This is an open book exam, and you are allowed access to books and notes. Please write your answers to the questions in the space allotted; if you use the back of a sheet make it clear which answer is where. And be sure to put your name on each page in case they become separated! There are seven questions! Good luck!

1. **10 pts)** You have isolated two mutant haploid strains of yeast, termed mutA and mutB, that are both petite and unable to carry out oxidation of glucose to CO$_2$ and H$_2$O. To understand the nature of these mutations (and you can safely assume that both mutA and mutB have mutations only in one gene) you cross these to wild-type (haploid) yeast, and observe that both the +/mutA and +/mutB diploids are not petite and carry out glucose oxidation normally. But after these diploid cells have been growing in culture for one week you observe that none of the +/mutA diploids are petite but in the +/mutB culture 2% of the cells are petite.

   **A. 5 pts)** Explain the different properties of the mutations that gave rise to the original mutA and mutB strains. In what types of genes could these mutations have occurred?

   MutB possesses a mutation in a mitochondrial gene (gene B), whereas mutA has a mutation in a nuclear gene (gene A). Crossing mutA with a wild type strain provides a wild type copy of gene A that will remain in the diploid through subsequent mitoses. However, mitochondria segregate randomly with mitoses, so over time a few progeny will contain only mutant mitochondria (the 2% in +/mutB). Mitochondrial mutations can only be rescued by complementation if other wild type mitochondria are present.

   **B. 5 pts)** You mate the original haploid mutA and mutB strains. Would or would not the resultant diploid be petite? Explain

   The resulting diploid is wild type, non-petite. Since we know the mutations are in separate genes, each haploid provides either a wild type copy of the gene or wild type mitochondria that the other haploid is lacking. Complementation has occurred.

2. **20 pts.)** You have isolated the cDNA that encodes reelin, an important cell surface membrane protein normally expressed by a set of cerebral nerve cells. The sequence of the protein predicted from the cDNA is mostly hydrophilic, but there are two stretches of 23 amino acids that are hydrophobic enough to form a membrane-
spanning segment, but because there are two charged amino acids in the middle of both of these stretches you are not certain whether or not either or both of these actually span a membrane. To explore the membrane orientation of reelin you use the reelin cDNA in an *in vitro* transcription system to synthesize reelin mRNA, then you translate reelin mRNA into protein in a cell-free system (containing radioactive amino acids) in the absence or presence of microsomes. You then prepare samples from these translation reactions in four different ways: (1) no treatment, (2) add trypsin (a protease), (3) add trypsin and detergent, and (4) treat reaction with detergent and add endo-glycosidase H (endo H), which removes N-linked sugars that are added in the ER. You analyze these samples by SDS polyacrylamide gel electrophoresis and autoradiography; the results are indicated in the table below.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Microsomes present?</th>
<th>Treatment of the reaction products</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NO</td>
<td>no treatment</td>
<td>One radioactive protein “band” of mw 40,000</td>
</tr>
<tr>
<td>2</td>
<td>NO</td>
<td>trypsin</td>
<td>No radioactive protein</td>
</tr>
<tr>
<td>3</td>
<td>NO</td>
<td>trypsin and detergent</td>
<td>No radioactive protein</td>
</tr>
<tr>
<td>4</td>
<td>NO</td>
<td>detergent and endo H</td>
<td>One radioactive protein “band” of mw 40,000</td>
</tr>
<tr>
<td>5</td>
<td>YES</td>
<td>no treatment</td>
<td>One radioactive protein “band” of mw 45,000</td>
</tr>
<tr>
<td>6</td>
<td>YES</td>
<td>trypsin</td>
<td>Two radioactive protein “bands,” one of mw 18,000 and the other of mw 21,000</td>
</tr>
<tr>
<td>7</td>
<td>YES</td>
<td>trypsin and detergent</td>
<td>No radioactive protein</td>
</tr>
<tr>
<td>8</td>
<td>YES</td>
<td>detergent and endo H</td>
<td>One radioactive protein “band” of mw 40,000</td>
</tr>
</tbody>
</table>

You conducted one additional experiment: you removed the two radioactive protein “bands,” from the experiment in sample 6, treated them with endoH, and reran them on a gel. You found no change in the molecular weight of either protein after endoH treatment.

A, 5 pts.) Is the protein normally glycosylated? Explain why or why not.

Yes, the protein is normally glycosylated upon entry into the ER. We know this because the protein in lane 1 is smaller than the protein in lane 5. The difference in treatment between the two lanes is that the protein in lane 5 had access to microsomes (miniature ER’s). Therefore, the protein was enlarged upon entry into the ER, which means it must have been glycosylated.
PLEASE NOTE- we apologize for the confusion caused by this problem. The structure of the protein, as described, is not solvable.

B. 5 pts.) Is there a cleaved signal sequence? Explain why or why not.

NO, there is no cleaved signal sequence, because the peptide is the same size in lanes 1 and 8. Lane 1 gives us the size of the protein before it enters any microsome. Lane 8 gives us the size of the protein after entry into the microsome, minus any sugar moieties that have been added. Since the proteins in both lanes are the same size, there was no cleavage of the protein, or it would appear smaller in lane 8.

C, 10 pts.) Draw the structure of reelin in the plasma membrane. Be sure to indicate where the N- and C- termini of the protein are located, and if you think the protein is glycosylated indicate where in the protein the sugars are attached. And explain why your diagram is consistent with the experimental data stated above.

Full credit (10 pts) was given to everyone for part c, regardless of answer.

3. 15 pts) As noted in lecture, the cytosolic protein tyrosine kinase JAK2 consists of three independently folded domains – an N- terminal domain that binds to the cytosolic domain of several cytokine receptors, a kinase- like domain that has no kinase activity and in fact no known function, and a C-terminal protein tyrosine kinase domain. You make several mutants of JAK2 and overexpress them (i.e. to a level 15- times that of the normal amount of JAK2) in a line of cultured human erythroleukemia cells (tumor of erythroid progenitor cells) in which a 3- hour incubation with erythropoietin (Epo) normally causes a 6- fold increase in β-globin mRNA.

The mutants of JAK2 you have made are as follows:

a. A deletion of the C- terminal kinase domain
b. A deletion of the N- terminal domain
c. A point mutation in the C- terminal kinase domain such that the kinase cannot bind the ATP substrate.
d. A tyrosine- to phenylalanine point mutation in the C- terminal kinase domain such that the kinase itself cannot become tyrosine- phosphorylated.

You observe that cells overexpressing mutant “a” are non-responsive to Epo, in that little or no β-globin mRNA is found in the cells after Epo addition. In contrast, cells overexpressing wild- type JAK2 undergo a slightly higher than normal induction of β- globin mRNA after Epo addition.

5 pts each for b, c, and d) Predict the properties of the cells overexpressing JAK2 mutants b, c, and d. Would they undergo normal induction of β-globin mRNA after Epo addition? Why or why not?
Mutant b, with a deletion of the N-terminal domain, will not bind to the cytosolic domain of the EPO receptor. The WT JAK2 will still bind and become activated upon receptor ligation, so no normal induction of EPO should be seen.

Mutant c, which is unable to bind the ATP substrate, will bind to the receptor but will not be able to phosphorylate its substrates, and therefore is unable to create the docking sites for STAT. It will interfere with the WT JAK2 since it will bind the receptor but be unable to function. The cells will be unresponsive to EPO due to the dominant negative effect of the mutant.

Mutant d will also be unresponsive to EPO due to a dominant negative effect of the mutant. In this case, the mutant will bind the receptor but will not be phosphorylated and will not be activated.

4. 5 pts) LK3 cells express JAK2, STAT3, and STAT5, but not the receptor for growth hormone (GH). These cells do express the prolactin receptor, and you find that after addition of prolactin they respond by tyrosine phosphorylation of JAK2, STAT3 and STAT5. You find that when, using standard recombinant DNA technology, you express the growth hormone receptor (GH-R) in these cells they respond to addition of GH by tyrosine phosphorylation of JAK2 and STAT3 but not STAT5.

Why does STAT5 become tyrosine phosphorylated after addition of prolactin but not GH?

STAT5 does not bind the GH receptor. STAT5 binds via SH2 domains to phospho tyrosines in a sequence-specific manner. This sequence is present in the prolactin receptor but not in the GH receptor.

5. 15 pts) Your medical colleagues have given you cultured cell lines from three unrelated patients, called A, B, and C (in order to protect their confidentiality) who suffer from diseases similar to, if not identical to, I-cell disease; these cells synthesize all lysosomal enzymes normally, but secrete them rather than direct them to lysosomes. By cell fusion techniques you can make heterokaryons of two types of cells. [Heterokaryons contain two nuclei, one from each of the parental cell types, and the cytosol is a mixture of that from both cells; heterokaryons do not grow and divide, but survive long enough so that biochemical experiments on them can be done.] You find, quite to your astonishment, that heterokaryons of lines A and B contain lysosomes filled with normal numbers of all lysosomal enzymes, as do heterokaryons of lines A and C as well as heterokaryons of lines B and C. Suggest the nature of the defects in lines A, B, and C, and explain why all three kinds of the heterokaryons have lysosomes that contain lysosomal enzymes while none of the parental cells A, B, or C do.

Lines A, B, and C most likely contain defects in:

1. GlcNAc phosphotransferase
2. Phosphodiesterase
3. M6P receptor
Partial credit was also given for listing a defect in clathrin.

The heterokaryons have lysosomes that contain lysosomal enzymes due to complementation (all three lines have defects in different genes).

6. (20 pts) A colleague of yours is experiencing difficulty in manufacturing CPA (an anti clotting) protein in a genetically engineered version of the mouse cell line Alpha. As is the case with manufacturing other proteins, the cells synthesize a large amount of CPA, but only ~10% of this becomes secreted; the remainder is retained in the ER and eventually degraded within the cell. Thinking that the level of the ER lumenal chaperone Hsc70 might be limiting, your student generates a recombinant derivative of the genetically engineered version of the Alpha cell line - called Alpha –Hsc, in which this Hsc70 protein is overexpressed such that the amount of Hsc70 produced is increased ten-fold over the original genetically engineered cell Alpha line. Much to your disappointment, the rate of CPA secretion is unchanged. Exploring the reasons for this, you find that the Alpha –Hsc cell line now secretes most (about 90%) of the ER lumenal chaperone Hsc70 it produces!

a. 10 pts) Why is the majority of the ER Hsc70 secreted, and not retained in the ER?

a) By overproducing the ER HSC70, the KDEL receptors are saturated, such that they can now bind and retrieve to the ER only a minority of this protein. The rest is secreted.

b. 5 pts) Why is the majority of the CPA not secreted?

b) The majority of the CPA is not secreted because it can’t be folded properly.

c. 5 pts) Where in the cell is the majority of newly-synthesized CPA degraded? Explain

c) Misfolded proteins are brought to the cytosol and degraded via the proteosome

7. 15 pts) This problem concerns dynamin, a protein involved in formation of clathrin-coated vesicles.

a. 8 pts) Dynamin was originally identified as the gene mutated in a temperature-sensitive strain of flies; the Drosophila were normal at 20°C but exhibited abnormalities in function of many synapses in their nervous system at 30°C. Explain why abnormalities in dynamin would adversely affect synapse function. Dynamin is required for synaptic vesicle recycling (clathrin-mediated endocytosis).

b. 7 pts) Dynamin itself is not involved in formation of COPI or COPII vesicles. You have just characterized a new yeast temperature sensitive (ts) mutant that you think is in a homolog of dynamin used in COPII vesicles. Describe a simple experiment you could do to confirm or deny this hypothesis.
Shift to restrictive temperature, look for defects in ER to Golgi vesicle movement (probably would see COPII coated vesicles that can not pinch off from ER, similar to loss of dynamin and clathrin-coated vesicles).