Bio3120 Cell and Molecular Biology
March 17, 2014
Dr. Kai F. Hung

Examination #1

Instructions:

1. This exam package is due by 8 am on Monday March 24. Late submissions will not be graded.

2. Students are required to work in groups. Each group will turn in 1 (one) copy of the finished homework package. Each homework package should consist of 1 (one) Summary Form and 1 set of answers.

3. Each student must complete the Peer Evaluation Form for each other group member. The peer evaluation forms will become available on Friday March 21.

4. All submissions (answer package and peer evaluation forms) must be in MS Word format and submitted through D2L (look under the Dropbox or the Exam1 folder). Submitted files that cannot be opened will not be graded.

5. You are allowed to use your textbook, your notes, and other resources to finish this homework. You may not, however, ask for help from other instructors or professors. Cited work must be properly accredited.

6. Each group must meet at least once. Further communications can be arranged through emails or phone.

7. Each member of the group should review the answers before submission.

Grading:

This exam package is worth 75 points of total course points. The credit for this package will be distributed as follows:

Summary Sheet and Answers	72 points
Peer Evaluation Sheets	3 points
Total	75 points

Summary Sheet

Member Roster: _____

_____

_____

_____

_____

No. of meetings: _____

Member Responsible for submitting the finished package: _____

Did all members review the answers? Yes No

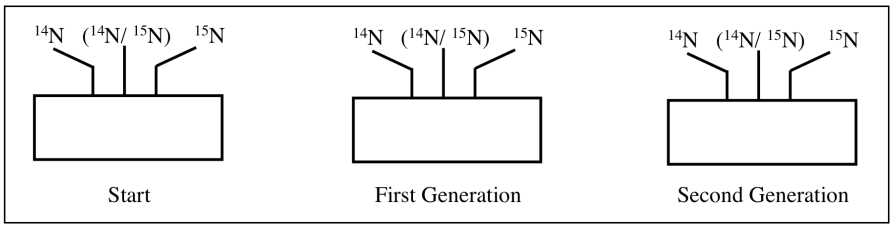
Question 1

The year is 2055 and you are now a research director at NASA's Astrobiology Unit (astrobiology is the study of biological organisms from outside of planet Earth). A mission to capture the water held under the frozen crust of Europa returns with great success. To everyone's excitement, a microscopic organism was identified in the water sample, and it is given the name *Europus novelus*. Preliminary studies have determined that this organism uses nucleic acids for transmission of heritable traits. Now it is up to your unit to find out more about this organism. Remarkably, *E. novelus* can be cultured and manipulated in the same way that cold-loving bacteria on Earth can be manipulated, so your team can grow and analyze this organism with the usual tools.

(A) You decide that you will use the Meselson-Stahl method to determine the mode of replication in *Europus novelus*. To refresh your own memory on the subject (it's been over 40 years since your Bio3120 class), you wrote out the original experimental procedure. Describe, in your own words and succinctly, what the key steps in this experiment are. (3 pts)

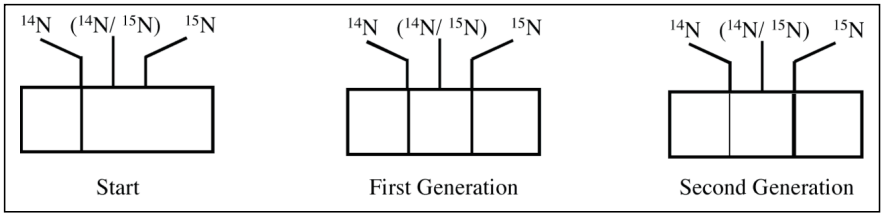
Kai Hung 3/19/14 16:18
Comment [1]: Parts A and B are meant to prime the students to think about the set of experiments that are needed to answer the question.

(B) After you refresh your memory on the experiment procedure, you draw out a summary of the original results from Messelson and Stahl. Depict what you would have drawn in the following diagram (2 pts):



(C) You proceed to ask one of your staff scientist to perform the Messelson-Stahl experiment on *Europus novelus*. The results are shown below:

Kai Hung 3/19/14 16:19
Comment [2]: This data deviate from what were presented in class. To answer this question, the students must be able to apply the principles of the published experiment in a new context.



As you look at your data, you realize that the scientist doing the experiment has made a mistake in following Messelson-Stahl's procedure. Explain what mistake that is and your evidence for making that conclusion (2 pts).

(D) Fortunately, despite the mistake described in part (C), the data can still be interpreted. Explain how you would adjust for the error described in (C). Based on this data, what mode of DNA replication does *Europus novelus* carry out? Justify your conclusion. (3 pts)

(E) With the help from a team of organic chemists and some biochemists, your unit determined that *E. novelus* does not use the same 4 bases as Earth organisms do. Instead of the G, C, A, and T bases, *E. novelus* appears to use I, G, C, A, and T. The inosine base is derived from adenine, but unlike adenine, it can form base pairs with C, A, or U. To determine how the inosine base behaves in the DNA of *E. novelus*, you decide to carry out a series of experiment similar to the ones done by Chargraff and colleagues. Experiments 1 to 4 are done in identical manners, except that they are done on 4 different days. You enter your data into the following table. Finish filling out the table. (2 pts)

	Bases					**Summary**		
Experiment	Inosine	Guanine	Adenine	Thymine	Cytosine	Purine	Pyrimidine	Total
1	2.1%	30.0%	19.8%	17.8%	30.1%			
2	2.4%	30.0%	20.1%	17.9%	30.2%			
3	2.1%	30.2%	20.1%	17.8%	30.2%			
4	1.8 %	28.9%	20.0%	18.2%	30.1%			

(F) Compare and contrast your data for *E. novelus* with the established Chargaff's rules for Earth organisms. (3 pts)

(G) Upon reviewing the data, you realize that there is a problem when it comes to understanding how *E. novelus* can manage to replicate their DNA faithfully from one generation to the next. Explain what the problem is. (3 pts)

(H) Design a <u>new</u> experiment to confirm that the problem you have identified in part (G) does indeed exist in *Europus novelus*. Provide a prediction on what the possible outcomes are, and how you can interpret these outcomes to help you confirm (or refute) this suspicion. (6 pts)

Kai Hung 3/19/14 16:20
Comment [3]: In actual organisms, the base inosine is not used at all. However, inosine is a real chemical compound. To answer this question, students must apply their knowledge about the Chargraff experiment in how and why bases interact with each other.

Kai Hung 3/19/14 16:21
Comment [4]: This prompts the students to identify the areas where the presence of this chemical compound might cause problems for living organisms. To answer this part, students must apply what they know of how normal organisms handle transmission of information.

Kai Hung 3/19/14 16:22
Comment [5]: This part builds on the answer from (G) and asks the students to formulate a new experiment to test their speculations.

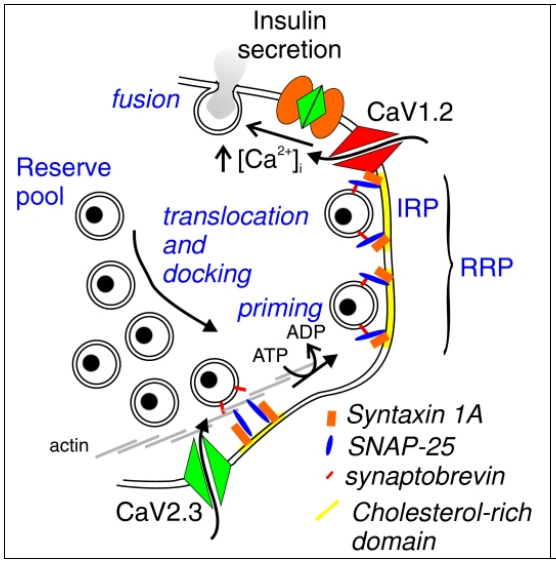
Question 3

Insulin is a key regulator of sugar metabolism in mammalian cells. In the specialized cells of the pancreas, insulin is synthesized and then, upon receiving the correct signal, released into the bloodstream. Insulin stimulates the uptake of glucose from the bloodstream and suppresses utilization of fat as energy source.

Kai Hung 3/19/14 17:18
Comment [6]: In class, we have not talked about the actions of insulin at all. This sets a context for the students so that they have be prepared to analyze the question critically.

The production of insulin has to undergo several modification steps. First, the gene is transcribed into an mRNA. The mRNA is then translated into a precursor form of insulin called the preproinsulin. After cleavage of a signal peptide from preproinsulin, the proinsulin is formed. Inside specialized vesicles in the pancreas cells, proinsulin is then further modified by other proteins into its final functional form, ready to be released through exocytosis.

The following diagram presents a summary view of the arrangement of insulin-containing vesicles in the pancreas. This diagram highlights the cellular components that are involved in the secretion of insulin.



CaV1.2 and CaV2.3 are both calcium channels. Syntaxin 1A, SNAP-25, and Synaptobrevin are all involved in the SNARE complex, mediating vesicular docking and targeting. You can ignore IRP and RRP.

Picture credit: Eliasson *et al.* (2008).

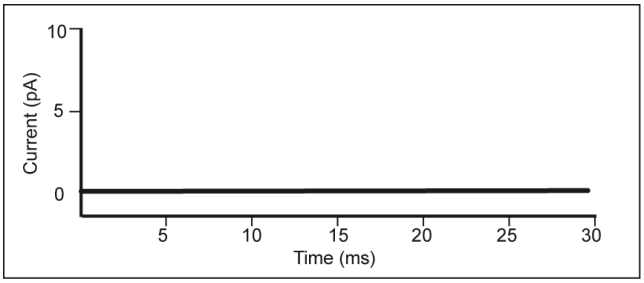
(A) Suppose there is a tissue culture cell line of these specialized pancreatic cells, which means that you can have access to these cells grown on a Petri dish. To study the activity of the calcium channels, you decided to do a Patch-Clamp experiment. Describe what a Patch-Clamp experiment is and the key steps in setting up a Patch-Clamp. (3 pts)

Kai Hung 3/19/14 17:19
Comment [7]: This part primes their thinking in the right direction.

Suppose that CaV1.2 is activated by cAMP and that CaV2.3 is activated by voltage (note: this is a thought exercise and CaV1.2 and CaV2.3 may or may not work this way in real life. The use of these two examples is to illustrate a biological principle in the context of this exam). You are

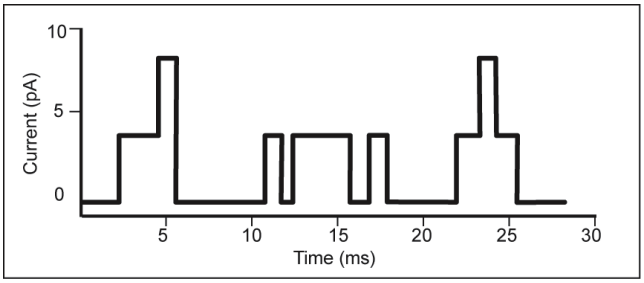
investigating the properties of these two channels using Patch-Clamp in collaboration with a pharmaceutical company that has discovered compound X, which disables CaV2.3 but not CaV1.2. In live mice injected with X, the mice tend to become obese and develop diabetes. The following diagrams represent the results you get from the Patch-Clamp experiments using the tissue culture cell lines.

(I)



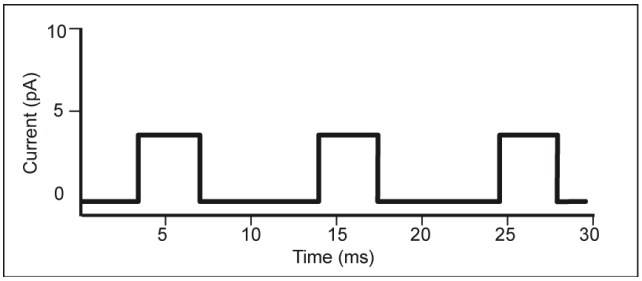
Condition
No cAMP.
No Voltage.
X added.
Ca^{2+} added.
K^+ added.

(II)



Condition
cAMP added.
Voltage applied.
No X.
Ca^{2+} added.
No K^+ added.

(III)



Condition
cAMP added.
Voltage applied.
X is added.
Ca^{2+} added.
No K^+ added.

(B) Based on the data in the experiments (I), (II), and (III), describe the behavior and characteristics (what cue do they respond to? How are their properties similar and different from each other?) of CaV1.2 and CaV2.3 under these testing conditions. Explain your reasoning. (8 pts)

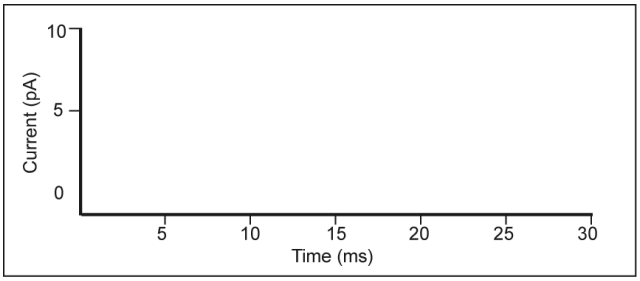
(C) In the following blank graph, draw out your prediction on what the data might look like if you run the experiment with cAMP added, voltage applied, no X, no Ca^{2+} added, and with K^+ added. Provide an explanation for your prediction with specific attention to

Kai Hung 3/19/14 17:21

Comment [8]: This part tests their ability to analyze critically the information given in the question and to apply what they know about Patch-Clamp experiments to interpret data sets that they have not encountered before. In class, we have covered what the graphs indicate with their axes and what the shapes of the curves mean in terms of cellular functions, but the students have not tackled "unknown" data sets in that format before.

which of the channels' properties might be relevant to the explanation. (4 pts) *(If you have a hard time drawing in MS Word, you can try drawing on a print-out, then scanning the image or taking a picture of it, crop it, and insert that as your answer.)*

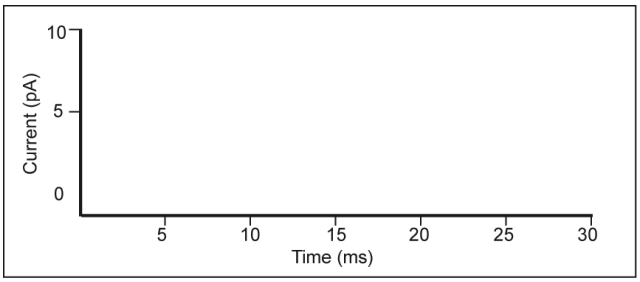
(IV)



Condition
cAMP added.
Voltage applied.
No X.
No Ca^{2+}.
K^+ added.

(D) In the following blank graph, draw out your prediction on what the data might look like if you run the experiment with no cAMP added, voltage applied, no X, Ca^{2+} added, and with K^+ added. Provide an explanation for your prediction. (4 pts) *(If you have a hard time drawing in MS Word, you can try drawing on a print-out, then scanning the image or taking a picture of it, crop it, and insert that as your answer.)*

(V)



Condition
No cAMP.
Voltage applied.
No X.
Ca^{2+} added.
K^+ added.

(E) Synaptobrevin (see diagram) is a member of the V-SNARE family and SNAP-25 is a member of the t-SNARE family. Together, in the context of insulin secretion, synaptobrevin and SNAP-25 help direct vesicles containing the mature form of insulin to the right location to be ready for fusion and subsequent release. Suppose there is a mutation in synaptobrevin that changes its property so that it no longer docks with SNAP-25. Instead, the mutated synaptobrevin now interacts with the t-SNARE on the surface of proteasomes, a type of organelle that degrades proteins. Will this mutation have a phenotype similar to the effects observed in live mice injected with Compound X? Why or why not? Explain your answer and be sure to include your reasoning. (5 pts)

Kai Hung 3/19/14 17:23

Comment [9]: This part actually requires the students to bring in 3 different concepts that they have encountered in 3 different contexts to synthesize the correct response. The course material had covered the functions of SNAPs/SNAREs in the context of cellular transport, the role of discrete regions within a protein for functions, and the nature of mutations, but not at once in the same context.