October 15, 2001

ID number _________

Grade: _____________

Biochemistry Cumulative Exam

This exam deals with a recent paper in last month's issue of *Science* (September 7, 2001): Chang and Roth, "Structure of MsbA from *E. coli*: A homolog of the Multi-drug Resistance ATP binding cassette transporters", *Science* **293**:1793

This is an important paper. It describes the structure of an example of a general class of membrane proteins that can transport a variety of molecules across biological membranes as well as flip the orientation of phospholipids ("flippases"). The structure of the protein goes a long way to showing how both these functions could be carried out. The rise of multi-drug resistant pathogenic bacteria is becoming increasingly worrisome. Knowing how these proteins work may be the first step in understanding how to solve the problem.

For each question, take as much space as is necessary to answer each one completely. **You may use any reference materials, including any book, journal articles or notes you may have made, but you may not consult anyone.**

1. What is the ABC cassette? What is the hallmark in the protein sequence for an ABC cassette (i.e. how would you know if a protein had one from its sequence alone)? How does it work? (I want more than "it binds ATP"…) What kinds of proteins have it? (i.e. just MDR-type membrane proteins?) What is the Walker motif? What does it do?

2. What does the phospholipid "flip-flop" function have to do with the multi-drug resistance? How are these 2 properties related? How can the same mechanism explain both. Draw a picture if you need to. First explain it to me as a scientist. Then write a second paragraph on the next page as if you were writing something to give to a journalist (i.e. someone scientifically naïve), and you want to explain how looking at basic science questions like asymmetry of biological membranes can have implications for "real-world" problems like the rise of multi-drug resistant bacteria.

3. A key feature of the model presented in this paper is that ATP hydrolysis is linked to movement of the protein. Is this feasible? Name at least 3 other proteins that couple movement of the protein (or part of the protein) to nucleotide binding and hydrolysis.

4. There is an ABC transporter in the yeast plasma membrane called Ste6. It is involved in the export of a farnesylated peptide pheremone, a-factor. The primary structure of the Ste6 protein shows that it has 6 TMDs (trans-membrane domains), an ABC cassette, followed by 6 more TMDs (trans-membrane domains) and another ABC cassette. As a graduate student, I was very surprised to see an experiment that someone tried — they split Ste6 into 2 polypeptides. They did this by putting a stop codon after the first ABC cassette, and then putting a new promoter, 5'UTR sequence and start codon, so that the C-terminal half would be expressed as a separate polypeptide. Amazingly (to me at the time), this produced a functional protein when both halves were co-expressed, but expression of one half alone would not do anything. From what you learned from this paper, explain this result fully. It is surprising to you? Why or why not? Try to formulate a general rule to answer this question: For different kinds of proteins, where would it be safe to split them into 2 polypeptides, so that they still function?

5. What is lipid A? Draw the structure of a lipid A molecule. Why is energy required to move it from one leaflet to the other in a lipid bilayer? An enzyme, like any catalyst, works by lowering the energy of the transition state. What does the transition state of this reaction correspond to? How does the ABC transporter lower its energy?

6. Examine the structure of the protein. A key point is the "ICD" domain that links the nucleotide-binding domain (NBD) to the transmembrane (TM) domain. Look at the TM and ICD helices and the junction between them. What sorts of residues are often there when the polypeptide chain makes a sharp bend. Does this make sense? Why?

However, there is often not a sharp bend or kink between a TM helix and the preceding or following ICD helix. Discuss the following argument:
"The distinction between the TM and ICD helices is mostly based on context — whether they are in the membrane or in the cytosolic phase. In almost every case, these can actually be considered to be a single helix that extends into the membrane bilayer."

Is this point relevant to the mechanism of action?

7. These are direct quotes from the paper. Briefly explain what each one means:

p. 1794: " Eco-msbA crystallized in space group P1 (a = 107.8 Å, b = 126.1 Å, c = 206.6 Å, $\alpha = 83.5°$, $\beta = 76.3°$, $\gamma = 84.1°$) using dodecyl-α-D-maltoside (α-DDM) (Table 1) (26). The native crystals diffracted to a resolution of ~6.2 Å using synchotron radiation but data was fairly anisotropic. In an effort to strengthen protein lattice contacts and decrease the disorder within these crystals, we applied a crystal refinement strategy that included the screening of an extensive matrix of detergents, detergent concentrations, salts, temperatures, organics, additives, deuterium, oxides and heavy metals."

p. 1795: "The structure refinement of Eco-msbA was complicated by a rapid decrease in the intensity of the diffraction pattern as a function of resolution, corresponding to an overall temperature factor of ~150 $Å^2$."

p. 1795: "In an effort to better simulate the data, 16 copies of the asymmetric unit (eight molecules per asymmetric unit) were simultaneously refined against the $OsCl_3$ data (Set 2) with very strict eightfold non-crystallographic harmonic constraints (2000 kcal/mol) between the monomers using the program XPLOR (*34–38*). After molecular dynamics refinement, an ensemble of very similar models (average root mean square deviation of C_α atoms among models of <1.4 Å) was achieved with a crystallographic R value of 27% and an R_{free} of 38%."