DISCUSSION SESSION ON LECTURE #4: STUDY QUESTIONS

ASSIGNED PAPER: Schwartz DC, Felberbaum R, Hochstrasser M (2007) The Ulp2 SUMO protease is required for cell division following termination of the DNA damage checkpoint. Mol. Cell Biol. 27: 6948-6961.

(1) What are the characteristic sequence and structural features that define a classical ubiquitin? In what ways is SUMO (small ubiquitin-like modifier) similar and in what ways is it different?

(2) What are the specific roles of the so-called E1, E2, and E3 in the process of ubiquitinylation? Are similar enzymes involved in the process of SUMOylation?

(3) What roles in cell biology is SUMOylation thought to play? Can proteins be both ubiquitinylated and SUMOylated? Can proteins be poly-SUMOylated? Are these modifications reversible? If so, how is ubiquitin removed from a ubiquitinylated protein? If so, how is SUMO removed from a SUMOylated protein?

(4) What is the DNA damage checkpoint? Assuming that repair is successful, how does the cell adapt to and recover from the block to the cell cycle and resume growth and proliferation? What novel insights about the events necessary to overcome the DNA damage checkpoint have been revealed by the work described in this paper?

Quantitative Question: You suspect that a protein important in your work might be regulated by ubiquitin-dependent degradation, so you want to measure the rate of its ubiquitinylation *in vitro* using an extract prepared from cells in which you have observed (by pulse-chase analysis *in vivo*) that your protein is very unstable. Your crude cell-free extract (source of the putative E3 that acts on your protein) contains 20 mg of protein per ml. A sample (10 μl) of the extract, when placed in a final reaction volume of 0.5 ml and assayed under optimum conditions— optimal pH, optimal salt concentration, saturating concentrations of the substrate (your protein), ATP, radioactive ubiquitin, E1, and a promiscuous E2 —catalyzed the attachment of 3 μmoles of ubiquitin to your protein in 1 min. **A**. Based on this information, calculate: (a) the initial velocity of the enzyme-catalyzed reaction in terms of μmoles product formed/liter-min; and, (b) the initial velocity of the enzyme-catalyzed reaction in terms of μmoles product formed/min. **B**. If another 10 μl sample of the exact same extract was assayed under the exact same conditions, but in a final volume of 1.0 ml, calculate: (a) the initial velocity of the enzyme-catalyzed reaction in terms of μmoles product formed/liter-min; and, (b) the initial velocity of the enzyme-catalyzed reaction in terms of μmoles product formed/min. **C**. Determine the concentration of the apparent E3 in the extract, in terms of units/ml (remember: the IUBMB sets 1 unit = 1 μmole of product formed per min at 25˚C). **D**. Determine the specific activity of the apparent E3 in the extract, in terms of units/mg protein.