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Student ID_____

Midterm 3

Biology 2020

Spring 2004

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1. (2 pts) The process by which a cell engulfs a large particle is called __phagocytosis___, whereas recycling of degenerating organelles is done by a process called __autophagy_.

2. (5 pts) What two organelles are involved in glycosylating proteins? What is the final destination of most glycosylated proteins? State one function of a glycosylated protein at its final destination.

er and golgi

plasma membrane / secreation

protection, signaling

3. (5 pts) State two important differences between an early endosome and a lysosome.

ph

degradation

4. (5 pts) What type of proteins are responsible for targeting vesicles for transcytosis rather than recycling? Briefly explain their functions.

snares

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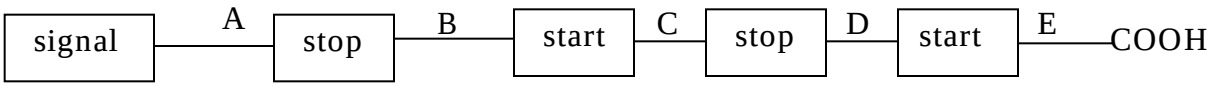
(2 pts) What is the final destination a protein with no signal, targeting or sorting sequences?

cytoplasm

6. (6 pts) **Membrane proteins**
You have cloned a gene with an N-terminal signal sequence directing the protein to the ER. This sequence is followed by a stop transfer sequence, a start transfer sequence, another stop transfer sequence and finally another start. Between these sequences are domains A, B, C, D and E as shown below. Draw a cartoon that depicts the final orientation of this protein in the ER **after the N terminal signal sequence is cleaved**. Be sure to include domains A, B, C, D, and E. Use the drawing of the ER membrane shown below.



see biology bldg

cytoplasm

ER lumen

7. (9 pts) **Signal sequences.** Match items in the right column with the appropriate organelle in the left column.

SOME LETTERS MAY BE USED MORE THAN ONCE, OTHERES NOT AT ALL AND SOME BLANKS MAY HAVE MORE THAN ONE LETTER.

__ea__ Mitochondria

_____ ER

__ed__ Chloroplast

___ea_ Nucleus

A. Amphipathic alpha helix
B. Clathrin
C. Basic sequence of lysine and arginine
D. alpha helix with hydrophobic core
E. Insertion into organelle is post-translational
F. Insertion into organelle is co-translational

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8. (4 pts) Which one or more of the following types of energy are directly required for protein import into mitochondria? Circle the correct answer.

I) Electro Chemical Gradient
II) High Energy Covalent Bonds
III) Reducing Power

A) I only

B) II only

C) I and II

D) I and III

E) I, II, and III

9. (9 pts) One day you happen to be browsing through the human genome sequence and come across a gene, previously unknown to you.

replication
A. You want to amplify the gene so that you can clone it, and you know the complete gene sequence so you use PCR. What normal cellular process, which is part of the 'central dogma', is this procedure based upon?

What equation describes the extent of amplification of the sequence of interest?

x^n

B. In order to clone your gene, you insert it into a plasmid DNA and then digest that plasmid DNA with BamH1 and EcoR1. This yields three fragments as pictured on next page (the largest fragment is the whole plasmid). You separate them by gel electrophoresis.

On the diagram on the next page draw in the location of the fragments on the gel following electrophoresis and label the fragments by size. Fill in + and – in the appropriate boxes beneath 'charge' to indicate the orientation of the electric field.

see biology bldg.

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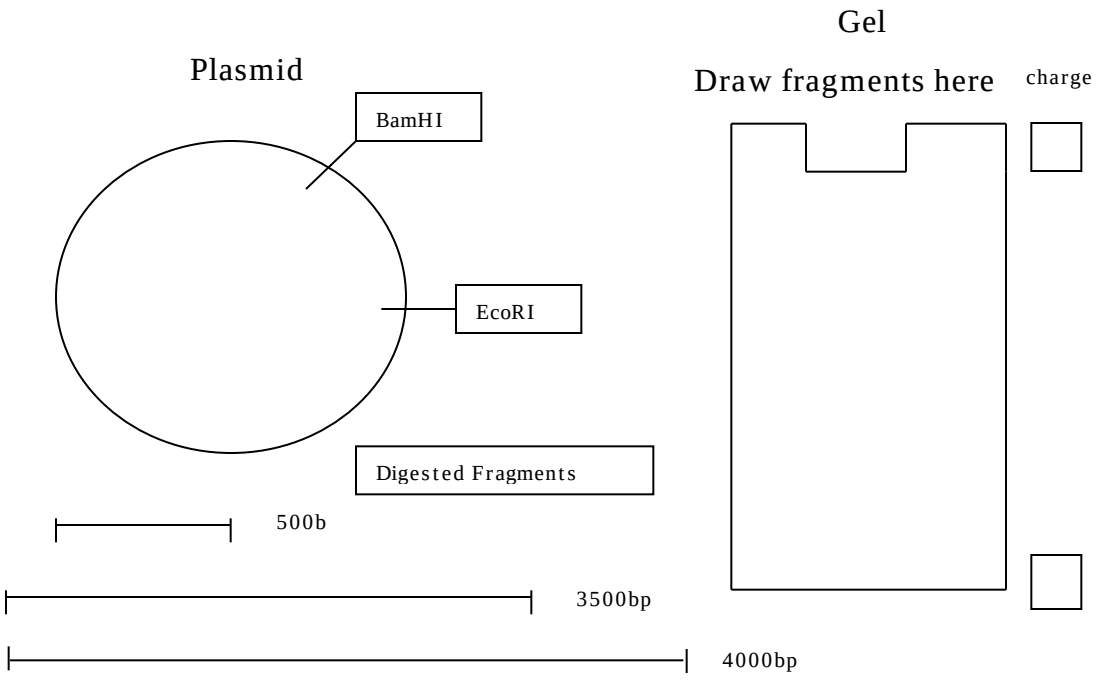

10. (8 pts) Patients with the genetic disease hypercholesterolemia have mutations in the gene coding for the LDL receptor. Describe what happens in a person in which the C terminal end of the protein is deleted, but the protein still inserts into the plasma membrane. Be sure to include a discussion of where the receptor is found, whether it binds LDL, whether the receptor cycles, whether it is loaded into coated pits.

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11. (9 pts) GMO Crops
Transgenic cotton plants containing the BT toxin gene are in wide use today. State ONE ADVANTAGE and ONE DISADVANTAGE of planting this genetically engineered crop.

decreases pesticides	cross pollination
	harmful to other insect population
increase havest yield	natural selection

The other major genetic modification of food crops is herbicide tolerance. Roundup is a trade name for the chemical __glyphosate__ which inhibits the synthesis of ___aromatic amino acids_____ by the shikimate pathway. To make Roundup Ready crops, a gene coding for EPSPS from __agrobacterium that does not bind Roundup is cloned into plant cells on a plasmid where it inserts into the genome. To confirm the presence of the plasmid, the cells are grown in the presence of _antiobiotic_____. The surviving cells are then regenerated into plants, let flower and seeds collected for marketing.

For my information only, do you think GMO crops should be regulated to a greater degree than they are currently? YES or NO
anything

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12. (10 pts) To study the intracellular pathways of protein Y, yeast cultures were grown in liquid media and harvested 10, 20, 30, and 40 minutes after expression of the tagged proteins was turned "On". The numbers in the table represent the percentage of the tagged proteins found in organelles at the time stated at the top of the column.

In initial experiments the scientists thought that protein Y was targeted for secretion, but later discovered that they had been working with a gene sequence carrying a deletion. When they reexamined localization of the entire protein, they obtained the following data:

Localization				
Harvest Time	10 Min	20 Min	30 Min	40 Min
Protein	Y	Y	Y	Y
Fraction				
Mitochondria	0	0	0	0
Rough ER	100	75	20	70
Golgi	0	25	80	30
Plasma membrane	0	0	0	0
Ribosomes	0	0	0	0
Cytosol	0	0	0	0
Nucleus	0	0	0	0
Secreted into Media	0	0	0	0

A. What sequence of protein Y was left out during the original cloning (be very specific). Explain your reasoning.

KDEL or KKXX — The difference between the original espts and the second indicate that a KDEL sequence was deleted from the protein.

B. Where in protein Y is this sequence located? Be specific.

c-terminus

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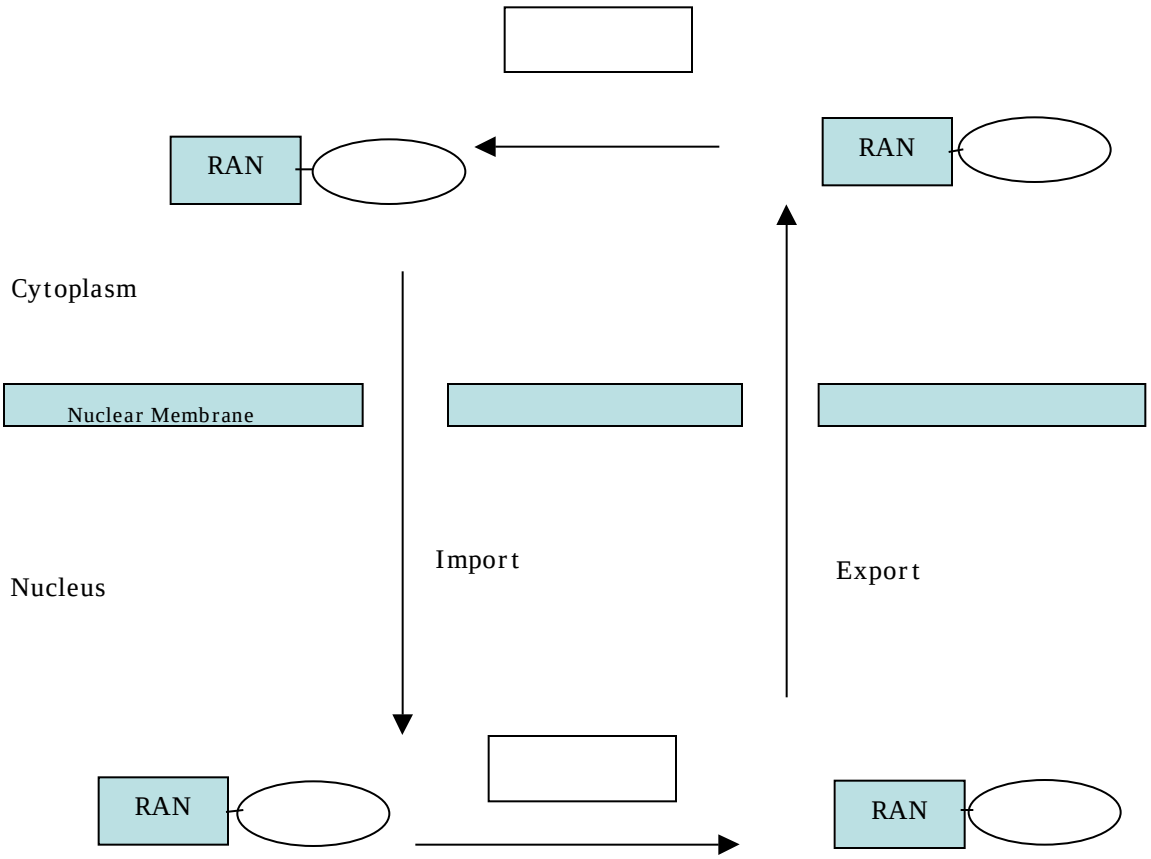
13. (8 pts) Nuclear import

A. Fill in the following diagram with nucleotides in the ovals and names of proteins in the rectangles.
see biology bldg.



Onto this drawing add two additional proteins, **importin** and a **cargo** molecule. Be sure to indicate any important targeting sequences. Show how importin cycles in and out of the nucleus by binding to other molecules. Show how the cargo molecule is specifically brought into the nucleus and what proteins it binds to during its voyage. Choose any shape you wish for importin and cargo.

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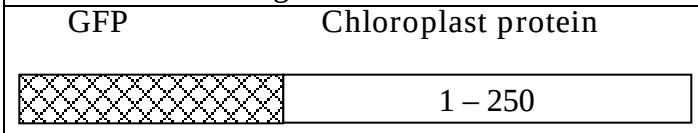
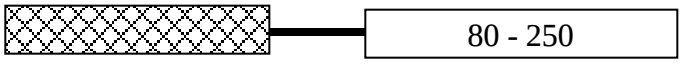
14. (10 pts) Chloroplast import

Your are studying targeting of a 250 amino acid chloroplast protein using protein fusions between this chloroplast protein and GFP shown below. The chloroplast protein is shown by a white box, numbers within the white box indicate which amino acids from this chloroplast protein are included in that protein fusion. GFP is shown by a shaded box and the black line indicates that a sequence in the protein is absent. The three columns to the right indicate the presence of the fusion protein in either the cytoplasm, chloroplast stroma or thylakoid lumen (+++ indicates presence, and --- indicates absence).

Protein fusion diagram	GFP in cytoplasm	GFP in lumen	GFP in stroma
	---	+++	---
	---	---	+++
	---	---	+++
	---	---	+++
	+++	---	---
	+++	---	---

A. Using the data in this table, identify where **the chloroplast targeting sequence for the stroma and the targeting sequence for the lumen are located in the 250 amino acid sequence. Explain your reasoning.**

Lumen= 1-80 import into lumen is dependent upon entering the stroma

Stroma=220-250 this is the minimal domain that allws the fragment to be imported

B. Do these experiments show that the sequences are **necessary** for import, **sufficient** for import, neither necessary nor sufficient, or both necessary and sufficient? WHY?

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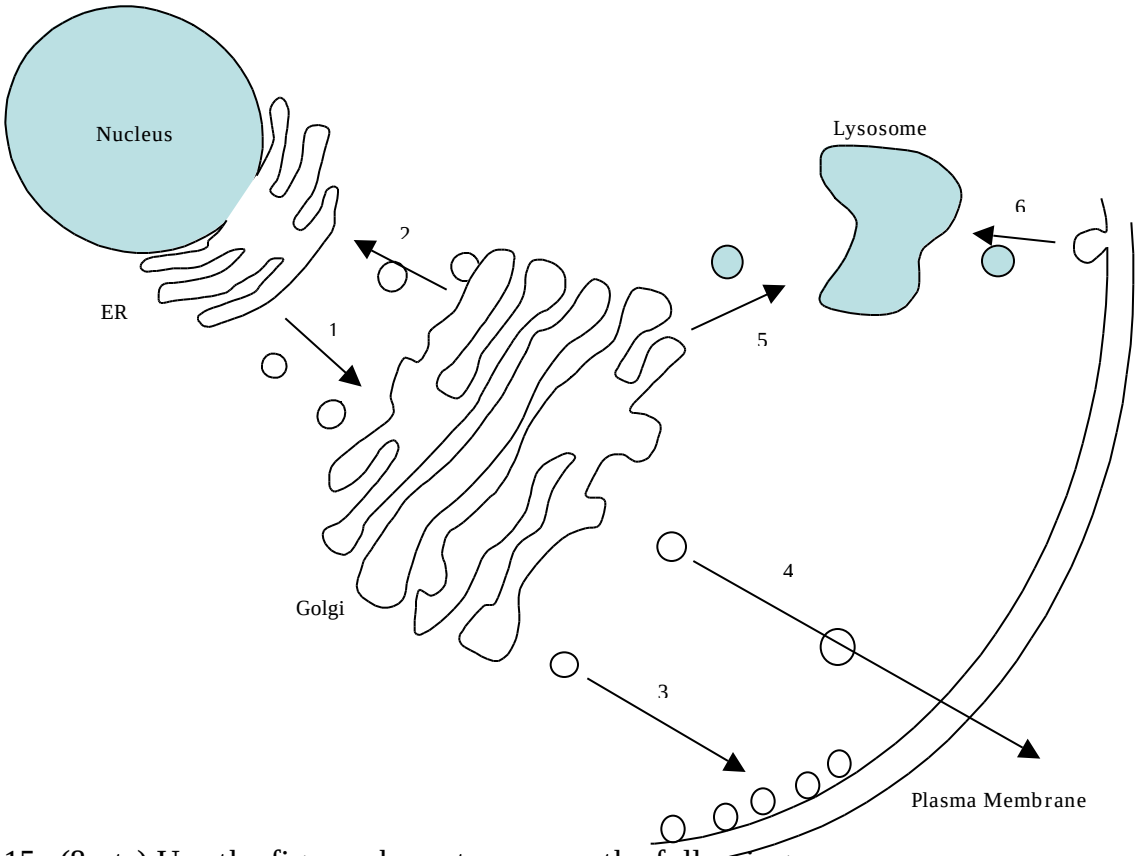

15. (8 pts) Use the figure above to answer the following.

1. Name secretory pathway 3. __regulated_____
2. Name secretory pathway 4. __constitutive_____
3. Name the GTPase involved in vesicle coating in pathway 1.

   _____sar1_____
4. Name the GTPase involved in vesicle formation in pathway 6. ___dynamin____
5. Name the protein that recruits cargo in pathway 1. ___copII____
6. What sequence does COPI recognize and bind in pathway 2? ____KKXX_____
7. In which pathway is fusion with the target membrane delayed until a signal is received? ____3_____
8. What is the address label for proteins targeted to pathway 5? _m-6-p_____

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9. SCORES

1._____

2. _____

3. _____

4. _____

5. _____

6. _____

7. _____

8. _____

9. _____

10._____

11._____

12._____

13._____

14._____

15._____

TOTAL_____