RESEARCH PROJECT

BACKGROUND:

Proteins are synthesized inside the cell and then sent to their final destination. Some remain inside the cell, some are transported out of the cell and thus must pass through the membrane surrounding the cell, and some proteins are sent to the membrane and remain there. Proteins that are exported from a cell use a specific secretion pathway in order to be transported through the membrane to the outside of the cell. For example, in *Escherichia coli*'s secretion system the most essential component for translocation is Sec A, an ATPase that can be in the cell or bound to the membrane and interacts with both the protein to be transported and the other components of the secretion system. The other components include Sec B, which acts as a chaperone inside the cell, and Sec D, E, F, and Y which are all membrane proteins. Sec YEG forms a heterotrimer which is thought to constitute the translocation channel (1,5).

In order for a protein to enter this secretory pathway, it needs a specific targeting signal. This is the signal peptide, which is an amino-terminal extension of the protein. This signal peptide is recognized by and interacts with the components of the secretion pathway. The signal peptide is normally cleaved off of the protein once its role is completed (4,5).

It is believed that proteins which are destined to remain in the cell's membrane use the same pathway to insert themselves in the membrane as those proteins that are excreted from the cell use to get across the membrane. Instead of the entire protein being translocated across the membrane and secreted, portions of the protein remain in the membrane. Thus membrane proteins have regions that are outside and inside the cell which are connected by one or more transmembrane segments (2).

Leader peptidase is an example of a membrane protein in *E. coli*. Both the amino-terminal and the carboxyl-terminal ends of the protein are in the area outside the cell, and there are two transmembrane segments connected by an intracellular loop. Leader peptidase does not have a cleavable signal peptide. It is believed that the second transmembrane segment acts as a signal for the protein and is needed for assembly in the membrane (6).

SPECIFIC AIM:

I will be investigating the reason why some membrane proteins have signal peptides and some do not. I will determine if the role of the signal peptide is to override sequence characteristics which would otherwise be unfavorable to membrane assembly in its absence.

PROJECT:

Leader peptidase is a useful protein for the purpose of this project since it does not usually have a signal peptide. Also, Gunnar von Heine's lab in Sweden has an inverted leader peptidase construct that can be utilized to test my hypothesis. The orientation of this leader peptidase mutant has been flipped by the addition of a cluster of charged residues (3).

It is difficult for a cell to transport positive charges through the membrane, and so in the leader peptidase mutant (Lep-inv) the orientation becomes N-in, C-in because of the addition of the positive charges near the N-terminus. I will insert a signal peptide at the N-terminus of this Lep-inv mutant and determine whether this overcomes the positive charges and produces the original orientation.

For this purpose, I have obtained the Lep and Lep-inv constructs and antiserum from Gunnar von Heijne in Sweden. From these I have begun making the new constructs I will need at the DNA level. I am also currently testing the parent plasmids from von Heijne for protein expression and using them to learn the assays I will need for the analysis of the leader peptidase's orientation.

Experimental analysis will include examining protein expression, signal peptide utilization and cleavage, and membrane integration. A particularly important experiment will involve determining the orientation of leader peptidase using a protease protection experiment. This involves disrupting the cell wall so the protease will be exposed to the parts of the protein that are outside the cell membrane. Proteins that are exposed to protease are cleaved, so the leader peptidase will be cut in different places depending on which part of the protein is exposed to the exterior of the cell. I will use SDS polyacrylamide gel electrophoresis to analyze the fragments produced. The size of the two fragments will differ depending on the orientation of the leader peptidase.

If the original experiment does not cause the lep-inv mutant to revert back to its normal orientation there are two things that can be changed. First, there are a number of different signal peptides from which I can choose to insert amino-terminal to leader peptidase. If the original one is not able to change the orientation I will use a more hydrophobic signal peptide. The stronger signal peptide may be able to overcome the positive charges a weaker one could not. A second alternative would be to put a polypeptide spacer between the signal peptide and the positive charges by inserting a linker sequence. The signal peptide may be able to function better further away from the positive charges than when it is right next to them.

PLAN OF STUDY

Spring '00

POLS 121	Introduction to Comparative Politics (3)
MATH 223Q	Geometry (3)
MCB 226W	Advanced Biochemistry Laboratory (4)
MCB 209	Structure and Function of Biological Macromolecules (3)
MCB 299	Independent Study (3)

For all three semesters I will continue taking Independent Study to work on my research project. In the spring I will take political science to fulfill my remaining requirement for a western culture class. Geometry will fulfill one if the last two classes I need for my math minor. Biochemistry laboratory will help me develop skills that will be useful in future experiments in my research and also will fill one of my remaining "W" requirements. MCB 209 will provide useful background information for my project. Being a University Scholar will allow me to engross myself in my area more deeply by taking more MCB courses, instead of having to give up at least one of these for a course in a related field.

Summer '00

Next summer I will find funding in order to be able to stay here at UCONN and continue with my research instead of going back to the summer job I otherwise would have.

Fall '00

MATH 215Q	Linear Algebra (3)
MCB 211	Basic Immunology (3)

MCB 312	Foundations of Structural Biochemistry (3)
MCB 299	Independent Study (3)

I plan to complete my minor in math by taking linear algebra next fall. From Immunology, I will gather a broader background in biology. As a university scholar I can take a graduate course I am interested in, MCB 312, which will let me get a better understanding of the behavior of the molecules I will be working with. Otherwise I would have had to take an undergraduate course in a different field as a related.

Spring ’01

ECON 219	Intermediate Microeconomic Theory (3)
MCB 325	Structure and Function of Biological Membranes (3)
MCB 380	Advanced Cell Biology (3)
MCB 292W	Senior Research Thesis in Molecular and Cell Biology (3)
MCB 299	Independent Study (3)

I was very interested by the microeconomics class I took and would therefore like to learn more about this subject by taking intermediate microeconomics. I will be able to get an in depth study of membrane proteins by taking the graduate level class MCB 325. I will also have the opportunity to take a graduate course in advanced cell biology, which will give me a better background for graduate school. I will also take MCB 292W and 299 to complete my research and a paper about it.

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6. Dalbey, R.E., Kuhn, A., and Wickner, W. (1987) *J Biol Chem* **262**: 13241-5