

Solution Key- 7.013 EXAM 3 (4 / 29 / 13)

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	Yes	Yes	No	N/A	Increases	Increases
#2	No	No	Yes	No	Decreases	Decreases
#3	Yes	No	Yes	No	Decreases	Decreases

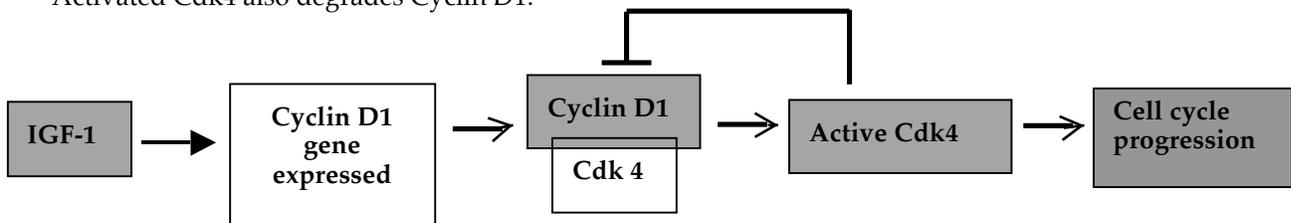
b) Although glucose is a small molecule it requires GLUT4 receptors to diffuse into the cell. **Explain** why is this so.

Glucose, being a monosaccharide, has multiple -OH groups that make it hydrophilic. So it cannot diffuse through the hydrophobic lipid bilayer but instead diffuses into the cell through the GLUT4 protein.

c) Identify the component in the schematic that causes feedback inhibition of insulin signaling. $p70^{S6k}$

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. *Cdk4*
- 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?

The cell will arrest in G1/ S checkpoint since the cell still has the diploid (2n) DNA content.

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.

Mutant A will show no cell proliferation since in the absence of its kinase domain Cdk4 will not phosphorylate its target protein and the cell will remain arrested in G1/ S phase. In comparison, since cyclin D1 in mutant 2 is not degraded it will constitutively activate Cdk4. Hence the cell will show uncontrolled cell proliferation.

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.

a) 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.

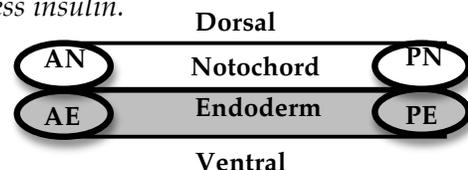
They are committed since after 4 days of culture they differentiate into insulin secreting pancreatic β cells.

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

It is an effector gene since it is expressed only in the functional β cells of the pancreas.

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.

*AN most likely, secretes an inducer (or a signaling factor), which binds to a specific receptor located on the cells of AE so that they become pancreatic β cells. **Note:** NO need to say anything about the other tissues.*

- 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?

The AE and PE can both respond to the AN, but the PE does not do so because it is far way from the AN. So the endoderm really PLAYS NO ROLE in restricting where the β cells form.

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...

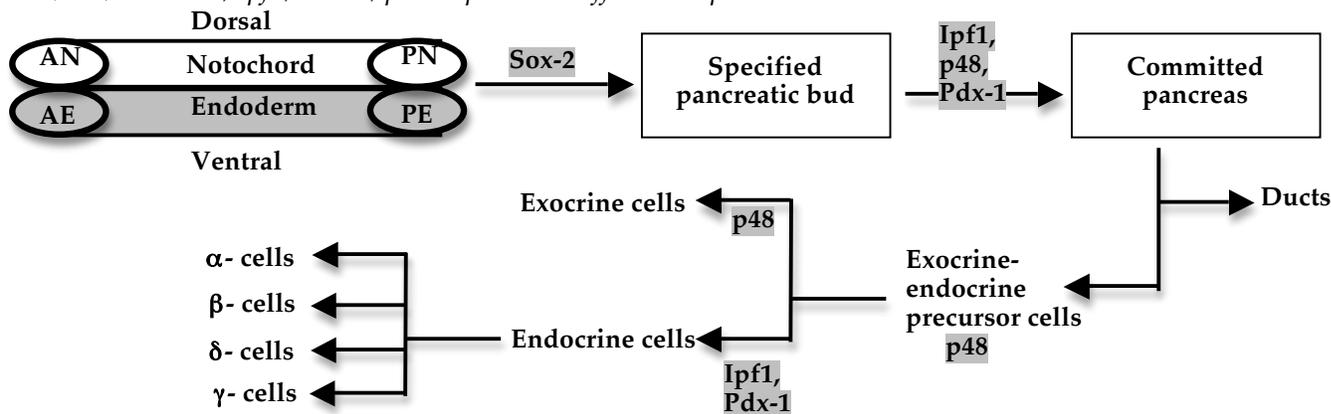
- Would you classify FGF as an inducer or determinant? Explain your choice.

FGF being secretory is an inducer and not a determinant.

- 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, Ipfl, 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.

It is multi-potent since it forms cells of multiple fates.

b) What would be the effect of loss-of-function mutation of Sox2 on pancreatic organogenesis?

In the absence of a functional Sox2, cell of the AE will not form the pancreatic bud and hence there will be NO 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 Ipfl leads to expression of p48 in the endocrine cells, which no longer go on to become pancreatic β cells.

i. What is the role of Ipfl in regulating p48 expression? *Ipfl-1 inhibits p48 expression.*

ii. What is the role of p48 in regulating pancreatic β cell formation?

P48 inhibits the differentiation of endocrine-exocrine precursor cells into endocrine pancreatic β cell.

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)

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.*

Since you need the live purified exocrine cell population for reprogramming you would use the antibody that specifically binds to the cell membrane protein and does not disrupt the integrity of the cell. The antibody specific to Pdx1 has to enter the inside of the cell to bind to Pdx1 and can do so only if the cells are permeabilized.

- 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**.

The mutation in mouse B is in the cells of the surrounding Niche. In the absence of the signals from the surrounding niche the introduced pancreatic β cells fail to function.

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.

No, since each individual undergoes a random arrangement of the antibody gene to produce B cells, each of which has a unique VDJ combination and is therefore producing a unique antibody that is specific to a unique antigen.

- 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?

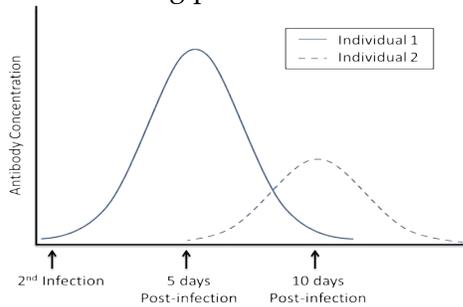
This is a weak virus and therefore causes either no or only mild symptoms compared to the flu virus.

- Allows it to provide protection against the flu virus?

The virus used as a heterologous vaccine has surface antigen common to the flu virus antigens. So the memory cells that are specific to the common antigens and are generated in an individual after receiving the heterologous vaccine will provide rapid and strong protection to the vaccinated individual against the flu virus.

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.

Unlike individual 2, individual 1 has had previous exposure to another virus/ pathogen that has surface antigens common to the new form of flu virus. So memory T and memory B cells that this individual already has, start proliferating to give rise to rapid and stronger secondary immune response.

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.

It would be the same since the B cell X and descending plasma cells are recognizing the same antigen. However, you may argue that the antibody gene in the plasma cells may have undergone somatic hypermutations to produce an antibody that has a higher affinity for the antigen compared to the surface antibody of the B cell X. Hence there may be slight variations.

- 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.

It is different due to alternative splicing thus producing a cell surface bound IgM and secretory IgG antibodies.

- 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.

If the virus is integrated, the viral proteins are not expressed and hence will not be presented on the surface of infected cells through MHC-I molecules and will therefore not be recognized by T_C cells. Hence the virus- infected cells remains unrecognized by the immune system and does not undergo T_C cells mediated killing.

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?

Question 5 continued

- 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	<i>Into the cell</i>	<i>Passive transport</i>	<i>Depolarization</i>
Na ⁺ K ⁺ pump	<i>Na⁺ out and K⁺ in</i>	<i>Active transport</i>	<i>All</i>

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 in the pore of the VG Na⁺ channel through which Na⁺ ions can pass (Yes/ No)? **Explain**.

No, allosteric modulator by definition binds to a site different from the substrate binding sites of the proteins or enzymes and thereby change their conformation. So AcotininE will bind to a different site instead of binding inside the pore of the VG Na⁺ channel through which Na⁺ ions can pass. Alternatively, occluding the pore would prevent Na⁺ ion passage.

- 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.

The frequency of the resulting action potential is reduced in the presence of AcotininE since it takes longer for the VG Na⁺ channels to close and reset.

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

- 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**.

No. In response to serotonin, since the 5-HT₂ receptors being metabotropic will first have to activate G proteins before the associated Cl⁻ channel can be opened. This takes more time than directly opening the 5-HT₃ receptors, which are ionotropic and themselves function as ligand ligated Na⁺ channels.

- 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.

No, the amplitude of action potential is always constant.

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

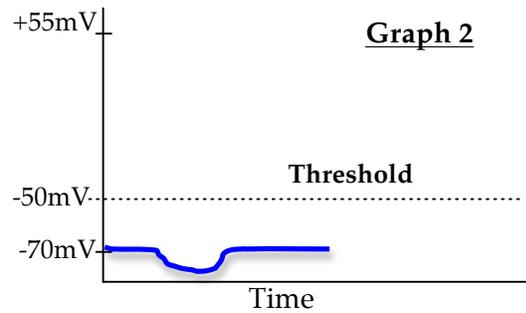
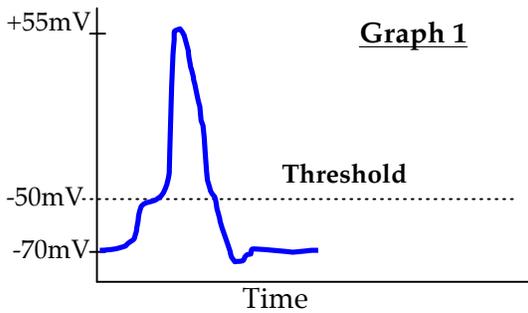
Yes, since voltage gated Ca²⁺ channels located at the axon terminus are involved in exocytosis of endorphins into the synaptic cleft.

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.

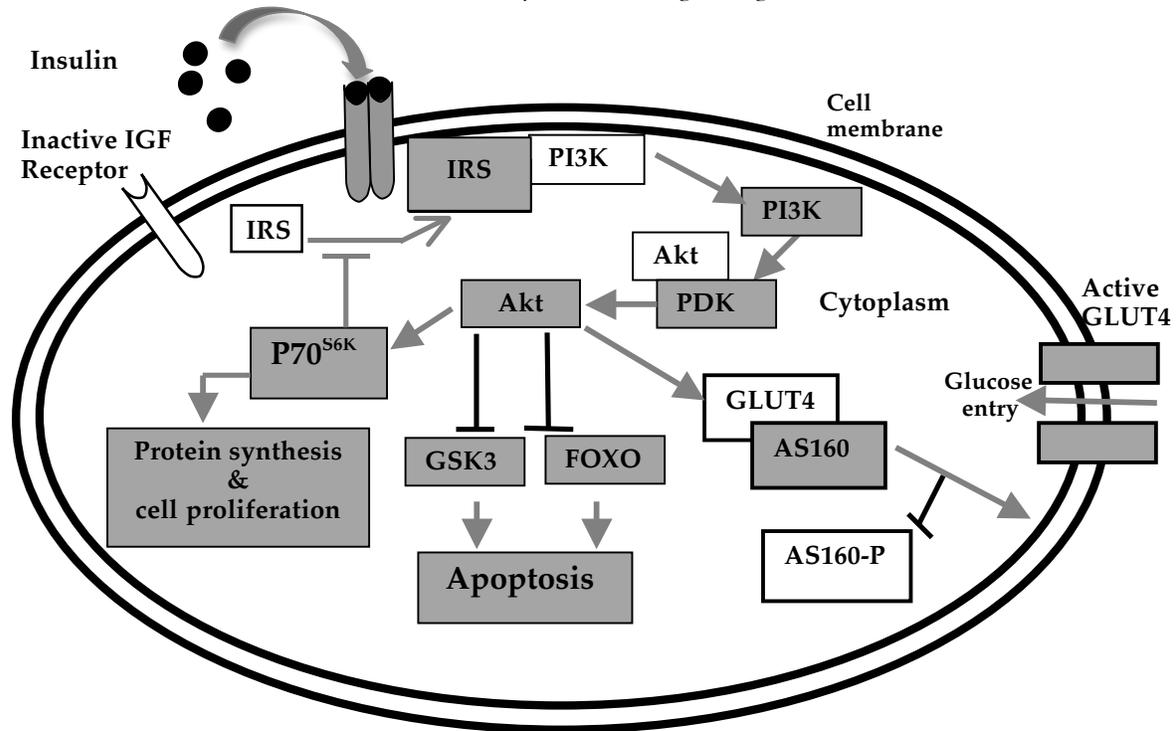


Explanation for Graph 1: Serotonin secreted by the pre-synaptic neuron will bind to its 5-HT₃ receptors on the post-synaptic neuron, which will open to allow Na⁺ ions to diffuse into the neuron thus depolarizing the membrane and resulting in an action potential.

Explanation for Graph 2: Glycine secreted by the pre-synaptic neuron will bind to its receptors on the post-synaptic neuron, which will open to allow Cl⁻ ions to move into the neuron thus hyperpolarizing the membrane and drifting the membrane potential even further away from threshold level.

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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