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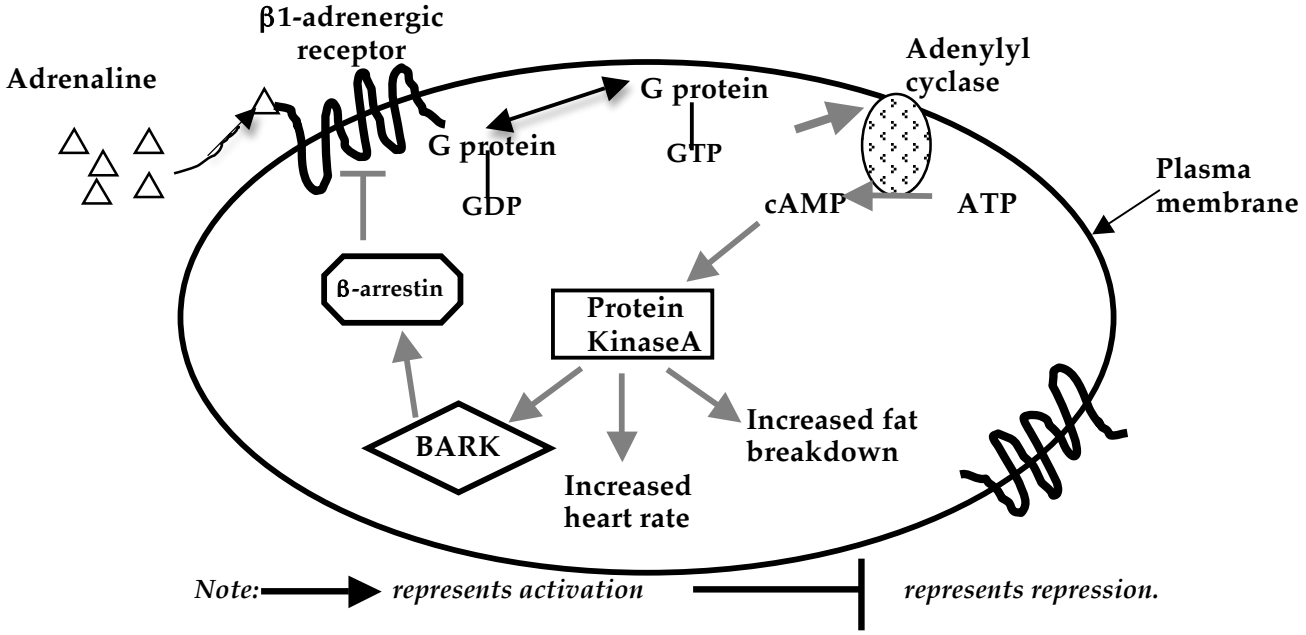
7.013 Problem Set 5- 2013

Question 1
During a summer hike you suddenly spot a huge grizzly bear. This emergency situation triggers a fight or flight response through a signaling pathway as shown below.



Some major features of this pathway are outlined below:

- *The small molecule adrenaline binds to its **β1-adrenergic membrane receptor**.*
- *Following the binding to adrenaline, this receptor acts as a kinase and converts the inactive GDP bound G proteins to the active **GTP bound G proteins**.*
- *The GTP bound G proteins **activate the adenylyl cyclase** enzyme.*
- *The GTP bound G proteins are **inactivated** by re-conversion to the GDP bound form.*
- *Adenylyl cyclase enzyme converts ATP to cyclic AMP (**cAMP**).*
- *The cAMP binds and activates **Protein Kinase A**.*
- *This results in an increased heart rate and breakdown of fat to trigger the "Fight or Flight" response.*
- *The Protein Kinase A enzyme also activates **BARK kinase**.*
- *The activated BARK **activates β-arrestin**, which inhibits **β1-adrenergic membrane receptor activity.***

a) Name the 1st and the 2nd ligand in this pathway and **give one reason that explains** why the 2nd messenger may be important in this pathway.

b) In order to further understand this cellular pathway you examine this pathway in a cell after the following perturbations.

Perturbation 1: Application of cholera toxin, which prevents G proteins from hydrolyzing GTP to GDP.
Perturbation 2: Treatment with nebivolol, a molecule that competes with adrenaline to bind to the ***β1-adrenergic membrane* receptor** but does not stimulate the pathway.

i. In which of the two treatments will the **adrenaline** cause a constitutive (continual) activation of adenylyl cyclase? **Explain.**

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Question 1 continued

ii. In which of the two treatments will you see very low or no activation of adenylyl cyclase **in the presence of adrenaline**? **Explain.**

c) Consider the following **perturbations** in different components of the above signaling pathway in a cell.

- *#1: BARK lacks its kinase domain.*
- *#2: β arrestin is constitutively (always) active.*
- *#3: β1-adrenergic membrane receptor lacks its ligand-binding domain.*

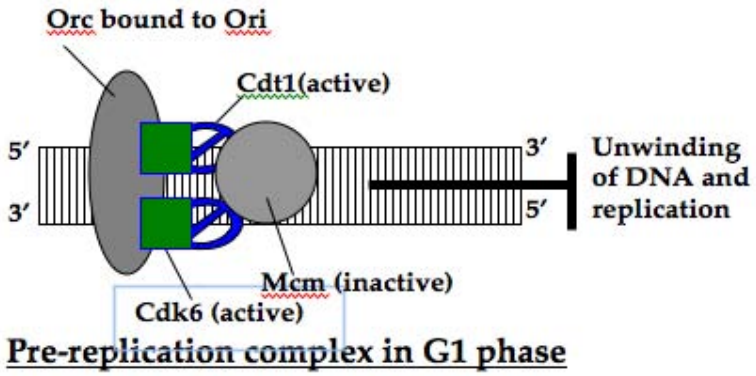
Complete the table for each of the following **perturbations** relative to unperturbed control cells in the **presence** of **adrenaline**. ***Note:*** *Consider each perturbation* ***independently*** *while answering the questions.*

Perturbation	Is cAMP produced?	Is Protein Kinase A activated?	Is β-arrestin activated?	Effect on heart rate and fat breakdown relative to unperturbed control cells in the presence of adrenaline (*choose from increased, decreased or unchanged*)? **Explain.**
1				
2			N/A	
3				

Question 2

The origin recognition complex (ORC) is a multi- subunit protein complex that binds to the **ori site(s)** and serves as a platform for the assembly of kinases like Cdk6 and Cdt1.

During the G1 phase of the cell cycle in yeast, ORC forms a pre- replication complex by recruiting Cdk6 and Cdt1 that bind to both strands of DNA. These factors bind and inhibit the Mcm protein that functions as a helicase as is shown in the schematic below. ***Note:*** *The activation is shown by an → and inhibition by ⊥ sign.*



Pre-replication complex in G1 phase

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Question 2 continued

a) Activation of the pre-replication complex occurs during the S phase and this requires its interaction with Cdk2 and Cyclin E proteins that degrade Cdk6 and Cdt1. This results in the replication of DNA. Draw a schematic, similar to the one on page 2, to show the regulatory interactions between ORC, Cdk6, Cdt1, Mcm, Cdk2 and Cyclin E proteins. ***Note:*** *Please indicate the activation by an → and inhibition by ⊥ sign.*

b) In a cell showing a Cdk2 **loss-of–function** mutation, in which phase (*choose from G1, S, G2, M, all or none*) would the cell arrest? **Explain** why you selected this option.

c) If the cdk-2 gene encodes Cdk-2 protein, in which phase (*choose from G1, S, G2, M, all or none*) will the cdk-2 gene be expressed? **Explain** why you selected this option.

d) If the cyclin E gene encodes Cyclin E protein, in which phase (*choose from G1, S, G2, M or all*) will the cyclin E gene be **optimally expressed**? **Explain** why you selected this option.

e) One method for studying the essential components that regulate different steps of the cell cycle involves the use of conditional temperature-sensitive mutants. You isolate temperature-sensitive Cdk2 yeast mutants. These mutant cells grow normally at the permissive temperature (25°C). However these cells arrest at a specific point in the cell cycle when the temperature is shifted to 36°C (non-permissive temperature).

i. Is the **primary structure** of the temperature-sensitive Cdk2 protein at 36°C ***same as*** or ***different*** from that at 25°C?

ii. Is the **three- dimensional conformation** of the temperature-sensitive Cdk-2 protein at 36°C ***same as*** or ***different*** from that at 25°C? **Explain** why you selected this option.

f) You grow the mutant cells, described in part (e), at 36°C or non- permissive temperature.

i. Predict the effect on **G1 phase** in the mutant cells grown at 36°C compared to those at 25°C.

ii. Predict the effect on the **S phase** in the mutant cells at 36°C compared to those at 25°C.

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Question 2 continued

g) You further create two mutant cells each having a mutation in Cdk-2 genes as described below.

- **Mutant cell- type 1:** The Cdk-2 protein lacks its kinase domain.
- **Mutant cell- type 2:** The Cdk-2 lacks its Cyclin E binding domain.

Predict what would happen to the **cell cycle** in…

i. **Mutant 1:**

ii. **Mutant 2:**

h) The proper development and functioning of different tissues and organs involves a tight regulation of cell division and cell death.

i. A cell can die by "**apoptosis"**. What does this term mean?

ii. Apoptosis may occur either via **extrinsic** or **intrinsic** signaling pathways. Based on what you have learnt in 7.013, if a cell makes the decision to die when subjected to hypoxia (low oxygen concentration) would you regard this as an example of extrinsic or intrinsic signaling pathway? **Explain** why you selected this option.

iii. Apoptosis involves the activation of Caspases. **Briefly explain** the function of these enzymes.

Question 3

Development of human and chicken hearts is thought to occur by very similar mechanisms. The heart develops from a layer of cells called the "mesoderm", which lies on top of another layer of cells called "endoderm".

a) The mesoderm forms the heart, the kidney and the blood.

i. What does the term "**potency**" mean?

ii. What term would describe the potency of the mesoderm (*choose from totipotent, multipotent or unipotent*)?

b) You want to know when the future heart cells decide to become such. You isolate a small piece of tissue (or explant), from the heart forming anterior mesoderm of a young embryo (stage 2), and also from a slightly older (stage 4) embryo (about 6 hours older than stage 2). You make sure to take the same relative region from both. You culture the tissues in tissue culture plates, examine them three days later and tabulate your results below.

Explant	Culture time	Observation
Stage 2 anterior mesoderm explant	3 days in culture	No change from original cells
Stage 4 anterior mesoderm explant	3 days in culture	Beating heart

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Question 3 continued

i. At the time of isolation, are the **stage 2 cells** determined, differentiated or undetermined compared to **Stage 4** cells? **Explain**.

ii. Are the beating heart cells determined, differentiated or neither compared to **Stage 4** cells? **Explain**.

c) You ask why the isolated Stage 2 cells did not become a heart when they were isolated. Since the anterior mesoderm cells lie on top of the "endoderm" layer, you therefore modify your experiment as shown in the table below and obtain the following results.

Tissue	Culture	Result
Stage 2 anterior mesoderm alone- labeled with green fluorescent protein (GFP)	3 days culture	Same as original GFP labeled cells
Stage 2 anterior mesoderm labeled with GFP) along with underlying endoderm that is unlabeled	3 days culture	Beating heart comprised of cells that fluoresce green
Endoderm alone that is unlabeled	3 days culture	Same as original unlabeled cells

Why does the stage 2 anterior mesoderm explant **when cultured with endoderm** make a heart, whereas the stage 2 anterior mesoderm explant alone does not?

d) You later observe that if Stage 2 cells are cultured for three days with a purified secreted protein, BMP4, they develop and form a beating heart. Based on all the data above, where in the embryo (*choose from mesoderm, endoderm or both*) would you expect BMP4 to be expressed if it directs normal heart formation? **Explain** why you selected this option.

e) Posterior mesoderm does not form the heart. To test whether BMP4 is able to turn posterior mesoderm into beating heart you culture the cells of the posterior mesoderm in the presence and absence of BMP4 and obtain the following results.

Explant	Culture	Result
Stage 2 posterior mesoderm explant	3 day culture	No Change
Stage 2 posterior mesoderm explant + BMP4	3 day culture	No change

You know that BMP4 is a secreted protein that activates its receptor via phosphorylation. The activated receptor phosphorylates a transcription factor, Smad1. Phospho-Smad1 moves to the nucleus to change transcription of target genes.

Using the above information, suggest why posterior mesoderm does not respond to BMP4.

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Question 3 continued

f) Anterior mesoderm cultured with BMP4 alone develops into a heart, while anterior mesoderm treated with BMP and fibroblast growth factor (FGF, another signaling protein) develops into kidney. You examine the combinatorial code required to determine heart or kidney, using tissue from these treatments, and obtain the following results.

Treatment	Regulatory genes expressed
Anterior mesoderm alone	A, B
Anterior mesoderm + BMP	A, C, D
Anterior mesoderm + FGF	A, E, F
Anterior mesoderm +BMP + FGF	A, C, D, E, G

i. Complete the following table:

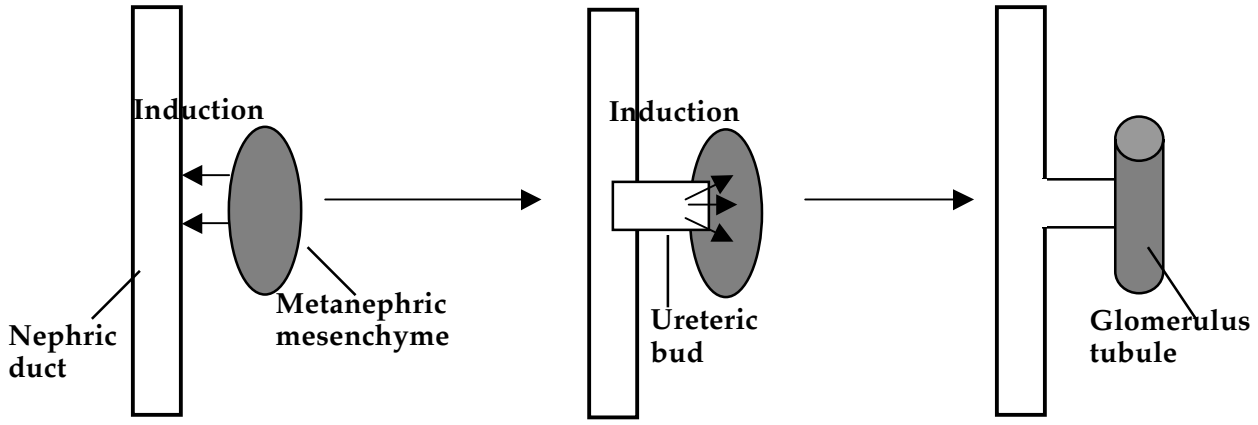
Tissue	Genes Present	Genes expressed
Heart		
Kidney		

ii. Give a possible role of Gene A in heart and kidney formation?

iii. What role might Gene B play in heart and kidney formation?

Question 4

Kidney is an organ that is comprised of 10 different cell types and has a specific 3- dimensional structure. Its formation involves the reciprocal interactions between the nephric duct (a tube) and the metanephric mesenchyme. First, the metanephric mesenchyme induces the duct to form a new tube called the ureteric bud. The ureteric bud induces the metanephric mesenchyme, which then forms the glomerulus, the principal filtration region of the kidney.



Kidney formation

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Question 4 continued

a) Briefly explain what is reciprocal induction and explain how it relates to the kidney development?

b) Formation of the ureteric bud, a tube, requires branching and lengthening of the existing nephric duct epithelium. List **two processes** that can lengthen an epithelium. Briefly **explain** what happens to cells during each process.

c) The metanephric mesenchyme condenses and becomes an epithelial tube.

i. Name the transition associated with this conversion?

ii. Of the two cell states associated with mesenchyme and epithelium, which can migrate?

iii. Give two possible changes that allow the mesenchymal cells to convert to a cell sheet?

d) During ureteric bud formation, the cells elongate to form the tubule. Predict the effect of each of the following events on ureteric bud formation.

i. Cells are treated with blebbistatin, a small molecule inhibitor of myosin function.

ii. Cells express a defective cadherin protein (*a protein involved in homotypic cell adhesion*) that lacks its extracellular domain.

Question 5

Stem cells are found in almost all organs of multi-cellular organisms. Stem cells can undergo mitotic cell division to form cell types that can differentiate into diverse specialized cells. Stem cells are believed to have immense therapeutic potential.

a) A stem cell is known to divide **asymmetrically**. When a stem cell divides asymmetrically, what are the possible fates of its two daughter cells?

b) Four human embryonic cell types, originally prepared from the **SAME embryo**, were tested for their potency **in vitro**. Based on the data below, complete the table by ranking the potency of these cell types.

Cell types	Cell types differentiated in vitro	Potency from 1-4 (1=most potent and 4= least potent).
A	motor	
B	motor, sensory, lateral, hippocampal	
C	sensory, lateral, hippocampal	
D	motor, sensory	

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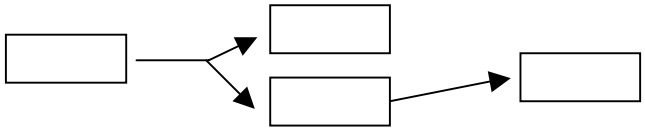
Question 5 continued

c) Draw a lineage tree for the cell types A-D using the information in the table on Page 7.



d) Do each of the above cell types have the same DNA (*Yes*/ *No*)? **Explain**.

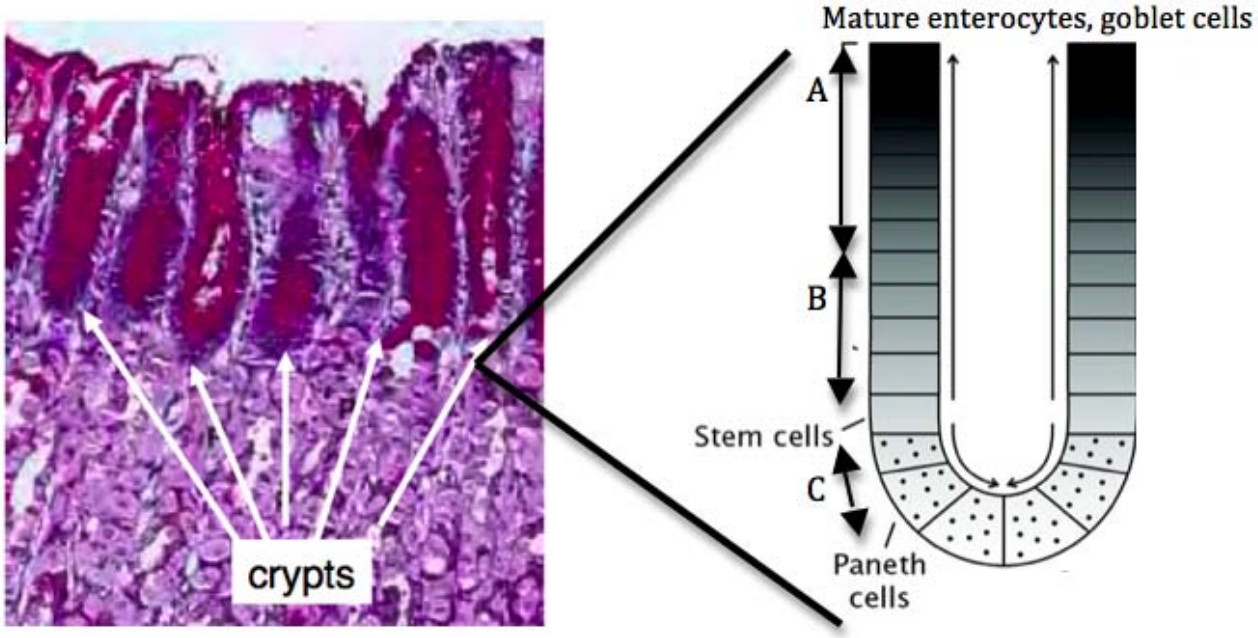
e) Induced pluripotent stem (iPS) cells hold great promise since they have the potential to differentiate into multiple cell types.

i. Which cell types do you start with while making iPS cells?

ii. If you would like to generate new kidney tissue for a patient would you start with iPS cells or the embryonic stem cells? Provide a brief explanation for the choice that you made.

f) Stem cells exist in most organs including bone marrow. Describe **one** experiment that proved that stem cells exist in the bone marrow.

g) The following schematic shows the cell lineage hierarchy in a small intestine crypt of a mouse. The epithelial surface of the intestine sloughs off continually and is renewed by fresh cells. Renewal occurs by movement of transit amplifying cells from invaginations called crypts.



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Question 5 continued
By doing a pulse chase experiment you determine that stem cells give rise to daughter stem cells and to transit amplifying cells. Transit amplifying cells can divide to produce daughter cells that ultimately differentiate into goblet cells and mature enterocytes. These differentiated cells die within 2-3 days and are shed from the epithelial surface. Briefly describe the underlying principle of a pulse chase experiment.

h) In the diagram on the right on Page 8,

i. Mark the region (*choose from A, B or C*) where you would find the transit amplifying cells?

ii. Are the transit amplifying cells uncommitted, committed or differentiated relative to the Stem cells? Choose one, and **explain** your choice.

iii. Accumulation of mutations in which cell type (*choose from stem cells, transit amplifying cells, goblet cells, enterocytes and paneth cells*) is potentially most harmful to the animal? **Explain** your choice.

iv. Stem cells give rise to specific cell types based on the signals they receive from the "niche". Looking at the diagram of a crypt, and taking into account the information given, indicate which are likely to be the niche cells of the crypt. **Explain** your choice.

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