Shree H.N.Shukla College of Science

Microbiology SEM-5

UNIT-5 BACTERIAL MEMBRANE & SIGNAL TRANSDUCTION

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UNIT-5 BACTERIAL MEMBRANE

Metabolic Transport

- A pure phospholipid bilayer is **permeable to water, gases (O_2, CO_2, N_2) and** small uncharged polar molecules (e.g. urea, ethanol), but is **impermeable to** large uncharged polar molecules (e.g. glucose), ions (Na^+, K^+, Cl^-, Ca^{2+}) and charged polar molecules (e.g. amino acids, ATP, glucose 6-phosphate).
- The first group of molecules can cross the membrane without an input of energy, whereas the latter group require the presence of **integral membrane transport proteins** and, in some cases, **an input of energy to travel through** impermeable membrane barrier.
- Thus the plasma membrane **maintaining** a distinct internal environment.

Passive transport

- The passive transport of molecules across a membrane does not require an input of metabolic energy. The rate of transport (diffusion) is proportional to the concentration gradient of the molecule across the membrane. There are two types of passive transport: **simple diffusion** and **facilitated diffusion**.

Simple diffusion

- Only relatively small uncharged or hydrophobic molecules (H2O, O2, CO2, other gases, urea and ethanol) cross the lipid bilayer by simple diffusion. No membrane proteins are involved, so there is no specificity.
- The molecule in aqueous solution on one side of the membrane dissolves into the lipid bilayer, crosses it, and then dissolves into the aqueous solution on the opposite side. The rate of diffusion is directly proportional to the concentration gradient of the molecule across the membrane and the process is not saturable.

Facilitated diffusion

- Unlike simple diffusion, the facilitated (or carrier-mediated) diffusion of a molecule across a biological membrane is dependent on **specific integral membrane proteins**, often called **uniporters** (see Topic E2). The molecule binds to the protein on one side of the membrane, the protein then undergoes a **conformational change**, transports the molecule across the membrane and then releases it on the other side. Molecules transported across membranes in this way include hydrophilic molecules such as glucose, other sugars and amino acids.
- The transport proteins are **specific** for one particular molecule or a group of structurally similar molecules. The transport proteins are capable of being saturated,

Active transport

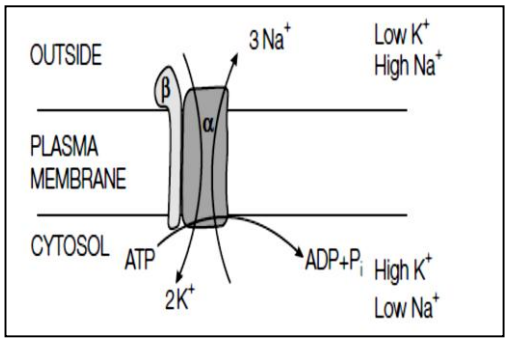
- The active transport of molecules requires an **input of metabolic energy**. This can be derived either from direct coupling to the **hydrolysis of ATP** or by coupling to the **movement of an ion** down its concentration gradient.

ATP-driven active transport

- In this case, the energy required for the transport of the molecule across the membrane is derived from the coupled hydrolysis of ATP, for example the movement of Na+ and K+ ions by the Na+/K+-ATPase. All cells maintain a high internal concentration of K+ and a low internal concentration of Na+. The resulting Na+/K+ gradient across the plasma membrane is important for the active transport of certain molecules, and the maintenance of the

membrane electrical potential. The movement across the membrane of Na+, K+, Ca2+ and H+, as well as a number of other molecules, is directly coupled to the hydrolysis of ATP.

Structure and action of the Na+/K+-ATPase



The Na+/K+-ATPase is an integral membrane protein consisting of 110 kDa _ and 55 kDa subunits. The functional unit is either a heterotetramer α orβ, more likely, a heterodimer. Upon hydrolysis of one molecule of ATP to ADP and Pi (the Pi transiently binds to an aspartyl residue in the protein), the protein undergoes a conformational change and three Na+ ions are pumped out of the cell across the plasma membrane and two K+ ions are pumped in the opposite direction into the cell. Both ions are being moved up their concentration gradients across the membrane; hence the requirement for an input of energy. No transport occurs unless ATP is hydrolyzed, and no ATP is hydrolyzed if there is no Na+ and K+ to transport (i.e. it is a **coupled system**).

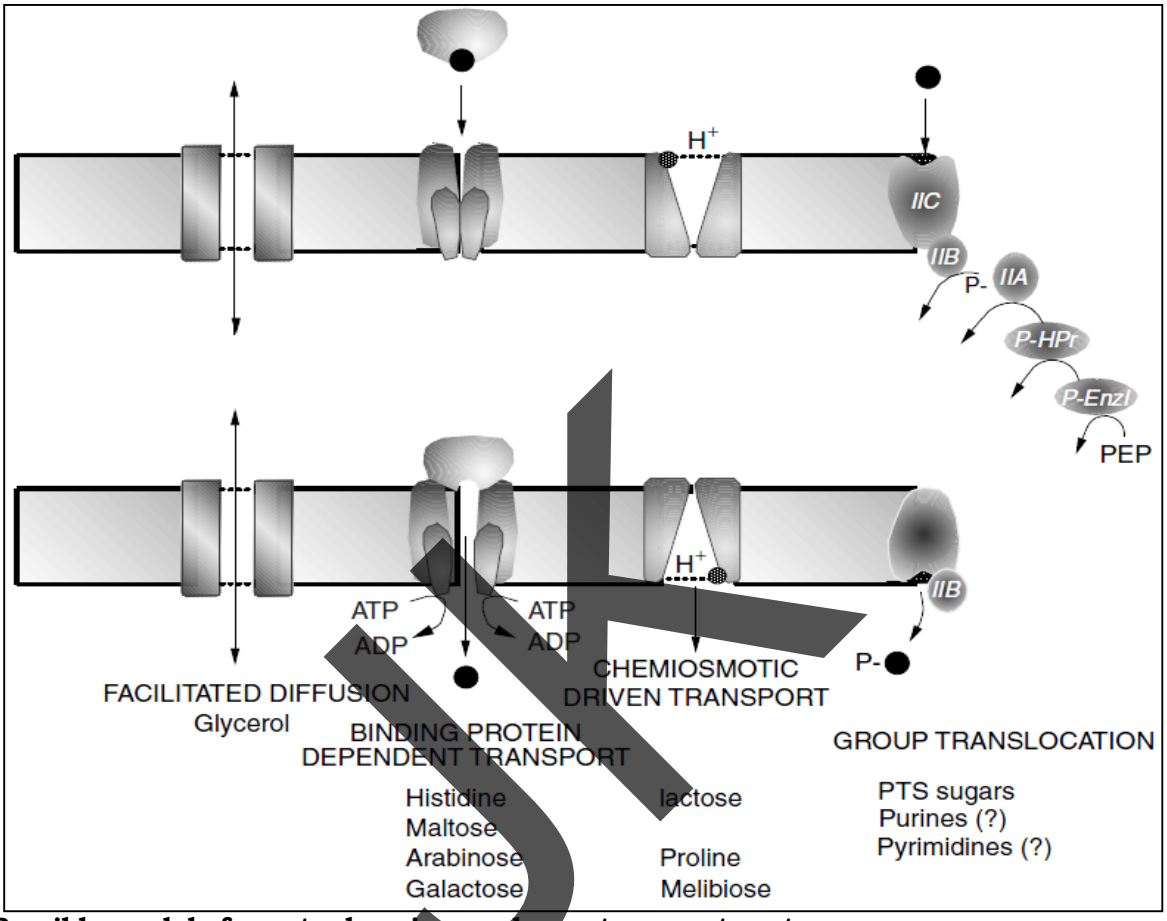
Mechanosensitive Channels

- ♣ Turgor pressure is defined as the outwardly directed pressure used by the cell to maintain shape and to allow for expansion of the cell membrane as the cell grows.
- ♣ The concentration of intracellular solutes (glutamate, potassium) determines the extent of turgor pressure (usually around 4 atm for *E. coli).*
- ♣ Upon transfer to medium that is lower in osmolarity, water will rapidly enter the cell.
- ♣ In the absence of mechanisms to reduce turgor, the pressure inside could rise to 11 atm, threatening the integrity of the cell.
- ♣ It has been proposed that catastrophic damage to the cell membrane is avoided by rapid activation of mechanosensitive channels (MscL, MscS).
- ♣ In contrast to **aquaporin, mechanosensitive** channels will permit inward or outward flow of ions.
- ♣ Transient opening of these channels allows K^+, glutamate, other compatible solutes, and ATP to exit and sodium and H^+ to enter. The result is a lowering of intracellular-compatible solute concentration and, thus, turgor

ATP-Binding Cassette Transporter Family

- ♣ Periplasmic proteins of gram-negative bacteria can be released into the surrounding medium if cells are subjected to osmotic shock. Among these periplasmic proteins are **binding proteins** for specific nutrients such as SO_4^{2-}, amino acids, sugars, and other compounds.
- ♣ In gram-positive bacteria, where no outer membrane is present to compartmentalize proteins, the binding proteins are present either as lipoproteins or as cell surface–associated proteins, bound to the external membrane surface via electrostatic interactions.
- ♣ The binding proteins function by transferring the bound substrate to a compatible membrane-bound complex of four proteins of which two are integral membrane proteins and two are peripheral, membrane-associated cytoplasmic proteins.
- ♣ It is remarkable that these transport systems, sometimes called **traffic ATPases.** The cytoplasmic members of these systems contain two specific ATP-binding motifs:
- ♣ The Walker A box which forms P-loop that interacts with the *γ phosphate of ATP.*
- ♣ Walker B box. As a result, these proteins are called ATP-binding cassette, or ABC proteins.

- The transport process is energized by ATP or other high-energy phosphate compounds such as acetyl phosphate. Approximately 40% of the substrates transported by *E. coli* involve periplasmic-binding protein and ABC transporter mechanisms.
- It appears with these systems that transfer of the substrate from the periplasmic-binding protein to the membrane complex triggers ATP hydrolysis, which in turn leads to the opening of a pore that allows unidirectional diffusion of the substrate to the cytoplasm.
- The **histidine permease of *E. coli*** is one of the best-studied examples of a traffic ATPase.



Possible models for cytoplasmic membrane transport systems.

Each of the systems probably has one or more membrane-spanning proteins that forms a specific channel in the cytoplasmic membrane. Facilitated diffusion and ion-driven transport systems require only one gene product, whereas binding protein-dependent transport and group translocation systems require several gene products. Facilitated diffusion allows entry and exit of substrate through a specific pore. Binding of substrate to specific sites accessible to the periplasmic side of the active transport systems coupled with an appropriate energy source allows a conformational change in the carrier proteins and substrate release inside the cell.

Chemiosmotic-Driven Transport

- These systems accomplish movement of a molecule across the membrane at the expense of a previously established ion gradient such a as proton motive or a sodium motive force.
- About 40% of the substrates that enter *E. coli* involve ion-driven transport.
- There are three basic types:
- **symport, antiport, and uniport.**
- **Symport** involves the simultaneous transport of two substrates in the same direction by a single carrier. For example, a proton gradient can allow symport of an oppositely charged ion or a neutral molecule. Transport of lactose by the LacY permease.

- **Antiport** is the simultaneous transport of two like-charged compounds in opposite directions by a common carrier. The Na^+/H^+ antiporters of *E. coli* are examples that are believed to be important for generating sodium motive force and maintaining neutral internal pH under alkaline growth conditions.
- **Uniport** occurs when movement of a substrate is independent of any coupled ion. Transport of glycerol is an example of uniport and was described above as facilitated diffusion.

Group translocation

- **Group translocation** couples transport of a substrate to its chemical modification (e.g., by attaching a phosphate or coenzyme A group to the substrate).
- This traps the substrate within the cell in a form different from the exogenous substrate, so the concentration gradient of unmodified substrate never equilibrates.
- The phosphotransferase system (pts) involved in the transport of many carbohydrates utilizes this approach.

Establishing Ion Gradients

- The establishment of ion gradients is of supreme importance to microorganisms.
- Proton and sodium gradients are important in various organisms for energy production and in transport.
- How the gradients are established and maintained ?
- The cell membrane is impermeable, for the most part, to charged ions, so the cell has an opportunity to control the flow of ions across the membrane through ion-specific transport systems.
- proton gradients are established principally by the electron transport systems that pump protons out of the cell as electrons are transferred down the system.
- There are also specific membrane-bound ATPases that can couple the hydrolysis of ATP with the export of H^+, Na^+, and K^+.
- There are a variety of antiport systems that can exchange ions such that one gradient can help build another (e.g., Na^+/H^+). So the picture that develops is of an interrelated series of ion circulations whose purpose is to provide energy to the cell.

SPECIFIC TRANSPORT SYSTEMS

ATP-Linked Ion Motive Pumps

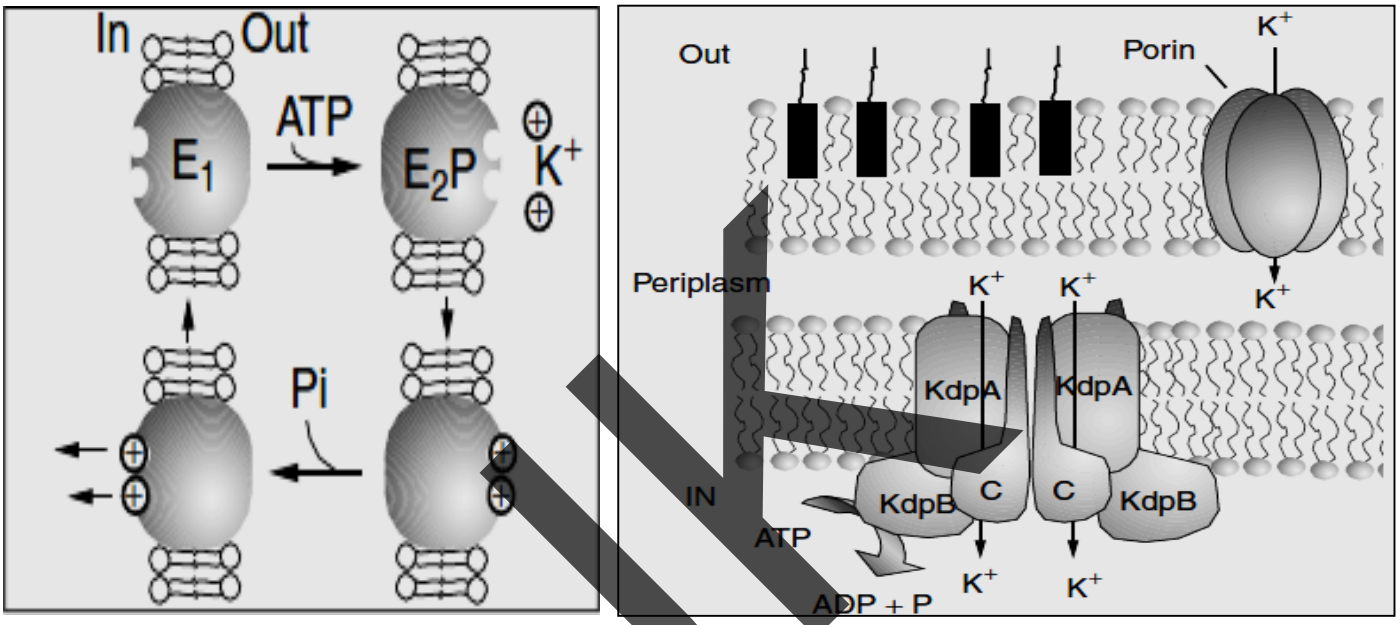
- The two major classes of these ion pumps are the F-type (F1F0) and P-type (E1E2) ATPases. The F-type ATPase is involved with pumping H+ out of the cell or in coupling H+ movement into the cell with the generation of ATP.
- The P-type ATPases are remarkably similar to each other despite the wide range of ions they transport.
- **They include potassium, magnesium, calcium, cadmium, and arsenate. All members have the following properties**:

  a phosphorylated intermediate two conformational forms of the phosphorylated intermediate, referred to as E1 and E2, which differ in reactivity to substrates and proteases a large (100 kDa) membrane-bound subunit with six to eight membrane-spanning regions and several regions of amino acid sequence homology.

- Most P-type ATPases only have this single large subunit. An exception is the Kdp ATPase.
- The **Kdp ATPase** is a three-subunit enzyme whose subunit ratio appears to be $A_2B_2C_2$, although the exact stoichiometry is unknown.

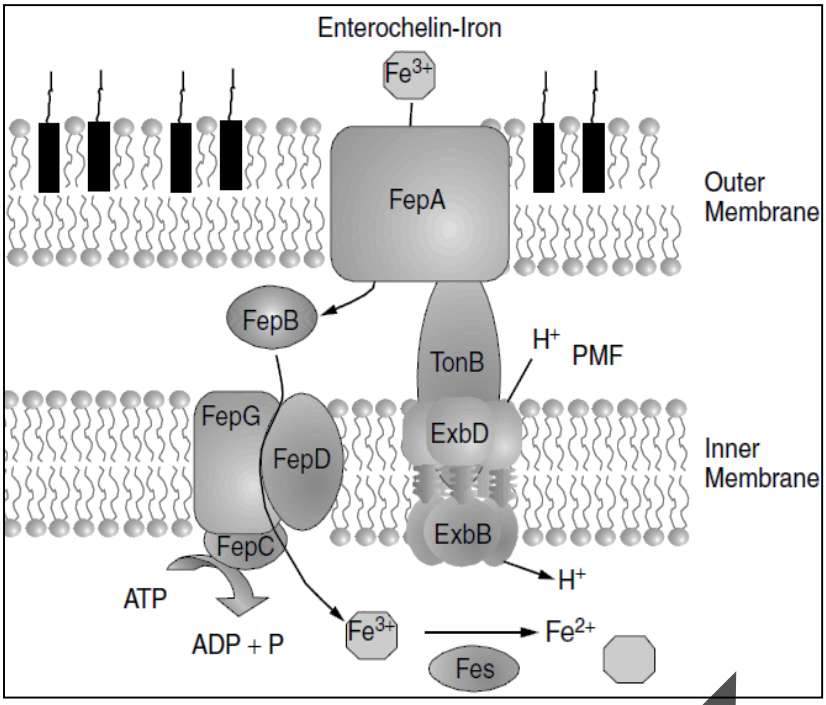
- All other P-type ATPases couple the export of one ion from the cytoplasm to the import of a different ion from outside the cell (e.g., Na^+K^+ ATPase).
- The Kdp ATPase is different from all other P-type ATPases in that it transports ions exclusively to the intracellular compartment.
- The low-energy state of the system (E_1) changes to high energy (E_2P) following phosphorylation
- In this state, sites on the exterior of KdpA are exposed and K^+ can enter.
- Opening of the KdpA membrane channel and dephosphorylation accompany movement of K^+ to the inner surface and a return of the complex to E_1. The number of ions transported per ATP hydrolyzed is not known.

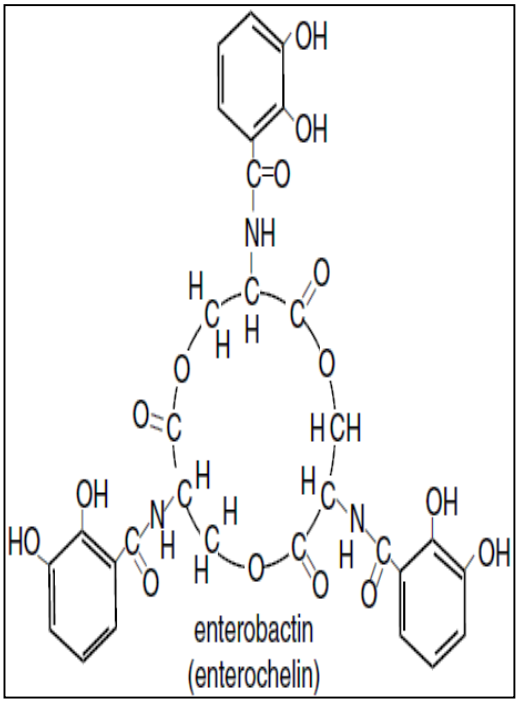
The Kdp potassium transport ATPase of *E. coli*



IRON TRANSPORT

- Iron is an essential nutrient for bacteria, serving as a cofactor for many enzymes and as a redox center in cytochromes and iron-sulfur proteins.
- However, iron is initially inaccessible to bacteria in the natural environment.
- Outside hosts, FeII rapidly oxidizes to FeIII and forms insoluble ferric hydroxide polymers.
- Bacteria have develop mechanisms to wrest iron from the environment.
- A common strategy, used also by our reference organism *E. coli*, involves the synthesis and secretion of small, high-affinity, iron-binding chelates (**siderophores**) that bind environmental iron.
- The iron chelate is then transported back into the cell by a specialized transport system and the iron is released.
- A major siderophore of *E. coli* is **enterochelin.** The mechanism by which desferrienterochelin is secreted remains a mystery.





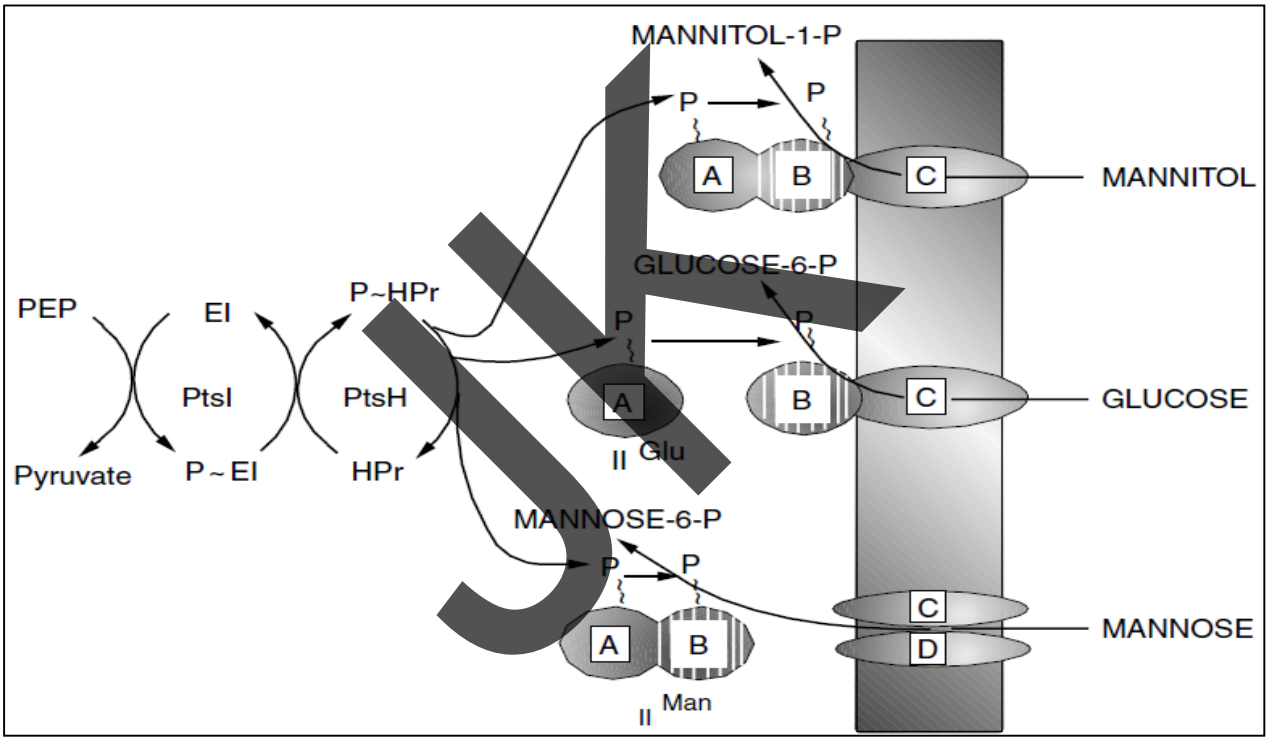
- Once in the environment, it will bind to Fe^{3+}, after which the products of the fep genes enable movement of ferrienterochelin back into the cell.

- The outer membrane FepA protein (79 kDa) functions as a monomer and enables transport of the complex across the outer membrane.
- It contains as many as 29 ß-sheet transmembrane spanning regions and an amino acid sequence called a **Ton box.**
- This region is predicted to contact the TonB inner membrane protein in a way that will "transduce" potential energy from the cytoplasmic membrane to FepA and drive transport of the siderophore across the outer membrane
- Two other inner membrane proteins, ExbB and ExbD, form a heterohexamer with a central channel that accommodates the transmembrane segment of TonB.

- PMF is harnessed by the passage of protons through the ExbDB hexamer. This in some way energizes TonB to interact with FepA, triggering a conformational change in FepA that facilitates ferrienterochelin transport to the periplasm.
- Next, a periplasmic ferrienterochelin-binding protein (FebB) delivers the complex to the inner membrane transport system comprised of FepG, FepD, and FepC.
- FepC is a membrane-associated ATPase that provides the energy required to transport of the complex through FepG and FepD and into the cytoplasm.
- Once inside the cell, the enterochelin backbone is cleaved by Fes esterase, reducing the affinity for iron.
- Regulation of this system is also interesting. The Fe^{3+} released in the cell is reduced by an unknown mechanism to Fe^{2+}. As ferrous iron accumulates in the cell, it is thought to bind to the regulatory protein **Fur** (ferric uptake regulator).
- Fur binds to a 17 bp consensus sequence (the IRON or FUR box) located in front of the seven transcriptional units in *E. coli* that contain the ent, fep, and fes genes and repress their expression.

PHOSPHOTRANSFERASE SYSTEM

- ♣ The phosphotransferase system (PTS) is involved in both the transport and phosphorylation of a large number of carbohydrates.
- ♣ In this group translocation transport system, carbohydrate phosphorylation is coupled to carbohydrate transport, the energy for which is provided by the EMP intermediate phosphoenolpyruvate (PEP).
- ♣ There are two proteins common to all of the PTS carbohydrates.
- ♣ They are enzyme I (ptsI in *E. coli)* and the histidine protein HPr (ptsH).
- ♣ They are soluble, cytoplasmic proteins that participate in the phosphorylation of all PTS carbohydrates in a given organism and therefore are called the **general PTS proteins.**
- ♣ In contrast, the enzyme IIs (EIIs) are carbohydrate specific. EIIs consist of three domains (A, B, and C) that may be combined in a single membrane-bound protein or split into two or more proteins (depending on the system) called IIA, IIB, and IIC.

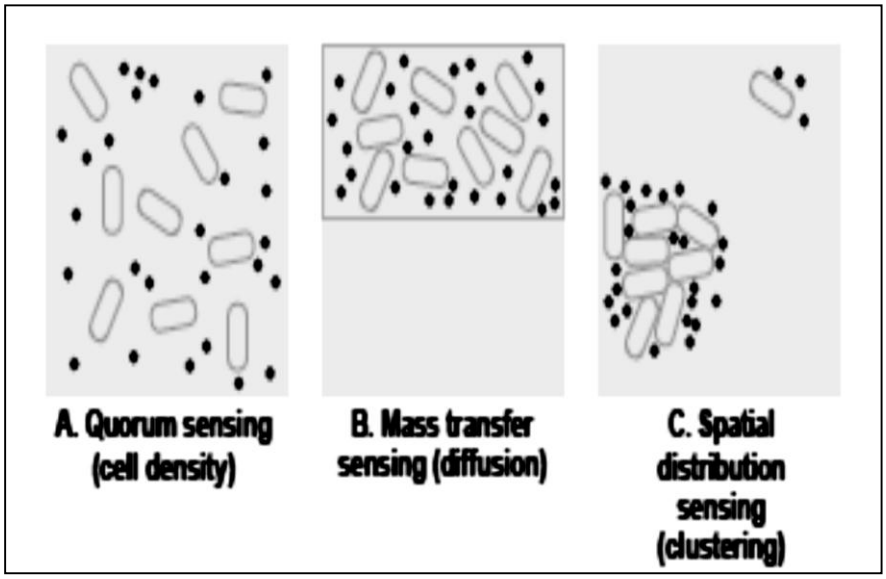


- ♣ The B and C domains may form one protein called IICB. Likewise, the A and B subunits may form one protein called IIAB.
- ♣ The phospho group from PEP is transferred to the incoming carbohydrate via phospho intermediates of EI, HPr, EIIA, and EIIB.
- ♣ EIIC forms the translocation channel and at least part of the specific carbohydrate-binding site.

Quorum Sensing

- ♣ QS Involves dedicated cellular systems for the production and detection of communication molecules, sometimes called quormones (quorum sensing pheromones). In bacterial species that employ QS, each cell secretes a basal amount of communication molecules at low cell density. As cell density increases, communication molecule concentration also increases, provided that the cells are not too far apart. Communication molecules bind to special receptors once their concentration exceeds a certain threshold. This in turn produces the physiological response.

- Secondly, bacteria may secrete SMs (Small molecules) that change their and other's behavior without QS (in the sense of cell density sensing) occurring (figure). It has been shown that in addition to population density, diffusion barriers can also be sensed in the same way. For example, **Staphylococcus aureus** can induce QS-dependent genes when confined in a host endosome. It has even been proposed that most of the pathways attributed to QS in fact are diffusion sensing pathways, although evidence for this is relatively scarce. As there seems to be no way to measure cell density that does not at the same time measure diffusion and spatial distribution, it may not be correct to assume that QS is always about cell density.



  - In bacteria, QS regulated phenotypes include bioluminescence, exo-polysaccharide production, virulence, conjugal plasmid transfer, antibiotic and exo-enzyme production, biofilm formation, and growth inhibition.

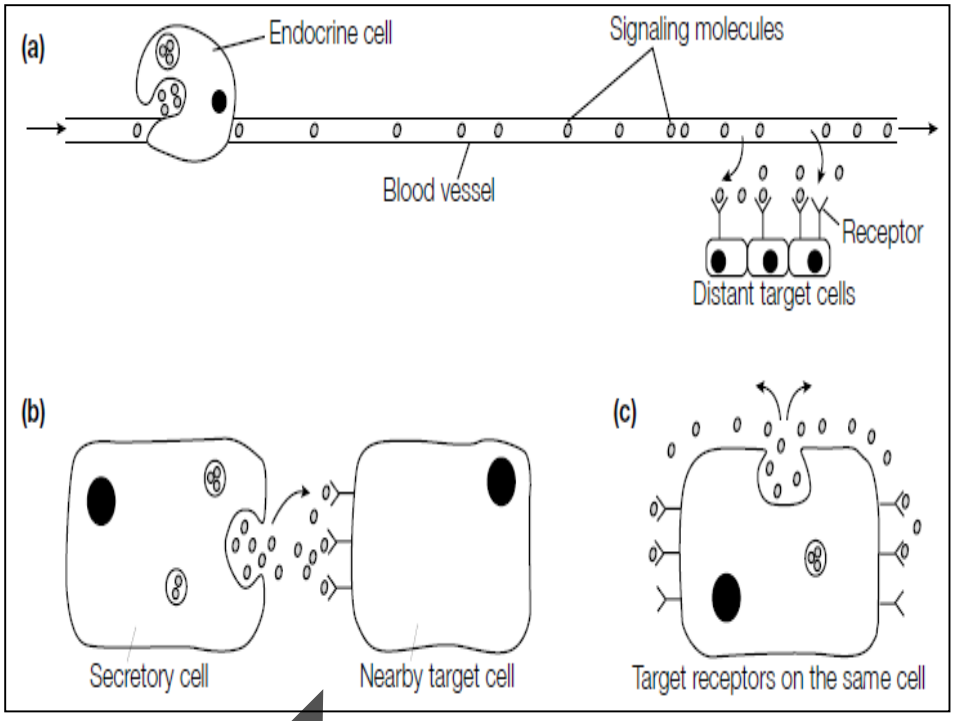
Acyl homoserine lactones

- Types of communication molecules discussed in this section are acyl homoserine lactones (AHLs), AI-2 molecules, and modified oligopeptides. AHLs mostly affect transcription via a one-component signal transduction system, where the protein domain that binds the SMs is fused to a DNA binding domain. Peptide communication molecules and AI-2, on the other hand, often affect transcription via two-component signal transduction systems composed of a histidine kinase and a response regulator protein
- The term 'autoinducer' conveys that the synthesis of AHLs is regulated by positive feedback, as has been discovered in the marine bacterium ***Vibrio fischeri***. AHLs are composed of a homoserine lactone ring with an attached fatty acid chain, AHLs are synthesised from **S-adenosyl methionine** and fatty acid carrier proteins by **LuxI** and its homologues.
- AHLs cross membranes by diffusion and bind **LuxR-like** response regulators. LuxR-like response regulators simultaneously act as sensors and transcription factors This group of signal transduction systems, where the signal binding domain and the transcription-regulating DNA-binding domain are fused, are referred to as one-component signal transduction systems. They have been shown to be the most common sort of bacterial signal transduction systems.

SIGNAL TRANSDUCTION

- Cells communicate with one another in multicellular organisms using extracellular signalin molecules or hormones. The hormone is secreted by the signaling cell and then binds to a receptor on the target cell, initiating a response in that cell.
- In endocrine signaling the hormone acts at a distant site in the body from where it was produced, in paracrine signaling the hormone acts on nearby cells, and in autocrine signaling the hormone acts on the same cell from which it was secreted.

- In **endocrine** signaling, the signaling molecule (e.g. insulin) acts on target cells distant from its site of synthesis in cells of an endocrine organ.
- In **paracrine** signaling, the signaling molecule affects only target cells close to the cell from which it was secreted. The communication from one nerve cell to another by chemical neurotransmitters is an example of paracrine signaling.
- The third type of cell signaling is **autocrine** signaling, where a cell responds to a molecule that it has produced itself.



Hormones

The signaling molecules or hormones can be classified based on their solubility and the location of their receptor.

Lipophilic hormones with intracellular receptors

- Small lipophilic (lipid-soluble) hormones diffuse across the plasma membrane and then interact with intracellular receptors in the cytosol or nucleus. The resulting hormone–receptor complex often binds to regions of the DNA and affects the transcription of certain genes. Small lipophilic hormones with intracellular receptors include the steroid hormones which are synthesized from cholesterol.

Lipophilic hormones with cell-surface receptors

- The principal lipophilic (lipid-soluble) hormones that bind to receptors located in the plasma membrane are the prostaglandins, a family of structurally similar compounds that are found in both vertebrates and invertebrates. Prostaglandins are synthesized from arachidonic acid (a 20-carbon fatty acid with four unsaturated double bonds) and act as paracrine signaling molecules. **Aspirin** and other anti-inflammatory agents inhibit the synthesis of prostaglandins.

Hydrophilic hormones with cell-surface receptors

- All hydrophilic (water-soluble) molecules (which cannot diffuse across the hydrophobic interior of the lipid bilayer) bind to receptors in the plasma membrane. There are two subclasses of hydrophilic hormones: (1) peptide hormones such as insulin and glucagon; and (2) small charged molecules, often biogenic amines, such as epinephrine (adrenalin) and histamine that are derived from amino acids and function as hormones and neurotransmitters.

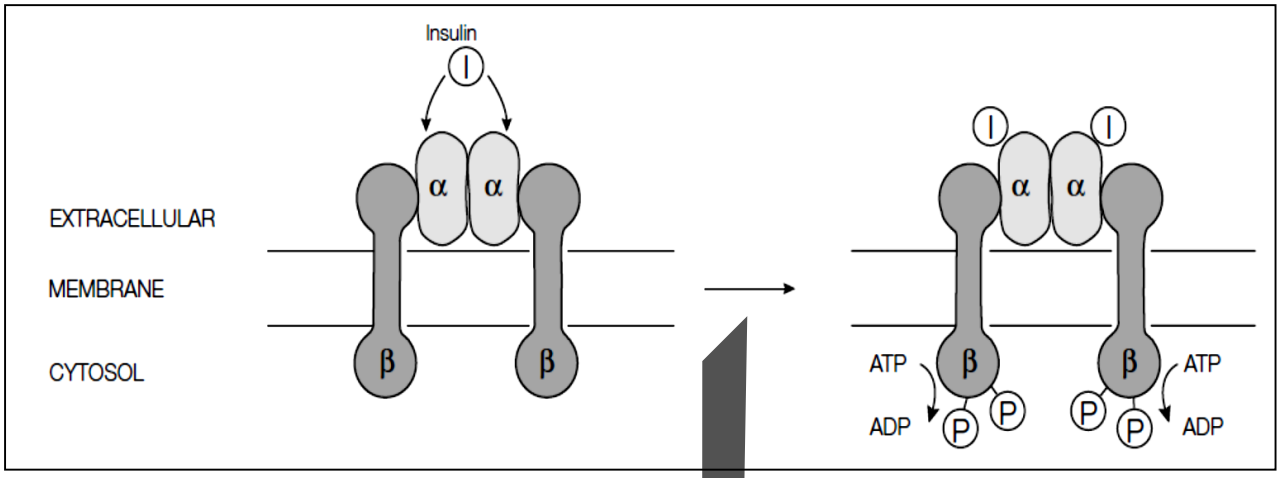
Cell-surface receptors

- Hydrophilic and some lipophilic hormones bind to cell-surface receptors. These are integral membrane proteins situated in the plasma membrane that bind the signaling molecule (ligand) with high affinity. The ligand binds to a specific site on the receptor in much the same way as a substrate binds to an enzyme. Binding of the ligand to the receptor causes a conformational change in the receptor that initiates a sequence of reactions in the target cell (often referred to as signal transduction) leading to a change in cellular function.

- Cell-surface receptors can be classified into three classes depending on how they transfer the information from the ligand to the interior of the cell: **enzyme-linked receptors, ion channel-linked receptors and G protein-linked receptors.**

Enzyme-linked receptors

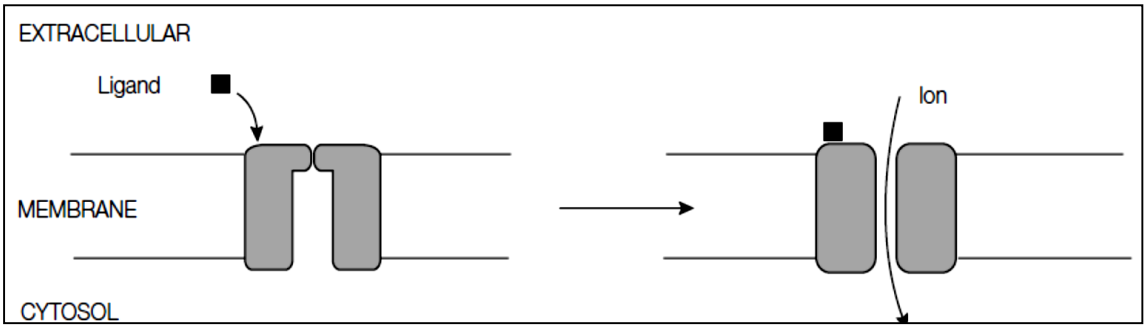
- On binding of the ligand to its extracellular face, the cell-surface receptor undergoes a conformational change and activates an intrinsic enzyme activity. In the case of the insulin receptor which is a complex of two α- and two β-subunits held together by disulfide bonds, the polypeptide hormone insulin (the ligand) binds to the extracellular face of the α-subunits.



- The receptor then undergoes a conformational change leading to the auto phosphorylation (self-phosphorylation) of the cytosolic domain of the β-subunit. Specifically the hydroxyl groups in the side chains of certain tyrosine residues are phosphorylated, with ATP being the phosphate donor. The phosphorylated receptor is then recognized by other proteins in the cytosol that in turn modulate various intracellular events, allowing the cell to respond to the hormone appropriately.

Ion channel-linked receptors

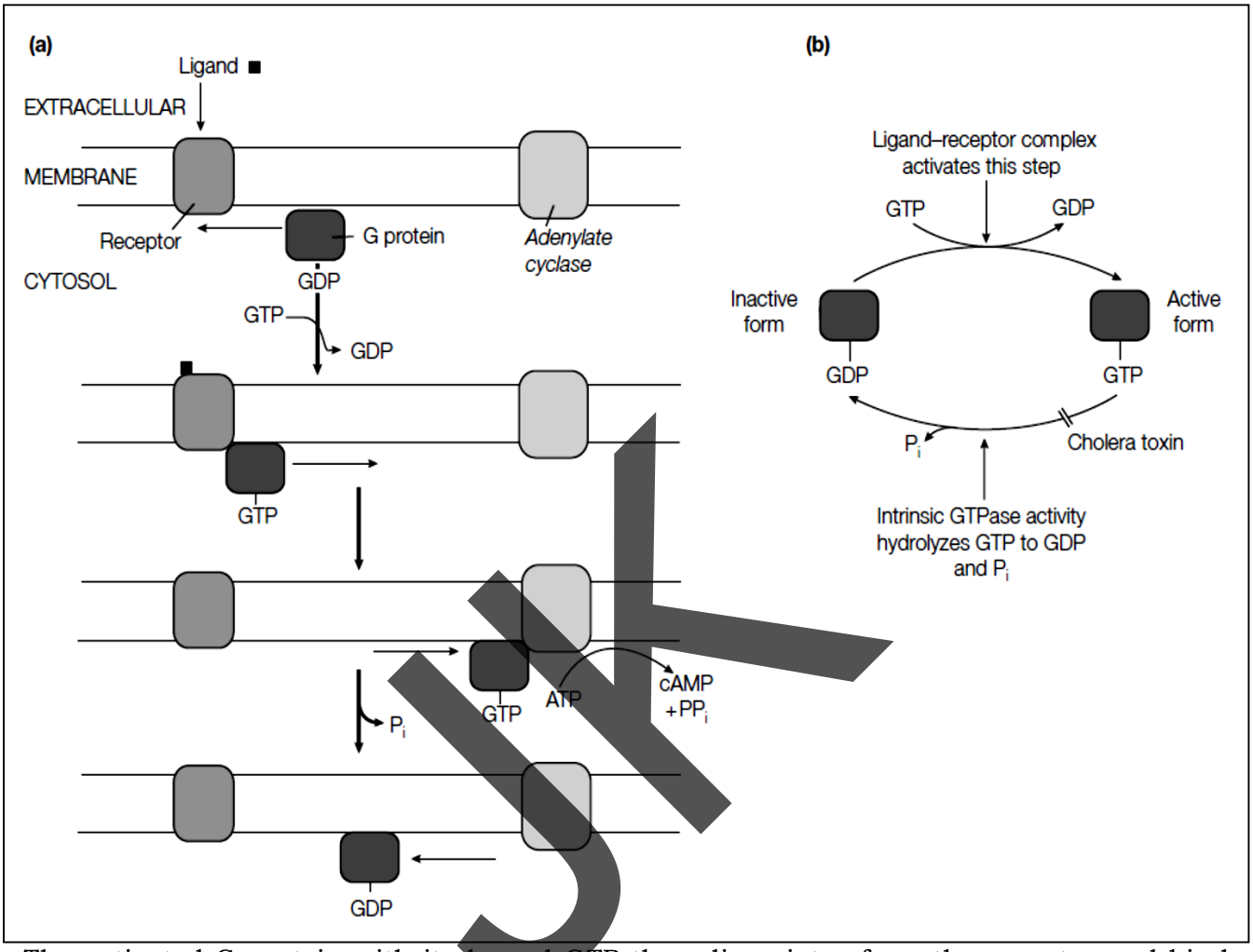
- Here binding of the ligand again causes a conformational change in the protein but this time such that a specific ion channel is opened. This allows a certain ion to flow through that subsequently alters the electric potential across the membrane. For example, at the nerve–muscle junction the neurotransmitter acetylcholine binds to specific receptors that allow Na+ ions to flow into and K^+ ions out of the target cell.



G protein-linked receptors

- On binding its ligand, a G protein-linked receptor activates G proteins [guanyl nucleotide (GTP)-binding proteins] which in turn activate or inhibit either an enzyme that generates a specific second messenger or an ion channel, causing a change in the membrane potential. Epinephrine and glucagon act through interaction with G protein-linked receptors.

- The majority of G protein-linked receptors contain seven transmembrane α-helices. Thus they have a similar overall shape to that of bacteriorhodopsin (which is not a receptor).
- G proteins are localized on the cytosolic face of the plasma membrane and act as on–off molecular switches. When it has guanosine diphosphate (GDP) bound, the G protein is in the 'off' state. The activated receptor causes it to release the GDP and exchange it for GTP, converting it to the 'on' state.



- The activated G protein with its bound GTP then dissociates from the receptor and binds to and activates an effector enzyme (e.g. adenylate cyclase) which in turn catalyzes the formation of a second messenger (e.g. cAMP). The G protein then hydrolyzes the bound GTP, causing it to revert back to the 'off' state.
- Cholera toxin acts by inhibiting the intrinsic GTPase activity of the G protein, with the result that once activated to the GTP-bound state the G protein cannot be turned off again.

Secondary messengers

- The binding of ligands to many G protein-linked receptors leads to a short lived increase in the concentration of certain intracellular signaling molecules called second messengers. (The hormone/ligand can be considered as the first messenger.)
- The major second messengers are 3',5'-cyclic AMP (cAMP), 3',5'- cyclic GMP (cGMP), inositol 1,4,5-trisphosphate (IP3), 1,2-diacylglycerol (DAG) and Ca2+.
- The elevation in the level of one or other of these second messengers then leads to a rapid alteration in cellular function. cAMP and cGMP are derived from ATP and GTP by the actions of adenylate cyclise. and guanylate cyclase, respectively. For example, the action of glucagon on glycogen metabolism is mediated through the second messenger cAMP.

- IP3 and DAG are derived from the membrane lipid phosphatidylinositol 4,5- bisphosphate (which is a phosphorylated derivative of phosphatidylinositol; by the action of phospholipase C which is also located in the plasma membrane and, like adenylate cyclase, is activated by G proteins. One of the main actions of the polar IP3 is to diffuse through the cytosol and interact with Ca2+ channels in the membrane of the ER, causing the release of stored Ca2+ ions which in turn mediate various cellular responses.
- The DAG produced by the hydrolysis of phosphatidylinositol 4,5-bisphosphate, along with Ca2+ ions released from the ER, activates protein kinase C, a membrane-bound enzyme that phosphorylates various target proteins, again leading to alterations in a variety of cellular processes.