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Question 1 (20 points)

Diabetes occurs either due to a reduced **insulin hormone** production by the pancreatic β cells or the resistance of target cells to insulin. As shown by the schematic on Page 9, insulin signaling mediates glucose uptake, protein synthesis, cell survival and proliferation. **Note: You can detach Page 9.**

a) Consider the following mutations in different components of insulin signaling.

1. The kinase $p70^{S6k}$ cannot bind to IRS.
2. PI3 kinase (PI3K) cannot activate PDK.
3. The promoter region of Akt gene has methylated cytosines (^{Me}C).

Complete the table for each of the above mutations relative to the wild-type cells in the **presence of Insulin**. **Note:** Consider each mutation independently.

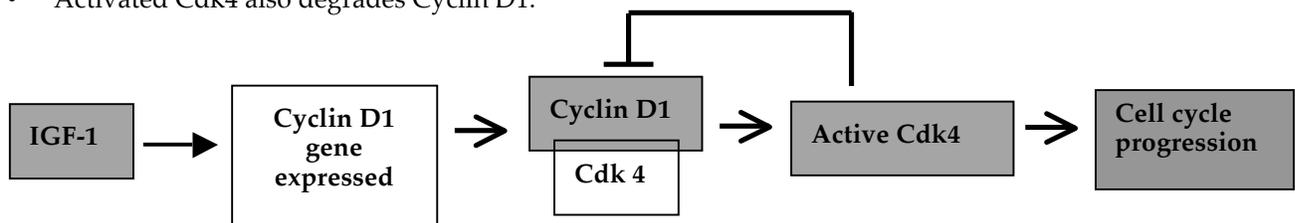
| Mutation | PDK active (Yes/No)? | Akt active (Yes/ No)? | FOXO active (Yes/No)? | $p70^{S6k}$ Active (Yes/No)? | Cell Survival (increases/ decreases/ remains the same)? | Cell proliferation (increased/ decreased/ remains the same)? |
|----------|----------------------|-----------------------|-----------------------|------------------------------|---|--|
| #1 | | | | N/A | | |
| #2 | | | | | | |
| #3 | | | | | | |

b) Although glucose is a small molecule it requires GLUT4 receptors to diffuse into the cell. **Explain** why is this so.

c) Identify the component in the schematic that causes feedback inhibition of insulin signaling.

d) The Insulin growth factor (IGF-1) promotes cell proliferation as shown below.

- IGF-1 activates a signaling cascade that finally activates Cyclin D1 gene expression.
- The Cyclin D1 protein binds to and activates Cyclin dependent kinase 4 (Cdk4).
- Active Cdk4 allows the cell to move to the next phase of the cell cycle.
- Activated Cdk4 also degrades Cyclin D1.



- i. On the schematic, **circle** the protein of IGF-1 signaling that is expressed during **ALL** the phases of cell cycle.
- ii. Using a temperature sensitive mutant of Cdk4, you find that the cells fail to divide at the non-permissive temperature and each arrested cell has a diploid DNA content (2n) in its nucleus. At what cell cycle checkpoint (*choose from G1/ S, S/G2 or G2/ M*) is the Cdk4 protein likely to act?

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Question 1 continued

iii. You identify the following Cdk4 mutants.

- **Mutant A** shows deletion of the kinase domain of Cdk4.
- **Mutant B** shows no degradation of Cyclin D1 by Cdk4.

Relative to the wild-type cells, which of these mutants (*choose from mutant A or B*) will show no cell proliferation? **Explain** why you selected this option over the other.

Question 2 (20 points)

Much research focuses on understanding how the pancreatic β cells form during embryogenesis in the hopes of regenerating these in the diabetic patients.

The pancreas endocrine cells, including the β cells, are formed from the anterior endoderm (AE). You isolate AE from embryos on days 5, 6 and 7 after fertilization and culture these explants *in vitro* (*in cell culture plates*) until each is 10 days old. Since insulin expression indicates differentiated pancreatic β cells, you measure the expression of insulin in each explant at the time of isolation and when they are 10 days old. You tabulate the results below.

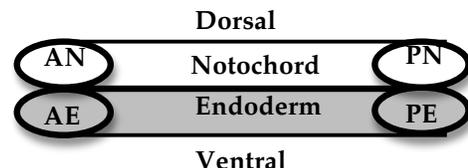
| Time of explant | Expresses insulin... | |
|-----------------|---------------------------|-------------------|
| | At the time of isolation? | When 10 days old? |
| 5 days | No | No |
| 6 days | No | Yes |
| 7 days | Yes | Yes |

i. At the time of isolation, are the **Day 6 explants** committed, differentiated, uncommitted compared to **Day 7 explants**? **Explain** why you selected this option.

ii. **Circle** the option that best categorizes the insulin gene (*choose from ubiquitous, restricted, effector gene*). **Explain** why you selected this option.

b) You want to study the interaction of endoderm cells with another group of cells called notochord. You isolate the **5 days old** anterior (head) endoderm (AE) and posterior (tail) endoderm (PE) from the embryo and culture them either alone or in combination with anterior notochord (AN) or posterior notochord (PN) until the cells are 10 days old. You measure the insulin expression in each culture to see if they have differentiated into pancreatic β cells. Your results are tabulated below. Note: The AE, PE, AN and PN were 5 days old at the time of isolation and did not express insulin.

| Explants | Insulin expression after 10 days ? |
|----------|------------------------------------|
| AE | No |
| PE | No |
| AE + AN | Yes |
| AE + PN | No |
| PE + AN | Yes |
| PE + PN | No |



i. Based on the data provided, **explain** the role of AN in the formation of pancreatic β cells.

ii. Based on the data provided, what is the role of the endoderm (AE, PE) in **restricting the formation** of the pancreatic β cells to the anterior of the embryo?

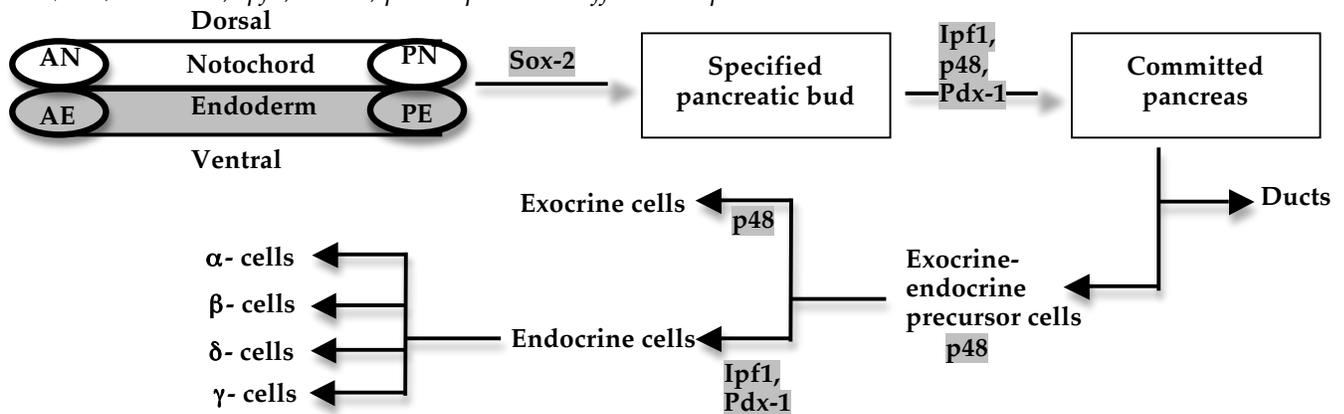
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Question 2 continued

- iii. The notochord **secretes fibroblast growth factor (FGF)**, which triggers a signaling cascade that activates the transcription factor Sox2. This promotes the formation of pancreatic β cells. Based on these data...
- Would you classify FGF as an *inducer* or *determinant*? **Explain** your choice.
 - Where would the receptors for FGF reside (*choose any from AN, PN, AE or PE*)?
 - Where would the **Sox2 gene be expressed** (*choose any from AN, PN, AE or PE*)?
 - Where would the **Sox2 gene be inactive** (*choose any from AN, PN, AE or PE*)?

Question 3 (20 points)

The following schematic represents the major steps of pancreatic organogenesis. Note: The transcription factors (TFs) i.e. *Hlx1, Ipf1, Pdx-1, p48* required at different steps are shown.



- a) **Circle** the option that best describes the potency of the cells of specified pancreatic bud that form the pancreas (*choose from multipotent, unipotent, totipotent*)? **Explain** why you selected this option.
- b) What would be the effect of **loss-of- function mutation of Sox2** on pancreatic organogenesis?
- c) The TF, p48 is expressed in early organogenesis and also later in the exocrine cells. However its expression is inhibited in the endocrine cells. A null mutant (complete knock out) of Ipf1 leads to expression of p48 in the endocrine cells, which no longer go on to become pancreatic β cells.
- What is the role of Ipf1 in regulating p48 expression?
 - What is the role of p48 in regulating pancreatic β cell formation?
- d) You identify a mutant where the cells fail to form epithelial sheets. **Circle** the correct characteristics of the cells from below that could produce the mutant phenotype. The cells in this mutant ...
- Show mesenchymal to epithelial transition (True or false)
 - Lack homotypic cell- cell junctions (True or false)
 - Fail to migrate (True or false)
 - Show apical and basal polarity (True or false)

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Question 3 continued

e) Researchers have shown that forcing the expression of three transcription factors (*Ngn3*, *Pdx1* and *Mafa*) can reprogram the exocrine cells of the pancreas in adult mice into insulin expressing pancreatic β cells.

i. You want to use the fluorescence activated cell sorter (FACS) to get a pure exocrine cell population for reprogramming. **Circle** the fluorescence tagged antibody that you would use for purification of these cells and **explain** why you selected this option.

- *Antibody specific to the Pdx1 transcription factor*
- *Antibody specific to cell surface protein X.*

ii. You successfully reprogram the exocrine cells into pancreatic β cells that secrete insulin and have wild-type morphology. You introduce these cells into two diabetic mice (mouse A & B).

- **Circle** the cell surface molecule that you would test to avoid the chances of immunological rejection of the introduced pancreatic β cells.

MHCI MHCII CD4 CD8 TcR IgG

- You find that the introduced pancreatic β cells reduce the symptoms of diabetes in Mouse A but not in Mouse B. Assuming there is no immunological rejection in either of the mice, which cells (*choose from original pancreatic β cells or cells in the surrounding niche*) in mouse B have the mutation. Provide a brief **explanation**.

Question 4 (22 points)

Vaccination is a very effective way of providing protection against pathogens.

a) You develop a **heterologous vaccine** that can provide immunity against a form of Flu virus. You vaccinate two healthy individuals with this vaccine.

i. Would the B cells with surface-bound antibodies specific to this form of flu virus in Individual 1 be the same as that in Individual 2? **Explain** why or why not.

ii. From the choices below **circle** the type(s) of immune responses that will be generated by this vaccine.

Humoral **Innate** **Cytotoxic** **All**

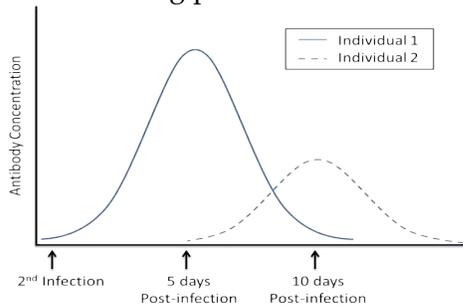
iv. What property of the virus used as a heterologous vaccine.....

- Prevents it from causing any severe symptoms in the recipient unlike the flu virus?
- Allows it to provide protection against the flu virus?

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Question 4 continued

b) Five years after being vaccinated with the heterologous vaccine, both Individuals encounter a new form of flu virus. You assay their humoral immune response specific to the new form of flu virus and obtain the following profile.



Explain why the immune response of Individual 1 to the new variant of flu virus differs from that of Individual 2.

c) You compare the antibody gene of a **B cell** (B cell X) that produces a membrane bound antibody that specifically binds to **the flu virus** with the antibody gene of the **memory B cells** and **plasma cells** descending from B cell X.

- i. Would the **antibody gene** be the *same or different* between the B cell X and the plasma cells? **Explain** why you selected this option.
- ii. Would the **mature mRNA transcript** be the *same or different* between the B cell X and the plasma cells? **Explain** why you selected this option.
- iii. **Circle** the cell type(s) (*choose from B cell X, memory B cells and plasma cells*) that will produce antibodies that can bind to and eliminate the virus.
- iv. **Circle** the cell type(s) (*choose from B cell X, memory B cells and plasma cells*) that is terminally differentiated.

d) You are working with a retrovirus that can integrate into the host genome and is not expressed. Briefly **explain** why a cell infected with this virus is not detected by the immune system.

Question 5 (18 points)

a) The voltage gated (VG) Na⁺ channel is comprised of two polypeptide chains and its activation results in the action potential.

- i. What is the **highest** order of protein structure (*choose from primary, secondary, tertiary or quaternary*) in a voltage gated Na⁺ channel?
- ii. Complete the table below for the VG Na⁺ channel and the Na⁺K⁺ pump.

| Channel / pump | Direction of flow of Na ⁺ ion (into the cell/ out of the cell)? | Example of active/ passive transport? | Active in which phase (<i>choose from resting, depolarization, repolarization or all</i>)? |
|-------------------------------------|--|---------------------------------------|--|
| VG Na ⁺ channels | | | |
| Na ⁺ K ⁺ pump | | | |

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Question 5 continued

b) AcotininE is a neurotoxin that specifically binds to the open / active conformation of VG Na⁺ channel and causes its persistent activation via **allosteric modulation**. Eventually the channels close and later reset so they are ready to depolarize.

- i. Would AcotininE mediate its effect by binding inside the pore of the VG Na⁺ channel through which Na⁺ ions can pass (*Yes/ No*)? **Explain**.

- ii. You stimulate a neuron with an excitatory neurotransmitter and measure the frequency of the resulting action potential. You next stimulate a similar neuron with the same neurotransmitter together with AcotininE. You observe that adding AcotininE reduces the frequency of the action potential. **Explain** why is this so.

c) Serotonin (5-HT) can serve both as an excitatory or inhibitory neurotransmitter based on the type of receptors to which it binds. The 5-HT₃ receptors act as ligand gated Na⁺ channels. In comparison, the 5-HT₂ receptors are ligand- gated Cl⁻ channels that are coupled to G proteins.

- i. Upon binding of serotonin to the 5-HT₃ and 5-HT₂ receptors would...

* Na⁺ ions flow *into* or *out* of the cell?

* Cl⁻ ions flow *into* or *out* of the cell?

- ii. In response to serotonin, would you expect both 5-HT₂ and 5-HT₃ receptors to activate ion flow with the same time course? **Explain**.

- iii. You are studying a neuron that expresses functional 5-HT₂ and 5-HT₃ receptors and secretes glycine in the synaptic cleft when stimulated. You stimulate this neuron with serotonin in the presence of latrotoxin, which induces a large Ca⁺⁺ influx specifically through VG Ca⁺⁺ channels located at the axon terminus of the neuron. Would this treatment have an effect on the...
 - Amplitude of action potentials elicited by this neuron (*Yes/ No*)? **Explain** your choice.

 - Secretion of neurotransmitter by this neuron (*Yes/ No*)? **Explain** your choice.

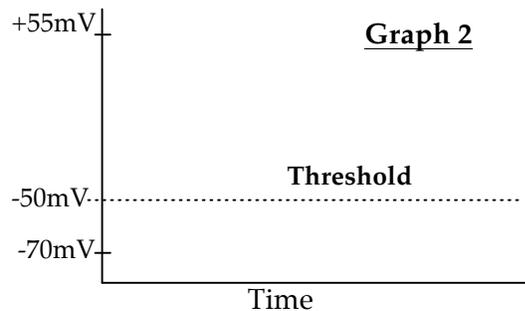
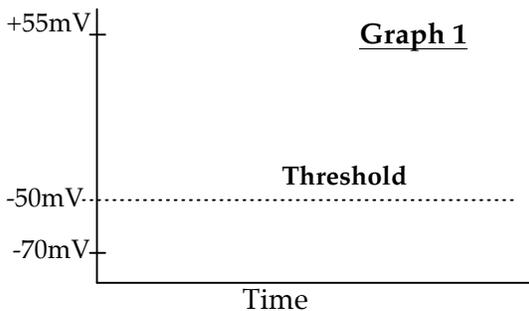
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Question 5 continued

d) You have the following information about two synapses.

| Synapses | Neurotransmitter (NT) secreted by the pre-synaptic neuron into the synapse | Effect of the released NT on the post-synaptic neuron | Ligand-gated receptors located on the post-synaptic neuron |
|----------|--|---|--|
| 1 | 5-HT | Excitatory | 5-HT ₃ (Na ⁺ channels) |
| 2 | Glycine | Inhibitory | Glycine receptors (Cl ⁻ channels) |

- i. In **synapse 1**, sketch the alteration in membrane potential of the post-synaptic neuron if your electrode is in the axon of the post-synaptic neuron. Use **Graph 1** below and **explain**. *Note: Assume maximal neurotransmitter release.*
- ii. In **synapse 2**, sketch the alteration in membrane potential of the post-synaptic neuron if your electrode is in the cell body of the post-synaptic neuron. Use **Graph 2** below and **explain**. *Note: Assume maximal neurotransmitter release.*



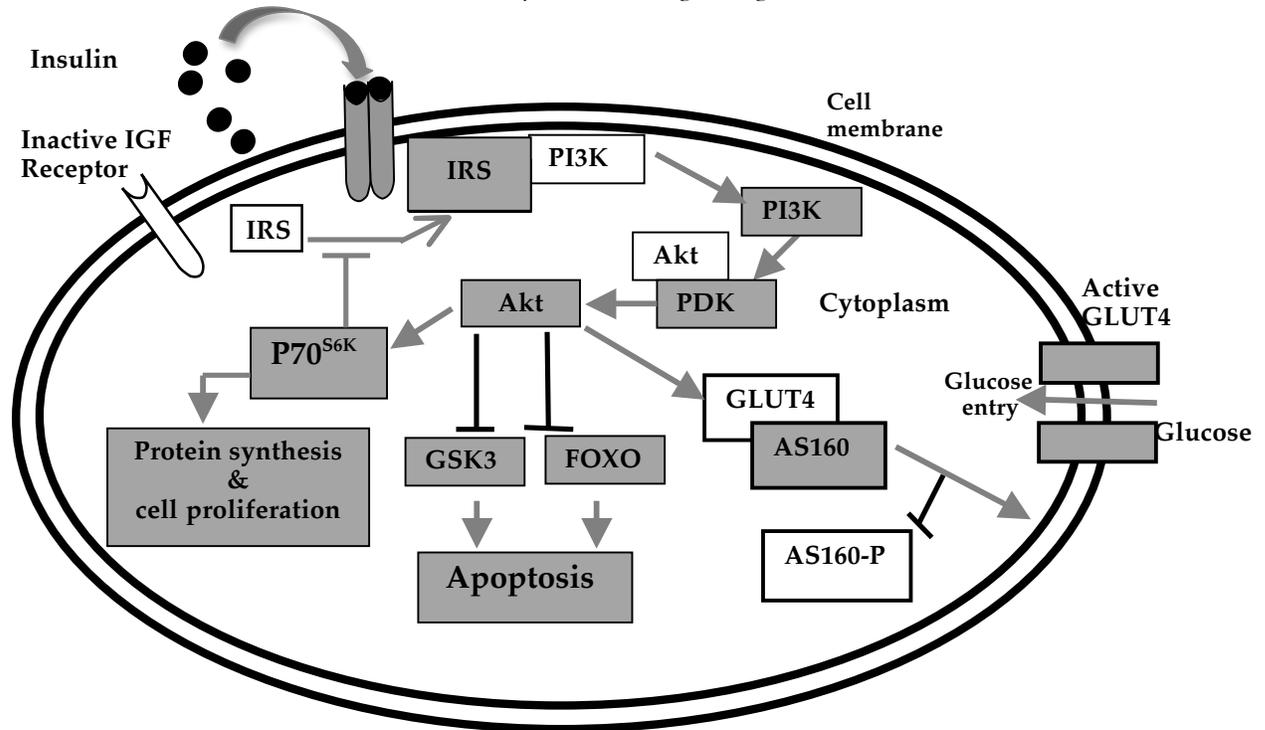
Explanation for Graph 1:

Explanation for Graph 2:

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Note: You can detach this page

Note: The arrow represents activation and a "T" represents inhibition. The shaded boxes show the signaling molecules in their active state and the blank boxes represent the signaling molecule in their inactive state.



The major steps of this pathway are given below:

- Insulin hormone binds to Insulin growth factor receptors (IGF receptor) and activates them through dimerization.
- Activated IGF receptors phosphorylate and activate insulin receptor substrate (IRS).
- Active IRS activates P13 kinase (PI3K), which phosphorylates and activates phosphoinositide- dependent kinase (PDK) through a series of steps.
- Activated PDK phosphorylates and activates Akt.
- **Activated Akt...**
 - ✓ Inhibits apoptosis by phosphorylating and inhibiting GSK3 and FOXO proteins.
 - ✓ Promotes protein synthesis by phosphorylating and activating P70^{S6K}. These newly synthesized proteins promote cell proliferation.
 - ✓ Phosphorylates and dissociates AS160 protein from GLUT4. The GLUT-4 then migrates to the cell membrane where it serves as a pore for Glucose entry.
- The activated p70^{S6K} also binds to and inhibits IRS.

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