Solution key- 7.013 EXAM 3

Question 1 (27 points)

During the cell cycle, the anaphase-promoting complex (APC) is necessary for progression from metaphase → anaphase as diagrammed and outlined on **Page 7**.

a) You create the following **homozygous** mutants in **diploid cells (ploidy: 2n).**

- **Mutant 1:** Tubulin loss-of-function mutation (where the spindle fiber does not form).
- **Mutant 2:** Cells lack the transcription factor (TF) required for the transcription of Separase.
- **Mutant 3:** M-Cdk lacks the M-Cyclin binding amino acid sequence.

Based on the information above, complete the table for each of the mutants during cell cycle.

Mutant	APC active (Yes/ No)?	Separase active (Yes/ No)?	Cell enters anaphase (Yes/ No)?	Ploidy of mutant cell (n/ 2n/ 4n)?
1	*Yes*	*Yes*	*No*	*4n (2pts)*
2	*Yes*	*No*	*No*	*4n (2pts)*
3	*No*	*No*	*No*	*4n (2pts)*

b) M-Cdk protein is inhibited by p27Kip1. You grow the M-Cdk temperature sensitive mutant at the permissive temperature (37^{O}C, Plate A) or non-permissive temperature (42^{O}C, Plate B) both in the presence of p27Kip1. Would the cells in Plate A grow **more rapidly/ less rapidly/ at the same rate** as the cells in Plate B? **Explain** why you selected this option.

They will grow at the same rate. The temperature sensitive mutant at 42^{O}C will be inactive regardless of p27Kip1 and it will inactivated by p27Kip1 at 37^{O}C. (4pts, 2pts for selection & 2 for explain)

c) Name the modification on the target protein that the proteasome recognizes: *Ubiquitin tags (2pts)*

d) The proteasome-mediated hydrolysis of the target protein is an example of a reaction that hydrolyzes **peptide bond/ hydrogen bond/ phosphodiester bond**? Circle as appropriate. *(2pts)*

e) You make a cell line that expresses M-Cdk as an in-frame GFP-Cdk fusion protein and M-cyclin as an in-frame RFP-M Cyclin fusion protein. ***Note:*** *GFP fluoresces green and RFP fluoresces red.*

i. Which part in a dividing cell would fluoresce **red**: **Cytoplasm/ Nucleus/ Cell membrane/ Golgi body/ Lysosomes**? *(2pts)*

ii. **Circle** the phase(s) of the cell cycle when the cells would fluoresce **green**: **G1/ S/ G2/ M/ all** *(2pts)*

f) If you had a mutant cell that shows a homozygous loss-of-function mutation of M-Cdk gene and a constitutively active APC protein, would you characterize it as oncogenic? **Explain. (*4pts,2 for explain)***

Yes, in this mutant, APC is constitutively active irrespective of the signals from M-Cdk-M Cyclin complex. Active APC protein activates separase by causing the proteasomal mediated degradation of securing. Active separase promotes cohesin degradation to promote the segregation of duplicated chromosomes thus promoting metaphase→ anaphase transition and cell cycle.

g) Classify the **M-Cyclin/ M-Cdk/ APC/ Securin/ Separase** genes as …*(5pts, 1 each)*

i. **Proto-oncogene(s):** ***M-Cdk, M-Cyclin, APC, separase***

ii. **Tumor suppressor gene(s):** ***Securin***

Question 2 (14 points)

a) Coal tar is a known carcinogen. Exposure to coal tar can form multiple tumors in mice. Would these tumor cells, when grown in culture plates, form a monolayer or multiple foci (piles of cells)? **Explain** why you selected this option. *They will form multiple foci since they lose the property of contact inhibition when exposed to carcinogens.* ***(2pts)***

Histidinal dehydrogenase (HD) is needed for histidine synthesis in bacteria**.** The following bacterial mutants have mutations in the DNA sequence for the amino acids 1-4 of HD. ***Note:*** *The start codon is underlined in wild type (WT), mutants 1 and 2. Each alternative codon is shaded. A codon chart is on* ***Page 7****.*

WT:	5′-A**ATG**ATAGATATG--3′ 3′-TTACTATCTATAC--5′
#1:	5′-A**ATG**TAGATATGG--3′ 3′-TTACATCTATACC--5′
#2:	5′-A**ATG**ATTGATATG--3′ 3′-TTACTAACTATAC--5′

b) Which DNA strand is the **template for transcription** in the WT DNA sequence: **Top or bottom?** ***Bottom*** ***(2pts)***

c) On the WT DNA, **show the direction of transcription** by an arrow. *Arrow is from left→ right* ***(2pts)***

d) Which bacterial mutant is His- such that it could be used in the Ames test: **1 OR 2**? **Explain** why you selected this mutant and not the other.

Mutant 2 has a silent point mutation. So it will make a functional HD resulting in a His+ bacteria. So it is not a good choice. Mutant 1 has a frame-shift mutation that generates a stop codon. So these mutants will be His- and hence good for the Ames test. ***(4pts, with 2 for explanation)***

e) You find that coal tar is **NOT** mutagenic per the standard Ames test although it is mutagenic and forms tumor in mice. Provide an **explanation** for this discrepancy.

Coal tar is likely a pro- mutagen. In its native form, it is not mutagenic. However when incubated with the liver extract in the modified Ames test, it is converted to a metabolic forms that are and which can convert His^- → His^+ bacteria in modified Ames test. ***(4pts)***

Question 3 (20 points)

a) Fill in the table below for a non-small cell lung carcinoma (NSLC) patient who has been treated with the following chemotherapeutic drugs. ***(4pts, 2 each)***

Drug	Drug activity	Process **targeted first**: **replication/ transcription/ translation/ mitosis**?
Taxol	Inhibits microtubules assembly	*Mitosis*
Cycloheximide	Inhibits eukaryotic ribosomal function	*Translation*

b) The NSLC patient in part (a) relapses with secondary tumors in lymph nodes and brain after 2 years.

i. Are the cancer cells of the primary tumor in ***epithelial (E)*** **OR mesenchymal (M)** state? *(2pts)*

ii. Which **"cell state transition"** did the cancer cells of the primary tumor undergo to cause the formation of secondary tumors: ***EMT*** **OR MET**? *(2pts)*

iii. Give **two** differences between the "cell state" in **part (i)** and the "cell state transition" in **part (ii).**

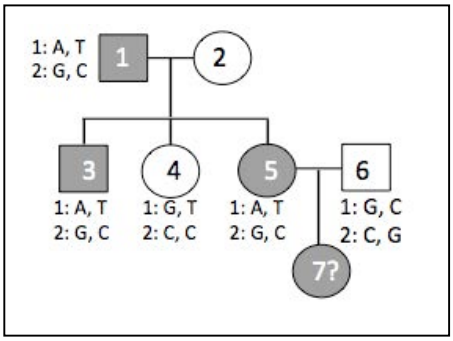
No cell-cell adhesion junctions, no apical-basal polarity, they migrate (2pts, 1 for each)

iv. Would the above patient following relapse have a good prognosis? **Why or why not?**

No, the cancer in this patient has already metastasized (2pts)

Question 3 continued

The following pedigree shows the inheritance of **predisposition** to NSLC that shows an **autosomal dominant mode** of inheritance**.** This disease is caused by a gain-of-function mutation in the *ALK* proto-oncogene**.** ***Note:*** *The profile of SNPs 1 and 2 that are in the ALK gene, for some individuals is shown.*



c) Give the genotype of the NSLC cells for **Individual 3** for the **ALK gene** using ALK+ for the wild-type allele and ALK^{M} for the mutant allele: ***ALK^{WT}/ ALK^{MUTANT}*** ***(2pts)***

d) The microarray shows the following SNP profile of Individual 7 **at birth**. **Explain** why the SNP profile of Individual 7 is different from his parents.

1: A, C
2: G, C

The gametes produced by #6 would all have SNP1 and 2 as (G, C (parental)/ C, G (parental)/ G, G (recombinant)/ C, C (recombinant)). When the gamete of #6 with C, C as SNP1 and 2 fuses with the a gamete from #5 (SNP1: A, SNP2: G you get #7 with the above SNP genotype. ***(4pts)***

e) Would this SNP profile predispose Individual 7 to NSLC? **Why or why not?**

Yes, since #7 gets A and G as SNP1 and SNP2 from #7, which is associated with cancer causing allele of Alk gene. So she is predisposed to developing NSLC. ***(2pts)***

Question 4 (25 points)

The lung develops from embryonic cells called 'endoderm' in the following stages described in mice.

- At day **E5:** The endoderm is present**.**
- At day **E8:** The endoderm forms a tube and expresses the transcription factor Osr1.
- From **E10** on: The dorsal (back) side of the tube makes pharynx and expresses Sox2, an essential transcription factor for pharynx development.
- From **E12** on: The ventral (belly) side of the tube makes lung buds and expresses Nkx2.1, an essential transcription factor for lung bud development.

a) You ask when endodermal cells are committed to form pharynx and lung buds. You isolate a small piece of tissue (explant) from **E5**, **E8** and **E9.5** endoderm of a mouse embryo and grow in a culture dish until each explant is 12 days old. Complete the table below. *(3pts, 1 each)*

Stage of isolated explants	Genes expressed at day E12.0	Committed tissue: **Pharynx/ lung/ both/ neither?**
E5.0	Osr1	*Neither*
E8.0	Sox2	*Pharynx*
E9.5	Nkx2.1	*Lung*

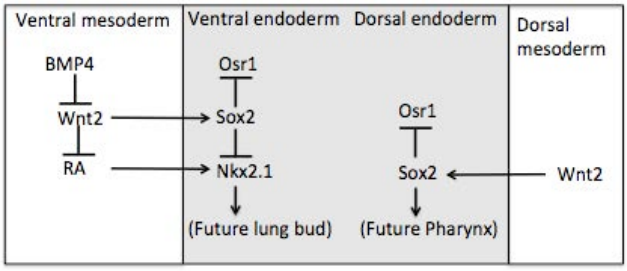
b) Give the potency of the endodermal cells that give rise to pharynx and lung bud. **Explain** your answer. *Bipotent, since it forms lung bud and pharynx, multi-potent since lung bud and pharynx may each have multiple cell types (2pts for explain)*

c) The endodermal tube is surrounded by cells called mesoderm. Following removal of the mesoderm at day E8, neither lung buds nor pharynx form. What role does mesoderm play in lung bud and pharynx formation?

It is an organizer or signaling center for pharynx and lung bud formation. (2pts)

Question 4 continued

The signaling pathways involved in lung bud and pharynx formation are shown below. Ventral mesoderm lies next to future lung bud, dorsal mesoderm next to future pharynx.



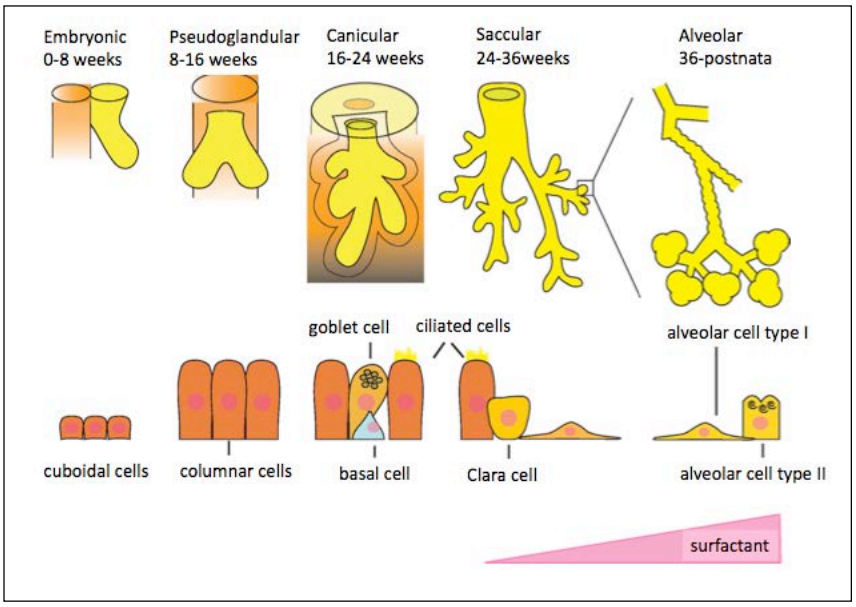
d) Where do you expect the BMP receptor to be expressed: **Ventral mesoderm OR Ventral endoderm**? **Explain** why you selected this option. *It is expressed in the ventral mesoderm, where it is targeting cells expressing Wnt2. (2pts, 1 for explain)*

e) You create the following **homozygous null (loss of function) mutants**. Predict what would happen to lung bud and pharynx formation in this mutant by choosing from **normal/ none/ excess.**

Mutant	**Lung bud? *(3pts)***	**Pharynx? *(3pts)***
BMP4	*None*	*Normal*
Wnt2	*Normal*	*None*
BMP4 and Wnt 2 double mutant	*Normal*	*None*

f) Is the formation of lung bud versus pharynx an example of **sequential induction/ co-induction/ migration? Why?** *Sequential induction, since pharynx is formed from endoderm tube at E10 followed by lung bud at E12. (2pts)*

The sequence of human lung development is shown below (*Sunday et al, 2016*). As the lung develops, the lung bud forms the trachea that splits into the bronchi and the lobules of the lung that contain alveoli. The alveolar cells secrete a surfactant protein into the fluid that prevents cells from sticking and allows inflation with air, and gas exchange.



Leibel S and Post M (2016) Endogenous and Exogenous Stem/Progenitor Cells in the Lung and Their Role in the Pathogenesis and Treatment of Pediatric Lung Disease. *Front. Pediatr.* 4:36. doi: 10.3389/fped.2016.00036. License CC-BY

g) For the physical transition of cuboidal to columnar cells, what part of the cell is most important: **Nucleus/ mitochondria/ cytoskeleton/ lysosome?** *(2pts)*

h) Between the pseudoglandular and cannicular stages there is huge expansion of the lung tubes. For each mutant below, indicate what cellular process(s) is impaired during lung tube expansion: **Apoptosis/ Cell division/ cell migration/ cell shape change**. Include as appropriate.

i. Null mutation in DNA polymerase: *Replication, cell division (2pt)*

ii. Partial loss of function of actin, a microfilament protein: *Cell shape, division, motility (2pt)*

Question 4 continued

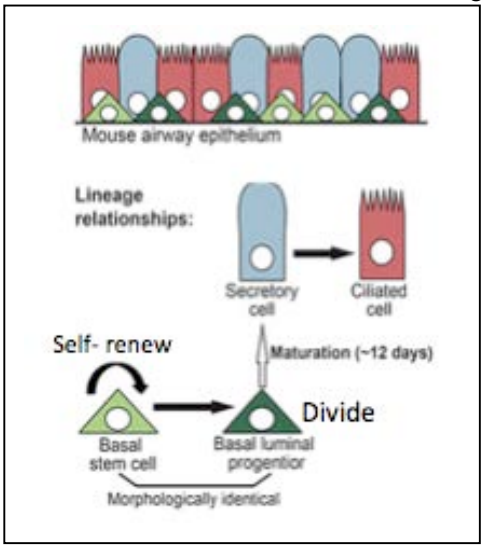
i) Explain why each of the following treatments, applied at the Saccular stage (24 weeks) will prevent or reduce **function of alveoli**, which are responsible for the gas exchange. *(2pts, 1 each)*

#1: Hydrourea, an S phase inhibitor *(for cell division to make enough cells to make alveoli)*

#2: An inhibitor of tight cell-cell junctions (*needed to hold alveolar epithelium together for an intact alveolus*)

Question 5 (14 points)

The schematic below shows the lung lining and respiratory stem cell lineage.



Watson, K., et al (2015). Clonal Dynamics Reveal Two Distinct Populations of Basal Cells in Slow-Turnover Airway Epithelium. Cell Reports 12, 90-101. http://x.doi.org/10.1016/.celrep.2015.06.011. License CC-BY

a) The Basal stem cells (BSC) express **Trp63** and **K15** cell surface markers. How would you use this information to purify the BSCs from a mixed cell population?
You would use antibodies specific to these cell surface markers to purify the CSC population by FACS or cell sorting. (3pts)

b) The BSCs lie next to 'stromal cells' and are usually quiescent (in G0). They enter the cell cycle in response to stress or inflammation and this activates Notch (ligand)-delta (receptor) signaling. Which cells would make the… *(2pts, 1 each)*

i. **Notch ligand:** BSCs or ***stromal***?
ii. **Delta:** ***BSCs*** or stromal cells?

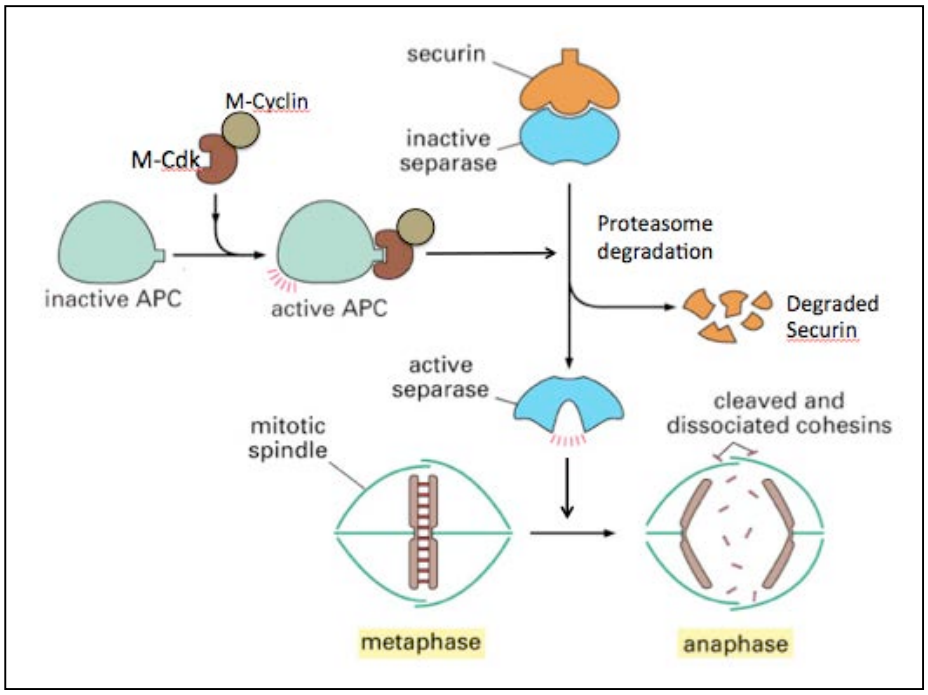
c) Which are potential transit amplifying cells? **Explain your choice.** *(2pts, 1 for explain)Basal laminar progenitor, they can divide and give rise to secretory and luminar cells.*

d) Cystic fibrosis (CF) is a genetic disorder that is caused by a mutation that inactivates the cystic fibrosis transmembrane receptor (cftr) gene. You want to alleviate the symptoms of CF in adult mice by replacing mutant cells with normal cells that have a wild-type copy of the cftr gene either by using iPS derived from this mouse or ES cells. Which of these two cells (iPS or ES) is a better option? **Why?**
You will NOT use iPS cells since they will be made from the adult differentiated cells of the CF mouse. You would rather use the ES cells that have a normal copy of the CFTR gene and which can potentially differentiate to form the normal, functioning cells. (3pts)

e) CF model mice are difficult to breed so you decide to propagate the CF mouse strain through somatic cell nuclear transfer (SCNT). Which nucleus would you preferentially use: **ciliated cell/ basal stem cell/ secretory cell/ stromal cell**? **Explain** your choice.
You would potentially use the nucleus from the basal stem cell since it is the most 'embryonic-type' of the options. It will have a chromatin structure most similar to the zygote and be easiest to reprogram. (2pts)

f) The trachea is the tube that directs air into the lungs, built of cartilage that is secreted by chondrocytes. The cartilage organizes into rings that keep the tube open. In order to get chondrocytes to form a tube, you can seed them into a strong, rigid plastic tube of the right diameter. You can also culture them on extracellular matrix from a decellularized trachea. Which option would you choose and **why**? *The ECM. This is a natural substrate for the cells, which can remodel it as they grow and a resulting normal trachea is more likely to form. The plastic tube may hamper oxygen flow to the cells, which will not survive well. (2pts)*

Diagram for Question 1 (You can detach this page)



1: The **M cyclin**, expressed at G2→ M checkpoint, phosphorylates and activate **M-Cdk**.

2. The M-cyclin-M-Cdk complex binds to and activates **APC**.

3: Activated APC causes the proteasomal-mediated degradation of **Securin**. This frees Separase from securing and activates **Separase**.

4: Active Separase degrades **Cohesin** proteins that hold the duplicated chromosomes together at the metaphase plate. So the duplicated chromosomes separate in anaphase and the dividing cell progresses *from metaphase → anaphase.*

Codon chart for Question 2d)

	U	C	A	G	
U	UUU **phe**	UCU **ser**	UAU **tyr**	UGU **cys**	U
	UUC **phe**	UCC **ser**	UAC **tyr**	UGC **cys**	C
	UUA **leu**	UCA **ser**	**UAA STOP**	**UGA STOP**	A
	UUG **leu**	UCG **ser**	**UAG STOP**	UGG **trp**	G
C	CUU **leu**	CCU **pro**	CAU **his**	CGU **arg**	U
	CUC **leu**	CCC **pro**	CAC **his**	CGC **arg**	C
	CUA **leu**	CCA **pro**	CAA **gln**	CGA **arg**	A
	CUG **leu**	CCG **pro**	CAG **gln**	CGG **arg**	G
A	AUU **ile**	ACU **thr**	AAU **asn**	AGU **ser**	U
	AUC **ile**	ACC **thr**	AAC **asn**	AGC **ser**	C
	AUA **ile**	ACA **thr**	AAA **lys**	AGA **arg**	A
	AUG met	ACG **thr**	AAG **lys**	AGG **arg**	G
G	GUU **val**	GCU **ala**	GAU **asp**	GGU **gly**	U
	GUC **val**	GCC **ala**	GAC **asp**	GGC **gly**	C
	GUA **val**	GCA **ala**	GAA **glu**	GGA **gly**	A
	GUG **val**	GCG **ala**	GAG **glu**	GGG **gly**	G

7.013 Introductory Biology

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