Homework 2 for Cell Biology--- 2017

1. Monomeric single-pass transmembrane proteins span a membrane with a single α helix that has characteristic chemical properties in the region of the bilayer. Which of the three 20-amino acid sequences listed below is the most likely candidate for such a transmembrane segment? Explain the reasons for your choice.

   A. I T L I Y F G V M A G V I G T I L L I S
   B. I T P I Y F G P M A G V I G T P L L I S
   C. I T E I Y F G R M A G V I G T D L L I S

2. Match each definition below with its term from the list below:
   A. An aqueous pore in a lipid membrane, with walls made of protein, through which selected ions or molecules can pass.
   B. The movement of a small molecule or ion across a membrane due to a difference in concentration or electrical charge.
   C. General term for a membrane-embedded protein that serves as a carrier of ions or small molecules from one side of the membrane to the other.
   D. Movement of a molecular across a membrane that is driven by ATP hydrolysis or other form of metabolic energy.
   E. Driving force for ion movement that is due to differences in ion concentration and electrical charge on either side of the membrane.

   (1) Active transport (2) Membrane-transport protein. (3) Channel. (4) Passive transport (facilitated diffusion) (5) Transporter (6) Electrochemical gradient.

3. Intracellular changes in ion concentration often trigger dramatic cellular events. For example, when a clam sperm contacts a clam egg, it triggers ion changes that result in the breakdown of the egg nuclear envelope, condensation of chromosomes, and initiation of meiosis. Two observations confirm that ionic change initiates these cellular events: (1) suspending clam eggs in seawater containing 60nM KCl triggers the same intracellular changes as do sperm; (2) suspending eggs in artificial seawater lacking calcium prevents activation by 60nM KCl What does the lack of activation by 60 mM KCl in calcium –free seawater suggest about the mechanism of KCl activation? What would you expect to happen if the calcium ionophore, A23187, which allow selective entry of Ca2+ was added to a suspension of eggs (in the absence of sperm (in either regular seawater or calcium –free seawater)?

4. Acetylcholine-gated cation channels do not discriminate among Na+, K+, and Ca2+ ions, allowing all to pass through freely. How is it, then, that when acetylcholine receptors in muscle cells open there is a large net influx principally of Na+?

5. A rise in the intracellular Ca2+ concentration causes muscle cells to contract. In addition to an ATP-driven Ca2+ pump, heart muscle cell, which contract quickly and regularly, have an antiporter that exchanges Ca2+ for extracellular Na+ across the plasma membrane. This

antiporter rapidly pumps most of the entering Ca2+ ions back out of the cell, allowing the cell to relax. Ouabain and digoxin, drugs that are used in the treatment of patients with heart disease, make the heart contract more strongly. Both drugs function by partially inhibiting the Na+—K+ pump in the membrane of the heart muscle cell. Can you propose an explanation for the effects of these drugs in patients? What will happen if too much of either drug is taken?

6. You have prepared lipid vesicles (spherical lipid bilayers) that contain Na+—K+ pumps as the sole membrane protein. Assume for the sake of simplicity that each pump transports one Na+ one way and one K+ the other way in each pumping cycle, as illustrated in figure below. All of the Na+—K+ pumps are oriented so that the portion of the molecule that normally faces the cytosol faces the outside of the vesicle. Predict what would happen under each of the following conditions.
   (1) The solution inside and outside the vesicles contains both Na+ and K+ ions, but no ATP.
   (2) The solution inside the vesicles contains both Na+ and K+ ions;
   (3) The solution outside contains both ions,as well as ATP.
   (4) The solution inside contains Na+; the solution outside contains Na+ and ATP.
   (5) The solution is as in B, but the Na+—K+ pump molecules are randomly oriented, some facing one direction, some the other.

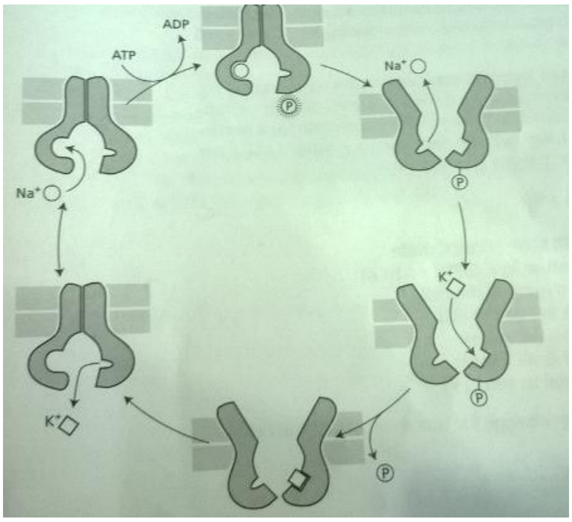

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Figure The Na+— K+ pump

7. Chloroplasts contain six compartments- outer membrane, Intermembrane space, inner membrane, stroma, thylakoid membrane, and thylakoid lumen - each of which is populated by specific sets of proteins. To investigate the import of nucleus-encoded proteins into chloroplasts, you have chosen to study ferredoxin, which is located in the stroma and plastocyanin, which is located in the thylakoid lumen, as well as two hybrid genes: ferredoxin with the plastocyanin signal peptide and plastocyanin with the ferredoxin signal peptide. You translate mRNAs from these four genes in vitro. Mix the translation products with isolated chloroplasts for a few minutes, reisolated the chloroplasts after protease treatment, and fractionate them to find which compartments the proteins have entered. The status of the

normal and hybrid proteins at each stage of the experiments is shown in the following Figure: each lane in the gels corresponds to a stage of the experiments as indicated alongside the experimental protocol in the following Figure.

(1). How efficient is chloroplast uptake of ferredoxin and plastocyanin in your in vitro system? How can you tell?

(2). Are ferredoxin and plastocyanin localized to their appropriate chloroplast compartments in these experiments? How can you tell?

(3). Are the hybrid proteins imported as you would expect if the N-terminal signal peptides determined their final location? Comment on any significant differences.

(4) Why are there three bands in experiments with plastocyanin and pcFD but only two bands in experiments with ferredoxin and fdPC? To the extend you can, identify the molecular species in the bands and their relationships to one another.

(5). Based on your experiments, propose a model for the import of proteins in the stroma and thylakoid lumen.

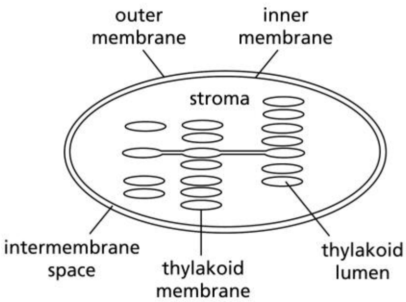

Figure 12-17 MBoC5: The Problems Book (© Garland Science 2008)

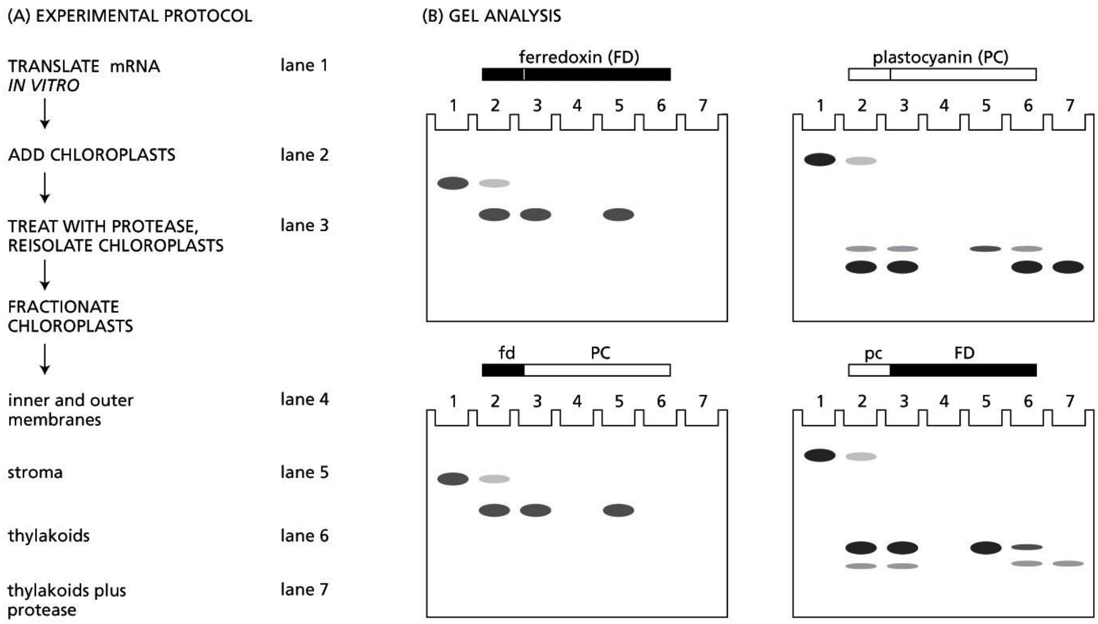

Figure 12-18 MBoC5: The Problems Book (© Garland Science 2008)