

Solution Key - Practice Questions

Question 1

a) A recent publication has shown that the fat stem cells (FSC) can act as bone stem cells to repair cavities in the skull, when transplanted into immuno-compromised mice. The FSC express the STRO-1+ cell surface protein. Briefly **explain** how you can use this information to purify the FSC from a mixed population that contains multiple cell types.

You can use fluorescent antibodies that specifically bind to the STRO-1+ cell surface protein expressed on the surface of FSC cells. Then you can separate the fluorescent FSC cells from the other cell types in the population using specific devices such as the fluorescence activated cell sorter (FACS).

b) In order to test the potency of mouse FSC over time you create chimera mice as specified below.

- **Chimera 1 (control):** You insert green fluorescent protein (GFP)- labeled FSC into a **wild-type embryo (at day 4, the inner cell mass stage)** and observe the formation of GFP labeled osteoblasts (that produce bone), and chondrocytes (that produce cartilage).
- **Chimera 2:** You insert the green fluorescent protein (GFP)- labeled FSC in a **wild-type embryo (at day 8, after the embryo has started to form organs)** and observe the formation of GFP labeled osteoblasts.

Provide an **explanation** for the fate of FSC in chimera 2 compared to chimera 1.

The regulatory factors provided by the cells in a day 8 are different compared to those in a day 4 embryo resulting in the difference in the number of fates that the FSC can acquire. At day 4, there may be more factors present (or different concentrations present) that can induce more fates than at day 8.

c) Normally, FSC are surrounded by “stromal cells”, which are believed to form the niche. You identify two mutants showing abnormal FSC potency, and want to determine if the gene in these mutants acts in FSC or in cells of the surrounding stromal region niche.

Mutant	Mutation	FSC proliferation?	Result
A	Fails to produce Fibroblast Growth Factor (FGF)	No	Early cell death
B	No RunX transcription factor produced	Yes	No differentiation

- iii. You transplant FSC from a normal mouse into the stromal region of a **mutant A** mouse. The transplanted cells fail to proliferate, similar to the mutant. Conversely, mutant A FSC transplanted into a normal mouse proliferate normally. Where would you predict FGF is produced (FSC/ osteoblasts/ stromal niche)? **Explain.**

It is produced in the stromal cell niche since both the wild- type and the mutant A cells fail to proliferate when transplanted into mutant A mice. Thus, there is something wrong with the mutant A niche, consistent with it producing FGF. This is supported by ability of a wild type niche to allow mutant A FSC to proliferate normally. This is also consistent with the secreted nature of FGF, acting in cells other than the ones producing it.

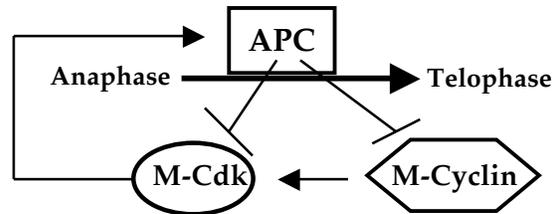
- iv. You transplant the normal FSCs to a **mutant B** mouse and find that the transplanted FSC behave normally. Conversely, mutant B FSC transplanted into a normal mouse proliferate normally but do not differentiate. Where would you predict **RunX** is expressed (FSC/ osteoblasts/ stromal niche)? **Explain.**

It is produced in the FSC since the mutant B FSC cannot differentiate in either WT or mutant niche. This says there is something acting cell autonomously in the FSC to control their fate. A transcription factor would be expected to do this.

Question 2

The cell cycle is regulated by multiple checkpoints, each operating at a specific phase of the cell division cycle.

- Anaphase-promoting protein complex (APC) is activated during Anaphase of mitosis.
- APC is necessary for the cells to enter Telophase and complete mitosis.
- APC is activated by mitotic Cyclin dependent kinase (M-Cdk).
- Activated APC degrades both M-Cdk and mitotic Cyclin (M-Cyclin).
- M-Cyclin phosphorylates and activates M-Cdk.



a) On the diagram above, use arrows and T-bars to diagram the regulatory interactions between APC, M-Cdk and M-Cyclin. **Please Note:** Indicate activation by an \rightarrow and inhibition by a T-bar.

b) Using the information from (a), complete the table below for each of the following mutants.

Mutant	Cell completes the M phase (Yes/ No)? Explain.
Mutant lacks M- cyclin.	<i>No, M-cyclin cannot activate M-Cdk and therefore APC is not activated. The cells will be arrested in the M phase of cell cycle.</i>
M-Cdk protein lacks the DEAD box domain that is required for its degradation.	<i>Yes, the cell cycle will still proceed normally. The APC cannot degrade M-Cdk, but can degrade M-Cyclin. In the absence of cyclin M-Cdk and hence the APC will not be activated.</i>

Question 3

Cells can become transformed (i.e., become capable of growing and dividing inappropriately) because of mutations in genes such as proto-oncogenes and tumor suppressor genes.

a) How is the function of a protein encoded by an oncogene different from that of the proto oncogene?
The function of a proto-oncogene is to promote the cell cycle when appropriate signals are received. The function of an oncogene promotes the cell cycle without regulation.

b) Five cell types are described below. Identify the normal version of Gene M, N, O, P or Ras as either a **tumor suppressor gene** or a **proto-oncogene** and give the phenotype of the cell that carries the two alleles described above. Choose **transformed** (i.e., has characteristics of a cancer cell) or **NOT transformed**.

Question 3 continued

Cell	Affected Gene	Normal function of protein	Allele1	Allele 2
1	Gene M	Protein M is an S-phase cyclin.	A mutation in the promoter such that cyclin is expressed at all stages of cell cycle	Wild-type
2	Gene N	Protein N inactivates a tumor suppressor protein	A mutation such that protein N has no kinase activity	Wild-type
3	Gene O	Protein O prevents exit from G1 phase.	A nonsense mutation that replaces the fourth codon with a stop codon	A deletion of the entire gene
4	Gene P	Protein P stimulates apoptosis when activated.	A mutation that gives a constitutively active protein P	A deletion of the entire gene
5	Ras	Ras turns on a signal cascade that promotes the cell cycle.	A loss of function mutation	Wild-type

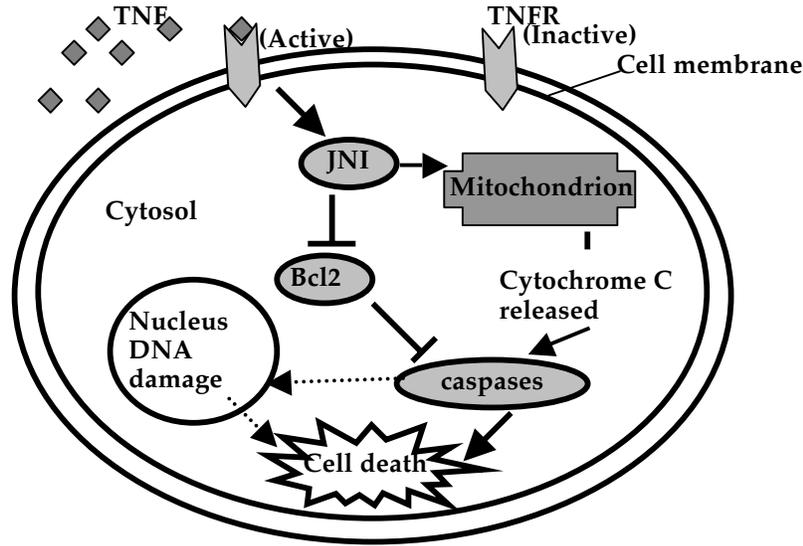
Cell	Normal Gene functions as	Phenotype of cell with alleles 1 and 2 above
1	Gene M: <i>proto-oncogene</i>	<i>Transformed</i>
2	Gene N: <i>proto-oncogene</i>	<i>NOT transformed</i>
3	Gene O: <i>tumor suppressor gene</i>	<i>Transformed</i>
4	Gene P: <i>tumor suppressor gene</i>	<i>NOT transformed</i>
5	Ras: <i>proto-oncogene</i>	<i>NOT transformed</i>

e) Cell lines are often used to test the oncogenic potential of the viruses. If cancer is a multi- step process, why can the introduction of a single active viral oncogene transform these cells?

Unlike the normal cells, the cell lines are immortal i.e. they can divide indefinitely and they are made so through the accumulation of mutations. Therefore the introduction of a single viral oncogene into a cell line essentially reflects the addition of one more mutation to a series of mutations that were preexisting in the cell line. Hence the transformation of a cell line by adding a single active viral oncogene does not counteract the statement that "cancer is a multi step process".

Question 4

Consider the following schematic of a signal transduction pathway that results in **apoptosis (programmed cell death)**. *Note: You may assume that this diagram represents the behavior of a single cell within an organism.*



- Tumor necrosis factor (TNF) binds to and activates its specific receptor (TNFR)
- The activated TNFR activates JNK1.
- The activated JNK1 activates caspases by releasing cytochrome C from mitochondria and also by inhibiting Bcl2. Bcl2, when active, promotes cell survival by inhibiting caspases.
- The active caspases result in DNA damage and cell death.
- In the schematic an arrow represents activation and a T represents inhibition.

a) Based on the signaling pathway shown in the schematic, what would be the effect of TNF on the cells that show a **loss-of-function** mutation of the following proteins? *Note: Consider each mutation independently.*

Cells showing a loss-of-function mutation of ...	Increased/ decreased cell death compared to TNF treated control wild - type cells? Explain why you selected this option.
TNFR	<i>Decreases cell death. In the absence of an activate TNF receptor, the downstream components of the signaling cascades will not be activated. Hence there will no DNA damage and hence no cell death.</i>
Bcl2	<i>Increases cell death. In absence of an active Bcl2, caspases will remain active and hence available to cause DNA damage thus promoting cell death.</i>
Caspases	<i>Decreases cell death. Caspases will not be active irrespective of the presence or absence of TNF. So they will be no DNA damage and hence no cell death.</i>

b) In the schematic above, list the component(s) of signaling pathway that may promote cancer cell growth if they had an oncogenic **gain- of- function** mutation.
Bcl2

c) In the schematic above, list the component(s) of signaling pathway that may be the product of tumor suppressor genes and that promote cancer cell growth if they had a **loss- of- function** mutation.
TNFR, JNK, Caspases, Cytochrome C release from mitochondrion

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