Transport in prokaryotic cells

Transport in prokaryotic cells: The transport system of a cell depends upon the substrate requirements of the cell, the bioavailability of the substrate and the environmental conditions. It also depends on the metabolic features and physiological state of the organism. Prokaryotic cells have simpler structure and mostly are unicellular. Hence their transport system is different from higher eukaryotes. Here we will study the transport in prokaryotic cells with respect to bacteria.

Membranes in bacteria: Membranes play a major role in transport. The different types of membrane found in bacteria are:

1. **Cytoplasmic membrane, in all bacteria**

The inner membrane of a cell is different from outer membrane of a cell. And the space between these membranes is called periplasam. The membrane is symmetrical, with an equal distribution of lipids (exclusively phospholipids, mainly phosphatidylethanolamine, phosphatidylglycerol and cardiolipin) among the inner and outer surface. Some of the functions associated with cytoplasmic membrane which has role in transport mechanism of cell are:

- Osmotic and permeability barrier
- Presence of transport system for various solutes
- Synthesis of membrane lipids
- Assembly and synthesis of extracytoplasmic proteins
- Coordination of DNA replication and segregation with septum formation and cell division
- Energy generation functions such as electron transport system, establishment of photon motive force and transmembrane ATP-synthesizing ATPase

2. Outer membrane, mostly in gram negative bacteria

The outer membrane is highly asymmetrical, with the inner leaflet, oriented to the periplasam. The outer leaflet, facing the external medium contains lipopolysaccharides (LPS) constituting of three parts: lipid A as anchor, the core oligosaccharide functioning as spacer element and an O-specific polysaccharide consisting of oligosaccharide repeating unit. Proteins are the integral components or associated with OM. Some of the functions associated with OM are:

- Involved in transport mechanism.
- Contribution of membrane integrity
- Serves as anchor for flagellae, fimbriae and pili. Hence important for locomotion, cell-cell interaction, adhesion to surfaces and formation of biofilms.
- LPS are major antigenic determinants, preventing entry of cell-damaging components and serve as receptor for a number of bacteriophages.

3. Cell walls of gram positive bacteria

The cell walls of gram positive bacteria are devoid of outer membrane but possess a thick murein layer. In Gram-positive bacteria, teichoic acids are covalently linked to peptidoglycan. Teichoic acids are polyol phosphate polymers with a strong charge. They are strongly antigenic and absent in Gram-negative bacteria. In some species, teichuronic acids are found as lipoteichonic acids which are composed of glycerol teichoic acid linked to glycolipid. Additional wall components can be polysaccharides, lipids and proteins.

4. Membrane that forms envelope in mycobacteria

Membrane that forms envelope in mycobacteria is characterized by their low permeability, which contributes resistance of the microbes to therapeutic agents. It contains two special features: an outer lipid barrier based on a monomer of mycolic acids and a capsule-like coat of polysaccharide and protein. The cell wall contains a large amount of C_{60}-C_{90} fatty acids, mycolic acids that are covalently linked to arabinogalactan.

Transport process:

Transport process can be divided into four classes on the basis of driving forces and modes of energy coupling (Milton H. Saier et al., 2000):

1. **Passive diffusion:**

The passive diffusion occurs along the concentration gradient and without the use of metabolic energy. Some solutes pass the permeability barrier of a lipid bilayer by passive diffusion. This is valid for small apolar molecules and small slightly polar but uncharged molecules like water and dissolved gases. Some other solutes are also transported via channels or channel type proteins to overcome in a diffusion-controlled movement.

2. **Primary active transport:**

Primary active transport is characterized by coupling translocation of solute directly to a chemical or photochemical reaction. Primary source includes pyrophosphate bond hydrolysis, methyl transfer and decarboxylation. Transport of Na^+ and K^+ by carrier protein, Na^+ - K^+ ATPase, is the most common example of primary active transport.

3. **Secondary active transport:**

In secondary active transport the translocation step across the membrane is coupled to the electrochemical potential of a given solute. The solute chemical potential created by primary active transport systems is the driving force, which allows an uphill transport of another solute, against its own concentration gradient. The uptake can be mediated as uniprot, symport and antiport. A common example of secondary active transport is the symport of Na^+ and glucose. The transmembrane protein Na^+ - glucose transporter, acts as a carrier, allows Na^+ and glucose to enter the cell together. The Na^+ flow down their concentration gradient while the glucose molecules are transported against their concentration gradient into the cell. Later the Na^+ is pumped back out of the cell by the Na^+ -K^+ ATPase.

4. Phosphophenolpyruvate: sugar phosphotransferase system (Pts):

Pts translocation process is exclusive to bacterial species which phosphorylates its carbohydrate substrates during transport.

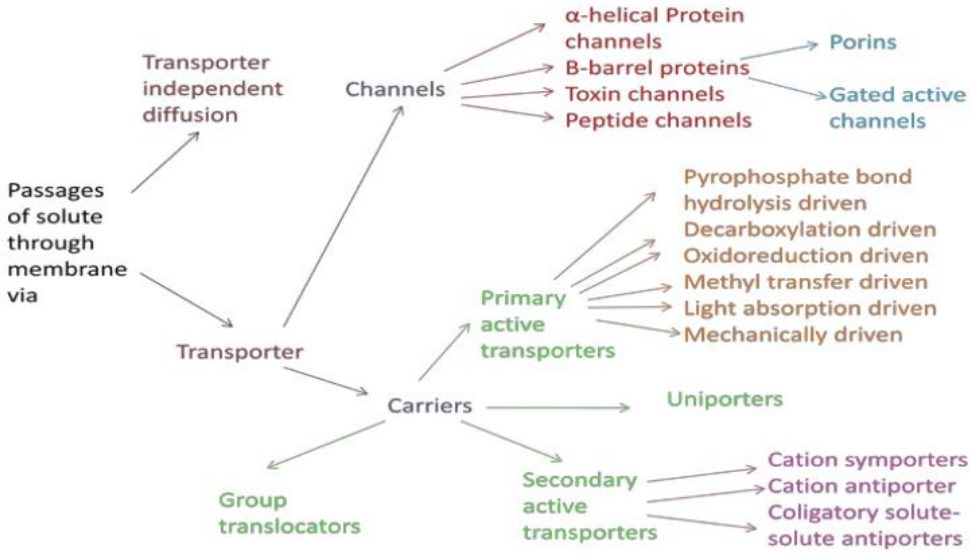

Figure 1: Classification of major types of transport mechanism across biological membranes based on function and phylogeny

The major transport mechanism based on the mode of transport, energy coupling mechanism and substrate specificity and protein phylogenetic grouping that reflects structure, function and its mechanism are:

1. Transport independent diffusion

   Gases (such as O_2 and CO_2); hydrophobic molecules (such as benzene) and small polar but uncharged molecules (such as H_2O and ethanol) are able to diffuse across the plasma membrane.

2. Transport dependent diffusion

   This transport takes allows polar and charged molecules such as carbohydrates, amino acids, nucleotides and ions, to cross the plasma membrane.

a. Channels

   Some examples are voltage gated channels which open in response to change in electric potentials; others called ligand gated channels open in response to the binding of the ligand.

(i) α-helical protein channel

(ii) β-barrel proteins

(iii)Toxin channels

(iv) Peptide channels

b. Carriers

The common example is the movement of glucose mediated by carrier protein called glucose transporter (GLUT).

(i) Primary active transport: Mechanically driven, Light absorption driven, Methyl transfer driven, Oxidoreduction driven, Decarboxylation driven, Pyrophosphate bond hydrolysis driven

(ii) Uniporters

(iii) Secondary active transport: Cation symporters, Cation antiporters, Solute solute antiporters

(iv) Group translocators

Some examples of transporter in bacteria can be studied with the following examples:

1. Phosphate transport:

Two major phosphate transport systems are involved in bacteria:

a. Low affinity Pit (phosphate inorganic transport) system

b. High affinity Pst (phosphate specific transport) system

Pit consists of a single trans-membrane protein and is constitutively expressed secondary transporter. This system is characterized by uptake of phosphate which is in the form of a neutral metal phosphate complex and is in symport with a proton. This transport of phosphate is achieved by binding and dissociation of the neutral metal phosphate complex and H^+ on the outer and inner surface of the trans-membrane protein carrier protein. Pit is reversible and therefore allows both import and export of divalent ions and phosphate. Also it has a relatively low specificity for both phosphate and arsenate (toxic analogue of phosphate).

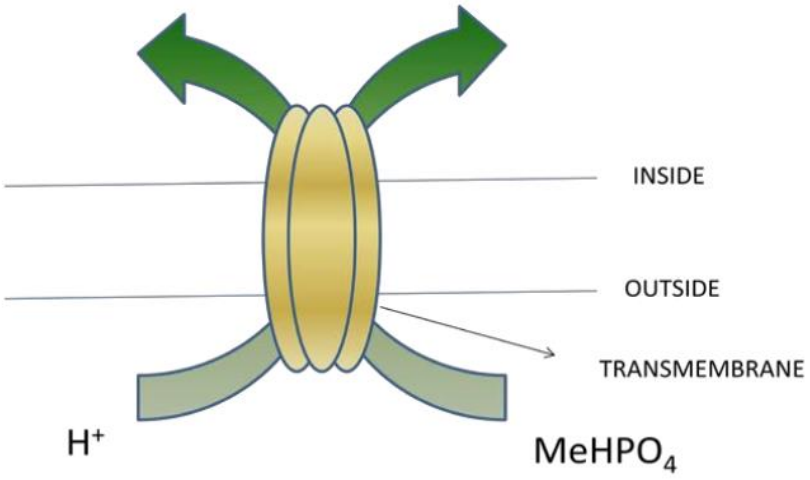

Figure 2: Phosphate transport by low affinity pit

In contrast, protein specific transport (Pst) is a periplasmic protein-dependent transporter. It consists of four subunits: a phosphate-binding protein located in the periplasmic space, two cytoplasmic associated proteins that contain six membrane spanning helices and a dimeric ATP binding protein. It operates as a primary transport mechanism i.e. unidirectional phosphate transport is coupled to a chemical reaction**.** Phosphate is transported in the form of $H_2PO_4^-$ and HPO_4^{2} in Pst system and has a relatively high substrate affinity.

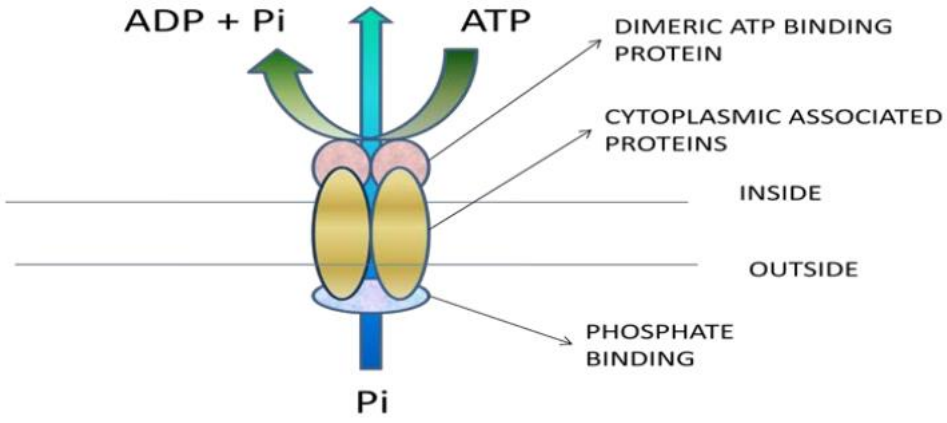

Figure 3: Phosphate transport by ATP dependent high affinity Pst system

Besides, phosphate also enters the cell in the form of esters such as *sn*-glycerol-3-phosphate, glucose-6-phosphate or mannose-6-phosphate. Other organic phosphate compounds may diffuse through the outer membrane before hydrolysis in the periplasm by phosphatases - allowing transport of Pi into the cytoplasm. Pi linked antiport systems of *sn*-glycerol-3-phosphate (GlpT) and glucose-6-phosphate (UhpT) mediate the translocation of organo-phosphate compounds across the cell membrane. Phosphate is also accepted as an analogue of organo-phosphate by these exchange systems; the affinity for phosphate is lower than for the organo-phosphate. PhoE pores are formed in *E. coli* cell membranes during phosphate limitation and have a preference for anions such as phosphate and phosphate-containing nutrients, facilitating the unspecific entry of phosphate into the cytoplasm by diffusion.

2. Arsenic transport:

It was studied that auqaporins facilitate the diffusion of metalloids such as arsenic (As) and antimony (Sb). The trivalent forms of these metalloids are structurally similar to glycerol at neutral pH and hence enter cells through aquaporins.

3. Magnesium transport:

Transport of Mg^{2+} into the cell is problematic, in spite of their largest hydrated radius, smallest ionic radius, and highest charge density. Transport systems for Mg^{2+} have been characterized well in *Salmonella typhimurium*. The CorA transport system is expressed constitutively and is the major Mg^{2+} transporter in Eubacteria and Archaea. It consists of three transmembrane domains, a large periplasmic domain, and no sequence homology to other proteins. The MgtE Mg^{2+} transporter also lacks sequence homology to other proteins, and it is unclear if Mg^{2+} transport is its primary function. The MgtA and MgtB Mg^{2+} transporters have sequence homology to P-type ATPases and closely related to the mammalian Ca^{2+} ATPases than to the prokaryotic P-type ATPases. Both transporters mediate Mg^{2+} influx with, rather than against its electrochemical gradient. Unlike CorA and MgtE, the MgtA and MgtC/MgtB loci are regulated, being induced by the two-component regulatory system PhoP/PhoQ. PhoQ is an Mg^{2+} membrane sensor kinase that phosphorylates the transcription factor PhoP under Mg^{2+} - limiting conditions. This factor then induces transcription of MgtA and MgtCB.

4. In hyperthermophillic Archaea, only tranporters of ABC type are useful in uptake of carbohydrates (e.g. glucose, cellobiose, maltotriose, arabinose, trahalose). This reflects an adaptation to the extreme habit, enabling organisms to acquire all available sugars very effectively.

Interesting facts:

- Transport system of a cell depends upon the substrate requirements of the cell, the bioavailability of the substrate, environmental conditions and membrane permeability.
- Phosphate can be transported either by low affinity pit or ATP dependent high affinity Pst system.
- In spite of largest hydrated radius, smallest ionic radius, and highest charge density of Mg^{2+}, its transport into the cell is problematic.
- Only tranporters of ABC type are useful in uptake of carbohydrates in hyperthermophillic Archaea.

Questions:

1. Transport of solutes across cells depends upon:
   a. Substrate requirements of the cell and bioavailability of the substrate.
   b. Environmental conditions and membrane permeability.
   c. Metabolic features and physiological state of the organism.
   d. All of the above.
2. The type of transport without any energy input in the cell is called:
   a. Passive transport
   b. Active transport
   c. Osmosis
   d. Plasymolysis
   e. Turgor pressure
3. Which of the following pieces of evidence would suggest that a substance entered a cell via active transport as opposed to passive transport?
   a. The substance moved from a high concentration to a low concentration.
   b. ATP was required for transport.
   c. The substance moved across the membrane via a carrier protein.
   d. None of the above.