Unit wise notes

PHARMACEUTICAL CHEMISTRY- IV
(Molecular Biology & Biochemistry)
B.PHARM

B.PHARM

IV SEMESTER, 2nd YEAR

SHAMBHUNATH INSTITUTE OF PHARMACY
JHAWLA ALLAHABAD

2015-2016

Prepared by
S.S.GAUTAM
(Assoc.Prof)

UNIT-I

Introduction and scope of molecular biology

The **history of molecular biology** begins in the 1930s with the convergence of various, previously distinct biological and physical disciplines: biochemistry, genetics,microbiology, virology and physics. With the hope of understanding life at its most fundamental level, numerous physicists and chemists also took an interest in what would become molecular biology.

In its modern sense, molecular biology attempts to explain the phenomena of life starting from the macromolecular properties that generate them. Two categories of macromolecules in particular are the focus of the molecular biologist: 1) nucleic acids, among which the most famous is deoxyribonucleic acid (or DNA), the constituent of genes, and 2) proteins, which are the active agents of living organisms. One definition of the scope of molecular biology therefore is to characterize the structure, function and relationships between these two types of macromolecules. This relatively limited definition will suffice to allow us to establish a date for the so-called "molecular revolution", or at least to establish a chronology of its most fundamental developments.

First isolation of DNA:-

Working in the 19th century, biochemists initially isolated DNA and RNA (mixed together) from cell nuclei. They were relatively quick to appreciate the polymeric nature of their "nucleic acid" isolates, but realized only later that nucleotides were of two types—one containing ribose and the otherdeoxyribose. It was this subsequent discovery that led to the identification and naming of DNA as a substance distinct from RNA.

Friedrich Miescher (1844–1895) discovered a substance he called "nuclein" in 1869. Somewhat later, he isolated a pure sample of the material now known as DNA from the sperm of salmon, and in 1889 his pupil, Richard Altmann, named it "nucleic acid". This substance was found to exist only in the chromosomes.

Chromosomes and inherited traits:-

In 1927 Nikolai Koltsov proposed that inherited traits would be inherited via a "giant hereditary molecule" which would be made up of "two mirror strands that would replicate in a semi-conservative fashion using each strand as a template".[8] Max Delbrück, Nikolay Timofeev-Ressovsky, and Karl G. Zimmer published results in 1935 suggesting that chromosomes are very large molecules the structure of which can be changed by treatment withX-rays, and that by so changing their structure it was possible to change the heritable characteristics governed by those chromosomes. In 1937 William Astbury produced the first X-ray diffraction patterns from DNA.

He was not able to propose the correct structure but the patterns showed that DNA had a regular structure and therefore it might be possible to deduce what this structure was.

In 1943, Oswald Theodore Avery and a team of scientists discovered that traits proper to the "smooth" form of the Pneumococcus could be transferred to the "rough" form of the same bacteria merely by making the killed "smooth" (S) form available to the live "rough" (R) form. Quite unexpectedly, the living R Pneumococcus bacteria were transformed into a new strain of the S form, and the transferred S characteristics turned out to be heritable. Avery called the medium of transfer of traits the transforming principle; he identified DNA as the transforming principle, and not protein as previously thought. He essentially redid Frederick Griffith's experiment.

Discovery of the structure of DNA:-

In the 1950s, three groups made it their goal to determine the structure of DNA. The first group to start was at King's College London and was led by Maurice Wilkins and was later joined by Rosalind Franklin and Eze Benjamin O . Another group consisting of Francis Crick and James Watson was at Cambridge. A third group was at Caltech and was led by Linus Pauling. Crick and Watson built physical models using metal rods and balls, in which they incorporated the known chemical structures of the nucleotides, as well as the known position of the linkages joining one nucleotide to the next along the polymer.

Helix structure:-

In 1948 Pauling discovered that many proteins included helical shapes. Pauling had deduced this structure from X-ray patterns and from attempts to physically model the structures. (Pauling was also later to suggest an incorrect three chain helical DNA structure based on Astbury's data.) Even in the initial diffraction data from DNA by Maurice Wilkins, it was evident that the structure involved helices. But this insight was only a beginning.

Complementary nucleotides:-

In their modeling, Watson and Crick restricted themselves to what they saw as chemically and biologically reasonable. Still, the breadth of possibilities was very wide. A breakthrough occurred in 1952, when Erwin Chargaff visited Cambridge and inspired Crick with a description of experiments Chargaff had published in 1947. Chargaff had observed that the proportions of the four nucleotides vary between one DNA sample and the next, but that for particular pairs of nucleotides — adenine and thymine, guanine and cytosine — the two nucleotides are always present in equal proportions.

Central Dogma:-

Watson and Crick's model attracted great interest immediately upon its presentation. Arriving at their conclusion on February 21, 1953, Watson and Crick made their first announcement on

February 28. In an influential presentation in 1957, Crick laid out the "central dogma of molecular biology",

Macromolecule & biological significance:-

A **macromolecule** is a very large molecule, such as protein, commonly created by polymerization of smaller subunits (monomers). They are typically composed of thousands or more atoms. The most common macromolecules in biochemistryare biopolymers (nucleic acids, proteins, carbohydrates and polyphenols) and large non-polymeric molecules (such as lipidsand macrocycles). Synthetic macromolecules include common plastics and synthetic fibres as well as experimental materials such as carbon nanotubes.

The term macromolecule (macro- + molecule) was coined by Nobel laureate Hermann Staudinger in the 1920s, although his first relevant publication on this field only mentions high molecular compounds (in excess of 1,000 atoms). At that time the phrase polymer, as introduced by Berzelius in 1833, had a different meaning from that of today.

According to the standard IUPAC definition, the term macromolecule as used in polymer science refers only to a single molecule. For example single polymeric molecule is appropriately described as a "macromolecule".

Structural feature:-

Because of the double-stranded nature of DNA, essentially all of the nucleotides take the form of Watson-Crick base pairs between nucleotides on the two complementary strands of the double-helix.

DNA is optimized for encoding information:-

DNA is an information storage macromolecule that encodes the complete set of instructions (the genome) that are required to assemble, maintain, and reproduce every living organism.

Proteins are optimized for catalysis:-

Proteins are functional macromolecules responsible for catalyzing the biochemical reactions that sustain life. Proteins carry out all functions of an organism, for example photosynthesis, neural function, vision, and movement.

RNA is multifunctional:-

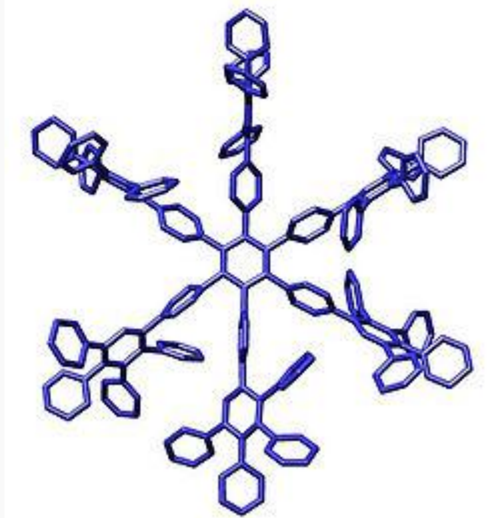
RNA is multifunctional; its primary function is to encode proteins, according to the instructions within a cell’s DNA. They control and regulate many aspects of protein synthesis in eukaryotes.

Branched biopolymers:-

Raspberry ellagitannin, a tannincomposed of core of glucose units surrounded by gallic acid esters and ellagic acid units.

Carbohydrate macromolecules (polysaccharides) are formed from polymers of monosaccharide. Because monosaccharides have multiple functional groups, polysaccharides can form linear polymers (e.g. cellulose) or complex branched structures (e.g. glycogen).

Synthetic macromolecules



Structure of a polyphenylene dendrimer macromolecule.

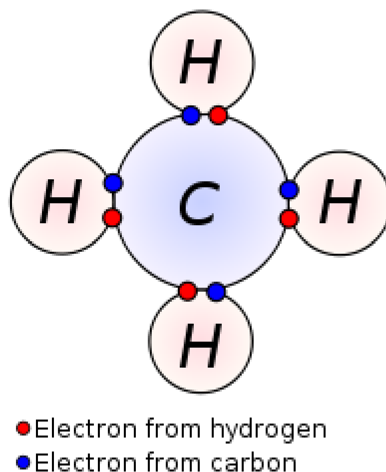
Some examples of macromolecules are synthetic polymers (plastics, synthetic fibers, and synthetic rubber), graphene, and carbon nanotubes.

Covalent bonds in macromolecules:-

The term **macromolecular assembly** (MA) refers to massive chemical structures such as virusesand non-biologic nanoparticles, cellular organelles and membranes and ribosomes, etc. that are complex mixtures of polypeptide, polynucleotide, polysaccharide or other polymericmacromolecules. They are generally of more than one of these types, and the mixtures are defined spatially (i.e., with regard to their chemical shape), and with regard to their underlying chemical composition and structure. Macromolecules are found in living and nonliving things, and are composed of many hundreds or thousands of atoms held together by covalent bonds; they are often characterized by repeating units (i.e., they are polymers). Assemblies of these can likewise be biologic or non-biologic, though the MA term is more commonly applied in biology, and the termsupramolecular assembly is more often applied in non-biologic contexts (e.g., in supramolecular chemistry and nanotechnology). MAs of macromolecules are held in their defined forms by non-covalent intermolecular interactions (rather than covalent bonds), and can be in either non-repeating structures (e.g., as in

the ribosome and or in repeating linear, circular, spiral, or other patterns (e.g., as in actin filaments and the flagellar motor, image). The process by which MAs are formed has been termed molecular self-assembly, a term especially applied in non-biologic contexts.

A covalent bond is a chemical bond that involves the sharing of electron pairs between atoms. These electron pairs are known as shared pairs or bonding pairs, and the stable balance of attractive and repulsive forces between atoms, when they share electrons, is known as covalent bonding.



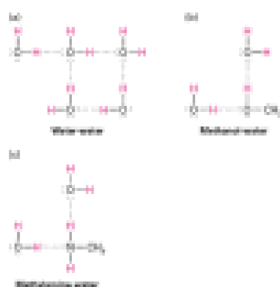
Non-covalent bonds in macromolecules:-

Carbohydrates illustrate the importance of subtle differences in covalent bonds in generating molecules with different biological activities. However, several types of noncovalent bonds are critical in maintaining the three-dimensional structures of large molecules such as proteins and nucleic acids. Noncovalent bonds also enable one large molecule to bind specifically but transiently to another, making them the basis of many dynamic biological processes.

The energy released in the formation of noncovalent bonds is only 1 – 5 kcal/mol, much less than the bond energies of single covalent bonds). Because the average kinetic energy of molecules at room temperature (25 °C) is about 0.6 kcal/mol, many molecules will have enough energy to break noncovalent bonds. Indeed, these weak bonds sometimes are referred to as interactions rather than bonds. Although noncovalent bonds are weak and have a transient existence at physiological temperatures (25 – 37 °C), multiple noncovalent bonds often act together to produce highly stable and specific associations between different parts of a large molecule or between different macromolecules).

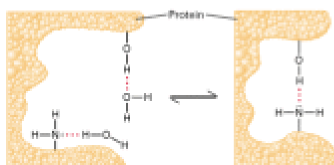
Hydrogen bond underlies water chemical and biological properties:-

Hydrogen bonding between water molecules is of crucial importance because all life requires an aqueous environment and water constitutes about 70–80 percent of the weight of most cells. The mutual attraction of its molecules causes water to have melting and boiling points at least 100 °C higher than they would be if water were nonpolar; in the absence of these intermolecular attractions, water on earth would exist primarily as a gas.



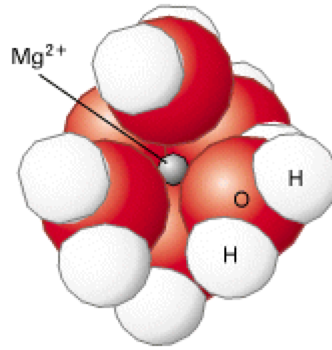
Hydrogen Bonds as a Stabilizing Force in Macromolecules:-

An important feature of all hydrogen bonds is directionality. In the strongest hydrogen bonds, the donor atom, the hydrogen atom, and the acceptor atom all lie in a straight line. Nonlinear hydrogen bonds are weaker than linear ones; still, multiple nonlinear hydrogen bonds help to stabilize the three-dimensional structures of many proteins. It is only because of the aggregate strength of multiple hydrogen bonds that they play a central role in the architecture of large biological molecules in aqueous solutions.



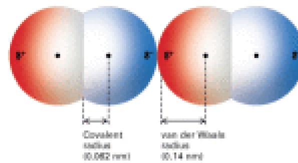
Ionic interactions are attractions between oppositely charged ions:-

In some compounds, the bonded atoms are so different in electronegativity that the bonding electrons are never shared: these electrons are always found around the more electronegative atom. In sodium chloride (NaCl), for example, the bonding electron contributed by the sodium atom is completely transferred to the chlorine atom.



Vander walls interactions are caused by transient dipoles:-

When any two atoms approach each other closely, they create a weak, nonspecific attractive force that produces a van der Waals interaction, named for Dutch physicist Johannes Diderik van der Waals (1837 – 1923), who first described it. These nonspecific interactions result from the momentary random fluctuations in the distribution of the electrons of any atom, which give rise to a transient unequal distribution of electrons, that is, a transient electric dipole.



Protein conformation and dynamics:-

Proteins are generally thought to adopt unique structures determined by their amino acid sequences, as outlined by Anfinsen's dogma. However, proteins are not strictly static objects, but rather populate ensembles of (sometimes similar) conformations. Transitions between these states occur on a variety of length scales (tenths of Å to nm) and time scales (ns to s), and have been linked to functionally relevant phenomena such as allosteric signaling and enzyme catalysis.

The study of protein dynamics is most directly concerned with the transitions between these states, but can also involve the nature and equilibrium populations of the states themselves. These two perspectives—kinetics and thermodynamics, respectively—can be conceptually synthesized in an "energy landscape" paradigm: highly populated states and the kinetics of transitions between them can be described by the depths of energy wells and the heights of energy barriers, respectively.

Local flexibility: atoms and residues:-

Portions of protein structures often deviate from the equilibrium state. Some such excursions are harmonic, such as stochastic fluctuations of chemical bonds and bond angles. Others are an harmonic, such as side chains that jump between separate discrete energy minima, or rotamers.

Evidence for local flexibility is often obtained from NMR spectroscopy. Flexible and potentially disordered regions of a protein can be detected using the random coil index. Flexibility in folded proteins can be identified by analyzing the spin relaxation of individual atoms in the protein. Flexibility can also be observed in very high-resolution electron density maps produced by X-ray crystallography particularly when diffraction data is collected at room temperature instead of the traditional cryogenic temperature (typically near 100 K).

Regional flexibility:-

Many residues are in close spatial proximity in protein structures. This is true for most residues that are contiguous in the primary sequence, but also for many that are distal in sequence yet are brought into contact in the final folded structure. Because of this proximity, these residues's energy landscapes become coupled based on various biophysical phenomena such as hydrogen bonds, ionic bonds, and van der Waals interactions.

When these coupled residues form pathways linking functionally important parts of a protein, they may participate in allosteric signaling. For example, when a molecule of oxygen binds to one subunit of the hemoglobin tetramer, that information is allosterically propagated to the other three subunits, thereby enhancing their affinity for oxygen. In this case, the coupled flexibility in hemoglobin allows for cooperative oxygen binding, which is physiologically useful because it allows rapid oxygen loading in lung tissue and rapid oxygen unloading in oxygen-deprived tissues (e.g. muscle).

Global flexibility:-

The presence of multiple domains in proteins gives rise to a great deal of flexibility and mobility, leading to **protein domain dynamics**.[1]Domain motions can be inferred by comparing different structures of a protein (as in Database of Molecular Motions), or they can be directly observed using spectra[7][8] measured by neutron spin echo spectroscopy. They can also be suggested by sampling in extensive molecular dynamics trajectories[9] and principal component analysis.[10] Domain motions are important for:-

- catalysis
- regulatory activity
- transport of metabolites
- formation of protein assemblies

- cellular locomotion

Helical to extended conformation:-

The interconversion of helical and extended conformations at the site of a domain boundary is not uncommon. In calmodulin, torsion angles change for five residues in the middle of a domain linking α-helix. The helix is split into two, almost perpendicular, smaller helices separated by four residues of an extended strand.

Shear motions:-

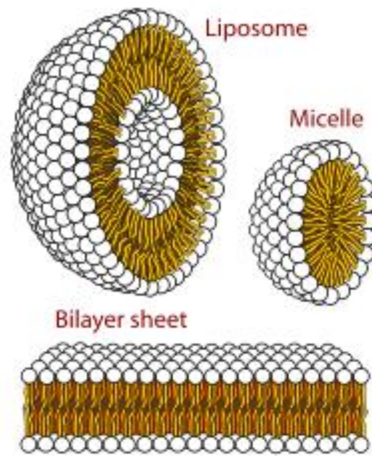
Shear motions involve a small sliding movement of domain interfaces, controlled by the amino acid side chains within the interface. Proteins displaying shear motions often have a layered architecture: stacking of secondary structures. The interdomain linker has merely the role of keeping the domains in close proximity.

Domain motion and functional dynamics in enzymes:-

The analysis of the internal dynamics of structurally different, but functionally similar enzymes has highlighted a common relationship between the positioning of the active site and the two principal protein sub-domains. In fact, for several members of the hydrolase superfamily, the catalytic site is located close to the interface separating the two principal quasi-rigid domains.Such positioning appears instrumental for maintaining the precise geometry of the active site, while allowing for an appreciable functionally oriented modulation of the flanking regions resulting from the relative motion of the two sub-domains.

Biological membrane:-

A **biological membrane** or **biomembrane** is an enclosing or separating membrane that acts as a selectively permeable barrier within living things. Biological membranes, in the form of cell membranes, often consist of a phospholipid bilayer with embedded, integral and peripheral proteins used in communication and transportation of chemicals and ions. Bulk lipid in membrane provides a fluid matrix for proteins to rotate and laterally diffuse for physiological functioning. Proteins are adapted to high membrane fluidity environment of lipid bilayer with the presence of an annular lipid shell, consisting of lipid molecules bound tightly to surface of integral membrane proteins. The cellular membranes should not be confused with isolating tissues formed by layers of cells, such as mucous membranes and basement membranes.



Composition:-

The lipid bilayer consists of two layers- an outer leaflet and an inner leaflet. The components of bilayers are distributed unequally between the two surfaces to create asymmetry between the outer and inner surfaces.] This asymmetric organization is important for cell functions such as cell signaling. The asymmetry of the biological membrane reflects the different functions of the two leaflets of the membrane.

Lipids:-

The biological membrane is made up of lipids with hydrophobic tails and hydrophilic heads. The hydrophobic tails are hydrocarbon tails whose length and saturation is important in characterizing the cell. Lipid rafts occur when lipid species and proteins aggregate in domains in the membrane. These help organize membrane components into localized areas that are involved in specific processes, such as signal transduction.

Proteins:-

Phospholipid bilayers contain different proteins. These membrane proteins have various functions and characteristics and catalyze different chemical reactions. Integral proteins span the membranes with different domains on either side. Integral proteins hold strong association with the lipid bilayer and cannot easily become detached. They will dissociate only with chemical treatment that breaks the membrane. Peripheral proteins are unlike integral proteins in that they hold weak interactions with the surface of the bilayer and can easily become dissociated from the membrane. Peripheral proteins are located on only one face of a membrane and create membrane asymmetry.

Oligosaccharides:-

Oligosaccharides are sugar containing polymers. In the membrane, they can be covalently bound to lipids to form glycolipids or covalently bound to proteins to formglycoproteins. Membranes

contain sugar-containing lipid molecules known as glycolipids. In the bilayer, the sugar groups of glycolipids are exposed at the cell surface, where they can form hydrogen bonds. Glycolipids provide the most extreme example of asymmetry in the lipid bilayer Glycolipids perform a vast number of functions in the biological membrane that are mainly communicative, including cell recognition and cell-cell adhesion. Glycoproteins are integral proteins. They play an important role in the immune response and protection.

Formation:-

The phospholipid bilayer is formed due to the aggregation of membrane lipids in aqueous solutions.[4] Aggregating is caused by the hydrophobic effect, where hydrophobic ends come into contact with each other and are sequestered away from water and hydrophilic ends are in contact with it. Less water is allowed to interact with the hydrophobic ends and, therefore, hydrogen bonding between hydrophilic heads and water is increased. This creates a favorable molecular arrangement by reducing unfavorable contact between hydrophobic tails and water and increasing hydrogen bonding between the hydrophilic heads and water.The increase in available hydrogen bonding increases the entropy of the system, creating a spontaneous process. Aggregation of non polar substances in water is, therefore, entropically driven and spontaneously occurring.] The aggregation formed due to the hydrophobic effect is partially responsible for the shape of biological membranes.

Function

Biological molecules are amphiphilic or amphipathic, i.e. are simultaneously hydrophobic and hydrophilic. The phospholipid bilayer contains charged hydrophilic headgroups, which interact with polar water. The lipids also contain hydrophobic tails, which meet with the hydrophobic tails of the complementary layer. The hydrophobic tails are usually fatty acids that differ in lengths. The interactions of lipids, especially the hydrophobic tails, determine the lipid bilayer physical properties such as fluidity.

Membranes in cells typically define enclosed spaces or compartments in which cells may maintain a chemical or biochemical environment that differs from the outside. For example, the membrane around peroxisomes shields the rest of the cell from peroxides, chemicals that can be toxic to the cell, and the cell membrane separates a cell from its surrounding medium. Peroxisomes are one form of vacuole found in the cell that contains by-products of chemical reactions within the cell. Most organelles are defined by such membranes, and are called "membrane-bound" organelles.

Selective Permeability:-

Probably the most important feature of a biomembrane is that it is a selectively permeable structure. This means that the size, charge, and other chemical properties of the atoms and

molecules attempting to cross it will determine whether they succeed in doing so. Selective permeability is essential for effective separation of a cell or organelle from its surroundings. Biological membranes also have certain mechanical or elastic properties that allow them to change shape and move as required.

Generally, small hydrophobic molecules can readily cross phospholipid bilayers by simple diffusion.

Particles that are required for cellular function but are unable to diffuse freely across a membrane enter through a membrane transport protein or are taken in by means of endocytosis, where the membrane allows for a vacuole to join onto it and push its contents into the cell. Many types of specialized plasma membranes can separate cell from external environment: apical, basolateral, presynaptic and postsynaptic ones, membranes of flagella, cilia, microvillus, filopodia and lamellipodia, the sarcolemma of muscle cells, as well as specialized myelin and dendritic spine membranes of neurons. Plasma membranes can also form different types of "supramembrane" structures such as caveola, postsynaptic density, podosome, invadopodium, desmosome, hemidesmosome, focal adhesion, and cell junctions. These types of membranes differ in lipid and protein composition.

Fluidity:-

The hydrophobic core of the phospholipid bilayer is constantly in motion because of rotations around the bonds of lipid tails.[1] Hydrophobic tails of a bilayer bend and lock together. However, because of hydrogen bonding with water, the hydrophilic head groups exhibit less movement as their rotation and mobility are constrained. This results in increasing viscosity of the lipid bilayer closer to the hydrophilic heads.

Below a transition temperature, a lipid bilayer loses fluidity when the highly mobile lipids exhibit less movement becoming a gel-like solid. The transition temperature depends on such components of the lipid bilayer as the hydrocarbon chain length and the saturation of its fatty acids. Temperature-dependence fluidity constitutes an important physiological attribute for bacteria and cold-blooded organisms. These organisms maintain a constant fluidity by modifying membrane lipid fatty acid composition in accordance with differing temperatures.

Membrane proteins

Membrane proteins are proteins that interact with, or are part of, biological membranes. They are one of the common ypes of protein along with soluble globular proteins, fibrous proteins, and disordered proteins. They are targets of over 50% of all modern medicinal drugs. It is estimated that 20–30% of all genes in most genomes encode membrane proteins.

Function:-

Membrane proteins perform a variety of functions vital to the survival of organisms:

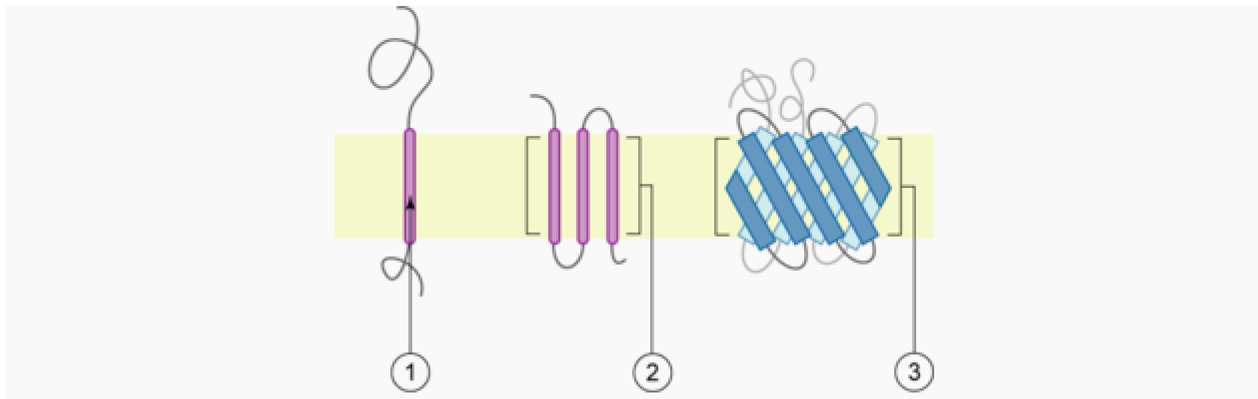
- Membrane receptor proteins relay signals between the cell's internal and external environments.
- Transport proteins move molecules and ions across the membrane. They can be categorized according to the Transporter Classification database.
- Membrane enzymes may have many activities, such as oxidoreductase, transferase or hydrolase.
- Cell adhesion molecules allow cells to identify each other and interact. For example, proteins involved in immune response.

Topology

The topology of an integral membrane protein describes the number of transmembrane segments, as well as the orientation in the membrane.[5] Membrane proteins have several different topologies:[6]

A slightly different classification is to divide all membrane proteins to integral and amphitropic. Amphitropic proteins exist in two alternative states: a water-soluble and a lipid bilayer-bound. The amphitropic protein category includes water-soluble channel-forming polypeptide toxins, which associate irreversibly with membranes, but excludes peripheral proteins that interact with other membrane proteins rather than with lipid bilayer.

Integral membrane proteins:-

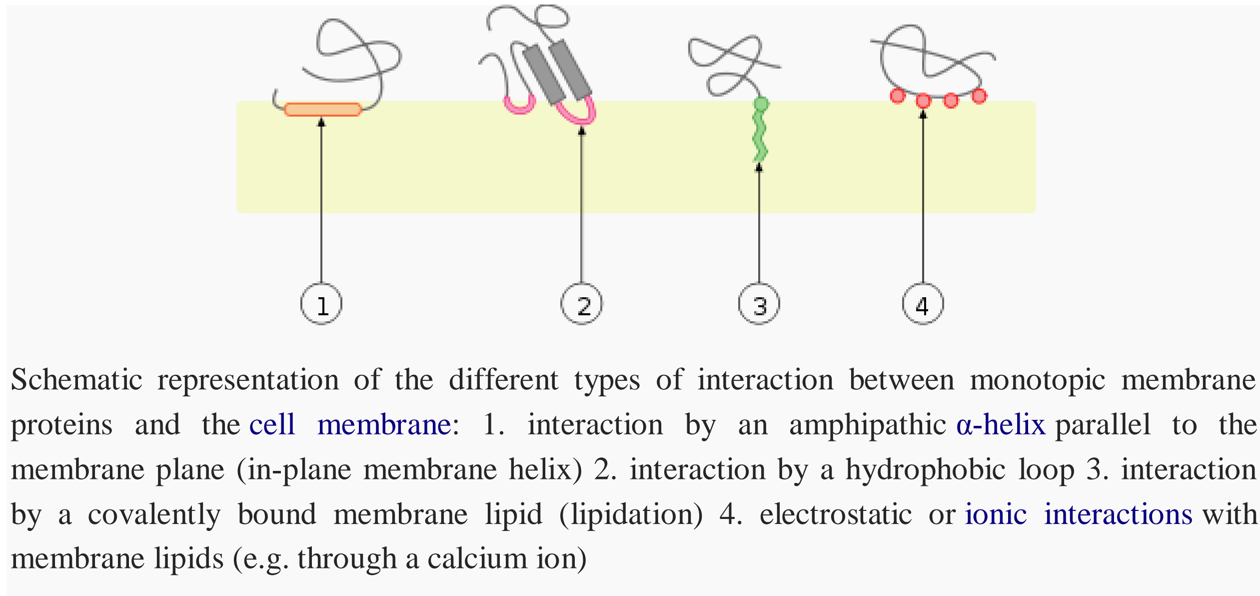


Schematic representation of transmembrane proteins: 1. a single transmembrane α-helix (bitopic membrane protein) 2. a polytopic transmembrane α-helical protein 3. a polytopic transmembrane β-sheet protein
The membrane is represented in light-brown.

Integral membrane proteins are permanently attached to the membrane. Such proteins can be separated from the biological membranes only using detergents, nonpolar solvents, or sometimes denaturing agents. They can be classified according to their relationship with the bilayer:

- Integral polytopic proteins, also known as "transmembrane proteins," are integral membrane proteins that span across the membrane at least once. They have one of two tertiary structures:
  - helix bundle proteins, which are present in all types of biological membranes;
  - beta barrel proteins, which are found only in outer membranes of Gram-negative bacteria, lipid-rich cell walls of a few Gram-positive bacteria, and outer membranes of mitochondria and chloroplasts.

Peripheral membrane proteins:-



Schematic representation of the different types of interaction between monotopic membrane proteins and the cell membrane: 1. interaction by an amphipathic α-helix parallel to the membrane plane (in-plane membrane helix) 2. interaction by a hydrophobic loop 3. interaction by a covalently bound membrane lipid (lipidation) 4. electrostatic or ionic interactions with membrane lipids (e.g. through a calcium ion)

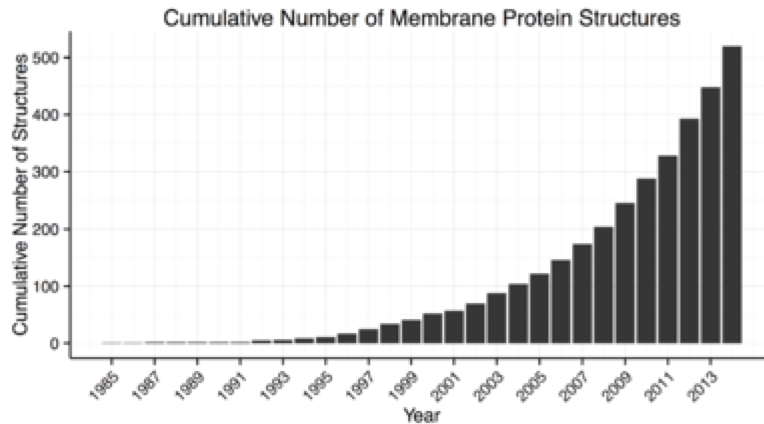
Peripheral membrane proteins are temporarily attached either to the lipid bilayer or to integral proteins by a combination of hydrophobic, electrostatic, and other non-covalent interactions. Peripheral proteins dissociate following treatment with a polar reagent, such as a solution with an elevated pH or high salt concentrations.

.

Polypeptide toxins:-

Polypeptide toxins and many antibacterial peptides, such as colicins or hemolysins, and certain proteins involved in apoptosis, are sometimes considered a separate category. These proteins are water-soluble but can aggregate and associate irreversibly with the lipid bilayer and become reversibly or irreversibly membrane-associated.

3D Structure



Increase in the number of 3D structures of membrane proteins known

The most common tertiary structures are helix bundle and beta barrel. The portion of the membrane proteins that are attached to the lipid bilayer (see annular lipid shell) are consisting of hydrophobic amino acids only. This is done so that the peptide bonds' carbonyl and amine will react with each other instead of the hydrophobic surrounding. The portion of the protein that is not touching the lipid bilayer and is protruding out of the cell membrane are usually hydrophilic amino acids.]

Membrane proteins have hydrophobic surfaces, are relatively flexible and are expressed at relatively low levels. This creates difficulties in obtaining enough protein and then growing crystals. Hence, despite the significant functional importance of membrane proteins, determining atomic resolution structures for these proteins is more difficult than globular proteins.

Membrane proteins in genomes:-

A large fraction of all proteins are thought to be membrane proteins. For instance, about 1000 of the ~4200 proteins of E. coli are thought to be membrane proteins, The membrane localization has been confirmed for more than 600 of them experimentally.] The localization of proteins in membranes can be predicted very reliably using hydrophobicity analyses of protein sequences, i.e. the localization of hydrophobic amino acid sequences.

Conjugated proteins

A **conjugated protein** is a protein that functions in interaction with other (non-polypeptide) chemical groups attached by covalent bonding or weak interactions.

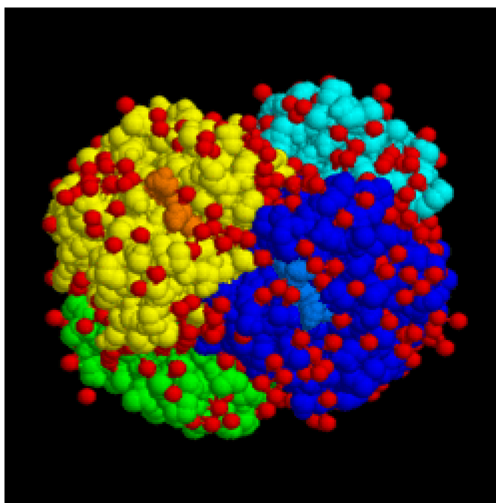
Many proteins contain only amino acids and no other chemical groups, and they are called simple proteins. However, other kind of proteins yield, on hydrolysis, some other chemical component in addition to amino acids and they are called conjugated proteins. The non-amino

part of a conjugated protein is usually called its prosthetic group. Most prosthetic groups are formed from vitamins. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups.

Someexamplesofconjugatedproteins are lipoproteins, glycoproteins, phosphoproteins, hemoproteins, flavoproteins,metalloproteins, phytochromes, cytochromes, opsins and chromoproteins.

Hemoglobin contains the prosthetic group known as heme. Each heme group contains an iron ion (Fe^{2+}) which forms a co-ordinate bond with an oxygen molecule (O_2), allowing hemoglobin to transport oxygen through the bloodstream. As each of the four protein subunits of hemoglobin possesses its own prosthetic heme group, each hemoglobin can transport four molecules of oxygen.

Glycoproteins are generally the largest and most abundant group of conjugated proteins. They range from glycoproteins in cell surface membranes that constitute the glycocalyx, to important antibodies produced by leukocytes.



Glycans & Biological function

The terms **glycan** and polysaccharide are defined by IUPAC as synonyms meaning "compounds consisting of a large number of monosaccharides linked glycosidically".[1]However, in practice the term glycan may also be used to refer to the carbohydrate portion of a glycoconjugate, such as a glycoprotein, glycolipid, or a proteoglycan, even if the carbohydrate is only an oligosaccharide.[2] Glycans usually consist solely of O-glycosidic linkages of monosaccharides. For example, cellulose is a glycan (or, to be more specific, a glucan) composed of β-1,4-linked D-glucose, and chitin is a glycan composed of β-1,4-linked N-acetyl-D-glucosamine. Glycans can be homo- or heteropolymers of monosaccharide residues, and can be linear or branched.

N-Linked glycans:-

Introduction

N-Linked glycans are attached in the endoplasmic reticulum to the nitrogen (N) in the side chain of asparagine in the sequon. The sequon is an Asn-X-Ser or Asn-X-Thr sequence, where X is any amino acid except proline and the glycan may be composed of N-acetyl galactosamine, galactose, neuraminic acid, N-acetylglucosamine, fucose,mannose, and other monosaccharides.

Assembly

In eukaryotes, N-linked glycans are derived from a core 14-sugar unit assembled in the cytoplasm and endoplasmic reticulum. First, two N-acetyl glucosamine residues are attached to dolichol monophosphate, a lipid, on the external side of the endoplasmic reticulum membrane. Five mannose residues are then added to this structure. At this point, the partially finished core glycan is flipped across the endoplasmic reticulum membrane, so that it is now located within the reticular lumen. Assembly then continues within the endoplasmic reticulum, with the addition of four more mannose residues. Finally, three glucose residues are added to this structure

Processing, modification, and diversity

Once transferred to the nascent peptide chain, N-linked glycans, in general, undergo extensive processing reactions, whereby the three glucose residues are removed, as well as several mannose residues, depending on the N-linked glycan in question. The removal of the glucose residues is dependent on proper protein folding. These processing reactions occur in the Golgi apparatus. Modification reactions may involve the addition of a phosphate or acetyl group onto the sugars, or the addition of new sugars, such asneuraminic acid. Processing and modification of N-linked glycans within the Golgi does not follow a linear pathway. As a result, many different variations of N-linked glycan structure are possible, depending on enzyme activity in the Golgi.

Functions and importance

N-linked glycans are extremely important in proper protein folding in eukaryotic cells. Chaperone proteins in the endoplasmic reticulum, such as calnexin and calreticulin, bind to the three glucose residues present on the core N-linked glycan. These chaperone proteins then serve to aid in the folding of the protein that the glycan is attached to. Following proper folding, the three glucose residues are removed, and the glycan moves on to further processing reactions. If the protein fails to fold properly, the three glucose residues are reattached, allowing the protein to re-associate with the chaperones.

O-Linked glycans

Introduction

In eukaryotes, O-linked glycans are assembled one sugar at a time on a serine or threonine residue of a peptide chain in the Golgi apparatus. Unlike N-linked glycans, there is no known consensus sequence yet. However, the placement of a proline residue at either -1 or +3 relative to the serine or threonine is favourable for O-linked glycosylation.

Assembly

The first monosaccharide attached in the synthesis of O-linked glycans is N-acetyl-galactosamine. After this, several different pathways are possible. A Core 1 structure is generated by the addition of galactose. A Core 2 structure is generated by the addition of N-acetyl-glucosamine to the N-acetyl-galactosamine of the Core 1 structure. Core 3 structures are generated by the addition of a single N-acetyl-glucosamine to the original N-acetyl-galactosamine. Core 4 structures are generated by the addition of a second N-acetyl-glucosamine to the Core 3 structure.

Functions and importance

Sialyl lewis x is important in ABO blood antigen determination.

SLex is also important to proper immune response. E-selectin release from Weibel-Palade bodies, on blood vessel endothelial cells, can be induced by a number of factors. One such factor is the response of the endothelial cell to certain bacterial molecules, such as peptidoglycan. E-selectin binds to the SLex structure that is present on neutrophils in the blood stream, and helps to mediate the extravasation of these cells into the surrounding tissue during an infection.

O-linked glycans, in particular mucin, have been found to be important in developing normal intestinal microflora. Certain strains of intestinal bacteria bind specifically to mucin, allowing them to colonize the intestine.

Examples of O-linked glycoproteins are:

- Glycophorin, a protein in erythrocyte cell membranes
- Mucin, a protein in saliva involved in formation of dental plaque
- Notch, a transmembrane receptor involved in development and cell fate decisions
- Thrombospondin
- Factor VII
- Factor IX
- Urinary type plasminogen activator.

Cell signaling

Cell signaling (**cell signalling** in British English) is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis. Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. By understanding cell signaling, diseases may be treated effectively and, theoretically, artificial tissues may be created. Traditional work in biology has focused on studying individual parts of cell signaling pathways. Systems biology research helps us to understand the underlying structure of cell signaling networks and how changes in these networks may affect the transmission and flow of information.

Classification:-

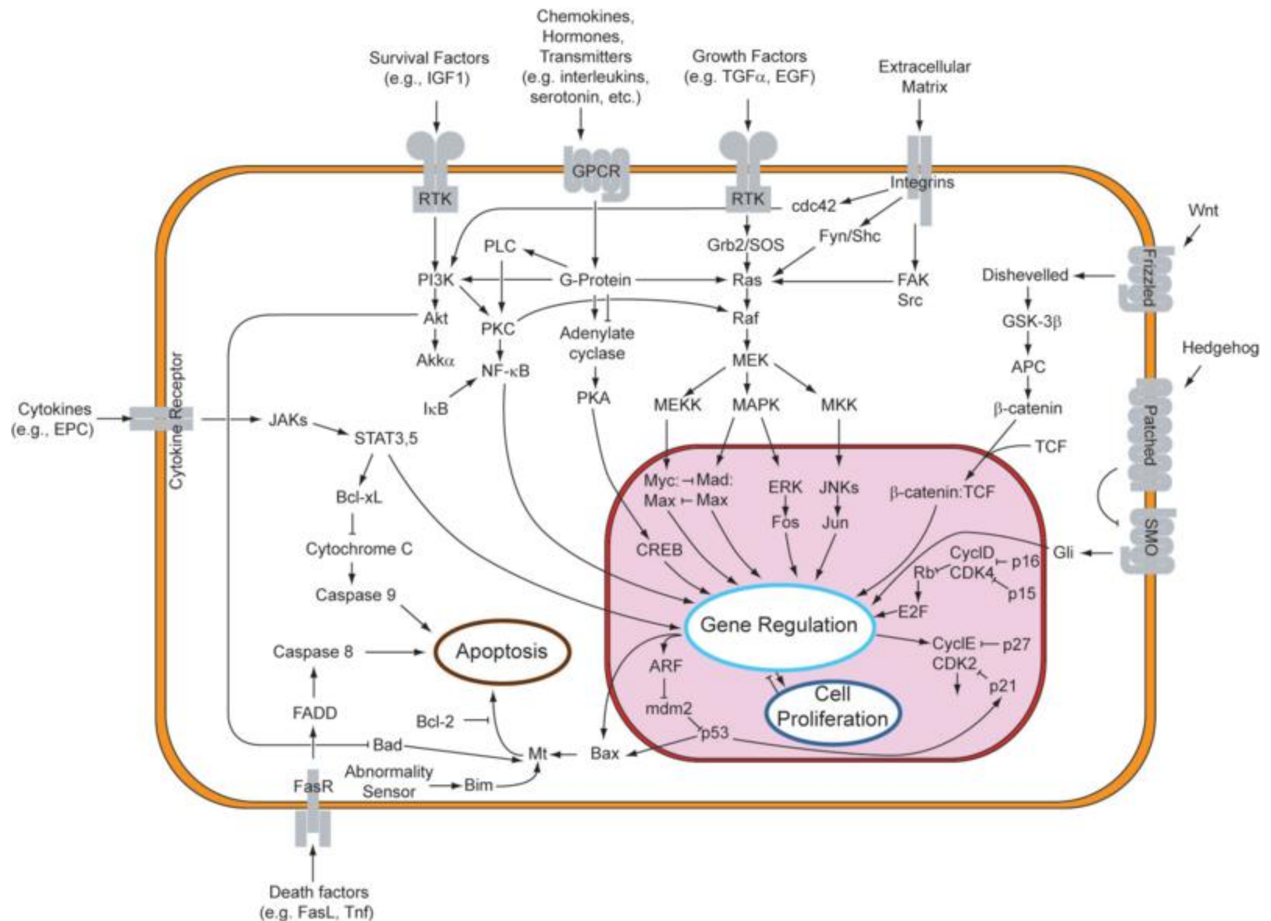
Cell signaling can be classified to be mechanical and biochemical based on the type of the signal. Mechanical signals are the forces exerted on the cell and the forces produced by the cell. These forces can both be sensed and responded by the cells.[10] Biochemical signals are the biochemical molecules such as proteins, lipids, ions and gases. These signals can be categorized based on the distance between signaling and responder cells. Signaling within, between, and among cells is subdivided into the following classifications:

- Intracrine signals are produced by the target cell that stay within the target cell.
- Autocrine signals are produced by the target cell, are secreted, and affect the target cell itself via receptors. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.
- Juxtacrine signals target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.
- Paracrine signals target cells in the vicinity of the emitting cell. Neurotransmitters represent an example.
- Endocrine signals target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body.

Signaling pathway:-

In some cases, receptor activation caused by ligand binding to a receptor is directly coupled to the cell's response to the ligand. For example, the neurotransmitter GABA can activate a cell surface receptor that is part of an ion channel. GABA binding to a $GABA_A$ receptor on a

neuron opens a chloride-selective ion channel that is part of the receptor. $GABA_A$ receptor activation allows negatively charged chloride ions to move into the neuron, which inhibits the ability of the neuron to produce action potentials.



UNIT-II

Enzymes are biocatalyst-s the catalysts life. A catalvsf is defined as a subsfance thatincreases ihe velocity or rate of a chemical reaction without itself undergoing any change in the overall process. Enzymes may be defined as biocatalysts synthesized by living cells. They are protein innature (exception - RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action.

Nomenclature and classification of enzyme

The Internat ionaUl nion of Biochemist r(yl UB) appointeda n EnzymeC ommissionin 1961.T hiscommittee made a thorough study of the existing enzymes and devised some basic principles for the classificationa nd nomenclatureo f enzymes. Since 1964, the IUB system of enzyme classification has been in force. Enzymes are divided into six major classes (in that order). Each

class on its own representtsh e generalt ype of reaction brought about by the enzymes of that class.

Class 1. Oxidoreductases.

To this class belong all enzymes catalysing oxidoreduction reactions. The substrate that is oxidized is regarded as hydrogen donor. The systematic name is based on donor:acceptor oxidoreductase. The common name will be dehydrogenase, wherever this is possible; as an alternative, reductase can be used. Oxidase is only used in cases where O_2 is the acceptor.

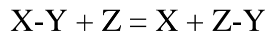
The second figure in the code number of the oxidoreductases, unless it is 11, 13, 14 or 15, indicates the group in the hydrogen (or electron) donor that undergoes oxidation: 1 denotes a -CHOH- group, 2 a -CHO or -CO-COOH group or carbon monoxide, and so on, as listed in the key.

The third figure, except in subclasses EC 1.11, EC 1.13, EC 1.14 and EC 1.15, indicates the type of acceptor involved: 1 denotes $NAD(P)^+$, 2 a cytochrome, 3 molecular oxygen, 4 a disulfide, 5 a quinone or similar compound, 6 a nitrogenous group, 7 an iron-sulfur protein and 8 a flavin. In subclasses EC 1.13 and EC 1.14 a different classification scheme is used and sub-subclasses are numbered from 11 onwards.

Class 2. Transferases.

Transferases are enzymes transferring a group, e.g. a methyl group or a glycosyl group, from one compound (generally regarded as donor) to another compound (generally regarded as acceptor). The systematic names are formed according to the scheme donor:acceptor grouptransferase. The common names are normally formed according to acceptor grouptransferase or donor grouptransferase. In many cases, the donor is a cofactor (coenzyme) charged with the group to be transferred. A special case is that of the transaminases

Some transferase reactions can be viewed in different ways. For example, the enzyme-catalysed reaction



$$X\text{-}Y + Z = X + Z\text{-}Y$$

Another problem is posed in enzyme-catalysed transaminations, where the $-NH_2$ group and -H are transferred to a compound containing a carbonyl group in exchange for the =O of that group, according to the general equation:

$$R^1\text{-}CH(\text{-}NH_2)\text{-}R^2 + R^3\text{-}CO\text{-}R^4 \qquad R^1\text{-}CO\text{-}R^2 + R^3\text{-}CH(\text{-}NH_2)\text{-}R^4.$$

The reaction can be considered formally as oxidative deamination of the donor (e.g. amino acid) linked with reductive amination of the acceptor (e.g. oxo acid), and the transaminating enzymes (pyridoxal-phosphate proteins) might be classified as oxidoreductases. However, the unique

distinctive feature of the reaction is the transfer of the amino group (by a well-established mechanism involving covalent substrate-coenzyme intermediates).

Class 3. Hydrolases.

These enzymes catalyse the hydrolytic cleavage of C-O, C-N, C-C and some other bonds, including phosphoric anhydride bonds. Although the systematic name always includes hydrolase, the common name is, in many cases, formed by the name of the substrate with the suffix -ase. It is understood that the name of the substrate with this suffix means a hydrolytic enzyme.

Some hydrolases (especially some of the esterases and glycosidases) pose problems because they have a very wide specificity and it is not easy to decide if two preparations described by different authors (perhaps from different sources) have the same catalytic properties, or if they should be listed under separate entries. An example is vitamin A esterase (formerly EC 3.1.1.12, now believed to be identical with EC 3.1.1.1). To some extent the choice must be arbitrary; however, separate entries should be given only when the specificities are sufficiently different.

.The second figure in the code number of the hydrolases indicates the nature of the bond hydrolysed; EC 3.1 are the esterases; EC 3.2 the glycosylases, and so on.

The third figure normally specifies the nature of the substrate, e.g. in the esterases the carboxylic ester hydrolases (EC 3.1.1), thiolester hydrolases (EC 3.1.2), phosphoric monoester hydrolases (EC 3.1.3); in the glycosylases the O-glycosidases (EC 3.2.1), N-glycosylases (EC 3.2.2), etc. Exceptionally, in the case of the peptidyl-peptide hydrolases.

Class 4. Lyases.

Lyases are enzymes cleaving C-C, C-O, C-N, and other bonds by elimination, leaving double bonds or rings, or conversely adding groups to double bonds. The systematic name is formed according to the pattern substrate group-lyase. The hyphen is an important part of the name, and to avoid confusion should not be omitted, e.g. hydro-lyase not 'hydrolyase'. In the common names, expressions likedecarboxylase, aldolase, dehydratase (in case of elimination of CO_2, aldehyde, or water) are used. In cases where the reverse reaction is much more important, or the only one demonstrated, synthase (not synthetase) may be used in the name. Various subclasses of the lyases include pyridoxal-phosphate enzymes that catalyse the elimination of a β- or γ-substituent from an α-amino acid followed by a replacement of this substituent by some other group. In the overall replacement reaction, no unsaturated end-product is formed; therefore, these enzymes might formally be classified as alkyl-transferases (EC 2.5.1...). However, there is ample evidence that the replacement is a two-step reaction involving the transient formation of enzyme-bound α,β(or β,γ)-unsaturated amino acids. According to the rule that the first reaction is indicative for classification, these enzymes are correctly classified as lyases. Examples are tryptophan synthase (EC 4.2.1.20) and cystathionine β-synthase (EC 4.2.1.22)..

Class 5. Isomerases.

These enzymes catalyse geometric or structural changes within one molecule. According to the type of isomerism, they may be called racemases, epimerases, cis-trans-isomerases, isomerases, tautomerases, mutases or cycloisomerases.

In some cases, the interconversion in the substrate is brought about by an intramolecular oxidoreduction (EC 5.3); since hydrogen donor and acceptor are the same molecule, and no oxidized product appears, they are not classified as oxidoreductases, even though they may contain firmly bound $NAD(P)^+$.

The subclasses are formed according to the type of isomerism, the sub-subclasses to the type of substrates.

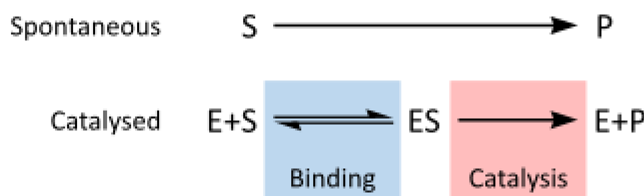
Class 6. Ligases. Ligases are enzymes catalysing the joining together of two molecules coupled with the hydrolysis of a diphosphate bond in ATP or a similar triphosphate. The systematic names are formed on the system X:Y ligase (ADP-forming). In earlier editions of the list the term synthetase has been used for the common names. Many authors have been confused by the use of the terms synthetase (used only for Group 6) andsynthase (used throughout the list when it is desired to emphasis the synthetic nature of the reaction). Consequently NC-IUB decided in 1983 to abandon the use of synthetase for common names. In a few cases in Group 6, where the reaction is more complex or there is a common name for the product, a synthase name is used (e.g. EC 6.3.2.11 and EC 6.3.5.1).

Enzyme kinetics:-

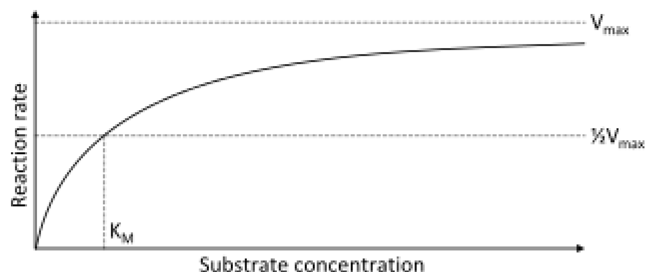
is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rateis measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Enzymes are usually protein molecules that manipulate other molecules — the enzymes' substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism

Michaelis–Menten kinetics



A chemical reaction mechanism with or without enzyme catalysis. The enzyme (E) binds substrate (S) to produceproduct (P).



Saturation curve for an enzyme reaction showing the relation between the substrate concentration and reaction rate.: Michaelis–Menten kinetics

As enzyme-catalysed reactions are saturable, their rate of catalysis does not show a linear response to increasing substrate. If the initial rate of the reaction is measured over a range of substrate concentrations (denoted as [S]), the reaction rate (v) increases as [S] increases, as shown on the right. However, as [S] gets higher, the enzyme becomes saturated with substrate and the rate reaches V_{max}, the enzyme's maximum rate.

The Michaelis–Menten kinetic model of a single-substrate reaction is shown on the right. There is an initial bimolecular reaction between the enzyme E and substrate S to form the enzyme–substrate complex ES but the rate of enzymatic reaction increases with the increase of the substrate concentration up to a certain level but then an increase in substrate concentration does not cause any increase in reaction rate as there no more E available for reacting with S and the rate of reaction becomes dependent on ES and the reaction becomes unimolecular reaction. Although the enzymatic mechanism for the unimolecular reaction $ES \xrightarrow{k_{cat}} E + P$ can be quite complex, there is typically one rate-determining enzymatic step that allows this reaction to be modelled as a single catalytic step with an apparent unimolecular rate constant k_{cat}. If the reaction path proceeds over one or several intermediates, k_{cat} will be a function of several elementary rate constants, whereas in the simplest case of a single elementary reaction (e.g. no intermediates) it will be identical to the elementary unimolecular rate constant k_2. The apparent unimolecular rate constant k_{cat} is also called turnover number and denotes the maximum number of enzymatic reactions catalysed per second.

The Michaelis–Menten equation[9] describes how the (initial) reaction rate v_0 depends on the position of the substrate-binding equilibrium and the rate constant k_2.

$$v_0 = \frac{V_{\mathbf{max}}[\mathrm{S}]}{K_M + [\mathrm{S}]}$$ (Michaelis–Menten equation)

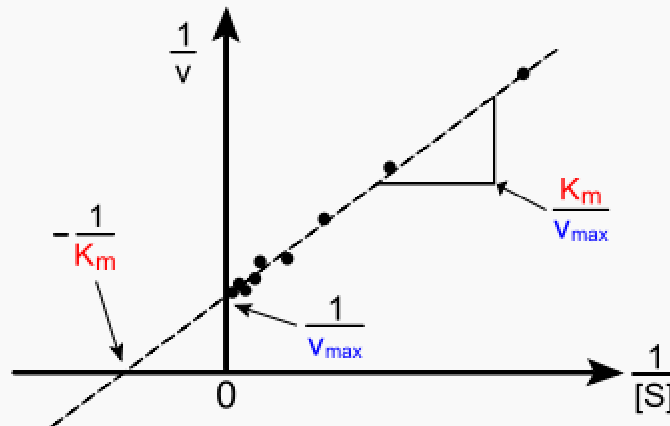
with the constants

$$K_M \stackrel{\text{def}}{=} \frac{k_2 + k_{-1}}{k_1} \approx K_D$$

$$V_{\max} \stackrel{\text{def}}{=} k_{cat}[E]_{tot}$$

This Michaelis–Menten equation is the basis for most single-substrate enzyme kinetics. Two crucial assumptions underlie this equation (apart from the general assumption about the mechanism only involving no intermediate or product inhibition, and there is no allostericity or cooperativity). The first assumption is the so-called quasi-steady-state assumption (or pseudo-steady-state hypothesis), namely that the concentration of the substrate-bound enzyme (and hence also the unbound enzyme) changes much more slowly than those of the product and substrate and thus the change over time of the complex can be set to zero . The second assumption is that the total enzyme concentration does not change over time, thus . A complete derivation can be found here.

$$d[ES]/dt \stackrel{!}{=} 0 [E]_{\text{tot}} = [E] + [ES] \stackrel{!}{=} \text{const}$$



Mechanism of Enzyme Action

Introduction - Enzyme Characteristics:

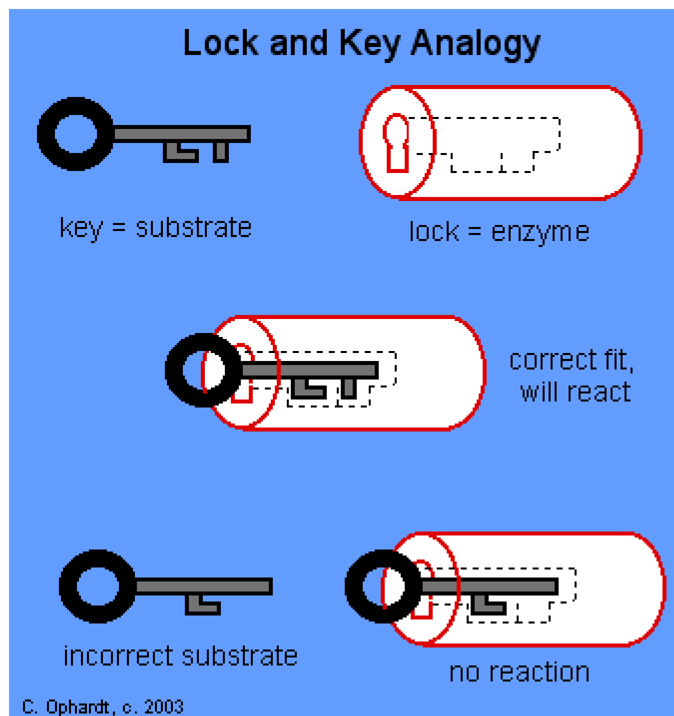
The basic mechanism by which enzymes catalyze chemical reactions begins with the binding of the**substrate** (or substrates) to the active site on the enzyme. The **active site** is the specific region of the enzyme which combines with the substrate. The binding of the substrate to the enzyme causes changes in the distribution of electrons in the chemical bonds of the substrate and ultimately causes the reactions that lead to the formation of products. The products are released from the enzyme surface to regenerate the enzyme for another reaction cycle.

The **active site** has a unique geometric shape that is complementary to the geometric shape of a substrate molecule, similar to the fit of puzzle pieces. This means that enzymes specifically react with only one or a very few similar compounds.

Lock and Key Theory:

The specific action of an enzyme with a single substrate can be explained using a **Lock and Key**analogy first postulated in 1894 by Emil Fischer. In this analogy, the lock is the enzyme and the key is the substrate. Only the correctly sized **key (substrate) fits** into the **key hole (active site)** of the**lock (enzyme)**.

Smaller keys, larger keys, or incorrectly positioned teeth on keys (incorrectly shaped or sized substrate molecules) do not fit into the lock (enzyme). Only the correctly shaped key opens a particular lock.

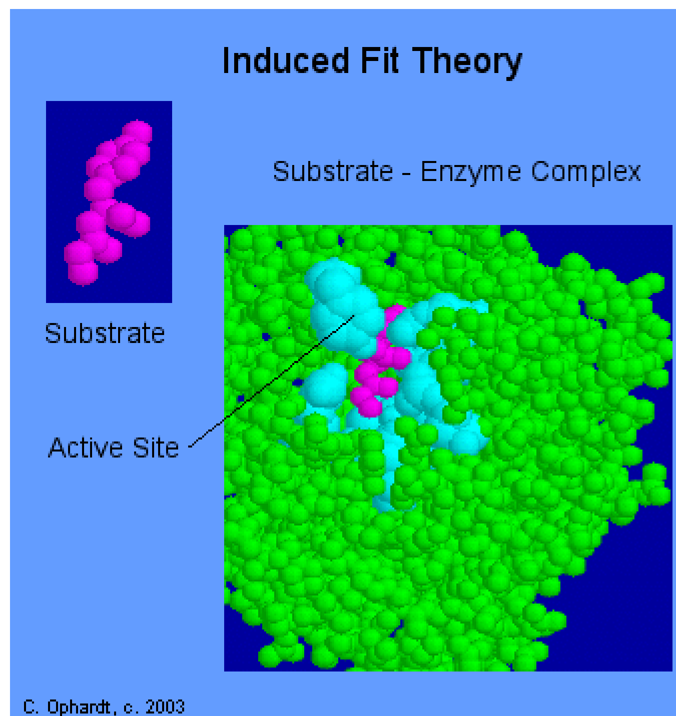


Induced Fit Theory:

Not all experimental evidence can be adequately explained by using the so-called rigid enzyme model assumed by the lock and key theory. For this reason, a modification called the induced-fit theory has been proposed.

The induced-fit theory assumes that the substrate plays a role in determining the final shape of the enzyme and that the enzyme is partially flexible. This explains why certain compounds can bind to the enzyme but do not react because the enzyme has been distorted too much. Other molecules may be too small to induce the proper alignment and therefore cannot react. Only the proper substrate is capable of inducing the proper alignment of the active site.

In the graphic on the left, the substrate is represented by the magenta molecule, the enzyme protein is represented by the green and cyan colors. The cyan colored protein is used to more sharply define the active site. The protein chains are flexible and fit around the substrate.



Mechanism of inhibition of enzyme

Competitive inhibition is a form of enzyme inhibition where binding of the inhibitor to the active site on the enzyme prevents binding of the substrate and vice versa.

Most competitive inhibitors function by binding reversibly to the active site of the enzyme. As a result, many sources state that this is the defining feature of competitive inhibitors. This, however, is a misleading oversimplification, as there are many possible mechanisms by which an enzyme may bind either the inhibitor or the substrate but never both at the same time. For example, allosteric inhibitors may display competitive, non-competitive, or uncompetitive inhibition.

Non-competitive inhibition is a type of enzyme inhibition where the inhibitor reduces the activity of the enzyme and binds equally well to the enzyme whether or not it has already bound the substrate.

The inhibitor may bind to the enzyme whether or not the substrate has already been bound, but if it has a higher affinity for binding the enzyme in one state or the other, it is called a mixed inhibitor. Non-competitive inhibition models a system where the inhibitor and the substrate may both be bound to the enzyme at any given time.

Allosteric regulation (or **allosteric control**) is the regulation of a protein by binding an effector molecule at a site other than the enzyme's active site.

The site to which the effector binds is termed the allosteric site. Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change involving protein dynamics. Effectors that enhance the protein's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors.

Allosteric regulations are a natural example of control loops, such as feedback from downstream products or feed forward from upstream substrates. Long-range allostery is especially important in cell signaling. Allosteric regulation is also particularly important in the cell's ability to adjust enzyme activity.

Isoenzyme In chemical diagnosis

isoenzymes or more generally as **Multiple forms of enzymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different K_M values), or different regulatory properties. The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or

developmental stage (for example lactate dehydrogenase (LDH)). In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes.

Introduction:-

Isozymes were first described by R. L. Hunter and Clement Markert (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual. This definition encompasses (1) enzyme variants that are the product of different genes and thus represent different loci (described as isozymes) and (2) enzymes that are the product of different alleles of the same gene.

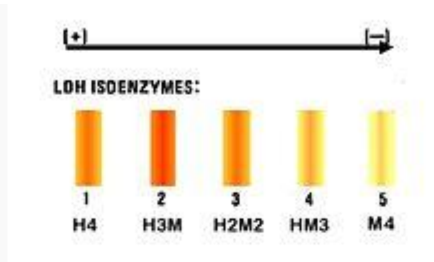
Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains identical to the original, then it is likely that one or the other will be lost as mutation accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of gene expression, then the two variants may both be favoured by natural selection and become specialised to different functions. For example, they may be expressed at different stages of development or in different tissues.

1. It is most likely that the new allele will be non-functional — in which case it will probably result in low fitness and be removed from the population by natural selection.
2. Alternatively, if the amino acid residue that is changed is in a relatively unimportant part of the enzyme (e.g., a long way from the active site), then the mutation may beselectively neutral and subject to genetic drift.
3. In rare cases, the mutation may result in an enzyme that is more efficient, or one that can catalyse a slightly different chemical reaction, in which case the mutation may cause an increase in fitness.

An example of an isozyme:-

An example of an isozyme is glucokinase, a variant of hexokinase which is not inhibited by glucose 6-phosphate. Its different regulatory features and lower affinity for glucose (compared to other hexokinases), allows it to serve different functions in cells of specific organs, such as control of insulin release by the beta cells of the pancreas, or initiation of glycogen synthesis by liver cells. Both of these processes must only occur when glucose is abundant, or problems occur.

The 5 isozyme of LDH



The differences between 5 isozyme when use Electrophoresis

The enzyme Lactate Dehydrogenase is made of two(H-form and M-Form) different sub units, combines in different Permutations and Combinations in depending on the tissue in which it is present as shown in below table,

	Composition	**Location**
LDH_1	HHHH	Heart and Erythrocyte
LDH_2	HHHM	Heart and Erythrocyte
LDH_3	HHMM	Brain and Kidney
LDH_4	HMMM	Skeletal Muscle and Liver
LDH_5	MMMM	Skeletal Muscle and Liver

COFACTORS

A **cofactor** is a non-protein chemical compound that is required for the protein's biological activity. These proteins are commonly enzymes, and cofactors can be considered "helper molecules" that assist in biochemical transformations.

Cofactors can be subdivided into either one or more inorganic ions, or a complex organic or metalloorganic molecule called a**coenzyme**; most of which are derived from vitamins and from required organic nutrients in small amounts. A cofactor that is tightly or even covalently bound is termed a **prosthetic group**. Additionally, some sources also limit the use of the term "cofactor"

to inorganic substances. An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is called a holoenzyme.

Some enzymes or enzyme complexes require several cofactors. For example, the multienzyme complex pyruvate dehydrogenase at the junction of glycolysis and the citric acid cycle requires five organic cofactors and one metal ion: loosely bound thiamine pyrophosphate (TPP), covalently bound lipoamide and flavin adenine dinucleotide (FAD), and the cosubstrates nicotinamide adenine dinucleotide (NAD^+) and coenzyme A (CoA), and a metal ion (Mg^{2+}).

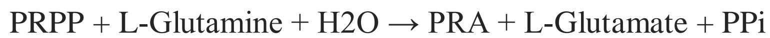
Cofactors can be divided into two broad groups: **organic cofactors**, such as flavin or heme, and **inorganic cofactors**, such as the metal ions Mg^{2+}, Cu^+, Mn^{2+}, or iron-sulfur clusters

Ion	Examples of enzymes containing this ion
Cupric	Cytochrome oxidase
Ferrous or Ferric	Catalase Cytochrome (via Heme) Nitrogenase Hydrogenase
Magnesium	Glucose 6-phosphatase Hexokinase DNA polymerase
Manganese	Arginase
Molybdenum	Nitrate reductase Nitrogenase
Nickel	Urease
Zinc	Alcohol dehydrogenase Carbonic anhydrase DNA polymerase

Biosynthesis of purine nucleotide (De Novo synthesis):-

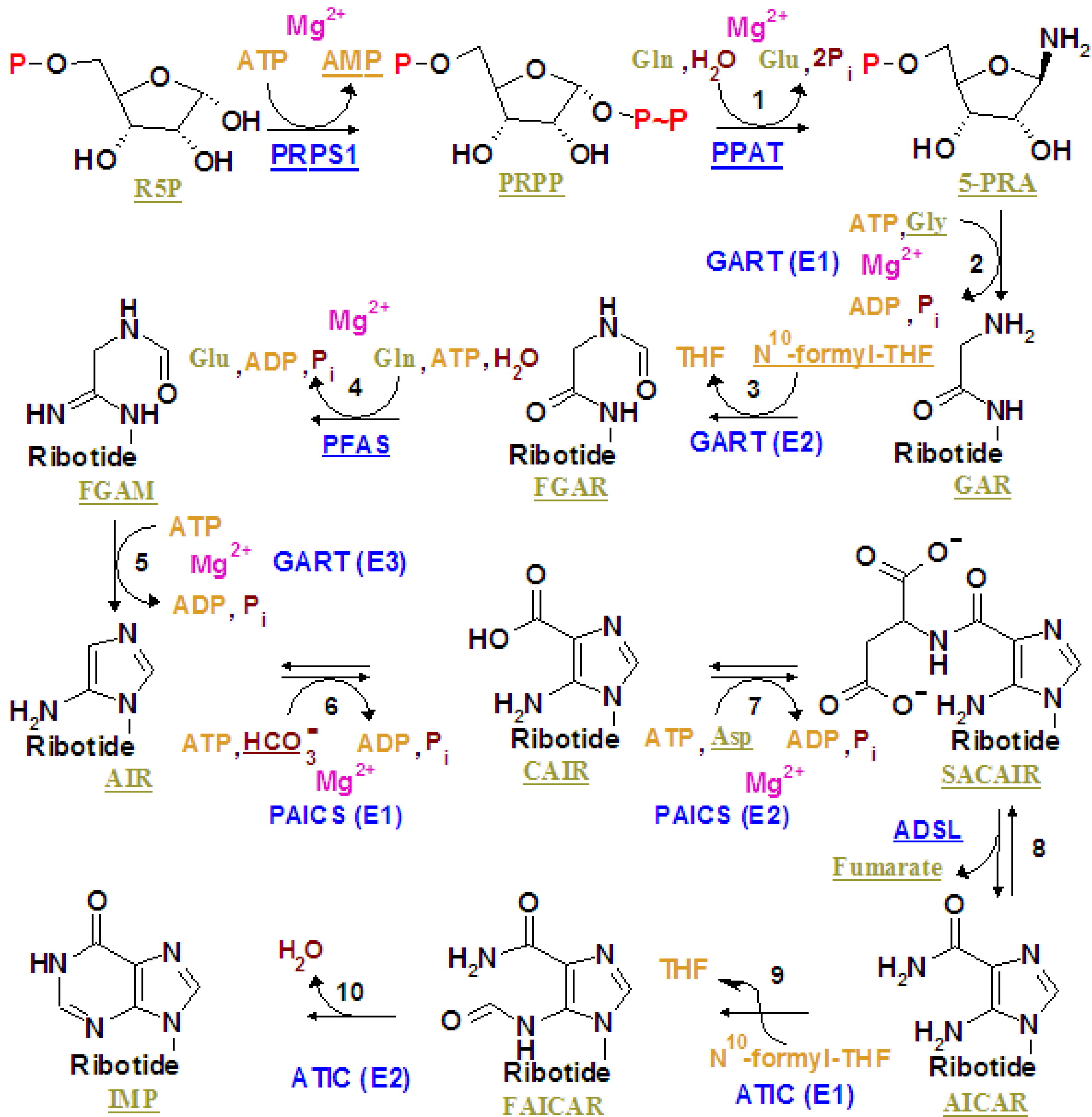
Purines are biologically synthesized as nucleotides and in particular as ribotides, i.e. bases attached to ribose 5-phosphate. A key regulatory step is the production of 5-phospho-α-D-ribosyl 1-pyrophosphate (PRPP) by ribose phosphate pyrophosphokinase, which is activated by inorganic phosphate and inactivated by purine ribonucleotides. It is not the committed step to purine synthesis because PRPP is also used in pyrimidine synthesis and salvage pathways.

The first committed step is the reaction of PRPP, glutamine and water to 5'-phosphoribosylamine (PRA),glutamate,and pyrophosphate -catalyzed by amidophosphoribosyltransferase, which is activated by PRPP and inhibited by AMP, GMP and IMP.


PRPP + L-Glutamine + H_2O → PRA + L-Glutamate + PPi

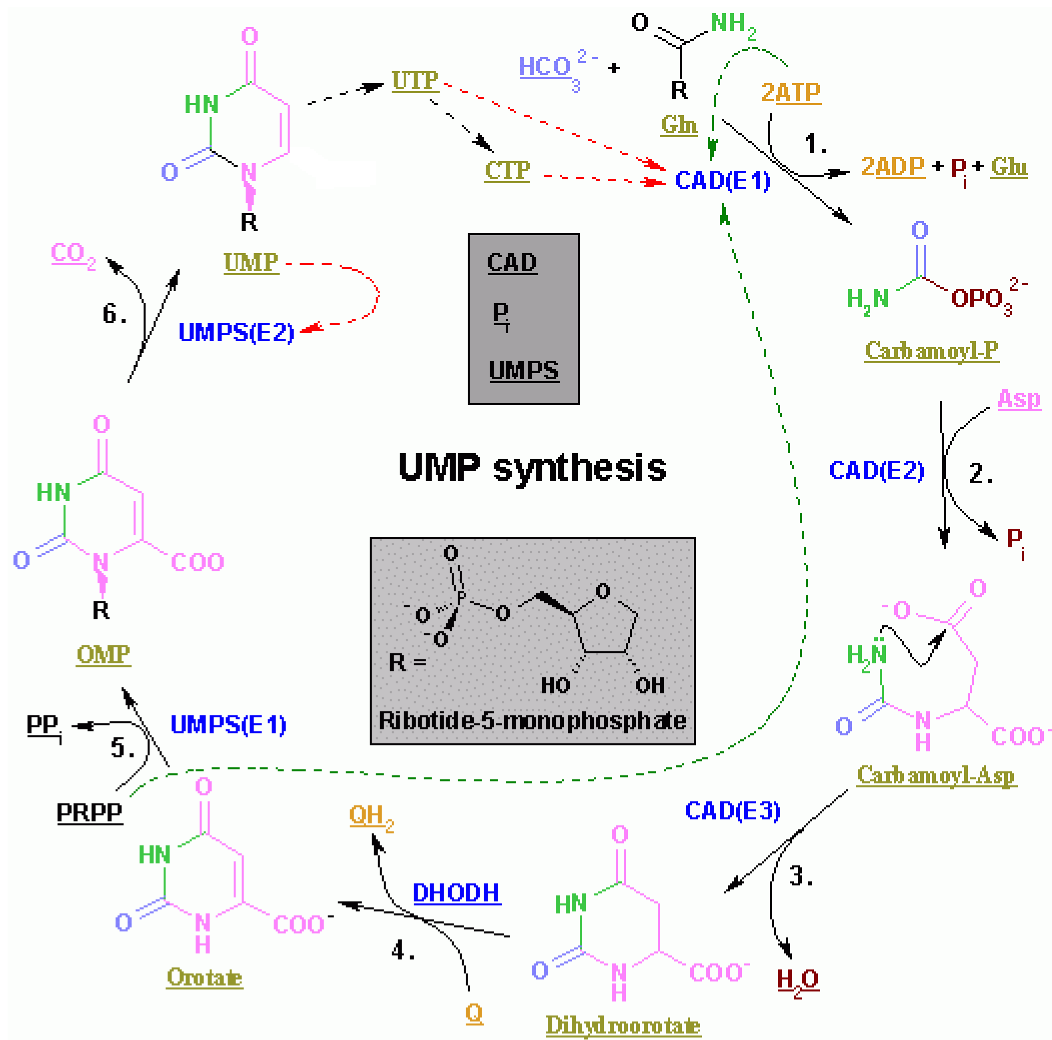
In the second step react PRA, glycine and ATP to create GAR, ADP, and pyrophosphate -catalyzed byphosphoribosylamine—glycine ligase (GAR synthetase). Due to the chemical lability of PRA, which has a half-life of 38 seconds at PH 7.5 and 37 °C, researchers have suggested that the compound is channeled from amidophosphoribosyltransferase to GAR synthetase.

inosine monophosphate is synthesized on a pre-existing ribose-phosphate through a complex pathway (as shown in the figure on the right). The source of the carbon andnitrogen atoms of the purine ring, 5 and 4 respectively, come from multiple sources. The amino acid glycine contributes all its carbon (2) and nitrogen (1) atoms, with additional nitrogen atoms from glutamine (2) and aspartic acid (1), and additional carbon atoms from formyl groups (2), which are transferred from the coenzyme tetrahydrofolate as 10-formyltetrahydrofolate, and a carbon atom from bicarbonate (1). Formyl groups build carbon-2 and carbon-8 in the purine ring system, which are the ones acting as bridges between two nitrogen atoms.



Biosynthesis of pyrimidine nucleotide

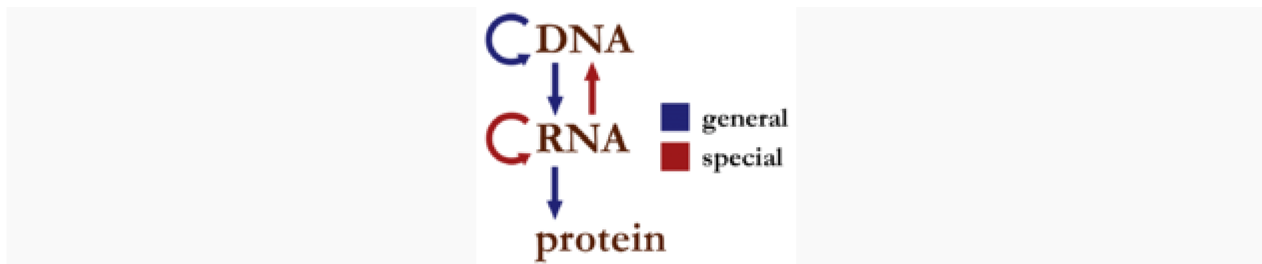
Pyrimidine is an aromatic heterocyclic organic compound similar to pyridine. One of the three diazines (six-membered heterocyclics with two nitrogen atoms in the ring), it has the nitrogen atoms at positions 1 and 3 in the ring. The other diazines arepyrazine (nitrogen atoms at the 1 and 4 positions) and pyridazine (nitrogen atoms at the 1 and 2 positions). In nucleic acids, three types of nucleobases are pyrimidine derivatives: cytosine (C), thymine (T), and uracil (U.



UNIT III

Central dogma of molecular biology

The **central dogma of molecular biology** is an explanation of the flow of genetic information within a biological system. It was first stated by Francis Crick in 1958 and re-stated in a Nature paper published in 1970.



Information flow in biological systems

> “ The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred back from protein to either protein or nucleic acid. ”

— Francis Crick

The central dogma has also been described as "DNA makes RNA and RNA makes protein," a positive statement which was originally termed the sequence hypothesis by Crick. However, this simplification does not make it clear that the central dogma as stated by Crick does not preclude the reverse flow of information from RNA to DNA, only ruling out the flow from protein to RNA or DNA. Crick's use of the word dogma was unconventional, and has been controversial.

The dogma is a framework for understanding the transfer of sequence information between information-carrying biopolymers, in the most common or general case, in living organisms. There are 3 major classes of such biopolymers: DNA and RNA (both nucleic acids), and protein. There are 3×3 = 9 conceivable direct transfers of information that can occur between these. The dogma classes these into 3 groups of 3: 3 **general transfers** (believed to occur normally in most cells), 3 **special transfers** (known to occur, but only under specific conditions in case of some viruses or in a laboratory), and 3 **unknown transfers** (believed never to occur). The general transfers describe the normal flow of biological information: DNA can be copied to DNA (DNA replication), DNA information can be copied into mRNA(transcription), and proteins can be synthesized using the information in mRNA as a template (translation.

General transfers of biological sequential information

Table of the 3 classes of information transfer suggested by the dogma

General	Special	Unknown
DNA → DNA	RNA → DNA	protein → DNA
DNA → RNA	RNA → RNA	protein → RNA

RNA → protein	DNA → protein	protein → protein

DNA Replications

In the sense that DNA replication must occur if genetic material is to be provided for the progeny of any cell, whether somatic or reproductive, the copying from DNA to DNA arguably is the fundamental step in the central dogma. A complex group of proteins called the replisome performs the replication of the information from the parent strand to the complementary daughter strand.

The replisome comprises:

- a helicase that unwinds the superhelix as well as the double-stranded DNA helix to create a replication fork
- SSB protein that binds open the double-stranded DNA to prevent it from reassociating
- RNA primase that adds a complementary RNA primer to each template strand as a starting point for replication
- DNA polymerase III that reads the existing template chain from its 3' end to its 5' end and adds new complementary nucleotides from the 5' end to the 3' end of the daughter chain
- DNA polymerase I that removes the RNA primers and replaces them with DNA.
- DNA ligase that joins the two Okazaki fragments with phosphodiester bonds to produce a continuous chain.

Replication process:-

DNA replication, like all biological polymerization processes, proceeds in three enzymatically catalyzed and coordinated steps: initiation, elongation and termination.

Initiation:-

For a cell to divide, it must first replicate its DNA.This process is initiated at particular points in the DNA, known as "origins", which are targeted by initiator proteins. In *E. coli* this protein is DnaA; in yeast, this is the origin recognition complex, Sequences used by initiator proteins tend to be "AT-rich" (rich in adenine and thymine bases), because A-T base pairs have two hydrogen bonds (rather than the three formed in a C-G pair) which are easier to unzip.

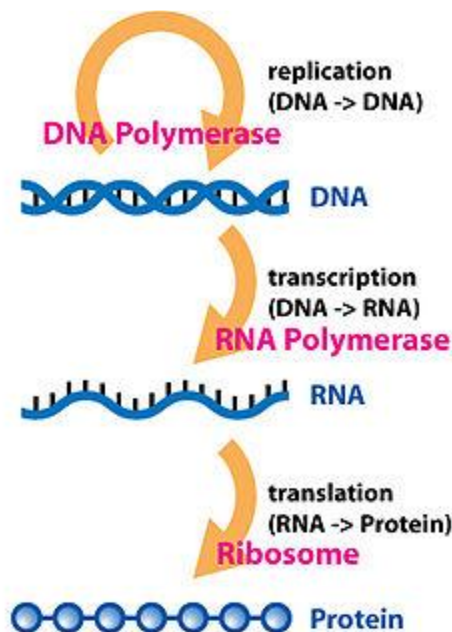
Elongation:-

DNA polymerase has 5'-3' activity. All known DNA replication systems require a free 3' hydroxyl group before synthesis can be initiated (Important note: the DNA template is read in 3' to 5' direction whereas a new strand is synthesized in the 5' to 3' direction—this is often confused). Four distinct mechanisms for initiation of synthesis are recognized:

Replication fork:-

The replication fork is a structure that forms within the nucleus during DNA replication. It is created by helicases, which break the hydrogen bonds holding the two DNA strands together. The resulting structure has two branching "prongs", each one made up of a single strand of DNA.

This process typically takes place during S phase of the cell cycle.



Transcription

Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA(mRNA) by the enzyme RNA polymerase.

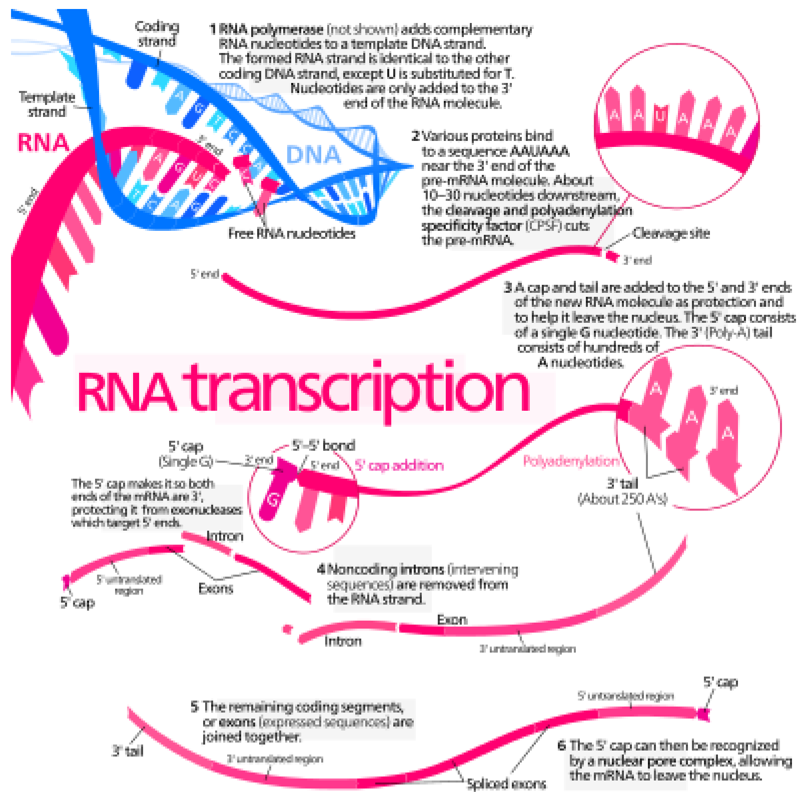
Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language. The two can be converted back and forth from DNA to RNA by the action of the correct enzymes. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallelRNA strand called a primary transcript.

Transcription proceeds in the following general steps:

1. One or more sigma factor protein binds to the RNA polymerase holoenzyme, allowing it to bind topromoter DNA.
2. RNA polymerase creates a transcription bubble, which separates the two strands of the DNA helix. This is done by breaking the hydrogen bonds between complementary DNA nucleotides.
3. RNA polymerase adds matching RNA nucleotides to the complementary nucleotides of one DNA strand.
4. RNA sugar-phosphate backbone forms with assistance from RNA polymerase to form an RNA strand.
5. Hydrogen bonds of the untwisted RNA-DNA helix break, freeing the newly synthesized RNA strand.
6. If the cell has a nucleus, the RNA may be further processed. This may include polyadenylation,capping, and splicing.
7. The RNA may remain in the nucleus or exit to the cytoplasm through the nuclear pore complex.

The stretch of DNA transcribed into an RNA molecule is called a transcription unit and encodes at least onegene. If the gene transcribed encodes a protein, messenger RNA (mRNA) will be transcribed; the mRNA will in turn serve as a template for the protein's synthesis through translation. Alternatively, the transcribed gene may encode for either non-coding RNA (such as microRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), or other enzymatic RNA molecules called ribozymes.[1] Overall, RNA helps synthesize, regulate, and process proteins; it therefore plays a fundamental role in performing functions within a cell.

In virology, the term may also be used when referring to mRNA synthesis from an RNA molecule (i.e., RNA replication). For instance, the genome of a negative-sense single-stranded RNA (ssRNA -) virus may be template for a positive-sense single-stranded RNA (ssRNA +). This is because the positive-sense strand contains the information needed to translate the viral proteins for viral replication afterwards.



DNA damage

DNA damage is an alteration in the chemical structure of DNA, such as a break in a strand of DNA, a base missing from the backbone of DNA, or a chemically changed base such as 8-OHdG. Damage to DNA that occurs naturally can result from metabolic or hydrolytic processes. Metabolism releases compounds that damage DNA includingreactive oxygen species, reactive nitrogen species, reactive carbonyl species, lipid peroxidation products and alkylating agents, among others, while hydrolysis cleaves chemical bonds in DNA Naturally occurring oxidative DNA damages arise at least 10,000 times per cell per day in humans and 50,000 times or more per cell per day in rats, as documented below.

DNA damage is distinctly different from mutation, although both are types of error in DNA. DNA damage is an abnormal chemical structure in DNA, while a mutation is a change in the sequence of standard base pairs.

DNA damage and mutation have different biological consequences. While most DNA damages can undergo DNA repair, such repair is not 100% efficient. Un-repaired DNA damages accumulate in non-replicating cells, such as cells in the brains or muscles of adult mammals and can cause aging. (Also see DNA damage theory of aging.) In replicating cells, such as cells lining the colon, errors occur upon replication of past damages in the template strand of DNA or during repair of DNA damages. These errors can give rise to mutations or epigenetic alteration.

DNA repair

DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode itsgenome. In human cells, both normal metabolic activities and environmental factors such as UV light and radiation can cause DNA damage, resulting in as many as 1 million individual molecular lesions per cell per day. Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene that the affected DNA encodes. Other lesions induce potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis. As a consequence, the DNA repair process is constantly active as it responds to damage in the DNA structure. When normal repair processes fail, and when cellular apoptosis does not occur,

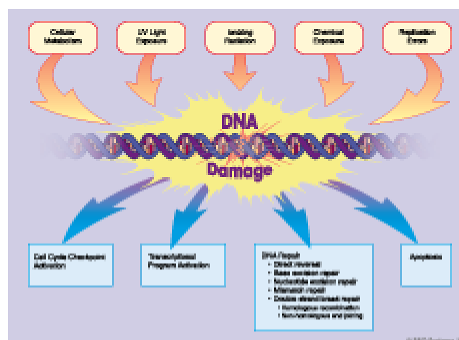
The rate of DNA repair is dependent on many factors, including the cell type, the age of the cell, and the extracellular environment. A cell that has accumulated a large amount of DNA damage, or one that no longer effectively repairs damage incurred to its DNA, can enter one of three possible states:

1. an irreversible state of dormancy, known as senescence
2. cell suicide, also known as apoptosis or programmed cell death
3. unregulated cell division, which can lead to the formation of a tumor that is cancerous

The DNA repair ability of a cell is vital to the integrity of its genome and thus to the normal functionality of that organism. Many genes that were initially shown to influence life span have turned out to be involved in DNA damage repair and protection.

Paul Modrich

The 2015 Nobel Prize in Chemistry was awarded to Tomas Lindahl, Paul Modrich, and Aziz Sancar for their work on the molecular mechanisms of DNA repair process.



Mechanism:-

Direct reversal

Cells are known to eliminate three types of damage to their DNA by chemically reversing it. These mechanisms do not require a template, since the types of damage they counteract can occur in only one of the four bases. Such direct reversal mechanisms are specific to the type of damage incurred and do not involve breakage of the phosphodiester backbone. The formation of pyrimidine dimers upon irradiation with UV light results in an abnormal covalent bond between adjacent pyrimidine bases. The photoreactivation process directly reverses this damage by the action of the enzyme photolyase, whose activation is obligately dependent on energy absorbed from blue/UV light (300–500 nm wavelength) to promote catalysis. Photolyase, an old enzyme present in bacteria, fungi, and most animals no longer functions in humans, who instead use nucleotide excision repair to repair damage from UV irradiation. Another type of damage, methylation of guanine bases, is directly reversed by the protein methyl guanine methyl transferase (MGMT), the bacterial equivalent of which is called ogt.

1. Base excision repair (BER) repairs damage to a single nitrogenous base by deploying enzymes called glycosylases.[18] These enzymes remove a single nitrogenous base to create an apurinic or apyrimidinic site (AP site). Enzymes called AP endonucleases nick the damaged DNA backbone at the AP site. DNA polymerase then removes the damaged region using its 5' to 3' exonuclease activity and correctly synthesizes the new strand using the complementary strand as a template.
2. Nucleotide excision repair (NER) repairs damaged DNA which commonly consists of bulky, helix-distorting damage, such aspyrimidine dimerization caused by UV light. Damaged regions are removed in 12-24 nucleotide-long strands in a three-step process which consists of recognition of damage, excision of damaged DNA both upstream and downstream of damage byendonucleases, and resynthesis of removed DNA region. NER is a highly evolutionarily conserved repair mechanism and is used in nearly all eukaryotic and prokaryotic cells. In prokaryotes, NER is mediated by Uvr proteins. In eukaryotes, many more proteins are involved, although the general strategy is the same.
3. Mismatch repair systems are present in essentially all cells to correct errors that are not corrected by proofreading. These systems consist of at least two proteins. One detects the mismatch, and the other recruits an endonuclease that cleaves the newly synthesized DNA strand close to the region of damage. In E. coli , the proteins involved are the Mut class proteins. This is followed by removal of damaged region by an exonuclease, resynthesis by DNA polymerase, and nick sealing by DNA ligase.

Inhibitor of protein synthesis

A **protein synthesis inhibitor** is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins.[1]

While a broad interpretation of this definition could be used to describe nearly any antibiotic, in practice, it usually refers to substances that act at the ribosome level (either the ribosome itself or the translation factor), taking advantages of the major differences between prokaryotic and eukaryotic ribosome structures.Toxins such as ricin also function via protein synthesis inhibition, Ricin acts at the eukaryotic 60S.

In general, protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins, like initiation, elongation (including aminoacyl tRNA entry,proofreading, peptidyl transfer, and ribosomal translocation) and termination:

Earlier stages

- Rifamycin inhibits prokaryotic DNA transcription into mRNA by inhibiting DNA-dependent RNA polymerase by binding its beta-subunit.

Initiation

- Linezolid acts at the initiation stage, probably by preventing the formation of the initiation complex, although the mechanism is not fully understood.

Aminoacyl tRNA entry

- Tetracyclines and Tigecycline[7] (a glycylcycline related to tetracyclines) block the A site on the ribosome, preventing the binding of aminoacyl tRNAs.

Proofreading

- Aminoglycosides, among other potential mechanisms of action, interfere with the proofreading process, causing increased rate of error in synthesis with premature termination.[8]

Peptidyl transfer

- Chloramphenicol blocks the peptidyl transfer step of elongation on the 50S ribosomal subunit in both bacteria and mitochondria.
- Macrolides (as well as inhibiting ribosomal translocation and other potential mechanisms) bind to the 50s ribosomal subunits, inhibiting peptidyl transfer.
- Quinupristin/dalfopristin act synergistically, with dalfopristin, enhancing the binding of quinupristin, as well as inhibiting peptidyl transfer. Quinupristin binds to a nearby site on the

50S ribosomal subunit and prevents elongation of the polypeptide, as well as causing incomplete chains to be released.

Ribosomal translocation

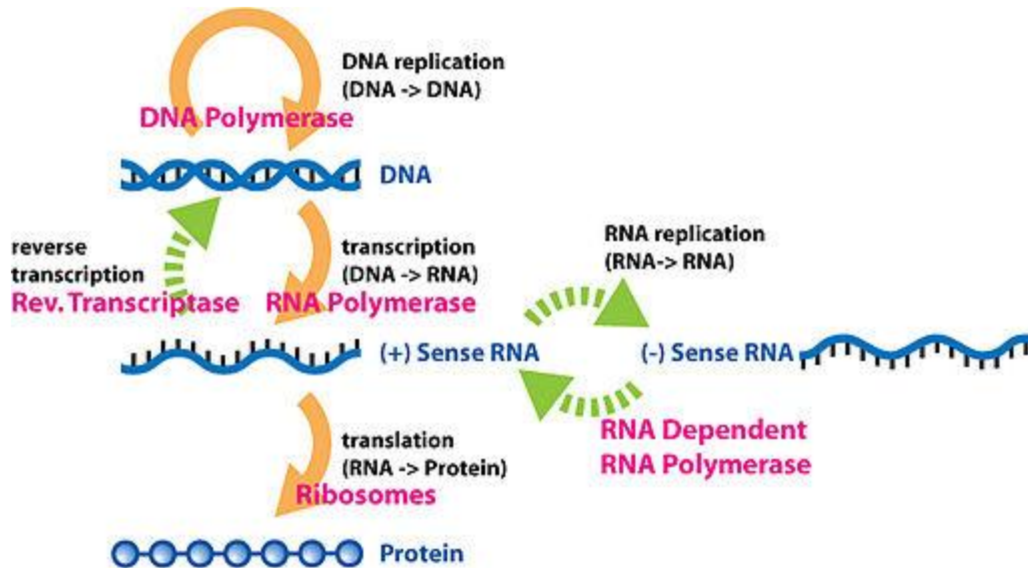
- Macrolides, clindamycin and aminoglycosides (with all these three having other potential mechanisms of action as well), have evidence of inhibition of ribosomal translocation.
- Fusidic acid prevents the turnover of elongation factor G (EF-G) from the ribosome.

Termination

- Macrolides and clindamycin (both also having other potential mechanisms) cause premature dissociation of the peptidyl-tRNA from the ribosome.
- Puromycin has a structure similar to that of the tyrosinyl aminoacyl-tRNA. Thus, it binds to the ribosomal A site and participates in peptide bond formation, producing peptidyl-puromycin. However, it does not engage in translocation and quickly dissociates from the ribosome, causing a premature termination of polypeptide synthesis.
- Streptogramins also cause premature release of the peptide chain.

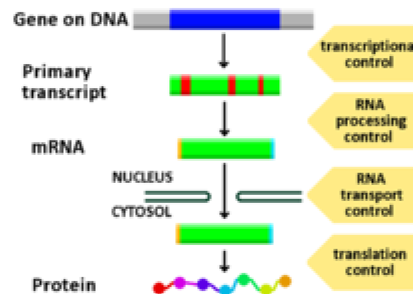
Translation

- The mature mRNA finds its way to a ribosome, where it gets translated. In prokaryotic cells, which have no nuclear compartment, the processes of transcription and translation may be linked together without clear separation. In eukaryotic cells, the site of transcription (the cell nucleus) is usually separated from the site of translation (the cytoplasm), so the mRNA must be transported out of the nucleus into the cytoplasm, where it can be bound by ribosomes. The ribosome reads the mRNA triplet codons, usually beginning with an AUG (adenine−uracil−guanine), or initiator methionine codon downstream of the ribosome binding site. Complexes of initiation factors and elongation factors bring aminoacylated transfer RNAs (tRNAs) into the ribosome-mRNA complex, matching the codon in the mRNA to the anti-codon on the tRNA. Each tRNA bears the appropriate amino acid residue to add to the polypeptide chain being synthesised. As the amino acids get linked into the growing peptide chain, the chain begins folding into the correct conformation. Translation ends with a stop codon which may be a UAA, UGA, or UAG triplet.



Regulation of gene expression

Regulation of gene expression includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (protein or RNA), and is informally termed gene regulation. Sophisticated programs of gene expression are widely observed in biology, for example to trigger developmental pathways, respond to environmental stimuli, or adapt to new food sources. Virtually any step of gene expression can be modulated, from transcriptional initiation, to RNA processing, and to the post-translational modification of a protein.



Lac operon (lactose operon) is an operon required for the transport and metabolism of lactose in Escherichia coli and many other enteric bacteria. Although glucose is the preferred carbon source for most bacteria, the lac operon allows for the effective digestion of lactose when glucose is not available. Gene regulation of the lac operon was the first genetic regulatory mechanism to be understood clearly, so it has become a foremost example of prokaryotic gene regulation.

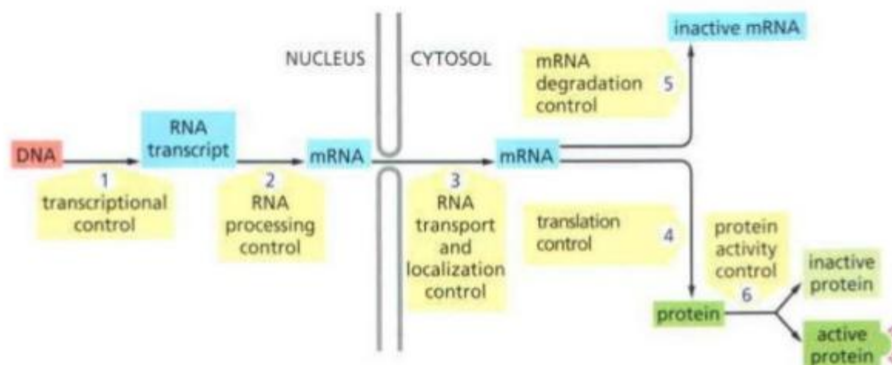
Bacterial operons are polycistronic transcripts that are able to produce multiple proteins from one mRNA transcript. In this case, when lactose is required as a sugar source for the bacterium, the three genes of the lac operon can be expressed and their subsequent proteins translated: lacZ, lacY, and lacA. The gene product of lacZ is β-galactosidasewhich cleaves lactose, a disaccharide, into glucose and galactose. lacY encodes lactose permease, a protein which becomes embedded in the cytoplasmic membrane to enable transport of lactose into the cell. Finally, lacA encodes galactoside O-acetyltransferase.

The lac operon consists of three structural genes, and a promoter, a terminator, regulator, and an operator. The three structural genes are: lacZ, lacY, and lacA.

- lacZ encodes β-galactosidase (LacZ), an intracellular enzyme that cleaves the disaccharide lactose into glucose andgalactose.
- lacY encodes lactose permease (LacY), a transmembrane symporter that pumps β-galactosides into the cell using a proton gradient in the same direction.
- lacA encodes galactoside O-acetyltransferase (LacA), an enzyme that transfers an acetyl group from acetyl-CoA to β-galactosides.

Gene regulation in Eukaryotes

- Absence of operon
- Regulation in each level of expression



(Alberts et al., 2008)

43

Cell cycle & its regulation

The cell cycle or **cell-division cycle** is the series of events that take place in a cell leading to its division and duplication of its DNA (DNA replication) to produce two daughter cells. In bacteria, which lack a cell nucleus, the cell cycle is divided into the B, C, and D periods. The B period extends from the end of cell division to the beginning of DNA replication. DNA replication occurs during the C period. The D period refers to the stage between the end of DNA replication and the splitting of the bacterial cell into two daughter cells.[2] In cells with a nucleus, as in eukaryotes, the cell cycle is also divided into three periods: interphase, the mitotic (M) phase, and cytokinesis. During interphase, the cell grows, accumulating nutrients needed for mitosis, preparing it for cell division and duplicating its DNA. During the mitotic phase, the cell splits itself into two distinct daughter cells. During the final stage, cytokinesis, the new cell is completely divided. To ensure the proper division of the cell, there are control mechanisms known as cell cycle checkpoints.

Cell cycle phage:-

State	**Description**	**Abbreviation**	
quiescent/ senescent	Gap 0	**G_0**	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	**G_1**	Cells increase in size in Gap 1. The G_1 checkpoint control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	**S**	DNA replication occurs during this phase.
	Gap 2	**G_2**	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The G_2 checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.

Cell division	Mitosis	**M**	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cell.

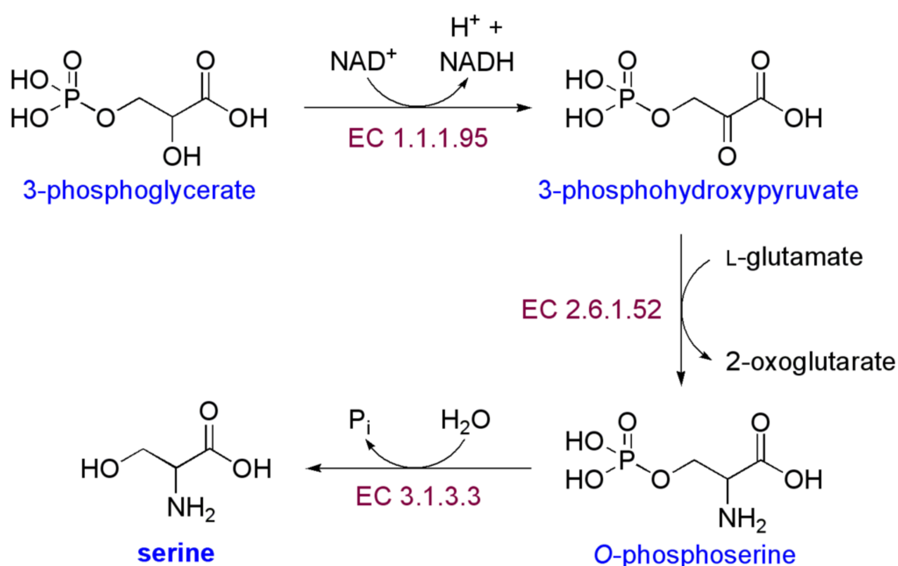
UNIT IV

Biosynthesis of serine

Serine (abbreviated as **Ser**) encoded by the codons UCU, UCC, UCA, UCG, AGU and AGC is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group (which is in the protonated $-NH_3^+$ form under biological conditions), acarboxyl group (which is in the deprotonated $-COO^-$ form under biological conditions), and a side chain hydroxyl group, classifying it as a polar amino acid. It is non-essential in humans, meaning the body can synthesize it.

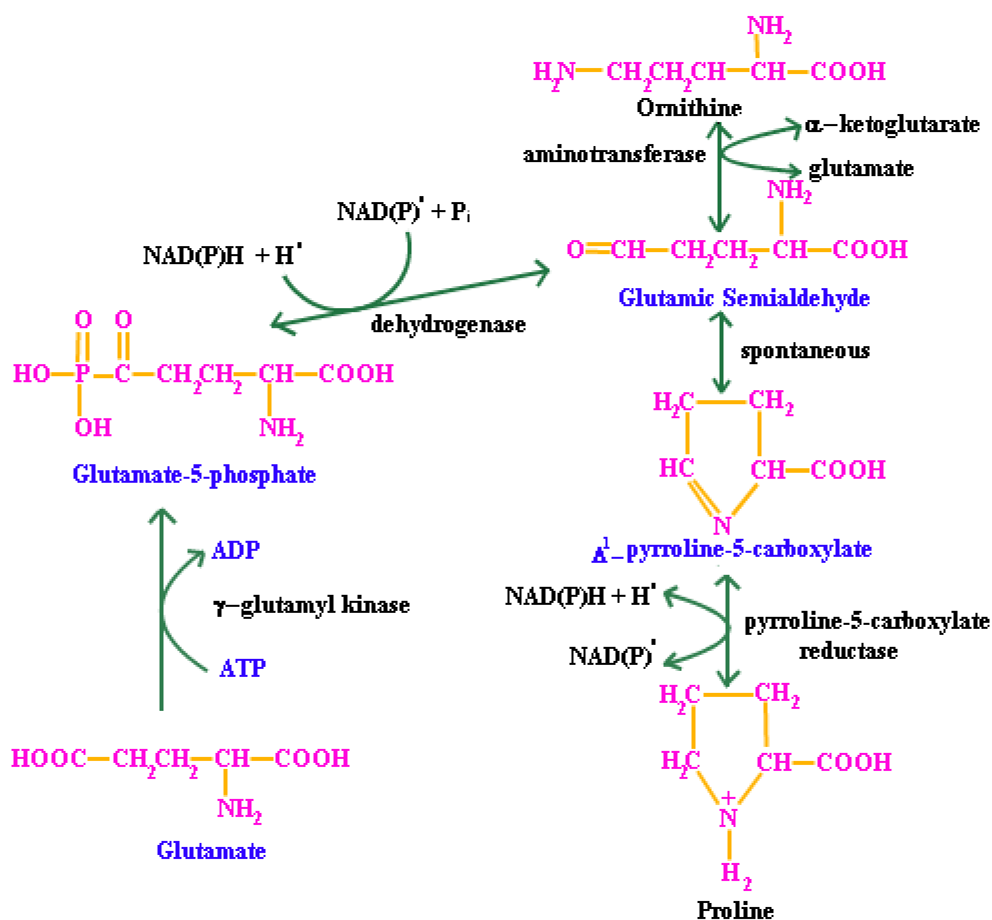
This compound is one of the naturally occurring proteinogenic amino acids. Only the L-stereoisomer appears naturally in proteins. It is not essential to the human diet, since it is synthesized in the body from other metabolites, including glycine. Serine was first obtained from silk protein.

The biosynthesis of serine starts with the oxidation of 3-phosphoglycerate (an intermediate from glycolysis) to 3-phosphohydroxypyruvate and NADH by phosphoglycerate dehydrogenase . Reductive amination (transamination) of this ketone by phosphoserine transaminase yields 3-phosphoserine (O-phosphoserine) which is hydrolyzed to serine by phosphoserine phosphatase.



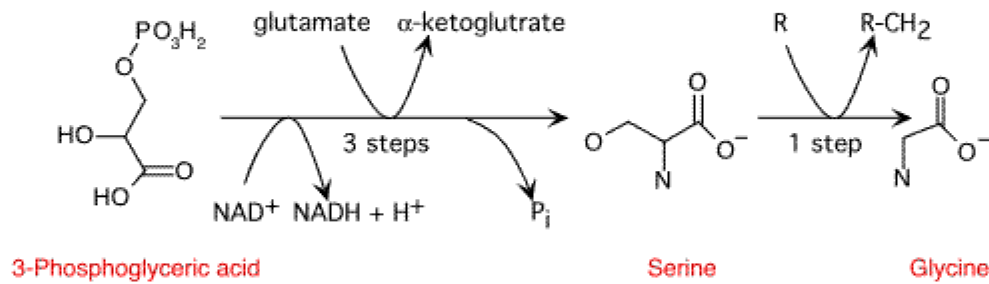
Biosynthesis of Proline

Proline (abbreviated as **Pro** or **P**; encoded by the codons CCU, CCC, CCA, and CCG) is an α-amino acid that is used in thebiosynthesis of proteins. It contains an α-amino group (which is in the protonated >NH_2^+ form under biological conditions), an α-carboxylic acid group (which is in the deprotonated $-COO^-$ form under biological conditions), and a side chain pyrrolidine, classifying it as a nonpolar(at physiological pH), aliphatic amino acid. It is non-essential in humans, meaning the body can synthesize it from the non-essential amino acid L-glutamate.



Biosynthesis of glycine

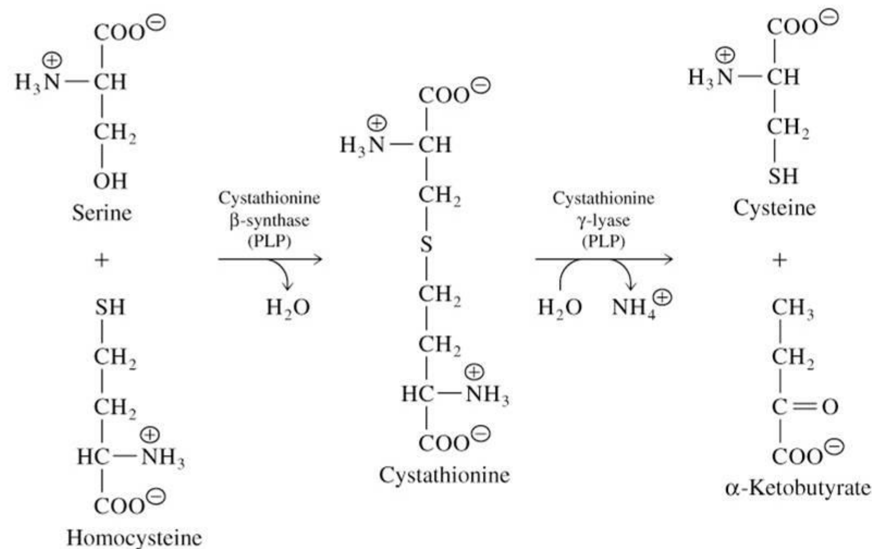
Glycine (abbreviated as **Gly** or **G**) is the smallest of the 20 amino acids commonly found in proteins, and indeed is the smallest possible (having a hydrogen substituent as its side-chain). The formula is NH_2CH_2COOH. Its codons are GGU, GGC, GGA, GGG of the genetic code.



Biosynthesis of Cysteine

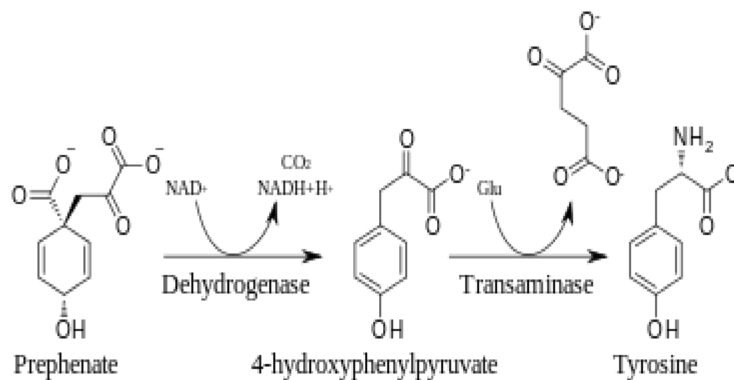
Cysteine (abbreviated as **Cys** or **C**) is a semi-essential[4] proteinogenic amino acid with the formula $HO_2CCH(NH_2)CH_2SH$>. It is encoded by the codons UGU and UGC. The thiol side chain in cysteine often participates in enzymatic reactions, as a nucleophile. The thiol is susceptible to oxidization to give the disulfide derivative cystine, which serves an important structural role in manyproteins.

Although classified as a non-essential amino acid, in rare cases, cysteine may be essential for infants, the elderly, and individuals with certain metabolic disease or who suffer from malabsorption syndromes.



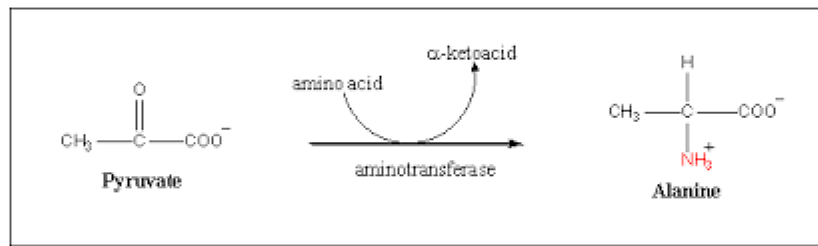
Biosynthesis of Tyrosine

Tyrosine (**Tyr** or **Y**) or **4-hydroxyphenylalanine** is one of the 22 amino acids that are used by cells to synthesize proteins. It is anon-essential amino acid with a polar side group. Its codons are UAC and UAU. The word "tyrosine" is from the Greek tyros, meaning cheese, as it was first discovered in 1846 by German chemist Justus von Liebig in the protein casein from cheese. It is called **tyrosyl** when referred to as a functional group or side chain. Tyrosine is a hydrophobic amino acid.



Biosynthesis Alanine

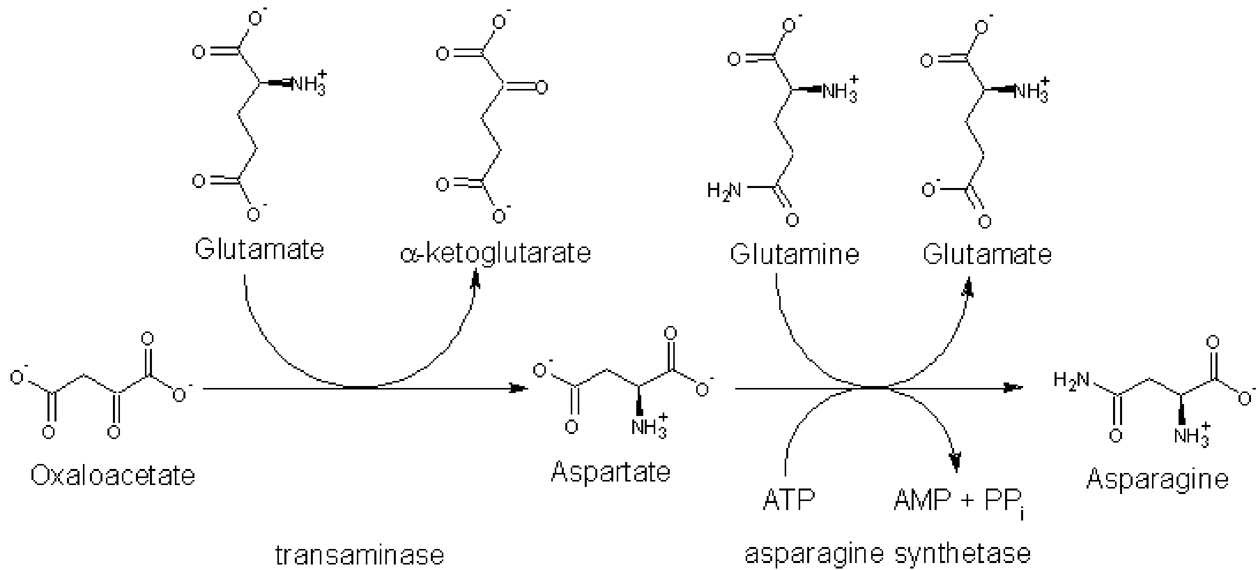
Alanine (abbreviated as **Ala** or **A**; encoded by the codons GCU, GCC, GCA, and GCG) is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group (which is in the protonated −NH3^{+} form under biological conditions), an α-carboxylic acid group (which is in the deprotonated −COO$^{−}$ form under biological conditions), and a side chain methyl group, classifying it as a nonpolar (at physiological pH), aliphatic amino acid. It is non-essential in humans, meaning the body can synthesize it.



Biosynthesis of asparagine

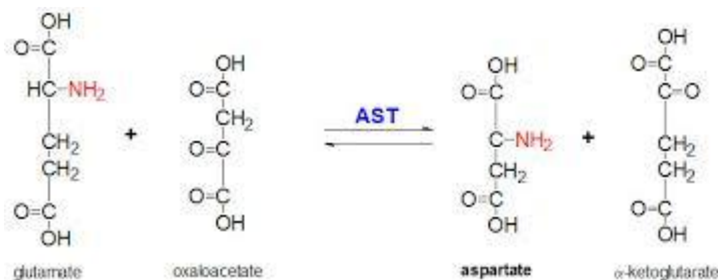
Asparagine (abbreviated as Asn or N) encoded by the codons **AAU** and **AAC**.[2] is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group (which is in the protonated−NH+3 form under biological conditions), an α-carboxylic acid group (which is in the

deprotonated −COO⁻ form under biological conditions), and a side chain carboxamide, classifying it as a polar (at physiological pH), aliphatic amino acid. It is non-essential in humans, meaning the body can synthesize it



Biosynthesis of aspartate

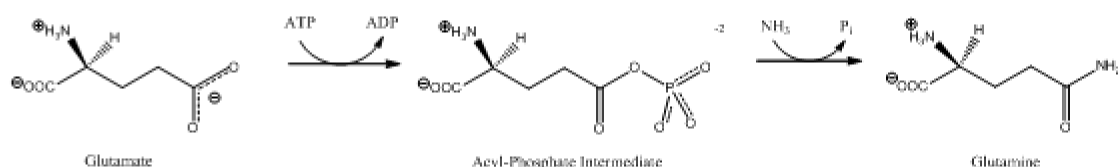
Aspartic acid (abbreviated as **Asp** or **D**; encoded by the codons [GAU and GAC]) is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group (which is in the protonated −NH+3 form under biological conditions), an α-carboxylic acid group (which is in the deprotonated −COO⁻ form under biological conditions), and a side chain CH_2COOH. Under physiological conditions in proteins the sidechain usually occurs as the negatively charged aspartate form, −COO⁻. It is semi-essential in humans, meaning the body can synthesize it from oxaloacetate.



Biosynthesis of Glutamine

Glutamine synthetase (GS) (EC 6.3.1.2)[3] is an enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine:

Glutamate + ATP + NH_3 → Glutamine + ADP + phosphate

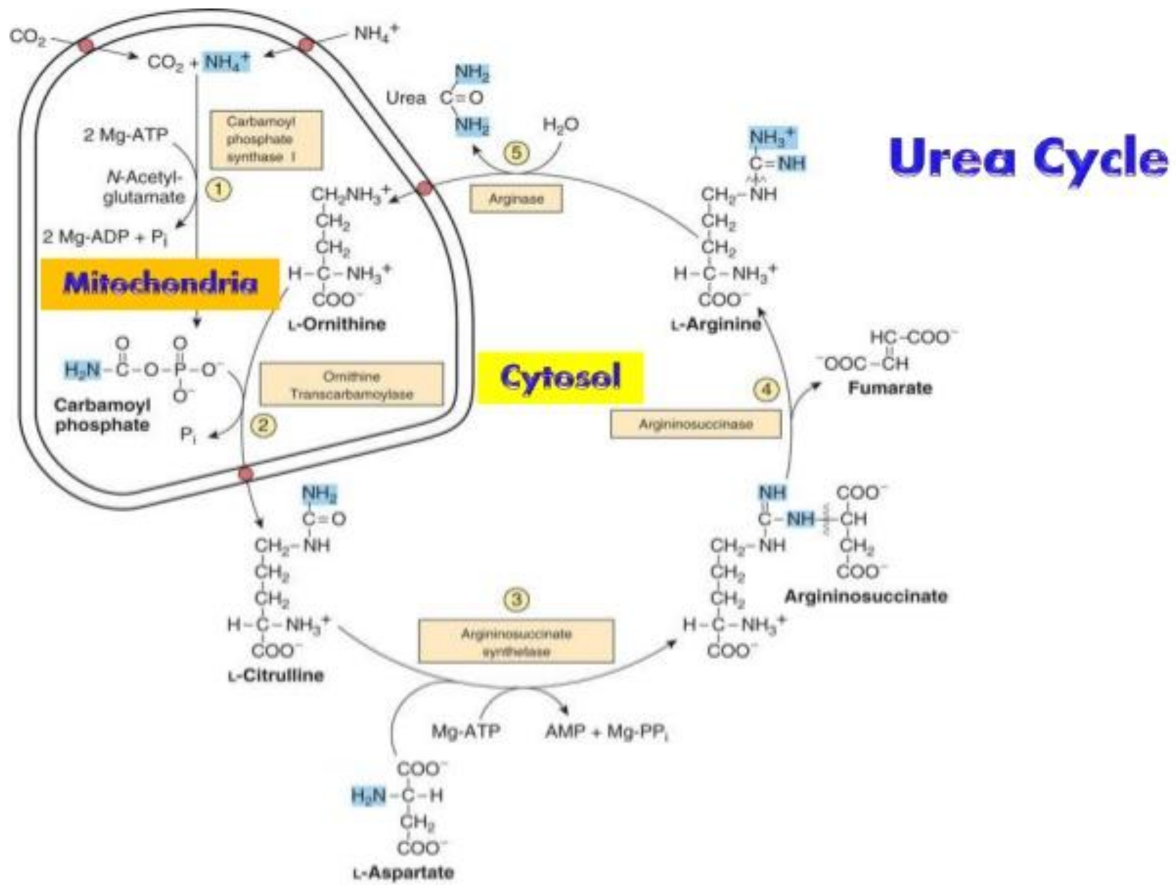


Urea Cycle & its function

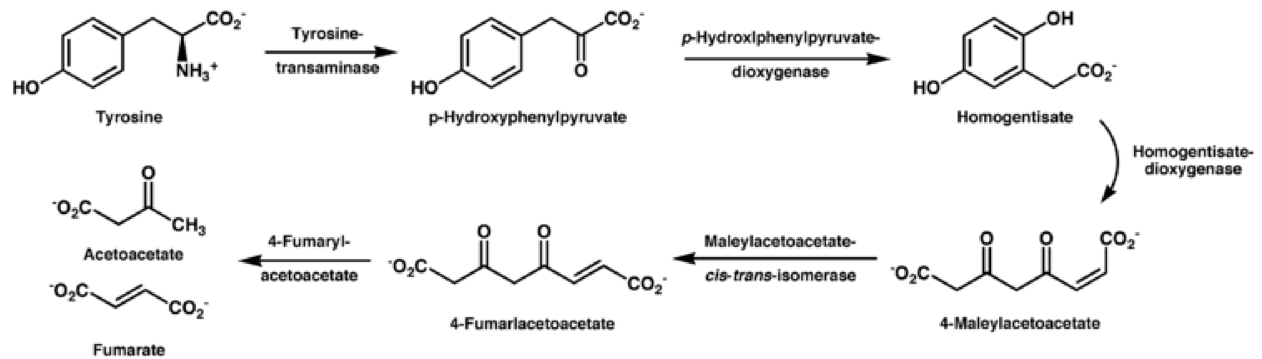
The **urea cycle** (also known as the **ornithine cycle**) is a cycle of biochemical reactions occurring in many animals that produces urea ($(NH_2)_2CO$) from ammonia (NH_3). This cycle was the first metabolic cycle discovered (Hans Krebs and Kurt Henseleit, 1932), five years before the discovery of the TCA cycle. In mammals, the urea cycle takes place primarily in the liver, and to a lesser extent in the kidney.

Function:-

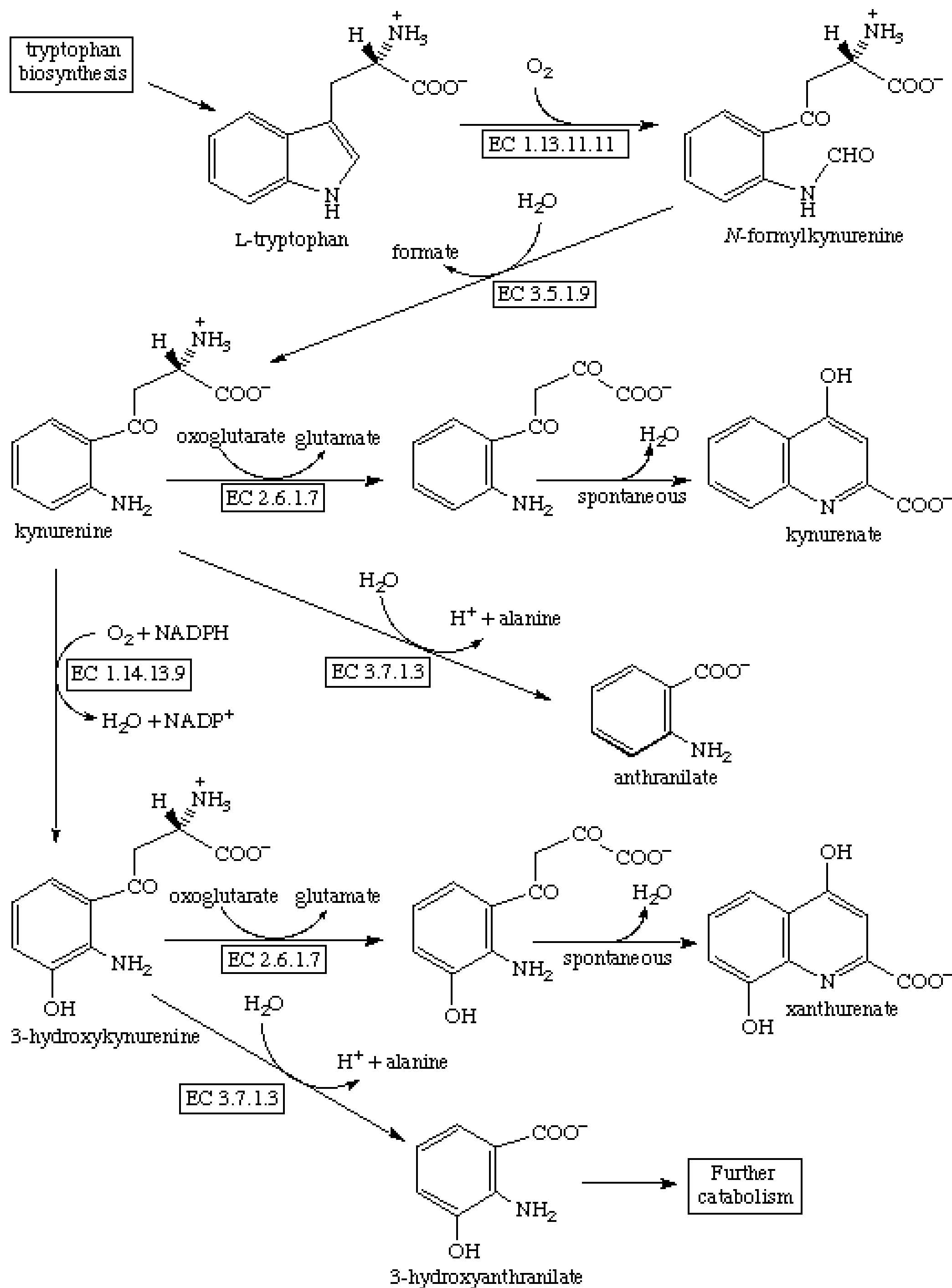
Organisms that cannot easily and quickly remove ammonia usually have to convert it to some other substance, like urea or uric acid, which are much less toxic. Insufficiency of the urea cycle occurs in some genetic disorders (inborn errors of metabolism), and in liver failure. The result of liver failure is accumulation of nitrogenous waste, mainly ammonia, which leads to hepatic encephalopathy.



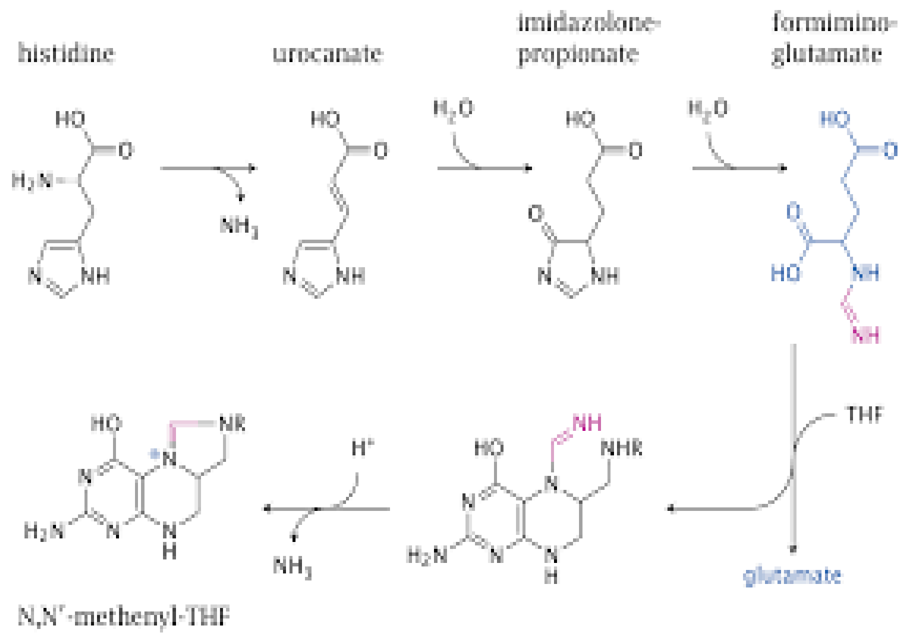
Catabolism of Tyrosine



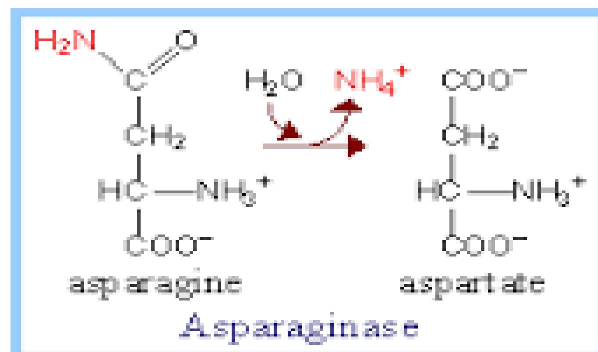
Catabolism of Tryptophan



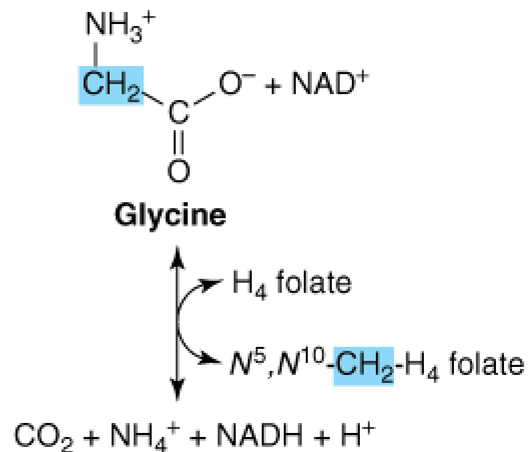
Catabolism of Histidine



Catabolism of Asparagine



Catabolism of glycine



Conversion of amino acids to specilized product

Epinephrine

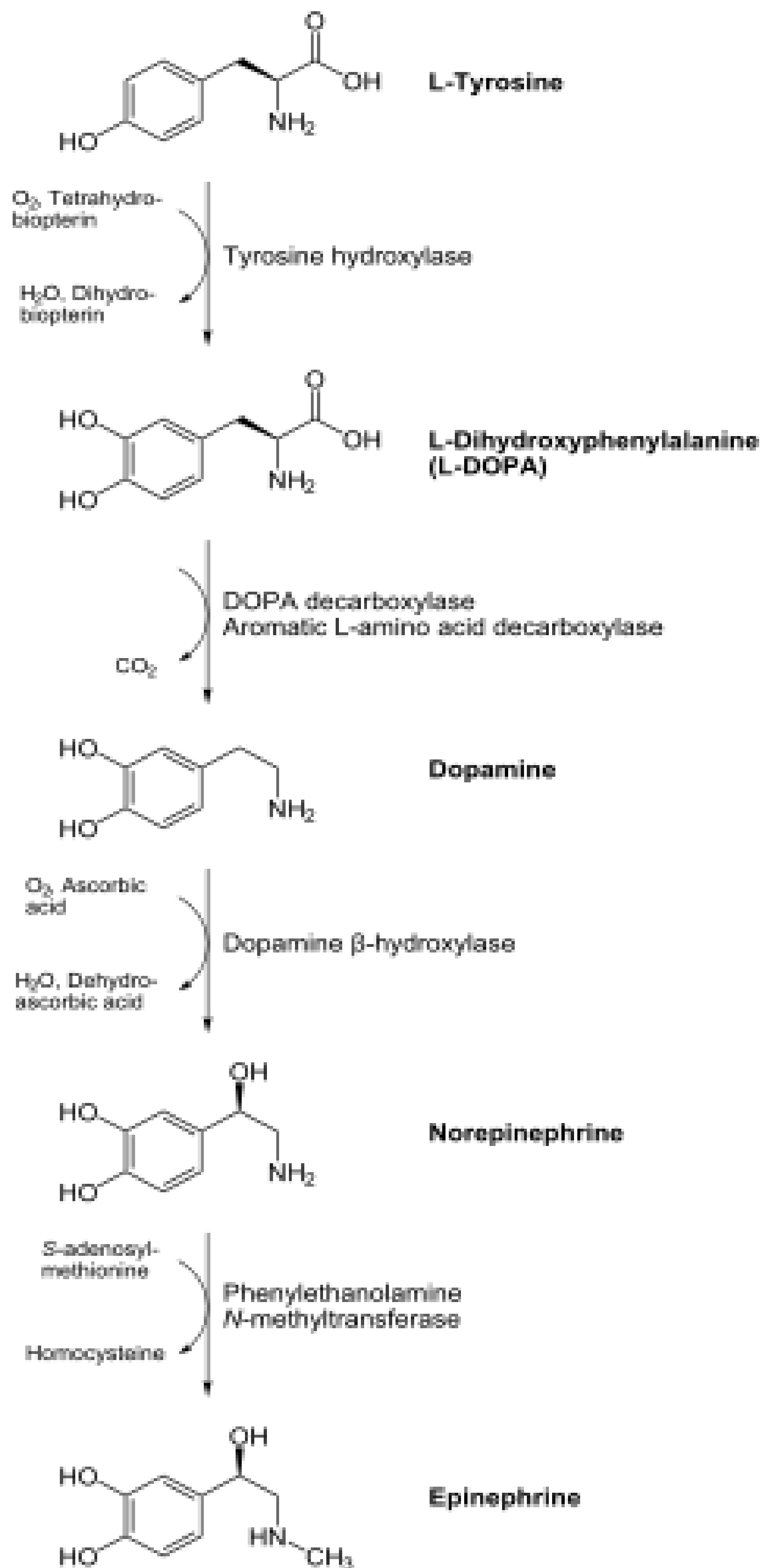
Epinephrine, also known as **adrenalin** or **adrenaline**, is primarily a medication and hormone. As a medication it is used for a number of conditions including: anaphylaxis, cardiac arrest, and superficial bleeding, Inhaled epinephrine may be used to improve the symptoms of croup, It may also be used for asthma when other treatments are not effective. It is given intravenously, by injection into a muscle, by inhalation, or by injection just under the skin.

Epinephrine is normally produced by both the adrenal glands and certain neurons, It plays an important role in the fight-or-flight response by increasing blood flow to muscles, output of the heart, pupil dilation, and blood sugar. Epinephrine does this by its effects on alpha and beta receptors.

Medicinal use:-

Epinephrine is used to treat a number of conditions including: cardiac arrest, anaphylaxis, and superficial bleeding. It has been used historically for bronchospasm and hypoglycemia, but newer treatments for these that are selective for β_2 adrenoceptors, such as salbutamol are currently preferred.

Biosynthesis:-

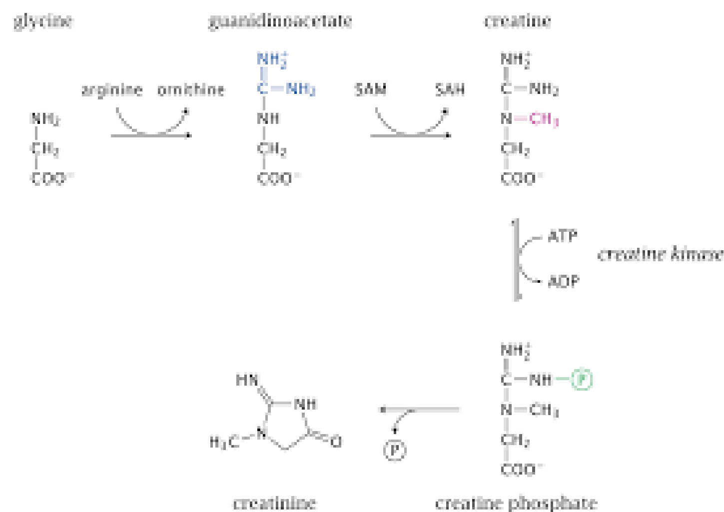

L-Tyrosine
HO
NH2
OH
O
O2, Tetrahydro-biopterin
Tyrosine hydroxylase
H2O, Dihydro-biopterin
L-Dihydroxyphenylalanine (L-DOPA)
DOPA decarboxylase
Aromatic L-amino acid decarboxylase
CO2
Dopamine
O2, Ascorbic acid
Dopamine β-hydroxylase
H2O, Dehydro-ascorbic acid
Norepinephrine
S-adenosyl-methionine
Phenylethanolamine N-methyltransferase
Homocysteine
Epinephrine
HN
CH3

Creatinine

Creatinine is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to all cells in the body, primarily muscle. This is achieved by increasing the formation of adenosine triphosphate (ATP). Creatine was identified in 1832 when Michel Eugène Chevreul isolated it from the basified water-extract of skeletal muscle. He later named the crystallized precipitate after the Greek word for meat, κρέας (kreas). Early analysis showed that human blood is approximately 1% creatine, and the highest concentrations are found in animal blood, brain (0.14%), muscle (0.50%), and testes (0.18%). The liver and kidney contain approximately 0.01% creatine. Today, creatine content (as a percentage of crude protein) can be used as an indicator of meat quality.

creatinine (a blood measurement) is an important indicator of renal health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine (also known as creatine phosphate), and adenosine triphosphate (ATP, the body's immediate energy supply).

Biosynthesis:-



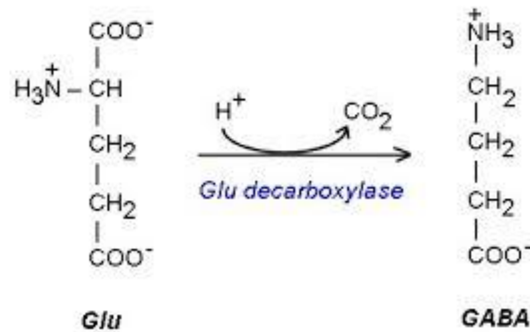
GABA

gamma-Aminobutyric acid (**GABA**) is the chief inhibitory neurotransmitter in the mammalian central nervous system. It plays the principal role in reducing neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone.

Although in chemical terms it is an amino acid, GABA is rarely referred to as such in the scientific or medical communities, because the term "amino acid," used without a qualifier, by

convention refers to the alpha amino acids, which GABA is not, nor is it considered to be incorporated into proteins.

While GABA is an inhibitory transmitter in the mature brain, its actions are primarily excitatory in the developing brain. The gradient of chloride is reversed in immature neurons, and its reversal potential is higher than the resting membrane potential of the cell; activation of a GABA-A receptor thus leads to efflux of Cl^- ions from the cell, i.e. a depolarizing current.

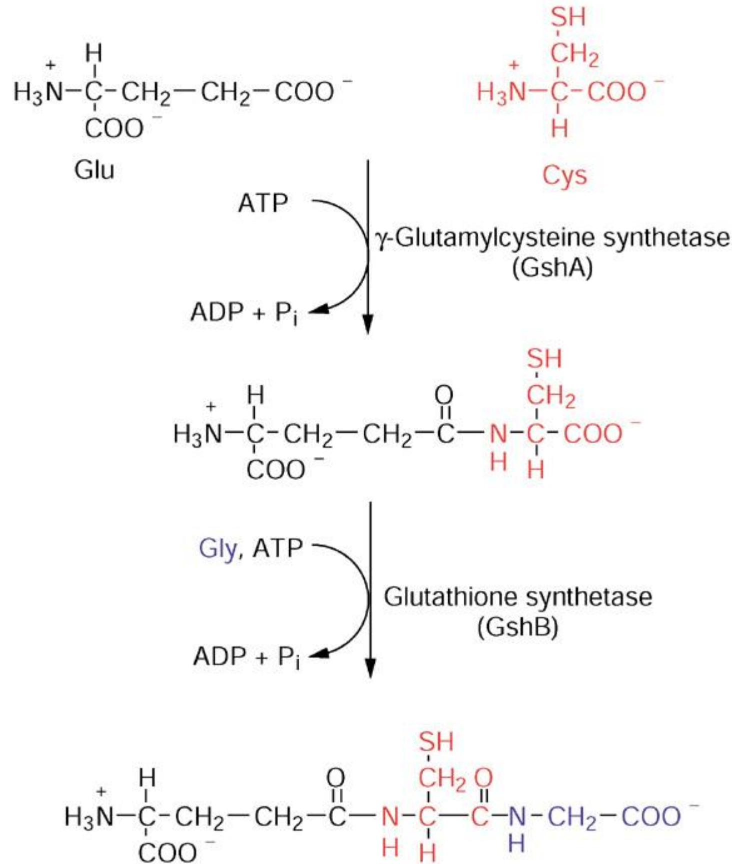


Glutathione

Glutathione (**GSH**) is an important antioxidant in plants, animals, fungi, and some bacteria and archaea, preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine (which is attached by normal peptide linkage to a glycine).

Thiol groups are reducing agents, existing at a concentration around 5 mM in animal cells. Glutathione reduces disulfide bondsformed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG), also called L-(–)-glutathione.

Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor.[3] The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity.



UNIT-V

Cholesterol, from the Ancient Greek chole- (bile) and stereos (solid) followed by the chemical suffix - for an alcohol, is an organic molecule. It is a sterol (or modified steroid), a lipid molecule and is biosynthesized by all animal cells because it is an essential structural component of all animal (not plant or bacterial) cell membranes that is required to maintain both membrane structural integrity and fluidity. Cholesterol enables animal cells to dispense with a cell wall to protect membrane integrity and cell viability, thus allowing them to change shape and move about (unlike bacteria and plant cells which are restricted by their cell walls).

Cholesterol functions as an insulating cover for the transmission of electrical impulses in the nervous tissue. Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxy group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty-acid chain of the other lipids.

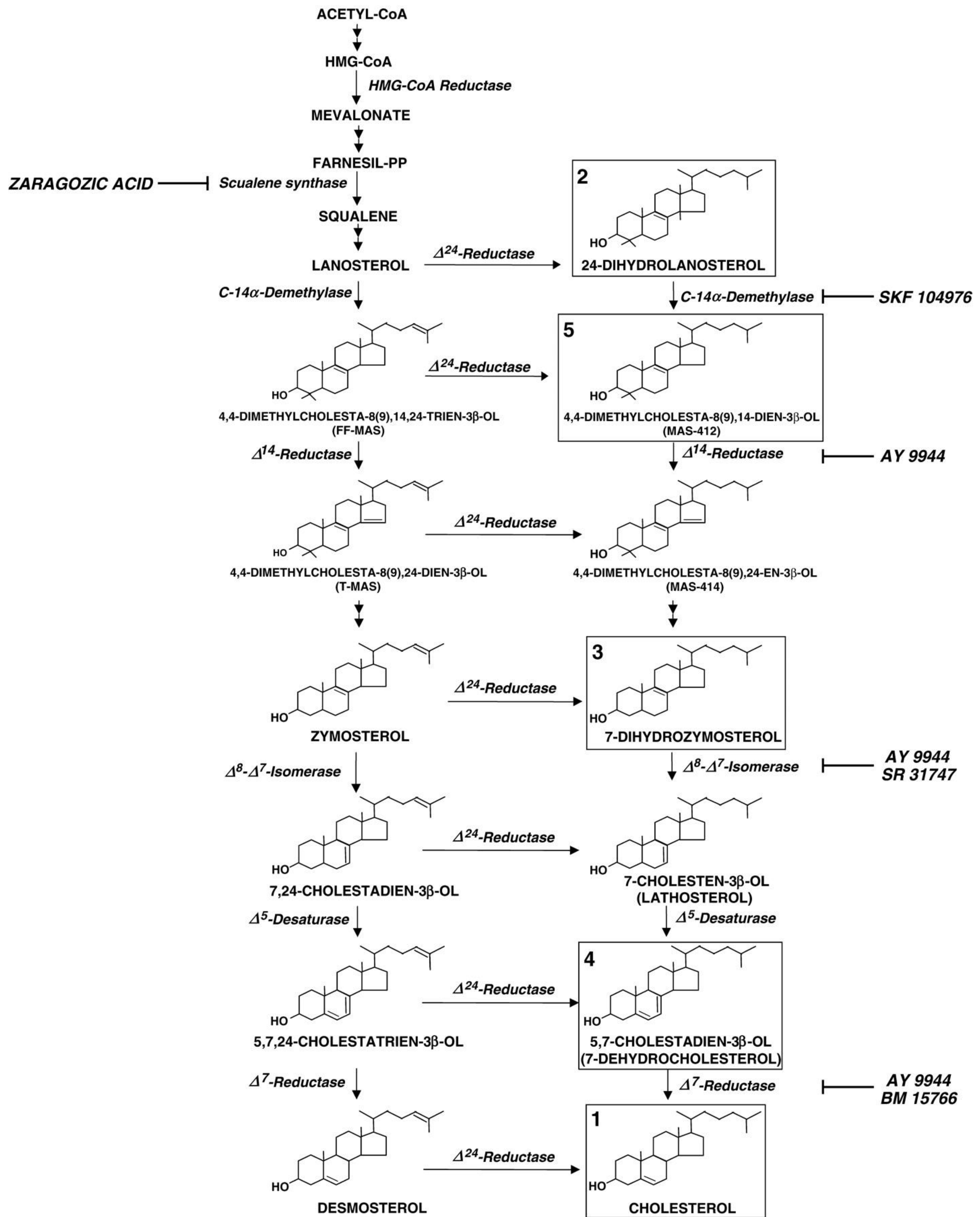
Dietery source:-

Animal fat are complex mixtures of triglycerides, with lesser amounts of phospholipids and cholesterol. As a consequence, all foods containing animal fat contain cholesterol to varying extents.[22] Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, fish, and shrimp.[23] Human breast milk also contains significant quantities of cholesterol.

Biosynthesis:-

All animal cells manufacture cholesterol for their use, with relative production rates varying by cell type and organ function. About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs. Synthesis within the body starts with the mevalonate pathway where two molecules of acetyl CoA condense to form acetoacetyl-CoA. This is followed by a second condensation between acetyl CoA and acetoacetyl-CoA to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA.

Mevalonate is finally converted to isopentenyl pyrophosphate (IPP) through two phosphorylation steps and one decarboxylation step that requires ATP. Three molecules of isopentenyl pyrophosphate condense to form farnesyl pyrophosphate through the action of geranyl transferase. Two molecules of farnesyl pyrophosphate then condense to formsqualene by the action of squalene synthase in the endoplasmic reticulum, Oxidosqualene cyclase then cyclizes squalene to form lanosterol. Finally, lanosterol is converted to cholesterol through a 19-step process.


ACETYL-CoA
HMG-CoA
HMG-CoA Reductase
MEVALONATE
FARNESIL-PP
ZARAGOZIC ACID
Scualene synthase
SQUALENE
LANOSTEROL
Δ24-Reductase
2
24-DIHYDROLANOSTEROL
C-14α-Demethylase
C-14α-Demethylase
SKF 104976
Δ24-Reductase
4,4-DIMETHYLCHOLESTA-8(9),14,24-TRIEN-3β-OL
(FF-MAS)
5
4,4-DIMETHYLCHOLESTA-8(9),14-DIEN-3β-OL
(MAS-412)
Δ14-Reductase
Δ14-Reductase
AY 9944
Δ24-Reductase
4,4-DIMETHYLCHOLESTA-8(9),24-DIEN-3β-OL
(T-MAS)
4,4-DIMETHYLCHOLESTA-8(9),24-EN-3β-OL
(MAS-414)
Δ24-Reductase
ZYMOSTEROL
3
7-DIHYDROZYMOSTEROL
Δ8-Δ7-Isomerase
Δ8-Δ7-Isomerase
AY 9944
SR 31747
Δ24-Reductase
7,24-CHOLESTADIEN-3β-OL
7-CHOLESTEN-3β-OL
(LATHOSTEROL)
Δ5-Desaturase
Δ5-Desaturase
Δ24-Reductase
5,7,24-CHOLESTATRIEN-3β-OL
4
5,7-CHOLESTADIEN-3β-OL
(7-DEHYDROCHOLESTEROL)
Δ7-Reductase
Δ7-Reductase
AY 9944
BM 15766
Δ24-Reductase
DESMOSTEROL
1
CHOLESTEROL
HO

Regulation of carbohydrate metabolism:-

Carbohydrate metabolism denotes the various biochemical processes responsible for the formation, breakdown and interconversion of carbohydrates in living organisms.

The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. Glucose and other carbohydrates are part of a wide variety of metabolic pathways across species: plants synthesize carbohydrates from carbon dioxide and water by photosynthesis storing the absorbed energy internally, often in the form of starch or lipids. Plant components are consumed by animals and fungi, and used as fuel for cellular respiration. Oxidation of one gram of carbohydrate yields approximately 4 kcal of energy, while the oxidation of one gram of lipids yields about 9 kcal. Energy obtained from metabolism (e.g., oxidation of glucose) is usually stored temporarily within cells in the form of ATP. Organisms capable of aerobic respiration metabolize glucose and oxygen to release energy with carbon dioxide and water as byproducts.

Carbohydrates can be chemically divided into complex and simple. Simple carbohydrates consist of single or double sugar units (monosaccharides and disaccharides, respectively). Sucrose or table sugar (a disaccharide) is a common example of a simple carbohydrate. Complex carbohydrates contain three or more sugar units linked in a chain, with most containing hundreds to thousands of sugar units. They are digested by enzymes to release the simple sugars. Starch, for example, is a polymer of glucose units and is typically broken down to glucose. Cellulose is also a polymer of glucose but it cannot be digested by most organisms. Bacteria that produce enzymes to digest cellulose live inside the gut of some mammals, such as cows, and when these mammals eat plants, the cellulose is broken down by the bacteria and some of it is released into the gut.

Doctors and scientists once believed that eating complex carbohydrates instead of sugars would help maintain lower blood glucose. Numerous studies suggest, however, that both sugars and starches produce an unpredictable range of glycemic and insulinemic responses. While some studies support a more rapid absorption of sugars relative to starches other studies reveal that many complex carbohydrates such as those found in bread, rice, and potatoes have glycemic indices similar to or higher than simple carbohydrates such as sucrose. Sucrose, for example, has a glycemic index lower than expected because the sucrose molecule is half fructose, which has little effect on blood glucose.[5] The value of classifying carbohydrates as simple or complex is questionable. The glycemic index is a better predictor of a carbohydrate's effect on blood glucose.

Carbohydrates are a superior short-term fuel for organisms because they are simpler to metabolize than fats or those amino acids (components of proteins) that can be used for fuel. In animals, the most important carbohydrate is glucose. The concentration of glucose in the blood is

used as the main control for the central metabolic hormone, insulin. Starch, and cellulose in a few organisms (e.g., some animals (such as termites and some microorganisms (such as protists and bacteria)), both being glucose polymers, are disassembled during digestion and absorbed as glucose. Some simple carbohydrates have their own enzymatic oxidation pathways, as do only a few of the more complex carbohydrates. The disaccharide lactose, for instance, requires the enzyme lactase to be broken into its monosaccharide components; many animals lack this enzyme in adulthood.

Carbohydrates are typically stored as long polymers of glucose molecules with glycosidic bonds for structural support (e.g. chitin, cellulose) or for energy storage (e.g. glycogen,starch). However, the strong affinity of most carbohydrates for water makes storage of large quantities of carbohydrates inefficient due to the large molecular weight of the solvated water-carbohydrate complex. In most organisms, excess carbohydrates are regularly catabolised to form acetyl-CoA, which is a feed stock for the fatty acid synthesispathway; fatty acids, triglycerides, and other lipids are commonly used for long-term energy storage. The hydrophobic character of lipids makes them a much more compact form of energy storage than hydrophilic carbohydrates. However, animals, including humans, lack the necessary enzymatic machinery and so do not synthesize glucose from lipids, though glycerol can be converted to glucose.

All carbohydrates share a general formula of approximately $C_nH_{2n}O_n$; glucose is $C_6H_{12}O_6$. Monosaccharides may be chemically bonded together to form disaccharides such assucrose and longer polysaccharides such as starch and cellulose.

Metabolic pathway:-

- Carbon fixation, or photosynthesis, in which CO_2 is reduced to carbohydrate.
- Glycolysis - the oxidation metabolism of glucose molecules to obtain ATP and pyruvate
  - Pyruvate from glycolysis enters the Krebs cycle, also known as the citric acid cycle, in aerobic organisms after moving through pyruvate dehydrogenase complex.
- The pentose phosphate pathway, which acts in the conversion of hexoses into pentoses and in NADPH regeneration, NADPH is an essential antioxidant in cells which prevents oxidative damage and acts as precursor for production of many biomolecules.
- Glycogenesis - the conversion of excess glucose into glycogen as a cellular storage mechanism; this prevents excessive osmotic pressure buildup inside the cell
- Glycogenolysis - the breakdown of glycogen into glucose, which provides a glucose supply for glucose-dependent tissues.
- Gluconeogenesis - de novo synthesis of glucose molecules from simple organic compounds. An example in humans is the conversion of a few amino acids in cellular protein to glucose.

Glucoregulation :- is the maintenance of steady levels of glucose in the body; it is part of homeostasis, and so keeps a constant internal environment around cells in the body.

- The hormone insulin is the primary regulatory signal in animals, suggesting that the basic mechanism is very old and very central to animal life. When present, it causes many tissue cells to take up glucose from the circulation, causes some cells to store glucose internally in the form of glycogen, causes some cells to take in and hold lipids, and in many cases controls cellular electrolyte balances and amino acid uptake as well. Its absence turns off glucose uptake into cells, reverses electrolyte adjustments, begins glycogen breakdown and glucose release into the circulation by some cells, begins lipid release from lipid storage cells, etc. The level of circulatory glucose (known informally as "blood sugar") is the most important signal to the insulin-producing cells. Because the level of circulatory glucose is largely determined by the intake of dietary carbohydrates, diet controls major aspects of metabolism via insulin. In humans, insulin is made by beta cells in the pancreas, fat is stored in adipose tissue cells, and glycogen is both stored and released as needed by liver cells. Regardless of insulin levels, no glucose is released to the blood from internal glycogen stores from muscle cell.

Role of sugar in nucleotide biosynthesis:-

Nucleotide sugars are the activated forms of monosaccharides. Nucleotide sugars act as glycosyl donors in glycosylation reactions. Those reactions are catalyzed by a group of enzymes called glycosyltransferases.

Biological importance & energetics :-

To act as glycosyl donors, those monosaccharides should exist in a highly energetic form. This occurs as a result of a reaction between nucleoside triphosphate (NTP) and glycosyl monophosphate (phosphate at anomeric carbon). The recent discovery of the reversibility of many glycosyltransferase-catalyzed reactions calls into question the designation of sugar nucleotides as 'activated' donors.

There are nine sugar nucleotides in humans which act as glycosyl donors and they can be classified depending on the type of the nucleoside forming them:[7]

- Uridine Diphosphate: UDP-α-D-Glc, UDP-α-D-Gal, UDP-α-D-GalNAc, UDP-α-D-GlcNAc, UDP-α-D-GlcA, UDP-α-D-Xyl

- Guanine Diphosphate:
- Cytosine Monophosphate it is the only nucleotide sugar in the form of nucleotide monophosphate.

In other forms of life many other sugars are used and various donors are utilized for them. All five of the common nucleosides are used as a base for a nucleotide sugar donor somewhere in nature. As examples, CDP-glucose and TDP-glucose give rise to various other forms of CDP and TDP-sugar donor nucleotides.

Normal metabolism of nucleotide sugars is very important. Any malfunction in any contributing enzyme will lead to a certain disease for example:

1. Inclusion body myopathy: is a congenital disease resulted from altered function of UDP-Glu epimerase .
2. Macular corneal dystrophy: is a congenital disease resulted from malfunction of Glc 6-sulfotransferase.
3. Congenital disorder in α-1,3 mannosyl transferase will result in a variety of clinical symptoms, e.g. hypotonia, psychomotor retardation, liver fibrosis and various feeding problems.

4. may be created as a result of the metabolism of synthetic triglycerides, such as triheptanoin.

Ketone bodies

Ketone bodies are three water-soluble molecules (acetoacetate, beta-hydroxybutyrate, and their spontaneous breakdown product, acetone) that are produced by the liver from fatty acids[1] during periods of low food intake (fasting), carbohydrate restrictive diets, starvation, prolonged intense exercise,[2]or in untreated (or inadequately treated) type 1 diabetes mellitus. These ketone bodies are readily picked up by the extra-hepatic tissues, and converted into acetyl-CoA which then enters the citric acid cycle and is oxidized in the mitochondria for energy.[3] In the brain, ketone bodies are also used to make acetyl-CoA into long-chain fatty acids. The latter cannot be obtained from the blood, because they cannot pass through the blood–brain barrier.

Ketone bodies are produced by the liver under the circumstances listed above (i.e. fasting, starving, low carbohydrate diets, prolonged exercise and untreated type 1 diabetes mellitus) as a result of intense gluconeogenesis, which is the production of glucose from non-carbohydrate sources (not including fatty acids).[1] They are therefore always released into the blood by the liver together with newly produced glucose, after the liver glycogenstores have been depleted. (These glycogen stores are depleted after only 24 hours of fasting.)

Acetoacetate consists of two acetyl-CoA molecules (without their -CoAs, or coenzyme A) combined in tandem. Beta-hydroxybutyrate is a reduced form of acetoacetate, in which the ketone group is converted into an alcohol (or hydroxyl) group (see illustration on the right). Both are 4-carbon molecules, that can readily be converted back into acetyl-CoA by most tissues of the body, with the notable exception of the liver. Acetone is the decarboxylated form of acetoacetate which cannot be converted back into acetyl-CoA except via detoxification in the liver where it is converted into lactic acid, which can, in turn, be oxidized into pyruvic acid, and only then into acetyl-CoA.

Production:-

Ketone bodies have a characteristic smell, which can easily be detected in the breath of persons in ketosis and ketoacidosis. It is often described as fruity or like nail polish remover (which usually contains acetone or ethyl acetate).

Fats stored in adipose tissue are released from the fat cells into the blood as free fatty acids and glycerol when insulin levels are low and glucagon and epinephrine levels in the blood are high. This occurs between meals, during fasting, starvation and strenuous exercise, when blood glucose levels are likely to fall. Fatty acids are very high energy fuels, and are taken up by all metabolizing cells which have mitochondria. This is because fatty acids can only be metabolized in the mitochondria.[1][5] Red blood cells do not contain mitochondria and are therefore entirely dependent on glycolysis (the fermentation of glucose intolactic acid) for their energy requirements. In all other tissues the fatty acids that enter the metabolizing cells are combined with co-enzyme A to form acyl-CoA chains. These are transferred into the mitochondria of the cells, where they are broken down into acetyl-CoA units by a sequence of reactions known as β-oxidation

Apart from the three endogenous ketone bodies, acetone, acetoacetic acid, and beta-hydroxybutyric acid, other ketone bodies like beta-ketopentanoateand beta-hydroxypentanoate may be created as a result of the metabolism of synthetic triglycerides.

Uses in heart , brain & muscle:-

Ketone bodies can be used for energy. Ketone bodies are transported from the liver to other tissues, where acetoacetate and beta-hydroxybutyrate can be reconverted to acetyl-CoA to produce energy, via the citric acid cycle. Ketone bodies cannot be used by the liver for energy, because the liver lacks the enzyme β-ketoacyl-CoA transferase, also called thiophorase.

The heart preferentially utilizes fatty acids for energy under normal physiologic conditions. However, under ketotic conditions, the heart can effectively utilize ketone bodies for energy.

Ketosis:-

Ketosis is a metabolic state in which most of the body's energy supply comes from ketone bodies in the blood, in contrast to a state of glycolysis in which blood glucose provides most of the energy. Ketosis is generally better known to the laypeople as **acetone breath** - a common symptom of progressing diabetes mellitus type II advanced stage. It is characterised by serum concentrations of ketone bodies over 0.5 mM, with low and stable levels of insulin and blood glucose.[1][2] It is almost always generalized with **hyperketonemia**, that is, an elevated level of ketone bodies in the blood throughout the body. Ketone bodies are formed by ketogenesis when liver glycogen stores are depleted (or from metabolising medium-chain triglycerides. The main ketone bodies used for energy are acetoacetate and β-hydroxybutyrate, and the levels of ketone bodies are regulated mainly byinsulin and glucagon. Most cells in the body can use both glucose and ketone bodies for fuel, and during ketosis, free fatty acids and glucose synthesis (gluconeogenesis) fuel the remainder.

Ketoacidosis:-

Ketone bodies are acidic, but acid-base homeostasis in the blood is normally maintained through bicarbonate buffering, respiratory compensation to vary the amount of CO_2 in the bloodstream, hydrogen ion absorption by tissue proteins and bone, and renal compensation through increased excretion of dihydrogen phosphate and ammonium ions. Prolonged excess of ketone bodies can overwhelm normal compensatory mechanisms, leading to acidosis if blood pH falls below 7.35.

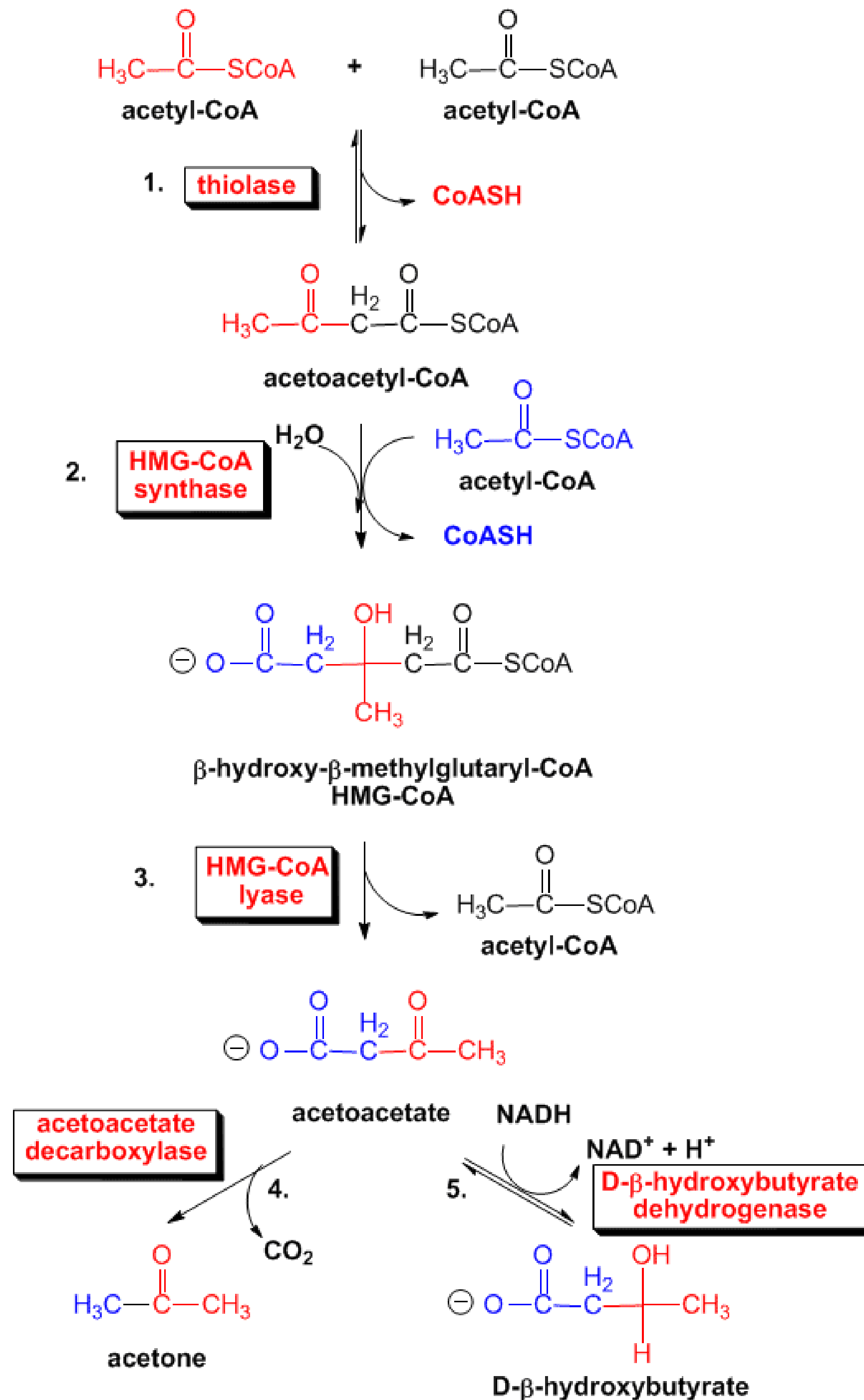
There are two major causes of ketoacidosis:

- Most commonly, ketoacidosis is diabetic ketoacidosis (DKA), resulting from increased fat metabolism due to a shortage of insulin. It is associated primarily with type I diabetes, and may result in a diabetic coma if left untreated.
- Alcoholic ketoacidosis (AKA) presents infrequently, but can occur with acute alcohol intoxication, most often following a binge in alcoholics with acute or chronic liver or pancreatic disorders. Alcoholic ketoacidosis occurs more frequently following methanol or ethylene glycol intoxication than following intoxication with uncontaminatedethanol.

Mechanism:-

Fats stored in adipose tissue are released from the fat cells into the blood as free fatty acids and glycerol when insulin levels are low and glucagon and epinephrine levels in the blood are high. This occurs between meals, during fasting, starvation and strenuous exercise, when blood glucose levels are likely to fall. Fatty acids are very high energy fuels, and are taken up by all metabolizing cells which have mitochondria. Fatty acids can only be metabolized in the mitochondria. Red blood cells do not contain mitochondria and are therefore entirely dependent

on glycolysis (the fermentation of glucose into lactic acid) for their energy requirements. The cells of the central nervous system, which although they do have mitochondria, can also not utilize fatty acids as these molecules cannot cross the blood brain barrier into the interstitial fluids that bathe these cells. In all other tissues the fatty acids that enter the metabolizing cells are combined with co-enzyme A to form acyl-CoA chain.



Respiratory chain, its role in energy capture & control:-

Electron transport chain of compounds that transfer electrons from electrondonors toelectron acceptors via redox reactions, and couples this electron transfer with the transfer of protons (H^+ions) across a membrane. This creates an electrochemical proton gradient that drives the synthesis ofadenosine triphosphate (ATP), a molecule that stores energy chemically in the form of highly strained bonds. The molecules of the chain include peptides, enzymes (which are proteins or protein complexes), and others. The final acceptor of electrons in the electron transport chain during aerobic respiration is molecular oxygenalthough a variety of acceptors other than oxygen exist in anaerobic respiration.

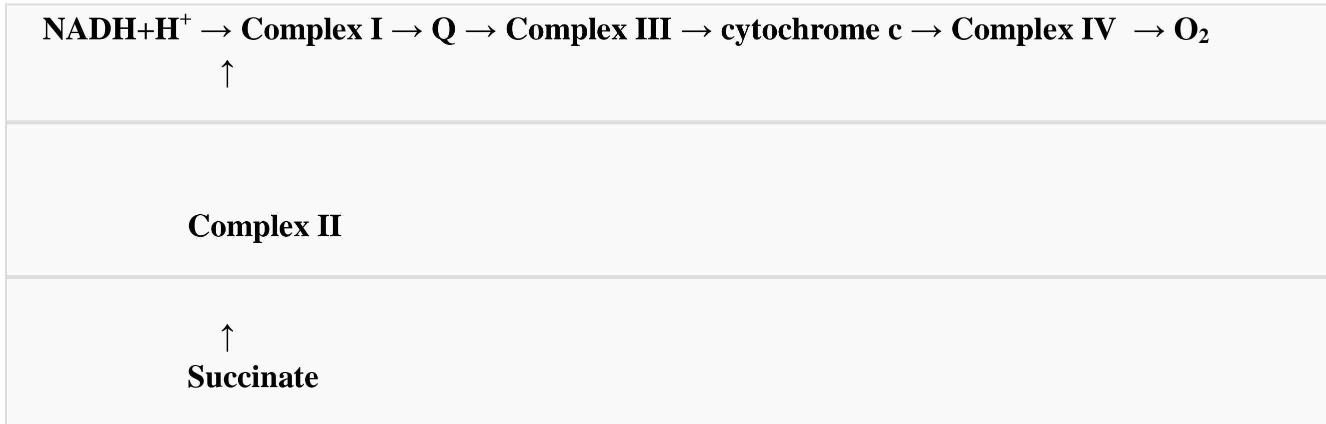
Electron transport chains are used for extracting energy via redox reactions from sunlight in photosynthesisor, such as in the case of the oxidation of sugars, cellular respiration. In eukaryotes, an important electron transport chain is found in the inner mitochondrial membrane where it serves as the site of oxidative phosphorylation through the use of ATP synthase. It is also found in the thylakoid membrane of the chloroplast in photosynthetic eukaryotes. In bacteria, the electron transport chain is located in their cell membrane.

In chloroplasts, light drives the conversion of water to oxygen and $NADP^+$ to NADPH with transfer of H^+ ions across chloroplast membranes. In mitochondria, it is the conversion of oxygen to water, NADH to NAD^+ andsuccinate to fumarate that are required to generate the proton gradient.

Electron transport chains are major sites of premature electron leakage to oxygen, generating superoxideand potentially resulting in increased oxidative stress.

Respiratory chain in mitochondria:-

Most eukaryotic cells have mitochondria, which produce ATP from products of the citric acid cycle, fatty acid oxidation, and amino acid oxidation. At the mitochondrial inner membrane, electrons from NADH and succinate pass through the electron transport chain to oxygen, which is reduced to water. The electron transport chain comprises anenzymatic series of electron donors and acceptors. Each electron donor passes electrons to a more electronegative acceptor, which in turn donates these electrons to another acceptor, a process that continues down the series until electrons are passed to oxygen, the most electronegative and terminal electron acceptor in the chain. Passage of electrons between donor and acceptor releases energy, which is used to generate a proton gradient across the mitochondrial membrane by actively "pumping" protons into the intermembrane space, producing a thermodynamic state that has the potential to do work. The entire process is called oxidative phosphorylation, since ADP is phosphorylated to ATP using the energy of hydrogen oxidation in many steps.



Complex I

In Complex I (NADH:ubiquinone oxidoreductase, NADH-CoQ reductase, or NADH dehydrogenase; EC 1.6.5.3), two electrons are removed from NADH and transferred to a lipid-soluble carrier, ubiquinone (Q). The reduced product, ubiquinol (QH_2), freely diffuses within the membrane, and Complex I translocates four protons (H^+) across the membrane, thus producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of superoxide.

The pathway of electrons is as follows:

NADH is oxidized to NAD^+, by reducing Flavin mononucleotide to $FMNH_2$ in one two-electron step. $FMNH_2$ is then oxidized in two one-electron steps, through a semiquinoneintermediate. Each electron thus transfers from the $FMNH_2$ to an Fe-S cluster, from the Fe-S cluster to ubiquinone (Q). Transfer of the first electron results in the free-radical (semiquinone) form of Q, and transfer of the second electron reduces the semiquinone form to the ubiquinol form, QH_2. During this process, four protons are translocated from the mitochondrial matrix to the intermembrane space.

Complex II

In Complex II (succinate dehydrogenase or succinate-CoQ reductase; EC 1.3.5.1) additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q. Complex II consists of four protein subunits: SDHA, SDHB, SDHC, and SDHD. Other electron donors (e.g., fatty acids and glycerol 3-phosphate) also direct electrons into Q (via FAD). Complex 2 is a parallel electron transport pathway to complex 1, but

unlike complex 1, no protons are transported to the intermembrane space in this pathway. Therefore, the pathway through complex 2 contributes less energy to the overall electron transport chain process.

Complex III

In Complex III (cytochrome bc_1 complex or CoQH_2-cytochrome c reductase; EC 1.10.2.2), the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH_2 at the Q_O site and sequentially transferred to two molecules of cytochrome c, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Q_i site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by one quinol (2H+2e-) oxidations at the Q_o site to form one quinol (2H+2e-) at the Q_i site. (in total four protons are translocated: two protons reduce quinone to quinol and two protons are released from two ubiquinol molecules).

Complex IV

In Complex IV (cytochrome c oxidase; EC 1.9.3.1), sometimes called cytochrome A3, four electrons are removed from four molecules of cytochrome c and transferred to molecular oxygen (O_2), producing two molecules of water. At the same time, eight protons are removed from the mitochondrial matrix (although only four are translocated across the membrane), contributing to the proton gradient. The activity of cytochrome c oxidase is inhibited by cyanide.

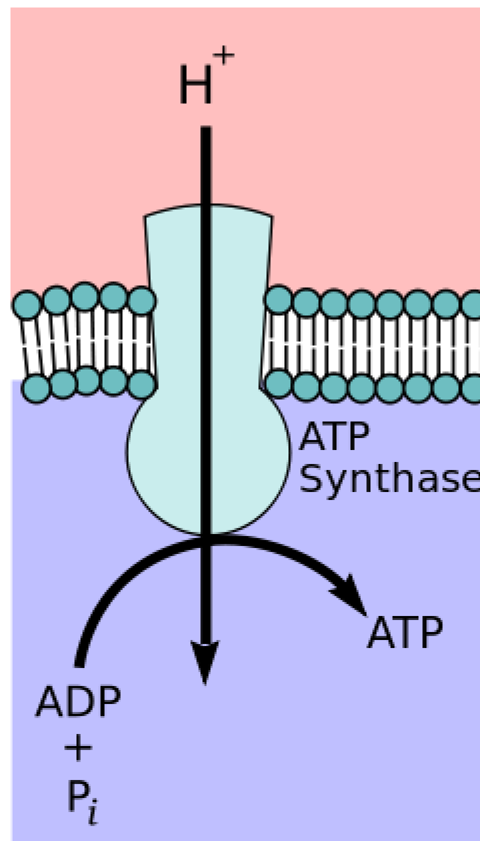
Oxidative phosphorylation:-

Oxidative phosphorylation (or OXPHOS in short) is the metabolic pathway in which cells use enzymes tooxidize nutrients, thereby releasing energy which is used to reform ATP. In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternativefermentation processes such as anaerobic glycolysis.

During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. Ineukaryotes, these redox reactions are carried out by a series of protein complexes within the inner membrane of the cell's mitochondria, whereas, in prokaryotes, these proteins are located in the cells' intermembrane space. These linked sets of proteins are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.

Coupling and oxidative phosphorylation:-

According to the chemiosmotic coupling hypothesis, proposed by Nobel Prize in Chemistry winner Peter D. Mitchell, the electron transport chain and oxidative phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane. The efflux of protons from the mitochondrial matrix creates an electrochemical gradient (proton gradient). This gradient is used by the F_OF_1 ATP synthase complex to make ATP via oxidative phosphorylation. ATP synthase is sometimes described as Complex V of the electron transport chain. The F_O component of ATP synthase acts as an ion channel that provides for a proton flux back into the mitochondrial matrix. This reflux releases free energy produced during the generation of the oxidized forms of the electron carriers (NAD^+ and Q). The free energy is used to drive ATP synthesis, catalyzed by the F_1 component of the complex. Coupling with oxidative phosphorylation is a key step for ATP production. However, in specific cases, uncoupling the two processes may be biologically useful. The uncoupling protein, thermogenin—present in the inner mitochondrial membrane of brown adipose tissue—provides for an alternative flow of protons back to the inner mitochondrial matrix. This alternative flow results in thermogenesisrather than ATP production. Synthetic uncouplers (e.g., 2,4-dinitrophenol) also exist, and, at high doses, are lethal.



Summary:-

In the mitochondrial electron transport chain electrons move from an electron donor (NADH or QH_2) to a terminal electron acceptor (O_2) via a series of redox reactions. These reactions are coupled to the creation of a proton gradient across the mitochondrial inner membrane. There are three proton pumps: I, III, and IV. The resulting transmembrane proton gradient is used to make ATP via ATP synthase.

The reactions catalyzed by Complex I and Complex III work roughly at equilibrium. This means that these reactions are readily reversible, by increasing the concentration of the products relative to the concentration of the reactants (for example, by increasing the proton gradient). ATP synthase is also readily reversible.