KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.

(For the candidates admitted from 2016 onwards)

DEPARTMENT OF BIOCHEMISTRY

STAFF NAME	**:**	**Dr. L. HARIPRASATH**		
SUBJECT	**:**	**PLANT BIOCHEMISTRY**		
SUBJECT CODE	**:**	**16BCU503A**		
SEMESTER	**:**	**V**	**CLASS**	**: III B.Sc. Biochemistry**

SCOPE

To provide sufficient knowledge about plant cell and its organelles basically, plant growth substances, plant nutrition and senescence.

OBJECTIVES

- Understanding of the plant cell organelles and their functions.
- To gain a wide knowledge about plant growth substances, plant nutrition and photo morphogenesis.

Unit I

Plant cell structure and Photosynthesis

Structure of Plasma membrane, Vacuole and tonoplast membrane, cell wall, plastids and peroxisomes. Photosynthesis - Structure of PSI and PSII complexes, Light reaction, Cyclic and non cyclic photophosphorylation.

Unit II

Carbon assimilation and Plant Respiration

Carbon assimilation - Calvin cycle and regulation; C4 cycle and Crassulacean acid metabolism (CAM). Respiration - Overview of glycolysis, Alternative reactions of glycolysis, Regulation of plant glycolysis, Translocation of metabolites across mitochondrial membrane, TCA cycle, Alternative NAD(P)H oxidative pathways; Cyanide resistant respiration and Photorespiration.

Unit III

Nitrogen metabolism

Biological Nitrogen fixation by free living and in symbiotic association, structure and function of enzyme Nitrogenase. Nitrate assimilation: Nitrate and Nitrite reductase. Primary and secondary ammonia assimilation in plants; ammonia assimilation by Glutamine synthetase- glutamine oxoglutarate amino transferase (GS-GOGAT) pathway. Seed storage proteins in legumes and cereals.

Unit IV

Regulation of plant growth and Plant tissue culture

Introduction to plant hormones and their effect on plant growth and development, Regulation of plant morphogenetic processes by light. Plant tissue culture - Cell and tissue culture techniques, types of cultures: organ and explants culture, callus culture, cell suspension culture and protoplast culture. Plant regeneration pathways: organogenesis and somatic embryogenesis. Applications of cell and tissue culture and somoclonal variation.

Unit V

Plant Secondary metabolites

Representatives alkaloid group and their amino acid precursors, function of alkaloids, Examples of major phenolic groups; simple phenylpropanoids, Coumarins, Benzoic acid derivatives, flavonoids, tannins and lignin, biological role of plant phenolics, Classification of terpenoids and representative examples from each class, biological functions of terpenoids.

Support Materials

T1: Lodish, H., Berk, A., Kaiser, C.A., & Krieger, M., (2012). *Molecular Cell Biology*, 7th edition. W.H. Freeman & Company, London.

T2: Nelson, D.L. and Cox, M.M., W.H. Freeman., Lehninger: *Principles of Biochemistry* (2013) 6th ed., and Company (New York), ISBN:13: 978-1-4641-0962-1 / ISBN:10:1-4641-0962-1.

T3: Murray, R.K., Bender, D.A., Botham, K.M., & Kennelly, P.J., (2012).Harper's illustrated Biochemistry, 29th edition. McGraw-Hill Medical. London.

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LECTURE PLAN

Sl. No	Duration of Period	Topics to be Covered	Books Referred	Page No	Web Page referred
		UNIT – I			
1.	1	Composition of biomembranes- prokaryotic, eukaryotic, neuronal and sub-cellular membranes.	T1	409-411	
2.	1	Study of membrane proteins. Fluid mosaic model with experimental proof.	T2	371-372	
3.	1	Polymorphic structures of amphiphilic molecules in aqueous solutions- micelles and bilayers.	T3 T1	423-428 412-415	
4.	1	Monolayer, planer bilayer and liposomes as model membrane systems	T3	423-426	
5.	1	CMC, critical packing parameter. Membrane asymmetry. Macro and micro domains in membranes.	T3	426-427	
6.	1	Membrane skeleton, lipid rafts, caveolae and tight junctions. RBC membrane architecture	T3	429-430	
7.	1	Lateral, transverse and rotational motion of lipids and proteins.	T1	416-420	
8.	2	Techniques used to study membrane dynamics - FRAP, TNBS labeling etc.	T2	381-385	
9.	1	Transition studies of lipid bilayer, transition temperature. Membrane fluidity, factors affecting membrane fluidity.	T2	380-381	
10.	1	Class test			

Total number of hours planned for Unit I: 11					
UNIT – II					
1.	1	Thermodynamics of transport. Simple diffusion and facilitated diffusion.	T3	433-435	
2.	1	Passive transport- glucose transporter, anion transporter and porins**.**	T1	441-446	
3.	2	Primary active transporters- P type ATPases, V type ATPases, F type ATPases.	T1	447-448	
4.	2	Secondary active transporters- lactose permease, Na^+-glucose symporter. ABC family of transporters- MDR, CFTR. Group translocation.	T1	452-456	
5.	2	Ion channels- voltage-gated ion channels (Na^+/K^+ voltage-gated channel), ligand-gated ion channels (acetyl choline receptor)	T2	410-416	
6.	2	Aquaporins, bacteriorhodopsin. Ionophores - valinomycin, gramicidin.	T2	406-408	
7.	1	Class test			
Total number of hours planned for Unit II: 11					
UNIT – III					
1.	2	Types of vesicle transport and their function- clathrin, COP I and COP II coated vesicles.	T1	592-598	
2.	2	Molecular mechanism of vesicular transport. Membrane fusion. Receptor mediated endocytosis of transferrin.	T1	606-612	
3.	1	Laws of thermodynamics, state functions, equilibrium constant, coupled reactions,	T3	88-89	
4.	1	Energy charge, ATP cycle, phosphorylation potential, phosphoryl group transfers.	T3	89-93	
5.	1	Chemical basis of high standard energy of hydrolysis of ATP, other phosphorylated compounds and thioesters.			
6.	1	Redox reactions, standard redox potentials and Nernst equation. Universal electron carriers.	T1 T3	59-60 94-95	
7.	1	Class test			
Total number of hours planned for Unit III: 9					
UNIT – IV					
1.	2	Mitochondria. Electron transport chain- its organization and function. Inhibitors	T2	691-701	

		of ETC and uncouplers.			
2.	1	Peter Mitchell's chemiosmotic hypothesis. Proton motive force.	T2	701-704	
3.	2	Fo F1ATP synthase, structure and mechanism of ATP synthesis. Metabolite transporters in mitochondria.	T2	708-714	
4.	2	Regulation of oxidative phosphorylation. ROS production and antioxidant mechanisms.	T2	716-718	
5.	2	Thermogenesis. Alternative respiratory pathways in plants.	T3	228-229	
6.	1	Class test			
Total number of hours planned for Unit IV: 10					
UNIT V					
1.	1	General features of photophosphorylation, historical background, Hills reaction, photosynthetic pigments,	T2	723-724	
2.	1	Light harvesting systems of plants and microbes and resonance energy transfer.	T2	725-730	
3.	2	Bacterial photophosphorylation in purple bacteria, Green sulfur bacteria and *Halobacterium salinarum*.	T2	730-736	
4.	1	Photophosphorylation in plants - structure of chloroplast,	T1	513-519	
5.	2	Molecular architecture of Photosystem I and Photosystem II, Z-scheme of photosynthetic electron flow	T1	519-524	
6.	2	Oxygen evolving complex and action of herbicides. Cyclic photophosphorylation and its significance.	T2	738-742	
7.	2	Photo inhibition. Evolution of oxygenic photosynthesis.	T2	742-744	
8.	1	Class test			
Total number of hours planned for Unit V: 12					
Previous year ESE Question Paper Discussion					
1.	2	Previous year ESE question paper discussion			
2.	2	Objective questions discussion			
3.	1	Revision			
Total Hours Planned: 53 + 5 = 58					

Support Materials

T1: Lodish, H., Berk, A., Kaiser, C.A., & Krieger, M., (2012). *Molecular Cell Biology*, 7th edition. W.H. Freeman & Company, London.

T2: Nelson, D.L. and Cox, M.M., W.H. Freeman., Lehninger: *Principles of Biochemistry* (2013) 6th ed., and Company (New York), ISBN:13: 978-1-4641-0962-1 / ISBN:10:1-4641-0962-1.

T3: Murray, R.K., Bender, D.A., Botham, K.M., & Kennelly, P.J., (2012).Harper's illustrated Biochemistry, 29th edition. McGraw-Hill Medical. London.

Unit 1
Plant cell structure and Photosynthesis

Plant cell structure and Photosynthesis
Structure of Plasma membrane, Vacuole and tonoplast membrane, cell wall, plastids and peroxisomes. Photosynthesis - Structure of PSI and PSII complexes, Light reaction, Cyclic and non-cyclic photophosphorylation.

Plasma Membrane Definition

The plasma membrane of a cell is a network of lipids and proteins that forms the boundary between a cell's contents and the outside of the cell. It is also simply called the cell membrane. The main function of the plasma membrane is to protect the cell from its surrounding environment. It is semi-permeable and regulates the materials that enter and exit the cell. The cells of all living things have plasma membranes.

Functions of the Plasma Membrane

A Physical Barrier

The plasma membrane surrounds all cells and physically separates the cytoplasm, which is the material that makes up the cell, from the extracellular fluid outside the cell. This protects all the components of the cell from the outside environment and allows separate activities to occur inside and outside the cell.

The plasma membrane provides structural support to the cell. It tethers the cytoskeleton, which is a network of protein filaments inside the cell that hold all the parts of the cell in place. This gives the cell its shape. Certain organisms such as plants and fungi have a cell wall in addition to the membrane. The cell wall is composed of molecules such as cellulose. It provides additional support to the cell, and it is why plant cells do not burst like animal cells do if too much water diffuses into them.

Selective Permeability

Plasma membranes are selectively permeable (or semi-permeable), meaning that only certain molecules can pass through them. Water, oxygen, and carbon dioxide can easily travel through the membrane. Generally, ions (e.g. sodium, potassium) and polar molecules cannot pass through the membrane; they must go through specific channels or pores in the membrane instead of freely diffusing through. This way, the membrane can control the rate at which certain molecules can enter and exit the cell.

Endocytosis and Exocytosis

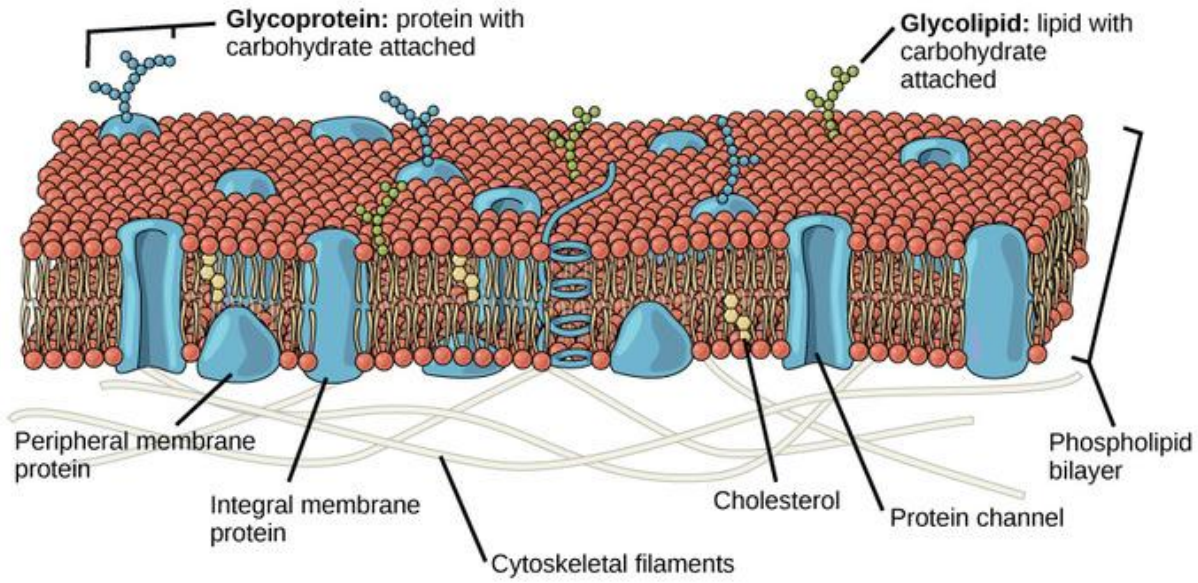
Endocytosis is when a cell ingests relatively larger contents than the single ions or molecules that pass through channels. Through endocytosis, a cell can take in large quantities of molecules or even whole bacteria from the extracellular fluid. Exocytosis is when the cell releases these materials. The cell membrane plays an important role in both of these

processes. The shape of the membrane itself changes to allow molecules to enter or exit the cell. It also forms vacuoles, small bubbles of membrane that can transport many molecules at once, in order to transport materials to different places in the cell.

Cell Signaling

Another important function of the membrane is to facilitate communication and signaling between cells. It does so through the use of various proteins and carbohydrates in the membrane. Proteins on the cell "mark" that cell so that other cells can identify it. The membrane also has receptors that allow it to carry out certain tasks when molecules such as hormones bind to those receptors.

Plasma Membrane Structure



Phospholipids

The membrane is partially made up of molecules called phospholipids, which spontaneously arrange themselves into a double layer with hydrophilic ("water loving") heads on the outside and hydrophobic ("water hating") tails on the inside. These interactions with water are what allow plasma membranes to form.

Proteins

Proteins are wedged between the lipids that make up the membrane, and these transmembrane proteins allow molecules that couldn't enter the cell otherwise to pass through by forming channels, pores or gates. In this way, the cell controls the flow of these molecules as they enter and exit. Proteins in the cell membrane play a role in many other functions, such as cell signaling, cell recognition, and enzyme activity.

Carbohydrates

Carbohydrates are also found in the plasma membrane; specifically, most carbohydrates in the membrane are part of glycoproteins, which are formed when a carbohydrate attaches to a

protein. Glycoproteins play a role in the interactions between cells, including cell adhesion, the process by which cells attach to each other.

Fluid Mosaic Model

Technically, the cell membrane is a liquid. At room temperature, it has about the same consistency as vegetable oil. Lipids, proteins, and carbohydrates in the plasma membrane can diffuse freely throughout the cell membrane; they are essentially floating across its surface. This is known as the fluid mosaic model, which was coined by S.J. Singer and G.L. Nicolson in 1972.

Structure of Vacuole and tonoplast membrane

Structure

In plant cells, the vacuole takes up a large amount of space. At times, this could be more than 90% of the plant cell space. It is said that they are usually formed by the fusion of many membrane vesicles. Due to this reason, a vacuole does not have any specific size or shape. Its structure is such that it complements its function. Many mature and grown plant cells usually have a single large vacuole, surrounded by a structure known as a tonoplast. This is said to be a very active and dynamic membrane of this all important part of plant cell structure.

The vacuole in its central, hollow region contains a fluid known as cell sap. This fluid contains different compounds, some of which are secretory, and some are excretory in nature. Also, the one in the middle, depending on the cell type and requirement, contains various concentrations of salts, sugars and different kinds of soluble pigments. The cell sap, which is a part of the central vacuole structure, also contains various enzymes that are even capable of digesting the cell itself. Although most mature plant cells contain a large single vacuole, when studying the cell biology in young plant cells, there are many vacuoles which slowly enlarge and eventually coalesce together. This eventually pushes the cytoplasm, nucleus and other such structures against the plasma membrane and the cell wall.

Functions

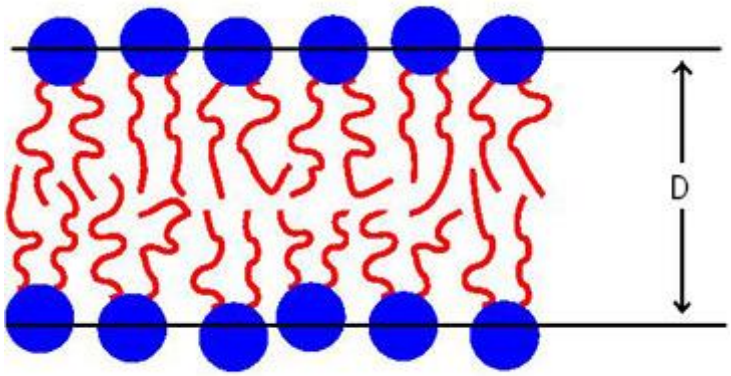
The vacuole structure is designed to aid this important cell organelle, which is one of the key plant and animal cell differences. Its membrane, the tonoplast, helps to separate its contents from those floating in the cytoplasm. Thus, this membrane does not allow harmful substances present in here from entering and harming the rest of the cell. As the tonoplast is selectively permeable in nature, it also tends to maintain the pH and ionic concentration of the cell, by regulating what travels in and out of the vacuole. Also, due to the structure being so large, it pushes the contents of the cell to the borders, near the cell wall and cell membrane and thus, helps maintain the turgor pressure of the cell. Furthermore, the vacuole sap contains many different digestive enzymes that are capable of destroying the cell (which come handy when there is a need for apoptosis). The tonoplast also aids in, along with maintaining turgor pressure, supporting the structures of the leaves and flowers of the plant.

This structure has been designed to aid in the vacuole function. There is usually a slightly acidic, that is, a low pH maintained inside this structure, because this helps in the functioning of the degradative enzymes present inside the it. In some cases, its structure may

differ slightly depending on the type of cell it is present in and its function, for example, in yeast cells, the vacuole is a very dynamic structure that can change its morphology. However, in general, it usually contains a tonoplast and the cell sap within it.

Tonoplast Structure

The tonoplast is unique to plant cells as animal cells do not have a central vacuole. The tonoplast is a **lipid bilayer**, typical of other cellular membranes. Recall that a lipid is part of the fats, oils, waxes, etc. group. A bilayer indicates that there are two layers, each with the hydrophillic (water loving) head facing out into the cellular cytosol and the vacuolar cytosol. **Cytosol** is a liquid component of a cell that surrounds organelles like the vacuole. The hydrophobic (water fearing) tails face inwards.



Here you can see the hydrophobic tails and hydrophillic tails. The tails keep away from the water where the heads turn towards them.

The tonoplast is **semi-permeable** as well. This means that certain molecules and substrates can pass through it but others cannot. This allows the tonoplast to maintain a balance of ions inside and outside of the central vacuole. Again, this maintains proper turgor pressure inside the cell.

Tonoplast function

One of the distinct organelles of plant cells is the vacuole which is also found in some protist and fungal cells. This organelle is essentially an enclosed compartment that is filled with water that contains both inorganic and organic molecules including enzymes in solution, although in certain cases it can contain solids that have been engulfed by the cell. Some of the major roles that vacuoles play in plant cells include turgor maintenance, accumulation of seed storage proteins, sequestration of deleterious compounds and storage of metabolites. Such functions are dependent directly or indirectly on proteins that are residing in the tonoplast, which is mainly the vacuolar membrane stacked together to form a larger unit, the vacuole.

The tonoplast contains numerous proteins that are responsible in the transport of small solutes and salts; and proteins that are involved in the fusion of membrane and remodelingthe

allowed delivery of macromolecules by vesicle traffic. In order to fulfill the said function, the proteins in the tonoplast need be synthesized correctly, sorted to the tonoplast and turned over. Since most mature plants normally have a single large central vacuole that occupies more than 30% of the total volume of the cell, and can occupy as much as 80% of certain cells' volume; the number of tonoplast in a single central volume is therefore dependent on its size, based on the given condition.

The term tonoplast is derived from the word origin Gktón(os), meaning "tension", "stretching", or "tone" and comb, meaning "molded". The tonoplast is also called as the vacuolar membrane, and it is a cytoplasmic membrane that surrounds a vacuole, thus separating the vacuolar contents from the cytoplasm of the cell. As a membrane, tonoplast is directly involved in the regulation of the movements of ions around the cell and isolating any possible harmful materials that is threatening to the cell.

Cell wall

Cell wall is the characteristic feature of all the plant cells. It is the outermost layer and covering of the plasma membrane. The cell wall is entirely lacking in animal cells. The cell wall is rather rigid, strong, thick, porousand non-living structure and is secreted by the living matter of the cell.

It is laid down at the telophase stage and is believed to be formed by the fragments of the endoplasmic reticulum. In young cells, the cell wall is thin, elastic and about 1 to 3 µ thick while in old and mature cells the cell wall becomes stiff and 5 to 10 µ thick. The cell wall of parenchymatous cells are comparatively thinner than those of collenchymas, sclerenchyma and xylem vessels.

Functions of cell wall

Cell wall performs the following important functions:

i. It protects the cell from adverse environment conditions.
ii. It provides a definite shape of the cell.
iii. It provides strength to the cell.
iv. It permits the entry of molecules of different sizes.
v. It determines the manner of cell division and growth.
vi. It possesses plasmodesmata through which cells remain connected with adjacent cells.
vii. It separates one cell from the other.

Structure of cell wall

The cell walls are complex and highly differentiated in some tissues. They also possess special sequence of their arrangement. The wall of a solitary cell can be differentiated into the following layers:

i. Primary cell wall
ii. Secondary cell wall
iii. Tertiary cell wall
iv. Middle lamella

Generally, primary and secondary cell walls are found in the cell. Tertiary cell walls are rare and are found in the xylem tracheids of Gymnosperms. These walls are deposited in layers one after the other during growth and differentiation.

1. **Primary cell wall**

The outermost wall layer of the cell is termed as primary cell wall and is regarded to be the first deposition product. It develops in the young growing or meristematic cells and parenchymatous cells. The primary cell wall is comparatively thin and permeable. Certain epidermal cells of leaf and the stem also possess the cutin and cutinwaxe which make the primary wall impermeable. At first the primary wall is rather elastic and able to extend as the cell grows, but when more cellulose is deposited, it becomes more rigid. In many fungi the cell wall is composed of chitin.

2. **Secondary cell wall**

Next to the primary cell wall is another layer known as secondary cell wall. It is comparatively thicker, than the primary wall. The secondary cell wall is generally found in the mature, permanent or non-growing cells. The outer surfaces of the epidermal cells with secondary wall of some leaves and the stem may have a cuticle rich in fats and waxes which tends to limit water loss.The primary cell wall is composed of cellulose but the secondary wall in addition to cellulose contains pectins, non-cellulose polysaccharides, lignin and a phenolic polymer which imparts hardness and mechanical rigidity to the wood. In certain cells, it is further differentiated into outer, middle and inner layers.

3. **Tertiary cell wall**

Rarely in certain cells a third layer is added inside the secondary wall which is known as tertiary cell wall. The presence of tertiary cell wall was described by Bucher (1953) in the xylem tracheids of Gymnosperms where it is quite thin and produces many warty outgrowths. The tertiary cell wall differs from the primary and secondary walls in its morphology, chemical composition and staining properties. Tertiary cell-wall is mainly composed of xylan instead of cellulose.

4. **Middle lamella**

The primary walls of the two adjacent cells are often separated by a layer or a structure known as middle lamella. During the development of cell-walls, the middle lamella is formed first. It is composed of calcium and magnesium pectates. The pectates are viscous and gelatinous. Middle lamella binds the adjoining cells firmly. In mature and aged cells the middle lamella is dissolved and consequently the cells are loosened. During the maturation of fruits the pectic substances of the middle lamella become soluble due to the action of pectolytic enzymes. The ripe fruits are therefore, soft.

Chemical composition of cell-walls

The primary cell wall consists of intertwined cellulose fibres. It may have a deposition of pectin, lignin, hemicelluloses etc. The cellulose molecules are polymers of disaccharide cellobiose having approximately 3000 glucose units. The glucose molecules are arranged in the form of chain which are joined by β 1→4 linkages. The cellulose fibrils are about 0.25 µ wide and up to 1 µ long. They are woven into an irregular net with a mesh of about 0.3 µ.

Many chains of cellulose molecules lie parallel to each other to form the bundles. A bundle of 100 cellulose molecular chains forms the elementary fibril known as micelle. The 20 parallely arranged micelles form another bundle known as microfibril. It is about 250 Aº thick. Similarly, 250 microfibrils form the large sized bundle known as macrofibril. The macrofibrils ultimately form the main framework of the cell-wall. The hemicelluloses are the polysaccharides of pentose as well as hexose sugars like arabinose, xylose, mannose and

galactose. The pectic substances are the long chains of repeating units of uronic acid derivatives of hexoses, glucuronic and galacturonic acids. Lignin is composed of coniferyl alcohols and cutin of many fatty acids. Chitin is a polymer of glucosamine.

The secondary wall is also composed of cellulose and contains lignin. The formation and structure of microfibrils and macrofibrils are the same as in case of primary cell wall. The only difference is in their arrangement. In the secondary walls the cellulose microfibrils in a macrofibril are comparatively more compactly arranged.

The tertiary wall is composed of xylan instead of cellulose and the middle lamella is composed of calcium and magnesium pectates.

The relative concentration of cellulose, hemicelluloses and pectic substances in the primary cell-wall is not constant in all the cells. It varies from one cell type to another cell-type. Bishop (1958) reported the presence of high concentration of hemicelluloses. Jensen (1960) found that the provascular cells had high concentrations of pectic substances and hemicelluloses in their cell-walls. The cells of the cortex and protoderm had lower concentrations.

Average analysis of primary cell wall

S.No	Substance	Percent of fresh weight
1	Water	60
2	Hemicelluloses	5-15
3	Cellulose	10-15
4	Pectic substances	2-8
5	Proteins	1-2
6	Lipids	0.5-30

In primary stages, the cell wall is thin (about 1-3µ) elastic and delicate. At the time of cell growth, it is stretched and new cell-wall forming materials are deposited upon it. As soon as the secondary growth is completed and the cell becomes mature, its thickness is increased to about 5-10 µ. The growth of cell-wall is of following two types:

1. Growth by intussusceptions

   The type of growth results in the increase in volume of the cell wall. During cell growth, at first the wall is stretched and a tension is created. Now, the new cell-wall material secreted from the protoplasm is filled in the intercellular spaces of the stretched primary wall. The type of growth takes place during the growth period of the cell.

2. Growth by opposition

   During the type of growth, the cell wall forming material secreted from the protoplasm is deposited towards the inner side of the primary cell-wall in the form of layers. Thus, the cell-wall becomes comparatively more thicker. Such layers are formed uniformly at all places of the cell-wall except at the pits.

Plasmodesmata

Different types of pores or pits are found on the cell-walls. When the cell-wall forming material is deposited on primary cell-wall, certain places are left as such. These are known as primary pit fields. Addition materials like cutin, suberin, calcium carbonate and calcium oxalate are also not deposited on these pits. The pores on the wall of living cells are usually very small and rounded. Through these pores the cytoplasm of adjacent cells remains

connected with each other. Such cytoplasmic connections are known as plasmodesmata. Through these connections, various substances are transported.

The plasmodesmata are found in multicellular plants with thick cell-walls and also in animal cells such as bone cells. Within the plasmodesmata, tubules have been observed in continuity with vacuoles or cisternae of endoplasmic reticulum.

Formation of plasmodesmata is related to the formation of cell plate or phragmoplast. At telophase of cell division the cell plate is crosses by vesicles and tubules of the endoplasmic reticulum that determine the location of the plasmodesmata.

Thickening of cell wall

The cell-wall of many cells like xylem etc., become very thick and hard during secondary growth. The reason behind this is that the lignin and other materials are deposited upon it. As soon as the lignin starts depositing on the primary wall the protoplasm of the cell starts decreasing. After deposition of lignin in great amount, the entire protoplasm of the cell is lost and the cell becomes dead. The deposition of lignin takes place in a definite sequence. It is first deposited on middle lamella, then on primary wall and then finally on secondary wall. Similarly, other materials are also deposited on the wall. Due to this deposition of lignin and other materials the cell-wall becomes very thick and the thickening may take following shapes:

i. Annular thickening: When the lignin is deposited in the form of rings e.g., thickening in the cells of protoxylem.
ii. Spiral thickening: When the lignin and other materials are deposited in the form of screw like strip.
iii. Scalariform or ladder like thickening: When the deposition of materials takes place in the form of transverse strips.
iv. Reticulate thickening: When deposition takes place in the form of a net.
v. Pitted thickening: When the deposition of materials takes place on entire cell-wall except at certain small places.

The pits are formed in pairs on opposite walls. The part of cell-wall which is found in between the opposite pits is known as closing membrane. In bordered pits, the closing membrane becomes slightly swollen and is called torus.

Types of pits: They are of following two types.

i. Simple pits: When the pitted portion contains only middle lamella and primary wall, the pit is called simple pit and these structures (middle lamella and primary wall) are called pit membranes. Simple pits are found in thick walled parenchymatous cells.
ii. Bordered pits: When the depositing material is deposited around the primary wall and forms a funnel like structure, the pit is called bordered pit. It is usually represented in the form of two circles. The smaller circle represents the pit-aperture and the larger the area of the border pit. The closing membrane, here, swells and forms the torus which functions as a value and controls the diffusion of liquids. When the pressure of the liquid is same on both the sides of torus, the pit remains open. If the pressure increases at any side, it becomes closed. Bordered pits are found in xylem tracheids and vessels.

Chemical changes in the Cell-wall

Following chemical changes may occur during the growth of the cell-wall

i. Lignification: The deposition of lignin on cell-wall or the conversion of cellulose into lignin is called lignifications.
ii. Cutinization: The conversion of cellulose into cutin in the cell-wall is called cutinization
iii. Suberization: The deposition of suberin on the cork cell-wall is known as suberization.
iv. Mucilaginous changes: Sometimes, the cellulose of cell-wall is converted into mucilage. This phenomenon is known as mucilaginous change.
v. Mineralization: The deposition of mineral substances like silica, calcium carbonate and calcium oxalate on the cell-wall is called as mineralization.

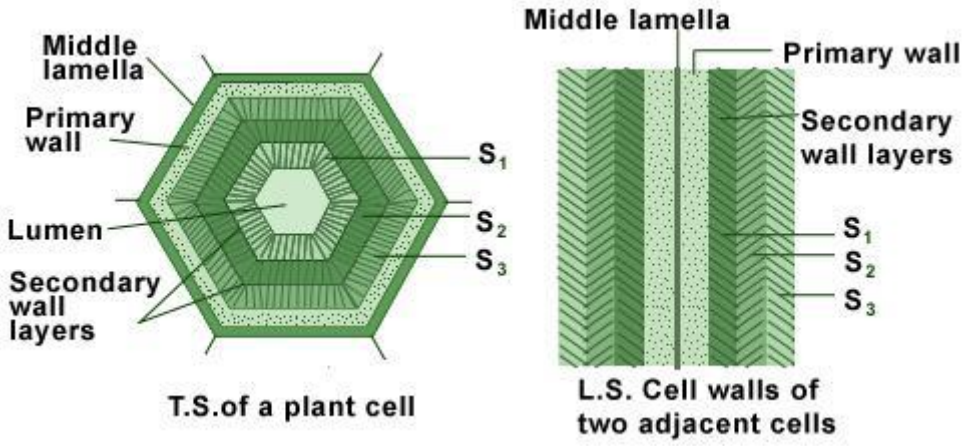
Intercellular spaces

They are found in permanent cells and are absent in meristematic cells. The spaces found in between the adjacent cells are called intercellular spaces. They contain either air or water in them. They are of following types:

i. Schizogenous cavities: When the intercellular spaces are formed by the contraction of the cell-walls of two adjacent cells, they are called Schizogenous cavities. They are small, narrow and remain filled with gases or liquids. They help in the diffusion of gases and osmosis of liquids. Resin ducts in the Pinus represents this type of schizogenous cavities.
ii. Lysigenous cavities: When the intercellular spaces are formed by the disintegration or degeneration of cells, they are called lysigenous cavities. They are comparatively larger, spherical or oval and contain liquid substances like oils. They are found in lemon, orange and other citrus species.
iii. Schizo-lysigenous cavities: When the intercellular spaces are formed jointly by the contraction and disintegration of cells, they are called schizo-lysigenous cavities. They are found in the xylem bundles of maize and are formed by protoxylem cells.

Origin of cell-wall

Cell-wall formation is initiated during the most advanced stage of mitosis called telophase. Endoplasmic reticulum plays an important role in its formation. During telophase the tubular fragments of endoplasmic reticulum migrate to the equatorial region. It is suggested by Northcote that the fragments of endoplasmic reticulum first form middle lamella which is the new cell-wall or starting of the cell-wall. As already pointed out the middle lamella is made up of calcium and magnesium pectates, the calcium pectate acts as an important cementing material and is found in most abundance. Soon after the formation of middle lamella by endoplasmic reticulum on either sides of it cellulose fibrils accumulate in concentric rows which finally form the primary cell-wall. The cytoplasm forms the plasmamembrane on the inner side of primary cell-wall. Later on, the deposition of cellulose, lignin, saccharides etc. takes place on the primary cell-wall which ultimately form the secondary cell wall.



PLASTIDS-TYPES AND FUNCTIONS

Plastids are characteristic structures of almost all the plant cells and a few animal cells like Euglena. They represents the largest cytoplasmic cell organelles and are intimately related with the synthesis and storage of carbohydrates, proteins and lipids.

Classification of plastids:

The plastids are generally classified into three main groups depending upon the presence or absence of colour contents.

(A) Leucoplasts

These plastids are devoid of pigments. They are mainly meant for storing the food materials like starch (carbohydrates), lipids and proteins. The leucoplasts vary in shape and size and are usually rod like spheroid. They are of common occurrence in embryonic cells, sex cells and meristematic cells. Depending upon the presence of storage material, they are further classified into following heads:

(i) **Amyloplasts** – The starch storing leucoplasts are known as amyloplasts. They are found in those cells which store the starch and particularly in storage tubers, cotyledons and endosperm.

(ii) **Elaioplasts** – The oil storing leucoplasts are known as elaioplasts. They are found in the seeds of both monocotyledonous and dicotyledonous plants. In most of the monocotyledonous plants the chloroplasts after losing their chlorophyll start storing oil. In the epidermal cells of orchidaceae and Liliaceae the disorganized plastids fuse to form oil droplets.

(iii) **Proteinoplasts**– The proteins storing plastids are known as proteinoplasts. They have been reported in the epidermal cells of Helleborus and seeds of Ricinus.

(B) Chromoplasts:

The coloured plastids are known as chromoplasts. They contain variety of pigments and most of them synthesize food through the process of photosynthesis. The chromoplasts are found in the cells of leaves, many flowers and fruits. Some of the common chromoplasts of plant cells are as follows:

(i) **Chloroplast** – The green plastids are known as chloroplasts. They contain the pigments chlorophyll A and chlorophyll B, DNA and RNA. The chloroplasts are found in green algae and higher plants where they play a role in the photosynthesis.

(ii) **Phaeoplast**– The brownish colour plastids are known as pheoplasts. They are found in the brown algae, diatoms and dinoflagellates. These appear brown due to masking effect of brown carotenoids. In brown algae, it is the pigment

fucoxanthin which is responsible for the absorption of light and transfer of energy to the chlorophyll.

(iii) **Rhodoplasts** – They are red colour plastids and contain the pigment phycoerythrin. This pigment plays the role similar to fucoxanthin. Rhodoplasts are found in the members of rhodophyceae, the red algae.

(C) Chromotophores:

In the cells of blue green algae, fungi and bacteria the pigments are not organized within a discrete plastid body but are often arranged on lamellar structures in concentric rings or plates. Various types of chromotophores are as follows:

(i) **Blue green chromotophores** – They are found in blue green algae which contain the pigments c-phycocyanin and c-phycoerythrin along with chlorophyll A and carotenoids. These accessory pigments impart bluish hue in addition to green colour and perform photosynthesis.

(ii) **Bacterial chromotophores** – Bacvterialchromotophores are found in certain bacteria, eg., purple sulphur bacteria and contain the pigment bacteriochlorophyll. The green sulphur bacteria contain the pigment bacterio-viridin in their chromotophores. Bacterio-chlorophyll is very important in the absorption of infra-red light.

(iii) **Carotenoids** – They are generally found in certain bacteria, fungi, red coloured ripe tomato and pepper, flowers and fruits. Usually the pigment capsanthin occurs in the carotenoids of bacteria and fungi. Other carotenoids contain certain other pigments like fucoxanthin, xanthophylls and carotenes either singly or along with chlorophyll in the algae and other plants.

The various types of plastids are apparently homologous with each other. They can be transformed from one to another.

Among of various plastids, the chloroplasts are of most common occurrence in plants and have greatest biological importance, since they produce most of the chemical energy used by the living organisms through photosynthesis.

Functions of chloroplasts

(i) Photosynthesis

The most important function of chloroplast is photosynthesis. During the process of photosynthesis, the chlorophyll contained in the chloroplasts traps energy of sunlight emitted as photons and transforms it into chemical energy. Because this process takes place inside the chloroplast, they are considered as the centres of photosynthetic activity. The chemical energy is stored in the chemical bonds that are produced during the synthesis of various food stuffs like carbohydrates, lipids and proteins.

The photosynthesis can be defined with the help of following equation:

$$nCO_2 + nH_2O \xrightarrow[\text{chlorophyll}]{\text{Light}} (CH_2O)n + nO_2$$

The equation shows that photosynthesis is the process in which carbon dioxide and water are converted into carbohydrates in presence of light and chlorophyll. The O_2 is evolved during the process.

The photosynthesis consists of two stages, the light or photochemical reaction and a dark or thermochemical reaction. The photochemical reaction is light sensitive and thermochemical (dark reaction) reaction is temperature sensitive. The photochemical reaction takes place in the following steps:

(i) Photophosphorylation, in which ATP is formed from ADP through a chain of electron carriers.

(ii) Hydrolysis and ionization of water, in which NADP is reduced to $NADPH_2$.

In dark reaction CO_2 is fixed and reduced by thermochemical mechanisms. This mechanism also involves a number of reactions in which the conversions of one product into another takes place in presence of certain specific enzymes. The initial enzyme, carboxydismutase, is responsible for the formation of phosphoglyceric acid molecules from ribulosediphosphate and CO_2.

(2) Protein synthesis

A special ribosomes are associated with chloroplasts and that chloroplasts contain a specific protein synthesizing system. The chloroplasts contain sufficient amounts of mRNA of its own. In the presence of CO_2, as the sole source of carbon, chloroplasts actively incorporate amino acids into proteins.

(3) Cytoplasmic heredity

The presence of DNA in chloroplasts has been related to the presence of a special non chromosomal genetic system. Thus, the chloroplasts act as carrier for genetic material.

(4) Role in Kreb's cycle and fatty acid synthesis

It is assumed that chloroplasts contain enzymes necessary for Kreb's cycle and for synthesis of fatty acids.

(5) Mutation

Some of the plastids even chloroplasts undergo mutation which is known as plastid-mutation. After mutation the plastids may perform altered functions. The genes of the plastids are known as plastogenes.

Peroxisome

Peroxisome, membrane-bound organelle occurring in the cytoplasm of eukaryotic cells. Peroxisomes play a key role in the oxidation of specific biomolecules. They also contribute to the biosynthesis of membrane lipids known as plasmalogens. In plant cells, peroxisomes carry out additional functions, including the recycling of carbon from phosphoglycolate during photorespiration. Specialized types of peroxisomes have been identified in plants, among them the glyoxysome, which functions in the conversion of fatty acids to carbohydrates.

Peroxisomes contain enzymes that oxidize certain molecules normally found in the cell, notably fatty acids and amino acids. Those oxidation reactions produce hydrogen peroxide, which is the basis of the name *peroxisome*. However, hydrogen peroxide is potentially toxic to the cell, because it has the ability to react with many other molecules. Therefore, peroxisomes also contain enzymes such as catalase that convert hydrogen peroxide to water and oxygen, thereby neutralizing the toxicity. In that way peroxisomes provide a safe location for the oxidative metabolism of certain molecules.

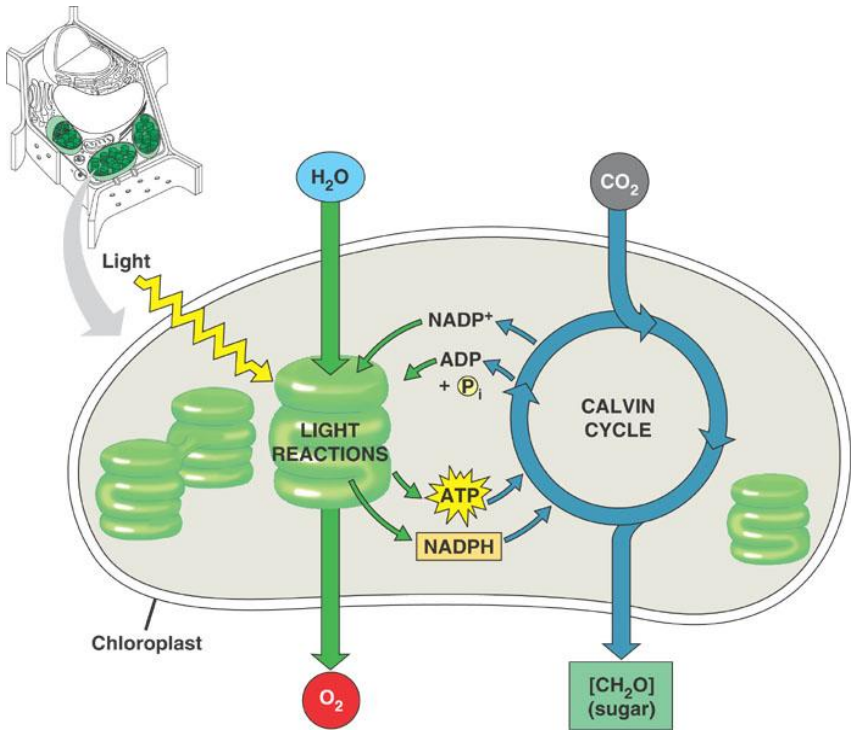
Plasmalogens are the primary ether lipids in humans (ether lipids contain one or more ether linkages, distinguishing them from other lipids, which typically contain ester linkages). Specialized enzymes in peroxisomes catalyze the synthesis of an ether phospholipid precursor. The precursor molecule undergoes further synthesis in the endoplasmic reticulum, resulting in the production of plasmalogen. Although the

physiological role of plasmalogens is unclear, defects in their biosynthesis, which occur as a result of peroxisomal disorders, are associated with severe developmental conditions, including rhizomelicchondrodysplasiapunctata (RCDP) and Zellweger syndrome.

Peroxisomal disorders are caused by mutations in genes that are involved in peroxisome biogenesis or that encode the enzymes and transporter proteins (which take up the enzymes from the cytoplasm) of the peroxisome. Peroxisomal disorders are congenital disorders, and they range from relatively moderate to severe in nature. The Zellweger spectrum, for example, includes Zellweger syndrome, neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease. Zellweger syndrome is characterized by complete absence or reduction in the number of peroxisomes. It is the most severe condition within the Zellweger syndrome. Mutations giving rise to Zellweger syndrome cause copper, iron, and substances called very long chain fatty acids to accumulate in the blood and in tissues, such as the liver, brain, and kidneys. Infants with Zellweger syndrome are often born with facial deformity and intellectual disability; some may have impaired vision and hearing and may experience severe gastrointestinal bleeding or liver failure. Prognosis is poor: most infants with Zellweger syndrome do not live beyond one year. Symptoms of NALD and infantile Refsum disease, by contrast, appear in late infancy or in childhood, and patients may survive to early adulthood. Likewise, patients with RCDP may survive into childhood or, in mild cases, early adulthood.

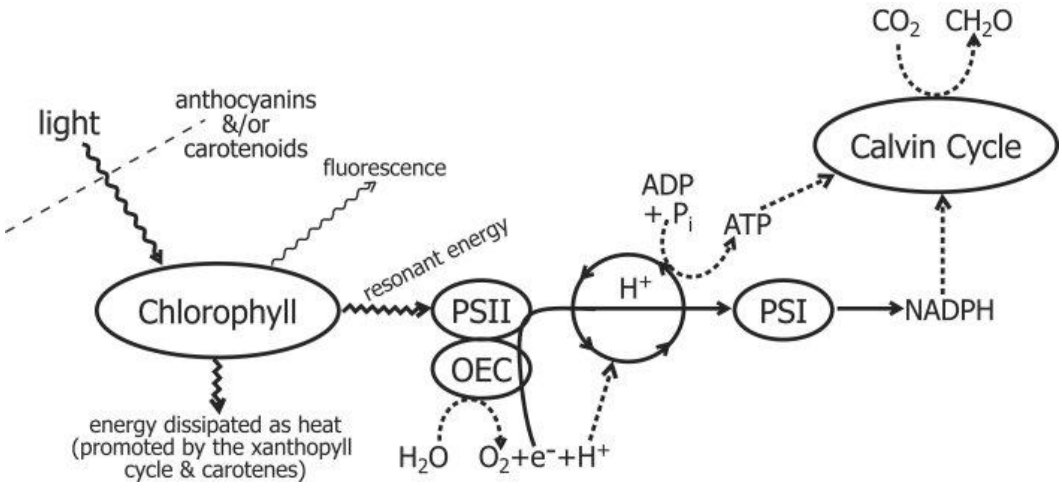
Peroxisomes were described in 1960 as part of the pioneering work of Christian René de Duve, who developed cell fractionation techniques. De Duve's method separated organelles on the basis of their sedimentation and density properties, and peroxisomes are denser than other organelles. He later coined the term *peroxisome*. De Duve shared the 1974 Nobel Prize for Physiology or Medicine with Albert Claude and George Palade for that work.

Photosynthesis-overview



Photosynthetic apparatus

Simplified diagram of photosynthetic apparatus. PS I and II are photosystem I and II, respectively; OEC is the oxygen-evolving complex; P_i is inorganic phosphate; e^- represents electrons; H^+ represents protons; CH_2O represents carbohydrate products of photosynthesis.



Reaction center

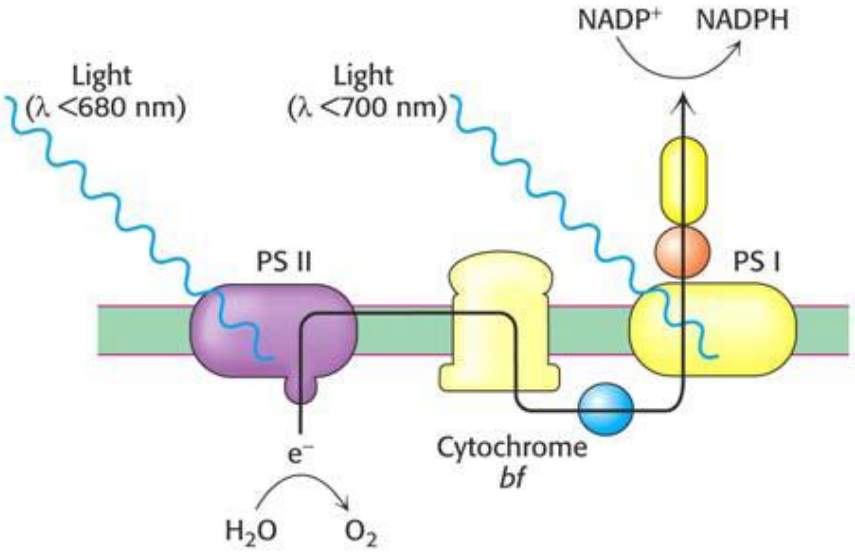
A photosynthetic reaction center is a complex of several proteins, pigments and other co-factors assembled together to execute the primary energy conversion reactions of photosynthesis.

The reaction center of photosystem I is P700

The reaction center of photosystem II is P680

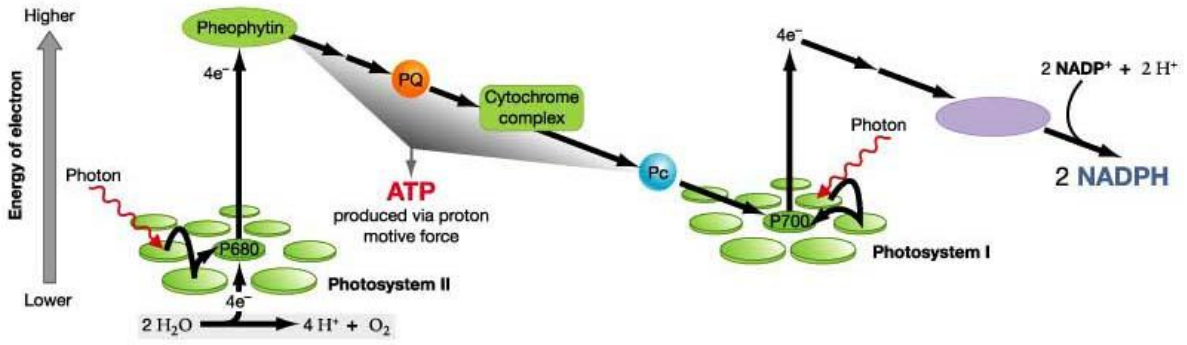
Photosystem I and II

Within the thylakoid membranes of the chloroplast, are two photosystems. Photosystem I optimally absorbs photons of a wavelength of 700 nm. Photosystem II optimally absorbs photons of a wavelength of 680 nm. The numbers indicate the order in which the photosystems were discovered, not the order of electron transfer. Under normal conditions electrons flow from PSII through cytochrome *bf* (a membrane bound protein analogous to Complex III of the mitochondrial electron transport chain) to PSI. Photosystem II uses light energy to oxidize two molecules of water into one molecule of molecular oxygen. The 4 electrons removed from the water molecules are transferred by an electron transport chain to ultimately reduce 2NADP+ to 2NADPH. During the electron transport process a proton gradient is generated across the thylakoid membrane. This proton motive force is then used to drive the synthesis of ATP. This process requires PSI, PSII, cytochrome *bf*, ferredoxin-NADP+ reductase and chloroplast ATP synthase.



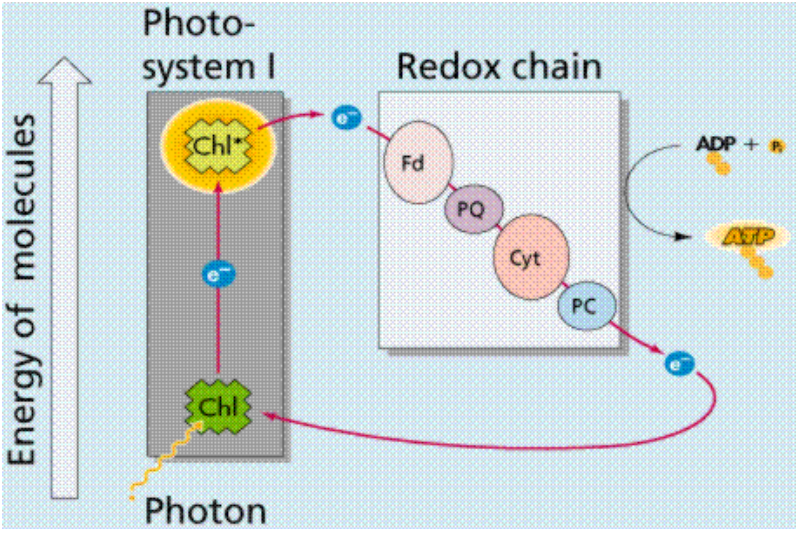
Cyclic and Non Cyclic Photophosphorylation

- Photophosphorylation is the process of creating ATP using a Proton gradient created by the Energy gathered from sunlight. The process of creating the Proton gradient resembles that of the electron transport chain of Respiration. But since formation of this proton gradient is light-dependent, the process is called Photophosphorylation.
- Non-cyclic photophosphorylation really refers to the ATP generated by Protons moved across the thylakoid membranes during the Z-scheme. The Cytb6-f complex acts as an electron transport chain. As the electrons lose energy (during a series of re/dox reactions) protons are moved into the Thylakoid space. This Proton gradient can be used to generate ATP chemiosmotically.



- During Cyclic Photophosphorylation the electrons are recycled, hence the name cyclic photophosphorylation. The excited electrons resulting from the absorption of light in photosystem I are received by the primary electron acceptor and then transferred to the cytb6-f complex which acts as an electron transport chain. The electrons return back to the reaction center of Photosystem I, where the cycle is ready to start all over.

The electrons are using to translocate Protons which the ATPase uses to synthesize ATP. No reduction of NADP+ occurs in Cyclic Photophosphorylation.



Evidences in support of light and dark reactions

1. Evidence from intermittent light indicates that the rate of dark reaction is reduced due to continuous supply of light.
2. From temperature coefficient – It also indicates that light and dark reactions are although independent but are interlinked.

From CO_2 reduction in dark – It indicates that this phase is definitely a dark phase

Photosynthesis

- Photosynthesis occurs in many kinds of bacteria and algae, and in the leaves and sometimes the stems of green plants.
- The energy used by most living cells comes ultimately from the sun, captured by plants, algae, and bacteria through the process of photosynthesis.
- Each day, the radiant energy that reaches the earth equals about 1 million Hiroshima-sized atomic bombs.
- Photosynthesis captures about 1% of this huge supply of energy, using it to provide the energy that drives all life.

Photosynthesis takes place in three stages:

(1)capturing energy from sunlight;

(2)using the energy to make ATP and reducing power in the form of a compound called NADPH; and

(3)using the ATP and NADPH to power the synthesis of organic molecules from CO_2 in the air (carbon fixation).

The first two stages take place in the presence of light and are commonly called the light reactions.

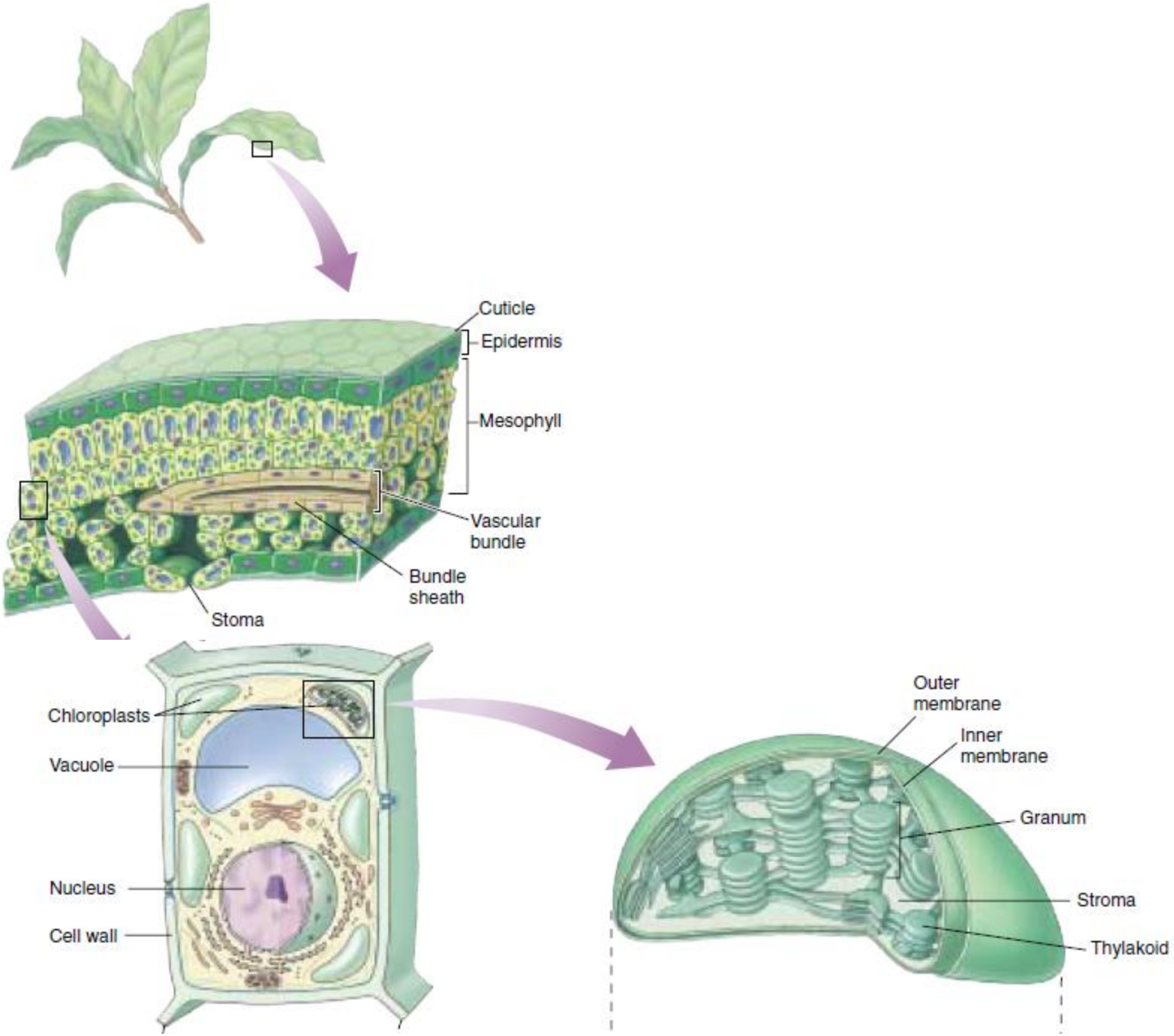
The third stage, the formation of organic molecules from atmospheric CO_2, is called the Calvin cycle.

As long as ATP and NADPH are available, the Calvin cycle may occur in the absence of light.

The following simple equation summarizes the overall process of photosynthesis:

$$6CO_2 + 12H_2O + \text{light} \longrightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

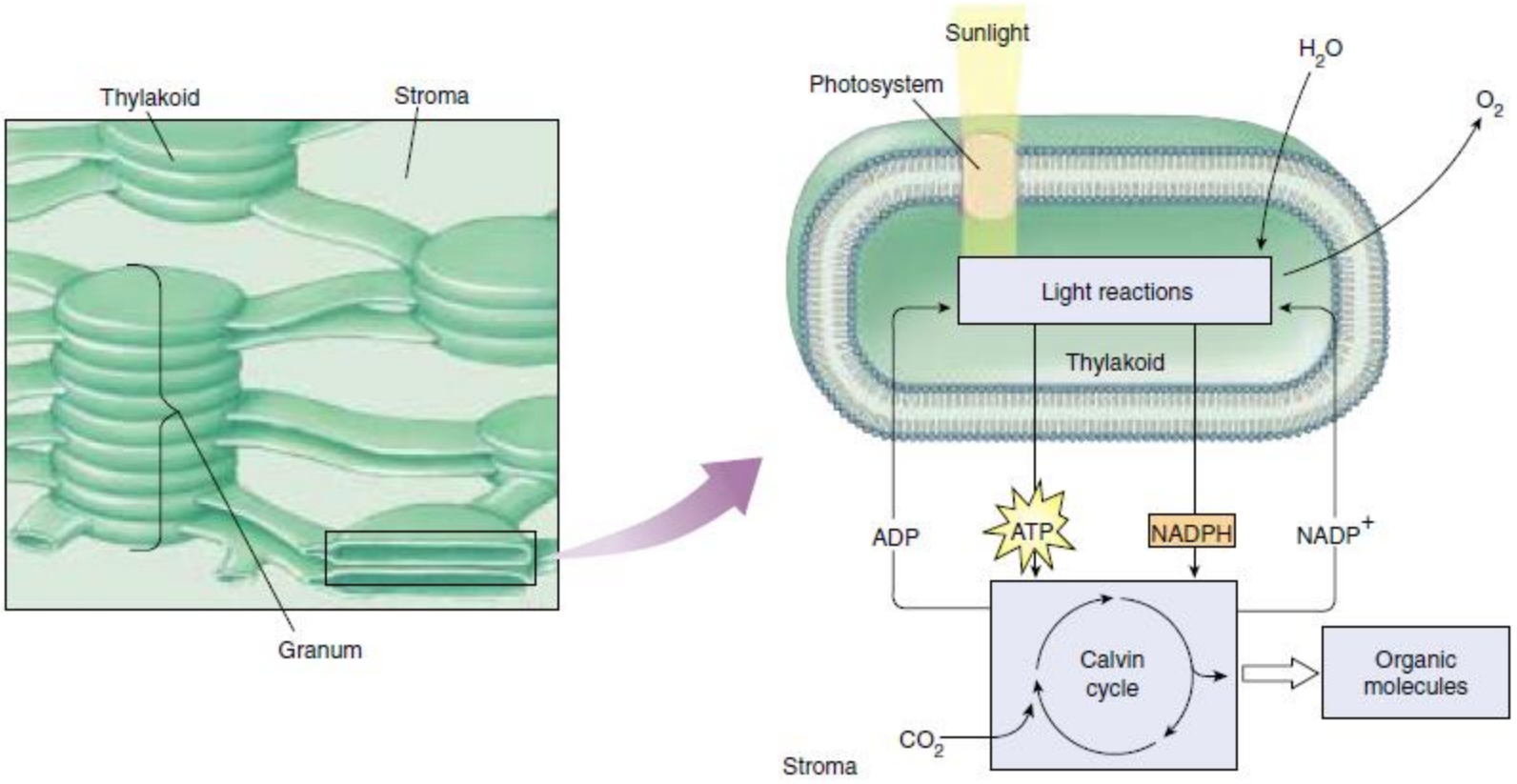
carbon water glucose water oxygen


Cuticle
Epidermis
Mesophyll
Vascular bundle
Bundle sheath
Stoma
Chloroplasts
Vacuole
Nucleus
Cell wall
Outer membrane
Inner membrane
Granum
Stroma
Thylakoid

Chloroplast

- ✓The internal membranes of chloroplasts are organized into sacs called *thylakoids,*
- ✓*Numerous thylakoids are* stacked on one another in columns called *grana.*
- ✓*The thylakoid* membranes house the photosynthetic pigments for capturing light energy and the machinery to make ATP.
- ✓Surrounding the thylakoid membrane system is a semiliquid substance called *stroma.*
- ✓*The stroma houses the enzymes* needed to assemble carbon molecules.
- ✓In the membranes of thylakoids, photosynthetic pigments are clustered together to form a **photosystem.**

- ✓ Each pigment molecule within the photosystem is capable of capturing *photons, which are packets of energy.*
- ✓ *A lattice* of proteins holds the pigments in close contact with one another.
- ✓ When light of a proper wavelength strikes a pigment molecule in the photosystem, the resulting excitation passes from one chlorophyll molecule to another.
- ✓ The excited electron is not transferred physically—it is the *energy* that passes from one molecule to another.
- ✓ Eventually the energy arrives at a key chlorophyll molecule that is touching a membrane-bound protein. The energy is transferred as an excited electron to that protein, which passes it on to a series of other membrane proteins that put the energy to work making ATP and NADPH and building organic molecules. The photosystem thus acts as a large antenna, gathering the light harvested by many individual pigment molecules.



The light reactions take place on the thylakoid membrane and generate the **ATP and NADPH** that **fuel the Calvin cycle**. The fluid interior matrix of a chloroplast, **the stroma, contains the enzymes that carry out the Calvin cycle**.

Absorption Spectra and Pigments

Electrons occupy discrete energy levels in their orbits around atomic nuclei. (*To boost an electron into a different energy level requires just the right amount of energy, just as reaching the next rung on a ladder requires you to raise your foot just the right distance).* A specific atom can, therefore, absorb only certain photons of light—namely, those that correspond to the atom's available electron energy levels.

As a result, each molecule has a characteristic **absorption spectrum, the range and efficiency of photons it is capable** of absorbing.

Molecules that are good absorbers of light in the visible range are called **pigments. Organisms have evolved a variety** of different pigments, but there are only two general types used in green plant photosynthesis: carotenoids and chlorophylls.

2. Wavelength – is measured in nanometers (nm)

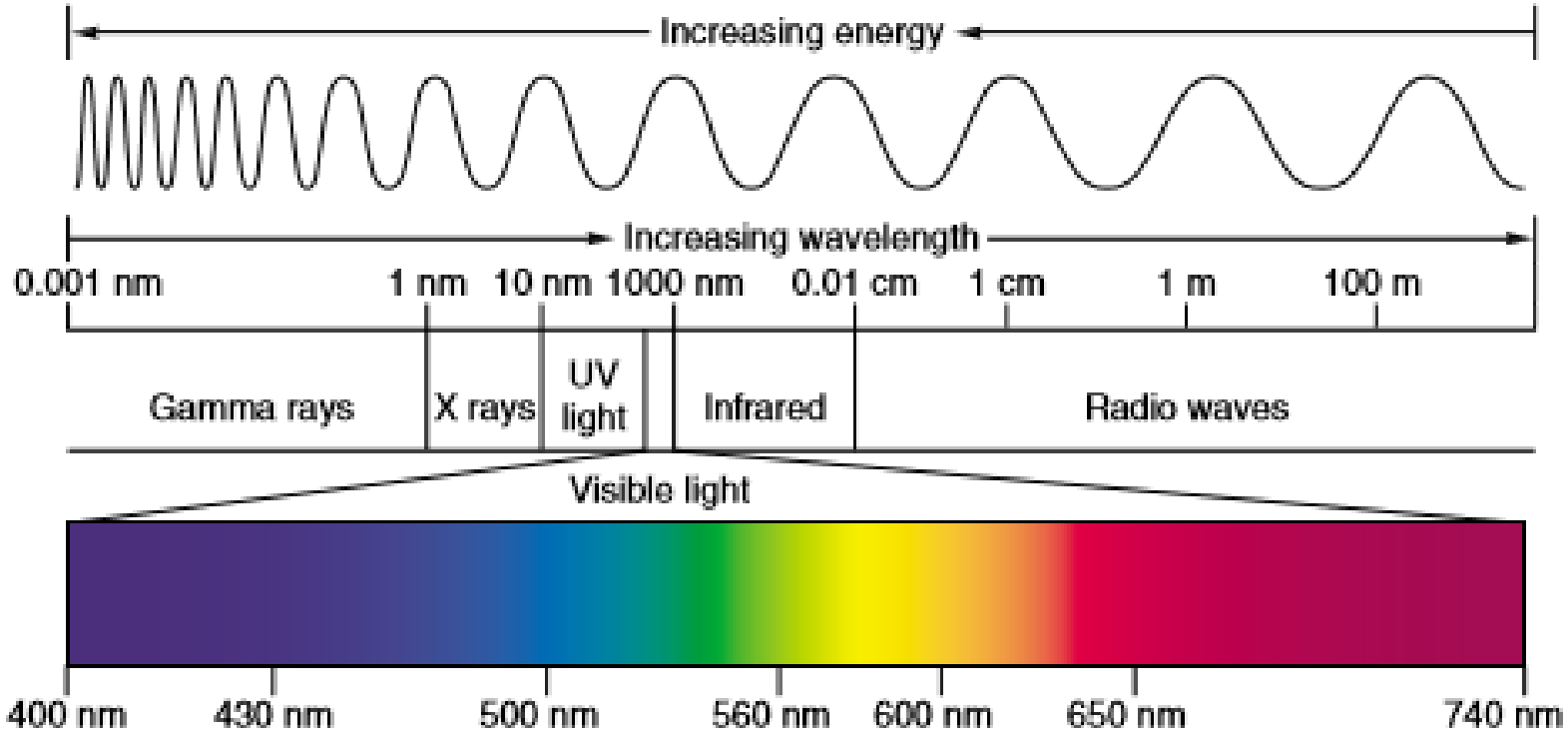
Visible Color Spectrum (nanometers; nm)

Red	Orange	Yellow	Green	Cyan	Blue	Violet
700nm	650nm	600nm	550nm	500nm	450nm	400nm

Wave lengths approximated for ease of understanding
Prepared by HydroGardeningTips.com

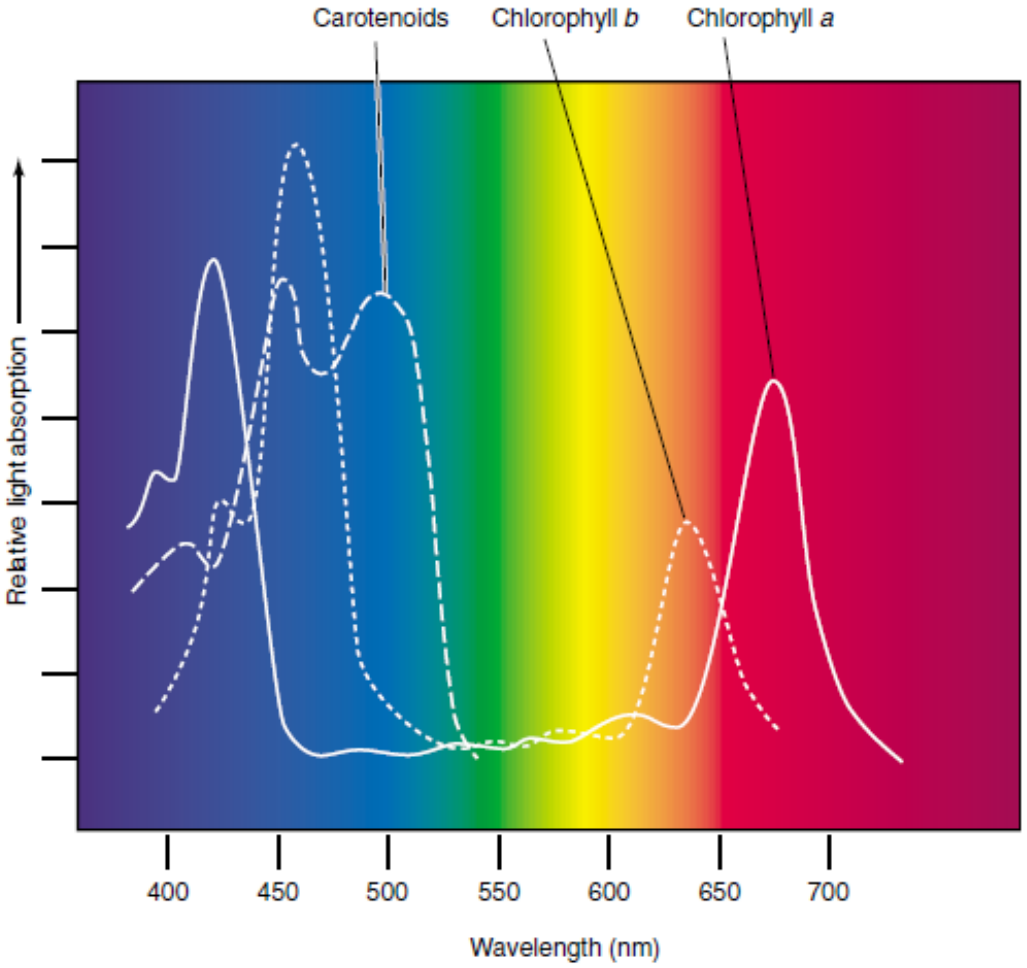
- **Blue light is best for growth/vegetative phase of plants**
- **i.e. >5000 ^{0}K or 400-500nm light emitting bulbs**

- **Red light is best for flowering phase of plants**
- **i.e. 2000 – 3000 ^{0}K or 600-700nm light emitting bulbs**



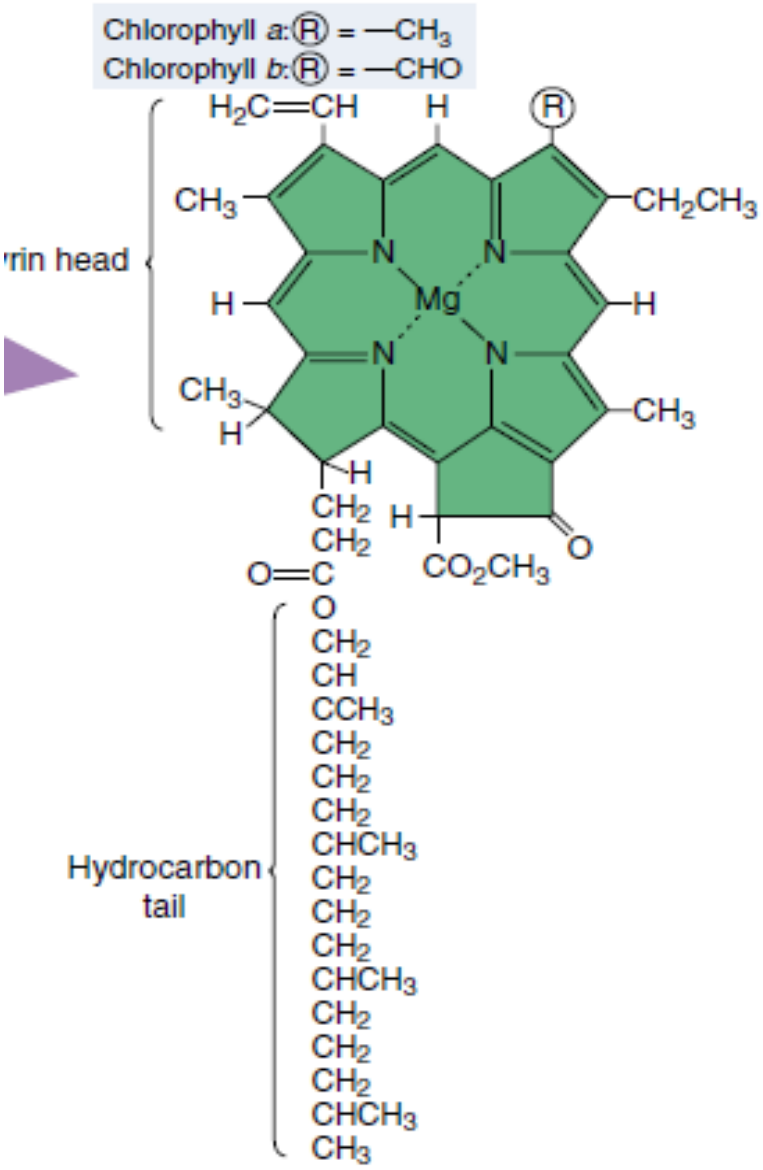
Light is a form of electromagnetic energy conveniently thought of as a wave. The shorter the wavelength of light, the greater its energy. Visible light represents only a small part of the electromagnetic spectrum between 400 and 740 nanometers.

- ✓ Chlorophylls absorb photons within narrow energy ranges. Two kinds of chlorophyll in plants, chlorophylls *a and b, preferentially absorb violet-blue and red* light.
- ✓ Neither of these pigments absorbs photons with wavelengths between about 500 and 600 nanometers, and light of these wavelengths is, therefore, reflected by plants.
- ✓ When these photons are subsequently absorbed by the pigment in our eyes, we perceive them as green.

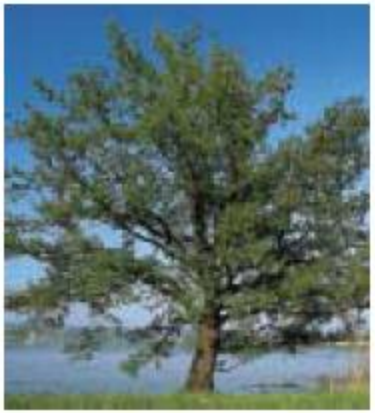


Carotenoids absorb mostly blue and green light and reflect orange and yellow light.

- ✓ Chlorophyll *a is the main photosynthetic pigment and is* the only pigment that can act directly to convert light energy to chemical energy.
- ✓ However, chlorophyll *b, acting as* an **accessory or secondary light-absorbing pigment, complements** and adds to the light absorption of chlorophyll *a.*
- ✓ Chlorophyll *b has an absorption spectrum shifted toward* the green wavelengths. Therefore, chlorophyll *b can absorb* photons chlorophyll *a cannot.*
- ✓ *Chlorophyll b therefore* greatly increases the proportion of the photons in sunlight that plants can harvest.
- ✓ An important group of accessory pigments, the carotenoids, assist in photosynthesis by capturing energy from light of wavelengths that are not efficiently absorbed by either chlorophyll.

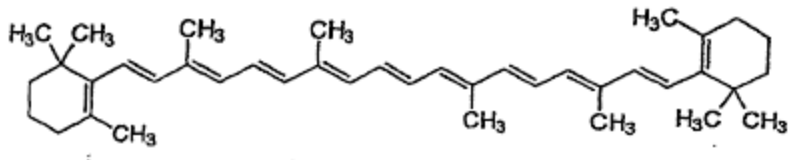


Chlorophyll molecules consist of a porphyrin head and a hydrocarbon tail that anchors the pigment molecule to hydrophobic regions of proteins embedded within the membranes of thylakoids. The only difference between the two chlorophyll molecules is the substitution of a —CHO (aldehyde) group in chlorophyll *b* for a —CH3 (methyl) group in chlorophyll *a.*



Fall colors are produced by carotenoids and other accessory pigments. During the spring and summer, chlorophyll in leaves masks the presence of carotenoids and other accessory pigments. When cool fall temperatures cause leaves to cease manufacturing chlorophyll, the chlorophyll is no longer present to reflect green light, and the leaves reflect the orange and yellow light that carotenoids and other pigments do not absorb.

- ✓ **Carotenoids consist of carbon rings linked to chains** with alternating single and double bonds. They can absorb photons with a wide range of energies, although they are not always highly efficient in transferring this energy.
- ✓ Carotenoids assist in photosynthesis by capturing energy from light of wavelengths that are not efficiently absorbed by chlorophylls.
- ✓ A typical carotenoid is β-carotene, whose two carbon rings are connected by a chain of 18 carbon atoms with alternating single and double bonds. Splitting a molecule of β-carotene into equal halves produces two molecules of vitamin A.
- ✓ Oxidation of vitamin A produces retinal, the pigment used in vertebrate vision. This explains why carrots, which are rich in β-carotene, enhance vision.



Trans Beta Carotene

Organizing Pigments into Photosystems

The light reactions take place **in four stages**:

1. **Primary photoevent: A photon of light is captured** by a pigment. The result of this primary photoevent is the excitation of an electron within the pigment.

2. **Charge separation: This excitation energy is transferred** to a specialized chlorophyll pigment termed a reaction center, which reacts by transferring an energetic electron to an acceptor molecule, thus initiating electron transport.

3. Electron transport: The excited electron is shuttled along a series of electron-carrier molecules embedded within the photosynthetic membrane. Several of them react by transporting protons across the membrane, generating a gradient of proton concentration.

Its arrival at the pump induces the transport of a proton across the membrane. The electron is then passed to an acceptor.

4. Chemiosmosis: The protons that accumulate on one side of the membrane now flow back across the membrane through specific protein complexes where chemiosmotic synthesis of ATP takes place, just as it does in aerobic respiration.

Architecture of a Photosystem

In chloroplasts light is captured by such photosystems. Each photosystem is a network of chlorophyll *a molecules, accessory pigments,* and associated proteins held within a protein matrix on the surface of the photosynthetic membrane.

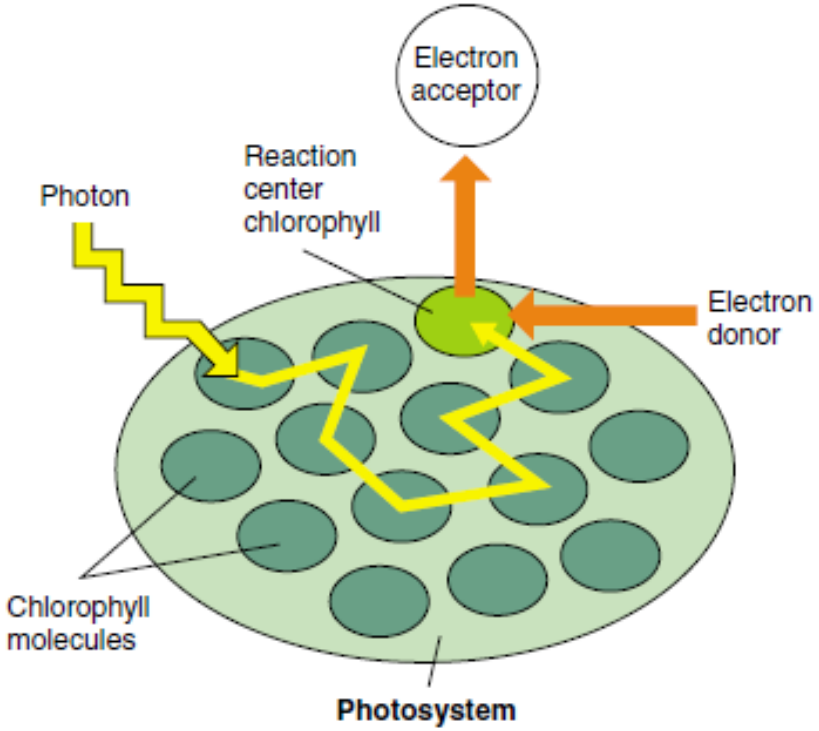
A photosystem thus consists of two closely linked components:

(1) an *antenna complex of hundreds of pigment* molecules that gather photons and feed the captured light energy to the reaction center; and

(2) a *reaction center, consisting of one or more chlorophyll a* molecules in a matrix of protein, that passes the energy out of the photosystem.

The Antenna Complex

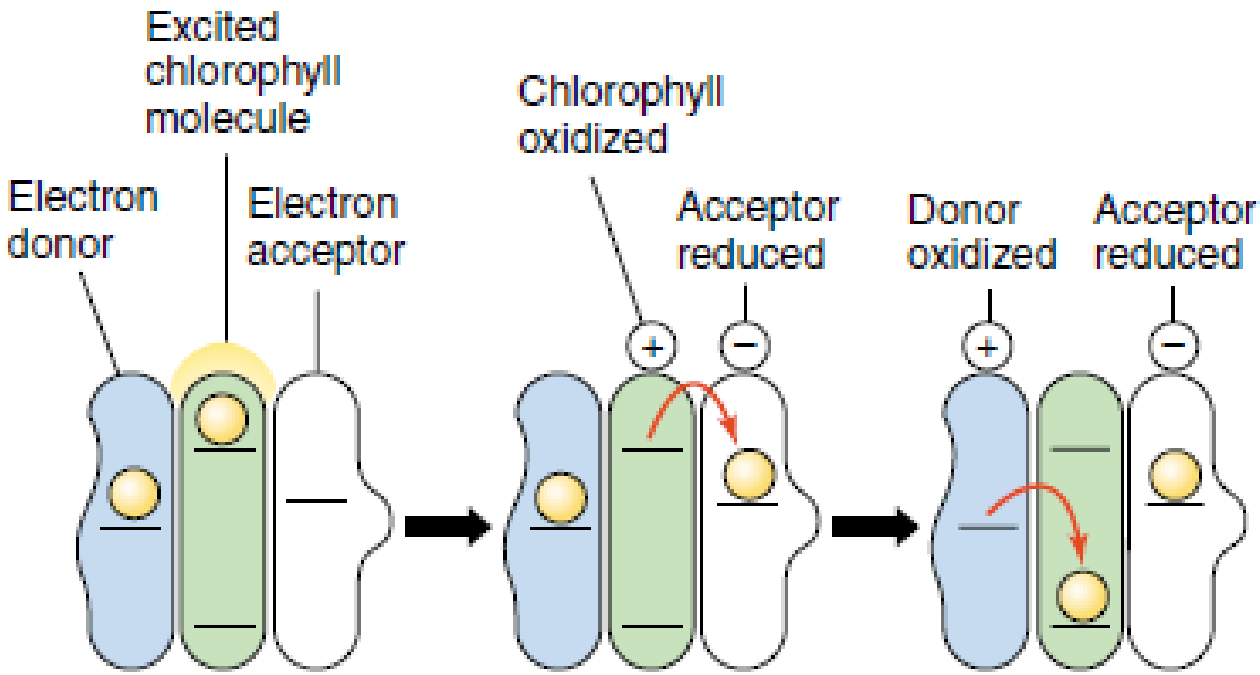
- The antenna complex captures photons from sunlight.
- In chloroplasts, the antenna complex is a web of chlorophyll molecules linked together and held tightly on the thylakoid membrane by a matrix of proteins.
- Varying amounts of carotenoid accessory pigments may also be present.



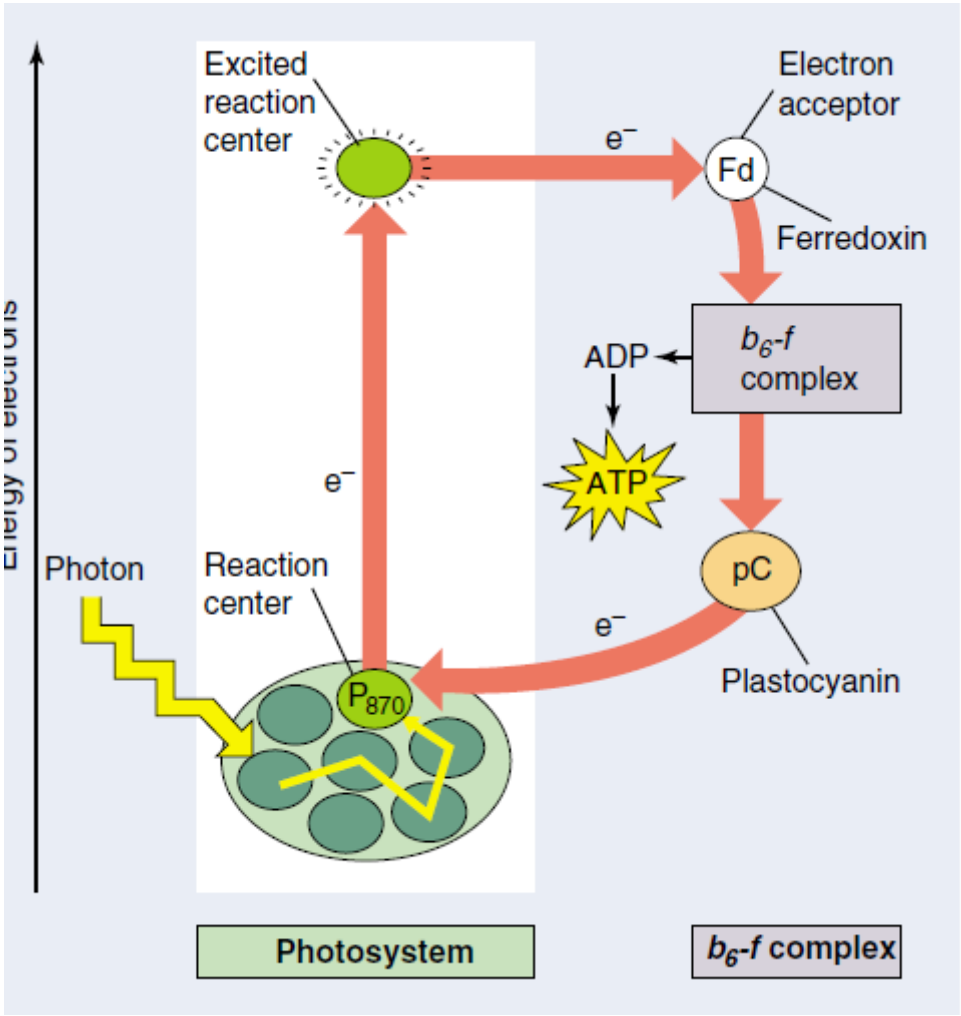
- The protein matrix serves as a sort of scaffold, holding individual pigment molecules in orientations that are optimal for energy transfer.
- The excitation energy resulting from the absorption of a photon passes from one pigment molecule to an adjacent molecule on its way to the reaction center.
- After the transfer, the excited electron in each molecule returns to the low-energy level it had before the photon was absorbed.
- Consequently, it is energy, not the excited electrons themselves, that passes from one pigment molecule to the next.
- The antenna complex funnels the energy from many electrons to the reaction center.

The Reaction Center

The reaction center is a transmembrane protein-pigment complex.

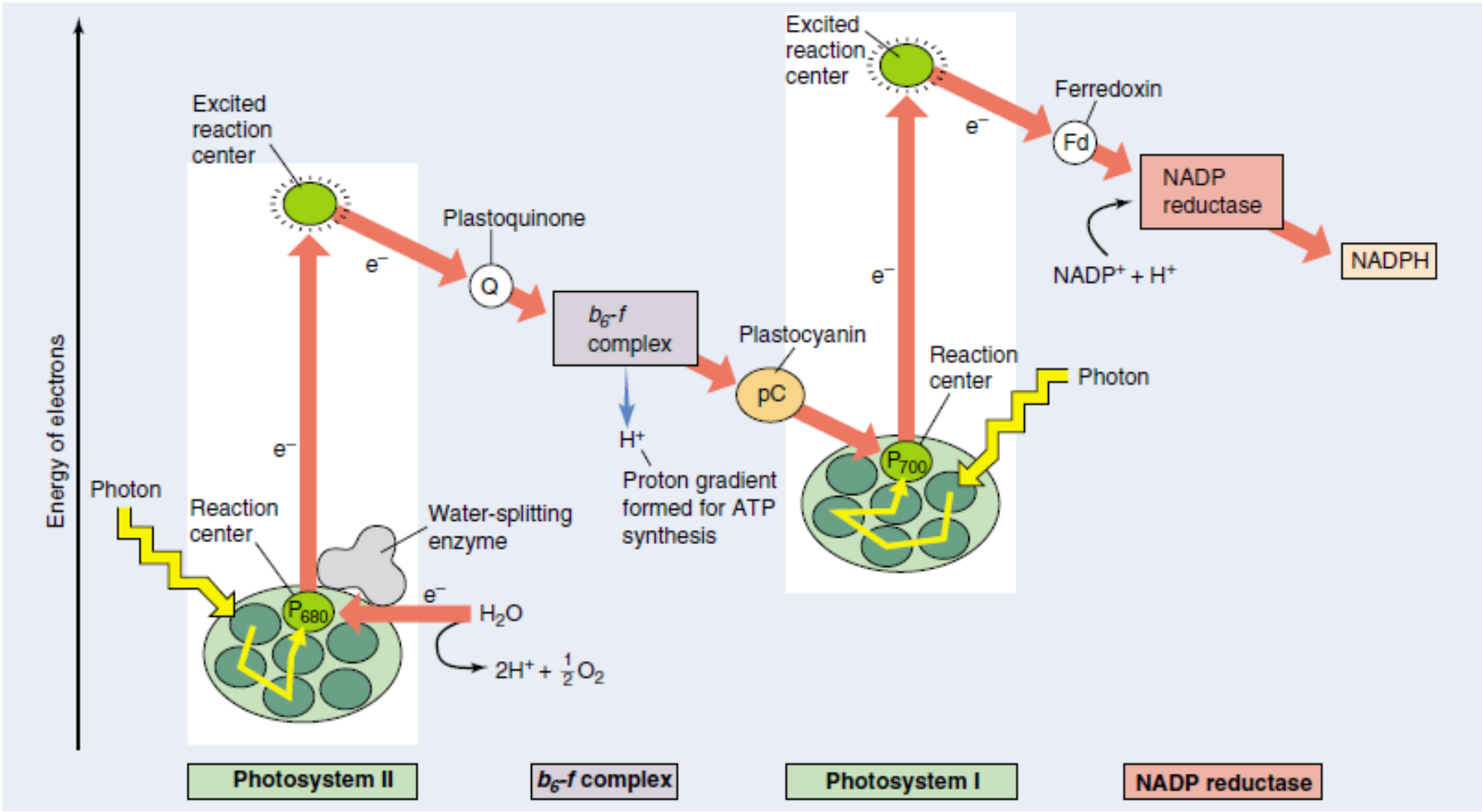


By energizing an electron of the reaction center chlorophyll, light creates a strong electron donor where none existed before. The chlorophyll transfers the energized electron to the primary acceptor, a molecule of quinone, reducing the quinone and converting it to a strong electron donor. A weak electron donor then donates a low-energy electron to the chlorophyll, restoring it to its original condition. In plant chloroplasts, water serves as the electron donor.



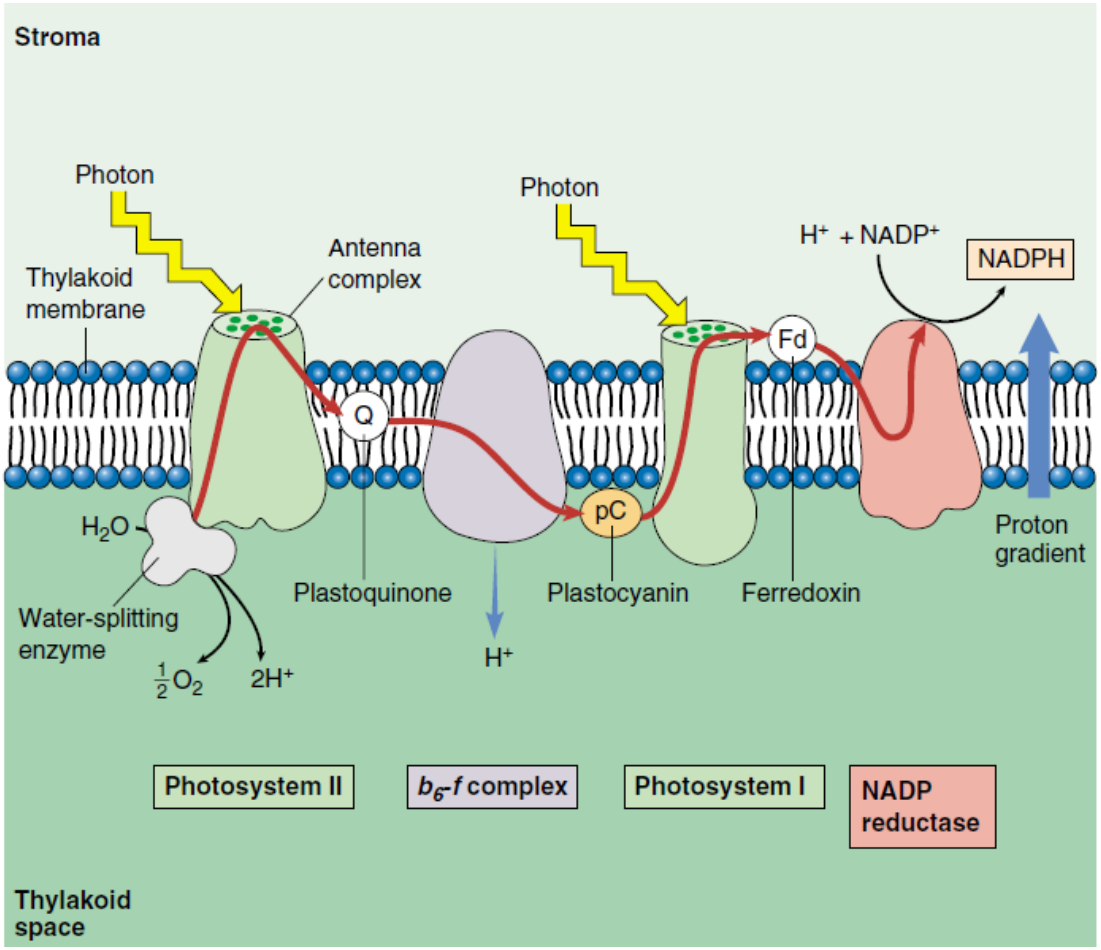
The path of an electron in purple sulfur bacteria. When a light-energized electron is ejected from the photosystem reaction center (P870), it passes in a circle, eventually returning to the photosystem from which it was ejected

Cyclic photophosphorylation



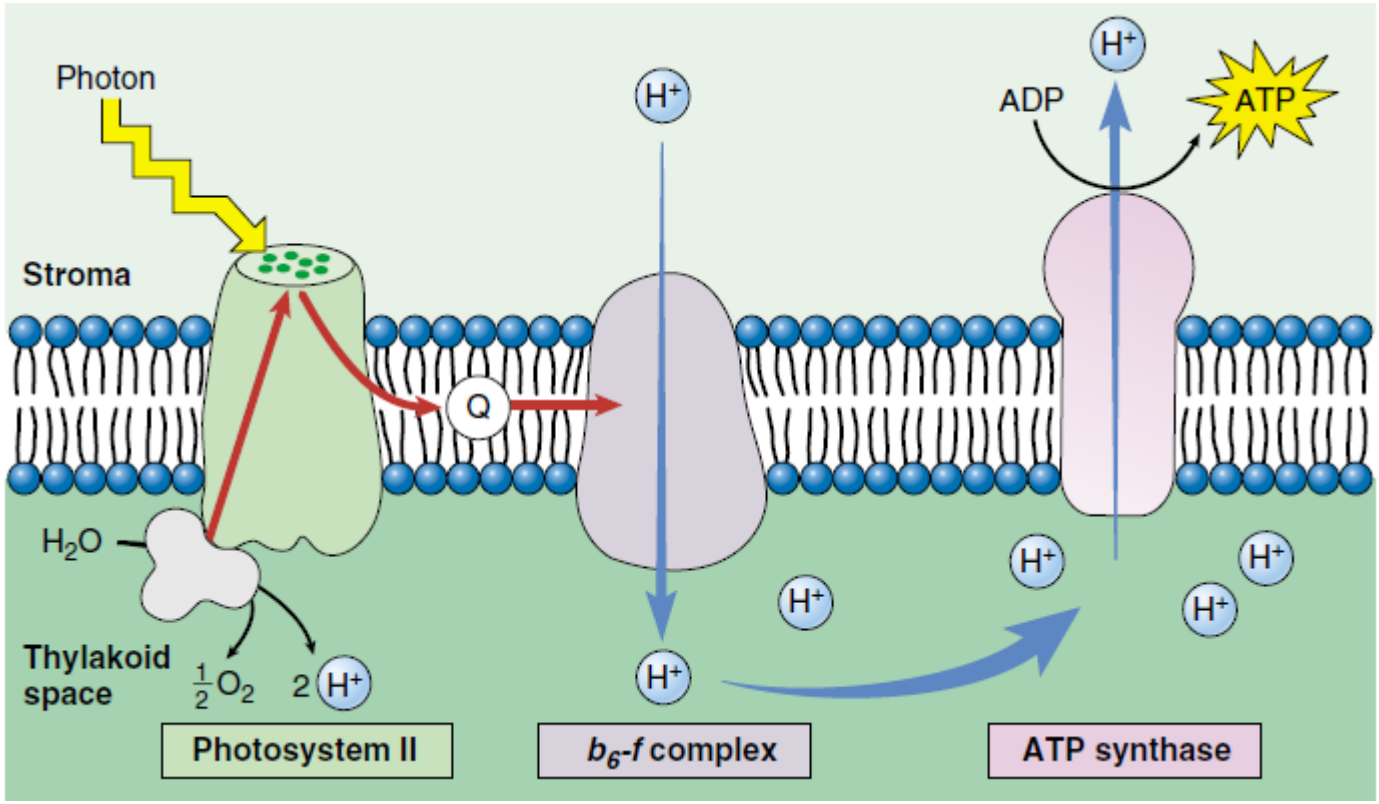
A Z diagram of photosystems I and II. Two photosystems work sequentially. First, a photon of light ejects a high-energy electron from
photosystem II; that electron is used to pump a proton across the membrane, contributing chemiosmotically to the production of a
molecule of ATP. The ejected electron then passes along a chain of cytochromes to photosystem I. When photosystem I absorbs a photon
of light, it ejects a high-energy electron used to drive the formation of NADPH.

The photosynthetic electron transport system.



When a photon of light strikes a pigment molecule in photosystem II, it excites an electron. This electron is coupled to a proton stripped from water by an enzyme and is passed along a chain of membrane-bound cytochrome electron carriers (*red arrow*). When water is split, oxygen is released from the cell, and the hydrogen ions remain in the thylakoid space. At the proton pump (*b6-f* complex), the energy supplied by the photon is used to transport a proton across the membrane into the thylakoid. The concentration of hydrogen ions within the thylakoid thus increases further. When photosystem I absorbs another photon of light, its pigment passes a second high-energy electron to a reduction complex, which generates NADPH.

Chemiosmosis in a chloroplast



The *b6-f* complex embedded in the thylakoid membrane pumps protons into the interior of the thylakoid. ATP is produced on the outside surface of the membrane (stroma side), as protons diffuse back out of the thylakoid through ATP synthase channels.

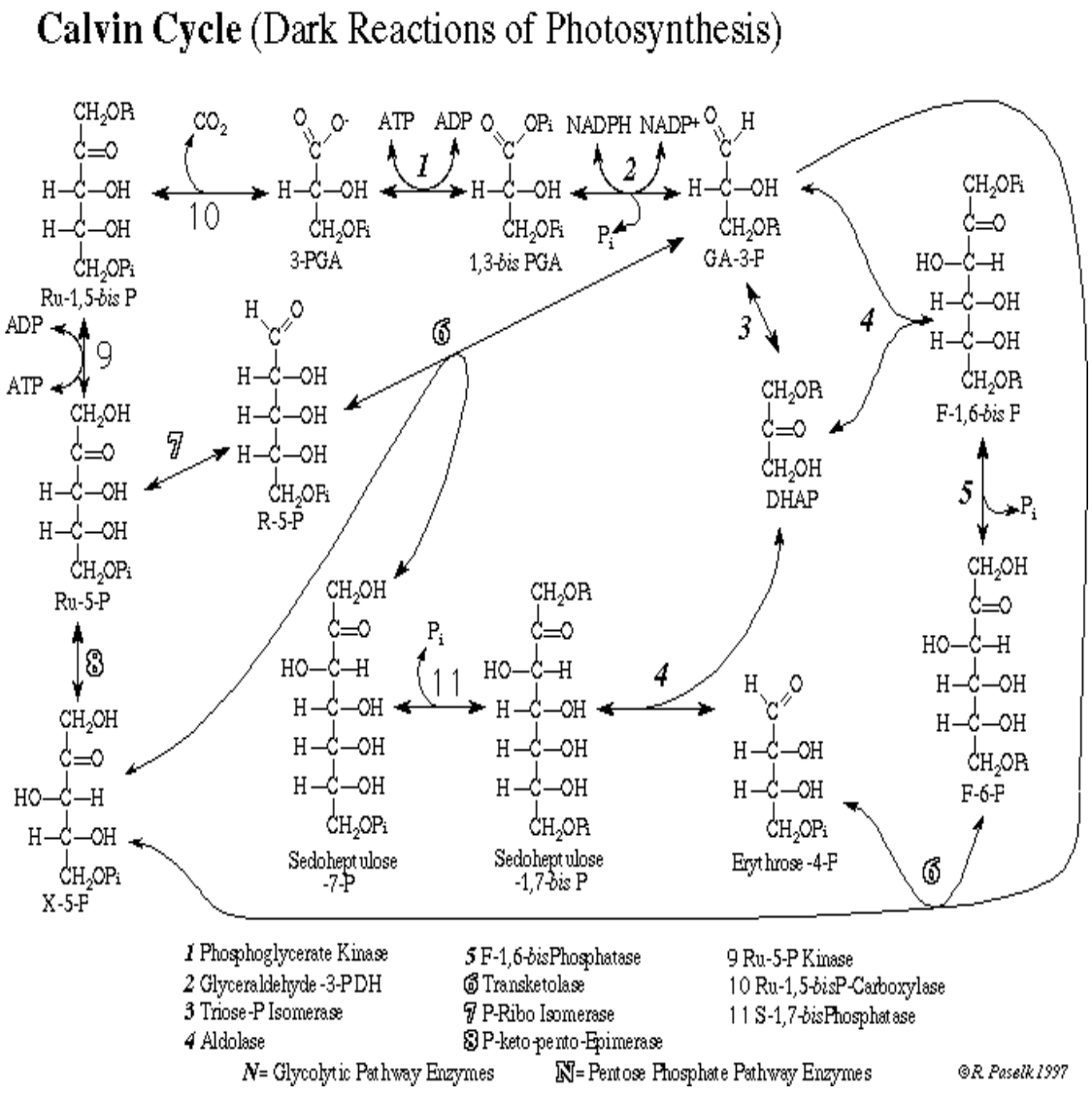
KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III BSc Biochemistry - Plant Biochemistry (16BCU503A)

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	Which of the following is important in the	Microfilaments	microtubules	plasmodesmata	plasmalemma	plasmodesmata
2	The neighboring cells are connected by	Plasmodesmata	Plasma membrane	cell wall	plasmalemma	plasmodesmata
3	The Main CHO constituent of cell wall is	Hemi Celluloses	cellulose	Pectin	Starch	cellulose
4	Micro fibril can only be digested by the	Cellulases	Amylase	Pectinase	proteases	Cellulases
5	Hemicellulose is found in	Cell wall	Amyloplast	Chloroplast	Leucoplast	Cell wall
6	The loosening of mature ripe fruits is due to	Unit membrane	Plasmodesmata	Middle lamella	Primary wall	Middle lamella
7	The pressure developed in a cell due to the	Diffusion pressure	Osmosis	Turgor pressure	Plasmolysis	Turgor pressure
8	Plants showing resistance to drought and	Lower osmotic pressure	Higher osmotic pressure	Diffusion pressure	Osmotic potential bars	Higher osmotic pressure
9	The most common method of water	Passive absorption	Active absorption	Negative absorption	diffusion	Passive absorption
10	The colourless plastids found in embryonic	Leucoplast	Amyloplast	Chromoplast	Rhodoplast	Leucoplast
11	In most of the plants water is transported by	Parenchyma	Symplast	Xylem	Phloem	Xylem
12	The outermost layer and covering of the	Cell wall	plasmalemma	cell sap	plasma desmata	Cell wall
13	The thickness of cell wall in young cells is	5 – 10 µ thick	1 – 3 µ thick	2 – 5 µ thick	10 – 15 µ thick	1 – 3 µ thick
14	The thickness of cell wall in mature cells is	5 – 10 µ thick	1 – 3 µ thick	2 – 5 µ thick	10 – 15 µ thick	5 – 10 µ thick
15	In many fungi the cell wall is composed of	Cellulose	hemicelluloses	chitin	pectin	chitin
16	Tertiary cell wall is mainly composed of	Cellulose	pectin	xylan	hemicelluloses	xylan
17	Middle lamella is composed of	Cellulose	calcium pectate	xylan	hemicelluloses	calcium pectate
18	The primary cell wall of the two adjacent	Intercellular spaces	middle lamella	cytoplasm	plasma membrane	middle lamella
19	The cytoplasmic connections of adjacent cells	plasmadesmata	middle lamella	plasma membrane	cell wall	plasmadesmata
20	The plastid involved in storing of food	chromoplast	leucoplast	phaeoplast	chromatophore	leucoplast
21	The starch storing leucoplasts are known as	amyloplast	elaioplast	proteinoplast	chromoplast	amyloplast
22	The oil storing leucoplasts are known as	amyloplast	elaioplast	proteinoplast	chromoplast	elaioplast
23	The plastid found in brown algae is	chloroplast	phaeoplast	chromoplast	amyloplast	phaeoplast
24	The pigment responsible for absorption of	xanthin	fucoxanthin	chlorophyll	phycoerythrin	fucoxanthin
25	The pigment present in rhodoplasts is	xanthin	fucoxanthin	chlorophyll	phycoerythrin	phycoerythrin
26	The pigment present in carotenoids of	bacteriochlorophyll	bcteriioviridin	capsanthin	fucoxanthin	capsanthin
27	The movement of free molecules of gases, liqu	Osmosis	diffusion	active transport	dialysis	diffusion
28	Dissolution of $KMnO_4$ particles in water is an	diffusion	osmosis	active transport	dialysis	diffusion
29	The rate of diffusion is maximum in	solids	liquids	gases	colloids	gases
30	The absorption of water through roots is	osmosis	active transport	dialysis	diffusion	diffusion
31	The diffusion of solvent molecules from the so	Diffusion	osmosis	passive transport	active transport	osmosis
32	Swelling up of dry raisins placed in water is	exosmosis	endosmosis	dialysis	diffusion	endosmosis
33	Collapsing of grapes, when placed in a medium	Exosmosis	endosmosis	dialysis	diffusion	Exosmosis
34	Osmosis occurs only in	solids	liquids	gases	colloids	liquids
35	The swelling of dry seeds when placed in	diffusion	osmosis	imbibitions	dialysis	imbibitions
36	The swelling of wooden windows and doors du	diffusion	osmosis	imbibitions	dialysis	imbibitions
37	Both Photo systems I and II are involved in	Cyclic	Non-cyclic	Pseudophotophosphory	photorespiration	Non-cyclic
38	Chlorophyll a and b can be excited to the	650-700nm	420-460	700-740nm	250-300nm	650-700nm
39	The reaction center of Photo system I is	P680	P700	P800	P750	P700
40	Which of the following chlorophylls is found	Chl a	Chl b	Chl c1	Chl c2	Chl b
41	Chlorophyll b differs from chlorophyll a	in having a formyl group	in having a formyl group	in having a formyl	in having a methyl group	in having a formyl group
42	The source of oxygen evolved during	water	CO2	Organic acid	glucose	water
43	Photosynthetic enhancement is referred as	Red drop	Quantum yield	Emerson effect	Hills reaction	Emerson effect
44	The central atom that is covalently and coordin	Mg++	Fe++	Fe3+	K+	Mg++
45	Chlorophyll is found in	Plasma membrane	Thylakoid membrane	Peroxisomes	Glyoxysomes	Thylakoid membrane
46	One of the following is not a photo synthetic	Chlorophyll	Carotenoid	Phycobilin	xanthin	xanthin
47	The Chlorophylls absorb light of wavelength	650 – 700nm	450 – 500nm	500 – 600 nm	350-400 nm	650 – 700nm
48	The Carotenoids absorb light in the	450 – 500nm	650 – 700nm	500 – 600nm	350-400nm	450 – 500nm
49	The Phycobilns absorb light in the wavelength	500 – 600nm	450 – 500nm	650 – 700nm	~ 450nm	500 – 600nm
50	The most potent reagents which block non-cyc	CMU & DCMU	Octyl guanidine &	urea	cyanogen bromide	CMU & DCMU
51	The primary electron donar in PS II is	P700	Cyt C	P680	Chl a	P680
52	In cyclic photo phosphorylation	Only PS I is functional	Both PSI and PSII are	O2 is evolved	NADPH2 is formed	Only PS I is functional
53	The main function of light harvesting	Increase the temperature	Capture solar energy	Enhance ATP	transfer of electrons	Capture solar energy
54	The skeleton of chlorophyll is made of a	pentanone	Tetrapyrrole	Per hydro	Hexagon	Tetrapyrrole
55	Food factory in a plant cell, where	cytoplasm	cellulose	chloroplast	vacuole	chloroplast
56	The shape of vacuole is similar to a	tube	bag	cylindrical	triangle	bag
57	Large storage for food, water and waste is	vacuole	tissue	organ	Cellulose	vacuole
58	Due to which reaction PGA is changed into	Oxidation	Reduction	Electrolysis	Hydrolysis	Reduction
59	Photosynthesis is	An Oxidation-Reduction r	Synthesis reaction	Organic reaction	Replacement reaction	An Oxidation-Reduction
60	What is the first step in photosynthesis:	Generation of ATP	Formation of NADPH	Through light,	Formation of Glucose	Through light, excitement

Unit 2
Carbon assimilation and Plant Respiration

Carbon assimilation and Plant Respiration
Carbon assimilation - Calvin cycle and regulation; C4 cycle and Crassulacean acid metabolism (CAM).Respiration - Overview of glycolysis, Alternative reactions of glycolysis, Regulation of plant glycolysis, Translocation of metabolites across mitochondrial membrane, TCA cycle, Alternative NAD(P)H oxidative pathways; Cyanide resistant respiration and Photorespiration

The Dark Reactions
(Calvin Cycle),



Regulation of the Calvin Cycle

Photosynthetic carbon assimilation, like any other important aspect of metabolism, must be regulated if it is to function effectively. Since Calvin cycle utilizes ATP and NADPH, cofactors produced in photochemical reactions, to reduce CO2, it is expected that the key enzymes of the pathway will be subject to regulation by light. Besides, light also regulates the activities of chloroplastic enzymes by a different mechanism. A number of good reviews have already appeared on regulatory aspects of Calvin cycle. The major modes of regulation of Calvin cycle are as under:

Photochemical changes

On illumination of chloroplast, photosynthetic electron transport reduces NADP to NADPH and phosphorylates ADP to ATP. As a result, NADPH/NADP ratio increases, which helps in the creation of reducing environment in the chloroplast. During active CO2 fixation, 40-50% of NADP is always in the reduced state. Phosphorylation of ADP to ATP increases the adenylate energy charge of the chloroplast stroma resulting in the increase in the levels of ATP and decrease in the levels of ADP, AMP and Pi. The ratio of ATP/ADP rises from 0.2-1.0 in the dark to 1.0 -5.0 in the light. Hence the rise in NADPH and ATP levels in light will exert major control on photosynthesis as the cycle cannot function without continuous supply of ATP and NADPH. Hence the reaction catalyzed by phosphoglycerate kinase, GAPDH and PRK requiring ATP/NADPH produced during photosynthetic electron transport will not occur in dark. Moreover, the two should also be produced in the required stoichiometry, as shortage of ATP leads to an accumulation of NADPH. This in turn switches on a series of events as discussed earlier by Stitt.

Illumination also increases the pH of the stroma from about 7.0 in the dark to about 8.0-8.5 in the light. This happens due to coupling of photosynthetic electron transport to proton uptake in thylakoid space, establishing proton gradient to drive phosphorylation. This is also accompanied by uptake of CI" and a flux of Mg2+ in the stroma. These events create an alkaline environment in the stroma and increase Mg2+ from about 1-3 mM in the dark to 3-6 mM in the light. These changes in the stromal environment favourably affect the number of enzymes of Calvin cycle as they function optimally in alkaline environment and many of them require Mg2+. The enzymes strongly activated include FBPase, SBPase and Rubisco .

Enzyme activation by light

Besides bringing favourable changes in stroma of chloroplast as discussed above, light directly activates some of the enzymes of Calvin cycle. However, different enzymes are not activated to the same extent and by the same mechanism. It is now well established that a small fraction of light generated electron flow is diverted to serve a signalling purpose. Indeed, the activities of several chloroplastic enzymes are enhanced or triggered by light. This effect is suppressed by addition of DCMU, a well known inhibitor of photosynthetic electron flow. The signalling system responsible for this effect is now well characterized and is called ferredoxin-thioredoxin system. In this system, the light signal is sensed by chlorophyll and is further transferred to protein called thioredoxin via the photosynthetic electron flow. The thioredoxins are ubiquitous low molecular weight (12 KDa) proteins which undergo reversible oxidation and reduction through changes in the disulfide group (S-

S -►2SH). The electron transfer between ferredoxin, the first soluble electron acceptor of the electron transfer chain and thioredoxins is mediated by ferredoxin-thioredoxin reductase (FTR). Two thioredoxin isoforms are present in chloroplasts, which have been named as m and / according to their specificities towards their initially defined target enzymes: NADP-malate dehydrogenase and FBPase, respectively. However, in recent studies, it has been shown that these specificities are much less strict than thought previously. In the reduced state, thioredoxins selectively activate enzymes of carbon metabolism including FBPase, SBPase, PRK and GAPDH. Additionally, thioredoxin also deactivates glu-6-P dehydrogenase, a regulatory enzyme of oxidative pentose phosphate pathway. A few other enzymes namely NADP-malate dehydrogenase and coupling factor (CFl-ATPase) are also regulated by this system in the chloroplasts.

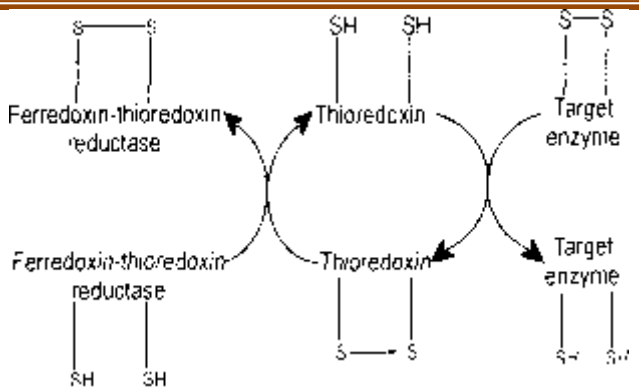
Light triggered changes in the activities of these enzymes are correlated with reduction of disulfides. This clue was obtained from the fact that dithiothreitol (DTT) could act as substitute for the whole photosynthetic electron transport chain in the reduction of thioredoxin and, in some cases, could even replace reduced thioredoxin in enzyme activation. Recent research has focussed on the intramolecular mechanism by which the reduction status of the chloroplastic enzyme affects its catalytic properties. Site-directed mutagenesis has been used to identify the regulatory cysteins involved (10). For some of the thiol-regulated enzymes, structure function studies have revealed that the complex conformational changes that occur in the process might be involved in disulfide isomerization and autoinhibition. Transgenic approaches have indicated that this regulatory mechanism constitutes a rapid means to adjust the enzyme activity to metabolic needs.

Regulation by transport

Regulation of the transport of Calvin cycle intermediates across the chloroplast envelope is another important factor in the control of the metabolism. This is due to the fact that majority of the intermediates of the Calvin cycle are also the intermediates in the pathway of sucrose synthesis or in the oxidative pentose phosphate pathway. Both these pathways are located in the cytosol of mesophyll cells. The inner envelope provides an effective barrier to the movement of most of the compounds between the two cell compartments. Carbon leaves the chloroplast in the form of DHAP in exchange for Pi through triose-P translocator whose properties have been studied in detail. The phosphate translocator with molecular mass of 29 KDa, amounts for 10-15% of the total proteins of the inner envelope, and there is strict counter exchange of Pi for 3-PGA or triose-P. The Km of the phosphate translocator for 3-PGA, triose-P and Pi is much low as compared to other sugar phosphates.

The phosphate translocator plays a crucial role in the control of carbon partitioning. Under steady state conditions, one sixth of the end-product of photosynthesis is either exported or stored in the chloroplast as starch. Hence for every three molecules of CO2 fixed to produce one molecule of triose-P, one molecule of Pi must be made available for incorporation into triose-P. Some Pi is released within the stroma when triose-P are utilized for

Light



Ferredoxin-thioredoxin system starch synthesis. However, starch synthesis is usually slower by a factor of 3 or 4 than maximal CO2 fixation. Hence, the remaining Pi enters the chloroplast in exchange for exported triose-P, which is ultimately converted to sucrose in cytoplasm. Pi released during sucrose synthesis is recycled to chloroplast. This may have its implications in regulation. Under suboptimal Pi, photosynthesis is limited due to increase in the ratio of [ATP]/[ADP] and depression of PGA reduction. Export is restricted due to limitation of Pi, and consequently the ratio of [PGA]/[Pi] in the chloroplast increases, which causes allosteric activation of ADP-glucose pyrophosphorylase (ADPGPPase), a regulatory enzyme in the process of starch biosynthesis. If external Pi is increased as during active sucrose synthesis, the balance between internal storage and export would drift towards export. On further increase in Pi, export would start to compete with regeneration as well as with internal storage, and photosynthesis would tend to decrease. Hence, the operation of Pi translocator is tuned to the rate of photosynthesis. Hence, the regulation of RPP pathway is linked to translocation and the rate of photosynthesis etc., which in turn is controlled by the availability of Pi. However, in recent years, it has been shown that fru-2,6-P2 co-ordinates the metabolism of sucrose, starch and CO2 fixation. In doing so, it links the metabolic processes of chloroplast with those of the cytosol.

Hatch-Slack (C4) Pathway of CO2 Fixation

Two Australian botanists Hatch and Slack (1966) discovered that there are two types of chloroplasts in sugarcane. One type restricted to bundle sheath cells have the normal grana. These chloroplasts carry on Hatch-Slack or C_4 cycle. Hence, Hatch-Slack cycle or C_4 cycle has been found in most monocots and some dicots. The plants having C_4cycle are known as C_4 plants, and the plants C_3 (Calvin cycle) are C_3 plants.

Photorespiration occurs in C_3 plants (Calvin cycle), which leads to a 25 percent loss of the fixed CO_2. Photorespiration occurs in C_3 plants only, as the enzyme Rubisco catalysis both carboxylation and oxygenation reactions of the initial acceptor molecule that is RuBP.

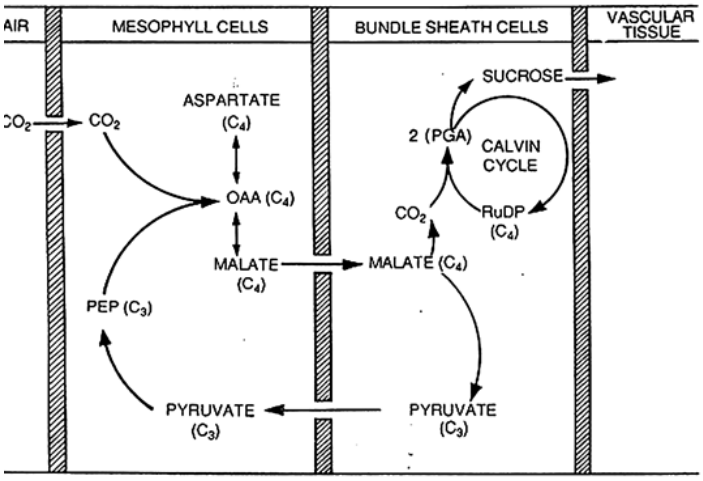


Fig. 5.11. Hatch Slack Pathway of CO_2 fixation.

In C_3 plants, photosynthesis occurs only in mesophyll cells. Photosynthesis has two types of reactions, i.e., light reactions and carbon or dark reactions

In light reactions, ATP and $NADPH_2$ are produced, and as a result of photolysis of water O_2 is released.

During carbon or dark reactions, CO_2 is assimilated and carbohydrates are produced.

As both light reactions and carbon (dark) reactions occur in mesophyll cells in C_3plants, it becomes essential for enzyme Rubisco to catalyse both oxygenation and carboxylation reactions of RuBP, simultaneously.

However, in category of C_4 plants, nature has evolved a mechanism to avoid occurrence of photorespiration, which is thought to be a harmful process.

C_4 pathway requires the presence of two types of photosynthetic cells, i.e., mesophyll cells and bundle sheath cells. The bundle sheath cells are arranged in a wreath like manner. This kind of arrangement of cells is called Kranz anatomy (Kranz: wreath). In Kranz anatomy, the mesophyll and bundle sheath cells are connected by plasmodesmata or cytoplasmic bridges.

The C_4 plants contain dimorphic chloroplasts. The chloroplasts in mesophyll cells are granal, whereas in bundle sheath cells they are agranal.

The granal chloroplasts contain thylakoids which are stacked to form grana, as formed in C_3 plants. However, in agranal chloroplasts of bundle sheath cells grana are absent and thylakoids are present only as stroma lamellae.

The presence of two types of cells (granal and agranal) allows occurrence of light and carbon (dark) reactions separately in each type.

Here, release of O_2 takes place in one type, while fixation of CO_2 catalysed by Rubisco enzyme occurs in another type of cells.

In C_4 plants (maize, sugarcane, etc.), light reactions occur in mesophyll cells, whereas CO_2 assimilation takes place in bundle sheath cells. Such arrangement of cells does not allow O_2 released in mesophyll cells to enter in bundle-sheath cells.

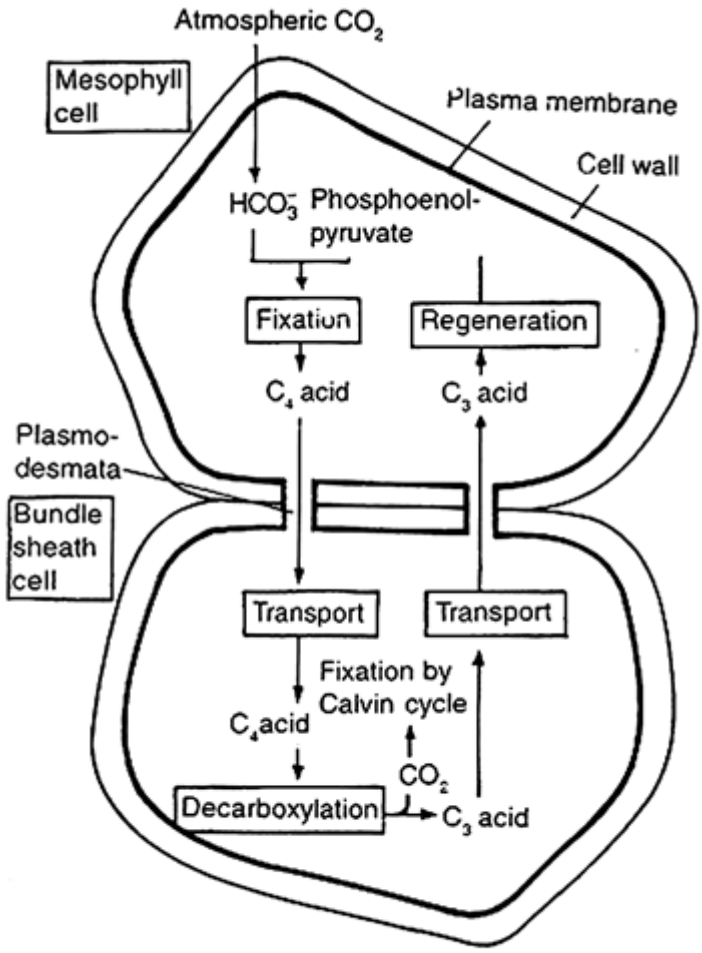


Fig. 5.12. C_4 photosynthetic carbon cycle.

Hence, Rubisco enzyme, which is present only in bundle-sheath cells, does not come into contact with O_2, and thus, oxygenation of RuBP is completely avoided.

In C_4 plants, a CO_2 concentrating mechanism is present which helps in reducing the occurrence of photorespiration (i.e., oxygenation of initial acceptor RuBP). This type of CO_2 concentrating mechanism is called C_4 pathway.

For operation of C_4 pathway, both mesophyll and bundle-sheath cells are required. The main objective of C_4 pathway is to build up high concentration of CO_2 near Rubisco enzyme in bundle- sheath cells. High concentration of CO_2 near Rubisco enhances carboxylation and reduces photorespiration.

C_4 photosynthetic Carbon Cycle:

In C_4 pathway, CO_2 from the atmosphere enters through stomata into the mesophyll cells and combines with phosphoenol pyruvate (3-carbon compound). This reaction is catalysed by an enzyme known as phosphoenol pyruvate carboxylase, i.e., PEPCase. With the result, a C_4 acid, oxaloacetic acid (OAA) is formed.

$$CO_2 + PEP + H_2O \xrightarrow[\text{enzyme}]{\text{PEPCase}} \text{Oxaloacetic acid (OAA)} + H_3PO_4$$

The above-mentioned reaction occurs in cytosol of the mesophyll cells and is called fixation of CO_2 or carboxylation.

Since this gives rise to the first stable product C_4 acid, and therefore, known as C_4pathway.

The next step of reaction is transport of oxalo acetic acid (OAA – 4 C compounds) from cytosol of mesophyll cells to chloroplasts of bundle-sheath cells, where it is decarboxylated to release fixed CO_2 and high concentration of CO_2 is generated near Rubisco.

The other product of decarboxylation reaction is a 3-carbon compound called pyruvic acid. Now, this is transported back to mesophyll cells, where if regenerates phosphoenol pyruvate to its own for continuation of C_4 pathway.

However, the C_4 pathway is more efficient than C_3 pathway due to absence of photorespiration in C_4 plants.

Differences between C(3) and C(4) Plants

C_3 Plants:

1. Plants that carry out C_3 cycle is known as C_3 plants.

2. CO fixation occurs one time in mesophyll cell.

3. RuBP (5-C compound) is CO_2 acceptor. It is a weak CO_2 acceptor.

4. First product of CO_2 fixation is PGA (3-C compound).

5. Leaf does not show Kranz anatomy.

6. Leaf with monomorphic chloroplasts.

7. Enzymes of Calvin cycle are found in all green cells.

8. Enzyme for carboxylation is RUBISCO.

9. Photorespiration occurs, oxygen functions as a inhibitor of photosynthesis.

10. CO_2 compensation point is 100 ppm.

11. CO_2: $NADPH_2$: ATP = 6 : 12 :18

12. Productivity moderate.

13. Temperature tolerance is less.

14. Glucose synthesis in mesophyll cells.

15. Utilize low CO_2, till it remains 50 – 100 ppm concentration in atmosphere.

16. Compensation point at high CO_2 concentration.

17. C_3 plants require 18 ATP for the synthesis of one mol of glucose.

C_4 Plants:

1. Plants that carry out C_4 cycle is known as C_4 plants.

2. CO_2 fixation occurs twice, one in mesophyll cells to form oxaloacetic acid (4C) and another in bundle sheath cells to form 3-phosphoglyceric acid (3C).

3. PEPA in mesophyll cells and RuBP in bundle sheath cells function as CO_2 acceptor. PEPA is a very strong CO_2 acceptor as compared to RuBP.

4. It is oxaloacetic acid (4-C compound) in mesophyll cells and PGA in bundle sheath cells.

5. Leaf with Kranz anatomy.

6. Leaf with dimorphic chloroplasts.

7. Enzymes of Calvin cycle are absent in mesophyll cells while present in bundle sheath cells.

8. Enzyme for carboxylation is PEP carboxylase (PEPCO).

9. Photorespiration is absent. There is no inhibitory effect of O_2.

10. CO_2 compensation point is 10 ppm.

11. CO_2: $NADPH_2$: ATP = 6: 12: 30.

12. Twice as compared to C_3 plants.

13. Temperature tolerance is good.

14. Glucose synthesis in bundle sheath cells.

15. Consume more CO_2 till it remains 10 ppm concentration in atmosphere.

16. Compensation point at very low CO_2 concentration.

17. C_4 plants require 30 ATP to synthesize one glucose molecule.

Crassulacean Acid Metabolism

Under natural conditions the acidity of green shoots of some non-halophytic succulents and semi-succulent plants increases at night and decreases during the following day. This diurnal change in acidity was first discovered in a Crassulacean plant Bryophyllumcalycinum hence, it has been termed as Crassulacean acid metabolism (CAM). Crassulacean acid metabolism occurs only in green organs and the plants which exhibit it belong to a number of different families. It is especially noticeable in leaves of Bryophyllum, Kalanchoe, Sedum, Kleinia, Crassula and fleshy green stems of Opuntia.

In CAM plants, the stomata are open at night and are usually closed during most of the day.

The distinctive diurnal fluctuation in acidity in plants showing CAM is predominantly due to changes in amounts of vacuolar malic acid in the mesophyll cells. During night, malic acid is synthesized utilising CO_2 (dark CO_2 fixation) which then accumulates in the vacuole and may account for about 85% of the total titrable acid content.

During the following day, this malic acid is consumed resulting in decrease of acidity. Besides malic acid, some other acids like citric acid and iso-citric acid also contribute to the total titrable acidity but their amount is negligible and moreover these do not show consistent diurnal pattern of fluctuation as exhibited by malic acid.

Diurnal Changes in Gaseous Exchanges during CAM:

During the dark synthesis of malate in CAM (resulting in the acidification), oxygen is absorbed continuously but, in the early stages, little or no CO_2 is evolved. This results in a respiratory quotient (R.Q.) of very low or even zero and sometimes a negative value.

When the accumulation of malate is complete, the CO_2 is evolved rapidly and the R.Q. is unity.

During light de-acidification, O_2 is continuously evolved but initially the absorption of CO_2 is very slow. This results in high values of photosynthetic quotient (ml. O_2 evolved/ml. CO_2 uptake). However, as the de-acidification slows down, the uptake of CO_2 increases rapidly until de-acidification is complete. In successive light periods, the vol. of CO_2 uptake equals to the vol. of O_2 given out and hence, the photosynthetic quotient falls to unity.

Factors influencing diurnal fluctuations in acidity:

The amplitude of the diurnal fluctuations in acidity varies with growth conditions and age of the plants. It increases with unfolding of the leaves until they are fully expanded and decreases when they enter senescence. Apart from these, the seasonal changes also have profound effect on it through changes in day and night temperatures, photosynthetic activity and at least in some plants in the length of the day.

Synthesis of malate during night or dark CO_2 fixation:

Large amounts of starch are consumed during acidification which indicates that carbohydrates are the source of malate synthesis, the overall process being represented by the following equation:

$C_6H_{12}O_6 + 2CO_2 \rightarrow 2C_4H_6O_5$ Malate

It is now generally believed that the malate is synthesized during night in reaction in which some product derived from carbohydrate reserves e.g., pyruvate or most likely phosphoenol pyruvate (PEP) is carboxylated to produce malate either directly or first forming oxaloacetic acid which is then reduced to malate according to the following reactions:

1. $Pyruvate + CO_2 + NADPH + H^+ \xrightleftharpoons{\text{Malic enzyme}} Malate + NADP^+$
2. $PEP + CO_2 + ADP \xrightleftharpoons{\text{PEP—Carboxykinase}} Oxaloacetate + ATP$
3. $PEP + CO_2 + H_2O \xrightarrow{\text{PEP—carboxylase}} Oxaloacetate + Pi$
4. $Oxaloacetate + NADH + H^+ \xrightleftharpoons{\text{Malate dehydrogenase}} Malate + NAD^+$

Consumption of malate in light deacidiflcation:

During the following day when acidified organs are exposed to light, rapid consumption of malate occurs resulting in de-acidification (due to CO_2 release from malate).

The malate may be decarboxylated in two ways:

(i) In some CAM plants, the malate is directly decarboxylated in the presence of $NADP^+$— malic enzyme into CO_2 and pyruvate.

$$\text{Malate} + \text{NADP}^+ \rightleftharpoons \text{Pyruvate} + CO_2 + \text{NADPH} + H^+$$

(ii) In other CAM plants, the malate is first oxidised to oxaloacetate by a malate dehydrogenase. The oxaloacetate is then converted into $CO_2$and phosphoenolpyruvate with the utilization of ATP by PEP—Carboxykinase.

$$\text{Malate} + \text{NAD}^+ \rightleftharpoons \text{Oxaloacetate} + \text{NADH} + H^+$$
$$\text{Oxaloacetate} + \text{ATP} \rightleftharpoons \text{PEP} + CO_2 + \text{ADP}$$

The CO_2 thus produced in either of the above two ways is then consumed in normal photosynthetic reaction sequence to yield carbohydrates. The pyruvate and phosphoenolpyruvate are probably also utilised for carbohydrates synthesis during the day. The pyruvate is first converted into phosphoenol pyruvate (PEP) in the presence of the enzyme pyruvate orthophosphate dikinase.

$$\text{Pyruvate} + \text{ATP} + \text{Pi} \rightarrow \text{PEP} + \text{AMP} + \text{PPi}$$

$$\text{AMP} + \text{ATP} \rightarrow \text{ADP} + \text{ADP}$$

The PEP in both types of CAM plants is converted into 3 PGA (3-phosphoglyceric acid) by reverse reactions of glycolysis. Thereafter, the 3-PGA is utilised in the Calvin cycle.

The outline of CAM is given in Fig. 11.28.

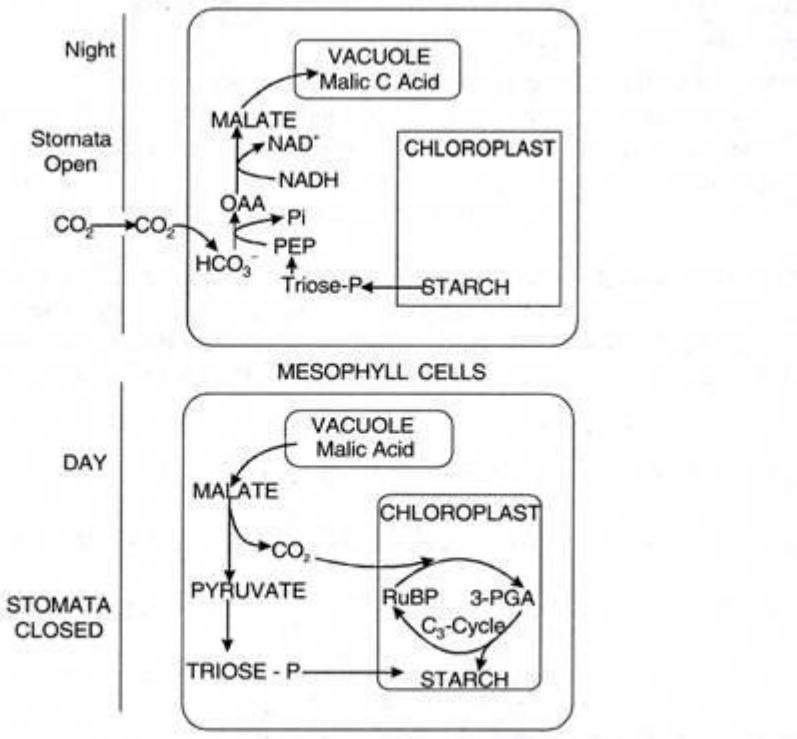


Fig. 11.28. Outline of CAM metabolism. Malate and malic acid are synonyms. OAA = oxaloacetate; PEP = phosphoenol pyruvate.

Distribution of CAM Plants:

About 24 families of angiosperms (including, Crassulacee, Portulacaceae, Cactaceae, Euphorbiaceae, Bromeliaceae, Aizoaceae, Agavaceae&Orchidaceae) are known to contain plants that exhibit CAM. Most of these families with the exception of Crassulaceae and Cactaceae, are not exclusively CAM, because they have both CAM and C_3 representatives. Some families may even have representatives showing all three modes of photosynthesis viz., C_3, C_4, and CAM.

Examples of commercially important plants which show CAM are, pine apple (Ananascomosus), agave (century plant), Cacti, and orchids.

Besides angiosperms, CAM also occurs in some pteridophytes such as Isoetes (Family Isoetaceae), some lithophytic and epiphytic ferns is (Family Polypodiaceae), and Welwitschia mirabilis (a gymnosperm). CAM is also known to occur in some aquatic species of plants.

Comparison between CAM and C_4 Plants:

The CAM and C_4 plants show some close similarities and also significant differences, and some scientists even think that CAM might be a variant of the C_4 syndrome.

A brief account of similarities and differences between the two follows:

Similarities:

i. Both CAM and C4 cycles, use cytoplasmic phosphoenolpyruvate carboxylase (PEPcase) to form 4-C acids from phosphoenolpyruvate (PEP) and bicarbonate ions (HCO_3^-) in mesophyll cells.

ii. In both the cases, the 4-C acids thus formed are subsequently decarboxylated to yield CO_2 for use in Calvin cycle or PCR-cycle.

Differences:

i. C_4-plants are found only in angiosperms, while CAM plants occur also in some pteridophytes, and a gymnosperm.

ii. Succulence is prerequisite for CAM, but not for C_4-cycle.

iii. Stomata are closed during day and open at night in CAM plants. Reverse is true for C4- plants.

iv. Krantz anatomy occurs in C4-plants, but not in CAM plants.

v. In CAM plants, carboxylation of PEP and decarboxylation of 4-C acids, occur in the same mesophyll cells but are temporally separated (i.e., separated in time), the former taking place in night and the latter during the following day. Contrary to it, in C_4 plants, carboxylation of PEP occurs in mesophyll cells while decarboxylation of 4-C acids takes place in bundle sheath cells in quick succession, and therefore, both these reactions are spatially separated but not temporally.

vi. In C_4 pathway, there is closed cycle of carbon intermediates (Fig. 11.22) while in CAM, it is not.

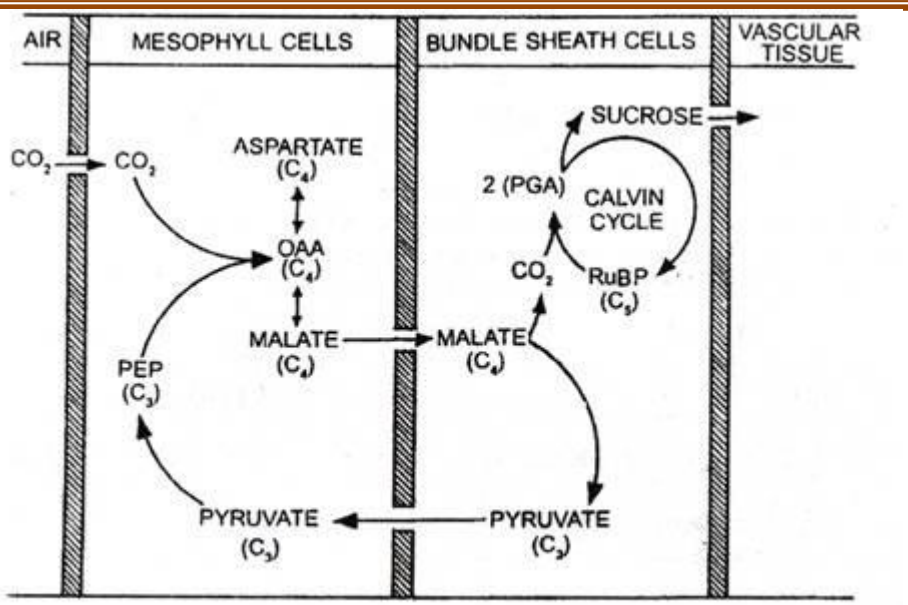


Fig. 11.22. Hatch-Slack Pathway of CO_2 fixation.

vii. The transpiration ratio is substantially lower in CAM plants than in C_4-plants.

Ecological Significance of CAM:

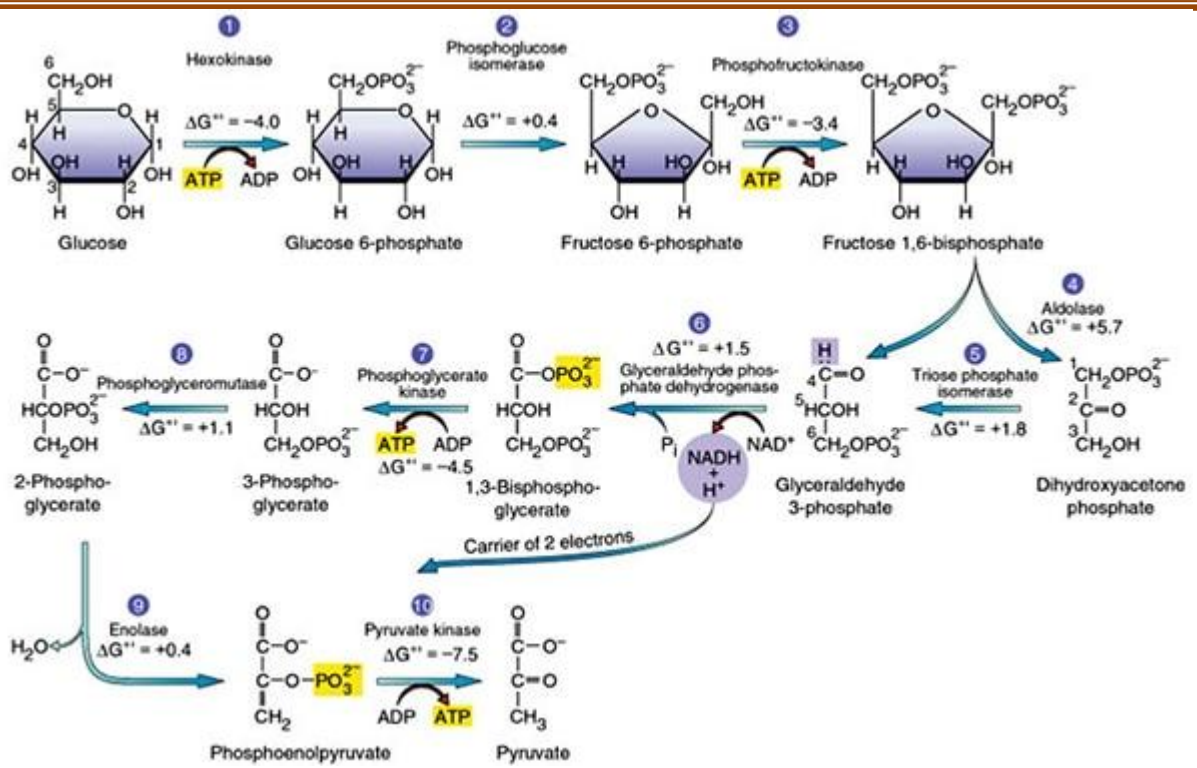
CAM plants are especially suited to dry habitats such as deserts. These plants have remarkable capacity to attain high biomass under conditions of high evaporation rate or scanty rainfall, which are otherwise insufficient for growth of crop plants.

Nocturnal opening of stomata in CAM plants allows uptake of atmospheric CO_2 when conditions for transpiration are at a minimum. During day time, when stomata are closed to check transpiration, photosynthesis can proceed by using CO_2 released from decarboxylation of malate. The transpiration ratio (i.e., the ratio of the wt. of water transpired to the wt. of dry matter produced) for CAM plants is substantially lower than either with those of C_3 or C_4 plants.

Typically, a CAM plant loses about 50-100 gm of water for every gm of CO_2 gained, as compared with 250-300 grams for C_4-plants and 400-500 grams for C_3-plants. Therefore, CAM-plants have definite competitive advantage over C_3 and C_4 plants in dry habitats such as in deserts. However, rates for daily carbon assimilation in CAM plants are only about half those of C_3-plants and one third those of C_4-plants.

Daylight closure of stomata in CAM plants to conserve water in dry habitats, may not be the unique basis for evolution of CAM. It is because, paradoxically, some aquatic plant species are also known to exhibit CAM. According to some scientists, CAM probably also increases acquisition of inorganic carbon in the form of bicarbonate ions (HCO_3^-) in aquatic habitats, when availability of CO_2 is restricted due to high resistance to gas diffusion.

Overview of glycolysis



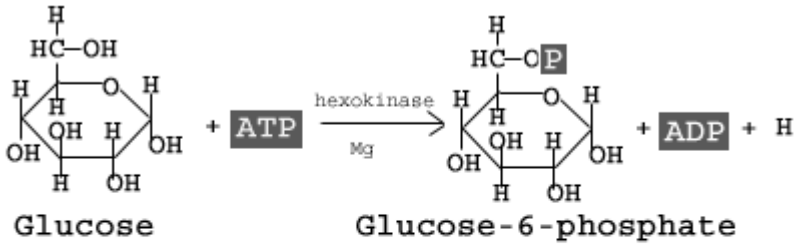
Glycolysis- 10 steps explained steps by steps with diagram

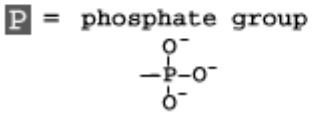
Glycolysis is the metabolic process that serves as the foundation for both aerobic and anaerobic cellular respiration. In glycolysis, glucose is converted into pyruvate. Glucose is a six- memebered ring molecule found in the blood and is usually a result of the breakdown of carbohydrates into sugars. It enters cells through specific transporter proteins that move it from outside the cell into the cell's cytosol. All of the glycolytic enzymes are found in the cytosol.

The overall reaction of glycolysis which occurs in the cytoplasm is represented simply as:

$C_6H_{12}O_6$ + 2 NAD^+ + 2 ADP + 2 P ——> 2 pyruvic acid, (CH_3(C=O)COOH + 2 ATP + 2 NADH + 2 H^+

Step 1: Hexokinase





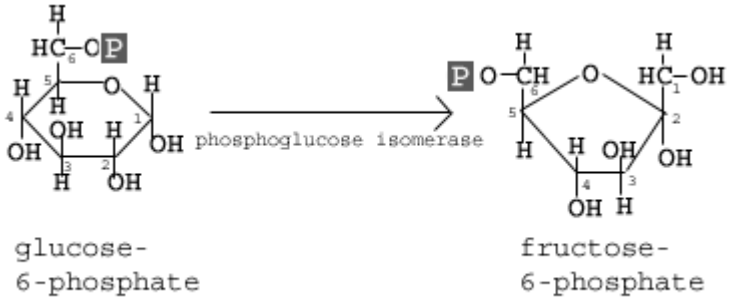
The first step in glycolysis is the conversion of D-glucose into glucose-6-phosphate. The enzyme that catalyzes this reaction is hexokinase.

Details:

Here, the glucose ring is phosphorylated. Phosphorylation is the process of adding a phosphate group to a molecule derived from ATP. As a result, at this point in glycolysis, 1 molecule of ATP has been consumed.

The reaction occurs with the help of the enzyme hexokinase, an enzyme that catalyzes the phosphorylation of many six-membered glucose-like ring structures. Atomic magnesium (Mg) is also involved to help shield the negative charges from the phosphate groups on the ATP molecule. The result of this phosphorylation is a molecule called glucose-6-phosphate (G6P), thusly called because the 6′ carbon of the glucose acquires the phosphate group.

Step 2: PhosphoglucoseIsomerase



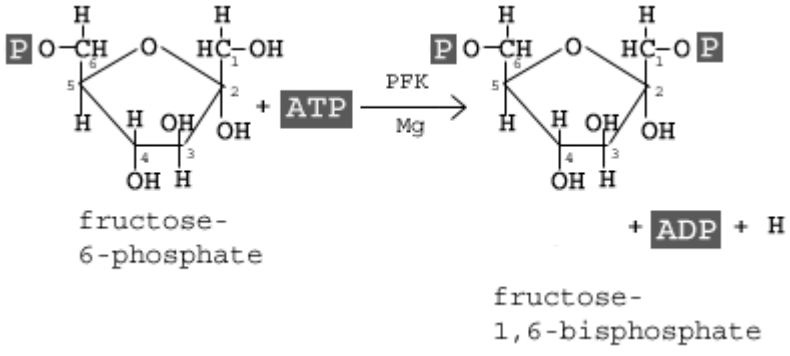
The second reaction of glycolysis is the rearrangement of glucose 6-phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphate isomerase (PhosphoglucoseIsomerase).

Details:

The second step of glycolysis involves the conversion of glucose-6-phosphate to fructose-6-phosphate (F6P). This reaction occurs with the help of the enzyme phosphoglucoseisomerase (PI). As the name of the enzyme suggests, this reaction involves an isomerization reaction.

The reaction involves the rearrangement of the carbon-oxygen bond to transform the six-membered ring into a five-membered ring. To rearrangement takes place when the six-membered ring opens and then closes in such a way that the first carbon becomes now external to the ring.

Step 3: Phosphofructokinase



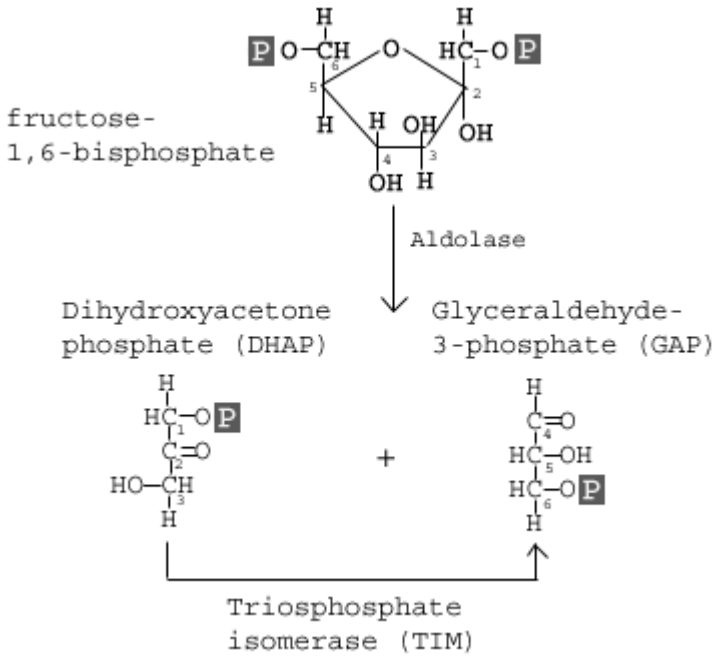
Phosphofructokinase, with magnesium as a cofactor, changes fructose 6-phosphate into fructose 1,6-bisphosphate.

Details:

In the third step of glycolysis, fructose-6-phosphate is converted to fructose- 1,6-*bi*sphosphate (FBP). Similar to the reaction that occurs in step 1 of glycolysis, a second molecule of ATP provides the phosphate group that is added on to the F6P molecule.

The enzyme that catalyzes this reaction is phosphofructokinase (PFK). As in step 1, a magnesium atom is involved to help shield negative charges.

Step 4: Aldolase

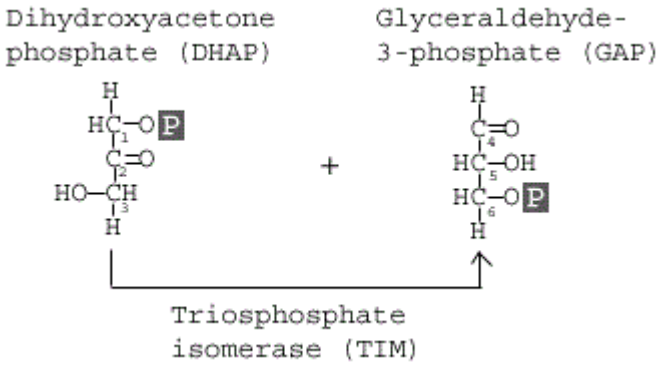


The enzyme Aldolase splits fructose 1, 6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetonephosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).

Details:

This step utilizes the enzyme aldolase, which catalyzes the cleavage of FBP to yield two 3-carbon molecules. One of these molecules is called glyceraldehyde-3-phosphate (GAP) and the other is called dihydroxyacetone phosphate (DHAP).

Step 5: Triphosphate isomerase

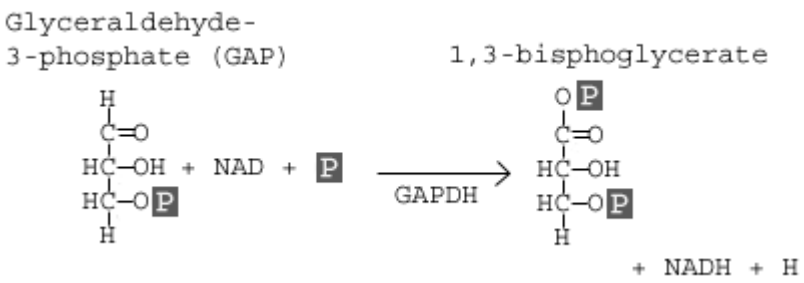


The enzyme triophosphateisomerase rapidly inter- converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). Glyceraldehyde phosphate is removed / used in next step of Glycolysis.

Details:

GAP is the only molecule that continues in the glycolytic pathway. As a result, all of the DHAP molecules produced are further acted on by the enzyme triphoshpateisomerase (TIM), which reorganizes the DHAP into GAP so it can continue in glycolysis. At this point in the glycolytic pathway, we have two 3-carbon molecules, but have not yet fully converted glucose into pyruvate.

Step 6: Glyceraldehyde-3-phosphate Dehydrogenase



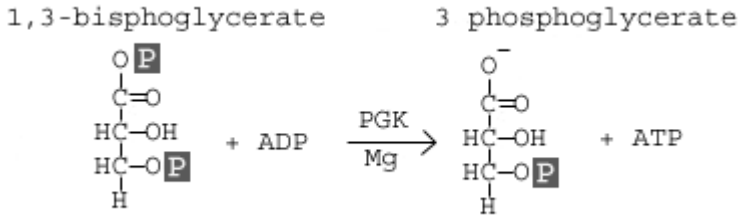
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dehydrogenates and adds an inorganic phosphate to glyceraldehyde 3-phosphate, producing 1,3-bisphosphoglycerate.

Details:

In this step, two main events take place: 1) glyceraldehyde-3-phosphate is oxidized by the coenzyme nicotinamide adenine dinucleotide (NAD); 2) the molecule is phosphorylated by the addition of a free phosphate group. The enzyme that catalyzes this reaction is glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

The enzyme GAPDH contains appropriate structures and holds the molecule in a conformation such that it allows the NAD molecule to pull a hydrogen off the GAP, converting the NAD to NADH. The phosphate group then attacks the GAP molecule and releases it from the enzyme to yield 1,3bisphoglycerate, NADH, and a hydrogen atom.

Step 7: Phosphoglycerate Kinase



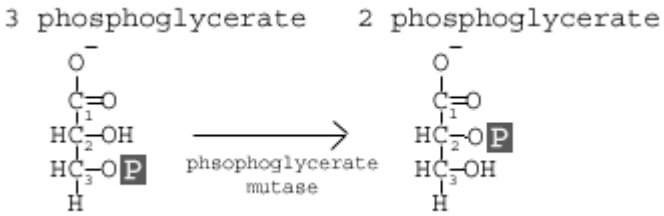
Phosphoglycerate kinase transfers a phosphate group from 1,3-bisphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.

Details:

In this step, 1,3bisphoglycerate is converted to 3-phosphoglycerate by the enzyme phosphoglycerate kinase (PGK). This reaction involves the loss of a phosphate group from the starting material. The phosphate is transferred to a molecule of ADP that yields our first molecule of ATP. Since we actually have two molecules of 1,3bisphoglycerate (because there were two 3-carbon products from stage 1 of glycolysis), we actually synthesize two molecules of ATP at this step. With this synthesis of ATP, we have cancelled the first two molecules of ATP that we used, leaving us with a net of 0 ATP molecules up to this stage of glycolysis.

Again, we see that an atom of magnesium is involved to shield the negative charges on the phosphate groups of the ATP molecule.

Step 8: PhosphoglycerateMutase



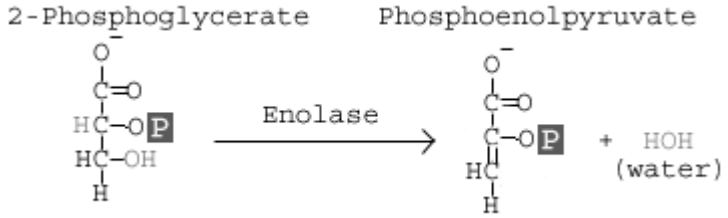
The enzyme phosphoglyceromutase relocates the P from 3- phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate.

Details:

This step involves a simple rearrangement of the position of the phosphate group on the 3 phosphoglycerate molecule, making it 2 phosphoglycerate. The molecule responsible for catalyzing this reaction is called phosphoglyceratemutase (PGM). A *mutase* is an enzyme that catalyzes the transfer of a functional group from one position on a molecule to another.

The reaction mechanism proceeds by first adding an additional phosphate group to the 2′ position of the 3 phosphoglycerate. The enzyme then removes the phosphate from the 3′ position leaving just the 2′ phosphate, and thus yielding 2 phsophoglycerate. In this way, the enzyme is also restored to its original, phosphorylated state.

Step 9: Enolase

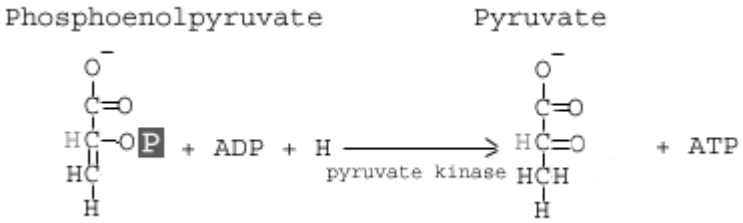


The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP).

Details:

This step involves the conversion of 2 phosphoglycerate to phosphoenolpyruvate (PEP). The reaction is catalyzed by the enzyme enolase. Enolase works by removing a water group, or *dehydrating* the 2 phosphoglycerate. The specificity of the enzyme pocket allows for the reaction to occur through a series of steps too complicated to cover here.

Step 10: Pyruvate Kinase



The enzyme pyruvate kinase transfers a P from phosphoenolpyruvate (PEP) to ADP to form pyruvic acid and ATP Result in step 10.

Details:

The final step of glycolysis converts phosphoenolpyruvate into pyruvate with the help of the enzyme pyruvate kinase. As the enzyme's name suggests, this reaction involves the transfer of a phosphate group. The phosphate group attached to the 2′ carbon of the PEP is transferred to a molecule of ADP, yielding ATP. Again, since there are two molecules of PEP, here we actually generate 2 ATP molecules.

Steps 1 and 3 = – 2ATP

Steps 7 and 10 = + 4 ATP

Net "visible" ATP produced = 2.

Immediately upon finishing glycolysis, the cell must continue respiration in either an aerobic or anaerobic direction; this choice is made based on the circumstances of the particular cell. A cell that can perform aerobic respiration and which finds itself in the presence of oxygen will continue on to the aerobic citric acid cycle in the mitochondria. If a cell able to perform aerobic respiration is in a situation where there is no oxygen (such as muscles under extreme exertion), it will move into a type of anaerobic respiration called homolactic fermentation. Some cells such as yeast are unable to carry

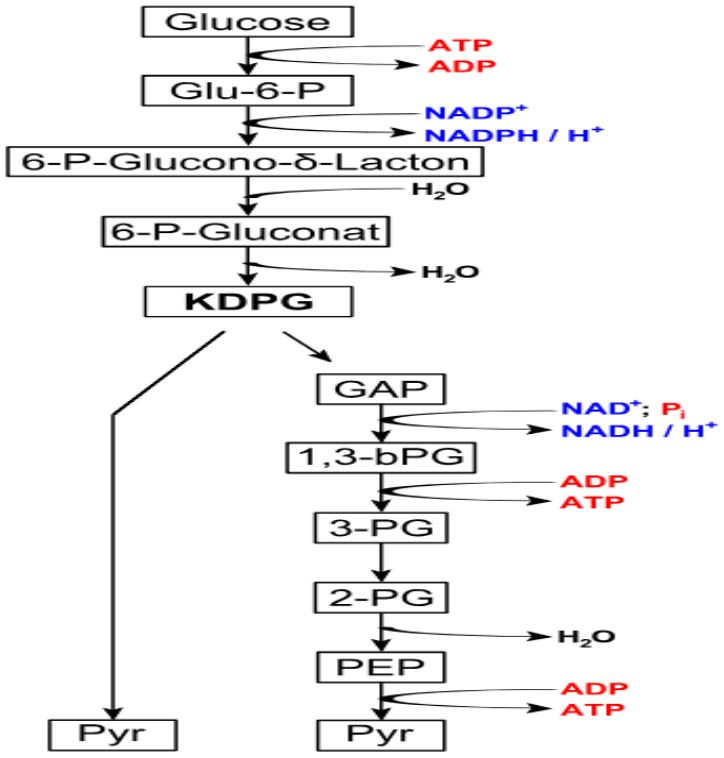
out aerobic respiration and will automatically move into a type of anaerobic respiration called alcoholic fermentation.

Alternatives to Glycolysis

The Entner–Doudoroff Pathway

The Entner–Doudoroff pathway is an alternate series of reactions that catabolize glucose to pyruvate.

The Entner–Doudoroff pathway describes an alternate series of reactions that catabolize glucose to pyruvate using a set of enzymes different from those used in either glycolysis or the pentose phosphate pathway. Glycolysis (from glycose, an older term for glucose + -lysis degradation) is the metabolic pathway that converts glucose C6H12O6, into pyruvate, CH3COCOO− + H+. The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide). Most bacteria use glycolysis and the pentose phosphate pathway. This pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.



The Entner–Doudoroff Pathway: This is a diagram of the Entner-Doudoroff pathway (KDPG: 2-keto-3-deoxy-6-phosphogluconate).

Microbes can use many different carbon sources for energy. The best known and perhaps most common example is glucose. Microbes can utilize hydrocarbons via a stepwise oxidation of a hydrocarbon by oxygen produces water and, successively, an alcohol, an aldehyde or a ketone, a carboxylic acid, and then a peroxide. Note the presence of oxygen, thus defining this as aerobic hydrocarbon oxidation. There are examples of anaerobic hydrocarbon oxidation, which will not be discussed here. This is of special interest as many of the environment pollutants released by human

industry are often hydrocarbon based. One of the best examples is oil spills. Understanding how microbes digest hydrocarbons has started the field of microbial biodegradation, a type of bioremediation. The goal of this is to find ways of using microbes to degrade hydrocarbon spills or waste into less dangerous byproducts such as alcohol.

Hydrocarbon utilizing microorganisms, mostly Cladosporiumresinae and Pseudomonas aeruginosa, colloquially known as "HUM bugs," are commonly present in jet fuel. They live in the water-fuel interface of the water droplets, form dark black/brown/green, gel-like mats, and cause microbial corrosion to plastic and rubber parts of the aircraft fuel system by consuming them, and to the metal parts by the means of their acidic metabolic products. They are also incorrectly called algae due to their appearance. FSII, which is added to the fuel, acts as a growth retardant for them. There are about 250 kinds of bacteria that can live in jet fuel, but fewer than a dozen are meaningfully harmful.

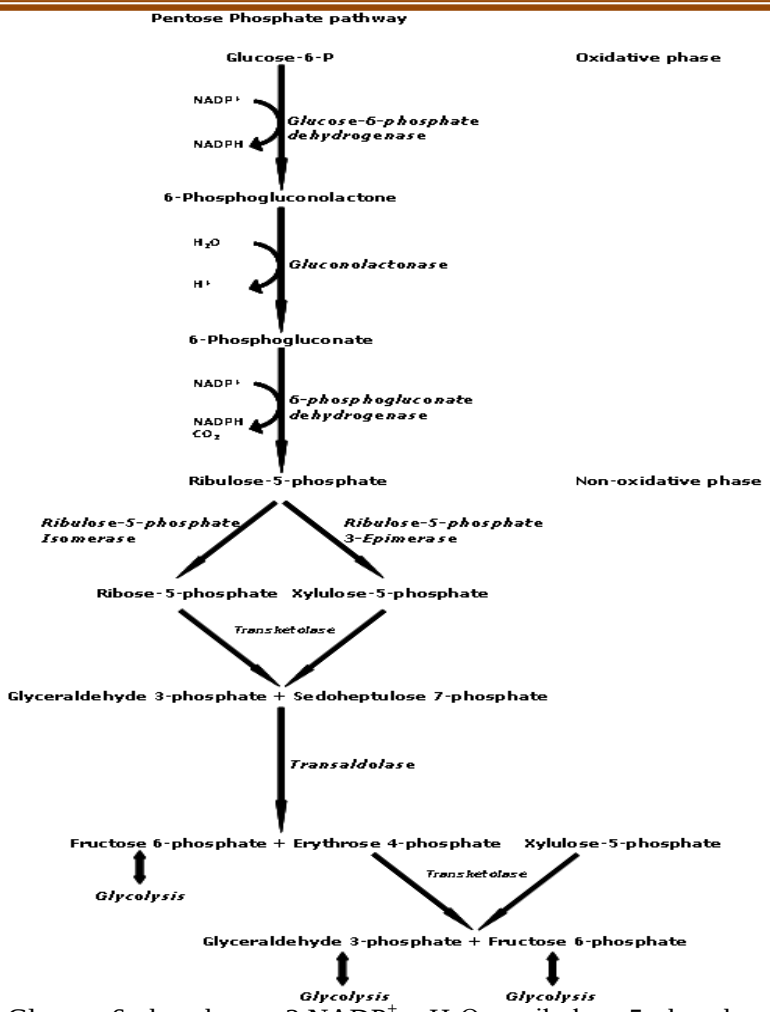
Biosurfactants are surface-active substances synthesized by living cells. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions, and potential applications in environmental protection. Biosurfactants enhance the emulsification of hydrocarbons, have the potential to solubilize hydrocarbon contaminants, and increase their availability for microbial degradation. The use of chemicals for the treatment of a hydrocarbon polluted site may contaminate the environment with their by-products, whereas biological treatment may efficiently destroy pollutants, while being biodegradable themselves. Therefore, biosurfactant-producing microorganisms may play an important role in the accelerated bioremediation of hydrocarbon-contaminated sites. These compounds can also be used in enhanced oil recovery and may be considered for other potential applications in environmental protection. Other applications include herbicides and pesticides formulations, detergents, healthcare and cosmetics, pulp and paper, coal, textiles, ceramic processing and food industries, uranium ore-processing, and mechanical dewatering of peat. Several microorganisms are known to synthesize surface-active agents; most of them are bacteria and yeasts. When grown on hydrocarbon substrate as the carbon source, these microorganisms synthesize a wide range of chemicals with surface activity, such as glycolipid, phospholipid, and others. These chemicals are synthesized to emulsify the hydrocarbon substrate and facilitate its transport into the cells. In some bacterial species such as Pseudomonas aeruginosa, biosurfactants are also involved in a group motility behavior called swarming motility.

The Pentose Phosphate Shunt

The pentose phosphate pathway (PPP) converts glucose-6-phosphate into NADPH and pentoses (5-carbon sugars).

The pentose phosphate pathway (PPP; also called the phosphogluconate pathway and the hexose monophosphate shunt) is a process that breaks down glucose-6-phosphate into NADPH and pentoses (5-carbon sugars) for use in downstream biological processes.

There are two distinct phases in the pathway: the oxidative phase and the non-oxidative phase. The first is the oxidative phase in which glucose-6-phosphate is converted to ribulose-5-phosphate. During this process two molecules of $NADP^+$ are reduced to NADPH. The overall reaction for this process is:



Glucose 6-phosphate + 2 $NADP^+$ + H_2O → ribulose-5-phosphate + 2 NADPH + 2 H^+ + CO_2

The second phase of this pathway is the non-oxidative synthesis of 5-carbon sugars. Depending on the body's state, ribulose-5-phosphate can reversibly isomerize to ribose-5-phosphate. Ribulose-5-phosphate can alternatively undergo a series of isomerizations as well as transaldolations and transketolations that result in the production of other pentose phosphates including fructose-6-phosphate, erythrose-4-phosphate, and glyceraldehyde-3-phosphate (both intermediates in glycolysis). These compounds are used in a variety of different biological processes including production of nucleotides and nucleic acids (ribose-5-phosphate), as well as synthesis of aromatic amino acids (erythrose-4-phosphate).

Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme in this pathway. It is allosterically stimulated by $NADP^+$. NADPH-utilizing pathways, such as fatty acid synthesis, generate $NADP^+$, which stimulates glucose-6-phosphate dehydrogenase to produce more NADPH. In mammals, the PPP occurs exclusively in the cytoplasm; it is found to be most active in the liver, mammary gland, and adrenal cortex. The ratio of NADPH:$NADP^+$ is normally about 100:1 in liver cytosol, making the cytosol a highly-reducing environment.

The PPP is one of the three main ways the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in humans. While the PPP does involve oxidation of glucose, its primary role is anabolic rather than catabolic, using the energy stored in NADPH to synthesize large, complex molecules from small precursors.

Additionally, NADPH can be used by cells to prevent oxidative stress. NADPH reduces glutathione via glutathione reductase, which converts reactive H_2O_2 into H_2O by glutathione peroxidase. For example, erythrocytes generate a large amount of NADPH through the pentose phosphate pathway to use in the reduction of glutathione.

Organic Acid Metabolism

Microbes can harness energy and carbon derived from organic acids by using a variety of dedicated metabolic enzymes.

Organic Acid Metabolism

A great many organisms generate organic acids (such as lactate) as a byproduct of fermentation. Some microbes are capable of utilizing such compounds as a sole source of energy.

The most commonly metabolized organic acids are the carboxylic acids, which are organic acids containing at least one carboxyl (-COOH) group. The general formula of a carboxylic acid is R-COOH, where R is a monovalent functional group. Many types of carboxylic acids can be metabolized by microbes, including:

- Fatty acids (carboxylic acids with long acyl tails)
- Amino acids (the building blocks of proteins)
- Straight-chained, saturated acids (e.g., formate, acetate, and palmitate)

FORMATE METABOLISM

Formate metabolism is important in methylotrophic organisms. It is vital in the catabolism of C_1 compounds such as methanol (see the "Methylotrophy and Methanotrophy" atom for more information on C_1 compound utilization). Methylotrophic microbes convert single-carbon compounds to formaldehyde, which is oxidized to formate by formaldehyde dehydrogenase. Degradation of formate is then catalyzed by formate dehydrogenase (FDH), which oxidizes formate to ultimately yield CO_2. It permits the donation of electrons to a second substrate (such as NAD+) in the process. This is a critical late step in the hydrocarbon utilization pathway. The ability to metabolize formate is also critical in bacterial anaerobic metabolism, in which case formate is also oxidized by an FDH enzyme but the electrons are donated to cytochromes (proteins involved in electron transport).

FATTY ACID METABOLISM

Many bacteria are capable of utilizing fatty acids of various tail lengths as sole energy and carbon sources. This process requires the β-oxidation pathway, a cyclic process that catalyzes the sequential shortening of fatty acid acyl chains to the final product, acetyl-CoA. The step-by-step process occurs as follows:

1. Fatty acid chains are converted to enoyl-CoA (catalyzed by acyl-CoA dehydrogenase).
2. Enoyl-CoA is converted to 3-hydroxyacyl-CoA (catalyzed by enoyl-CoA hydratase).
3. 3-hydroxyacyl-CoA is converted to 3-ketoacyl-CoA (catalyzed by 3-hydroxyacyl-CoA dehydrogenase).
4. 3-ketoacyl-CoA is thiolated (by 3-ketoacyl-CoA thiolase) to yield one molecule of acetyl-CoA and a derivative of the original input fatty acid that is now shorter by two carbons.

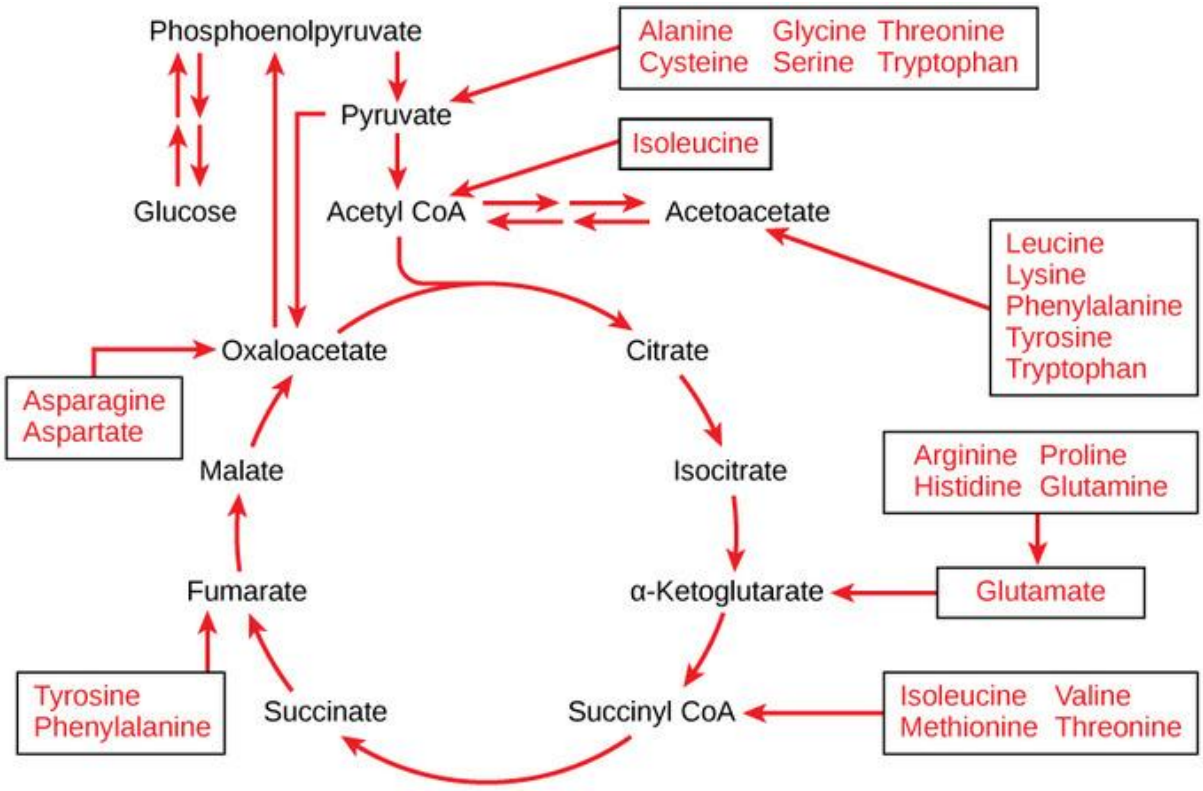
The fatty acid chain that is left over after the thiolation step can then reenter the β-oxidation pathway, which can cycle until the fatty acid has been completely reduced to acetyl-CoA. Acertyl-CoA is the entry molecule for the TCA cycle. The TCA cycle is the process used by all aerobic organisms to generate energy.

Connecting Proteins to Glucose Metabolism

Excess amino acids are converted into molecules that can enter the pathways of glucose catabolism.

Metabolic pathways should be thought of as porous; that is, substances enter from other pathways and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways. Proteins are a good example of this phenomenon. They can be broken down into their constituent amino acids and used at various steps of the pathway of glucose catabolism.

Proteins are hydrolyzed by a variety of enzymes in cells. Most of the time, the amino acids are recycled into the synthesis of new proteins or are used as precursors in the synthesis of other important biological molecules, such as hormones, nucleotides, or neurotransmitters. However, if there are excess amino acids, or if the body is in a state of starvation, some amino acids will be shunted into the pathways of glucose catabolism



Each amino acid must have its amino group removed (deamination) prior to the carbon chain's entry into these pathways. When the amino group is removed from an amino acid, it is converted into ammonia through the urea cycle. The remaining atoms of the amino acid result in a keto acid: a carbon chain with one ketone and one carboxylic acid group. In mammals, the liver synthesizes urea from two ammonia molecules and a carbon dioxide molecule. Thus, urea is the principal waste product in mammals produced from the nitrogen originating in amino acids; it leaves the body in urine. The keto acid can then enter the citric acid cycle.

When deaminated, amino acids can enter the pathways of glucose metabolism as pyruvate, acetyl CoA, or several components of the citric acid cycle. For example, deaminated asparagine and aspartate are converted into oxaloacetate and enter glucose catabolism in the citric acid cycle. Deaminated amino acids can also be converted into another intermediate molecule before entering the pathways. Several amino acids can enter glucose catabolism at multiple locations.

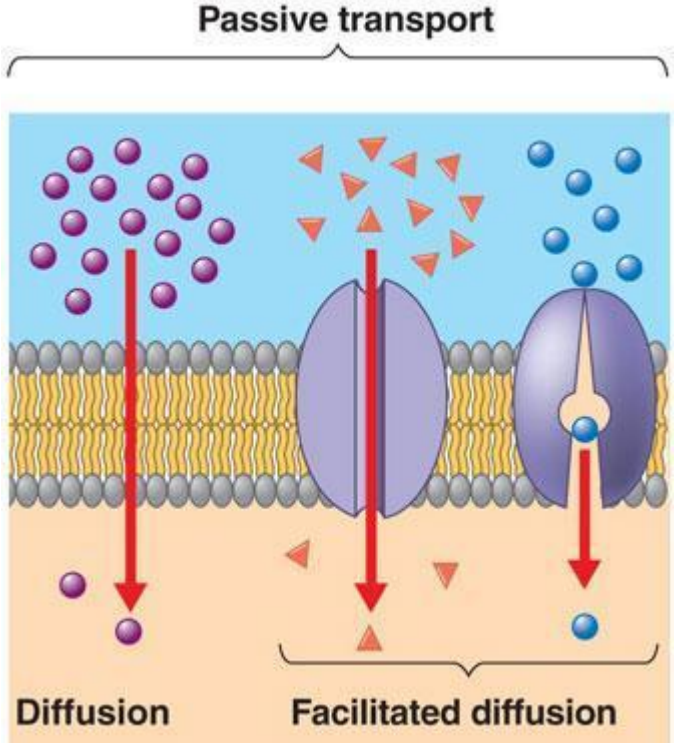
Membrane transport system-Passive and Active transport

Membrane transport system is the transport system by which various molecules enter into and out of cell across cell membrane. Cells have various transport mechanism. Based on whether the molecules pass directly through lipid bilayer or via membrane channel, whether or not the molecules is altered as it passes through membrane , whether or not the process require energy, membrane transport system is categorized into two major groups.

1. **Passive transport**
2. **Active transport**

1. Passive transport:

- Passive transport mechanism does not require cellular energy to transport molecules across cell membrane. So it is a passive process.
- In this transport system, molecules are transported from its higher concentration to the lower concentration until concentration gradient is diminished.
- Passive transport does not work against concentration gradient.



- Passive transport includes-

i) Simple diffusion or passive diffusion

ii) Osmosis

iii) Facilitated diffusion

i. Simple diffusion:

- Simple diffusion is the transport or movement of molecules from higher concentration to lower concentration without expenditure of energy.
- In this process, molecules simply diffuses through the pore of cell membrane.
- Simple diffusion do not require transporter protein.
- When the concentration of molecules is different inside and outside of the cell membrane, concentration gradient is established. Then the molecules moves from higher concentration to lower concentration until equilibrium is maintained.
- When the concentration of molecules becomes equal on both side of the membrane, transport process stops.
- In some case, the molecules after entering the cell transform metabolically, preventing to build up concentration of transported molecules, hence the concentration gradient remain established.
- The rate of diffusion is determined by concentration gradient and permeability of cytoplasmic membrane.
- Greater the concentration gradient and permeability of the cell membrane, greater will be the rate of passive diffusion.
- Examples: water or gases enter the bacterial cell by simple diffusion.

ii. Osmosis:

- The movement of solvent (water) across membrane in response to the concentration gradient of solute is known as Osmosis.
- Bacterial cytoplasm have generally higher solute concentration than its surrounding.
- There are 3 types of solution based on comparison to the bacterial cytoplasmic concentration.
- In **isotonic** solution, water move equally in both direction inside and outside of cell.
- In **hypertonic** solution, water moves out of the cell so that cell shrinks. The process is known as **plasmolysis.**
- In **hypotonic** solution, water moves inside the cell so that cell swells up. The process is known as **plasmotysis.**

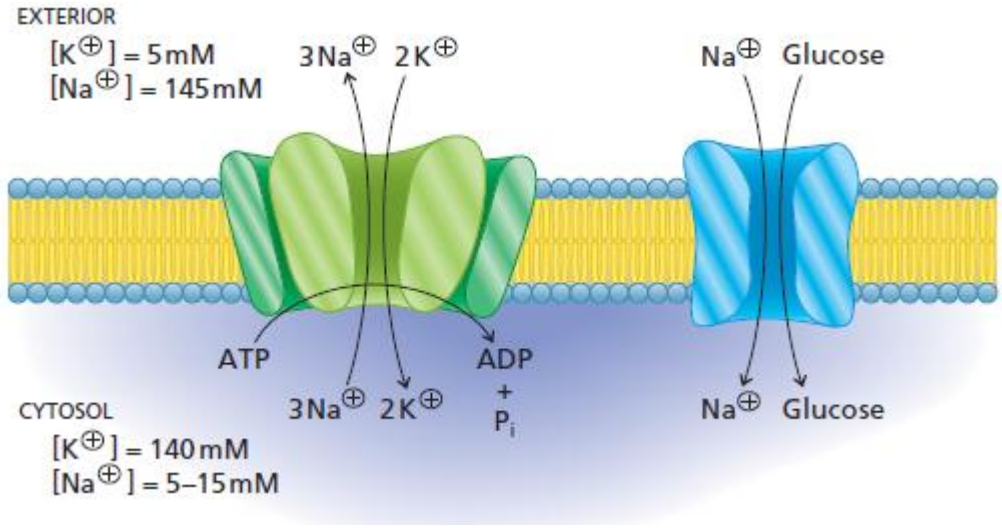
iii. Facilitated diffusion:

- The process of facilitated diffusion is similar to simple diffusion as the molecules flows from higher concentration to lower concentration but it is different from simple diffusion because it requires transporter protein for the process.
- The transporter protein is known as Permease or Porter or carrier protein.
- The transporter protein are specific however some can transport multiple compounds.

- At first solute molecule binds with the transporter protein and changes the 3D structure of the transporter protein and this change in shape allows the solute to carried across the membrane.

2. Active transport:

- Active transport requires transporter protein and continuous supply of cellular energy for the transport of molecules across concentration gradient of the membrane.
- Active transport is very important to transport the molecules which are present in very low concentration in the medium.
- In active transport permease or transporter protein carries the molecules across the membrane and the energy required to transport is obtained by ATP or Ion gradient.
- The substances transported by active transport are glucose, aminoacids, organic acids and inorganic ions (SO4–, PO4–, K+ etc).
- Examples: Lac operon (transport of lactose in E. coli), Na-K pump



- Active transport system includes-

i) Primary active transport

ii) Secondary active transport

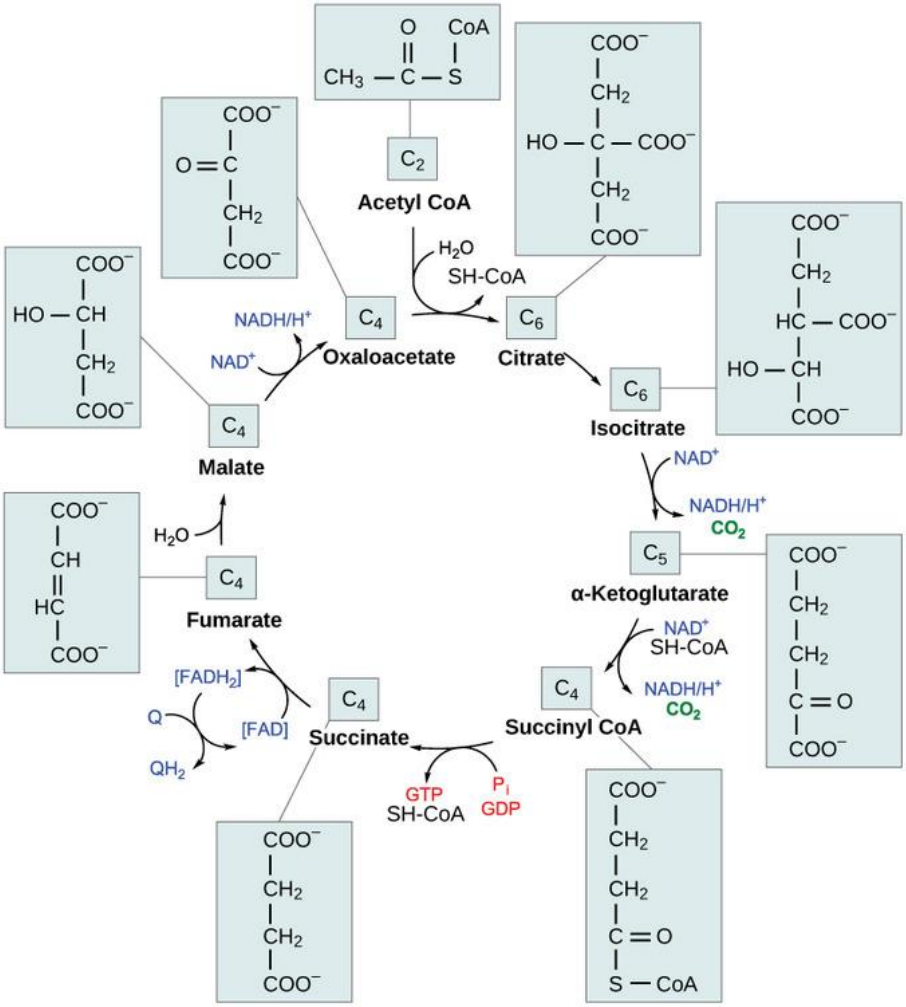
i. Primary active transport:

- In primary active transport, hydrolysis of energy rich molecules such as ATP provide energy required for transport of molecules form lower concentration to higher concentration across membrane.

ii. Secondary active transport:

- In secondary active transport, one type of molecule migrates from higher concentration to lower concentration, releasing energy. This released energy is used to transport other molecule from its lower concentration to higher concentration across cell membrane.

Tricarboxylic acid cycle (The Citric Acid Cycle)



Citric Acid Cycle (Krebs Cycle)

Like the conversion of pyruvate to acetyl CoA, the citric acid cycle takes place in the matrix of the mitochondria. Almost all of the enzymes of the citric acid cycle are soluble, with the single exception of the enzyme succinate dehydrogenase, which is embedded in the inner membrane of the mitochondrion. Unlike glycolysis, the citric acid cycle is a closed loop: the last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one GTP/ATP, and reduced forms of NADH and FADH2. This is considered an aerobic pathway because the NADH and FADH2 produced must transfer their electrons to the next pathway in the system, which will use oxygen. If this transfer does not occur, the oxidation steps of the citric acid cycle also do not occur. Note that the citric acid cycle produces very little ATP directly and does not directly consume oxygen.

Steps in the Citric Acid Cycle

Step 1. The first step is a condensation step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. CoA is bound to a sulfhydryl group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. The rate of this reaction is controlled by

negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

Step 2. Citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

Steps 3 and 4. In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD+ to NADH. This step is also regulated by negative feedback from ATP and NADH and by a positive effect of ADP. Steps three and four are both oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. α-Ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

Step 5. A phosphate group is substituted for coenzyme A, and a high- energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6. Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. The energy contained in the electrons of these atoms is insufficient to reduce NAD^+ but adequate to reduce FAD. Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

Step 7. Water is added to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced.

Products of the Citric Acid Cycle

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not necessarily contain the most recently-added carbon atoms. The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide. Each turn of the cycle forms three NADH molecules and one $FADH_2$ molecule. These carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is amphibolic (both catabolic and anabolic).

CYANIDE RESISTANT RESPIRATION AND ITS SIGNIFICANCE

The flow of electrons in the usual mitochondrial electron transport chain (in both animals and plants) during aerobic respiration is blocked by the presence of cyanides which inhibit the activity of cytochrome oxidase. This type of respiration is therefore, known as cyanide sensitive respiration.

Plant mitochondria, however, differ from the animal mitochondria in having an alternate oxidase system pathway through which terminal oxidation of reduced coenzyme continues even in the presence of cyanides. This type of respiration is known as cyanide resistant (or cyanide insensitive) respiration.

In cyanide resistant respiration, the flow of electrons from reduced coenzymes to Ubiquinone is the same as in usual mitochondrial electron transport chain. But after this point (branch point) the electrons pass from UQ to a flavoproteinFPma (with a mid-range E'_0(= + 0.02 V) and a large absorbance change on redox change), and from there to a cyanide resistant or alternate oxidase (designated as X) and finally to O_2 (Fig. 16.17). Usually the reduction of O_2 should result in the formation of H_2O but present evidences indicate the possibility of H_2O_2 being formed instead of H_2O. The H_2O_2 can easily be converted into water arid oxygen then by the enzyme catalase.

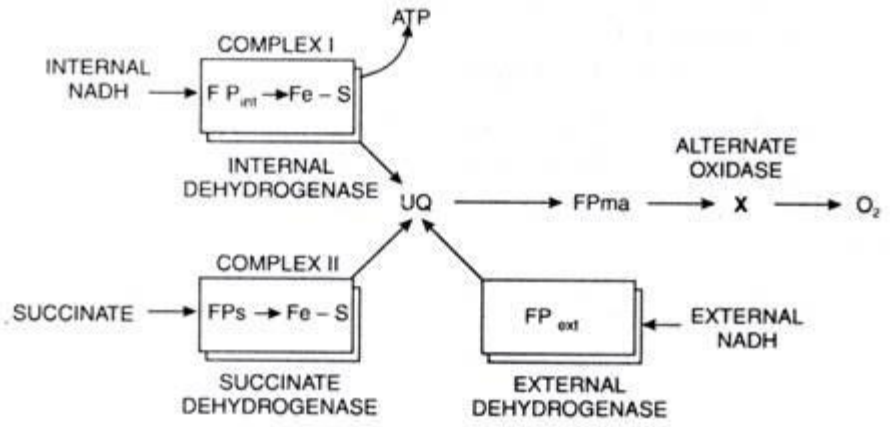


Fig.16.17. Electron transport chain in cyanide resistant respiration (only in plants). See text for abbreviations.

(The exact nature of alternate terminal oxidase X is not clearly understood. It is probably an iron-containing protein which is neither a hemoprotein nor an iron-sulphur (Fe-S) protein. The activity of alternate oxidase is inhibited by m-CLAM (= m-chlorobenzhydroxamic acid).

P/O ratio (i.e. no. of ADP molecules converted into ATP molecules per O atom) in cyanide resistant respiration is one. As in conventional electron transport chain, the first phosphorylation site is coupled with electron transport chain in cyanide resistant respiration also.

Physiological Significance of Cyanide Resistant Respiration:

The physiological significance of cyanide resistant respiration is not very clear. Following roles are usually attributed to it.

1. Cyanide resistant respiration is believed to be responsible for the climacteric in fruits (i.e., remarkable increase in respiration during and just before ripening). The climacteric is induced by ethylene and the latter may act to implement the cyanide resistant respiration in ripening fruit, (production of H_2O_2 and superoxide increases the oxidation and breakdown of membrane which are necessary activities in the ripening process).

2. Cyanide resistant respiration is known to generate heat in thermogenic tissues. Thermogenecity is observed in the flowers or inflorescences of some plants such as water lily (Victoria), arum lilies, Arum maculatum, Symplocarpusfoetidus (skunk cabbage) etc. The excessive heat produced in the inflorescence of Arum etc. is used to volatilize the odiferous compounds such as amines &indoles which are produced in them and which serve to attract pollinating insects. The amount of heat

produced in thermogenic tissues may be as high as 51°C with an atm. temp, of 15°C (e.g., in appendix of Arum italicum).

(In cyanide resistant respiration, most of the energy liberated in the oxidation of respiratory substrate is lost as heat and only little of it is consumed in the production of ATPs. For instance the P/O ratio for 1 NADH molecule is only one in cyanide resistant respiration while in cyanide sensitive or usual respiration it is 3).

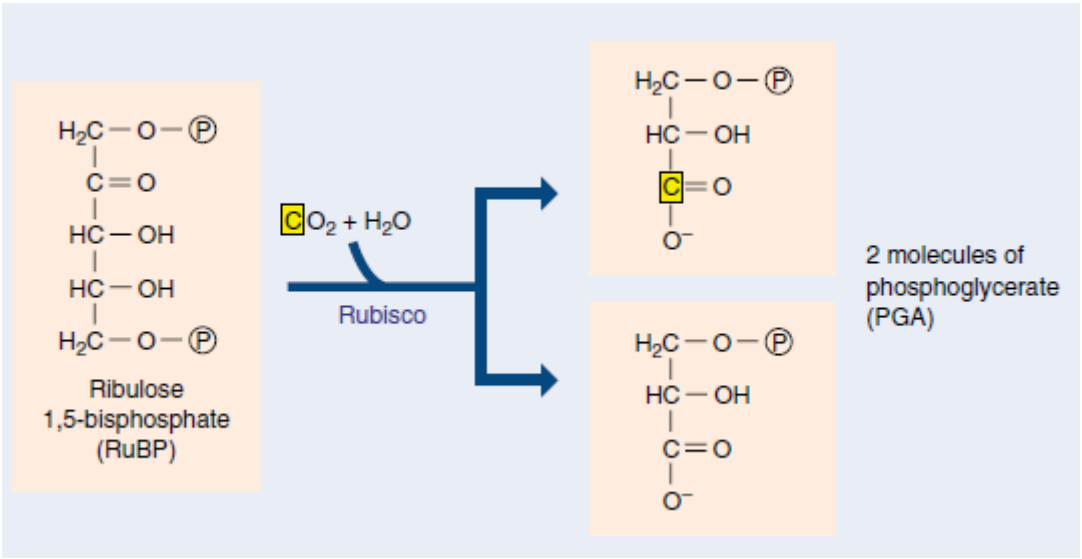
3. If ATPs generated in usual respiration in mitochondria are not sufficiently drained off, they may inhibit the Krebs' cycle (TCA cycle) via the stoppage of electron flow in electron transport chain. Therefore, cyanide resistant respiration may provide continued oxidation of NADH and operation of TCA cycle though the energy demand is lesser. The operation of TCA cycle is important because TCA cycle intermediates are precursors for cellular components.

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III BSc Biochemistry - Plant Biochemistry (16BCU503A)

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	A compound which is found in all living	ADP	ATP	chlorophyll	granum	ATP
2	Phases of Calvin cycle involves	carbon fixation	reduction	regeneration	All of Above	All of Above
3	Enzyme which catalyze reaction of	phosphatase	catalase	rubisco	amylase	rubisco
4	The final product of the Calvin cycle is	RuPB	PGA	ATP	G3P	G3P
5	The dark reaction in photosynthesis is	CO2, temperature, and	CO2, light, and water	water, temperature, and	oxygen, water, and temperature	CO2, temperature, and
6	What energy-rich organic compound is	NADPH	glucose	ATP	H2O	glucose
7	How many carbon atoms are in a	five	one	two	three	five
8	Production of one molecule of 3-	1	2	3	6	3
9	The immediate products of C3 and C4	ribulose 1,5-	malate; carbon dioxide	3-phosphoglycerate;	glyceraldehyde 3-phosphate; phospho-	3-phosphoglycerate;
10	Light is required for the light dependent	it is the source for	it splits the water molecule	it energizes electrons in the	it splits ATP molecules which	it energizes electrons in
11	During catabolism, only about 40% of	is lost as heat	is used to reduce NADP	remains in the products of	is stored as fat.	is lost as heat
12	The released energy obtained by	a concentration gradient	ADP	ATP	NAD+	ATP
13	A kinase is an enzyme that	removes phosphate	uses ATP to add a phosphate	uses NADH to change the	removes water from a double bond	uses ATP to add a
14	Which of the following enzyme catalyzes tl	Hexokinase	Pyruvate kinase	Glucokinase	Phosphofructokinase-1	Hexokinase
15	Cleavage of Fructose 1, 6-biophosphate	Two aldoses	Two ketoses	An aldose and a ketose	Only a ketose	An aldose and a ketose
16	The substrate used in the last step of	Glyceraldehyde 3-	Pyruvate	Phosphoenolpyruvate	1, 3-bisphosphoglycerate	Phosphoenolpyruvate
17	High concentration of glucose 6-	Hexokinase	Pyruvate kinase	Glucokinase	Phosphofructokinase-1	Hexokinase
18	The product formed in the first substrate	Pyruvate	3-phosphoglycerate	1, 3-bisphosphoglycerate	2-phosphoglycerate	3-phosphoglycerate
19	Glycolysis converts	Glucose into pyruvate	Glucose into	Fructose into pyruvate	Fructose into phosphoenolpyruvate	Glucose into pyruvate
20	For every one molecule of sugar glucose	1	2	3	4	2
21	The enzymes of glycolysis in a eukaryotic	intermembrane space	plasma membrane	cytosol	mitochondrial matrix	cytosol
22	In C_4 cycle oxalo acetic acid is converted	malic dehydrogenase	malate decarboxylase	malic oxidase	transaminase	malic dehydrogenase
23	In C_4 plants malic enzyme is present in	mesophyll cells	bundle sheath cells	xylem	phloem	bundle sheath cells
24	CAM cycle is observed in all the plants	cactus	orchid	chlorella	bryophyllum	chlorella
25	In C_4 cycle the enzyme involved in	malic enzyme	malate dehydrogenase	transaminase	malate decarboxylase	malate dehydrogenase
26	Only mesophyll cells are involved in	C3 cycle	C4 cycle	CAM cycle	C2 cycle	C3 cycle
27	In CAM plants carbohydrate synthesis	night time	day time	both night and day time	only in the evening	day time
28	Which of the following is formed during	H2O2	O2	OH- ions	O3	H2O2
29	In photo respiration glyoxylic acid is	transaminase	decarboxylase	dehydrogenase	reductase	transaminase
30	In photo respiration the enzyme involved	catalase	decarboxylase	transaminase	dehydrogenase	catalase
31	When the atmospheric CO_2 is less than	3 phospho glyceric acid	glycolic acid	glyoxylic acid	glycine	glycolic acid
32	In hydration, fumarate is converted by	L-Malate	D-Malate	A-Malate	C-Malate	L-Malate
33	Last step of citric acid cycle is named as	decarboxylation	oxidation	hydrogenation	aldol condensation	aldol condensation
34	In citric acid cycle, the number of NADH	6	5	4	3	6
35	The other names for citric acid cycle are	kerb's cycle	tricarboxylic acid cycle	both A and B	kerbs cycle	tricarboxylic acid cycle
36	In a eukaryotic cell, most of the enzymes	inner mitochondrial	mitochondrial matrix	outer mitochondrial	cytosol	mitochondrial matrix
37	Most of the ATP made during cellular	glycolysis	photophosphorylation	oxidative phosphorylation	substrate-level phosphorylation	oxidative
38	Pyruvate, the end product of glycolysis,	acetaldehyde	acetyl CoA	lactic acid	acetic acid	acetyl CoA
39	Energy that is released from glucose	H2O	heat	CO2	ADP	heat
40	Which of the following reactions is not a	Isocitrate dehydrogenase	a-Ketoglutarate	Malate dehydrogenase	Pyruvate dehydrogenase	Malate dehydrogenase
41	The following reactions in the citric acid	Citrate synthase	Fumarase	Succinic dehydrogenase	Succinyl-CoA synthetase	Citrate synthase
42	The process of respiration in green plants	only when stomata are	only when photosynthesis	only when photosynthesis	At all times	At all times
43	Respiratory enzymes are located in	mitochondrial matrix	cristae	perimitochondrial space	outer membrane	cristae
44	The site of glycolysis in a cell is	chloroplast	nucleus	cytoplasm	mitochondria	cytoplasm
45	Respiration is	Anabolic process	Exothermic process	Endothermic process	Endergonic process	Exothermic process
46	The annual plant exchange of gases takes	Leaf scars	lenticels	stomata	stem	stomata
47	Kreb's cycle take place in	vesicles of ER	Mitochondrial matrix	lysosomes	Dictyosomes	Mitochondrial matrix
48	cellular activity	NAD	ATP	DNA	RNA	ATP
49	Photorespiration involves	Glycolate cycle	Kreb's cycle	Calvin cycle	CAM cycle	Glycolate cycle
50	In succulent xerophytes the R.Q is	Zero	unity	less than one	more than one	Zero
51	R.Q. of fatty substances is generally	unity	Zero	more than one	less than one	less than one
52	alcohol is facilitated by the enzymes	a) carboxylase	b)phosphatase	c) dehygrogenase	d) carboxylase and dehygrogenase	d) carboxylase and dehygrogenase
53	to	ethanol and water	ethanol and oxygen	ethanol and CO2	lactic acid and CO2	ethanol and CO2
54	oxidation of one glucose molecule in a	38 ATP molecules	30 ATP molecules	36 ATP molecules	24 ATP molecules	36 ATP molecules
55	electron transport chain is	Water	Oxygen	Hydrogen	Cytochrome b	Oxygen
56	the help of	lysosomes	Peroxisomes	glyoxisomes	microtubules	glyoxisomes

The Calvin Cycle

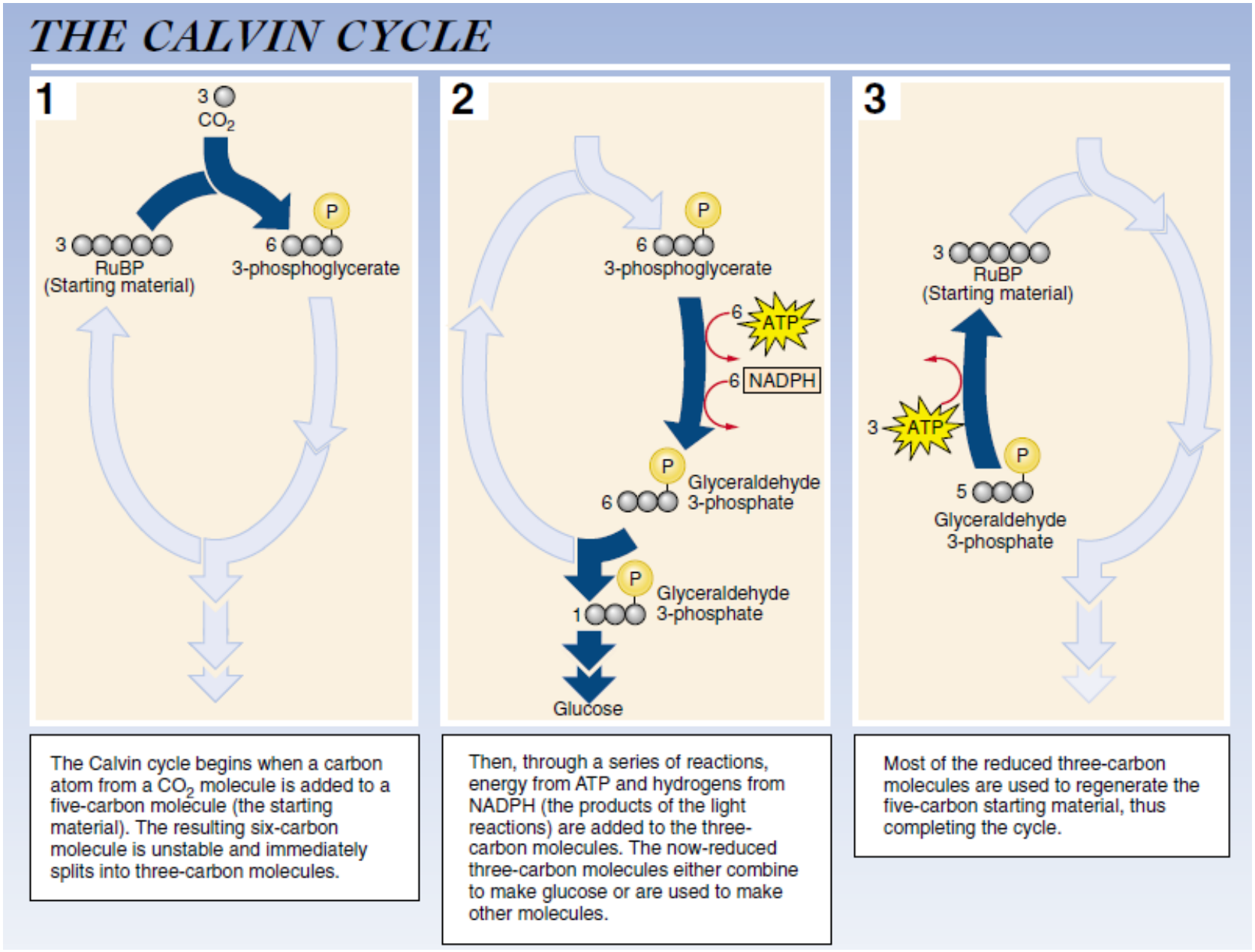
Calvin cycle is used by plants that are called C3 because of the 3-Carbon molecules that are made



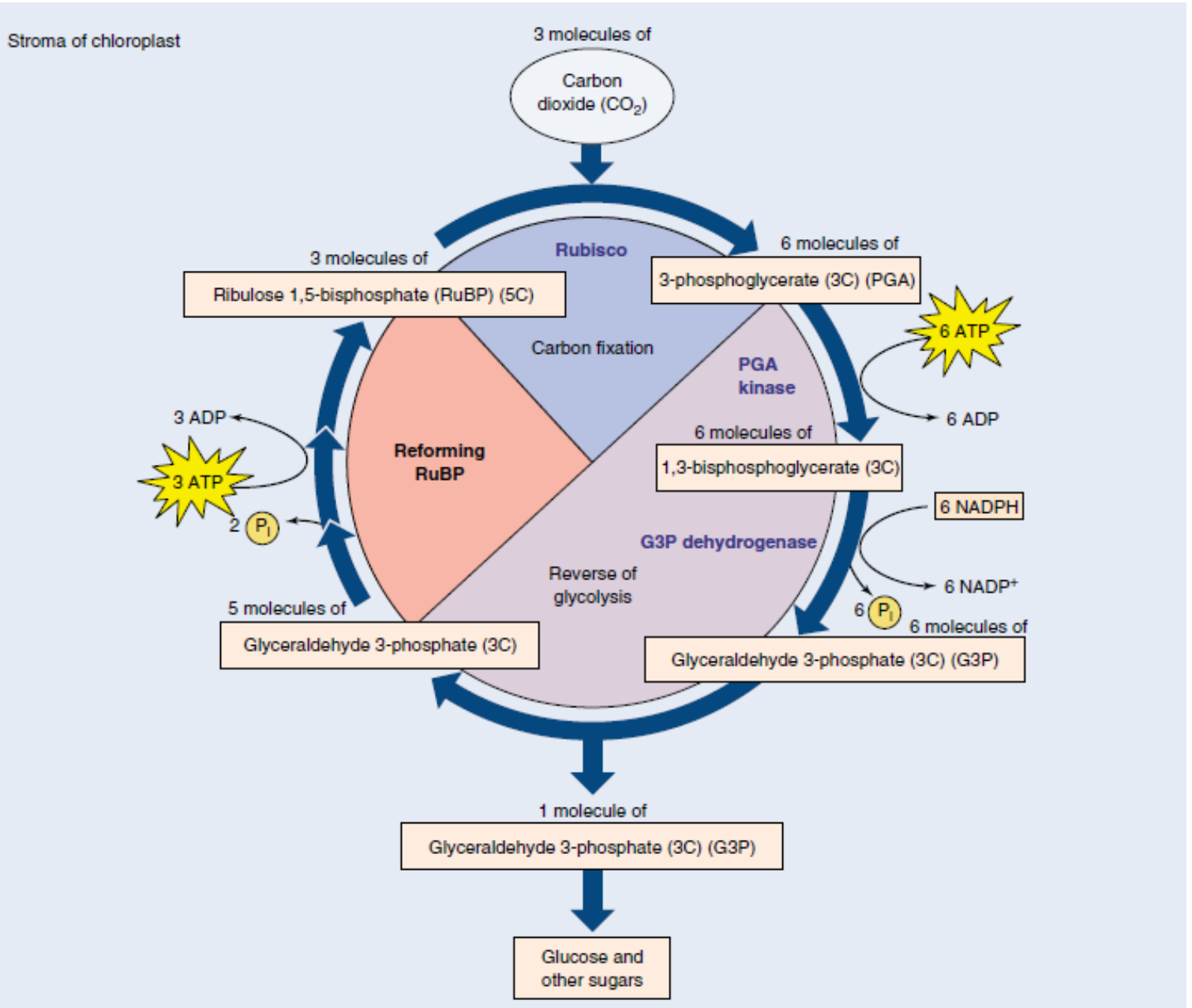
Melvin Calvin and his coworkers at the University of California worked out the first step of what later became known as the Calvin cycle. They exposed photosynthesizing algae to radioactive carbon dioxide (14CO2). By following the fate of a radioactive carbon atom, they found that it first binds to a molecule of ribulose 1,5-bisphosphate (RuBP), then immediately splits, forming two molecules of phosphoglycerate (PGA). One of these PGAs contains the radioactive carbon atom. In 1948, workers isolated the enzyme responsible for this remarkable carbon-fixing reaction: RUBISCO.

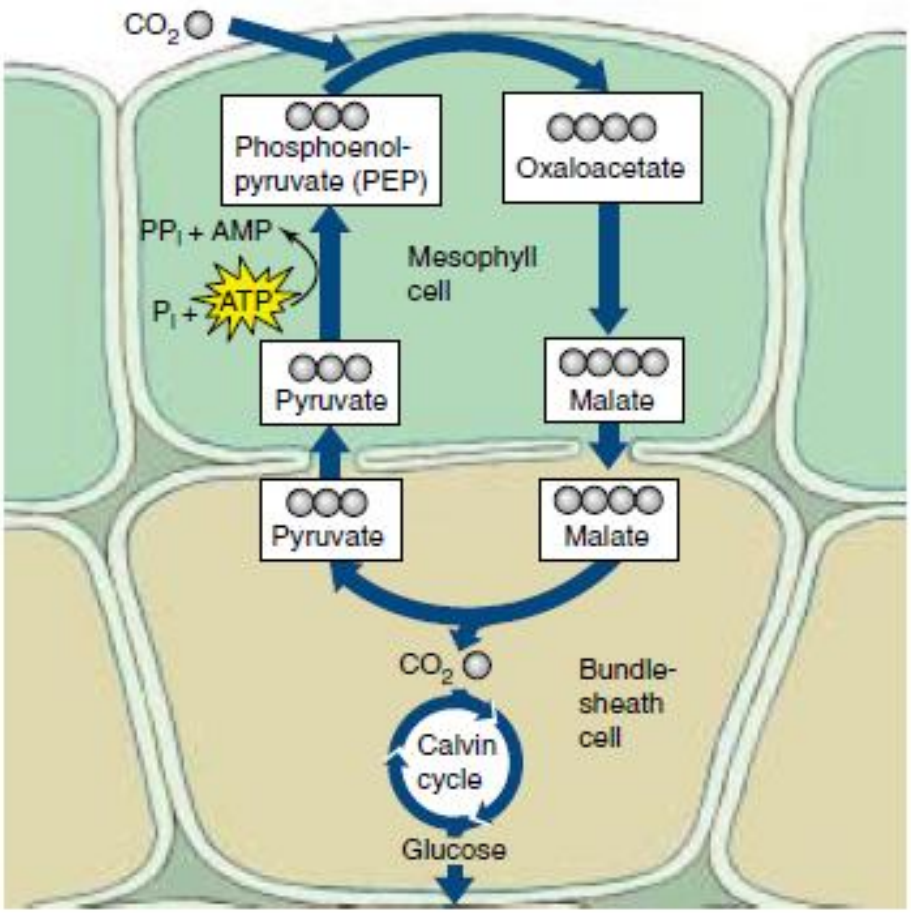
Photosynthesis

- Light-independent reaction Reaction)
  - Does not require light
- Calvin Cycle
    - Occurs in stroma of chloroplast
    - Requires CO_2
    - Uses ATP and NADPH as fuel to run
    - Makes glucose sugar from CO_2 and Hydrogen



CO2 + 9 ATP + 6 NADPH + water —→
glyceraldehyde 3-phosphate + 8 Pi + 9 ADP + 6 NADP+

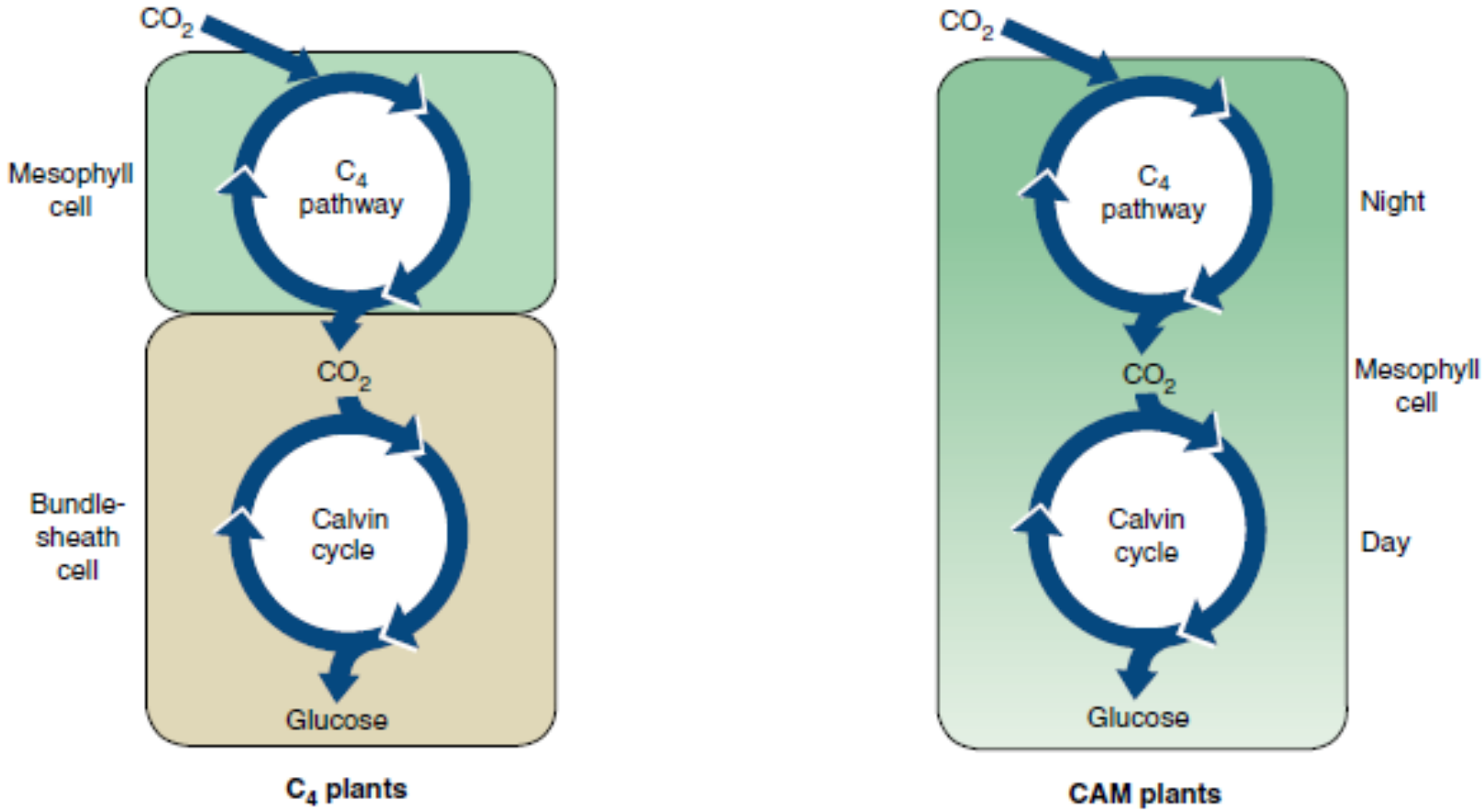

Stroma of chloroplast
3 molecules of
Carbon dioxide (CO_2)
Rubisco
3 molecules of
Ribulose 1,5-bisphosphate (RuBP) (5C)
6 molecules of
3-phosphoglycerate (3C) (PGA)
6 ATP
6 ADP
Carbon fixation
PGA kinase
3 ADP
3 ATP
2 P_i
Reforming RuBP
6 molecules of
1,3-bisphosphoglycerate (3C)
6 NADPH
G3P dehydrogenase
Reverse of glycolysis
6 NADP+
6 P_i
5 molecules of
Glyceraldehyde 3-phosphate (3C)
6 molecules of
Glyceraldehyde 3-phosphate (3C) (G3P)
1 molecule of
Glyceraldehyde 3-phosphate (3C) (G3P)
Glucose and other sugars



Rubisco, the enzyme that catalyzes the key carbonfixing reaction of photosynthesis, provides a decidedly suboptimal solution. This enzyme has a second enzyme activity that interferes with the Calvin cycle, *oxidizing* ribulose 1,5-bisphosphate. In this process, called **photorespiration,** O2 is incorporated into ribulose 1,5-bisphosphate, which undergoes additional reactions that actually release CO2

C4 plants: corn, sugarcane, sorghum, and a number of other grasses.

Carbon fixation in C4 **plants.** This process is called the C4 pathway because the starting material, oxaloacetate, is a molecule containing four carbons.



A comparison of C4 and CAM plants. Both C4 and CAM plants utilize the C4 and the C3 pathways. In C4 plants, the pathways are separated spatially: the C4 pathway takes place in the mesophyll cells and the C3 pathway in the bundle-sheath cells. In CAM plants, the two pathways are separated temporally: the C4 pathway is utilized at night and the C3 pathway during the day.

A second strategy to decrease photorespiration in hot regions has been adopted by many succulent (water-storing) plants such as cacti, pineapples, and some members of about two dozen other plant groups. This mode of initial carbon fixation is called **crassulacean acid metabolism (CAM),** after the plant family Crassulaceae.

Unit 3
Nitrogen metabolism

Nitrogen metabolism
Biological Nitrogen fixation by free living and in symbiotic association, structure and function of enzyme Nitrogenase. Nitrate assimilation: Nitrate and Nitrite reductase. Primary and secondary ammonia assimilation in plants; ammonia assimilation by Glutamine synthetase-glutamine oxoglutarate amino transferase (GS-GOGAT) pathway. Seed storage proteins in legumes and cereals

Nitrogen fixation is of two types

Non Biological Fixation

Atmospheric nitrogen fixation (or Natural) by lightning:- It contributes about 10% of the total nitrogen fixation. Usually atmospheric nitrogen fixation (or Natural) by lightning occurs in rainy season during lightning or thunder storms.

Industrial fixation through the Haber - Bosch process and combustion. Some ammonia is also produced industrially by the Haber-Bosch process. When nitrogen (dinitrogen) combines with hydrogen in the presence of an iron-based catalyst, at a pressures of 35-100 MPa and fairly high temperature. Usually fossil fuels are used both as a source of energy and hydrogen. Most nitrogenous fertilizers are now derived from atmospheric nitrogen through this type of fixation process.

Biological fixation by certain microbes - alone or in a symbiotic relationship with some plants and animals. Biological nitrogen fixation was discovered by the Dutch microbiologist MartinusBeijerinck. It contributes 60% of total nitrogen fixation. But the major conversion of atmospheric N2 into salts of nitrogen, and then into proteins, is achieved by microorganisms (prokaryotes) such as bacteria, fungi and algae in the process called biological nitrogen fixation (or dinitrogen fixation). Microorganisms that fix nitrogen are called diazotrophs.

- Free living or non-symbiotic nitrogen fixation: The fixation of free nitrogen of the soil by all the microorganisms living freely or outside the plant cell is called non-symbiotic biological N2 fixation. It is performed by the aerobic and anaerobic bacteria and blue green algae.
- By bacteria Nitrogen fixing bacteria: which are present in the soil convert free nitrogen into soluble compound which are absorbed from the soil by plants. The nitrogen fixing bacteria are of four types:-
- Free living non-photosynthetic aerobic nitrogen fixing bacteria e.g., Azotobacter, Beijerinckia and Derxia.
- Free living non-photosynthetic anaerobic nitrogen fixing bacteria e.g., Clostridium.

- Free living photosynthetic nitrogen fixing bacteria e.g, Chromatium, Rhodopseudomonas, Rhodospirillum.
- Free living chemosynthetic nitrogen fixing bacteria e.g., Desulfovibrio
- By free living nitrogen fixing Blue-Green algae. About 15 genera of photosynthetic cyanobacteria (blue-green algae) are found freely in the soil where they fix free N2 into nitrogenous and ammonium compound. Mostly they are heterocysts. e.g, *Nostoc*, *Anabaena*, *Aulosira*, *Cylindrospernum*, *Calothrix*. Nitrogen fixation occurs in special thick walled cells termed heterocysts or heterocytes (H) which occur at intervals along the cyanobacterial filaments. This separation of cellular functions is necessary because cyanobacteria have oxygen-evolving photosynthesis but the nitrogen-fixing enzyme, nitrogenase, is unstable in the presence of oxygen. This problem is overcome because the heterocysts contain only part of the photosynthetic apparatus, termed photosystem I, which can be used to generate energy (as ATP). But the heterocysts do not contain photosystem II, which is used to split water into hydrogen (for combination with CO2 to produce organic products) and oxygen. Non-heterocystous nitrogen fixing blue-green algae are less in number e.g., Oscillatoria, Phormidium, Gleocapsa.
- Free-living, non-photosynthetic bacteria depend on soil organic matter as a food source whereas the photosynthetic microorganisms may derive their food from the products of photosynthesis.
- The nitrogen fixing activity of free-living, non-photosynthetic, aerobic bacteria is strongly dependent on favorable moisture conditions, oxygen, and an organic food source. Anaerobic representatives (Clostridium) predominate in grassland and waterlogged soils and soil aggregates where moisture conditions and organic substrates are available but oxygen supply to the micro-environment of the bacteria is severely restricted.

Symbiotic

Some N_2-fixing organisms develops loose (associative) symbiosis with plants or animals (Acetobacter and sugarcane), or establish longer-term relationships within specialized structures provided by their host (Rhizobium and the legume nodule). To provide them with sugars, supplying both a source of energy and a source of carbon for the bacterium's own synthetic reactions. Symbiosis is a close ecological relationship between the individuals of two (or more) different species. Sometimes a symbiotic relationship benefits both species, sometimes one species benefits at the other's expense, and in other cases neither species benefits.

The fixation of free nitrogen of the soil by N_2-fixing organisms living symbiotically inside plants is known as symbiotic biological nitrogen fixation.

Nitrogen fixation through nodule formation in Leguminous plants

The bacteria responsible for the formation of root nodules in leguminous plants belong to the genus Rhizobium. Rhizobium also lives free in soil but only fixes N_2 when inside plant The symbiotic Bacteria Rhizobia (from the Greek words Riza = Root and Bios = Life) are soil bacteria that fix nitrogen (diazotrophy) after becoming established inside root nodules of legumes. According to host specificity and growth of bacteria have been divided into three groups:

Genus	Species	Plant host
Rizobium	leguminosarum	Peas
Rizobium	Meliloti	Lucerne
Rizobium	Trifolii	Clover
Rizobium	phaseoli	Beans
Rizobium	Lupine	Lupins

Bradyrhizobiumjaponicum is the group of slow growing symbionts of Soybeans (plant host).

Azorhizobiumcaulinodans is a bacterium that forms stem nodules in Sesbania (plant host).

The bacteria "invade" the plant and cause the formation of a nodule by inducing localised proliferation of the plant host cells. Root nodules acts as a site of Nitrogen fixation. The root nodules contain a pigment called leghaemoglobin (serving the same function as the oxygen-carrying haemoglobin in blood).The heme (oxygen-binding) portion is produced by the bacterium, while the globin (protein) portion is produced by the host plant, again showing the closeness of the symbiotic relationship. The function of this molecule in nodules is to reduce the amount of free oxygen, and thereby to protect the nitrogen-fixing enzyme nitrogenase, which functions only under anaerobic conditions. Nitrogenase is the only enzyme that can split nitrogen molecule for nitrogen fixation.

Nitrogen fixation through nodule formation in None-Leguminous plants :- There are many plants belonging non- Leguminosae families, specially shrubs and plants which produces root nodules. Example: Frankia is a genus of the bacterial group termed actinomycetes - filamentous bacteria.

Frankia form nitrogen-fixing root nodules (sometimes called actinorhizae) with numerous genera of non-leguminous angiosperms, such as alder (Alnus species), sea buckthorn (Hippophaerhamnoides, which is common in sand-dune environments) and Casuarina (a Mediterranean tree genus).

Alder and the other woody hosts of Frankia are typical species that invade nutrient-poor soils. These plants probably benefit from the nitrogen-fixing association, while supplying the bacterial symbiont with photosynthetic products.

Rhizobiums also form nitrogen-fixing root nodules with genus Parasponia.

Sometimes nodules are also formed in the roots of certain gymnosperms e.g., Podocarpus and in the leaves of Pavettazinumermanniana and Chomelia.

Nitrogen fixation through non-nodulation: In some plants root nodules are not formed but symbiotic nitrogen fixation takes place. Examples: Lichens live as symbionts with photosynthetic cyanobacteria (blue green algae or Green chlorophyllous and with fungi.

Anthroceros (Bryophte): It contains Blue green alga Nostoc inside mucilage cavities present on ventral side.

Azolla: The water fern, Azolla lives symbiotically with the nitrogen-fixing cyanobacteria (Anabaena azollae) Azolla is grown in rice paddies early in the season. As the rice grows above the water surface, it shades out the fern, which dies, releasing the stored nitrogen. In this way, the paddy is fertilized without application of chemical fertilizers.

Cycas (gymnosperms) It contains cyanobacteria (blue-green algae) Anabaena or Nostoc. Aerial roots contain a nitrogen-fixing cyanobacterialsymbiont.

Gunneramacrophylla (angiosperms): Its stem contains Nostoc.

Associative Symbiotic Nitrogen Fixation

When bacteria form a close association with the roots of cereals and grasses and fix nitrogen, the association is of loose mutualism type and known as loose (associative) symbiosis and this type of nitrogen fixation is known as associative symbiotic nitrogen fixation. The bacteria grow in the rhizosphere in close contact with the roots, sometimes invade the outer cortical regions of the roots, and fix nitrogen. Azospirillumbransilense (= Spirillumlipoferum) a bacterium discovered J. Dobereiner (Edomonds, 1978), is the bacterium forming associate-symbiosis with the cereal roots. Others are Pseudomonas azotogensis, Enterobacter, Bacillus, Klebsiella etc.

Rates of symbiotic N_2 fixation in legumes vary with plant species and cultivator, growing season, and soil fertility. Some forage legumes can fix 600 kilograms per hectare per year but more common values are 100 to 300 kilograms per hectare per year. Rates for grain legumes are often lower. Inclusion of legumes in crop rotations is generally thought to improve soil nitrogen levels, but benefits depend on the level of N_2 fixed and the amount of nitrogen removed in grain or forage. A good soybean crop might fix 180 kilograms per hectare but remove 210 kilograms per hectare in the grain. Non-symbiotic bacteria fix only upto 5 kg of nitrogen per hectare in one year

Formation of Nodule

- Rhizobia is the group of genera of alpha-proteobacteria (family Rhizobiaceae) which includes all of the nitrogen-fixing species that produce nodules with legumes such as clover and soybean, Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Phyllobacterium, Rhizobium, and Sinorhizobium, as well as the plant pathogen Agrobacterium.

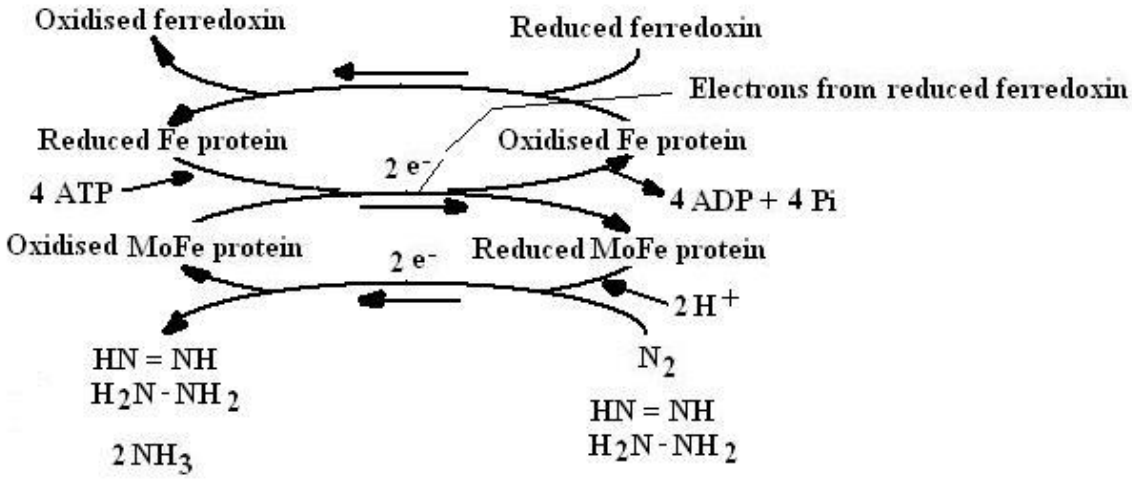
- Rhizobia produce stem or root nodules on their host(s), and within these nodules receive protection from external stresses and energy for growth and N2 fixation. The host receives most of the nitrogen it needs for growth.
- Only infection via root hairs is considered here. Plants of legume family secrete flavonoids which are recognized by bacterial NodD protein When NodD binds flavonoid it activates other nodulation genes.
- Rhizobium secretes Nod factors (Some nod genes encode enzymes that make Nod factors) the leguminous plant recognizes Nod factors.
- Then growing root hair of a leguminous plant comes in contact with the bacterium - Rhizobium, the growing root hairs curl and form a pocket for the rhizobia. The bacteria invade the plant by a newly formed infection thread growing through it. The root hair cell wall.
- Simultaneously, cortical cells are mitotically activated, giving rise to the nodule primordium.
- lnfection threads grow toward the primordium, and the bacteria are then released into the cytoplasm of the host cells, surrounded by a plant derived peribacteroid membrane (PBM). This separation is usually seen as a mechanism to suppress plant defense responses likely to harm the bacteria.
- With the production of the infection threads, bacteria produce cytokinins (type of plant hormone). Cytokinins promote division of plant cells to form nodules and nodules begin to form in the root hairs.
- The nodule primordium thereupon develops into a mature nodule, while the bacteria differentiate into their endosymbiotic form, which is known as the bacteroid. Bacteroids, together with the surrounding PBMs, are called symbiosomes.
- Cell division now sets in, in the infected tissue leading to nodule formation. The area of active N2 fixation is either pink or red in color due to the presence of leghaemoglobin needed for oxygen transport. The nodule thus formed establishes a direct vascular connection with the host for the exchange of nutrients.
- All of the steps of nodule development involve the expression of nodule-specific plant genes, the so called nodulin genes. The early nodulin genes encode products that are expressed before the onset of nitrogen fixation and are involved in infection and nodule development. The products of the late nodulin genes are involved in the interaction with the endosymbiont and in the metabolic specialization of the nodule.

Mechanism of nitrogen fixation

The bacteroids within the nodules formed on legume roots fix nitrogen.

Structure and Operation of Nitrogenase

The nitrogenase is an enzymatic complex, which converts atmospheric nitrogen (N2) to ammonia. Then nitrogenase complex exists in both free living nitrogen fixing organisms as well as in symbiotic nitrogen fixing bacteria. Nitrogenase is a complex of two separately isolated proteins- an iron protein or dinitrogenreductase and a molybdenum-iron protein or dinitrogenase. The proteins have a negative redox potential. The MoFe protein (Iron-Molybdenum protein), is a heterotetramer composed of two alpha subunits and two beta subunits. The protein contains two copies of each of two types of clusters: P clusters and FeMo cofactors. Each P cluster contains 8 iron atoms and 7 sulfides linked to the protein by 6 cysteinate residues. Each FeMo cofactor contains one molybdenum atom, 7 iron atoms, 9 sulfides. This protein is responsible for reducing atmospheric nitrogen to ammonia via a series of electron transfers within the protein to the substrate molecule. The reaction requires the addition of six electrons for each nitrogen molecule that is split into two ammonia molecules. The Fe protein (Nitrogenase Reductase - NR) is a dimer and formed by 2 subunits of polypeptide chains linked by a 4Fe-4S cluster. Each monomer contains an ATP binding site.



The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to H2N-NH2, and this in turn is reduced to 2NH3.The reactions occur while N2 is bound to the nitrogenase enzyme complex.

The favorable condition for nitrogen fixation is

- Presence of enzymes nitrogenase and hydrogenase in the nitrogen fixing cells or organisms.
- Presence of leghaemoglobin which protect the enzyme nitrogenase from oxygen.
- Ferredoxin which supplies electrons for this process.
- A source of hydrogen (strong reducing agent) like NADPH or FMNH2

- Constant supply of ATP to transfer hydrogen atoms to dinitrogen.ATP is provided by aerobic respiration of sugars, ultimately produced by photosynthesis. Phosphorous is an important component of the biochemical energy source, ATP (adenosine triphosphate). Thus, for legumes to fix N, there must be adequate available soil P.
- Presence of coenzymes and cofactors.
- Compounds for trapping ammonia formed by the reduction of dinitrogen (N2). Nitrogen fixation is controlled by plant nod genes and bacterial nod, nif and fix gene cluster. Biological nitrogen fixation by free living and symbiotic bacteria is carried out by step by step progressive reduction of dinitrogen (N2) molecules by the addition of of a pair of hydrogen atoms. Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation. In the heterocystous bacteria the primary electron donor to nitrogenase is also a ferrredoxin, but it receives electrons produced by the action of light on the photosynthetic apparatus. The electrons are supplied via ferredoxin to nitrogenasereductase and then nitrogenase. The reductase donates 8 electrons in succession to the nitrogenase cofactor, a molybdenum-iron containing active center which catalyses the actual reduction of dinitrogen. Iron (Fe+3) and molybdenum (Mo+4) of enzyme nitrogenase takes part in attachment of a dinitrogen molecule (N2) and weaken the bonds between the two atoms of the nitrogen. The weakened molecule of nitrogen is reduced by the reducing agent (NADPH, FMNH2). It produces dimide (N2H2), hydrazine (N2H4) and then ammonia (2NH3) Where one molecule of N2 is reduced in the presence of protons to 2 NH3, and H2 as a byproduct. Semi-activated nitrogenase can reduce easy substrates such as acetylene. In the typical reaction, two molecules of ATP are consumed for each electron transferred. 16 ATP's are needed to fix a single nitrogen molecule in nitrogen fixation, the plant regulates the nitrogenase's activity and expression according to reduced nitrogen availability and oxygen presence.
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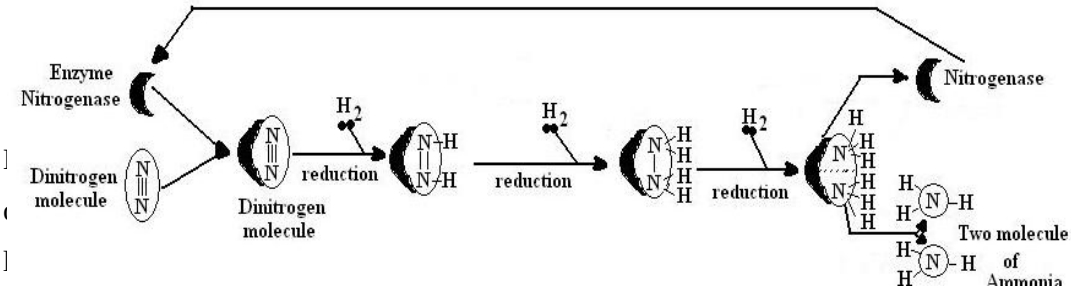


ecular oxygen is a strong inhibitor of the nitrogenase Mo-Fe cofactor and is removed by the plant oxygen binding protein leghemoglobin in the root nodules. Some of thecyanobacteria have yet another mechanism for protecting nitrogenase: nitrogen fixation occurs in special cells (heterocysts) which possess only photosystem I (used to generate ATP by light-mediated

reactions) whereas the other cells have both photosystem I and photosystem II (which generates oxygen when light energy is used to split water to supply H2 for synthesis of organic compounds). Nitrogenase also converts hydrogen ions to hydrogen gas at the same time thus consuming even more ATP in the process.

Nitrogen metabolism

Nitrogen metabolism All of the nitrogen in a plant, whether derived initially from nitrate, nitrogen fixation, or ammonium ions, is converted to ammonia, which is rapidly incorporated into organic compounds through a number of metabolic pathways.

Nitrogen reduction or Nitrification

Ammonia formed as a result of nitrogen fixation is used for the synthesis of amino acids. Amino acids are transported through phloem to other parts for the synthesis of proteins .Ammonium ions can be taken by higher plants but plants are more adapted to absorb nitrate ($NO3^-$) than ammonium ions ($NH4^+$) from soil. Nitrification is an aerobic microbial process by which specialized bacteria oxidize ammonium to nitrite and then to nitrate. It is accomplished by nitrifying bacteria like nitrosomonas, nitrosococcus and nitrobacter. Nitrification is a two-step process. The first stage is the oxidation of ammonium (NH4+) to nitrite (NO2-), a function carried out by bacteria in the genus Nitrosomonas. The nitrite formed is rapidly oxidized to nitrate (NO3-) by bacteria in the genus Nitrobacter.

The nitrifying bacteria nitrosomonas, nitrosococcus and nitrobacter are chemoautotrophs. They gain their energy by chemical oxidations (chemo-) and they are autotrophs (self-feeders)

because they do not depend on pre-formed organic matter. As

they derive energy for synthesis of organic food by oxidising inorganic materials ammonia. Nitrification is an autotrophic process during which energy is liberated from the oxidation of ammonium with the biosynthesis of simple inorganic molecules such as carbon dioxide and water into organic compounds and oxygen is an electron acceptor. Nitrifying bacteria, gain their energy by oxidising ammonium, while using CO2 as their source of carbon to synthesize organic compounds

Nitrogen assimilation

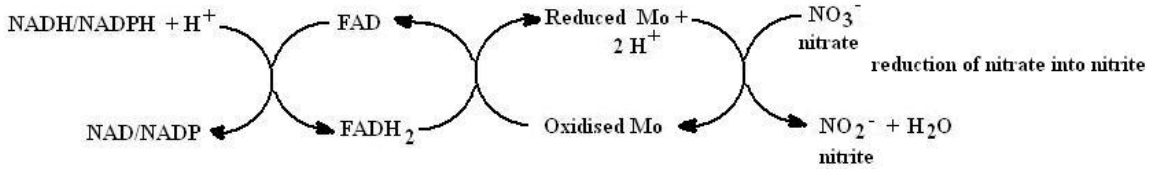
Nitrogen assimilation is the conversion of inorganic nitrogen (such as nitrate) into an organic form of nitrogen like, for example, an amino acid. Nitrate is reduced for this purpose by enzymes first to nitrite (by nitrate reductase), then to ammonia (by nitrite reductase). Ammonia is incorporated into

amino acids. The process of nitrate reduction to ammonia is accomplished in two steps, each mediated by specific enzymes.

Reduction of nitrate to nitrite

The nitrate serves as a terminal electron acceptor for anaerobic respiration. The nitrate is reduced to nitrite by enzyme nitrate reductase. It is co-enzyme NADH/NADPH-dependent according to organism.

The enzyme is a flavoprotein that contains iron and molybdenum serves as an electron carrier. FAD receives hydrogen from reduced co-enzyme NADH/NADPH +H+ (serves as hydrogen donor) for the reduction of nitrate



Reduction of nitrite to ammonia

Nitrite reductase reduces the nitrite ions to ammonium ions. Nitrite reductase does not require molybdenum and may contain copper and iron. Ferredoxin is the direct source of electrons for nitrite reduction, which occurs in higher plants mostly in the leaves.

The nitrite ions formed in other parts of the plant are also transported to leaves and reduced to ammonia. The reduced coenzyme NADPH +H+ or NAD + H+ serves as hydrogen donor for the reduction of nitrite.

Ammonia thus formed as a result of nitrogen fixation is not given out .It is highly toxic and used for the synthesis of amino acids. Amino acids are the building blocks for the synthesis of proteins. The amino acids are transported through phloem to other parts of the plant for the synthesis of proteins.

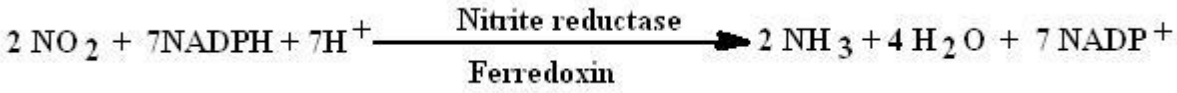
Amino acids are the initial products of nitrogen assimilation. An amino acid molecule consists of at least one carboxyl (-COOH) groups and one or several amino (-NH2) groups. Majority of amino acids are synthesized in plants by two main processes.

Reductive animation

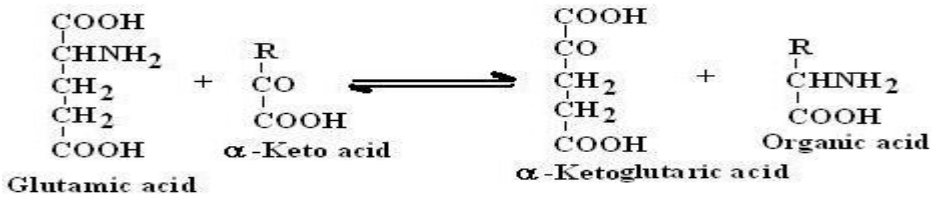
In this process, ammonia reacts with alpha-ketoglutaric acid to form glutamic acid in the presence of enzyme glutamate dehydrogenase. A reduced coenzyme NADPH in leaves, NADH in roots is required.

Transamination

Glutamic acid is the main amino acid from which other 17 amino acids are formed through transamination. With the help of enzyme transaminase, amino group of an amino acid (-CHNH2) is



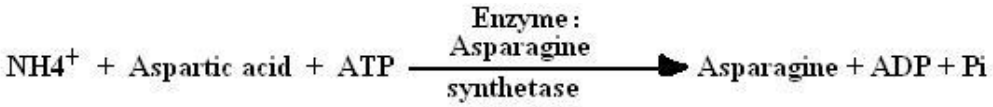
exchanged with keto group (-CO) of ketoacid. Pyridoxal phosphate is required as coenzyme which is obtained from a vitamin.

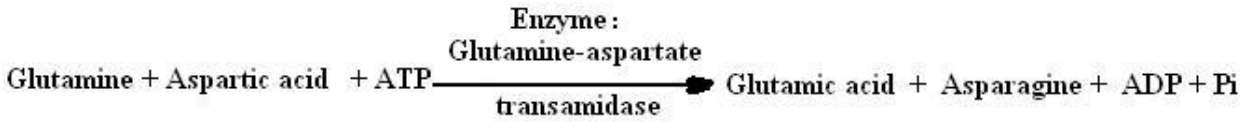


In most of transamination glutamic acid is present as one of the reactants. The other reactant may be any one of a number of alpha-keto acids, which alpha keto acid receives the amino group from glutamic acid is determined by the specificity of the enzyme.

Amides

Amides are nitrogen-containing organic compounds and contain more nitrogen than amino acids. Amides are formed when amino acids bond to each other to form proteins. Amino acids in which hydroxyl group (-OH) of carboxylic group is replaced by amino group (-NH2) from ammonia, or another amino acid. ATP is required. The two important amides found in plants are asparagine and glutamine.





They are formed from two amino acids namely glutamic acid and aspartic acid. This reaction takes place in the presence of the enzymes glutamine synthetase or asparagine synthetase.

The glutamate synthase or GS-GOGAT cycle

For many years it was thought that bacteria and higher plants assimilate ammonia into glutamate via the GDH pathway, as in certain fungi and yeasts. However, in bacteria it became clear in 1970 that an alternative pathway of ammonia assimilation [involving glutamine synthetase (GS)

[EC 6.3.1.2] and an NADPH-dependent glutamine:2-oxoglutarate amidotransferase (GOGAT) [EC 1.4.1.13] or glutamate synthase, must be operating when ammonia is present in the growth medium at low levels (Tempest et al, 1970). Thus, N-starvation leads to derepression and activation of GS (with a high affinity for NH_3) and derepression of GOGAT, and repression of GDH (with a relatively low affinity for NH_3) (Tempest et al, 1970). High ammonia availability leads to repression and deactivation of GS and induction of GDH.

GDH

$$NH_3 + 2\text{-oxoglutarate} + NADPH + H^+ \text{<---> glutamate} + NADP^+$$

GS-GOGAT

$$NH_3 + \text{glutamate} + ATP \text{ ---> glutamine} + ADP + Pi$$

$$\text{glutamine} + 2\text{-oxoglutarate} + NADPH + H^+ \text{ ---> 2 glutamate} + NADP^+$$

Both the GDH and GS-GOGAT pathways produce 1 mole of glutamate from 1 mole each of NH_3, 2-oxoglutarate and NADPH. But note that the GS-GOGAT pathway is energetically more costly than the GDH pathway, consuming 1 ATP.

Escherichia coli is now known to have two primary pathways for glutamate synthesis. The GS-GOGAT pathway is essential for glutamate synthesis at low ammonium concentrations and for regulation of the glutamine pool, and is used when the cell is not under energy limitation. The GDH pathway is used in glutamate synthesis when the cell is limited for energy (and carbon; i.e. glucose-limited growth) but ammonium and phosphate are present in excess. *Synechocystis* sp. strain PCC 6803 utilizes the GS-GOGAT pathway as the primary pathway of ammonia assimilation, but the presence of GDH appears to offer a selective advantage for the cyanobacterium under nonexponential growth conditions. These dual pathways may be common to bacteria, cyanobacteria, algae, yeasts and fungi.

Re-examination of ammonia assimilation in yeasts and fungi now reveals the operation of alternative pathways of glutamate synthesis, independent of NADPH-GDH:

- Mutants of *Aspergillusnidulans* lacking NADP-GDH activity grow more poorly than wild-type strains on ammonium as a sole nitrogen source. The leaky growth of these mutants is indicative of an alternative pathway of ammonium assimilation and glutamate biosynthesis. *A. nidulans* mutants disrupted in the *gltA* encoding GOGAT, were found to be dispensable for growth on ammonium in the presence of NADP-GDH. However, a strain carrying

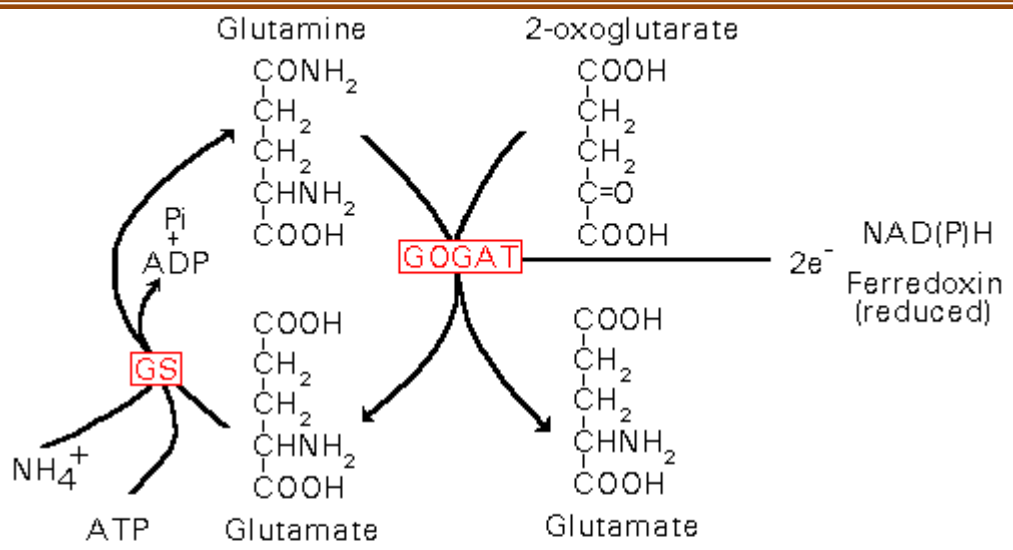
the *gltA* inactivation together with an NADP-GDH structural gene mutation (*gdhA*) was unable to grow on ammonium or on nitrogen sources metabolized via ammonium.

- *Schizosaccharomycespombe* mutants lacking either NADPH-GDH or GOGAT are still able to grow on ammonium as sole nitrogen source. Complete lack of growth on ammonium as sole N source is seen only in double mutants lacking both NADPH-GDH and GOGAT.

- In contrast to *Candida utilis*, analysis of ^{15}N-ammonium assimilation in actively growing mycelium of *Agaricusbisporus* indicates participation of the GS-GOGAT pathway, and no participation of NADP-GDH. $^{13}NH_3$ tracer studies indicate that the GS-GOGAT pathway is the major route of ammonium assimilation in *Candida albicans* and also in nitrogen-starved cultures of *Saccharomyces cerevisiae* and *Candida tropicalis*.

The yeast *Saccharomyces cerevisiae* synthesizes glutamate through the action of either NADP-glutamate dehydrogenase (NADP-GDH), encoded by *GDH1* (under conditions of ammonia excess), or through the combined action of glutamine synthetase (GS) and glutamate synthase (GOGAT), encoded by *GLN1* and *GLT1* (under conditions of ammonia limitation). Dynamic modeling indicates that the GS-GOGAT pathway plays a more important physiological role in yeast than is generally assumed. However, a double mutant of *S. cerevisiae* lacking NADP-GDH and GOGAT activities was able to grow on ammonium as the sole nitrogen source and thus to synthesize glutamate through a third pathway. A computer search for similarities between the *GDH1* nucleotide sequence and the complete yeast genome led to the discovery that *GDH1* showed high identity to an open reading frame (*GDH3*) on chromosome I. Triple mutants impaired in *GDH1*, *GLT1*, and *GDH3* are strict glutamate auxotrophs, indicating that *GDH3* plays a significant physiological role, providing glutamate when *GDH1* and *GLT1* are impaired. This appears to be the first example of a microorganism possessing three pathways for glutamate biosynthesis.

Following the discovery of glutamate synthase (GOGAT) in bacteria, a similar activity was sought in plants. A ferredoxin-dependent glutamate synthase [EC 1.4.7.1] was discovered in photosynthetic tissues of higher plants in 1974, and an NADH-dependent "glutamate synthetase" in non-photosynthetic plant tissues in the same year.



The Glutamate Synthase Cycle Lea *et al* (1992)

This pathway was termed the glutamate synthase cycle by Rhodes *et al* (1980). [Rhodes D, Sims AP, Folkes BF (1980) Pathway of ammonia assimilation in illuminated *Lemna minor*. Phytochemistry 19:357-365.]

Evidence in favor of the operation of the GS-GOGAT cycle as the primary pathway of ammonia assimilation in higher plants has been reviewed by several authors (e.g. Miflin and Lea, 1980; Lea et al, 1992). This evidence includes:

- almost complete inhibition of $^{15}NH_4^+$ assimilation by the glutamine synthetase (GS) inhibitor, methionine sulfoximine (MSX).
- quantitative analysis of $^{15}NH_4^+$ in *Lemna minor* is consistent with incorporation of ^{15}N primarily into glutamine-amide, followed by transfer to glutamate and the amino-group of glutamine via the action of GOGAT and GS, respectively, provided that it is assumed that the GS-GOGAT cycle is compartmentilized in the chloroplast, and that a second site of glutamine synthesis occurs in the cytoplasm.
- the maize *gdh1*-null mutant exhibits about 5% of the total GDH enzyme activity of wildtype plants. Although this mutant exhibits a slightly reduced total rate of $^{15}NH_4^+$ assimilation, when methionine sulfoximine (MSX), a potent inhibitor of GS is supplied, this completely blocks $^{15}NH_4^+$ assimilation in both the mutant and wildtype roots and shoots. The contribution of GDH to net ammonia assimilation is small in comparison to that catalyzed by the GS-GOGAT cycle.

- mutants of *Arabidopsis* and barley defective in GS or GOGAT exhibit markedly impaired ammonia assimilation, especially under photorespiratory conditions.

Enzyme kinetic considerations also suggest a role for the GS-GOGAT pathway in ammonia assimilation at low tissue/cell ammonia concentrations. GS has a much higher affinity for ammonia than GDH and is viewed as a scavenger of ammonia in bacteria and in plants.

The major role of GDH in tissue cultured cells is the oxidation of glutamate to provide sufficient carbon skeletons for effective functioning of the TCA cycle.

In wildtype *Arapidopsis*, *GDH1* mRNA accumulates to high levels in dark-adapted or sucrose-starved plants; light or sucrose treatment each repress GDH1 mRNA accumulation. These results suggest that the GDH1 gene product functions in the direction of glutamate catabolism under carbon-limiting conditions. Low levels of GDH1 mRNA present in leaves of light-grown plants can be induced by exogenously supplied ammonia. Under such conditions of carbon and ammonia excess, GDH1 may function in the direction of glutamate biosynthesis. The recessive *Arabidopsis* glutamate dehydrogenase-deficient mutant allele *gdh1-1* cosegregates with the *GDH1*. The *gdh1-1 mutant* displays a conditional phenotype; seedling growth is specifically retarded on media containing exogenously supplied inorganic nitrogen, suggesting that GDH1 plays a nonredundant role in ammonia assimilation under conditions of inorganic nitrogen excess. This is consistent with the fact that the levels of mRNA for GDH1 and chloroplastic glutamine synthetase (GS2) are reciprocally regulated by light.

Seed Storage Proteins

Plants are the primary source of food for humans and feed for livestock. A major part of the human diet all over the world consists of cereals and legumes. 70% of human food comprises cereals and legumes and the remaining 30% comes from animals. Plants accumulate storage substances such as starch, lipids and proteins in certain phases of development. Storage proteins accumulate in both vegetative and reproductive tissues and serve as a reservoir to be used in later stages of plant development. Seed storage proteins are a group that comprises proteins generated mainly during seed production and stored in the seed that serve as nitrogen sources for the developing embryo during germination. The average protein content of cereal grains is 10-15 % of their dry weight that of leguminose seeds 20-25, while it is only 3-5 % in normal leaves. Besides seeds, storage proteins can also be found in root and shoot tubers. No clear definition what a storage protein is exists. The term was coined for all those proteins whose share in the total protein amount of the cell is greater than 5%. Typically, seed storage proteins have the following properties

- The proteins have no enzymatic activities.
- They serve as nitrogen sources for the germinating seed.
- They occur normally in an aggregated state within a membrane surrounded vesicle (protein bodies, aleuron grains).
- They are often built from a number of different polypeptide chains.

Seed proteins were empirically classified by T.B. Obsorne based on their solubility as follows:

- Albumins: Water extractable (1.6S-2S);
- Globulins: Extractable in dilute salt solutions (7S-13S);
- Prolamins: Extractable in aqueous alcohol;
- Glutelins: Most difficult to solubilize; Extractable by weakly acidic or alkaline or dilute SDS solution

Seed storage proteins are important for human nutrition so an interest in the production of mutants with increased protein content or an increased amount of essential amino acids (lysine, methionine tryptophan etc.) exists. Leguminoses contain mostly two types of storage proteins, legumin and vicelin. The legumins - as well as the vicelins - are very similar in the different leguminose species. Gramineae contain a third type: prolamin and, depending on the origin, it is distinguished between the zeines (from Zea mays), the hordeines (from Hordeumvulgare) etc. In contrast to legumins and vicelins that are mainly located in the cotyledons of seeds, prolamines are found in the endosperm. The first detailed studies on plant storage proteins were carried through by Obsorne. Legumins occur in the seeds of many dicot families and that a compound similar to legumin is also produced in monocots. These molecules are usually polymer. They are typically constructed from two subunits, the acidic and the basic polypeptides. The quaternary structure is composed of six acidic and six basic polypeptides that are linked by disulfide bonds. The accumulation of seed storage protein is beneficial for the survival of plants and also an important source of dietary plant proteins

Seed proteins are generally incomplete in nutrition due to their deficiency in several essential amino acids, for example, lysine and tryptophan in cereals and methionine and cysteine in legumes. Attempts to breed crops with increased levels of lysine and methionine have been less than satisfactory. Modern biotechnology offers alternative approaches for rectifying this nutrition deficiency. Several transgenic strategies aimed at modifying the amino acid composition of plant proteins and enhancing the content of specific essential amino acid for nutrition improvement have been developed which include synthetic proteins, modification of protein sequences, over-expression of heterologous or homologous proteins, and metabolic engineering of the free essential amino acid pool and protein sink.

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III BSc Biochemistry - Plant Biochemistry (16BCU503A)

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	Chlorosis of leaves is due to the deficiency of	Carbon	nitrogen	sulphur	phosphorus	nitrogen
2	The release of NH^{4+} ion in the soil throueh the decon	Nitrification	biological nitrogen fixatio	Ammonification	denitrification	Ammonification
3	The conversion of ammonium ions into nitrites is per	Nitrobacter	nitrococcus	azotobacter	clostridium	nitrococcus
4	Oxidation of nitrites into nitrates is performed by	Nitrobacter	nitrosomonas	nitrococcus	clostridium	Nitrobacter
5	The process by which NH3 is converted to nitrates is	Nitrification	denitrification	ammonification	nitrogen fixation	Nitrification
6	Nitrate reductase is found in	Mitochondria	cytoplasm	vacuoles	golgi complex	cytoplasm
7	The electron transfer in the enzyme nitrite reductase	Mo	FAD	sirohaem	cyt b557	sirohaem
8	Which of the following is a free living chemo synthet	Chlorobium	clostridium	azotobacter	desulphovibrio	desulphovibrio
9	Which of the following is a free living anaerobic nitr	Desulphovibrio	clostridium	chlorobium	azotobacter	clostridium
10	The growth factor secreted by the roots of pisum sati	Serine	homoserine	glycine	alanine	homoserine
11	Which is the symbiotic nitrogen fixing bacteria?	Rhizobium	azotobacter	clostridium	nitrosomonas	Rhizobium
12	The pink colour of nitrogen fixing nodule is due to th	Xanthophylls	carotenoids	leghaemoglobin	bacteroids	leghaemoglobin
13	The conversion of nitrogen to ammonia or nitrogenou	Nitrogen assimilation	Nitrogen fixation	Denitrification	Nitrification	Nitrogen fixation
14	Which of the following N2 fixer is involved in sumbi	Rizobium	azotobacter	Rodospirillum	clostridium	Rizobium
15	To fix one molecule of nitrogen	6 ATP melecules are req	12 ATP melecules are req	16 ATP melecules are r	20 ATP melecules are re	16 ATP melecules are required
16	Conversion of NO2- to NO3- is carried out by	Nitrosomonas	Nitrosococcus	Nitrobacter	Clostridium	Nitrobacter
17	The process of conversion of soil NO3- to N2 is calle	Nitrification	Denitrification	Ammonification	nitrogen fixation	Ammonification
18	Leghaemoglobin creates	Anaerobic condition for	Aerobic condition for opt	Required oxygen concer	Suitable environment for	Anaerobic condition for optimum activity of nitrogenase
19	The nodule forming bacteria are:	Azotobacter	Nitrobactor	Clostridium	Rhizobium	Rhizobium
20	The conversion of gaseous nitrogen into the nitrates v	biological oxygen fixatio	atmospheric oxygen fixat	atmospheric nitrogen fix	biological nitrogen fixati	biological nitrogen fixation
21	The nitrites are transformed with the help of bacteria	nitrites	nitrates	ammonia nitrous	ammonium nitrate	nitrates
22	The bacteria which is responsible for ammonification	nitrogenous virus	nitrogenous bacteria	ammonifying virus	ammonifying bacteria	ammonifying bacteria
23	Dinitrogenase reductase is a	Monomer	Dimer	Trimer	Tetramer	Dimer
24	The reaction of glutamate and NH4+ to yield glutami	Uridylyltransferase	Adenylyltransferase	Glutamate synthase	Glutamine synthase	Glutamine synthase
25	The process in which nitrites and nitrates are reduce	ammonification	assimilation	nitrification	denitrification	denitrification
26	The bacteria which is responsible for ammonification	nitrogenous virus	nitrogenous bacteria	ammonifying virus	ammonifying bacteria	ammonifying bacteria
27	Plants absorb nitrates from soil and convert them int	urea	ammonia	nitrogen	Uric acid	ammonia
28	Which of the following is a common nitrogen acceptc	α-keto glutarate	pyruvate	oxaloacetate	fumarate	α-keto glutarate
29	The two nitrogen of urea are derived from	Aspartate and ammonia	Glutamate and ammonia	Argino succinate and an	Alanine and ammonia	Aspartate and ammonia
30	How many molecules of ATP are hydrolysed to form	10	5	16	15	16
31	The nitrogen atoms of urea produced in the urea cycl	nitrate	ammonia and aspartic aci	nitrite	ammonia	ammonia and aspartic acid
32	The products of urea cycle are	1 molecule of urea, 1 mo	1 molecule of fumaric aci	1 molecule of aspartic a	None of the above	1 molecule of fumaric acid, 1 molecule of urea, 1 molecule of AMP, 2 molecules of ADP
33	Which of the following is used as carbon atom sourc	Arginine	Aspartic acid	Carbon dioxide	Glucose	Carbon dioxide
34	Uridylylation and deuridylylation of PII are brought a	Uridylyltransferase	Adenylyltransferase	Glutamate synthase	Dinitrogenase	Uridylyltransferase
35	Dinitrogenase reductase is a	Monomer	Dimer	Trimer	Tetramer	Dimer
36	Dinitrogenase is a	Monomer	Dimer	Trimer	Tetramer	Tetramer
37	The reaction of glutamate and NH4+ to yield glutami	Uridylyltransferase	Adenylyltransferase	Glutamate synthase	Glutamine synthase	Glutamine synthase
38	An intermediate of the citric acid cycle that undergoe	a-ketoglutarate	Glutamine	NADPH	H+	a-ketoglutarate
39	Which of the following catalyzes reactions that incor	Glutamine amidotransfe	Adenylyltransferase	Glutamate synthase	Glutamine synthase	Glutamine amidotransferase
40	Conversion of nitrogen to ammonia or nitrogenous co	Nitrogen fixation	Nitrification	Denitrification	Nitrogen assimilation	Nitrogen fixation
41	Formation of organic nitrogen compounds like amino	Nitrogen fixation	Nitrification	Denitrification	Nitrogen assimilation	Nitrogen assimilation
42	Ammonia or ammonium is oxidized to nitrite followe	Nitrogen fixation	Nitrification	Denitrification	Nitrogen assimilation	Nitrification
43	Nitrate is reduced and ultimately produces N2 throug	Nitrogen fixation	Nitrification	Denitrification	Nitrogen assimilation	Denitrification
44	How many ATP and electrons are required for the red	8 ATP and 8 electrons	16 ATP and 8 electrons	16 ATP and 16 electron	8 ATP and 16 electrons	16 ATP and 8 electrons
45	In the process of nitrification, nitrate reductase conve	nitrate; nitrogen	nitrate; ammonia	nitrate; nitrite	nitrate; nitric oxide	nitrate; nitrite
46	What enzyme is responsible for the incorporation of a	glutamine synthetase	glutamine synthase	glutamate synthetase	glutamate synthase	glutamine synthetase
47	What amino acid is attached to the pyridoxal-5'-phos	His	Arg	Gln	Lys	Lys

Unit 4

Regulation of plant growth and Plant tissue culture

Regulation of plant growth and Plant tissue culture
Introduction to plant hormones and their effect on plant growth and development, Regulation of plant morphogenetic processes by light. Plant tissue culture - Cell and tissue culture techniques, types of cultures: organ and explants culture, callus culture, cell suspension culture and protoplast culture. Plant regeneration pathways: organogenesis and somatic embryogenesis. Applications of cell and tissue culture and somoclonal variation.

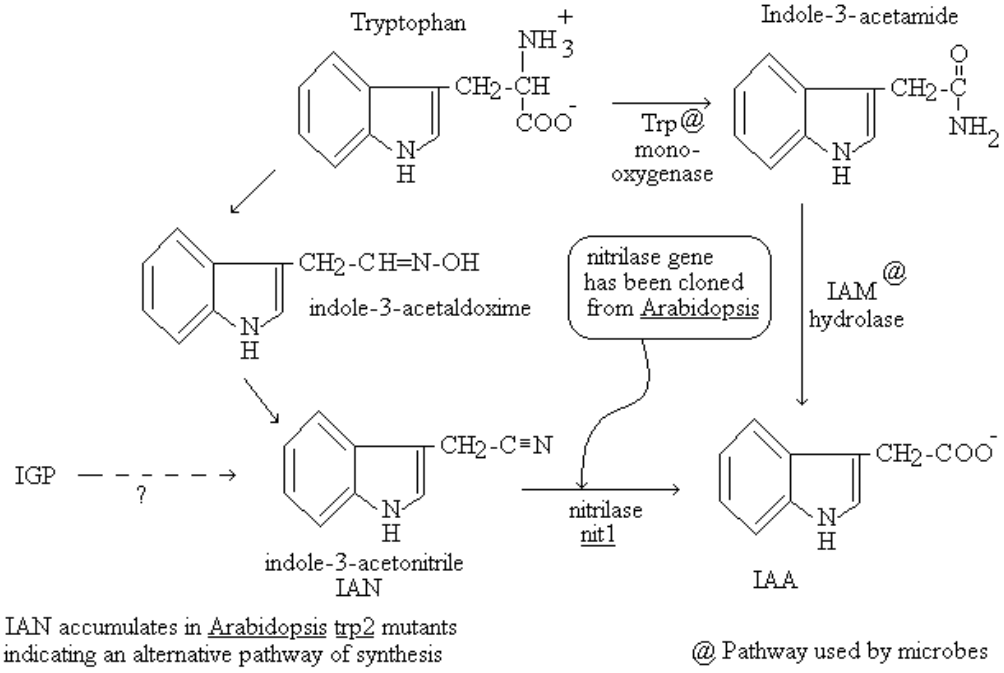
Plant growth substances

Plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development, though other processes, such as stomatal movement, may also be affected. Similarly, the effects of plant hormones depend largely on the target tissues and the chemical environment in which these tissues find themselves.

Auxin

Chemistry: Indole-3-acetic acid (IAA) is the main auxin in most plants.

Biosynthesis: IAA is synthesized from tryptophan or indole primarily in leaf primordia and young leaves, and in developing seeds.



Mechanism of Action

The mechanism by which the plant hormone auxin regulates plant growth has puzzled scientists since Darwin's time. Auxin is known to regulate gene expression by binding to its receptor TIR1 and promoting ubiquitin-dependent degradation of Aux/IAA repressor proteins. Now the determination of the crystal structures of TIR1 in complexes with three different auxins and an Aux/IAA peptide shows auxin to act as a 'molecular glue' promoting interactions between the receptor and proteins targeted for degradation. As well as revealing auxin's mechanism, this work establishes the first structural model of a plant hormone receptor. Also, the discovery that a small molecule like auxin can regulate ubiquitin ligases suggests a novel strategy for developing therapeutics for human disorders associated with ubiquitin ligase defects. On the cover, auxin (shown as a spacefilling model) is seen in the cavity between TIR1 (blue) and IAA7 peptide (orange).

Physiological Effects

- Cell enlargement - auxin stimulates cell enlargement and stem growth.
- Cell division - auxin stimulates cell division in the cambium and, in combination with cytokinin, in tissue culture.
- Vascular tissue differentiation - auxin stimulates differentiation of phloem and xylem.
- Root initiation - auxin stimulates root initiation on stem cuttings, and also the development of branch roots and the differentiation of roots in tissue culture.
- Tropistic responses - auxin mediates the tropistic (bending) response of shoots and roots to gravity and light.
- Apical dominance - the auxin supply from the apical bud represses the growth of lateral buds.
- Delayed leaf senescence.
- Leaf and fruit abscission - auxin may inhibit or promote (via ethylene) leaf and fruit abscission depending on the timing and position of the source.
- Delayed fruit ripening.
- In several systems (e.g., root growth) auxin, particularly at high concentrations, is inhibitory. Almost invariably this has been shown to be mediated by auxin-produced ethylene. If the ethylene synthesis is prevented by various ethylene synthesis inhibitors, then auxin is no longer inhibitory.

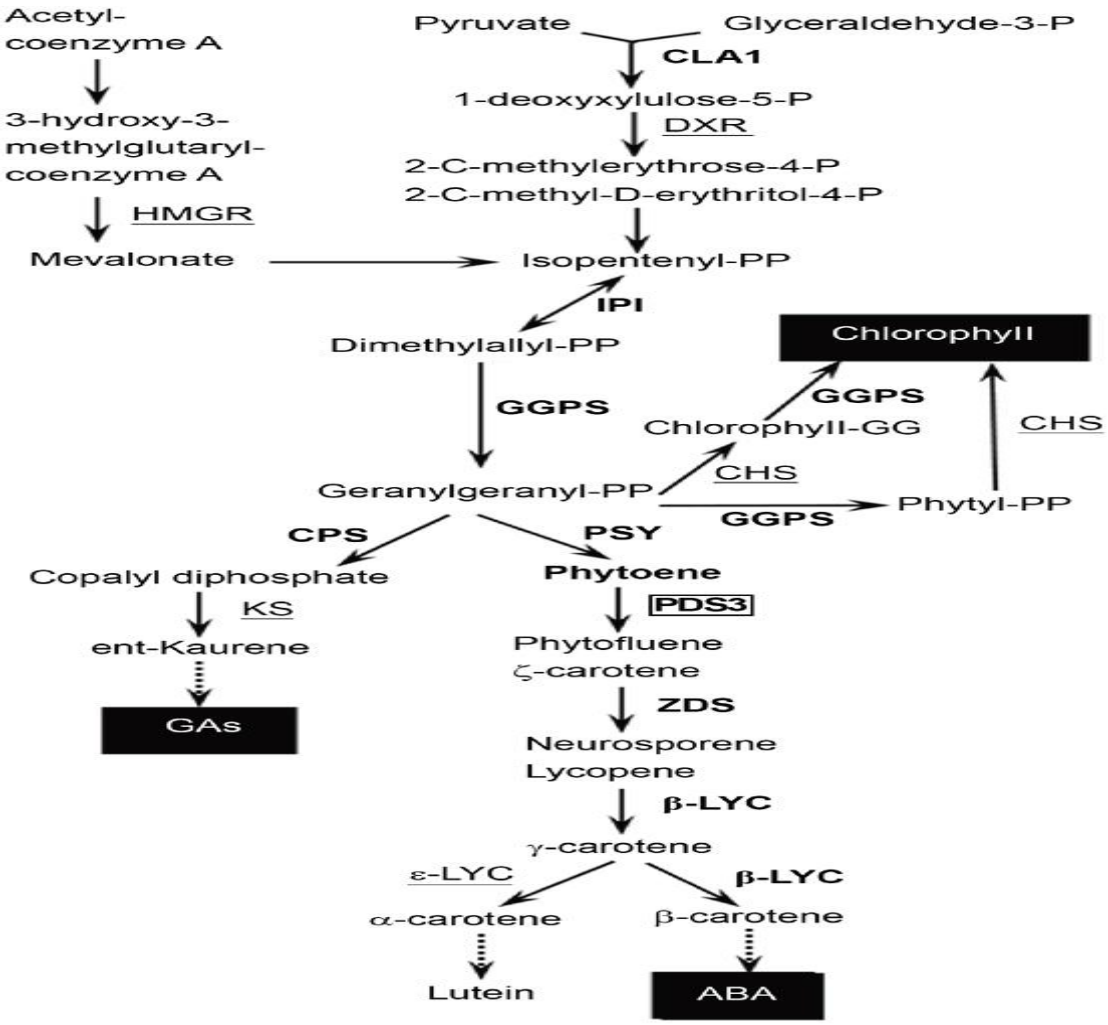
Gibberillin

Gibberellins are a plant growth substance (phytohormone) involved in promotion of stem elongation, mobilisation of food reserves in seeds and other processes. Its absence results in the dwarfism of some plant varieties. Chemically all known gibberellins are gibberellic acids, a family of diterpene acids that are synthesized by the terpenoid pathway in plastids and then modified in the

endoplasmic reticulum and cytosol until they reach their biologically active form. Gibberellin was first isolated in 1926 by Japanese scientists. It was derived from the Gibberella fungus.

Chemistry: The most widely available compound is GA3, or gibberellic acid, which is a fungal product. The most important GA in plants is GA1, which is the GA primarily responsible for stem elongation.

Biosynthesis: GAs are synthesized from mevalonic acid in young tissues of the shoot (exact location uncertain) and developing seed. Gibberellin biosynthesis can be divided into three parts: The first part is localized in proplastids and forms a C_{20}-precursor (*ent*-kaurene). The second part covers oxidation reactions that are located at the endoplasmic reticulum. Finally, in the cytoplasm of the plant cell gibberellin plant hormones are formed by 2-oxoglutarate dependent dioxygenases, enzymes that are also involved in inactivation of the plant hormone.



Physiological Effects

- Stimulates shoot and cell elongation
- Delays senescence of leaves
- Inhibits root growth

- Inhibits adventitious root growth
- Produces seed germination
- Antagonist promotes root growth and GA reverses this
- Promotes root initiation in low concentration in pea cuttings
- Stimulates bolting and flowering in biennials
- Regulates production of hydrolytic enzymes for digesting starches
- Inhibits CK bud growth on calluses
- Inhibits bud formation
- Inhibits leaf formation

Cytokinins

Nature: CKs are adenine derivatives characterized by an ability to induce cell division in tissue culture (in the presence of auxin). The most common cytokinin base in plants is zeatin.

Biosynthesis: CK biosynthesis is through the biochemical modification of adenine. It occurs in root tips and developing seeds.

Mode of action: The action of CKs is still poorly understood and insufficient evidence exists to conclusively identify any biochemical point of action.

Transport: CK transport is via the xylem from roots to shoots.

Physiological Effects

- Cell division - applications of CKs induce cell division in tissue culture in the presence of auxin.
- The presence of CKs in tissues with actively dividing cells (e.g., fruits, shoot tips) indicates that CKs may naturally perform this function in the plant. Morphogenesis - in tissue culture, CKs promote shoot initiation.
- Growth of lateral buds - CK applications can cause the release of lateral buds from apical dominance. Leaf expansion - resulting solely from cell enlargement.
- This is probably the mechanism by which the total leaf area is adjusted to compensate for the extent of root growth, as the amount of CKs reaching the shoot will reflect the extent of the root system.
- CKs delay leaf senescence.
- CKs may enhance stomatal opening in some species.
- Chloroplast development - the application of CK leads to an accumulation of chlorophyll and promotes the conversion of leukoplasts into chloroplasts.

Abscisic Acid

Nature: The name abscisic acid is rather unfortunate. The first name given was "abscisin II" because it was thought to control the abscission of cotton bolls. By a compromise the name abscisic acid was

coined. It now appears to have little role in either abscission or bud dormancy, but we are stuck with this name. As a result of the original association with abscission and dormancy, ABA has become thought of as an inhibitor. While exogenous applications can inhibit growth in the plant, ABA appears to act as much as a promoter (e.g., storage protein synthesis in seeds) as an inhibitor, and a more open attitude towards its overall role in plant development is warranted.

Biosynthesis: ABA is synthesized from mevalonic acid in roots and mature leaves, particularly in response to water stress. Seeds are also rich in ABA which may be imported from the leaves or synthesized.

Transport: ABA is exported from roots in the xylem and from leaves in the phloem. There is some evidence that ABA may circulate to the roots in the phloem and then return to the shoots in the xylem.

Physiological Effects

- Stomatal closure - water shortage brings about an increase in ABA which leads to stomatal closure.
- ABA inhibits shoot growth (but has less effect on, or may promote, root growth).
- This may represent a response to water stress. ABA induces storage protein synthesis in seeds.
- ABA counteracts the effect of gibberellin on a-amylase synthesis in germinating cereal grains.
- ABA affects the induction and maintenance of some aspects of dormancy in seeds.
- It does not, however, appear to be the controlling factor in "true dormancy" or "rest," which is dormancy that needs to be broken by low temperature or light.

Ethylene

Nature: The gas ethylene (C_2H_4) is synthesized from methionine in many tissues in response to stress. It does not seem to be essential for normal vegetative growth. It is the only hydrocarbon with a pronounced effect on plants.

Sites of Biosynthesis: Ethylene is synthesized by most tissues in response to stress. In particular, it is synthesized in tissues undergoing senescence or ripening.

Transport: Being a gas, ethylene moves by diffusion from its site of synthesis.

Physiological Effects

- Release from dormancy.
- Shoot and root growth and differentiation.
- Adventitious root formation.
- Leaf and fruit abscission.
- Flower induction in some plants.
- Induction of femaleness in dioecious flowers.
- Flower opening.

- Flower and leaf senescence.
- Fruit ripening.

THE EFFECT OF LIGHT IN A PLANT'S LIFE CYCLE

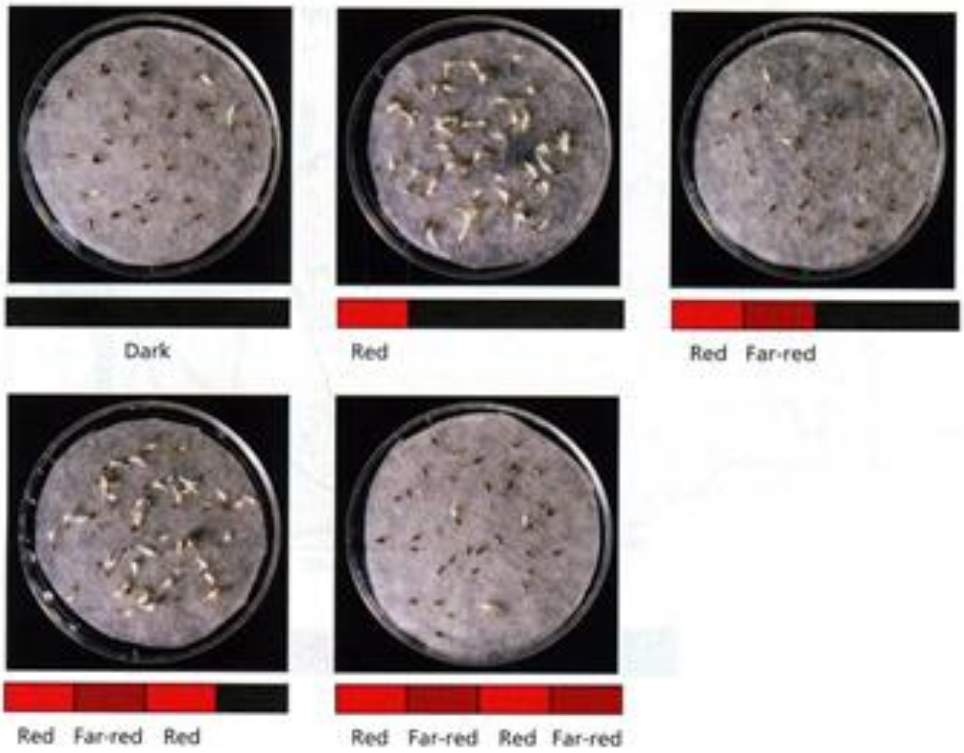
The regulation of plant development by light, or *photomorphogenesis*, is a central theme in plant development. In order to acquire and interpret the information that is provided by light, plants have developed sophisticated photosensory systems comprised of light-sensitive *photoreceptors and signal transduction pathways*. A photoreceptor "reads" the information contained in the light by selectively absorbing different wavelengths of light. Absorption of light normally induces a conformational change in the pigment or an associated protein. Whatever the nature of the primary event, absorption of light by the photoreceptor sets into motion a cascade of events that ultimately results in a developmental response.

There are four classes of photoreceptors in plants. The **phytochromes** absorb red (R) and far-red (FR) light (ca. 660 and 735 nm, respectively) and have a role in virtually every stage of development from seed to germination to flowering. **Chryptochromes and phototropin** detect both blue (400-450 nm) and UV-A light (320-440 nm). The chryptochromes appear to play major roles during seedling development, flowering, and resetting the biological clock. Phototropin mediates phototropic responses, or differential growth in a light gradient. A fourth class of photoreceptors that mediate responses to low levels of UV-B (280-320 nm) light have not yet been characterized.

Characteristics of phytochrome-induced responses

A key breakthrough in the history of phytochrome was the discovery that the effects of red light (650–680 nm) on morphogenesis could be reversed by a subsequent irradiation with light of longer wavelengths (710–740 nm), called far-red light. This phenomenon was first demonstrated in germinating seeds, but was also observed in relation to stem and leaf growth, as well as floral induction.

The initial observation was that the germination of lettuce seeds is stimulated by red light and inhibited by far-red light. But the real breakthrough was made many years later when lettuce seeds were exposed to alternating treatments of red and far-red light. Nearly 100% of the seeds that received red light as the final treatment germinated; in seeds that received far-red light as the final treatment, however, germination was strongly inhibited.



Lettuce seed germination is a typical photoreversible response controlled by phytochrome (source: Taiz L., Zeiger E., 2010)

Two interpretations of these results were possible. One is that there are two pigments, a red light–absorbing pigment and a far-red light–absorbing pigment, and the two pigments act antagonistically in the regulation of seed germination. Alternatively, there might be a single pigment that can exist in two interconvertible forms: a red light–absorbing form and a far-red light–absorbing form.

In dark-grown or etiolated plants, phytochrome is present in a red light-absorbing form, referred to as *Pr*because it is synthesized in this form. Pr, which to the human eye is blue, is converted by red light to a far-red light-absorbing form called *Pfr*, which is blue-green. Pfr, in turn, can be converted back to Pr by far-red light. Known as *photoreversibility*, this conversion/reconversion property is the most distinctive property of phytochrome. The interconversion of the Pr and Pfr forms can be measured *in vivo or in vitro*.

Evidence such as this has led to the conclusion that Pfr is the physiologically active form of phytochrome. In cases in which it has been shown that a phytochrome response is not quantitatively related to the absolute amount of Pfr, it has been proposed that the ratio between Pfr and Pr, or between Pfr and the total amount of phytochrome, determines the magnitude of the response.

Phytochrome responses can be distinguished by the amount of light required to induce them. The amount of light is referred to as the *fluence*, which is defined as the number of photons impinging on a unit surface area. The remarkable effects of vanishingly low levels of illumination are called *very low-fluence responses*, and they are *nonphotoreversible*. *Low-fluence responses* include most of the red/far-red *photoreversible* responses, such as the promotion of lettuce seed germination and the regulation of leaf movement.

Phytochromesignaling pathways

All phytochrome-regulated changes in plants begin with absorption of light by the pigment. After light absorption, the molecular properties of phytochrome are altered, probably affecting the interaction of the phytochrome protein with other cellular components that ultimately bring about changes in the growth, development, or position of an organ.

Molecular and biochemical techniques are helping to unravel the early steps in phytochrome action and the signal transduction pathways that lead to physiological or developmental responses. These responses fall into two general categories:

1. Ion fluxes, which cause relatively rapid turgor responses
2. Altered gene expression, which result in slower, long-term processes

Phytochrome can rapidy alter the properties of membranes, within seconds of a light pulse. Such rapid modulation has been measured in individual cells and has been inferred from the effects of red and far-red light on the surface potential of roots and oat coleoptiles, in which the lag between the production of Pfr and the onset measurable hyperpolarization (membrane potential changes) is 4.5 seconds. Changes in the bioelectric potential of cells imply changes in the flux of ions across the plasma membrane and suggest that some of the cytosolic responses of phytochrome are initiated at or near the plasma membrane.

Circadian rhythms

Various metabolic processes in plants, such as oxygen evolution and respiration, cycle alternately through high-activity and low-activity phases with a regular periodicity of about 24 hours. These rhythmic changes are referred to as *circadian rhythms*.

The period of a rhythm is the time that elapses between successive peaks or troughs in the cycle, and because the rhythm persists in the absence of external controlling factors, it is considered to be endogenous. The endogenous nature of circadian rhythms suggests that they are governed by an

internal pacemaker, called an *oscillator*. The endogenous oscillator is coupled to a variety of physiological processes. An important feature of the oscillator is that it is unaffected by temperature, which enables the clock to function normally under a wide variety of seasonal and climatic conditions. The clock is said to exhibit temperature compensation.

Light is a strong modulator of rhythms in both plants and animals. Although circadian rhythms that persist under controlled laboratory conditions usually have periods one or more hours longer or shorter than 24 hours, in nature their periods tend to be uniformly closer to 24 hours because of the synchronizing effects of light at daybreak, referred to as entrainment. Both red and blue light are effective in *entrainment*. The red-light effect is photoreversible by far-red light, indicative of phytochrome; the blue-light effect is mediated by blue-light photoreceptor(s).

Phytochrome enables plants to adapt to changing light conditions

The presence of a red/far-red reversible pigment in all green plants, from algae to dicots, suggests that these wavelengths of light provide information that helps plants adjust to their environment.

Compared with direct daylight, there is relatively more far-red light during sunset, under 5 mm of soil, or under the canopy of other plants (as on the floor of a forest). The canopy phenomenon results from the fact that green leaves absorb red light because of their high chlorophyll content but are relatively transparent to far-red light.

An important function of phytochrome is that it enables plants to sense shading by other plants. Plants that increase stem extension in response to shading are said to exhibit a shade avoidance response. As shading increases, the R:FR ratio decreases. The greater proportion of far-red light converts more Pfr to Pr, and the ratio of Pfr to total phytochrome (Pfr/Ptotal) decreases. When simulated natural radiation was used to vary the farred content, it was found that for so-called sun plants (plants that normally grow in an open-field habitat), the higher the far-red content (i.e., the lower the Pfr:Ptotal ratio), the higher the rate of stem extension.

For a “sun plant” or “shade-avoiding plant” there is a clear adaptive value in allocating its resources toward more rapid extension growth when it is shaded by another plant. In this way it can enhance its chances of growing above the canopy and acquiring a greater share of unfiltered, photosynthetically active light. The price for favoring internode elongation is usually reduced leaf area and reduced branching, but at least in the short run this adaptation to canopy shade seems to work.

The responses to blue light signals are distinct from phytochrome responses

Plants utilize light as a source of energy and as a signal that provides information about their environment. A large family of blue-light responses is used to sense light quantity and direction. These blue-light signals are transduced into electrical, metabolic, and genetic processes that allow plants to alter growth, development, and function in order to acclimate to changing environmental conditions. Blue light responses include phototropism, stomatal movements, inhibition of stem elongation, gene activation, pigment biosynthesis, tracking of the sun by leaves, and chloroplast movements within cells.

The physiology of blue-light responses varies broadly. In phototropism, stems grow toward unilateral light sources by asymmetric growth on their shaded side. In the inhibition of stem elongation, perception of blue light depolarizes the membrane potential of elongating cells, and the rate of elongation rapidly decreases. In gene activation, blue light stimulates transcription and translation, leading to the accumulation of gene products that are required for the morphogenetic response to light.

Plants can be classified according to their photoperiodic responses

As we have seen, the circadian clock enables organisms to determine the time of day at which a particular molecular or biochemical event occurs. *Photoperiodism*, or the ability of an organism to detect day length, makes it possible for an event to occur at a particular time of year, thus allowing for a seasonal response. Circadian rhythms and photoperiodism have the common property of responding to cycles of light and darkness. Perhaps all plant photoperiodic responses utilize the same photoreceptors, with subsequent specific signal transduction pathways regulating different responses.

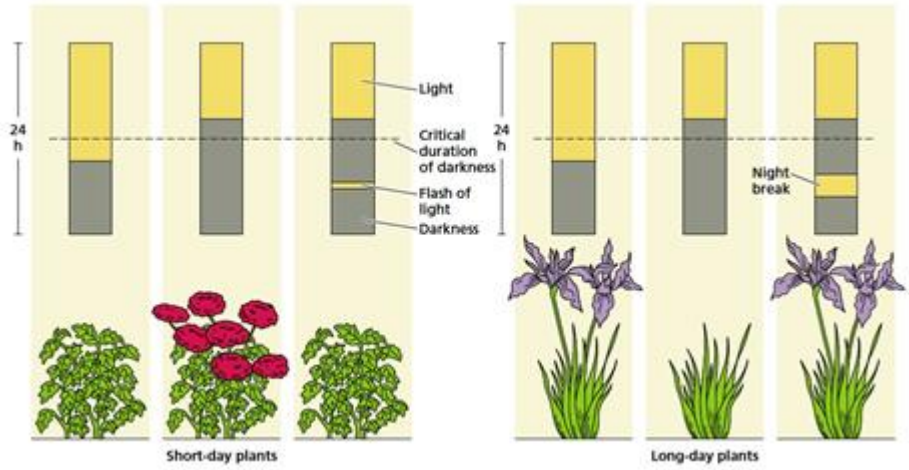
The classification of plants according to their photoperiodic responses is usually based on flowering, even though many other aspects of plants' development may also be affected by day length. The two main photoperiodic response categories are short-day plants and long-day plants:

1. *Short-day plants* (SDPs) flower only in short days (qualitative SDPs), or their flowering is accelerated by short days (quantitative SDPs).synthesized during long days.

2. *Long-day plants* (LDPs) flower only in long days (qualitative LDPs), or their flowering is accelerated by long days (quantitative LDPs).

The essential distinction between long-day and short-day plants is that flowering in LDPs is promoted only when the day length exceeds a certain duration, called the *critical day length*, in every 24-hour cycle, whereas promotion of flowering in SDPs requires a day length that is less than the critical day

length. The absolute value of the critical day length varies widely among species, and only when flowering is examined for a range of day lengths can the correct photoperiodic classification be established.



The photoperiodic regulation of flowering: effects on short-day and long-day plants (source: Taiz L., Zeiger E., 2010)

Long-day plants can effectively measure the lengthening days of spring or early summer and delay flowering until the critical day length is reached. Many varieties of wheat (*Triticumaestivum*) behave in this way. SDPs often flower in fall, when the days shorten below the critical day length, as in many varieties of *Chrysanthemum morifolium*. However, day length alone is an ambiguous signal because it cannot distinguish between spring and fall.

Finally, species that flower under any photoperiodic condition are referred to as day-neutral plants. *Day-neutral plants* (DNPs) are insensitive to day length. Flowering in DNPs is typically under autonomous regulation – that is, internal developmental control. Some day-neutral species, such as *Phaseolus vulgaris* (common bean) evolved near the equator where the daylength is constant throughout the year.

Vernalization: promoting flowering with cold

Plants exhibit several adaptations for avoiding the ambiguity of day length signal. One is the coupling of a temperature requirement to a photoperiodic response. Certain plant species, such as winter wheat, do not respond to photoperiod until after a *cold period (vernalization or overwintering)* has occurred.

Plants differ considerably in the age at which they become sensitive to vernalization. Winter annuals, such as the winter forms of cereals (which are sown in the fall and flower in the following summer),

respond to low temperature very early in their life cycle. They can be vernalized before germination if the seeds have imbibed water and become metabolically active. Other plants, including most biennials (which grow as rosettes during the first season after sowing and flower in the following summer), must reach a minimal size before they become sensitive to low temperature for vernalization.

The effective temperature range for vernalization is from just below freezing to about 10°C, with a broad optimum usually between about 1 and 7°C. The effect of cold increases with the duration of the cold treatment until the response is saturated. The response usually requires several weeks of exposure to low temperature, but the precise duration varies widely with species and variety.

Vernalization appears to take place primarily in the shoot apical meristem. Localized cooling causes flowering when only the stem apex is chilled, and this effect appears to be largely independent of the temperature experienced by the rest of the plant. Excised shoot tips have been successfully vernalized, and where seed vernalization is possible, fragments of embryos consisting essentially of the shoot tip are sensitive to low temperature.

PLANT TISSUE CULTURE

Meaning of Plant Tissue Culture:

Plant tissue culture is the technique of maintaining and growing plant cells, tissues or organs especially on artificial medium in suitable containers under controlled environmental conditions.e

The part which is cultured is called explant, i.e., any part of a plant taken out and grown in a test tube, under sterile conditions in special nutrient media. This capacity to generate a whole plant from any cell/explant is called cellular toti-potency. In fact, the whole plant can be regenerated from any plant part (referred to as explant) or cells. Gottlieb Haberlandt first initiated tissue culture technique in 1902.

Hormones used in Plant Tissue Culture:

1. Auxinsneoline (Indole-3-acetic acid, Indole-3-butyric acid, Potassium Salt— Naphthalene acetic acid 2, 4-Dichlorophenoxyacetic acid p-Chloro-phenoxy acetic acid)

2. Cytokinins (6-Benzylaminopurine, 6-Dimethylallylaminopurine (2ip), Kinetin)

3. Gibberellins (Gibberellic Acid)

4. Abscisic Acid (ABA) (Abscisic Acid)

5. Polyamines (Putrescine, Spermidine)

Environmental Conditions:

There are three important aspects in vitro (outside the living organism and in an artificial environment) culture namely:

(i) nutrient medium,

(ii) aseptic conditions and

(iii) aeration of the tissue

1. Nutrient Medium:

The composition of plant tissue culture medium can vary depending upon the type of plant tissues or cell that are used for culture. A typical (generalized) nutrient consists of inorganic salts (both micro and macro elements), a carbon source (usually sucrose), vitamins (e.g., nicotonic acid, thiamine, pyridoxine and myoinositol), amino acids (e.g., arginine) and growth regulators (e.g., auxins like 2,4-D or 2,4-dichlorophenoxyacetic acid and cytokinins such as BAP = benzlaminopurine and gibberellins). Other compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice, etc. may be added for specific purposes.

Plant hormones play important role in growth and differentiation of cultured cells and tissues. An optimum pH (usually 5.7) is also very important. The most extensively used nutrient medium is MS medium which was developed by Murashige and Skoog in 1962. Usually a gelling agent agar (a polysaccharide obtained from a red algae Gelidiumamansi) is added to the liquid medium for its solidification.

2. Aseptic Conditions (Sterilization):

Nutrient medium contains ample sugar which increases growth of microorganisms such as bacteria and fungi. These microbes compete with growing tissue and finally kill it. It is essential to maintain aseptic conditions of tissue culture. Thus sterilization means complete destruction or killing of microorganisms so that complete aseptic conditions are created for in vitro culturing.

3. Aeration of the Tissue:

Proper aeration of the cultured tissue is also an important aspect of culture technique. It is achieved by occasionally stirring the medium by sterring or by automatic shaker.

Plant Material—the Explant:

Any part of a plant taken out and grown in test tube under sterile conditions in special nutrient media is called explant.

Methods of Plant Tissue Culture:

Plant tissue culture includes two major methods:

(A) Type of in vitro growth-callus and suspension cultures.

(B) Type of explant— single cell culture, shoot and root cultures, somatic embryo culture, meristem culture, anther culture and haploid production, protoplast culture and somatic hybridisation, embryo culture, ovule culture, ovary culture, etc.

Types of Plant Tissue Culture:

Callus and Suspension Cultures:

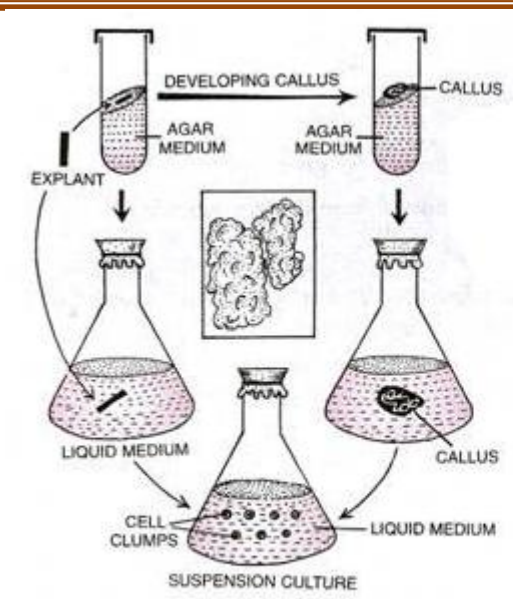
In callus culture, cell division in explant forms a callus. Callus is irregular unorganised and undifferentiated mass of actively dividing cells. Darkness and solid medium gelled by agar stimulates callus formation. The medium ordinarily contains the auxin, 2,4-D, (2, 4- dichlorophenoxy acetic acid) and often a cytokinin like BAP (Benzyl aminopurine). Both are growth regulators. This stimulates cell divison in explant. Callus is obtained within 2-3 weeks.

A suspension culture consists of single cells and small groups of cells suspended in a liquid medium. Usually, the medium contains the auxin 2,4-D. Suspension cultures must be constantly agitated at 100-250 rpm (revolutions per minute). Suspension cultures grow much faster than callus culture.

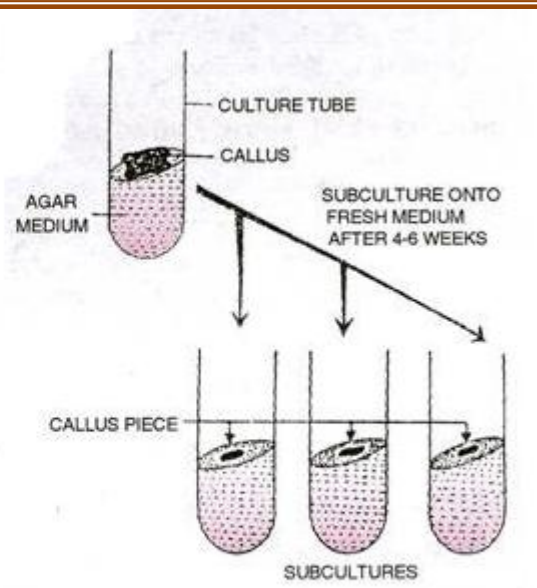
Sub culturing:

If tissue cultures are kept in the same culture vessel, they die in due course of time. Therefore, cells/tissues are regularly transferred into new culture vessels containing fresh media. This process is called sub culturing. It is important to note that during subculture; only a part of the culture from a vessel is transferred into the new culture vessel.

The callus and suspension cultures may be used to achieve cell biomass production, regeneration of plantlets, production of transgenic plants and isolation of protoplasts.



Initiation of callus and suspension cultures.



Schematic representation of subculturing.

Single Cell Culture (Cell Cloning):

As stated earlier, cells derived from a single cell through mitosis constitute a clone and the process of obtaining clones is called cloning (asexual progeny of a single individual make up a clone). There are two popular techniques for single cell culture.

1. Bergmann's Plating Technique:

This is widely used technique. The cells are suspended in a liquid medium at a cell density that is twice the desired density in the plate. Sterilized agar (Ca 1%) medium is kept malted in a water bath at 35°C. Equal volumes of the liquid and agar media are mixed and spread in Ca 1 mm thick layer in a petridish. The cells remain embedded in the soft agar medium which is observable under a microscope. When large colonies develop they are isolated and cultured separately.

2. Filter Paper Raft Nurse Tissue Technique:

Single cells are placed on small pieces (8×8 mm) of filter paper, which are placed on top of callus cultures several days in advance. This allows the filter papers to be wetted by the callus tissues. The single cells placed on the filter paper derive their nutrition from the callus. The cells divide and form macroscopic colonies on the filters. The colonies are isolated and cultured.

Shoot and Root Cultures:

Shoot culture is promoted by a cytokinin like BAR However; root culture is promoted by an auxin like NAA (naphthalene acetic acid). The shoot and root cultures are generally controlled by auxin-cytokinin balance. Usually, an excess of auxin promotes root culture, whereas that of cytokinin promotes shoot culture. Roots culture from the lower end of these shoots to give complete plantlets.

Somatic Embryo Culture:

A somatic embryo develops from a somatic cell. The pattern of development of a somatic embryo is comparable to that of a zygotic embryo. Somatic embryo culture is induced by a high concentration of an auxin, such as 2,4-D. These embryos develop into mature embryos. Mature somatic embryos or embryoids germinate to give complete plantlets.

Establishment in the Field:

The plantlets are removed from culture vessels and established in the field. This transfer is done by specific procedures called hardening. During hardening, plantlets are kept under reduced light and high humidity. Hardening procedures make the plantlets capable of tolerating the relatively harsher environments outside the culture vessels.

Endosperm Culture:

Tissue culture methods are also used for culturing endosperm. It is unique because it supplies nutrition to the developing embryo. It is also triploid in its chromosome constitution. Triploid plants are used for the production of seedless fruits (e.g., apple, banana etc.). The technique of endosperm culture involves the following:

(i) The immature seeds are dissected under aseptic condition. Endosperms along with embryos, are excised. Sometimes, mature seeds can also be used.

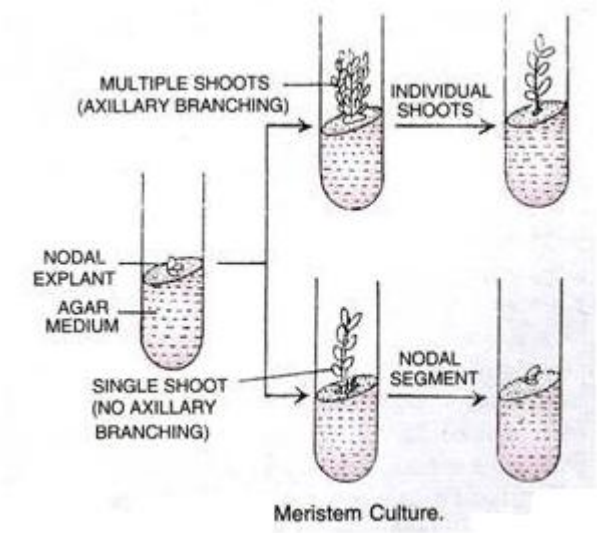
(ii) The excised endosperms are cultured on a suitable medium and embryos are removed after initial growth.

(iii) The initial callus phase is developed.

(iv) The shoots and roots may develop and complete triploid plants are formed for further use.

Meristem Culture:

Meristem is a localized group of cells, which are actively dividing and undifferentiated but ultimately giving rise to permanent tissue. Although the plant is infected with a virus, yet the meristem is free of virus. Therefore, meristem can be removed and grown in vitro to obtain virus free plants. Cultivation of axillary or apical shoot meristems is called meristem culture. The apical or axillary meristems are generally free from virus. Meristem culture involves the development of an already existing shoot meristem and subsequently, the regeneration of adventitious roots from the developed shoots.

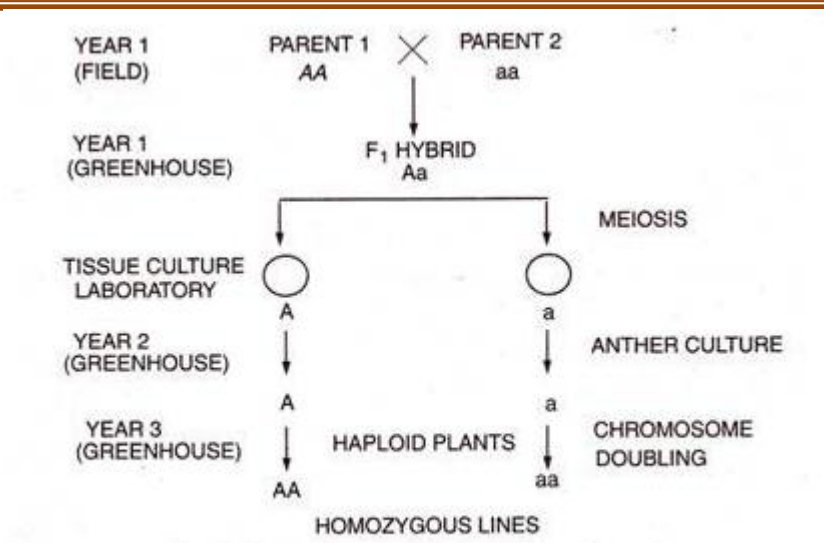


Meristem Culture.

It usually does not involve the regeneration of a new shoot meristem. The explants commonly used in meristem culture are shoot tips and nodal segments. These explants are cultured on a medium containing a cytokinin (generally BAP). The plantlets thus obtained are subjected to hardening and, ultimately, established in the fiddi Meristem culture is carried out in Potato, Banana, Cardamom, Orchids (protocorm stage), Sugarcane, Strawberry, Sweet Potato, etc. It is used in (i) Production of virus-free plants like potato, sugarcane, banana and apple, (ii) Germplasm conservation, (iii) Production of transgenic plants, (iv) Rapid clonal multiplication.

Anther Culture and Haploid Production:

An individual/cell having the chromosome number found in the gametes of the species is called haploid. Formation of haploid is called haploid production. Thus haploid individuals arise from the gametes. A haploid has only one copy of each chromosome. Haploids are sterile and of no direct value.



Production of homozygous lines using anther culture.
The two parents are shown to differ for only one gene, *i.e.*, AA and aa.

When the chromosome number of a haploid plant is doubled, the plants of normal chromosome number for particular species are obtained. These plants are homozygous and are produced in 2-3 years. The chromosome number of these haploid plants is doubled by using colchicine to obtain homozygous plants.

In nature, haploid plants originate from unfertilized egg cells, but in laboratory, they can be produced from both male and female gametes. Anther is the part of the flower of Angiosperms producing pollen (microspores), borne at the end of the stamens and usually consisting of four sporangia. When anthers of some plants are cultured on a suitable medium to produce haploid plants, it is called anther culture.

The technique was developed by Guha and Maheshwari (1964) who cultured mature anthers of Daturainnoxia. It is highly useful for the improvement of many crop plants. It is also useful for immediate expression of mutations and quick formation of purelines. This technique was first used in India to produce haploids of Datura. In many plants, haploids are also produced by culturing unfertilized ovaries/ovules. Sometimes, pollen grains are separated from anthers and cultured on suitable medium.

Embryo Culture:

Culturing young embryos on a nutrient medium is called embryo culture. Young embryos are obtained from the developing seeds. The embryos complete their development on the medium and grow into seedlings. In general, older embryos are more easily cultured in vitro than young embryos.

Embryo culture is useful as follows:

(i) Orchid seeds do not have any form of stored food. Embryos of such seeds can be cultured to obtain seedlings and maximum seedling formation can be achieved. Embryo culture in orchids can be applied for rapid clonal propagation.

(ii) In certain species, inhibitors present in the endosperm or seed coat make the seed dormant. Such embryos can escape dormancy by culturing on a suitable medium.

(iii) In certain hybrid seeds developed after interspecific crosses, the endosperm degenerates at an early stage and the young embryo is left with no food, consequently it also dies. Such young embryos can be excised from the seeds and cultured on the nutritive medium. Getting nutrition, they develop into seedlings which can be transplanted in the field.

(iv) A popular example includes hybridization of barley and wheat with Hordeumbulbosum leading to the production of haploid barley and haploid wheat respectively. Haploid wheat plants have also been successfully obtained through culture of hybrid embryos from wheat x maize crosses.

Ovule Culture:

Ovule culture technique is utilized for raising hybrids which normally fail to develop due to the abortion of the embryos at an early stage. Ovules can easily be excised from the ovary and cultured on the basal medium. The loss of a hybrid embryo due to premature abscission of fruits may be prevented by ovule culture. In some cases, addition of fruit/vegetable juice increase the initial growth.

Ovary culture:

Ovary culture technique has also been successfully employed to raise interspecific hybrids between sexually incompatible species, Brassica campestris and B. oleracea. Ovaries are excised from the flowers and cultured at the zygote or two-celled proembryo stage for obtaining normal development on culture medium.

Sometimes coconut milk when used as a supplement to the medium promote formation of fruits that are larger than those formed in vivo (within the living organism). In Anethum, addition of kinetin in the medium caused polyembryony which gave rise to multiple shoots.

Micro propagation:

Micropropagation is the tissue culture technique used for rapid vegetative multiplication of ornamental plants and fruit trees by using small sized explants. Because of minute size of the

propagules in the culture, the propagation technique is named as mircopropagation. This method of tissue culture produces several plants. Each of these plants will be genetically identical to the original plant from where they were grown.

The genetically identical plants developed from any part of a plant by tissue culture/micropropagation are called somaclones. The members of a single somaclone have the same genotype. This micropropagation is also known as somaclonal propagation. It is the only process adopted by Indian plant biotechnologists in different industries mainly for the commercial production of ornamental plants like lily, orchids, Eucalyptus, Cinchona, Blueberry, etc. and fruit trees like tomato, apple, banana, grapes, potato, citrus oil palm, etc.

There are four defined steps in micro propagation method. These are:

(i) Initiation of culture from an explant like shoot tip on a suitable nutrient medium.

(ii) Shoot formation multiple shoots formation from the cultured explant.

(iii) Rooting of shoots rooting of in vitro developed shoots.

(iv) Transplantation the hardening of tissue culture raised plants and subsequent transplantation to the field.

Advantages of Micro propagation:

These are as follows:

1. It helps in rapid multiplication of plants.

2. A large number of plantlets are obtained within a short period and from a small space.

3. Plants are obtained throughout the year under controlled conditions, independent of seasons.

4. Sterile plants or plants which cannot maintain their characters by sexual reproduction are multiplied by this method.

5. It is an easy, safe and economical method for plant propagation.

6. In case of ornamentals, tissue culture plants give better growth, more flowers and less fall-out.

7. Genetically similar plants (somaclones) are formed by this method. Therefore, desirable characters (genetope) and desired sex of superior variety are kept constant for many generations.

8. The rare plant and endangered species are multiplied by this method and such plants are saved.

Regeneration of Plantlets:

1. Preparation of Suitable Nutrient Medium:

Suitable nutrient medium as per objective of culture is prepared and transferred into suitable containers.

2. Selection of Explants:

Selection of explants such as shoot tip should be done.

3. Sterilisation of Explants:

Surface sterilization of the explants by disinfectants and then washing the explants with sterile distilled water is essential.

4. Inoculation:

Inoculation (transfer) of the explants into the suitable nutrient medium (which is sterilized by filter-sterilized to avoid microbial contamination) in culture vessels under sterile conditions is done.

5. Incubation:

Growing the culture in the growth chamber or plant tissue culture room, having the appropriate physical condition (i.e., artificial light; 16 hours of photoperiod), temperature (-26°C) and relative humidity (50-60%) is required.

6. Regeneration:

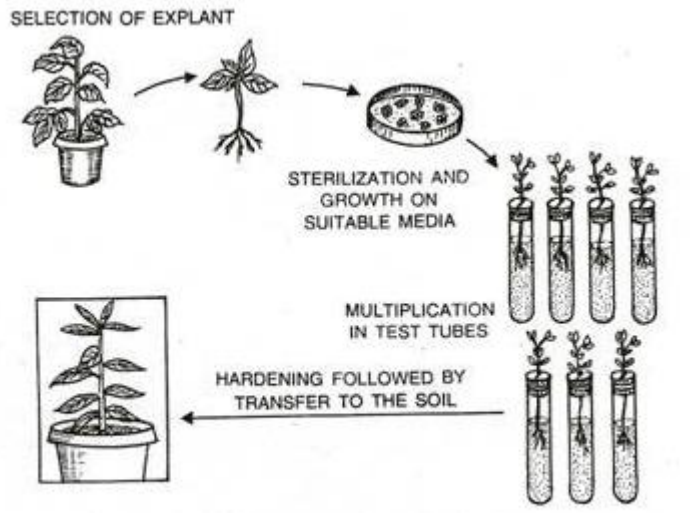
Regeneration of plants from cultured plant tissues is carried out.

7. Hardening:

Hardening is gradual exposure of plantlets to an environmental conditions.

8. Plantlet Transfer:

After hardening plantlets transferred to the green house or field conditions following acclimatization (hardening) of regenerated plants.



Regeneration of whole plants using tissue culture technique.

Protoplast Culture and Somatic Hybridisation:

When a hybrid is produced by fusion of somatic cells of two varieties or species, it is known as somatic hybrid. The process of producing somatic hybrids is called somatic hybridisation. First, the cell wall of the plant cells is removed by digestion with a combination of pectinase and cellulase. The plant cells without cell wall are called protoplasts.

The protoplasts of the two plants are brought together and made to fuse in a solution of polyethylene glycol (PEG) or sodium nitrate. The fusion of protoplasts with the help of chemicals is called chemo-fusion. Fusion of protoplasts with the help of high voltage pulse is known as electro-fusion. The fusion of protoplasts not only involves the fusion of their cytoplasm but also their nuclei. The fused protoplasts are allowed to grow on culture medium. Soon they develop their own walls when they are called somatic hybrid cells.

The hybrid cells give rise to callus. Callus later differentiates into new plant which is somatic hybrid between two plants. Somatic hybrids in plants were first obtained between two species of Tobacco (Nicotianaglauca and N. langsdorfit) by Carlson et al in 1972. Successful somatic hybrids have also been got from different species of Brassica, Petunia, and Solanum.

Pomato is somatic hybrid between Potato and Tomato that belong to two different genera and Bomato is somatic hybrid between Brinjal and Tomato. Somatic hybrids are also produced between rice and carrot. The hybrid plant bears both fruits and tubers of the two parents.

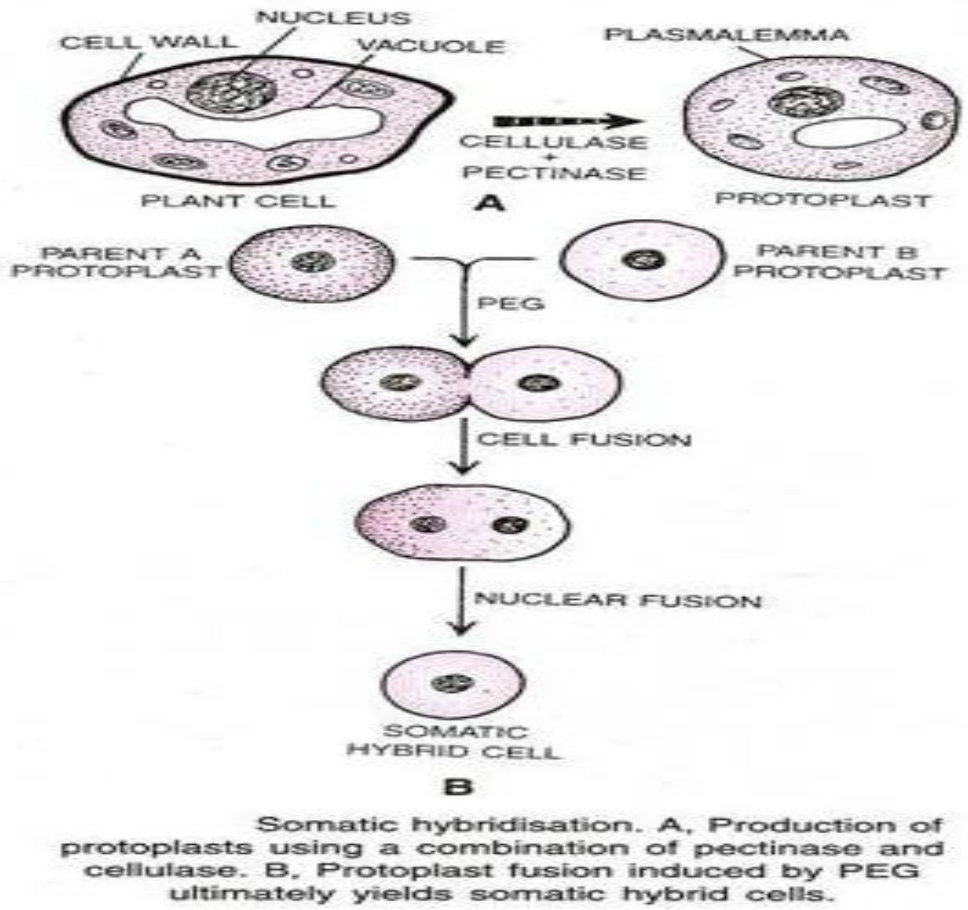
(a) Protoplast technology has opened up avenues for development of hybrids of even asexually reproducing plants.

(b) There is a distinct possibility of development of new crop plants, e.g., Pomato.

(c) Somatic hybrids may be used for the production of useful allopolyploids (Individuals produced by interspecific polyploidy).

(d) Genetic manipulations can be carried out more rapidly when plant cells are in protoplast state. New genes can be introduced (e.g., male sterility, herbicide resistance). Mutations will be easier.

If we conclude, plant tissue culture is a broad term used to define different types of in vitro plant culture. It may be recognized in the following types. Each type can result in a whole plant. (1) Callus culture — culture of differentiated tissue from an explant that dedifferentiates. (2) Cell culture — culture of cells or cell aggregates (small clumps of cells) in liquid medium. (3) Protoplast culture — culture of plant cells with their cell walls removed. (4) Embryo culture — culture of isolated embryos. (5) Seed culture — culture of seeds to generate plants. (6) Organ culture — culture of isolated plant organs such as anthers, roots, buds and shoots.



Somatic hybridisation. A, Production of protoplasts using a combination of pectinase and cellulase. B, Protoplast fusion induced by PEG ultimately yields somatic hybrid cells.

Artificial Seeds:

There are many plants which neither have seeds nor produce a small quantity of seeds. To overcome this problem the concept of artificial seeds has become popular, where somatic embryos are encapsulated in a suitable matrix composed of sodium alginate, along with substances like mycorrhizae, herbicides, fungicides and insecticides. The technique involved in the production of artificial seeds is based on cellular totipotency and somatic embryogenesis.

An artificial seed is a bead of gel containing a somatic embryo (or shoot bud) and the nutrients, growth regulators, antibiotic, etc. needed for the development of a complete plantlet. Artificial seeds may be produced using one of the following two ways: desiccated systems and hydrated systems. In the desiccated systems the somatic embryos (SEs) are first hardened to withstand desiccation and then are encapsulated.

In the hydrated systems, the beads become hardened as calcium alginate is formed, after about 20-30 minutes the artificial seeds are removed, washed with water and used for planting. Hydrated artificial seeds become dry rapidly in the open air. Therefore, hydrated artificial seeds have to be planted soon after they are produced.

In India, this technique of synthetic seeds is being done for sandalwood and mulberry at BARC (Bhaba Atomic Research Centre), Mumbai.

Advantages (i) They can be directly sown in the soil like natural seeds, (ii) They can be stored upto a year without loss of viability, (iii) They are easy to handle, and useful as units of delivery.

The only disadvantage of artificial seeds is the high cost of their production.

Practical Applications of Plant Tissue Culture:

The use of plant cells to generate useful products and/or services constitutes plant biotechnology. In plant biotechnology, the useful product is a plantlet. The plantlets are used for the following purposes.

1. Rapid Clonal Propagation:

A clone is a group of individuals or cells derived from a single parent individual or cell through asexual reproduction. All the cells in callus or suspension culture are derived from a single explant by mitotic division. Therefore, all plantlets regenerated from a callus/suspension culture generally have

the same genotype and constitute a clone. These plantlets are used for rapid clonal propagation. This is done in oil palm.

2. Somaclonal Variation:

Genetic variation present among plant cells of a culture is called somaclonal variation. The term somaclonal variation is also used for the genetic variation present in plants regenerated from a single culture. This variation has been used to develop several useful varieties.

3. Transgenic Plants:

A gene that is transferred into an organism by genetic engineering is known as transgene. An organism that contains and expresses a transgene is called transgenic organism. The transgenes can be introduced into individual plant cells. The plantlets can be regenerated from these cells. These plantlets give rise to the highly valuable transgenic plants.

4. Induction and Selection of Mutations:

Mutagens are added to single cell liquid cultures for induction of mutations. The cells are washed and transferred to solid culture for raising mutant plants. Useful mutants are selected for further breeding. Tolerance to stress like pollutants, toxins, salts, drought, flooding, etc. can also be obtained by providing them in culture medium in increasing dosage. The surviving healthy cells are taken to solid medium for raising resistant plants.

5. Resistance to Weedicides:

It is similar to induction of mutations. Weedicides are added to culture initially in very small concentrations. Dosage is increased in subsequent cultures till the desired level of resistance is obtained. The resistant cells are then regenerated to form plantlets and plants.

ORGANOGENESIS:

From cells of tissue culture various organs, such as, roots, stems, leaves or flowers may be initiated. This is called organogenesis. Such organ development does not require any pre-existing initials. These new organs are formed in two stages.

In the first phase (dedifferentiation) cells of the explant divide and form undifferentiated cells. In the second phase cell differentiation takes places. Organ primordia are formed from single cells or small groups of differentiated cells. These cells form small meristem with cells containing dense cytoplasm and large nuclei.

According to Torrey (’66) meristemoids (i.e., meristematic zones) occur near the tissue medium interface.

Root formation in culture is called rhizogenesis and shoot initiation is called caulogenesis.

Root formation on culture has been noted in several cases. In culture of carrot cells root formation was first observed by Nobecourt (’39b). Explants taken from any part of a plant may produce roots. Like roots shoot buds are also formed frequently. Leaves develop less frequently than roots and shoots.

Root formation on culture of Jerusalem artichoke is influenced by mineral salts, auxin, sugar, temperature and light.

Root formation stops after several subcultures. These may be due to (a) some substances required for root initiation may be exhausted, (b) culture tissue is incapable of rhizogenesis or (c) epigenetic changes of some genes may occur.

Few layers of epidermal or sub-epidermal cells from various plants under regulated condition on culture can produce organs. In Begonia rex explants from epidermal or sub-epidermal layers near the midveins of leaves can produce roots or shoots rapidly. Root initiation occurs in a medium supplemented with zeatin and NAA. Shoot initiation takes place in presence of zeatin but in absence of auxin.

In short term cultures organization of the new meristem bears a relationship with the original organization of the explant. In culture of tobacco stalk shoot primordium arises from the external phloem.

In culture of Convolvulus roots shoot primordium originates near the protoxylem. In culture of carrot cells root primordium arises in association with the protoxylem strands and when this is transferred to an agar medium, it forms a complete plant.

In long term cultures shoot and bud primordia develop exogenously. But in some cases, as in Convolvulus callus shoot primordia develop either exogenously or endogenously. Culture of explants from lower internodes produce vegetative buds, whereas explants from young upper parts or from inflorescence produce flower buds.

For flower formation high level of nitrogen, presence of cytokinin and various constituents of nucleic acid in the medium, are necessary. Presence of auxin, gibberellin and organic nitrogenous compounds have an inhibitory effect on flower formation.

Factors Influencing Organogenesis:

(1) Age of Culture

(2) Ploidy Level

(3) Phytohormons

(4) Phosphate Concentration

(5) Photoperiodism and Vernalization

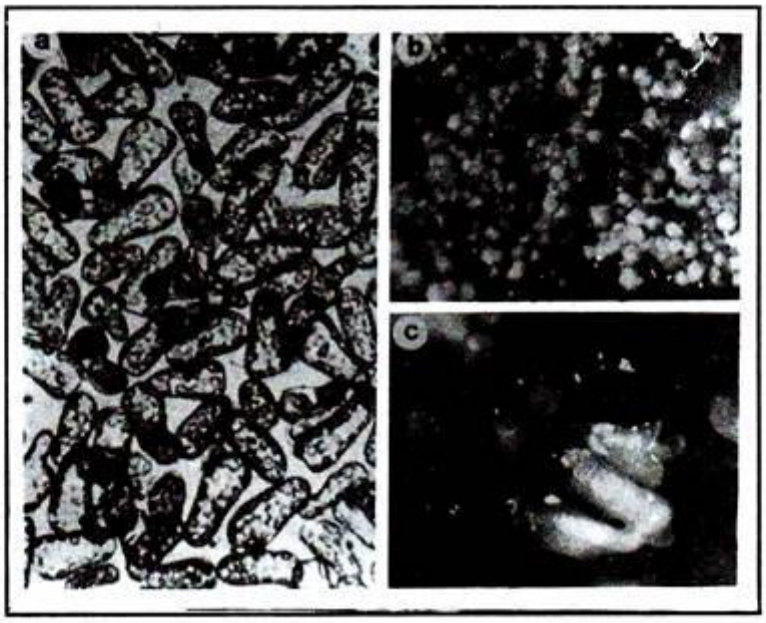
SOMATIC EMBRYOGENESIS

Plant cells undergoing somatic embryogenesis are either pro-embryonic determined cells (PEDC) or induced embryogenic determined cells (IEDC). There have been reports on the induction of somatic embryos frequently from various tissues like seedlings, shoot meristem, young inflorescence and zygotic embryos. In addition, other tissues such as root, nucellus has also yielded somatic embryos.

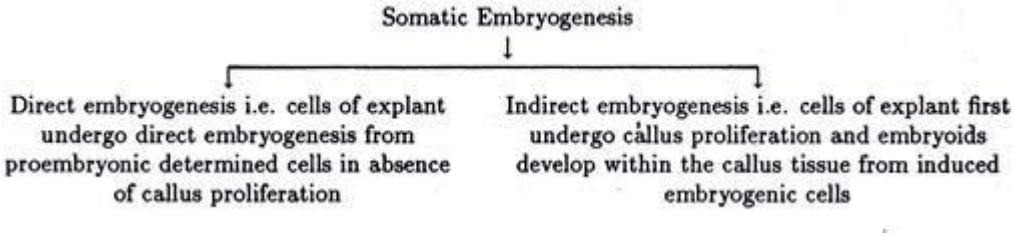
The favorable responses of a few of the above tissues actually contain proembryogenic determinant cells (PEDC) or these cells may require minor reprogramming to enter embryogenic state. The first report on the production of somatic embryos in carrot suspension cells was published by Steward and co-workers in 1958. Thereafter reports were flooded on the production of somatic embryos in plants.

Somatic embryogenesis may be initiated in two different ways:

1. In some cultures somatic embryogenesis occurs directly in absence of any callus production from "pro-embryo genic determined cells" that are already programmed for embryo differentiation. For instance, somatic embryos has been developed directly from leaf mesophyll cells of orchard grass (Dactyhsglomerata L.) without an intervening callus tissue.



Explants, made from the basal portions of two innermost leaves of orchard grass were cultured on a Schenk and Hildebrandt medium supplemented with 30 µM 3, 6-dichloro-O-anisic acid (dicamba). Plant formation occurred after sub culturing the embryos on the same medium without dicamba (Conger et al., 1983).



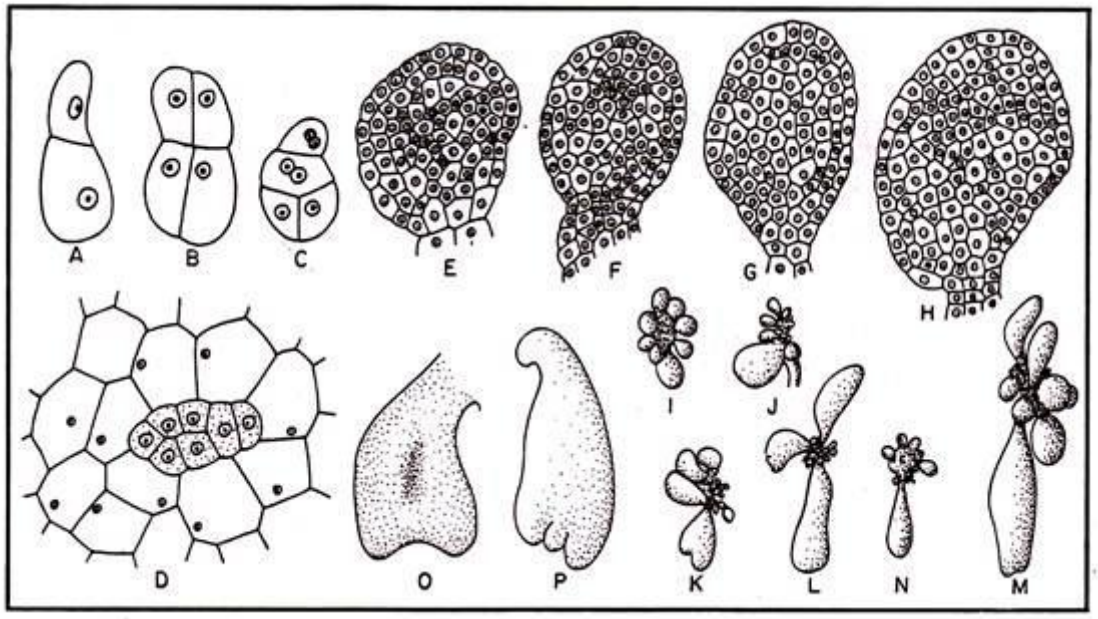
2. The second type of somatic embryo development needs some prior callus formation and embryoids originate from "induced embryo genic cells" within the callus tissue.

In most of the cases, indirect embryogenesis occurs. For indirect somatic embryogenesis where it has been induced under in vitro condition, two distinctly different types of media may be required—One medium for the initiation, of the embryonic cells and another for the subsequent development of these cells into embryoids.

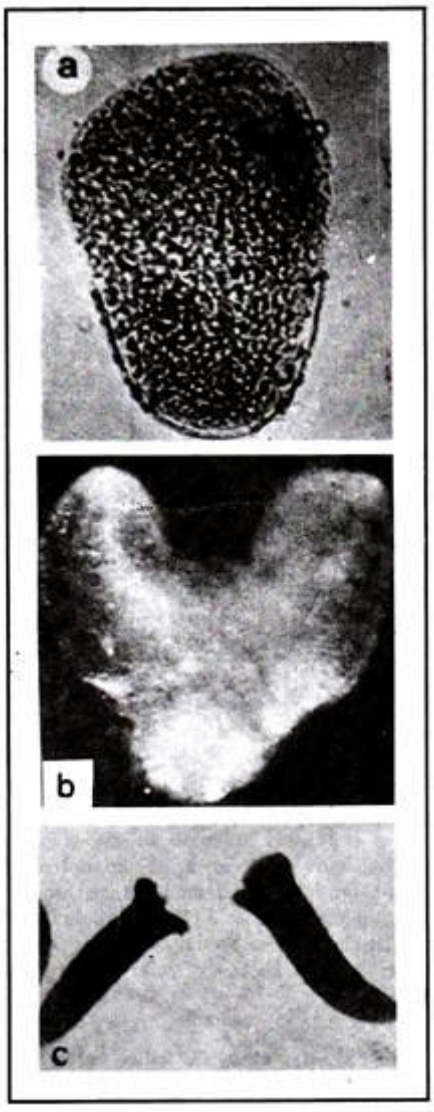
The first or induction medium must contain auxin in case of carrot tissue and somatic embryogenesis can be initiated in the second medium by removing the hormone or lowering its concentration. With some plants, however, both embryo initiation and subsequent maturation and subsequent maturation occur on the first medium and a second medium is required for plantlet development.

In some cases, a given culture may differentiate the embryo genic cells, but their further growth may be blocked by an imbalance of nutrition in the culture medium. According to Kohlenback, (1978), abnormalities known as embryonal budding and embryo genic clump formation may occur, if relatively high level of auxin is present after the embryo genic cells have been differentiated.

Embryoids are generally initiated in callus tissue from the superficial clumps of cells (primordia) associated with enlarged vacuolated cells that do not take part in embryogenesis. The embryo genic cells are generally characterised by dense cytoplasmic contents, large starch grains, a relatively large nucleus with a darkly stained nucleolus. In suspension culture, embryoids do not form suspended single cell, but form cells lying at or near the surface of the small cell aggregates.



Each developing embryoid of carrot passes through three sequential stages of embryo formation such as globular stage, heart-shape stage and torpedo stage. The torpedo stage is a bipolar structure which ultimately gives rise to complete plantlet. The culture of other plants may not follow such sequential stages of embryo development.



In general, somatic embryogenesis occurs in short-term culture and this ability decreases with increasing duration of culture. But there are some exceptional cultures where embryogenesis has been reported from the callus tissue maintained over a period of year. According to Smith and Street, (1974), changes in ploidy of the cultured cell may lead to loss of embryo genic potential in long term culture. The loss of embryo genic potential in long term culture may also result from loss of certain biochemical properties of the cell.

In callus culture or in suspension culture, embryoid formation occurs asynchronously. Some progress has been made in inducing synchronization of somatic embryogenesis in cell suspension culture. A high degree of synchronization has been achieved in a carrot suspension culture by sieving the initial cell population.

Protocols for Inducing Somatic Embryogenesis in Culture:

The plant material Daucuscarota represents the classical example of somatic embryo- genesis in culture.

The protocol is described below:

1. Leaf petiole (0.5-1 cm) or root segments from seven-day old seedlings (1 cm) or cambium tissue (0.5 cm^3) from storage root can be used as explant. Leaf petiole and root segment can be obtained from aseptically grown seedlings (Cambium tissue can be obtained from surface sterilized storage tap root 2. Following aseptic technique, explants are placed individually on a semi-solid Murashige and Skoog's medium containing 0.1 mg/L 2, 4-D and 2% sucrose. Cultures are incubated in the dark. In this medium the explant will produce sufficient callus tissue.

3. After 4 weeks of callus growth, cell suspension culture is to be initiated by transferring 0.2 gm. of callus tissue to a 250 ml of Erlenmeyer flask containing 20-25 ml of liquid medium of the same composition as used for callus growth (without agar). Flasks are placed on a horizontal gyratory shaker with 125-160 rpm at 25°C. The presence or absence of light is not critical at this stage.

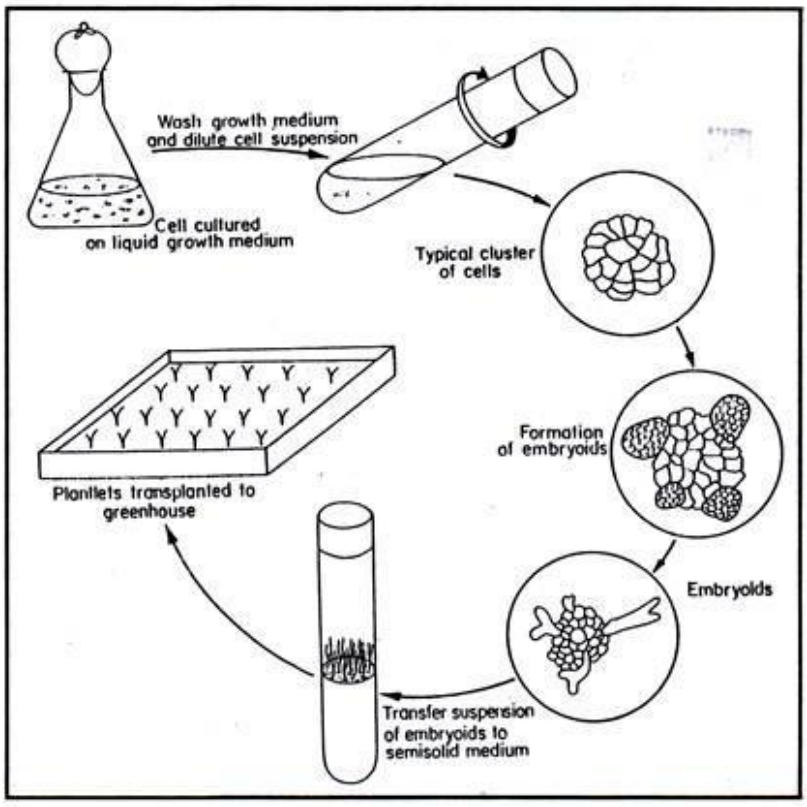
4. Cell suspensions are sub-cultured every 4 weeks by transferring 5 ml to 65 ml of fresh liquid medium.

5. To induce a more uniform embryo population, cell suspension is passed through a series of stainless steel mesh sieves. For carrot, the 74 µ sieve produces a fairly dense suspension of single cell and small multiple clumps. To induce somatic embryogenesis, portions of sieved cell suspension are transferred to 2, 4-D free liquid medium or cell suspension can be planted in semi-solid MS medium devoid of 2, 4-D. For normal embryo development and to inhibit precocious germination especially root elongation, 0.1- 1 µM ABA can be added to the culture medium. Cultures are incubated in dark.

6. After 3-4 weeks, the culture would contain numerous embryos in different stages of development.

7. Somatic embryos can be placed on agar medium devoid of 2, 4-D for plantlet development.

8. Plantlets are finally transferred to Jiffy pots or vermiculite for subsequent development.



Importance of Somatic Embryogenesis:

The potential applications and importance of in vitro somatic embryogenesis and organogenesis are more or less similar. The mass production of adventitious embryos in cell culture is still regarded by many as the ideal propagation system. The adventitious embryo is a bipolar structure that develops directly into a complete plantlet and there is no need for a separate rooting phase as with shoot culture.

Somatic embryo has no food reserves, but suitable nutrients could be packaged by coating or encapsulation to form some kind of artificial seeds. Such artificial seeds produce the plantlets directly into the field. Unlike organogenesis, somatic embryos may arise from single cells and so it is of special significance in mutagenic studies.

Plants derived from asexual embryos may in some cases be free of viral and other pathogens. For an example, Citrus plant propagation from embryo genic callus of nuclear origin are free of Virus. So it is an alternative approach for the production of disease-free plants.

APPLICATIONS OF CELL AND TISSUE CULTURE

Micropropagation /Clonal Propagation

Clonal propagation refers to the process of asexual reproduction by multiplication of genetically identical copies of individual plants. The vegetative propagation of plants is labour-intensive, low in productivity and seasonal. The tissue culture methods of plant propagation, known as 'micropropagation' utilizes the culture of apical shoots, axillary buds and meristems on suitable nutrient medium.The regeneration of plantlets in cultured tissue was described by Murashige in 1974. Fossard (1987) gave a detailed account of stages of micropropagation. The micropropagation is rapid and has been adopted for commercialization of important plants such as banana, apple, pears, strawberry, cardamom, many ornamentals (e.g. Orchids) and other plants.The micropropagation techniques are preferred over the conventional asexual propagation methods because of the following reasons: (a) In the micropropagation method, only a small amount of tissue is required to regenerate millions of clonal plants in a year., (b) micropropagation is also used as a method to develop resistance in many species., (c) in vitro stock can be quickly proliferated as it is season independent,. (d) long term storage of valuable germplasm possible.

The steps in micropropagation method are: a) Initiation of culture - from an explant like shoot tip on a suitable nutrient medium, b) multiple shoots formation from the cultured explant, c) rooting of ***in vitro*** developed shoots and, d) transplantation - transplantation to the field following acclimatization. The factors that affect micropropagation are: (a) genotype and the physiological status of the plant e.g. plants with vigorous germination are more suitable for micropropagation., (b) the culture medium and the culture environment like light, temperature etc. For example an illumination of 16 hours a day and 8 hours night is satisfactory for shoot proliferation and a temperature of 250C is optimal for the growth.

The benefits of micropropagation this method are:

a) rapid multiplication of superior clones can be carried out through out the year, irrespective of seasonal variations.

b) multiplication of disease free plants e.g. virus free plants of sweet potato ***(Ipomeabatatus***), cassava (***Manihotesculenta***)

c) multiplication of sexually derived sterile hybrids

d) It is a cost effective process as it requires minimum growing space.

Somaclonal variation

The genetic variations found in the ***in vitro*** cultured cells are collectively referred to as somaclonal variation and the plants derived from such cells are called as 'somaclones'. It has been observed that the long-term callus and cell suspension culture and plants regenerated from such cultures are often associated with chromosomal variations. It is this property of cultured cells that finds potential application in the crop improvement and in the production of mutants and variants (e.g. disease resistance in potato).

Larkin and Scowcroft (1981) working at the division of Plant Industry, C.S.I.R.O., Australia gave the term 'somaclones' for plant variants obtained from tissue cultures of somatic tissues. Similarly, if the tissue from which the variants have been obtained is having gametophytic origin such as pollen or egg cell, it is known as 'gametoclonal' variation.They explained that it may be due to: (a) reflection of heterogeneity between the cells and explant tissue, (b) a simple representation of spontaneous mutation rate, and (c) activation by culture environment of transposition of genetic materials.

Shepard et al. (1980) also contributed by screening about 100 somaclones produced from leaf protoplasts of Russet Burbank. They found that there was a significant amount of stable variation in compactness of growth habit, maturity, date, tuber uniformity, tuber skin colour and photoperiodic requirements.

Somaclonal Variations has been used in plant breeding programmes where the genetic variations with desired or improved characters are introduced into the plants and new varieties are created that can exhibit disease resistance, improved quality and yield in plants like cereals, legumes, oil seeds tuber crops etc. Somaclonal variation is applicable for seed

APPLICATIONS OF SOMACLONAL VARIATIONS

- Methodology of introducing somaclonal variations is simpler and easier as compared to recombinant DNA technology.
- Development and production of plants with disease resistance e.g. rice, wheat, apple, tomato etc.
- Develop biochemical mutants with abiotic stress resistance e.g. aluminium tolerance in carrot, salt tolerance in tobacco and maize.
- Development of somaclonal variants with herbicide resistance e.g. tobacco resistant to sulfonylurea.

- Development of seeds with improved quality e.g. a new variety of ***Lathyrussativa*** seeds (Lathyrus Bio L 212) with low content of neurotoxin.
- Bio-13 – A somaclonal variant of ***Citronella java*** (with 37% more oil and 39% more citronellon), a medicinal plant has been released as Bio-13 for commercial cultivation by Central Institute for Medicinal and Aromatic Plants (CIMAP), Lucknow, India.
- Supertomatoes- Heinz Co. and DNA plant Technology Laboratories (USA) developed Supertomatoes with high solid component by screening somaclones which helped in reducing the shipping and processing costs.

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III BSc Biochemistry - Plant Biochemistry (16BCU503A)

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	Auxin is found in which region of tha	Meristematic region	mature tissue	flowers	fruits	Meristematic region
2	Chemically auxin is	Indole pyruvic acid	Indole 3 acetic acid	Indole butyric acid	2,4 dichloro phenoxy acetic acid	Indole 3 acetic acid
3	Auxin isolated from human urine is	Indole acetic acid	auxonotriolic acid	auxonolonic acid	alpha naphthalene acetic acid	auxonotriolic acid
4	The only true natural auxin of higher	Indole 3 acetic acid	alpha naphthalene acetic acid	2,4 dichloro phenoxy acetic a	Indole butyric acid	Indole 3 acetic acid
5	The precursor of indole acetic acid is	Tyrosine	methionine	tryptophan	phenyl alanine	tryptophan
6	The enzyme involved in conversion o	indole pyruvic acid decarb	indole acetaldehyde decarboxyl	amino transferase	tryptophan decarboxylase	amino transferase
7	In tryptamine pathway tryptophan is	tryptophan decarboxylase	indole pyruvic acid decarboxyl	indole acetaldehyde decarbox	amino transferase	tryptophan decarboxylase
8	The indole acetaldoxime pathway is	cruciferae	solanacea	marvacea	malvacea	cruciferae
9	The only non indole auxin is	phenyl pyruvic acid	phenyl acetic acid	alpha naphthalene acetic acid	2,4 dichloro phenoxy acetic acid	phenyl acetic acid
10	Phenyl alanine is converted to phenyl	aromatic amino transferas	indole pyruvic acid decarboxyl	indole acetaldehyde decarbox	amino transferase	aromatic amino transferase
11	Absorption of water is increased by	gibberellins	auxin	cytokinin	ethylene	auxin
12	Shortening of internodes and product	gibberellins	auxin	cytokinin	ethylene	auxin
13	The hormone used widely to break se	gibberellins	auxin	cytokinin	ethylene	auxin
14	_____ are weed killers	gibberellins	auxin	cytokinin	ethylene	auxin
15	Early flowering and fruiting is induc	gibberellins	auxin	cytokinin	ethylene	auxin
16	The precursor for gibberellin biosynt	mevalonic acid	tyrosine	tryptophan	alanine	mevalonic acid
17	Genetic dwarfism is overcome by	gibberellins	auxin	cytokinin	ethylene	gibberellins
18	Bolting and flowering in *Brassica* is	gibberellins	auxin	cytokinin	ethylene	gibberellins
19	Light induced inhibition of stem grow	gibberellins	auxin	cytokinin	ethylene	gibberellins
20	The production of parthenocarpic fru	gibberellins	auxin	cytokinin	ethylene	gibberellins
21	Reduction in number of male flowers	gibberellins	auxin	cytokinin	ethylene	gibberellins
22	Chemically gibberellins are	triterpenoid acids	sesquiterpenoid acids	diterpenoid acids	monoterpenoid acids	diterpenoid acids
23	Seed germination is promoted by	gibberellins	auxin	cytokinin	ethylene	gibberellins
24	Cytokinin is a derivative of	6 furfuryl amino purine	pyrimidine	isopentenyl pyro phosphate	geranyl pyro phosphate	6 furfuryl amino purine
25	Kinetin is formed from	adenosine	guanosine	deoxy adenosine	deoxy guanosine	deoxy adenosine
26	Cell enlargement is induced by all th	abscisic acid	auxin	cytokinin	ethylene	abscisic acid
27	Enzyme synthesis in plants is regulat	abscisic acid	auxin	cytokinin	ethylene	cytokinin
28	Sex reversal is induced by	abscisic acid	auxin	cytokinin	ethylene	cytokinin
29	RNA synthesis in elongating zones o	abscisic acid	auxin	cytokinin	ethylene	cytokinin
30	Who is the father of tissue culture?	Bonner	Haberlandt	Laibach	Gautheret	Haberlandt
31	The production of secondary metabo	protoplast	cell suspension	meristem	auxillary buds	cell suspension
32	Synthetic seed is produced by encaps	sodium chloride	sodium alginate	sodium acetate	sodium nitrate	sodium alginate
33	Hormone pair required for a callus to	auxin and cytokinin	auxin and ethylene	auxin and absiccic acid	cytokinins and gibberlin	auxin and cytokinin
34	DMSO (Dimethyl sulfoxide) is used	Gelling agent	alkaylating agent	Chelating agent	Cryoprotectant	Cryoprotectant
35	The most widely used chemical for p	Manitol	Sorbitol	Mannol	Poly ethylene glycol (PEG)	Poly ethylene glycol (PEG)
36	Cybrids are produced by	Fusion of two different nu	Fusion of two same nuclei from	Nucleus of one species but cy	None of the above	Nucleus of one species but cytoplasm from both the parent species
37	Callus is	Tissue that forms embryo	An insoluble carbohydrate	Tissue that grows to form emb	Un organised actively dividing mass o	Un organised actively dividing mass of cells maintained in cultured
38	Part of plant used for culturing is call	Scion	Explant	Stock	Callus	Explant
39	Growth hormone producing apical do	Auxin	Gibberellin	Ethylene	Cytokinin	Auxin
40	A medium which is composed of che	Natural media	Synthetic media	Artificial media	None of these	Synthetic media
41	To obtain haploid plant, we culture	Entire anther	Nucleus	Embryo	Apical bud	Entire anther
42	Somaclonal variations are the ones	Caused by mutagens	Produce during tissue culture	Caused by gamma rays	Induced during sexual embryogeny	Produce during tissue culture
43	Which of the following plant cell will	Xylem vessels	Sieve tube	Meristem	Cork cells	Meristem
44	Which vector is mostly used in crop	Plasmid	Cosmid	Phasmid	Agrobacterium	Agrobacterium
45	A(n) _____ is an excised piece	microshoot	medium	explant	scion	explant
46	Protoplasts can be produced from sus	cellulotyic enzymes	pectolytic enzymes	both cellulotyic and pectolytic	proteolytic enzymes	both cellulotyic and pectolytic enzymes
47	What is meant by 'Organ culture' ?	Maintenance alive of a wh	Introduction of a new organ in	Cultivation of organs in a labo	The aspects of culture in community	Maintenance alive of a whole organ, after removal from the organism by partial immersion in a nutrient fluid
48	Which method of plant propagation i	Grafting	Cuttings	Layering	Micropropagation	Layering
49	Who is the father of tissue culture?	Bonner	Haberlandt	Laibach	Gautheret	Haberlandt
50	The production of secondary metabo	protoplast	cell suspension	meristem	auxillary buds	cell suspension
51	Somaclonal variations are the ones	Caused by mutagens	Produce during tissue culture	Caused by gamma rays	Induced during sexual embryogeny	Produce during tissue culture
52	To obtain haploid plant, we culture	Entire anther	Nucleus	Embryo	Apical bud	Entire anther
53	Growth hormone producing apical do	Auxin	Gibberellin	Ethylene	Cytokinin	Auxin
54	Part of plant used for culturing is call	Scion	Explant	Stock	Callus	Explant
55	Which breeding method uses a chem	Mass selection	Protoplast fusion	Transformation	Transpiration	Protoplast fusion
56	The response of a plant to the	phytochrome	photoperiod	cytochrome	photoreceptor	photoperiod
57	The protein pigment that absorb red a	cytochrome	phytochrome	photochlorophyllide	bacterial chlorophyllide	phytochrome
58	The photo period of short day plants	less than 12 hours	less than 10 hours	less than 15 hours	less than 5 hours	less than 12 hours
59	The photoperiod required by long da	more than 12 hours	more than 10 hours	more than 15 hours	more than 5 hours	more than 12 hours
60	The plants require a photoperiod of 1	short day plant	long day plant	photo neutral plant	intermediate plant	intermediate plant

Unit 5
Plant Secondary metabolites

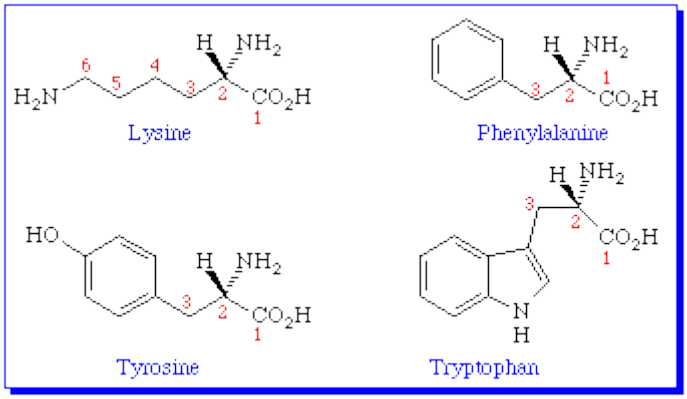
Plant Secondary metabolites
Representatives alkaloid group and their amino acid precursors, function of alkaloids, Examples of major phenolic groups; simple phenylpropanoids, Coumarins, Benzoic acid derivatives, flavonoids, tannins and lignin, biological role of plant phenolics, Classification of terpenoids and representative examples from each class, biological functions of terpenoids

REPRESENTATIVES ALKALOID GROUP AND THEIR AMINO ACID PRECURSORS

Introduction

In our introduction to the chemistry of amines, we defined alkaloids as natural products that contain an amino group. The name is derived from the fact that aqueous solutions of these compounds are slightly basic, i.e. alkaline, due to the presence of the amino group. The reactions that produce alkaloids generally involve the secondary metabolism of amino acids. In particular, most alkaloids are derived from four different amino acids; lysine, phenylalanine, tyrosine, and tryptophan. The structures of these amino acids are shown in Figure 1. Some of the carbon atoms are numbered for reference to structures in later figures.

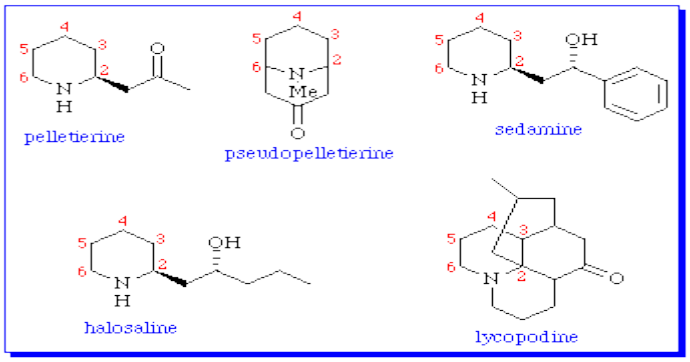
Amino Acids as Alkaloid Precursors



Alkaloids Derived from Lysine

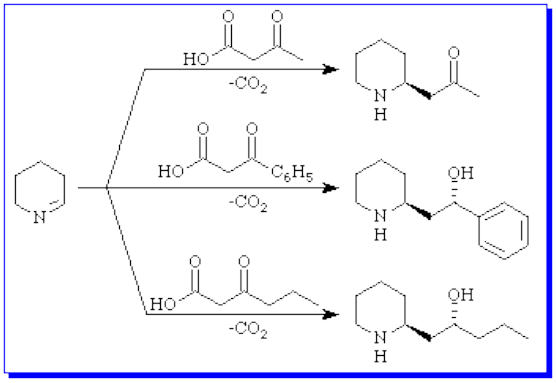
The structures of five lysine-derived alkaloids are shown in Figure 2. Pelletierine and pseudopelletierine are found in the bark of pomegranate trees. Sedamine is one of over 600 alkaloids isolated from the genus *Sedum*, a common garden plant,whilehalosaline is a minor constituent from the species *Haloxyonsalicornicum*. Lycopodine is obtained from various species of the moss *Lycopoodium*.

Lysine-Based Alkaloids



omparison the structures of pelleterine, sedamine, and halosaline suggests that each of these molecules arises from the reaction of a common intermediate with a different b-ketoacid as shown in Figure 3. The most likely intermediate is thought to be D^1-piperideine, a compound that we invoked during our discussion of the role of the Mannich reaction in the biosynthesis of nicotine.

The Origin of Three Species



Alkaloids Derived from Phenylalanine and Tyrosine

The simplest members of this group of alkaloids are compounds that contain the b-phenylethylamine, $C_6H_5CH_2CH_2NH_2$, skeleton. Figure 4 presents the structures of several members of this family whose names should be familiar to you.

A Familiar Family

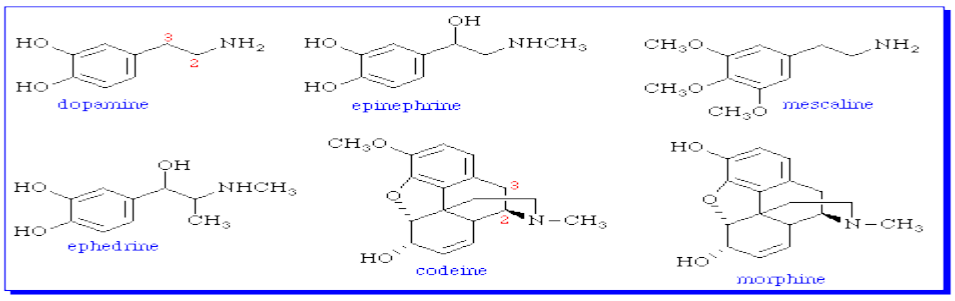
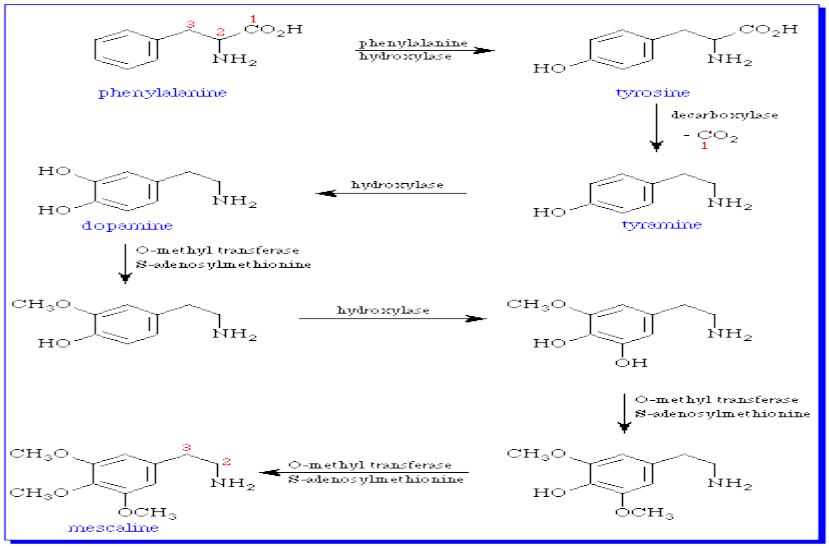


Figure 5 delineates the biosynthetic path from phenylalanine to mescaline, 3,4,5-trimethoxyphenylethylamine. Mescaline, a mild hallucinogen, is obtained from the peyote cactus, *Lophophorawilliamsi*.

The Biosynthesis of Mescaline

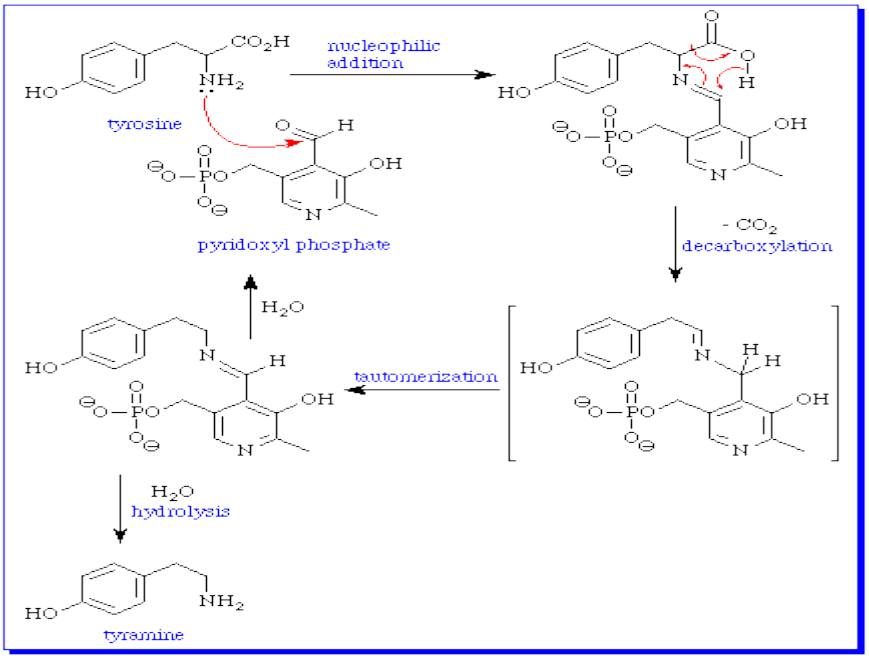


All of the reactions summarized in Figure are enzyme catalyzed. Investigation of the details of biosynthetic transformations such as those shown in Figure involve isotopic labeling studies. One approach involves *in vivo* labeling, wherein compounds with isotopicallylabeled atoms (the most common isotopes are ^{2}H, ^{3}H, ^{13}C, ^{14}C, and ^{15}N) are fed to seedlings. The alkaloid of interest is then isolated from the mature plant and the positions of the labeled atoms are determined, either by degradation of the alkaloid to simple compounds that are easily identified or by NMR spectroscopy. For example, when $^{14}C_1$-phenylalanine is fed to *L. williamsi*, the mescaline isolated from the plant does not contain any radioactivity. However, when $^{15}N_2$-phenylalanine is used, the thelabeled nitrogen ends up in the amino group of the mescaline.

Because plant feeding experiments take a long time, *in vitro* methods are often used. This approach involves addition of the labeled compounds to cell-free extracts from the organism of interest. These extracts contain the enzymes-hydroxylases, decarboxylases, methyl transferases, etc.-that are responsible for transformations like those shown in Figure.

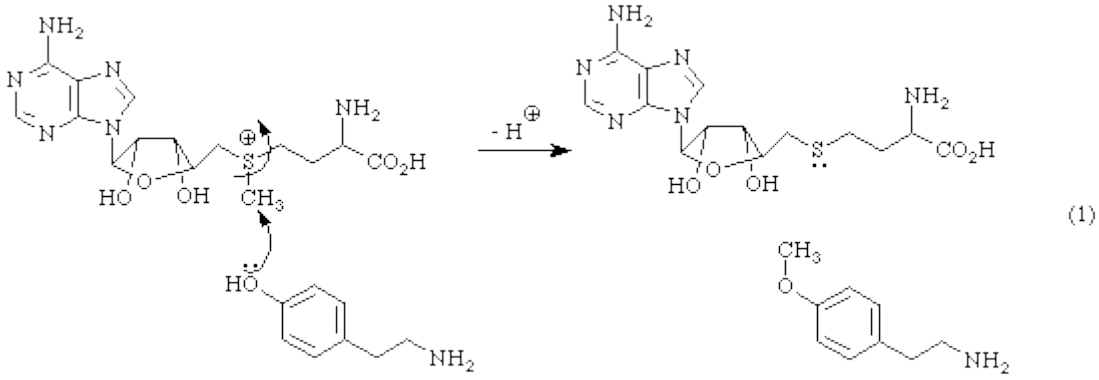
While the reactions shown in Figure are catalyzed by enzymes, the mechanisms of those reactions are similar to those deduced from investigations of non-enzymatic systems. For example, the decarboxylation of tyrosine is thought to proceed by a pathway similar to that outlined in Figure. The transformation begins with the nucleophilic addition of the amino group of tyrosine to the aldehyde function of an enzyme-bound molecule of pyridoxyl phosphate. The N=C unit of the resulting imine presumably activates the carboxylic acid group for an intramolecular proton transfer similar to that which occurs with b-keto acids. Elimination of CO_2 generates the imine shown in brackets, tautomerization of which produces a third imine, that upon hydrolysis yields tyramine, while at the same regenerating the pyridoxyl phosphate.

Decarboxylation of an Amino Acid



It is interesting to note that tyramine occurs in many foods such as aged cheese, smoked fish, and sausage. It also occurs in some beers and wines. Elevated levels of tyramine, due to ingestion of these foods and beverages, cause blood vessels to constrict and blood pressure to rise. In fact, tyramine is suspected as a possible cause of migraine headaches.

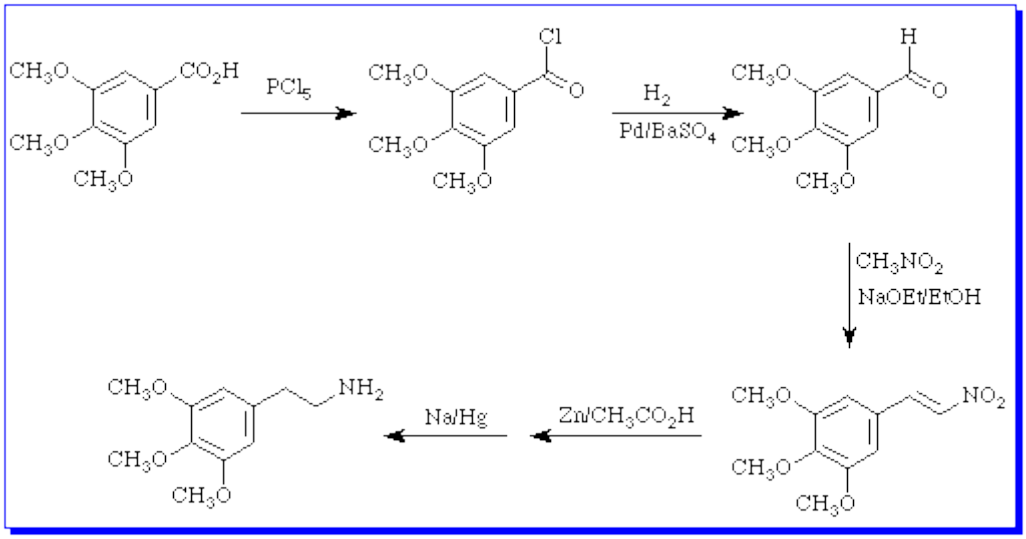
Referring back to Figure 5, the O-methylation reactions all involve the transfer of a methyl group from S-adenosylmethionine as shown in general terms in Equation 1.



(1)

This reaction is an excellent example of a nucleophilic aliphatic substitution reaction. The S-adenosylmethionine is a biological equivalent of methylating agents such as methyl iodide or methyl bromide.

The First Total Synthesis of Mescaline

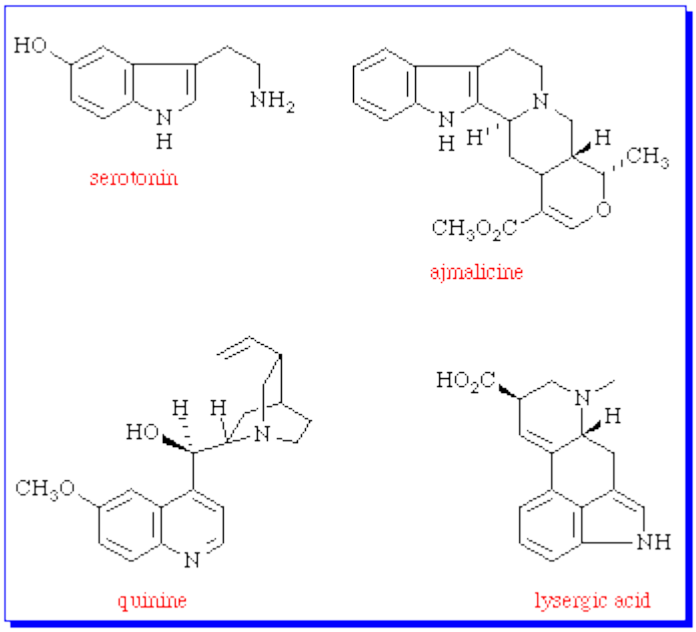


While many of the reagents shown in the above Figure may be unfamiliar, you should be able to select a modern day counterpart that would achieve the same outcome for each of the steps shown in the figure.

Alkaloids Derived from Tryptophan

Presents a small sampling of alkaloids that are derived from tryptophan.

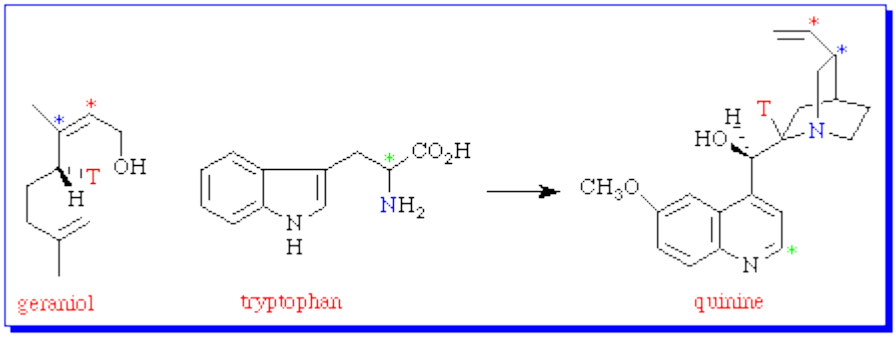
A Small Selection of Tryptophan-Based Alkaloids



While the connection between tryptophan and serotonin is obvious, it is not apparent that quinine is derived from this amino acid. This is, in part, because the quinine skeleton includes

atoms derived from non-amino acid sources, in particular the terpenegeraniol. However, feeding experiments using isotopically labelled geraniol and tryptophan have shown that molecular rearrangements must also be involved. Figure 9 presents a composite of the results of several such experiments. Since many of the steps along the biosynthetic pathway to quinine are uncertain, we will not elaborate further. However, it should be obvious that the biosynthetic pathway involved in the synthesis of quinine is a twisted one indeed.

A Proposed Pathway for the Biosynthesis of Quinine



Functions of alkaloids in plants:

- The possible functions of alkaloids in plants or the reason why they occur in plants can be assessed as follows.
- They at as poisonous agents and protect the plants against insects and herbivores.
- They may be the end products of detoxification reactions representing a metabolic locking up of compounds otherwise harmful to the plant.
- They may act as regulatory growth factors.
- They may be reserve substances, capable of supplying nitrogen or other substances.
- In some cases alkaloids could provide means of storage or transportation of plant substances in soluble form.
- Current research is demonstrating that the alkaloids do participate in plant metabolism but daily variation in alkaloidal contents (both qualitative & quantitative) shows that presence of alkaloids is not vital to the plant.
- As they participate in metabolic sequences, they are not solely the waste end products of metabolism.

PHENOLIC GROUPS

Phenol, any of a family of organic compounds characterized by a hydroxyl (−OH) group attached to a carbon atom that is part of an aromatic ring. Besides serving as the generic name for the entire family, the term *phenol* is also the specific name for its simplest member, monohydroxybenzene (C_6H_5OH), also known as benzenol, or carbolic acid.

Phenols are similar to alcohols but form stronger hydrogen bonds. Thus, they are more soluble in water than are alcohols and have higher boiling points. Phenols occur either as colourless liquids or white solids at room temperature and may be highly toxic and caustic.

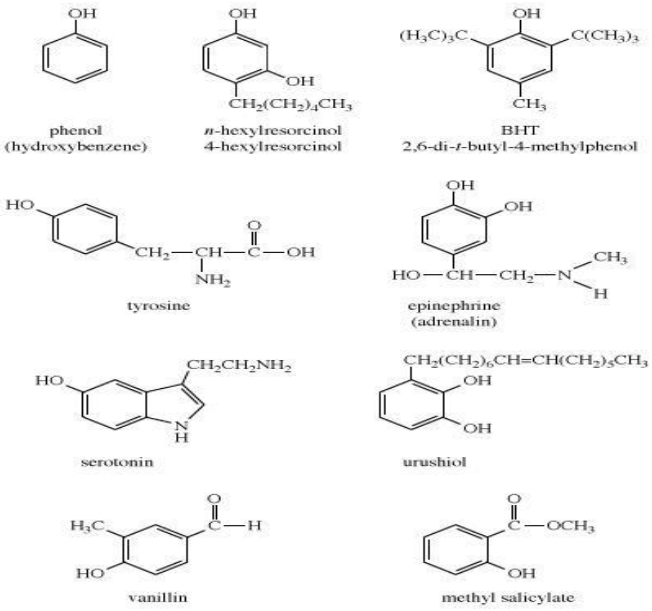
Phenols are widely used in household products and as intermediates for industrial synthesis. For example, phenol itself is used (in low concentrations) as a disinfectant in household

cleaners and in mouthwash. Phenol may have been the first surgical antiseptic. In 1865 the British surgeon Joseph Lister used phenol as an antiseptic to sterilize his operating field. With phenol used in this manner, the mortality rate from surgical amputations fell from 45 to 15 percent in Lister's ward. Phenol is quite toxic, however, and concentrated solutions cause severe but painless burns of the skin and mucous membranes. Less-toxic phenols, such as *n*-hexylresorcinol, have supplanted phenol itself in cough drops and other antiseptic applications. Butylatedhydroxytoluene (BHT) has a much lower toxicity and is a common antioxidant in foods.

In industry, phenol is used as a starting material to make plastics, explosives such as picric acid, and drugs such as aspirin. The common phenol hydroquinone is the component of photographic developer that reduces exposed silver bromide crystals to black metallic silver. Other substituted phenols are used in the dye industry to make intensely coloured azo dyes. Mixtures of phenols (especially the cresols) are used as components in wood preservatives such as creosote.

Natural Sources of Phenols

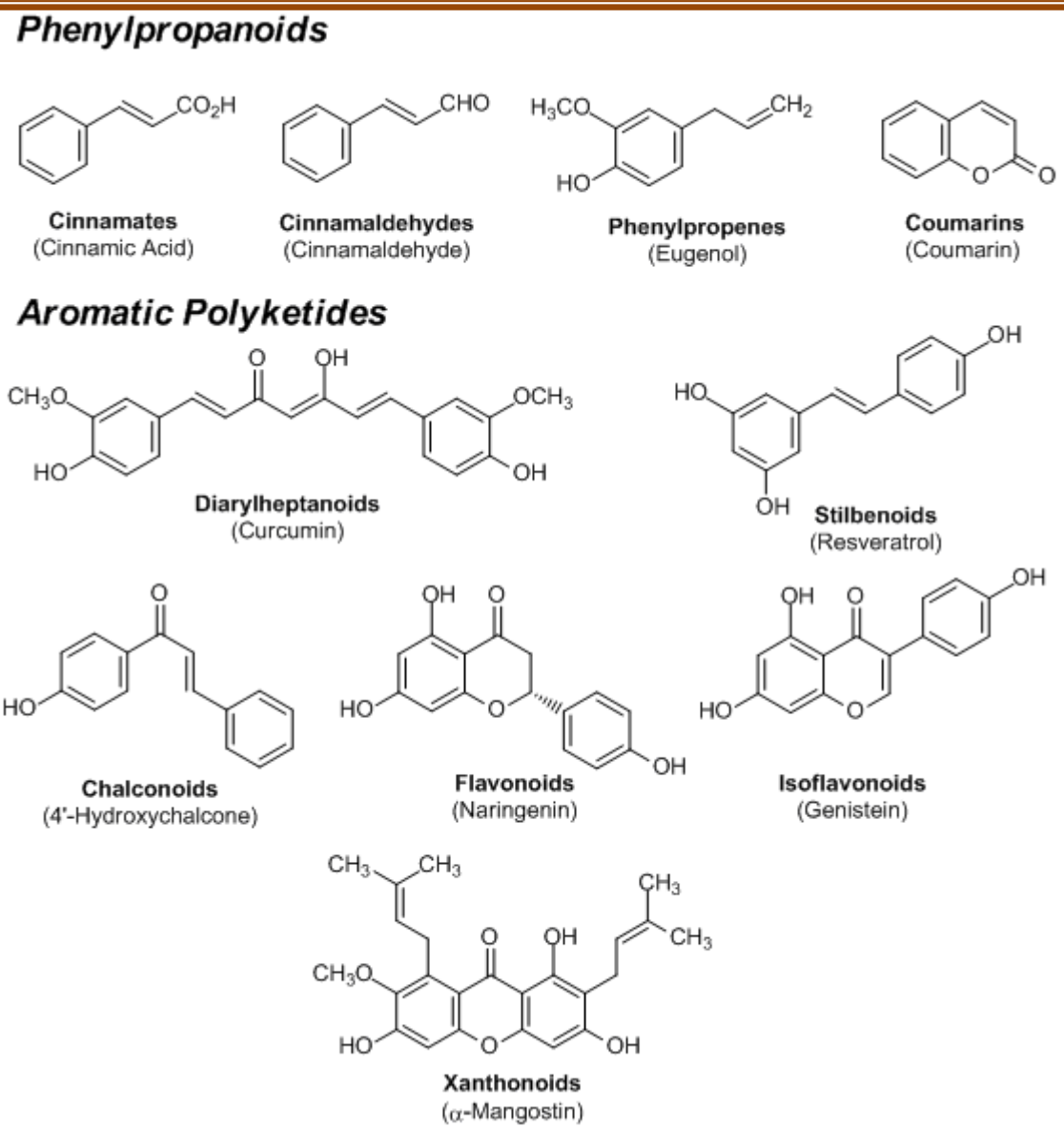
Phenols are common in nature; examples include tyrosine, one of the standard amino acidsfound in most proteins; epinephrine (adrenaline), a stimulant hormone produced by the adrenal medulla; serotonin, a neurotransmitter in the brain; and urushiol, an irritant secreted by poison ivy to prevent animals from eating its leaves. Many of the more complex phenols used as flavourings and aromas are obtained from essential oils of plants. For example, vanillin, the principal flavouring in vanilla, is isolated from vanilla beans, and methyl salicylate, which has a characteristic minty taste and odour, is isolated from wintergreen. Other phenols obtained from plants include thymol, isolated from thyme, and eugenol, isolated from cloves.



Phenol, the cresols (methylphenols), and other simple alkylated phenols can be obtained from the distillation of coal tar or crude petroleum.

Phenylpropanoids, Aromatic Polyketides

Both phenylpropanoids and aromatic polyketides are natural organic compounds of plant origin biosynthesized via the shikimic acid pathway. Phenylalanine and tyrosine are their precursors.[1)] Phenylpropanoids are classified in the group of compounds in which side chains with three carbons are attached to a benzene ring. They are ingredients of essential oils obtained from anis, cinnamon bark, and clove and are used for fragrances and aromatherapy. Aromatic polyketides are designated as compounds in which carbon chains are extended with malonyl-CoA onto phenylpropanoids.[2)] Diarylheptanoids are biosynthesized from two cinnamyl-CoA units and one malonyl-CoA. Their two aromatic rings are connected with an aliphatic seven-carbon chain. Stilbenoids, chalconoids, flavonoids and isoflavonoids are formed from a cinnamyl-CoA with three malonyl-CoA units. Chalconoids, flavonoids and isoflavonoids possess a C_6-C_3-C_6 skeleton whereas stilbenoids have a C_6-C_2-C_6 skeleton which arises by decarboxylation during the biosynthesis. Most of them have phenolic hydroxy groups and show antioxidative activity. Some of them show physiologic activities towards plants, such as phytoalexins, with the budding of seeds and adjusting of growth. Isoflavonoids are a unique compound group: they are biosynthesized via the phenyl group migration from flavonoids.[2)] To date, they are found only in the Leguminosae/Fabacaeae plant family. This distinct biosynthesis triggered researchers to investigate detailed biosynthetic mechanisms.



Structures of Phenylpropanoids and Aromatic Polyketides

Solubility

They are generally soluble in many organic solvents. They can be rather diffi cult to dissolve in non-polar solvents such as hexane but dissolve well in high polar solvents such as chloroform, methanol and DMSO. Compounds with carboxyl or phenolic hydroxy groups are soluble in aqueous alkaline solutions. Since they are easily oxidized in the liquid state, we suggest you to use them within a short period of time after preparation.

Storage

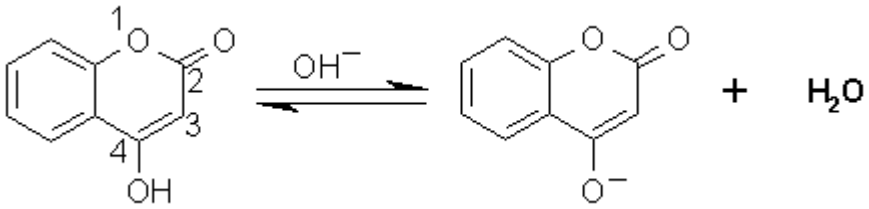
Precautions As long as no special remark is mentioned in the catalogues or labels, they can be stored at room temperature. Solids can be stored longer than liquid compounds or solutions. Note should be taken that compounds with phenolic hydroxy groups are labile to oxidation and can gradually change color from brown to black while being stored. Compounds with aldehyde groups are also apt to be oxidized to carboxylic acids. After unsealing these labile

reagents, they should be stored refrigerated or frozen under an inert gas such as nitrogen/argon.

Coumarins

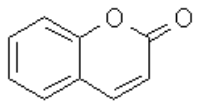
Structure

Coumarin and its derivatives are principal oral anticoagulants. Coumarin is water insoluble, however 4-hydroxy substitution confers weakly acidic properties to the molecule that makes it water soluble under slightly alkaline conditions (Equation below).

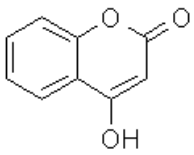


The structures of coumarin and its derivatives are as shown below. Warfarin is marketed as the sodium salt. It has one chiral center. The S(-) isomer is about 5 - 8 times more potent than the R(+) isomer, however, commercial warfarin is a racemic mixture. Click on the appropriate hyperlinks to visualize the three-dimensional structures of individual coumarins.

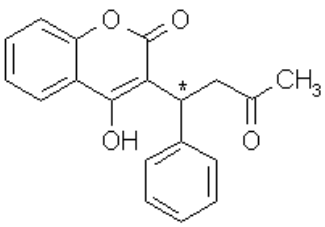
1. Coumarin
2. 4-Hydroxycoumarin
3. Warfarin
4. Dicoumarol



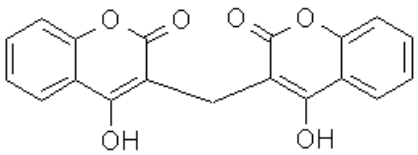
coumarin



4-hydroxycoumarin



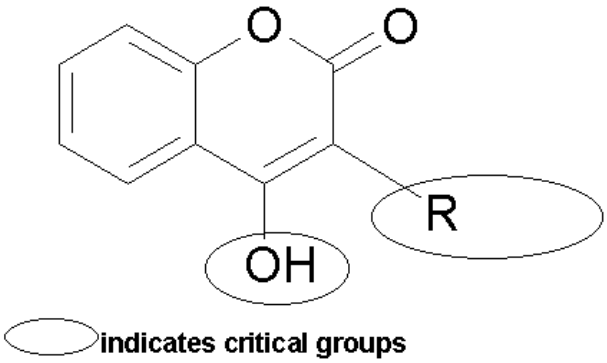
warfarin
* chiral center



bishydroxycoumarin
(dicoumarol)

Structure - Activity Relationships

Coumarin and 4-hydroxycoumarin do not possess anticoagulant activity. Link, who pioneered the isolation and characterization of bihydroxycoumarin (dicoumarol) from sweet clover, concluded that the minimal requirements for anticoagulant activity are 4-hydroxy group, a 3-substituent, and a bis molecule (see below).



Pharmacology

Coumarins exert their effect in vivo only after a latent period of 12 to 4 hours and their effect lasts for 1.5 to 5 days. The observed slow onset may be due to the time required to decrease predrugprothrombin blood levels, whereas the long duration of action observed with warfarin may be due to the lag time required for the liver to resynthesize prothrombin to predrug blood levels.

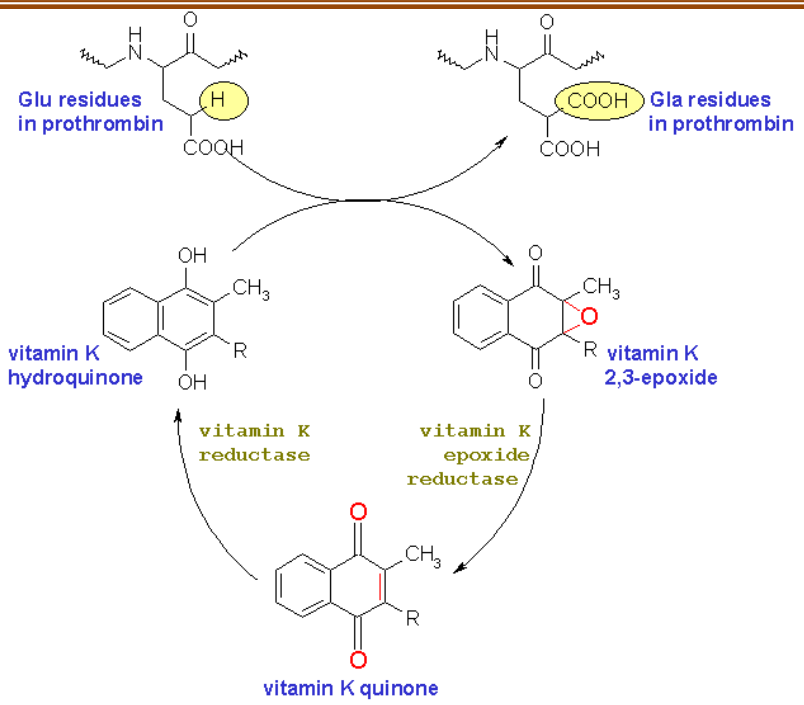
Coumarins and 1,3-indandiones (later section) interact with certain drugs. For example, the action of oral anticoagulants can be enhanced by drugs such as phyenylbutazone and salicylates while antagonized by barbiturates and vitamin K.

Biochemical Mechanism of Action

Coumarins are competitive inhibitors of vitamin K in the biosynthesis of prothrombin.

The coagulation cascade relies on the conversion of prothrombin to thrombin in a very important step. However, this conversion depends on the presence of 10 g-carboxyglutamic acid (GLA) residues in the N-terminus of prothrombin. The multiple Gla residues form a binding site for Ca^{+2}. Under normal circumstances 10 glutamic acid (Glu) residues of prothrombin are converted to Gla residues in a post-translational modification.

This post-translation modification is catalyzed by an enzymes vitamin K reductase and vitamin K epoxide reductase. Vitamin K is a co-factor in this conversion reaction. Thus it cycles between a reduced form and an epoxide form. Because of their structural similarity with vitamin K coumarins are thought to bind the enzymes, vitamin K reductase and vitamin K epoxide reductase, without facilitating the conversion of Glu residues of prothrombin to Gla. Thus prothrombin cannot be acted upon by factor Xa.



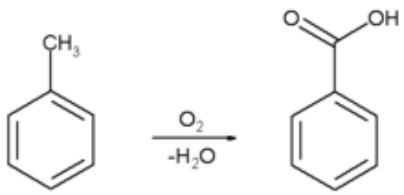
Benzoic acid,

Benzoic acid, $C_7H_6O_2$ (or C_6H_5COOH), is a colorless crystalline solid and the simplest aromatic carboxylic acid. The name derived from gum benzoin, which was for a long time the only source for benzoic acid. This weak acid and its salts are used as a food preservative. Benzoic acid is an important precursor for the synthesis of many other organic substances.

Production

Industrial preparations

Benzoic acid is produced commercially by partial oxidation of toluene with oxygen. The process is catalyzed by cobalt or manganese naphthenates. The process uses cheap raw materials, proceeds in high yield, and is considered environmentally attractive.



U.S. production capacity is estimated to be 126 000 tonnes per year, much of which is consumed domestically to prepare other industrial chemicals.

Historical preparations

The first industrial process involved the reaction of benzotrichloride (trichloromethyl benzene) with calcium hydroxide in water, using iron or iron salts as catalyst. The resulting calcium benzoate is converted to benzoic acid with hydrochloric acid. The product contains significant amounts of chlorinated benzoic acid derivatives. For this reason, benzoic acid for

human consumption was obtained by dry distillation of gum benzoin. Even after the discovery of other synthesis methods, it was forbidden to use benzoic acid of other source than gum benzoin.

Alkyl substituted benzene derivatives give benzoic acid with the stoichiometric oxidants potassium permanganate, chromium trioxide, nitric acid.

Uses

Food preservative

Benzoic acid and its salts are used as a food preservative, represented by the E-numbers E210, E211, E212, and E213. Benzoic acid inhibits the growth of mold, yeast[4] and some bacteria. It is either added directly or it is created from reactions with its sodium, potassium or calcium salt. The mechanism starts with the absorption of benzoic acid in to the cell. If the intracellular pH changes to 5 or lower the anaerobic fermentation of glucose through phosphofructokinase is decreased by 95 percent. The effectivity of benzoic acid and benzoate is thus dependent on the pH of the food.Acidic food and beverage like fruit juice (citric acid), sparkling drinks (carbon dioxide), soft drinks (phosphoric acid), pickles (vinegar) or other acidified food are preserved with benzoic acid and benzoates.

Concern has been expressed that benzoic acid and its salts may react with ascorbic acid (vitamin C) in some soft drinks, forming small quantities of benzene.

Synthesis of other chemicals

Benzoic acid is used to make a large number of chemicals, important examples:

- Benzoyl chloride, $C_6H_5C(O)Cl$, is obtained by treatment of benzoic with thionyl chloride, phosgene or one of the chlorides of phosphorus. $C_6H_5C(O)Cl$ is an important starting material for several benzoic acid derivates like benzyl benzoate, which is used as artificial flavours and insect repellents.

- Benzoyl peroxide, $[C_6H_5C(O)O]_2$, is obtained by treatment with peroxide. The peroxide is a radical starter in polymerization reactions and also a component in cosmetic products.

- Benzoate plasticizers, such as the glycol-, diethylengylcol-, and triethyleneglycol esters are obtained by transesterification of methyl benzoate with the corresponding diol. Alternatively these species arise by treatment of benzoylchloride with the diol. These plasticizers are used similarly to those derived from terephthalic acid ester.

- Phenol, C_6H_5OH, is obtained by oxidative decarboxylation at 300-400°C. The temperature required can be lowered to 200°C by the addition of catalytic amounts of copper(II) salts. The phenol can be converted to cyclohexanol, which is than starting material for nylon synthesis.

Medicinal

Benzoic acid is a constituent of Whitfield Ointment which is used for the treatment of fungal skin diseases such as tinea, ringworm and athlete's foot. It is also considered an effective treatment for acne.

Purification

Benzoic acid is purified by a method called recrystallisation. Process starts with crystalline material, removes all the impurities and forms new crystals.

Biology and health effects

Gum benzoin contains up to 20 percent of benzoic acid and 40 percent benzoic acid esters.

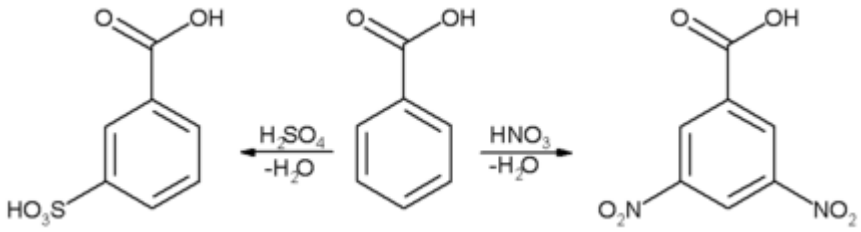
Benzoic acid is present as part of hippuric acid (N-Benzoylglycine) in urine of mammals, especially herbivores (Gr. *hippos* = horse; *ouron* = urine). Humans produce about 0.44 g/L hippuric acid per day in their urine, and if the person is exposed to toluene or benzoic acid it can rise above that level.

For humans the WHO's International Programme on Chemical Safety (IPCS) suggests a provisional tolerable intake would be 5 mg/kg body weight per day. Cats have a significantly lower tolerance against benzoic acid and its salts than rats and mice. Lethal dose for cats can be as low as 300 mg/kg body weight. The oral LD_{50} for rats is 3040 mg/kg, for mice it is 1940-2263 mg/kg.

Chemistry

Reactions of benzoic acid can occur at either the **aromatic ring** or the **carboxylic group**:

Aromatic ring



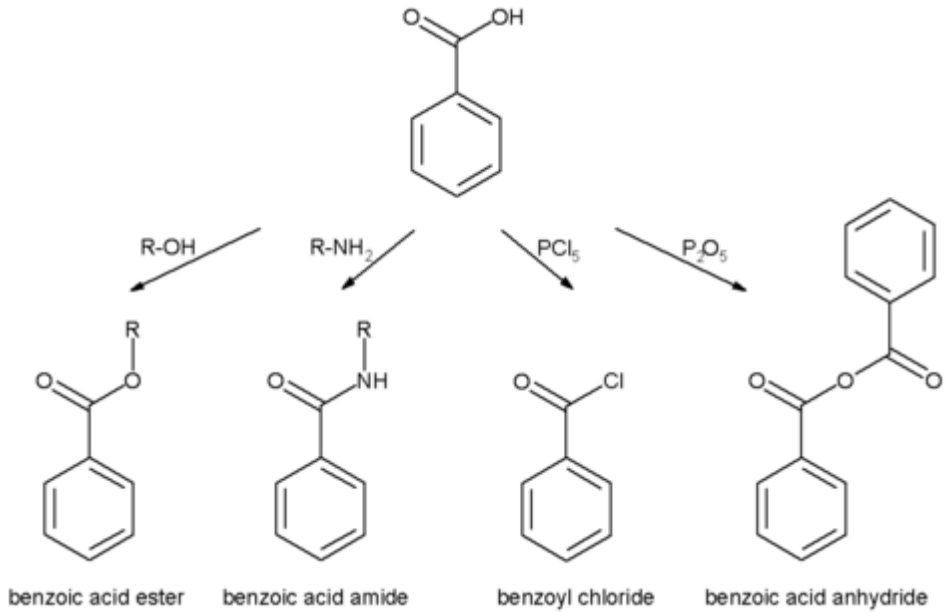
Electrophilic aromatic substitution reaction will take place mainly in 3-position to the electron-withdrawing carboxylic group.

The second substitution reaction (on the right) is slower because the first nitro group is deactivating. Conversely, if an activating group (electron-donating) was introduced (eg alkyl), a second substitution reaction would occur more readily than the first and the disubstituted product might not accumulate to a significant extent.

Carboxylic group

All the reactions mentioned for carboxylic acids are also possible for benzoic acid.

- Benzoic acid esters are the product of the acid catalysed reaction with alcohols.
- Benzoic acid amides are more easily available by using activated acid derivatives (such as benzoyl chloride) or by coupling reagents used in peptide synthesis like DCC and DMAP.
- The more active benzoic anhydride is formed by dehydration using acetic anhydride or phosphorus pentoxide.
- Highly reactive acid derivatives such as acid halides are easily obtained by mixing with halogenation agents like phosphorus chlorides or thionyl chloride.
- Orthoesters can be obtained by the reaction of alcohols under acidic water free conditions with benzonitrile.
- Reduction to benzaldehyde and benzyl alcohol is possible using DIBAL-H, $LiAlH_4$ or sodium borohydride.
- The copper catalysed decarboxylation of benzoate to benzene may be effected by heating in quinoline. Alternatively, Hunsdieckerdecoarboxylation can be achieved by forming the silver salt and heating.



Laboratory preparations

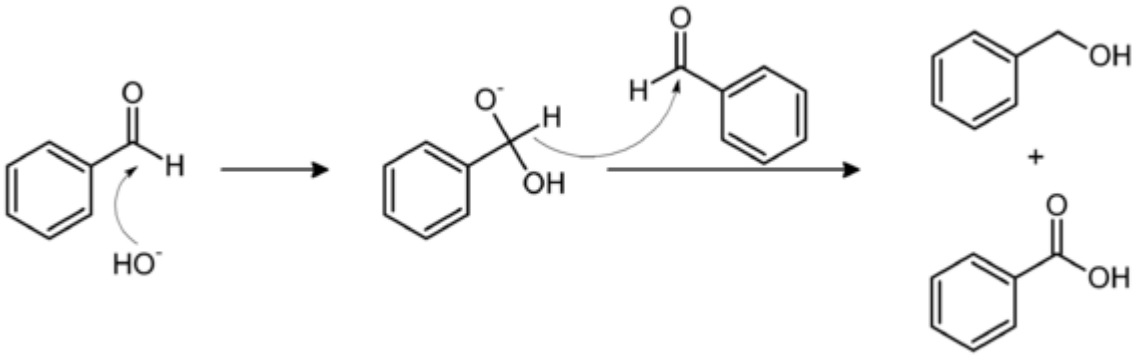
Benzoic acid is cheap and readily available, so the laboratory synthesis of benzoic acid is mainly practiced for its pedogical value. It is a common undergraduate preparation and an unusual feature of the compound is that its melting point equals its molecular weight (122). For all syntheses, benzoic acid can be purified by recrystallization from water owing to its high solubility in hot and poor solubility in cold water. The avoidance of organic solvents for the recrystallization makes this experiment particularly safe.

By hydrolysis

Like any other nitrile or amide, benzonitrile and benzamide can be hydrolyzed to benzoic acid or its conjugate base in acid or basic conditions.

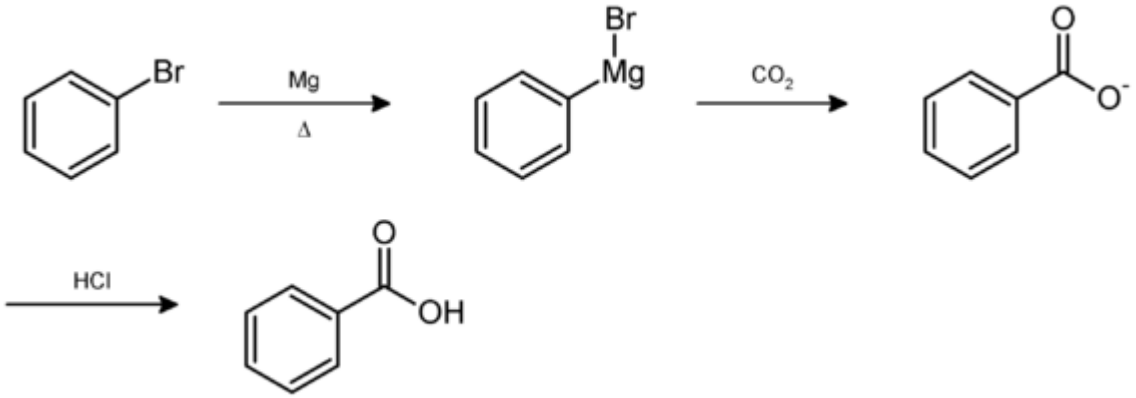
From benzaldehyde

The base-induced disproportionation of benzaldehyde, the Cannizzaro reaction, affords equal amounts of benzoate and benzyl alcohol; the latter can be removed by distillation.



From bromobenzene

Bromobenzene in diethyl ether is stirred with magnesium turnings to produce phenylmagnesium bromide (C_6H_5MgBr). This Grignard reagent is slowly added to dry ice (solid carbon dioxide) to give benzoate. Dilute acid is added to form benzoic acid.



From benzyl alcohol

Benzyl alcohol is refluxed with potassium permanganate or other oxidizing reagents in water. The mixture hot filtered to remove manganese oxide and then allowed to cool to afford benzoic acid.

Simple Plant Phenolics:

The phenolic compounds which contain basic carbon skeleton as C_6, $C_6 - C_1$, $C_6 - C_2$, $C_6 - C_3$ and $C_6 - C_4$ are sometimes grouped as simple phenolics. Many of these compounds occur in plants as secondary metabolites e.g., caffeic acid, ferulic acid; coumarins such as umbelliferone; furanocoumarins such as psoralen; benzoic acid derivatives such as salicylic acid etc. Structures of some of these compounds are given in Fig. 24.16.

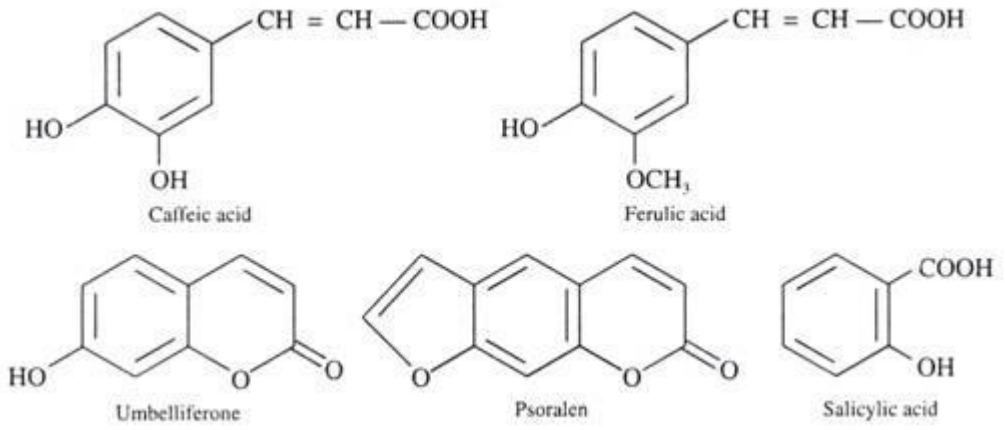


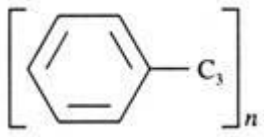
Fig. 24.16. Structures of some simple phenolic compounds occurring as secondary metabolites in plants.

Some of these phenolic compounds such as furanocoumarins are phototoxic and have defensive role in plants (especially the members of the family Umbelliferae) against insect herbivores and fungi. Some phenolic compounds such as caffeic acid and ferulic acid have allelopathic activity.

These compounds are released by some plants into the soil which inhibit germination and growth of other neighbouring plants and thus act as agents of plant-plant competition. Salicylic acid and its methyl ester methyl salicylate are known to be involved in systemic acquired resistance (SAR) to plant pathogens.

Lignin:

Lignin is highly complex and branched polymer of simple phenolic compounds with $C_6 - C_3$ basic carbon skeleton.



The structure of lignin is obscure. However, three phenyl propanoid alcohols viz., coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol (Fig. 24.17) are believed to be building blocks of lignin. These three building units of lignin are not joined in a simple repeating manner but have a highly branched and complex arrangement to form lignin. The proportion, of these three compounds in lignin varies among species, plant organs and even in different regions of the same cell wall.

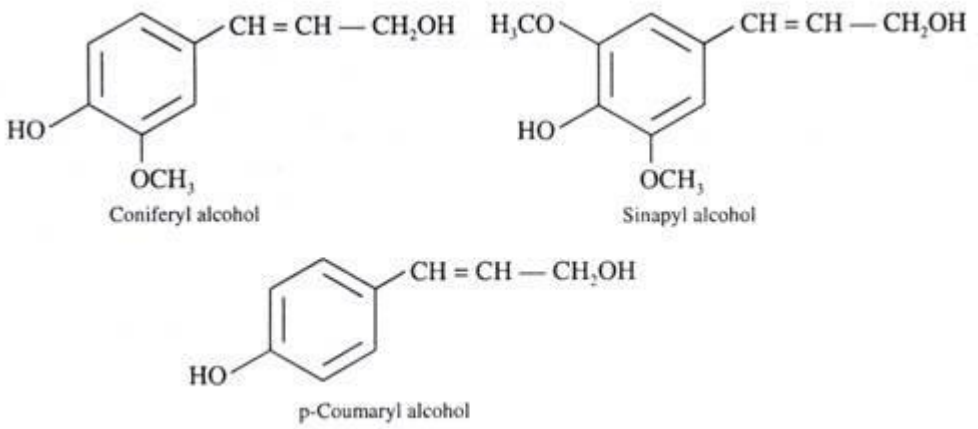


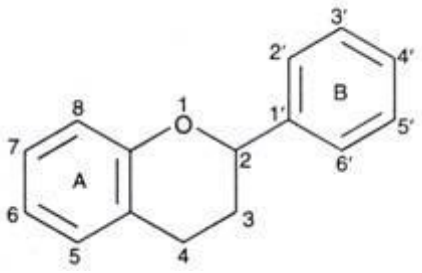
Fig. 24.17. Structures of phenolic subunits found in lignins.

Lignin is a strengthening material which occurs chiefly in secondary walls of supporting and conducting tissue especially vessels and tracheids of xylem in all vascular plants. It may also occur in middle lamella and primary wall along with celluloses and other cell wall polysaccharides. After cellulose, lignin is second most abundant organic substance in higher plants. It comprises 15-25% of dry weight of many woody plant species.

Primary function of lignin is to provide mechanical support to plant. Besides this, lignin also protects the cell walls from physical, chemical and biological attack. Lignin is considered to have great evolutionary significance as an important adaptation of primitive plants to terrestrial environment.

Flavonoids:

Flavonoids are 15-C phenolic compounds widely distributed in plants and consist of $C_6 - C_3 - C_6$ basic carbon skeleton. Positions on this carbon skeleton are numbered as shown in Fig. 24.18.



The two aromatic carbon rings at the left and right sides of the flavonoid molecule are designated as A and b rings respectively. The second ring B and 3-carbons (at positions 2, 3, 4) of the middle ring are derived from the shikimic acid pathway, whereas the ring A and oxygen of the middle ring are derived entirely from acetate units provided by acetyl-CoA through malonic acid pathway.

Flavonoids usually occur as glycosides and are soluble in water and mostly coloured being red, crimson, purple, blue and yellow. They accumulate in vacuole although they are syntheiszed outside the vacuole. Sometimes, flavonoids may also occur in chromoplasts and chloroplasts.

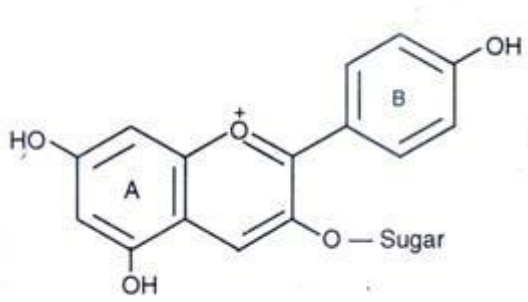
Hydroxyl groups (- OH) are usually present in flavonoids especially at positions 3′ and 4′ of ring B or positions 5 and 7 of ring A or 3rd position of central ring. In the latter cases, they serve as points of attachment of various sugars which make them soluble in water. Flavonoids perform variety of functions in plants including defense and pigmentation.

Based on degree of oxidation at various positions of the central ring, the flavonoids are classified into many subgroups. However, three of these subgroups are of particular interest in plant physiology viz., anthocyanins, flavonols& flavones and isoflavones (isoflavonoids).

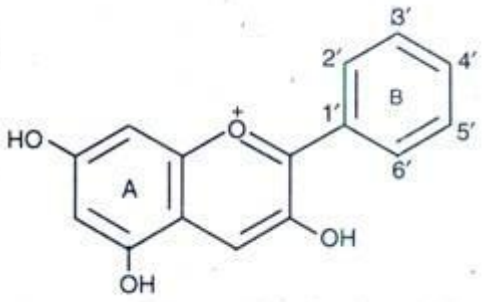
(i) Anthocyanins:

The anthocyanins (from Greek anthos = flower; kyanos = dark blue) are coloured flavonoid pigments commonly found in blue, purple and red flowers. Sometimes, they may also occur in other parts of plants such as some fruits, stems, leaves and even roots. Although anthocyanins provide different colours to vast majority of flowers and fruits, but sometimes the colouration of flowers and fruits may be due to carotenoid pigments (tetraterpenes) such as in some yellow flowers and tomato fruits.

Anthocyanins are found dissolved in cell sap in vacuole as glycosides. Without sugar molecule, the rest part of anthocyanin (i.e., itsaglycone) is called as anthocyanidin. Basic structures of anthocyanin and anthocyanidin are shown in Fig. below.



Anthocyanin



Anthocyanidin (a flavynium cation)

Anthocyanidins contain hydroxyl groups at 3rd position of central ring and 5th and 7th positions of ring A. Sugars usually in the form of one or two glucose or galactose units are mostly attached at 3rd position of central ring or 5th position of ring A to form its glycoside i.e., anthocyanin. Sometimes sugar may be attached at 7th position of ring A.

Anthocyanidins also contain one or more hydroxyl groups (- OH) in ring B at 3′, 4′ and 5′ positions some of which may be methylated. These substituent groups on ring B give characteristic colour to the anthocyanidins.

Besides this, anthocyanin colour is also influenced by other factors such as:

(i) Occurrence of different anthocyanins in the same flower or plant organ,

(ii) Association among anthocyanins especially at high concentration,

(iii) pH of the vacuoles,

(iv) Co-existence of anthocyanins with other flavonoids such as flavonols and flavones (i.e., copigmentation) and

(v) Association of anthocyanins with chelated metal ions.

More than 22 different anthocyanidins are known. Of these, three are most common viz. pelargonidin, delphinidin and cyanidin. The colours and structural features of these and a few other anthocyanidins are given in Table below.

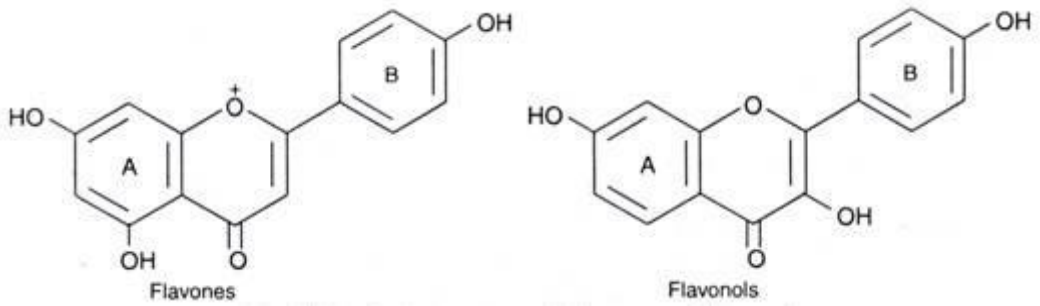
Anthocyanidin	Colour	Substituents and their positions on ring B.
Pelargonidin	Scarlet (bright red)	4′ – OH
Delphinidin	Bluish purple	3′ – OH, 4′ – OH, 5′ – OH
Cyanidin	Crimson (deep red)	3′ – OH, 4′ – OH
Peonidin	Rosy red	3′ – OCH_3, 4′ – OH
Petunidin	Purple	3′ – OCH_3, 4′ – OH, 5′ – OH
Malvidin	Mauve (Purplish)	3′ – OCH_3, 4′ – OH, 5′ – OCH_3

Main role of anthocyanins in flowering plants is to attract insects and other animals for pollination and dispersal of fruits and seeds.

Anthocyanins and other flavonoids present in related species of a genus provide useful information to plant taxonomists in classifying and determining lines of plant evolution. These pigments are also of interest to many plant geneticists because sometimes it is possible to correlate many morphological differences in closely related species of a genus with the types of anthocyanins and other flavonoids which are found in them.

(ii) Flavones and Flavonols:

These are closely related in structure to anthocyanins except that they differ in central ring of their molecules as shown in Fig. below.



Flavones or flavonols are mostly yellowish and ivory coloured pigments which are widespread in flower petals and may contribute to flower colour. Some of these pigments are colourless and appear to give ‘body’ to white, cream and ivory-coloured flowers. Flavones &flavonols are also widespread in leaves.

These pigments perform many biological functions in plants:

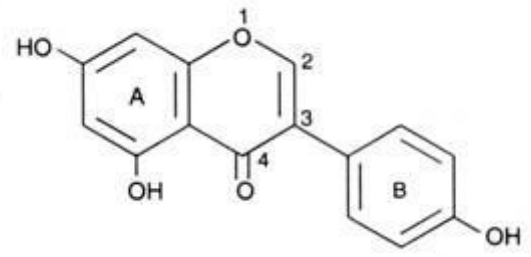
(i) Colourless flavones &flavonols absorb UV radiation and thus provide protection to cells against UV rays,

(ii) These may also be feeding deterrents to herbivores,

(iii) These pigments are not visible to human eye because they absorb UV light, however insects such as bees can see into UV range of spectrum and therefore, may respond to these pigments as attractant cues.

(iii) Isoflavonoids (Isoflavones):

These are found mostly in leguminous plants and differ from other flavonoids in structure in that the aromatic ring B is shifted and is attached to carbon at 3rd position of central ring instead to carbon at second position (Fig. below).



Isoflavonoids or isofloavones perform many biological functions:

(i) Some of them such as rotenoids have strong insecticidal properties (rotenone, an isoflavonoid from the roots of Derris elliptica, is widely used insecticide),

(ii) Isoflavonoids resemble in structure to some animal hormones estrogens such as estradiol, and cause infertility in mammals especially sheep,

(iii) Isoflavonoids are also known to act as phytoalexins. (Phytoalexins are antimicrobial substances which are produced in plants as a result of fungal or bacterial infection. Besides isoflavonoids, many sesquiterpenes in plants especially members of family solanaceae, are produced as phytoalexins).

Tannins:

The term “tannins” was introduced in 1796 to describe a group of compounds occurring in some plants which could tan animal skins or hides to produce leather. Tannins bind with collagen proteins of animal skins to make the latter more resistant to heat, water and microbial attack.

Tannins are plant phenolic polymers which are widely distributed in higher plants. Bark, leaves, wood and unripe fruits of some plants are good sources of tannins. The compounds having a mol., wt. between 500 – 3000 and containing sufficient phenolic hydroxylic groups (1- 2% M.W.) to form effective cross links with proteins are considered as good tannin agents. An extract from oak bark (Quercus spp.) is a common tanning agent.

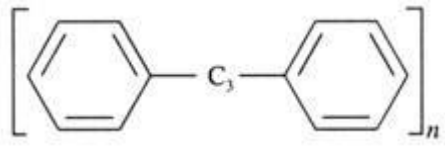
Tannins are divided into two main groups, condensed tannins and hydrolysable tannins.

(i) Condensed tannins (flavolans):

These are polymers of 15-C phenolic compound with flavone or C_6-C_3-C_6 type of basic carbon skeleton.

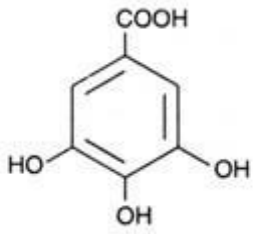
Condensed tannins are not easily hydrolysed:

On treatment with hydrolytic agents, they do not yield significant amounts of compounds of low mol. wt. Instead, they tend to be polymerised especially in acid, to form amorphous red coloured compounds called phlobaphenes.



(ii) Hydrolysable tannins:

These are heterogeneous polymers containing phenolic acids particularly gallic acid (Fig. 24.22) and simple sugars such as glucose. They have smaller mol. wts. in comparison to condensed tannins and can be hydrolysed comparatively more easily.



The simplest example of hydrolysable tannins is Chinese tannin from sumac (Khus sp.) which consists of eight Gallic acid resides connecting in various ways to one another and glucose. The hydrolysable tannins which consist of gallic acids as phenolic residues are also called as gallotannins.

Biological functions of tannins:

(i) Tannins act as feeding deterrents against herbivores because they cause astringency and also because they interfere with digestion and utilization of foods.

(ii) Tannins provide protection to plants against microbial attack.

(iii) Many gallotannins are known to inhibit plant growth and some of them act as allelopathic agents.

Biological Functions of Plant Phenolics:

(i) Some of them act as chemical deterrents against herbivores and pathogens.

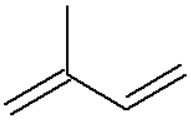
(ii) Plant phenolics such as lignins provide mechanical strength to the plants and have significant proective functions in them.

(iii) Some phenolics play important role in plants in attracting pollinators and fruits & seeds dispersers.

(iv) Some plant phenolics play important role in allelopathy (Greek, allelon = of one another; pathos = diseases). Allelopathy is the influence of chemicals released by one plant species on another plant or animal with resulting benefits to the species which contains them.

TERPENOIDS

The terpenoids (aka isoprenoids) are a large (estimated 60% of known natural products) and diverse group of lipids derived from five-carbon isoprene units assembled in thousands of combinations. Technically a terpenoid contains oxygen, while a terpene is a hydrocarbon. Often the two terms are used to refer collectively to both groups.



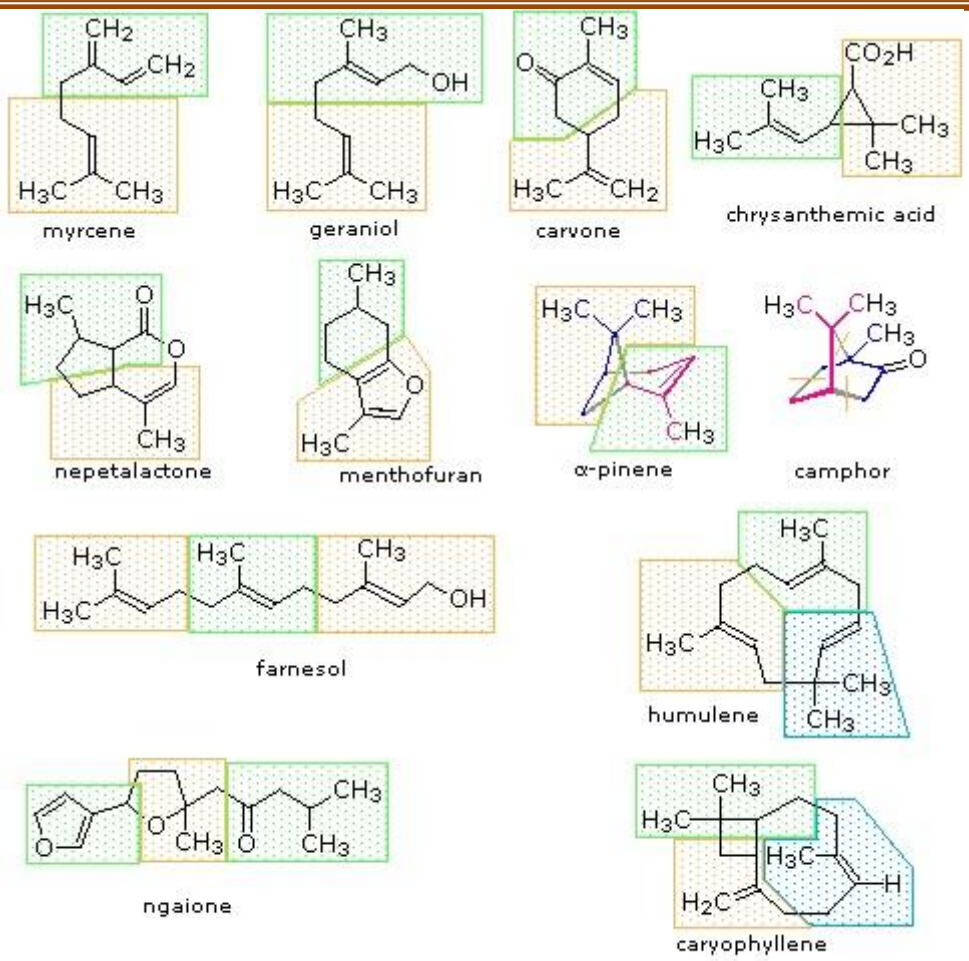
isoprene

Isoprene Rule

Compounds classified as terpenes constitute what is arguably the largest and most diverse class of natural products. A majority of these compounds are found only in plants, but some of the larger and more complex terpenes (e.g. squalene&lanosterol) occur in animals. Terpenes incorporating most of the common functional groups are known, so this does not provide a useful means of classification. Instead, the number and structural organization of carbons is a definitive characteristic. Terpenes may be considered to be made up of isoprene (more accurately isopentane) units, an empirical feature known as the isoprene rule. Because of this, terpenes usually have 5n5n carbon atoms (nn is an integer), and are subdivided as follows:

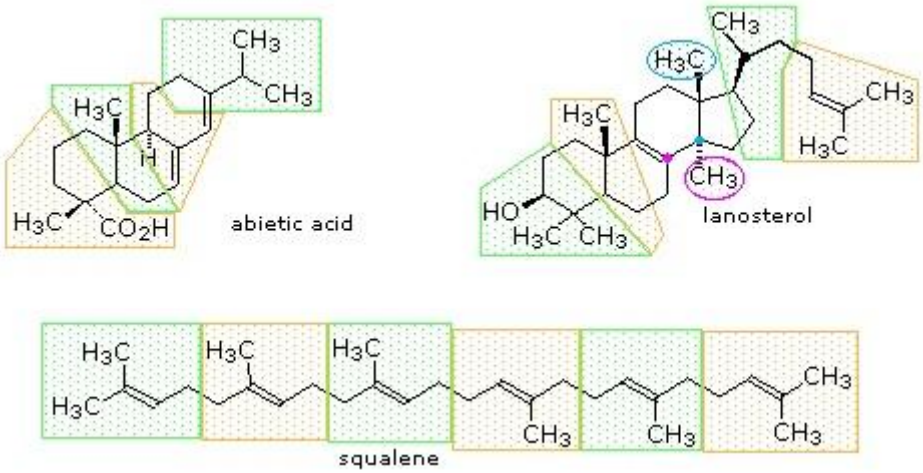
Classification	Isoprene Units	Carbon Atoms
monoterpenes	2	C_{10}
sesquiterpenes	3	C_{15}
diterpenes	4	C_{20}
sesterterpenes	5	C_{25}
triterpenes	6	C_{30}

Isoprene itself, a C_5H_8 gaseous hydrocarbon, is emitted by the leaves of various plants as a natural byproduct of plant metabolism. Next to methane it is the most common volatile organic compound found in the atmosphere. Examples of C10 and higher terpenes, representing the four most common classes are shown in the following diagrams. Most terpenes may be structurally dissected into isopentane segments. How this is done can be seen in the diagram directly below.



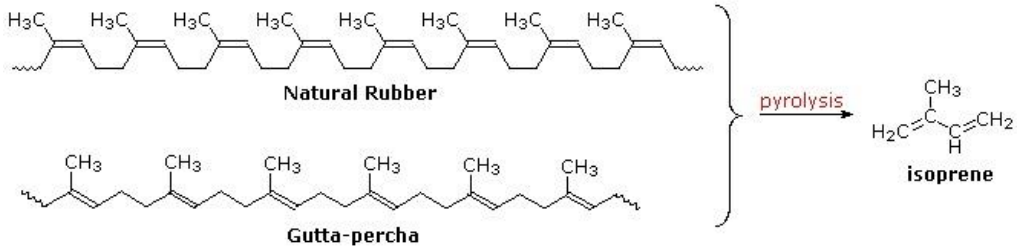
Monoterpenes and sesquiterpenes

The isopentane units in most of these terpenes are easy to discern, and are defined by the shaded areas. In the case of the monoterpene camphor, the units overlap to such a degree it is easier to distinguish them by coloring the carbon chains. This is also done for alpha-pinene. In the case of the triterpenelanosterol we see an interesting deviation from the isoprene rule. This thirty carbon compound is clearly a terpene, and four of the six isopentane units can be identified. However, the ten carbons in center of the molecule cannot be dissected in this manner. Evidence exists that the two methyl groups circled in magenta and light blue have moved from their original isoprenoid locations (marked by small circles of the same color) to their present location. This rearrangement is described in the biosynthesis section. Similar alkyl group rearrangements account for other terpenes that do not strictly follow the isoprene rule.



Triterpenes

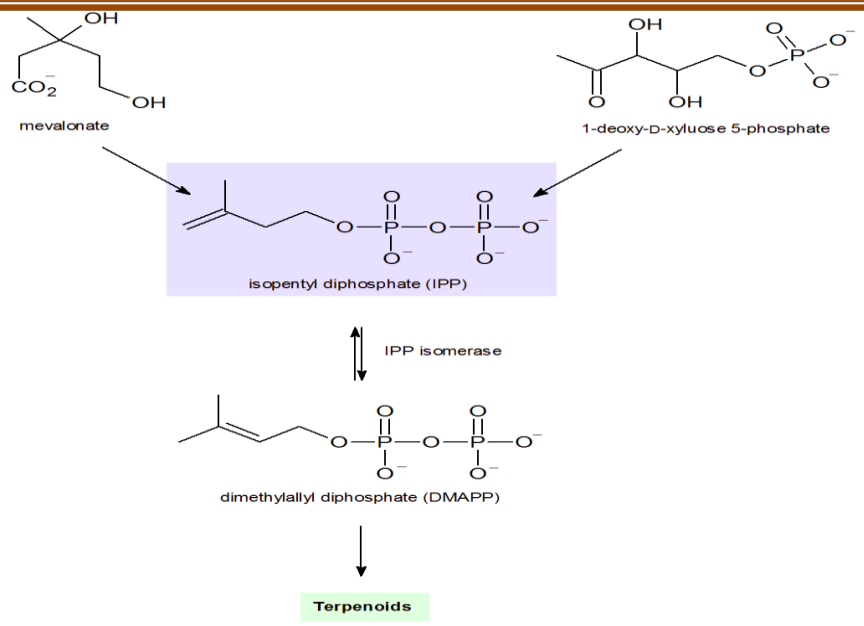
Polymeric isoprenoid hydrocarbons have also been identified. Rubber is undoubtedly the best known and most widely used compound of this kind. It occurs as a colloidal suspension called latex in a number of plants, ranging from the dandelion to the rubber tree (*Heveabrasiliensis*). Rubber is a polyene, and exhibits all the expected reactions of the C=C function. Bromine, hydrogen chloride and hydrogen all add with a stoichiometry of one molar equivalent per isoprene unit. Ozonolysis of rubber generates a mixture of levulinic acid ($CH_3COCH_2CH_2CO_2H$) and the corresponding aldehyde. Pyrolysis of rubber produces the diene isoprene along with other products.



The double bonds in rubber all have a Z-configuration, which causes this macromolecule to adopt a kinked or coiled conformation. This is reflected in the physical properties of rubber. Despite its high molecular weight (about one million), crude latex rubber is a soft, sticky, elastic substance. Chemical modification of this material is normal for commercial applications. Gutta-percha (structure above) is a naturally occurring E-isomer of rubber. Here the hydrocarbon chains adopt a uniform zig-zag or rod like conformation, which produces a more rigid and tough substance. Uses of gutta-percha include electrical insulation and the covering of golf balls.

Terpenoid Biosynthesis

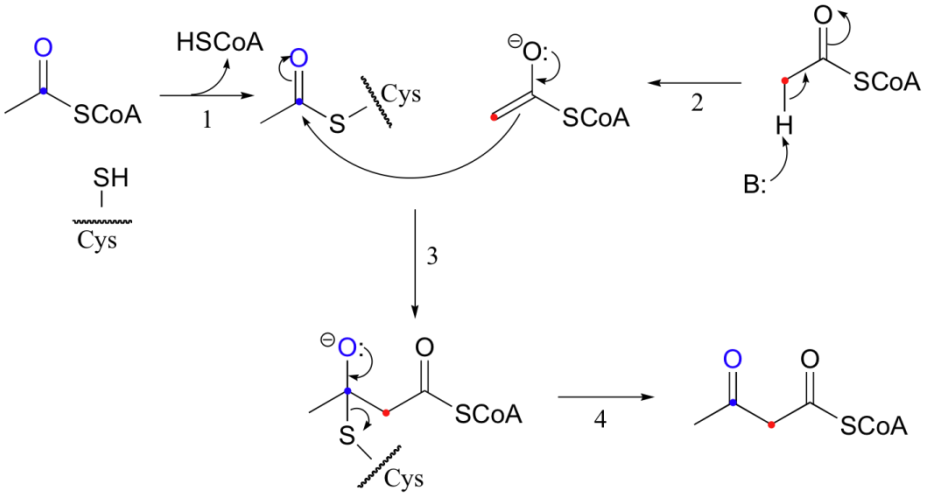
While we can identify isoprene units within a terpenoid structure and use that in its classification, the building block for terpenoid synthesis in nature is isopentenyldiphosphate (formerly called isopentenyl pyrophosphate and abbreviated IPP). There are two major routes to the synthesis of IPP; namely (1) the mevalonate pathway and (2) the 1-deoxyxylulose pathway.



Mevalonate Pathway

Step 1 - Claisen Condensation

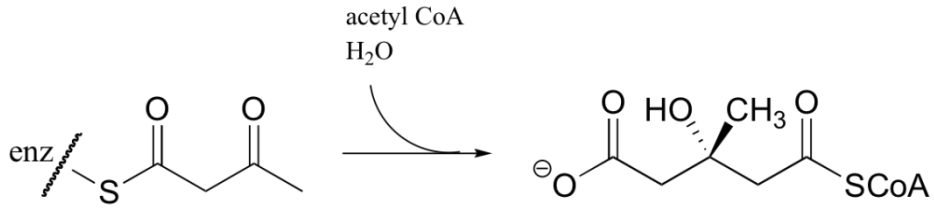
An early step in the biosynthesis of cholesterol and other 'isoprenoid' compounds is a Claisen condensation between two acetyl CoA molecules. An initial trans-thioesterase process transfers the acetyl group of the first acetyl CoA to an enzymatic cysteine (Reaction 1). In the Claisen condensation phase of the reaction, the alpha-carbon of a second acetyl CoA is deprotonated, forming an enolate (Reaction 2).



The enolate carbon attacks the electrophilic thioester carbon, forming a tetrahedral intermediate (Reaction 3) which quickly collapses to expel the cysteine thiol (Reaction 4) and produce acetoacetyl CoA.

Step 2 - Aldol Condensation

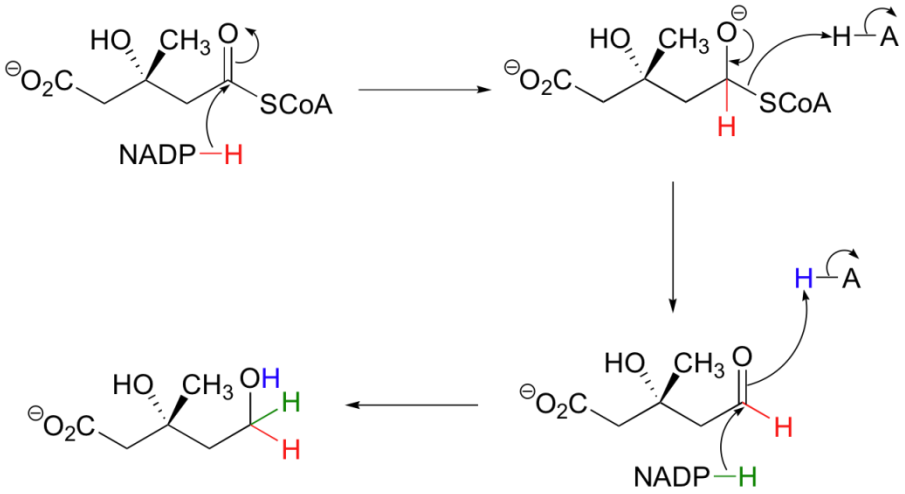
Acetyl CoA then reacts with the acetoacetyl CoA in an aldol-like addition. Subsequent hydrolysis produces (3*S*)-3-hydroxy-3-methylglutaryl CoA (HMG-CoA).



Generating HMG-CoA

Step 3 - Reduction of the Thioester

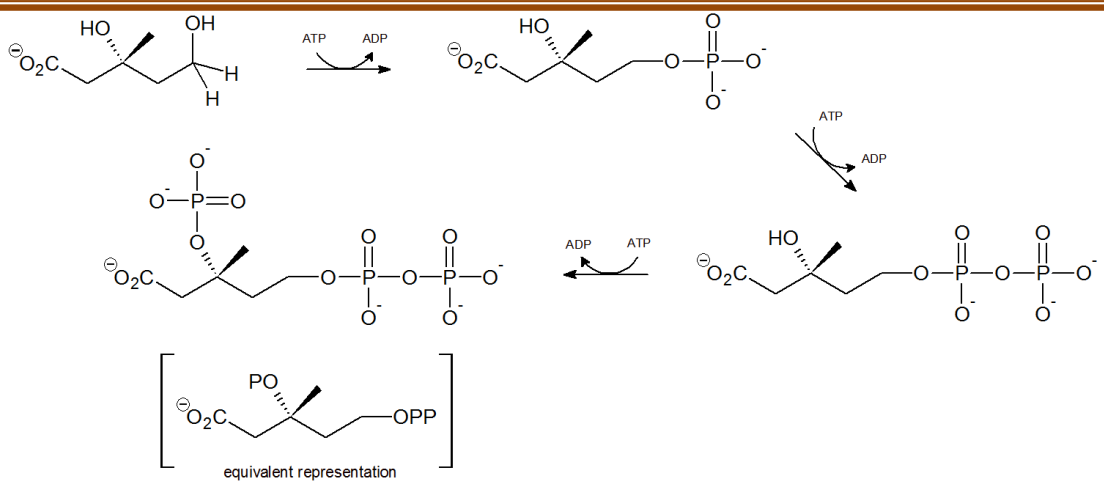
The thioester is reduced first to an aldehyde, then to a primary alcohol by two equivalents of NADPH producing (*R*)-mevalonate. The enzyme catalyzing this reaction is the target of the statin family of cholesterol-lowering drugs.



Generating (*R*)-Mevalonate

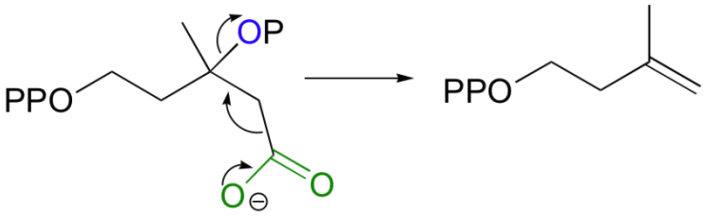
Step 4 - Mevalonate Phosphorylation

Two phsophorylations by adenosine triphosphate (ATP) occur at the terminal hydroxyl/phosphorus group through nucleophilic substitution, followed by a third ATP phosphorylation of the tertiary hydroxyl group.



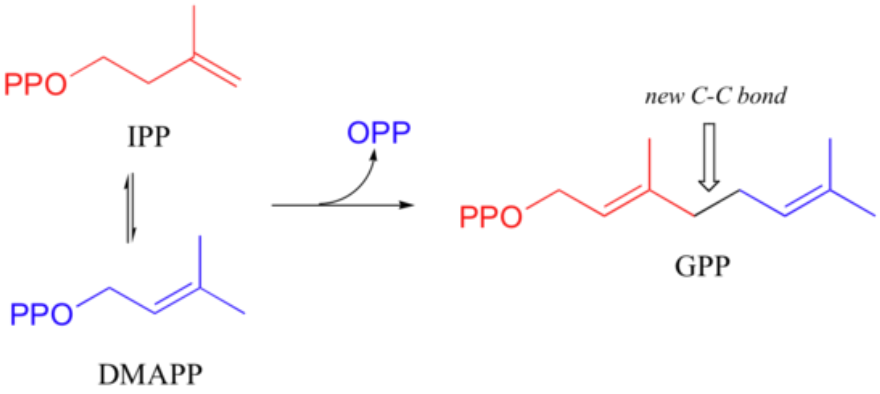
Step 5 - Decarboxylation

Finally isopentenyldiphosphate (IPP), the 'building block' for all isoprenoid compounds, is formed from a decarboxylation-elimination reaction.

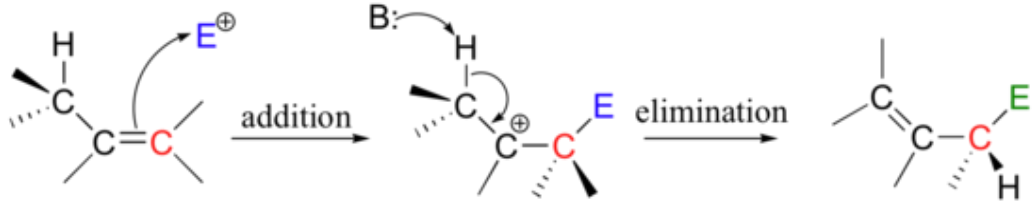


Conversion of IPP to Terpenoids

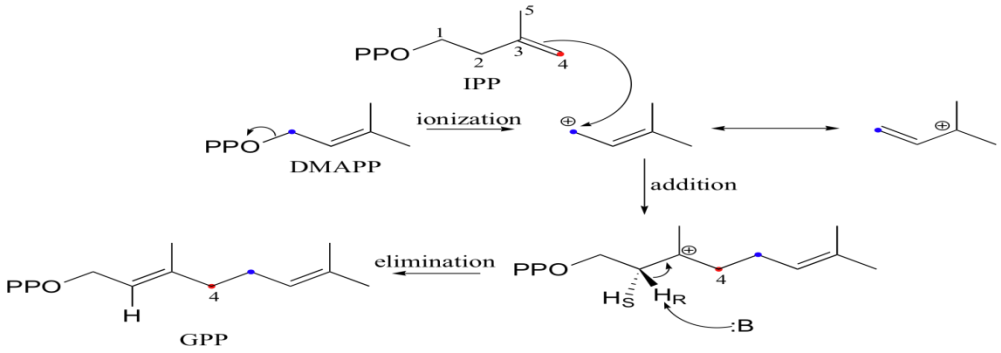
The electrophilic double bond isomerization catalyzed by IPP isomerase is a highly reversible reaction, with an equilibrium IPP:DMAPP ratio of about 6:1. In the next step of isoprenoid biosynthesis, the two five-carbon isomers condense to form a 10-carbon isoprenoid product called geranyldiphosphate (GPP).



This is a nice example of an electrophilic addition/elimination mechanism:

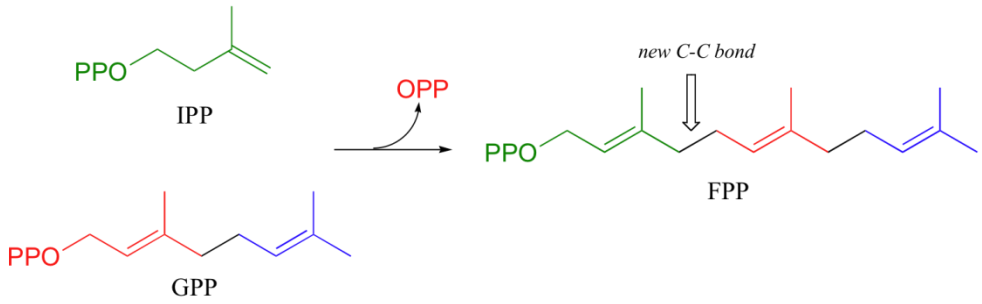


The first step is ionization of the electrophile - in other words, the leaving group departs and a carbocation intermediate is formed. In this case, the pyrophosphate group on DMAPP is the leaving group, and the electrophilic species is the resulting allylic carbocation.

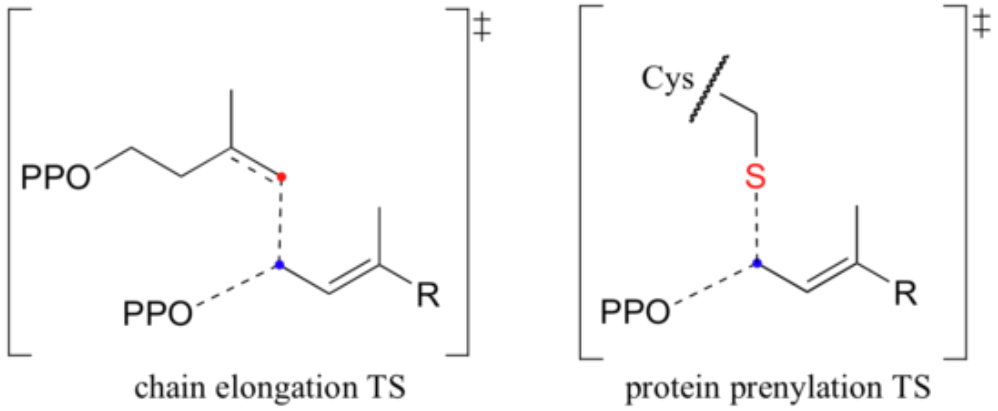


In the condensation (addition) step, the C_3-C_4 double bond in IPP attacks the positively-charged C_1 of DMAPP, resulting in a new carbon-carbon bond and a second carbocation intermediate, this time at a tertiary carbon. In the elimination phase, proton abstraction leads to re-establishment of a double bond in the GPP product. Notice that the enzyme specifically takes the *pro-R* proton in this step.

To continue the chain elongation process, another IPP molecule can then condense, in a very similar reaction, with C_1 of geranyldiphosphate to form a 15-carbon product called farnesyldiphosphate (FPP).



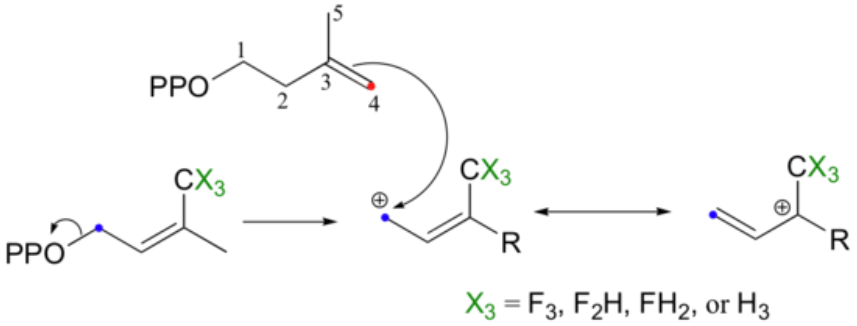
How do we know that these are indeed S_N1-like mechanisms with carbocation intermediates, rather than concerted S_N2-like mechanisms? First of all, recall that the question of whether a substitution is dissociative (S_N1-like) or associative (S_N2-like) is not always clear-cut - it could be somewhere in between, like the protein prenyltransferase reaction. The protein prenyltransferase reaction and the isoprenoid chain elongation reactions are very similar: the electrophile is the same, but in the former the nucleophile is a thiolate, while in the latter the nucleophile is a pi bond.



chain elongation TS protein prenylation TS

This difference in the identity of the nucleophilic species would lead one to predict that the chain elongation reaction has more S_N1-like character than the protein prenylation reaction. A thiolate is a very powerful nucleophile, and thus is able to *push* the pyrophosphate leaving group off, implying some degree of S_N2 character. The electrons in a pi bond, in contrast, are only weakly nucleophilic, and thus need to be *pulled* in by a powerful electrophile - *ie*. a carbocation.

So it makes perfect sense that the chain elongation reaction should more S_N1-like than S_N2-like. Is this in fact the case? We know how to answer this question experimentally - just run the reaction with fluorinated DMAPP or GPP substrates and observe how much the fluorines slow things down.

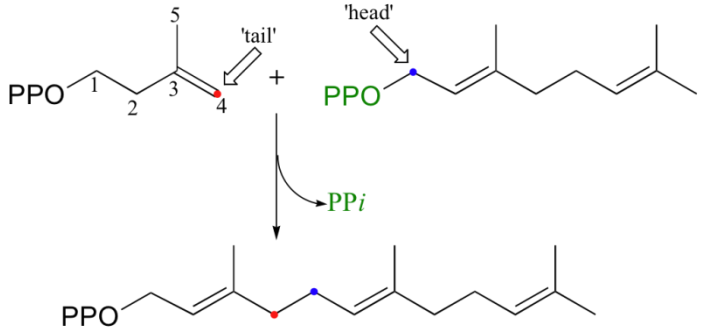


$X_3 = F_3, F_2H, FH_2$, or H_3

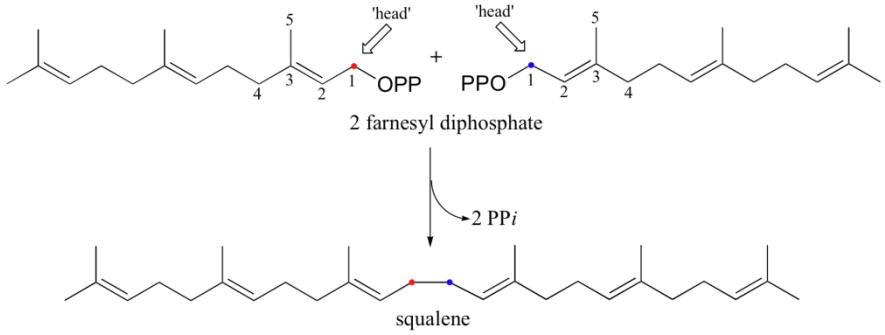
If the reaction is S_N1-like, the electron-withdrawing fluorines should destabilize the allylic carbocation intermediate and thus slow the reaction down considerably. If the mechanism is S_N2-like, the fluorine substitutions should not have a noticeable effect, because a carbocation intermediate would not be formed. When this experiment was performed with FPP synthase, the results were dramatic: the presence of a single fluorine slowed down the rate of the reaction by a factor of about 60, while two and three fluorines resulted in a reaction that was 500,000 and 3 million times slower, respectively (*J. Am. Chem. Soc*. **1981**, *103*, 3926.) These results strongly suggest indicate the formation of a carbocation intermediate in an S_N1-like displacement.

In this section, we will briefly examine the reaction catalyzed by an enzyme called squalene synthase, an important enzymatic transformation that involves some very interesting and unusual electrophilic additions, rearrangements, and reactive intermediates. This particular enzyme is also of interest because it represents a potential new target for cholesterol-lowering drugs.

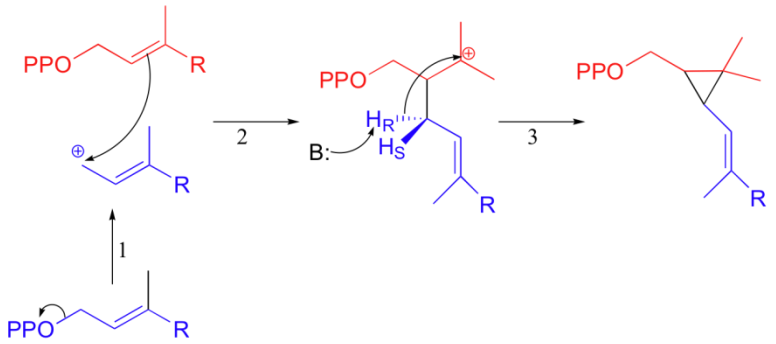
Cholesterol, as we discussed earlier in this chapter, is derived from a 30-carbon isoprenoid molecule called squalene. Squalene, in turn, is derived from the condensation of two molecules of farnesyldiphosphate (FPP), a 15-carbon isoprenoid. You may recall that FPP is the product of the C_4 to C_1, or 'head to tail' electrophilic condensation of isoprenoid chains:



The condensation of two molecules of FPP to form squalene, however, is something different: this is a 'head to head' condensation, where C_1 of the first molecule forms a bond to C_1 of the second. The chemistry involved is quite a bit more complicated.

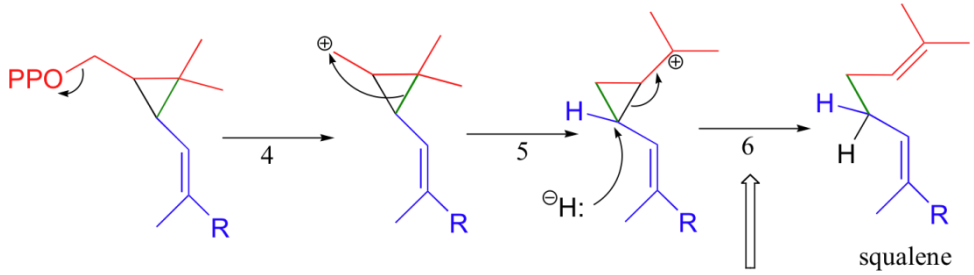


The first two steps are familiar: first, the pyrophosphate on one FPP molecule leaves (step 1), resulting in an allylic carbocation that is attacked by the C_2-C_3 π bond of the second molecule (step 2).

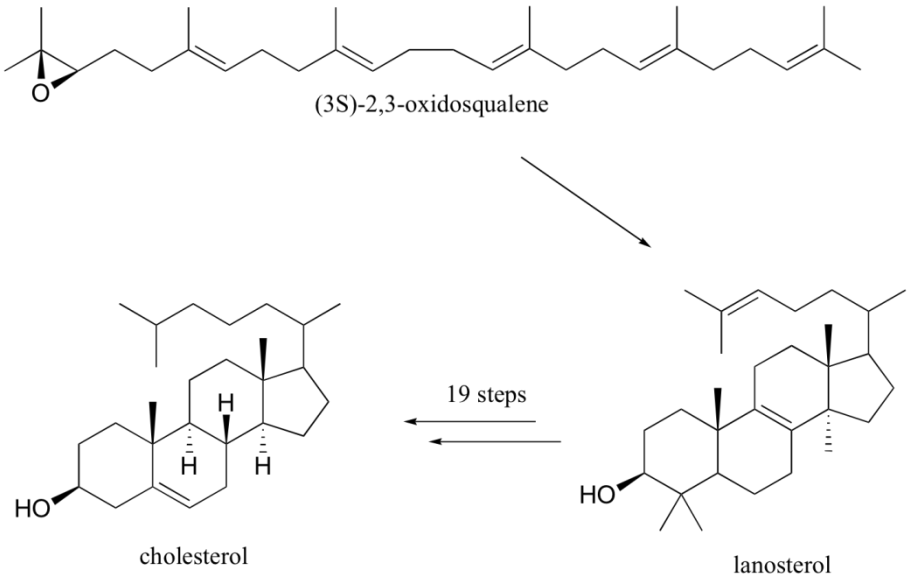


This results in a new carbon-carbon bond between the two FPP molecules, but with incorrect C_1 to C_2 connectivity (remember, the overall reaction is a C_1 to C_1 condensation). In step 3, a proton is abstracted and the electrons from the broken C-H bond bridge across a 2-carbon gap to form a cyclopropyl intermediate.

In the second stage of squalene synthesis, the second pyrophosphate group leaves, generating a cyclopropylcarbinylcation (step 4). Because this is a primary carbocation, you probably are wondering about how stable it could be (and thus how likely an intermediate). As it turns out, such carbocations are remarkably stable, due to favorable interactions between the empty orbital and orbitals on the three-membered ring (the level of bonding theory needed to really understand this idea is beyond the scope of this text, but you may learn about it if you take a class in advanced organic chemistry). What occurs next is an alkyl shift leading to a tertiary carbocation (step 5).



Discussion of the final step (step 6) will need to be put off - this is a reduction with a hydride nucleophile derived from a coenzyme called NADPH. Although this may seem like an extremely convoluted (and perhaps unlikely!) mechanism, there is much experimental evidence to back it up.



KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III BSc Biochemistry - Plant Biochemistry (16BCU503A)

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	Auxin is found in which region of tha	Meristematic region	mature tissue	flowers	fruits	Meristematic region
2	Chemically auxin is	Indole pyruvic acid	Indole 3 acetic acid	Indole butyric acid	2,4 dichloro phenoxy acetic acid	Indole 3 acetic acid
3	Auxin isolated from human urine is	Indole acetic acid	auxonotriolic acid	auxonolonic acid	alpha naphthalene acetic acid	auxonotriolic acid
4	The only true natural auxin of higher	Indole 3 acetic acid	alpha naphthalene acetic acid	2,4 dichloro phenoxy acetic a	Indole butyric acid	Indole 3 acetic acid
5	The precursor of indole acetic acid is	Tyrosine	methionine	tryptophan	phenyl alanine	tryptophan
6	The enzyme involved in conversion o	indole pyruvic acid decarb	indole acetaldehyde decarboxyl	amino transferase	tryptophan decarboxylase	amino transferase
7	In tryptamine pathway tryptophan is	tryptophan decarboxylase	indole pyruvic acid decarboxyl	indole acetaldehyde decarbox	amino transferase	tryptophan decarboxylase
8	The indole acetaldoxime pathway is	cruciferae	solanacea	marvacea	malvacea	cruciferae
9	The only non indole auxin is	phenyl pyruvic acid	phenyl acetic acid	alpha naphthalene acetic acid	2,4 dichloro phenoxy acetic acid	phenyl acetic acid
10	Phenyl alanine is converted to phenyl	aromatic amino transferas	indole pyruvic acid decarboxyl	indole acetaldehyde decarbox	amino transferase	aromatic amino transferase
11	Absorption of water is increased by	gibberellins	auxin	cytokinin	ethylene	auxin
12	Shortening of internodes and product	gibberellins	auxin	cytokinin	ethylene	auxin
13	The hormone used widely to break se	gibberellins	auxin	cytokinin	ethylene	auxin
14	_____ are weed killers	gibberellins	auxin	cytokinin	ethylene	auxin
15	Early flowering and fruiting is induc	gibberellins	auxin	cytokinin	ethylene	auxin
16	The precursor for gibberellin biosynt	mevalonic acid	tyrosine	tryptophan	alanine	mevalonic acid
17	Genetic dwarfism is overcome by	gibberellins	auxin	cytokinin	ethylene	gibberellins
18	Bolting and flowering in *Brassica* is	gibberellins	auxin	cytokinin	ethylene	gibberellins
19	Light induced inhibition of stem grow	gibberellins	auxin	cytokinin	ethylene	gibberellins
20	The production of parthenocarpic fru	gibberellins	auxin	cytokinin	ethylene	gibberellins
21	Reduction in number of male flowers	gibberellins	auxin	cytokinin	ethylene	gibberellins
22	Chemically gibberellins are	triterpenoid acids	sesquiterpenoid acids	diterpenoid acids	monoterpenoid acids	diterpenoid acids
23	Seed germination is promoted by	gibberellins	auxin	cytokinin	ethylene	gibberellins
24	Cytokinin is a derivative of	6 furfuryl amino purine	pyrimidine	isopentenyl pyro phosphate	geranyl pyro phosphate	6 furfuryl amino purine
25	Kinetin is formed from	adenosine	guanosine	deoxy adenosine	deoxy guanosine	deoxy adenosine
26	Cell enlargement is induced by all th	abscisic acid	auxin	cytokinin	ethylene	abscisic acid
27	Enzyme synthesis in plants is regulat	abscisic acid	auxin	cytokinin	ethylene	cytokinin
28	Sex reversal is induced by	abscisic acid	auxin	cytokinin	ethylene	cytokinin
29	RNA synthesis in elongating zones o	abscisic acid	auxin	cytokinin	ethylene	cytokinin
30	Who is the father of tissue culture?	Bonner	Haberlandt	Laibach	Gautheret	Haberlandt
31	The production of secondary metabo	protoplast	cell suspension	meristem	auxillary buds	cell suspension
32	Synthetic seed is produced by encaps	sodium chloride	sodium alginate	sodium acetate	sodium nitrate	sodium alginate
33	Hormone pair required for a callus to	auxin and cytokinin	auxin and ethylene	auxin and absiccic acid	cytokinins and gibberlin	auxin and cytokinin
34	DMSO (Dimethyl sulfoxide) is used	Gelling agent	alkaylating agent	Chelating agent	Cryoprotectant	Cryoprotectant
35	The most widely used chemical for p	Manitol	Sorbitol	Mannol	Poly ethylene glycol (PEG)	Poly ethylene glycol (PEG)
36	Cybrids are produced by	Fusion of two different nu	Fusion of two same nuclei from	Nucleus of one species but cy	None of the above	Nucleus of one species but cytoplasm from both the parent species
37	Callus is	Tissue that forms embryo	An insoluble carbohydrate	Tissue that grows to form emb	Un organised actively dividing mass o	Un organised actively dividing mass of cells maintained in cultured
38	Part of plant used for culturing is call	Scion	Explant	Stock	Callus	Explant
39	Growth hormone producing apical do	Auxin	Gibberellin	Ethylene	Cytokinin	Auxin
40	A medium which is composed of che	Natural media	Synthetic media	Artificial media	None of these	Synthetic media
41	To obtain haploid plant, we culture	Entire anther	Nucleus	Embryo	Apical bud	Entire anther
42	Somaclonal variations are the ones	Caused by mutagens	Produce during tissue culture	Caused by gamma rays	Induced during sexual embryogeny	Produce during tissue culture
43	Which of the following plant cell will	Xylem vessels	Sieve tube	Meristem	Cork cells	Meristem
44	Which vector is mostly used in crop	Plasmid	Cosmid	Phasmid	Agrobacterium	Agrobacterium
45	A(n) _____ is an excised piece	microshoot	medium	explant	scion	explant
46	Protoplasts can be produced from sus	cellulotyic enzymes	pectolytic enzymes	both cellulotyic and pectolytic	proteolytic enzymes	both cellulotyic and pectolytic enzymes
47	What is meant by 'Organ culture' ?	Maintenance alive of a wh	Introduction of a new organ in	Cultivation of organs in a labo	The aspects of culture in community	Maintenance alive of a whole organ, after removal from the organism by partial immersion in a nutrient fluid
48	Which method of plant propagation i	Grafting	Cuttings	Layering	Micropropagation	Layering
49	Who is the father of tissue culture?	Bonner	Haberlandt	Laibach	Gautheret	Haberlandt
50	The production of secondary metabo	protoplast	cell suspension	meristem	auxillary buds	cell suspension
51	Somaclonal variations are the ones	Caused by mutagens	Produce during tissue culture	Caused by gamma rays	Induced during sexual embryogeny	Produce during tissue culture
52	To obtain haploid plant, we culture	Entire anther	Nucleus	Embryo	Apical bud	Entire anther
53	Growth hormone producing apical do	Auxin	Gibberellin	Ethylene	Cytokinin	Auxin
54	Part of plant used for culturing is call	Scion	Explant	Stock	Callus	Explant
55	Which breeding method uses a chem	Mass selection	Protoplast fusion	Transformation	Transpiration	Protoplast fusion
56	The response of a plant to the	phytochrome	photoperiod	cytochrome	photoreceptor	photoperiod
57	The protein pigment that absorb red a	cytochrome	phytochrome	photochlorophyllide	bacterial chlorophyllide	phytochrome
58	The photo period of short day plants	less than 12 hours	less than 10 hours	less than 15 hours	less than 5 hours	less than 12 hours
59	The photoperiod required by long da	more than 12 hours	more than 10 hours	more than 15 hours	more than 5 hours	more than 12 hours
60	The plants require a photoperiod of 1	short day plant	long day plant	photo neutral plant	intermediate plant	intermediate plant