Bioinorganic Chemistry

Unit I: Metal ion storage and transport

Contents: Factors responsible for storage and transport of iron, Ferritin and Transferrin (structure and function). Biomineralization of Fe, Si, P and Ca. Transport of Ca^{2+}. Active ion transport across cell membrane.

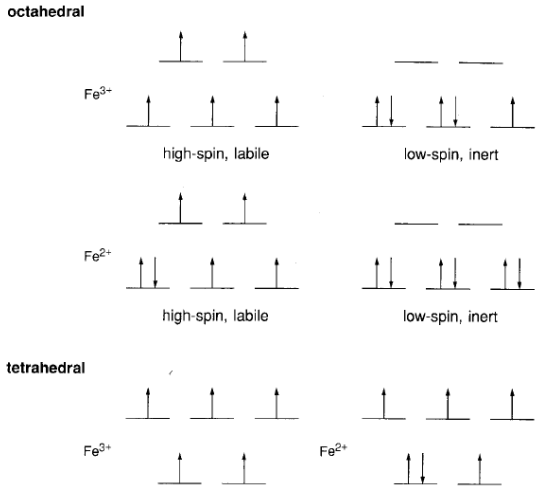
Lecture 1,2

Biochemical role of Fe

- Fe is the most abundant transition metal in biology.
- All plants, animals, and bacteria use Fe, except for lactobacillus.
- Its two oxidation sates viz. Fe (II) and Fe (III) are interconvertible
- Fe is crucial to the survival of living organisms and plays role in
  - (i) Ribonucleotide reduction (DNA synthesis),
  - (ii) Energy production (respiration)
  - (iii) Energy conversion (photosynthesis)
  - (iv) Nitrogen reduction
  - (v) Oxygen transport (respiration, muscle contraction)
  - (vi) Oxygenation (steroid synthesis, solubilization and detoxification of arenes)

Chemical Properties Relative to Storage and Transport

- Transport behavior of Fe is mostly governed by its redox chemistry, hydrolysis, and the solubility in various complexes, particularly the hydroxides.
- The variation in the concentration of Fe in rivers (depending on water source) is the consequential effects of solubility
- Amount of dissolved iron in the form of free Fe (III) ion or its hydrolysis product (irrespective of water source) is extremely low.
- The concentration of free Fe (III) ion is extremely low at neutral pH ($K_s \sim 10^{-18}$ M).
- Different iron-coordination environments alter the chemical properties of iron (ligand field can strongly alter the structural and ligand exchange properties)
- The centers, both Fe (II) and Fe (III) in high spin state (with bioligands) are quite labile and complexation occurs rapidly
- Their low spin (an axially ligated porphyrin) complexes are inert
- Depending on the environment, they can adopt both Td and Oh geometries.
- Bulky ligands such as those provided by metalloprotein and enzyme sites, can cause a tetrahedral environment, where both Fe (II) and Fe (III) ions form HS complexes



- The distribution of Fe in specific Fe-complexes depends strongly on their function.
- About 70% of total Fe is present in Hb (oxygen-transport) and Mb (muscle oxygen-storage)
- It is also incorporated in other metalloproteins (catalase, peroxidase, Fe-S protein, cytochromes)
- Fe is also present in ferritin and transferrin
- 70% of daily requirement is required in biosynthesis of blood (not absorbed from diet)
- Destruction of RBC gives about 20 mg Fe and rest comes from Fe-storage site
- Redox potential of Fe(III)/Fe(II) couple can be monitored (0.77 to -0.50 V) by changing the ligand environment
- For example, the standard reduction potential for Fe(III) ion in acid solution is 0.77 V (ferric ion is a good oxidant). In contrast, cytochrome c has a redox potential of 0.25 V
- In polynuclear ferredoxin (each Fe is tetrahedrally coordinated by S), reduction potential ~ 0.4 V

Protein	**MW (kDa)**	**Function**	**Coordination sphere**
Hemoglobin, Fe (II)	64.5	Plasma O_2 transport	Heme (4-Fe)
Myoglobin, Fe (II)	17	Muscle O_2 transport	Heme (1-Fe)
Transferrin, Fe (III)	76	Plasma Fe transport	Non-heme (2-Fe)
Ferritin, Fe (III)	444	Cell Fe-storage	Non-heme (4500-Fe)
Hemosiderin, Fe (III)		Cell Fe-storage	Non-heme (5000-Fe)
Catalase, Fe (II)	280	H_2O_2 metabolism	Heme (1-Fe)
Cytochrome c, Fe (II/III)	12.5	Electron transport	Heme (1-Fe)

In-vivo storage and transport of iron

- Two main problems

(i) Insolubility of Fe (III): At physiological O_2 concentrations, Fe (II) is readily oxidized to Fe (III), which is highly insoluble in aqueous solution at normal pH.

(ii) Toxicity of free Fe species through the generation of free radicals causing severe cell damage.

- Nature has developed sophisticated chemical system to execute and acquisition to its subsequent transport, storage, and utilization in tissue.

- ❖ Fe storage system must be able to respond to 'supply and demand' (store the excess, and must mobilize & release when needed), as the amount of Fe in the diet is variable.
- ❖ Ferritin stores and transferrin transports Fe in mammals.
- ❖ Siderophore stores and transports Fe in microorganisms.

Storage of Iron

- ❖ Three properties of Fe accounts for its extensive use in biological processes

(i) facile redox reactions of iron ions;

(ii) an extensive range of redox potentials available by ligand substitution

(iii) abundance and availability under conditions apparently existing when terrestrial life began

- ❖ The combination of the reactivity of Fe (II) ion and the relatively large amounts of Fe used by cells have necessitated its storage
- ❖ The transition in the atmosphere (about 2.5 billion years ago) resulted in drop in bioavailability of Fe thereby increasing the need for its storage.
- ❖ Comparison of the solubility of Fe^{3+} at physiological conditions (~10^{-18} M) to the Fe content of cells (~ 10^{-5} to 10^{-8} M) emphasizes the difficulty of acquiring sufficient Fe

Ferritin

- ❖ Ferritin is a storage protein for Fe in non-toxic form.
- ❖ It is present in liver, spleen, and bone marrow, and in plants and bacteria.
- ❖ Ferritin is known to release Fe to the developing fetus
- ❖ It consists of Fe-mineral core (hydrated Fe (III) oxide) surrounded by a protein coat/sheath (apoferritin) with varying amount of phosphate
- ❖ The diameter of the core is ~ 80Å and ~ 4500 Fe atoms can be reversibly stored inside the protein coat.
- ❖ The lipophilic sheath makes the Fe (III)-complex soluble in biological fluid.
- ❖ Apoferritin allows controlled access to the core though eight hydrophilic channels (enter) and six hydrophobic channels (leave)
- ❖ Fe (III) ion is of high spin nature and is subjected to strong antiferromagnetic coupling
- ❖ The role of the stored iron in ferritin varies (intracellular use for biosynthesis of Fe-proteins or mineralization, long term storage and detoxification of excess Fe)
- ❖ Iron regulates the synthesis of ferritin; large amounts of ferritin is associated with Fe excess and its small amounts is associated with Fe deficiency.
- ❖ Ferritin is also known to be a precursor of several forms of Fe in living organisms (hemosiderin in lysosomes of animals – Fe complex with protein is insoluble).
- ❖ Magnetite (Fe_3O_4) is another form of biological Fe derived from the Fe in ferritin. It plays a role in the behavior of magnetic bacteria, bees, and homing pigeons

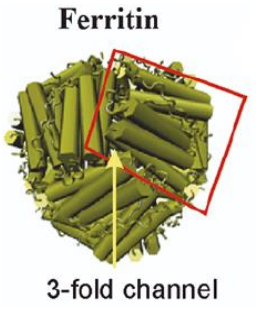
Structure of ferritin

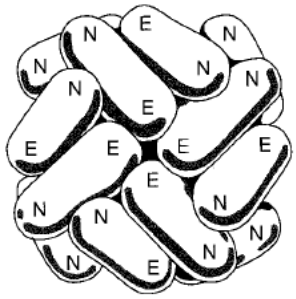
- ❖ The structure of ferritin can be considered to consist of three units: the protein coat, iron-protein interface, and Fe-core

Protein coat/sheath (Apoferritin)

- ❖ The protein sheath consists of subunits of 24 polypeptide chains (about 175 amino acid) folded into ellipsoids (lozenge like shape; MW=20000 D).

- Each subunit is approximately cylindrical & linked together to form a hollow sphere with two-fold, three-fold and four-fold axes.
- The hollow sphere is about 100 Å, the organic surface is about 10 Å thick.
- Two ends of the apoferritin subunit are designated as N (the polar N-terminal end of the protein) and E (the non-polar helical segment)
- At 8 places, three subunits meet with their N-ends to form a polar channel of three-fold symmetry (C_3) through which Fe-can be transferred in or out.
- These three-fold polar channels are lined up with hydrophilic aspartate or glutamate residues
- There are 6 non-polar channels of C_4 symmetry produced by the meeting of four subunits with their E-ends.
- These 4-fold non-polar channels are lined up with hydrophobic amino acid residues.
- The protein coat is stable with or without Fe, and hence the center of the hollow sphere may be filled with solvent and/or $Fe_2O_3.H_2O$.
- Amino acids required to form the shape of the protein coat & the ligands for Fe-core is not established (perhaps tyrosine acts as an Fe (III)-ligand)





Iron-Protein interface

- At the Fe-protein interface, smaller clusters of Fe(III) centers (bridged to each other by oxo/hydroxo bridges) are attached to the protein.
- The coordination of Fe to the protein occurs via carboxyl groups from glutamic (Glu) & aspartic (Asp) acids (EXAFS - Extended X-ray Absorption Fine Structure & Mossbauer spectra)

Iron core

- The composition of the microcrystalline core is $[(FeOOH)_8(FeO).H_2PO_4]$
- The core is primarily consists of a sheet structure of Fe(III)-oxide, similar to the mineral ferrihydrite ($5Fe_2O_3.9H_2O$)
- All the Fe-centers are octahedrally surrounded by oxygen
- The core provides a close packed matrix of O^{2-} ions with Fe(III) ions randomly distributed in the octahedral holes (EXAFS-study)
- The HS Fe(III) centers undergo strong antiferromagnetic coupling.
- Hydroxide and phosphate groups present in the core helps in counterbalancing the charge and binding at the protein surface
- Fe(III) atom in ferritin is surrounded by six O-atoms at a distance of 1.95 Å and six Fe- atoms at distances of 3.0 to 3.3 Å (Mossbauer spectroscopy and EXAFS study)
- Ferritin cores vary in their degree of structural, magnetic ordering and/or the level of hydration.

- Structural differences in the iron core have been associated with variations in the anions present (phosphate or sulfate), and with the electrochemical properties of iron.
- Formation of ferritin core is a representative example of biomineralization process. This biomineral is insoluble but remains soluble in biological fluids as a complex having protein sheath.

Mechanism of Fe-core formation

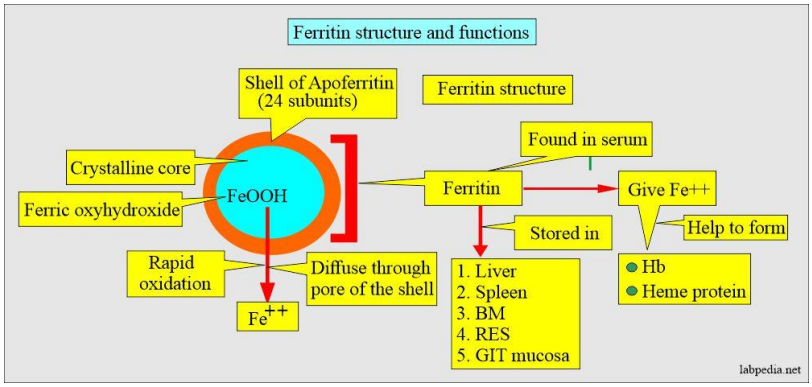
- Fe(II) and dioxygen are required for core formation especially in the early stages.
- The core is formed from aqueous Fe(II) & its oxidation to Fe(III) follows its incorporation.
- Fe-gets into the core through channels in the protein and then transferred into the cavity to form first diiron-oxo dimers.
- Aggregates and clusters are formed via a progression of oligomers related to iron hydrolysates.
- Oxidation to Fe(III) and hydrolysis produce one e^- and 2.5 H^+ for incorporation of each Fe-atom into the iron core (for 4500 Fe-atoms, 4500 e^- & 11250 H^+)
- The protons are released & electrons are transferred to dioxygen (the relative rates of proton release, oxo-bridge formation, & electron transfer are not known in detail).
- If all the protons were retained, the pH would drop to 0.4
- When large numbers of Fe(II) atoms are added, the protein coat appears to stabilize the encapsulated Fe(II).
- An Fe(III)-tyrosinate (Fe(III)-ligand) complex is proposed to be a transient precursor to polynuclear cluster formation (UV-Vis and resonance Raman spectroscopy)

Iron storage mechanism (proposed)

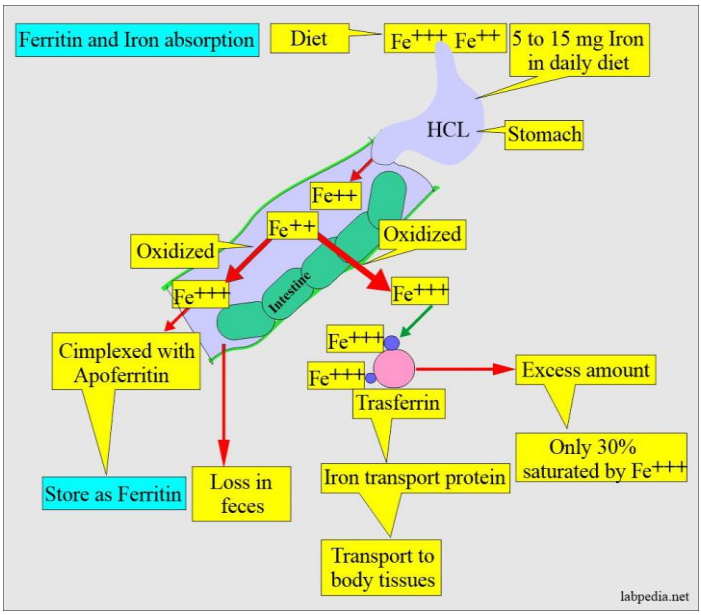
- The mechanism for the reversible incorporation of Fe in ferritin involves its transport in and out as Fe(II).
- There are 8 hydrophilic and 6 hydrophobic pores of apoferritin.
- The 24 subunits arranged in such a way that at 8 places, 3 subunits meet with their N-ends to form a polar channel (hydrophilic pores) through which Fe can be transferred in or out.
- There are also 6 non-polar channels (hydrophobic pores) produced by the meeting of four subunits with their E-ends.
- Iron is taken up in the labile ferrous form prior to oxidation.
- Release of iron requires reduction of the core (Fe(III) to Fe(II)) with a biological reductant (NADH – nicotinamide adenine dinucleotide + hydrogen)
- The mobile Fe(II) is oxidized to Fe(III) at specific di-iron binding sites known as ‘ferroxidase centers’, present in each of the subunits.
- Oxidation to Fe(III) involves the coordination of O_2 and inner sphere electron transfer:

$$2Fe(II) + O_2 + H^+ \rightarrow 2Fe(III) + H_2O_2$$

Summary of Ferritin structure and functions



Summary of ferritin and Iron absorption

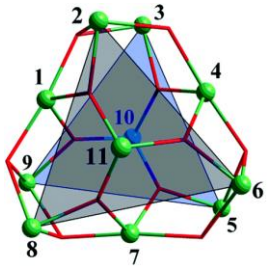


Hemosiderin

- Hemosiderin is also an Fe-storage protein in several mammalian organs.
- It is proposed to be a degradation product of ferritin and probably functions like ferritin
- It contains larger amount of iron hydroxide than ferritin with variable constitution
- In liver and spleen, it exists as insoluble iron-protein granules.
- It also contains apoprotein called aposiderin
- The iron core in hemosiderin is similar to the ferrihydrite cores of ferritin
- In the erratic metabolism (due to genetic disorder), excessive deposits of Fe-occur in the form of insoluble hemosiderin granules in liver & spleen in different diseases like hemosiderosis, hemochromatosis
- In hemochromatosis, deposition of iron occurs in liver, spleen, pancreas, skin etc. due to breakdown of premature RBC. It leads to cirrhosis of liver, pancreatic fibrosis and bronze pigmentation on skin. This is why; this disease is called bronze diabetes.

Synthetic iron-oxo aggregates

- By controlling the extent of hydrolysis of Fe(III) in variety of oxo-and hyroxobridged oligomers, polynuclear iron-oxo units (high nuclearity iron-oxo clusters) found in biological systems have been reported.
- The crystal structure of the cluster $[Fe_{11}O_6(OH)_6(O_2CPh)_{15}]$ has been well documented.
- The 11 iron atoms, which are found at the vertices of a distorted, pentacapped trigonal prism with three-fold symmetry, are stacked along the three-fold axis.
- Six triply bridging O-atoms connect the Fe-atoms leading to 18 short Fe-O bonds and 6 pyramidal triply bridging O-atoms further link the Fe-atoms in layers, giving rise to 18 Fe-OH bonds.
- The structure is completed by 15 bridging groups giving a further 30 Fe-O bonds and each of the 11 Fe-atoms has a distorted octahedral coordination geometry.



Iron transport -Transferrin (Tf)

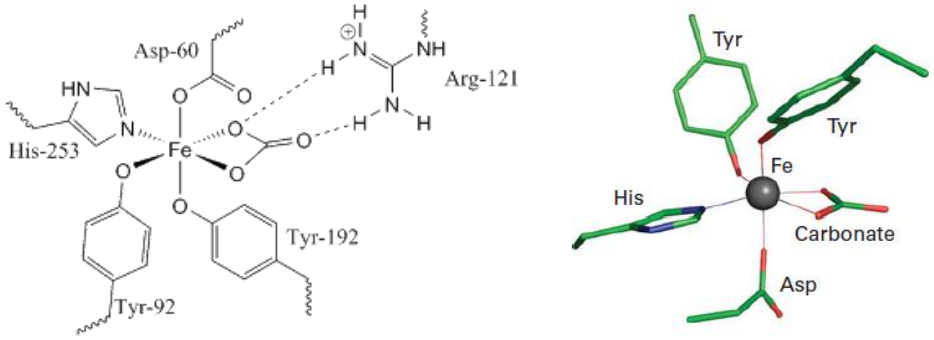
- Transport of Fe-from ferritin or hemosiderin occurs via the serum-transport protein transferrin.
- Serum transferrin is a monomeric glycoprotein with high MW ≈ 80000 D
- Serum transferrin (blood plasma - most studied), conalbumin or ovatransferrin (egg white) and lactoferrin (mother's milk) are examples of Fe-transport protein.
- Serum transferrin mainly functions to transport iron from the Fe-core and breakdown cells of RBC to the place of biosynthesis of RBC. Excess iron is also transported back to the ferritin.
- Tf is also present in tears, serving to cleanse eyes after irritation.
- Ovatransferrin acts as antibacterial agent, lactoferrin is a potent antibacterial Tf (protects from infectious disease) and serum Tf is a potential Fe-transporter.
- Transferrin is bilobal, with each lobe reversibly (independently) binding Fe(III) ion (binding constant $>10^{20}$ M^{-1})
- This complexation of the metal cation occurs via prior complexation of a synergistic HCO_3^- or CO_3^{2-} ion.
- LMCT from phenolate to Fe accounts for salmon pink color of transferrin
- Binding affinity for Fe(III) decreases progressively with decreasing pH
- Tf binds only two Fe-atoms (efficient) and delivers iron by interacting with the tissues where required

Apo-transferrin

- Transferrin not bound to iron (free Tf)
- It has two iron binding sites per molecule and are similar but not identical.
- For each site, the binding constant (K_A) under physiological condition is ~10^{26}.
- It binds Fe(III) so strongly that no other protein can snatch the iron effectively from it.
- Although the two iron-binding sites of transferrin are different, their coordination environments are quite similar.

Structure of Transferrin

- Fe site in human lactoferrin has been determined by XRD (E. Baker *et al.*)
- Transferrin is an ellipsoidal protein with two subdomains or lobes (almost similar), each of which binds iron.
- Polypeptide chain contains 679 amino acids.
- Serum transferrin contains about 6% carbohydrates linked to the protein, and affect the recognition & conformation of the native protein.
- Fe-atom in Tf is coordinated to four amino acid residues viz. two tyrosines (one phenolate oxygen each), one histidine (N-atom) & one aspartate (O-atom of carboxylate group).
- A bidentate CO_3^{2-} ion occupies the remaining two sites of the distorted octahedral environment.
- It sits in a pocket between the Fe-atom & two positively charged protein chains viz. an arginine side chain and a helix N-terminal.
- Sometime, a carboxylate ligand can also bind instead of CO_3^{2-} ion.
- Fe is buried deep in cleft between two protein domains, with two polypeptide strands behind it.
- Flexing of these strands alters the conformation providing driving force for Fe-release



Binding and transport/release of Fe

- Fe(II) in stomach is oxidized to Fe(III), catalyzed by ceruloplasmin (Cu-protein)
- On passing from stomach to blood (pH=7.4), oxidation of Fe(II) occurs
- Fe(III) binds to apo transferrin effectively (no other bioligand can compete)
- Fe(III) complexation involves binding of CO_3^{2-} and release of proton.

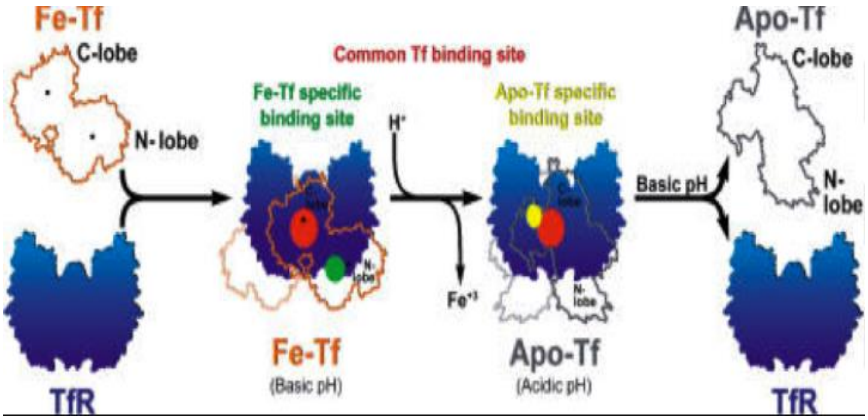
$$\text{apo-Tf} + \text{Fe(III)} + CO_3^{2-} \rightarrow \text{Fe(III)-Tf-}CO_3^{2-} + H^+$$

- The protein chain provides four binding sites with two cis-sites vacant for the synergistic CO_3^{2-} ion (bidentate coordination, may also be linked to Fe(III) release)
- The release of the Fe from Tf occurs at low pH of the endosome (inside the cell), and Apo-Tf is returned to the outside of the cell for delivering of another pair of Fe-atoms (millions of Fe-atoms per cell per minute)
- The reduction potential of Tf (-0.05V) is too negative to be reduced by common biological reducing agents

Mechanism of Transport/release

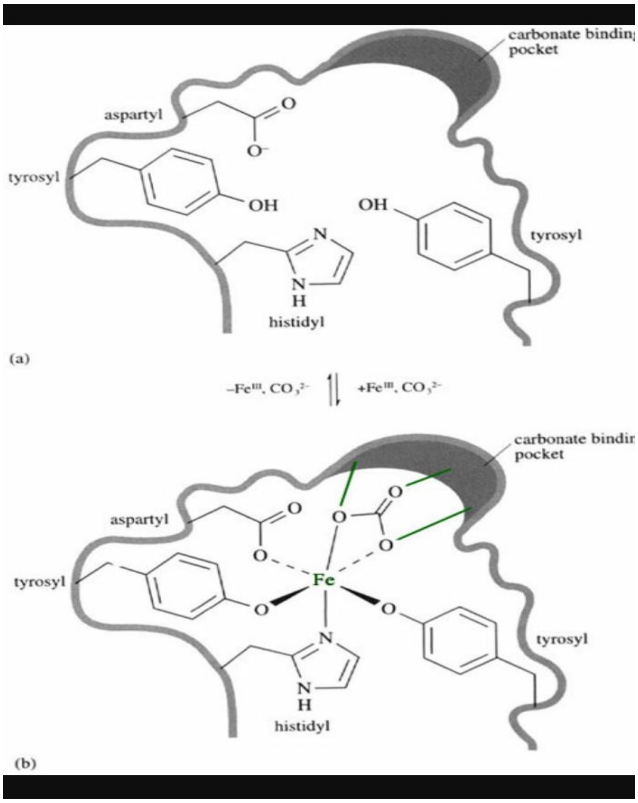
- A transferrin receptor (glycoprotein) influences the stability of Fe-transferrin complex on the cell binding site
- The complex binds to receptor and gets transported into the cell in a vesicle by receptor-mediated 'endocytosis'.

- The pH of the vesicle is reduced (~ 5.5) by H^+ pumps (structural variation in protein), causing transferrin to release its Fe ions thereby forming a complex with cell receptors
- The vesicle then splits and the Tf-receptor complex is returned to the plasma membrane by exocytosis, and Fe(III) is released to the cytoplasm.
- The path of Fe from the endosome to Fe-proteins has not been established; and the form of transported intracellular iron is not known.



Synergistic action of CO_3^{2-}/HCO_3^-

- Without CO_3^{2-} within the coordination sphere of Fe(III), transferrin fails to retain Fe(III)
- CO_3^{2-} establishes the stabilizing interaction from H-bonding (folding protein chain) and coordination of Fe(III) in the pocket
- The anion bridges between Fe(III) and the cationic sites of encircling protein (Fe-O hard-hard interaction).
- This coordinated synergistic anion minimizes the electrostatic repulsion between the metal center and the cationic sites of the protein chain.
- Removal of CO_3^{2-}/HCO_3^- from Tf destabilizes Fe-Tf interaction, a key step in Fe-release
- This explains the synergistic function of the anion.



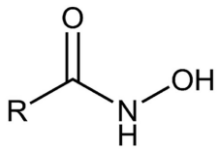
Fe-storage and transport in lower organisms

- Almost all microorganisms including plants have Fe as an essential element
- In reducing environment, Fe was substantially available as Fe(II) compounds (relatively soluble at ~ neutral pH). Availability of Fe(II) state was one of the factors leading to its incorporation in several metabolic processes.
- In oxidizing environment, microorganisms were forced to deal with insoluble Fe(III)-hydroxide
- On Fe deficiency, a high-affinity iron binding site called siderophores comes into play
- Siderophores capture Fe and transfer it through the cell wall.
- Siderophore-mediated iron-uptake usually occurs in aerobic conditions.
- In *E. coli*, Fe-uptake can occur under anaerobic conditions

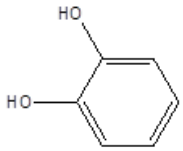
Siderophores

- Siderophores are small polydentate ligand with low MW (500-1000 D)
- They have peptide backbones and are strong chelating agents.
- It solubilizes and transports iron as Fe(III) and have high affinity for Fe.
- They sequester Fe to give a soluble complex that re-enters the organism at a specific receptor. Once inside the cell, the Fe is released.
- They are grouped into two categories viz. one having hydroxamate ligands (ferrichromes, ferrioxamines) & other having catecholate (o-dihydroxybenzene) ligands (enterobactin).
- Hydroxamates occur mainly in fungi and yeast, while the catecholates mainly occur in bacteria,
- These molecules are polydentate ligands with many potential ligating atoms (HSAB principle -hard O-donor sites) to form chelate.
- Readily form extremely stable octahedral complexes with high spin Fe(III).

- Although stable, the complexes are labile (HS, d^5, no CFSE) enough to allow transport and transfer of iron within the bacteria



hydroxamate



catecholate

- At the site of iron release, Fe(III) is reduced to Fe(II) which does not bind so strongly with the siderophores.
- Iron containing siderophores look red brown and this is why, siderophores are also named as siderochromes.
- The colour originates from LMCT (ligand to Fe(III)) band
- Enterobactin-mediated iron uptake in *E. coli* is one of the best-characterized of the siderophore-mediated iron-uptake processes
- The ferric-enterobactin complex interacts with a specific receptor in the outer cell membrane, and the complex is taken into the cell by active transport.

Lecture 3

Biomineralization

- Biomineralization is the process of formation of minerals by organisms from simple substances
- Formation of structural constituents involve biomineralization process
- Biomineralization leads to crystallization under the biological control
- Calcium, iron, silicon, phosphorous and alkaline earth metals are an important constituent of mineralized biological tissues.
- Teeth (for breaking food), bones (for support), the shell (snail, mussels, oysters etc., protect the animal from predators) are common example of biominerals
- Biomineralization occurs in the ocean (Ca in shell, Si in coral reef) & on land in both plants (Si in grass) & animals/birds (Ca in bone and teeth, Fe in ferritin, Fe in magnetite).
- These materials have characteristics optical, mechanical and magnetic properties which are exploited by the organism for different purposes

Iron biomineralization

- Iron oxide is present as biominerals in teeth of chiton (continual deposition of single-crystal ferric oxide)
- Three types of biological iron oxides have distinct relationships to the proteins, lipids, or carbohydrates associated with their formation and with the degree of crystallinity.
- Magnetotactic bacteria use Fe_3O_4 (magnetite) as a magnetic compass to navigate the proper direction and orientation. These bacteria possess organelles called magnetosome vesicles that contain the magnetic crystals of magnetite. These crystals help them to navigate directions
- Migratory birds, homing pigeons and some bees use magnetite in their brain as the magnetic compass in determining the direction of their journey
- Magnetite is also present in lepidocrocite-containing teeth, molluscs, fishes and even in humans
- Ferrihydrite (FeO(OH) in ferritin of mammals) exists as large single crystals or collections of small crystals

- Iron oxides in some ferritins that have large amounts of phosphate are very disordered.
- Goethite [αFeO(OH)] and lepidocrocite [γ-FeO(OH)] form as small single crystals in a complex matrix of carbohydrate & protein in the teeth of some shellfish (limpets & chitons)
- The differences in the iron-oxide structures during formation of the mineral depends on nature of co-precipitating ions, organic substrates/boundaries, surface defects, inhibitors, pH, and temperature.
- Magnetite can form in both lipid and protein or carbohydrate environments, and can be derived from amorphous or semicrystalline ferrihydrite-like material.
- After controlled oxidation a water containing Fe(III) oxide precipitates, dehydration first leads to ferrihydrite, and then under partial reduction to magnetite.
- Synthetic iron complexes have provided models for two stages of ferritin iron storage and biomineralization:

  (a) In the early stage, small numbers of clustered Fe-atoms are bound to the ferritin protein coat, and

  (b) the final stages, where the bulk iron is a mineral with relatively few contacts to the protein coat.

Calcium biomineralization

- Ca is a major component of the structural materials like bone & shell
- The formation of calcified tissue-shells, bone, and teeth are complex (biomineralization)
- The pattern of calcification differs in shells, bone, teeth, and other mineralized tissues.
- Calcite, $CaCO_3$ is important in shell (sea shells, egg shells) formation (structural component) in aquatic life.
- It is used as a minor component of vertebrate bones.
- Formation of $CaCO_3$ (calcification) through biomineralization is favorable at low partial pressure of CO_2 and high concentration of Ca^{2+}
- The pH of the medium is also important for calcite formation
- $CaCO_3$ exists in three different forms (calcite, aragonite and vaterite) and only calcite is thermodynamically stable.
- Apatite, $[Ca_{10}(PO_4)_6(OH)_2]$ is involved in teeth and bone formation.
- Formation of hydroxyapatite, $Ca_5(OH)(PO_4)_3$ is also an important example of biomineralization, the most important constituent of bone tissues in vertebrate skeleton.
- Inorganic matter of bone and teeth resembles apatite minerals $[Ca_5(OH)(PO_4)_3]$.
- Bone and teeth are the examples of bio-nanocomposite materials of hydroxyapatite and fibrous protein collagen
- In bone and teeth, hydroxyapatite nanocrystals constitute the inorganic nanoplatelets as the filler dispersed in an ordered way into the organic collagen polymer matrix (bricks and mortar structural model, inorganic nanoplatelets = brick, organic matrix = mortar)
- Replacement of OH of hydroxyapatite by F gives fluorapatite, strengthening of teeth
- Fluorapatite $3[Ca_3(PO_4)_2].CaF_2$ (hydroxy group replaced by fluoride) is required in the formation of enamel on teeth, more resistant to organic acids (prevents dental caries).
- Because of this preventive action, NaF and SnF_2 are used in commercial toothpastes
- Low concentration of fluoride is beneficial and at high concentration dental fluorosis (mottling of teeth) occurs
- Dental enamel is made up of much larger and uniform thin crystals

Anion	Formula	Crystal form	Occurrence	Function
Carbonate	$CaCO_3$	Calcite, Aragonite Valerite	Sea corals, molluscs	Exoskeleton, Ca-store, eye lens
Oxalate	$Ca(COO)_2.H_2O$ $Ca(COO)_2.2H_2O$	Whewellite Weddellite	Insect egg, vertebrate stones	Deterrent, cytoskeleton, Ca-store
Phosphate	$Ca_{10}(PO_4)_6(OH)_2$	Hydroxyapatite	Bones, teeth, shells	Skeletal, Ca-storage
Sulphate	$CaSO_4.H_2O$	Gypsum	Jelly fish, plants	Gravity device, S and Ca store

- Bone functions as a structural support and central store of calcium.
- Bone is being continuously dissolved and reformed
- Both Ca^{2+} and PO_4^{3-} ions must be concentrated in cells or organelles bordering on the regions where mineralization is to take place.
- During pregnancy (huge demand of calcium), demineralization of bore occurs to supply the required calcium.
- Some women after menopause suffer from osteoporosis (decalcification of bone). This loss of bone mass with increasing age, makes bones more susceptible to breaking.
- Fresh layers of bone matrix are formed by a continuously replenished layer of cells called *osteoblasts*, which, in addition to apatite crystallites, also secrete collagen & large specific proteins (*osteonectin, osteocalcin*, proteoglycans & phosphoproteins)
- In tissues undergoing rapid mineral deposition, the crystallites appear to be formed in vesicles that may have peeled off from the adjacent cell layers
- *Osteoblast cells* handle bone formation while *osteoclasts cells* can erode it.
- These macrophage-like cells can form deep tunnels in a bone matrix, and the cavities left behind are rapidly invaded by other cells forming blood vessels and new layers of osteoblasts.
- Osteoclast cells may secrete calcium-chelating organic anions (such as citrate), to assist in the solubilization of the bones and extracellular proteases that degrade the organic part of the matrix.
- When the rates of these two counteracting processes are not in balance, the result may be decalcification, or *osteoporosis*, which seriously reduces the strength of the bone.

Silicon biomineralization

- Silicon is required to produce stable structural materials in living organisms.
- Biomineralization of Si occurs in single-celled organisms (diatoms), in lower Metazoa like sponges and in higher plants
- The cell wall of diatoms consists of polymerized silicic acid, an amorphous material without any crystalline structure.
- Some plants produce silica-based (SiO_2) protective devices like hairs or spines against the prediatory herbivores.
- Silica (SiO_2) is also present in grass and in the shells of small invertebrates such as Radiolara
- Si is required for skeletal development in chicks and rats

- The silicate (SiO_4^{4-}) materials are the infinite covalent network

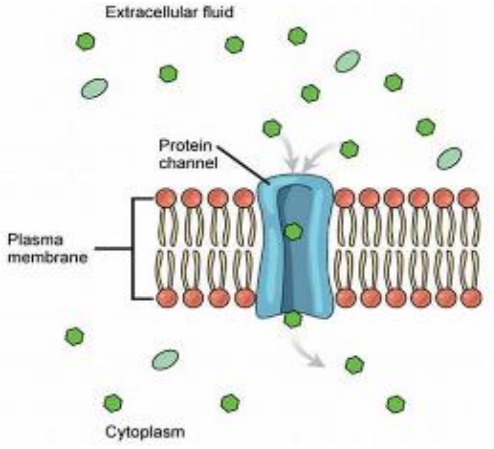
Phosphate Biomineralization

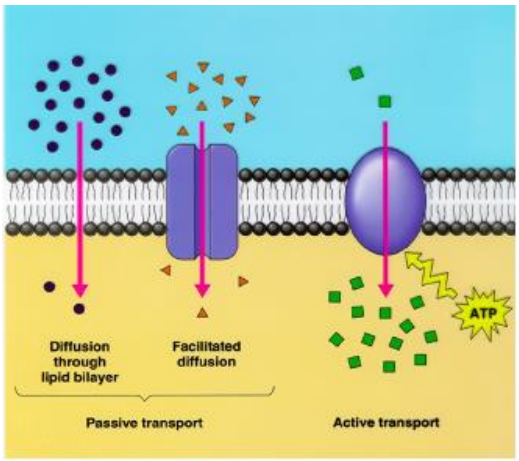
- Inorganic phosphate is a vital constituent of cells and cell membranes, body fluids, and hard tissues, and is important for bone health.
- Availability of adequate Ca^{2+} and PO_4^{3-} in the right proportions is essential for biomineralization, and maintenance of mass and strength of the skeleton.
- Phosphate biomineralization is a complex, dynamic and lifelong process by which precipitations of inorganic Ca^{2+} and PO_4^{3-} occur to form biological hard tissues (bone, cementum, dentin, and enamel)
- Calcium phosphate biomineralization is essential to the formation of bones (hydroxyapatite) and teeth (fluorapatite), and other pathological calcifications.
- Calcium phosphate was proposed to mineralize following a non-classical crystal growth pathway of pre-nucleation cluster aggregation.

Lecture 4,5

Transport of ions

- Active ion transport involves the movement of ions across a cell membrane against their concentration gradient, requiring energy.
- This process is typically facilitated by membrane proteins such as ion pumps.
- Transport of ions across cell membranes is a critical process for various cellular functions, including signal transduction, muscle contraction, & regulation of enzyme activities.
- Ions are transported across cell membranes through channels and pumps.





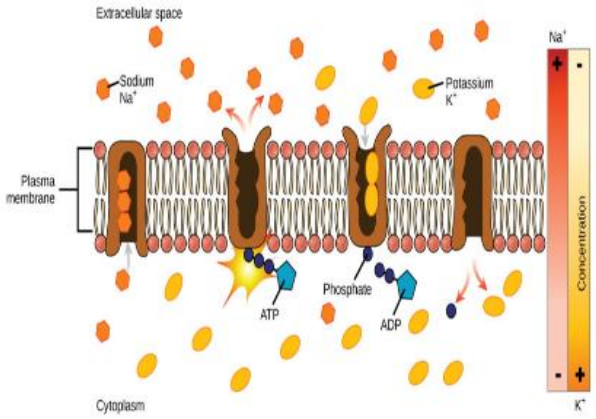
Channels vs Pumps

- Channels & pumps are both proteins that help transport ions across cell membranes.
- Channels allow ions to passively diffuse down their concentration gradient (high to low) and pumps actively transport ions against their concentration gradient (low to high) by consuming energy.
- Channels are like gates that open & close to allow ions to flow through and pumps are like revolving doors that move ions across the membrane.
- The main difference between channels & pumps is that channels require only a single gate, while pumps require at least two gates that should never be open at once.

Membrane Pumps (ATPases)

- Membrane pumps play a crucial role in maintaining the appropriate ion concentrations inside and outside cells, contributing to the overall function and homeostasis of cells.

- There are different types of membrane pumps.
- Primary active transporters and secondary active transporters
- Primary active transport is a mechanism of active transport that directly uses energy derived from the hydrolysis of ATP to transport ions or molecules across a cell membrane against their concentration gradient
- This process is typically mediated by specific proteins known as ATPases or pumps.
- Three types of ATPase or pumps are involved in active transport of ions
  (a) Sodium-Potassium Pump (Na^+/ K^+ ATPases)
  (b) Sarco/Endoplasmic Reticulum Calcium ATPase (SERCA)
  (c) Proton Pump (H^+/K^+ ATPase)
- These three pumps are designated P-type ion transporters because they use the same basic mechanism; a conformational change in the proteins as they are reversibly phosphorylated by ATP.
- In other words, if the pumped ions are allowed to diffuse back through the membrane complex, ATP can be synthesized from ADP and inorganic phosphate.



Thermodynamics of membrane transport and membrane potential

- Movement of a solute across the membrane from its higher concentration region to its lower concentration region is associated with the free energy change (ΔG).
- If ΔG is negative, the process is called passive transport (thermodynamically downhill transport)
- On the other hand, movement of a solute species against its concentration gradient is called active transport, ΔG is positive in this case (thermodynamically uphill transport)
- Passive transport is thermodynamically favorable (spontaneous)
- Active transport is non-spontaneous and needs to be coupled with another thermodynamically favorable process (to make resultant ΔG negative)
- Generally, ATP hydrolysis (ΔG = -30.5 kJ/mol) is coupled with an active transport system
- ΔG for transporting 1 mol of an uncharged solute (say A) from side 1 (its molar concentration C_1) to side 2 (its molar concentration C_2) is given by
  $\Delta G = G_A$ (side 2) – G_A (side 1) = RT ln(C_2/C_1)
  G_A is chemical potential or partial molar free energy of A
- For $C_1 > C_2$; ΔG < 0; the transfer of A from side 1 to side 2 is spontaneous (passive)
- For $C_1 < C_2$; ΔG > 0; the transfer of A from side 1 to side 2 is non-spontaneous (active)

- If the transported species is charged (Na^+, Cl^-), the unequal distribution of charge across the membrane generates an electrical potential difference (ΔV) across the membrane
- This electric potential will electrostatically resist the movement of the ion
- Thus, both concentration gradient factor and electrical factor are to be considered for the free energy change (ΔG) during transport of the charged species
- The free energy change (ΔG) for the transfer of 1 mol of the ion is given by

$$\Delta G = RT \ln(C_2/C_1) + ZF\Delta V$$

and for the transport of 1 mol of an ionic charge Z from side 1 to side 2

$$E \text{ (total potential)} = \Delta G/ZF = (RT/ZF) \ln(C_2/C_1) + \Delta V$$

where Z is the electrical charge of the transported species, ΔV is the electrical potential different (in V) across the membrane and F is Faraday (= 96500 C/mol = 23.06 kcal/V)

- The electric potential (ΔV) across the membrane is called the membrane potential
- The first term in ΔG is the free energy contribution arising from concentration gradient and the second term is the free energy contribution due to the transport of electrical charge
- The sum of these two is called electrochemical gradient which produces the total potential
- At equilibrium, both these factors counterbalance each other and hence $\Delta G = 0$
- $\Delta G = 0 = RT \ln(C_2/C_1) + ZF\Delta V$
- or, $\Delta V = (RT/ZF) \ln(C_1/C_2)$; (Nernst Equation)
- At equilibrium, the membrane potential is given by the Nernst equation and is also called Nernst potential or equilibrium potential at this condition (arises from the transmembrane concentration gradient of the ionic substance
- If the molar concentration of a particular ion (say Na^+) is C_{out} and C_{in} from outside and inside the cell membrane, ΔV for $Na^+_{out} \leftrightarrow Na^+_{in}$ is
- $V_{eq} = \Delta V = (RT/ZF) \ln (C_{out}/C_{in}) = (RT/F) \ln (C_{out}/C_{in})$
- For a typical mammalian muscle cell, $[K^+]_{in}$ =155mM and $[K^+]_{out}$ = 4mM, Nernst or equilibrium potential a normal human body temperature is -97.6mV
- For Na^+ with $[Na^+]_{out}$ = 143mM and $[Na^+]_{in}$ = 14mM, the equilibrium potential is +62 mV
- Across the membrane, different types of ions may be distributed and then sum of the Nernst or equilibrium potentials due to each type of ion determined by its charge and concentration gradient across the membrane is called the net membrane potential ($\Delta\Psi$)

$$\Delta\Psi = \text{sum of } \Delta V_i = \Sigma \Delta V_i$$

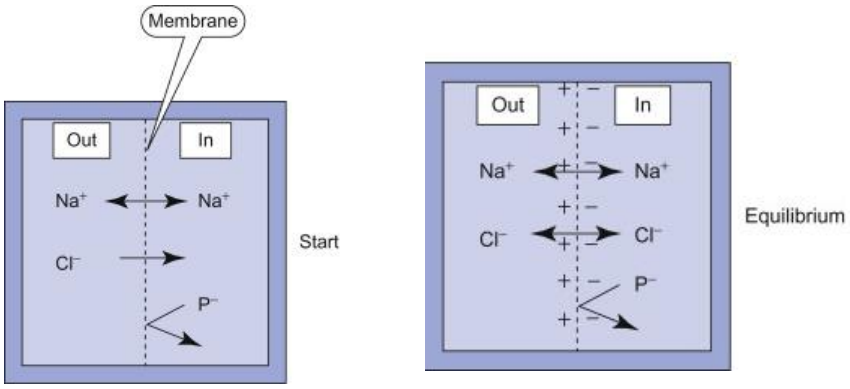
Different types of ATP driven transporters

- Considering the stoichiometry of the transport process, these are classified as
- **Uniport**: movement of only one type of substance in certain direction
- **Symport**: movement of two different types of substances in the same direction (Na^+-glucose transporter - indirectly powered by ATP hydrolysis)
- **Antiport**: movement of two different types of substances in the opposite directions
- These transporters are directly or indirectly powered by the ATP hydrolysis
- Transporters directly powered by ATP hydrolysis: Ca^{2+} pump (uniport), Na^+-K^+ pump (antiport)
- (Na^+-K^+ pump - directly powered by ATP hydrolysis,

- Transporter indirectly powered by ATP hydrolysis: Na^+-Ca^{2+} exchanger (antiport) , Na^+-glucose transporter (symport)
- Uphill flow - directly powered by ATP hydrolysis
- Downhill flow - indirectly powered by ATP hydrolysis (cotransporter or secondary transporter)

Gibb's-Donnan Equilibrium or Donnan Equilibrium

- Let us consider a semipermeable membrane to separate two electrolytes (NaCl and NaR) where the membrane is permeable to Na^+ and Cl^- and impermeable to R^-. C_1 and C_2 be the concentration of NaCl and NaR at side I and side II respectively
- At initial stage when, $C_1 > C_2$ and hence Na^+ and Cl^- ions with move from side I to side II
- For electroneutrality, amount of Na+ and Cl^- ions passing through the SPM will be equal



- If $[Na^+]_1 = [Cl^-]_1 = C_1\text{-}x$ at the side I then $[Na^+]_2 = C_2+x$, $[Cl^-]_2 = x$ and $[R^-] = C_2$ at side II when equilibrium is reached. At equilibrium

  $\Delta G = \Delta G_{Na+} + \Delta G_{Cl-} = 0 = RT \ln(C_2/C_1) + ZF\Delta V$

  where ΔG = change in Gibbs free energy in moving the ions from side I to side II

  $\Delta G_{Na+} = RT \ln([Na^+]_2/[Na^+]_1) + ZF\Delta V$

  $\Delta G_{Cl-} = RT \ln([Cl^-]_2/[Cl^-]_1) - ZF\Delta V$

  $\Delta G = \Delta G_{Na+} + \Delta G_{Cl-} = 0 = RT \ln([Na^+]_2/[Na^+]_1) + RT \ln([Cl^-]_2/[Cl^-]_1)$

  Or, $[Na^+]_2[Cl^-]_2 = [Na^+]_1[Cl^-]_1$,
- The product of concentrations of the two diffusible ions on both sides of the membrane will be equal (Gibb's Donnan equilibrium or Donnan equilibrium)
- The percentage of ion migrated = $(x/C_1) \times 100$
- Transmembrane potential at Donnan equilibrium, $\Delta V = (RT/ZF) \ln([Na^+]_2/[Na^+]_1) = (RT/ZF) \ln([Cl^-]_2/[Cl^-]_1)$

Illustration, Say $[NaR] = C_2 = 0.01M$, $[NaCl] = C_1 = 0.05M$

Then, $[Na+]_2 = 0.01+ x$, $[Na+]_1 = 0.05 – x$, $[Cl-]_1 = 0.05 – x$ and $[Cl-]_2 = x$

At Donnan equilibrium,

$[Na^+]_2[Cl^-]_2 = [Na^+]_1[Cl^-]_1$,

Or, $(0.01 + x)(x) = (0.05 – x)(0.05-x)$

Or, $x = 0.023$

Thus, percentage of NaCl migrated from side I to side II is $(0.023/0.050) \times 100 = 46$ %

Sodium potassium pump

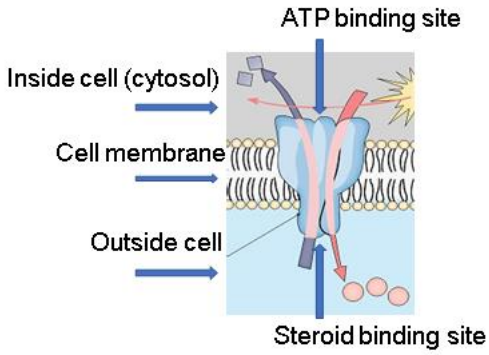
- Ion pump maintains the active transport of ions across the cell membrane.
- The concentration gradient of Na^+ and K^+ ions across the cell-membrane is achieved by an energy repairing pump known as Na^+-K^+ pump (antiport).
- The pump transports three Na^+ out of the cell in exchange for two K^+.
- The pump is driven by an integral enzyme, Na^+/K^+ ATPase (P-type)
- The energy for required for pumping these ions is obtained from hydrolysis of intracellular ATP catalyzed by Mg^{2+}-ions.

$$3Na^+_{intra} + 2K^+_{extra} + Mg^{2+}\text{-}ATP^{4-} + H_2O \rightarrow 3Na^+_{extra} + 2K^+_{intra} + Mg^{2+}\text{-}ADP^{3-} + H_3PO_4^{2-} + H^+$$

- Different Na^+/K^+ ratio (and the correct concentrations of Na^+ and K^+) inside and outside the cell develops an electrical potential across the membrane (essential for functioning of nerve & muscle cells).

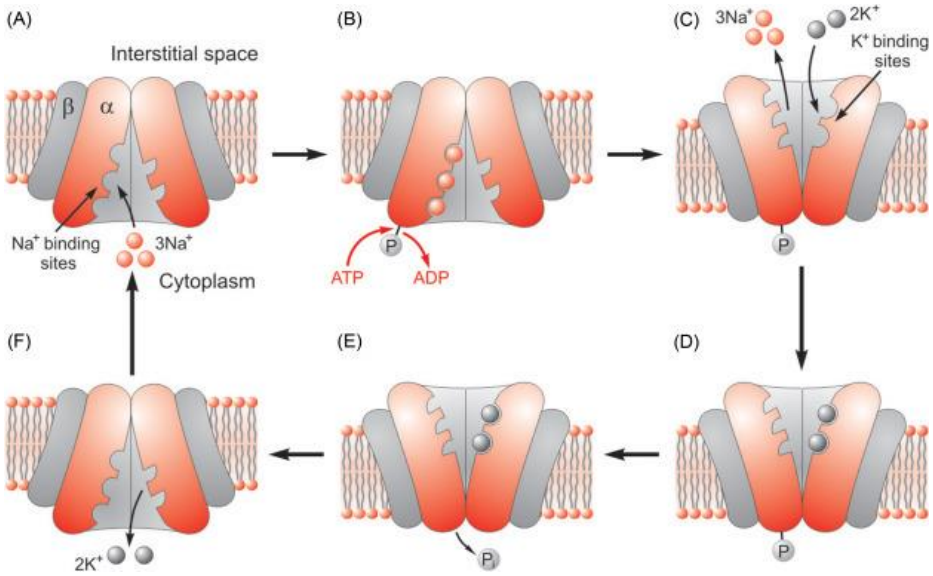
Na^+/K^+ ATPase

- The Na^+/K^+ ATPase exists in two forms, depending on its orientation to the interior or exterior of the cell and its affinity for either Na^+ or K^+ ions.
- The enzyme Na^+/K^+ ATPase (280 kD) is a tetrameric ($\alpha_2\beta_2$) protein.
- The larger unit (two α units, 100 kD) contains the ATP binding site (acts as revolving door, pass through plasma membrane).
- The α-chains contain the selective metal binding sites and phosphorylation sites (one end).
- Other end of α-chains has the steroid inhibitor binding site
- The α-chains traverse the plasma membrane
- The smaller unit (two β units, 40 kD) primarily contains carbohydrate.



Mechanism of sodium potassium pump

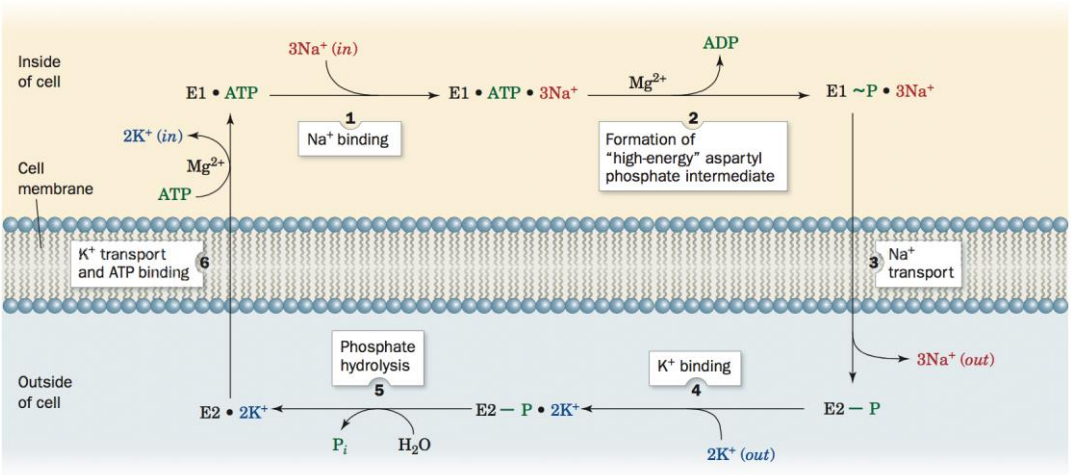
- Na^+/K^+ ions are pumped against their concentration gradients by the enzyme Na^+/K^+ ATPase coupled with hydrolysis of ATP catalyzed by Mg^{2+}-ions.
- In the function of Na^+/K^+ pump, one cycle involves the transport of $3Na^+$ ions from inside the cell to the outside and $2K^+$ ions from outside the cell to inside the cell
- Binding of $3Na^+$ ions with the protein (α_2 unit) changes the local polarities to facilitate the binding of ATP, α_2 unit is phosphorylated and ADP is released after hydrolysis



- The phosphorylation changes the conformation (eversion) of protein (E1)
- In this conformation, the Na^+-binding sites become open and three Na^+ is released to the extracellular fluid
- The open channel binds two K^+ from outside causing dephosphorylation from the protein chain.
- Conformational changes (eversion) then again occur (E2), opening the K^+-binding site to cytosol finally leading to release of two K^+
- This leads to the original conformation of enzyme to initiate a new cycle again.
- The overall process of the uphill transport of Na^+ and K^+ ion is

$$3Na^+_{intra} + 2K^+_{extra} + Mg^{2+}\text{-}ATP^{4-} + H_2O \rightarrow 3Na^+_{extra} + 2K^+_{intra} + Mg^{2+}\text{-}ADP^{3-} + H_3PO_4^{2-} + H^+$$

- E1 projects the ion binding sites towards the cytosol, E2 projects the same outside the cell
- Na^+ binding triggers phosphorylation (E1) and K^+ binding triggers dephosphorylation (E2)



Role of Mg^{2+} ion:

- Mg^{2+} plays two crucial roles viz. catalyzes the ATP hydrolysis and structure forming effect to change the protein conformation.

Importance of Na^+-K^+ pump

- Extrusion of Na^+ from the cytosol of the cell by this pump is important for animal cells to control water content osmotically
- If $[Na^+]$ inside the cell were not depleted, the animal cells lacking in cell walls would swell and burst because the cell membrane is permeable to water
- The concentration gradient $[Na^+]_{out}/[Na^+]_{in} \approx 15$ maintained by this pump powers

  (a) the transport (symport) of glucose and amino acids in some cells

  (b) The transport of Ca^{2+} through the Ca^{2+}-Na^+ exchanger (antiport)
- The electrochemical gradient developed by this pump is responsible for the generation and propagation of nerve impulses

Cardiac glycoside or cardiotonic steroids

- Digitalis and Ouabain are well known cardiac drugs, used in the treatment of congestive heart failure
- These are the natural products and can intensify the heart muscle contracting force
- Digitalis and Ouabain cardiotonic steroids can inhibit the Na^+-K^+-ATPase through binding to the externally exposed side of the Na^+-K^+ pump (the said drugs are ineffective when injected into the cell) and inhibit the dephosphorylation step (generation of the E2 conformation is prevented)

  $$E2\text{-}OPO_3H + H_2O \rightarrow (K+)_2\text{-}E2 + P_i$$

  (inhibited by the cardiotonic steroids)
- Inhibition of the Na^+-K^+-ATPase enzyme by the cardiotonic steroids leads to the higher level of Na^+ concentration inside the cell
- This reduces the $[Na^+]_{out}/[Na^+]_{in}$ concentration gradient and consequently the extrusion of Ca^{2+} from the cytosol through the Na^+-Ca^{2+} exchanger (antiport) is reduced
- The release of Ca^{2+} from the sarcoplasmic reticulum (SR) elevates the cytosolic Ca^{2+} level to trigger the muscle contraction
- In presence of the cardiotonic steroids arresting the Na^+-K^+ pump, the increased cytosolic Ca^{2+} level intensifies the normal force of the muscle contraction

Active transport of Na^+ and K^+ ions and Nerve impulse: Na^+ and K^+ channels

- A nerve cell, neuron has a much higher concentration of K^+ ions inside the cell, while Na^+ ions are more concentrated outside the cell at resting condition
- These concentration gradients are maintained at the cost of metabolic energy.
- The equilibrium membrane potentials $V_{eq} = \Delta V = RT/nF(C_{out}/C_{in})$ for Na^+ and K^+ are approximately +0.60 mV and -0.95 mV respectively
- At rest, the membrane is rather, permeable to K^+ compared to Na^+
- Cl^- and other anions present inside the cell maintain the electroneutrality
- K^+ ions tend to leave the cell to attain the equilibrium concentration, but this leaves behind an excess of anions (prevents the tendency towards concentration equilibrium)
- At rest, the concentration gradient generates an electric membrane potential of about -60 mV which is close to the equilibrium potential for K^+ because at rest some K^+ channels are open
- The electrical membrane potential (ΔV) can be expressed in terms of permeabilities of the involved ions by Goldman-Hodgkin-Kartz (GHK) equation

  $$\Delta V = \Delta\Psi = (RT/F)\ \ln(\Sigma P_c[C_{out}] + \Sigma P_a[A_{in}])/(\Sigma P_c[C_{in}] + \Sigma P_a[A_{out}])$$

  where C and A denotes cations and anions respectively

Pc and Pa are the respective permeability coefficients for the monovalent cations and anions

- Considering the monovalent ions K^+, Na^+ and Cl^- ions to be involved in the process, ΔV is given by

$$\Delta V = \Delta\Psi = (RT/F) \ln (P_K[K^+_{out}] + P_{Na}[Na^+_{out}] + P_{Cl}[Cl^-_{in}])/(P_K[K^+_{in}] + P_{Na}[Na^+_{in}] + P_{Cl}[Cl^-_{out}])$$

- The permeability changes on excitation as follows

$P_K : P_{Na} : P_{Cl} \approx 1 : 0.04 : 0.45$ (resting) and $P_K : P_{Na} : P_{Cl} \approx 1 : 20 : 0.45$ (excited)

- Thus, at rest, the potential (-0.60 mV) arises due to the permeability of K^+ while at the excited state, it is due to the permeability of Na^+ ion
- This is why; the resting potential is called the K^+ potential while the potential at the excited state is called the Na^+ potential (+30mV)
- Since the concentration gradient of Na^+ is opposite to that of K^+, the K^+ potential and Na^+ potential is opposite in nature
- Thus, on excitation, there is a swing from the K^+ potential and Na^+ potential
- This swing occurring over a time about 1ms is the nerve impulse, which is an electric signal
- Transmission of the nerve impulse across the synaptic gap occurs though the neurotransmitter acetylcholine

K^+ ion channels

- K^+ ion channels contribute significantly to the establishment and maintenance of the resting membrane potential in excitable cells such as neurons and muscle cells.
- The resting membrane potential is defined as the electrical potential difference across the cell membrane when the cell is not actively transmitting signals.
- K^+ ion channels exhibit high selectivity for K^+ ions. They allow the movement of K^+ ions while preventing the passage of other ions, contributing to the specificity of ion flow across the membrane.
- The proteins that manage the neuronal system are called potassium voltage-gated ion channels (some drugs target K^+ channels to modulate their activity)
- Rapid re-opening and closing of these channels release ions, moving electrical impulses from the brain in a wave to their destination in the body.
- Without K^+ and Na^+ (specific for Na^+ and selectively allow its passage) channels, neurons could not generate electrical signals and hearts could not beat rhythmically.

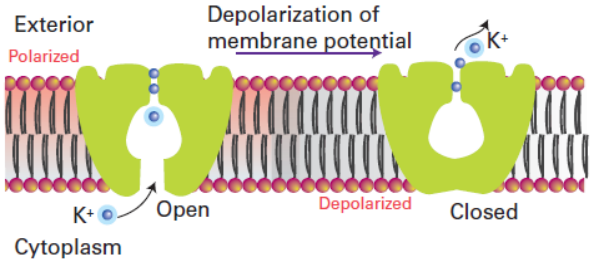
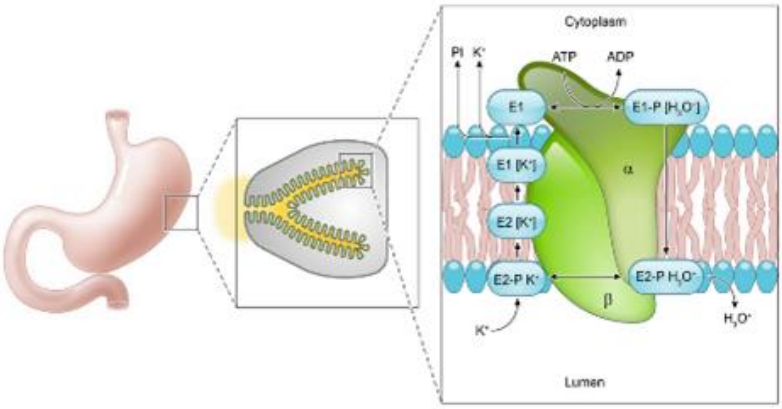


Figure 27.6 Proposed mechanism of action of the K^+ channel. The potential difference across the membrane is sensed by the protein, which causes the pore to open, allowing hydrated ions to enter the cavity. After shedding their hydration sphere, K^+ ions pass up the selectivity filter at rates close to diffusion control.

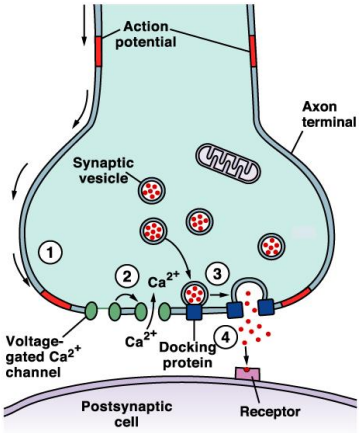
- Permeability of ions through Na^+ and K^+ ion channels largely depends on their size.

- Relatively smaller ions prefer Na^+ channels while relatively larger ions prefer K^+ channels
- However, bulky protonated ions (NH_3OH^+, $NH_3NH_2^+$) are permeable in the Na+ channels and are not permeable in the K^+ channels
- This is probably due to the electrostatic attraction experienced by larger cations and H-bonding with the negatively charged carboxylate (COO^-) group present in the inner surface of Na^+ channels
- Sometime presence of non-polar group on the ion prevents H-bonding ($CH_3NH_3^+$) and hence cannot pass though Na^+-channels
- The Na^+ channels bearing the negatively charged carboxylate (COO^-) groups projected towards the center of the channel select the Na^+ ion of smaller size. K^+ ions cannot pass so comfortably through the Na^+ channels because of their larger size
- Na^+ permeability via K^+-channels is not favorable, because in the K^+-channel, the carbonyl group of peptide linkage experiences better interaction with K^+ ion



Synaptic transmission of nerve signal

- Nerve impulse transmission occurs through the neuron by an electrical mechanism controlled by the relative permeabilities of Na+ and K+ through the voltage gated cation channels.
- But when the action potential reaches the axon terminal point (presynaptic point), the nerve signal is transmitted through the synaptic gap or synaptic cleft to the postsynaptic end of another neuron through the chemical mechanism.
- The neurotransmitter, acetylcholine participates in the chemical transmission of nerve impulse from one neuron to another neuron through the synaptic gap.
- Acetylcholine (ACh) is synthesized at the presynaptic end through the transfer of an acetyl group from acetyl-CoA to choline in a reaction catalyzed by choline acetyltransferase.



Proton pump (H^+/K^+ pump)

- Gastric digestion in our stomach requires a high acidity (pH ≈ 1)
- Proton pumping from the mucosal parietal cells into the lumen of the stomach maintains the acidity
- Carbonic anhydrase catalyzed intracellular hydration of $CO_{2\ is}$ the source of proton
- Cl^- ions are also passively transported from cytosol of parietal cell to the lumen of stomach to maintain the electroneutrality (In stomach lumen, it exists as HCl)
- The proton pump involves the electroneutral H+-K+ antiport run by H+-K+-ATPase
- The proton pumping produces a pH difference by about 6.5 unit
- Such a huge H^+ gradient is really a unique phenomenon

Proton pump (H^+/K^+ ATPase)

- The H^+/K^{+-}ATPase is primarily found in the membrane of the parietal cells in the gastric glands of the stomach lining.
- The primary function of the H^+/K^{+-}ATPase is to actively pump protons into the stomach lumen (contributes to the acidic environment of the stomach)
- The acidic environment created by the H^+/K^+-ATPase is essential for the activation of pepsin, an enzyme that plays a key role in breaking down proteins during digestion.
- The pump operates by exchanging H^+ from the cytoplasm of the parietal cell for K^+ ions from the gastric lumen. The exchange is facilitated by the energy derived from the hydrolysis of ATP.
- The H^+/K^+ ATPase utilizes the energy released from ATP hydrolysis to drive the active transport of ions across the cell membrane.
- Dysregulation of this pump leads to disorders related to acid secretion in the stomach.

Mechanistic steps

- CA catalyzed hydration of CO_2 is the source of proton (dissociation of H_2CO_3)
- The H^+ ions produced within the cell are actively pumped into the lumen of stomach through the electroneutral H^+-K^+ antiport (K^+ enter into the parietal cell while H^+ moves away from the cell into the lumen of stomach). The enzyme H^+/K^{+-}ATPase runs the pump and is phosphorylated during the transport process.
- Thus, the K^+ ion is accumulated within the parietal cell diffuse passively into the lumen of stomach
- To maintain the electroneutrality into the lumen side where H^+ ions are accumulated, Cl^- ions passively diffuse from the parietal cell into the lumen side
- Through an exchanger (HCO_3^--Cl^- exchanger) port located on the opposite face of the parietal cell, it balances the loss of Cl^- from the parietal cell by importing Cl^- ions from the blood in exchange of the HCO_3^- ions generated within the cell by the action of CA

Proton pump inhibitors as the drug in treatment of ulcers

- When inbuilt protective mechanism fails to protect the gastric mucosa, ulceration takes place due to attack of the stomach acid on gastric mucosa
- The bacteria *H. Pylori* cause the infection on the peptic ulcers
- The proton pump inhibitors such as omeprazole, pantoprazole, lansoprazole directly inhibit the H^+-K^+-ATPase through covalent interaction with the enzyme
- This proton pump inhibition actually inhibits the stomach acid secretion

Important function of the high acidity of gastric juice

- Production of the active digestive enzyme (pepsin) from the inactive proenzyme
- Breakdown of the ingested tissues to favor the attack by the digestive enzyme in stomach
- Maintenance of the low pH required for the function of digestive enzyme
- Killing the invading microorganisms in the stomach

Presence of HCl cannot harm the digestive tissues (mucosa)

- HCl present in the stomach lumen cannot attack mucosa because mucosa can secret large quantities of protective mucus that line the mucosal surface
- The mucus enriched with the basic electrolyte, HCO_3^- can neutralize HCl, it penetrates the mucus layer
- When the acidic contents of stomach reach the small intestine, the acidity is neutralized by the addition of HCO_3^- supplied by the pancreas

Calcium pump (Serca Pump) and Ca^{2+}-ATPase

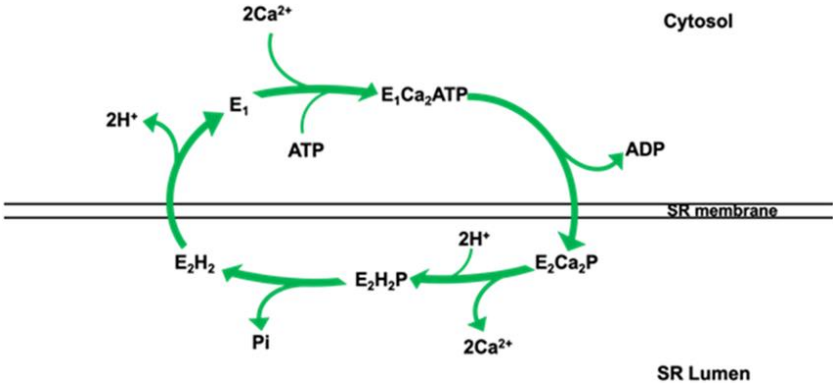
- It transports Ca^{2+} against the concentration gradient (uphill flow).
- The Ca^{2+} pump located on the plasma membrane transport Ca^{2+} from cytosol to outside the plasma membrane
- The Ca^{2+} pump located on the sarcoplasmic reticulum (SR, a special form of endoplasmic reticulum, ER) membrane pumps/transports Ca^{2+} ion from cytosol (sarcoplasm, outside) to SR-lumen (inside) against concentration gradient

  Ca^{2+} (cytosol) → Ca^{2+} (outside the plasma membrane or lumen of SR/ER)
- The Ca^{2+} pump depending on the location is of two types viz. plasma membrane Ca^{2+}-ATPase and ER (SR) membrane Ca^{2+}-ATPase
- SR is present in the muscle fiber and acts as a reservoir of Ca^{2+} ion within the cell
- At rest, Ca^{2+} ions pumped (ATP driven pump) pump into SR, so that the concentration of Ca^{2+} ion increases inside the SR to more than 10^{-3} mol/L and its concentration in the surrounding cytoplasm of muscle reduces to less than 10^{-6} mol/L
- A protein called calsequestrin (40 high affinity binding sites) binds the Ca^{2+} ion inside the SR
- Excitation of SR membrane by a nerve impulse cause to release large amounts of Ca^{2+} ion from SR to sarcoplasm and this release triggers the muscle contraction
- Troponin and tropomyosin mediate the Ca^{2+} ion regulation in muscle contraction.
- The interaction of actin and myosin is inhibited by troponin and tropomyosin in absence of Ca^{2+} ion
- The transport of Ca^{2+} ion into the SR or plasma is carried out by the ATP driven Ca^{2+} pump
- The pump has two protein subunits.
- Larger subunit (MW ≈100 kD) of the pump traverses the membrane and it possesses the phosphorylation site (an aspartate residue). The unit also bears the Ca^{2+} binding sites
- Smaller subunit (MW ≈ 55 kD) is a glycoprotein
- The accumulation of Ca^{2+} ion that occurs through Ca^{2+} pump is active transport while during excitation, the release of Ca^{2+} ion occurs through the hydrophobic receptor mediated channels (passive transport)

Mechanism:

- The enzyme is phosphorylated at the aspartate site in the presence of Ca^{2+} and Mg^{2+} ions (also described as Ca^{2+}-Mg^{2+}-ATPase) and undergoes dephosphorylation in the next step

- During phosphorylation and dephosphorylation, it transports two Ca^{2+} ion for each molecule of ATP hydrolyzed
- The protein changes its conformation during phosphorylation and dephosphorylation
- Ca^{2+} pump can exists in two conformations (E_1 and E_2)
- E_1 conformation possesses two high affinity Ca^{2+} ion binding sites projected towards the cytoplasmic side while the E_2 conformation possesses the low affinity Ca^{2+} ion binding sites projected towards the SR luminal side
- E_1 binds with Ca^{2+} and ATP followed by phosphorylation on an aspartate site. It leads to high Ca^{2+}-affinity conformation $(E_1\text{-}Pi)(Ca^{2+})_2$ which changes to low affinity conformation $(E_2\text{-}Pi)(Ca^{2+})_2$
- This conformation change is assisted by Ca^{2+} and Mg^{2+} ions which are known to have strong structure forming effect.
- $(E_2\text{-}Pi)(Ca^{2+})_2$ releases Ca^{2+} ions inside the cell
- The release of Ca^{2+} ion triggers the dephosphorylation.
- The dephosphorylated protein (E_2) changes to the stable conformation E_1.
- E_2 form is stabilized by phosphorylation while E_1 form is stabilized by dephosphorylation



Overview of the primary active transport of Ca^{2+} ions by the SERCA pump (Ca-ATPase):

(a) Binding of Ca^{2+} ions: The SERCA pump has a high-affinity binding site for Ca^{2+} ions in the cytoplasm of the cell.

(b) ATP hydrolysis: ATP is hydrolyzed by the SERCA pump, providing the energy needed for the transport process.

(c) Conformational changes: The energy from ATP hydrolysis induces conformational changes in the pump.

(d) Transport of Ca^{2+} ion into endoplasmic reticulum: Because of these conformational changes, the pump undergoes a series of steps that allow it to transport Ca^{2+} ions from the cytoplasm into the lumen of the endoplasmic reticulum.

(e) Resetting the pump: After transporting Ca^{2+} ions, the pump resets its conformation, making it ready for another cycle of calcium transport.

- In humans, uptake of Ca^{2+} from food occurs in the small intestine, and transport is regulated by a metabolite of vitamin D, calcitriol.
- Mammalian females supply Ca^{2+} to the fetus during pregnancy and to the newborn child through the mother's milk.

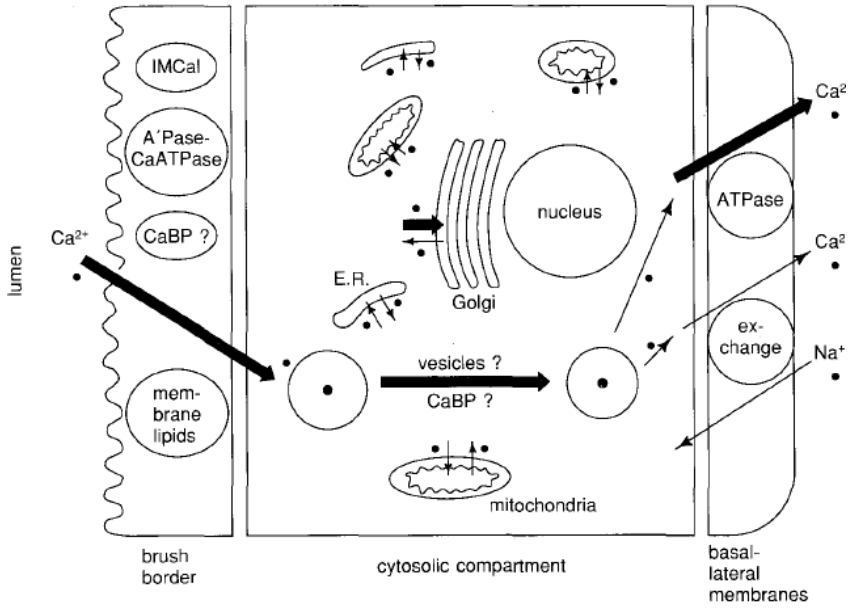
Lecture 6,7

Transport and regulation of Calcium in higher organisms

- All living organisms need calcium, which must be taken up from the environment
- In higher organisms (humans), the blood plasma level of total calcium is constant (~2.45 mM), and there must be a mechanism for regulating this concentration.
- On cellular level, cytoplasmic Ca^{2+} concentration is low & in certain organelles (endoplasmic/sarcoplasmic reticulum or mitochondria) it is considerably higher.
- For a suitable intracellular "messengers", Ca^{2+} levels in the cytoplasm have to be increased rapidly as a result of some stimulus.
- Ca^{2+} ions may enter the cytoplasm either from the extracellular pool or from the Ca^{2+}-rich organelles inside the cell (or both).
- Ca^{2+} channels being regulated by chemical signaling, either by a hormone acting directly on the channel or by a small molecule released intracellularly when a hormone is attached to a membrane-bound receptor.
- Some channels may be switched on by voltage gradients, and both these mechanisms may operate simultaneously.
- Increased intracellular Ca^{2+} levels must eventually be brought back to the basal levels quickly
- The ions could be transported out of the cell or back into the Ca^{2+}-rich organelles.
- This transport will be against an electrochemical potential gradient, and thus requires energy.
- There are many possibilities for different forms of Ca^{2+} transport and regulation in living systems

A. Ca^{2+} uptake and secretion

- In human, Ca^{2+} uptake occurs in the small intestine, and its transport is regulated by a metabolite of vitamin D, calcitriol (l,25-dihydroxy vitamin D_3).
- About 50% of the calcium content in diet is not absorbed (associated with loss).
- To maintain homeostasis and keep the Ca^{2+}-level in blood plasma constant, excess Ca^{2+} is excreted through the kidney.
- This phenomenon in vertebrates is controlled by the level of the parathyroid hormone that acts on kidney (increases Ca^{2+} resorption), on bone, and indirectly, via stimulated production of calcitriol, on the intestinal tract (increases Ca^{2+} uptake).
- Calcium enters the cells from outside (intestinal lumen), by traveling through the brush-border membrane of the intestinal epithelial cells, through the cytosolic interior of these cells, and into the body fluids through the basal lateral membranes of the same cells.
- The Ca^{2+} transport process across intestinal epithelial cells is shown in figure.
- Transport across the brush-border membrane is assumed to be passive or to be facilitated by a carrier, and is also influenced by vitamin D.
- Transport through the cell may be in vesicles and/or in association with Ca^{2+} binding proteins (CaBP), notably calbindins D9k (mammals) or D28k (avians).
- Temporary storage of Ca^{2+} may occur through cytosolic CaBPs, mitochondria, endoplasmic reticula (ER), or other organelles.
- Transport of Ca^{2+} out of the cell through the basal-lateral membranes is energetically uphill, & primarily accomplished by a Ca^{2+}-ATPase & by a Na^{2+}-Ca^{2+} antiport to some extent.
- An alternative mechanism is vesicular transport.



- In chicken intestine, lysosomes are the only epithelial organelles that increased in Ca^{2+} content as a result of calcitriol treatment.
- The result supports to a transport mechanism involving Ca^{2+} uptake across the brush-border membrane by endocytic vesicles, fusion of these vesicles with lysosomes, and possibly also delivery of Ca^{2+} to the basal lateral membrane of the epithelial cell by exocytosis.
- This process would also explain the vitamin-D-induced alterations in brush-border-membrane lipid compositions because of preferential incorporation of certain types of lipids into the vesicles
- The lysosomes in the chicken studies also contained high levels of calbindin D28k-a type of vitamin-D-induced Ca^{2+}-binding protein that acts as a "receptor" for Ca^{2+} at the brush-border membrane and upon Ca^{2+} binding could become internalized in endocytic vesicles.
- The basal lateral plasma membrane contains at least two types of Ca^{2+} pumps that also may play a role in Ca^{2+} uptake, one ATP-driven, one driven by a concurrent flow of Na^{+} ions into the cytoplasm (i.e., a Na+-Ca^{2+} antiport (above figure)

Ca^{2+} transport in placenta

- Intestinal Ca^{2+} transport is somewhat analogous to that occurring in the placenta.
- Transplacental movements of Ca^{2+} increase dramatically during the last trimester of gestation.
- In mammalian placental trophoblasts, high concentrations of calbindin D9K are found.
- The protein synthesis in this tissue also occurs under calcitriol regulation.
- Ca^{2+} ions is supplied by mammalian females, not only to the fetus during pregnancy, but also to the newborn child through the mother's milk.
- The molecular details of Ca^{2+} transport in the mammalian glands is not known
- In milk, Ca^{2+} is bound mainly to micelles of casein, and the average Ca^{2+} content is reported to be 2.5 g/liter.

B. Intracellular Ca^{2+} transport

- Knowledge of intracellular Ca^{2+} transport is imperative for understanding of the role of Ca^{2+} as regulator of cellular function
- The major pathways for Ca^{2+} transport across cellular membranes involve three membrane systems

(a) the plasma membrane
(b) the inner mitochondrial membrane, and
(c) the membrane of the endoplasmic reticulum (ER) (or, in striated muscle cells, a specialized form sarcoplasmic reticulum (SR).

- Two of the membrane bound transport systems are Ca^{2+}-ATPases (derive their main energy from ATP hydrolysis of ATP (1 and 2 in the figure, their properties differ in other aspects).

1. Ca^{2+}-ATPases

- The plasma membrane **Ca^{2+}-ATPases** of erythrocytes was first recognized by Schatzmann in 1966 - was isolated in pure form by Niggli *et at.* in 1979
- The sarcoplasmic reticulum Ca^{2+}-ATPase (SR Ca^{2+}-ATPase) was first purified by MacLennan in 1970. Presently it is the best characterized Ca^{2+}-ATPase.
- The primary active transport of calcium ions is mediated by a class of proteins called calcium pumps or calcium ATPases.
- These pumps use the energy derived from the hydrolysis of ATP to actively transport calcium ions across cell membranes against their concentration gradient.
- The cell membrane contains at least two types of Ca^{2+} pumps that also play some role in Ca^{2+} uptake, one ATP-driven, and one driven by a concurrent flow of Na^{+} ions into the cytoplasm

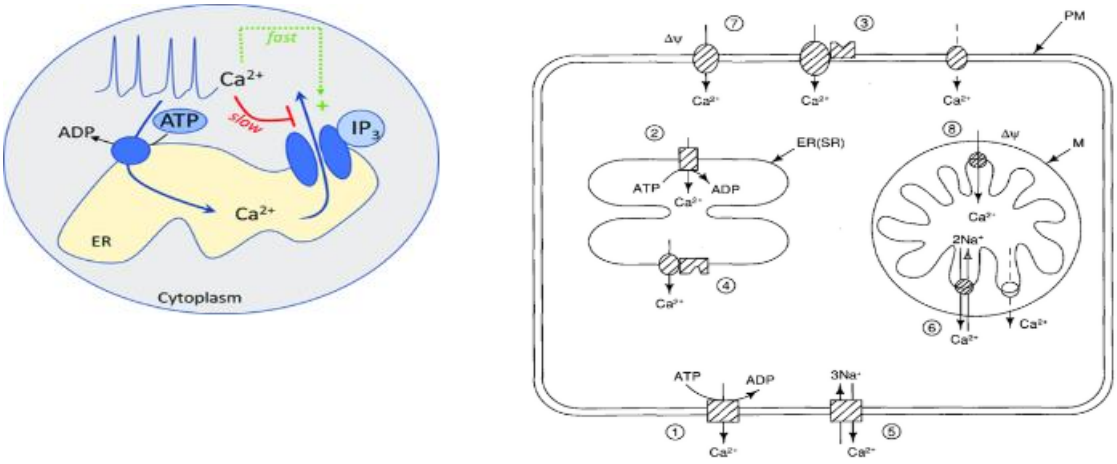


Figure: Schematic representation of the major pathways for the transport of Ca^{2+} across cellular membranes; The transport proteins shown are: 1 and 2, Plasma membrane (PM) and ER(SR) Ca^{2+}-ATPases; 3 and 4, PM and ER(SR) receptor-mediated Ca^{2+} channels; 5 and 6, PM and M (inner-membrane) Na+/Ca^{2+} exchangers; 7 and 8, PM and M voltage-sensitive Ca^{2+} channels.

Transporters

- The Ca^{2+}-ATPases
- The Na +/Ca^{2+} exchanger of the plasma membrane
- Mitochondrial Ca^{2+} transport: influx
- Mitochondrial Ca^{2+} transport: efflux
- Ca^{2+} efflux from non-mitochondrial stores
- Other voltage-gated or receptor-activated Ca^{2+} channels

Thermodynamic limits of the transport

- Let us define an "inside" and an "outside" separated by a membrane (Fig. 3.13), where $[Ca^{2+}]$ and Ψ denote activities and membrane potentials, respectively.
- The difference in electrochemical potential, $\Delta\mu$, across the membrane for a Ca^{2+} ion is given by $\Delta\mu_{Ca2+} = + RT \ln ([Ca^{2+}]_{out}/[Ca^{2+}]_{in}) + 2F\Delta\Psi$, where F is Faraday's constant, T the temperature and R is the gas constant
- At SR, $\Delta\Psi = 0$; the free energy change ΔG for transferring Δn moles of Ca^{2+} across the membrane at RT can be calculated using $\Delta G = -\Delta n \times \Delta\mu_{Ca2+}$
- If $[Ca^{2+}]_{out}/[Ca^{2+}]_{in} = 10^{-3}$, $\Delta G = \Delta n \times 4.1$ kcal/mol
- If $[Ca^{2+}]_{out}/[Ca^{2+}]_{in} = 10^{-4}$, $\Delta G = \Delta n \times 5.4$ kcal/mol
- Under the pertinent cellular conditions, ΔG associated with ATP hydrolysis has been calculated by Tanford to be – 13 to – 14 kcal/mol

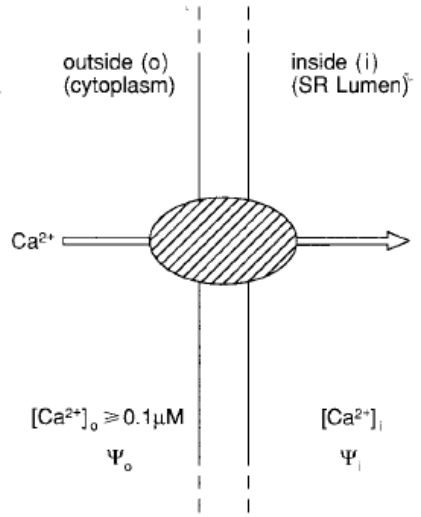


Figure 3.13
Schematic representation of Ca^{2+} transport through a membrane by a Ca^{2+}-ATPase molecule. Ψ denotes membrane potentials.

- In the absence of a membrane potential, it is thus possible to transport two Ca^{2+} ions for every ATP molecule hydrolyzed against a concentration (or activity) gradient of 10^4 or more.
- In the specialized cells of muscle tissue, the SR may contain much calcium (free, as high as high as 30 mM)
- This would cause an osmotic pressure difference across the membrane, as well as put a high demand on the SR Ca^{2+}-ATPase.
- A lowering of the free Ca^{2+} concentration inside the SR would be clearly beneficial

2. The Na^+/Ca^{2+} exchanger of the plasma membrane

- In heart plasma-membrane vesicles, the exchanger has the following characteristics: Km = 1.5-5 μM for Ca^{2+} and ~ 20 nM for Na^+; Vmax = 20 nmol Ca^{2+}/mg protein.
- The stoichiometry is at least 3:1 Na^+/Ca^{2+}
- Let us consider the thermodynamic framework for an Na^+/Ca^{2+} exchanger (Fig.). The difference in electrochemical potential for Na^+ and Ca^{2+} across the membrane is:

$$\Delta\mu_{Ca2+} = + RT \ln ([Ca^{2+}]_{out}/[Ca^{2+}]_{in}) + 2F\Delta\Psi,$$

$$\Delta\mu_{Na+} = + RT \ln ([Na^+]_{out}/[Na^+]_{in}) + F\Delta\Psi$$

- The free-energy change, $\Delta G_t Ca^{2+}$, associated with a transfer of Δn_{Ca2+} moles of Ca^{2+} from the inside to the outside is $\Delta G_t Ca^{2+} = -\Delta n_{Ca2+} \times \Delta\mu_{Ca2+}$
- The corresponding change associated with the movement of Δn_{Na+} moles of Na+ from the inside to the outside is $\Delta G_t Na^+ = -\Delta n_{Na++} \times \Delta\mu_{Na+}$
- If these free-energy changes are coupled via the exchanger, there will be a net flux of Ca^{2+} as long as the free-energy difference,

$$\Delta\Delta G = \Delta G_t Ca^{2+} - \Delta G_t Na^+ = (-\Delta n_{Ca2+} \times \Delta\mu_{Ca2+}) - (\Delta n_{Na++} \times \Delta\mu_{Na+})$$
$$= 2.303RT[\log([Ca^{2+}]_{out}/[Ca^{2+}]_{in}) - \Delta n_{Na+} \log([Na^+]_{out}/[Na^+]_{in})] + (2 - \Delta n_{Na+})F\Delta\Psi$$

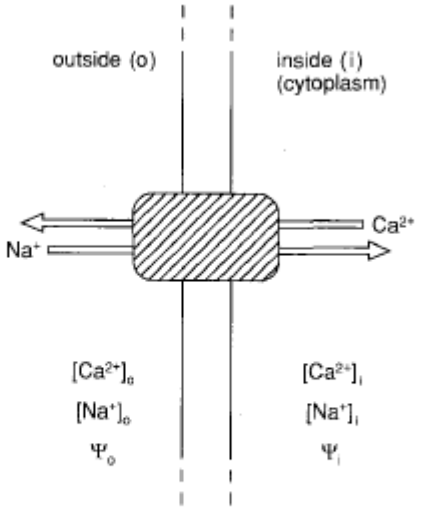


Figure 3.14
Schematic representation of the Ca^{2+}/Na^+ exchanger of the plasma membrane. Ψ denotes membrane potentials.

- Equating ion activities with concentrations, in a typical mammalian cell $[Na^+]_{out}$ = 110-145 mM, and $[Na^+]_{in}$ = 7-15 mM, or $[Na^+]_{out}[Na^+]_{in} = 10$.
- In the absence of a membrane potential difference $\Delta\Psi = 0$, above equation is simplified to

$$\Delta\Delta G = 2.303RT[\log([Ca^{2+}]_{out}/[Ca^{2+}]_{in}) - \Delta n_{Na+}]$$

- To pump one Ca^{2+} ion out of a cell against a concentration gradient of about 10^3 ($1\ \mu M \rightarrow 1$ mM) requires that at least 3 Na^+ ions pass in the opposite direction, thus maintaining $\Delta\Delta G < 0$.
- What then will be the effect of a membrane potential difference?
- Most animal cells, particularly excitable cells such as nerve and muscle cells, have resting potential differences, $\Delta\Psi$, over the plasma membrane of 30 to 90 mV (cytoplasm negative).
- For this value, the change in free energy, $\Delta\Delta G$, for the transport of one mol Ca^{2+} to be

$$\Delta\Delta G = 2.303RT[(\log([Ca^{2+}]_{out}/[Ca^{2+}]_{in}) - \Delta n_{Na+})] + (2 - \Delta n_{Na+})\ 0.1F$$

- Thus, for $\Delta n_{Na+} > 2$, we have $\Delta\Delta G < 0$, and the transport of Ca^{2+} against a concentration gradient of about 10^3 will be promoted.
- This is another good reason for having a Na^+/Ca^{2+} exchange stoichiometry of 3:1

3. Mitochondrial Ca^{2+} transport: influx

- Mitochondria isolated from various types of animal cells can rapidly accumulate exogenous Ca^{2+}.

- The transporter is located in the inner membrane and the driving force behind the Ca^{2+} transport appears to be the high potential difference across this membrane ($\Delta\Psi$ = 150 to 180 mV, negative in the inner matrix).
- This potential difference is fairly closely maintained by the pumping out of H^+ from the matrix by cell respiration.
- For the transport of 1 mol Ca^{2+} from the "outside" (= cytoplasm) to the "inside" (= inner mitochondrial matrix), the free-energy change ΔG may be written (~nCa^{2+} = - 1)

$$\Delta G = - RT \ln ([Ca^{2+}]_{out}/[Ca^{2+}]_{in}) - 2F\Delta\Psi$$

- From this analysis it may be inferred that the limiting Ca^{2+} concentration (or activity) ratio that can be achieved by this **electrogenic** pump (i.e., $\Delta G = 0$) is

$$[Ca^{2+}]_{out}/[Ca^{2+}]_{in} = \exp (-2F\Delta\Psi/RT)$$

- With $\Delta\Psi$ =150 mV, this ratio is calculated to be $8.4x10^{-6}$ at 25ºC
- The Ca^{2+} **uniporter** has a very high pumping potential if the Ca^{2+} influx does not lower the membrane potential difference.
- The pumping rate, V_{max}, are indeed high (>10 nmol/mg protein) and probably limited only by the rate of electron transport and H^+ extrusion in the mitochondria.
- Mitochondria may accumulate large quantities of Ca^{2+}, probably to maintain electroneutrality.
- To prevent the buildup of high concentrations of free Ca^{2+} (and of osmotic pressure), phosphate ions are also transported into the inner matrix, where amorphous calcium phosphate or a phosphocitrate is formed.
- The equilibrium concentration of free Ca^{2+} in the mitochondrial matrix may as a result be comparatively low (on the order of 1 μM).

4. Mitochondrial Ca^{2+} transport: efflux

- Mitochondria, as well as SR, release Ca^{2+} ions by mechanisms other than "back leakage" through the pumps.
- In mitochondria from excitable cells, the efflux occurs mainly through an antiport, where $2Na^+$ ions are transported inward for every Ca^{2+} ion departing for the cytosolic compartment.
- In other cells there is evidence for the dominance of a $2H^+$-Ca^{2+} antiport
- The Ca^{2+}-efflux is regulated, possibly by the redox state of pyridine nucleotides in the mitochondria.

5. Ca^{2+} efflux from non-mitochondrial stores

- Release of Ca^{2+} from ER and SR presently appears to be the prime effect of the new intracellular messenger 1,4,5-triphosphoinositol (l,4,5-IP_3) released into the cytoplasm because of an external hormonal stimulus
- The receptors for 1,4,5-IP_3 have been established on ER, and that the binding of 1,4,5-IP_3 causes a release of Ca^{2+} stored in this organelle.
- In addition to the receptor-controlled Ca^{2+} efflux, there may be other pathways for Ca^{2+} release, and Ca^{2+} mobilization may be regulated by other intracellular entities, including the Ca^{2+} ions

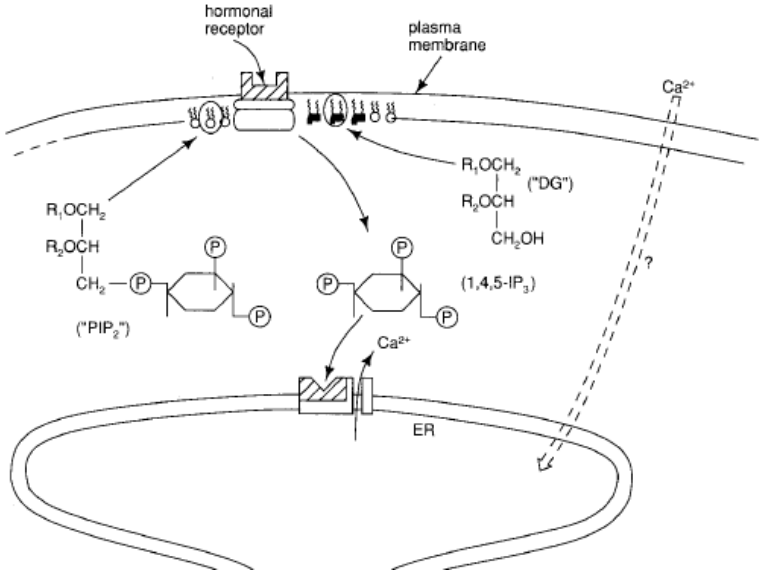
6. Other voltage-gated or receptor-activated Ca^{2+} channels

- Some cells seem to have Ca^{2+} channels in the plasma membrane that can be opened by the action of an agonist on a receptor or that are gated in response to changes in membrane potential.
- For example, Ca^{2+} channels can be opened by nicotinic cholinergic agonists or by the excitatory amino acid N-methyl-D-aspartate (NMDA).

- Endocrine cells and also some muscle and neuronal cells have voltage-sensitive Ca^{2+} channels.

C. Inositol Trisphosphate and the Ca^{2+} Messenger System

- A "second" messenger is an entity that inside a cell mediates the action of some hormone at the plasma membrane, the hormone being considered the "first" messenger.
- The discovery of the first such second messenger, and the very molecule that led to the formulation of the whole concept-was cyclic AMP.
- The intracellular release of Ca^{2+} ions also accompanied hormonal stimuli, and the Ca^{2+} ion slowly became regarded as a second messenger (Rasmussen in early 1970s).
- In the mid-1970s Calmodulin (Ca^{2+}-binding protein) protein was shown to be a Ca^{2+}-dependent regulator of several Ca^{2+}-dependent enzymes, transport proteins, etc., establishing a molecular basis for Ca^{2+} action in cells.
- Although a transitory increase in intracellular Ca^{2+} concentration in response to the binding of a hormone or transmitter substance to a surface receptor could result from extracellular Ca^{2+} being released into the cytoplasm, the muscle cells that the main Ca^{2+} source was the sarcoplasmic reticulum (SR).
- "Ca^{2+}-induced Ca^{2+} release," i.e., that upon stimulation of the cell, a small amount of Ca^{2+} entered into the cytoplasm and triggered the release of greater amounts of Ca^{2+} from the SR.
- For some cell types transient increases in intracellular Ca^{2+} could occur even when extracellular Ca^{2+} was removed, although *prolonged* responses required the presence of extracellular Ca^{2+}
- Release of Ca^{2+} into the cytoplasm from intracellular stores appears to be important.
- The intracellular Ca^{2+} is released in response to the formation of a new type of intracellular messenger: 1,4,5-IP_3 (several evidences).
- Receptors for this messenger is found in the membranes of intracellular organelles, and binding of 1,4,5-IP_3 to these receptors results in the release of Ca^{2+} ions.
- 1,4,5-IP_3 is formed as a product in the hydrolysis of a special phospholipid (phosphatidyl-inositol-4,5-bisphosphate.) present in the cell membrane
- The newly formed 1,4,5IP_3 is assumed to diffuse into the cytoplasm, and eventually reach intracellular 1,4,5-IP_3 receptors on the ER, thereby triggering the release of Ca^{2+}
- A diacylglycerol (DG) is also formed in the hydrolysis step. DG can also act as an intracellular messenger, and stimulates the activity of a membrane-bound protein kinase, known as *protein kinase* C (PKC).
- PKC may phosphorylate certain key proteins and influence their activity. PKC is also activated by Ca^{2+} ions.
- 1,4,5-IP_3 is either directly degraded in a series of enzymatic steps back to inositol, which is then used to resynthesize the phospholipid, or it may be further phosphorylated to inositol-l,3,4,5-tetraphosphate (1,3,4,5-IP_4)' which may undergo de phosphorylation to form inositol-1,3,4-trisphosphate (l,3,4-IP_3)
- The intracellular levels of Ca^{2+} are restored back to the normal low resting values (l00 to 200 nM) via transport back into the SR, and/or into mitochondria, or out through the plasma membrane by the pumping mechanisms.
- Depriving a cell of extracellular Ca^{2+} will make the cell incapable of prolonged responses to external stimuli.
- The intracellular Ca^{2+} stores may become depleted if not replenished.



Outline of the presumed role of inositol phosphates in the intracellular mobilization of Ca^{2+}.

- The intracellular ER Ca^{2+} pool has a direct route of access to the extracellular pool, a route that is closed when the ER pool is full.
- Ca^{2+} seems to have been downgraded by the inositol phosphates from a "second" to a "third" messenger; however, the pivotal role of Ca^{2+} as a regulator of cellular activities is undisputed.

Summary

- The fluxes of Ca^{2+} ions & their regulation in micro- and higher organisms, depend on several transport proteins in addition to vesicular and gated processes.
- Ca^{2+}-ATPase is an important class of transport proteins (in muscle cells)
- These proteins translocate Ca^{2+} ions against large activity (or concentration) gradients through the expenditure of ATP.
- Transport of Ca^{2+} ions against activity gradients across membranes may also be accomplished by coupled transport of other ions (Na^{+}), with a gradient in the opposite direction.
- As a result of some external stimulus-the action of a hormone, for example the "free" Ca^{2+}-ion concentrations in the cytoplasm of many cell types may transiently increase
- This increase largely results from the release of Ca^{2+} from intracellular stores (ER, SR)
- The activity of Ca^{2+}-transport proteins eventually restores the Ca^{2+} concentration levels to resting levels.
- This sequence of events forms the basis for Ca^{2+}'s role in the regulation of a wide variety of cellular activities

Molecular aspects of Ca^{2+}-regulated intracellular processes

- Many biological processes (muscle contraction, transport processes, cell division and growth, enzyme activities, and metabolic processes) are regulated by intracellular Ca^{2+} concentration
- For the purpose, Ca^{2+} ions must interact with different proteins, known as intracellular Ca^{2+} receptors
- Characteristics of intracellular Ca^{2+}-receptor proteins

(a) Their Ca^{2+}-affinity must be such that their Ca^{2+}-binding sites are unoccupied at resting levels of free Ca^{2+} (~10^{-7} M) and occupied at levels reached upon stimulus (10^{-5} - 10^{-6} M). The binding constant, K_B^{Ca2+} is ~10^6 M^{-1}

(b) Ca^{2+} must exert its function in the presence of several other ions (in mammalian cells, intracellular Mg^{2+} and K^+ ions are present). The receptors must therefore have an adequate selectivity for Ca^{2+}

(c) In response to Ca^{2+} binding, a Ca^{2+} receptor must undergo conformation change to alter its interaction with other molecules and changes its activity if it is an enzyme.

(d) Finally, the receptors must be able to interact swiftly (in mS) with incoming Ca^{2+} ions, & the ions must also be able to depart almost as rapidly.

- This class of Ca^{2+} receptors is often called the "calmodulin superfamily" and includes troponin C (regulating muscle contraction) and calmodulin (regulation of many cellular processes).
- Amino-acid sequence, X-ray and 2D ^1H NMR studies have revealed a strong homology between the regulatory Ca^{2+}-binding proteins.
- The Ca^2 -binding sites are located in a loop flanked by two helices, and the Ca^{2+} ions are ligated with approximately octahedral or pentagonal bipyramidal symmetry.
- The ligands are six or seven oxygen atoms that are furnished by sidechain carboxylate or hydroxyl groups, backbone carbonyls, and water molecules.
- Pairs of these Ca^{2+} sites appear to be the functional unit, and a common consequence of their arrangement is cooperative Ca^{2+} binding.
- Ca^{2+} binding to the intracellular receptor proteins is accompanied by structural changes that expose hydrophobic patches on their surfaces and enable them to bind to target proteins.

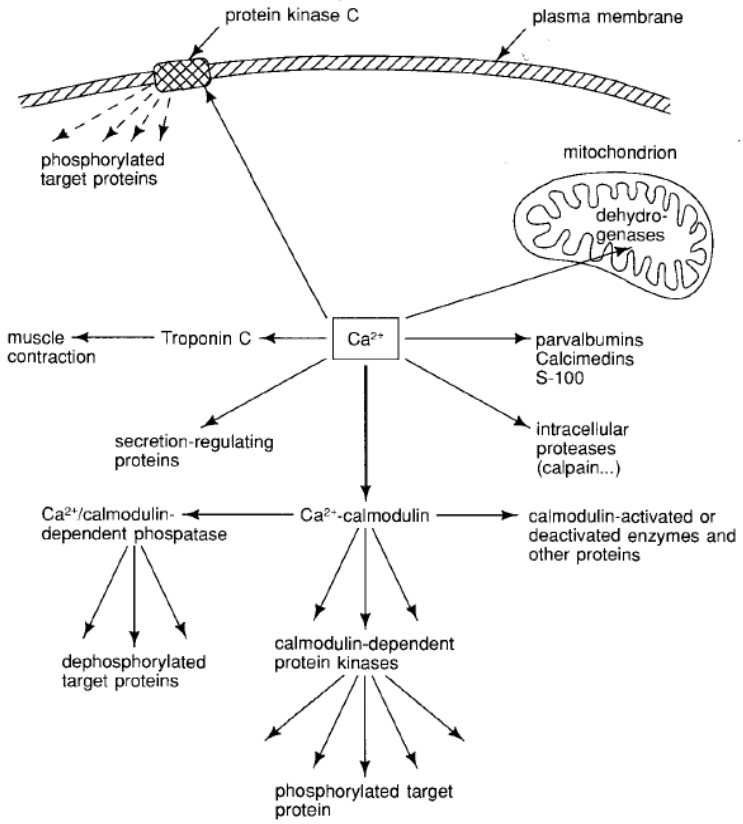


Figure 3.16
Condensed overview of the interaction of Ca^{2+} with intracellular proteins.

Calmodulin (CaM, MW 17kDa)

- It is a calcium modulating protein present in eucaryotic cells
- Acts as a regulatory protein to stimulate many enzymes, proteins, transporters and many cellular signaling systems
- It possesses a dumble like shape with motif globular domains connected by an 8-turn helix
- There is no direct contact between the domains and each domain possesses two structurally similar Ca^{2+}-binding sites (total 4 Ca^{2+} binding site)
- Each of the 4 Ca^{2+} binding site represents a helix-loop-helix motif (known as EF hand: E-forefinger, F-thumb)
- In this structural motif, the loop possesses the Ca^{2+} binding site and two helices are positioned like the forefinger (E-helix) and thumb (F-helix) of the right hand
- In this helix loop-helix structural motif, one helix lies in the plane of the finger while the other helix is projected perpendicular to the plane
- The remaining fingers create the Ca^{2+} binding loop.
- This structural motif is found in many other Ca^{2+} binding proteins like troponin, parvalbumin
- Ca^{2+} present in the loop of CaM maintains the 6 or 7-coordination number (CaO_6 or CaO_7 unit, hard-hard interaction)
- For 7 coordination number, the coordination sites are 5 from carboxylate groups of aspartate and glutamate moieties, 1 from the backbone carbonyl groups and 1 from H_2O molecule ion
- CaM can selectively bind Ca^{2+} in presence of other metal ions or even in the presence of abundant Mg^{2+} (due to small size it does not fit properly to the loop)
- The first two Ca^{2+} ion bind in a cooperative manner with higher binding constant ($10^5 M^{-1}$) and the other two bind weakly (10^4 M^{-1})
- CaM changes its conformation when it binds with Ca^{2+}
- To coordinate Ca^{2+}, the coordinating sites (aspartate, glutamate) get projected towards Ca^{2+} position in the loop of EF-motif.
- It brings the hydrophobic residues from inside to the outside of the domains.
- These exposed hydrophobic patches on the surface of the domain can interact with many other biomolecules (proteins, enzymes like CaM–dependent kinases) to stimulate them
- Ca^{2+} free CaM cannot recognize these biomolecules

  CaM (Inactive) + $4Ca^{2+}$ → CaM*$(Ca^{2+})_4$ (active) → Ca*$(Ca)_4$.Enz (activated enzymes)
- Thus, many enzymes (NO synthase, phosphates which carry outs dephosphorylation, protein kinase which can carry out the phosphorylation process), transporting pumps (Ca –ATPase) and many other target proteins are activated by Ca^{2+} loaded calmodulin
- The enzyme, myosin kinase which mediates the myosin-actin interaction is activated by Ca^{2+}- loaded CaM.
- The tranquilizer like phenothiazine and a wide variety of insect venom proteins disturbs the physiological process by inhibiting the calmodulin function.
- Calmodulin inhibition by Zn(II) salts may produce the anti-inflammatory action and also ant sickling activity in the treatment of sickle cell anemia

Troponin C

- Troponin complex consists of troponin I (Tn-I), troponin T (Tn-T) and troponin C (Tn-C)

- Tn-C (MW = 18kDa) is a Ca^{2+}-binding protein that acts as the central regulatory protein of muscle contraction in higher vertebrates.
- Troponin complex (Ca^{2+}-free condition), blocks the myosin binding sites on the actin filament.
- Ca^{2+} binding with Tn-C causes its conformational change and the troponin complex is removed from the actin filament and then the actin-myosin interaction needed for the muscle contraction becomes possible
- Skeletal muscle troponin C (4 Ca^{2+}-binding sites) is structurally similar to calmodulin
- Tn-C also possesses two domains and each domain contains two Ca^{2+} binding sites.
- Tn-C domain possesses the same helix-loop-helix structural motif as in CaM.
- In cardiac muscle troponin C, one of the four Ca^{2+} binding sites has been modified and it binds with three Ca^{2+} ions.

Parvalbumin and Calbindins D9K and D28K

- Parvalbumin (MW = 12 kDa) is a Ca^{2+}-binding intracellular protein belong to the CaM-TnC family in terms of the nature of Ca^{2+}-binding sites (helix-loop-helix motif described as EF-hand structure).
- It is found in the lower vertebrates (muscles of chicken, turkey and rabbit) probably does not show any direct regulatory function.
- It shows two Ca^{2+} binding sites (CaM, Tn-C having 4 Ca^{2+} binding sites)
- Calbindin D_{9K} (MW = 9 kDa) and Calbindin D_{28K} (MW = 28 kDa)
- These calbindins (binding proteins) also belong to the CaM-Tn-C family in terms of the nature of Ca^{2+}-binding sites.
- These intracellular Ca^{2+}-binding proteins do not show the direct regulatory function.

Sarcoplasmic Calcium-Binding Protein from *Nereis diversicolor*

- The calmodulin superfamily of proteins also includes sarcoplasmic Ca^{2+}-binding proteins (SCPs) that are vertebrate and invertebrate muscle.
- They originally contained four helix-loop-helix Ca^{2+}-binding domains.
- Ca^{2+} binding has been preserved in the first and third domains of all known SCPs, but only one, if any, of domains II and IV is functional.
- The C-terminal half (domains III and IV) of the molecule contains two Ca^{2+} binding EF-hands similar to calbindin D9k and the globular domains of troponin C and calmodulin.
- The N-terminal half markedly different from the normal helix-loop-helix geometry.
- The domain I binds Ca^{2+} with a novel helix-loop-helix conformation, whereas domain II lacks calcium-binding capacity.
- The two halves are packed closely together, and are connected by a solvent-exposed α-helix.

Membrane Cytoskeleton and Phospholipid Binding Proteins

- These are some intracellular Ca^{2+} phospholipid-binding proteins that appear to be distinct from the calmodulin superfamily
- These include *lipocortin, endonexin, calelectrin, p36,* and *calpactin*
- These membrane-binding proteins are collectively called *annexins,* and contain repeated domains distinct from EF-hands.
- The Ca^{2+} sites are very similar to that observed in phospholipase A_2, as shown by the recently determined X-ray structure of annexin V.

- In annexin, the three Ca^{2+}-binding sites are located on the side of the molecule that is involved in membrane binding.

Ca^{2+}-Dependent Proteases

- Ca^{2+}-activated intracellular protease (calpain), contains four distinct domains.
- The first and third domains have no clear sequence homologies with known protein sequences, but the second domain has a high homology with the proteolytic enzyme papain, and the fourth domain is highly homologous to calmodulin.
- This fourth domain thus has four EF-hand-type Ca^{2+}-binding sites, although the third site has a somewhat unusual loop sequence.

Protein Kinase C

- Protein kinase C (PKC) is an important Ca^{2+}-regulated kinase phosphorylating enzyme
- The activity of this enzyme appears to be regulated by three factors: phospholipids, in particular phosphatidylserine; diacyl-glycerol, one of the products of inositol lipid breakdown; and Ca^{2+} ions.
- The high-activity form of PKC, which appears responsible for much of the phosphorylation activity of many cells, is presumably membrane-bound, whereas the low-activity form may be partly cytosolic.
- In rabbit PKC (MW = 77 kDa), the Ca^{2+} site(s) are presumably in the regulatory domain.
- No typical "EF-hand" pattern has been found in the amino-acid sequence.
- A protein kinase that requires Ca^{2+} but not phospholipids nor calmodulin for activity has been purified from soybean.
- From the amino-acid sequence the protein appears to have a calmodulin-like Ca^{2+}-binding domain, very much as in calpain.
- Its activity is markedly increased in the presence of Ca^{2+}, and it has a high calcium-binding constant in the presence of diacylglycerol or **phorbol esters**

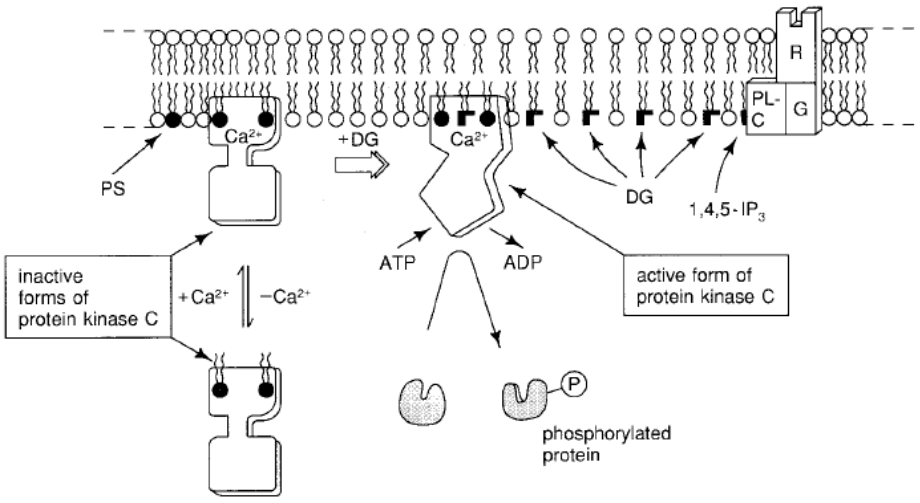


Figure 3.27
Outline of the cellular events that result in the activation of protein kinase C (PKC). The enzyme apparently exists in at least two states. Recent sequence work indicates that it has a Ca^{2+}-binding site of the EF-hand type. When no Ca^{2+} ion is bound, and when the ''concentration'' of diacylglycerol (DG) in the inner layer of the plasma membrane is low, the kinase exists in a low-activity form, possibly dissociated from the membrane. When a hormone binds to a plasma-membrane receptor (R), cleavage of phosphoinositol into 1,4,5-IP_3 and DG is induced. The latter lipid may bind to and activate the calcium-loaded form of PKC. The active form of protein kinase C will now phosphorylate other cytoplasmic proteins, and in this way modify their biochemical properties. R = receptor; PL-C = phospholipase C; G = a GTP-binding protein that is assumed to act as an intermediary between the receptor and the membrane bound PL-C.

In summary,

- C*almodulin* (CaM, best intracellular Ca^{2+} receptors) is present in all eukaryotic cells. Most of the cellular responses elicited by Ca^{2+} appear to result from interactions between the Ca^{2+}-calmodulin complex and various other target enzymes and proteins.
- Another important Ca^{2+}-receptor protein is *troponin* C (TnC), which occurs in muscle cells and is instrumental in mediating muscle contraction.
- These two are highly homologous, and are the members of a superfamily (the calmodulin superfamily) of closely related intracellular Ca^{2+}-binding proteins.
- All members of the superfamily are not Ca^{2+}-receptors (*parvalbumins* and *calbindins* have some role in intracellular transport and/or Ca^{2+}-buffering)

Extracellular Ca^{2+}-Binding proteins

- The Ca^{2+}-concentration in extracellular fluids is usually orders of magnitude higher than intracellular concentrations.
- Ca^{2+} ions in extracellular fluids play a very different role from that inside cell.
- To ensure Ca^{2+} binding the macromolecular binding sites need have only a modest Ca^{2+} affinity and since extracellular Ca^{2+} does not seem to have a signaling function, the rates of Ca^{2+} association or dissociation in protein binding sites need not be very high.
- One particularly important aspect of Ca^{2+} in mammals is its role in the blood coagulation system.

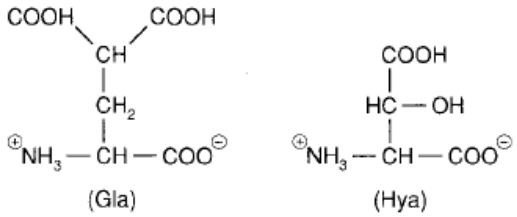


Figure 3.29
Chemical structures of two novel amino acids believed to bind calcium in, e.g., blood-clotting proteins.

Consulted Books

1. Bioinorganic Chemistry by Valentine, Gray, Lippard and Bertini
2. Bioinorganic Chemistry by Das and Das
3. Inorganic Chemistry by Huheey, Keiter, Huheey and Medhi
4. Inorganic Chemistry by Atkins
5. Inorganic Chemistry by Missler and Tar
6. EPG-Pathshala
7. IGNOU-MSc Materials
8. Bioinorganic Chemistry by Fenton