Title: Active Transport Lab

Purpose

- To explore how substances are transported across membranes against a concentration gradient
- To investigate the effects of amino acid concentration and ATP on amino acid transport.

Hypothesis:

This lab will allow you to determine how amino acid concentration and ATP affect amino acid transport and develop *hypotheses* about the effects as *your conclusion*.

Materials:

OnLine Active Transport Interface

(See <u>below</u> for background information on using the Active Transport Interface)

Procedure:

- Go to http://www.mhhe.com/biosci/genbio/biolink/j_explorations/ch03expl.htm
- Click Skip Intro
- Note that green spheres represent amino acids, blue spheres represent Na⁺ ions, and the yellow spheres represent ATP.

Part A: No ATP expenditure

- 1. Set ATP Expenditure to 0.
- 2. Click and drag the sliding indicator in the *initial amino acids levels* box in the lower left corner to the right to 1000 (1000/1 ratio of amino acids levels outside the cell relative to those inside.
- 3. Click Start and let run for 60 sec.
- 4. Repeat with initial amino acids levels set to 0.001.
- 5. Record your data/observations in Data Table A.
- 6. Do any amino acids enter the cell in the time interval? Why or why not?
- Part B: Low ATP Expenditure
 - 1. Set ATP expenditure to low.
 - 2. Set initial amino acid levels to 0.001.
 - 3. Click Start
 - 4. Record amino acid transport rate and amino acid concentrations at 60 sec.
 - 5. Repeat for initial amino acid levels indicated in Data Table B.

Part C: Medium ATP Expenditure

- 1. Set ATP expenditure to medium.
- 2. Set initial amino acid levels to 0.001.
- 3. Click Start
- 4. Record amino acid transport rate and amino acid concentrations at 60 sec.
- 5. Repeat for initial amino acid levels indicated in Data Table C.
- Part D: High ATP Expenditure
 - 6. Set ATP expenditure to high.
 - 7. Set initial amino acid levels to 0.001.
 - 8. Click Start
 - 9. Record amino acid transport rate and amino acid concentrations at 60 sec.
 - 10. Repeat for initial amino acid levels indicated in Data Table C.
- Construct a multiline graph of initial amino acid levels (x-axis) vs amino acids concentrations at 60 sec (y-axis) using <u>Create a Graph</u> (<u>http://nces.ed.gov/nceskids/graphing</u>).



Results:

Data Chart A: No ATP Expenditure				
Initial AA Levels	Amino Acid Movement			
1000				
0.001				
Explanation				

Data Table B: Low ATP Expenditure						
Initial AA Levels	AA Transport Rate	Amino Acid Concentration at 60 sec				
0.001						
0.01						
0.1						
1.0						
10						
100						
1000						

Data Table D: High ATP Expenditure						
Initial AA Levels	AA Transport Rate	Amino Acid Concentration at 60 sec				
0.001						
0.01						
0.1						
1.0						
10						
100						
1000						

Data Table C: Medium ATP Expenditure						
Initial AA Levels	AA Transport Rate	Amino Acid Concentration at 60 sec				
0.001						
0.01						
0.1						
1.0						
10						
100						
1000						

Construct a multiline graph of initial amino acid levels (x-axis) vs amino acids concentrations at 60 sec (y-axis) using <u>Create a Graph</u> (http://nces.ed.gov/nceskids/graphing).



Discussion:

(Summarize what you did, describe your results, indicate whether or not you met your purpose or supported your hypothesis, indicate sources of error, and suggest improvements in the experimental design)

Answer the following questions as wel!

- 1. How is this remarkable system affected by the concentration of amino acids available and the amount of ATP used?
- 2. How important is the sodium/potassium pump?
- 3. What kinds of phenomena influence its behavior?
- 4. What would happen if the sodium/potassium pump was damaged or inoperable?

Conclusion: (Use the following statements and fill in the blanks for each enzyme) 2 TESTABLE statements about the effects of amino acid concentrations outside the cell and ATP expenditure on amino acid transport.

Reflection:

(Personal commentary about what you learned from the lab simulation)

How to Use the Active Transport OnLine Lab

This interactive exercise will allow you to explore how substances are transported across membranes against a concentration gradient (that is, toward a region of higher concentration). The exercise uses a diagram of a coupled channel within a membrane through which amino acids are pumped into the cell. By altering ATP concentrations, you will be able to speed or slow the operation of the ATP-driven sodium/potassium pump and explore the consequences for amino acid transport. Similarly, you can alter the cellular or extracellular levels of amino acids and investigate the effect on cellular expenditure of ATP. Because the amino acid transport channel is coupled to the ATP-driven sodium/potassium pump, you will hopefully discover that both ATP and amino acid levels have important influences.

Click on the Skip Intro button, located in the lower right-hand corner, to start the Exploration.

On the screen you see a cutaway of a cell membrane: large globular proteins are embedded in a double layer of two-tailed phospholipid molecules. The top and bottom of the screen are the external and internal environments, respectively. The floating spheres represent different kinds of molecules: the blue are sodium ions, the yellow are potassium ions, and the green are amino acids, which are the building blocks of protein. Now suppose this cell requires a supply of amino acids so that it can construct proteins. It must obtain them from the outside, concentrating them within the cell. This requires energy for active transport, since diffusion would otherwise spread them out evenly. In our cells, the energy for this process comes from ATP. Using the mouse, click and drag the sliding indicator in the "initial amino acids levels" box in the lower left corner to the right as far as desired to increase amino acids levels outside the cell, relative to those inside. Click on "start." Now that you have provided an abundance of amino acids outside, two things are going to happen that will get them into the cell: first, the sodium/potassium pump will use the energy of ATP to pump sodium ions out; second, the sodium ions will diffuse back in - but to get back in they must pass through a coupled channel paired with an amino acid!

To set this into motion, click and drag the sliding indicator in the "ATP expenditures" box to the right and click on "start." This powers your project by supplying ATP. The meters in the upper left corner of the screen monitor the rate of amino acid transport and the relative concentrations of amino acids inside and outside the cell. Once ATP is made available, it fuels the sodium/potassium pump, illustrated on the left edge of the cell membrane. Energy from ATP, represented as a yellow star burst, is used to actively pump sodium, the blue spheres, out of the cell, and potassium, the yellow spheres, into the cell. This creates a concentration gradient, with more sodium outside the cell than inside the cell. Following the laws of diffusion, this sodium will tend to reenter the cell, distributing itself more evenly. Now look at what happens: the sodium ions "hitch a ride," passing through the coupled channel hand-in-hand with amino acids. In effect, the steep sodium ion concentration gradient pulls amino acids into the cell, even though they're already more amino acids inside than outside. The trick is that the cell spends ATP energy to ensure that the ion gradient is steeper than the amino acid gradient.

Modified from http://www.mhhe.com/biosci/genbio/biolink/j_explorations/ch03expl.htm

