Name: _____

7.016 EXAM 3
November 16, 2018

TA:_____ Recitation: _____

The Exam starts at 10:05 am and ends at 10:55 am.

Write your name on this page and your initials on all the other pages in the space provided. This exam has **7** pages including the coversheet. Check that you have all the pages **1-7**.

NOTE: Page 7 has a schematic of the signaling pathway for Question 1.

Only answers on the **FRONT** of each page will be graded. You may use the backs of the pages, but only as scratch paper.

Questions	Points	Score
1	38	
2	27	
3	10	
4	15	
5	10	
TOTAL	100	

Initials: _____

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.

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

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

i. Include a nuclear localization sequence: _____

ii. Serve as transcription factors: _____

iii. Are translated on the membrane of the endoplasmic reticulum: _____

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

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.**

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

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.**

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).

i. **NF1: _____**

ii. **ETS: _____**

iii. **AKT: _____**

iv. **Gene that induces apoptosis: _____**

f) Where in this cell is the FGFR protein translated? **Explain** how translation is targeted to this site.

g) You have a mutant cell line in which FGFR is mislocalized to the cytosol and degraded into peptides by the proteasome.

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

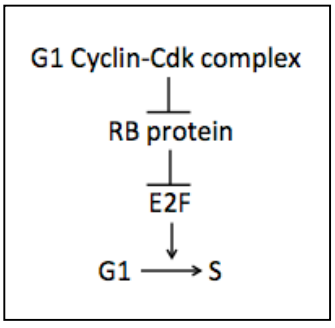
II. Name the most likely type of non-covalent interaction that causes misfolded or partially-folded proteins to aggregate (which can result in diseases such as neurodegeneration): _____

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

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.

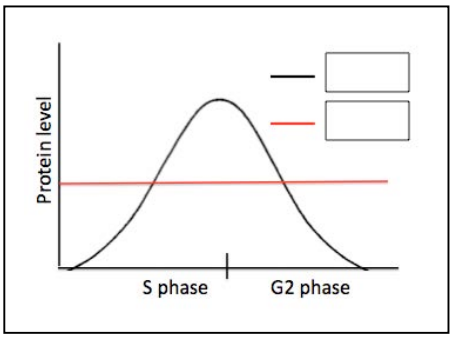


a) You isolate a version of E2F that has a **Lys^{33}→Glu^{33}** mutation in its DNA-binding domain. How might this mutation affect the binding of E2F to DNA and the G1➔ S transition? ***Note:*** *The structures of Lys and Glu are shown on the last page.*

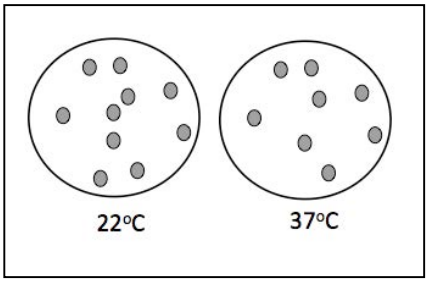
b) Human papilloma virus (HPV) infections can result in cervical, head and neck cancer. Once the virus infects the target cell, the viral protein (E7) binds to and inhibits the RB protein in the target cell. Would you classify the E7 protein the product of an **oncogene**, **tumor suppressor**, or **caretaker**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

Question 2 continued

c) On the schematic below, 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.



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.

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

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!)

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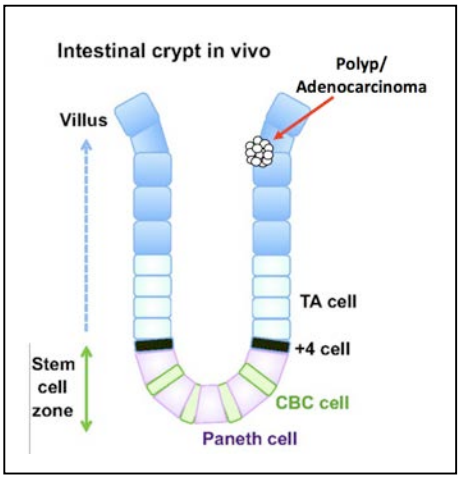
Question 3 (10 points)
The following is a schematic of a primary tumor in the intestinal crypt. All the cells in a normal, healthy crypt originate from stem cells (SC) located at the base of the crypt and migrate up towards the lumen, which they are shed into after 3-5 days.



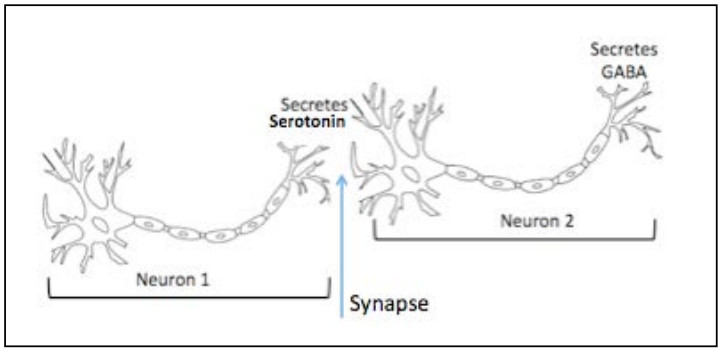
a) Explain why inhibition of cell migration along the lining of the crypt results in a polyp or adenoma.

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.

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.

a) Plate A is treated with **Celexa** (an inhibitor of serotonin reuptake from synaptic cleft).

b) Plate B is treated with **Jingzhaotoxin III** from spiders (prevents the opening of voltage gated Na^+ channels)

c) Plate C is treated with a drug that **inhibits GABA receptor.**

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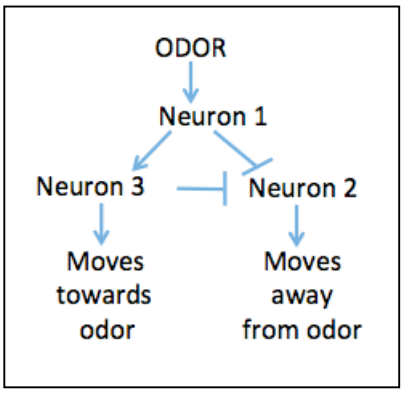
Question 5 (10 points)

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).

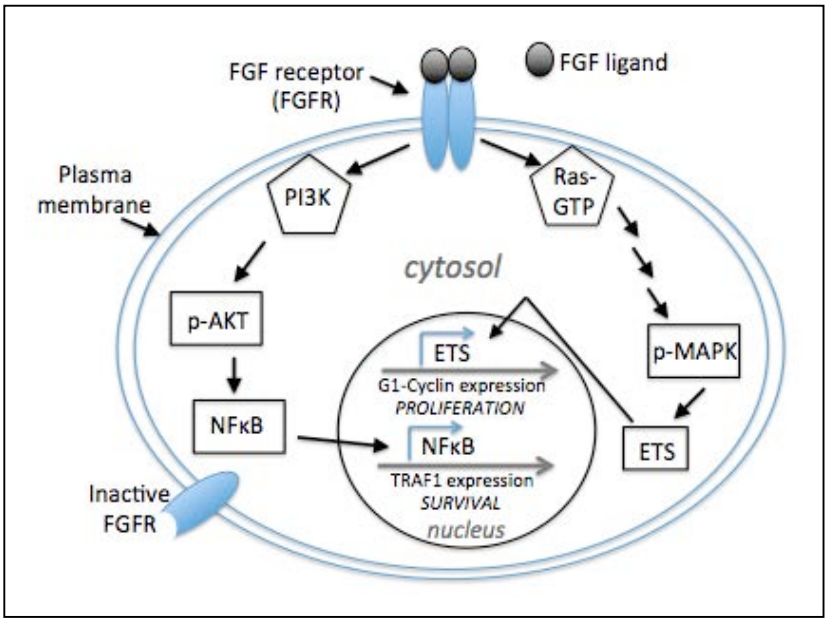
What response would you predict on stimulation with blue light and **why**?

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**?

Please detach this Page

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.

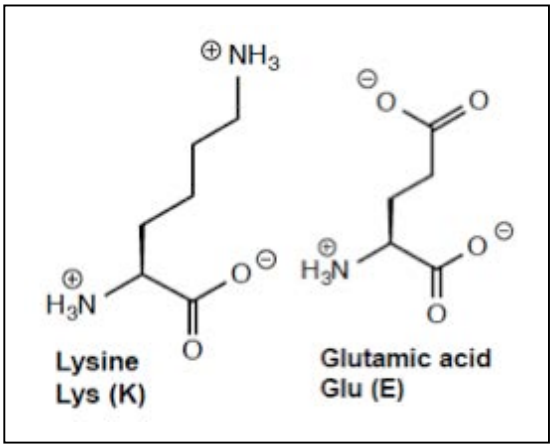
- Active ETS turns on the expression of the G1-cyclin gene, which results in cell proliferation.

- 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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