CHAPTER: 23 FATTY ACIDS

a fatty acid is a carboxylic acid often with a long unbranched aliphatic tail (chain), which is either saturated or unsaturated. Carboxylic acids as short as butyric acid (4 carbon atoms) are considered to be fatty acids, while fatty acids derived from natural fats and oils may be assumed to have at least 8 carbon atoms, e.g. caprylic acid (octanoic acid). Most of the natural fatty acids have an even number of carbon atoms, because their biosynthesis involves acetyl-CoA, a coenzyme carrying a two-carbon-atom group

Types of fatty acids

Saturated fatty acids: Saturated fatty acids do not contain any double bonds or other functional groups along the chain. The term "saturated" refers to hydrogen, in that all carbons (apart from the carboxylic acid [-COOH] group) contain as many hydrogens as possible. In other words, the omega (ω) end contains 3 hydrogens (CH3-) and each carbon within the chain contains 2 hydrogen Saturated fatty acids form straight chains and, as a result, can be packed together very tightly, allowing living organisms to store chemical energy very densely. The fatty tissues of animals contain large amounts of long-chain saturated fatty acids. In IUPAC nomenclature, fatty acids have an [-oic acid] suffix. In common nomenclature, the suffix is usually -ic. The shortest descriptions of fatty acids include only the number of carbon atoms and double bonds in them (e.g. C18:0 or 18:0). C18:0 means that the carbon chain of the fatty acid consists of 18 carbon atoms and there are no (zero) double bonds in it, whereas C18:1 describes an 18-carbon chain with one double bond in it. Each double bond can be either in a cis- or trans- conformation and in a different position with respect to the ends of the fatty acid, therefore, not all C18:1s, for
example, are identical. If there is one or more double bonds in the fatty acid, it is no longer considered saturated, rather it makes it mono- or polyunsaturated

Unsaturated fatty acids:-Unsaturated fatty acids are of similar form, except that one or more alkenyl functional groups exist along the chain, with each alkene substituting a singly-bonded " -CH2-CH2-" part of the chain with a doubly-bonded "-CH=CH-" portion (that is, a carbon double bonded to another carbon).The two next carbon atoms in the chain that are bound to either side of the double bond can occur in a cis or trans configuration.

Trans fatty acids

A trans fatty acid (commonly shortened to trans fat) is an unsaturated fatty acid molecule that contains a trans double bond between carbon atoms, which makes the molecule less 'kinked' in comparison to fatty acids with cis double bonds. These bonds are characteristically produced during industrial hydrogenation of plant oils. Research suggests that amounts of trans fats correlate with circulatory diseases such as atherosclerosis and coronary heart disease more than the same amount of non-trans fats, for reasons that are not well understood.

Free fatty acids

Fatty acids can be bound or attached to other molecules, such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. The uncombined fatty acids or free fatty acids may come from the breakdown of a triglyceride into its components (fatty acids and glycerol). Free fatty acids are an important source of fuel for many tissues since they can yield relatively large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose. In particular, heart and skeletal muscle prefer fatty acids. The brain cannot use fatty acids as a source of fuel; it relies on

glucose, or on ketone bodies. Ketone bodies are produced in the liver by fatty acid metabolism during starvation, or during periods of low carbohydrate intake.

Carbon skeleton	*Structure**	*Systematic name*†	*Common name (derivation)*
12:0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic acid	Lauric acid (Latin *laurus*, "laurel plant")
14:0	$CH_3(CH_2)_{12}COOH$	*n*-Tetradecanoic acid	Myristic acid (Latin *Myristica*, nutmeg genus)
16:0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic acid	Palmitic acid (Latin *palma*, "palm tree")
18:0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic acid	Stearic acid (Greek *stear*, "hard fat")
20:0	$CH_3(CH_2)_{18}COOH$	*n*-Eicosanoic acid	Arachidic acid (Latin *Arachis*, legume genus)
24:0	$CH_3(CH_2)_{22}COOH$	*n*-Tetracosanoic acid	Lignoceric acid (Latin *lignum*, "wood" + *cera*, "wax")
16:1(Δ^9)	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	*cis*-9-Hexadecenoic acid	Palmitoleic acid
18:1(Δ^9)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	*cis*-9-Octadecenoic acid	Oleic acid (Latin *oleum*, "oil")
18:2($\Delta^{9,12}$)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-9,12-Octadecadienoic acid	Linoleic acid (Greek *linon*, "flax")
18:3($\Delta^{9,12,15}$)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-,*cis*-9,12,15-Octadecatrienoic acid	α-Linolenic acid
20:4($\Delta^{5,8,11,14}$)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$	*cis*-,*cis*-,*cis*-,*cis*-5,8,11,14-Icosatetraenoic acid	Arachidonic acid

Nomenclature: There are two different ways to make clear where the double bonds are located in molecules.

For example: * cis/trans-Delta-x or cis/trans-Δx: The double bond is located on the xth carbon-carbon bond, counting from the carboxyl terminus. The cis or trans notation indicates whether the molecule is arranged in a cis or trans conformation. In the case of a molecule having more than one double bond, the notation is, for example, cis, cis-Δ^9,Δ^{12}.

Omega-x or ω-x: A double bond is located on the xth carbon-carbon bond, counting from the ω, (methyl carbon) end of the chain. Sometimes, the symbol ω is substituted with a lowercase letter n, making it n-6 or n-3.

- Alpha-linolenic, docosahexaenoic, and eicosapentaenoic acids are examples of omega-3 fatty acids. Linoleic acid and arachidonic acid are omega-6 fatty acids. Myristoleic is omega-5 fatty acid, palmitoleic is omega-7 fatty acid, and oleic and erucic acid are omega-9 fatty acids. Stearic and oleic acid are both 18 C fatty acids. They differ only in that stearic acid is saturated with hydrogen, while oleic acid is an unsaturated fatty acid with two fewer hydrogens.

Essential fatty acids

The human body can produce all but two of the fatty acids it needs. These two, **linoleic acid (LA) and alpha-linolenic acid (LNA),** are widely distributed in plant oils. In addition, fish oils contain the longer-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Other marine oils, such as from seal, also contain significant amounts of docosapentaenoic acid (DPA), which is also an omega-3 fatty acid.

Although the body to some extent can **convert LA and LNA** into these longer-chain omega-3 fatty acids, the omega-3 fatty acids found in marine oils help fulfil the requirement of essential fatty acids (and have been shown to have wholesome properties of their own).

Since they cannot be made in the body from other substrates and must be supplied in food, they are called essential fatty acids. Mammals lack the ability to introduce double bonds in fatty acids beyond carbon 9 and 10.Hence **linoleic acid (18:2:$\Delta^{9,12}$)and linoleinic acid(18:3;$\Delta^{9,12,15}$)are essential fatty acids** for humans. In the body, essential fatty acids are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection.

Essential fatty acids are polyunsaturated fatty acids and are the parent compounds of the omega-6 and omega-3 fatty acid series, respectively. They are essential in the human diet because there is no synthetic mechanism for them. Humans can easily make saturated fatty acids or monounsaturated fatty acids with a double bond at the omega-9 position, but do not have the enzymes necessary to introduce a double bond at the omega-3 or omega-6 position. The essential fatty acids are important in several human body systems, including the immune system and in blood pressure regulation, since they are used to make compounds such as prostaglandins. The brain has increased amounts of linolenic and alpha-linoleic acid derivatives. Changes in the levels and balance of these fatty acids due to a typical Western diet rich in omega-6 and poor in omega-3 fatty acids is alleged [citation needed] to be associated with depression and behavioral change, including violence. The actual connection, if any, is still under investigation. Further, changing to a diet richer in omega-3 fatty acids, or consumption of supplements to compensate for a dietary imbalance, has been associated with reduced violent behavior and increased attention span, but the mechanisms for the effect are still unclear. So far, at least three human studies have shown results that support this: two school studies as well as a double blind study in a prison Fatty acids play an important role in the life and death of cardiac cells because they are essential fuels for mechanical and electrical activities of the heart.

Essential fatty acids, or EFAs, are fatty acids that cannot be constructed within an organism from other components (generally all references are to humans) as there are no known biochemical pathways capable of producing them. They can only be obtained in the diet if they are to be incorporated into human biological processes. The term refers to those involved in biological processes, and not fatty acids, which may just play a role as fuel. As many of the compounds created from essential fatty acids can be taken directly in the diet, it is possible that the amounts required in the diet (if any) are overestimated. It is also possible they can be underestimated as organisms can still survive in unideal, malnourished conditions.

There are two families of EFAs: ω-3 (or omega-3 or n-3) and ω-6 (omega-6, n-6.) Fats from each of these families are essential, as the body can convert one omega-3 to another omega-3, for example, but cannot create an omega-3 from scratch.

Functions

The biological effects of the ω-3 and ω-6 fatty acids are largely mediated by their mutual interactions,

In the body, essential fatty acids serve multiple functions. In each of these, the balance between dietary ω-3 and ω-6 strongly affects function

* They are modified to make

- ❖ the eicosanoids (affecting inflammation and many other cellular functions)
- ❖ the endogenous cannabinoids (affecting mood, behavior and inflammation)
- ❖ the lipoxins from ω-6 EFAs and resolvins from ω-3 (in the presence of aspirin, downregulating inflammation.)
- ❖ the isofurans, isoprostanes, hepoxilins, epoxyeicosatrienoic acids (EETs) and Neuroprotectin D

* They form lipid rafts (affecting cellular signaling)

* They act on DNA (activating or inhibiting transcription factors such as NFκB, which is linked to pro-inflammatory cytokine production)

Rancidification

Rancidification is the decomposition of fats and other lipids by hydrolysis and/or oxidation. Hydrolysis will split fatty acid chains away from the glycerol backbone in glycerides. These free fatty acids can then undergo further auto-oxidation.

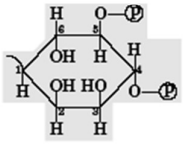
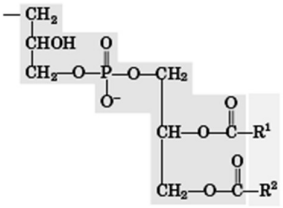
Oxidation primarily occurs with unsaturated fats by a free radical-mediated process. These chemical processes can generate highly reactive molecules in rancid foods and oils, which are responsible for producing unpleasant and obnoxious odors and flavors. These chemical processes may also destroy nutrients in food. Under some conditions, rancidity, and the destruction of vitamins, occurs very quickly.

Antioxidants are often added to fat-containing foods in order to retard the development of rancidity due to oxidation. Natural anti-oxidants include flavonoids, polyphenols, ascorbic acid (vitamin C) and tocopherols (vitamin E). Synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ethoxyquin. The natural antioxidants tend to be short-lived, so synthetic antioxidants are used when a longer shelf life is preferred. The effectiveness of water-soluble antioxidants is limited in preventing direct oxidation within fats, but is valuable in intercepting free radicals that travel through the watery parts of foods. A combination of water-soluble and fat-soluble antioxidants is ideal, usually in the ratio of fat to water. Additionally, rancidification can be decreased, but not completely eliminated, by storing fats and oils in a place with little exposure to oxygen or free radicals, low temperature, and away from light, since light and heat accelerate the rate of reaction of fats with oxygen.

Phospholipids are a class of lipids, and a major component of all biological membranes, along with glycolipids, cholesterol and proteins.

Types:

In phosphoglycerides, the carboxyl group of each fatty acid is esterified to the hydroxyl groups on carbon-1 and carbon-2 of the glycerol molecule. The phosphate group is attached to carbon-3 by an ester link. This molecule, known as a phosphatidate, is present in small quantities in membranes, but is also a precursor for the other phosphoglycerides.

Name of glycerophospholipid	Name of X	Formula of X
Phosphatidic acid	—	— H
Phosphatidylethanolamine	Ethanolamine	— CH_2—CH_2—$\overset{+}{N}H_3$
Phosphatidylcholine	Choline	— CH_2—CH_2—$\overset{+}{N}(CH_3)_3$
Phosphatidylserine	Serine	— CH_2—$\underset{COO^-}{CH}$—$\overset{+}{N}H_3$
Phosphatidylglycerol	Glycerol	— CH_2—$\underset{OH}{CH}$—CH_2—OH
Phosphatidylinositol 4,5-bisphosphate	*myo*-Inositol 4,5-bisphosphate	
Cardiolipin	Phosphatidyl-glycerol	

In phosphoglyceride synthesis, phosphatidates must be activated first. Phospholipids can be formed from an activated diacylglycerol or an activated alcohol.

* **Phosphatidyl serine and phosphatidyl inositol** are formed from a phosphoester linkage between the hydroxyl of an alcohol (serine or inositol) and cytidine diphosphodiacylglycerol (CDP-diacylglycerol).

* In the synthesis of **phospatidyl ethanolamine**, the alcohol is phosphorylated by ATP first, and subsequently reacts with cytidine triphosphate (CTP) to form the activated alcohol (CDP-ethanolamine). The alcohol then reacts with a diacylglycerol to form the final product. Phosphatidyl ethanolamine. Phosphatidyl ethanolamine is the major component of cephalin

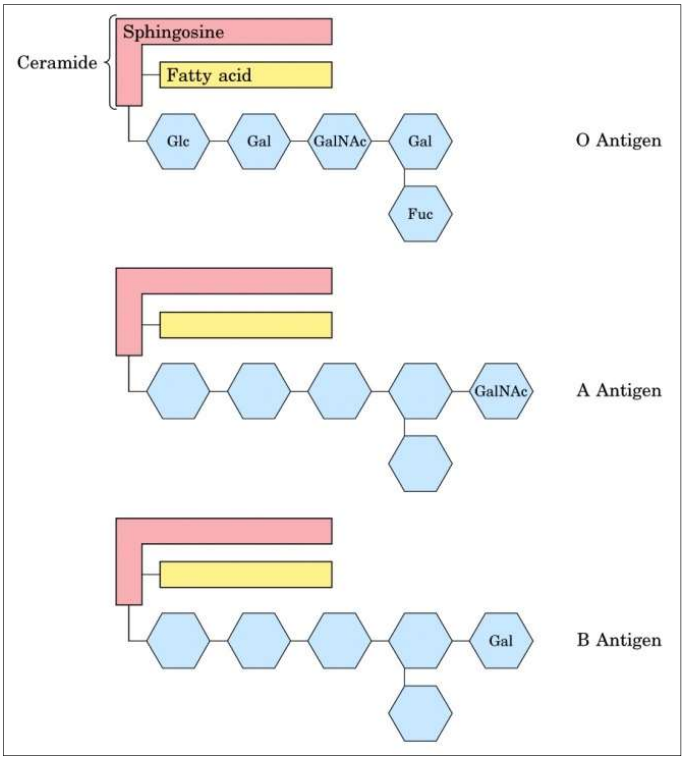
- In mammals**, phosphatidyl choline** can be synthesized via two separate pathways; a series of reactions similar to phosphatidyl ethanolamine synthesis, and the methylation of phosphatidyl ethanolamine, which is catalyzed by phosphatidyl ethanolamine methyltransferase, an enzyme produced in the liver.Phosphatidyl choline is the major component of lecithin. It is also a source for choline in the synthesis of acetylcholine in cholinergic neurons.

Sphingomyelin

The backbone of sphingomyelin is sphingosine, an amino alcohol formed from palmitate and serine. The amino terminal is acylated with a long-chain acyl CoA to yield ceramide. Subsequent substitution of the terminal hydroxyl group by phosphatidyl choline forms sphingomyelin.

Sphingomyelin is also present in all eukaryotic cell membranes, especially the plasma membrane, and is particularly concentrated in the nervous system because sphingomyelin is a major component of myelin, the fatty insulation wrapped around nerve cells by Schwann cells or oligodendrocytes.

Multiple sclerosis is a disease characterised by deterioration of the myelin sheath, leading to impairment of nervous conduction.



Amphipathic character: Due to its polar nature, the head of a phospholipid is hydrophilic (attracted to water); the lipophilic tails are not attracted to water.

When placed in water, phospholipids form one of a number of lipid phases. In biological systems this is restricted to bilayers, in which the lipophilic tails line up against one another, forming a membrane with hydrophilic heads on both sides facing the water. This allows it to form liposomes spontaneously, or small lipid vesicles, which can then be used to transport materials into living organisms and study diffusion rates into or out of a cell membrane. This membrane is partially permeable, capable of elastic movement, and has fluid properties, in which embedded proteins and phospholipid molecules are able to move laterally in it. Such movement can be described by the Fluid Mosaic Model, that describes the membrane as a mosaic of lipid molecules that act as a solvent for all the substances and proteins within it, so proteins and lipid molecules are then free to diffuse laterally through the lipid matrix and migrate over the membrane.

However, this model has now been superseded, as through the study of lipid polymorphism it is now known that the behavior of lipids under physiological (and other) conditions is not simple.

SOME IMP PROSTAGLANDINS

1. Thromboxane:

* Produced primarily by platelets.
* Promotes platelet aggregation.
* Decreases formation of cAMP.
* Vasoconstriction
* Mobilizes intracellular calcium.
* Contraction of Smooth Muscle

2. Prostaglandin E_2:

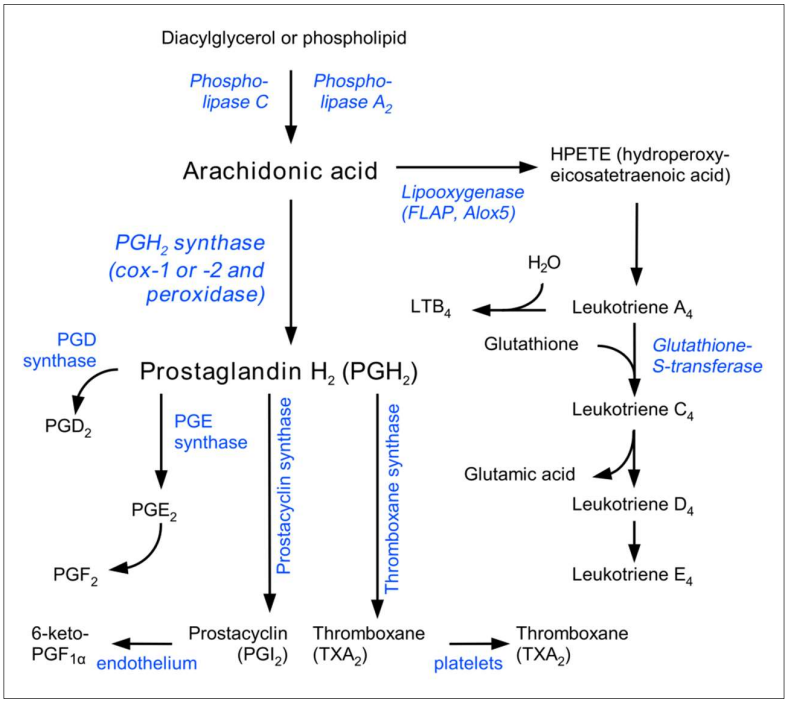
* Produced by most tissues, especially kidney.
* Vasodilation
* Relaxes smooth muscle
* Used to induce labor

3. Prostacyclin:

* Produced primarily by endothelium of vessels.
* Vasodilation
* Inhibit platelet aggregation
* Increases formation cAMP.

4. Leu Kotriene A_4 → C_4→D_4→E_4

* contraction of smooth muscle
* Broncho constriction
* Vasoconstriction
* Increased vascular permeability.
* Components of slow reacting substance of anaphylaxis (SRS-A)

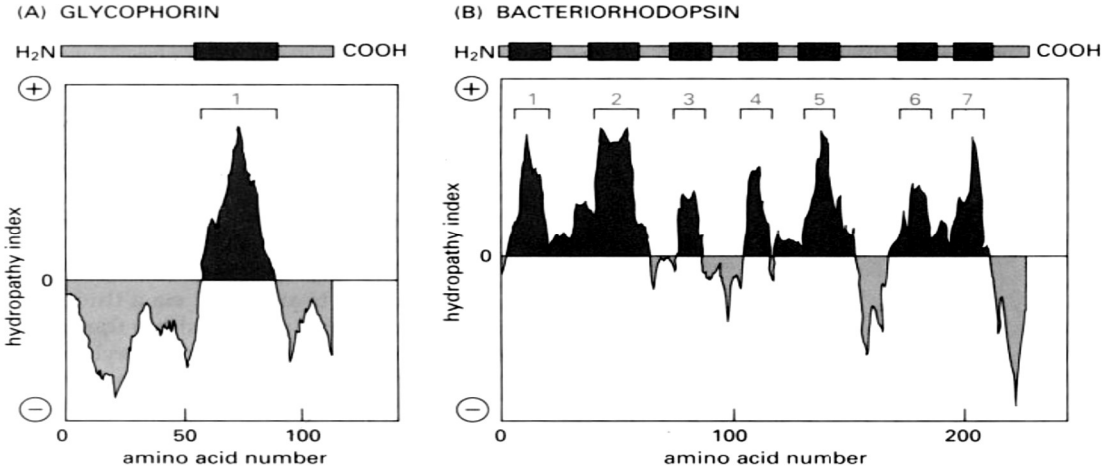


hydrogen bonding between peptide bonds is maximized because of α-helix form of transmembrane domain (in most of the Transmembrane protein this is usual structure).

Hydropathy curve

- The free energy needed to transfer successive segment of a polypeptide chain from a nonpolar solvent to water(polar solvent) is calculated. And **a plot is drawn between Aminoacid segment** of a fixed size (usually 10 Aminoacid) beginning of protein chain **and free energy**. **The "hydropathy index" of the segment is plotted on the "Y-axis" as a function of its location in its chain.**
- A positive value indicates that free energy is required for transfer to water (hydrophobic) and the value assigned is an index of amount of energy needed.
- **We can predict the α-helix by finding out Amino acid sequence But, to predict which segment span membrane as α helix hydropathy plot is used.**
- **By this method it is found out that 20-30 Amino acid with a high degree of hydrophobicity are long enough to span a membrane as a α-helix.**
- **Peaks in the hydropathy index appear at the positions of hydrophobic segments in Aminoacid sequence**.

- **<u>For Glycophorin (Single pass transmembrane protein) there will be a single peak</u>**
- In case of multipass transmembrane proteins (like channel protein or Band 3 protein) there will be multiple peaks

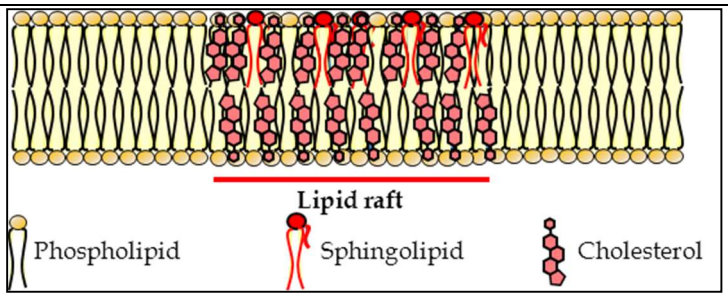


LIPID RAFT

- The plasma membrane of cells is made of a combination of glycosphingolipids and protein receptors organized in **glycolipoprotein microdomains termed lipid rafts.**
- These specialized membrane micro domains compartmentalize cellular processes by serving as **<u>organizing centers for the assembly of signaling molecules, influencing membrane fluidity and membrane protein trafficking, and regulating neurotransmission and receptor trafficking.</u>**
- **<u>Lipid rafts are more ordered and tightly packed than the surrounding bilayer, but float freely in the membrane bilayer.</u>**

<u>Properties of lipid rafts</u>

lipid rafts generally contain twice the amount of cholesterol found in the surrounding bilayer. Also, lipid rafts are enriched in sphingolipids such as sphingomyelin, which is typically elevated by 50% compared to the plasma membrane. To offset the elevated sphingolipid levels, phosphatidylcholine levels are decreased which results in similar choline-containing lipid levels between the rafts and the surrounding plasma membrane.



⇨Cholesterol interacts preferentially, although not exclusively, with sphingolipids due to their structure and the saturation of the hydrocarbon chains. Although not all of the phospholipids within the raft are fully saturated, the hydrophobic chains of the lipids contained in the rafts are more saturated and tightly packed than the surrounding bilayer.
Cholesterol is the dynamic "glue" that holds the raft together. **Due to the rigid nature of the sterol group, cholesterol partitions preferentially into the lipid rafts where acyl chains of the lipids tend to be more rigid and in a less fluid state**

⇨lipid rafts can be extracted from a plasma membrane. The extraction would take advantage of lipid raft resistance to non-ionic detergents, such as **Triton X-100, Brij-98 or CHAPS** at low temperatures (e.g., 4 °C). When such a detergent is added to cells, the fluid membrane will dissolve while the lipid rafts may remain intact and could be extracted.⇨Because of their composition and detergent resistance, lipid rafts are also called detergent-insoluble glycolipid-enriched complexes (GEMs) or DIGs or **Detergent Resistant Membranes (DRMs).**
⇨DRMs can be isolated from cells at higher temperatures following extraction with other non-ionic detergents, such as Lubrol WX and detergents of the Brij series
⇨DRMs can also be isolated on sucrose gradients

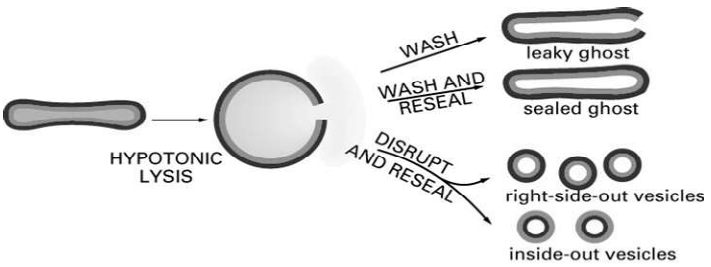
⇨**Lipids in the plasma membrane, with its high sterol and sphingolipid content, are expected to be more ordered than those in intracellular membranes,** even if phase separation and raft formation do not occur. Thus weak detergents might not fully solubilize the plasma membrane even if no rafts were present Detergent concentration, and the ratio of detergent to cellular proteins and lipids, can also affect the degree to which proteins associate with DRMs . That is, even proteins with low inherent affinity for ordered membranes can be detected in DRMs prepared with low amounts of TX100 . It is important in DRM studies to include non-raft-loving proteins (such as the transferrin receptor) as negative controls

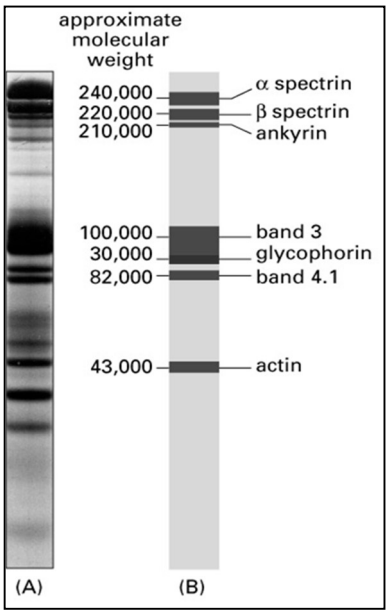
- **Caveolae** are **invaginated lipid raft domains** of the plasma membrane that have roles in cell signaling and membrane internalization.
- **Caveolin** is a protein associated with the cytosolic leaflet of the plasma membrane in caveolae.
- Caveolin interacts with **cholesterol** and self-associates as **oligomers** that may contribute to deforming the membrane to create the unique morphology of caveolae.

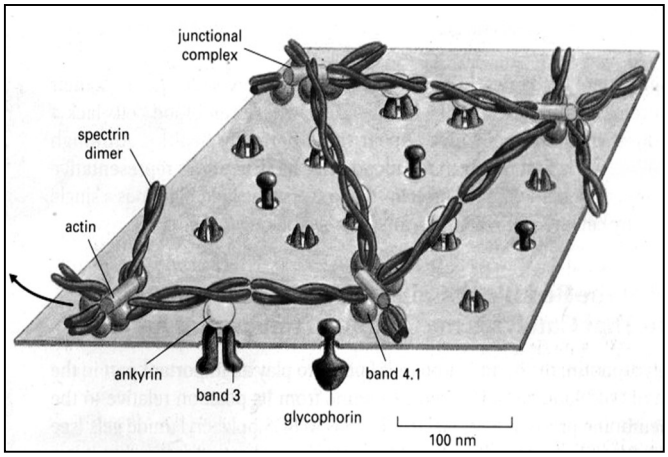
Studying the membrane structure

RBCs are preferred for membrane studies because

1. available in large numbers, relatively uncontaminated by other cell type.
2. No nucleus, No internal organells so No contamination by internal membranes.
3. inside out is possible (in Ghost cells)
4.







In RBCs membrane approximately 15 major protein bands are detected in SDS-PAGE, out which 3 are most important **Spectrin, Glycophorin and Band 3.**

►**Spectrin α & β (Band 1 & 2)** they are long thin flexible rod about 100 nm length that constitute about 25% of the membrane associated protein mass. They are heterodimer formed from two large structurally similar subunits. Heterodimer and 10 or fever residues are sufficient to traverse lipid bilayer as **an extended β-strand.**

- The great majority of Transmembrane proteins are Glycosylated at noncytoplasmic side of membrane.
- Interchain & intrachain (S-S) bond found on non-cytoplasmic side of membrane because they can not be formed at cytosolic side because of reducing environment.
- **Self associated head to head to form 200 nm long tetramer tail end of 4-5 tetramer are linked together by binding to short actin filament and to other cytoskeleton protein (band 4.1) in a functional complex.**
- ⇨***They maintain structural integrity and biconcave shape of RBCs. In their absence RBCs will be spherical and abnormally fragile-causing anaemia.***
- ⇨Binding of Spectrin to membrane is through ankyrin which is intracellular attachment protein which attach to Band 3 on other side. **This is how ankyrin also reduces the rate of diffusion of these band 3 molecules in the lipid bilayer**.
- Spectrin cytoskeleton is also attached with band 4.1 which also bind to both **band 3 & Glycophorin**

►**Glycophorin:**

This 131 amino acid protein was the **first integral protein to be sequenced** .

The polypeptide chain of glycophorin consists of three domains:

1. An N-terminal region on the extracellular side of the membrane that contains all the N- and O-linked **glycosylation sites**;

2. A hydrophobic central region that is buried in the hydrophobic core of the bilayer; and

3. A C-terminal region rich in polar and charged residues that is exposed on the cytosolic side of the membrane

As with the majority of transmembrane proteins, the hydrophobic membrane spanning region consists mainly of amino acid residues with hydrophobic side-chains that are folded in an α-**helical conformation**. **10^6 per cells, function unknown, cell appear healthy even if they are absent.**

►**Band 3:** 930 Aminoacid long, Multipass trans membrane protein, helping in carrying CO_2 from tissue to lung by acting as **antiporter** by allowing HCO_3^- to cross membrane is exchange for Cl^-. Thus by making RBC membrane freely permeable to HCO^-_3 this transporter increases the amount of CO_2 that the blood can deliver to the lung

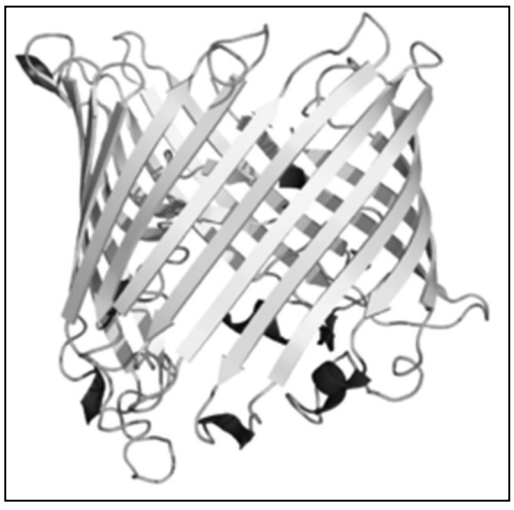
►Multiple membrane-spanning proteins

- Some integral proteins have multiple membrane spanning α-helices.
- Bacteriorhodopsin, a protein found in a photosynthetic bacterium, captures energy from light and uses it to pump protons across the bacterial membrane. Like numerous other integral proteins, the polypeptide chain of bacteriorhodopsin loops back wards and forwards across the lipid bilayer seven times.
- Each of the seven transmembrane α-helices is linked to the next by a short hydrophilic region of the polypeptide chain that is exposed either on the extracellular or cytosolic side of the membrane.
- In contrast, **the anion exchange band 3 protein of the erythrocyte plasma membrane that transports Cl- and HCO_3^- across the membrane loops backwards and forwards across the lipid bilayer 12-14 times**

►Porin:

- Multipass transmembrane segment arranged as β-sheet (αβ barrel). Found in outer membrane of bacteria (negative) allowing hydrophilic solutes of upto 600 dalton to diffuse across the outer lipid bilayer.
- It consist of trimer in which each monomer forms a tubular -barrel, which traverses the lipid bilayer and has a water filled pore at its center.
- The barrel is formed from a 16-standard anti-parallel β-sheet which is sufficiently curved to roll up into a cylindrical structure. Polar side chain line the aqueous channel on the inside, while nonpolar side chains project from the outside of the barrel to interact with the hydrophobic core of the lipid bilayer.

Membrane protein purification and reconstitution:



1. The first step in the **purification** of an integral membrane protein is to disrupt its interactions with other integral proteins and the lipids in the membrane. This is commonly achieved by adding a **detergent** which solubilizes the membrane.
2. In order to solubilize the membrane but not denature the protein, gentle detergents such as Triton X-100 or octyl glucoside are used, rather than the harsh detergent SDS. As the detergent molecules are themselves amphipathic they readily intercalate into the lipid bilayer and disrupt the hydrophobic interactions.
3. Once solubilized, the hydropnobic region of the integral protein is coated with a layer of detergent molecules which enables the protein to remain in solution.
4. The solubilized protein can then be purified as for a water-soluble globular protein as long as detergent is kept in the buffers to prevent aggregation and loss of the protein.
5. Once purified, an integral protein can be reincorporated into artificial lipid vesicles (liposomes) in order to study its function.
6. If phospholipids are added to the protein in detergent solution and the detergent dialyzed away, phospholipid vesicles containing the protein will spontaneously form. **The function of the protein can then be studied.** For example, if the Ca^{2+}-ATPase is reincorporated into lipid vesicles, its function (i.e. transport of Ca^{2+} upon ATP hydrolysis) can be studied by monitoring Ca^{2+} on the inside of the vesicle upon addition of Ca^{2+} and ATP to the outside .

Detergents are polar lipids that are soluble in water. The presence of both a hydrophobic and hydrophilic portion makes these compounds very useful for lysis of lipid membranes, solubilization of antigens, and washing of immune complexes.

Types of Detergents

Anionic	SDS, Cholate, Deoxycholate
Cationic	CTAB
Amphoteric	LysoPC, CHAPS, Zwittergent
Nonionic	Octylglucoside, Digitonin, Lubrol, Triton X-100, Nonidet P-40, Tween 80

The following detergents have detrimental properties for some procedures:

1. **Phenol containing detergents** (ie., Triton X-100 and NP40) have a high absorbance at 280nm and thus Interfere with protein monitoring at that OD. Lubrol may be substituted. Phenol-containing detergents also induce precipitation in the Folin (Lowry) protein assay. They can also be iodinated...and thus should not be used if iodinating proteins.
2. **High micellar molecular weight** interferes with gel filtration and not easily removed by dialysis.
3. Sodium cholate and sodium deoxycholate are insoluble below pH 7.5. Above an ionic strength of 0.1%SDS they will often crystalize.
4. **Ionic detergents interfere with nondenaturing electrophoresis and isoelectric focusing**.

CHAPTER: 15

REGENERATION

Regeneration is of three types:

1. Physiological Regeneration

There is a constant loss of many kinds of calls due to wear and tear caused by day-to-day activities. The replacement of these cells is known as physiological regeneration

Example:

⇨Replacement of R.B.C's

The worn out R.B.C's are deposited in the spleen and new R.B.C's regularly produced from the bone marrow cells, since the life span of R.B.C's is only 120days.

⇨Replacement of Epidermal Cells of the Skin

The cells from the outer layers of epidermis are regularly peeled off by wear and tear. These are constantly being replaced by new cells added by the malpighian layer of the skin.

2. Reparative Regeneration

This is the replacement of lost parts or repair of damaged body organs. In this type of regeneration, wound is repaired or closed by the expansion of the adjoining epidermis over the wound.

Example:

⇨Regeneration of limbs in salamanders

⇨Regeneration of lost tail in lizard

⇨Healing of wound

⇨Replacement of damaged cells.

3. Autotomy

In some animals like starfish, some part of the body is broken off on being threatened by a predator. This phenomenon of self-mutilation of the body is called autotomy

Example:

⇨Crabs break off their leg on approaching of the enemy

⇨Holothurians throw off their internal viscera

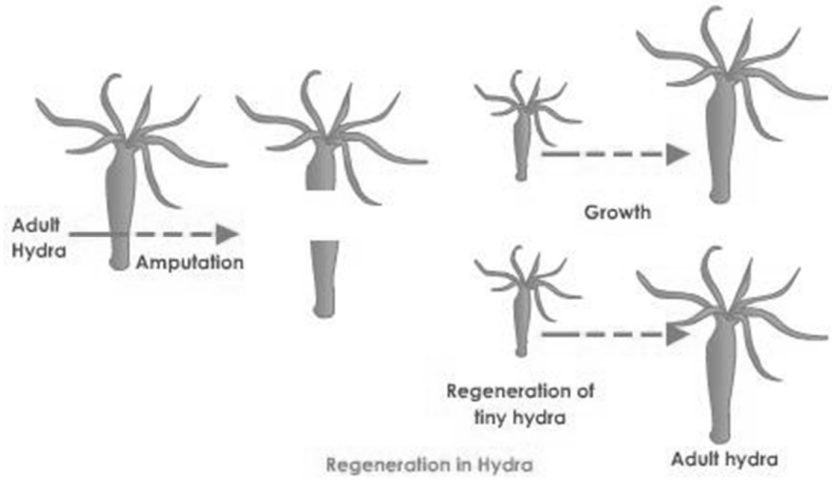
⇨Starfish breaks off an arm

Regenerative capacity in Animal Group

The capacity of regeneration varies in its extent in various animal groups. Regenerative capacity is very high among the protozoan, sponges and coelenterates.

Invertebrates

- **In sponges**, the entire body can be reconstructed from isolated body cells. The cells rearrange and reorganize to form bilayered sponge body wall.
- **Regeneration was first discovered in hydra by Tremble (1740). Even 1/1000th part of the body regenerate into new organisms.**
- **In hydra and planaria**, small fragments of the body can give rise to a whole animal. When a hydra or a planaria is cut into many pieces, each individual part regenerates into a whole individual.
- **Some annelids like earthworms** are able to regenerate some segments removed from the anterior and posterior ends of the body.
- **Some molluscs can regenerate** only the eyes and heads while squids can also regenerate their arms.



- Many arthropods (e.g., spiders, crustaceans, insect larvae, etc) can regenerate limbs only. Regeneration is faster in the young than in the adults. Regenerated part may not always be similar to the part lost. This type of regeneration is called heteromorphosis.
- Echinoderms (like starfish, brittle star, sea lilly)exhibit autotomy. They can regenerate arms and parts of te body.
- **Vertebrates**
- **Fishes:** Lamprey can regenerate its lost tail. Some fishes have the ability to regenerate parts of its fins.
- **Amphibians:** The regeneration power is well marked in urodel amphibians like salamanders, newts and their axolotl larvae. They can regenerate limbs, tail, external gills, jaws, parts of eye like lens and retina. Tail and limb regeneration is found in the larval stages of frogs and toads.
- **Reptiles:** Lizards exhibit autotomy. When threatened, the lizard detaches its tail near the base to confuse its predator and later regenerates a new tail. The new tail differs from the old one in its shape, absence of vertebrae and the kind of scales covering it.
- **Birds:** Regeneration is restricted to parts of the beak.
- **Mammals:** Regeneration is restricted to tissues only. External parts are not regenerated. Skin and skeletal tissues possess great power of regeneration. The liver has the maximum capacity of regeneration. If one kidney is damaged or removed, the other enlarges to compensate the lost kidney. This is called as compensatory hypertrophy.
- **Regeneration is an usual form of asexual reproduction in several lower groups of animals.**

Three Types of Regeneration based on Cellular Mechanism

1. The first mechanism involves the dedifferentiation of adult structures of form an undifferentiated mass of cells that then becomes respecified. This type of regeneration is called **epimorphosis** and is characteristic of regenerating limbs.
2. **The second mechanism is called morphallaxis**. Here, regeneration occurs through the re-patterning of existing tissues, and there is little new growth. Such regeneration is seen in hydras.
3. A third type of regeneration is an intermediate type, and can be through of as **compensatory regeneration**. Here, the cells divide, but maintain their differentiated functions. They produce cells similar to themselves and do not form a mass of undifferentiated tissue. **This type of regeneration is characteristic of the mammalian liver.**

1. Epimorphosis:

In contrast to morphallaxis, **epimorphosis requires active cellular proliferation prior to the replacement of the lost body part.**
Epimorphosis can be further subdivided into dedifferentiation-*dependent* and dedifferentiation-*independent* subclasses. Planarian, which are flatworms, regenerate using a dedifferentiation-*independent* mechanism in which preexisting stem cells, known as neoblasts, begin to proliferate and migrate to the injured site in response to injury.

These cells then form a mass of proliferating cells, known as the regeneration blastema, that will later differentiate into the specialized cells that comprise the regenerated structure.

Most tissue regeneration in mammals also belongs to the dedifferentiation-*independent* subclass. For example, mammals can regenerate their muscle, bone, epithelia of the skin and gut, blood, and some neurons by activating preexisting stem cells or progenitor cells.

Vertebrate limb regeneration involves cell dedifferentiation and growth.

Regeneration of a Limb of a Newt

When an adult salamander limb is amputated, the remaining cells are able to reconstruct a complete limb, with all its differentiated cells arranged in the proper order. In other words, the new cells construct only the missing structures and no more. For example, when a wrist is amputated, the salamander forms a new wrist and not a new elbow.

⇨Upon limb amputation, a plasma clot forms, and within 6 to 12 hours, epidermal cells from the remaining stump migrate to cover the wound surface, forming the **wound epidermis**. This single-layered structure is required for the regeneration of the limb, and it proliferates to form the **apical ectodermal cap**. Thus, in contrast to wound healing in mammals, no scar forms, and the dermis does not move with the epidermis to cover the site of amputation. The nerves innervating the limb degenerate for a short distance proximal to the plane of amputation

⇨During the next 4 days, the cells beneath the developing cap undergo a dramatic dedifferentiation: bone cells, cartilage cells, fibroblasts, myocytes, and neural cells lose their differentiated characteristics and become detached from one another The formerly well-structured limb region at the cut edge of the stump thus forms a proliferating mass of indistinguishable, dedifferentiated cells just beneath the apical ectodermal cap. This dedifferentiated cell mass is called the **regeneration blastema**. These cells will continue to proliferate, and will eventually redifferentiate to form the new structures of the limb

⇨The creation of the blastema depends upon the formation of single, mononucleated cells.It is probable that the macrophages that are released into the wound site secrete metallo-proteinases that digest the extracellular matrices holding epithelial cells together The proliferation of the salamander limb regeneration blastema is dependent on the presence of nerves. a minimum number of nerve fibers must be present for regeneration to take place. It is thought that the neurons release mitosis-stimulating factors that increase the proliferation of the blastema cells

⇨**Glial growth factor** (**GGF**) is known to be produced by newt neural cells, is present in the blastema, and is lost upon denervation. When this peptide is added to a denervated blastema, the mitotically arrested cells are able to divide again

⇨**A fibroblast growth factor** may also be involved. FGFs infused into denervated blastemas are able to restore mitosis.

⇨Another important neural agent necessary for cell cycling is **transferrin**, an iron-transport protein that is necessary for mitosis in all dividing cells (since ribonucleotide reductase, the rate-limiting enzyme of DNA synthesis, requires a ferric ion in its active site). When a hindlimb is severed, the sciatic nerve transports transferrin along the axon and releases large quantities of this protein into the blastema. Neural extracts and transferrin are both able to stimulate cell division in denervated limbs, and chelation of ferric ions from a neural extract abolishes its mitotic activity regeneration blastema resembles in many ways the progress zone of the developing limb.

⇨The dorsal-ventral and anterior-posterior axes between the stump and the regenerating tissue are conserved, and cellular and molecular studies have confirmed that the patterning mechanisms of developing and regenerating limbs are very similar. By transplanting regenerating limb blastemas onto developing limb buds, Muneoka and Bryant (1982) showed that the blastema cells could respond to limb bud signals and contribute to the developing limb

⇨Retinoic acid appears to play an important role both in the dedifferentiation of the cells to form the regeneration blastema and in the respecification processes as the cells redifferentiate. If regenerating animals are treated with sufficient concentrations of retinoic acid (or other retinoids), their regenerated limbs will have duplications along the proximal-distal axis This response is dose-dependent and at maximal dosage can result in a complete new limb regenerating (starting at the most proximal bone), regardless of the original level of amputation. Dosages higher than this result in inhibition of regeneration. It appears that the retinoic acid causes the cells to be respecified to a more proximal position

⇨ In regenerating limbs, retinoic acid is present in a distinct pattern & is higher in concentration in more distal blastemas. Retinoic acid is synthesized in the regenerating limb wound epidermis and is seen to form a gradient along the proximal-distal axis of the blastema. This gradient of retinoic acid is thought to activate genes differentially across the blastema, resulting in the specification of pattern in the regenerating limb.

⇨ **Exposure to retinoic acid changes the positional value of a blastema to more proximal ones, such that elements proximal to the cut as well as those distal will be generated. Wounded epidermis is a strong source of retinoic acid**

⇨Following limb amputation, regeneration always occurs in the distal direction, regardless of the position of the cut or the orientation of the limb. This is shown by the following experiments:

- If the limb is amputated at the wrist, only a new hand is regenerated, but if it is amputated at the shoulder, an entire new limb will form.
- If the limb is amputated at the wrist, and then the cut surface of the stump is inserted into the flank of the body, a blood supply is established and the animal has a 'closed limb'. If this limb is then cut in the middle of the humerus, both cut surfaces produce a blastema and regenerate distal structures, even though the proximodistal axis of one of the limbs is inverted.

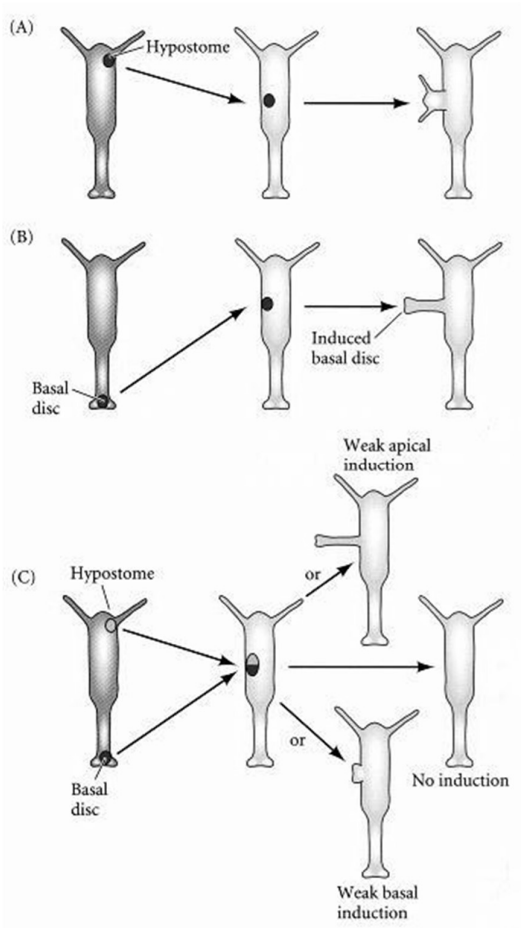
These experiments indicate that the cells in the urodele limb retain a set of positional values along the proximodistal axis. Following limb amputation, the cells at the site of amputation use their own positional identity as a reference to generate a new set of positional values in the blastema, representing the more distal limb elements. Furthermore, the positional values established in the regeneration blastema are autonomous. If the blastema is removed from a regenerating limb and grafted to another region of the body that supports its growth, it will give rise to the same set of distal structures.

Table: Some key differences between limb development and limb regeneration

Context	Regenerating limb	Developing limb
Role of mesoderm	Blastema proliferates and produces all mesodermal structures *including muscles*	Progress zone proliferates and produces skeletal derivatives. Muscles form from immigrating myoblasts.
Role of ectoderm	AEC **apical ectodermal cap** induces dedifferentiation and maintains proliferation of blastema.	AER **apical ectodermal ridge** maintains proliferation of progress zone.
Role of nervous system	Required to initiate regeneration.	None.
Heterochronic mesenchyme grafts	Intercallary regeneration occurs when distal blastema grafted to proximal stump.	No intercallary growth occurs when older progress zone grafted to younger limb bud. Proximal elements missing from limb.
Proximodistal axis specification	Exposure of cells to retinoic acid specifies positional values? Downstream activation of *HoxA* genes.	Length of time cells remain in the progress zone specifies positional values. Downstream activation of *HoxA* genes.

2. Morphallactic Regeneration in Hydras

When a hydra is cut in half, containing the head will regenerate a new basal disc, and the half containing the basal disc will regenerate a new head. Moreover, if a hydra is cut into several portions, the middle portions will regenerate both heads and basal discs at their appropriate ends.



▶The head activator gradient

The polarity of the hydra is coordinated by a series of morphogenetic gradients that permit the head to form only at one placed and the disc to form only at another.

When hypostome tissue from one hydra is transplanted into the middle of another hydra, it forms a new apical-basal axis, with the hypostome extending outward. When a basal disc is grafted to the middle of a host hydra, a new axis also forms, but with the opposite polarity, extending a basal disc. When cells from both ends are transplanted simultaneously into the middle of a hoist, no new exis is formed, or the new axis has little polarity.

The head inhibitor gradient. Normal regeneration of the hypostome is inhabited when an intact hypostome is grafted adjacent to the amputation site. This finding suggested that one hypostome can inhibit the formation of another. Extra heads do not form in the hydra because the presence of the hypostome prevents the formation of nay other hypostome. The head inhabitor gradient can be measured by inserting a subhypostomal region into various regions along the trunks of host hydras. This region will not produce a head when implanted into the apical area of an intact host hydra. However, it will form a head if the host's head has been removed.

Aging can be defined as the time-related deterioration of the physiological functions necessary for survival and fertility. Characteristics of aging – as distinguished from diseases of aging affect all the individuals of a species.

3. Compensatory Regeneration in the Mammalian Liver

The standard assay for liver regeneration is to remove (after anesthesia) specific lobs of the liver, leaving the others intact. The removed lobe does not grow back, but the remaining lobes enlarge to compensate for the loss of the missing liver tissue. Even in humans, the amount of liver regenerated is equivalent to the amount of liver removed. The liver regenerates by the proliferation of the existing tissues.

As in the regenerating salamander limb, there is a return to an embryonic condition in the regenerating liver. Fetal transcription factors and products are made, as are the cyclins that control cell division. But the return to the embryonic state is not as complete as in the amphibian limb.

One of the most important proteins for returning live cells to the cell cycle is hepatocyte growth factor (HGF). Within an hour after partial hepatectomy, the blood level of HGF has risen 20-fold. The trauma of partial hepatectomy may activate metalloproteinases that digest the extracellular matrix and permit the hepatocytes to separate and proliferate. **These enzymes also may cleave HGF to its active form**

CHAPTER: 16

AGING

Almost all living organisms pass through a sequence of changes, characterized by growth, development, maturation and senescence

- **Gerontology**--study of all aspects of individual aging and its consequences
- **Geriatrics**--subdivision of medicine that is concerned with old age and its diseases
- **Senescence**--the constellation of changes in structure and function of an organism that can be observed after sexual maturation; a deteriorative change that causes an increase in mortality with age.
- **Senility.** The endpoint of senescence
- **Life Expectancy**--average length of life from birth
- **Maximum Life Span Potential**--longest life an individual can live
- **Life Endurancy**--age at which 1% of original cohort still alive
- **Active Life Expectancy**--end point is the loss of independence in daily life. The end point of active life is measured with a battery of tests referred to as **ADLs** (activities of daily living--feeding, bathing, mobility, etc.).

Causes of Aging

Oxidative damage

Aging is a by-product of normal metabolism; no mutations are required. About 2-3% of the oxygen atoms taken up by the mitochondria are reduced insufficiently to reactive oxygen species (ROS). These ROS include the superoxide ion, the hydroxyl radical, and hydrogen peroxide. ROS can oxidize and damage cell membranes, proteins, and nucleic acids.

The evidence for ROS involvement for ROS involvement in mammalian aging is not as clear. Mutations in mice that result in the lack of certain ROS – degrading enzymes do not cause premature aging.

Another type of evidence does suggest that ROS may be important in mammalian aging : aging in mammals can be slowed caloric restriction. Also, vitamins E and C are both ROS inhibitors, and vitamin E increases the longevity of flies and nematodes when it is added to their diet. However, results in mammals are not as easy to interpret, and there is no clear evidence that ROS inhibitors work as well as in invertebrates.

General wear-and-tear and genetic instability

As one gets older, small traumas to the body build up. Point mutations increase in number, and the efficiencies of the enzymes enclosed by our genes decrease. Moreover, if a mutation occurred in a part of the protein synthetic apparatus, the cell would make a large percentage of faulty proteins. If mutations arose in the DNA-synthesizing enzymes, the rate of mutations would be expected to increase markedly. Likewise, DNA repair may be important in preventing senescence, and species whose members' cells have more efficient DNA repair enzymes live longer.

▶Mitochondrial General Damage

The mutation rate in mitochondria is 10-20 times faster than the nuclear DNA mutation rate. It is thought that mutations in mitochondria could

(1) lead to defects in energy production,
(2) lead to the production of ROS by faculty electron transport, and/or
(3) induce apoptosis. Age-dependent declines in mitochondrial function are seen in many animals, including humans.

▶**Telomere Shortening.** Telomerase adds the telomere onto the chromosome at each cell division. Most mammalian somatic tissues lack telomerase, so it has been proposed that telomere shortening could be a "clock" that eventually prohibits the cells from dividing any more. When human fibroblasts are cultured, they can divide only a certain number of times, and their telomeres shorten. If these cells are made to express telomerase, they can continue dividing.

CHAPTER: 4 DNA REPAIR

DNA in the living cell is subject to many chemical alterations (a fact often forgotten in the excitement of being able to do DNA sequencing on dried and/or frozen specimens
If the genetic information encoded in the DNA is to remain uncorrupted, any chemical changes must be corrected.
A failure to repair DNA produces a mutation.

Agents that Damage DNA

- ►Certain wavelengths of **radiation**
  - ionizing radiation such as gamma rays and x-rays
  - ultraviolet rays, especially the UV-C rays (~260 nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield
- ►Highly-reactive **oxygen radicals** produced during normal cellular respiration as well as by other biochemical pathways
- ►Chemicals in the **environment**
  - many hydrocarbons, including some found in cigarette smoke
  - some plant and microbial products, e.g. the aflatoxins produced in moldy peanuts
- ►Chemicals used in **chemotherapy**, especially chemotherapy of cancers

Types of DNA Damage

1. All four of the bases in DNA (**A, T, C, G**) can be covalently modified at various positions.
   - One of the most frequent is the loss of an amino group ("deamination") — resulting, for example, in a **C** being converted to a **U**.
2. **Mismatches** of the normal bases because of a failure of proofreading during DNA replication.
   - Common example: incorporation of the pyrimidine **U** (normally found only in RNA) instead of **T**.
3. **Breaks** in the backbone.
   - Can be limited to one of the two strands (a single-stranded break, **SSB**) or
   - on **both strands** (a **d**ouble-**s**tranded **b**reak (**DSB**).
   - Ionizing radiation is a frequent cause, but some chemicals produce breaks as well.
4. **Crosslinks** Covalent linkages can be formed between bases
   - on the same DNA strand ("intrastrand") or
   - on the opposite strand ("interstrand").

Several chemotherapeutic drugs used against cancers crosslink DNA

Repairing Damaged Bases

Damaged or inappropriate bases can be repaired by several mechanisms:
A. Direct chemical reversal of the damage
B. Excision Repair, in which the damaged base or bases are removed and then replaced with the correct ones in a localized burst of DNA synthesis. There are three modes of excision repair, each of which employs specialized sets of enzymes.

1. Base Excision Repair (**BER**)
2. Nucleotide Excision Repair (**NER**)
3. Mismatch Repair (**MMR**)

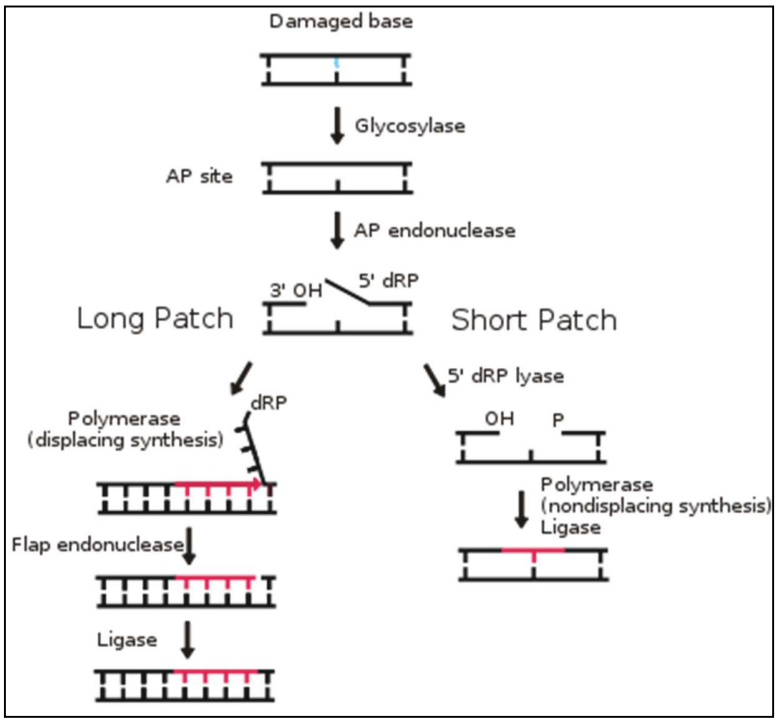
A. Direct Reversal of Base Damage

Perhaps the most frequent cause of point mutations in humans is the spontaneous addition of a methyl group (CH_3-) (an example of alkylation) to C followed by deamination to a T. Fortunately, most of these changes are repaired by enzymes, called glycosylases, that remove the mismatched T restoring the correct C. This is done without the need to break the DNA backbone (in contrast to the mechanisms of excision repair described below).
Some of the drugs used in cancer chemotherapy ("chemo") also damage DNA by alkylation.
Some of the methyl groups can be removed by a protein encoded by our **MGMT** gene. However, the protein can only do it once, so the removal of each methyl group requires another molecule of protein.
This illustrates a problem with direct reversal mechanisms of DNA repair: they are quite wasteful. Each of the myriad types of chemical alterations to bases requires its own mechanism to correct. What the cell needs are more general mechanisms capable of correcting all sorts of chemical damage with a limited toolbox. This requirement is met by the mechanisms of **excision repair**.

B1. Base Excision Repair (BER)

The steps and some key players:

1. removal of the damaged base (estimated to occur some 20,000 times a day in each cell in our body) by a DNA glycosylase. We have at least 8 genes encoding different DNA glycosylases each enzyme responsible for identifying and removing a specific kind of base damage.
2. removal of its deoxyribose phosphate in the backbone, producing a gap. We have two genes encoding enzymes with this function.
3. replacement with the correct nucleotide. This relies on **DNA polymerase beta**, one of at least 11 DNA polymerases encoded by our genes.
4. ligation of the break in the strand. Two enzymes are known that can do this; both require ATP to provide the needed energy.



B2. Nucleotide Excision Repair (NER)

NER differs from BER in several ways:

- It uses different enzymes.
- Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage.

The steps and some key players:

1. The damage is recognized by one or more protein factors that assemble at the location.
2. The DNA is unwound producing a "bubble". The enzyme system that does this is **Transcription Factor IIH**, **TFIIH**, (which also functions in normal transcription).
3. Cuts are made on both the 3' side and the 5' side of the damaged area so the tract containing the damage can be removed.
4. A fresh burst of DNA synthesis — using the intact (opposite) strand as a template — fills in the correct nucleotides. The DNA polymerases responsible are designated polymerase **delta** and **epsilon**.
5. A **DNA ligase** covalent binds the fresh piece into the backbone.

Xeroderma Pigmentosum (XP)

XP is a rare **inherited** disease of humans which, among other things, predisposes the patient to

- pigmented lesions on areas of the skin exposed to the sun and
- an elevated incidence of skin cancer.

It turns out that XP can be caused by mutations in any one of several genes — all of which have roles to play in **NER**. Some of them:

- **XPA**, which encodes a protein that binds the damaged site and helps assemble the other proteins needed for NER.

- **XPB** and **XPD**, which are part of TFIIH. Some mutations in XPB and XPD also produce signs of premature aging.
- **XPF**, which cuts the backbone on the 5' side of the damage
- **XPG**, which cuts the backbone on the 3' side.

Transcription-Coupled NER

Nucleotide-excision repair proceeds most rapidly

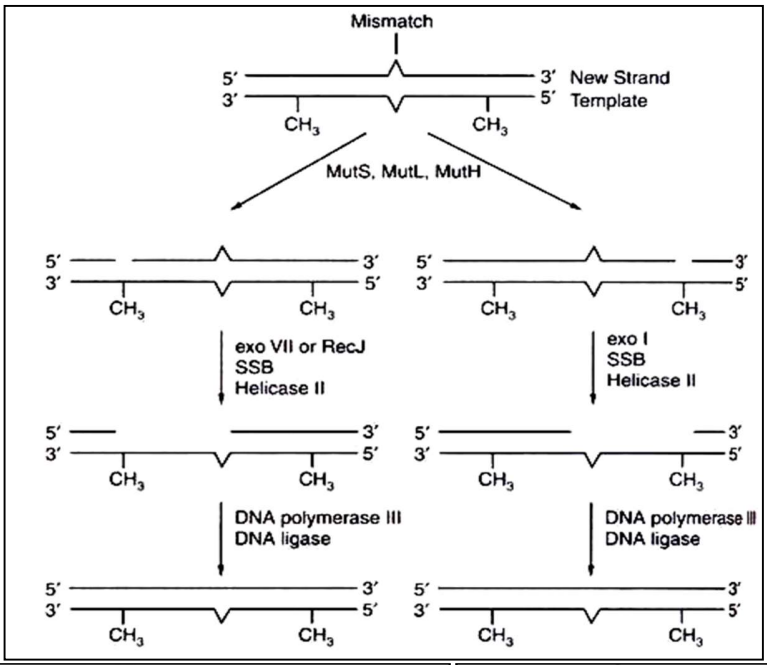
- in cells whose genes are being actively transcribed
- on the DNA strand that is serving as the template for transcription.

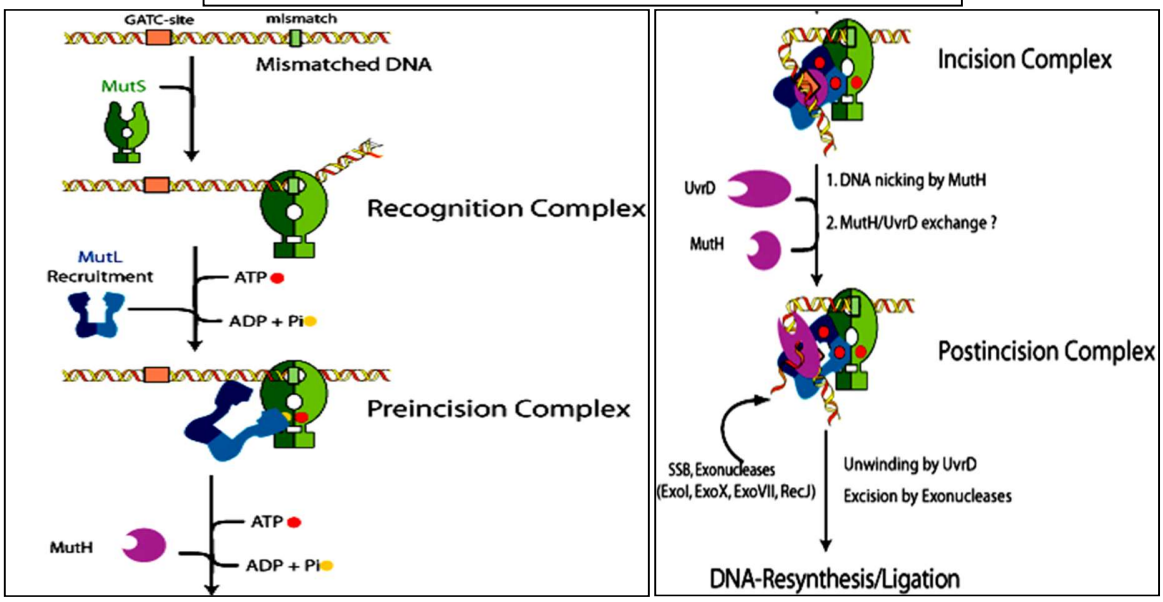
This enhancement of NER involves XPB, XPD, and several other gene products. The genes for two of them are designated **CSA** and **CSB** (mutations in them cause an inherited disorder called **Cockayne's syndrome**).

The CSB product associates in the nucleus with **RNA polymerase II**, the enzyme responsible for synthesizing **messenger RNA** (mRNA), providing a molecular link between transcription and repair.

One plausible scenario: If RNA polymerase II, tracking along the template (antisense) strand), encounters a damaged base, it can recruit other proteins, e.g., the CSA and CSB proteins, to make a quick fix before it moves on to complete transcription of the gene.

B3. Mismatch Repair (MMR)





Mismatch repair deals with correcting mismatches of the **normal bases**; that is, failures to maintain normal **Watson-Crick base pairing (A•T, C•G)** Many incorrectly inserted nucleotides that escape detection by proofreading are corrected by *mismatch repair*

⇨ Incorrectly paired bases distort the three-dimensional structure of DNA, and mismatch repair enzymes detect these distortions. In addition to detecting incorrectly paired bases, the mismatch-repair system corrects small unpaired loops in the DNA, such as those caused by strand slippage in replication.

⇨After the incorporation error has been recognized, mismatch-repair enzymes cut out the distorted section of the newly synthesized strand and fill the gap with new nucleotides, by using the original DNA strand as a template.

⇨The proteins that carry out mismatch repair in *E. coli* differentiate between old and new strands by **the presence of methyl groups on special sequences of the old strand.** After replication, adenine nucleotides in the sequence GATC are methylated by an enzyme called Dam methylase. **The process of methylation is delayed and so, immediately after replication, the old strand is methylated and the new strand is not** .

In *E. coli*, the proteins MutS, MutL, and MutH are required for mismatch repair.

→ **MutS** binds to the mismatched bases and forms a complex with **MutL and MutH;** this complex is thought to bring an unmethylated GATC sequence in close proximity to the mismatched bases. MutH nicks the unmethylated strand at the GATC site and exonucleases degrade the unmethylated strand from the nick to the mismatched bases

⇢ DNA polymerase and DNA ligase fill in the gap on the unmethylated strand with correctly paired nucleotides

⇨It can enlist the aid of enzymes involved in both **base-excision repair (BER) and nucleotide-excision repair (NER) as well as using enzymes specialized for this function.**

⇨Mutations in either of these genes predisposes the person to an inherited form of colon cancer. So these genes qualify as **tumor suppressor genes.**

Question: How does the MMR system know which is the incorrect nucleotide?

In E. coli, certain adenines become methylated shortly after the new strand of DNA has been synthesized. The MMR system works more rapidly, and if it detects a mismatch, it assumes that the nucleotide on the already-methylated (parental) strand is the correct one and removes the nucleotide on the freshly-synthesized daughter strand. How such recognition occurs in mammals is not yet known.

Synthesis of the repair patch is done by the same enzymes used in NER: **DNA polymerase delta** and **epsilon**. Cells also use the MMR system to enhance the fidelity of recombination; i.e., assure that only homologous regions of two DNA molecules pair up to crossover and recombine segments (e.g., in meiosis).

Repairing Strand Breaks

Ionizing radiation and certain chemicals can produce both single-strand breaks (**SSB**s) and double-strand breaks (**DSB**s) in the DNA backbone.

1. **Single-Strand Breaks (SSBs)**
   Breaks in a single strand of the DNA molecule are repaired using the same enzyme systems that are used in Base-Excision Repair (BER).
2. **Double-Strand Breaks (DSBs)**
   There are two mechanisms by which the cell attempts to repair a complete break in a DNA molecule:
3. **Direct joining** of the broken ends. This requires proteins that recognize and bind to the exposed ends and bring them together for ligating. They would prefer to see some complementary nucleotides but can proceed without them so this type of joining is also called **Nonhomologous End-Joining** (**NHEJ**).

- Errors in direct joining may be a cause of the various **translocations** that are associated with cancers.
- **Examples**: **Burkitt's lymphoma , the Philadelphia chromosome in chronic myelogenous leukemia (CML), B-cell leukemia**

4. **Homologous Recombination.** Here the broken ends are repaired using the information on the intact **sister chromatid** (available in G_2 after chromosome duplication), **or** on the **homologous chromosome** (in G_1; that is, before each chromosome has been duplicated). This requires searching around in the nucleus for the homolog — a task sufficiently uncertain that G_1 cells usually prefer to mend their DSBs by **NHEJ**. **or** on the **same chromosome** if there are duplicate copies of the gene on the chromosome oriented in opposite directions (head-to-head or back-to-back).
   Two of the proteins used in homologous recombination are encoded by the genes **BRCA1** and **BRCA2**. Inherited mutations in these genes predispose women to breast and ovarian cancers.

Meiosis also involves DSBs

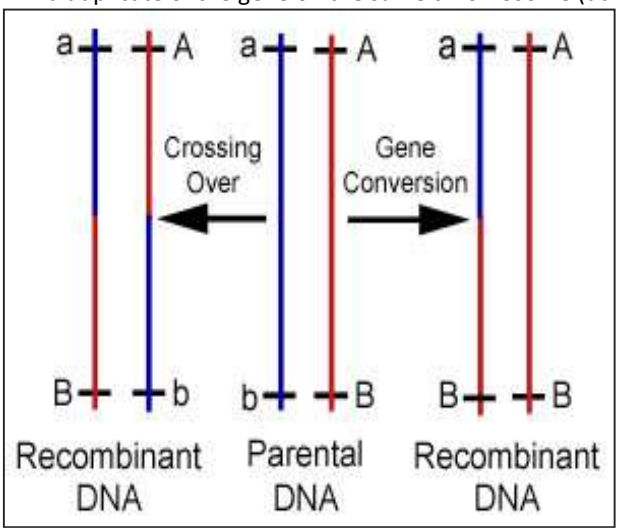
- Recombination between homologous chromosomes in meiosis I also involves the formation of DSBs and their repair. So it is not surprising that this process uses the same enzymes
- Meiosis I with the alignment of homologous sequences provides a mechanism for repairing damaged DNA; that is, mutations. in fact, many biologists feel that the main function of sex is to provide this mechanism for maintaining the integrity of the genome.
- However, most of the genes on the human Y chromosome have no counterpart on the X chromosome, and thus cannot benefit from this repair mechanism. They seem to solve this problem by having multiple copies of the same gene — oriented in opposite directions. Looping the intervening DNA brings the duplicates together and allowing repair by homologous recombination.

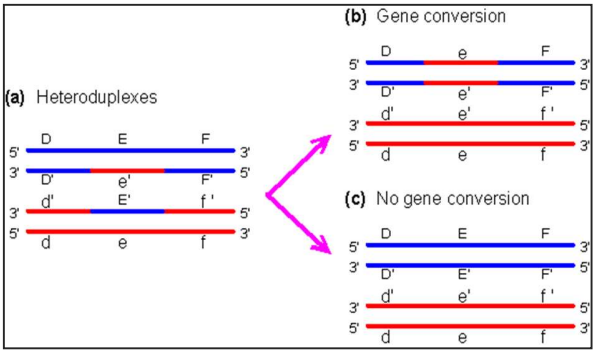
Gene Conversion

If the sequence used as a template for repairing a gene by homologous recombination differs slightly from the gene needing repair; that is, an allele, the repaired gene will acquire the donor sequence. **This nonreciprocal transfer of genetic information is called gene conversion.**

The donor of the new gene sequence may by:

- the homologous chromosome (during meiosis)
- the sister chromatid (also during meiosis)
- a duplicate of the gene on the same chromosome (during mitosis)





Gene conversion during meiosis alters the normal mendelian ratios. Normally, meiosis in a heterozygous (**Aa**) parent will produce gametes or spores in a 1:1 ratio; e.g., 50% **A**; 50% **a**. However, if gene conversion has occurred, other ratios will appear. If, for example, an **A** allele donates its sequence as it repairs a damaged **a** allele, the repaired gene will become **A**, and the ratio will be 75% **A**; 25% **a**.

Genotoxicity

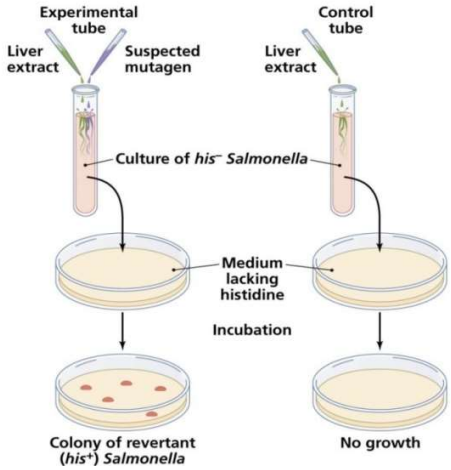
►This is a recently developed branch of toxicology which identifies mutagens in the environment. Most agents that cause cancer, carcinogens, are also mutagens. Identification of mutagens is recognised as important in many areas including pharmaceuticals, food additives, agriculture, pollution analysis and in many industrial processes.

►For this reason it has been necessary to develop tests to identify dangerous compounds. Early testing systems relied on small mammals such as rats or mice, but these tests were time consuming, very expensive and attracted considerable ethical criticism.

AMES TEST:-

A number of *in vitro* tests have been developed which use bacteria, or animal cells grown in tissue culture. In many countries there are legal requirements for new chemicals to be tested by a series of different tests before being licensed by government agencies. **The best known test is the Ames Test**. This is a very rapid test for mutagens.

►It utilizes bacteria of the species *Salmonella typhimurium* that have a mutation in the histidine operon, and hence cannot synthesize histidine. **They are referred to as histidine auxotrophs (his).**



►Compounds are tested to determine whether they can induce reversion of this mutation. The test can be carried out, very simply, by plating out *his*⁻ mutants on an agar plate that contains only a trace of histidine. A crystal, or a filter disc containing a solution of the compound to be tested, is placed on the surface of the agar. The bacteria grow for a short time until the histidine is depleted. After that point, the only bacteria capable of continuing growth to form colonies are those which have undergone reversion and are capable of synthesizing their own histidine, histidine prototrophs *(his+).* If the test compound is not mutagenic a few revertant colonies will be found randomly scattered across the agar plate. If it is mutagenic then the number of colonies will be increased and they will be clustered around the point where the compound was placed on the plate.

►Obviously the test can be constructed in a more quantitative manner to give a dose response curve for any compound under test. In order to identify the maximum number of mutagens two types of *his*-mutants are used, one is a single base substitution and the other a frameshift mutation. This allows detection of mutagens which have different effects on DNA. It is also possible to genetically alter both the permeability of the bacteria to test
compounds, and to decrease their ability to repair damaged DNA. This again increases the likelihood of detecting mutagenic activity.

►Some compounds which are known to cause cancer are only capable of doing so after they have been converted to mutagens by the action of enzymes within the body. These are known as procarcinogens- Enzyme action converts them to ultimate carcinogens. If procarcinogens are used in the Ames Test they will give a negative result, however the test can be adapted to take account of this. The liver is a rich source of activating enzymes. Liver extracts, containing these enzymes, can be added along with the test compound. Activation of a procarcinogen will then occur resulting in increased numbers of revertants.

► The Ames Test is rapid; it can be carried out in 48 hours. It is cheap and easily quantifiable. It has identified many compounds as mutagens including certain hair dyes, flame-retardants and food colorings.

►One deficiency of the Ames Test is that the target organism is a bacterium rather than a mammal. For this reason a number of tests have been developed using cultured animal cell lines. These are very similar to the Ames Test but use different selection systems and test genes.

►**Agents that damage chromosomes are known as clastogens**. These are also identified by test systems. These tests can be carried out on cell lines, in laboratory animals or even in plants. The tests consist of scoring chromosome aberrations such as breaks, exchanges, ring chromosomes, dicentries and trans locations. The frequency of aberration is often low.

►An alternative to enumeration of such gross chromosomal aberrations is to count **sister chromatid exchanges (SCE).** SCE involves exchange of material between two chromatids of the same chromosome, and is a process that takes place spontaneously at low frequencies in all cell types. It can occur both in mitosis and meiosis.

►Its usefulness is that the frequency of SCE increases much more rapidly than gross chromosomal aberrations, as a response to clastogen treatment. SCE can be detected by a number of procedures, all of which are dependent on the semi-conservative nature of DNA replication. The essence of the technique is to make sister chromatids stain differently so that exchanges can easily be observed.

This is achieved by a complex staining method after culturing cells for two rounds of division in the presence of the thymidine analog bromodeoxyuridme (BrdU). This is incorporated into the newly synthesized DNA in place of thymidine and alters the staining properties of the chromatid so that one stains dark and the other light

CHAPTER: 5 DNA RECOMBINATION

Biological Roles for Recombination

a) Generating new gene/allele combinations (crossing over during meiosis)
b) Generating new genes (e.g., Immuno- globulin rearrangement)
c) Integration of a specific DNA element
d) DNA repair

Practical Uses of Recombination

1. Used to map genes on chromosomes (recombination frequency proportional to distance between genes)
2. Making transgenic cells and organisms

Types of Recombination

1. **Homologous** - occurs between sequences that are nearly identical (e.g., during meiosis)
2. **Site-Specific** - occurs between sequences with a limited stretch of similarity; involves specific sites
3. **Transposition** – DNA element moves from one site to another, usually little sequence similarity involved

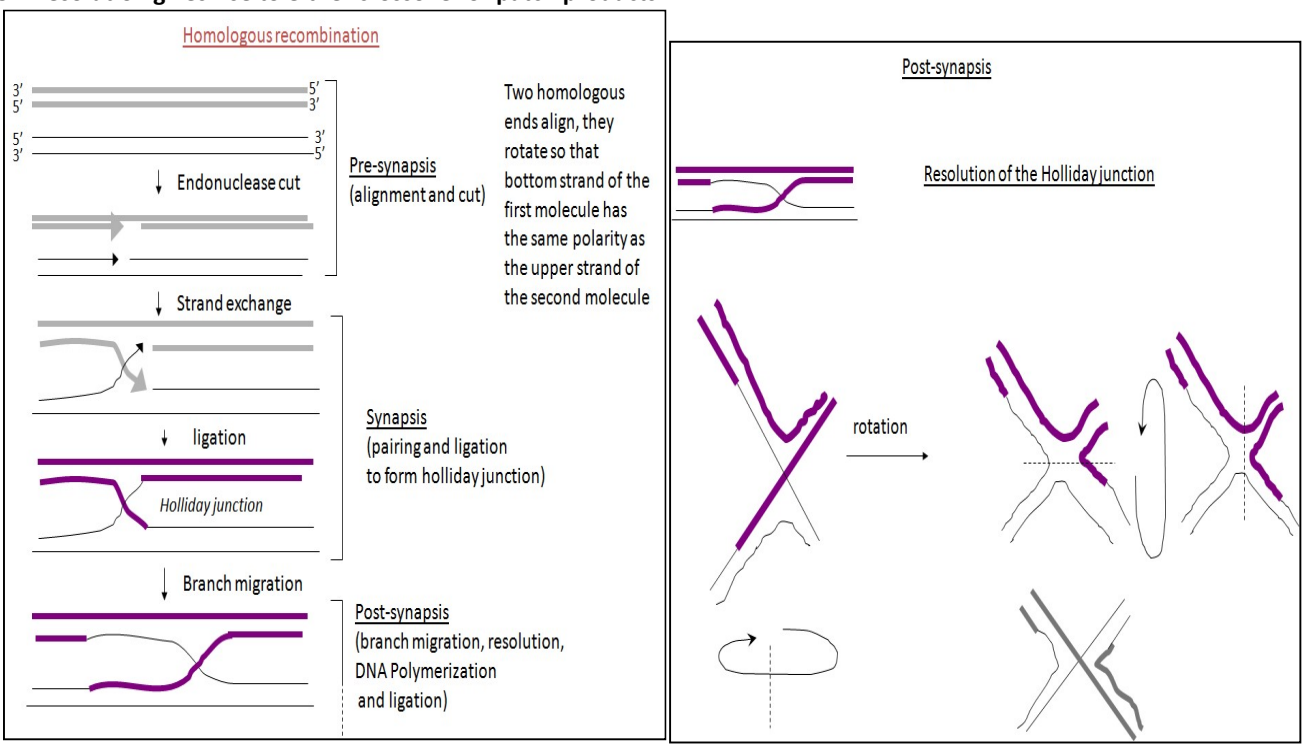
HOMOLOGOUS RECOMBINATION

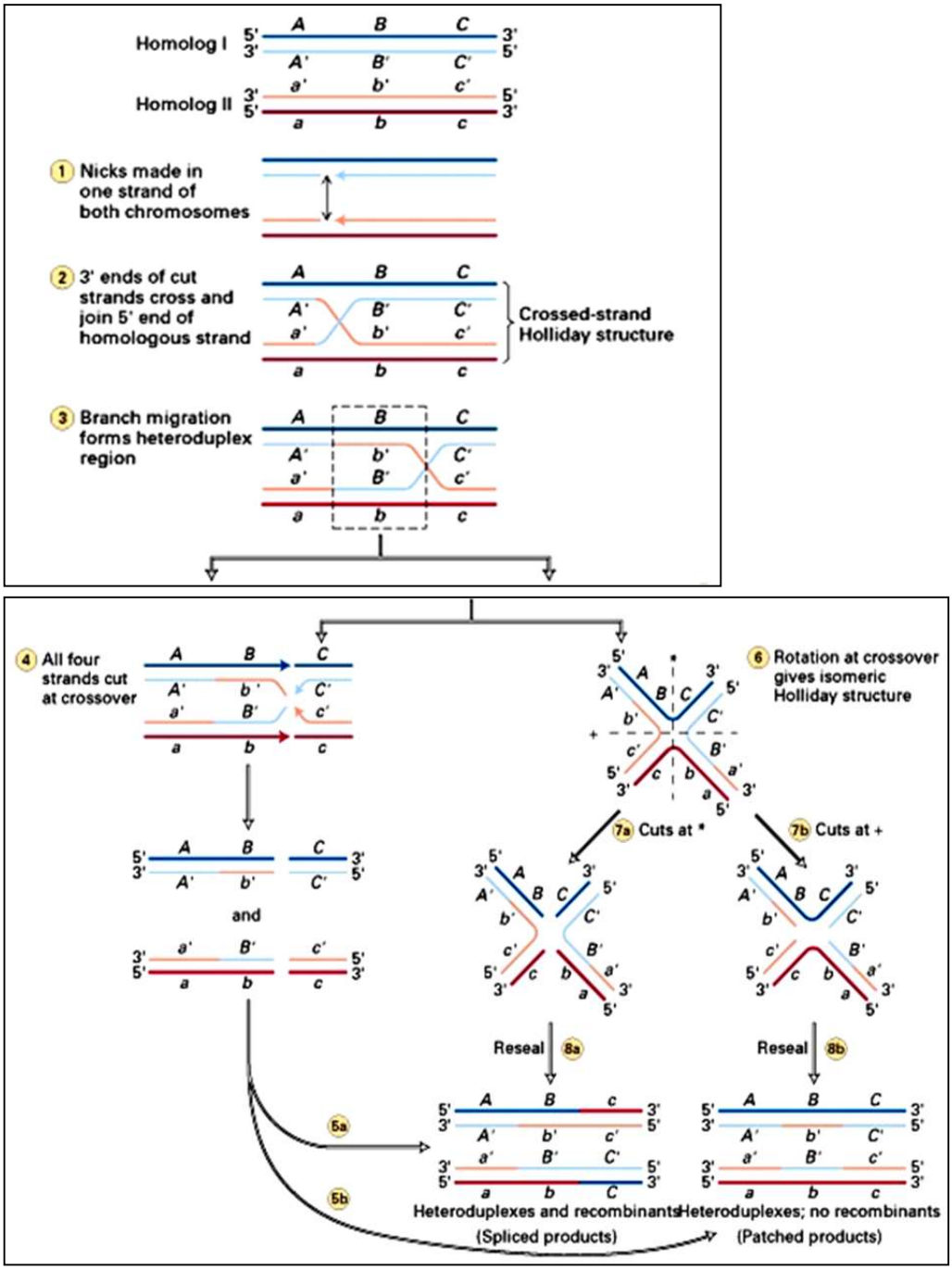
- Occurs randomly between homologous sequences
- occurs in bacteria as well as in eukaryotes.
- In Bacteria, homologous recombination is used **to repair double stranded breaks in the DNA**, Restart collapsed replication forks and to allow chromosome recombination with bacteriophage genome or via conjugation.
- In Eukaryotes, homologous recombination is also used for DNA repair, to restart collapsed replication forks and to ensure variation in genes needed to be passed on to the next generation.
- Homologous recombination Occurs at "four strand" stage of meiosis, involves two of four strands between 2 homologous DNA molecules. At least 100bp need to be identical. Within homologous regions, there are going to be different alleles of the same gene.

Models that describe putative ways in which homologous recombination takes place

Holliday Model

1. **Homologous chromosomes align.**
2. **A single DNA strand breaks in each duplex.**
3. **Strand invasion takes place.**
4. **Branch migration takes place**
5. **Resolution gives rise to either crossover or patch products.**





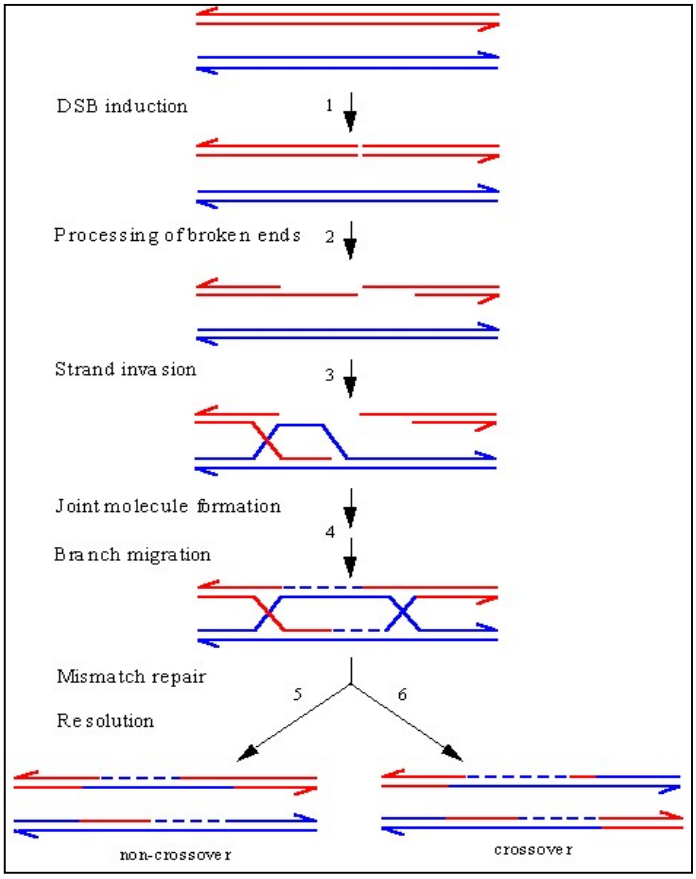
Double-Strand break (DSB) repair Model

1. Homologous chromosomes align.
2. **<u>Double strand breaks occur on one DNA duplex.</u>**
3. Degradation of the breaks occur to create single stranded 3' extensions (DNA tails). The 3' ends serve as primers for DNA synthesis.
4. Strand invasion takes place on intact chromosome and base pairing with complementary stand occurs.
5. **Two holiday junctions are generated**.
6. Branch migration takes place
7. Resolution gives rise to either crossover or patch products

Because double strand breaks occur relatively frequently, the DSB model is the most attractive model.

Double-stranded (dsDNA) breaks

1. **Meiosis: -** Created by topoisomerase-like enzymes
2. **Mitosis :-** Radiation., Mutagens (e.g. chemicals), Stalled replication forks, Specialized endonucleases (eg site-specific HO endonuclease in switching of yeast matting type (*MAT*) genes)



The RecBCD pathway is the best understood homologous recombination event. It occurs in E. coli and it follows the proposed DSB repair Model events

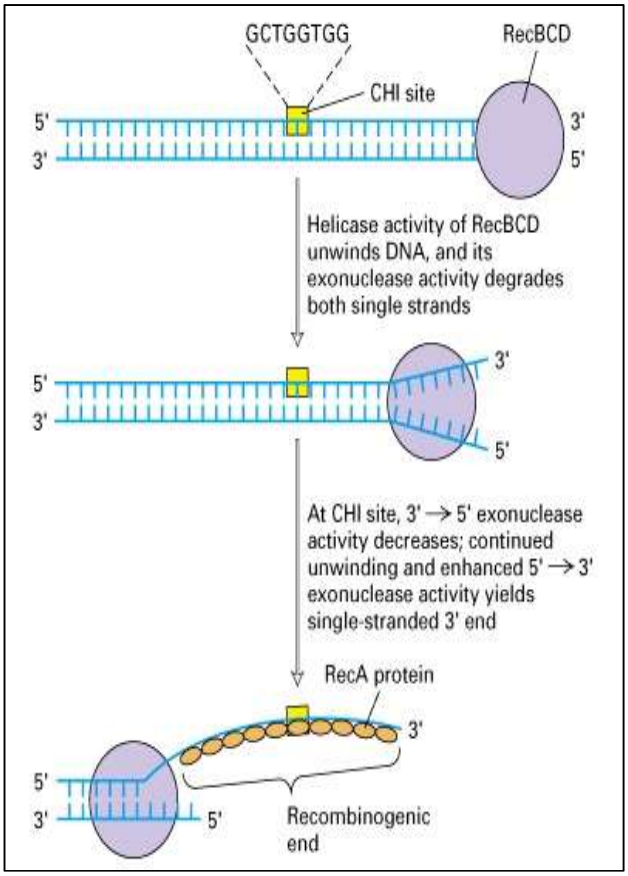
1. RecB, RecC and RecD proteins are components of RecBCD enzyme, which is also called as **ExoV.**
2. RecBCD has an ATP dependent dsDNA and ssDNA **exonuclease** activity. The RecBCD complex is a nuclease that cleaves single stranded DNA and degrades it. It has an ATP-dependent unwinding activity on linear dsDNA producing ssDNA tails or long ssDNA. **Endonuclease** subunits (*recBCD*) that cut one DNA strand close to Chi sequence. **RecD** is a DNA helicase that moves on the 5'ending strand. **RecB** is a DNA helicase that moves on the 3'ending strand.

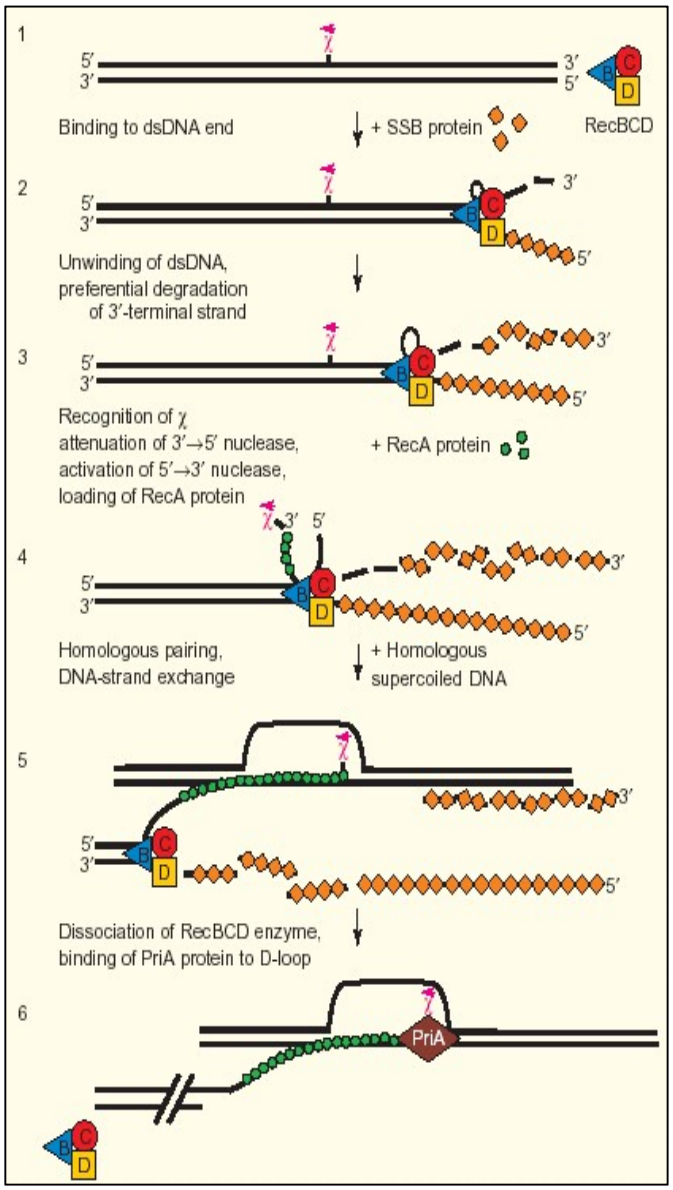
   This is essential for 99% of recombination events occurring at double-stranded breaks in bacteria Where it binds double stranded break and unwinds DNA to generate SS regions and degrades DNA
3. During unwinding from right to left it Pauses at *chi* sequence and makes ss nicks near *chi* site (5'-GCTGGTGG-3'). [They are present near sites of recombination in E. coli and act as crossover hotspot instigators).] and then **Loads RecA on 3' ssDNA extensions.**
4. **Upon reaching the chi site, RecBCD changes enzymatic activity because RecD seems to be inactivated or lost.**
5. **RecBCD** interacts with RecA to promote assembly of RecA on single stranded DNA. Thus, **RecA** coats the single stranded DNA tail.
6. RecA is 38 kDa protein requires ATP for binding and polymerising to ssDNA rapidly and cooperatively in 5'-3' direction to generate a nucleo-protein filament (RecA bound ssDNA).
7. RecA is a strand exchange protein, that is, it **induces strand invasion.** Strand exchange proteins of the RecA family are present in all forms of life.
8. The nucleo-protein filament binds non-specifically to dsDNA (binding to Ds DNA, but not as strongly as SS) to generate DNA network. In these networks, ssDNA synapses with homologous dsDNA sequence. Thus, RecA promotes pairing and strand exchange.

9. After strand invasion is complete and the Holliday junction forms, **RuvA** (a Holliday junction specific DNA binding protein) recruits RuvB. **RuvB** is an ATPase. **RuvB promotes branch migration.**
10. **RuvC** is the Holliday junction resolving **endonuclease.**
11. DNA ligase then joins the 5′ phosphoryl group with the 3′ OH.

RecBCD Pathway of Homologous Recombination

***RecA* Function Dissected** 3 steps of strand exchange
Pre-synapsis: *recA* coats single stranded DNA (accelerated by SSB)
Synapsis: alignment of complementary sequences in SS and DS DNA (paranemic or side-by-side structure)
Post-synapsis or strand-exchange: SS DNA replaces the same strand in the duplex to form a new DS DNA (requires ATP)





(i) **Alluvial soil** :- It is brought through the water. This is highly fertile soil,
{ii**) Colluvial Soil** :- It is brought by gravity
(iii) **Galacial soil :-** Soil is brought by ice
(iv) **Eolian soil** :- This soil is brought through wind.

Particles name	**Diameter range (mm)**
Clay	Less than 0.002
Silt	0.002—0.02
Fine sand	0.02—0.2
Coarse sand	0.2—2
Stones and Gravel	Above 2.0

Soil profile

Soils are described and identified by reference to their profiles. Soil profile is the sequence and nature of the horizons (layers) superimposed one above the other and exposed in a pit-section dug- through the soil mantle.

The smallest three dimensional volume of a soil needed to give full representation of horizontal variability of soil is termed a pedon. Although profiles of different types of soil differ markedly in respect of their physiochemical and biological properties.

The soil profile consists of the following main horizons: -

(1) The 'O' horizons—These are the organic horizons forming above the surface of the mineral matrix; mainly composed of fresh or partially decomposed organic matter. This horizon is well developed in forests and may be completely absent in grassland. This horizon divided into following two sub layers—

(A) O_1 (A_oo) region—This is the uppermost layer consisting of freshly fallen dead organic matter as dead leaves, branches, flowers and fruits, dead parts of animals.

(B) O_2 (A_0) region—It's just below the O, region in which decomposition has begin. Thus organic matter is found under different stages of decomposition and micro-organisms like bacteria,fungi actinomycetes are frequently found. Upper layers contain detritus in initial stage of decomposition, where the material can be faintly recognised, whereas the lower layers contain fairly decomposed matter called duff.

(2) The 'A' horizons—These are the mineral horizons formed either at or adjacent to the surface. They are rich in organic matter. This is also known as zone of eluviation which means downward loss or leaching. This horizon is divided into two sublayers—

(A) A_1 region—It is dark and rich in organic matter. The amorphous and organic matter becomes mixed with the mineral matter, which is now known as 'humus', which is dark brown or black coloured. In forest soils this region is less deeper than those of the grasslands.

(B) A_2 region—This region is of light colour in which the mineral particles of large size as sand are more, with little amount of organic matter. Chiefly in areas with heavy rainfall, the mineral elements and organic chemicals are rapidly lost downwards (eluviation) in this region making it light coloured. This is thus also known as podsolic or eluvial zone or zone of leaching . **The E horizon near the surface of a podzol is a good example of an eluvial horizon.**

(3) The 'B' horizons:.

(a) Enrichment with in washed iron, aluminium, manganese or organic matter.

(b) Residual enrichment with sequioxides or silicate clays, which has occurred other than by the removal of carbonates or readily soluble salts.

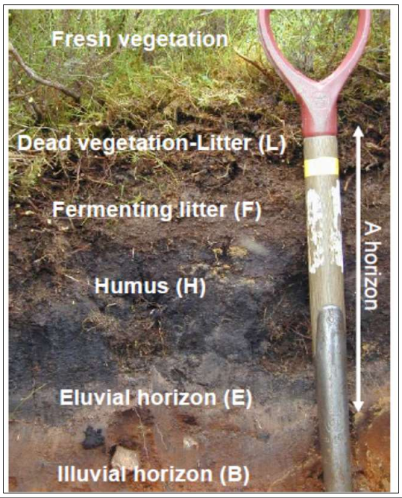
(c) Sequioxides coating of mineral grains sufficient to give a more intense colour than horizon above or below.

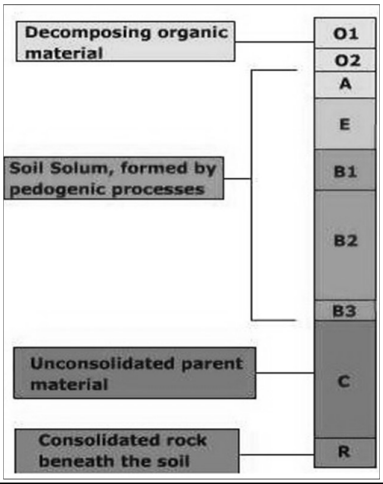
This is just below the 'A' horizon and can be divided into B_1(A_3), B_2 and B_3 regions depending upon the stages of soil development.

It is also known as zone of illuviation or illuvial zone the collection of leached material like silicates, clay, hydrated oxides of aluminium, iron, etc from E horizon tis is why this is dark coloured and coarse textured **.** This zone is poorly developed in dry areas.A_1,A_2 and B horizons collectively known as mineral soil or '**solum**.' **This is the zone of maximum accumulation-** The B horizon is the zone of illuviation,

(4) The 'C' horizons—These are the mineral horizons below the 'B' horizons but excluding true bedrock and without any characteristics of 'A' and 'B' horizons. It consists of completely weathered, large masses of rocks.

(5) The 'R' horizons—This is the parent, unweathered bedrock, upon which there is collected water.





Brownish black and dark brown colours in the A horizon indicate high organic matter. Very pale brown to reddish colours of B horizon are characteristics of well drained soil, whereas dark brown to blackish colours of B horizon indicate poor drainage. Red soils derive their colour from presence of iron oxides. The bright colours indicate good drainage and aeration. Red and yellow colours increase from temperate to the equator. Grayish colour denotes permanently saturated soils in which iron is in ferrous form. The colours of soils are determined by the use of standardized colour charts known as **Munsell colour charts**. **SOIL COMPONENTS**

(1) Soil Water—Soil water plays important role in the plant growth. Plant absorbs a small quantity of rain water and dew directly from their surfaces but most of water absorbed by them comes from the soil. Water in the soil comes mainly through infiltration of precipitated water (snow, rain, sleet and hail) and irrigation whereas it is lost from the soil chiefly through transpiration, percolation, stream and evaporation. The quantity of water available in the soil varies from place to place and also depends upon the quality of soil. In loamy, clay and silty soil, the amount of water is greater than that in coarse sandy soil. Water is available in the soil in the following forms—

(a) Capillary water—**It is most important form of water to plants**. The amount of water present around the soil particles at saturation stage, when gravitational water has drained away through capillaries or channels, is called capillary capacity or field capacity and water which is held by surface tension and attraction force of water molecules as thin film around soil particles in the capillary space is called capillary water. It moves in the direction where capillary tension is more.

(b) Gravitational water—This is the water that moves downwards through a moist soil in response to gravity. After complete water saturation of soils the excess water displaces air from the pore spaces between soil particles and percolates downwardly under gravitational influence and finally it is accumulated in the pore spaces. This excess water is called gravitational water. The amount of water held in the soil **(when all pores are filled and when drainage is restricted)** is maximum water holding capacity. When the gravitational water percolates down and reaches to the level of parental rock it is called ground water, the upper surface of which is water table.

(c) Combined water—It is water of chemical compounds held by chemical forces of molecules as (e.g., $CuSO_4$-$5H_2O$). It can be driven out from the compounds only at bright red heating. Combined water is present as hydrated oxides of aluminium, silicon, iron in the soil.

(d) Hygroscopic water—Water which is absorbed on the soil particles and held on the surface of soil particles by forces of attraction and cohesion of its molecules is called hygroscopic water. This water can not be easily removed by the plants.

The field capacity is the amount of water remaining in the soil a few days after having been wetted and after free drainage has ceased. The larger pores drain first so gravity drainage, if not restricted, may only take hours, whereas in clay soils (without macropores); gravity drainage may take two to three days. The volumetric soil moisture content remaining at field capacity is about 15 to 25% for sandy soils, 35 to 45% for loam soils, and 45 to 55% for clay soils.

The permanent wilting point is the water content of a soil when most plants (corn, wheat, sunflowers) growing in that soil wilt and fail to recover their turgor upon rewetting. The matric potential at this soil moisture condition is commonly estimated at -15 bar. Most agricultural plants will generally show signs of wilting long before this moisture potential or water content is reached (more typically at around -2 to -5 bars) because the rate of water movement to the roots decreases and the stomata tend to lose their turgor pressure and begin to restrict transpiration. This water is strongly

retained and trapped in the smaller pores and does not readily flow. The volumetric soil moisture content at the wilting point will have dropped to around 5 to 10% for sandy soils, 10 to 15% in loam soils, and 15 to 20% in clay soils**.**

The total available water (holding) capacity is the portion of water that can be absorbed by plant roots. By definition it is the amount of water available, stored, or released between field capacity and the permanent wilting point water contents. The average amount of total available water in the root zone for a loam soil.

For a particular soil, certain soil water proportions are defined which dictate whether the water is available or not for plant growth. These are called the soil water constants, which are described below.

• **Saturation capacity:** this is the total water content of the soil when all the pores of the soil are filled with water. It is also termed as the maximum water holding capacity of the soil. At saturation capacity, the soil moisture tension is almost equal to zero.

• **Field capacity:** this is the water retained by an initially saturated soil against the force of gravity. Hence, as the gravitational water gets drained off from the soil, it is said to reach the field capacity. At field capacity, the macro-pores of the soil are drained off, but water is retained in the micropores. Though the soil moisture tension at field capacity varies from soil to soil, it is normally between 1/10 (for clayey soils) to 1/3 (for sandy soils) atmospheres.

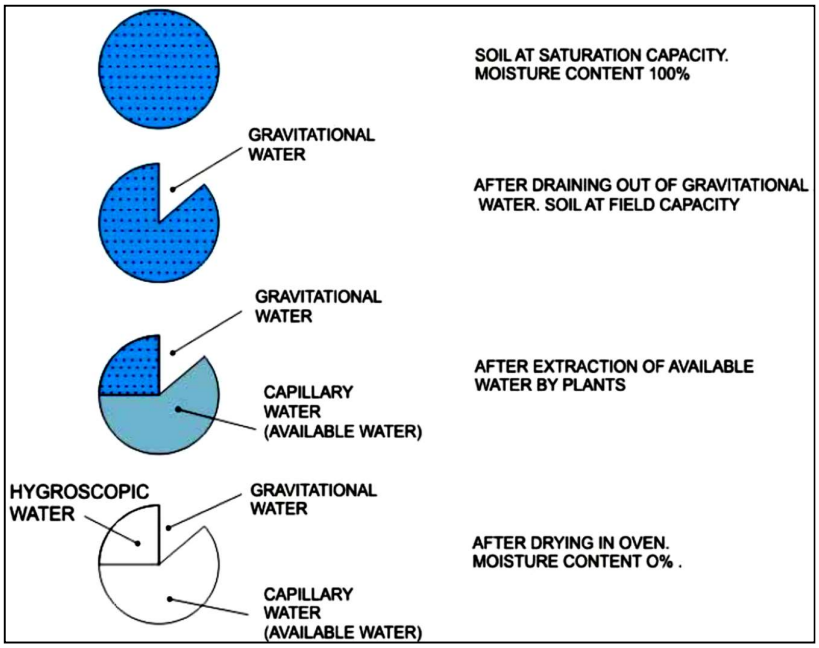
$$\text{Field capacity} = \frac{\text{Weight of water held by soil per unit area}}{\text{Weight of soil per unit area}}$$

• **Permanent wilting point:** plant roots are able to extract water from a soil matrix, which is saturated up to field capacity. However, as the water extraction proceeds, the moisture content diminishes and the negative (gauge) pressure increases. At one point, the plant cannot extract any further water and thus wilts.

Two stages of wilting points are recognized and they are:

• **Temporary wilting point:** this denotes the soil water content at which the plant wilts at day time, but recovers during right or when water is added to the soil.

• **Ultimate wilting point:** at such a soil water content, the plant wilts and fails to regain life even after addition of water to soil.



(2) Mineral components—The constituents of minerals at the soil are derived from the parental rock or regolith. They may be found in the form of particles of various sizes. The minerals present about 90% of the total weight of the soil. Important elements available in compound state are Oxygen (O_2), C, H, Si, Fe, N, K, P, Al etc. In a given sample of soil, there may be present different sized particles in different proportions. Depending upon their size, the **International Society of Soil Science** has given different names to these mineral particles, as follows—

(3) Organic matter or Humus: Organic matter is the main source of mineral's return to soil. It can hold minerals as ions by absorption and binding clay particles into aggregates. **Soil organic matter (humus) represents the equilibrium between input, originating from primary photosynthetic production, and the degradative and resynthetic processes associated with soil organisms.** The input is largely of carbohydrates, fats and proteins, lignin compounds, resin,

alkaloids etc. Soluble sugars, lipids, proteins, amino acids, organic acids etc. are minor constituents of organic matter. However the major constituents of humus are humic complexes, which make the greatest proportion of it in most soils. When the plants and animals die, their dead remains are acted upon by a number of micro-organisms and finally degraded or decomposed into simple organic compounds. A product of this microbial decomposition is humus which is a dark coloured, jelly-like amorphous substance and not soluble in water. Humus is present in the soil in the form of organic colloids. Humus percentage in the soil is affected by climatic and biological factors. It is less in arid soils and very high in humid soils.

Soil development

Soil continues to develop over time due to climate, vegetation and other factors. Major processes in soil development are podsolization, laterization, calcification, gleyization and salinization.

Podsolization denotes the ash colour of the soil due to depletion of bases from surface layers to the B horizon. The organic horizon is a mor with a layer of fermented litter on top of a layer of humus unmixed with mineral soil. Such soils are highly acidic and develop under coniferous forests in cool humid regions.

Laterization is common in humid sub tropical and tropical forested regions, where rainfall is heavy and temperature is high. Weathering is entirely chemical brought by water and its dissolved substances. Bases, silica, Al, hydrated aluminosilicates and iron oxides become soluble and carried down ward. The weathered end product is composed of silicate, hydrous oxides, clays deficient in bases and low in plant nutrients. The large amount of residual Fe and Al hydroxides gives bright reddish colour in upper part of E horizon, which may be especially deep.

Calcification: The sub humid to arid and temperate to tropical regions supporting grassland vegetation show calcification. Vegetative material above ground and part of root system turned back to soil as organic matter every year and thus developing soil is high in organic matter. Rainfall is generally not sufficient to remove calcium and magnesium carbonates and grasses help in maintaing of soil a high calcium content of soil by redepositing during decomposition. In this process of soil development A horizon is distinct and $CaCO_3$ and leaches to the B horizon where calcium nodules are formed, which are called kanker.

Gleyization: Under poor drainage condition of cold wet situation and where water table is high upto B and C horizons, compact structureless horizons develop and this process is called gleyization. Organic matter is high due to reduced rate of humification and soil has dull gray to bluish colour. Iron stays in reduced ferrous compound most of the time.

Salinization: In arid and semi arid regions with sparse vegetation and scant precipitation, slightly weathered and slightly leached soils are formed. The horizons are very thin and soils contain very high amounts of soluble salts. Due to high rate of evaporation a more or less cemented horizon of Na, Ca and Mg salts is formed below surface. This horizon is called **caliche**. Soils are low is humus and high in base content.

Humus:- The organic matter is present in soil called humus. Total of the organic compounds in soil exclusive of undecayed plant and animal tissues, their "partial decomposition" products, and the soil biomass. The term is often used synonymously with soil organic matter. Carbohydrates, fats & proteins, amino-acids and humic complex (lignin, resin and phenolic polymers) are found in humus.

Litter: The surface layer of the forest floor which is not in an advanced stage of decomposition, usually consisting of freshly fallen leaves, needles, twigs, stems, bark, and fruits.

Humus is formed by decomposition of litter. Partially decomposed litter is called duff.

Types of humus in terrestrial enviroment are following:

MOR A thick mat of undecomposed to partially decomposed litter that is not significantly incorporated into the mineral soil, present in coniferous forests**.**

This humus arise under conditions of **low-biological activity in soil**. The **mineralization** of organic matter proceed **slowly and create layers**, which maintain a structure of vegatable material.Acidophilic fungi and low active invertebrates participates in transformations of plant residues. **It is characterized by the presence of a thick litter (L)* layer with thin or no fermentation (F)* and humus (H) * layers.** C/N ratio of mor humus is always more than 20, or even 30-40. This is raw humus and it is formed in acidic soil (pH 3.8 - 4.0).

Mor humus is characteristic of the Fire-Dependent forest/Woodland System.

MODER is a transitional form of humus between mor and mull, characteristic Undecomposed and partially decomposed remains of broad-leaved deciduous forest litter that is shallowly incorporated into the mineral soil . The organic horizons with moder humus consist of low-thicknessed litter (2-3 cm**),** which gradually, without bounds, pass on to humus-accumulative horizons. Moder is a type of medium humified humus.

Bacteria, Acidophilic fungi and arthropodan participates in transformations of plant residues.

C/N ratio equal 15-25. Produced mineral-organic complexes are labile and weakly bounded with mineral portion of soil **. It is characterized by the presence of litter (L) *, fermentation (F) *, and humus (H) * layers. The humus layer gradually grades into the mineral topsoil.**
Moder humus is characteristic of the Mesic Hardwood Forest System without worms.

MULL
Well-decomposed organic matter that is mixed deeply into the mineral soil. It is characterized by the presence of a thin litter (L) * layer comprised only of remnants from the previous fall and a thick topsoil layer.**This type of humus arise under grass vegetation. Mull is a well humified organic matter, which is produce in very biologically active habitat.**
This type of humus is characterized by neutral pH, C/N ratio nearing to 10 and ability to creation stable mineral-organic complexes. Mull is a type of humus which occurs in soils under cultivation

❋NON TERRESTRIAL HUMUS
➡MUCK
Highly decomposed organic matter in which the original plant parts are not recognizable. It contains more mineral matter, is darker in color than peat, and is characterized by the presence of a thick humus (H)* layer. Muck is physically and chemically distinct from the humus of upland communities in its ability to absorb water, adsorb metals toxic to plants, and release nutrients.

Muck Humus Is Characteristic Of The Wet Forest System Where The Hydrologic Regime Fluctuates Annually Between Aerobic And Anaerobic Conditions.

Three Peat Humus Types Sapric, Hemic, And Fibric Form In Wetlands With Continuous Water Saturation. Organic Matter (Peat) Accumulates Because Anaerobic Conditions And Low Temperatures Prevent Decomposition.

Sapric Peat
The Most Decomposed Class Of Peat Characterized By Less Than 1/3 Recognizable Plant Fibers (Compare To Hemic And Fibric Peat).

Hemic Peat
A Moderately Decomposed Class Of Peat Characterized By 1/3 To 2/3 Recognizable Plant Fibers (Compare To Sapric And Fibric Peat). The Source Of Organic Matter Is Mosses, Sedges, Or Grasses.

Fibric Peat The Least Decomposed Class Of Peat Characterized By More Than 2/3 Recognizable Plant Fibers (Compare To Hemic And Sapric Peat). The Source Of Organic Matter Is Sphagnum Moss And Woody Plant Debris

Role of humus in the soil
(i) It makes the soil fertile,
(ii) Humus makes the soil porous, thus increases the aeration and percolation which make the soil more suitable for the plant growth,
(iii) It provides nutrients to the plants and micro-organisms.
(iv) Availability of humus in the soil increases the rate of absorption in plants.
(v) On complete decomposition, it forms several organic acids which serve as solvents for soil materials. Thus humus increases the availability of minerals in dissolved state to plants,
(vi) It functions as weak cement thus binds the sand particles.
(vii) Humus is highly porous, therefore, it has got high capacity for retaining water.

(4) Soil atmosphere—Gases found in the soil profile are said to form the atmosphere which is considered to be the most important component of the soil. The important factors which bring about changes in the soil atmosphere are temperature,atmospheric pressure, wind and rainfall.
The soil atmosphere contains three main gases namely oxygen, carbondioxide and nitrogen. In the atmosphere nitrogen is approximately 79%, oxygen is 20% and carbon dioxide is 0.15 to 0.65% by volume. In the cultivated land, percentage of CO_2 is much higher than that of atmospheric CO_2, but oxygen content in such soil is poorer than the percentage of oxygen in atmospheric air.**Do you know an ideal soil should have 25 % soil air**

Important aspects of Soil atmosphere
* Oxygen of soil is absorbed by plant roots and soil micro-organisms in respiration and CO_2 is given out which accumulates in spaces.

* The amount of CO_2 increases with the increase in depth of the soil due to decomposition of accumulated organic matter and abundance of plant roots.
* The accumulation of soluble nutrients in the soil makes it more productive.
* If the soil is deficient in oxygen, the rates of microbial activities are slowed down and may be eliminated.
* Temperature and atmospheric pressure cause expansion and contraction of the soil air.
* Wind helps the soil in sucking the air in and rainwater displaces in soil air.

(5) Soil micro-organisms: Soil flora and soil fauna constitute soil organisms which are listed below—

Soil flora	**Soil fauna**
1. Soil bacteria	Nematodes
2. Soil fungi	Protozoa
3. Soil actinomycetes	Insects and mites
4. Root, rhizoid and	burrowing vertebrates rhizome bearing higher plants
5. Algae	Rodents and earthworms

These soil organisms are variable in behaviour, because many of these like Protozoa, mites and insects are moving organisms, as they move in or on the surface of soil in search of food. Earthworms by burrowing habit make the soil loose and fertile.
The majority of the soil fungi are found in acidic soils. Actinomycetes prefer saline soils and soil bacteria prefer neutral soil highly supplied with organic nutrients. Algae are found in the top layer of soil. The soil microflora bacteria form about 90% of the total microbe population.

Role of Soil Organisms

*They help in mixing of soil. *They improve the soil aeration.
*They decompose the dead organic matter and increase plant nutrients in available forms.
*They help in the production of toxins.
*They produce growth stimulating hormones.
*They influence nitrogen fixation in the soil.
*Some microbes become parasites of higher plants and cause considerable damage.
*They help in the improvement in the aggregation of Soil particles or Soil binding.

Examples of nitrogen-fixing bacteria (* denotes a photosynthetic bacterium)			
Free living		**Symbiotic with plants**	
Aerobic	**Anaerobic**	**Legumes**	**Other plants**
Azotobacter ***Beijerinckia*** ***Klebsiella*** **(some)** Cyanobacteria **(some)***	***Clostridium* (some)** ***Desulfovibrio*** **Purple sulphur bacteria*** **Purple non-sulphur bacteria*** **Green sulphur bacteria***	***Rhizobium***	***Frankia*** ***Azospirillum***

Adaptations to Environmental Factors

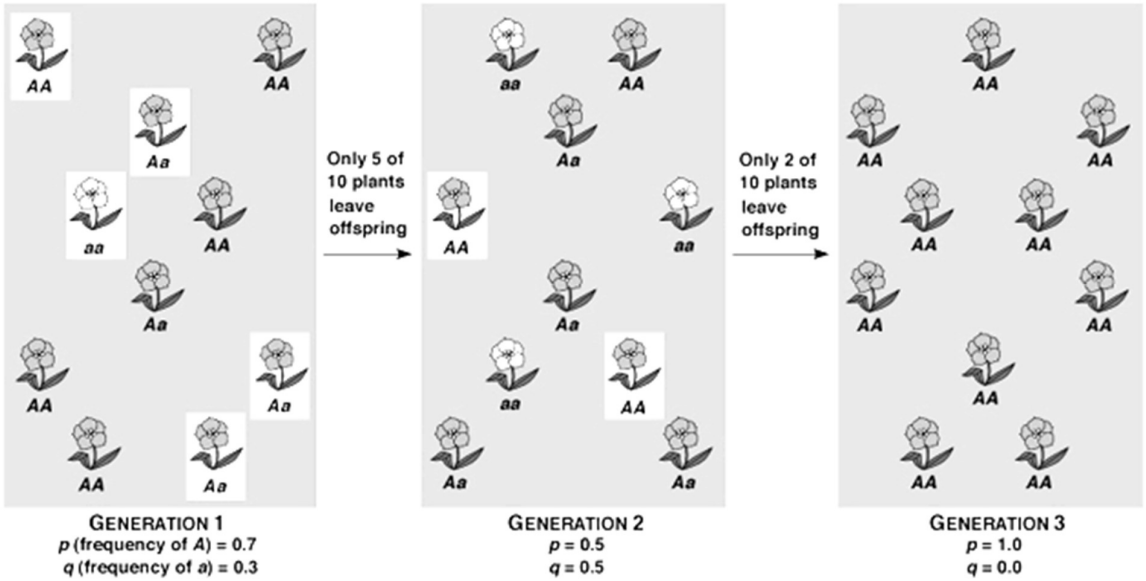
Any environmental factor that inhibits the growth of plant either through its deficiency or excess is said to be a limiting factor. For example cold temperature restricts plant growth at higher elevations and water availability in desert. Limiting factors for terrestrial plants are light, moisture and temperature. **Liebig's Law of minimum** states that growth of plants is dependent on the amount of nutrient present in minimum quantity. Shelford's **Law of tolerance** suggests an ecological minimum and an ecological maximum and the range between these two conditions represents the tolerance range of plants. The plants with narrow range of tolerance to temperature are called **stenothermal** and those with wide range of tolerance are called **eurythermal**.
A plant does not do equally well throughout its whole range of distribution. There is a range of environmental condition, where it does better and this is referred as the **range of the optimum**. When some important feature of environmental condition changes, the plant changes in response. These changes keep certain important aspects of the

CHAPTER: 10 GENETIC DRIFT

Random Fluctuations' in the gene frequencies in a small population generation after generation purely by chance. **Evolution is a change in allele/gene frequencies over time that occurs as less adapted alleles are replaced with better adapted ones. Genetic drift is a random change in gene frequency arising through chance, and tends to be more apparent in small populations. The allele frequencies in a population experiencing significant genetic drift can be observed to 'drift' away from their starting values over time.** Because the changes are at random and not under the influence of natural selection, frequencies will alter in a non directional way, increasing, decreasing or fluctuating up and down.

Drift occurs because of the influence of chance on which genes are transmitted from one generation to the next. Not all individuals mate and not all of the gametes produced by an individual contribute to reproduction. Those gametes that do result in offspring may not be representative, in terms of the frequencies of the alleles they carry, of the parents. In a large population these so-called sampling effects will tend to cancel each other out; if the offspring of one pair of parents under-represents an allele it is likely that others will over represent it. This is less likely to be the case in a small population and the result will be a different allele frequency in the offspring population than in the parents. These random changes can cause alleles to become lost from the population completely such that only one allele remains at that locus. Successive fixation events such as these will lead to the progressive loss of genetic variation from the population.

Random mortality will have the same effect as the above; the genotypes of the survivors may not be representative of the population before mortality occurred and unrepresentative genes will be passed to the next generation. This effect of mortality explains why random genetic drift is also apparent in asexual populations.



Salient Features of Genetic Drift

1. Genetic drift is an evolutionary force operating in small populations.
2. Gene frequency in small populations changes by chance.
3. In small populations some genes may be lost or reduced and other may increase by sheer chance irrespective of their selective advantage or disadvantage.
4. By genetic drift a new mutation arising in a small population may either be fixed or lost irrespective of its adaptive value.
5. In small population herertozygosity tends to change to homozyosity by chance.
6. Genetic drifts may fix some nonadaptive traits in small populations.
7. Genetic drifts tend to preserve or eliminate genes without distinction (opposite to natural selection which selects and favours genes of adaptive value).
8. Isolated small populations of large population come to possess some unusual characteristics not shown by the large parental population.

Genetic Drift and Evolution

1. Most breeding populations of animals are usually small.
2. Even a widely ranging, broad based population is isolated into small subgroups, called demes, either on account of ecological or geographical discontinuities, homing instinct or territoriality. The size of these small demes or subpopulations is such that they appear to be affected by chance events underlying genetic drift.

3. Seasonal, annual or cyclical fluctuations are observed in population size in many species, For example-
   (i) Huge populations of insects that appear in the warm spring and summer months experience high mortality during cold winter months. The few that survive the following spring form a much smaller gene pool.
   (ii) Because of the limited size of the small breeding populations, the gene pool of their new generations may not be the representative of the parental gene pools because of change changes or action of genetic drift. The altered gene pools in due course of time lead to new species.

Thus genetic drift in small isolated populations leads to -

i. Variations among population by fixing or climinating certain in alleles.
ii. Fixation or elimination of gene mutations.
iii. Fixation of unfavourable, neutral or favourable characteristics in populations.
Establishing reproductive isolation between different demes of a large population and origin of new species

Genetic Bottleneck:-If a population undergoes an abrupt contraction of its numbers, say as a result of environmental catastrophe or overexploitation. it is said to have gone through a bottleneck. This is accompanied by a change in gene frequencies and decline in total genetic variation. The change in population size overtime for a population undergoing a bottleneck and then recovery..

If the population remains small, gene frequencies will be subject to genetic drift in successive generations, genetic variation will be lost and the population may become extinct. Alternatively, population numbers could recover after the crash

This has happened in the northern elephant seal, *Mirounga angustirostris,* whose numbers were reduced to about 20 in the 1890s due to over hunting. There are now approximately 30 000 northern elephant seals and studies have demonstrated an extremely low level of genetic variation in this species with fixation for one allele at all of the 24 loci examined. Once genetic variation has been lost from a population it can only be restored through mutation (which will take many generations) or through mixing with another, genetically different population. The intensity of shading indicates the accumulation of genetic variation through mutation.

Founder effect The establishment of a new population in vacant habitat may involve only one or a few individuals The level of genetic variation and the presence of particular genes in the new colony will be entirely dependent on the genotype of the colonists. This can cause founder populations to have low levels of genetic variation and alleles that are unrepresentative of the parent population. In the extreme case, a new population may be founded by a single pregnant female or a single seed of a self-compatible plant. lf a colonist is carrying alleles that are rare in the parent population these may become common in the founder population.

The clearest example comes from humans. The Afrikaner population of South Africa is mainly descended from one shipload of 20 immigrants who landed in 1652. **One of the original colonists was a Dutch male carrying the gene for Huntington's disease; the high incidence of this gene in the present day population is attributed to this founder effect.**

CHAPTER: 11 TYPES OF EVOLUTION

SEQUENTIAL AND DIVERGENT EVOLUTION

(i) Minor changes in the gene pool of a population from one generation to the next, with the result that no new populations are formed, but the descendent population is not genetically identical with its predecessor. This is known as sequential evolution.

(ii) The changes which result in the evolution of new populations, species, families, groups or classes represent as divergent evolution.

1. MICROEVOLUTION

The evolution, which results from the interaction of the elemental forces of evolution (i.e. mutation, variations, recombination, natural selection and the genetic drifts) to producer relatively small changes in the population or populations, is known as microevolution.

Microevolutionary forces operating for a shorter period produce sequential evolution, whereas when continued for generations together result in the evolution of new populations from the existing one. The origin of new populations can occur in two different ways-

(i) in a successional manner, and

(ii) in a divergent manner.

The successional microevolution is the evolution within a single population which results in the successional replacement of the pre-existing populations by the new once. This could be seen in successive strata of palaeontological series. It leads the micro-evolution to the formation of clines, when characters of a population seem to change gradually across its place of distribution. The formation of clines is an example of gradual changes in response to gradual changes in the climate.

The divergent microevolution results in the splitting of parental population into two or more new populations with the appearance of genetic divergence. Isolation is the additional factor operating to establish genetic divergence in the related populations.

2. MACROEVOLUTION (ADAPTIVE RADIATION)

The evolution, which results in the production of new adaptive types through a process of population fragmentation and genetic divergence, is known as macroevolution. It operates above the species level and results in the splitting of the population of species into several subgroups, each of which exhibits changes in a definite adaptive direction. These changes are known as adaptive trends and the phenomenon as the adaptive radiation or macroevolution. It means macroevolution is actually adaptive radiation.

Mechanism of macroevolution - Macroevolution operates above species level and results in the establishment of new genera, families and orders. The changes in the organization occur on account of sudden mutations of large size, which are named “macro mutations” or “systematic mutation” by **Goldsehmidt. Macroevolution** occurs in a group of individuals which have entered a new adaptive zone free of competition.

3. MEGAEVOLUTION

Megaevolution has been described as the origin or evolution of new types of biological organization as a result of general adaptation from its predecessor, resulting in the formation of new classes, groups of phyta. Megaevolutionary changes are rare and have occurred only a few times in the evolutionary history of persist without extinction (with few exceptions).

MECHANISM OF MEGAEVOLUTION

During mega-evolution the organisms of the ancestral stock attempt to entera new zone, which is uninhabited by these forms and its devoid of competition. These exhibit varied modifications in different directions until one of these is found suitable to the new zone. It means a group of individuals of the parental stock develops certain generalized preadaptaions which unable them to enter the new zone. Therefore, these make a break - through into the new adaptive zone and start radiating into all the available habitats, thereby developing more specialized which are known as postadaptations.

►PATTERENS OF EVOLUTION

When morphological changes undergone by evolving higher taxa are plotted through time, distinctive patterns similar to those displayed in speciation are found. These are as follows-

1. **Adaptive Divergence or Divergent evolution**- When lineages split and evolve along separate adaptive

pathways showing increased morphological differences in a given biospace, it is called adaptive divergence or divergent evolution. Divergent evolution is also seen when separate lineages follow different morphological solutions to the same problems.

2. **Adaptive radiation or Radiation evolution**- It shows splitting up of a number of lineages from some primitive group and these lineages modify and evolve to exploit a number of distinctive biospaces. This results in multiple divergence. Such evolutionary changes represent radiation evolution. Diversification of stegocephalian amphibians of various mammalian orders from their primitive ancestral forms represent adaptive radiation.

3. **Parallel evolution**- In parallel evolution two or more distinct lineages evolve along similar lines under the influence of similar environmental opportunities or requirements. As a result these exhibit similar morphological changes. best example of parallel evolution is found in arthropods. Arthropods are said to exhibit polyphylectic origin and it is presumed that 2 to 4 separate lineages of annelid - like worms have separately undergone arthropodization.

4. **Iterative evolution**- In iterative evolution similar sequences of morphological codification appear successively from the basic stock. For example, irregularly coiled ammonites (heterostrophic) have arisen three or more times from coiled ancestors.

5. **Adaptive convergence or Convergence evolution**- In this case separate lineages assumes similar morphology under the influence of similar environmental factors. For example, dolphins and whales (mammals) have fish-like appearance to lead a successful aquatic life.

► Monophylectic And Polyphylectic Species

Taxa whose members have descended from a common ancestor are called **monophylectic**. All members or species of monophylectic taxon descend either from the same parents or same population or same species i.e. the new species is the temporal extension of the parent species.

Taxa whose members are descended from diverse ancestral lineages are called **polyphylectic**.

ANAGENESIS represent change in character of a lineage through time or linear succession of lineages through time i.e. succession of one species by other in due course of time. It is characterized by the replacement of one lineage by another.

Anagenesis creates organisms with novel characters and abilities, beyond those of their ancestors.

CLADOGENESIS represents divergent evolution in which parental population of parental lineage splits or branches into several lineages. Lineage branches resulting from cladogenesis are called clades and are monophbylectic. This ensures rapid origin of new species.

STASIGENESIS is a condition in which lineages neither split nor changes but persist unchanged. For example, turtles, Sphenodon, Coelacanths all represent stasigenesis.

George Gaylord Simpson noted two modes of evolution-

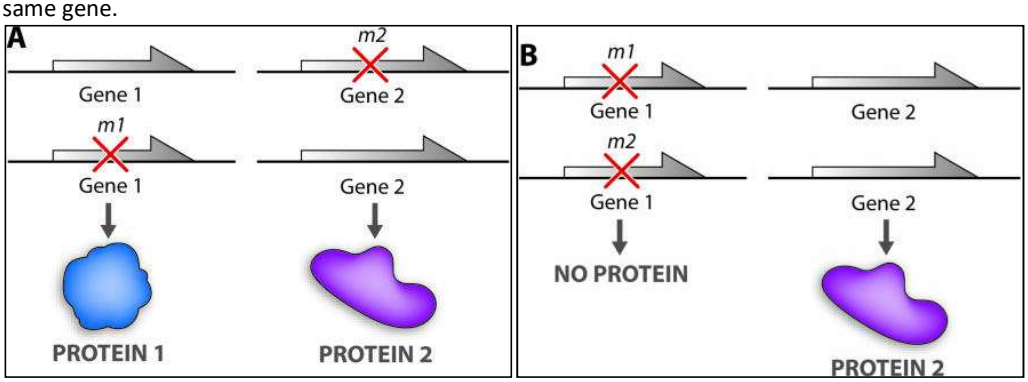
Bradytelic evolution- (G. brady, means slow) - it includes evolution of new species rather slowly and gradually in relatively minor ways. This can also be called gradualism.

Tachytelic evolution (G. tachy, means fast) - it includes origin of species by abrupt changes. As a result, the intermediate fossil forms are absent. This type of evolution is called punctuationism.

CHAPTER: 7 Complementation Test: (Complementation analysis)

A test of gene function where two genotypes with recessive alleles are combined by a cross to test whether the genotype of one parent can supply the function absent in the genotype of the other parent.

▶Consider two independently isolated mutations, *m1*and *m2*. Mutation *m1* and mutations *m2* are both fully recessive, and *m1/ m2* homozygotes produce similar mutant phenotypes. ***If m1 and m2 are in the same gene, double heterozygotes (m1/ m2) will possess only mutant copies of the gene,*** and thus produce only the mutant phenotype. If the mutant phenotype is observed, we say that *m1* and *m2* "fail to complement each other" and that they define the same gene.



▶***Suppose, however, that m1 and m2 are not in the same gene.*** These double heterozygotes have a wildtype phenotype because they carry one wildtype allele of both the m1 and m2 gene. In this instance, the two mutants are said to "complement each other" and to define different genes.

A more formal version of this test is the so-called *cis-trans* test. Using this test, two mutants are said to define the same functional unit (cistron) or gene

A complementation analysis asks if two putative alleles, **when in the** same cell **and** acting independently**, can supply all** functions necessary **for a** wild-type phenotype**. Complementation is therefore a test of function. The superscripts in this definition are explained below:**

1. The "two putative alleles" means **two versions of the same region of the chromosome, each of which separately confer a mutant phenotype.**
2. The two alleles can either be present on the chromosome or on extra-chromosomal elements. If either version is in more than one copy, there can be both regulatory complications (e.g. titration of a regulatory factor) and difficulties in interpretation (e.g. you do not know if a positive result is due to inappropriate quantities of the product encoded by the multi-copy gene).
3. Care must be taken that the mutations cannot recombine to form a wild-type genotype so that typically Rec- strains are used.
4. Only functions absolutely necessary for the desired phenotype, under the conditions used, are "demanded" by a complementation test. **Mutations affecting genes whose products are not essential for the desired phenotype will not be tested for in complementation analysis.**

▶ When the two mutations in the separate mutant alleles affect the same gene, then neither is capable of generating a wild-type product of that gene and the resultant merodiploid strain is mutant in phenotype.

▶ On the other hand, if the two mutations affect different genes, so that each copy of the region is able to generate some of the gene products required (and between them all necessary gene products are synthesized) then the resulting strain is phenotypically wild-type.

Rules for using the complementation test

1. ***The complementation test can be done ONLY when both mutants are fully recessive*.** Because the complementation test works by revealing the presence or absence of a normal allele, it will work only for recessive mutants.
2. ***The complementation test does not require that the two mutants have the same phenotype***.
3. There are cases where the phenotype of a double heterozygote is more extreme than that of either homozygote. There are several well-described instances in which the phenotype of a double heterozygote for two mutants within a given gene is considerably more extreme than that of either of the two homozygotes. This phenomenon is referred to as "negative complementation" . Negative complementation presumably reflects the ability of the two abnormal protein products to form a dimmer or multimer that is not only non-functional (as are homodimers of the two mutant proteins) but poisonous as well.

Complementation in bacteriophage:

1. Benzer's fine-structure mapping of phage T4 used similar experiments involving the *rII* gene.
   a. Different *rII* mutations of T4 were used, each with the characteristic large clear plaques and limited host range.
   b. T4 with the wild-type r^+gene infects *E. coil* strains *B* and *K12(λ).* For *rII* T4, strain *B* is permissive but *K12(λ)* is nonpermissive.

2. The T4 rII region has two genes, rIIA and rIIB. A mutation in either gene produces the rII phenotype for both plaque morphology and host range

In Benzer's work, nonpermissive strain K12(λ) was infected with pairs of rII mutants. Neither can grow alone in this strain.

a. If progeny are produced, the two mutants have complemented each other by providing different gene functions, either by genetic recombination (producing a few plaques) or complementation (lysing the entire lawn)

i. Infect bacterium with two phage genomes. Genotype of one is rIIA$^+$ rIIB, and of the other is rIIA rIIB$^+$.

ii. One phage provides the rIIA product, the other the rIIB product, and so the phage lytic cycle occurs.

b. If no progeny are produced, both mutations are in the same functional unit. Both mutants produce the same defective product (e.g., the rIIA product), and so the phage lytic cycle cannot occur.

c. Benzer's work showed two functional units for the rII phenotype, the complementation groups rIIA and rIIB. Both gene products must be produced for the lytic cycle to occur.

i. The fine-structure map indicates the boundaries of rIIA and rIIB.

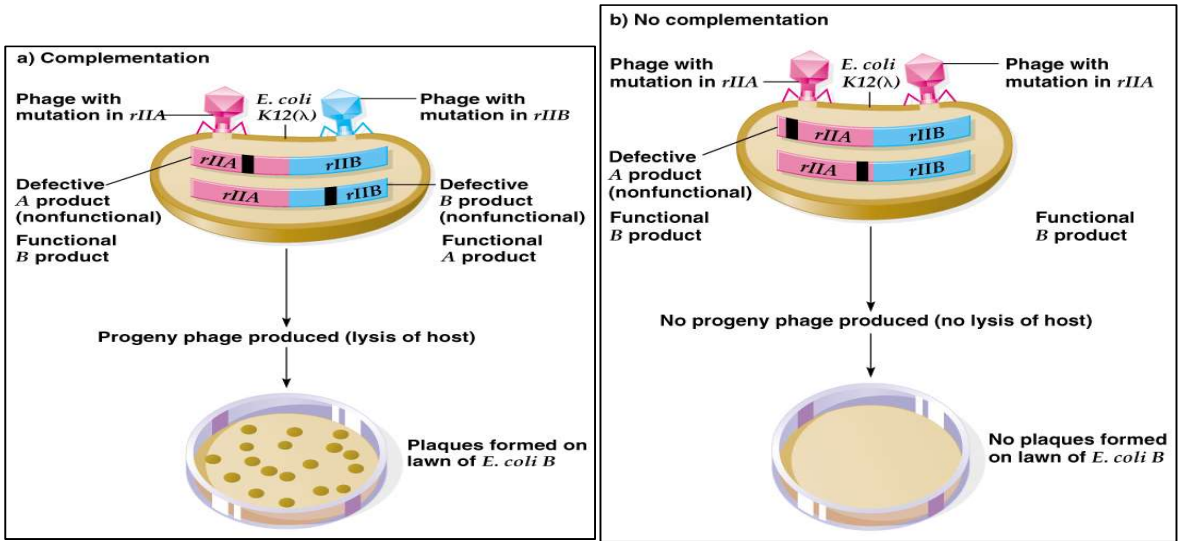
ii. Point mutants and deletion mutants in both rIIA and rIIB give the same results in complementation tests.

iii. Deletions that span rIIA and rIIB do not complement either rIIA or rIIB.

d. Alleles may be arranged two different ways in cis-trans complementation experiments:

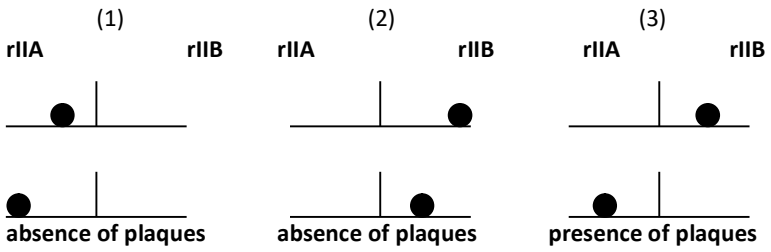
i. When the mutant alleles are on two different chromosomes, as in the complementation experiment above, they are in the trans configuration.

ii. A control for the experiment is to coinfect *E. coli* K12(λ) with an rII mutant carrying both mutations, and a wild-type T4 (expected result is wild-type). This rII mutant phage carries the mutations in the cis configuration. Benzer called the genetic unit of function defined by a cis-trans complementation test a cistron. Defined as the smallest segment of DNA encoding an RNA, cistrons are now usually referred to as genes.



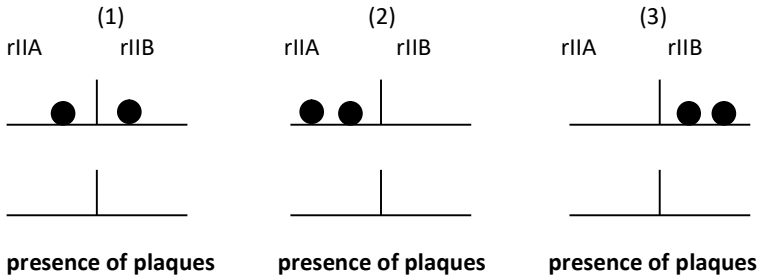
▶If plaque develop on the **K12λ** strain , it indicate that the two rII mutations must be in different cistrons. If plaque do not develop , it indicate that the two rII mutation are in the same cistron

▶A cistron code for a specific gene product. Cistron A is specific for synthesis of polypeptide A and Cistron B is specific for synthesis of polypeptide B. Both polypeptide s are necessary for the formation of a functional protein. these relationships are shown with the following diagram



Situation 1 is a trans arrangement, that is, the two mutations are on separate DNA strands. No plaque formation will occur on $K_{12\lambda}$ in this case because there is no way to generate a normal polypeptide A for the protein to be functional. **Situation 2** is also a trans arrangement. No plaque formation will occur on $K_{12\lambda}$ again because there is no way to generate a normal polypeptide B for the protein to be functional.
Situation 3 is also a trans arrangement, however, phage multiplication can occur on $K_{12\lambda}$ because a normal polypeptide A is specified from one viral DNA strand, while polypeptide B is specified by the other viral DNA strand. A functional protein will then result because of complementation, and the viral mutants will lyse $K_{12\lambda}$. The experimentation is known as complementation testing.

▶**The rII mutations can also be brought together into a cis arrangement**. With these mixed infections, one would always expect lysis to occur on $K_{12\lambda}$. The cis test serves as a control in these experiments. All combination in cis should yield plaques.



If one rII mutant was initially and arbitrarily designated as rIIA or rIIB, all others could be assigned to one cistron or the other based upon these mixed infection results. Benzer worked with hundreds of rII mutants in this way, and he was able to categorize al of them as residing in either the rIIA or rIIB cistron. From this analysis, support is added to the concept that the specification of a polypeptide is the unit of genetic function.
Problem: Pairs of rII mutants of the T4 phage were tested in both cis and trans by mixed infections of *E* coli $K_{12\lambda}$. Comparisons were made as to the number of phage particles that were reproduced per host bacterium, called burst size. Results of six different rII mutants, r-1, r-2, r-3 , r-4, r-5, and r-6, are as follows :

Mixed infection	cis Burst size	trans Burst size
r-1 × r-3	260	220
r-2 × r-6	211	0
r-3 × r-6	235	236
r-4 × r-5	241	233
r-5 × r-2	244	0
r-6 × r-4	229	240

If we assign mutation r-4 to the A cistron, what are the locations of the other rII mutations relative to the A and B cistrons?
Solution: All of the cis results are expected to show a normal viral burst because a wild-type chromosome is always available in such arrangements to effect a lysis.
The trans burst results yield the following information.

r-4 = A (given)
then r-5 = B (r –5 and r-4 complement)
r-6 = B (r –6 and r-5 do not complement)
r-3 = A (r –3 and r-6 complement)
r-2 = B (r –2 and r-4 complement)
r-1 = B (r –1 and r-2 do not complement)

CHAPTER: 8 Cytoplasmic Inheritance/ Maternal Inheritance

Chloroplasts: Variegation in Four O'Clock Plants

Correns discovered a variant of the four o'clock plant, Mirabilis jalapa, that had branches with either white, green or variegated leaves.

	Location of Ovule		
Source of Pollen	**White branch**	**Green branch**	**Variegated branch**
White branch	White	Green	White, green or variegated
Green branch	White	Green	White, green or variegated
Variegated branch	White	Green	White, green or variegated

In the above table, All possible combinations of crosses are strictly determined by the phenotype of the ovule source. For example, if the seeds (representing the progeny) were derived from ovules on branches with green leaves, all progeny plants bore only green leaves, regardless of the phenotype of the source of the pollen.

Correns concluded that inheritance was transmitted through the cytoplasm of the maternal parent because the pollen, which contributes little or no cytoplasm to the zygote, had no apparent influence on the progeny phenotypes.

Chloroplast mutations in *Chlamydomonas reinhardii*

This haploid eukaryotic organism has a single large chloroplast over 50 copies of a circular double-stranded DNA molecule. Matings that reestablish diploidy are immediately followed by meiosis, and the various stages of the life cycle are easily studied in culture in the laboratory. **The first cytoplasmic mutation, streptomycin resistance (*str*^{R}), was reported in 1954 by Ruth Sager.**

Although Chlamydomonas' two mating types– *mt*$^+$ and *mt*$^-$ —appear to make equal cytoplasmic contributions to the zygote, **Sager determined that the *str* phenotype is transmitted only through the *mt*+ parent, which is expressed in all offspring. One half of the offspring are *mt*$^+$ and one half of them are *mt*$^-$, indicating that mating type is controlled by a nuclear gene that segregates in a Mendelian fashion.**

str^R mt$^+$ × str^S mt$^-$

↓

1/2 mt$^+$ 1/2 mt$^-$

all str^R

str^S mt$^+$ × str^R mt$^-$

↓

1/2 mt$^+$ 1/2 mt$^-$

all str^s

Mitochondrial Mutations: The Case of *poky* in Neurospora

In 1952, Mary B.Mitchell and Hershell K. Mitchell studied the bread mold ***Neurospora crassa***. They discovered a slow-growing mutant strain and named it *poky* (*mi*-1 for maternal inheritance). **Slow growth is associated with impaired mitochondrial function, specifically in relation to certain cytochromes essential for electron transport.** Results of genetic crosses between wild type and *poky* strains suggest that the trait is maternally inherited.

If the female parent is *poky* and the male parent is wild type, all progeny colonies are *poky* . **The reciprocal cross produces normal wild-type colonies.**

Poky ♀ × wild-type ♂ ⟶ progeny all poky

Wild-type ♀ × poky ♂ ⟶ progeny all wild type

Studies with *poky* mutants show that occasionally hyphae from separate mycelia fuse with one another, giving rise to structures containing two or more nuclei of different genotypes, the structure is called a heterokaryon. The cytoplasm will contain mitochondria derived from both initial mycelia. A heterokaryon may give rise to haploid spores, or conidia, that produce new mycelia.

Heterokaryons produced by the fusion of *poky* and wild-type hyphae initially show normal rates of growth and respiration. However, mycelia produced through conidia formation become progressively more abnormal until they show the *poky* phenotype. This occurs despite the presumed presence of both wild-type and *poky* mitochondria in the cytoplasm of the hyphae.

To explain the initial growth and respiration pattern, we assume that the wild-type mitochondria support the respiratory needs of the hyphae. The subsequent expression of the *poky* phenotype suggests that the presence of **the *poky* mitochondria may some how prevent or depress the function of these wild type mitochondria.**

MAJOR IMMUNOGLOBULINS

1. Immunoglobulin G(IgG):

This is the major Ig in the normal human serum. Almost 70-75% of the immunoglobulins pool is IgG. IgG is found in both intravascular and extravascular pools.

The IgG molecule consist of 2 γ heavy chains and 2 *k* or 2λ light chains. In humans, there are 4 sub-classes of IgG, distinguished by differences in γ-chain sequence & numbered according to their decreasing average serum concentration. –IgG1, IgG3 & IgG4.

These sub classes can be distinguished from one another, according to **size of hinge region**, **number & position of the interchain disulphide bonds between the heavy chains**. Amino acid differences between the sub-classes of IgG, affects the biological activity of the molecule.

- IgG_1, IgG_3 & IgG_4 readily cross the placenta & protects the developing foetus.

IgG_3 is the most important complement activator. IgG_1 & IgG_2 are less efficient & IgG_4 is not able to activate complement at all. **The binding site for complement component Cl q is in the C_H2domain.**

- IgG_1 & IgG_3 bind with high affinity to Fc receptors on the phagocytic cells and thus mediate opsonization.
- Most IgG subclasses have a molecular weight of 150 kDa; IgG3 is slightly larger.
- Most IgG has a half-life of approximately 21 days; IgG3 has a half-life of only 7 day
- IgG_2 has low affinity for Fe receptors & IgG_4 has intermediate affinity.
- IgG molecules of all sub-class cross the placenta and confers passive immunity to new borns.
- Effective antitoxic immunity is exclusively IgG.
- IgG is the major opsonizing immunoglobulin.

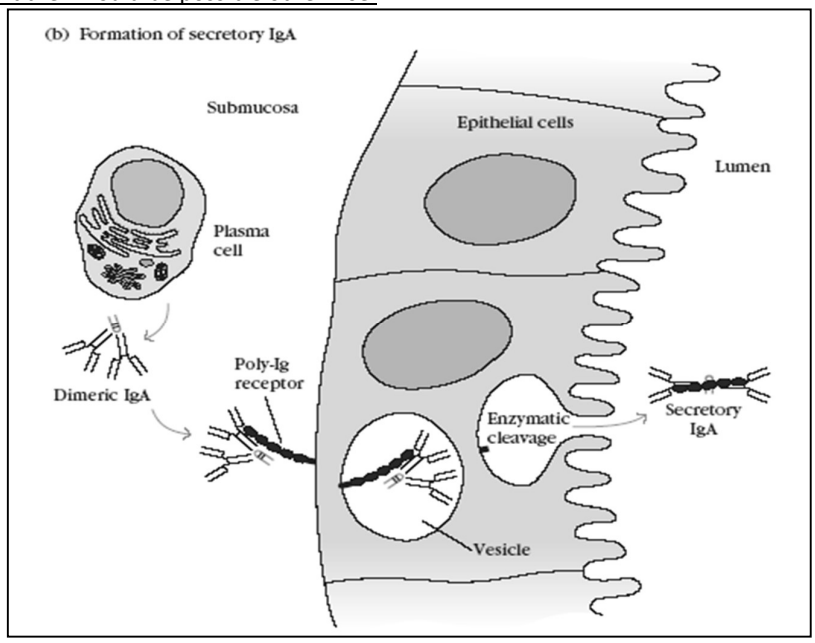
2. Immunoglobulin M (IgM):-

- Antibody out 5-10% of the Ig pool is IgM in human serum. It is seen only in the intravascular pool. Monomeric IgM, with a molelular weight of 180,000, is expressed as membrane bound Ig on B-cells. It is **secreted by plasma cells as a pentamer** in which five monomer units are held together by disulfide bonds. The 5 monomer sub-units are arranged with their Fc region in the center of the pentamer and 10 Antigen binding sites on the periphery of the molecule.
- Each pentamer contains an additional Fc linked polypeptide called the J (joining) chain, required or polymerization of the monomers to form pentameric IgM.
- **IgM is the 1st immunoglobulin produced in an primary response to an Antigen Therefore, elevated levels of IgM indicate recent infection by new Antigen. And**
- IgM is also first and predominant antibody produced by the fetus. An elevated IgM level in the cord blood of a newborn (the normal level is approximately 10 mg/dl) may indicate that the fetus was infected before birth. IgM has a serum half-life of approximately 10 days.
- **An IgM molecule can bind 10 small hapten molecules**; but because of stearic hindrance only 5 or fewer molecules of larger Antigen can bound simultaneously. Pentameric IgM is more efficient in binding Antigen because of its high valency than other isotypes
- IgM is also more efficient than IgG at activating complement, because complement activation requires 2 Fc regions in close proximity & IgM fulfills this requirement.
- Because of its large size, IgM doesnot diffuse well and therefore is found in very low concentration in the intercellular tissue fluid. **IgM also plays an important accessory role as a secretory Ig.**
- The presence of the J chain allows IgM to bind to receptors on secretory cells. Which transport it across epithelial linings to enter the external secretions that bathe mucosal surface.

3. Immunoglobulin A (IgA):

- ► Although IgA constitutes only 10-15% of the total Ig pool in serum**, it is the pre-dominant Ig class in external secretions such as breast milk, saliva tears & mucous of the bronchial, genitourinary and digestive tracts.**
- ► IgA exists primarily in monomeric forms but polymeric forms are also exists sometimes with a J chain polypeptide.
- ► **Secretory IgA,** IgA of external secretion, consist of a dimmer or tetramer. J-chain polypeptide and a polypeptide chain called **secretory component** derived from the receptors responsible for transportation of IgA.
- ► J-chain polypeptide facilitates the polymerization of IgA. Secretory component is produced by epithelial cells of mucous membrane and consist of 5 immunoglobulin like domains.
- ► The daily production of secretory IgA is greater than any other Ig. IgA secreting plasma cells are concentrated along mucous membrane surfaces.
- ► IgA producing plasma cells migrate to sub-epithelial tissue, where the secreted IgA binds to a receptor **for polymeric Ig.** This poly Ig receptor is expressed on mucosal epithelia i.e. lining of digestive, respiratory & genital tracts & on glandular epithelia in the mammary, salivary & lachrymal glands. After polymeric IgA binds

to the receptor this receptor-IgA complex is transported across the epithelial barrier to lumen. This transport involves receptor-mediated endocytosis into coated pits and directed transport of the vesicle across the epithelial cell to luminal membrane, where vesicle fuses with plasma membrane. The poly Ig receptor is then cleaved enzymatically from the membrane becomes secretory component which is bound to & released together with polymeric IgA into mucous secretions. The secretory component masks sites susceptible to protease cleavantigene in the hinge region of IgA, allowing the molecule to exist longer in protease rich environment then would be possible otherwise.



The poly Ig-receptor interact with J chain of IgA & IgM antibodies.

► Secretory IgA serves an important effector function at mucous membrane surfaces & restricts the entry of most pathogenic organisms.

► Secretory IgA binds to viral and bacterial surface Antigens, prevents attachment of pathogen to mucosal cells and thus inhibiting infections.

► Secretory IgA provides an important line of defence against bacteria such as *Salmonella, N.gonorrhoeae*, *polio, influenza & reoviruses.*

► Breast milk with IgA help to protect newborn antigenainst infection during 1st month of life, because their immune-system is non-functional during this period of time.

4. Immunoglobulin E (IgE):-

► This is found in very low concentration i.e. 0.3 µg/ml. in serum. Actual identification of IgE was accompalished by K. & T. Ishizako in 1966. They immunized rabbit with serum of an allergic individual to prepare anti isotype antiserum. The rabbit antiserum react with human Antibody & anti isotype Antibody were precipitated and removed from rabbit anti serum. Remained anti-isotype Antibody completely block the P-K reaction & named as IgE.

► These Antibody mediate hypersensitivity reactions responsible for symptoms of hay fever, asthma and anaphylactic shocks. This was 1st demonstrated by **K. Prausnitz and H. Kustner in 1921**, who injected intra-dermaly the serum from allergic person to non-allergic one. When the appropriate Antigen was later injected at the same site a **wheal & flare reaction** developed there, which is P-K reaction, was the basis of the 1st biological assay for IgE activity.

[**The wheal is a sharply delineated soft swelling** surrounded by **the flare — a reddened area**.Both are caused by the release of leukotrienes at the site, which increase the flow of blood to the site making it swollen and red.]

- **The IgE binds to Fc receptors on the basophils and mast cells.**
- Cross linked antigen of receptor bound IgE molecules to Antigen/allergen induces a person known as degranulation i.e. basophils & mast cells translocate their granules to plasma membrane & release their contents to extra cellular environment.
- Due to this, a variety of pharmacologically active mediators are released and give rise to allergic manifestations. Localized mast-cell degranulation also may release mediators that build up various cells necessary for antiparasitic defense. IgE may also be important in immunity to certain helminthic parasites. **IgE is unable to activate complement via the classical pathway.**
- IgE is associated with atopic diseases (e.g., asthma and anaphylaxis).

- IgE is homocytotropic; that is, it has an affinity for cells ("cytotropic") of the host species that produced it ("homo"). This affinity is particularly strong for tissue mast cells and blood basophils. Fixation to these cells occurs via a **cell membrane bound $F_c\varepsilon R$** (i.e., receptor for the Fc portion of the **ε** chain of IgE) reacting with the Fc fragment (CH3and CH4domains).
- On combining with allergens, IgE antibodies trigger the release of histamine and other mediators of atopic disease from the cells.
- IgE may also be important in immunity to certain helminthic parasites.
- IgE is unable to activate complement via the classical pathway.
- IgE has a vascular half-life of 2 to 3 days and is heat-labile at 56°C

Production: IgE is produced by B cells and plasma cells in the spleen, in lymphoid tissue of the tonsils and adenoids, and in the respiratory and gastrointestinal mucosa. IgE does not cross the placenta. IgE production begins in the fetus early in gestation.

5. Immunoglobulin D (IgD):-

It was first discovered in a patient developed multiple myeloma. They have serum concentrate of 30 µg/ml (approximately 3 to 5 mg/dl).and constitute about .2% of human serum. They are present extensively on PM of B-cells. Their exact function is unknown but probably they play a role in Antigen dependent B-cells differentiation i.e. it eliminates B-cells which can produce self reactive antibodies**. IgD exists as a monomer**

CHAPTER: 6 MONOCLONAL ANTIBODIES

An antibody produced by a single clone of cells (specifically, a single clone of hybridoma cells) and therefore a single pure homogeneous type of antibody. Monoclonal antibodies can be made in large amounts in the laboratory and are a cornerstone of immunology. The term "monoclonal" pertains to a single clone of cells, a single cell and the progeny of that cell.

Köhler and Milstein found a way to combine

- the unlimited growth potential of myeloma cells with
- the predetermined antibody specificity of normal immune spleen cells.

They did this by literally fusing myeloma cells with antibody-secreting cells from an immunized mouse. The technique is called **somatic cell hybridization**. The result is a **hybridoma**

The procedure

Mix the following spleen cells from a mouse that has been immunized with the desired antigen with myeloma cells. Use an agent to facilitate fusion of adjacent plasma membranes like PEG. Even so, the success rate is so low that there must be a way to **select for** the rare successful **fusions.**

So, use myeloma cells that have:

- **lost** the ability to synthesize **hypoxanthine-guanine-phosphoribosyltransferase** (**HGPRT**).

This enzyme enables cells to synthesize purines using an extracellular source of **hypoxanthine** as a precursor. Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an alternate pathway that they can use to synthesize purines.

However, ***when cells are exposed to aminopterin (a folic acid analog), they are unable to use this other pathway and are now fully dependent on HGPRT for survival.***

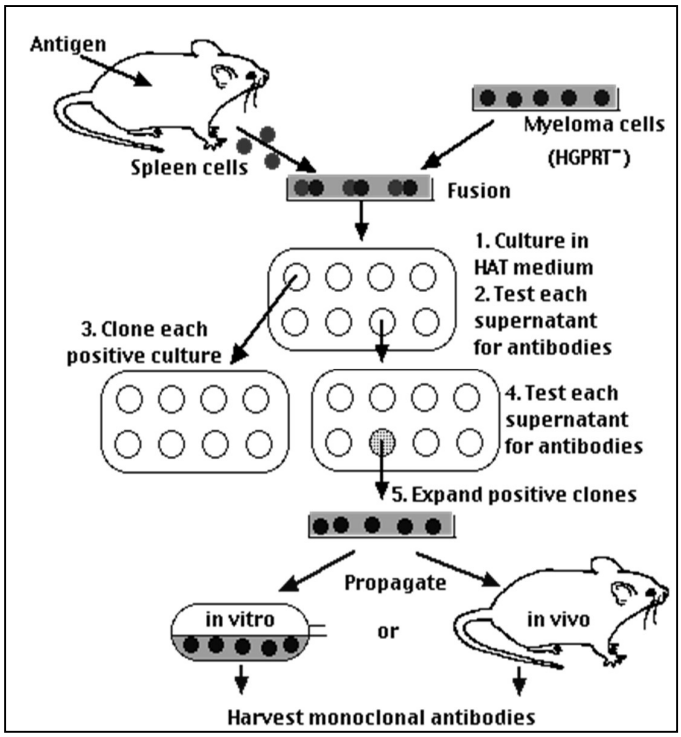
- **lost** the ability to synthesize any antibody molecules of their own (so as not to produce a hybridoma producing two kinds of antibody molecules).

1. The first property is exploited by transferring the cell fusion mixture to a culture medium - called **HAT medium** because it contains:

- **h**ypoxanthine
- **a**minopterin
- the pyrimidine **t**hymidine

The logic:

- Unfused myeloma cells cannot grow because they lack HGPRT.
- Unfused normal spleen cells cannot grow indefinitely because of their limited life span. However,
- Hybridoma cells (produced by successful fusions) are able to grow indefinitely because the spleen cell partner supplies HGPRT and the myeloma partner is immortal.



2. Test the supernatants from each culture to find those producing the desired antibody.
3. Because the original cultures may have been started with more than one hybridoma cell, you must now isolate single cells from each antibody-positive culture and subculture them.
4. Again, test each supernatant for the desired antibodies. Each positive subculture having been started from a single cell - represents a clone and its antibodies are monoclonal. That is, each culture secretes a single kind of antibody molecule directed against a single determinant on a preselected antigen.
5. Scale up the size of the cultures of the successful clones.
Hybridoma cultures can be maintained indefinitely:

- ***in vitro***; that is, in culture vessels. The yield runs from 10-60 µg/ml.
- ***in vivo***; i.e., growing in mice. Here the antibody concentration in the serum and other body fluids can reach 1-10 mg/ml. [When the hybridoma cells are injected in mice (in the peritoneal cavity, the gut), they produce tumors containing an antibody-rich fluid called **ascites fluid**.]

***Catalytic Antibodies*:** Antibodies have been selected for their affinity for the antigen rather than for the transition state of any reaction the antigen might undergo. If the immunogen were a transition state or a transition-state analogue, however, antibodies should catalyze the appropriate reaction. If this were the case, it should be possible to make antibodies with catalytic activity to order.
Monoclonal antibodies directed against various transition-state analogues have been found to have some of the expected catalytic activities and specificities. Such antibodies have many of the characteristics of enzymes in that they accelerate reactions up to 10^5-fold over the noncatalyzed rate, show comparable substrate specificities, exhibit a Michaelis K_m values for substrates, and are subject to competitive inhibition. The catalytic activities of the antibodies generated thus far are still lower than of natural enzymes, however, probably as a result of deficiencies in the way the analogues mimic the true transition states.
Other approaches can be taken to generate catalytic antibodies. Bisubstrate inhibitors can generate antibodies that bind the two individual substrates and enhance reaction between them simply due to their proximity in the antibody combining sites. Reactive groups can be generated in combining sites by using the appropriate immunogen, by mutation of the antibody gene, or by chemical modification of the antibodies.

CHAPTER: 24 AUXIN

Some special type of chemical substances are present in plants which are lies in extremely low concentration at growth points. They are called "Growth hormones". They promote or inhibit the growth of the plant. First of all idea of growth hormone was introduced by **"Julius Von Sachs"** Thimann termed them "Phytohormones" and defined as those complex organic substance synthesized in one region and transferred to other regions and affect the growth in very low concentration.

Classification of Hormones:-

Generally, they are divided into two categories:-

[A] Growth Promoters - IAA, GA, CK

[B] Growth Inhibitors - ABA, Ethylene

Growth Promoters:- Such hormones which increase the growth of plant are called "growth promoters". The following hormones come under this category:-

AUXINE:

It is the first discovered plant hormones & first growth regulator. **"Charles Darwin" and "Francis Darwin"** conducted experiment on ***Phalaris canariensis*** & wrote the book "The Power of Movement in Plants" (1880). According to Darwin the coleoptile bends towards the light, if light is provided from one direction , some chemical (stimulus) move away from light region so more growth occurs in dark region. So it bends towards the light, i.e., coleoptile shows phototactic response. Darwin also explained that apical region of coleoptile receive light stimulus. If coleoptile is removed or it is covered by metal cap then coleoptile does not shows curvature towards the light.They further found that the apical part regulates the growth of basal part of the plant and light affects this growth.

Boysen & Jensen :- They performed their experiment on oat coleoptile [*Avena sativa*] and found that if coleoptile tip is removed then its phototrophic response is destroyed. If excised coleoptile is tip again placed on decapitated part its tropic stimulus is again resumed. They explained, phototropism is not a physical process but it is due to the presence of certain chemical (material substance) in the apical region.

Paal :- He proved that chemicals present in the apical region, are growth regulators. He also found that the substances produced in the apex are soluble in water. **Unequal distribution of growth hormone suggested by Paal.**

F.W. Went :-The credit for discovery of Auxin has been given to F.W. Went. (1928)Went did his experiment on oat coleoptile. He placed excised coleoptile tip on agar block and divided the agar block into smaller blocks which were placed on decapitated coleoptile kept in dark, this it shows curve in opposite direction. He proved that a substance present in the apex of coleoptile which is diffused into the agar block. Due to this substance stump of coleoptile shows curvature. Went has given the name **"Auxin"** to this substance. He found that the curvature is proportional to the concentration of auxin. Went found that the amount of auxin was 57% in dark region and 27% in lighted region about 16%. **Auxin is transfer basipetaly or photooxidised**.

Chemical Nature of Auxin :-

Kogl and H. Smit isolated Auxin like substance from the human urine. They called it as "Auxin-a" [Auxenotriolic acid]. Its chemical formula is $C_{18}H_{32}O_5$. **Kogl** Axlerben and H. Smit isolated Auxin-b [Auxenolonic acid] from corn germoil, its chemical formula is $C_{18}H_{30}O_4$. They also isolated an other substance from human urine which was named Hetero auxin by Thimann. Now a days it is called as IAA [Indole-3 Acetic Acid]. Its chemical formula is $C_{10}H_9O_2N$. Indole acetic Acid [IAA] is a natural Auxin which is found in plants.

Some natural Auxin which are derivatives of IAA are as follows:-

(i) Indoie-3 Acetaldehyde (ii) Indole-3 pyruvic Acid, (iii) Indole - 3 acetonitrile

Natural Auxin are synthesized in the apical meristems (75% in stem apex). Synthesis of natural Auxin [IAA] takes place in the meristematic region by **tryptophar amino acid,** Natural Auxin mostly moves from apex to the base. Their transport is **basipetal & Polar** Their concentration is maximum in apical meristems. Photo oxidation of Auxin takes place in the high light intensity & (also in blue light). It means they are destroyed or they are accumulated in the shady part of the plant. "Zn is essential for the synthesis of Auxin". Auxin is destroyed in old tissues by an enzyme IAA-oxidase.

Synthetic Auxins:

*• Some synthetic chemical compounds also behave as Auxin. They are called synthetic Auxins such as

(i) **NAA = Naphthalene Acetic Acid :-** Its common name is **"Horotomone"**

(ii) **IBA** - **Indole Butyric Acid** :- Its common name is **"Rootone"**

(iii) **2-4D = 2-4-dichlorophenoxy Acetic Acid**

(iv) **2, 4, 5-T (2, 4, 6-Trichlorophenoxy acetic acid.)**

(v) Picloram = 2, 3, 5-trichloro- 4-amino picolinic Acid. (Tordon)

(vi) **Delapon = 2, 2-dichloro Propionic Acid.**

Non polar transport" is found in synthetic Auxin.

They spread in whole of the plant. This is also called **as "systemic transport".**

Auxin is not transported by vascular bundle but transport takes place through the diffusion from one cell to another cell. Rate of Auxin transport is 1-1.6 cm/hr.

►Auxin is the only plant growth hormone that is transported polarly. Because the shoot apex serves as the primary source of Auxin for the entire plant, polar transport contributes to the formation of an Auxin gradient from the shoot to the root. The longitudinal gradient of Auxin is thought to affect various developmental processes, including stem elongation, apical dominance would healing, and leaf senescence.

►Polar transport proceeds in a cell-to-cell fashion, rather than through the symplast. That is, Auxin exits the cell through the plasma membrane, diffuses across the two primary walls, and enters the cell below through its plasma membrane. The loss of Auxin from cells is termed Auxin efflux; the entry of Auxin into cells is called Auxin uptake or influx. These processes require metabolic energy, as evidenced by the sensitivity of polar transport to O2 deprivation and metabolic inhibitors. The rate of polar transport is about 1 cm h^{-1}- ten times slower than the rate of transport in the phloem. Neither inactive Auxin analogs nor Auxin metabolites are transported polarly, suggesting that polar transport involves protein carries on the plasma membrane that can recognize the hormone and its active analogs.

►Most of the Auxin in the root is transported acropetally (from root base to root tip) and transport occurs mainly in the stele. The Auxin in the root vascular cylinderis thought to play an important role in stimulating the pericycle to form branch roots.

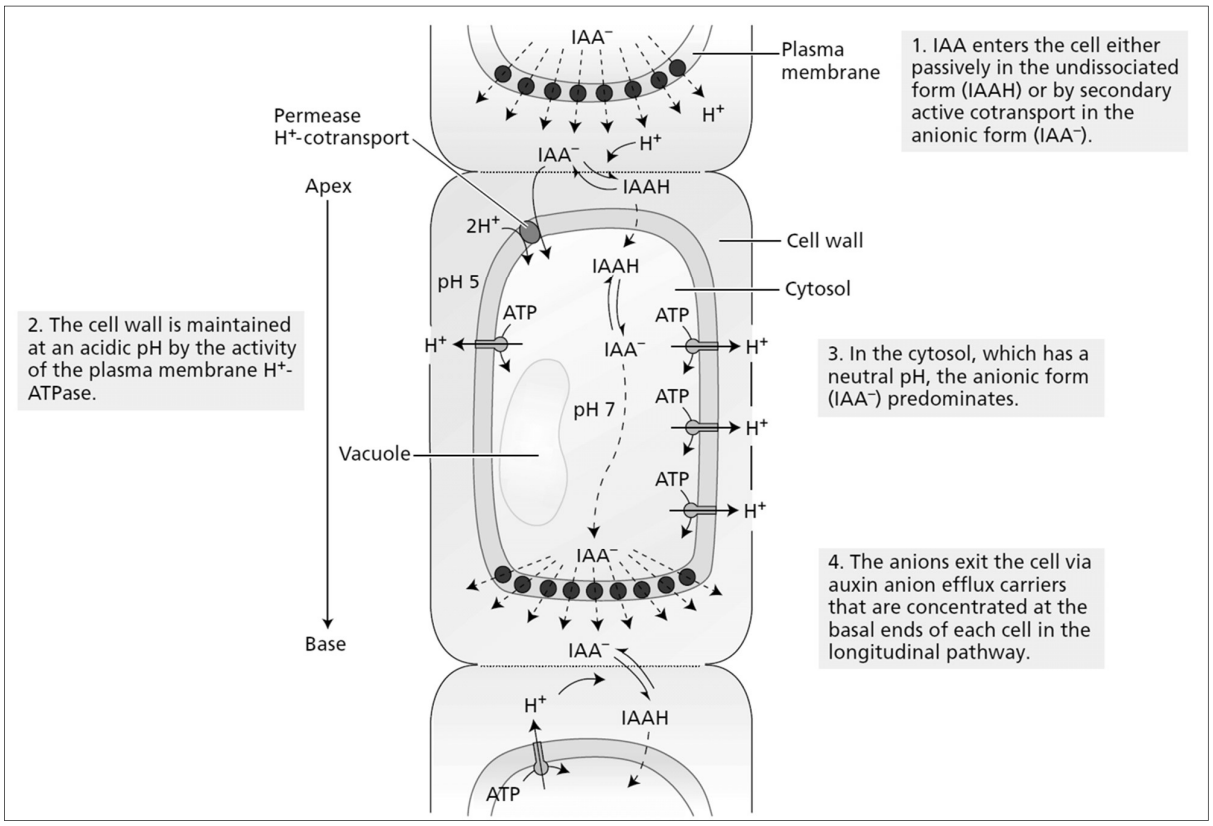
A Chemiosmotic Model Explains Polar Auxin Transport

►According to the widely accepted chemiosmotic model for polar Auxin transport, the total proton motive force (ΔE+Δ PH) across the plasma membrane drives the uptake of Auxin, whereas transport of Auxin from the cell is driven solely by the membrane potential, ΔE. A crucial feature of the polar transport model is that the Auxin efflux carriers are localized predominantly at the basal ends of the conducting cells.

►The first step in polar transport is Auxin influx. Auxin enters plant cells by either of two mechanisms: passive diffuxion across the phospholipids bilayer or secondary active transport via a proton symporter. The dual pathway of auxin uptake arises because the passive permeability of the membrane to Auxin depends strongly on the apoplastic pH. The undissociated form of indole-3-acetic acid, in which the carboxyl group is protonated, is lipophilic and readily diffuses across lipid bilayer membranes.

►In contrast, the dissociated form of IAA is negatively charged and therefore does not cross membranes unaided. Since the plasma membrane H^-ATPase normally maintains the cell wall solution at about pH 5, about half of the Auxin (pKa = 4.75) in the apoplast will be in the undissociated form, and will diffuse passively across the plasma membrane down a concentration gradient. Expermental support for pH-dependent, passive Auxin uptake was first provided by Rubery and Sheldrake, who showed that IAA uptake by plant cells increases as the extracellular pH is lowered from a neutral to a more acidic value.

►**An H^+-IAA symporter contransports two protons along with the Auxin anion.** This secondary active transport of Auxin allows for greater Auxin accumulation than simple diffusion does since it is driven across the membrane by the proton motive force.



►**Once IAA enters the cytosol, which has a pH of approximately 7.2, nearly all of it will be in the anionic from. Because the membrane is less permeable to IAA than to IAAH, Auxin will tend to accumulate in the cytosol.** However, much of the Auxin that enters the cell escapes via an Auxin efflux carrier. Transport of IAA out of the cell is driven by the inside negative membrane potential. As noted earlier, a crucial feature of the chemiosmotic model for polar transport is that IAA efflux takes place preferentially at the basal end of each cell. According to the model, the repetition of Auxin uptake at the apical end of the cell and preferential release from the base of each cell in the pathway gives rise to the total polar transport effect.

►Auxin is Transported Nonpolarly in the Phloem

Most of the IAA that is synthesized in mature leaves appears to be transported to the rest of the plant non-polarly via the phloem, can move from these leaves up or down the plant at velocities much higher than those of polar transport. Phloem transport is largely passive, not requiring energy directly.

⇨Most IAA in the Plant is in a Covalently Bound Form

Although free IAA is the biologically active form of the hormone, the vast majority of Auxin in plants is found in a covalently bound state. These conjugated, or "bound," auxins have been identified in all higher plants and are generally regarded as hormonally inactive. IAA has been found to be conjugated to both high and low molecular-weight compounds. Examples of low-molecular-weight conjugated auxins include esters of IAA with glucose or myo-inositol and amide conjugates such as IAA-N-aspartate. High molecular weight IAA conjugates include IAA-glucan (7-50 glucose units per IAA) and IAA-glycoproteins found in cereal seeds. The compound to which IAA is conjugated and the extent of the conjugation depend on the specific conjugating enzymes. The best-studied reaction is the conjugation of IAA to glucose in Zea mays.

►The highest concentrations of free Auxin in the living plant are in the apical meristems of shoots and in young leaves, since these are the primary sites of Auxin synthesis.

►There Are Two Subcellular Pools of IAA: The Cytosol and the Chloroplasts

The distribution of IAA in the cell appears to be regulated largely by pH. Because the dissociated form does not cross membranes unaided, whereas the protonated form readily diffuses across membranes, Auxin tends to accumulate in the more alkaline compartments of the cells.

►In the wild-type cells, about one-third of the IAA is fond in the chloroplast, and the remainder is located in the cytosol. IAA conjugates are located exclusively in the cytosol.

A Possible Auxin Receptor Has Been Identified

►Auxin receptor was located on the plasma membrane. Auxin-binding sites were subsequently identified in the endoplasmic reticulum (site I), the plasma membrane (site II), and the tonoplast (site III). However, most of the Auxin-binding activity was associated with site I. Besides representing the bulk of the binding activity, the affinity of site I binding for Auxin analogs roughly parallels the Auxin activity of the analogs.

►The ABP1 gene encodes a hydrophilic glycoprotein containing a signal sequence at its N terminus; the C terminus contains the amino acid sequence that acts as an ER retention signal, lysine-aspartic acid-glutamic acid leucine (K-D-E-L using the single letter amino acid code). The presence of a signal sequence indicates the ABP1 is synthesized on the ER and translocated into the ER lumen. The presence of the KDEL sequence indicates that, rather than being transported to the Golgi. ABP1 is retained in the ER. This retention is consistent with binding studies showing that the ER is the richest source of ABP1.

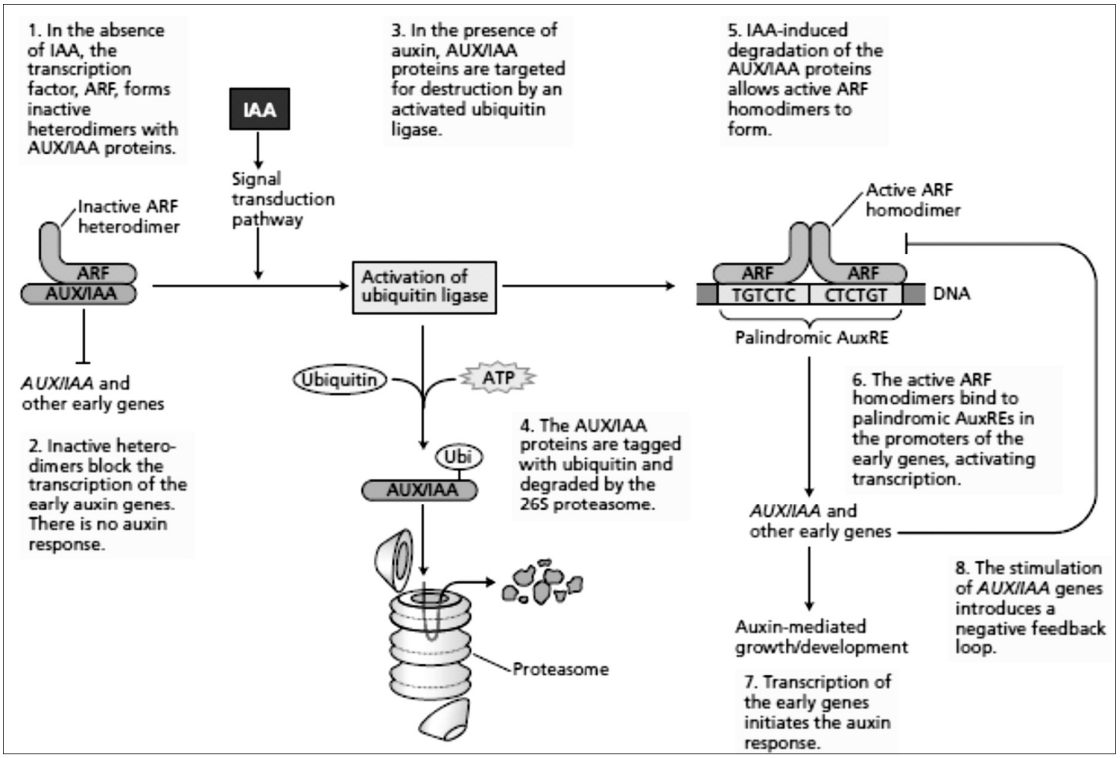
►The localization of ABP1 in the ER seems to contradict the original assumption that the Auxin receptor is located on the plasma membrane. This location was supported by studies using forms of Auxin that are unable to cross the membrane and enter the cell.

The MAP Kinase Cascade May Mediate Auxin Effects

►Both Auxin and Cytokinin are required for the completion of the cell cycle, When tobacco cells are deprived of Auxin, they arrest at the end of either the G1 or the G2 phase and cease dividing; if Auxin is added back into the culture medium, the cell cycle resumes. Auxin appears to exert its effect on the cell cycle primarily by stimulating the synthesis of the major cyclin-dependent protein kinase (CDK) Cdc2.

►Pathway, required for Auxin action, involving the ubiquitination of nuclear proteins. The first enzyme in the ubiquitin pathway is E1, which binds to and activates ubiquitin using ATP. The gene involved in the Auxin-resistant axr1 mutation of Arabidopsis, which results in defects in many Auxin responses, including gravitropism and gene expression, was found to encode an enzyme related to the N-terminal half of E1. AXR1 is related to a gene in yeast, AOS1, which also lacks the C-terminal half of E1.

►The two proteins AXR1 and ECR1 appear to form an E1-like heterodimer similar to that of the yeast Aos1p-Uba2p heterodimer. However, rather than binding to ubiquitin, the AXR1-ECR1 heterodimer binds to a family of small, ubiquitin-related proteins called RUBs. Binding of AXR1-ECR1 to RUB activates RUB and initiates a pathway similar to the ubiqutination pathway in which RUB is covalently attached to a target protein.



►However, unlike ubiquinated proteins, proteins tagged with RUB are usually activated rather than marked for destruction. AXR1 - ECR1 heterodimer mediates the transfer of RUB to an E3 enzyme complex of the ubiquitination pathway, thereby activating the E3 complex. According to this model, Auxin increases the activity of AXR1-ECR1, either by inducing its synthesis or by activating a preexisting enzyme. Since the AXR1 protein has been localized to the nuclei of dividing and elongating cells, Estelle and colleagues have suggested that this Auxin-induced ubiqutination occurs in the nucleus, resulting in the degradation of nuclear proteins by the 26S proteasome.

Effect of Auxin

[1] Cell Elongation: Higher concentration of Auxin stimulates cell elongation in shoot. Therefore, shoot is a positively phototropic and negatively geotropic. Cell elongation is due to:-