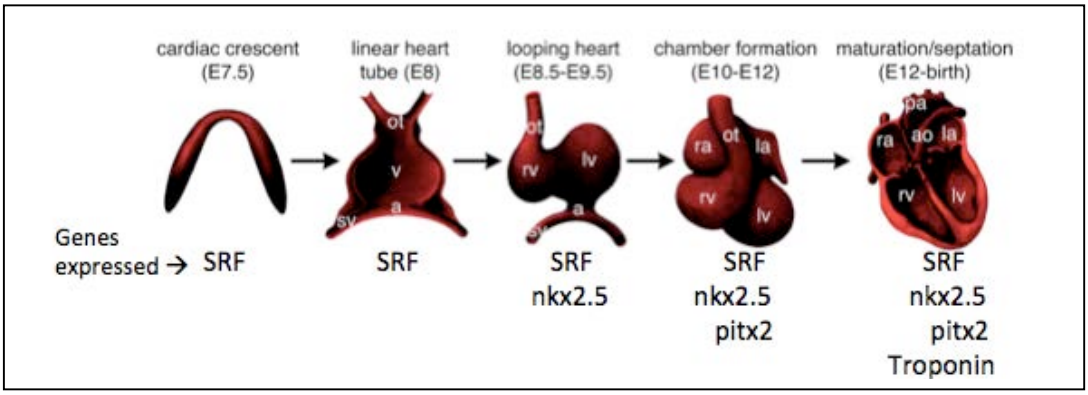
7.013 Problem Set 6- 2018

Introduction
The heart pumps oxygenated blood through the body and sends deoxygenated blood to the lungs for re-oxygenation. Heart disease is a major killer in the US. Malformations of the heart are a common birth anomaly. This makes understanding development and regeneration of the heart of great interest. This problem set focuses on mechanisms underlying cardiac development and strategies for repair.

The mouse heart develops over time as diagrammed in Figure 1 (*from Bruneau, 2002*) and explained below.

- A group of cells form a crescent shaped region (on embryonic day 7.5 (E7.5))
- Future heart cells form a tube (E8)
- The tube loops (E9.5)
- Heart chambers form (atria (a) and ventricles (v)) (E10)
- Heart walls form their final fate: 'cardiac muscle', which beats (from E12 onward)
- Gene expression changes over time. Genes expressed at each stage are shown below.



Question 1
In order to understand when the cardiac muscle 'knows' what it is going to form, scientists performed an isolation experiment, where future ventricle cells (v) were removed at E7.5 and E8.5. These isolated cells (called 'explants') were allowed to grow and develop in the lab. The explants were assessed 6 and 5 days later respectively, when embryos from which they were removed would have reached 13.5 days old.

Days at which explants were isolated	Days for which explants were grown in the lab (until the embryos would be 13.5 days old)	Beating cardiac muscle formed?
E7.5	6 Days	No
E8.5	5 Days	Yes

a) From this experiment, when do cells commit to form cardiac muscle?

b) From this experiment, at what stage do heart cells differentiate?

Question 1 continued

c) What is the combinatorial gene expression code for heart development?

d) SRF, nkx2.5 and pitx2 are all transcription factors. Classify…

i. **SRF** as ubiquitous/ regulatory (restricted)/ effector (cell-type specific) protein**. Circle one.**

ii. **nkx2.5** as a regulatory (restricted) OR effector (cell-type specific) protein. **Circle one.**

e) You make mouse mutants that have homozygous loss-of-function mutations in nkx2.5, pitx2 and troponin genes. You observe the following changes in RNA of the developing heart at E13.5. ***Note:*** *the row showing the wild type is shaded.*

Mutants	RNA profile in the future heart
Control (wild type)	SRF, nkx2.5, pitx2, troponin
SRF	None
nkx2.5	SRF
Pitx2	SRF and nkx2.5
Troponin	SRF, nkx2.5, pitx2

Based on the table, draw a flowchart to account for how SRF, nkx2.5, pitx2 and troponin genes regulate one another

f) Interestingly, you identify a mutant that has the RNA for nkx2.5 but there is no Nkx2.5 protein. You examine the nkx2.5 RNA and see it is larger (in terms of the numbers of bases) than that observed in the wild type. **Give one plausible explanation for a lack of Nkx2.5 protein production.**

g) BMP signaling is required for heart formation.

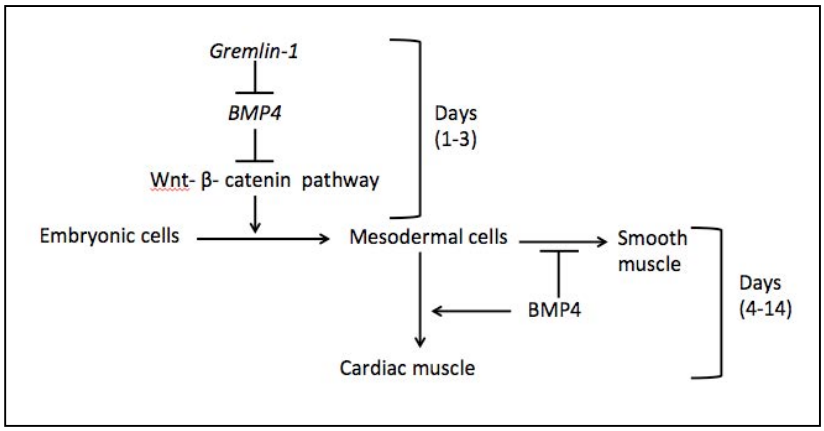
- BMP ligand binds to a receptor on the target cell membrane.
- The receptor is activated via phosphorylation.
- Activated receptor phosphorylates the Smad1 transcription factor.
- Phospho-Smad1 changes transcription of target genes, including SRF.

i. On what cells do you expect to find the BMP receptor? **Explain** your choice.

ii. Where would phospho-Smad1 be localized: **Cytoplasm/ plasma membrane/ nucleus**? **Explain** your choice.

iii. In a BMP receptor mutant lacking a signal sequence, briefly **explain** what would happen to heart formation?

Question 1 continued
Cardiac muscle is one type of contractile cell. Smooth muscle that lines blood vessels is another. The heart is largely cardiac muscle, but blood vessels entering and leaving the heart must make smooth muscle. There is a therefore a choice of which muscle type is made, shown by the gene regulatory network below *(Reference: Umezawa et al PLos 2008)*.



- Gremlin-1 promotes Wnt-β catenin signaling by inhibiting BMP4.
- This promotes the formation of mesoderm cells from the embryonic cells
- Mesodermal cells either form cardiac muscle if BMP4 is active or skeletal muscles when BMP4 is inactive.

Predict whether **cardiac muscle/ smooth muscle/ neither/ both** will be formed if … …

i. In a *Gremlin-1* homozygous null mutant?

ii. If the cells in the mesoderm have a heavily methylated *BMP4* gene promoter?

iii. In a constitutively active β-catenin?

Question 2
There are five cell types in the heart:

- Cardiomyocytes (form cardiac muscle)
- Endocardial cells (line the heart chambers)
- Epicardial cells (form a surrounding sheath)
- Pacemaker cells (set heartbeat rhythm)
- Purkinje neurons (control contraction)

a) During heart development, cardiomyocytes and endocardial cells appear simultaneously. Is this consistent with **migration/ co-induction/ sequential induction**? Circle the correct option(s) and **explain** why you selected this option(s).

b) Later, the other cell types appear. If you remove the cardiomyocytes, no pacemaker cells appear. Is this consistent with **migration/ co-induction/ sequential induction**? Circle the correct option(s) and **explain** why you selected this option(s).

Question 2 continued

c) Formation of the heart tube requires lengthening of the existing epithelium.

~~i.~~ What is an epithelium?

ii. Name three processes that can lengthen an epithelium, and briefly **explain** how each does so.

d) Predict the effect of each of the following events on the formation of the heart tube. **Offer a reasonable mechanism to account for your prediction.**

i. Cells express a defective cadherin protein that lacks its extracellular domain.

ii. Cells are treated with nocodazole, a small molecule inhibitor of microtubule function.

e) The cardiac epicardium migrates into the heart region to set up a protective cell sheet around the developing heart.

~~i.~~ What is the name for the cell state that can migrate?

ii. What is the process called by which migratory cells convert to a cell sheet? Give two changes that occur during this process.

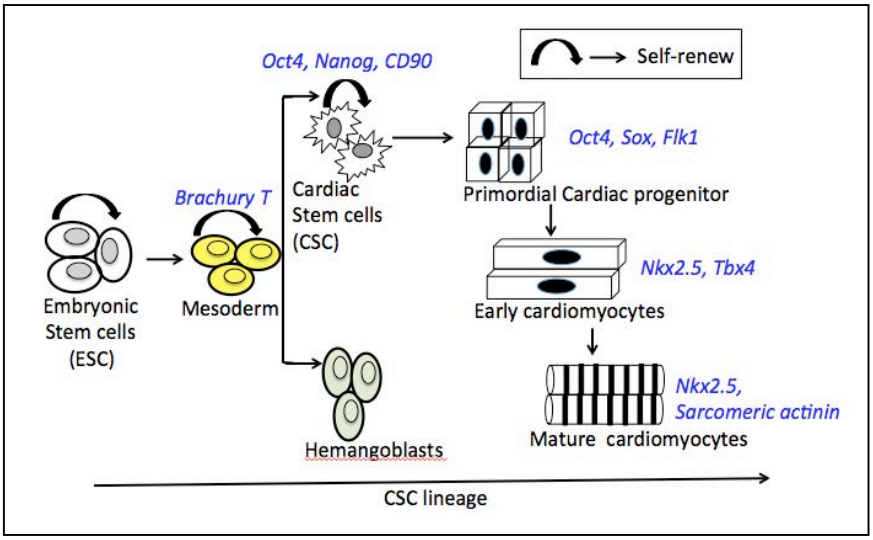
f) Troponin protein is critical for heart muscle contraction and functions in cytoplasm. **Explain** what mutation of troponin gene that might give the results described below. Where multiple answers are possible, **list only one** for each of the following parts.

i. Cells secrete Troponin protein.

ii. Cell lacks the mRNA corresponding to the troponin protein.

Question 3

Stem cells are being considered as a means to treat heart failure. Cardiac stem cells (CSC) can be prepared starting from embryonic stem cells (ESC) as shown in the following lineage. ***Note:*** *Genes expressed in each cell type are italicized and shown in blue.*



a) Are all the cells in this lineage likely to have **IDENTICAL** histone acetylation patterns? Why or why not?

b) List **two** characteristics of the CSC that qualifies them as stem cells.

c) CSC transcription factors include **Oct4 and Nanog** and the cell surface protein **CD90**. Which of these proteins would be most useful to purify living CSC from a mixed population that has multiple cell types by FACS (Florescence activated cell sorter)? **Explain** your choice.

d) CSCs are also naturally found in the adult heart (at very low levels). You want to determine the half-life of CSC in mice by FACS analysis. What technique would allow you to do so? Briefly describe an experiment using this technique that will let you make the determination.

e) Both ESCs and induced pluripotent stem cells (iPSC) can produce 'mesoderm' cells that are a precursor of CSCs. In terms of their origin, how does the ESC differ from iPS cells?

Question 3 continued

f) In adults, CSCs reside in a defined region of the heart called a ”niche”. CSCs require growth factor ligands such as EGF, from the niche for cell division and differentiation. You analyze two mouse mutants: ***Note:*** Mutant Mouse strain 1 expresses EGF whereas Mutant mouse strain 2 does not.

Experiment A: You isolate CSCs from GFP-labeled mutant mouse strain 1. You transplant them into the cardiac region of mutant mouse strain 2 that is not GFP-labeled. After waiting for four days, you **do not observe** any GFP labeled differentiated cardiomyocytes in mutant mouse strain 2.

Experiment B: You isolate CSCs from GFP-labeled mutant mouse strain 2. You transplant them into the cardiac region of mutant mouse strain 1 that is not GFP-labeled. After waiting for four days, you **observe** GFP labeled differentiated cardiomyocytes in mutant mouse strain 1.

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i. Which of these is likely to have mutations in the genes that encode the EGF receptor? **Explain** your choice.

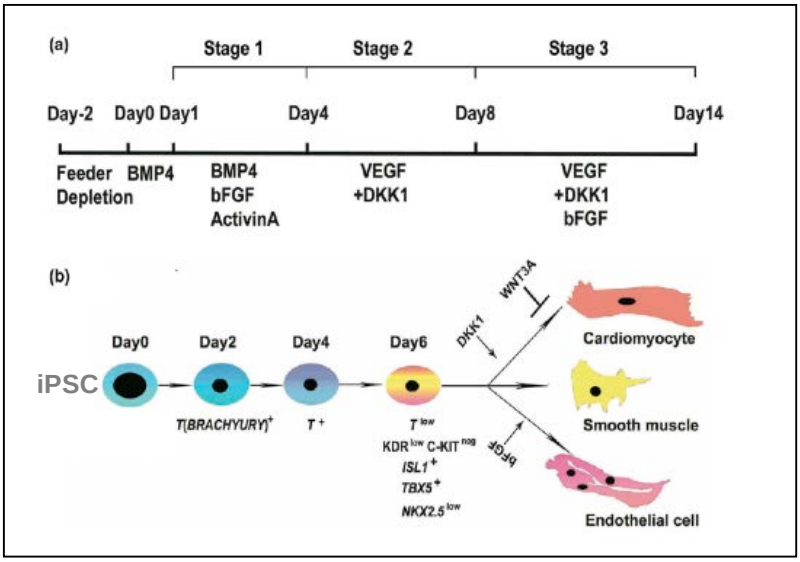
ii. Why did you use GFP-labeled cells?

Question 4

Cardiomyopathy refers to disease of cardiac muscle. The goal of regenerative medicine is to provide cells that can repair diseased cardiac muscle. You begin by preparing human cardiomyocytes from iPSCs cells.

a) Starting with human skin cells, briefly **explain** how you would generate iPSCs.

b) Would you class iPSC as totipotent, pluripotent, bipotent? **Explain** your answer.



The lineage diagram below shows formation of cardiomyocytes from iPSC (***Reference:*** Lu and Yang Stem Cell Research & Therapy 2011) ***Note:*** BMP4, bFGF, Activin A, VEGF, DKK and WNT3A are signaling factors. *T(Brachyury), KDR, C-KIT, ISL1 and TBX5* are *transcription factors.* Based on the schematic:

i. Which transcription factor(s) would you hypothesize is essential for initiating the progression towards cardiomyocytes?

Question 4 continued

ii. Give an experiment that would test your hypothesis. What would you expect if your hypothesis is supported? (1-2 sentences)

iii. Which transcription factor(s) are likely essential for promoting differentiation of cardiomyocytes, smooth muscle and endothelial cells?

iv. Why are the signaling (growth) factors sequentially applied during the culture period?

v. If you wanted to maximize production of cardiomyocytes…

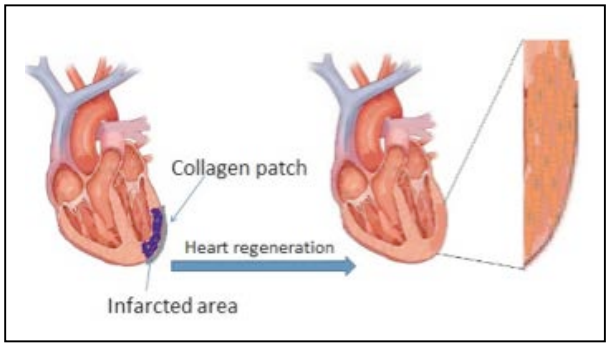
- What growth factor(s) would you apply at day 8? Justify each of your answers.

- What would you NOT apply? Justify each of your answers.

vi. At the start of the scheme, what does 'feeder' refer to? Why would you want to deplete this before the differentiation scheme begins?

c) How would you determine whether you had produced functional cardiomyocytes after the culture period?

d) One approach to cure cardiomyopathy is to use tissue engineering. In the diagram below, a collagen patch is placed into the damaged area of a heart.



i. What is the function of the collagen patch?

ii. Why is collagen a good choice of material?

Question 4 continued

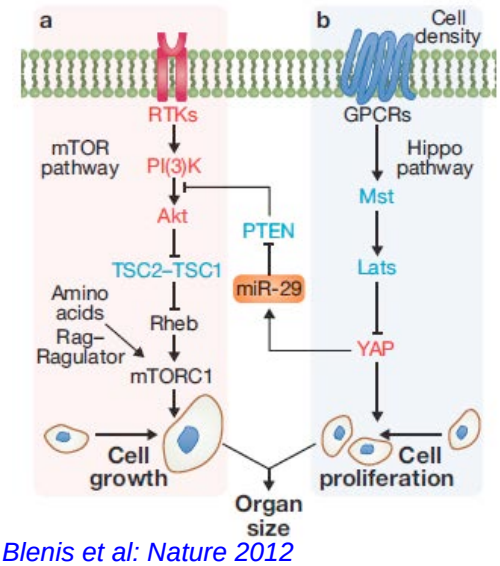
e) As a model for cardiomypathy, you define a mouse mutant with some characteristics of cardiomyopathy, however, the phenotype is variable between animals. You decide to perform somatic cell nuclear transfer (SCNT) to clone animals with more uniform characteristics. For this use of SCNT you have the option of using the nucleus from ***adult CSCs, embryonic CSCs or adult cardiomyocytes.***

i. Categorize the options as 1-3 with 1 being the best and 3 being the worst and provide an explanation **for your** classification.

ii. 5-aza-cytosine (5-azaC) is a nucleotide that prevents DNA methylation. Recent results show that CSCs grown in 5-azaC are more efficient in animal cloning than untreated adult CSCs. How can you explain this?

Question 5

The following is a schematic of signaling pathway crosstalk that regulates the size of organs and may be altered in tumor growth and tumor size of many cancers. The major steps of the cross-talking pathways are outlined below.



Blenis et al: Nature 2012

<u>mTOR pathway:</u>

1. Growth factors bind and stimulate receptor tyrosine kinases (RTKs).

2. RTKs can activate the PI(3)K–Akt signaling pathway.

3. The activated Akt, a serine threonine kinase, inhibits the TSC1–TSC2 complex, allowing Rheb to activate mTORC1.

4. In parallel, amino acids activate the mTORC1 pathway through a mechanism requiring the Rag–Ragulator complex.

<u>Hippo pathway:</u>

1 The binding of the ligand activates G-protein-coupled receptors (GPCRs), which activate Mst and Lats.

2. YAP activity is modulated by phosphorylation of Mst and Lats. YAP upregulates miR-29, which in turn downregulates PTEN, an inhibitor of PI(3)K and Akt. So, the two pathways crosstalk and coordinate cell number and growth.

Consider the following **mutations** in different components of the signaling pathway.

- #1: A heterozygous gain- of function mutation that results in a constitutively active YAP.
- #2: A homozygous loss-of-function mutation in PTEN.
- #3: A constitutively active mTORC1

Question 5 continued
Complete the table for each of the above **mutations** relative to wild type reference cells that are treated with ligands that bind to RTK and GPCR. ***Note:*** *Consider each mutation* ***independently****.*

Mutations	**Oncogen/ tumor suppressor gene**	**mTORC1 active (*Yes/No*?)**	**YAP active (*Yes/No*?)**	**Cell growth (More/ less)?**	**Organ size (bigger or smaller)?**
#1					
#2					
#3					

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