7.06 Cell Biology
EXAM #3 KEY
May 2, 2006

This is an OPEN BOOK exam, and you are allowed access to books, a calculator, and notes
BUT NOT computers or any other types of electronic devices.

Please write your answers in PEN (not pencil) to the questions in the space allotted.

Please write only on the FRONT SIDE of each sheet, as we will Xerox all of the exams and thus only grade writing on the front.

And be sure to put your NAME ON EACH PAGE in case they become separated!
There are SEVEN pages including this cover sheet.

Question 1. 24 pts _______
Question 2. 15 pts _______
Question 3. 27 pts _______
Question 4. 19 pts _______
Question 5. 15 pts _______

TOTAL 100 pts _______
Question 1. (24 pts.)
For each of the following engineered proteins, predict which location in the cell they would be targeted to. Assume that each targeting sequence is at the site along the protein (N terminus, C terminus, internal) where it would normally be found. Also assume that the only targeting sequences possessed by each protein are those listed.

Choose from the choices: the cytoplasm, the nucleus, the nuclear membrane, the peroxisome, the peroxisomal membrane, the outer mitochondrial membrane, the intermembrane space of the mitochondria, the inner mitochondrial membrane, the mitochondrial matrix, the lumen of the ER, or the membrane of the ER.

(a, 4 pts) an NES and a mitochondrial matrix targeting sequence

The mitochondrial matrix. The NES would have no effect on the protein’s localization, because a protein cannot be targeted for export from the nucleus if it is never targeted to the nucleus in the first place. Thus an NES is meaningless without an NLS.

(b, 4 pts) a cleavable signal sequence and a stop transfer sequence

The membrane of the ER. The signal sequence at the N terminus would be recognized by SRP. SRP would bind the sequence, halt translation, and target the protein to the ER. Part of the way through translation, the ribosome would synthesize the stop transfer sequence. This sequence would diffuse out of the translocon and imbed in the ER membrane. The C terminus of the protein would then be made and remain in the cytosol.

(c, 4 pts) a mitochondrial matrix targeting sequence and a stop transfer sequence

The inner mitochondrial membrane. The mitochondrial matrix targeting sequence at the N terminus would be recognized by the import receptor, which would target the protein to the matrix. Part of the way through translocation, the stop transfer sequence would start to go through the translocon. This sequence would diffuse out of the translocon and imbed in the inner mitochondrial membrane. The rest of the protein would remain in the intermembrane space. This could not be an outer mitochondrial membrane protein, because those proteins require a special outer membrane localization sequence such that the protein does not go all the way through the translocon to the matrix.

(d, 4 pts) a mitochondrial matrix targeting sequence and a sequence for phosphorylation of mannose residues on the 6 carbon atom
The mitochondrial matrix. A mannose-6-P residue is a sugar that allows proteins to be targeted to the lysosome from the trans Golgi. However, proteins can never get to the trans Golgi unless they have a signal sequence. This protein does not have a signal sequence, so it would be synthesized in the cytoplasm. The MTS sequence would then bind to the import receptor and direct the protein to the mitochondrial matrix.

(e, 4 pts) a PTS1 targeting sequence and an internal signal anchor sequence

The membrane of the ER. The PTS1 sequence is normally found on the C terminus. This sequence would never have a chance to find Pex5 so that it is targeted to the peroxisome. This is because the protein is synthesized by the ribosome in the N-to-C direction. Thus the ribosome would reach the internal signal anchor sequence first. SRP would bind to this sequence, halt translation, and target the protein to the ER. The rest of the protein (including the C terminus) would be made as it was being translocated into the ER.

(f, 4 pts) a KDEL sequence and an NLS sequence

The nucleus. A KDEL sequence is a sequence that allows proteins to be returned to the ER from the cis Golgi. However, proteins can never get to the ER or Golgi unless they have a signal sequence. This protein does not have a signal sequence, so it would be synthesized in the cytoplasm. The NLS sequence would then bind to importin and direct the protein to the nucleus.

Question 2. (15 pts.)
You are interested in identifying a ligand-gated channel involved in the nematode C. elegans’ ability to sense a compound called compound X. If you place compound X on the center of a petri plate on which worms are crawling, they move away from the center of the plate. You isolate a mutant that does not crawl away from the center of the plate, and identify the gene mutated in that strain. You name the gene ROX. For each finding you make that is stated below, outline an experiment you could have conducted that would enable you to draw the conclusion. Include a description of the specific result that would be obtained from the experiment you outlined in order to make the conclusion.

(a, 5 pts) ROX is a ligand-gated Na+ channel that is gated by compound X.

You would do patch clamping in one of two ways. You could patch clamp neurons from a wild-type worm and from a ROX mutant worm, to show that the activity you are seeing in the patch clamping assay comes specifically from ROX. Alternatively, you can patch clamp onto frog oocytes that are expressing only the ROX channel. Then you know automatically that the activity you see is due to ROX, because frog
oocytes do not have their own channels, so ROX would be the only channel on the membrane.

To determine that ROX is a Na+ channel, you need to make sure that the only ion in your patch electrode (which is filled with solution) is Na+. To see that the channel is gated by compound X, you need to either add or not add compound X to the solution in the patch electrode.

The result you see is that, in the absence of compound X, NO current would flow and you would get a reading from your patch clamping experiment that was a flat line. In the presence of compound X, you would see opening and closing of the channels. Given that ROX is an Na+ channel, it leads to excitation of the sensory neuron, and the sensory neuron should be sending action potentials when X is present but not when X is absent. Thus the channel should be open in the presence of X and closed in its absence.

(b, 5 pts) ROX protein is expressed in only one specific pair of sensory neurons ("Neurons AL and AR") in the head.

To look at expression in a whole worm, you must use light microscopy. You can either make a translational ROX-GFP fusion and look at expression in live worms, or you can fix worms and do immunofluorescence on them. The result you should get is that only the AL and AR neurons stain for ROX.

It is not at all plausible to use immuno-gold EM staining, because you have to consider that you are looking at a whole organism (which is 1mm long) to see that staining is only in 2 cells out of 1000. To show that ROX is only expressed in 2 out of 1000 cells in an organism, you would have to examine 1000s of sections of the worm. You must always remember the difference in resolution of light and electron microscopy when choosing one or the other for an experiment. You cannot use EM to look at an organism that is macroscopic. By using light microscopy, it would be immediately obvious by a single image that the protein is only expressed in the head in exactly two places.

(c, 5 pts) Neurons AL and AR are responsible for the ability of the worm to sense compound X.

In this experiment, you need to first somehow destroy the AL and AR neurons, and then see if the worm still crawls away from the center of a plate containing compound X in the middle of the plate. The way you can destroy specific neurons is either to laser ablate them (shine a laser at that specific neuron) or express a protein toxin in the worm under a promoter that is specific to the AL and AR neurons. The result you should see is, when AL and AR neurons are destroyed, the worm does not avoid the center of the plate.
Question 3. (27 pts.)
You are studying signal peptidase in yeast. In yeast, a single gene encodes signal peptidase, and its gene product is a soluble protein localized to the lumen of the ER.

(a, 4 pts) Would you expect that signal peptidase itself is synthesized with a signal sequence? If yes, which enzyme would remove the signal sequence from signal peptidase?

Yes, by signal peptidase already present in the ER lumen. All cells come from other cells, so when a eukaryotic cell is born, it already has ER that came from the mother cell. This ER already has old signal peptidase, so when new signal peptidase is synthesized and targeted to the ER, its signal sequence is cleaved off by old signal peptidase.

(b, 4 pts) How could you mutate the gene encoding signal peptidase such that full-length signal peptidase would now reside in the cytoplasm?

You could replace the signal sequence of signal peptidase with a sequence that does not target it to the ER (for instance, by mutating some of the hydrophobic residues to alanine).

(c, 4 pts) Suppose this mutant signal peptidase from part (b) had normal enzymatic activity. What (if any) phenotype would this strain of yeast have?

The phenotype would be lethal/dead. There is no signal peptidase in the ER, where it needs to be found. Thus no proteins that make it to the ER will ever have their signal sequences removed. Thus all of the secretory proteins will have extra amino acids on their N termini, which can impede with folding. However there is also signal peptidase in the cytoplasm which is active. Thus other secretory pathway proteins may never even get to the ER to begin with, because the signal peptidase active in the cytoplasm might cleave off the signal sequences of these proteins in the cytoplasm, preventing SRP from ever binding to these proteins and targeting them to the ER.

(d, 4 pts) Suppose this mutant signal peptidase from part (b) did NOT have normal enzymatic activity. Explain why you think the mutant signal peptidase was not active.

There are two main reasons. First, signal peptidase will be full length, because you have mutated its signal sequence such that it is in the cytoplasm, where there is no active signal peptidase, so the N-terminal sequence that is usually cut off from signal peptidase cannot be cut off. This means that there will be extra amino acids on the N terminus of signal peptidase, which may interfere with its folding. Second, the cytoplasm is a very different environment than the ER. Thus signal peptidase will
probably not be active because it cannot be glycosylated or have disulfide bonds, so it most likely will not fold properly.

(e, 3 pts) Name a gene you could conditionally inactivate such that full-length wild-type signal peptidase would now reside in the cytoplasm at the non-permissive temperature.

SRP or Sec61. To get full-length signal peptidase in the cytoplasm, you need to mutate one of the genes required to target a protein to the secretory pathway. If the protein can never be targeted to the secretory pathway, it will made in the cytoplasm.

(f, 4 pts) How would you generate a yeast strain in which signal peptidase was localized both to the ER lumen and to the matrix space of the mitochondrion?

To have signal peptidase protein in both locations, you need to have two copies of the gene encoding signal peptidase. (You cannot have one copy of the gene that includes both an MTS and a signal sequence, because then the protein would be torn between two targeting pathways and not know where to go.) You can make a yeast with two copies of the signal peptidase gene in two ways. One is to transform a haploid yeast with a plasmid containing one gene, making it diploid only for that gene. The other way is to mate two haploids together to get a diploid. For the plasmid method, you can simply transform wild-type yeast with a plasmid containing signal peptidase whose signal sequence has been replaced with a mitochondrial matrix targeting sequence. The protein made from the chromosomal copy of the gene would go to the ER, and the protein made from the plasmid copy of the gene would go to the mitochondrial matrix. The other way is to mate a wild-type haploid yeast to a haploid yeast whose signal peptidase gene has been mutated such that the signal sequence has been replaced with a mitochondrial matrix targeting sequence.

(g, 4 pts) Suppose signal peptidase in both locations in the strain from part (f) had normal enzymatic activity. What (if any) phenotype would this strain of yeast have?

The phenotype would be wild-type. There is signal peptidase in the ER, where it needs to be found. However there is also signal peptidase in the mitochondrial matrix. However this signal peptidase:
-- will probably not be active because it cannot be glycosylated or have disulfide bonds, so it most likely will not fold properly
-- will not affect any proteins because the sequence it recognizes and cleaves is a signal sequence, and no proteins in the mitochondrial matrix contain signal sequences.
Question 4.  (19 pts.)
Below are true statements about the process of importing proteins into the peroxisome. For each statement, outline an experiment that could be used to come to that conclusion. Include an illustration of the specific result (that depicts pictorially the actual data you would obtain from the experiment outlined) that would lead you to make each conclusion.

(a, 4 pts) A PTS1 sequence is sufficient for targeting a protein to the peroxisome.

You take a protein that is normally localized to the cytoplasm, and fuse it to a peroxisomal targeting sequence like PTS1. You can then proceed with an experiment in cells or an in vitro experiment.

The experiment in cells is as follows. You see that cells expressing this protein have the protein in the peroxisomes. You could do this by immunogold EM microscopy using gold-conjugated antibodies that react with your targeted protein. You could also do this with fluorescence microscopy, if you do immunofluorescence using an antibody (conjugated to a fluorescent compound) that reacts with your protein. The way you know the protein is really in peroxisomes is by doing co-localization studies with a known peroxisomal protein. If you do not do co-localization studies, it would be impossible to say whether your protein was actually in peroxisomes or not, because at the resolution of a light microscope, peroxisomes just look like little dots scattered around the cytoplasm.

The in vitro protease protection assay is as follows. You place your engineered protein, peroxisomes, and cytosolic extract into a test tube. You then wait for translocation to occur, and assay whether it has occurred or not using a protease protection assay. Your result would be that you do get translocation, so your protein is protected from protease. You would add protease, and then run the sample on an SDS-PAGE gel and then do a Western blot using an antibody against your protein. You would see a band in the lane, because your protein was protected. If your protein had not been translocated, you would have seen no band, because your protein was outside of the peroxisomes and thus not protected from protease and thus degraded.

(b, 5 pts) Proteins are imported into peroxisomes post-translationally.

For this problem, you must add together in a test tube: an already synthesized protein that is targeted to peroxisomes, peroxisomes, and cytosolic extract. You then wait for translocation to occur, and assay whether it has occurred or not using a protease protection assay. (See the description of the assay from part (a) and the possible results above.) Your result would be that you do get translocation, so your protein is protected from protease, so you would see a band on the gel. You cannot
do this experiment in cells, so localization experiments using microscopy are not really feasible options. You CANNOT add the mRNA encoding your protein, everything necessary for translation, peroxisomes, and cytosolic extract and then do this assay. That experiment would NOT distinguish between co-translational translocation and post-translational translocation.

(c, 5 pts) Proteins are imported into peroxisomes after they have been folded.

In order to show that a folded protein can be translocated into the peroxisomes, you need to ensure that your peroxisomal-targeted protein is really folded. We know that DHFR must be folded whenever methotrexate is present. Thus you can express a version of DHFR fused to a PTS1 sequence. You do the experiment plus and minus methotrexate, and you should get the same result – that the protein is translocated either way.

You could do this experiment in cells (in which case you would visualize uptake by microscopy as outlined in part (a)) or you could do this experiment using an in vitro peroxisome uptake assay (in which case you would visualize uptake by a protease digestion assay as outlined in part (a), and you would see a band on the gel).

(d, 5 pts) The receptor Pex14 and the Pex2/10/12 translocon proteins are the only proteins that need to be present in the peroxisome to allow for translocation of a peroxisomal protein into the lumen.

In order to show that these 4 proteins are the ONLY proteins that need to be present to get translocation, you need to create a membrane-bound compartment that only has these 4 proteins in it. Thus you can incorporate purified Pex14, Pex2, Pex10 and Pex12 proteins into liposomes. You can then place your liposomes, a protein that is targeted to peroxisomes (like catalase), and cytosolic extract into a test tube. You then wait for translocation to occur, and assay whether it has occurred or not using a protease protection assay. (See the description of the assay from part (a) and the possible results above.) Your result would be that you do get translocation, so your protein is protected from protease, so you would see a band on the gel. You cannot do this experiment in cells, so localization experiments using microscopy are not really feasible options.

You CANNOT do this experiment with “peroxisome ghosts” because peroxisome ghosts contain many other membrane proteins too, so you could not conclude that these 4 proteins listed above are the ONLY proteins that need to be present in order to get translocation. Also, you CANNOT do this experiment with microsomes, because microsomes are little pieces of ER that contain all of the ER membrane proteins normally found there, so again, the four proteins listed above would not be the only proteins present.
Question 5. (15 pts.)
The *Drosophila* versions of many ion channels and other proteins involved in the neural system were isolated in mutant hunts for temperature sensitive mutants that did not move properly due to the inactivation of the gene product at high temperature. Screens have been done for mutants that are paralyzed at high temperature, and for mutants that shake uncontrollably at high temperature.

(a, 5 pts) Into which one of the two categories of mutants would the nicotinic Ach receptor fall and why?

A fly with a temperature sensitive mutation in the nicotinic Ach receptor would be paralyzed when transferred to high temperature. This is because muscle cells could not sense any of the acetylcholine released by the pre-synaptic neurons. (This receptor is found on muscle cells, not on neurons.) If this ligand-gated Na+ channel cannot function, then the muscle cell will never depolarize slightly so that the cell reaches threshold, which is the trigger for the voltage-gated Na+ channels to open. This means that the voltage-gated calcium channels will never open. Thus calcium would never be released into the muscle cell cytoplasm, so muscles would never contract. Note that the nicotinic Ach receptor does NOT lead to action potential formation – instead, it slightly depolarizes the muscle cell so that the voltage-gated Na+ channels open.

(b, 5 pts) Into which one of the two categories of mutants would acetylcholine esterase fall and why?

A fly with a temperature sensitive mutation in acetylcholine esterase would start to shake uncontrollably when transferred to high temperature. This is because the fly could not degrade the acetylcholine in the synaptic cleft of the neuromuscular junction if acetylcholine esterase were inactivated. Thus the muscle cells would be receiving more acetylcholine signals than they should.

(c, 5 pts) Into which one of the two categories of mutants would the voltage-gated Ca$^{2+}$ channel fall and why?

A fly with a temperature sensitive mutation in the voltage-gated calcium channels would be paralyzed when transferred to high temperature. The reasoning depends on which voltage-gated calcium channels were inactivated. If the channels that live on the plasma membrane of neurons were inactivated, then when an action potential reached the axon terminus of the neuron, no neurotransmitter-filled vesicles could fuse with the pre-synaptic cell’s membrane. Thus no neurotransmitter would be released into the synaptic cleft, and the muscle cells would receive no signals. If the channels that live on the SR membrane inside the muscle cells were inactivated, then calcium would never be released into the muscle cell cytoplasm, so muscles would never contract.