

### PROBLEM SET 3

1. You have obtained immortalized liver cells from a patient who died of Wilson's disease, an inherited disorder of copper metabolism marked by neuronal degeneration and hepatic cirrhosis. The Wilson protein is a copper-transporting P-type ATPase that localizes to the trans Golgi network. People with this disease have a mutant (and thus misfolded) version of this copper transporter in the ER.

A) It is unclear if this mutant protein is non-functional or if this mutant triggers the unfolded protein response. Design an experiment that could determine this.

B) There are several chaperone proteins in the ER. You would like to know if BiP binds the Wilson protein. You find that you can isolate some Wilson protein bound to BiP when ADP is added to the cell extracts but not when ATP is added to the extract. Explain this result based on the mechanism for BiP binding substrate proteins.

C) You over express a mutant form of IRE-1 that does not bind BiP. Would this make the Wilson phenotype (i.e. the degradation of Wilson protein) more severe or less severe? Explain your reasoning.

D) You discover that if the transcription factor Hac1 is not activated, the Wilson protein will be transported to its proper location and function normally. Considering the mechanism of Hac1 activation, describe two ways you could inhibit Hac1's function.

2. You are studying the EGFR signaling pathway in a tumor cell line isolated from a melanoma. These cells have uncontrolled proliferation. They continue to grow in the absence of EGF or any other growth factor. Another group determined this was due to constitutive activation of the Ras-MAP kinases pathway.

A) You compare the levels of EGFR to wild type cells and see no difference. How would you experimentally compare EGFR levels? What could account for this difference in proliferation but not in overall receptor protein levels?

B) You hypothesize that the Raf kinase may be constitutively active. You are given purified MEK protein. How could you test the state of kinase activation in these cells?

C) You find that the Raf kinase is not constitutively active. What was necessary in the previous assay to show any Raf kinase activity?

D) You hypothesize that either Ras or MAK kinase must be constitutively active. Design experiments to prove which hypothesis is correct.

**3.** You are studying the secretory pathway in budding yeast using a combination of genetic, biochemical and cell biological techniques.

a) You are interested in performing a screen for temperature sensitive secretory mutants in budding yeast. What property of cells blocked in the secretory system will you exploit in this screen? How will you use this property to isolate mutants?

b) You isolate 5 classes of mutants in this screen. These classes are listed below, along with the location where proteins accumulate. Describe what function is defective in each class, and briefly explain your answer.

*a. Class A – Cytoplasm*

*b. Class B – Endoplasmic Reticulum*

*c. Class C – ER to Golgi Vesicles*

*d. Class D – Golgi*

*e. Class E – Secretory Vesicles*

- c) What protein might be mutated in Class A mutants? What protein might be mutated in Class B mutants?
- d) You isolate a mutant in which proteins that are normally targeted to the endosome instead accumulate in the Golgi. What protein might be defective in these mutants? What other process might also be affected in these mutants?
- e) An *in vitro* assay to reconstitute transport processes was discussed in class. Briefly describe how this assay works.
- f) You are using this system to study retrograde transport. Give an example of a protein that must be included in order to reconstitute retrograde transport.





6. What are the roles of the following in the glycolytic pathway:

- Dolichol
- Oligosaccharyl transferase
- Tunicamycin
- Calnexin/Calreticulin

b) CD62E is a transmembrane glycoprotein made by cells lining the inside of blood vessels.

- What point mutation would abolish the glycosylation sites of this protein while minimally affecting the rest of its folding properties?
- Please describe the likely fate of such a mutated version of CD62E.
- Does your answer change if these mutations are seen in a cell defective in calnexin/calreticulin? Why or why not?

c) CD62E binds to the sialyl-Lewis<sup>X</sup>, a carbohydrate expressed on neutrophils. These are white blood cells that are one of the first ones to respond to pathogen-induced inflammation. Neutrophils, which normally circulate in the bloodstream, must slow down and stop in blood vessels closest to the site of inflammation, and then squeezing out of the blood vessels altogether in order to approach and engulf the invading pathogens.

- Using this information, hypothesize as to the function of CD62E in the immune response.
- Given the localization of this protein, do you think its surface expression is constitutive or induced? Why?

d) What are some other functions of oligosaccharide modifications?