Solution key- 7.016 EXAM 3 (November 16, 2018)

Question 1 (38 points)
The fibroblast growth factor (FGF) is secreted by one cell and triggers a signaling pathway by binding to the FGF receptor (FGFR) in the membrane of a nearby target cell.

This signaling pathway is outlined on Page 7. ***Note:*** *You can detach Page 7.*

a) Circle the correct option**.** FGF-mediated signaling represents **autocrine/ paracrine/ juxtacrine/ endocrine** signaling. *(2 points)*

b) Choosing from **FGF/ FGFR/ Ras/ PI3K/ AKT/ MAPK/ ETS/ NFκB/ TRAF1/ G1-cyclin**, identify the proteins in the signaling pathway that… *(8 points)*

Note: *Parts (i)-(iv) may have more than one correct answer. Provide* ***ALL*** *correct answers.*

i. Include a nuclear localization sequence: **ETS & NFκB**

ii. Serve as transcription factors: **ETS & NFκB**

iii. Are translated on the membrane of the endoplasmic reticulum: **FGF & FGFR**

iv. Have a lipid component that is post-translationally attached in order to reach the final cellular destination: **Ras & PI3K**

c) Consider the following **homozygous mutations** in components of this signaling pathway. In each case, FGF is present outside the cell.

Mutant #1: FGFR mutant that is constitutively (always) dimerized.

Mutant #2: Overexpression of a gene/protein (**NF1**) that promotes **Ras protein GTP hydrolysis (GTP→GDP)**.

Mutant #3: ETS mutant that lacks the MAPK binding domain.

Complete the table for each mutation **in the presence of FGF ligand.** *(6 points, 2 per row)*

Mutation	MAPK active (*Yes/ No*)?	Proliferation (*Yes/ No*)?	AKT active (*Yes/ No*)?	Survival (*Yes/ No*)?
#1	Yes	Yes	Yes	Yes
#2	No	No	Yes	Yes
#3	Yes	No	Yes	Yes

d) You identify a mutant cell line that is homozygous for mutation 1 and mutation 2. Would these cells show **increased**/ **decreased**/ **no change** in **(1) Cell survival** and **(2) Cell proliferation** compared with wild-type cells when treated with FGF ligand? **Explain.** *(6 points, 3 for survival and 3 for proliferation part)*
Since dimerized FGFR (mutation 1) is constitutively active independent of the FGF ligand, the PI3K→AKT→NFκB pathway will always remain active resulting in increased cell survival.
Since the Ras gene is not expressed (due to mutation 2), active FGFR will not be able to activate the RAS→ MAPK→ ETS→ M-Cyclin pathway and hence there will be no cell proliferation.

Question 1 continued

e) Mutations in the genes encoding the proteins of this signaling pathway are observed in many cancers. Indicate whether each of the genes below is classified as a **proto-oncogene**, **tumor suppressor**, **caretaker**, or **none of the above**. ***Note:*** NF1 promotes Ras protein GTP hydrolysis (GTP→GDP). *(8 points, 2 for each)*

i. **NF1:** ***Tumor suppressor gene***

ii. **ETS:** ***Proto-oncogene***

iii. **AKT:** ***Proto-oncogene***

iv. **Gene that induces apoptosis:** ***Tumor suppressor gene***

f) Where in this cell is the FGFR protein translated? **Explain** how translation is targeted to this site. *FGFR is translated on the ribosomes attached to the ER membrane. Targeting to this site is controlled by an N- terminal signal sequence on the newly translated protein (2 points, 1 for explanation)*

g) You have a mutant cell line in which FGFR is mislocalized to the cytosol and degraded into peptides by the proteasome. *(6 points, 2 each)*

I. What post-translational modification on the mutant FGFR protein makes it a target of the proteasome? *Poly-ubiquitination*

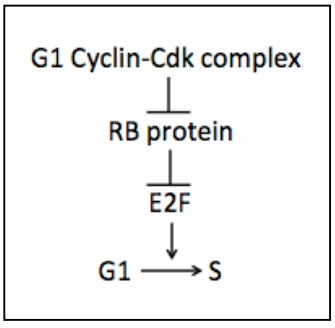
II. Name the most likely type of non-covalent interaction that causes misfolded or partially-folded proteins to aggregate (which can result in diseases such as neurodegeneration): *Hydrophobic interaction*

III. Name the class of cellular proteins that prevents aggregation of misfolded proteins and allows them to fold correctly: *Chaperones*

Question 2 (27 points)

Retinoblastoma is a pediatric cancer that results from mutation of the Retinoblastoma (*RB*) tumor suppressor gene.

The following regulatory network shows that the RB protein binds to the transcription factor E2F and prevents the E2F-mediated G1 ➔ S transition. The G1 cyclin-Cdk complex inactivates the RB protein, which promotes G1 ➔ S entry.

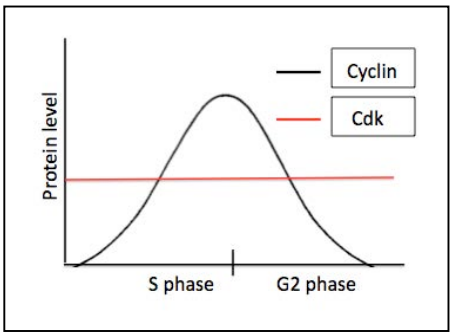


a) You isolate a version of E2F that has a **Lys^{33}→Glu^{33}** mutation in its DNA-binding domain. How might this mutation affect the binding of E2F to DNA and the G1➔ S transition? ***Note:*** *The structures of Lys and Glu are shown on the last page.*
Mutating the positively- charged Lys to a negatively- charged Glu will cause E2F to dissociate from negatively-charged DNA by electrostatic repulsion. The impaired binding will inhibit G1➔ S transition causing a G1 arrest. (4 points)

b) Human papilloma virus (HPV) infections can result in cervical, head and neck cancer. Once the virus infects the target cell, the viral protein (E7) binds to and inhibits the RB protein in the target cell. Would you classify the E7 protein the product of an **oncogene**, **tumor suppressor**, or **caretaker** gene? Briefly **explain** your answer. *(4 points)*
E7 protein promotes growth by repressing RB protein function. Therefore, it is an oncogene.

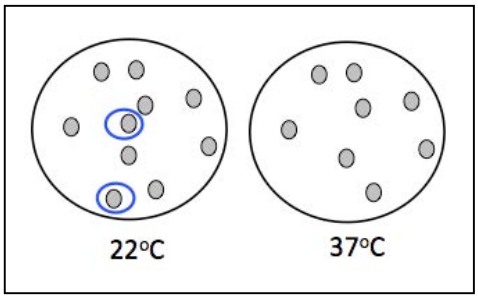
Question 2 continued

c) On the schematic to the left, identify the profile that represents the levels of S-cyclin and Cdk by filling in the boxes. **Explain** how this promotes progression through the cell cycle i.e. G1➔S➔G2➔M.



Phase-specific Cyclin-Cdk complexes tightly regulate the transition from one phase of the cell cycle to the next. Although Cdks are expressed in all phases of the cell cycle, they are activated only once they bind to specific cyclins. The cyclins, unlike the corresponding Cdks show a transient expression in a specific phase then they are degraded. (6 points, 2 for boxes and 4 for explanation)

d) You create temperature sensitive *cell division cycle (cdc)* mutants by treating yeast cells with chemical mutagen. You plate the cells at 22°C, then replica-plate the cells at 36°C, as shown in Figure below.



i. Circle the colonies that represent temperature sensitive mutants in the Figure above. *(3 points)*

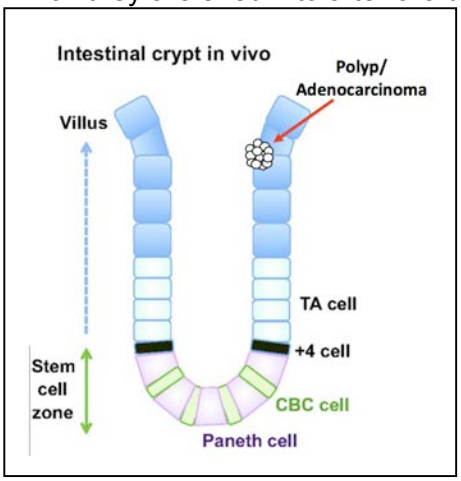
ii. **Explain** why you would treat yeast cells with a chemical mutagen rather than X-rays, which generate chromosomal deletions.

Temperature sensitive mutants are usually missense mutations that sensitize a protein to temperature rather than X rays, which generate chromosomal deletions. (3 points)

e) It has been suggested that *cdc* genes are evolutionarily conserved across species. Outline the steps of an experiment that you could perform to test this hypothesis. **Note:** Your approach should be experimental, not computational (Sorry CS majors!)

You can transform the yeast with the Temperature sensitive cdc mutant with a wild-type variant of human cdc gene. Then plate the yeast on growth media and see if the cells divide at restrictive temperature (36°C). If they divide and form colonies at 36°C then the human cdc rescues the function of the mutant cdc suggesting functional conservation (i.e. wild-type phenotype). (7 points)

Question 3 (10 points)
The following is a schematic of a primary tumor in the intestinal crypt. All the cells in a normal, healthy crypt originate from stem cells (SC) located at the base of the crypt and migrate up towards the lumen, which they are shed into after 3-5 days.



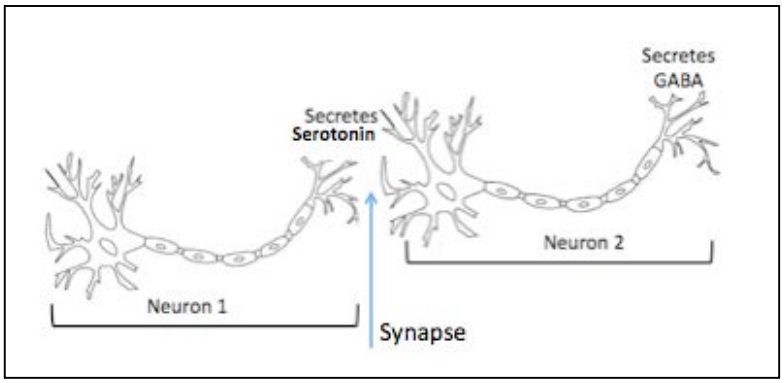
a) Explain why inhibition of cell migration along the lining of the crypt results in a polyp or adenoma. *(5 points)*
These are the cells that go through rapid divisions and thus will have acquired mutations. So if instead of being shed, they persist, they can grow and divide uncontrollably to form a primary tumor.

b) The intestinal stem cells (ISC) have significantly fewer mutations than their descendants in the same lineage. **Explain** why this is so and what is its significance. *(5 points)*
Each time a cell divides there is a probability that it will acquire mutation(s).The ISCs have fewer mutations since they are dividing slower and therefore have lower probability of acquiring a mutation. Transit amplifying cells (TA cell) divide faster and therefore have higher probability of acquiring mutation(s).

Question 4 (15 points)
Note: The concentrations of Na^+, Cl^- and Ca^{2+} are high in the exoplasm. In contrast, the concentration of K^+ is high in the cytoplasm of the neuron.

To the right is a schematic of a synapse between neurons 1 and 2 in the cell culture plates A, B and C.

- Neuron 1 secretes serotonin (excitatory neurotransmiter)
- Neuron 2 secretes GABA (inhibitory neurotransmiter) and responds to serotonin (i.e. has serotonin receptors, ligand-gated Na^+ channels)



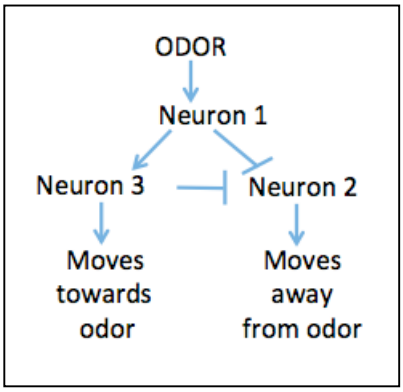
You stimulate Neuron 1 in the presence of different inhibitors. For each plate, select whether Neuron 2 secretes **higher**, **lower**, or **the same level of GABA** (compared to control plates without inhibitor) and **explain** your reasoning. *(15 points, 5 each)*

a) Plate A is treated with **Celexa** (an inhibitor of serotonin reuptake from synaptic cleft).
Since serotonin continues to exist at the synapse (less signal termination), it will bind to the receptors on the dendrites of Neuron 2 for longer resulting in GABA release.

b) Plate B is treated with **Jingzhaotoxin III** from spiders (prevents the opening of voltage gated Na^+ channels)
The inhibitor will prevent the opening of voltage gated Na+ channels in neuron 1 and neuron 2. So there is no depolarization and no AP and hence there is no/ less GABA release.

c) Plate C is treated with a drug that **inhibits GABA receptor.**
Inhibiting the GABA receptor will inhibit downstream neuronal transmission but will have no effect on GABA release (same level).

Question 5 (10 points)
The worm *C. elegans* moves towards or away from an odor using the neuronal circuitry shown below.



- When Neuron 1 activates Neuron 3, the worm moves towards the odor.

- When Neuron 2 is active, the worm moves away from the odor.

- When Neuron 1 is stimulated by odor, it inhibits Neuron 2 preventing movement away from the odor.

Note: *The concentrations of Na^+, Cl^- and Ca^{2+} are high in the exoplasm. In contrast, the concentration of K^+ is high in the cytoplasm of the neuron.*

Optogenetics experiments can be applied to the worm to investigate the roles of specific neurons in odor sensing. For a) and b) below, choose from the following options: ***worm moves towards the odor/ worm moves away from the odor/ worm is unresponsive to the odor*** and **explain** why you selected the response.

a) The light-activated channel rhodopsin (ChR1) opens in response to blue light and allows passage of Na^+ ions across the membrane. You construct a *C. elegans* where the ChR1 channel is expressed exclusively in *Neuron 2*. Then you expose the worm to an odor (ethanol).

What response would you predict on stimulation with blue light and **why**?
Blue light causes influx of Na^+ ions through the ChR1 channel and activates neuron 2. So the worm moves away from the odor. (5 points, 3 for explanation)

b) The light-activated Halorhodopsin channel (NpHR) opens in response to yellow light and allows passage of Cl^- ions across the membrane. You construct a *C. elegans* where the NpHR is exclusively expressed in *Neuron 1.* Then you expose the worm to an odor (ethanol).

What response would you predict on stimulation with yellow light and **why**?

Yellow light hyperpolarizes the neuron 1. So the worm would be unresponsive to an odor (5 points, 3 for explanation)

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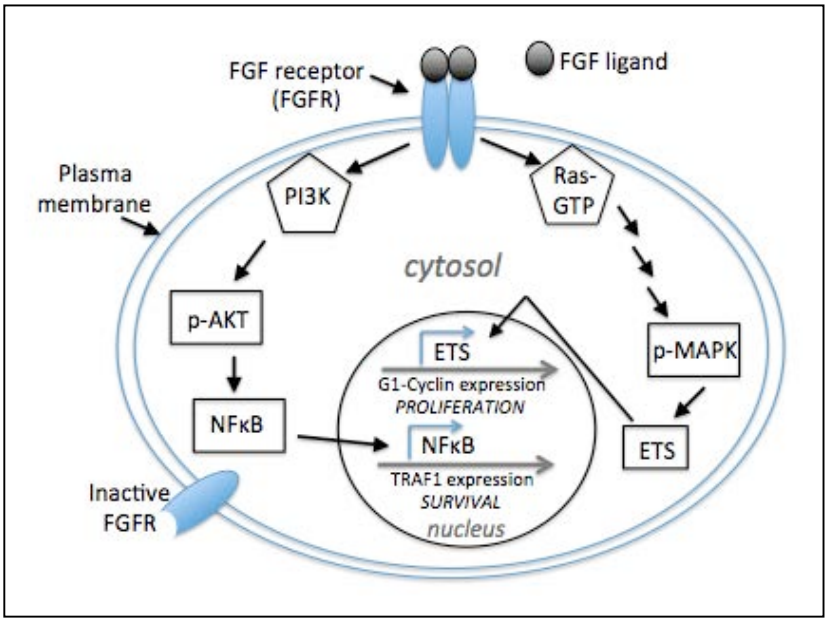
Fibroblast growth factor (FGF) signaling pathway for Question 1



- The FGF ligand binds the FGF receptor (FGFR). The binding allows the FGFR to dimerize and become active.

- Active FGFR converts the plasma membrane bound Ras from the inactive GDP-bound form (not shown) to the active GTP-bound form.

- Ras-GTP activates MAP kinase (MAPK), which in turn activates ETS.

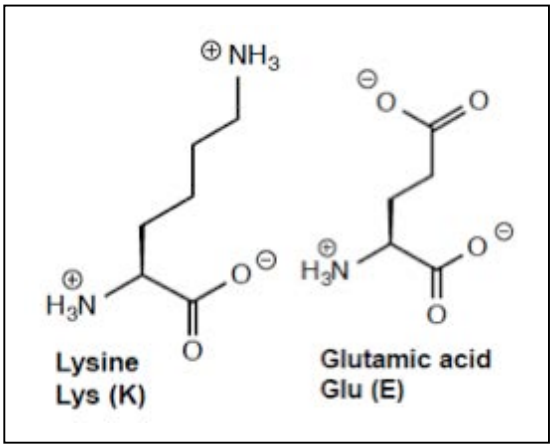
- Active ETS turns on the expression of the G1-cyclin gene, which results in cell proliferation.

- Active FGFR also activates the plasma membrane bound PI3 kinase (PI3K), which activates AKT kinase.

- Activated AKT (p-AKT) kinase activates NFκB.

- Active NFκB turns on the expression of the TRAF1 gene, which results in cell survival.

Amino acids for Question 2, Part (a)



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