

College of Engineering, Pune

(An Autonomous Institute of Government of Maharashtra)

Applied Science Department

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CT16002 – Biology for Engineers

UNIT I: Biomolecules and Biopolymers

Structure and Function: Organic and inorganic molecules; Unique Properties of Carbon; Carbohydrates, Amino Acids and proteins, Lipids, Nucleic Acids, Vitamins and Minerals; The Rise of Living Systems.

1) BIOPOLYMERS & MACROMOLECULES

A living organism's body is built of and run by thousands of different types of molecules. As these are made chiefly by the living organisms they are known as biomolecules. Biomolecules have distinct properties and functions responsible for their selection and continuation in the course of evolution.

Many of the small molecules with low molecular weight, simple structure and high solubility are known as **micromolecules** (or monomers e.g. water, mineral, simple sugars, nucleotide etc.) form the building units for larger **macromolecules** (or polymers e.g. protein, lipids etc.). The biomolecules are classified into organic and inorganic types based on their composition.

Thus, all cells are made up of biomolecules, these are organized in physico -chemical organizations and in isolation they do not have living characteristics. Biomolecules produce, maintain and perpetuate the living state and are continuously transformed i.e. synthesized and broken down.

Water, minerals and gases are important groups of inorganic bimolecules while lipids, carbohydrates, proteins and nucleic acids are the four important classes of organic compounds.

WATER

Physical & Chemical Properties of Water:

- Water is cohesive & adhesive
- Water has high specific heat
- Water has high thermal conductivity
- Water has high boiling point
- Water is good evaporative coolant
- Water has high freezing point and is less dense as a solid than liquid

In the biological reactions, two important features are observed,

- **polarity** (+ ve charge for H and – ve for O extend polarity to water molecule; water molecules form cluster around electrically charged molecules like PO_4 or COOH , that are

water soluble hence known as **hydrophilic** while water does not react with non charged molecules like lipids that are insoluble known as **hydrophobic**) and

- **ionization ability** (water molecule dissociates to form H and OH ions)

Significance of water in living system

- Life has doubtless origin from the water.
- Water is the most abundant substance in living system making up more than 70% of the weight for most of the living organisms.
- Water provides liquid medium for colloidal protoplasm for chemical reactions and transport mechanism in the cell.
- The water molecule and its ionization products, H and OH influence the structure, properties and self assembly of all cellular components.
- Aqueous solutions of weak acids & bases with their salts act as buffer in pH change in biological system. It facilitates chemical reactions in the cells.
- The non covalent interactions responsible for the strength & specificity of biomolecules are decisively influenced by the solvent property of water. It is known as Universal solvent for most of the organic & inorganic molecules.
- It absorbs heat and maintains body temperature.
- In green plants, it is a source for H + ve ions as a source of energy.
- Removal of waste material thus helps in maintaining **homeostasis**

MINERALS

Minerals are the nutrients required especially for the growth of plants that are absorbed from the soil. Some of these minerals are required in larger quantity and some in trace levels for the plant growth. Accordingly they are known as **micro** or **macronutrients** respectively. The role of some minerals in the cell metabolism is as follows,

Mineral	Function	Mineral	Function
N, S	Synthesis of Amino acids, Proteins	P	Present in compounds like phospholipids, ATP, nucleotides etc.
K, Na	Constituents of Body fluids, nerve cells, blood plasma	Ca	Plays significant role in Blood coagulation & cell wall formation, propulsion of nerve impulses
Fe	Formation of haemoglobin	Mg	Formation of chlorophyll, enzymes, structural integrity of ribosomes
I	Functioning of thyroid glands	Cu, Mo	Activation of enzymes

Ions are required to maintain osmotic concentration of cellular as well as extra cellular fluids.

Gasses are significant for the basic cellular processes.

Gas	Function
O ₂	Essential for respiration for all aerobic bacteria, combustion process, photosynthesis byproduct
N ₂	Constituents of proteins, nucleic acid, fixation & release of nitrogen by bacteria for plants
CO ₂	Used in photosynthesis, excess is dissolved in water

Carbohydrates –These are hydrates of carbon made up of C, H, O

Reducing sugars – Sugars with free aldehyde / ketone group

Non- reducing sugars- e.g. aldehyde region of glucose reacting with ketone region of fructose – form glycosidic bond – non – reducing sugar as free aldehyde / ketone groups are masked.

Aldoses: Glucose, Ribose, Deoxiribose, Mannose, Galactose etc.

Ketoses: Fructose, Ribulose, Xylulose etc.

According to number of monomers present in carbohydrate molecule

Monosaccharide: Water soluble

- Trioses (Dihydroxy acetone, glyceraldehydes)
- Tetroses (Threose, Erythrose)
- Pentoses (Ribose, Deoxyribose, Xylose, Ribulose, Arabinose)
- Hexoses (Glucose – also called blood sugar, grape sugar and Dextrose can be polymerized into glycogen in animals and starch in plants; Fructose – Fruit sugar; Galactose, Mannose)
- Heptose (Sedoheptulose)

Oligosaccharides : 2-9 monomers

- Disccharides (Maltose, Sucrose, lactose etc)
- Trisaccharides (Raphinose, Pectin, Inulin)
- Polysaccharides (Starch, Cellulose, Glycogen, Chitin, Agar)

Homo-polymers: All the monomers same in given polysaccharides (Starch, Hemicellulose, Cellulose, Glycogen)

Hetero-polymers: Two or more monomers in given polysaccharides (Agar, Chitin)

Monomers are linked by glycosidic bond during polymerization

Types & Function of Polysaccharides:

Storage polysaccharides: *Starch, inulin* stored in roots, tubers of plants; *Glycogen*: In animals and bacteria

Inulin is the smallest polysaccharide: not metabolized in human body filtered through kidney. ————— used in kidney testing.

Structural polysaccharides: *Cellulose, Hemicellulose, Pectin* – (in plants), Chitin (plant fibres & animal exoskeleton like insects, spiders, crabs etc.)

Chondrin sulphate in cartilage, tendon ligament

Hyaluronic acid – (glucoronic a.+ acetyl glucosamine) cementing subs. between animal cells. In diff body fluid – vitreous humor of eye,

sinusoidal fluid CSF e.g. *Keratan Sulphate* in cornea, skin, cartilage, bone, hair, nail

Mucopolysaccharide – slimy substances e. g. *Hyaluronic acid*

Agar – used in culture media, medicine, capsules and chromatography

Algin –used in Ice creams, cosmetics.

Carrageenin –used as a emulsifier, clearing agent –fruit juice.

Funori –used as adhesive in hair curling

Heparin –used in blood bank as blood anti -coagulant

Husk of *Plantago ovata* –used as purgative / laxative

Aloe gel – used as inflammation - relief, in hand lotion, shampoo, hair conditioner, sunscreen lotion.

PROTEINS:

Proteins make up more than 50 % of the dry mass of animals and bacteria and perform important functions in living organisms. They contain the elements carbon, oxygen, hydrogen, nitrogen and usually sulfur that makes a monomer of protein i.e. amino acid. All organisms contain 20 common amino acids as biological molecules.

Essential amino acids: cannot be synthesized by animals, so must be taken in diet. In man such amino acids are 8, in other animals are 7.

Non-Essential Amino acids: Can be synthesized by animals, so may not be taken in diet
Each amino acid (AA) has a carboxyl group (-COOH), amino group (-NH₂) and a hydrogen atom bounded to a central carbon atom. The sequence of amino acids (linked by peptide bond) determines the overall shape and properties of proteins. Depending on number of amino acids in a chain oligopeptide (1 -10 AA), Polypeptides (11 -50 AA) and protein (>50 AA).

Various categories made for the classification of proteins based on the composition, structure etc. are as follows;

Structural organization of proteins:

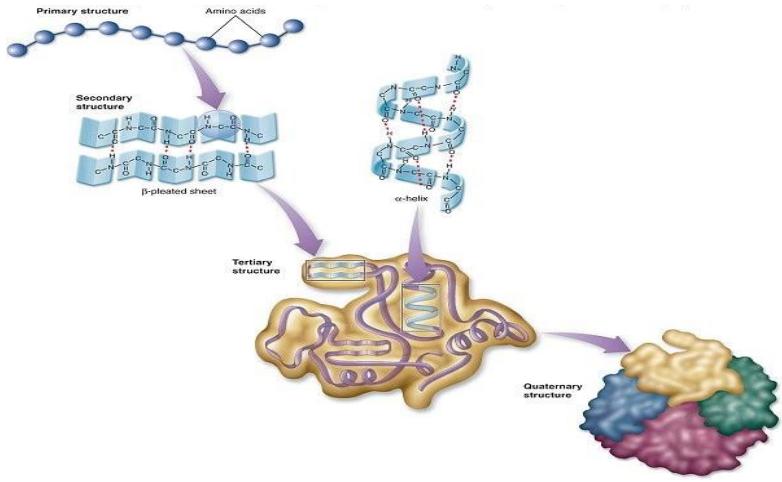
Primary Proteins: two dimensional, simple chain of AA with peptide (covalent) bond e.g. Insulin

Secondary Proteins: Various functional groups exposed on outer surface interact with hydrogen bonds

- α-helix – e.g. keratin, hair, fur, claws, hooves
- β- pleated – B. keratin of feathers, silk fibroin
- Collagen helix – 3 α-helices coiled around one another

Tertiary Proteins: Additional bonds between functional groups, twisting of secondary protein, weak covalent and high energy disulphide bonds are formed e.g. Myoglobin

Quaternary Proteins: Formed as a result of 2 -more polypeptide chain and have specific orientation



Types of proteins according to structure:

Fibrous – collagen fibres, keratin, elastin, fibrin, fibroin, actin, myosin, bl. clot.

Globular – glutelin, protamine, globulin, albumin, glutenin, orygemin.

Intermediate – (myosin), fibrinogen.

Types of proteins according to chemical nature

Simple – only a.a. Albumin, globulin, protamine, fish, prolamine (corn, pl, wheat), histone (corn, wheat), glutelin (glutenin), keratin.

Conjugated – protein + non protein (prosthetic group) e.g. **Nucleoprotein** (nucleic acid), **chromoprotein** (Hb, cytochrome), **metallo** (with metals Zn, Fe), **lipoprotein**, **glycoprotein** etc.

Properties of proteins:

- Number: According to length,, number & types of polypeptides – thousands of proteins
- Specificity: High specificity in the individual but shared with related species or group
- Molecular weight – ACTH (4500 daltons) to Pyruvate Dehydrogenase (4,600,000 daltons)
- Solubility: Some are insoluble due to large size, many form colloidal solution with water
- Amphoteric nature: Show both acidic & basic properties.
- Electrical reaction: Isoelectric point at which pH is neutral (Curdling of milk at pH 4.7 due to isoelectric point at acidic pH 4.7)

- Denaturation: Permanent or temporary loss of three dimensional structure caused due to UV, heat, strong acid & alkali, high salt concentration; within limit renaturation occur.

Role of protein:

Type of protein	Example	Function
Enzymes	Amylase	Converts starch into sugar
Structural	Keratin, Collagen	Hair, wool, nail, horn, hoofs, tendons, cartilage
	Haemoglobin	Blood clotting
Hormones	Insulin, glucagons	Regulate glucose metabolism
Contractile	Actin, myosin	Contractile filaments in muscle, cilia & flagella (in lower organisms)
Amphoteric	All proteins	Maintain acid-base equilibrium
Storage	Ferritin, albumin Casin	Stores iron in spleen & egg yolk Milk
Transport	Haemoglobin Serum albumin	Carried oxygen in blood Carries fatty acid in blood
Energy	All proteins	Provides energy stored in peptide bonds
Metaloprotein	Cytochrome	Electron transport
Receptor	Adrenalin	Conduction of nerve stimulus
Nucleoprotein	Histones & non-histone	Stabilization of DNA coiling
Immunological	Antibodies	Forms complexes with foreign proteins
Toxins	Venum (Neurotoxin)	Blocks the nerve function

Proteins are masterpieces of molecular engineering and they are tailored to their functions by millions of years of natural selection.

LIPIDS:

Lipids are the organic compounds that share a distinguishing property of non polarity and so do not dissolve in water. They mostly contain carbon and hydrogen with very small portion of oxygen compare to carbohydrates. Some of them also incorporate phosphorus and nitrogen. Basically they are polymers of fatty acids & glycerol.

As lipids are insoluble in water they are vital components of the membrane that separate living cells from each other and their surrounding.

Lipids offer unique way to store energy as they possess very high proportion of energy rich carbon-hydrogen bonds in a concentrated form within the cells. They contain six times more energy than the carbohydrates and have become increasingly important as food reserves for organisms. (e.g. migratory birds).

Fatty Acids: Simplest form of lipids consisting of a long hydrocarbon chain (non polar hydrophobic) with a carboxyl group at the end (which is hydrophilic). Because of this characteristic orientation, fatty acids significantly contribute in the structure of cell wall.

Fats & Oils: These are the energy store reserves for the plant & animal cells. Fats are formed by the condensation of fatty acid molecules and are characteristically non polar. They are classified into **saturated** (butter, coconut oil) which are solid at room temperature and without double bond and **unsaturated** (from olive, corn, safflower, peanut etc.) which are liquid at room temperature and with double bond. Usually, animals use saturated fatty acids against the plants with unsaturated fatty acids.

Phospholipids: These are similar to fats except one or two fatty acids are replaced by phosphate group which in turn are linked to nitrogen containing group.

Steroids: They differ from lipids in structure but insoluble in water. Cholesterol is most commonly known steroid forming essential component of animal cell membrane. It also served as a raw material for the production of vitamin D and steroid hormones.

In general the steroids carry chemical messages between the cells.

Properties:

- Saturated & unsaturated
- Insoluble in water and soluble in organic solvents like alcohol
- Low specific gravity hence float on water

- On hydrolysis give fatty acids and glycerol
 - Neutral fats or triglycerides are colour less, odourless, tasteless
 - Rancidity: Naturally occurring unsaturated fats undergo partial hydrolysis by the action of enzyme lipase. Oxidation at double bond produces aldehydes and carboxylic acids.
- This develops foul smell and odour to the fats. **Types of Lipids –**

1. Simple Lipids – These are neutral or true fats. Solid at room temperature, on hydrolysis give three fatty acids and one glycerol e.g. waxes

R C O R ← esters of fatty acids with different alcohols.

e.g. tripalmitin, dipalmitin are hard fats, solid at room temp.

2. Compound / Conjugated lipids –

Phospholipids – Cephalin – act as insulation for nerves

Lecithin – cell permeability

Glycolipids – Cerebrosides – brain cells – cell mem. gangliosides – grey matter.

Sphingomyelins – in myelin sheath.

Sphingosine → amino alcohol.

Lipoproteins – found in milk, egg yolk, blood plasma, tissues, cell surfaces.

Cutin – from cuticle.

Suberin – due to its cell wall impermeable to H₂O.

Chromolipids – e.g. carotenoids.

3. Derived Lipids – Formed from hydrolysis of simple & comp. lipids, include f.a., steroids, prostaglandins, terpenes.

Prostaglandins – Hormone – like unsaturated fatty acids / local hormones, present in amniotic and tissue fluid

- Circulate in blood
- Cause acid production in stomach
- Stimulate contraction of smooth muscles.

Steroids – solid wax like alcohols e.g. ergosterol – yeast. Cholesterol - animal cell mem., blood, bile. When bl. level of chole. rises – Cholesterol and its esters form bond with fats secreted by endothelium of arteries. And thus deposited on wall of arteries.

It is precursor for hormone progesterone, testosterone, cortisol, estradiol, androsteron
Produces bile salts, vitamin D by action o f U V rays of sunlight.

- React with protein in nucleus
- Trigger changes in gene expression and metabolism

Role of lipids:

- Reserved food: In plants oilseeds like groundnuts, mustard, coconut are the stores of fats. Animals contain adipocytes which are the cells containing the fat droplets as stored food.
- Structural component: Phospholipids, glycolipids and sterols are the structural components of the cell membranes.
- Synthesis: Take part in the synthesis of steroids, hormones, Vit D etc.
- Energy source: Rich source of energy. 9.3 kcal/gram
- Insulation: Provide electrical and thermal insulation. Deposited below the skin and around the internal organs to lessen the heat loss. Also work as shock absorbers.
- Solvent: Fats are the solvents for fat soluble vitamins like A, D, K, E.
- Waxes are water proof agents e.g. fur, feathers, insect exoskeleton, bee wax, ear wax (cerumen), skin wax (sebum), paraffin wax & plant waxes

NUCLEIC ACIDS :

1st reported by Friedrich Miescher. from pus cells nuclei. Called them nuclein. Altman called N.A. Feulgen developed staining tech. of N.A. with fusch.

DNA – Deoxyribo nucleic acid

Made up of three components –

i) Deoxyribose sugar – (pentagonal shape with 5 C atoms)

ii) Nitrogen containing bases –

Purine – Adenine (A), Guanine (G).

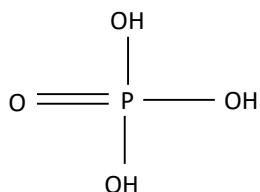
Pyrimidine – Cytosine (C), Thymine (T)

Pentose sugar + N base □□□ nucleoside

Glycosidic bond between 1st C of sugar and nitrogen at 3rd position in pyrimidine base and 9th position in purine base.

iii)) Phosphoric acid –

OH – 3 acid groups.

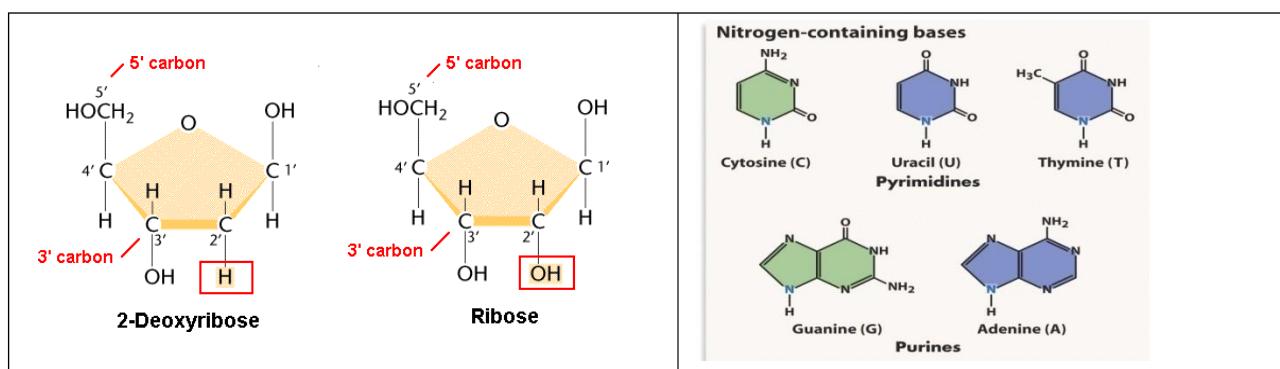


Nucleoside + P group at 5' position by **phosphor-diester bond**.

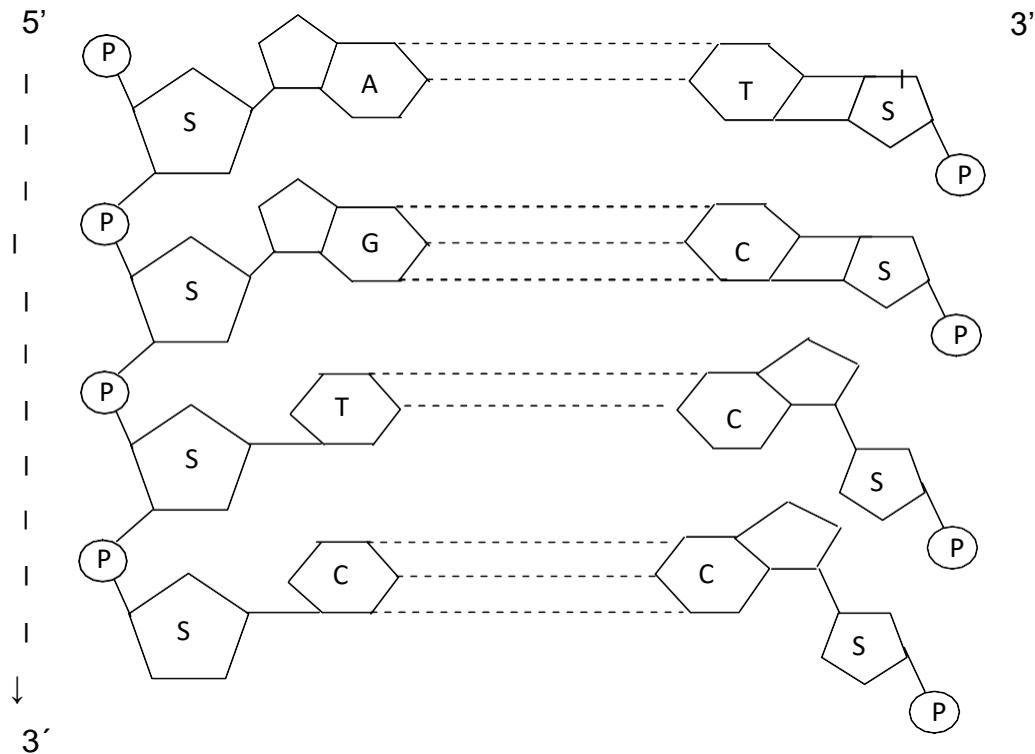
Nucleoside + Phosphate group □□□ nucleotide.

Amount of DNA measured by picogram = 10^{-12} g., 1 Pg DNA has 31 cm length.

Human cell – contains 5.6 Pg DNA – 174 cm long.



Chain of nucleotides – **poly nucleotide chain**

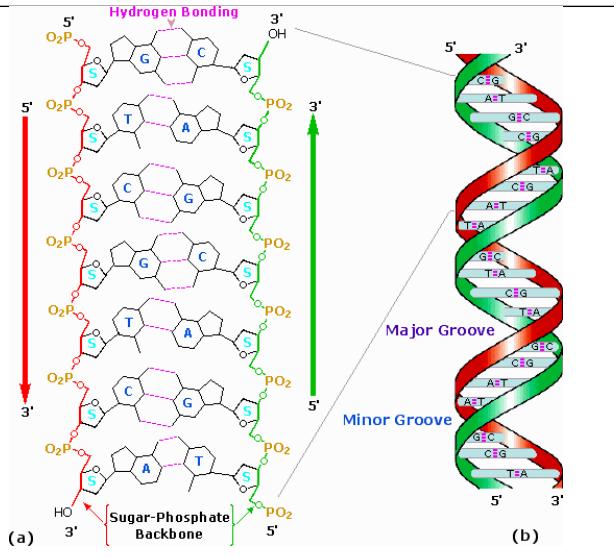


Characteristics / Properties – DNA

- It has several thousand Nucleotides.
- Back bone of it by alternate d sugar and PO_4 gr.
- Nitrogen bases are inside at right angle to longitudinal axis.
- PO_4 gr. Attached 5th, 3rd C atom.
- By phosphodiester bond.
- 2 chains joined by weak H bond – A = T, G = C specific pairing. H of one base linked to O₂ / N₂ of another base.
- 2 strands anti parallel i.e. 3', 5' phosphor ----- link in opp. direction.
- Pairing specific — 2 chain complementary.
i.e. sequence of N₂ bases in one chain will decide it on other chain.
- Diameter of DNA – 20 \AA

- **Erwin Chargaff's rule** – regardless of source - purine, pyrimidine components occur in equal amounts in a DNA mole.

- 1) A = T, G = C from this it is also seen
- 2) $\frac{A}{T} = \frac{C}{G} = 1$
- 3) A + G = T + C
- 4) A + C = G + T but
- 5) A + T not always G + C necessarily.



James Watson & Francis Crick – suggested three dimensional molecular model based on X ray crystallography technique; according to this model DNA comprises of

- 1) 2 right Handed helices.
- 2) Each turn has – 10 nitrogen base pairs
- 3) One spiral each 3.4 \AA°
- 4) Distance between 2 nitrogen bases 3.4 \AA°

Denaturation and Renaturation of DNA –

- 1) If DNA solution heated / exposed to alkaline PH or acidic PH, H bonds break and 2 strands uncoil this is known as **denaturation** or **DNA melting**.
- 2) If above solution gradually cooled / neutralized – new base pair formation begins, it becomes thermally / chemically stable finally double stranded DNA formed which is called as **renaturation**.

Linear DNA with ends free with histones (eukaryotes) and circular r DNA 2 ends covalently linked **without histones** (prokaryots).

Repetitive DNA –

- The part of DNA which contains same sequence of N bases repeated several times in tandem (one behind another)
e.g. A A T C G G A A T C G G A A T C G G
- It occurs specifically near telomeres (ends), centromeres,
- Area with long sequence of repetitive DNA is called satellite DNA as it separates out during density gradient ultra centrifugation.
- Microsatellite DNA—1–10 base pairs repeat units
Minisatellite DNA—11 – 60 base pairs repeat units, it is hypervariable (it is known as VNTR variable Number of Tandom Repeats discovered by Jeffreys et al., specific for each individual therefore used in DNA finger printing.)

Palindromic DNA –

DNA duplex has areas with sequence of nucleotides same reading forward or backward from central axis of symmetry G A C T G C G T C A G

↑————→
AND MADAM DNA

(Restriction endo -nuclease commonly recognize DNA sequences that are palindromes.

RNA – Ribo Nucleic Acid –

It is also made up of three components;

- i) Ribose sugar – Pentose sugar
- ii) Nitrogen containing bases – **Purine** – Adenine, Guanine & **Pyrimidine** – Cytosine, Uracil.

Sugar + N. B. —→ Nucleoside

Genetic in some pl. viruses TMV yellow MV animal viruses – influenza, poliomyelitis, HIV;

Animal, Plant viruses —→ single stranded

Reovirus of some plant —→ Double stranded.

Non- genetic RNA –

Mainly in nucleolus, cytoplasm, ribosome, mitochondria, chloroplast, in association with chromo.

Found both in pro & eukaryots

Synthesis in Non one of the DNA strand by transcription.

Thus carries genetic inf. from DNA.

Structure – Single stranded. Hence does not follow Chargaff's rule.

Types – three types of RNA - all are synthesized in nucleus

1) m – RNA / messenger / template : linear, longest molecule with 900 – 1500 nucleotides

Function: To carry genetic information in the form of codons from DNA to site of protein synthesis i.e. ribosomes.

2) r – RNA / ribosomal RNA – folded.

Function:

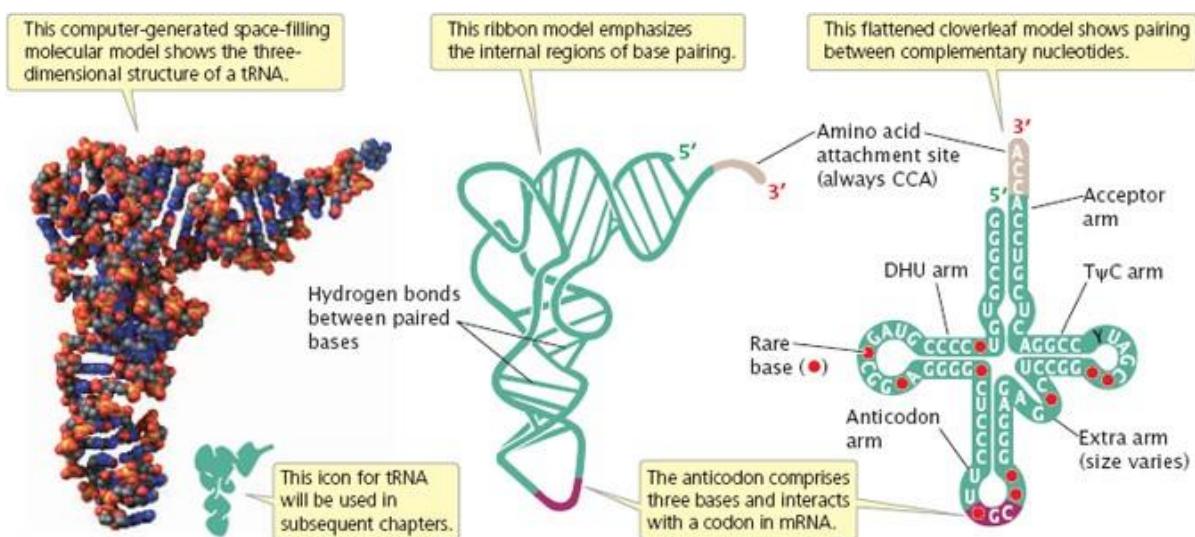
- Proper orientation of mRNA
- Formation of ribosomal complex by the attachment of smaller & larger subunit and further ribosomal complex with m RNA
- release of t RNA from ribosome complex after transfer of AA to polypeptide chain

3) t - RNA/ transfer RNA/ soluble RNA (can't be precipitated by ultracentrifugation)

Structure: According to shape two models are explained viz. clover leaf and hair pin

Function:

- To bring AA at the site of protein synthesis
- Transfer of AA to polypeptide chain





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UNIT II: Levels of Organization of Life

Cell as basic unit of life, prokaryotic and eukaryotic cells, microbes, plant and animal cells; Cell organelles – structure and function; Levels of organization of life - tissues, organs, systems and organism.

LEVELS OF ORGANIZATION OF LIFE

Cell as basic unit of life, prokaryotic and eukaryotic cells, microbes, plant and animal cells;

CELL ORGANELLES

Present in all eukaryotic cells. Absent in prokaryotic cells, secondarily lost in mammalian RBC.

MITOCHONDRION

Also called as power houses, energy coins. Present in all eukaryotic cells, except mammalian RBC where secondarily lost.

No. per cell variable, 1 in primitive eukaryotes, 5,00,000 in insect flight muscles.

Size 1.5—10 μm in length, 0.25 μm in diameter.

Shape cylindrical common, may be spherical, tubular, branched, discoidal.

Ultrastructure : --

- 1) 2 membranes : **Outer** – limiting, permeable, smooth, **Inner** – selectively permeable thrown into folds called cristae / trabeculae.
- 2) In between two membranes peri – mitochondrial space, filled with homogenous fluid called cytosol, contains H_2O , minerals.
- 3) Inner mitochondrial cavity has dense, homogenous gel like matrix with high conc. of soluble proteins, nucleotides, lipids, circular DNA called mitochondrial/mt DNA, ribosomes of 70s type, K^+ , HPO_4^{2-} , Mg^{++} , Mn^{++} , Cl^- , SO_4^{2-} , RNA (3 types), riboflavin vitamin.
- 4) Inner cavity divided into many compartments due to cristae, which are more in active cells. Inner membrane has 2 faces, outer face called C/cytosol face, inner M/matrix face. On inner surface of inner membrane i.e. at M face, numerous knob like elementary particles / F_1 particles / oxysomes / Fernandez – Moran subunits.

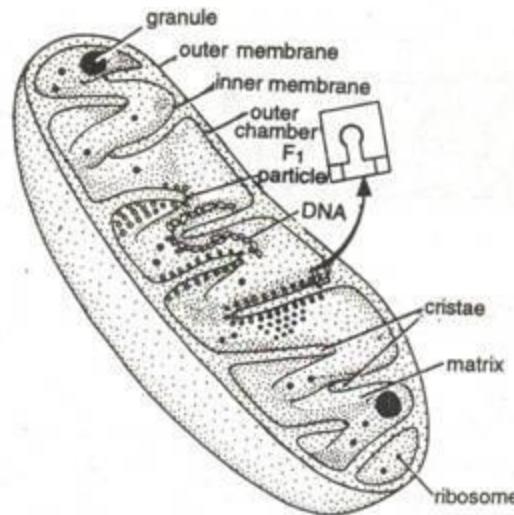


Fig. 2 Structure of mitochondrion cut longitudinally.

Oxysome :- composed of base, stalk, head piece.

Head piece – contains F₁ – subunit, spherical, contains enzyme ATPase / ATP synthatase

Function – oxidative phosphorylation, oxidation of food, ATP release.

Base – contains Fo – particle / subunit , rectangular ,embedded in inner mitochondrial membrane, contains coenzymes of ETC.

Stalk – contains F5, F6 subunit.

Mitochondria:- self duplicating, New one formed by division of existing one.

Semi autonomous organelle –

Mitochondria – have own genetic information, in mitochondria DNA is independent of cell's nuclear DNA., capable of self replication, capable of forming 3 types of RNA.

Mitochondria has its own ribosomes. Hence can, form its own structural proteins. Few subunits of mitochondria & enzymes are formed by itself from ribosomes. Remaining subunits from cytosol. Hence mitochondrion is a semiautonomous organelle.

Functions:-

- 1) Power house / storage batteries / ATP mills of cells.
- 2) Bring about oxidation of carbohydrates, fats., proteins.
- 3) Capable of self –replication.
- 4) Site for synthesis of haemoglobin(protein in blood), myoglobin (protein in muscles).
- 5) Site for thermogenesis (heat production).

PLASTIDS – FOOD FACTORIES & STORE HOUSES

On the basis of colour pigments plastids are classified into chloroplasts (green), chromoplast (Yellow, orange etc.) and amyloplasts (White)

Chloroplast: Present in green parts of plant like leaves, skin of raw fruits, flower in bud condition, young stem.

Shape–Cup shaped, Spiral ,Girdle,Branched,Starlike ,Reticulate,Spherical,Oval, Discoidal in higher plants. **Number** –1 to several hundreds. Size – 4 – 6 μm .

Ultra structure –

- 1) Covered by 2 membranes. Outer one permeable with less proteins. Inner one semi permeable with more proteins.
- 2) Periplastidial space of 25 – 75 Å between 2 membranes.
- 3) Matrix / stroma – Ground substance, colourless, granular with proteins, lipids, 70 S ribosomes, circular DNA, (called as chloroplast/ct – DNA), RNA (3 types), enzymes.
- 4) In stroma no. of membranous sheets called lamellae. Lamellae form closed oval sacs called thylakoids.
- 5) Each thylakoid has intra thylakoid space / loculus. In loculus no. of para crystalline rounded bodies called quantosomes present which trap quantum of light. Each quantosome contains 230 chlorophyll pigment molecules. In higher plants quantosomes contain chlorophyll a & b, carotene, xanthophyll. Thylakoids also contain various electron carriers like cytochrome f, b, ferredoxin, plastocyanin, plastoquinone.

In eukaryotes – thylakoids are superimposed like a pile of coins and form grana. In each grana 10 – 100 (average 20 – 50) thylakoids. In each chloroplast about 40 – 60 grana. Adjacent grana interconnected by stroma lamellae / frets / intergranal lamellae.

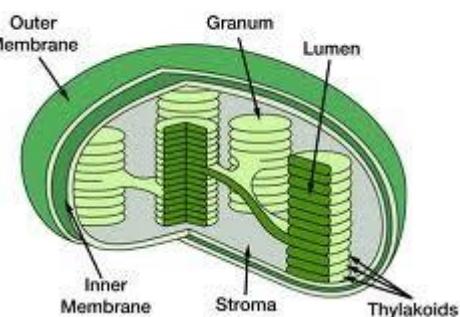
Semi – autonomous organelles

Circular DNA, 70 S ribosome, RNA (3 types) present, hence can form another chloroplast using some enzymes from cytoplasm.

Functions- 1. Photosynthesis.

2. O₂ replenished in atmosphere.
3. Starch storage.
4. Natural greenery

Chloroplast



Endoplasmic Reticulum – (ER)

ER has interconnected membrane bound vacuoles / cavities , concentrated in endoplasmic portion of cytoplasm (Cytoplasm has 2 regions – outer homogenous--ectoplasm, inner granular – endoplasm), hence called ER,

Occurrence –

Well developed in fully differentiated, metabolically active eukaryotic cells – e.g. liver, pancreas. Absent in prokaryotic cells, secondarily lost in matured mammalian erythrocytes (RBC).

Ultra structure – Composed of 3 shapes

- 1) Cisternae – Near nucleus. Long, flattened, saclike, unbranched tubules. Lie one upon the other, interconnected & studded with ribosomes.
- 2) Vesicles – oval / rounded, vacuolar structures, scattered in cytoplasm.
- 3) Tubules – branched, form reticular structure along with cisternae and vesicles. Near cell membrane. .

Types:-

1) Agranular / Smooth ER – SER

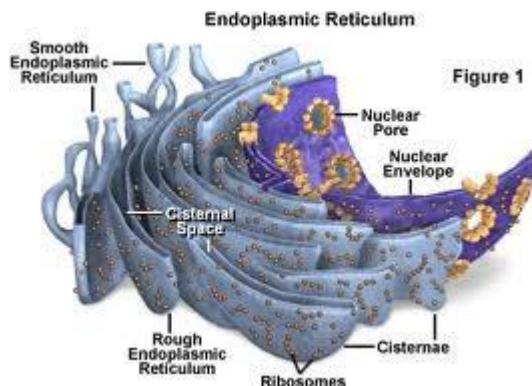
Ribosomes absent on outer membrane.
Present near cell membrane. Generally in the form of tubules.

2) Granular / Rough ER / RER –

Ribosomes attached to outer membrane.
Generally in the form of cisternae.

Functions of Endoplasmic Reticulum –

- 2) Fluid filled vacuolar system. Acts as endoskeleton; gives support to colloidal protoplasm.
- 3) Active, passive transport of material.
- 4) Divides cytoplasm into many compartments, thus cell activities take place separately in each compartment. Various organelles remain stationed.
- 5) Increase surface area for absorption / chemical reactions within cell.
- 6) Contain variety of enzymes.



Golgi Complex: Molecular sorting & finishing area

Ultrastructure -

Present in three shapes / forms --

a) Cisternae - Flat / curved , piled up one above other, with swollen ends. Outer convex surface associated with nuclear membrane/ ER. It is called – forming / cis / entry face Inner concave surface, called maturing / trans / exit face.

b) Vacuoles –Formed by fusion of small vesicles / large parts of broken cisternae. Generally associated near concave surface.

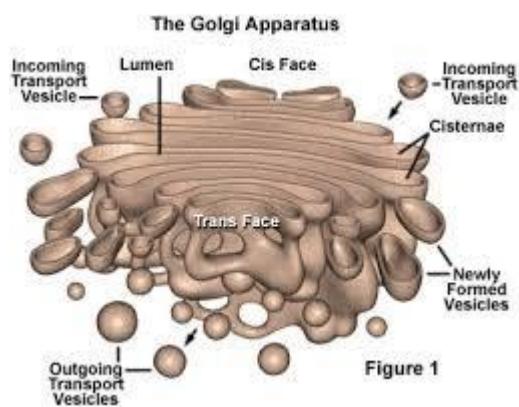
c) Vesicles – Pinched off from edges of cisternae hence near edges / concave surface.

Chemical composition – Proteins – 60%, Phospholipids – 40% , Enzymes.

Origin – mostly from SER as cisternae connected to E.R.

Functions –

- 1) Secretion – Mainly secretion of enzymes, hormones, glycoprotein, Ab(antibody).
- 2) Storage and Synthesis – Store proteins, lipids in the form of glycoprotein & glycolipid.
- 3) Packing and forwarding center for enzymes, mucus, hormones in small vesicles.
- 4) Cell plate formation in cell division.
- 5)Formation of primary lysosomes – Hydrolytic enzymes are formed in ER, then come to cisternae, packed and budded off as primary lysosome.



Lysosomes : Sacs of hydrolytic enzymes

Structure – These are small membrane bound (unit membrane) vesicles. Contain hydrolytic enzymes.

*** Hydrolytic enzymes are stored in crystalline / fluid form. Membrane of lysosome is impermeable to enzyme. But ruptures during O₂ deficiency / exposure to poisonous substances. Then enzymes are released and cell itself is destroyed. Hence lysosomes are also known as suicidal bags of cells.***

Types of Lysosomes

- 1) Primary lysosome - / storage granules – Derived from G.C. Contain only hydrolytic enzymes in inactive form. In the form of small vesicles.
- 2) Secondary lysosome / Digestive vacuoles / Heterophagosomes – Pinosome (vacuole with liquid) / phagosome (vacuole with solid) fuse with primary lysosome. Hence contain enzyme + material to be digested.
- 3) Residual Bodies/Tertiary lysosome/Telolysosome – Undigested material remain in. Now called residual body. Come near plasma membrane, throw out their contents outside through ephagy / exocytosis. If contents not discharged, the cells are loaded with it, cause nephritis, hepatitis, arthritis, gout, lung fibrosis.
- 4) Autophagosomes / Autolysosomes – Cell organelles like ER, Mitochondria get worn out. Its degradation by lysosome called as autophagy. Primary lysosome + worn out cell organelle form autophagosomes.

Function –

- 1) Digestion – by hydrolytic enzymes.

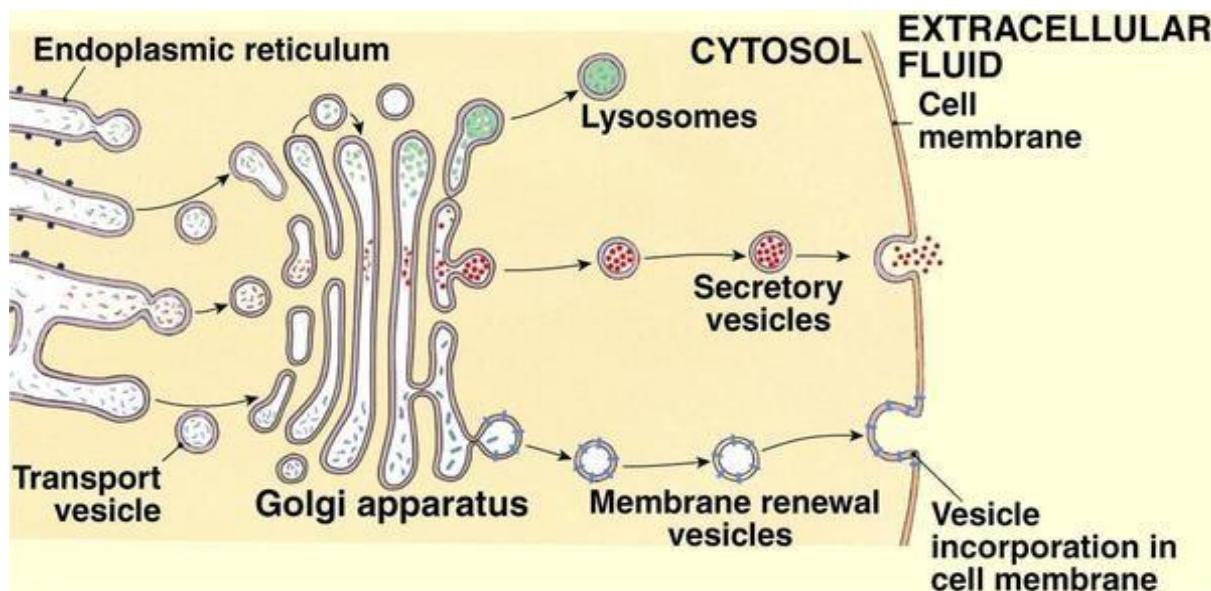
Extracellular – enzymes are released in surrounding medium by exocytosis.

Intracellular – by formation of secondary lysosomes or autophagosomes. E.g. phagocytes in higher animals, degeneration of tail in tadpole larva of frog by enzyme cathepsin.

Heterophagy – digestion of foreign substance,

Autophagy – digestion of self substances. Thus lysosomes are self disposal units, also bring about physiological rejuvenation. Digestion of reserve food during starvation is also called as Autophagy.

- 2) Initiate cell division by removing repressors of this process.
- 3) By breaking thyroglobulins, thyroid hormone(thyroxin) is produced.
- 4) In joint disorder like gout, arthritis --macrophages come here & release lysosomes which causes inflammation.
- 5) Accidental / pathological release of lysosome enzyme causes chromosome breakage, abnormal distribution of chromosomes during mitosis, which may lead to blood cancer.



Ribosomes: Work benches for protein analysis

Occurrence -- both in pro and eukaryotic cells, except mature RBC.

Types of Ribosomes – According to size, sedimentation coefficient ($S = 1 \times 10^{-13} \text{ cm/sec}$ / dyne / gm) 2 types.

- 1) 70 S ribosomes – found in mitochondria, chloroplast of eukaryotic & prokaryotic cells
- 2) 80 S ribosomes – “ in eukaryotic plant & animal cells.

Structure – Not covered by unit membrane, but porous, hydrated, 2 subunits. Larger & smaller. 70 S ribosome has 50 S and 30 S subunits & 80 S ribosome has 60 S and 40 S subunits which are separated by a narrow cleft. 2 subunits remain separated, join only

during protein synthesis. In high conc. of Mg⁺⁺ ions → 2 subunits remain united & called as dimer. Smaller subunit fits like a cap on larger subunit. Larger subunit – dome-shaped, 2 binding sites Peptidyl / P site / donor site, Amino – acyl / A site / acceptor site. It has protuberance, ridge and stalk. Smaller subunit – ellipsoidal shape, cap like. It has a platform, cleft, head & base.

Polyribosome / polysomes – It is chain of ribosomes as formed during protein synthesis on m-RNA.

Functions –

- 1) Protein factories / engines of cell as site of protein synthesis.
- 2) Free ribosome produce non-secretory proteins like enzymes for intra cellular use (e.g. in muscle cells, skin cells)
- 3) Bound ribosome like present on RER synthesize secretory proteins e.g. enzymeA
After synthesis of proteins, proper folding of proteins is assisted by specific proteins **chaperons** which also assist transport of proteins into organelles like mito chondria

Nucleus: Genetic message centre

Ultrastructure -

Contains nuclear membrane, nucleolus, nucleoplasm, chromatin

Nuclear membrane/karyotheca/Nuclear envelop/Nucleolemma.

- It is an outer envelop
- Present in all eukaryotic N Absent during late cell division.
- Consists of 2 unit membrane, between them perinuclear space of 75 Å °.
- Outer membrane continuous with RER, studded with ribosome on outer side.
- Nuclear openings or pores in it to maintain nucleo – cytoplasmic connection.
- Outer membrane called as ectokaryotheca, inner called as endokaryotheca.
- Each nuclear pore has cylindrical annulus with pore complex.
- Through pore complex / basket movement of substances takes place.
- mRNA come out through them into cytoplasm.
- Dissociates during early cell division, reappears at end of cell division.

Nucleus –

Appears spherical, dense, colloidal, no limiting membrane. No. 2 -- 5

Parts – i) Granular region -- protein granules ii) Fibrillar region – proteinaceous fibrils
iii) Amorphous matrix – less dense called ‘pars amorpha’. iv) Chromatin fibres are perinucleolar and intranucleolar.

Nucleoplasm – nuclear sap / nucleoplasm / karyolymph.

Transparent., semi – solid, granular, acidophilic. Composed of – Nucleic acids, enzymes, minerals.

Chromatin –

Hereditary part. Network of fibres. During cell division organizes as chromosome.

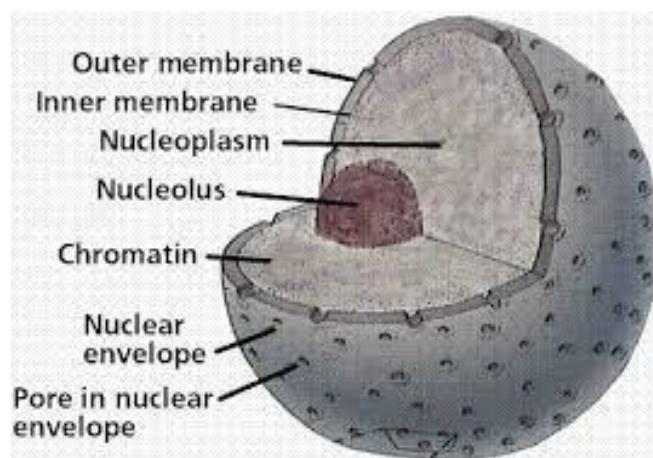
Heterochromatin – Shows thick regions, darkly staining where DNA is condensed. Lies near nuclear membrane. Contain late replicating genes. Inactive genetically.

Euchromatin – Thin regions, less darkly staining, DNA loose, genetically active.

Chromatin thread composition – DNA, RNA, proteins (histones., non – histones.)

Functions --

- 1) Contain hereditary material in the form of chromosomes
- 2) Transfer genetic characters from one generation to another
- 3) Control cell division
- 4) Control all physiological activities of the cell.



EVOLUTION OF BIOLOGICAL MACHINES

Major changes that occurred when prokaryotes gave rise to eukaryotes -

- Cells acquired more DNA.
- DNA folded compactly into discrete complexes with specific proteins to divide it equally between daughter cells at cell division.
- Specialized proteins stabilize folded DNA (chromosomes).
- A system of intracellular membranes and a double membrane surrounding the DNA was developed.
- Early eukaryotic cells enveloped aerobic bacteria or photosynthetic bacteria to form endosymbiotic associations that became permanent. Some aerobic bacteria evolved into mitochondria of modern eukaryotes and some photosynthetic bacteria became plastids like chloroplasts and likely ancestors of modern plant cells.
- It was advantageous to cluster together for acquiring greater motility, efficiency, or reproductive success than their free-living, single-celled competitors.
- Specialization within the colony – to cellular differentiation.
- It led to even more complex and highly differentiated organisms, in which some of them carried out the sensory functions, others the digestive, photosynthetic or reproductive functions so forth.

Principles of generating diverse body plans and design in nature :

The major events include the changes in

- a. Size of organisms
- b. Form and complexity
- c. Expansions in diversity
- d. Production of many shapes of macroscopic life.

The evidences for the process of evolution are usually obtained from fossil records which is also the data at the time of origin.

Inferences about direction of evolution:

- a. Multicellularity evolved independently many times and in all parts of life i.e. plants, animals and microorganisms.
- b. Multicellularity evolved from different unicellular ancestors.
- c. These multicellular organisms have new body plans and physiologies
- d. They represented more complex features.

These complex forms then diversified so that varied kinds appeared over a long period.

Figure: Evolution of eukaryotes through endosymbiosis:

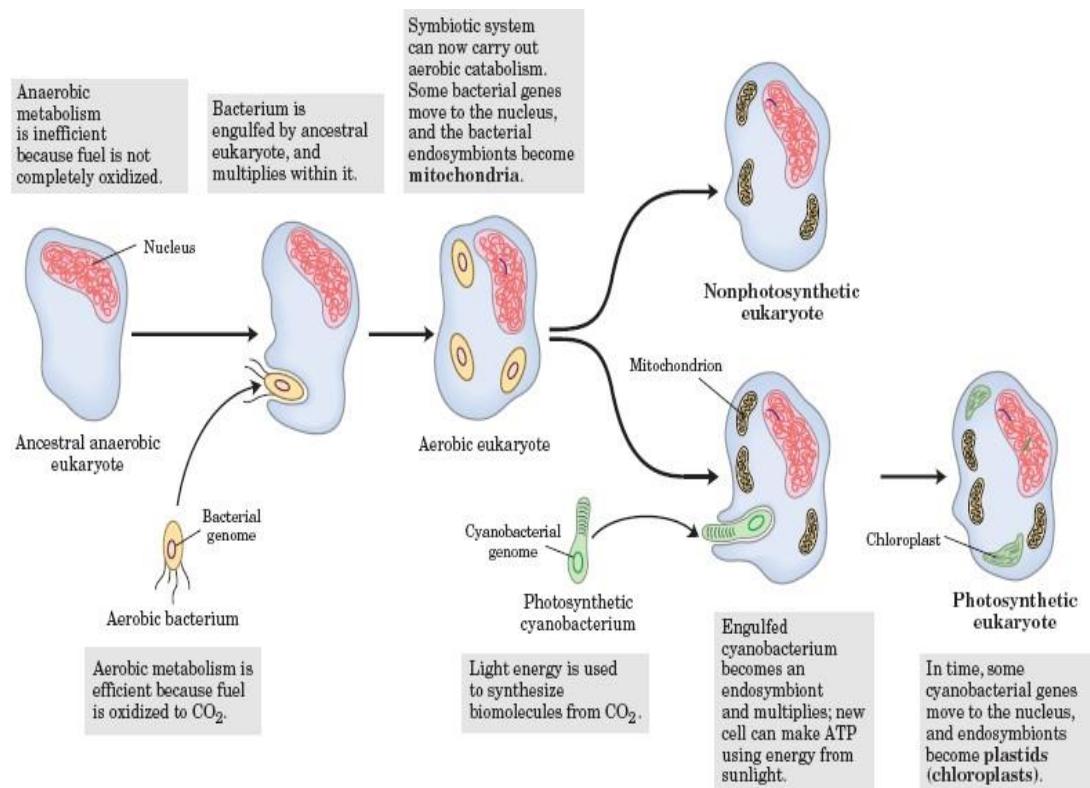


FIGURE 1-36 Evolution of eukaryotes through endosymbiosis. The earliest eukaryote, an anaerobe, acquired endosymbiotic purple bacteria (yellow), which carried with them their capacity for aerobic catabolism and became, over time, mitochondria. When photosynthetic

cyanobacteria (green) subsequently became endosymbionts of some aerobic eukaryotes, these cells became the photosynthetic precursors of modern green algae and plants.

Size and multicellularity:

For first 2500 million years of life on the earth, most species were generally much smaller and rarely exceeded 1mm in size. The bacterial microfossils obtained from 3500 million years had 5mm diameter.

The early microfossils of eukaryotes were 40 -200 mm in size for first 600 -800 million years

Cellular dimension are limited by oxygen diffusion:

A bacterial cell is 1 -2 mm long and animal/plant cell is 5 -100 mm long. The upper limit of cell size is set by the rate of diffusion of solute molecules in aqueous system.

Consider the example of a bacterial cell -

It depends upon oxygen consuming reactions for energy production. So it has to obtain molecular oxygen by diffusion across its plasma membrane. The cell is small and the ratio of its surface area to its volume is large hence every part of its cytoplasm is easily receiving the diffused oxygen.

But as cell size increases, surface-to-volume ratio decreases. The rate of consumption of oxygen is faster than that of its diffusion because of the metabolism of the cell. So when the cell size goes on increasing, the oxygen demand for metabolism increases to such a point that the metabolism becomes impossible. This puts a theoretical upper limit for the cell size and cell cannot increase above this point.

Complexity: It is referred to as number of different cell types or the no./ functional specialization of parts.

There are four types of complexity –

1. the number of different physical parts e.g. genes, cells, organs and organisms in a system..
2. the no. of different interactions between the above mentioned parts
3. the no. of levels
4. the no. of parts or interactions in a specific condition

Diversity: Actually the diversity of life has expanded from its origin but it doesn't cause continuous increase. For the organisms those are made entirely of soft tissues or of small size, it cannot be said whether the total diversity increased or decreased over a long period of time.

Levels of Organization

Within multi-cellular organisms there is division of labor. Division of labor means that the work (labor) of keeping the organism alive is divided (division) among the different parts of the body. Each part has a job to do and as each part does its special job, it works in harmony with all the other parts.

The arrangement of specialized parts within a living thing is referred to as levels of organization.

First Level :-Cells

Cells of course, are the first level of organization

Second Level:- Tissues

Tissues are the second level of organization. In any multi-cellular organism, cells rarely work alone. Cells that are similar in structure and function are usually joined together to form tissues. There are four basic/major types of tissues in the human body: Muscle tissue (skeletal, smooth, cardiac muscles), nerve tissue (brain, spinal nerves, cranial nerves), connective tissue (bone, cartilage, blood), and epithelial tissue (skin, other body parts coverings).

Third Level :- Organs

Organs are the third level of organization.

When a bunch of different types of tissues work together, they form an organ. E.g. Brain, liver, stomach, heart etc.

Fourth Level :- Organ System

Organ systems are the fourth level of organization.

Each organ in human body is a part of an organ system, a group of organs that work together to perform a major function. E.g. heart, blood vessels are parts of circulatory system, likewise digestive, excretory, respiratory systems.

Fifth Level :-- Organism/ Individual

Organisms with many systems form fifth level of organization.

Single cell to multi cellular organism

- Unicellular organisms formed colonies by remaining together after each cell division.
- Division of labor, made it possible to exploit resources in better way.
- For formation of multicellular organism, cells remain bound together. In animals extracellular organic matrix binds cells together as cell wall, plasmodesmata are absent.
- Such fundamental arrangement is seen in epithelial tissue sheets.
- From a group of cells, some cells differentiated from others and adopt different structure, chemistry, function usually in response to cues from neighbouring cells.
- Cells have memory i.e. cell and its progeny usually persist in their differently specialized state even after disappearance of original stimuli.
- Final character of animal not determined by its final environment but entire sequences of influences to which cells are exposed during development.
- As body grows and matures, progressively finer details of the adult body pattern become specified, complex organisms are formed in long developmental history.
- Though more and more complex organisms are formed, early developmental stages very similar though adult stage radically different.
- Specialization of cells depend on gene expression and not on loss or acquisition of genes. As specialization also involves loss of genetic material. E.g. RBC – lost nucleus during differentiation.
- In eukaryotes sophisticated mechanisms for controlling gene expression has evolved.
- Groups of genes activated or repressed in response to external and internal signals.
- Radical differences of character between cell types reflect stable changes in gene expression.



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CT16002 – Biology for Engineers

UNIT III: Bioenergetics and Metabolism

1. Energy Dynamics in Biology –

- a. Photosynthesis and energy assimilation: aerobic and anaerobic systems.
Applications
- b. Respiration and Electron Transport Chain: Mitochondria and respiration,
ATP generation.

2. Bioenergetics: Thermodynamic principles applied to biology, negative entropy changes in biological systems, Free Energy, Chemical Equilibrium

1) PHOTOSYNTHESIS

(PHOTOSYNTHETIC ELECTRON TRANSFER, CALVIN CYCLE)

Introduction:

Solar energy is the prime source of energy to entire living world. 2] All living organisms require energy for their life processes. 3] Solar energy can't be utilized by organisms.

Important Features of Photosynthesis:

1) It is an intracellular process. 2) It takes place only in green cells of plant 3) During this process organic food is synthesized. 4) It utilizes solar or light energy. 5) It uses CO_2 & H_2O as raw materials. 6) Solar energy is converted into chemical energy. 7) Only 1 to 5% of solar energy received by earth is utilized in photosynthesis. 8) It is redox reaction where water is oxidized to oxygen & CO_2 is reduced to carbohydrates. 9) At first simple sugar like glucose is formed & then complex food like starch, proteins, fats are formed. 10) Chlorophyll acts as a catalyst.

Definition:- It is a biochemical process in which organisms prepare complex organic food from simple, inorganic substances like CO_2 & H_2O with the help of chlorophyll & light energy, releasing O_2 as by product.

Overall reaction: -



Photosynthesis is an anabolic (biosynthetic) & endergonic (E dependent) process.

Pigments and their role : --1] Photosynthetic pigments of chloroplast in higher plants are chlorophyll & carotenoids.

2] Chlorophyll : - Each chlorophyll molecule looks like a kite or tennis racket with head & tail. Head is made up of 4 pyrrol rings with Mg in center. It is hydrophilic Tail is made up of phytol which is long chain alcohol. It is lipophilic, hydrophobic.

3] Chlorophyll a : - It is bluish green, with molecular formula $\text{C}_{55}\text{H}_{72}\text{O}_6\text{N}_4\text{Mg}$, absorbs – blue, yellow, red wave lengths of light.

4] Chlorophyll b – It is yellowish green, molecular formula $\text{C}_{55}\text{H}_{70}\text{O}_6\text{N}_4\text{Mg}$, absorbs – blue, orange wave lengths of light.

5] Carotenoids are carotenes & xanthophylls. Carotenes are yellowish orange with molecular formula C₄₀H₅₆. Xanthophylls are yellow with molecular formula C₄₀H₅₆O₂. Carotenoids are long chain hydrocarbons. They don't have definite shape.

6] All photosynthetic pigments trap light energy in form of photons.

7] Chlorophyll b, Carotenes & Xanthophylls transfer trapped light energy to chlorophyll a by resonance transfer, do not participate actively in photosynthesis. Hence they are called as accessory pigments/antennae pigments/light gatherers. These accessory pigments avoid photo-oxidation of reaction center in intense light.

8] Chlorophyll a collects light energy from these pigments. It also absorbs light energy. It uses light energy for formation of ATP. Hence it is called as active pigment. It acts as a center of chemical reaction. It shows fluorescence.

9] Middle region of quantosomes are called as photocentres or reactive centers.

10) Chlorophyll a has two pigment systems called photosystems i.e. PSI , PSII are involved . PS I absorbs far red light of wavelength 700 nm & PSII absorbs short red light of wavelength 680 nm. Each system has its own type of chlorophyll a i.e. P₇₀₀ & P₆₈₀. Both system work in cooperation to capture radiant energy.

PS I – 670 , 683, 700. P – 700 is Reaction center. PSII – 680, 673. P – 680 is Reaction center. PSI – lies on outer surface of thylacoid, PSII – lies in inner surface of thylacoid.

Mechanism of Photosynthesis --

Reaction of photosynthesis takes place in two phases i.e. photochemical phase & biochemical phase.

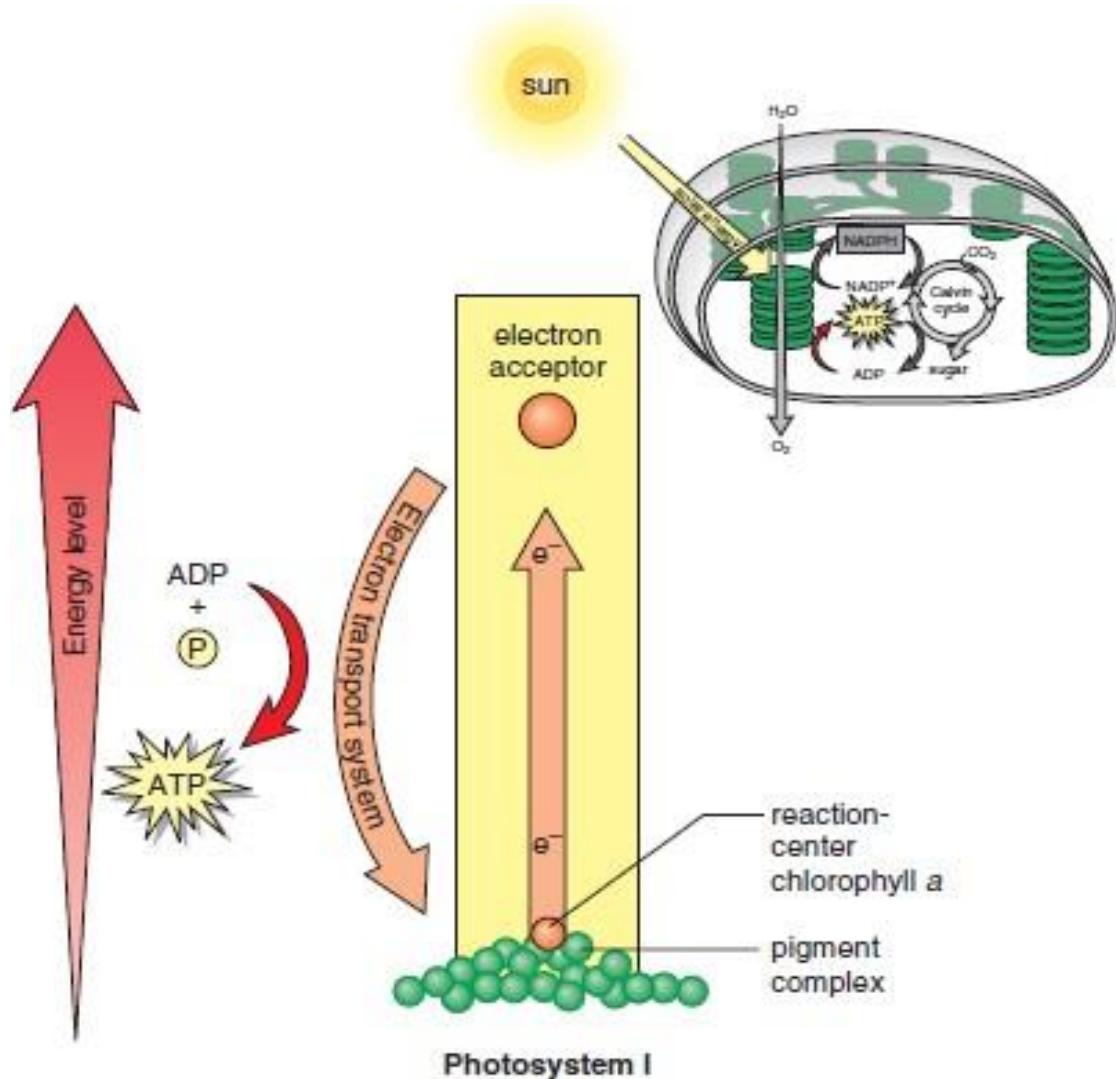
I] Primary Process / Photochemical phase / Light reaction

- 1) It takes place in presence of solar energy i.e. light & only in granna of chloroplast. Hence it is light reaction.
- 2) Light energy is converted into chemical energy with formation of ATP & NADPH₂. Hence it is photochemical phase.
- 3) ATP is formed by addition of 1 inorganic phosphate to ADP with the help of energy. This is called as phosphorylation.
- 4) Energy required for phosphorylation is obtained from light in form of photons. Hence it is called as photophosphorylation.
- 5) During this process e⁻ s are transferred through a system of e⁻ acceptors.
- 6) Two pathways are there of e⁻ transfer i.e. cyclic & non cyclic

(A) Cyclic e⁻ transfer / Cyclic photophosphorylation

(1) It involves PS I (Pigment system I). (2) Light strikes chlorophyll-a i.e. P – 700 trap (3) It absorbs quantum of light energy. (4) As a result it is excited i.e. its energy level increases. (5) Hence it emits a pair of high energy electrons. (6) Energy rich e⁻ leave chlorophyll molecule & hence the chlorophyll molecule becomes +vely charged (ionized) i.e. unstable. (7) Electrons move through various electron acceptors such as FRS(Z), ferredoxin, cytochrome b6, cytochrome f & plastocyanin. (8) As energy rich electrons move through electron acceptors, they loose some of their energy which is used for synthesis of ATP from ADP & inorganic phosphate. (9) Finally de-energised electrons return to unstable chlorophyll a molecule which becomes stable. (In one millionth of a second) (10) Thus the electrons lost by chlorophyll molecules return to the same chlorophyll molecule. Hence it is called as cyclic electron transfer.

Cyclic electron transfer occurs when light intensity is low, CO₂, O₂ low.



B] Non cyclic photophosphorylation / Non cyclic e⁻ transfer

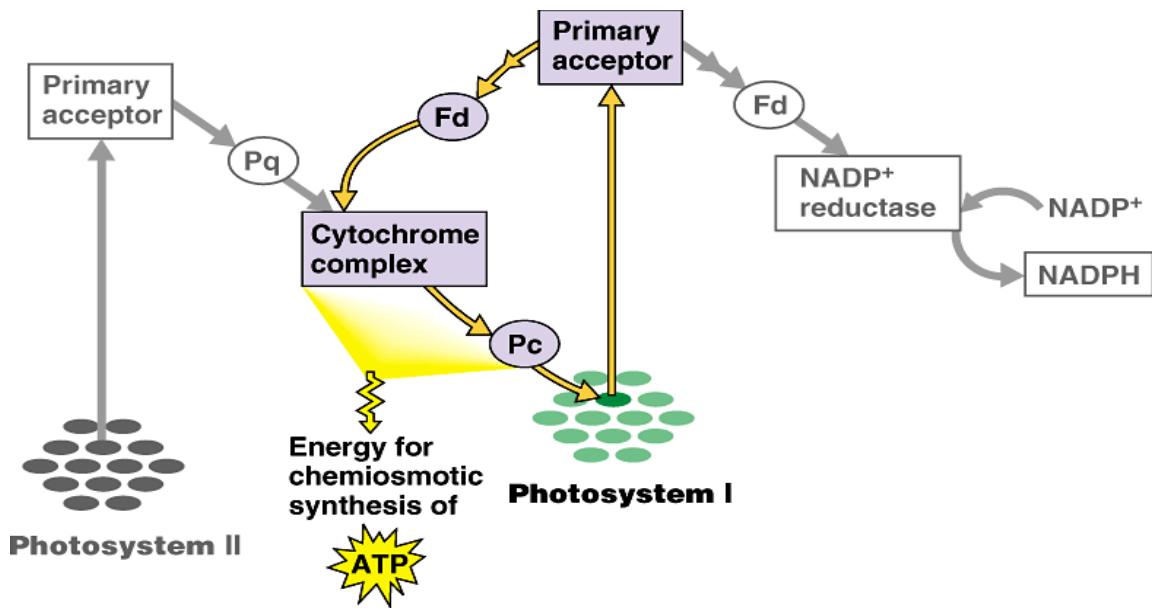
- 1) It involves pigment system I & II i.e. PS I & PS II
- 2) Light strikes chlorophyll – a of PS I & PSII i.e. P₇₀₀ trap & P₆₈₀ trap.
- 3) They absorb quantum of light energy .
- 4) As a result they are excited i.e. their energy level increases.
- 5) Hence they emit a pair of high energy electrons
- 6) Energy rich e⁻s leave chlorophyll a molecule which becomes + very charged (Ionised) i.e. unstable.
- 7) These e⁻s from PS II system, which is the primary electron donor, move through various e⁻ acceptors such as FRS, ferredoxin and finally accepted by NADP, that from PS I through plastoquinone, cytochrome b₆, Cytochrome f, plastocyanin.
- 8) As energy rich electrons move through electron acceptors, they lose some of their energy which is used for synthesis of ATP from ADP & inorganic phosphate.
- 9) In presence of light & chlorophyll a molecule photolysis of water takes place & two electrons , two H⁺ ions & O₂ are released. O₂ is released outside. These electrons are accepted by chlorophyll –a of PS II & it becomes stable. H ions are accepted by NADP.
- 10) Two electrons emitted from PSII are accepted by chlorophyll- a of PS I after it emits two e⁻s when light strikes on it. Thus chlorophyll-- a of PS I becomes stable.
- 11) Finally two H⁺ ions released by photolysis & two e⁻s released by PSI combine together & reduce NADP to NADPH₂
- 12) Thus e⁻s emitted from one pigment system don't return to it. Hence it is called as non cyclic e⁻ transfer.

FRS – ferredoxin reducing system

Ferredoxin – Fe containing flavo protein

Cytochromes – Fe containing proteins

Plastocyanin – Cu “ “



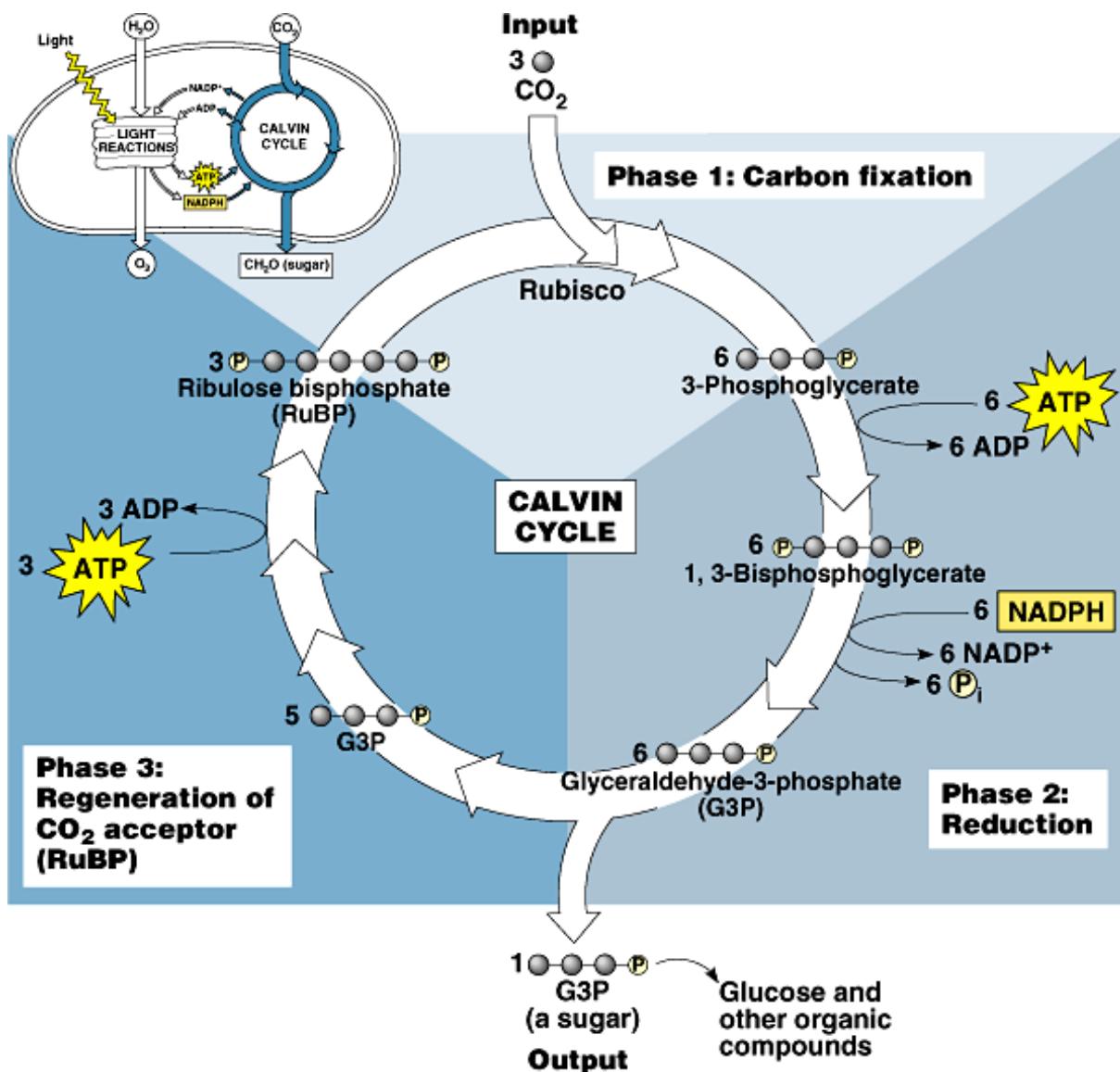
Significance of Non cyclic e⁻ transfer :-

- 1] In this pathway ATP & NADPH₂ are formed.
- 2] ATP acts as an energy donar in dark reaction
- 3] NADPH₂ acts as a hydrogen donar in dark reaction
- 4] Photolysis of water takes place.
- 5] Oxygen is liberated as bi product.
- 6] It is more efficient than cyclic photophosphorylation.

Cyclic Photophosphorylation	Non cyclic Photophosphorylation
<ol style="list-style-type: none"> 1. e⁻s return to same chlorophyll molecule from which they are emitted. 2. Photolysis of water doe not take place. 3. O₂ is not evolved 4. NDPH₂ is not formed 5. NADP doesn't take part. 6. Only pigment system I is involved 7. Less efficient as less energy is formed. 8. Primary acceptor is FRS (Z) 9. It takes place in photosynthetic bacteria 10. Occurs in low intensity light, anaerobic condition, less CO₂ available 	<ol style="list-style-type: none"> 1. e⁻s don't return to same chlorophyll molecule from which they are emitted. 2. Photolysis of water take place. 3. O₂ is evolved as bi product. 4. NADPH₂ is formed 5. NADP takes part as e - acceptor 6. Both pigment systems I & II are involved. 7. More efficient as more energy is formed. 8. Primary acceptor is FRS (Z) & plastoquinone . 9. It takes place in green plants. 10. Occurs in normal light, aerobic condition, sufficient CO₂

II) Secondary Process / Biochemical Phase / Dark Reaction

- 1) It takes place in stroma of chloroplast, independent of chlorophyll
- 2) Light is not required for it. Hence it is known as dark reaction.
- 3) ATP, NADPH₂ formed during light reaction are used in dark reaction for reducing & fixing CO₂ in carbohydrate i.e. hexose sugar. Hence it is also known as CO₂ fixation or synthetic phase.
- 4) Energy in ATP is used for various reactions.
- 5) Enzymes & coenzymes necessary for dark reaction are present in stroma of chloroplast.
- 6) Blackman in 1950 observed this reaction first. Hence it is also called as Blackman's reaction



- 7) Melvin Calvin & Benson traced path of carbon during dark reaction. They were awarded Nobel prize in 1961.
- 8) During their experiment they fed unicellular algae chlorella & Scenedesmus with radio active carbon isotope i.e. C¹⁴O₂. Algae were allowed to carry photosynthesis. At different time intervals algal cell extract was chemically analysed by paper chromatography to find out compound containing C¹⁴. On the basis of products obtained they suggested a cycle for dark reaction which is called as Calvin Cycle. It has three phases

A] Carboxylation Phase B] Reduction Phase C] Regeneration & Synthetic Phase.

A] Carboxylation Phase.

- i) Atmospheric CO₂ is taken by stroma of chloroplast.
- ii) RuMP (Ribulose Mono Phosphate) – 5 carbon compound is present in stroma. It is phosphorylated into RuDp (RuDP—Ribulose Di Phosphate) It is called as CO₂ acceptor
- iii) RuDP absorbs atmospheric CO₂ & forms unstable 6 C compound.
- iv) 6 C compound immediately undergoes hydrolysis & splits into 2 molecules of 3C compound i.e. 3 PGA. This is first stable compound in Calvin Cycle. Hence it is also called as C₃ Cycle.

B] Reduction Phase –

- i) 3PGA is phosphorylated to 1, 3 DPGA(Di Phospho Glyceric Acid)
- ii) 1, 3 – DPGA is reduced to 3 – PGAL (Phospho Glyceraldehyde) by using hydrogen from NADPH₂ & in this reaction one inorganic phosphate is released. The process is reverse of oxidation in glycolysis. Hence it is known as glycolytic reversal.

C] Regeneration & Synthetic Phase

- i) 10 molecules of 3 – PGAL are used for regeneration of RuMP through various phases with formation of 10C, 9C, 8C compounds.
- ii) Two molecules of 3PGAL are used to form hexose sugar i.e. glucose which is converted into starch by polymerization.

Action spectrum – rate of photosynthesis at different wavelengths of light.

Absorption spectrum – absorption of light of different wave lengths.

Quantum requirement – no. of photons/quanta required to release 1 molecule of O₂

Emerson & Lewis showed that it is 8 quanta.

Red drop – sudden fall in photosynthesis yield beyond red region of spectrum. Showed by Emerson & Lewis.

Emerson's enhancement effect – if simultaneously shorter & longer wave lengths are provided, rate of photosynthesis is higher than total rate from the beams separately.

RESPITATION

Introduction - Living beings need regular supply of energy for vital functions or activities like cell division, transport of materials, locomotion, digestion etc .

Definition :- It is an intracellular oxidation -- reduction reaction in which complex organic substances are broken down stepwise to release chemical energy in the form of ATP & CO₂ & H₂O are given out as byproducts.

Important features :-- Overall Reaction



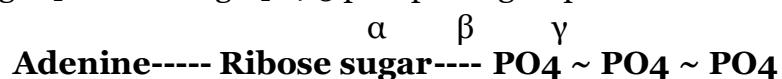
- In biochemical process the reaction is not so simple.
- Free molecular oxygen does not combine directly with substrate like in combustion.
- Hydrogen is gradually removed from the substrate & the electrons released ($\text{H}_2 \rightarrow 2\text{H}^+$ + 2e^-) are transferred through a series of e^- carriers to generate energy in the form of ATP (exergonic -- energy producing, catabolic – breakdown process)
- The process occurs at cellular temperature.
- Gases are exchanged in liquid medium by blood, tissue fluid etc.
- CO₂ & H₂O are given out as byproducts.
- All energy in glucose molecule is not converted into ATP but some of it is lost as heat energy.

ATP –The Energy Currency of the Cell

These are bio-molecules which store energy in biologically usable form.

ATP – Adenosine Tri Phosphate

It is composed of a) Adenosine – which is made up of adenine [Nitrogen base] + Ribose Sugar [Pentose sugar] b) 3 phosphate groups



Second & Third phosphate groups are attached to ribose sugar by high energy bonds, when cell needs energy it breaks the third high energy bond & even the second phosphate bond of ATP forming ADP & AMP respectively



All living cells generate ATP by using energy trapped in glucose molecule during photosynthesis. During this process glucose molecule is oxidized. In this reaction CO_2 & H_2O are given out as by products. This is called as cellular respiration. Energy released is trapped in ATP molecule by attaching phosphate group. This is called as phosphorylation. As glucose molecule is oxidized it is called as oxidative phosphorylation.

Significance : 1] It stores energy in biological usable form. 2] It supplies energy in

various cellular activities by breaking phosphate bond in between 2nd & 3rd and even 1st & 2nd phosphate groups. 3] It acts as a phosphate donor in various biochemical reactions.

- Cellular respiration is oxidation of food material into CO_2 and H_2O .
- During this oxidation E released is trapped in ATP (1ATP traps 7.28 k cal) for using in all cell activities.

This oxidation occurs in three phases.

1) **Glycolysis** – Breakdown of glucose to pyruvic acid (pyruvate). It occurs in cytosol i.e. cytoplasm. Hence mitochondria are not necessary. It occurs in prokaryotes as well as eukaryotes. As O_2 is also not required it is common to aerobic as well as anaerobic organisms. In glycolysis 2 ATP and 2 NADH + H^+ also formed.

2) Citric Acid Cycle / Krebs cycle -

- Decarboxylation of pyruvic acid to CO_2 and H_2O along with formation of NADH + H^+ and FADH_2 .
- In eukaryotes it occurs in mitochondrial matrix. Matrix has complex mixture of soluble enzymes for decarboxylation of pyruvic acid.

At the end $3\text{CO}_2 \uparrow$, 4 NADH + H^+ , 1 FADH_2 are formed (when 2e^- are removed from malic acid transferred to NAD^+ reducing it to NADH + H^+ , same way 2e^- removed from succinic acid and reduces FAD. To FADH_2)

Outer membrane – contains many complexes of integral membrane proteins that form channels – porins through which many molecules and ions move in and out of mitochondria.

- e^- from NADH and $FADH_2$ are transferred to next phase i.e. respiratory chain.

3) Electron Transport Chain / Respiratory Chain -

- Inner membrane of mitochondria contains complexes of integral membrane proteins.

NADH dehydrogenase complex

Succinate dehydrogenase complex

Cytochrome c reductase complex

Cytochrome c oxidase complex

ATP synthase complex

- It also consists ubiquinone, cytochrome a,b,c, which shuttle electrons from one complex to another.

Inner membrane is selectively permeable.

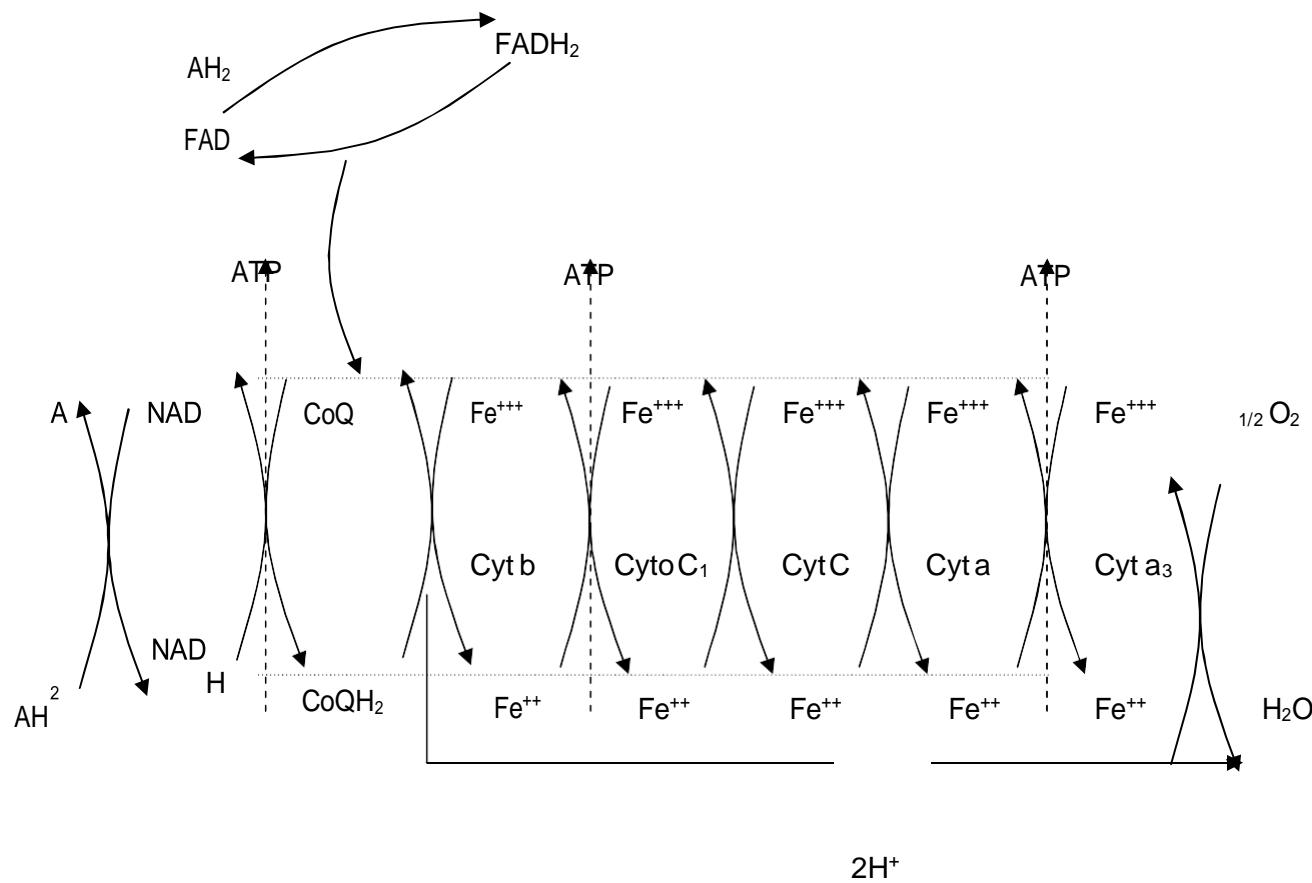
(NADH carries e^- from catabolic reactions to respiratory chain.

NADPH supplies e^- to anabolic reactions.)

- Stepwise transfer of e^- from NADH, Ubiquinone, Cytochromes and then finally to O_2 to form H_2O takes place. Neither NADH nor NADPH can cross inner mitochondrial membrane, but the e^- carried by them can shuttle across.
- During e^- transfer E is released.
- It is used to transfer/ pump H^+ (protons) from matrix into inter membrane space by active transport.
- Thus matrix becomes – very charged and inter membrane space +very charged.
- Gradient of protons formed across the inner membrane forms a miniature battery. (Mitochondria contain 3 classes of cytochromes – a,b,c which absorb different light spectra)

[Plastoquinone is like ubiquinone. Ubiquinone (Coenzyme Q) → small, hydrophobic, hence freely diffusible in lipid bilayer of inner mitochondrial membrane and can shuttle reducing equivalents between other less mobile electron carriers in the membrane. Cytochromes → proteins with iron – heme group; show strong absorption of visible light.)

- In mitochondrial respiratory chain, e⁻ move as follows – NADH / FADH₂ → Co.Q → Cytochrome b → Cytochrome C₁ → Cytochrome C → Cytochrome a → Cytochrome a₃ → O₂.

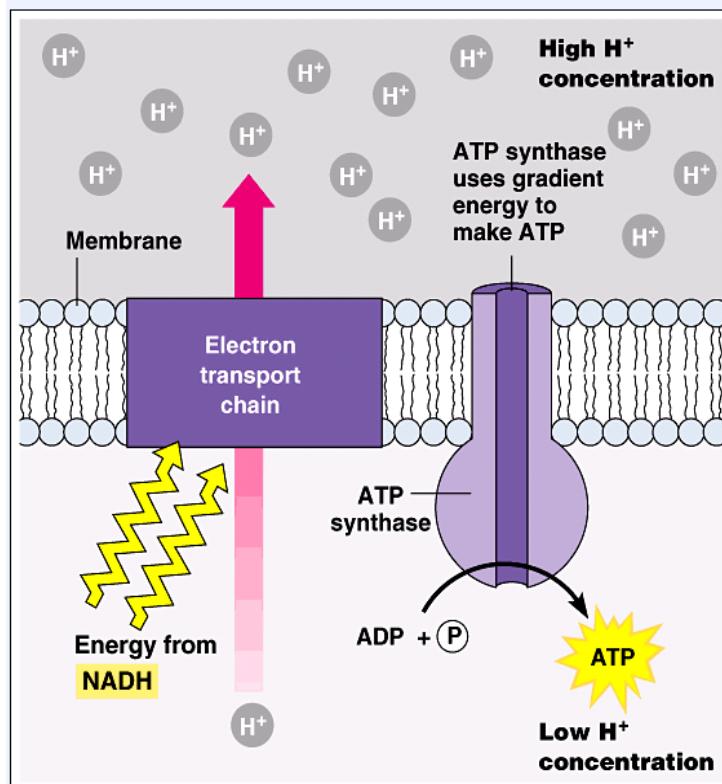


Chemiosmosis in Mitochondria:-

- As e⁻ pass from NADH + H⁺ / FADH₂ down the gradient to O₂, E is released.
- This E is used to pump H⁺ (protons)from matrix into inter membrane space against conc. / electrochemical gradient by active transport
- As proton conc. increases in inter membrane space; a strong diffusion gradient is set up.

- These protons can only exit through ATP synthase complex into matrix .

- E is released as protons flow down their conc. gradient through specific protein channels in inner membrane. This free E is utilized for ATP synthesis. The process catalyzed by a membrane protein complex ATP synthase. ATP synthase is present in elementary particles of inner membrane. Mitochondrial ATP synthase is an F-type ATP ase . ATP synthase has two distinct components : F₁ → peripheral membrane protein & F₀ → integral to above membrane. F₀ has a proton pore through which protons leak as fast as they are pumped by e⁻ transport. Without a proton gradient the F₁ depleted vesicles can't make ATP. On the other hand isolated F₁ catalyze ATP hydrolysis (reversal of synthesis) hence originally called as F₁ ATP ase. When purified F₁ is added back to depleted vesicles, it reassociates with F₀ plugging its proton pore & restoring membrane's capacity to couple e⁻ transfer & ATP synthesis.
- This transfer of protons along concentration gradient from inter membrane space to matrix is called chemiosmosis. It is an example of facilitated diffusion.



- Peter Mitchell proposed chemiosmotic model. According to this model transmembrane differences in proton concentration are the reservoir for E extracted biological oxidation reactions.

- Inhibitors of e⁻ transfer to O₂, like cyanide, CO, antimycin A, block ATP synthesis and vice versa. (oligomycin inhibits ATP synthase activity.) Thus these two processes show obligatory coupling.

Mitochondrial DNA:-

- Human mitochondrion contains 5 – 10 circular DNA molecules – mt – DNA.
- Mutation in mt – DNA causes human diseases; affecting mainly brain and muscles.
- In mammals 99.99% of mt DNA is inherited from mother. This is because in zygote paternal mitochondria are only about 100, while maternal are 100,000.

2) BIOENERGETICS

Living cells and organisms must perform work to stay alive and to reproduce themselves. The synthetic reactions that occur within cells, like synthetic processes in any factory, require the input of energy. Energy is also consumed in the motion of a bacterium or an Olympic sprinter.

Although the characteristic composition of an organism changes little through time, the population of molecules within the organism is far from static. Small molecules, macromolecules, and supra-molecular complexes are continuously synthesized and then broken down in chemical reactions that involve a constant flux of mass and energy through the system. The hemoglobin molecules carrying oxygen from your lungs to your brain at this moment were synthesized within the past month; by next month they will have been degraded and entirely replaced by new hemoglobin molecules. The amounts of hemoglobin in the blood remain nearly constant because the rate of synthesis balances the rate of its breakdown, the constancy of concentration is the result of a dynamic steady state, a steady state that is far from equilibrium. Maintaining this steady state requires the constant investment of energy; when the cell can no longer generate energy, it dies and begins to decay toward equilibrium with its surroundings.

Metabolism –The sum of all chemical transformations taking place in a cell /organism.

Metabolic pathways – A series of enzyme catalyzed reactions. Each step in it brings about specific, small chemical change like removal, transfer / addition of a particular atom / functional group.

Metabolites – Metabolic intermediates which convert precursors into products.

Intermediary metabolism – Combined activities of all metabolic pathways that interconvert precursors, metabolites & products of low molecular weight

Catabolism --The degradative phase of metabolism in which organic nutrient molecules (carbohydrates, fats, and proteins) are converted into smaller, simpler end products (such as lactic acid, CO₂, NH₃). Catabolic pathways release energy, some of which is conserved in the formation of ATP and reduced electron carriers (NADH, NADPH, and FADH₂); the rest is lost as heat.

Anabolism –(also called biosynthesis) Small, simple precursors are built up into larger and more complex molecules, including lipids, polysaccharides, proteins, and nucleic acids. Anabolic reactions require an input of energy.

Bioenergetics - The quantitative study of the energy transductions that occur in living cells and study of the nature and function of the chemical processes underlying these transductions. Biological energy is not in the form of heat mechanical or light energy therefore word thermodynamics is not used but word bioenergetics is used. This energy is termed as free energy and defined as energy available for work. It symbolizes change in energy and not the absolute energy.

Two approaches to study physical or chemical processes:

Kinetic molecular approach: In this, process is studied in terms of molecules and atoms.

Thermodynamic approach: Process is studied by considering energy changes involved. Thermodynamics means study of heat flow. But actually not only relation between heat and work but also deals with all kinds of inter conversion of one kind of energy in to the other. Most of the energy forms are ultimately converted in to heat.

Thermodynamics help to forecast whether certain physical or chemical transformations are possible or not. Under given set of conditions of temperature, pressure, concentration etc. But it can not give any information of time required for completion of change as well as rate of reaction.

If the system exchanges neither matter nor energy with its surroundings, it is said to be **isolated**. If the system exchanges energy but not matter with its surroundings, it is a **closed system**; if it exchanges both energy and matter with its surroundings, it is an **open system**.

A living organism is an open system; it exchanges both matter and energy with its surroundings. Living organisms derive energy from their surroundings in two ways:

- 1) They take up chemical fuels (such as glucose) from the environment and extract energy by oxidizing them; or
- 2) They absorb energy from sunlight.

Homogenous system – System with same chemical composition throughout.

Heterogenous system – System with two or more phases which are homogenous themselves but separated from each other by definite boundary. (ice and water)

State of a system: Variable of a state are temperature, pressure, volume, composition

Gas Equation: PV = nRT

Therefore if 2 values are known the third can be determined thus state of a simple homogenous system can be defined. Physical properties of a system are of two types:

Extensive properties: Depend on quantity of matter in the system under consideration e.g. mass, volume, energy

Intensive properties: Depend on nature of substance and independent of its amount e.g. temperature, pressure, viscosity, refractive index

Thermodynamic equilibrium: It is said to be achieved when observable properties like temperature, pressure, volume does not change with time. For thermodynamic studies a system must be in 3 types of equilibria which must exist simultaneously.

a) Thermal equilibrium

b) Chemical equilibrium

c) Mechanical equilibrium: No movement of particles of the constituents of system itself and between itself and surroundings.

Isothermal process: Temperature remains same

For Exothermal process – heat evolved give out immediately to surroundings to maintain the temperature. For endothermic process required amount of heat enters the system from surroundings to maintain the temperature.

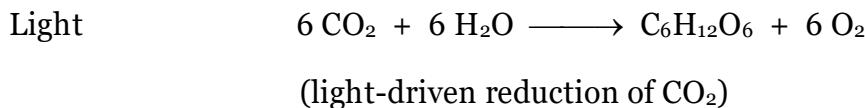
Adiabatic process – heat neither enters nor leaves the system during the process.

Thermodynamic laws and living organisms

The molecular complexity and orderliness of structure of living organisms is much higher in contrast to the randomness of non living matter.

The first law of thermodynamics, fully valid for biological systems

Photosynthetic cells absorb light energy and use it to drive electrons from water to carbon dioxide, forming energy-rich products such as glucose ($C_6H_{12}O_6$), starch, and sucrose and releasing O_2 into the atmosphere:



Non – photosynthetic cells and organisms obtain the energy they need by oxidizing the energy-rich products of photosynthesis and then passing electrons to atmospheric O₂ to form water, carbon dioxide, and other end products, which are recycled in the environment:



(energy-yielding oxidation of glucose)

DNA, RNA, and proteins are informational macromolecules. In addition to using chemical energy to form the covalent bonds between the subunits in these polymers, the cell must invest energy to order the subunits in their correct sequence. It is extremely improbable that amino acids in a mixture would spontaneously condense into a single type of protein, with a unique sequence. This would represent increased order in a population of molecules; but according to the second law of thermodynamics, the tendency in nature is toward ever- greater disorder in the universe: the total entropy of the universe is continually increasing. To bring about the synthesis of macromolecules from their monomeric units, free energy must be supplied to the system (in this case, the cell).

Second law of thermodynamics

How living organisms can create and maintain their intricate orderliness in an environment that is relatively disordered and becoming more with the time ? Living organisms do not constitute exceptions to thermodynamic laws. Their high degree of molecular orderliness must be paid for in some way since it can not arise spontaneously from disorder.

Living organisms have following characteristic properties such as,

- 1) **Use free energy:** Living organisms absorb useful form of energy that is free energy from surrounding under specific temperature and pressure and return less useful form of energy to the environment in equal amount. The useful form of energy returned by the living organisms is heat or other form that is quickly randomized in the environment and thus increase the entropy.
- 2) **Open system:** living organisms are not in equilibrium with the environment
- 3) **Steady state:** Cell is non equilibrium open system, a machine for extracting free energy from the environment which it causes to increase in randomness. The

rate of transfer of energy and matter from environment in to system is equal to transfer of energy and matter from system to environment.

4) **Non equilibrium:** Open system in steady state can do work in non equilibrium. Process under non equilibrium can be regulated. This is orderly state of an open system.

5) **Isothermal system:** The living system is essentially isothermal that is at any given time all parts of the cell have the same temperature. Furthermore, there are no significant differences in pressure between one part of the cell and another. For this reason, cells are unable to use heat as a source of energy, since, heat can do work at constant pressure only if it passes from a higher to a lower temperature zone.

6) **Isothermal chemical engines:** energy absorbed from environment is transformed to carry out synthesis of cell components, osmotic work, transport of material into cell, nerve conduction, muscle contraction etc. which takes place at constant struggle against the tendency to produce entropy. Synthesis of large and information rich macromolecules, the information of intricately structured cells, development of an organization, all these are powerful anti entropic doom imposed on all natural phenomena. Under the second law of thermodynamics, living organisms choose the least evil – they produce entropy at a minimum rate by maintaining steady state.

An attempt to produce a machine which could produce more mechanical work than the equivalent energy used is failed. This compels to accept the first law of thermodynamics in biological systems.

Mathematical formulation of first law

Suppose some amount of heat is put in the system, Since heat can not be lost it must remain either partial or whole in the system, or can be used up by the system in doing mechanical work.

In general case, when both happen,

Heat absorbed = increase of internal energy + work done by the system

If final and initial internal energy of the system is E_2 and E_1 respectively, then increase internal energy is $\Delta E = E_2 - E_1$

If heat absorbed is q and work done is w then

$\Delta E = E_2 - E_1 = q - w$ (this is first law of thermodynamics)

Although the heat absorbed / the work done by the system might vary the path by which the change is affected ΔE is always same.

Second law of thermodynamics:

1. First law explains the equivalence between heat and work but imposes no condition on their mutual convertibility. It never explains under what circumstances and to what extent it is possible to convert one form of energy in to other.
2. It also explains about the amount of heat lost by a hot body must be equivalent to the gain by cold body. But it does not explain that heat has to flow spontaneously from hot to cold body and not in reverse direction.
3. Different forms of energy can be readily and completely converted in to heat but not possible to convert back heat completely in to work. Hence, there must be some other law besides the first law that governs the direction of flow of heat and extent of its convertibility in to work. This limitation forms the basis for second law of thermodynamics. **The total entropy of a system must increase if the process has to occur spontaneously.**

Entropy: The quantitative expression for randomness or disorder of the components of a chemical system is expressed as entropy, S.

When the products of a reaction are less complex & more disordered than the reactants, the reaction proceeds with a gain in entropy. Any change in randomness of the system is expressed as **entropy change**, ΔS , which by convention has a positive value when randomness increases. **J. Willard Gibbs**, who developed the theory of energy changes during chemical reactions, showed that the **free energy content**, G, of any closed system can be defined in terms of three quantities:

Enthalpy, H – heat content of reacting system, reflecting the number and kinds of chemical bonds in the reactants & products; **Entropy, S**; and the **absolute temperature, T** (in degrees Kelvin).

The definition of free energy is $G = H - TS$.

When a chemical reaction occurs in biological system at constant temperature & pressure, the free-energy change, ΔG , is determined by the enthalpy change, ΔH , reflecting the kinds and numbers of chemical bonds and non covalent interactions broken and formed, and the entropy change, ΔS , describing the change in the system's randomness:

$$\Delta G / \Delta F = \Delta H - T \Delta S \quad (F \rightarrow \text{Helmholtz free E}, T \rightarrow \text{absolute temp.})$$

$$\text{Also } \Delta G / \Delta F = \Delta E - T \Delta S \quad (E/Q \rightarrow \text{internal energy})$$

Hence total energy of the system is $\Delta E = \Delta G + T \Delta S$

If **ΔG is negative**, the reaction would proceed spontaneously with loss of free energy that is exergonic reaction. If in addition, ΔG is of great magnitude, the reaction goes virtually to completion and is essentially irreversible.

If **ΔG is positive**, the reaction can not occur spontaneously and would proceed only if the free energy can be gained that is endergonic.

If **ΔG is zero**, the system is at equilibrium and no net change takes place.

Relationship between equilibrium constant and standard free energy change in a model reaction. Thus, in a reaction, $A + B \leftrightarrow C + D$



$$\Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]}$$



When the concentration of $[A]$ $[B]$ $[C]$ $[D]$ ΔG is 0.1 M, ΔG° known as standard free energy change. At equilibrium, $\Delta G^\circ = 0$



$$\text{i.e. } \Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]}$$

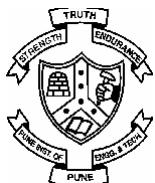


For biochemical reactions, a standard state is defined as having a pH of 7. the standard free energy change at this standard state is denoted by ΔG° since the equilibrium constant under standard condition is;

$$K'_{\text{eq}} = [C] [D] / [A] [B]$$

$$\text{Substitution gives } \Delta G^\circ = -RT \ln K'_{\text{eq}}$$

Thus, the standard free energy change can be calculated from the equilibrium constant K'_{eq} it is important to note that ΔG may be larger or smaller than ΔG° depending on the concentration of various reactants.



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CT16002 – Biology for Engineers

UNIT IV: Expression and transmission of Genetic Information

DNA replication, Enzyme driven process of DNA cloning, Protein synthesis-Transcription & translation Techniques for optimization: a. At molecular level: Recombinant DNA Technology, DNA hybridization, PCR, DNA microarray

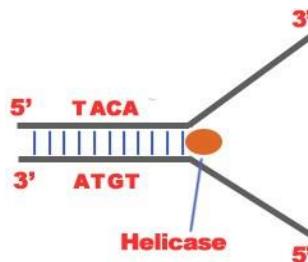
DNA Replication

DNA is capable of self reproduction. Parent DNA produces two daughter DNA molecules which are exact copies / replicas of parent DNA in N₂ base sequence. Hence DNA duplication is called as DNA replication.

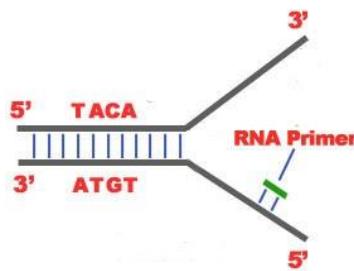
Definition-- It is making exact copies of parent DNA. It is bidirectional.

Steps of DNA Replication --

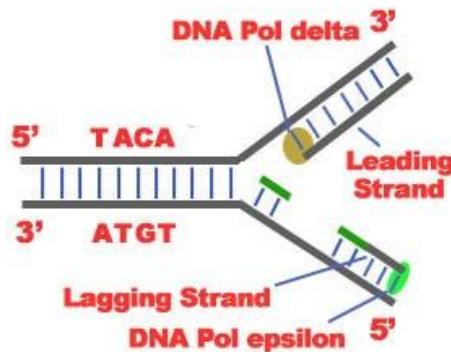
- 1) The first major step for the **DNA Replication** to take place is the breaking of hydrogen bonds between bases of the two antiparallel strands. The unwinding of the two strands is the starting point.
- 2) Activation of deoxyribonucleotides by energy & enzyme **Phosphorylase**.
- 3) Enzyme **Endonuclease** makes cut to one of the strands of DNA. The splitting happens in places of the chains which are rich in A-T. That is because there are only two bonds between Adenine and Thymine (there are three hydrogen bonds between Cytosine and Guanine). **Helicase** is the enzyme that splits the two strands. The initiation point where the splitting starts is called "origin of replication". The structure that is created is known as "**Replication Fork**".
- 4) **Topoisomerase, helix destabilizing protein** stabilize replication fork.



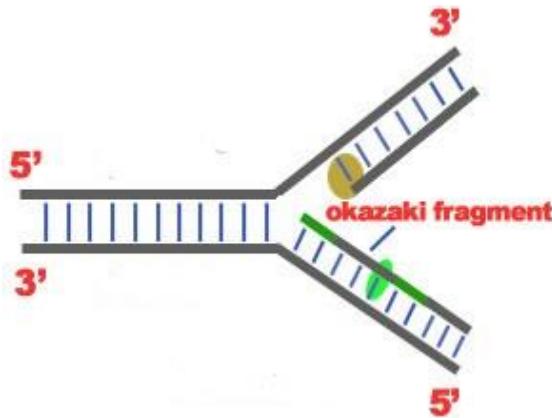
- 5) **Primase** synthesizes RNA primer that attaches at 3' end. RNA primer functions as 5' end of new strand.



- 6) Two separated strands function as templates. Initiation of replication occurs at 3' end.
- 7) DNA polymerase adds nucleotides in 5' → 3'direction continuously complementary to the nucleotides of the template, e.g. A—T. This strand is called **leading strand**.
- 8) On the other strand DNA polymerase adds nucleotides in 5' → 3'direction in short fragments. Hence it is called as **lagging strand**. The newly synthesized DNA fragments are called as **Okazaki fragments**. These fragments are **joined by DNA ligase & become continuous**.



- 9) Once replication is completed, RNA primer is removed& DNA nucleotides are synthesized by **DNA polymerase**.
- 10) **Proof reading** – Mismatched N₂ bases are removed by **endonuclease** & appropriate N₂ bases are introduced by **DNA polymerase**.
- 11) In daughter DNA, 2 strands coil around each other to form helix.



12) Thus in daughter DNA one strand is old i.e. conserved & another strand is new. Hence it is known as "**semiconservative replication**".

Protein Synthesis:

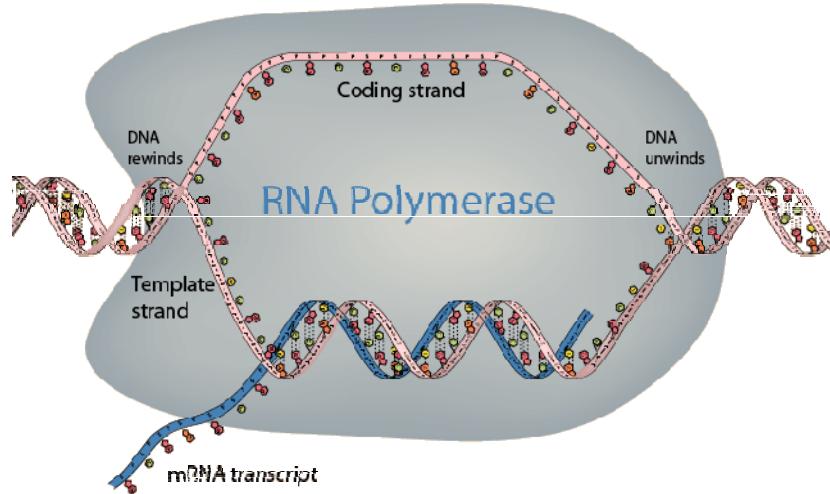
Protein synthesis is the main mechanism in body with respect to growth and changes in the cell. It results in the production of amino acid chains which are for proteins (important component in body). But for short term reactions importance of protein synthesis is the production of variety enzymes for different reactions as needed by the body for that moment. Since we cannot exist without enzymes, protein synthesis is needed for our existence. The process of protein synthesis translates the codons (nucleotide triplets) of the messenger RNA (mRNA) into the 20-symbol code of amino acids that build the polypeptide chain of the proteins.

DNA \longrightarrow mRNA \longrightarrow PROTEIN is known as CENTRAL DOGMA in the process of protein synthesis. It has 2 steps— transcription (DNA \longrightarrow mRNA) and translation (mRNA PROTEIN).

TRANSCRIPTION

The first step in protein synthesis is the transcription of mRNA from a DNA gene in the nucleus. In this phase, one strand of DNA double helix acts as a template to synthesize its complimentary strand i.e. mRNA. Transcription starts with an enzyme called **polymerase** copying the DNA sequence to a similar molecule called messenger RNA (mRNA). This synthesis takes place in a specific portion of DNA which is known as transcription bubble. In the bubble, DNA strands are separated or unzipped

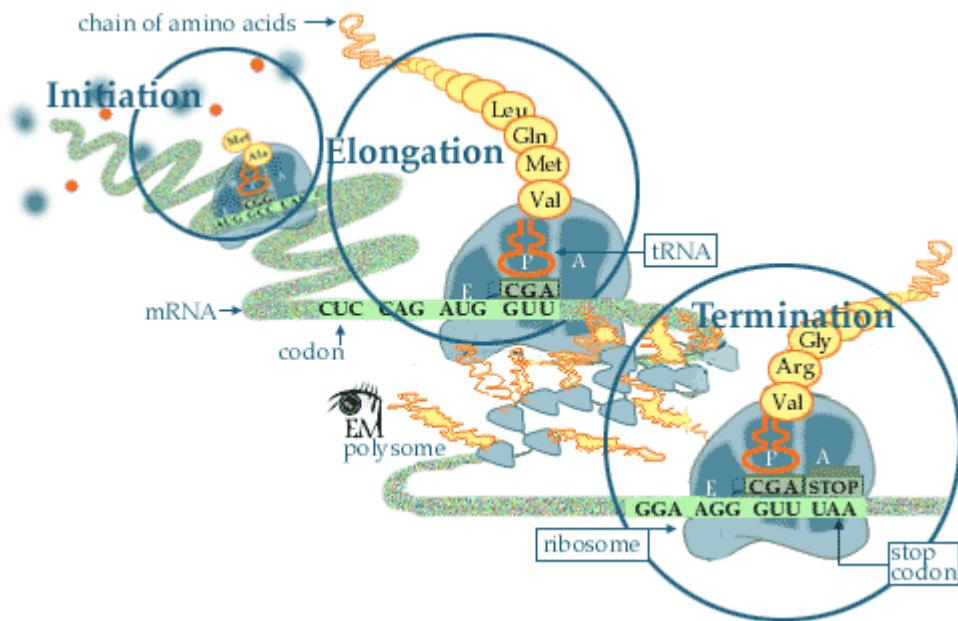
The process of mRNA translation begins from its 5'-end towards its 3'-end as the polypeptide chain is synthesized from its amino-terminal (N-end) to its carboxyl-terminal (C-end). It replaces T with U (Uracil), a helper base, making it clear that the mRNA is a copy. The bases (A, T, G, C) on one strand of the DNA specify the order of bases on the new strand of mRNA (A, U, G, C). At the end of transcription, DNA stays inside the nucleus while the RNAs migrate from the nucleus into the cytoplasm.



TRANSLATION

This is the second phase of protein synthesis where the ribosomes in the cytoplasm use transfer RNA (tRNA) to attach to the mRNA and translate the bases into amino acids. tRNA molecules bring the specified amino acids that the ribosome links together to make a protein. Translation has four steps viz. Activation and charging, Initiation, Elongation and Termination

Before the actual translation, **activation**, which is not part of translation in technical sense, occurs. In this step, the correct amino acid (AA) is joined to the correct tRNA. It is required for translation to proceed. When the activated tRNA has an amino acid linked to it, it is termed as "**charged**". The AA is joined by its carboxyl group to the 3' OH of the tRNA by an ester bond.



Initiation: In the cytoplasm, protein synthesis is actually initiated by the AUG codon on mRNA. The AUG codon signals both the interaction of the ribosome with mRNA and also the tRNA with the anticodons (UAC). The tRNA which initiates the protein synthesis has N-formyl-methionine attached. The formyl group is really formic acid converted to an amide using the -NH₂ group on methionine (left most graphic)

The next step is for a second tRNA to approach the mRNA (codon -CCG). This is the code for proline. The anticodon of the proline tRNA which reads this is GGC. The final process is to start growing peptide chain by having amine of proline to bond to the carboxyl acid group of methionine (met) in order to elongate the peptide.

Elongation: Elongation of the peptide begins as various tRNA's read the next codon. In the example on the left the next tRNA to read the mRNA is tyrosine. When the correct match with the anticodons of a tRNA has been found, the tyrosine forms a peptide bond with the growing peptide chain. The proline is now hydrolyzed from the tRNA. The proline tRNA now moves away from the ribosome and back into the cytoplasm to reattach another proline amino acid.

Elongation and Termination: When the stop signal on mRNA is reached, the protein synthesis is terminated. The last amino acid is hydrolyzed from its t-RNA. The peptide chain leaves the ribosome. The N-formyl-methionine that was used to initiate the protein synthesis is also hydrolyzed from the completed peptide at this time.

The ribosome is now ready to repeat the synthesis several more times. The events following translation include post-translational modification and protein folding. During

and after synthesis, polypeptide chains often fold to attain secondary, tertiary and quaternary structures. This process is known as **maturation of protein** and it takes place in Golgi Complex.

GENETIC ENGINEERING

Recombinant DNA Technology, Genetic modification/manipulation (GM) and Gene splicing are terms that apply to the direct manipulation of an organism's genes. Genetic engineering is different from traditional breeding, where the organism's genes are manipulated indirectly.

Definition --Genetic engineering uses the techniques of molecular cloning and transformation to alter the structure and characteristics of genes directly.

Genetic engineering techniques have found some successes in numerous applications. Some examples are in improving crop technology, the manufacture of synthetic human insulin through the use of modified bacteria, the manufacture of erythropoietin in hamster ovary cells, and the production of new types of experimental mice such as the oncomouse (cancer mouse) for research.

Definition Clone: A clone is a group of identical copies. A clone of a cell is a group of cells of a single type isolated and allowed to reproduce to create a population of identical cells. A clone of a DNA molecule is an isolated DNA molecule which is multiplied number of times to produce a large amount of identical copies.

Definition Cloning : It is a method of producing identical copies of cells / molecules/organisms.

There are a number of ways through which genetic engineering and cloning together is accomplished. Essentially, the process has following steps :-

1. Isolation of the gene/ DNA fragment of interest (known function) from an organism (**A**) . It is known as an **insert**.
2. Enzymatic cleavage (**B**) and joining (**C**) of insert DNA to another DNA molecule (cloning vector) to form recombinant DNA (rDNA) i.e. vector + insert DNA (**D**)
3. Transformation of a host cell that includes transfer and maintenance of rDNA molecule in the host organism (**E**)
4. Identification of transformed cells (with rDNA) and their selection from non transformants
5. Amplification of rDNA to get multiple copies in a cell (**F**)
6. Cell multiplication (**G**) to get clones (population of genetically identical individuals carrying multiple copies of foreign DNA)

Isolation of gene of interest:

- Isolation is achieved by identifying the gene of interest that the user wishes to insert into the organism, usually on the basis of existing knowledge about various functions of genes. This segment of DNA is the molecule is used for the cloning process.
- Isolation is carried out by using variety of Restriction Endonucleases or Restriction Enzymes (e.g. *EcoR1*) that recognize the site of cleavage (cut) on DNA at specific palindromic sequences.

- Broadly these enzymes are categorized in three categories Type I, II and III. Recognition site and cleavage site is same in Type II while it is different in Type I and III. Thus, type II enzymes are widely used in genetic engineering especially for gene manipulation.

The details of these groups are as follows

Type I <i>EcoB, EcoK</i>	Recognition site for type I enzyme is 15 bp long and cleavage site 1000 bp away; the enzyme has restrictive subunit, modification subunit and specificity subunit; requires Mg ⁺⁺ , S-adenosyl methionine and ATP as cofactors
Type II <i>EcoR1/R2</i> <i>Hae III</i> <i>BamH1</i>	The length of recognition site for type II enzyme varies from 4/5/6/8 or more bp long and cleavage site 1000 bp away; the enzyme has restrictive subunit, modification subunit and specificity subunit; more stable and requires Mg ⁺⁺ as cofactors
Type III <i>MboII</i> <i>FokI</i>	Made up of 2 subunits for recognition and cleavage; requires ATP for energy and Mg as co factor; recognition site is non palindromic and cleavage site 25-27 bp away

- As a result of these enzymes, the DNA from donor cell is cut in such a way that it has sticky/staggered cuts or sometimes blunt ends.

Insertion of the above mentioned gene of interest into a cloning / transfer vector

- This can be done with the opening of vector DNA molecule with a cut by the same restriction enzyme (i.e. producing similar kind of the sticky or blunt ends).
- Thus the foreign DNA and the vector DNA (plasmid) now share the complementarities at these ends.
- Then the addition of DNA ligase joins the two DNA molecules by ligating their nucleotides with each other. This prepares the recombinant DNA vector i.e. the recombinant plasmid.
- A vector is a cloning vehicle i.e. the agent used to multiply the isolated gene. It itself is a synthesized DNA molecule that carries the isolated gene into a host where it can replicate producing many of its copies.
- Hence the same number of the copies the gene of interest are generated. The product thus generated is called the **Recombinant DNA** (rDNA) and the technique is called gene cloning.
- A variety of vectors which are actually the cloning vehicles have been developed which allow the multiplication of the inserted gene.
- The most common vectors are plasmids. Other vectors can also be used, such as viral vectors, and non-prokaryotic ones such as liposomes, or even direct insertion using DNA guns.

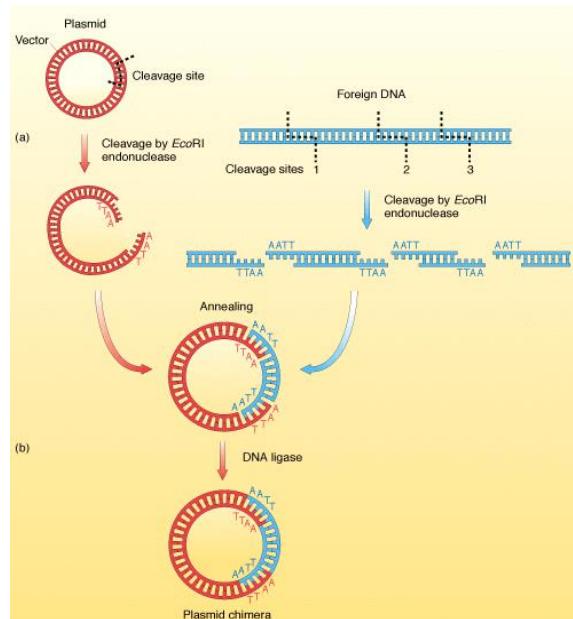
Commonly used vectors:

- Plasmids:** Extra-chromosomal, closed, circular, double stranded DNA molecules present in bacterial cells. May present independently or integrated in the bacterial chromosome. Capable of independent replication and

transmission. Many times

specify number of properties of the host. e.g. antibiotic resistance, heavy metal resistance, nitrogen fixation. Plasmids are used with *E. coli* cells. They are introduced in the bacterial cells by the process of transformation. The *E. coli* cells and plasmid DNA are incubated together at 0°C in CaCl₂ solution, then subjected to the heat shock by rapidly changing the temperature to 43°C. Few cells of the bacteria take up the plasmid DNA. Plasmid DNA can also be introduced in *E. coli* cells by electroporation during which the mixture of the cells and the plasmid is subjected to a high voltage pulse. The cell membrane becomes permeable and the plasmid can enter the cells.

- **Bacteriophages:** Also called phages which are the viruses that infect bacteria. They have the ability to attach the host (bacterium) and transfer their own DNA to the host. Most frequently used phage vectors are lambda (λ) phage and M13. The genome of λ phage is a linear double stranded with 12 bases long single stranded stretch at 5' end which can be used as cohesive/sticky ends. Also there is a large non-essential region in its DNA which can be replaced with the foreign DNA. Such λ vectors can accommodate large DNA inserts around 40,000 – 53,000 b p (base pair) long. These vectors can then be packaged into the infectious phage particles. The infectious phages bring about the lysis of the host cell.
- **BACs:** Bacterial artificial chromosomes, A **bacterial artificial chromosome (BAC)** is a DNA construct, from plasmids. They can contain very long DNA inserts (100,000-300,000) as a foreign DNA.
- **YACs:** **Yeast artificial chromosome** (short YAC) is a vector used to clone large DNA fragments (larger than 100 kb and up to 3000 kb). It is an artificially constructed chromosome and contains the sequences needed for replication and preservation in yeast cells.
- **Shuttle vectors:** these are the plasmid DNAs that can be propagated in the cells of 2 or more different species.



Transfer of the vector by transformation of the host cell is the next step. The commonest type of host organism used for introducing the vectors (plasmids and viral vectors) is *Escherichia coli*. Its DNA metabolism is well-understood and plasmids are the naturally occurring circular DNA molecules in the *E. coli*.

Transformation is the process by which the *E. coli* are made receptive by some heat shock or electric shock and the recombinant plasmid DNA is kept in the surrounding medium of such competent *E. coli* cells. The recombinant plasmid DNA enters the *E. coli* cells. Such organisms are called **GMO or Genetically Modified Organisms**.

Selection of the transformed organisms is required to find out only those organisms which have received the recombinant from those which have not. The selection is carried out by various ways –

a) Direct Selection:

- If the cloned DNA itself codes for resistance to the antibiotic ampicillin (amp^R), the recombinants can be allowed to grow on the minimal medium containing ampicillin.
- Thus only such recombinants will grow and develop colonies on medium, which contain amp^R gene on its plasmid vector.
- But with this method one can not segregate the transformed cells with re-ligated plasmid (i.e. cut by endonuclease & joined again)vector from the recombinant plasmid.

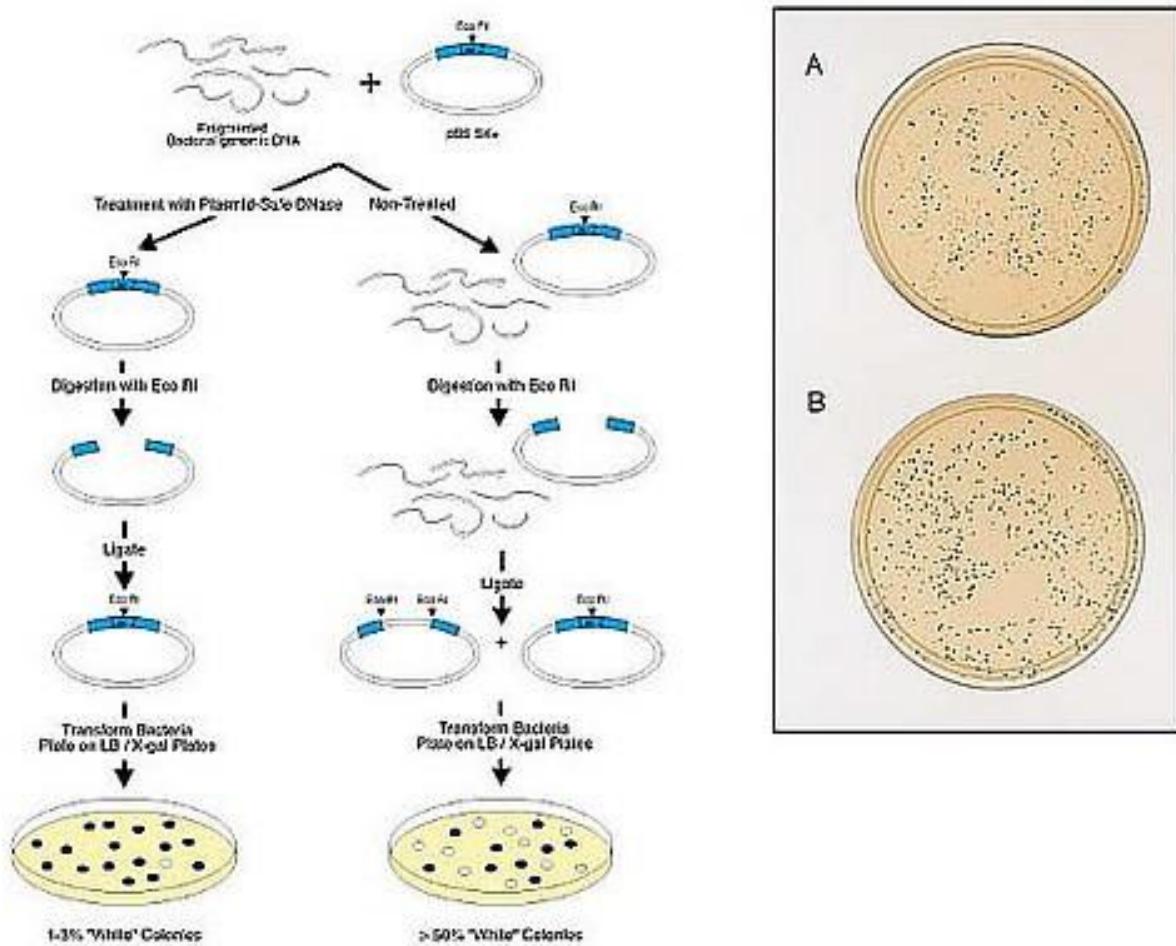
b) Insertional Selection Inactivation:

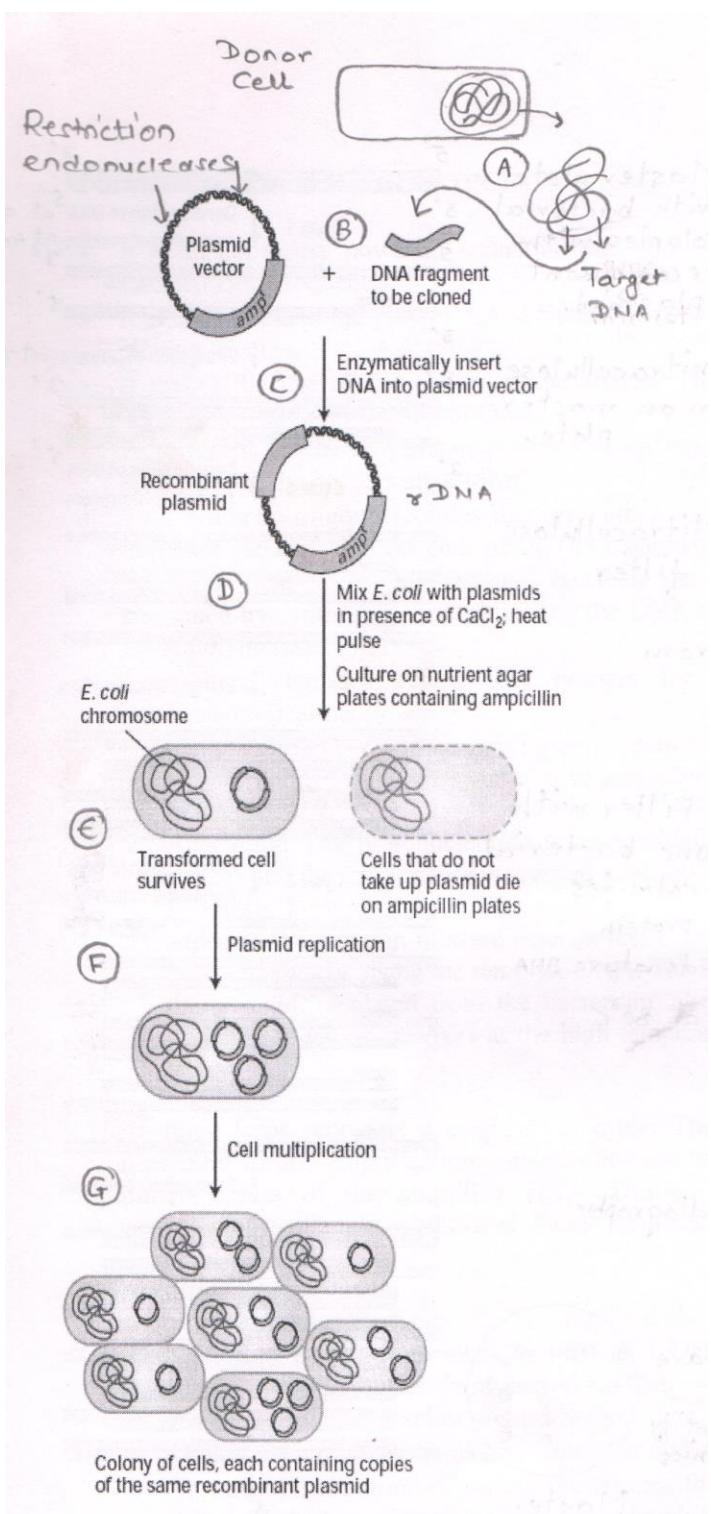
- In this method, as discussed earlier, the vector plasmid is constructed with some specific DNA sequences which confer the antibiotic resistance to the host cell to two different antibiotics (e.g. ampicillin and tetracycline).
- It is designed in such a way that the foreign gene gets inserted in this part of the plasmid disrupting one of these genes. As a result the resistance for one antibiotic is lost by the recombinant plasmid whereas the plasmid DNA without the insert (foreign DNA) has the intact resistance for both the antibiotics.
- Thus the host cells that have not taken the plasmid cannot grow on the medium containing both the antibiotics.
- Those cells which get transformed by the non-recombinant DNA have intact resistance for both the antibiotics.
- The *E. coli* cells that get transformed by the recombinant plasmid lose resistance for one of the antibiotics and retain for the other.
- Hence the cells are first grown on medium with ampicillin and then on medium with tetracycline.
- This strategy can be exploited for identifying the host cells that have taken the foreign gene. These cells are then grown in cultures to produce multiple copies of the foreign gene (as explained in the diagrammatic representation).

c) Blue White Colony

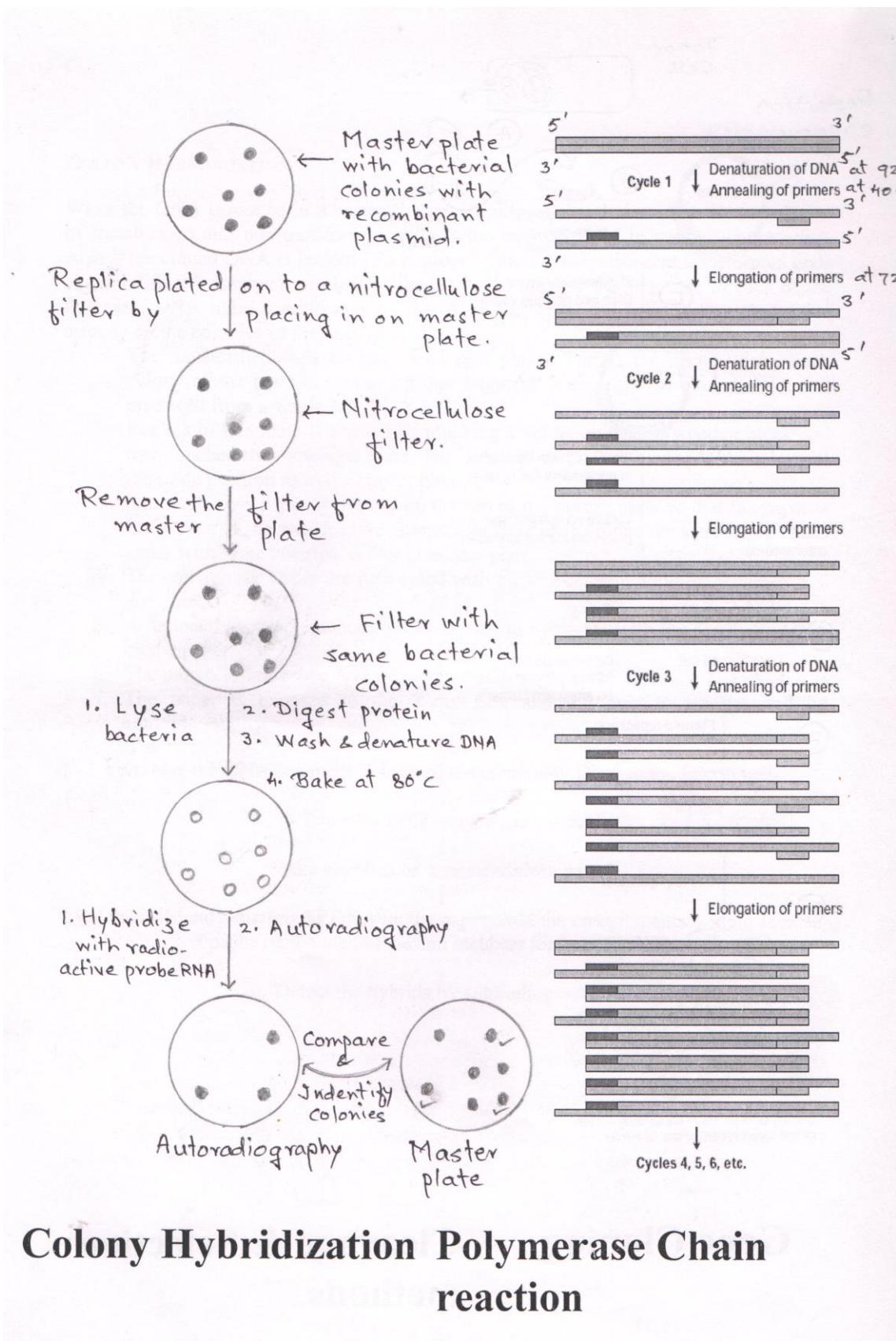
- In this method instead of antibiotic resistance gene, a lacZ gene is constructed which is responsible for the synthesis of beta-galactosidase enzyme.

- The enzyme reacts with chemical B galactose and develops blue colour.
- In the recombinant plasmid, the introduced gene disrupts(alter function) lacZ gene and thus prevents further reaction if grown on the medium containing B galactose.
- Against this, the non recombinant plasmid certainly develops blue colour on the growing plate and can easily be differentiated from white colonies of recombinant plasmids which use glucose from the medium.



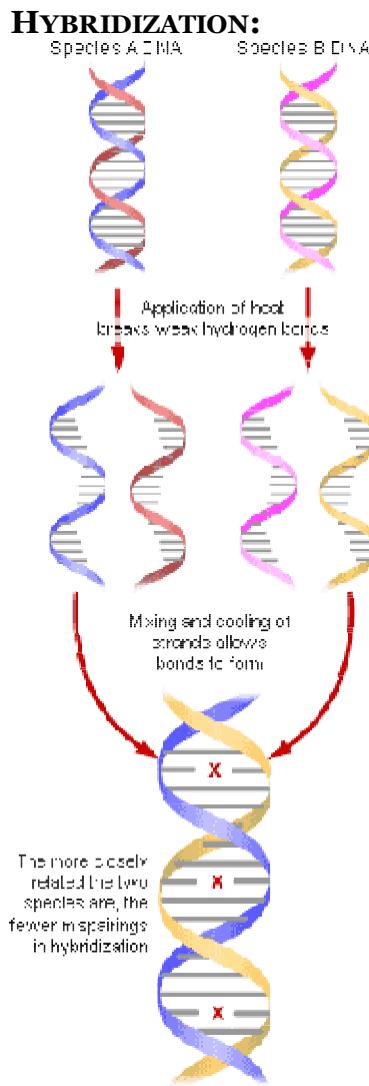


Gene Cloning



Colony Hybridization Polymerase Chain reaction

DNA



- Hybridization technique makes use of the ability of DNA molecule to renature or reanneal when it is converted to single stranded structure.
- It is the method that detects DNA by its hydrogen bonding capability - we call this "probing" the DNA, and we say that the probe "hybridizes" to the target sequence.
- Also if any two DNA molecules share any complementarity, they can bind each other to form a double stranded DNA molecule.
- When any two different DNA samples are to be compared they are first denatured. They are then mixed and the mixture is cooled i.e. allowed to renature.
- During renaturation, the two DNA strands of the original molecule anneal and also the strands of the two different molecules anneal if they have significant similarity. Depending upon the extent of the similarity between them, they will form DNA duplexes of various lengths. Thus they form hybrids (if the renatured DNA has formed from the two different sources present in the mixture).
- These hybrids are the partial duplexes.
- This technique is highly useful in detecting similarity between two genes or DNA molecules from different organisms or species etc.
- Similarly, a small complementary probe also can hybridize with the small region of the DNA to be detected or studied. This probe is usually labeled with radioactive or fluorescent label.

The level of the radioactivity or fluorescence is detected after the two DNA solutions are mixed and allowed to renature. The intensity of the label is directly proportional to the complementarity of the two DNA molecules. This method can be implemented to detect a gene sequence.

Like DNA – DNA hybridization RNA –DNA hybridization can also be carried out.

The overall process can be summarized as follows,

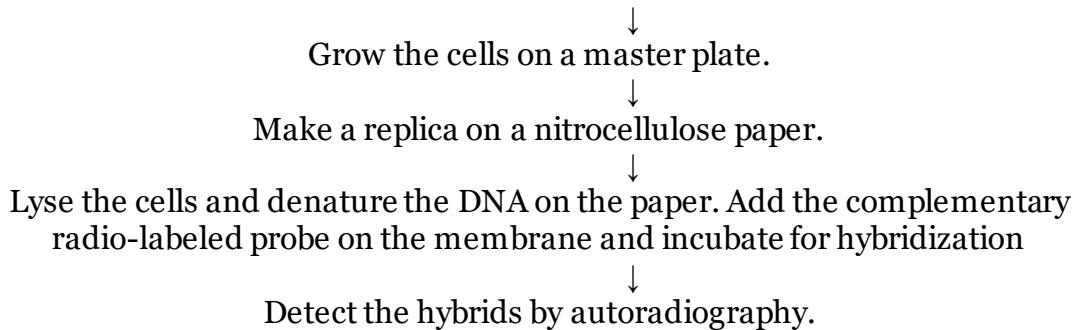
Denatured DNA sample 1 + Denatured DNA sample 2 → Cool for renaturation and/or hybridization → Hybrid DNA duplexes → Screen the label by its radioactivity or fluorescence.

COLONY HYBRIDIZATION

When the DNA is cloned in a bacterial host, identifying the cloned DNA in the mixture of transformed and non-transformed cells is the step involved in genetic engineering. Also if the cloned DNA is broken into number of pieces, then different transformed cells contain different fragment or piece of the DNA. To identify the sequence of each of the fragment, DNA hybridization is very useful. In this case, the hybridization is carried directly on the colonies of the bacterial cells.

1. The transformed cells are grown on agar plates. This is the master plate. Each colony of the plate is containing one fragment of the original cloned DNA and produced from a single cell.
2. Replica of this plate is created by touching a velvet cloth or a wooden block and then touched to a new agar plate. The new replica plate is having same colony at the same position as in the master plate.
3. Nitro-cellulose paper is pressed on the top of the master plate so that the paper is the replica of the master plate. Some cells from each colony are transferred to the paper with same position as that in master plate.
4. The cells on the paper are now lysed with alkali treatment and also their DNA is denatured.
5. A radioactive complementary probe is used to hybridize with the denatured DNA on the paper.
6. The unhybridized probe is removed by washing.
7. The paper is exposed to the X-ray film and the hybrids are detected by autoradiography.

Introduce the DNA fragment in form of a recombinant DNA in the E. coli cells



POLYMERASE CHAIN REACTION (PCR)

- It is an extremely powerful technique that allows a million fold amplification of selected DNA sequence.
- It is used to clone the given DNA sequence *in vitro* without using living cells during cloning process.
- It makes the use of the ability of the enzyme DNA polymerase to carry out the semi-conservative replication of DNA.
- PCR results in the amplification of a chosen region of DNA molecule when the sequences of the borders are known.
- Two synthetic oligonucleotides that are complementary to end parts of the chosen sequence can anneal to the ends of the DNA segment.
- The amplification of this segment between the two defined ends can be then continued by synthesizing or replicating the DNA with the help of a special enzyme DNA polymerase.
- The oligonucleotides act as the primers for the enzyme to complete the synthesis/replication.
- This amplification is achieved by a repetitive series of cycles involving three steps.
 1. Denaturation: The DNA sample to be amplified (template DNA) is denatured by heating at 92°C.
 2. Annealing: The oligonucleotide primers added to the separated template DNA strands and the temperature is reduced to 40-60 °C.
 3. Synthesis by extending the primers: At 72°C DNA polymerase that has been added in the reaction mixture extends the 3' ends of the oligonucleotide primers complementarily using the template DNA. The DNA polymerase used in PCR is a thermostable, isolated from the bacterium *Thermus aquaticus*. It is called Taq polymerase which survives at the high temperature required during denaturation step.

These three steps represent a single PCR cycle. The products of the first cycle are replicated for further amplification. This reaction can be performed many times to supply unlimited copies of the amplified DNA. During repetition of the cycle regular denaturation of the freshly synthesized double stranded DNA molecules is carried out.

Applications of PCR

- After 25 cycles the target DNA is amplified about 10^6 fold.
- PCR can detect and amplify as little as 1 DNA molecule. It allows successful cloning of DNA even from samples which are more than 40,000 years old or mummified human bodies, extinct animals like wooly mammoth or dinosaurs.
- Highly useful in new fields like molecular archaeology, molecular palaeontology
- To trace the evolution of pathogenic viruses, forensic medicine, detection of viral infections before causing the disease or showing the symptoms, prenatal diagnosis of genetic disorders

It was used for Human Genome Project.

DNA Microarray

- **DNA** microarrays are solid supports, usually of glass or silicon, upon which DNA is attached in an organized pre-determined grid fashion.
- Each spot of DNA, called a probe, represents a single gene.
- DNA microarrays can analyze the expression of tens of thousands of genes simultaneously.
- There are several synonyms of DNA microarrays such as DNA chips, gene chips, DNA arrays, gene arrays, and biochips.

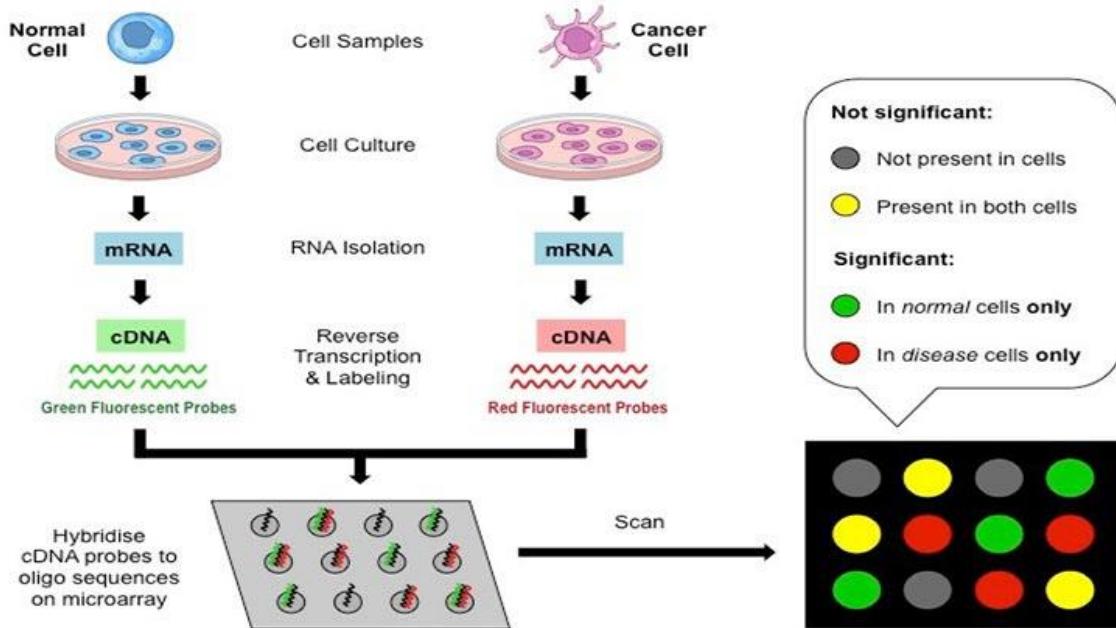


Image Source: [BioNinja](#)

Principle of DNA Microarray Technique

- The principle of DNA microarrays lies on the hybridization between the **nucleic acid** strands.
- The property of complementary nucleic acid sequences is to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs.
- For this, samples are labeled using fluorescent dyes.
- At least two samples are hybridized to chip.
- Complementary nucleic acid sequences between the sample and the probe attached on the chip get paired via hydrogen bonds.
- The non-specific bonding sequences remain unattached and washed out during the washing step of the process.
- Fluorescently labeled target sequences that bind to a probe sequence generate a signal.
- The signal depends on the hybridization conditions (ex: temperature), washing after hybridization etc while the total strength of the signal, depends upon the amount of target sample present.
- Using this technology the presence of one genomic or cDNA sequence in 1,00,000 or more sequences can be screened in a single hybridization.

Steps Involved in cDNA based Microarray

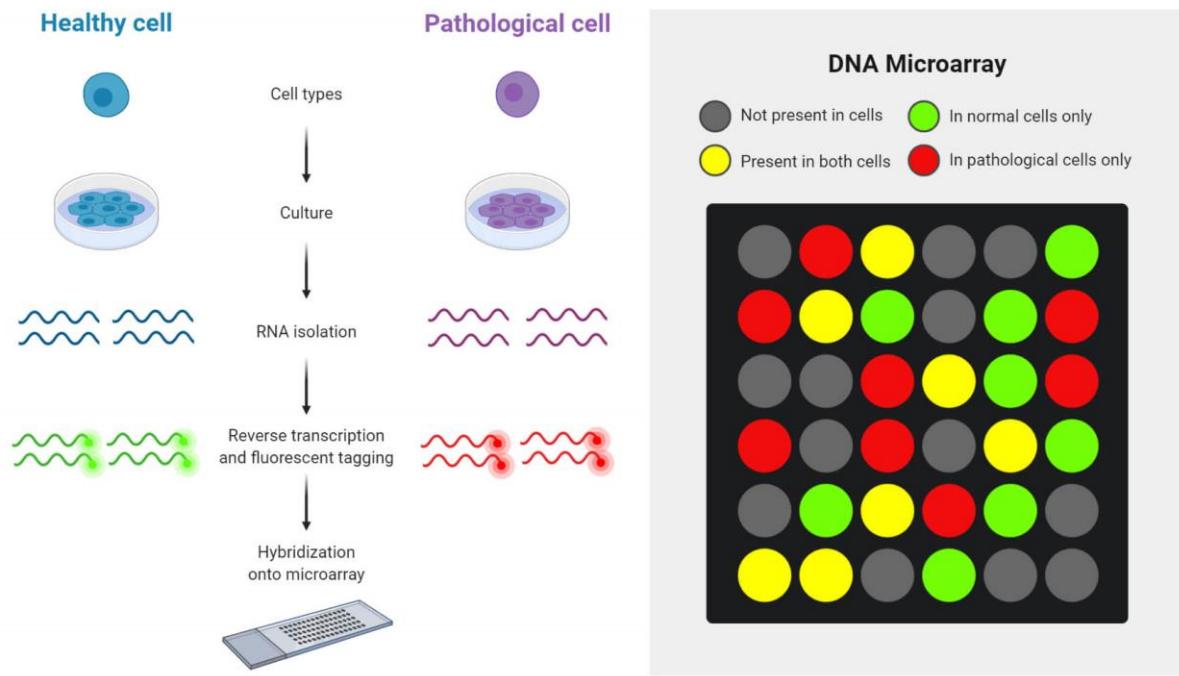


Image By Sagar Aryal, created using biorender.com

The reaction procedure of DNA microarray takes places in several steps:

1. Collection of samples

- The sample may be a cell/tissue of the organism that we wish to conduct the study on.
- Two types of samples are collected: healthy cells and infected cells, for comparison and to obtain the results.

2. Isolation of mRNA

- RNA is extracted from the sample using a column or solvent like phenol-chloroform.
- From the extracted RNA, mRNA is separated leaving behind rRNA and tRNA.
- As mRNA has a poly-A tail, column beads with poly-T-tails are used to bind mRNA.
- After the extraction, the column is rinsed with buffer to isolate mRNA from the beads.

3. Creation of labeled cDNA

- To create cDNA (complementary DNA strand), reverse transcription of the mRNA is done.
- Both the samples are then incorporated with different fluorescent dyes for producing fluorescent cDNA strands. This helps in distinguishing the sample category of the cDNAs.

4. Hybridization

- The labeled cDNAs from both the samples are placed in the DNA microarray so that each cDNA gets hybridized to its complementary strand; they are also thoroughly washed to remove unbounded sequences.

5. Collection and analysis

- The collection of data is done by using a microarray scanner.
 - This scanner consists of a laser, a computer, and a camera. The laser excites fluorescence of the cDNA, generating signals.
 - When the laser scans the array, the camera records the images produced.
 - Then the computer stores the data and provides the results immediately. The data thus produced are then analyzed.
 - The difference in the intensity of the colors for each spot determines the character of the gene in that particular spot.
-

Applications of DNA Microarray

- In humans, they can be used to determine how particular diseases affect the pattern of gene expression (the expression profile) in various tissues, or the identity (from the expression profile) of the infecting organism. Thus, in clinical medicine alone, DNA microarrays have huge potential for diagnosis.

Besides, it has applications in many fields such as:

- Discovery of drugs
 - Diagnostics and genetic engineering
 - Alternative splicing detection
 - Proteomics
 - Functional genomics
 - DNA sequencing
 - Gene expression profiling
 - Toxicological research (Toxicogenomics)
-

Advantages of DNA Microarray

- Provides data for thousands of genes in real time.
 - Single experiment generates many results easily.
 - Fast and easy to obtain results.
 - Promising for discovering cures to diseases and cancer.
 - Different parts of DNA can be used to study gene expression.
-

Disadvantages of DNA Microarray

- Expensive to create.
- The production of too many results at a time requires long time for analysis, which is quite complex in nature.
- The DNA chips do not have very long shelf life.



College of Engineering, Pune

(An Autonomous Institute of Government of Maharashtra)

Applied Science Department

CT16002 – Biology for Engineers

UNIT V: Complex processes- Transport, communication and Defense

1. Transport Phenomena in Biological Systems:

Membrane, channels and ion channels; Fluid flow and mass transfer

- a. In plants: Xylem and Phloem
- b. In animals: Blood and Lymph
- c. Transport of molecules and gases (Oxygen and Carbon dioxide)
- d. Heat Transport - Body temperature regulation

2. Communication: Cell junctions, Cell-cell communications – cell signaling, Hormones, Pheromones; Chemotaxis. Communication in living systems by photo, bio, chemotactic methods.

3. Defense mechanisms in plants and animals:

- a. In plants: Herbivory, secondary metabolites.
 - b. In animals: Innate and Adaptive immune systems.
-

Plasma Membrane

Background:

Also called as biomembrane, plasmalemma., unit membrane, cell membrane.

Cell membrane & cell organelle membrane have same ultra structure.

Plasma membrane is thin, transparent, elastic, porous, semi fluid, dynamic, living, protective, semi permeable, regenerative, 5-8 nm thick

Chemical composition:-- Vary in different cells. But generally

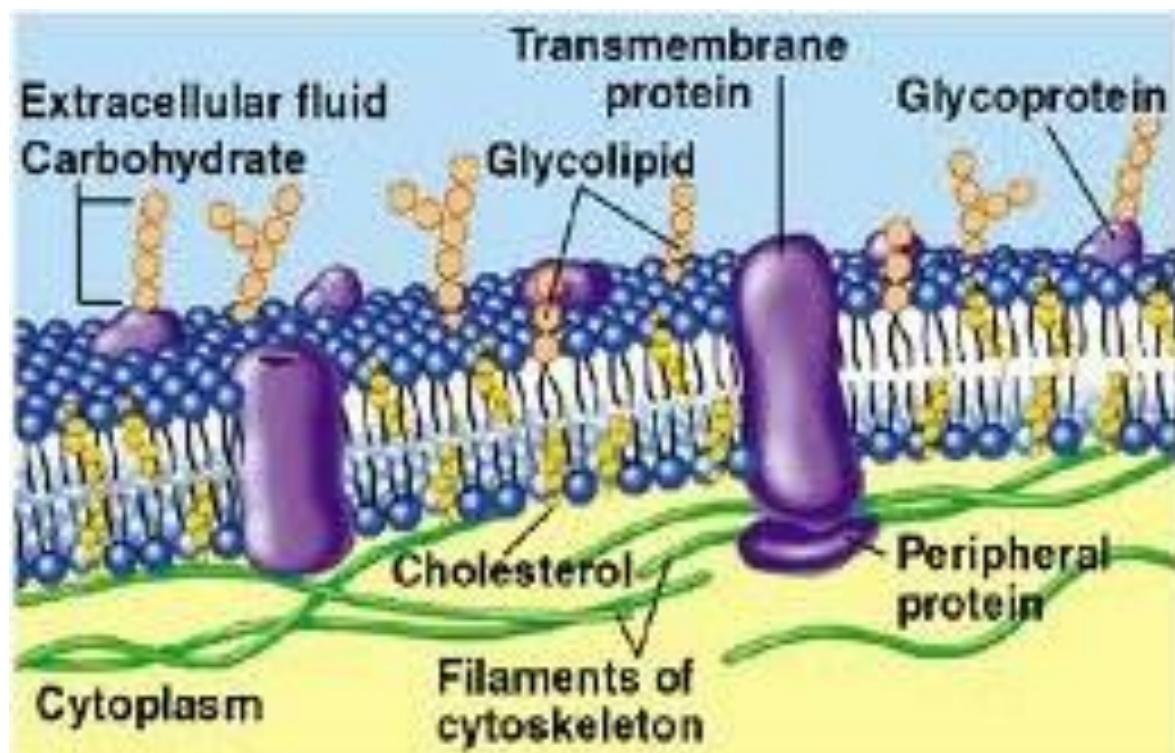
Proteins –58–75% Structural –form backbone of cellmem.

Carrier (trans membrane) –for transport of substances

Lipids – 20 – 40 % e.g. lecithin, cholesterol, galactolipid, phospholipid

Carbohydrates – 2 -5 % glucose, glucosamine.

Fluid Mosaic Model by Jonathan Singer and Garth Nicholson.



1. Most accepted model up till now. Describes as “**protein icebergs in lipid sea**”. Based on chemical analysis, biophysical properties of membrane
2. Plasma membrane is lipoprotein, trilamellar / trilaminar in nature.
3. Phospholipid bilayer – same as in above 2 models. It is fluid in nature. Phospholipid molecules exhibit 3 types of movements.
 - a. **Transition movement** – molecules move laterally in the same monolayer changing position within same layer. It is frequent.
 - b. Rotational movement – individual lipid molecules rotate very rapidly about their long axis.
 - b. **Flip – flop movement** – molecules of 2 layers inter change position. It is not so frequent
4. Proteins are globular, of 3 types. i. e. **Extrinsic / peripheral proteins** – lie outside to the phospholipid layer, loosely / non-covalently attached to phospholipid mole. of exoplasmic surface of pl. mem., hence can be easily removed in aqueous soln. Present 30 %. e. g. Acetylcholinesterase, ATPase, Spectrin.
 - ii. **Intrinsic proteins** – covalently attached to phospholipid mole. of cytosolic surface of pl. mem., hence strongly held to these mole., not easily separable. These are 70 %, if separated, become insoluble in aqueous soln. e. g. Cytochrome oxidase (from mitochondria.), rhodopsin (from retinal rod cells)
 - iii. **Channel / Tunnel / Transmembrane / Integral proteins** – Large sized, extend across bilayer on both surfaces of phospholipid bilayer as single α helix / β sheet or barrels E. g. glycoproteins. Act as charged channels for transport of H_2O – sol. Materials, ions and H_2O along conc. gradient & against conc. gradient. e. g. enzymes in mitochondria. Receptors in hormones. Permease in selective transport
- Function -- Proteins provide structural, functional specificity to cell mem., elasticity, mechanical support . Proteins on both surfaces show functional differences.
6. Oligosaccharides – molecules covalently attached with outer surface and form glycolipids with lipids. e.g. sialic acid. & glycoproteins with proteins, may extend in extra cellular fluid & form a zone called glycocalyx. Due to these molecules membrane becomes asymmetric.

Function – cell recognition, imp. role in blood grouping, blood clotting , immune response, cell to cell adhesion, tissue rejection, inflammation process, prevent unwanted protein protein interaction between adjacent cells, protect cells from mechanical & chemical damage.

7. In animal membrane sterol molecule – like cholesterol between phospholipids that are rigid and provide stability & maintain fluidity of membrane, tighten packing of lipids..

8. Cytoskeletal filaments of actin present towards cytosolic surface.

Function -- give support to membrane & restrict diffusion of membrane proteins.

Importance of above model ---

1. Quasi – fluid state of biomembrane. Hence membrane can undergo dynamic changes. (renewed, removed, folded), variations in form, size, shape are possible.

2. Two distinct surfaces of plasma membrane because phospholipids in outer layer with oligosaccharides

Cytosolic – facing the cytoplasm,

Exoplasmic – facing the inter cellular atmosphere

Glycoproteins, Glycolipids form glycocalyx which is found only on exoplasmic surface.

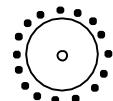
3. As proteins are in mosaic pattern membrane is not rigid.

Experiment to demonstrate fluid nature of cell membrane

Mouse cell + green fluorescent Ab. —————→ cell with green fluorescent Ab on surface



A



Human cell + red fluorescent Ab. —————→ cell with red fluorescent Ab on surface



B

Fusion of A + B —————→ —————→

i.e. Somatic hybridization

Heterokaryon Heterokaryon

At 0 C At 37 C

No mixing of Ab Mixing of Ab as
as lipids are solid lipids are liquid at
at 0 C 37 C

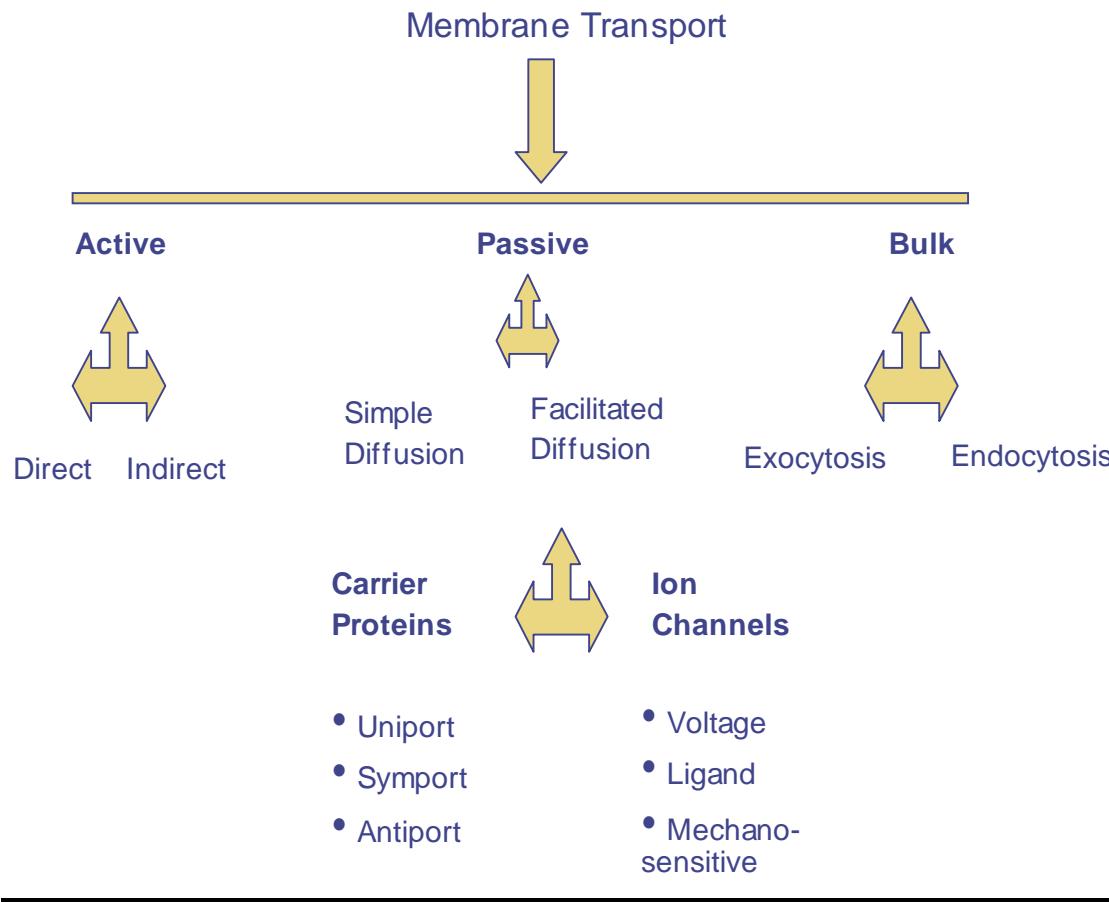
(Heterokaryon – hybrid cell with fused cytoplasm, but nuclei separate.)

When lipids are mixed with water 3 types of lipid aggregates can form ----

- 1) Micelles – spherical structures with monolayer
- 2) Bilayer – as in pl. membrane
- 3) Liposomes – lipid bilayer folded with aqueous cavity inside

Functions of Plasma membrane :

- 1) Maintain individuality, forms cell, organelles.
- 2) Cell becomes dynamic, regulate flow of material, energy in and out of cell.
- 3) Cell contents separated from external environment and cell environment.
- 4) Protect from injury
- 5) Forms new sub – cellular organelles like endoplasmic reticulum, golgi complex.
- 6) Controls cellular interactions req. for tissue formation, defense against microbes entry.
- 7) Imp. role in blood groups, immune response, organ transplant.
- 8) Selective permeability – due to enzymatic activity.
- 9) Helps in maintaining transport and permeability of cell through – osmosis, diffusion, active transport.
- 10) Concern with cell adhesion, maintaining cell shape, cell rigidity,
- 11) Pseudopodia, cilia, flagella formed of plasma membrane help in cellular movement.
- 12) Release of secretions, wastes by exocytosis.
- 13) Nerve impulse transmission.
- 14) Inner membrane of mitochondria, mesosomes contain electron transport chain of respiration.
- 15) Infolds carry endocytosis, pinocytosis, phagocytosis. Outfolds increase absorptive surface area.

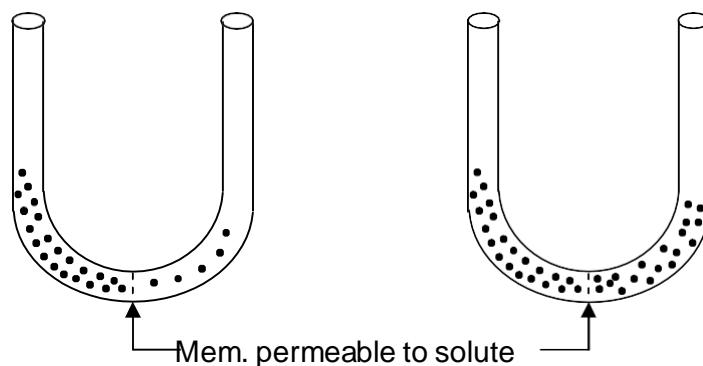


Transport Mechanism: Passive, Active and Bulk

A] Passive Transport –

- Bidirectional, along conc. gradient, no E. used, slow process
- 1) **Diffusion -**
- Dependent upon no. of particles per unit vol., density of med., distance thro' which it occurs, temp., pressure.
 - If 2 subs. don't react, then diffuse independently.
Gas mole. move along pressure gradient
Non – electrolytes move along - conc. gradient
Ions move along electro–chemical gradient
 - Most of the substances move across membranes in the form of one ion / molecule e.g. Na^+ , K^+ , Ca^{++} , Cl^- , H^+ , sugars, amino acids, nucleotides.
 - Concentration of these substances most of the times higher inside cell / cell organelle than outside.

- For cell, membrane transport is important because 20 % of genes in E.coli are identified as involved in some aspect of transport.
- Diffusion is always movement toward equilibrium i.e. it tends toward minimum free energy.
(As against chemical reactions and physical processes which always proceed in the direction of decreasing freeenergy according to 2nd law of thermodynamics.)



(In membrane transport free energy change depends on conc. / electrochemical gradient but also on heat, pressure, entropy. There diffusion always proceeds from regions of higher to lower free energy. At thermodynamic equilibrium when free energy of the system is at minimum no further net movement occurs.)

- Thermodynamically diffusion is exergonic process as requiring no input of metabolic E
E.g. Dialysis, Filtration, respiration in animals due to diffusion of gases from blood to alveoli and vice versa, Uniform distribution of materials in cytoplasm. In animals sexual act, territory marking due to diffusion of pheromones. (special hormones in animals)

2) Osmosis –

Special diffusion of H₂O or solvent

- Operates always in liquid media
- Only solv. part of solution diffuses, not solute, through differentially permeable membrane.
- Depends on no. of solute particles.
- Opposed by turgor or hydrostatic pressure.

- Types of Osmosis –
1. Endosmosis – entry of solvent into cell, animal cell swells & bursts due to excessive endosmosis –i.e. plasmolysis, plant cell becomes swollen/turgid, due to cell wall it does not burst
 2. Exosmosis – exit of solvent from cell, animal cell shrinks, plasma membrane of plant cell shrinks from cell wall due to excessive exosmosis & turgid / swollen cell shrinks / becomes flaccid.

Hydrostatic / Turgor pressure –

Due to endosmosis pressure exerted by protoplast on pl. membrane, cell wall is called as turgor pressure.

Osmotic pressure – Maximum hydrostatic pressure required to resist osmotic flow of H_2O into solution., when solution. is separated from pure H_2O by differentially / selectively permeable membrane. Higher is solute conc. in soln. higher is osmotic pressure. It is measured by osmometer.

Tonicity – Tension / stress developed in a system due to presence of osmotically active substance in it.

Isotonic solution – solute conc. of extra and intra cellular fluid is same, hence no osmosis. e.g. mammalian RBC (other than human) in 0.9 % NaCl solution, human RBC in 5 % glucose solution.

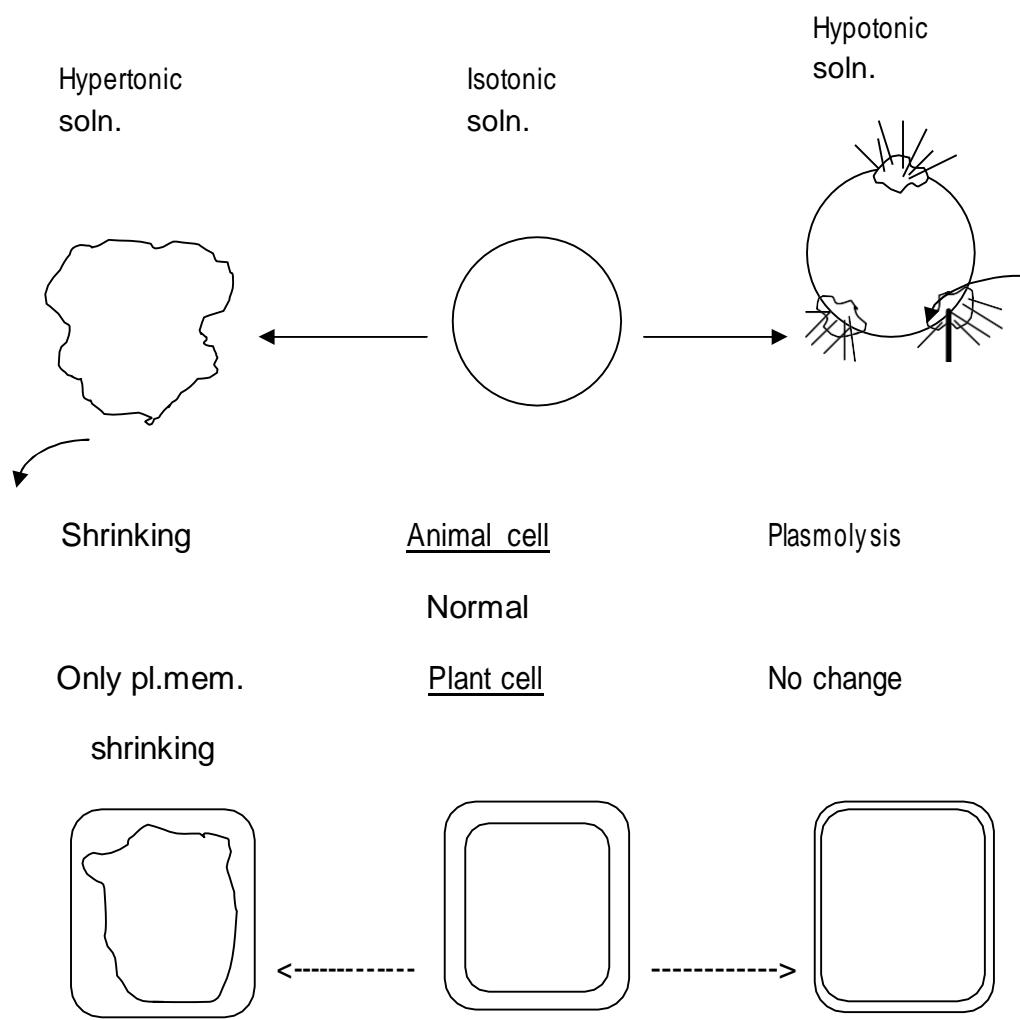
Hypertonic solution – solute concentration of extra cellular fluid > intra cellular fluid, hence exosmosis occurs. e.g. Cells shrinks in 2 % NaCl, 10 % Glucose solution. e.g. plasmolysis, wrinkles, crenation.

Hypotonic solution –

solute conc. of extra cellular fluid < intra cellular fluid, hence endosmosis occur, cell swell e.g. RBCs in distilled H_2O swell, finally burst , result in Ghost of RBCs. 0.5 % NaCl, 3 % Glucose.

♣ Significance of osmosis -

- 1) Soil H_2O enters root cells.
- 2) Turgidity in living cells maintained.
- 3) Due to turgidity, soft parts like leaves, fruits., flowers remain stretched.
- 4) Germination of seed occurs, resulting in growth of radicle, plumule.
- 5) Plant move.
- 6) Opening, closing of stomata(openings in leaves)



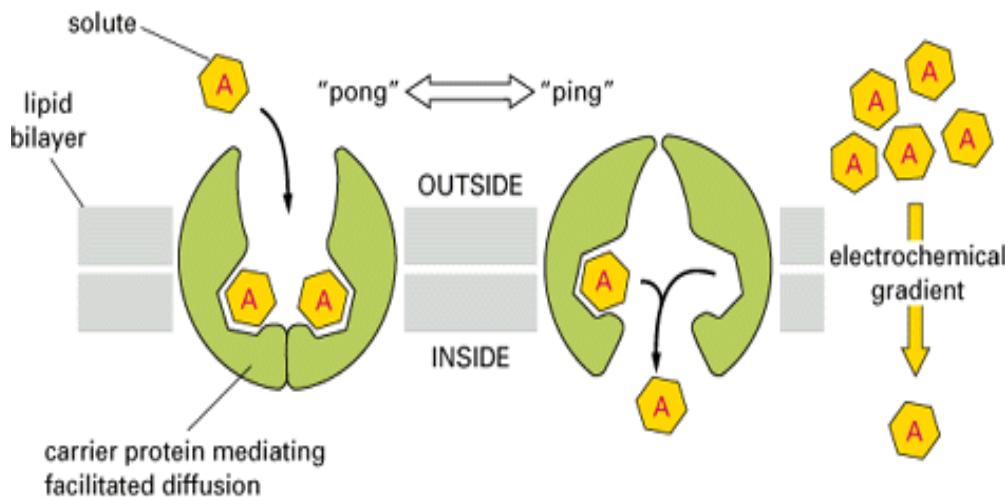
- Substances too large / too polar can move into / out of cell / cell organelle at appreciable rates only with the help of transport protein. If solutes still diffuse down the conc. gradient without input of energy then it is facilitated diffusion.
E.g. conc. of glucose higher in blood than in RBC, so transport of glucose across the plasma membrane of cell is no doubt passive, but glucose is too large, too polar to diffuse across the membrane unaided; hence transport protein is required to facilitate its inward movement.
- Transport proteins are transmembrane segments, hence traverse (turn horizontally) the membrane several times.

2 Types –

1) Carrier proteins / Transporters / Permeases –

- Bind 1 / more solute molecules on one side of membrane, then undergo conformation (structure) change to transfer the solute on other side of membrane. (proposed by S.J. Singer)

- Probably carrier protein shields the polar / charged group of solute from non-polar interior of the membrane.

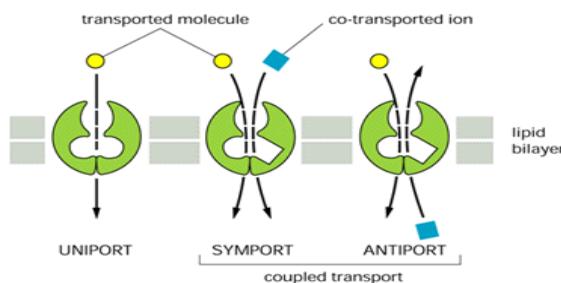


Types of Carrier Protein Transport-

- 1) **Uniport Transport** – transport of single solute.
- 2) **Cotransport /coupled transport** – simultaneous transport of 2 solutes, both solutes necessary for transport i.e. obligatory. Types
 - a) **Symport** – if both solutes move in same direction (as shown in diagram)
 - b) **Antiport** – if both solutes move in opposite direction.(as shown in diagram)

Both ways transport of H₂O takes place.

(Facilitated diffusion is possible in animal cells due to low glucose concentration because incoming glucose is quickly phosphorylated (addition of PO₄) to glucose -6-PO₄ by hexokinase with ATP as PO₄ donor and energy source.)



Erythrocyte Anion Exchange Protein – Antiport Carrier

- By antiport carrier protein chlorid – bicarbonate exchange facilitates reciprocal exchange of Cl^- and HCO_3^- ions across the membrane in 1 : 1 ratio in obligatory manner.
- In cell if HCO_3^- conc. is high., binding site of anion exchange protein binds to HCO_3^- ion on inner surface and Cl^- ion on outer surface; reverse will happen when in cell HCO_3^- conc. is low.
- Same way transport of CO_2 from tissues into erythrocytes by converting it into HCO_3^- ions takes place.

2) Channel Proteins –

- Form hydrophilic channels through membrane to allow the passage of solutes without any change in conformation of protein.
- E.g. pores found in outer membrane of bacteria, mitochondria, chloroplast. They are formed by transmembrane proteins e.g. porins, allow selected hydrophilic solutes with molecular wt. up to 600 Dalton to pass through.
- Most of these channel proteins involved in transport of ions rather than molecules, hence called as **ion channels**.
- As no conformational changes necessary, movement of solutes through ion channels is much rapid than carrier proteins..
- **Channel Proteins Facilitate Diffusion by Forming Hydrophilic Transmembrane Channels**
- By forming hydrophilic transmembrane channels, channel proteins allow ions to move across the membrane directly.

3 Types of Channel Proteins – ion channels, porins and aquaporins.

1) Ion channels-

- Ion channels are highly selective, allow passage of only one kind of ion. Hence separate channels for each in Cl^- , Ca^{++} , Na^+ , K^+ transport.
- Selectivity is due to binding sites & constricted center acting as a filter.
- A single channel can conduct almost 1000000 ions / sec.
- Most ion channels are gated i.e. they can be opened and closed by conformational changes in the protein, thus regulate ions flow through channel.
- In animal cells 3 kinds of stimuli control opening and closing of gated channels.
 - I Membrane potential – Control voltage gated channels.

II Specific substances – Control ligand gated channels.

III Mechanical force – Control mechano sensitive channels.

2) Porins –

- Pores in outer membrane of bacteria, chloroplast, mitochondria are formed by porins.
- X – ray crystallography reveals that porins present in the form of β sheet called β barrel and not α helix has a water filled pore at center, polar side chains are lining inside pore, non-polar side chains at outside of barrel that interact with hydrophobic interior of the membrane.

3) Aquaporins –

- Allow all H_2O moles to pass but not any other polar subs.
- Or continual movement of membrane lipids create transient holes in lipid monolayers to allow H_2O molecules to move first through monolayer and then through the others.
- Facilitate rapid movement of H_2O molecules into or out of cells in specific tissues (not all) e.g. PCT (present in kidney), erythrocytes.
- Size 0.3 nm. (just large enough to pass H_2O molecule one at time.)

Active Transport – (Protein Mediated Movement Up the Gradient)

It moves solutes away from thermodynamic equilibrium (i.e. Up/against the concentration or electrochemical gradient) and hence always requires an input of energy. Thus thermodynamically it is unfavorable (endergonic) and occurs only when coupled with an exergonic process. Therefore membrane proteins involved in active transport must provide mechanism for moving desired solute molecules across the membrane as well as for coupling such movements to energy yielding reactions.

- Due to active transport following processes take place
- 1) Uptake of essential nutrient from surrounding fluid / environment against concentration gradient.
 - 2) Removal of waste products, secretory products from cell or cell organelles against concentration gradient also.
 - 3) Non equilibrium intracellular concentration of specific inorganic ions, notably K^+ , Na^+ , Ca^{++} , H^+ maintained constant.

Indirect Active Transport –

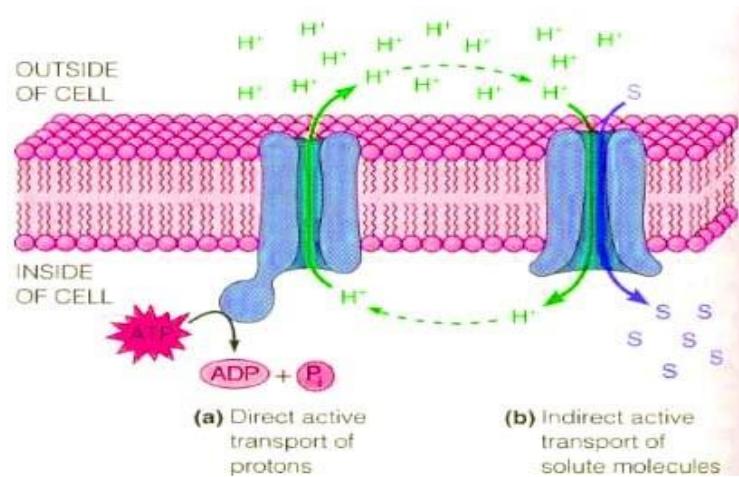
- It is co-transport of 2 solutes, one down its gradient, another up its gradient.
- Out of 2 solutes, mostly one is an ion (usually Na^+ / K^+) which moves exergonically down its electrochemical gradient driving second solute (amino acid, monosaccharide against its concentration gradient.)
- For animal cells Na^+ symport is usual option.

Direct Active Transport –

In this transport accumulation of solute molecules /ions on one side membrane is coupled directly to an exergonic reaction, most commonly hydrolysis of ATP.

Transport proteins driven directly by ATP hydrolysis are called transport ATPases or ATPase pumps. 4 types of transport ATPases – P-type, V – type, F – type, ABC – type

- Above 4 types differ in structure, mechanism, localization and role but mostly move Na^+ outward across plasma membrane of the cell.

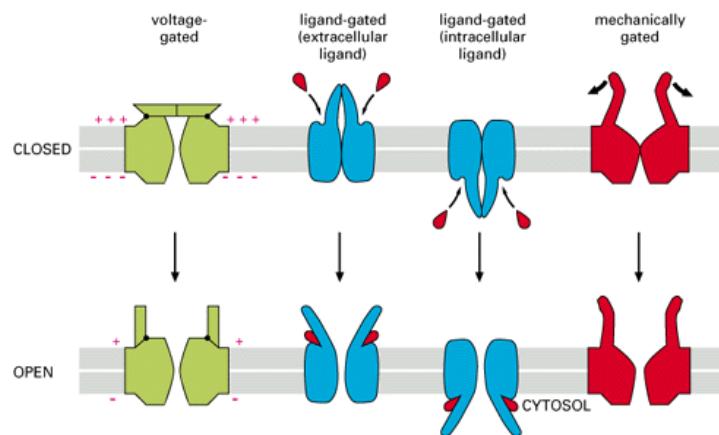


Applications of cell membrane:

A) Based on biosignaling:

1. Detection and identification of biological warfare agents – artificial ion channels are made which are sensitive to pathogen and detect and identify the pathogen. E.g. *Yersinia pestis* (causes plague), *Bacillus anthracis* (causes anthrax)

2. Drugs for modulating (regulating) ion channels with therapeutic (pertaining to therapy) potential – voltage gated ion channels are potential therapeutic agents in cardiovascular disorders and ligand gated in nervous system disorders.



3. Developing molecular device for live cell kinetic method for screening of neuro -transmitter uptake by cell.

4. Ion channel assay (potency determination)system using ion selective optical nano -sensors is used to determine drug potency.

B) Liposome based:

1. Liposome kit for DDS – Custom-made synthesis of highly purified synthetic phospholipids provide liposome kit for DDS(Drug Delivery System)

2. Delivering anti-cancer drugs like vincristin in liposomes to treat lymphoma and other cancers for more efficient targeting and delivery.

3. Cell membrane modifier – Using biocompatible anchor formembrane, cell membrane can be modified without causing damage and cells can be immobilize alive on surface of various materials.

4. Gene therapy – For targeting cells with defect, liposomes containi ng specific antigen (disease marker) & immunostimulatory DNA molecule is used.

5. Cosmetic liposomes – They have good stability in cosmetic products, excellent dry feeling on skin, enhance penetration into skin. Physiologically active materials such as ant iageing, skin whitening, moisturing agents are stably encapsulated.

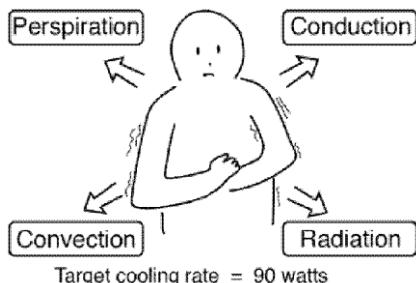
Different mechanisms transport substances over short or long distances

Given the diversity of substances that move through plants and the great range of distances and barriers over which such substances must be transported, it is not surprising that plants employ a variety of transport processes. Before examining these processes, however, we will look at the two major pathways of transport: the apoplast and the symplast.

Plant tissues may be viewed as having two major compartments —the apoplast and the symplast. The **apoplast** consists of everything external to the plasma membranes of living cells and includes cell walls, extracellular spaces, and the interior of dead cells such as vessel elements and tracheids. The **symplast** consists of the entire mass of cytosol of all the living cells in a plant, as well as the plasmodesmata, the cytoplasmic channels that interconnect them.

The compartmental structure of plants provides three routes for transport within a plant tissue or organ: the apoplastic, symplastic, and transmembrane routes. In the *apoplastic route*, water and solutes (dissolved chemicals) move along the continuum of cell walls and extracellular spaces. In the *symplastic route*, water and solutes move along the continuum of cytosol. This route requires substances to cross a plasma membrane once, when they first enter the plant. After entering one cell, substances can move from cell to cell via plasmodesmata. In the *transmembrane route*, water and solutes move out of one cell, across the cell wall, and into the neighboring cell, which may pass them to the next cell in the same way. The transmembrane route requires repeated crossings of plasma membranes as substances exit one cell and enter the next. These three routes are not mutually exclusive, and some substances may use more than one route to varying degrees.

Temperature Regulation of the Human Body



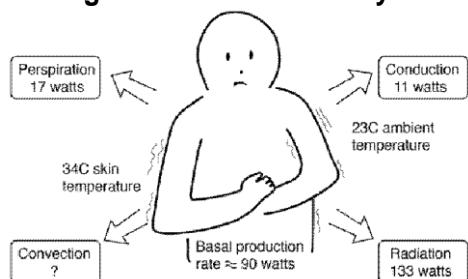
The human body has the remarkable capacity for regulating its core temperature somewhere between 98°F and 100°F (36.6°C to 37.7°C) when the ambient temperature is between approximately 68°F and 130°F (20.0°C to 54.4°C) according to Guyton (This presumes a nude body and dry air).

The external heat transfer mechanisms are **radiation**, **conduction**, **convection** and **evaporation of perspiration**. The process is far more than the passive operation of these heat transfer mechanisms, however. The body takes a very active role in temperature regulation. The temperature of the body is regulated by **neural feedback mechanisms** which operate primarily through the **hypothalamus**. The hypothalamus contains not only the control mechanisms, but also the key temperature sensors. Under control of these mechanisms, sweating begins almost precisely at a skin temperature of 37°C and increases rapidly as the skin temperature rises above this value. The heat production of the body under these conditions remains almost constant as the skin temperature rises.

If the skin temperature drops below 37°C a variety of responses are initiated to conserve the heat in the body and to increase heat production. These include

- Vasoconstriction to decrease the flow of heat to the skin.
- Cessation of sweating.
- Shivering to increase heat production in the muscles.
- Secretion of norepinephrine, epinephrine, and thyroxine to increase heat production
- In lower animals, the erection of the hairs and fur to increase insulation.

Cooling of the Human Body



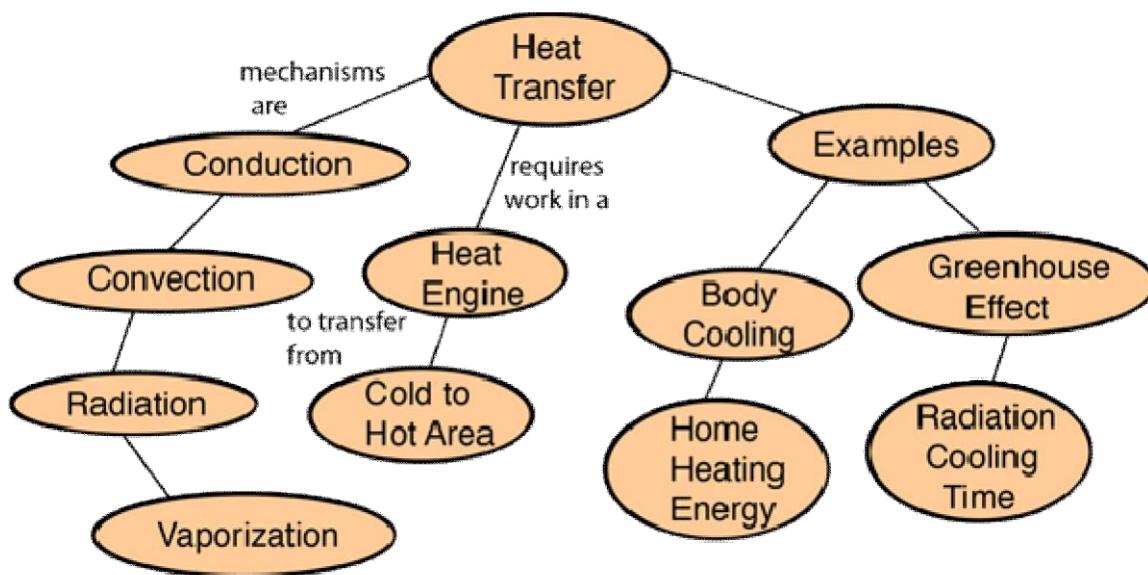
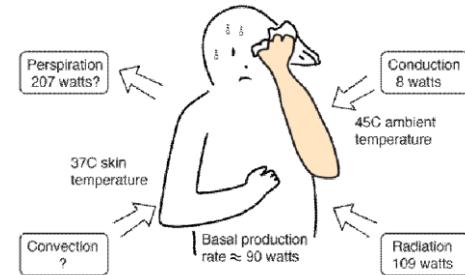
This is a simplified model of the process by which the human body gives off heat. Even when inactive, an adult male must lose heat at a rate of **about 90 watts** as a result of his basal metabolism. One implication of the model is that radiation is the most important heat transfer mechanism at ordinary room temperatures. This model indicates that an unclothed person at rest in a room temperature of 23°C or 73 Fahrenheit would be

uncomfortably cool. Select one of the cooling mechanisms for further details about how the model numbers were obtained. The skin temperature of 34°C is a typical skin temperature taken from physiology texts, compared to the normal core body temperature of 37°C. Even when inactive, an adult male must lose heat at a rate of about 90 watts as a result of his basal metabolism. This becomes a problem when the ambient temperature is above body

temperature, because all three standard heat transfer mechanisms work against this heat loss by transferring heat into the body. Our ability to exist in such conditions comes from the efficiency of cooling by the **evaporation of perspiration**.

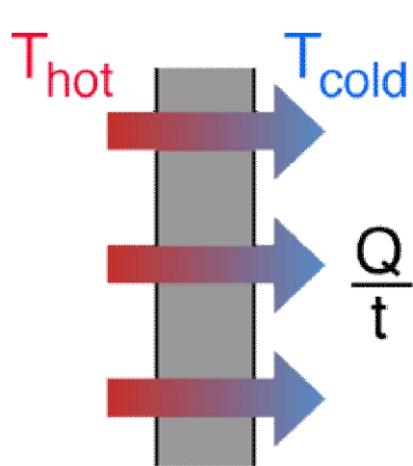
At a temperature of 113 Fahrenheit (45°C) the evaporation process must overcome the transfer of heat into the body and give off enough heat to accomplish a 90 watt net outward flowrate of energy. Because of the body's temperature regulatory mechanisms, the skin temperature would be expected to rise to 37°C at which point perspiration is initiated and increases until the evaporation cooling is sufficient to hold the skin at 37°C if possible. With those assumptions about the temperatures, the Stefan-Boltzmann law for an area of 2 m^2 and emissivity 0.97 gives a net input power of 109 watts to the body. The perspiration cooling must overcome that and produce the net outflow of 90 watts for equilibrium.

The transfer of heat is normally from a high temperature object to a lower temperature object. Heat transfer changes the internal energy of both systems involved according to the 1st law of Thermodynamics.



Heat Conduction

Conduction is heat transfer by means of molecular agitation within a material without any motion of the material as a whole. If one end of a metal rod is at a higher temperature, then energy will be transferred down the rod toward the colder end because the higher speed particles will collide with the slower ones with a net transfer of energy to the slower ones. For heat transfer between two plane surfaces, such as heat loss through the wall of a house, the rate of conduction heat transfer is:



$$\frac{Q}{t} = \frac{\kappa A(T_{hot} - T_{cold})}{d}$$

Q = heat transferred in time = t

κ = thermal conductivity of the barrier

A = area

T = temperature

d = thickness of barrier

Stefan-Boltzmann Law

The thermal energy radiated by a blackbody radiator per second per unit area is proportional to the fourth power of the absolute temperature and is given by

$$\frac{P}{A} = \sigma T^4 \text{ j/m}^2\text{s} \quad \text{Stefan-Boltzmann Law}$$

$$\sigma = 5.6703 \times 10^{-8} \text{ watt/m}^2\text{K}^4$$

For hot objects other than ideal radiators, the law is expressed in the form:

$$\frac{P}{A} = e\sigma T^4$$

where e is the emissivity of the object ($e = 1$ for ideal radiator). If the hot object is radiating energy to its cooler surroundings at temperature T_c , the net radiation loss rate takes the form

$$P = e\sigma A(T^4 - T_c^4)$$

The Stefan-Boltzmann relationship is also related to the energy density in the radiation in a given volume of space.

Heat Radiation

Thermal radiation is energy transfer by the emission of electromagnetic waves which carry energy away from the emitting object. For ordinary temperatures (less than "red hot"), the radiation is in the infrared region of the electromagnetic spectrum. The relationship governing the net radiation from hot objects is called the Stefan-Boltzmann law:

$$P = e\sigma A(T^4 - T_C^4)$$

P = net radiated power

e = emissivity ($=1$ for ideal radiator)

A = radiating area

T = temperature of radiator

σ = Stefan's constant

T_C = temperature of surroundings

$$\sigma = 5.6703 \times 10^{-8} \text{ watt/m}^2 \text{ K}^4$$

While the typical situation envisioned here is the radiation from a hot object to its cooler surroundings, the Stefan-Boltzmann law is not limited to that case. If the surroundings are at a higher temperature ($T_C > T$) then you will obtain a negative answer, implying net radiative transfer to the object.

Heat Convection

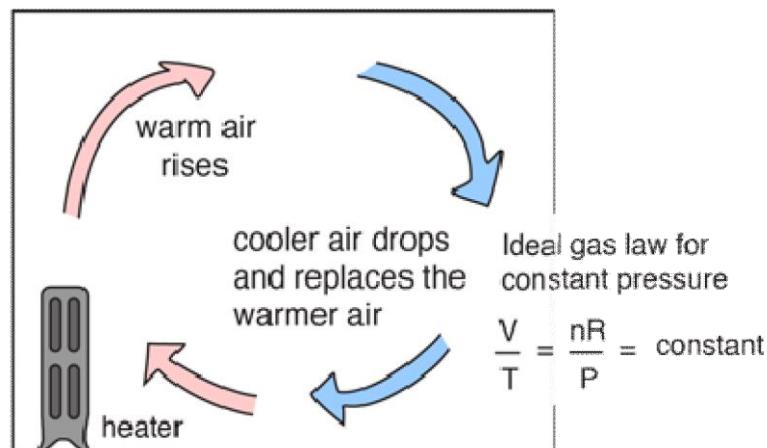
Convection is heat transfer by mass motion of a fluid such as air or water when the heated fluid is caused to move away from the source of heat, carrying energy with it. Convection above a hot surface occurs because hot air expands, becomes less dense, and rises. Hot water is likewise less dense than cold water and rises, causing convection currents which transport energy.

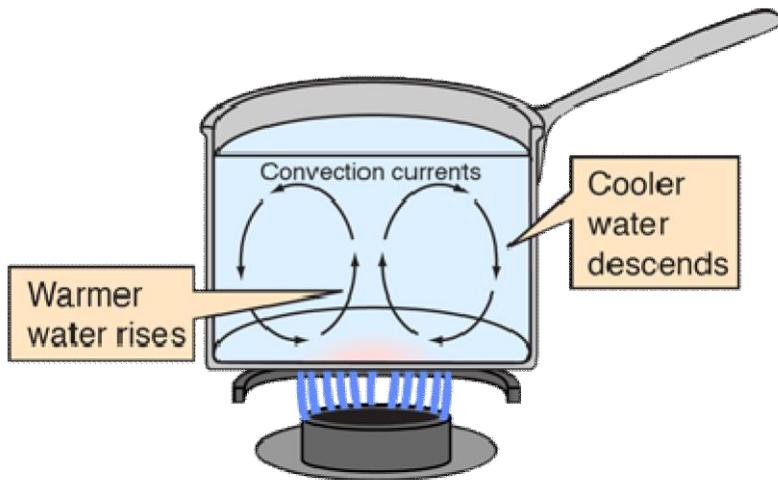
If volume increases,
then density decreases,
making it buoyant.

$$\rho = \frac{m}{V}$$

$$\frac{V}{T} = \text{constant}$$

If the temperature
of a given mass of
air increases, the
volume must increase
by the same factor.



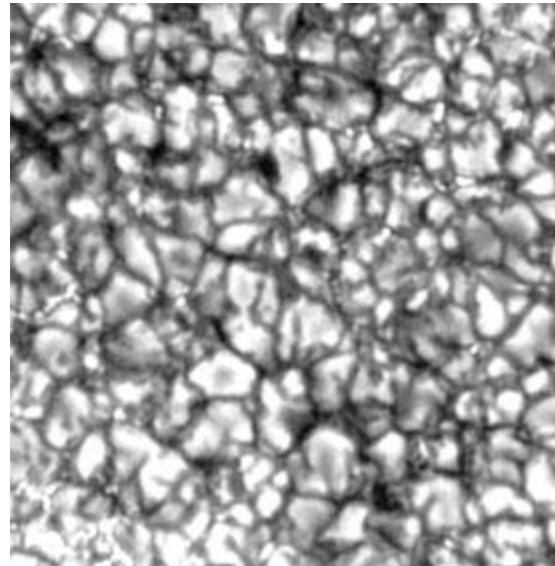


Convection can also lead to circulation in a liquid, as in the heating of a pot of water over a flame. Heated water expands and becomes more buoyant. Cooler, more dense water near the surface descends and patterns of circulation can be formed, though they will not be as regular as suggested in the drawing.



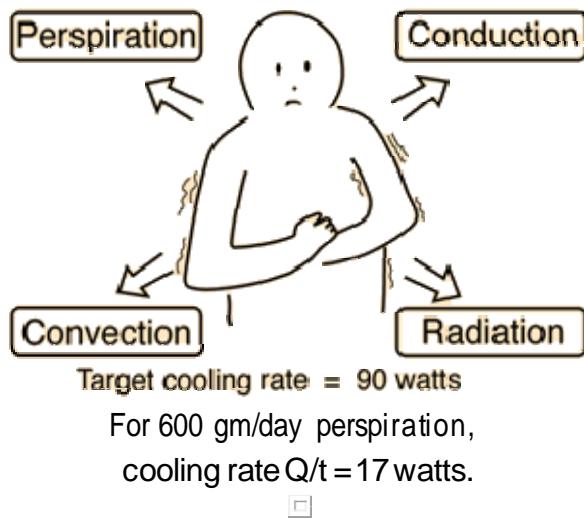
Convection cells are visible in the heated cooking oil in the pot at left. Heating the oil produces changes in the index of refraction of the oil, making the cell boundaries visible. Circulation patterns form, and presumably the wall-like structures visible are the boundaries between the circulation patterns.

Convection is thought to play a major role in transporting energy from the center of the Sun to the surface, and in movements of the hot magma beneath the surface of the earth. The visible surface of the Sun (the photosphere) has a granular appearance with a typical dimension of a granule being 1000 kilometers. The image at right is from the NASA Solar Physics website and is credited to G. Scharmer and the Swedish Vacuum Solar Telescope. The granules are described as convection cells which transport heat from the interior of the Sun to the surface.



In ordinary heat transfer on the Earth, it is difficult to quantify the effects of convection since it inherently depends upon small nonuniformities in an otherwise fairly homogeneous medium. In modeling things like the cooling of the human body, we usually just lump it in with conduction.

Perspiration Cooling of Body



When the ambient temperature is above body temperature, then radiation, conduction and convection all transfer heat into the body rather than out. Since there must be a net outward heat transfer, the only mechanisms left under those conditions are the evaporation of perspiration from the skin and the evaporative cooling from exhaled moisture. Even when one is unaware of perspiration, physiology texts quote an amount of about 600 grams per day of "insensate loss" of moisture from the skin.

The cooling effect of perspiration evaporation makes use of the very large heat of vaporization of water. This heat of vaporization is 540 calories/gm at the boiling point, but is even larger, 580 cal/gm, at the normal skin temperature.

$$\frac{Q}{t} = \left(600 \frac{\text{gm}}{\text{day}} \right) \left(580 \frac{\text{cal}}{\text{gm}} \right) \left(4.186 \frac{\text{J}}{\text{cal}} \right) \left(\frac{1 \text{ day}}{24 \text{ hr}} \right) \left(\frac{1 \text{ hr}}{3600 \text{ s}} \right) = 17 \text{ watts}$$

As part of the physiological regulation of body temperature, the skin will begin to sweat almost precisely at 37°C and the perspiration will increase rapidly with increasing skin temperature. Guyton reports that a normal maximum perspiration rate is about 1.5 liters/hour, but that after 4 to 6 weeks of acclimatization in a tropical climate, it can reach 3.5 liters/hr! You would have to just sit around drinking constantly, just to keep from getting dehydrated! That maximum rate corresponds to a maximum cooling power of almost 2.4 kilowatts!

CELL TO CELL JUNCTION

Cell forms multi cellular vertebrates. To form tissues & organs cells organize into sheets During development, maintaining tissues in mature organism, cells express informational and junction molecules on their surface. As a result cells recognize and adhere to like cells.

Cell – cell junctions – 3 functional types:-

- Occluding Junction / Tight Junction
- Adhesive Junction / Anchoring Junction
- Communicating Junction / Channel Forming Junction

Occluding junctions / Tight junction:-

- Seal cells together in epithelial sheet.
- Found near free surface of cell.
- Define apical, basal sides of epithelial cells.
- Imp. In intestine, bladder, glands.
- Segregate active, passive glucose transporters for directional glucose uptake from lumen of the gut.
- Prevent passage of water and water soluble substances.
- Responsible for electrical resistance across - epithelia.
- Look like honey – comb.

Adhesive junctions

- Adherens Junctions.
- Desmosomes Junctions – due to association between to cadherin protein.

1) Adherens Junctions

- Hold cells in fixed position, tightly together, give mechanical strength.
- Prominent in epithelial(covering) tissues □□□ Which is always subjected to stress.

- Also found in cardiac muscles □□□ always subjected to stress, cell layers covering organs, lining cavities.
- Belt like junctions located just below tight junction.
- Cadherins cross pl. mem. and get linked to cytoskeleton by linker proteins.
- Different cell types produce different types of cadherin proteins.
- These junctions join actin bundles in one cell to actin bundles in an adjoining cell.
- Cadherins linked actin microfilaments extend out of cell and bind to cadherins of an adjoining cell.
- These junctions form belt around cell, button like bundle.
- Actin microfilaments can contract, therefore involved in invaginations during development.

2) Desmosomes

- Link intermediate filaments of adjoining cells.
- Cadherins linked to keratin intermediate filaments inside the cell, then extend across the pl.mem. to associate with identical cadherins of an adjacent cell.
- Cadherins and keratin filaments are anchored to a dense mixture of attachment proteins forming button like structure, called as plaque.
- Present abundantly in skin, heart, neck of uterus ||| at these places there is need of withstanding mechanical stress.
- Also maintain cell position during development.

3) Hemidesmosomes

- Link keratin intermediate filaments to basal lamina.
- Integrins are linked to keratin filaments inside the cell and extend across the pl.mem to bind to lamina in the basal lamina.

Communicating junctions:-

- Direct connections between cytoplasms of adjoining cells.

- Allow exchange of small molecules as signals between adjacent cells.

Types:-

Gap junctions:-

- Form channels across the pl. mem.s of adjoining animal cells.
- Hexamer complex of proteins called connexon in each of two adjacent cells are aligned, forming an aqueous channel between them.
- Opening of channel is regulated by cell.
- Thro' these channels usually rapid diffusion of small molecules and ions allowed.
- Found commonly in smooth and cardiac muscles.
- Blocking gap junctions can disrupt development..

Plasmodesmata:-

- Found in plant cells.
- Form channel thro' cell wall , connect adjoining cells.
- Thro' channel allow move. of metabolites, ions, hormones, proteins RNA.
- Continuous connection of cytoplasm of adjoining cells.
- Function – same as gap junction in animal cell.

Cell Adhesion Molecules – (CAMs):-

- CAMs allow cells to bind to other cells or to extracellular matrix.
- Most imp. function in choreographing tissues and organ formation during embryogenesis.
- Play imp. role in host defense, repair.

Experiment. – chick embryo dissociated into single cells, these cells placed in a culture dish.

Observation – strong tendency of cells to reaggregate into clusters based on their tissue of origin

Conclusion – specific adhesion mole. present in cell.

- All adhesion mole. are integral membrane proteins
- They have cytoplasmic, transmembrane, extracellular domains.
- Cytoplasmic tail often interacts with cytoskeletal proteins which are actual anchors within the cell.

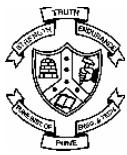
Homophilic binding :- Same type of adhesion molecules bind.

Heterophilic binding:- binding of different types of molecules or binding to an intermediary linker which it self binds to other adhesion molecules.

Major Families:-

- 1) **Cadherins** – adhesion via homophilic binding between z cadherins in a calcium dependent manner. (therefore removal of extracellular Ca⁺⁺ disrupts binding) Play imp. role in segregating embryonic cells into tissues, in desmosomes, adherens junctions, cell adhesion by cadherins is thro' cytoplasmic actin and intermediate filaments.
- 2) **Immuno globulin like adhesion molecules** –large gr. of molecules generated from a smaller no. of genes by alternative RNA splicing. Cause adhesion by both homophilic and heterophilic binding. Best e.g. neural cell adhesion molecules (N – CAMs) □□□ predominatly expressed in nervous tissue, intercellular cell adhesion molecules. (I – CAM's)
- 3) **Integrins** – large gr. of heterodimeric glycoproteins, two subunits alpha and beta. Imp. in binding and interactions of cells with components of extracellular matrix such as fibronectin. Imp. function – facilitate communication between cytoskeleton and extracellular matrix, allow each to influence orientation and structure of other. Imp. feature – some integrins exist in both active and inactive state. E.g. integrins responsible for binding of W.B.C. to endothelium are normally inactive, hence W.B.C. circulate freely in blood, become active in response to inflammatory mediators, as a result W.B.C. pulled out from blood into inflamed tissues. If such integrins are less it results in abnormal inflammatory response in certain diseases.
- 4) **Selectins** – expressed primarily on leukocytes and endothelial cells. Imp. in many host – defense mechanism involving those cells. In contrast to other adhesion molecules, selectins bind to carbohydrate ligands on cell, the resultant binding forces are relatively weak e.g. selectin mediated interactions between leukocytes and endothelial cells promote rolling of leukocytes along endothelium, while integrin binding allows leukocytes to be stopped in place.

* Functions of adhesion molecules are confirmed by spontaneous and induced mutations of their encoding genes.



College of Engineering, Pune

(An Autonomous Institute of Government of Maharashtra)

Applied Science Department

CT16002 – Biology for Engineers

UNIT VI: Engineering perspectives of biological sciences

1. Biology and engineering crosstalk
 2. Optimization in biological systems
 - A. At cell level: Hybridoma technology
 - B. Optimization At tissue level: Plant Tissue Culture, Animal Tissue Culture;
 - C. Tissue Engineering: Principles, methods and applications
 3. Introduction to Biomimetics and Biomimicry, nanobiotechnology
-

ENGINEERING PERSPECTIVES OF BIOLOGICAL SCIENCES

Bioengineering or Biomedical Engineering is a discipline that advances knowledge in engineering, biology, and medicine -- and improves human health through cross-disciplinary activities that integrate the engineering sciences with the biomedical sciences and clinical practice. Bioengineering/ Biomedical Engineering combines engineering expertise with medical needs for the enhancement of health care. It is a branch of engineering in which knowledge and skills from the existing methodologies in such fields as molecular biology, biochemistry, microbiology (study of microorganism), pharmacology (study of drugs and medicines), cytology (cell biology), immunology (study of immune system) and neuroscience (neurology) are utilized and applied to the design of medical devices, diagnostic equipment, biocompatible materials, and other important medical needs.

Bioengineering is not limited to the medical field. Bioengineers have the ability to exploit new opportunities and solve problems within the domain of complex systems. They have a great understanding of complexity within the living systems which can be applied to many fields including entrepreneurship. Those working within the bioengineering field are of service to people, work with living systems, and apply advanced technology to the complex problems of medical care.

Bioengineering may be categorized as:

- Biomedical engineering;
- Biomedical technology
- Biomedical Diagnosis
- Biomedical Therapy
- Biomechanics
- Biomaterials.
- Genetic engineering
- Cell engineering

Biomedical engineering (BME): By combining biology and medicine with engineering, biomedical engineers develop devices and procedures that solve medical and health-related problems. Biomedical engineers may be called upon to design instruments and devices, to bring together knowledge from many sources to develop new procedures, or to carry out research to acquire knowledge needed to solve problems. Many do research, along with life scientists, chemists, and medical scientists, to develop and evaluate systems and products for use in the fields of biology and health, such as artificial organs, prostheses (artificial devices that replace missing body parts), instrumentation, medical information systems, and health management and care delivery systems. Bioengineers design devices used in variety of medical procedures, such as the computers used to analyze blood or the laser systems used in corrective eye surgery. They develop artificial organs, imaging systems such as magnetic resonance, ultrasound, and x-ray, and devices for automating insulin injections or controlling body functions. Most engineers in this specialty require a sound background in one of the basic engineering specialties, such as mechanical or electronics engineering, in addition to specialized biomedical training. Some specialties within bioengineering or biomedical engineering include biomaterials, biomechanics, medical imaging, rehabilitation engineering, and orthopedic engineering.

Examples of work done by biomedical engineers include:

- designing and constructing cardiac pacemakers, defibrillators, artificial kidneys, blood oxygenators, hearts, blood vessels, joints, arms, and legs.
 - designing computer systems to monitor patients during surgery or in intensive care, or to monitor healthy persons in unusual environments, such as astronauts in space or underwater divers at great depth.
 - designing and building sensors to measure blood chemistry, such as potassium, sodium, O₂, CO₂, and pH.
 - designing instruments and devices for therapeutic uses, such as a laser system for eye surgery or a device for automated delivery of insulin.
- developing strategies for clinical decision making based on expert systems and artificial intelligence, such as a computer-based system for selecting seat cushions for paralyzed patients or for managing the care of patients with severe burns or for diagnosing diseases.

- designing clinical laboratories and other units within the hospital and health care delivery system that utilize advanced technology. Examples would be a computerized analyzer for blood samples, ambulances for use in rural areas, or a cardiac catheterization laboratory.
- designing, building and investigating medical imaging systems based on X-rays (computer assisted tomography), isotopes (positron emission tomography), magnetic fields (magnetic resonance imaging), ultrasound, or newer modalities.
- constructing and implementing mathematical/computer models of physiological systems.
- designing and constructing biomaterials and determining the mechanical, transport, and biocompatibility properties of implantable artificial materials.
- implementing new diagnostic procedures, especially those requiring engineering analyses to determine parameters that are not directly accessible to measurements, such as in the lungs or heart.
- investigating the biomechanics of injury and wound healing.

Specialty Areas

By combining biology and medicine with engineering, biomedical engineers develop devices and procedures that solve medical and health-related problems. Many do research, along with life scientists, chemists, and medical scientists, to develop and evaluate systems and products for use in the fields of biology and health, such as artificial organs, prostheses (artificial devices that replace missing body parts), instrumentation, medical information systems, and health management and care delivery systems. Some of the well established specialty areas within the field of biomedical engineering are bioinstrumentation, biomechanics, biomaterials, systems physiology, clinical engineering, and rehabilitation engineering.

Bioinstrumentation

Bioinstrumentation is the application of electronics and measurement principles and techniques to develop devices used in diagnosis and treatment of disease. Computers are becoming increasingly important in bioinstrumentation, from the microprocessor used to do a variety of small tasks in a single purpose instrument to the extensive computing power needed to process the large amount of information in a medical imaging system.

Biomechanics

Biomechanics is mechanics applied to biological or medical problems. It includes the study of motion, of material deformation, of flow within the body and in devices, and transport of chemical constituents across biological and synthetic media and membranes. Efforts in biomechanics have developed the artificial heart and replacement heart valves, the artificial kidney, the artificial hip, as well as built a better understanding of the function of organs and musculoskeletal systems. Biomaterials describes both living tissue and materials used for implantation. Understanding the properties of the living material is vital in the design of implant materials. The selection of an appropriate material to place in the human body may be one of the most difficult tasks faced by the biomedical engineer. Certain metal alloys, ceramics, polymers, and composites have been used as implantable materials. Biomaterials must be nontoxic, noncarcinogenic, chemically inert, stable, and mechanically strong enough to withstand the repeated forces of a lifetime.

Systems Physiology

Systems physiology is the term used to describe that aspect of biomedical engineering in which engineering strategies, techniques and tools are used to gain a comprehensive and integrated understanding of the function of living organisms ranging from bacteria to humans. Modeling is used in the analysis of experimental data and in formulating mathematical descriptions of physiological events. In research, models are used in designing new experiments to refine our knowledge. Living systems have highly regulated feedback control systems which can be examined in this way. Examples are the biochemistry of metabolism and the control of limb movements.

Clinical Engineering

Clinical engineering is the application of technology for health care in hospitals. The clinical engineer is a member of the health care team along with physicians, nurses and other hospital staff. Clinical engineers are responsible for developing and maintaining computer databases of medical instrumentation and equipment records and for the purchase and use of sophisticated medical instruments. They may also work with physicians on projects to adapt instrumentation to the specific needs of the physician and the hospital. This often

involves the interface of instruments with computer systems and customized software for instrument control and data analysis. Clinical engineers feel the excitement of applying the latest technology to health care.

Rehabilitation Engineering

Rehabilitation engineering is a new and growing specialty area of biomedical engineering. Rehabilitation engineers expand capabilities and improve the quality of life for individuals with physical impairments. Because the products of their labor are so personal, often developed for particular individuals or small groups, the rehabilitation engineer often works directly with the disabled individual. These specialty areas frequently depend on each other. Often the bioengineer, or biomedical engineer, who works in an applied field will use knowledge gathered by bioengineers working in more basic areas. For example, the design of an artificial hip is greatly aided by a biomechanical study of the hip. The forces which are applied to the hip can be considered in the design and material selection for the prosthesis. Similarly, the design of systems to electrically stimulate paralyzed muscle to move in a controlled way uses knowledge of the behavior of the human musculoskeletal system. The selection of appropriate materials used in these devices falls within the realm of the biomaterials engineer. These are examples of the interactions among the specialty areas of biomedical engineering.

Major Advances in Bioengineering

Artificial Joints

In 1994, a National Institutes of Health Consensus Panel declared that total hip replacement (THR) is one of the most successful surgical procedures, providing immediate and substantial improvement in a patient's pain, mobility, and quality of life. THR involves removing diseased or damaged bone in the upper end of the thigh bone (femur) and the section of the lower pelvis into which the femur fits. The bone is then replaced with prosthesis, usually made of a metal alloy or polyethylene (plastic) components. Successful replacement of deteriorated, arthritic, and severely injured hips has contributed to enhanced mobility and comfortable, independent living for many people who would otherwise be substantially disabled.

Magnetic Resonance Imaging (MRI)

In 1952, the Nobel Prize in Physics was awarded for the discovery of nuclear magnetic resonance, which laid the groundwork for one of the most unique and important inventions in medical imaging since the discovery of the X-ray. Magnetic resonance imaging (MRI) is a method of looking inside the body without using surgery, harmful dyes or radiation. The method uses magnetism and radio waves to produce clear pictures of the human anatomy. Although MRI is used for medical diagnosis, it uses a physics phenomenon discovered in the 1930s in which magnetic fields and radio waves, both harmless to humans, cause atoms to give off tiny radio signals. Different kinds of animal tissue emit response signals of differing length e.g. response signals between cancerous and non-cancerous tissue, and among the response times of other kinds of diseased tissue.

Heart Pacemaker

The invention and development of the heart pacemaker illustrates the merging of medicine and engineering. The device is a result of the collective efforts and collaboration of people and organizations from both engineering and medicine, and both public and private institutions. The pacemaker was the first electronic device ever surgically implanted inside a human. First developed in the 1960s, pacemaker typically refers to a small, battery-powered device that helps the heart beat in a regular rhythm. Small electrical charges travel to one or multiple electrodes placed next to the heart muscle. Originally pacemakers sent one steady beat to the heart through a single electrode. Today's pacemakers can sense when a heart needs help and delivers just the right amount and duration of impulse --- sometimes through multiple electrodes --- that maintain steady heart rate, even during physical activity. While most pacemakers today are permanent implants, some are used as temporary therapy for recovering heart patients.

Arthroscopy

Arthroscopy is a surgical procedure orthopedic surgeons use to visualize, diagnose and treat problems inside a joint. The word arthroscopy comes from two Greek words, "arthrō" o"

(joint) and "skopein" (look), and literally means "to look within the joint." In an arthroscopic examination, an orthopedic surgeon makes a small incision in the patient's skin and then inserts pencil -sized instruments that contain a small lens and lighting system to magnify and illuminate the structures inside the joint. Light is transmitted through fiber optics to the end of the arthroscope that is inserted into the joint. By attaching the arthroscope to a miniature television camera, the surgeon is able to see the interior of the joint through this very small incision. The camera attached to the arthroscope displays the image of the joint on a television screen, allowing the surgeon to look, for example, throughout the knee -- at cartilage and ligaments, and under the kneecap. The surgeon can determine the amount or type of injury, and then repair or correct the problem, if necessary.

Angioplasty

Insertion of a catheter into a patient's coronary artery and inflated a tiny balloon, opening a blockage and restoring blood flow to a human heart is known as coronary angioplasty. It accounts a most common medical intervention in the world. Although this procedure was first envisioned as simply an alternative to open heart bypass surgery in only a handful of patients, today angioplasty accounts for more than half of the treatments for coronary artery disease. Biomedical engineering and advances in technology have not only optimized basic balloon angioplasty, but also added the use of stents, lasers and other interventional devices that restore normal blood flow while minimizing damage to the heart muscle.

Bioengineered Skin

The burgeoning field of tissue engineering promises to be one of the most significant biomedical areas of the new century. The hope is that, eventually, whole organs could be manufactured to replace those that are injured or diseased. The field's first contribution to health care took a big step toward fulfilling these promises by producing artificial version of the body's largest organ, skin. Skin is a difficult organ to transplant because of its inherently strong immune defense system. Nevertheless, it has a relatively simple structure, making it a good testing ground for the talents of tissue engineers. Patients can

have skin made to order that combines collagen as a binder with living human cells. This is placed onto a wound, usually a chronic ulcer or a burn, and its cells become activated and gradually integrate with those of the patient.

Kidney Dialysis

Considerable human population on the Earth currently lives with chronic kidney failure resulting from disease, birth defect or injury. Virtually all these patients would die if not for the aid of ongoing kidney dialysis. Kidney dialysis artificially filters and removes waste products and excess water from blood, a process normally performed by the kidneys. Although often referred to as an artificial kidney, kidney dialysis is not a cure. The procedure can, however, give damaged kidneys a rest and a chance to recover normal function, or be used until the patient receives a transplant. For many patients, kidney dialysis is a way of life. Kidney dialysis was first developed by a Dutch physician, Willem Kolff, M.D., Ph.D. In the early 1940s, he began searching for a way to use dialysis, the process by which particles pass through a membrane, to treat patients with kidney failure. A severe shortage of materials due to the war forced Kolff to improvise, especially when it came to a suitable membrane, the key component to the filtering process.

Today, research to find more efficient, low -cost methods of treatment remains a priority for biomedical engineers. Current efforts include not only improving the components of dialysis, such as better dialysates and membranes, but also developing alternatives to dialysis, such as a true artificial kidney, xenotransplantation and replacement kidneys through tissue engineering.

Heart-lung Machine

One of the truly revolutionary pieces of medical equipment has been the invention and development of the heart -lung machine. Before its introduction to medicine in the 1950s, heart surgery was unheard of; there was no way to keep a patient alive while working on the heart. During an open -heart surgery, such as bypass surgery, the heart -lung machine takes over the functions of the heart and lungs and allows a surgeon to carefully stop the heart while the rest of the patient's body continues to receive oxygen -rich blood. The surgeon can then perform delicate work on the heart without interference from bleeding or

the heart's pumping motion. Once the procedure is over, the surgeon restarts the heart and disconnects the heart -lung machine.

A typical biomedical engineering department does the corrective and preventive maintenance on the medical devices used by the hospital , except for those covered by a warranty or maintenance agreement with an external company. All newly acquired equipment is also fully tested. That is, every line of software is executed, or every possible setting is exercised and verified. Most devices ar e intentionally simplified in some way to makethe testing process less expensive, yet accurate. Many biomedical devices need to be sterilized. This creates a unique set of problems, since most sterilization techniques can cause damage to machinery and materials. Most medical devices are either inherently safe, or have added devices and systems so that they can sense their failure and shut down into an unusable, thus very safe state. A typical, basic requirement is that no single failure should cause the therapy to become unsafe at any point during its life -cycle.

TISSUE ENGINEERING

Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions. In practice the term is closely associated with applications that repair or replace portions of or whole tissues (i.e., bone, cartilage, blood vessels, bladder, etc.). The term **regenerative medicine** is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to produce tissues.

Stem cells are cells found in most, if not all, multi-cellular organism. They are characterized by the ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types. The two broad types of mammalian stem cells are: **embryonic stem cells** that are isolated from the inner cell mass of blastocysts, and **adult stem cells** that are found in adult tissues.

Stem cells can now be grown and transformed into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies. Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies.

Powerful developments in the multidisciplinary field of tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell, and developmental biology, as well as materials science and bioinformatics.

In 2003, a report entitled "The Emergence of Tissue Engineering as a Research Field" has been published, which gives a thorough description of the history of this field.

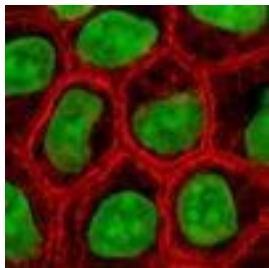
Examples

- Bioartificial liver device — several research efforts have produced hepatic assist devices utilizing living hepatocytes.
- Artificial pancreas — research involves using islet cells to produce and regulate insulin, particularly in cases of diabetes.
- Artificial bladders — Anthony Atala (Wake Forest University) has successfully implanted artificially grown bladders into seven out of

approximately 20 human test subjects as part of a long-term experiment.

- Cartilage — lab-grown tissue was successfully used to repair knee cartilage.
- Doris Taylor's heart in a jar
- Tissue-engineered airway
- Artificial skin constructed from human skin cells embedded in collagen
- Artificial bone marrow

Cells as building blocks



Stained cells in culture

Tissue engineering utilizes living cells as engineering materials. Examples include using **living fibroblasts** in **skin** replacement or repair, **cartilage** repaired with living **chondrocytes**, or other types of cells used in other ways.

Cells became available as engineering materials when scientists discovered how to extend telomeres in 1998, producing immortalized cell lines. Before this, laboratory cultures of healthy, noncancerous mammalian cells would only divide a fixed number of times, up to the **Hayflick limit**.

Extraction

From fluid tissues such as blood, cells are extracted by bulk methods, usually centrifugation or apheresis. From solid tissues, extraction is more difficult. Usually the tissue is minced, and then digested with the enzymes trypsin or collagenase to remove the extracellular matrix that holds the cells. After that, the cells are free floating, and extracted using centrifugation or apheresis. Digestion with trypsin is very dependent on temperature. Higher temperatures digest the matrix faster, but create more damage. **Collagenase** is less temperature dependent, and damages fewer cells, but takes longer and is a more expensive reagent.

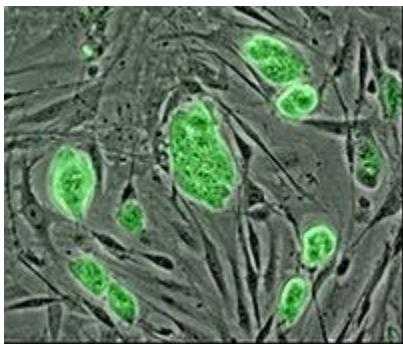
Types of cells

Cells are often categorized by their source:

- **Autologous** cells are obtained from the same individual to which they will be reimplanted. The distinguishing features of Autologous cells are:
 1. They have the very few problems with rejection and pathogen transmission however in some cases might not be available. For example in genetic disease suitable autologous cells are not available. Also very ill or elderly persons, as well as patients suffering from severe burns, may not have sufficient quantities of autologous cells to establish useful cell lines. Moreover since this category of cells needs to be harvested from the patient, there are also some concerns related to the necessity of performing such surgical operations that might lead to donor site infection or chronic pain. Autologous cells also must be cultured from samples before they can be used: this takes time, so

autologous solutions may not be very quick. Recently there has been a trend towards the use of mesenchymal stem cells from bone marrow and fat.

2. These cells can differentiate into a variety of tissue types, including bone, cartilage, fat, and nerve.
3. A large number of cells can be easily and quickly isolated from fat, thus opening the potential for large numbers of cells to be quickly and easily obtained. Several companies have been founded to capitalize on this technology, the most successful at this time being Cytori Therapeutics.



Mouse embryonic stem cells.

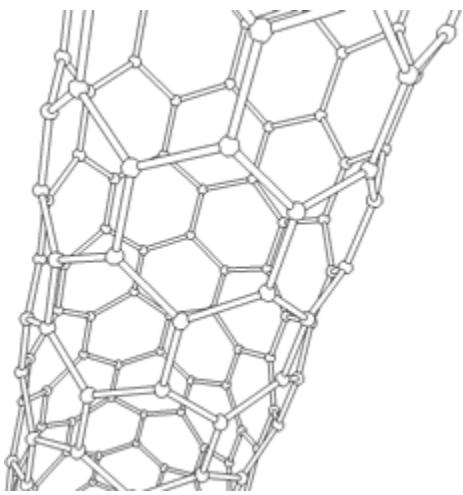
- **Allogenic** cells come from the body of a donor of the same species. While there are some ethical constraints to the use of human cells for *in vitro* studies, the employment of dermal fibroblasts from human foreskin has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin.
- **Xenogenic** cells are those isolated from individuals of another species. In particular animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants.
- **Syngenic** or **isogenic** cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models.

Primary cells are from an organism.

Secondary cells are from a cell bank.

Stem cells are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells. According to their source stem cells are divided into "adult" and "embryonic" stem cells, the first class being multipotent and the latter mostly pluripotent; some cells are totipotent, in the earliest stages of the embryo. While there is still a large ethical debate related with the use of embryonic stem cells, it is thought that stem cells may be useful for the repair of diseased or damaged tissues, or may be used to grow new organs.

Scaffolds/Extracellular matrices



Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds, are often critical, both *ex vivo* as well as *in vivo*, to recapitulating the *in vivo* milieu and allowing cells to influence their own microenvironments. The purposes served by the scaffolds are:

- Allow cell attachment and migration
- Deliver and retain cells and biochemical factors
- Enable diffusion of vital cell nutrients and expressed products
- Exert certain mechanical and biological influences to modify the behaviour of the cell phase

This rotating Carbon nanotube shows its 3D structure. Carbon nanotubes are

1. Among the numerous candidates for tissue engineering scaffolds
2. They are biocompatible
3. Resistant to biodegradation
4. Can be functionalized with biomolecules.
5. However, the possibility of toxicity with non-biodegradable nano-materials is not fully understood.

Also the requirements of an ideal scaffold are:

- A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients.
- Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal.
- The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation: this means that while cells are fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body and eventually it will break down leaving the neotissue, newly formed tissue which will take over the mechanical load.
- Injectability is also important for clinical uses.

Materials: They are usually functionally customized and the ideal properties are:

- | | |
|--------------------------|-----------------------|
| a. injectability, | c. biocompatibility |
| b. synthetic manufacture | d. non-immunogenicity |

- e. transparency
- f. nano-scale fibers
- g. low concentration
- h. desired resorption rates

The different types of materials:

1. **Natural or constructed from natural materials** - different derivatives of the extracellular matrix. Proteic materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans (GAGs), are all suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains. Among GAGs hyaluronic acid, possibly in combination with cross linking agents (e.g. glutaraldehyde, water soluble carbodiimide, etc...), bioresorbable sutures like collagen
2. **Synthetic** – PuraMatrix, PLA - polylactic acid. This is a polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body; polyglycolic acid (PGA) and polycaprolactone (PCL): their degradation mechanism is similar to that of PLA, but slightly slower.

The materials can be biodegradable or non-biodegradable.

Synthesis

A number of different methods has been described in literature for preparing porous structures to be employed as tissue engineering scaffolds. Each of these techniques presents its own advantages, but none is devoid of drawbacks.

- **Nanofiber Self-Assembly:** Molecular self-assembly is the method to create biomaterials with properties similar in scale and chemistry to that of the natural in vivo extracellular matrix (ECM). The polymers are immersed in the hydrogels and assemble on their own thus known as self assembly. These are hydrogel scaffolds, superior in vivo toxicology and biocompatibility.
- **Textile technologies:** these techniques include the preparation of non-woven meshes of different polymers. e.g. non-woven polyglycolide structures. Such fibrous structures are useful to grow different types of cells. The drawbacks - difficulties of obtaining high porosity and regular pore size.
- **Solvent Casting & Particulate Leaching (SCPL):** the preparation of porous structures with regular porosity, but with a limited thickness.
 1. the polymer is dissolved into a suitable organic solvent (e.g. polylactic acid could be dissolved into dichloromethane),
 2. the solution is cast into a mold filled with porogen particles of inorganic salt like sodium chloride, crystals of saccharose, gelatin spheres or paraffin spheres. The size of the porogen particles and the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure.
 3. The solvent is allowed to fully evaporate,
 4. the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen. Once the porogen has been fully dissolved a porous structure is obtained.

The drawback of SCPL - its use of organic solvents which must be fully removed to avoid any possible damage to the cells seeded on the scaffold.

- **Gas Foaming:** to overcome the necessity to use organic solvents and solid porogens a technique using gas as a porogen has been developed.
 1. disc shaped structures made of the desired polymer are prepared by means of compression molding using a heated mold.
 2. The discs are then placed in a chamber where are exposed to high pressure CO₂ for several days.
 3. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that abandon the polymer, resulting in a sponge like structure.

The drawbacks: prohibits the incorporation of any temperature labile material into the polymer matrix; the pores do not form an interconnected structure.
- **Emulsification/Freeze-drying:** this technique does not require the use of a solid porogen like SCPL.
 1. a synthetic polymer is dissolved into a suitable solvent (e.g. polylactic acid in dichloromethane),
 2. water is added to the polymeric solution
 3. the two liquids are mixed in order to obtain an emulsion.
 4. the emulsion is cast into a mold
 5. quickly frozen by means of immersion into liquid nitrogen.
 6. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure.

Drawbacks -it still requires the use of solvents, pore size is relatively small and porosity is often irregular.)
- **Thermally Induced Phase Separation (TIPS):** similar to the previous technique, (this phase separation procedure requires the use of a solvent with a low melting point that is easy to sublime. For example dioxane could be used to dissolve polylactic acid, then phase separation is induced through the addition of a small quantity of water: a polymer-rich and a polymer-poor phase are formed. Following cooling below the solvent melting point and some days of vacuum-drying to sublime the solvent a porous scaffold is obtained.) Liquid-liquid phase separation presents the same drawbacks of emulsification/freeze-drying.
- **CAD/CAM Technologies:** since most of the above described approaches are limited when it comes to the control of porosity and pore size, computer assisted design and manufacturing techniques have been introduced to tissue engineering. First a three-dimensional structure is designed using CAD software, then the scaffold is realized by using ink-jet printing of polymer powders or through Fused Deposition Modeling of a polymer melt.

Assembly methods

One of the continuing, persistent problems with tissue engineering is mass transport limitations. Engineered tissues generally lack an initial blood supply, thus making it difficult for any implanted cells to obtain sufficient oxygen and nutrients to survive, and/or function properly.

Self-assembly may play an important role here, both from the perspective of encapsulating cells and proteins, as well as creating scaffolds on the right physical scale for engineered tissue constructs and cellular ingrowth.

It might be possible to print organs, or possibly entire organisms. A recent innovative method of construction uses an ink-jet mechanism to print precise layers of cells in a matrix of thermoreversible gel. Endothelial cells, the cells that line blood vessels, have been printed in a set of stacked rings. When incubated, these fused into a tube.

Tissue culture

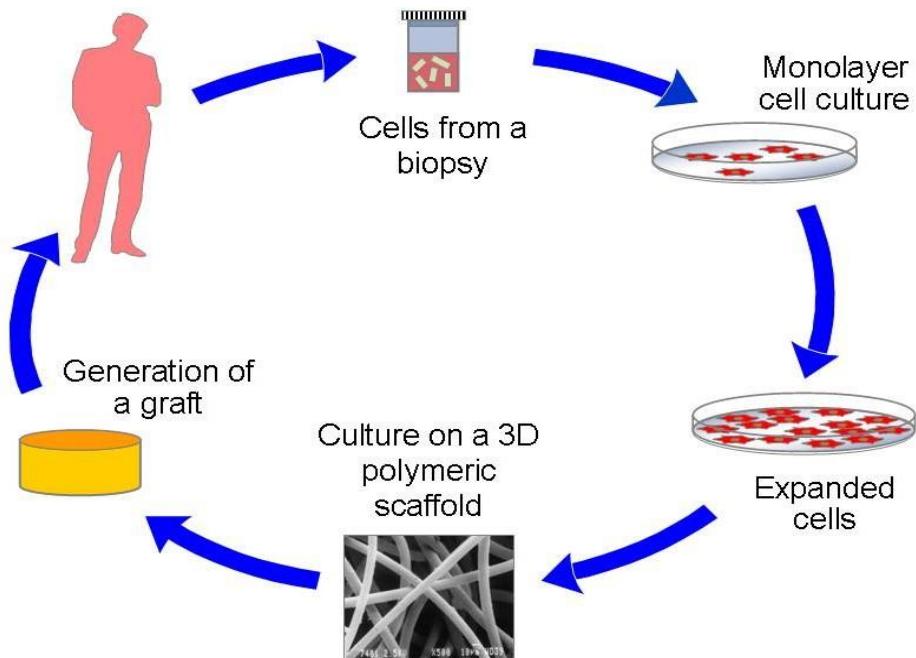
In many cases, creation of functional tissues and biological structures *in vitro* requires extensive culturing to promote survival, growth and inducement of functionality. In general, the basic requirements of cells must be maintained in culture, which include oxygen, pH, humidity, temperature, nutrients and osmotic pressure maintenance.

Tissue engineered cultures also present additional problems in maintaining culture conditions. In standard cell culture, diffusion is often the sole means of nutrient and metabolite transport. However, as a culture becomes larger and more complex, such as the case with engineered organs and whole tissues, other mechanisms must be employed to maintain the culture.

Another issue with tissue culture is introducing the proper factors or stimuli required to induce functionality. In many cases, simple maintenance culture is not sufficient. Growth factors, hormones, specific metabolites or nutrients, chemical and physical stimuli are sometimes required. For example, certain cells respond to changes in oxygen tension as part of their normal development, such as chondrocytes, which must adapt to low oxygen conditions or hypoxia during skeletal development. Others, such as endothelial cells, respond to shear stress from fluid flow, which is encountered in blood vessels.

Basic principle of Tissue engineering is illustrated in the following figure. Cells can be isolated from the patient's body, and expanded in a petridish in laboratory. Once we have enough number of cells, they can be seeded on a polymeric scaffold material, and cultured *in vitro* in a bioreactor or incubator. When the construct is matured enough, then it can be implanted in the area of defect in patient's body.

Basic principles of Tissue engineering



The application of the principles & methods of engineering & life sciences towards the fundamental understanding of structure, function & relationships in normal & pathological mammalian tissue & the development of biological substitutes to restore, maintain & improve tissue function.

... Symposium on Tissue Engineering (1988)

Defect	Organ	Function
Mechanical	Cartilage	Resist compression
Metabolites	Liver	Nitrogen metabolism
Synthetic	Pancreas	Insulin production
Communication	Nerve	Coordination
Combination	Skin	prevents water loss immunologic barrier

Biomimicry, Biomimetics, Biologically Inspired Design : much more than inspiration

There are many human inventions which were inspired by observations of the living (and non-living) systems that surround us. The systematic study of the systems of nature with the aim of helping engineers has started to receive greater interest since the 60s: terms such as bionics and biomimetics were created to refer to these approaches aiming specifically at using the knowledge gathered from living systems to improve human-created technology. The idea behind these two terms was that copying or mimicking some function, some characteristic of the natural systems would be useful for improving technical systems. The publication of the book "Biomimicry, Innovation inspired by Nature" in 1997 by the American science writer and lecturer Janine Benyus gave a new impulsion for the so-called biologically inspired approach or biomimicry which is defined as "a new science that studies nature's models and then imitates or takes inspiration from these designs and processes to solve human problems" [1].

Numerous associations of biomimicry practitioners and people interested in the approach were created: BIORON (Germany), the Biomimicry Institute (co-founded in the US by Benyus), Biomimicry Europa (Belgium and France), Biomimicry NL (Netherlands), Biomimicry IL (Israel), Biomimicry UK. In France, a dedicated research center is being built in the town of Senlis (CEEBIOS). And the number of publications on the biomimicry / biomimetic field keeps growing [2].

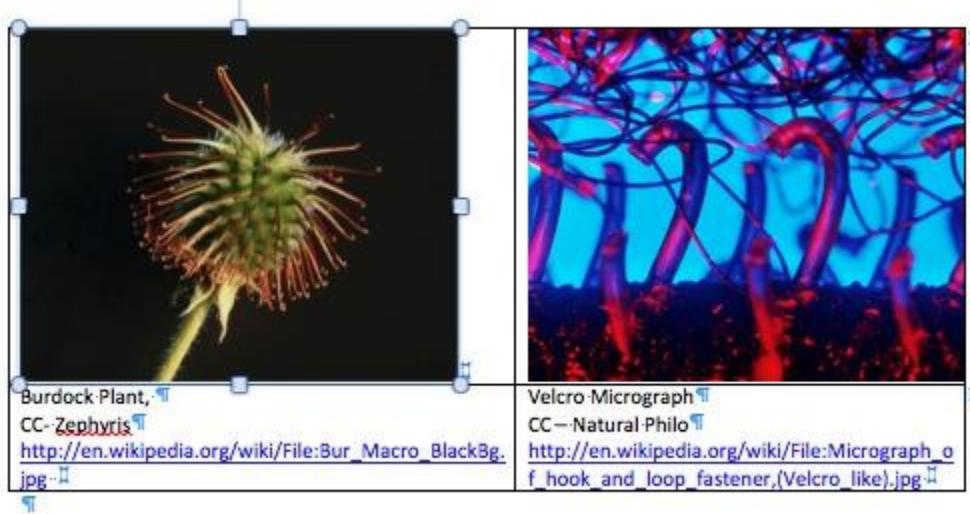
This article presents some examples of biologically inspired products and recent developments and also some perspectives about the use of biological inspiration in innovative design.

Some "successful" examples

There are numerous publications related to biologically inspired developments. The two books of Y. Bar-Cohen [3,4] for example, give a wide set of examples spanning different technological fields in which biological inspiration has been applied to. The most emblematic examples, gathered from literature on biological inspiration are:

1) George de Mestral's Velcro®

George de Mestral, a swiss engineer, observed that the seeds of burdock plant got caught in his dog's fur [5], being easily removed with a light force. He analyzed these seeds and attributed the gripping to the tiny hooks that cover the seeds surface. Velcro was then created as a two sided zip, which contains in one side stiff hooks (as in the burdock seeds) and on the other loops to which the hooks get attached. The name Velcro came from the contraction of the words *velour* (velvet) and *crochet* (hook) [6].



2) Self-cleaning surfaces

Lotus-leaves have long been recognized as a symbol of purity, as it grows in muddy environments and always stay clean. Two German researchers, Barthlott & Neinhuis, while doing some studies on plants surfaces, unveiled the mechanism that allowed these plants to stay clean. They discovered that leaves such as the lotus, had a rough surface covered with a hydrophobic coating [7]. This allowed the development of microstructured hydrophobic coatings being employed as paints for rendering surfaces self-cleaning (ex. Lotusan, developed by Sto AG)[8].



3) Eastgate Center

The Eastgate Center, in Zimbabwe, was projected by the Zimbabwean architect Mick Pearce. He drew its inspiration from the termite mounds of southern Africa termite mounds, which were believed to provide ventilation and temperature control for the colony. The ventilation system of the building was built using two models of termite

mound ventilation: the thermosiphon and the induced flow, and steady temperatures are achieved without massive energy consumption for air conditioning. However, Turner and Soar [9] pointed out that "there is no evidence that termites regulate nest temperature" and the ventilation has no or little to do with the temperature regulation. This shows that even *not fully understood* biological phenomena can lead to interesting biologically inspired developments.

	
<p>Termite mound, Lichfield National Park [1] CC- David King [2] http://www.asknature.org/strategy/0b6de7e76091446430d275b2c52473dd</p>	<p>Eastgate Centre [1] CC- David Brazier [2] http://en.wikipedia.org/wiki/File:Eastgate_Centre,_Harare,_Zimbabwe.jpg</p>

4) Flectofin®

Flectofin® is the result of collaboration between German engineers, architects and botanists. Reducing complexity of deployable systems in architecture is a challenge for architects, as in general deployability is achieved using "rigid elements connected with technical hinges" [10]. In Nature, this property is achieved using different mechanisms, such as the elastic deformations in plants. This was the starting point of a screening process for finding plants movements that would be useful for technical applications. The bird of paradise flower petals movements were identified as a torsional buckling mechanism, known to engineers, but usually considered as a failure mode, to be prevented. The plants movements showed how to use this mechanism, which was abstracted in a "thin shell element attached to a beam" [11]. Further studies on the materials properties and simulations allowed the development of Flectofin, described as a façade shading system formed by bending lamellas that is also adaptable to curved geometries.



5) Nature-inspired algorithms for optimization

Steer, Wirth and Halgamuge, in a review about these algorithms [12] propose that natural systems may exhibit behavior that is optimum-seeking, which could be used in artificial applications, while others act like "metaphor" inspiring, providing a framework for problem understanding and for the production of solutions. Their review included the following examples of nature-inspired algorithms :

- Evolutionary algorithms, inspired by the Darwin's theory of Evolution by Natural Selection ("evolutionary operation", "genetic algorithms", "evolution strategies" and "evolutionary programming").
- Particle Swarm Optimization, inspired by social behavior in nature.
- Ant Colony Optimization, inspired by the "recruitment strategy of ants which use chemical markers to mark the source of a rich food source".
- Artificial Neural Networks, inspired by the central nervous system of many organisms.

These examples show that the biologically inspired designs are rarely a copy of the inspiring natural system, they in fact involve an interaction between the knowledge from biology and the technical knowledge: normally observing the biological systems allow an activation of knowledge that would not otherwise be activated: for example, who would think that roughness could produce self-cleaning surfaces? Or that a materials failure mode could be the key for improving deployability?

Including biological inspiration in R&D

The growing number of publications involving biomimetics in different scientific fields and the innovations some of them brought, attracted the interest of many companies, seeking to be more innovative.

The first and more classic use of the biological knowledge in companies can be as a means of stimulating idea generation. Asking engineers: "how our problem is solved in nature?", can stimulate them to analogically generate new ideas for their problems. Nevertheless,

the examples mentioned above highlight that only the analogy may not be sufficient for finding the disruptive path: some further research in the biological knowledge may be necessary. For example, in the flectofin case, a screening process of different plants mechanisms was necessary, in the Eastgate centre, the two ventilation mechanisms needed to be uncovered.

As a consequence, engineers may require an easy access to the biological knowledge, which will allow them to lately contact specialists. This access can be achieved by using databases containing biological phenomena, such as Asknature.org [13] or by searching in biological literature, as proposed in the Natural Language Approach [14]. There are also computational tools being developed for facilitating the transfer between the biological knowledge and the technical knowledge [15]. Therefore, the encounters between engineers and specialists may produce some interesting mutual inspirational interactions: engineers will propose biologists new questions about their work and biologists may propose new interpretations or regards about engineers' problems. These questions are currently being studied at the chair "Design Theory and Methods for Innovation", with the C-K theory as a framework for understanding the different roles of biological knowledge in the biologically inspired design process and for demonstrating that biomimetics indeed allows designers to go beyond mimicking or inspiration.

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