Synthetic Circuits for the Targeting and Differentiation of Cardiomyocytes to Repair Infarcted Cardiac Tissue

Regenerative Sciences Institute iGEM Team 2008

Bay Area RSI iGEM 2008 Team

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Vision

Develop new ways to treat disease by programming cells to recognize and repair damaged tissue

Myocardial Infarction (MI)

1,200,000 people suffer a new or recurrent MI every year, and about 40% of them die as a result of the attack. This means that roughly every 65 seconds, an American dies of a coronary event.

Need for Better MI Treatment

There is an urgent need to repair MI damaged hearts

Fact - Human and other mammalian hearts do not regenerate after damage

What to do? -> Direct cells to repair hearts.

Fact - Promise of ES and adult stem cells are all based on "Do the right thing"

Fact - State of the art: direct adult mesenchymal-derived stem cells to the injured myocardium by injection or bispecific antibody targeting and then **hope** that they do the "right thing."

Fact - Most studies report benefit, albeit modest ~ 5-8% improved LV function

Problems With Current Stem Cell Therapies

- Adult stem cells do not easily recognize damaged heart
- When localized to the heart, adult stem cells do not efficiently differentiate into cardiomyocytes
- Differentiated cardiomyocytes introduced into the heart die
- Adult stem cells do not efficiently stimulate new blood vessel formation
- Therapies are limited to the injured surface. Damaged cells beneath the surface are not targeted
- Current stem cell based therapies do not work very well and provide only modest improvement in function

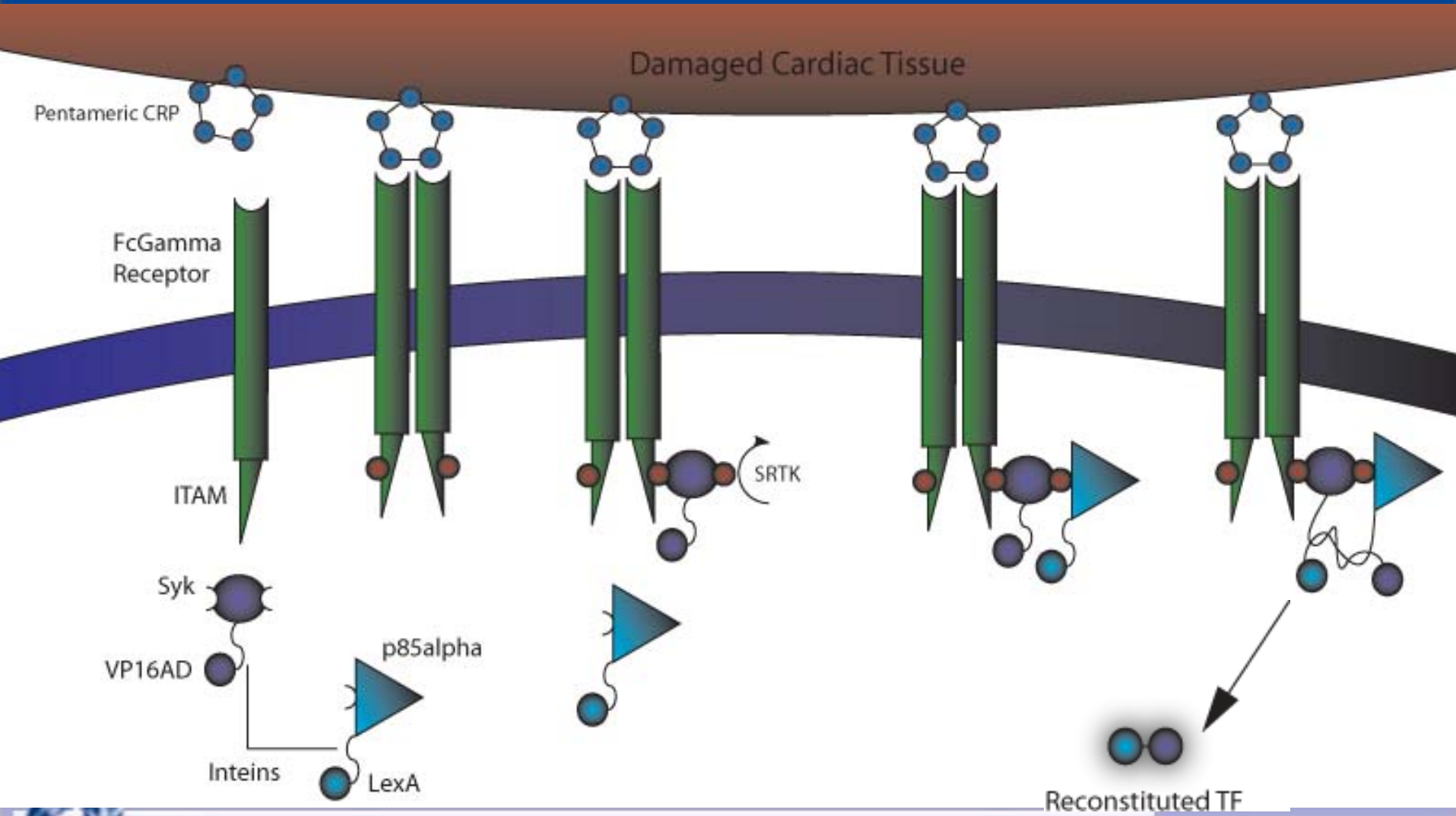
iGEM 2007 Targeting Circuits to Damaged Cardiac Tissue

In 2007 we designed novel chimeric receptor-coupled intein-mediated signaling circuits to target damaged cardiac tissue and activate effector genes that aid in integration, anti-apoptosis and alteration of the microenvironment, and the results from our initial experiment with the CRP circuit were very promising.

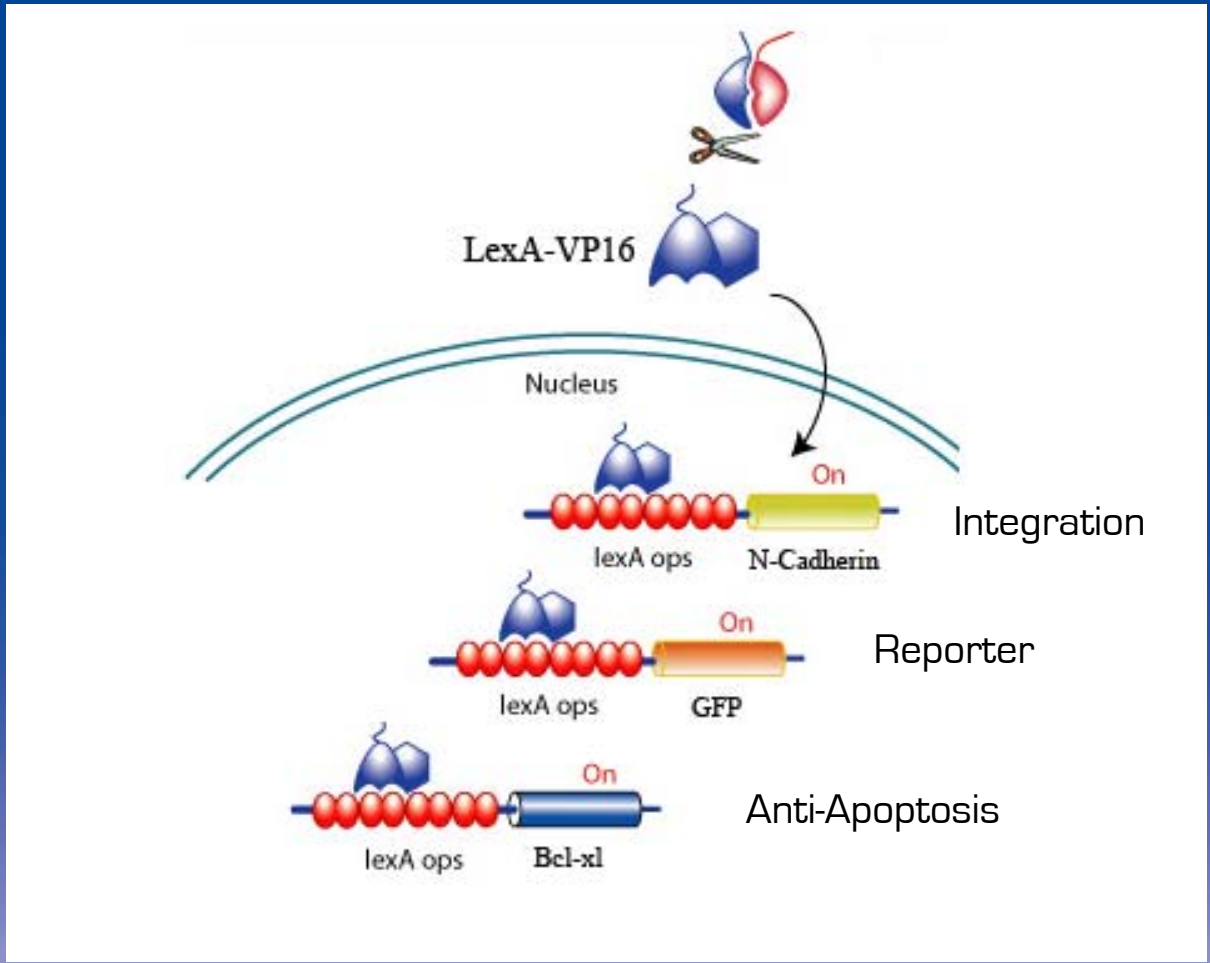
Progress Since iGEM 2007

- CRP Circuit Targets Damaged Cardiomyoblasts In Vitro
- CRP Receptor Relays Signal To Activate Effectors Upon CRP Receptor Relays Signal To Activate Effectors Upon Binding
- Complementary Circuit for the Efficient Induction of Cardiomyocyte Differentiation

CRP Targeting Circuit

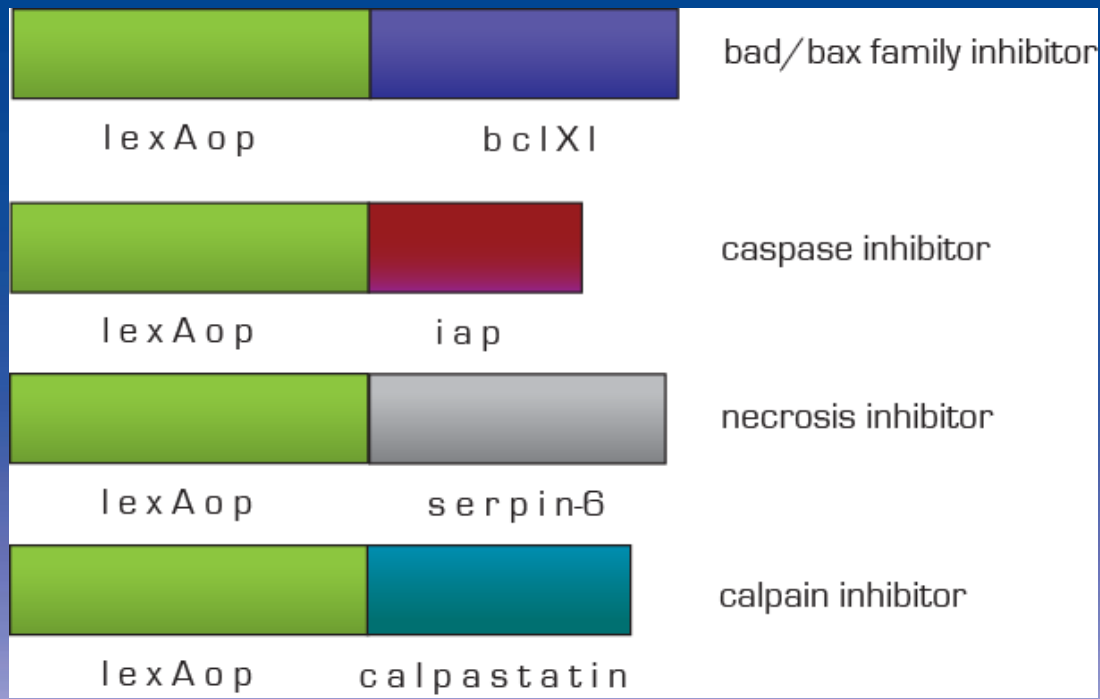


Activation of Effector Genes



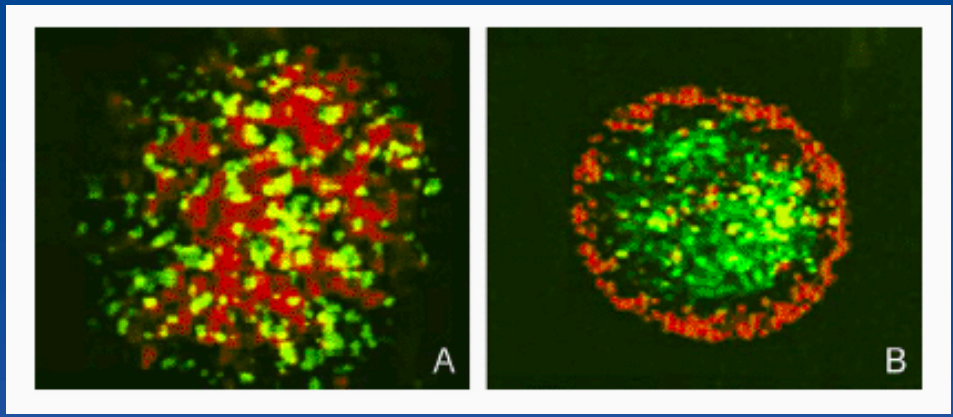
Effectors: Inhibitors of Endogenous Cell Death Pathway

- Major problem with current stem cell therapies for the heart = cell death of stem cells/cardiomyocytes
- Block endogenous apoptosis and necrosis with proteins:

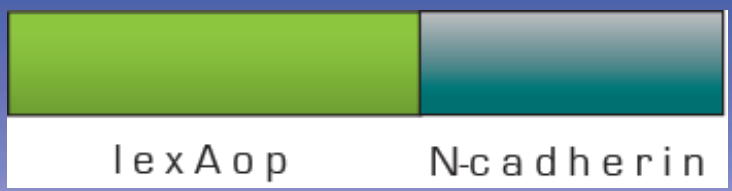


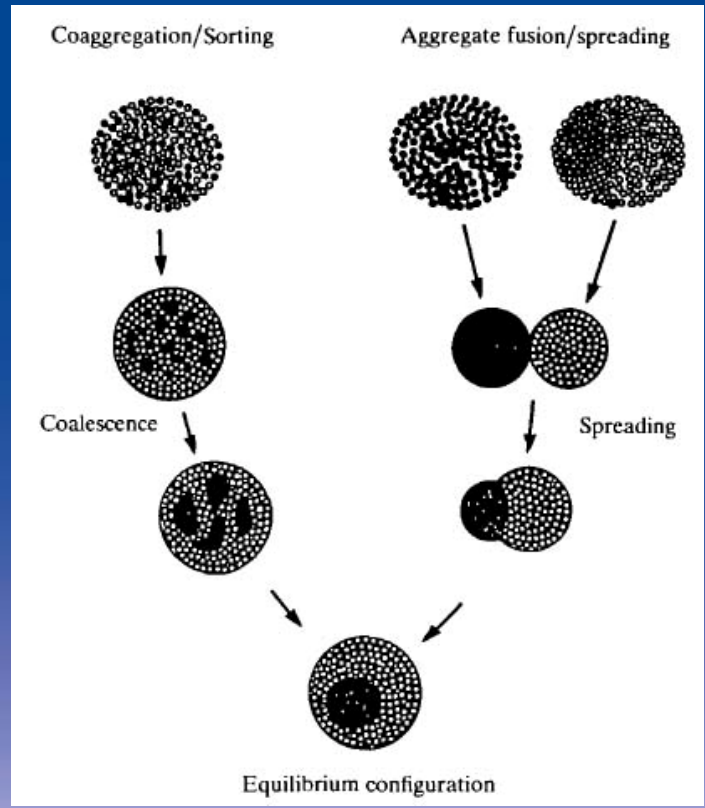
Effectors: Tissue Pattern Formation

Increase tissue integration via altering cell-cell interactions and patterning (Differential Adhesion Hypothesis)



Sorting of Transfected L cells expressing N-Cad at 2.4 (green):1 (red) ratio. Foty and Steinberg, 2005





Effectors: Tissue Microenvironment

Pro-Angiogenesis

lexAop VEGF

VEGF Promotes the formation of new blood vessel

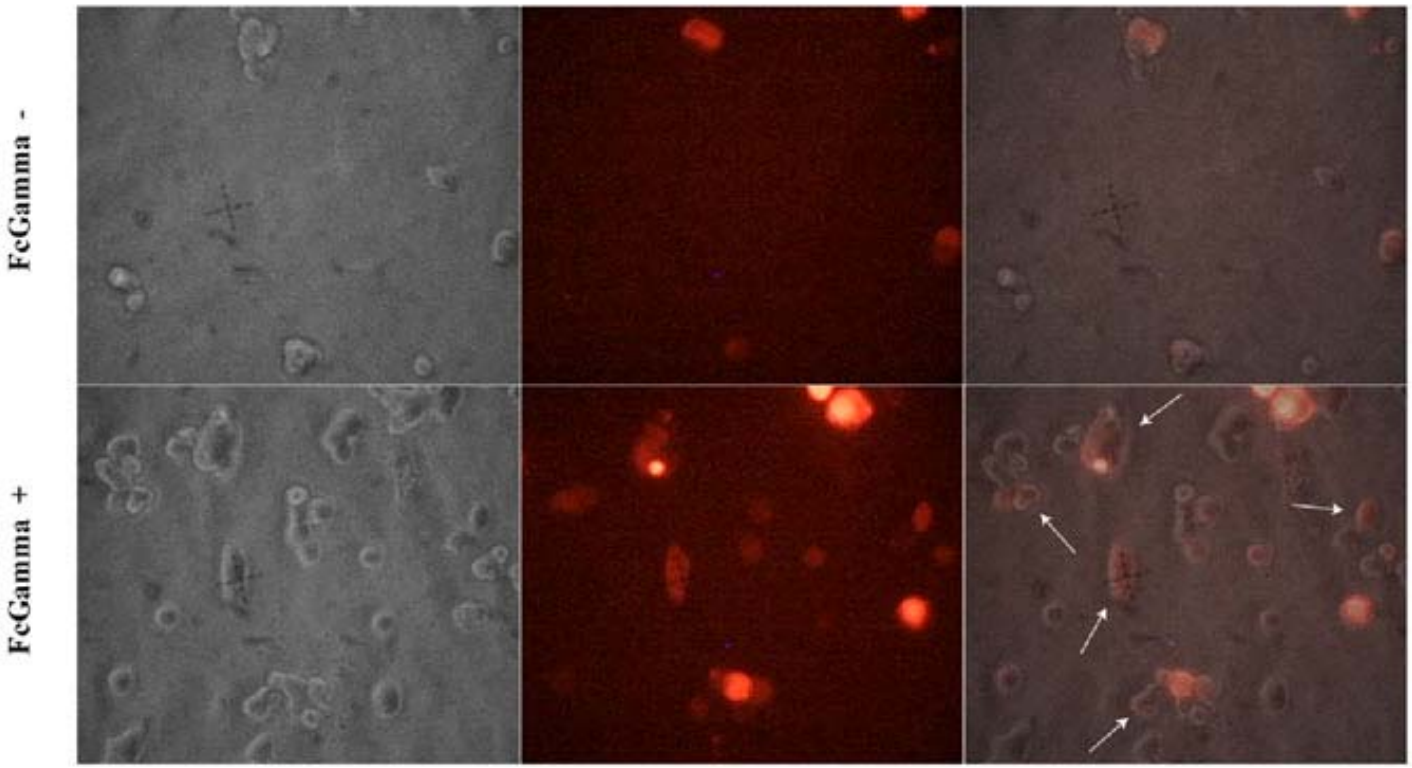
Anti-Scarring

lexAop HGF

HGF Inhibits apoptosis of nearby cells and fibrosis via competing signal to TGF-β

FCGamma Receptor Binds Immobilized CRP on Damaged Cardiomyocytes In Vitro

FcGammaRIIa positive cells (red) bound the CRP-coated ischemic H9C2 cells with much greater affinity than FcGammaRIIa negative cells

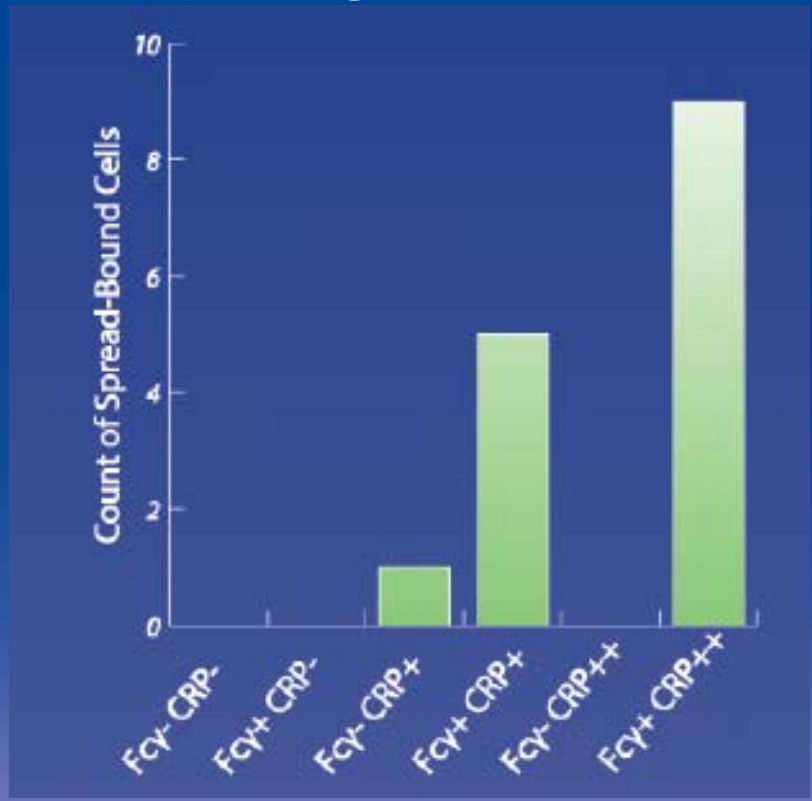


Arrows indicate flattened attached 293T FcGamma positive cells on CRP bearing MI rat H9C2 cardiomyocytes

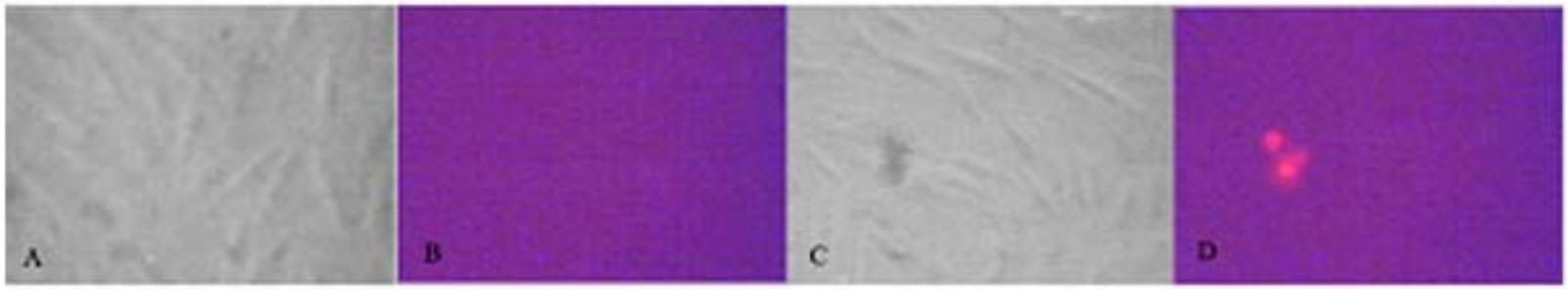
FCGammaRIIa Expressing Cells Bind Ischemic Myocardiocytes In Presence of Competing CRP

FCGamma Receptor	Serum CRP	Count of Spread Bound cells
-	-	0
+	-	0
-	+	1
+	+	5
-	+ competitive	0
+	+ competitive	9

FCGammaRIIa expressing cells bind in the presence of 150ug/ml competitor CRP, which simulates elevated CRP serum levels in vivo

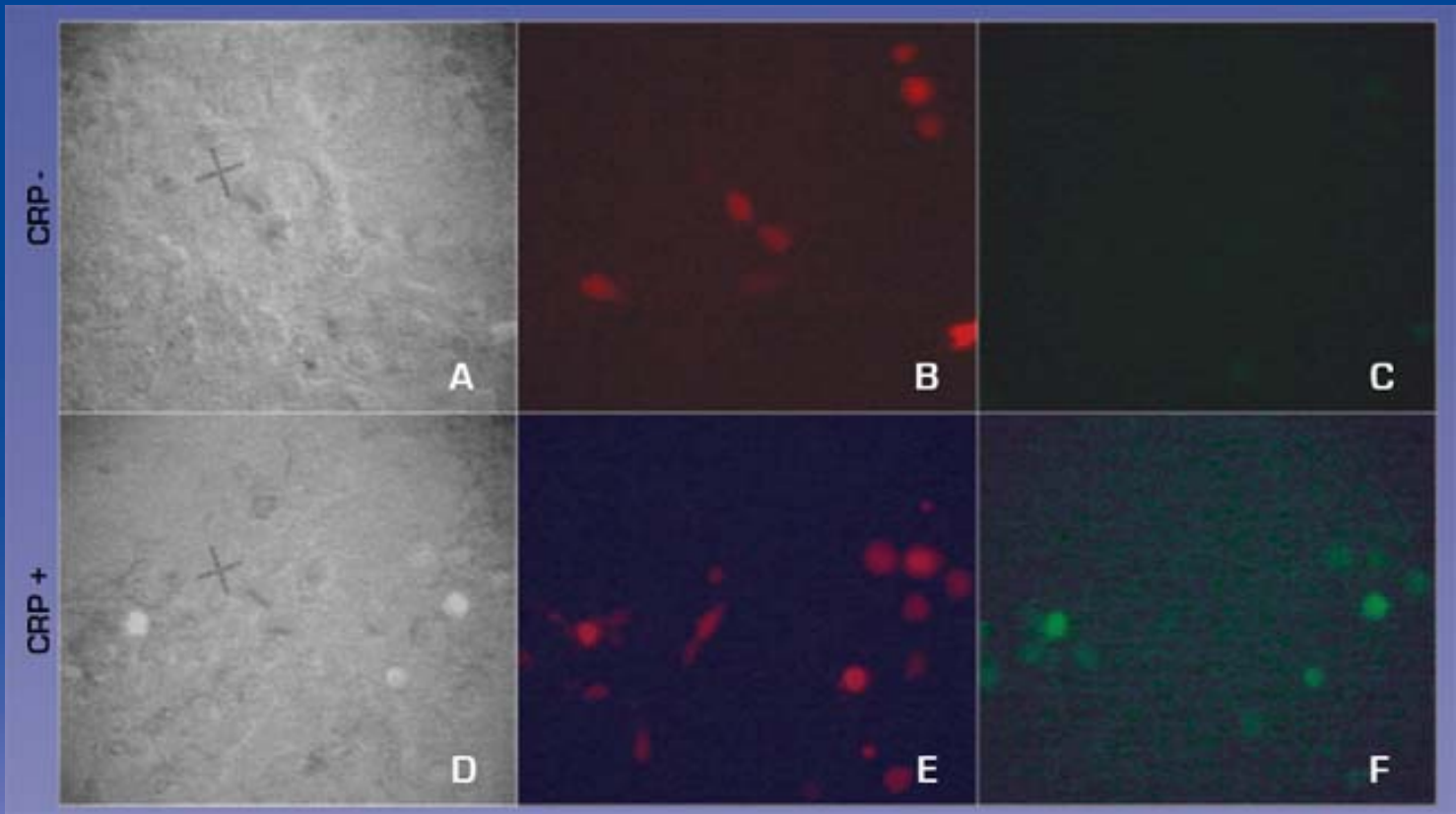


H9C2-FCGammaRIIa cells bind to Immobilized CRP on Damaged Cardiomyocytes In Vitro



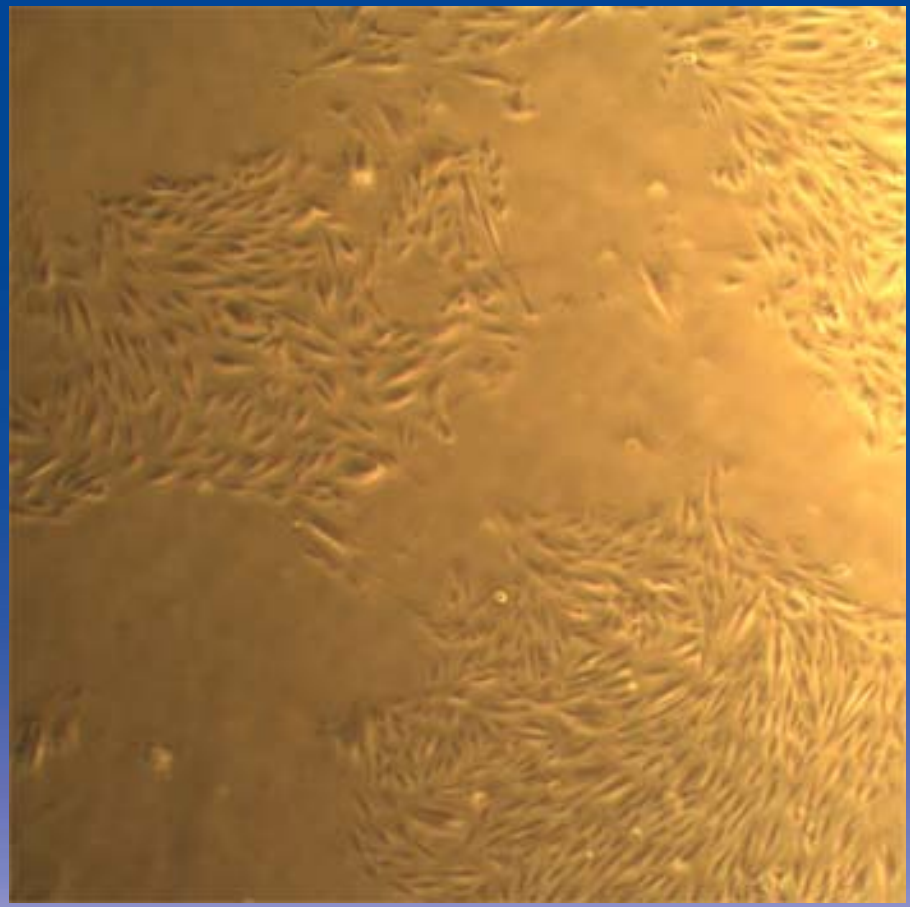
A,B Cells infected with control vector **C,D** Cells infected with FCGammaIIa. Red cells in **D** demonstrate binding to ischemic CRP positive cardiomyocytes.

CRP activates GFP signaling upon FCGamma Binding



150ug/ml CRP activates GFP effector in HEK293T cells transfected with CRP Circuit

Generation of Clonal H9C2-FCGamma Cell Lines



CRP Targeting Circuit Advantages

Advantages

1) Selectively binds damaged cardiomyocytes

2) Neutralizes CRP, reducing inflammation

3) Adaptable for other CRP-associated pathologies

4) Signaling system uses endogenous pathway

5) Limited potential for cross-talk

CRP Targeting Circuit Concerns

Concerns

1) FcGammaRIIa also binds serum IgG. To minimize this, we used a mutant, FcGammaRIIa-R131, which exhibits greater selectivity for CRP. By linking the FcGammaR circuit with another receptor-based circuit, we can implement an AND gate to achieve even greater sensitivity.

2) Basal CRP serum levels may interfere with signaling. However, even in the presence of soluble CRP, preliminary experiments have shown that cells bearing FcGammaRIIa bind ischemic cardiomyocytes.

CRP Circuit Future Directions

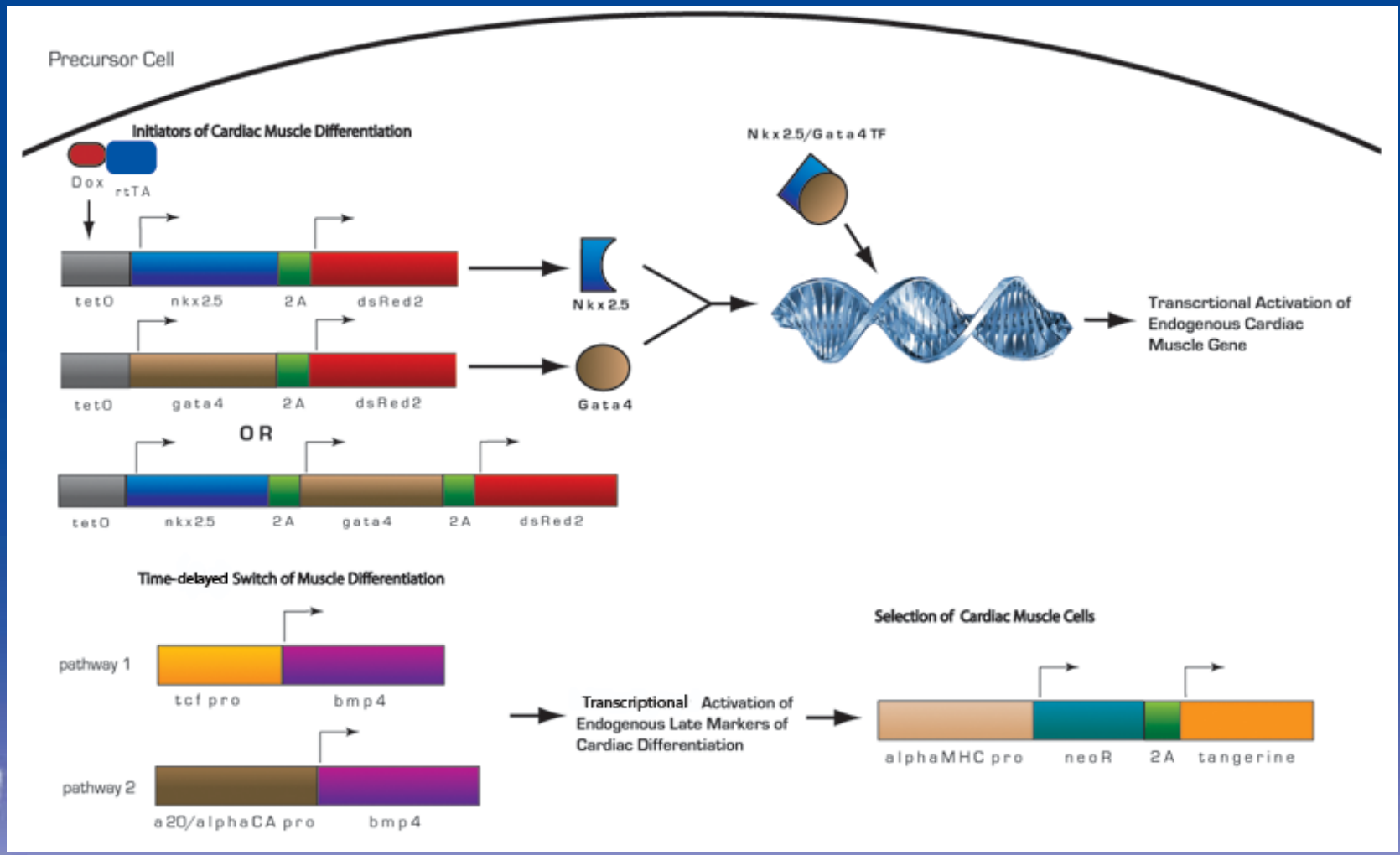
- Tune Sensitivity of FCGammaR2a Receptor in clonal lines
- LexA mutants for reduced affinity
- Next Generation Universal Receptor to target different tissues

Part II. Cardiomyocyte Differentiation

Problems with Current Differentiation Strategies

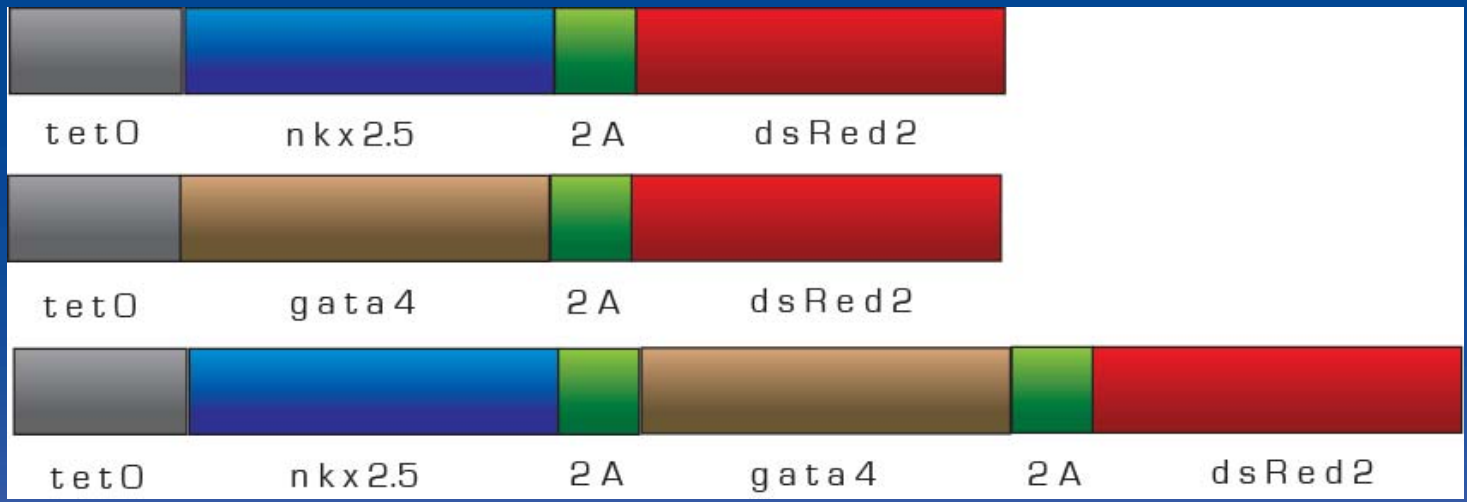
- Low efficiencies
- Long differentiation time
- No cardiomyocyte selection from precursor cell population

Differentiation Circuit

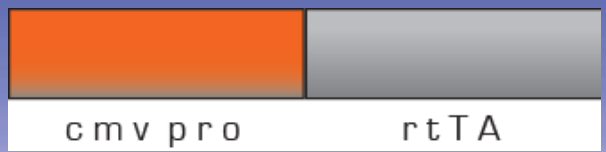


Doxycycline Inducible System of Cardiomyocyte Differentiation - Nkx-2.5, Gata4, rtTA

Inducible expression of Nkx-2.5 and Gata4 through separate constructs OR in one construct

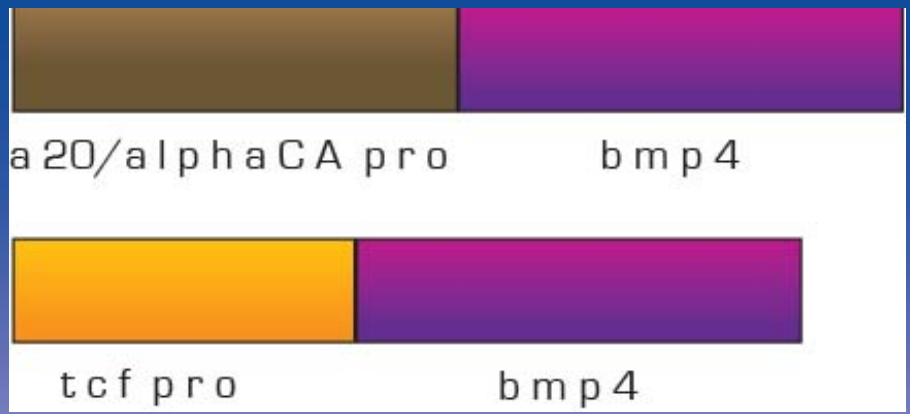


Constitutive Tet Transactivator, rtTA, for Tet-On

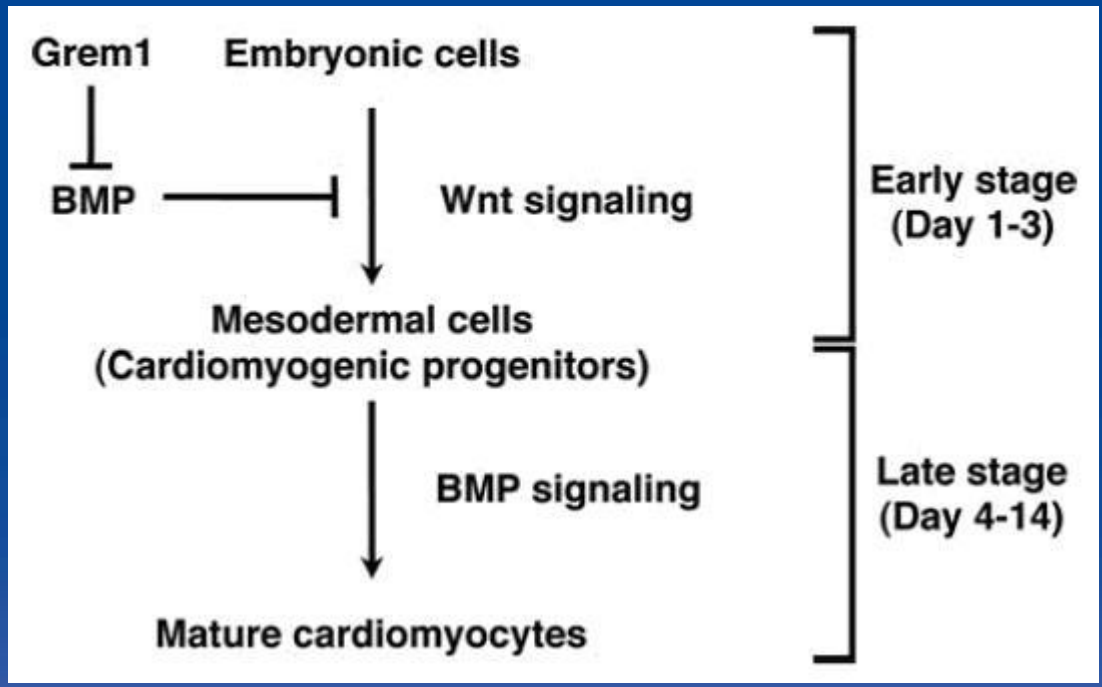


Delayed Bmp4 Expression: Choice of Two Promoters

- Early induction of Bmp4 has inhibitory effects
- Downstream target of Nkx-2.5 and Gata4
- AlphaCA promoter induced directly by Nkx-2.5 and Gata4
- TCF promoter induced through TCF buildup in the beta-catenin pathway.



Delayed Bmp4 Expression: TCF Promoter



Kami D, Shiojima I, Makino H, Matsumoto K, Takahashi Y, et al. (2008) Gremlin Enhances the Determined Path to Cardiomyogenesis BMP4 expression at early stages inhibits cell from entering into the cardiomyogenic pathway.

Selection of Differentiated Cardiomyocytes

- Neomycin resistance gene under the control of a very late marker of cardiac differentiation, alpha cardiac myosin heavy chain

Differentiation Circuit Advantages

1) After induction with doxycycline, our circuit works autonomously.

2) We utilize key endogenous transcription factors from the cardiomyogenic pathway

3) Circuit timing is tightly controlled with early and delayed expression of key regulatory proteins

4) The NeoR gene selects for pure populations of cardiomyocytes

Differentiation Circuit Concerns

Stem cells may turn cancerous when injected.

In our circuit, differentiation and selection of precursor cells will occur prior to targeting. Thus, cardiomyocytes are post-mitotic, reducing the possibility of cancer arising from our therapy.

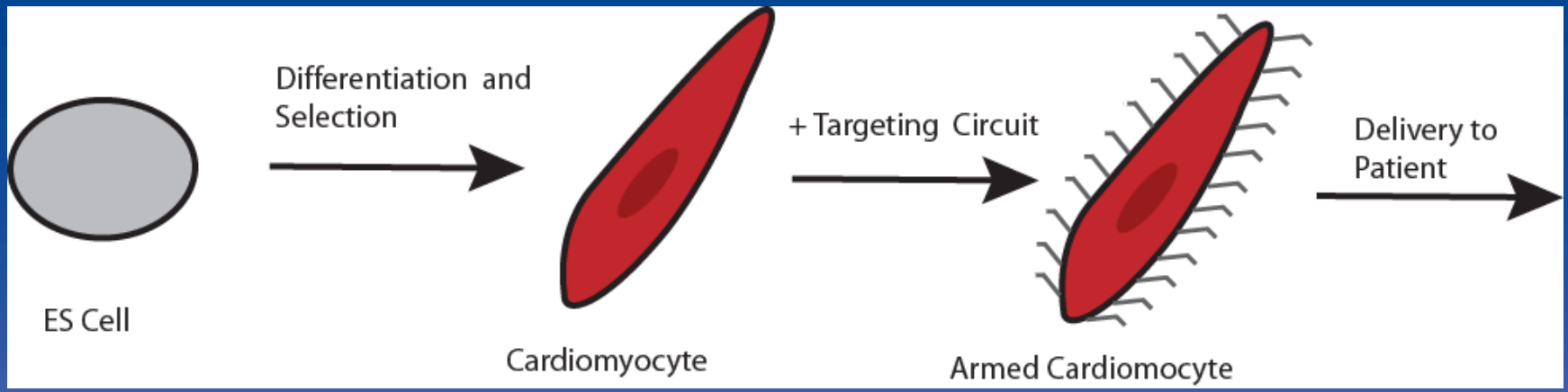
Secondly, our system need not utilize stem cell precusors. From the literature, there is a good possibility that this therapy can be used on cardiac fibroblasts or non-cardiac cells such as adipocytes or leukocytes via transdifferentiation.

Differentiation Future Directions

- Transfer of constructs to lentiviral vectors and HSV amplicon
- Characterization of differentiation circuit
- Integration of both targeting and differentiation circuits in culture
- In vivo animal model tests
- Clinical trials

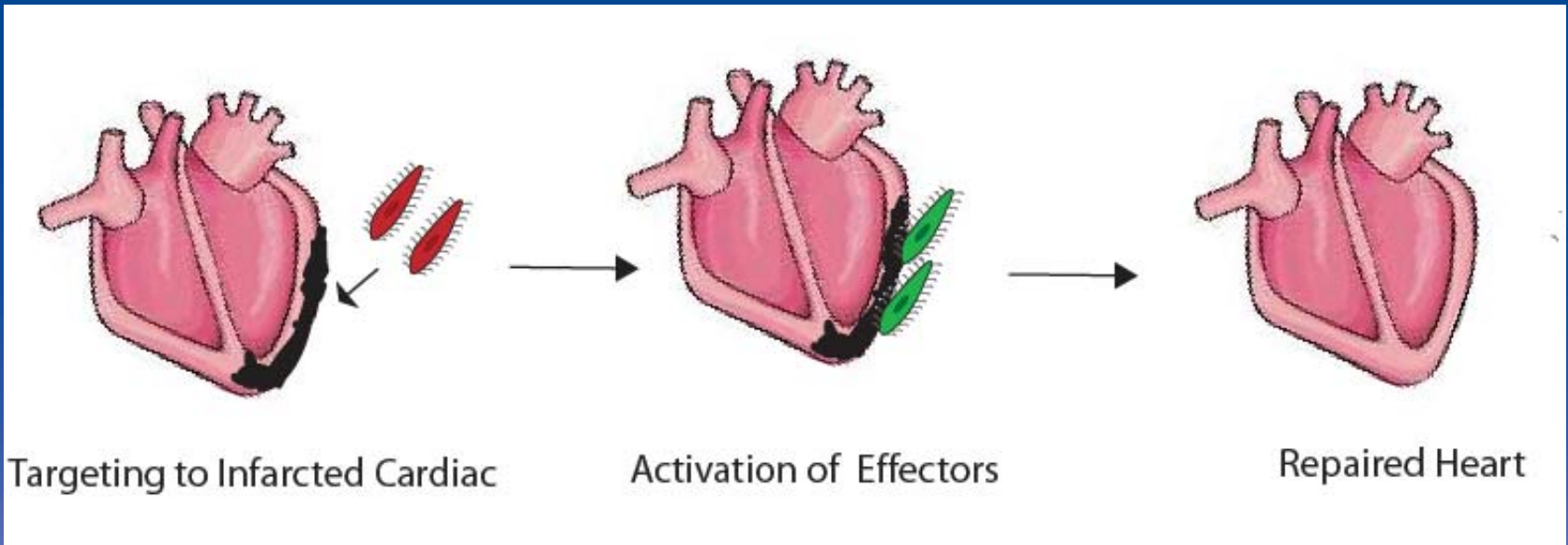
Synthetic Biology and Gene Therapy

Differentiation and Arming



Synthetic Biology and Gene Therapy

Targeting and Repair



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