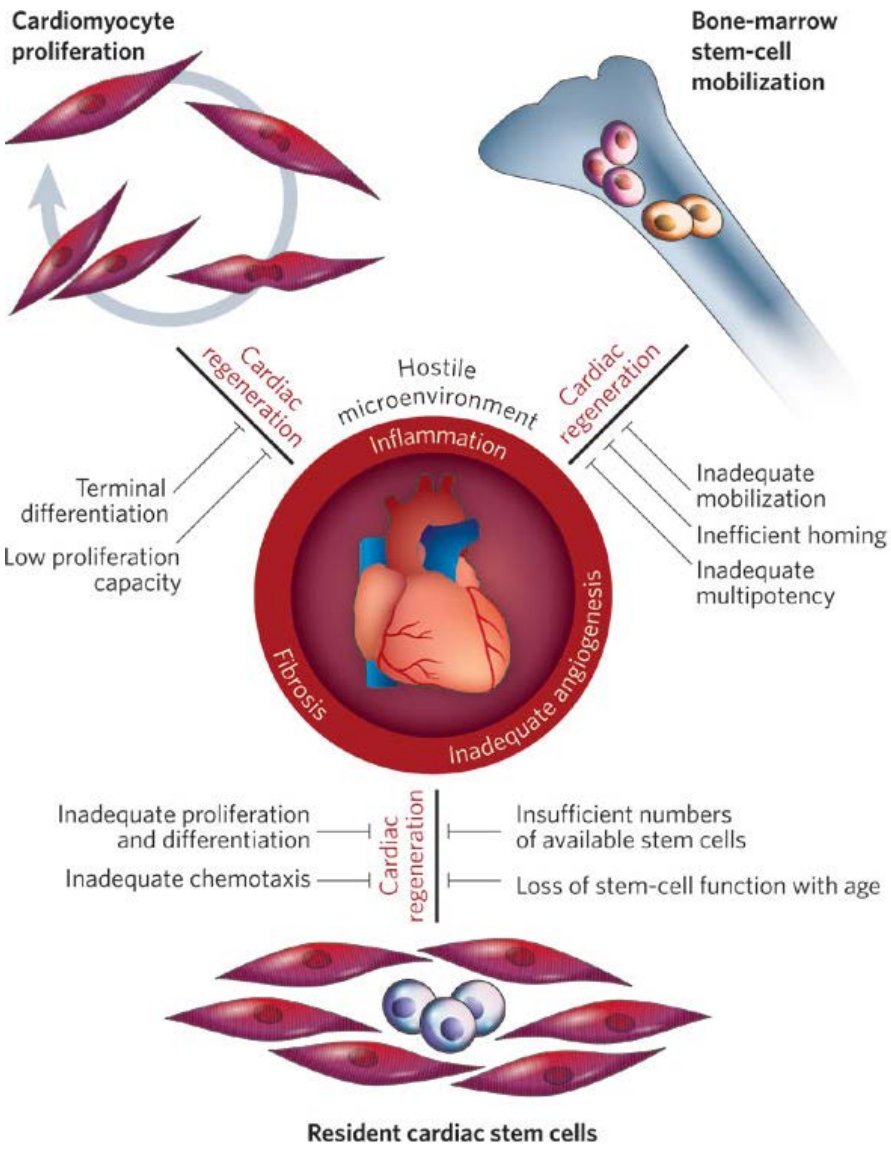
Adult stem cells
Autologous and off-the-shelf (allogeneic)

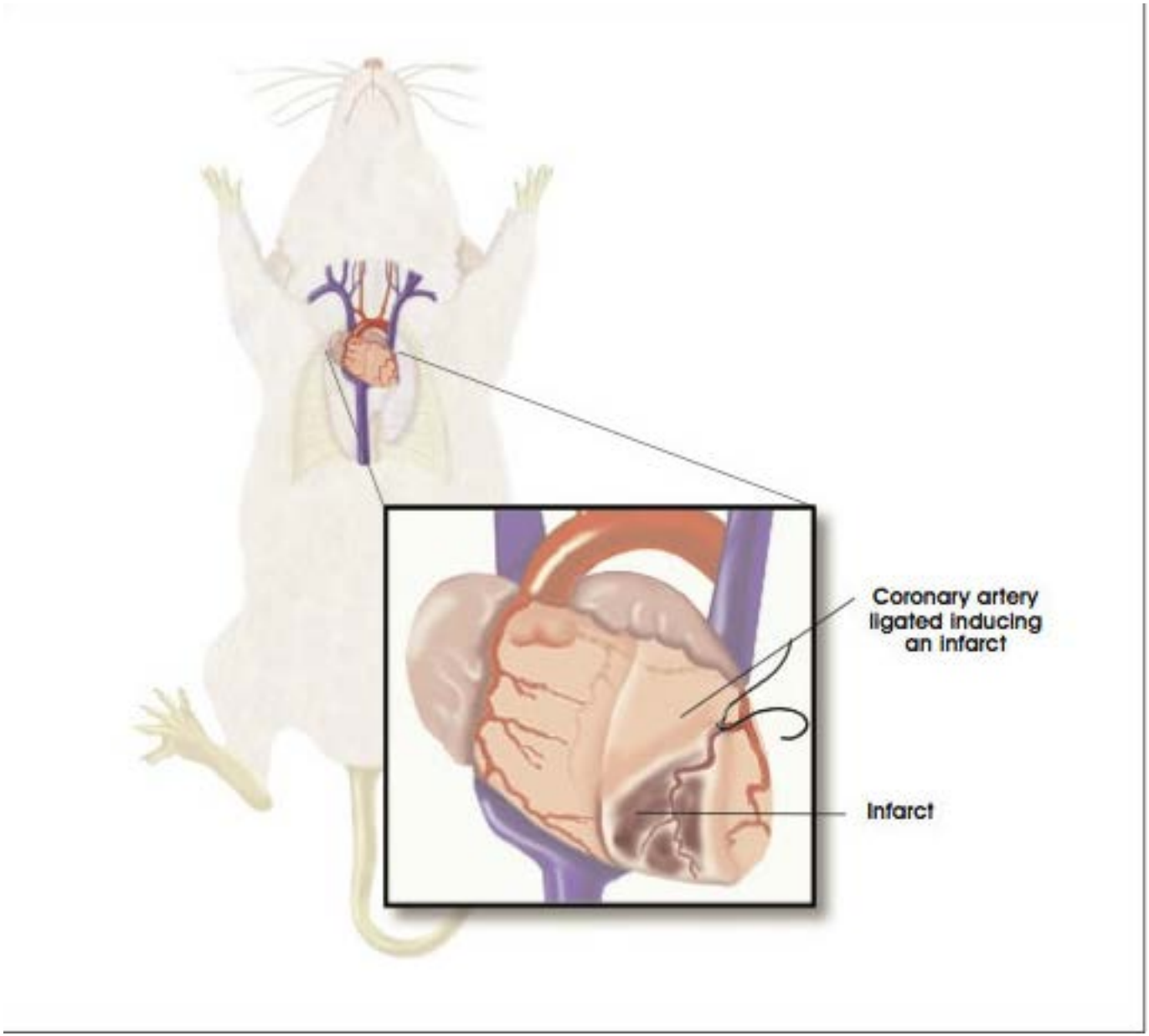
Hematopoietic cells autologous CD34+ (vascular progenitors, the concept of myocardial differentiation appears to be obsolete) increased perfusion and function

Mesenchymal stem cells (bone marrow-derived), off-the-shelf or allogeneic,
Autologous interventions in patients with ventricular dysfunction

Cardiosphere-derived cells, autologous, endomyocardial biopsy

Pre-clinical studies in pigs
Phase I/II clinical trials around the Globe
Reduction of infarct size, safe, improvement LV function
Intracoronary/intramyocardial delivery
Cannot trace the cells
Short-term effect (retention of cells and cytokines)
Difficult to recruit patients with AMI, but easier to recruit patients with chronic heart failure
Future treatment: combination of paracrine recombinant protein (rFGF) and adult stem cells


Cardiomyocyte proliferation
Bone-marrow stem-cell mobilization
Cardiac regeneration
Hostile microenvironment
Cardiac regeneration
Terminal differentiation
Low proliferation capacity
Inflammation
Fibrosis
Inadequate angiogenesis
Inadequate mobilization
Inefficient homing
Inadequate multipotency
Inadequate proliferation and differentiation
Inadequate chemotaxis
Cardiac regeneration
Insufficient numbers of available stem cells
Loss of stem-cell function with age
Resident cardiac stem cells


Coronary artery
ligated inducing
an infarct
Infarct

Pluripotent stem cells

Embryonic stem cells:

Ethical concerns, technical concerns, failure of SCNT in human eggs
(Hwan South Korea, who has cloned dogs and now is now cloning coyotes and mammoths)

A proper reprogramming occurs only in the presence of a non-enucleated cell. In this context, the somatic nucleus is reprogrammed.

Currently, nuclear reprogramming is possible, with “premium quality” eggs. DNA methylation and transcriptome profiles of NT ESCs corresponds closely to those of IVF ESCs.

Important problem: accessibility to eggs. Although hESC research can be done with the use of federal money, The National Academy of Sciences (NAS) recommends that no compensation should be involved in the donation of eggs for stem cell research.

Tumorigenic potential

Induced pluripotent stem cells (iPSCs): circumvents ethical and technical concerns, but
still have problems:
-Low efficiency
-Tumorigenic potential
-Despite of being autologous (patient-specific) there still may be immunoreaction
-Differentiation protocols vary from lab to lab, or from colony to colony (epigenetic memory)
-Incomplete reprogramming

Several methods:
Yamanaka factors, retrovirus (Yamanaka), lentivirus (Thomson), adenovirus, single polycystronic vectors, small molecules (Ding), modified-mRNA (Morrisey), miRNA

Problems associated with differentiation into CMs:
-Maturation (electrical coupling)
-Automaticity (arrhythmias)
-Teratoma potential

Potential solutions:
-Scaffolds/matrix/tissue engineering -Mathematical/computerized modeling programs to mimic topology
-Electrical connections between CMs but not on the side
-Co-culture with endothelial cells and cardiac fibroblasts

Safety studies:

Arrhythmias, tumorigenic potential

conducted in large animals

Swine (pigs) and dogs

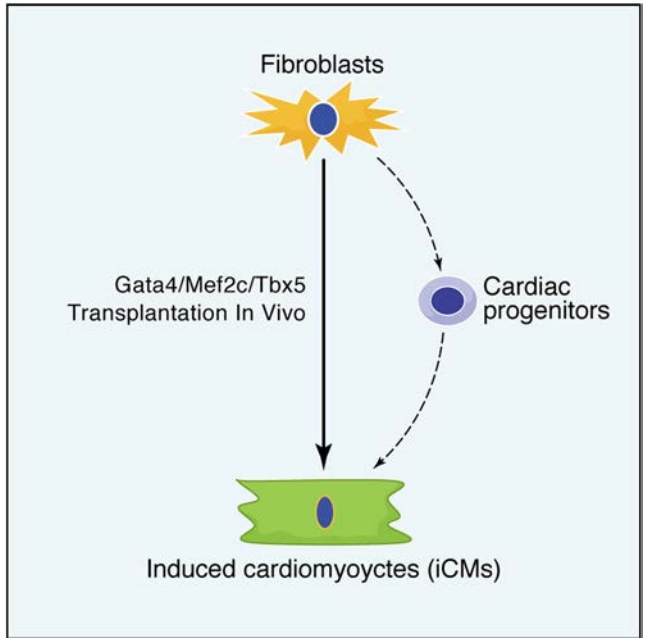
Main application of iPSCs

Modeling system in Petri dish:
-CMs Long QT-syndrome -ALS (Eggan)
-Motor neurons (Eggan)
-Pancreatic beta-cells
-Astrocytes
-Oligodendrocytes
-Hematopoietic precursors
Thomson: large scale and 98% purity (selection with blasticidin)

NOVEL CONCEPT:

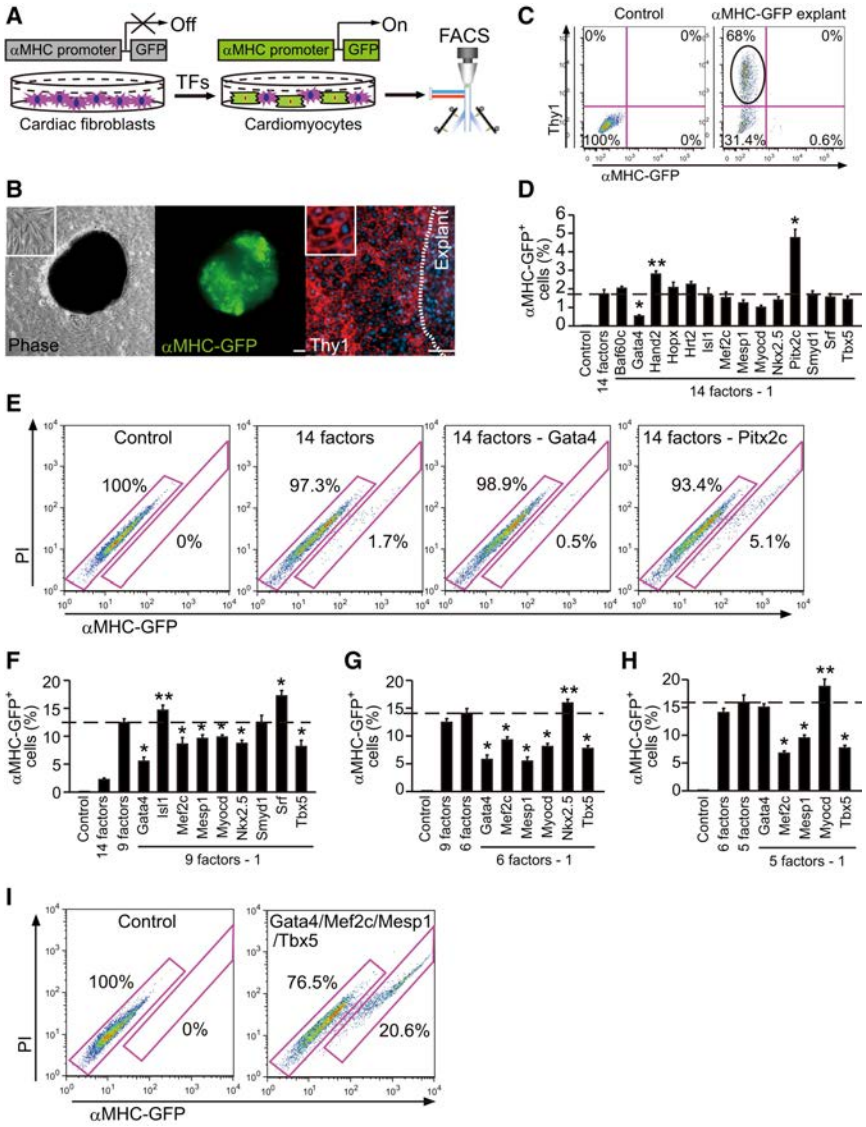
TRANSDIFFERENTIATION

Example of a rigorous study to demonstrate transdifferentiation



Transdifferentiation: from cardiac fibroblasts to cardiac myocytes

Screening for Cardiomyocyte-Inducing Factors



(A) Schematic representation of the strategy to test candidate cardiomyocyte-inducing factors.

(B) Morphology and characterization of fibroblast-like cells migrating from αMHC-GFP heart explants. Phase contrast (left), GFP (middle), and Thy-1 immunostaining (right). Insets are high-magnification views. See also Figure S1.

(C) Thy-1^{+}/GFP^{-} cells were FACS sorted from explant cultures for reprogramming.

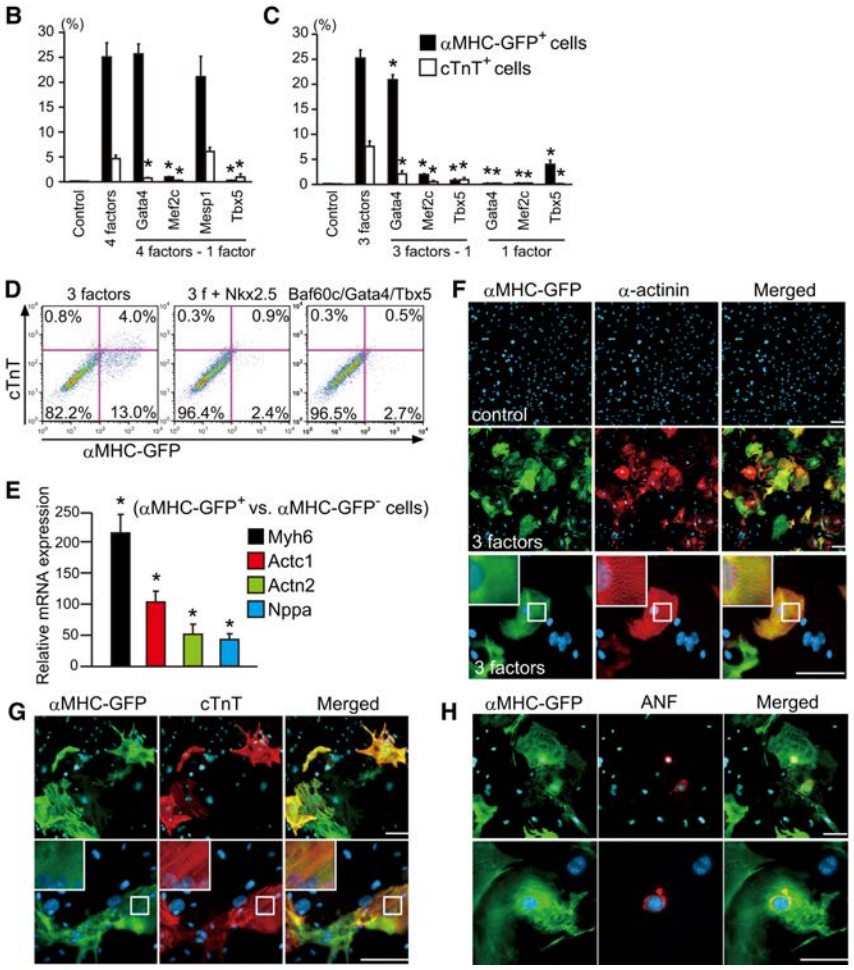
(D) Summary of FACS analyses for α-MHC-GFP^{+} cells. Effect on GFP^{+} cell induction with 14 factors or the removal of individual factors from the pool of 14 factors (n = 3). Removal of Baf60c, Hand2, Hopx, Hrt2, or Pitx2c did not decrease the percent of GFP^{+} cells and were excluded for further analyses. See also Figure S2.

(E) FACS plots for analyses of GFP^{+} cells. GFP^{+} cells were analyzed 1 week after 14 factor transduction. The number of GFP^{+} cells were reduced by removal of Gata4, but increased by removal of Pitx2c from 14 factors.

(F–H) Effect on GFP^{+} cell induction of the removal of individual factors from the pool of 9 (F), 6 (G), or 5 (H) factors (n = 3). Factors that did not decrease efficiency upon removal were excluded from further study.

(I) GFP^{+} (20%) cells were induced from fibroblasts by the combination of four factors, Gata4, Mef2c, Mesp1, and Tbx5. Representative data are shown in each panel. PI, propidium iodine. All data are presented as means ± SD. $^{*}p < 0.01$; $^{**}p < 0.05$ versus relevant control. Scale bars represent 100 μm.

Combination of Three Transcription Factors Induces Cardiac Gene Expression in Fibroblasts



(B) Quantitative data of GFP⁺ cells and cTnT⁺ cells in (A) (n = 3).

(C) Effect of the transduction of pools of three, two, and one factors on GFP⁺ and cTnT⁺ cell induction (n = 3).

(D) FACS analyses for α-MHC-GFP and cTnT expression. Effects of GMT plus Nkx2.5 and Baf60c/Gata4/Tbx5 transduction are shown.

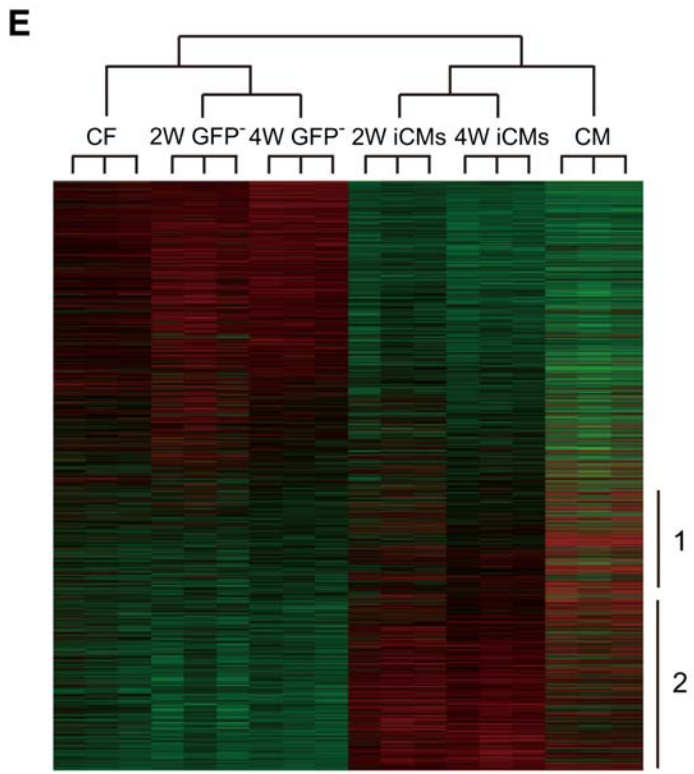
(E) The mRNA expression in GFP⁺ and GFP⁻ cells 7 days after GMT transduction was determined by qPCR (n = 3).

(F) Immunofluorescent staining for GFP, α-actinin, and DAPI. The combination of the three factors, GMT, induced abundant GFP, and α-actinin expression in cardiac fibroblasts 2 weeks after transduction. High-magnification views in insets show sarcomeric organization. See also figs1IMAGE-DOWNSAMPLED and figs2IMAGE-DOWNSAMPLED.

(G) Induced cardiomyocytes expressed cTnT by immunocytochemistry with clear sarcomeric organization 4 weeks after transduction. Insets are high-magnification views.

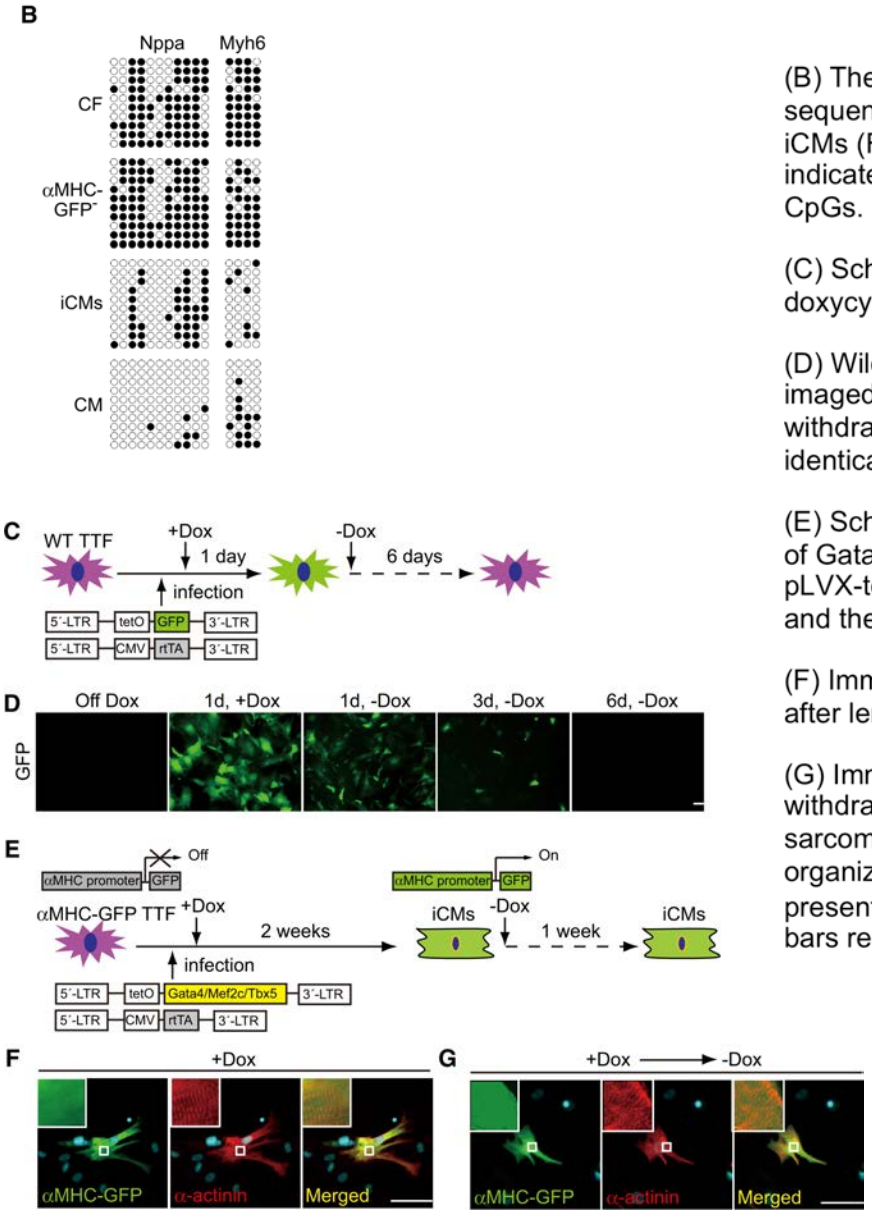
(H) Induced cardiomyocytes expressed ANF at perinuclear sites 2 weeks after transduction. All data are presented as means ± SD. *$p < 0.01$ versus relevant control. Scale bars represent 100 μm.

Gene expression of induced cardiac myocytes is globally reprogrammed



(E) Heatmap image of microarray data illustrating differentially expressed genes among CF, α-MHC-GFP⁻, iCMs (FACS sorted 2 and 4 weeks after transduction), and CM (n = 3 in each group). The scale extends from 0.25- to 4-fold over mean (−2 to +2 in log2 scale). Red indicates increased expression, whereas green indicates decreased expression. Group 1 includes the genes upregulated only in CM, and group 2 includes the genes upregulated in CM and 4W-iCMs compared to CF. Lists of genes are shown in Table S1 and Table S2. All data are presented as means ± SD. $^{*}p < 0.01$; $^{**}p < 0.05$ versus relevant control. See also Figure S4 for endogenous and exogenous expression of reprogramming factors and Table S1 and Table S2 for differentially expressed genes.

Fibroblasts are stably reprogrammed into iCMs by Gata4, Mef2c and Tbx5



(B) The promoters of *Nppa* and *Myh6* were analyzed with bisulfite genomic sequencing for DNA methylation status in CF, α-MHC-GFP⁻ cells, α-MHC-GFP⁺ iCMs (FACS sorted 4 weeks after transduction), and neonatal CM. Open circles indicate unmethylated CpG dinucleotides; closed circles indicate methylated CpGs.

(C) Schematic representation of the strategy to test expression kinetics of the doxycycline (Dox)-inducible lentiviral system.

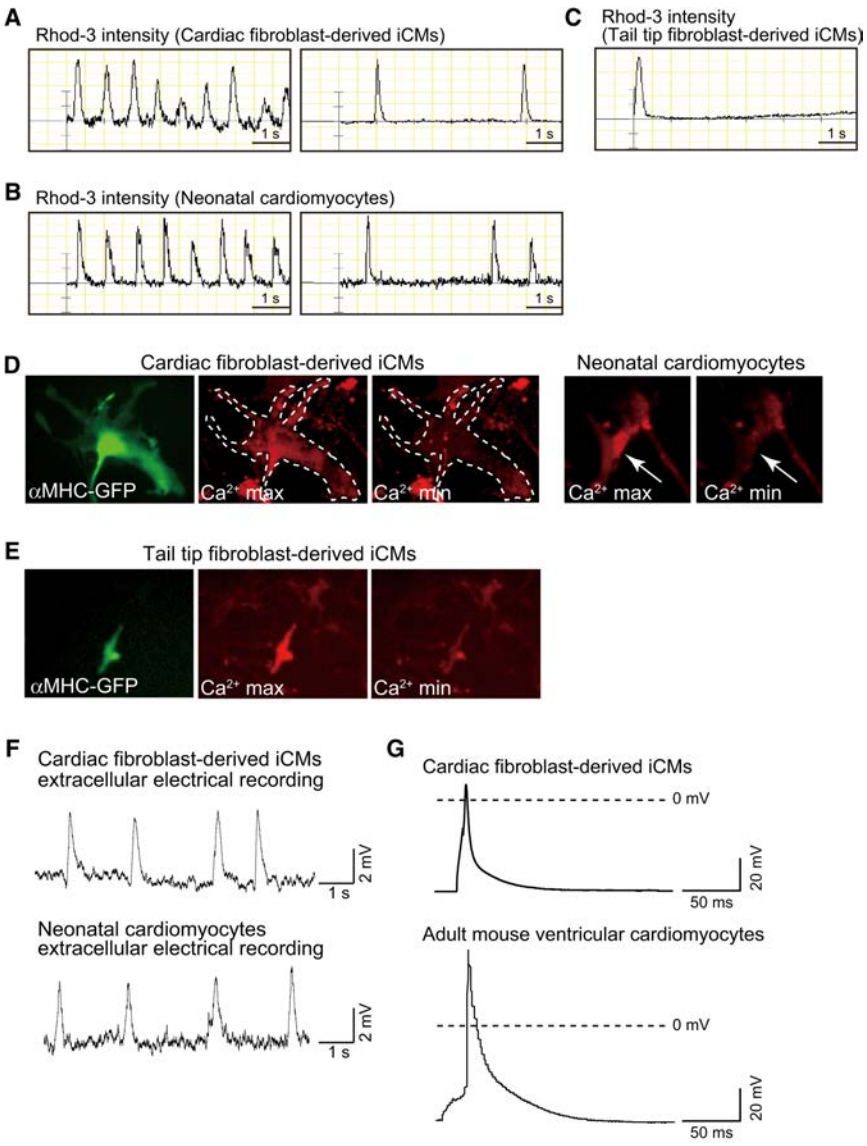
(D) Wild-type TTFs were infected with pLVX-tetO-GFP and pLVX-rtTA and imaged before (off Dox), 1 day after Dox addition, and at time points after Dox withdrawal (–Dox). All images were taken using constant exposure times and identical camera settings.

(E) Schematic representation of the strategy to determine temporal requirement of Gata4/Mef2c/Tbx5 in reprogramming. Thy1⁺/GFP⁻ TTF were infected with the pLVX-tetO-GMT and pLVX-rtTA lentiviruses, and Dox was added for 2 weeks and thereafter withdrawn for 1 week.

(F) Immunofluorescent staining for GFP, α-actinin, and DAPI in iCMs 2 weeks after lentiviral infection and Dox induction.

(G) Immunofluorescent staining for GFP, α-actinin, and DAPI 1 week after Dox withdrawal. iCMs maintained α-MHC GFP expression and had α-actinin positive sarcomeric structures. High-magnification views in insets show sarcomeric organization. Representative data are shown in each panel. All data are presented as means ± SD. $^{*}p < 0.01$; $^{**}p < 0.05$ versus relevant control. Scale bars represent 100 μm.

Induced CMs exhibit spontaneous Ca2+ Flux, Electrical Activity and beating



Induced Cardiomyocytes Exhibit Spontaneous Ca^{2+} Flux, Electrical Activity, and Beating

(A and B) Cardiac fibroblast (CF)-derived iCMs showed spontaneous Ca^{2+} oscillation with varying frequency (A), similar to neonatal cardiomyocytes (B). Rhod-3 intensity traces are shown.

(C) Tail-tip dermal fibroblast (TTF)-derived iCMs showed spontaneous Ca^{2+} oscillation with lower frequency. The Rhod-3 intensity trace is shown.

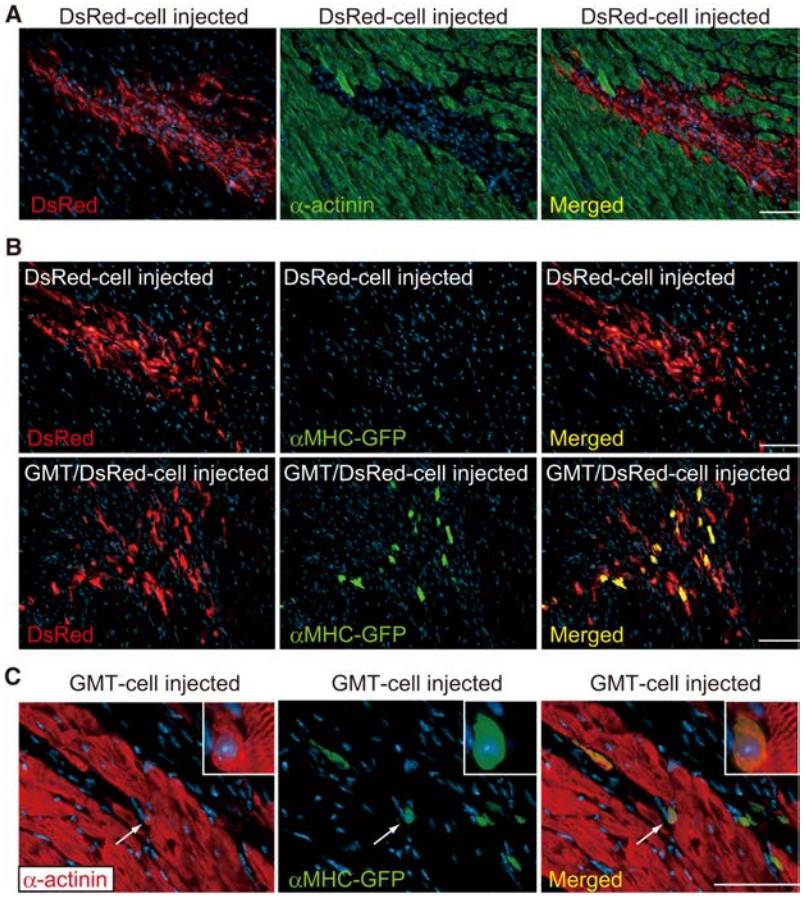
(D) Spontaneous Ca^{2+} waves observed in CF-derived α-MHC-GFP^{+} iCMs (white dots) or neonatal cardiomyocytes (arrows) with Rhod-3 at Ca^{2+} max and min is shown. Fluorescent images correspond to the Movie S1.

(E) Spontaneous Ca^{2+} oscillation observed in the TTF-derived α-MHC-GFP^{+} iCMs with Rhod-3 at Ca^{2+} max and min is shown. Fluorescent images correspond to the Movie S2.

(F) Spontaneously contracting iCMs had electrical activity measured by single cell extracellular electrodes. Neonatal cardiomyocytes showed similar electrical activity.

(G) Intracellular electrical recording of CF-derived iCMs cultured for 10 weeks displayed action potentials that resembled those of adult mouse ventricular cardiomyocytes. Representative data are shown in each panel (n = 10 in A–F, n = 4 in G). See also Figure S5 and Movies S1, S2, S3 and S4.

Transplanted Cardiac Fibroblasts Transduced with Gata4/Mef2c/Tbx5 Can be reprogrammed To Cardiomyocytes in Vivo



Transplanted Cardiac Fibroblasts Transduced with Gata4/Mef2c/Tbx5 Can Be Reprogrammed to Cardiomyocytes In Vivo

(A) DsRed infected cardiac fibroblasts (DsRed-cell) were transplanted into NOD-SCID mouse hearts 1 day after infection and cardiac sections were analyzed by immunocytochemistry after 2 weeks. Transplanted fibroblasts marked with DsRed did not express α-actinin (green).

(B) Cardiac fibroblasts infected with DsRed or Gata4/Mef2c/Tbx5 with DsRed (GMT/DsRed-cell) were transplanted into NOD-SCID mouse hearts 1 day after infection and visualized by histologic section. Note that a subset of GMT/DsRed cells expressed α-MHC-GFP. Data were analyzed 2 weeks after transplantation.

(C) Gata4/Mef2c/Tbx5-transduced cardiac fibroblasts (GMT-cell) were transplanted into mouse hearts and histologic sections analyzed. A subset of induced GFP^+ cells expressed α-actinin (red) and had sarcomeric structures. Insets are high-magnification views of cells indicated by arrows. Data were analyzed 2 weeks after transplantation.

Representative data are shown in each panel (n = 4 in each group). Scale bars represent 100 μm. Note that GMT/DsRed or GMT-infected cells did not express GFP at the time of transplantation (Figure 4A).