BIO-372 "MICROBIOLOGY" EXERCISES (WEEK 5)

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EXERCISE 1 "TRANSPORT ACROSS THE BACTERIAL CELL ENVELOPE" :

1. Bacteria use three types of transmembrane import events: uniport, symport, and antiport. Explain how they work.

Uniport. A single substrate is transported. The substrate travels down its concentration gradient across the membrane (high outside ➔ low inside).

Symport. A substrate and a co-substrate are transported simultaneously in the same direction. The substrate travels up its concentration gradient (low outside ➔ high inside). The co-substrate travels down its concentration gradient (high outside ➔ low inside).

Antiport. A substrate and a co-substrate are transported simultaneously in opposite directions. The substrate travels up its concentration gradient (low outside ➔ high inside). The co-substrate travels down its concentration gradient (high inside ➔ low outside).

2. Bacteria also use three classes of transmembrane importer systems: simple transporters, group translocators, and ABC transporters. Explain how they work.

All three classes of transporters have channel proteins with 12 trans-membrane alpha-helices surrounding a central aqueous channel through which the transported substrate passes. Group translocators and ABC transporters also include additional proteins.

Simple transporter. These transporters comprise only the protein that forms the aqueous channel through which the transported substrate passes. Transport is driven by concentration gradients of the transported substrates. Unlike group translocators and ABC transporters, simple transporters do not involve hydrolysis of high-energy phosphate bonds. Example: the Pit system that imports inorganic phosphate.

Group translocator. These transporters covalently modify the transported substrate by phosphorylation during the transport process. The high-energy phosphate group is donated by a phosphorelay chain of proteins located on the cytoplasmic side of the membrane. The phosphorelay ultimately derives the phosphate group from phosphoenolpyruvate as the phosphate donor. Example: the phosphotransferase system that imports glucose, which modifies the substrate to glucose-6-phosphate during the transport process.

ABC transporter. In addition to the *transmembrane domain (TMD) protein* that comprises the substrate channel, these transporters include a *substrate-binding protein (SBP)* located on the periplasmic or extracellular side of the cytoplasmic membrane, which has extremely high affinity and specificity for the transported substrate, and a *nucleotide-binding domain (NBD) protein* on the cytoplasmic side of the membrane. The SBP binds the substrate outside the cell and delivers it to the TMD protein, which then passes the substrate through the channel and releases it into the cytoplasm. Transport of the substrate through the channel is driven by conformational changes of the channel protein from an outward-facing high-affinity conformation (which takes the substrate from the substrate binding protein on the periplasmic or extracellular side of the membrane) to an inward-facing low-affinity conformation (which releases the substrate into the cytoplasm). These conformational cycles are driven by cycles of ATP binding, hydrolysis, and release by the NBD protein. Example: the Pst system that imports inorganic phosphate.

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3. Transmembrane importer systems can import solutes (such as nutrients) against their concentration gradients, which requires energy. For each of the three types of importer systems, what is the energy source and how does it drive transmembrane transport?

Simple transporter. Energy is stored in the form of a concentration gradient of a substrate (or co-substrate) across the cytoplasmic membrane and released when the substrate (or co-substrate) flows down its concentration gradient. For uniporters, the imported substrate simply flows down its concentration gradient; this is essentially facilitated diffusion. For symporters, import of the substrate up its concentration gradient is coupled to import of a co-substrate down its concentration gradient. For antiporters, import of the substrate up its concentration gradient is coupled to export of a co-substrate down its concentration gradient. For symporters and antiporters, an energetically unfavorable transport event (import of the substrate up its concentration gradient) is made possible by coupling it to an energetically favorable transport event (import or export of the co-substrate down its concentration gradient).

Group translocator. Import of the substrate (e.g., glucose) is coupled to its phosphorylation. The ultimate donor of the high-energy phosphate group is phosphoenolpyruvate, which has an even higher phospo-transfer potential than ATP. Thus, an energetically unfavorable transport event (import of the substrate up its concentration gradient) is made possible by coupling it to an energetically favorable chemical reaction (phosphoenolpyruvate hydrolysis).

ABC transporter. Import of the substrate is coupled to cycles of ATP hydrolysis and release by the nucleotide binding domain (NBD) protein located on the cytoplasmic side of the membrane. Thus, an energetically unfavorable transport event (import of the substrate up its concentration gradient) is made possible by coupling it to an energetically favorable chemical reaction (ATP hydrolysis).

4. Bacteria typically have two different import systems for uptake of essential nutrients. As an example, uptake of inorganic phosphate (Pi) is mediated by the Pit system *and* the Pst system. Compare and contrast these systems. Why are two Pi-uptake systems necessary? In what conditions would Pit-mediated Pi uptake dominate? In what conditions would Pst-mediated Pi uptake dominate?

The Pit system is an example of a simple transporter (a symporter) that imports inorganic phosphate up its concentration gradient (low outside ➔ high inside) by coupling it to import of a proton down its concentration gradient (high outside ➔ low inside). The Pit system has high transport velocity but low substrate affinity and it is energetically inexpensive (one proton per phosphate transported). The Pit system functions optimally when external phosphate concentrations are relatively high but it is unable to supply the cell's nutritional needs when external phosphate concentrations are very low due to its low substrate affinity.

The Pst system is an example of an ABC transporter that imports inorganic phosphate up its concentration gradient (low outside ➔ high inside) by coupling it to cyclic conformational changes of the transport channel driven by cycles of ATP binding, ATP hydrolysis, and ADP + Pi release. The Pst system has low transport velocity but high substrate affinity and it is energetically expensive (at least one ATP hydrolyzed per phosphate transported, although the exact stoichiometry is not known). The Pst system functions optimally when external phosphate concentrations are very low but it is inefficient when external phosphate concentrations are relatively high due to the high transport cost.

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5. Bacteria use the phosphotransferase system (PTS) for uptake of sugars (like glucose) from the environment. Why is the PTS necessary? How does the PTS work? What are the sugar-specific and sugar-nonspecific components of the PTS and what are their functions?

The PTS is an example of a group translocator system. It is necessary because glucose, being a highly hydrophilic molecule, cannot readily cross the cytoplasmic membrane by simple diffusion. Also, the concentration of glucose is generally higher inside the cell than outside the cell, so energy is required to import glucose up its concentration gradient. The PTS works by phosphorylating glucose during the transport process and converting it to glucose-6-phosphate, which can then enter into central metabolism via glycolysis. The high-energy phosphate is ultimately derived from hydrolysis of phosphoenolpyruvate and passed through a phospho-relay system comprising four proteins before being transferred to glucose. The trans-membrane channel and the two proteins of the phosphorelay that are proximal to the channel are sugar-specific. The two proteins of the phosphorelay that are distal to the channel, including the protein that accepts the high-energy phosphate group from phosphoenolpyruvate, are sugar-non-specific. The proteins of the phosphorelay chain also function in regulation of central carbon metabolism. Their regulatory activities are controlled by their phosphorylation status, which is, in turn, controlled by the rate of glucose uptake. If glucose uptake is slow (low concentration in the environment) then the proteins in the phosphorelay chain will be hypo-phosphorylated. If glucose uptake is fast (high concentration in the environment) then the proteins in the phosphorelay chain will be hyper-phosphorylated

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EXERCISE 2 "TRANSPORT ACROSS THE BACTERIAL CELL ENVELOPE" :

*My "rubric answers" use the proper names of the relevant proteins for convenience. It isn't important for you to memorize the **names** of the relevant proteins. You should, however, remember the **functions** of the relevant proteins.*

1. The Sec system is responsible for secretion of proteins into the periplasmic space (in Gram-negative bacteria) or the extracellular space (in Gram-positive bacteria). Explain how this process works (you may want to use diagrams). Secretion of proteins requires energy; where does this energy come from?

As the nascent protein emerges from the ribosome it is bound by a **chaperone** (SecB) that maintains it in the unfolded state. This is important because the Sec system can transport proteins only in their *unfolded* state, not in their *folded* state. When translation is finished, the SecB-bound protein is delivered to the **motor protein** (SecA) that drives the translocation of the unfolded protein through the **secretory channel** (SecYEG and other proteins). The energy for protein translocation comes from cycles of ATP binding, hydrolysis, and release by SecA. The proton motive force is also required, although its precise role is unclear. When the entire protein has been translocated it folds into its final configuration in the periplasmic space (Gram-negative) or extracellular (Gram-positive) space.

2. The Sec system is also responsible for insertion of transmembrane proteins into the cytoplasmic membrane (in Gram-negative and Gram-positive bacteria). Explain how this process works (you may want to use diagrams). Insertion of proteins into the cytoplasmic membrane requires energy; where does this energy come from?

As the nascent protein emerges from the ribosome it is bound by a **chaperone** (SRP for "signal recognition particle") that pauses translation. The complex of nascent protein-SRP-ribosome then docks with an **adaptor protein** (FtsY), which also interacts with the **secretory channel** (SecYEG and other proteins). The SRP disengages from the ribosome-FtsY-SecYEG complex and protein translation resumes, with the ribosome serving as the **motor protein** for translocation of the exported protein. As the protein passes through the SecYEG channel, hydrophobic segments of the protein are inserted into the membrane while hydrophilic segments of the protein pass through to the other side of the membrane into the aqueous periplasmic space or extracellular space. The energy for protein translocation comes from cycles of GTP binding, hydrolysis, and release by the ribosome as part of the normal translation cycle. The proton motive force is also required, although its precise role is unclear. Thus, for transmembrane proteins, the ribosome functions as both a *protein synthesis machine* and a *motor protein*. When the entire transmembrane protein has been synthesized and translocated, hydrophobic segments of the protein reside in the membrane and hydrophilic segments of the protein reside in the aqueous milieu in the cytoplasm or periplasmic/extracellular space.

3. Exported proteins can be secreted into the periplasmic/extracellular space *or* inserted into the cytoplasmic membrane. How does the cell "decide" whether an exported protein should be secreted or inserted into the cytoplasmic membrane?

For both classes of exported proteins (secreted proteins and transmembrane proteins), the final location of the protein is dictated by a "signal peptide" at the N-terminus of the protein. The signal peptide is both necessary and sufficient to specify the cellular "address" of the protein to which it is fused; for example, a cytoplasmic protein can be forced into the secretory pathway by simply fusing a signal peptide to its N-terminus. The signal peptide for

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transmembrane proteins is longer and more hydrophobic than the signal peptide for secreted proteins, which determines which chaperone (SRP or SecB) the nascent protein interacts with as it emerges from the ribosome. After translocation is complete, the signal peptide is usually cleaved off by a signal peptidase. Thus, the signal peptide is usually not part of the mature exported protein.

4. Transmembrane proteins typically contain distinct domains localized to the cytoplasm, membrane, and periplasmic/extracellular space. How does the cell "decide" where to put each domain of a transmembrane protein?

The location of a transmembrane protein segment is dictated by its relative hydrophobicity or hydrophilicity. During the translocation process, hydrophobic segments of protein are inserted into the membrane while hydrophilic segments of protein either remain in the hydrophilic milieu of the cytoplasm or pass through the secretory channel to the hydrophilic milieu of the periplasmic space or extracellular space. Thus, the ultimate cellular "address" of a protein segment is encoded in the amino acid sequence of the protein.

5. Some secreted proteins must fold into their final three-dimensional configurations in the cytoplasm prior to export. Examples include proteins that incorporate cofactors that are not available outside the cell and proteins that cannot fold in the oxidizing environment outside the cell. Would the Sec system be useful for exporting such proteins? Why or why not? How do bacteria export such proteins?

The Sec system transports proteins only in their unfolded state. It cannot transport proteins in their folded state. Thus, the answer is "No" – the Sec system is not useful for exporting proteins that must fold into their mature configuration in the cytoplasm. Such proteins are instead exported by the so-called "twin arginine translocator" (Tat) system, which is capable of transporting folded proteins, although it is energetically very expensive.