CHAPTER

11

BIOENERGETICS

Animation 11: Bioenergetics
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Bioenergetics is the quantitative study of energy relationships and energy conversions in biological systems. Biological energy transformations obey the laws of thermodynamics.

All organisms need free energy for keeping themselves alive and functioning. All life on this planet Earth is powered, directly or indirectly, by solar energy. But no organism can make direct use of sunlight as source of energy for metabolism; all can use chemical energy in the food such as sugars etc. The chloroplasts of the plants capture light energy coming from the sun and convert it into chemical energy that gets stored in sugar and then in other organic molecules.

With the emergence of photosynthesis on earth, molecular oxygen began to accumulate slowly in the atmosphere. The presence of free oxygen made possible the evolution of respiration. Respiration releases great deal of energy, and couples some of this energy to the formation of adenosine triphosphate (ATP) molecules. ATP is a kind of chemical link between **catabolism** and **anabolism**.

The process of photosynthesis helps understand some of the principles of energy transformation (Bioenergetics) in living systems. Photosynthetic organisms (higher land plants for instance) use solar energy to synthesize organic compounds (such as carbohydrates) that can not be formed without the input of energy. Energy stored in these molecules can be used later to power cellular processes and can serve as the energy source for all forms of life. Whereas photosynthesis provides the carbohydrate substrate, **glycolysis** and respiration are the processes whereby the energy stored in carbohydrate is released in a controlled manner. So the photosynthesis acts as an energy-capturing while respiration as an energy releasing process.

PHOTOSYNTHESIS
(CONVERSION OF SOLAR ENERGY INTO CHEMICAL ENERGY)

Photosynthesis can be defined as the process in which energy-poor inorganic oxidised compounds of carbon (i.e. $C0_2$) and hydrogen (i.e. mainly water) are reduced to energy-rich carbohydrate (i.e. sugar-glucose) using the light energy that is absorbed and converted into chemical energy by chlorophyll and some other photosynthetic pigments. The process of photosynthesis in green plants can be summarised as:

$$\underset{\text{(carbon dioxide)}}{6CO_2} + \underset{\text{(water)}}{12H_2O} + \text{light} \xrightarrow{\text{chlorophyll}} \underset{\text{(glucose)}}{C_6H_{12}O_6} + \underset{\text{(oxygen)}}{6O_2} + \underset{\text{(water)}}{6H_2O}$$

Photosynthetic Reactants and Products

From above overall reaction of photosynthesis it becomes evident that carbon dioxide, water and light are the reactants while glucose and oxygen are the products. Water appears on both sides of the equation because water is used as reactant in some reactions and released as product in other. However, because there is no net yield of H_20, we can simplify the summary equation of photosynthesis for purpose of discussion:

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

This is almost exactly opposite to the overall equation of aerobic respiration ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$).Photosynthesis uses the products of respiration and respiration uses the products of photosynthesis. There is another important difference between the two processes : Photosynthesis occurs only during day time, whereas respiration goes on day and night. During darkness leaves (and other actively metabolizing cells) respire and utilize oxygen and release carbon dioxide. At dawn and dusk, when light intensity is low, the rate of photosynthesis and respiration may, for a short time, equal one another. Thus the oxygen released from photosynthesis is just the amount required for cellular respiration. Also, the carbon dioxide released by respiration just equals the quantity required by photosynthesizing cells. At this moment there is no net gas exchange between the leaves and the atmosphere. This is termed as **compensation point**. As the light intensity increases, so does the rate of photosynthesis and hence the requirement for more carbon dioxide increases which respiration alone cannot supply. Similarly, the oxygen produced during photosynthesis is more than the need of the respiring cells, so the result is the net release of oxygen coupled with the uptake of carbon dioxide.

Water and Photosynthesis

Oxygen released during photosynthesis comes from water, and is an important source of atmospheric oxygen which most organisms need for aerobic respiration and thus for obtaining energy to live. In 1930s, Van Niel hypothesized that plants split water as a source of hydrogen, releasing oxygen as a by-product. Niel's hypothesis was based on his investigations on photosynthesis in bacteria that make carbohydrate from carbon dioxide, but do not release oxygen.

Niel's hypothesis that source of oxygen released during photosynthesis is water and not carbon dioxide, was later confirmed by scientists during 1940s when first use of an isotopic tracer (O^{18}) in biological research was made. Water and carbon dioxide containing heavy-oxygen isotope O^{18} were prepared in the laboratory. Experimental green plants in one group were supplied with H_20 containing O^{18} and with $C0_2$ containing only common oxygen O^{16}. Plants in the second group were supplied with H_20 containing common oxygen O^{16} but with $C0_2$ containing O^{18}.

It was found that plants of first group produced O^{18} but the plants of second group did not.

$$\text{Group-1 Plants: } CO_2 + 2H_2O^{18} \longrightarrow CH_2O + H_2O + O_2^{18}$$

$$\text{Group-2 Plants: } CO_2^{18} + 2H_2O \longrightarrow CH_2O^{18} + H_2O^{18} + O_2$$

Water is thus one of the raw materials of photosynthesis, other being carbon dioxide. Hydrogen produced by splitting of water reduces NADP to $NADPH_2$ ($NADPH + H^+$).

NADPH is the "reducing power" which, along with ATP also formed during **'light reactions',** is used to reduce $C0_2$ to form sugar during **'dark reactions'.**

CHLOROPLASTS - THE SITES OF PHOTOSYNTHESIS IN PLANTS

All green parts of a plant have chloroplasts, but the leaves are the major sites of

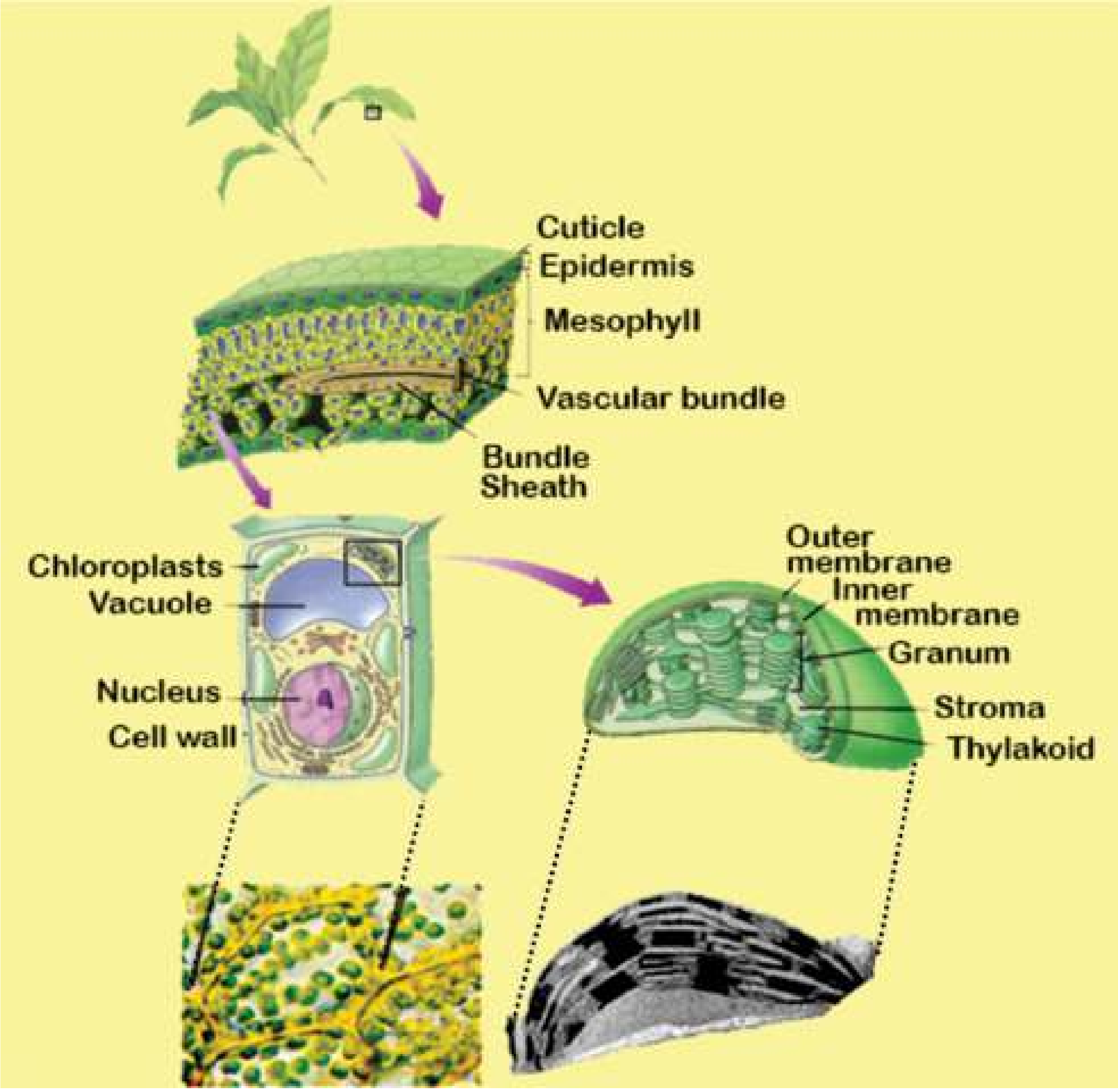


Fig. 11.1 A plant possesses thick layer of mesophyll cells rich in chloroplasts. Thylakoids in chloroplasts are stacked into grana. Light reactions take place on the grana, and dark reactions in the stroma.

photosynthesis in most plants. Chloroplasts are present in very large number, about half a million per square millimeter of leaf surface. Chloroplasts are present mainly in the cells of mesophyll tissue inside the leaf (Fig. 11.1). Each mesophyll cell has about 20-100 chloroplasts. Chloroplast has a double membrane envelope that encloses dense fluid-filled region, the **stroma** which contains most of the enzymes required to produce carbohydrate molecules. Another system of membranes is suspended in the stroma. These membranes form an elaborate interconnected set of flat, disc like sacs called **thylakoids**. The thylakoid membrane encloses a fluid-filled **'thylakoid interior space'** or **lumen**, which is separated from the stroma by thylakoid membrane. In some places, thylakoid sacs are stacked in columns called **grana** (sing **granum**). Chlorophyll (and other photosynthetic pigments) are found embedded in the thylakoid membranes and impart green colour to the plant. Electron acceptors of photosynthetic 'Electron Transport Chain' are also parts of these membranes. Thylakoid membranes are thus involved in ATP synthesis by **chemiosmosis**.

Chlorophyll (and other pigments) absorb light energy, which is converted into chemical energy of ATP and NADPH, the products which are used to synthesize sugar in the stroma of chloroplast.

Photosynthetic prokaryotes lack chloroplasts but they do have unstacked photosynthetic membranes which work like thylakoids.

PHOTOSYNTHETIC PIGMENTS

Light can work in chloroplasts only if it is absorbed. Pigments are the substances that absorb visible light(380-750 nm in wave length). Different pigments absorb light of different wave lengths (colours), and the wave lengths that are absorbed disappear. An instrument called **Spectrophotometer** is used to measure relative abilities of different pigments to absorb different wavelengths of light. A graph plotting absorption of light of different wave lengths by a pigment is called **absorption spectrum** of the pigment.

Thylakoid membranes contain several kinds of pigments, but **chlorophylls** are the main photosynthetic pigments. Other, accessory photosynthetic pigments present in the chloroplasts include yellow and red to orange **carotenoids; carotenes** are mostly red to orange and **xanthophylls** are yellow to orange. These broaden the absorption and utilization of light energy.

Chlorophylls

There are known many different kinds of chlorophylls. Chlorophyll a, b, c and d are found in eukaryotic photosynthetic plants and algae, while the other are found in photosynthetic bacteria and are known as **bacteriochlorophylls.**

Chlorophylls absorb mainly violet-blue and orange-red wave lengths. Green, yellow and indigo wave lengths are least absorbed by chlorophylls and are transmitted or reflected, although the yellows are often masked by darker green colour. Hence plants appear green, unless masked by other pigments (Fig. 11.4).

A chlorophyll molecule has two main parts : One flat, square, light absorbing hydrophilic head and the other long, anchoring, hydrophobic hydrocarbon tail. The head is complex **porphyrin ring** which is made up of 4 joined smaller pyrrole rings composed of carbon and nitrogen atoms. An atom of magnesium is present in the centre of porphyrin ring and is coordinated with the nitrogen of each pyrrole ring (Fig. 11.2) That is why magnesium deficiency causes yellowing in plants.

Haem portion of haemoglobin is also a porphyrin ring but containing an ironatom instead of magnesium atom in the centre.

Long hydrocarbon tail which is attached to one of the pyrrole rings is **phytol** ($C_{20}H_{39}$). The chlorophyll molecule is embedded in the hydrophobic core of thylakoid membrane by this tail.

Chlorophyll a and chlorophyll b differ from each other in only one of the functional groups bonded to the porphyrin; the methyl group ($-CH_3$) in chlorophyll a is replaced by a terminal carbonyl group (-CHO) in chlorophyll b.

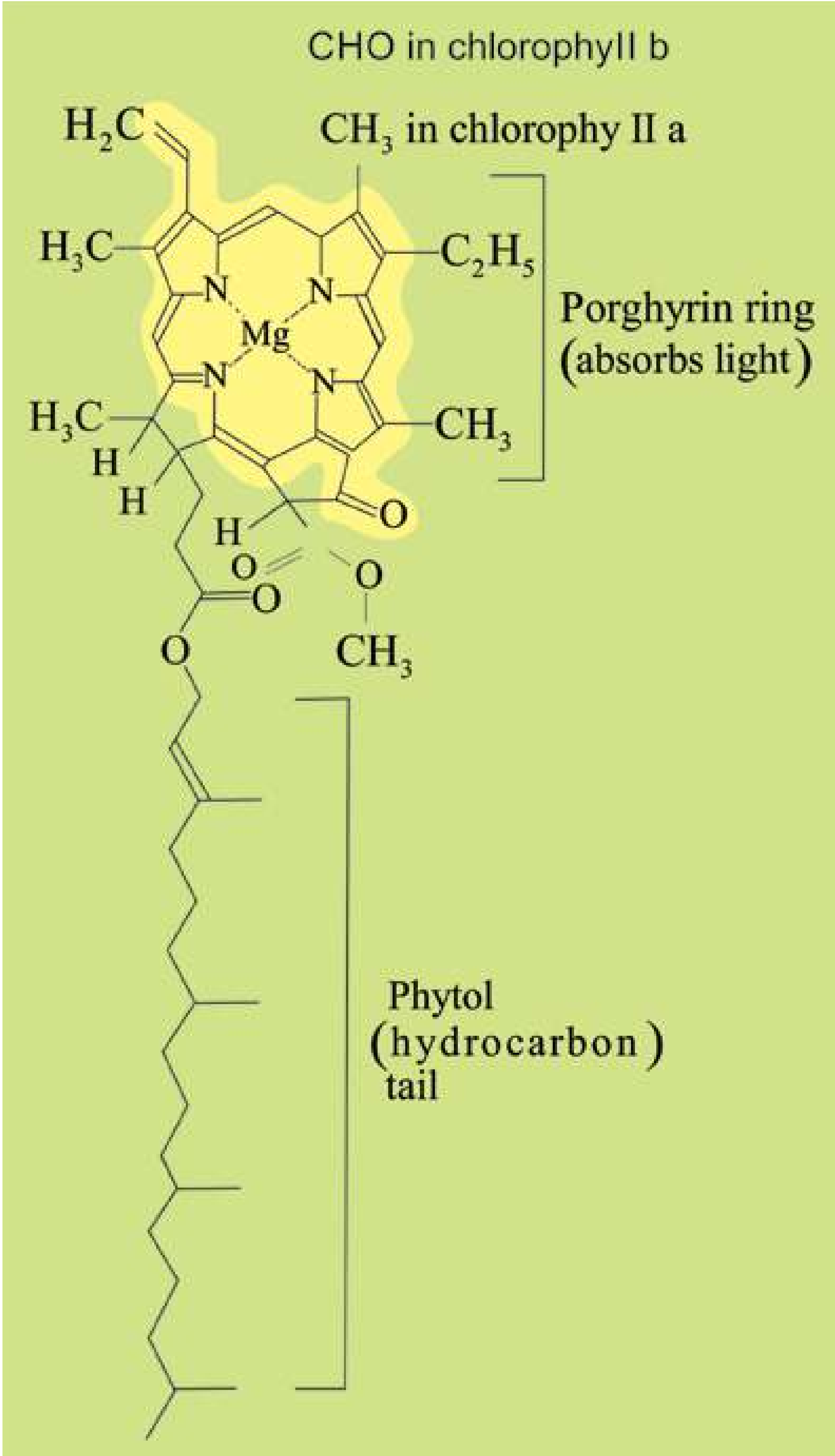


Fig. 11.2 A molecule of chlorophyll

The molecular formulae for chlorophyll a and b are:

Chlorophyll a : $C_{55} H_{72} 0_5 N_4$ Mg

Chlorophyll b : $C_{55} H_{70} 0_6 N_4$ Mg

Due to this slight difference in their structure, the two chlorophylls show slightly different absorption spectra and hence different colours. Some wave lengths not absorbed by chlorophyll a are very effectively absorbed by chlorophyll b and vice-versa. Such differences in structure of different pigments increase the range of wave lengths of the light absorbed. Chlorophyll a is blue-green while chlorophyll b is yellow-green.

Of all the chlorophylls, chlorophyll a is the-most abundant and the most important photosynthetic pigment as it takes part directly in the light-dependent reactions which convert solar energy into chemical energy. It is found in all photosynthetic organisms except photosynthetic bacteria. Chlorophyll a itself exists in several forms differing slightly in their red absorbing peaks e.g. at 670, 680, 690, 700 nm.

Chlorophyll b is found alongwith chlorophyll a in all green plants (embryophytes) and green algae.

Chlorophylls are insolube in water but souble in organic solvents, such as carbon tetrachloride, alcohol etc.

Carotenoids-accessory pigments

Carotenoids are yellow and red to orange pigments that absorb strongly the blueviolet range, different wave lengths than the chlorophyll absorbs. So they broaden the spectrum of light that provides energy for photosynthesis.

These and chlorophyll b are called **accessory pigments** because they absorb light and transfer the energy to chlorophyll a, which then initiates the light reactions. It is generally believed that the order of transfer of energy is:

$$\text{carotenoids} \longrightarrow \text{chlorophyll b} \longrightarrow \text{chlorophyll a}$$

Some carotenoids protect chlorophyll from intense light by absorbing and dissipating excessive light energy, rather than transferring energy to chlorophyll. (Similar carotenoids may be protecting human eye).

LIGHT-THE DRIVING ENERGY

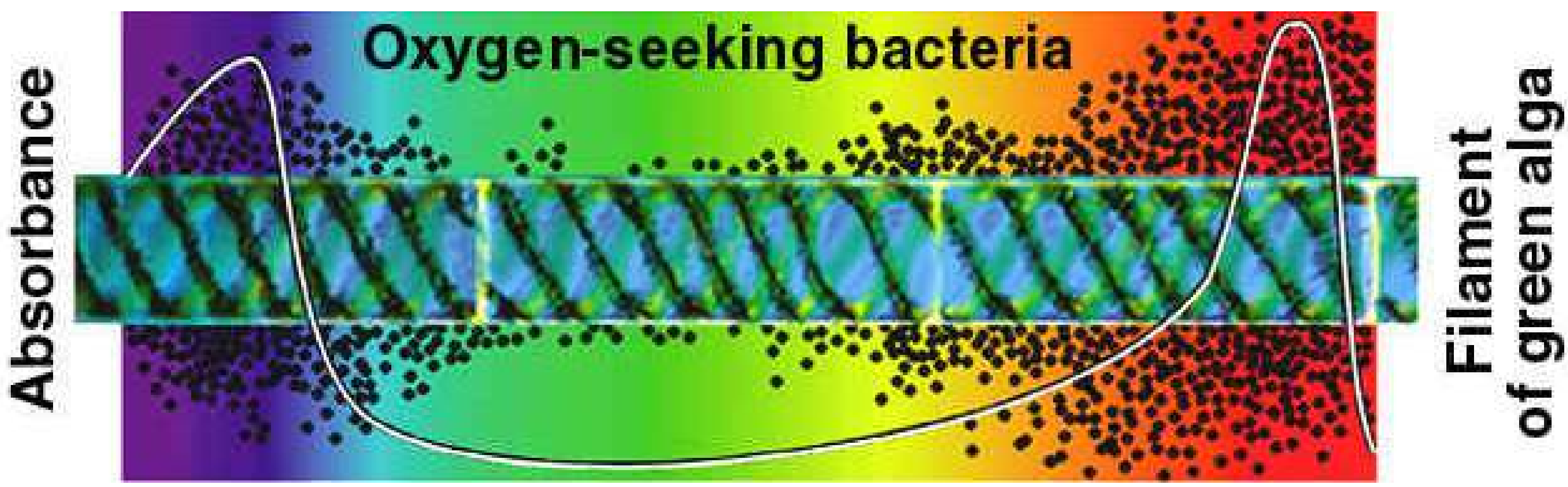
Light is a form of energy called electromagnetic energy or radiations. Light behaves as waves as well as sort of particles called **photons**. The radiations most important to life are the **visible light** that ranges from about 380 to 750 nm in wave length.

It is the sunlight energy that is absorbed by chlorophyll, converted into chemical energy, and drives the photosynthetic process. Not all the. light falling on the leaves is absorbed. Only about one percent of the light falling on the leaf surface is absorbed, the rest is reflected or transmitted.

Absorption spectrum for chlorophylls (Fig. 11.4) indicates that absorption is maximum in blue and red parts of the spectrum, two absorption peaks being at around 430 nm and 670 nm respectively. Absorption peaks of carotenoids are different from those of chlorophylls.

Different wavelengths are not only differently absorbed by photosynthetic pigments but are also differently effective in photosynthesis. Graph showing relative effectiveness of different wavelengths (colours) of light in driving photosynthesis is called **action spectrum** of photosynthesis (Fig. 11.4)

The first action spectrum was obtained by German biologist, T.W.Engelmann in 1883. He worked on Spirogyra.



Fi.g 11.3 Engel man illuminated a filament of Spirogyra with light that had been passed through a prism. Aerobic bacteria moved toward the portions of the algal filament emitting the most oxygen, along the cells in blue and red portion of the spectrum.

Action spectrum can be obtained by illuminating plant with light of different wavelengths (or colours) and then estimating relative $C0_2$ consumption or oxygen release during photosynthesis.

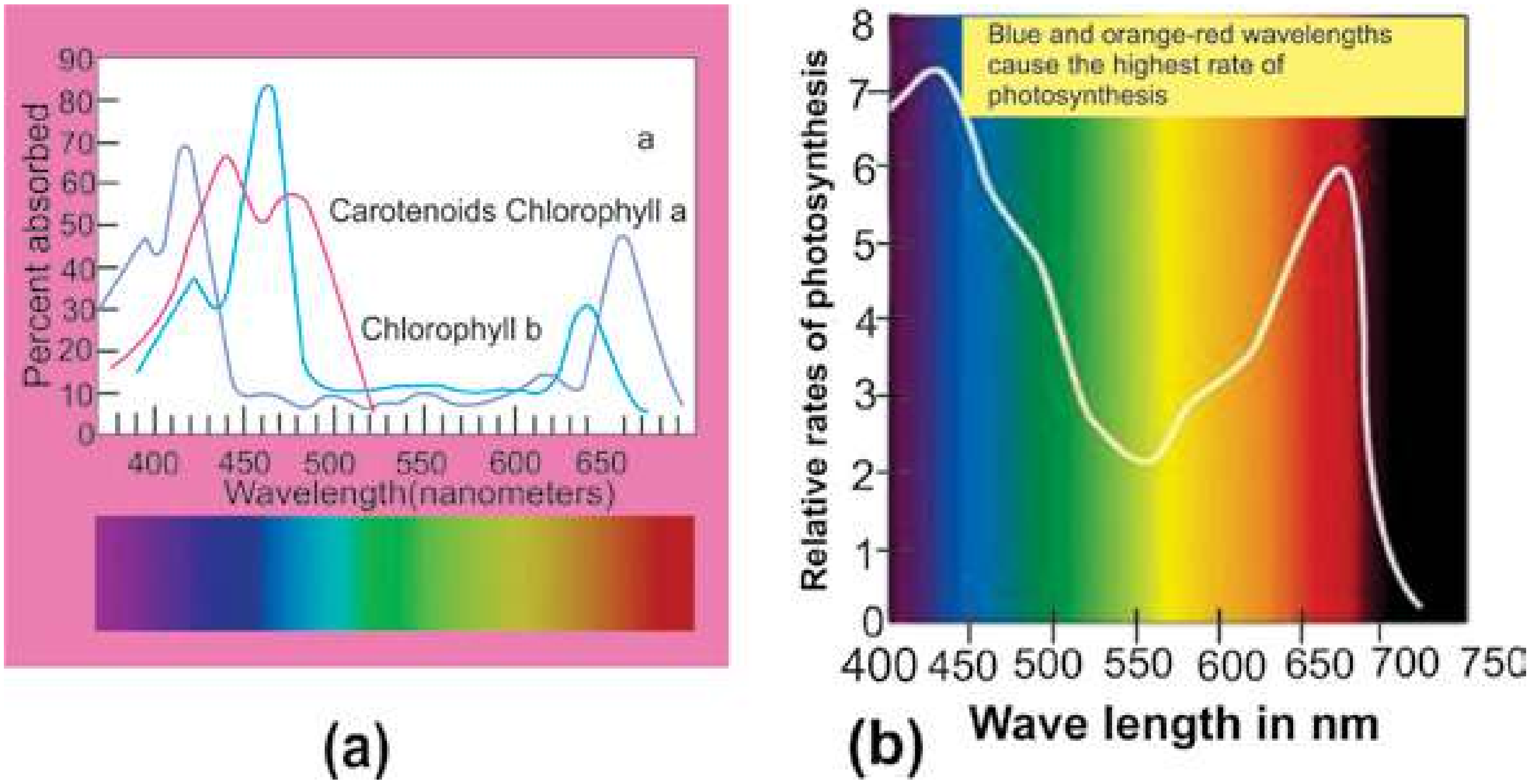


Fig. 11.4 (a) Absorption spectrum of chlorophyll and carotenoids.
(b) Action spectrum for photosynthesis.

As is evident from above figure 11.4, action spectrum of photosynthesis corresponds to absorption spectrum of chlorophyll. The same two peaks and the valley are obtained for absorption of light as well as for CO_2 consumption. This also shows that chlorophyll is the photosynthetic pigment.

However, the action spectrum of photosynthesis does not parallel the absorption specturm of chlorophyll exactly. Compared to the peaks in absorption spectrum, the peaks in action spectrum are broader, and the valley is narrower and not as deep.

(Photosynthesis in the most absorbed range is more than the absorption itself. Likewise, photosynthesis in 500-600 nm (including green light) is more than the absorption of green light by the chlorophyll). This difference occurs because the accessory pigments, the carotenoids, absorb light in this zone and pass on some of the absorbed light to chlorophylls which then convert light energy to chemical energy. When equal intensities of light are given, there is more photosynthesis in red than in blue part of spectrum.

ROLE OF CARBON DIOXIDE : A PHOTOSYNTHETIC REACTANT

Sugar is formed during **light - independent reactions** of photosynthesis by the reduction of CO_2, using ATP and NADH, the products of **light - dependent reactions**. Obviously photosynthesis does not occur in the absence of CO_2.

About 10 percent of total photosynthesis is carried out by terrestrial plants, the rest occurs in oceans, lakes and ponds. Aquatic photosynthetic organisms use dissolved CO_2, bicarbonates and soluble carbonates that are present in water as carbon source. Air contains about 0.03 - 0.04 percent CO_2. Photosynthesis occurring on land utilizes this atmospheric $C0_2$.

Carbondioxide enters the leaves through **stomata** and gets dissolved in the water absorbed by the cell walls of mesophyll cells. Stomata are found in a large number in a leaf; their number being proportional to the amount of gas diffusing into the leaf. Stomata cover only 1 - 2 percent of the leaf surface but they allow proportionalety much more gas to diffuse.

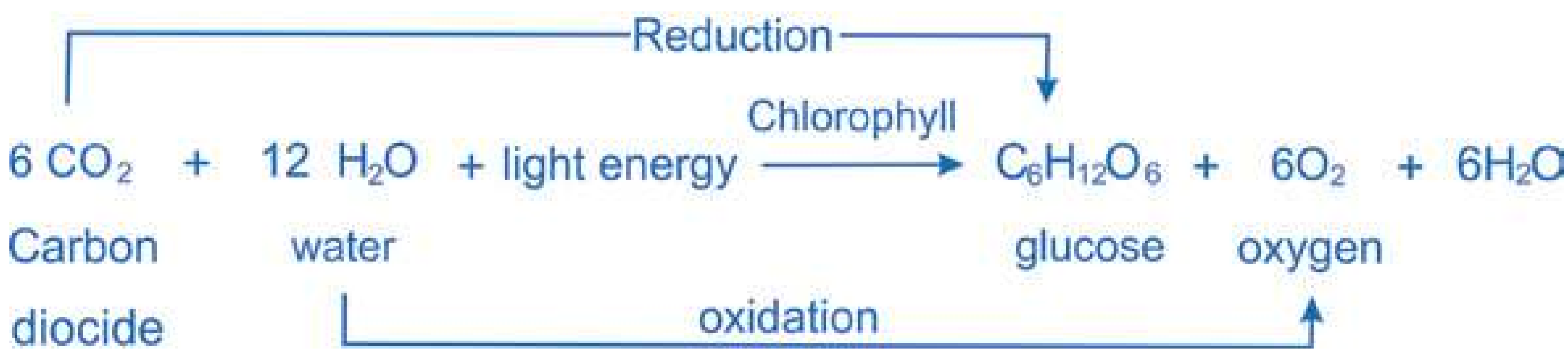
The entry of $C0_2$ into the leaves depends upon the opening of stomata. The guard cells guarding the stoma, because of their peculiar structure and changes in their shape, regulate the opening and closing of stomata.

Stomata are adjustable pores that are usually open during the day when CO_2 is required for photosynthesis and partially closed at night when photosynthesis stops.

Daily rhythmic opening and closing of stomata is also due to an internal clock located in the guard cells. Even if a plant is kept in a dark closet, stomata will continue their daily rhythm of opening and closing.

REACTIONS OF PHOTOSYNTHESIS

Photosynthesis is a **'redox process'** that can be represented by the following simplified summary equation:



However, it is not a simple, single step process, but is a complex one that is completed by a series of simple steps or reactions. These reactions of photosynthesis consist of two parts:

The light-dependent reactions (light reactions) which use light directly and

The light-independent reactions (dark reactions) which do not use light directly.

Light dependent reactions constitute that phase of photosynthesis during which light energy is absorbed by chlorophyll and other photosynthetic pigment molecules and converted into chemical energy. As a result of this energy conversion, **reducing** and **assimilating power** in the form of NADPH (NADPH + H^+) and ATP, are formed, both temporarily storing energy to be carried alongwith H to the light independent reactions.

NADPH provides energized electron (and H^+), while ATP provides chemical energy for the synthesis of sugar by reducing CO_2, using reducing power and chemical energy of NADPH and ATP respectively, produced by light reactions. The energy is thus stored in the molecules of sugar. This phase of photosynthesis is also called **dark reactions** because these reactions do not use light directly and can take place equally well both in light and dark provided $NADPH_2$ and ATP of light reactions are available.

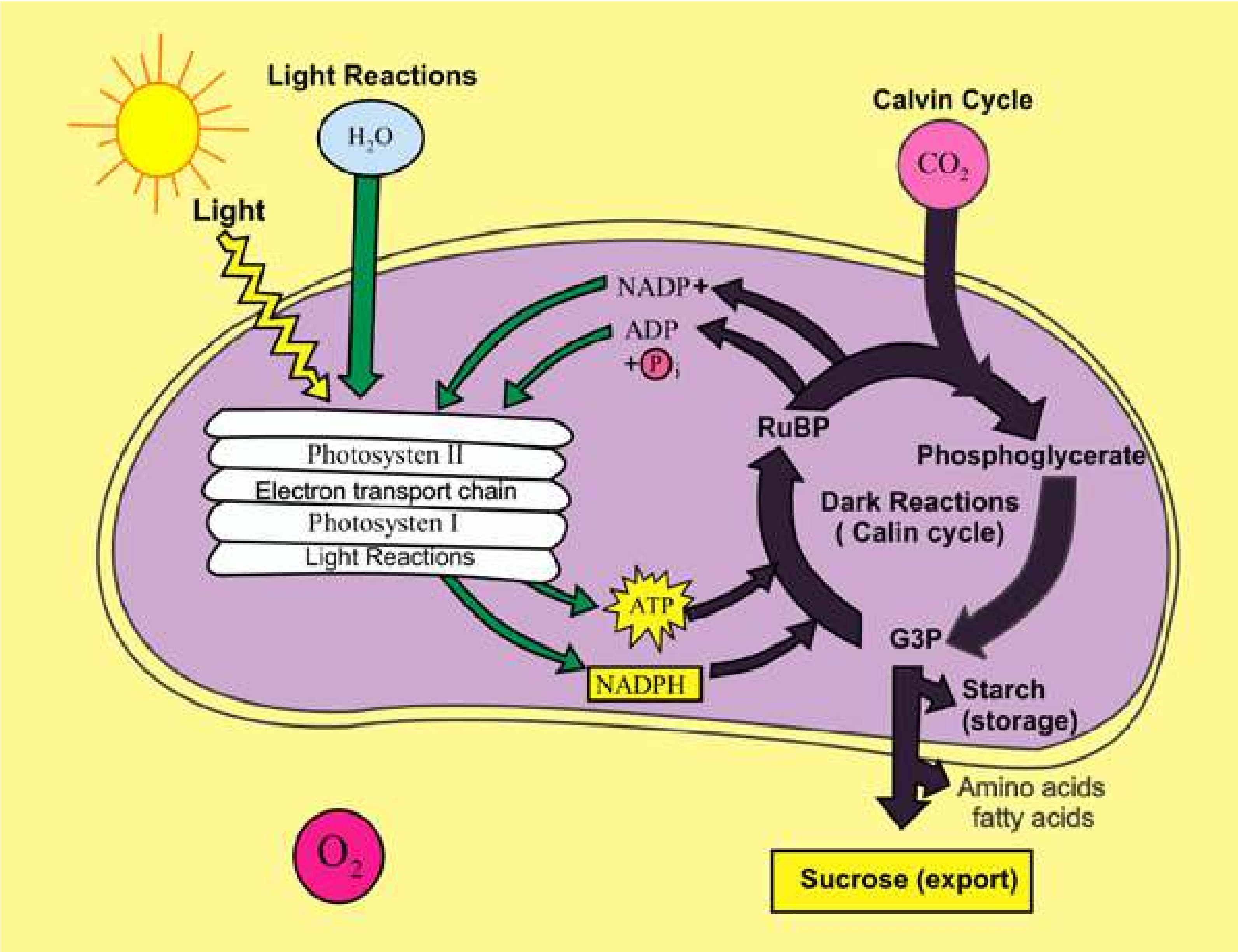


Fig. 11.5 An overview of photosynthesis : Light - Dependent Reactions (Energy - conversion) and Light - Independent Reactions (Energy - conservation)

Light dependent Reactions
(Energy-conversion phase; formation of ATP and NADPH)

As has been described previously, sunlight energy which is absorbed by photosynthetic pigments drives the process of photosynthesis. Photosynthetic pigments are organized into clusters, called **photosystems**, for efficient absorption and utilization of solar energy in thylakoid membranes (Fig. 11.6).

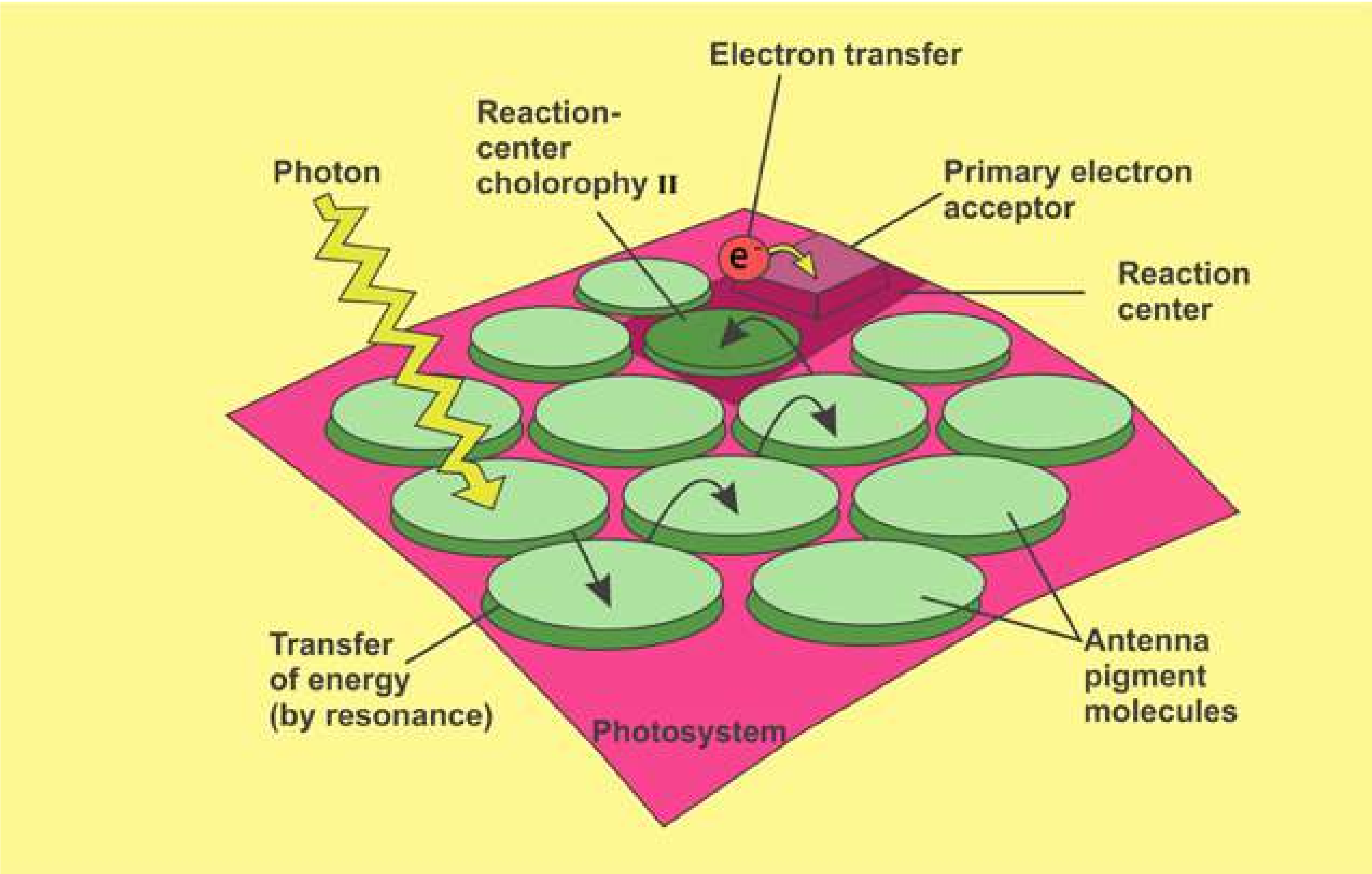


Fig. 11.6: Light harvesting photosystem. Energy of light (photon) absorbed by photosynthetic pigment molecules is transferred from molecule to molecule, and finally reaches the reaction centre where actual energy conversion begins.

Each **photosystem** consists of a light-gathering **'antenna complex'** and a **'reaction center'**. The antenna complex has many molecules of chlorophyll a, chlorophyll b and carotenoids, most of them channeling the energy to reaction center. Reaction center has one or more molecules of chlorophyll a along with a **primary electron acceptor**, and associated electron carriers of **'electron transport system'**. Chlorophyll a molecules of reaction center and associated proteins are closely linked to the nearby electron transport system. Electron transport system plays role in generation of ATP by **chemiosmosis** (which will be discussed in later section). Light energy absorbed by the pigment molecules of antenna complex is transferred ultimately to the reaction center. There the light energy is converted into chemical energy.

There are two photosystems, **photosystem I (PS I)** and **photosystem II (PS II)**. These are named so in order of their discovery. **Photosystem I** has chlorophyll a molecule which absorbs maximum light of 700 nm and is called **P_{700},** whereas reaction center of photosystem II has P_{680}, the form of chlorophyll a which absorbs best the light of 680 nm. A specialized molecule called, **primary electron acceptor** is also associated nearby each reaction center. This acceptor traps the high energy electrons from the reaction center and then passes them on to the series of electron carriers. During this energy is used to generate ATP by chemiosmosis.

In predominant type of electron transport called **non-cyclic electron flow,** the electrons pass through the two photosystems. In less common type of path called **cyclic electron flow** only photosystem I is involved. Formation of ATP during non-cyclic electron flow is called **non-cyclic phosphorylation** while that during cyclic electron flow is called **cyclic phosphorylation**.

Non-cyclic Phosphorylation

1. When photosystem II absorbs light, an electron excited to a higher energy level in the reaction center chlorophyll P_{680} is captured by the primary electron acceptor of PS II. The oxidized chlorophyll is now a very strong oxidizing agent; its electron "hole" must be filled.

2. This hole is filled by the electrons which are extracted, by an enzyme, from water. This reaction splits a water molecules into two hydrogen ions and an oxygen atom, which immediately combines with another oxygen atom to form O_2. This water splitting step of photosynthesis that releases oxygen is called **photolysis**. The oxygen produced during photolysis is the main source of replenishment of atmospheric oxygen.

3. Each photoexcited electron passes from the primary electron acceptor of photosystem II to photosystem I via an **electron transport chain.** This chain consists of an electron carrier called

plastoquinone (Pq), a complex of two cytochromes and a copper containing protein called **plastocyanin** (Pc).

4. As electrons move down the chain, their energy goes on decreasing and is used by thylakoid membrane to produce ATP. This ATP synthesis is called **photophosphorylation** because it is driven by light energy. Specifically, ATP synthesis during non-cyclic electron flow is called **non-cyclic photophosphorylation**. This ATP generated by the light reactions will provide chemical energy for the synthesis of sugar during the Calvin cycle, the second major stage of photosynthesis.

5. The electron reaches the "bottom" of the electron transport chain and fills an electron "hole" in P_{700}, the chlorophyll a molecules in the reaction center of photosystem I. This hole is created when light energy is absorbed by molecules of P_{700} and drives an electron from P_{700} to the primary acceptor of photosystem I.

6. The primary electron acceptor of photosystem I passes the photoexcited electrons to a second electron transport chain, which tmasmits them to **ferredoxin (Fd)**, an iron containing protein. An enzyme called NADP reductase then transfers the electrons from **Fd** to **NADP**. This is the redox reaction that stores the high-energy electrons in NADPH. The NADPH molecule will provide reducing power for the synthesis of sugar in the Calvin cycle.

The path of electrons through the two photosystems during non-cyclic photophosphorylation is known as **Z-scheme** from its shape.

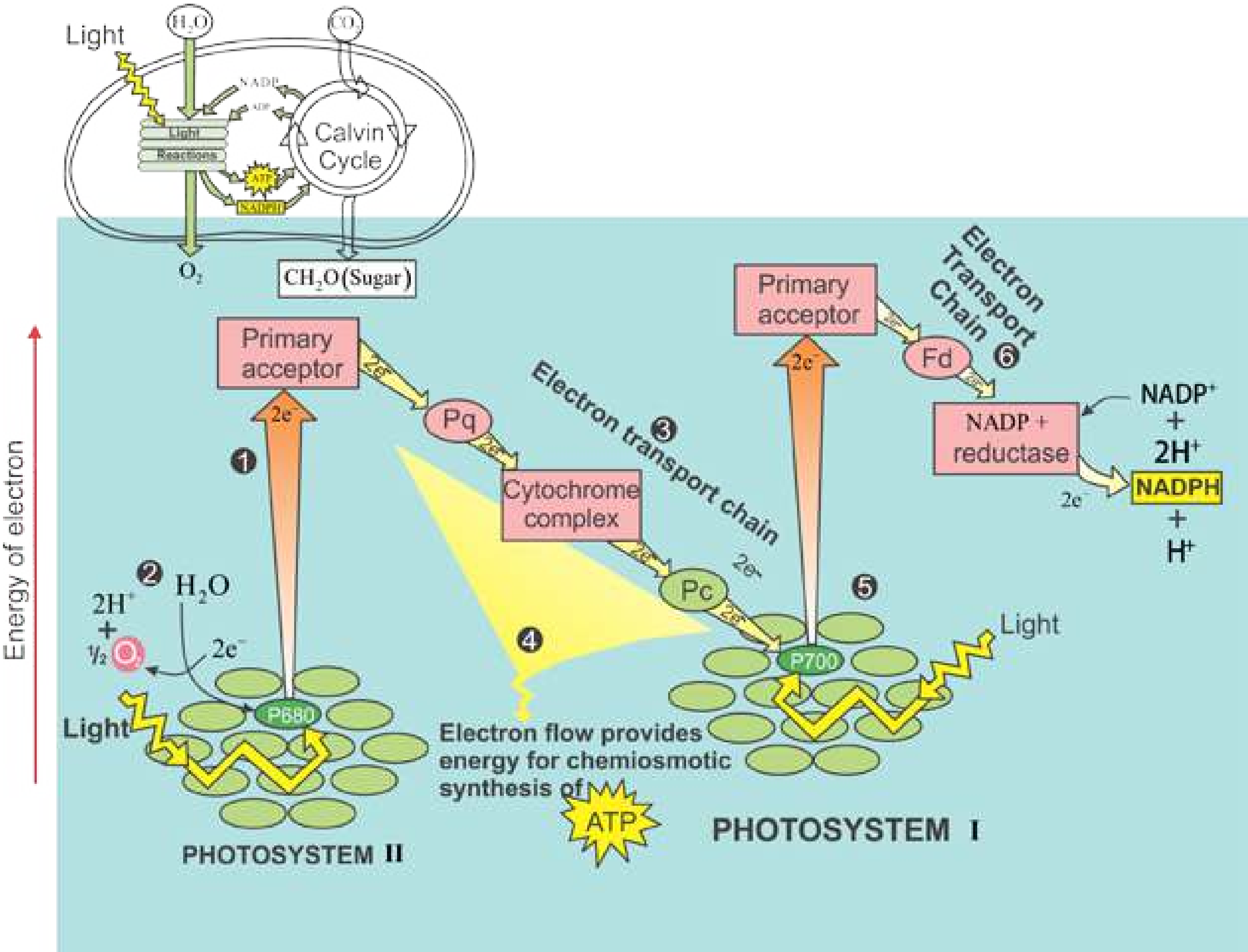


Fig 11.7: Non-cyclic electron flow during photosynthesis ATP, NADPH and oxygen are generated. The arrows trace the current of light-driven electrons from water to NADPH. Each photon of light excites single electron, but the diagram tracts two electrons at a time, the number of electrons required to reduce NADP+. The numbered steps are described in the text.

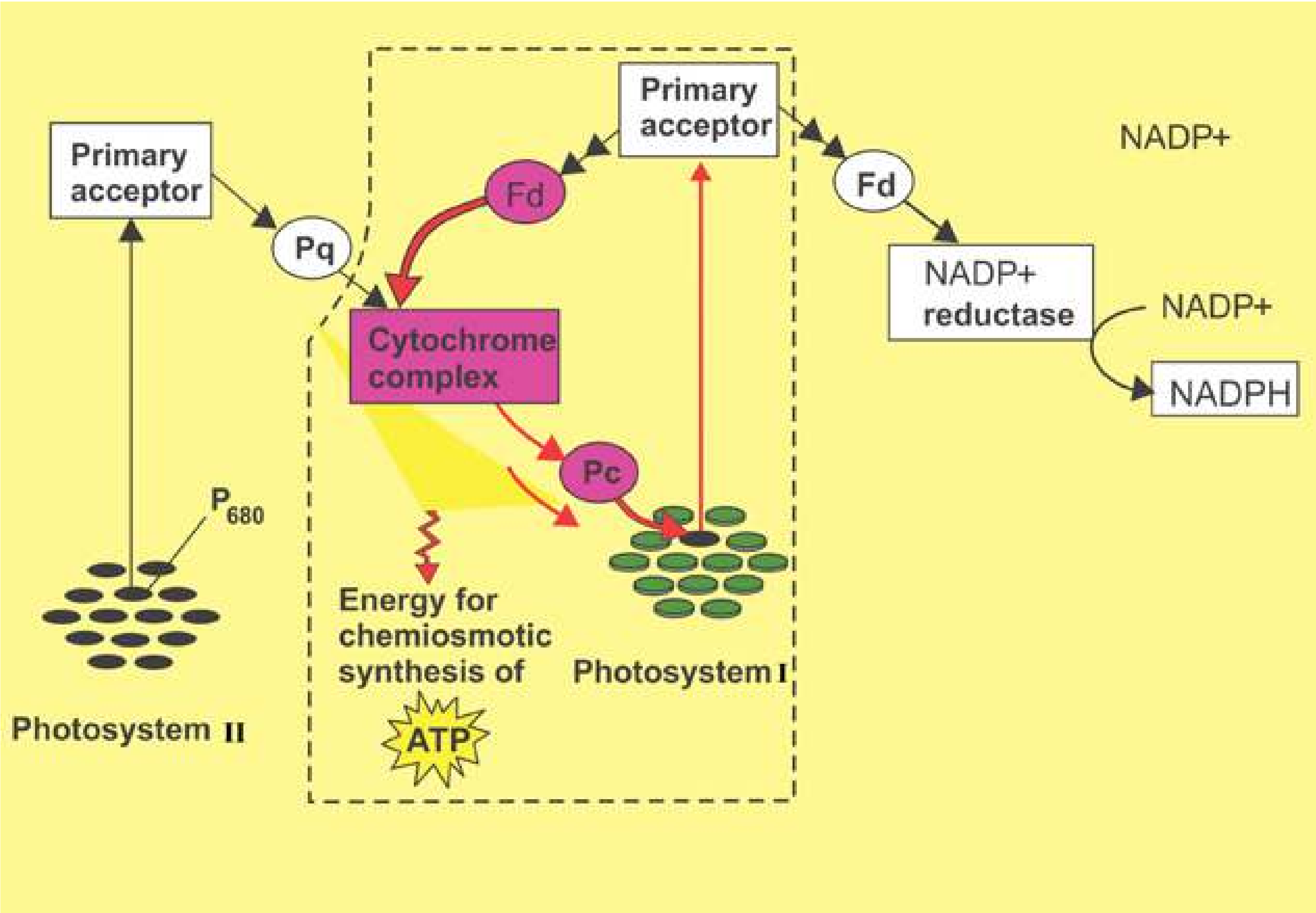


Fig. 11.8: Cyclic electron flow in box. Only PS I involved. ATP is generated but no NADPH and oxygen.

Chemiosmosis

In both cyclic and non-cyclic photophosphorylation, the mechanism for ATP synthesis is **chemiosmosis**, the process that uses membranes to couple redox reactions to ATP production. Electron transport chain pumps protons (H^+) across the membrane of thylakoids in case of photosynthesis into the thylakoids space. The energy used for this pumping comes from the electrons moving through the electron transport chain. This energy is transformed into potential energy stored in the form of H^+ gradient across the membrane. Next the hydrogen ions move down their gradient through special complexes called **ATP synthase** which are built in the thylakoid membrane. During this diffusion of H^+ the energy of electrons is used to make ATP (Fig. 11.9).

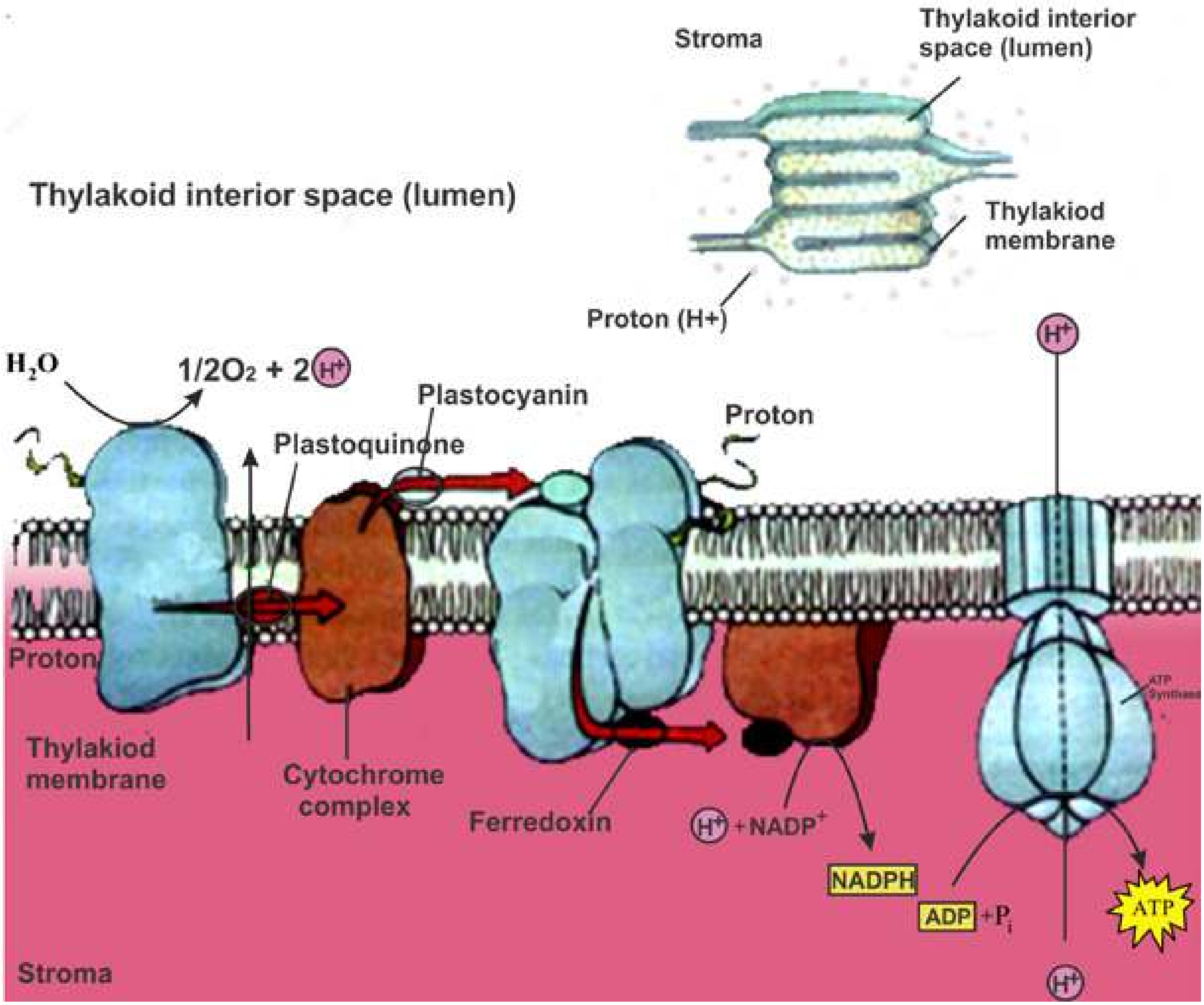


Fig. 11.9 Electron Transport chain and chemiosmosis, coupling of ETC and formation of ATP by chemiosmosis.

Light independent (or Dark) Reactions

Calvin cycle : carbon fixation and reduction phase, synthesis of sugar

The **dark reactions** take place in the stroma of chloroplast. These reactions do not require light directly and can occur in the presence or absence of light provided the assimilatory power in the form of ATP and NADPH, produced during light reactions is available. Energy of these compounds is used in the formation of carbohydrates from $C0_2$, and thus stored their in. These reactions can be summarised as follows (Fig. 11.10):

$$3CO_2 + 6NADPH + 9ATP \longrightarrow \underset{\text{(carbohydrate)}}{(CH_2O)_3} + 6NADP + 9ADP + 9Pi + 3H_2O$$

The details of path of carbon in these reactions were discovered by Melvin Calvin and his colleagues at the University of California. Calvin was awarded Nobel Prize in 1961.

The cyclic series of reactions, catalyzed by respective enzymes, by which the carbon is fixed and reduced resulting in the synthesis of sugar during the dark reactions of photosynthesis is called **Calvin Cycle**.

The **Calvin cycle** can be divided into three phases: Carbon fixation, Reduction, and Regeneration of CO_2 acceptor (RuBP) (Fig 11.10).

Phase 1: Carbon fixation: Carbon fixation refers to the initial incorporation of CO_2 into organic material. Keep in mind that we are following three molecules of CO_2 through the reaction (because 3 molecules of CO_2 are required to produce one molecule of carbohydrate, a triose). The Calvin cycle begins when a molecule of CO_2 reacts with a highly reactive phosphorylated five - carbon sugar named **ribulose bisphosphate (RuBP).** This reaction is catalyzed by the **enzyme ribulose bisphosphate carboxylase**, also known as Rubisco (it is the most abundant protein in chloroplasts, and probably the most abundant protein on Earth). The product of this reaction is an highly unstable, six - carbon intermediate that immediately breaks into two molecules of three - carbon compound called **3 - phosphoglycerate (phosphoglycerie acid-PGA).** The carbon that was originally part of CO_2 molecule is now a part of an organic molecule; the carbon has been “fixed”. Because the product of initial carbon fixation is a three - carbon compound, the Calvin cycle is also known as **C_3 pathway**.

Phase 2: Reduction: Each molecule of (PGA) receives an additional phosphate from ATP of light reaction, forming **1,3 - bisphosphoglycerate** as the product. 1,3 bisphosphoglycerate is reduced to **glyceraldehyde 3-phosphate(G3P)** by receiving a pair of electrons donated from NADPH of light reactions. G3P is the same three-carbon sugar which is formed in glycolysis (first phase of cellular respiration) by the splitting of glucose. In this way fixed carbon is reduced to energy rich G3P with the energy and reducing power of ATP and NADPH (both the products of light-dependent reactions), having the energy stored in it. Actually **G3P**, and not glucose, is the carbohydrate produced directly from the Calvin cycle. For every three molecules of CO_2 entering the cycle and combining with 3 molecules of five-carbon RuBP, six molecules of G3P (containing 18 carbon in all) are produced. But only one molecule of G3P can be counted as a net gain of carbohydrate. Out of every six molecules of G3P formed, only one molecule leaves the cycle to be used by the plant for making glucose, sucrose starch or other carbohydrates, and other organic compounds; the other five molecules are recycled to regenerate the three molecules of five-carbon RuBP, the CO_2 acceptor.

Phase 3: Regeneration of CO2 acceptor, RuBP: Through a complex series of reactions, the carbon skeletons of five molecules of three-carbon G3P are rearranged into three molecules of **five-carbon ribulose phosphate (RuP)**. Each RuP is phosphorylated to ribulose bisphosphate (RuBP), the very five-carbon $C0_2$ acceptor with which the cycle started. Again three more molecules of ATP of light reactions are used for this phosphorylation of three RuP molecules. These RuBP are now prepared to receive $C0_2$ again, and the cycle continues.

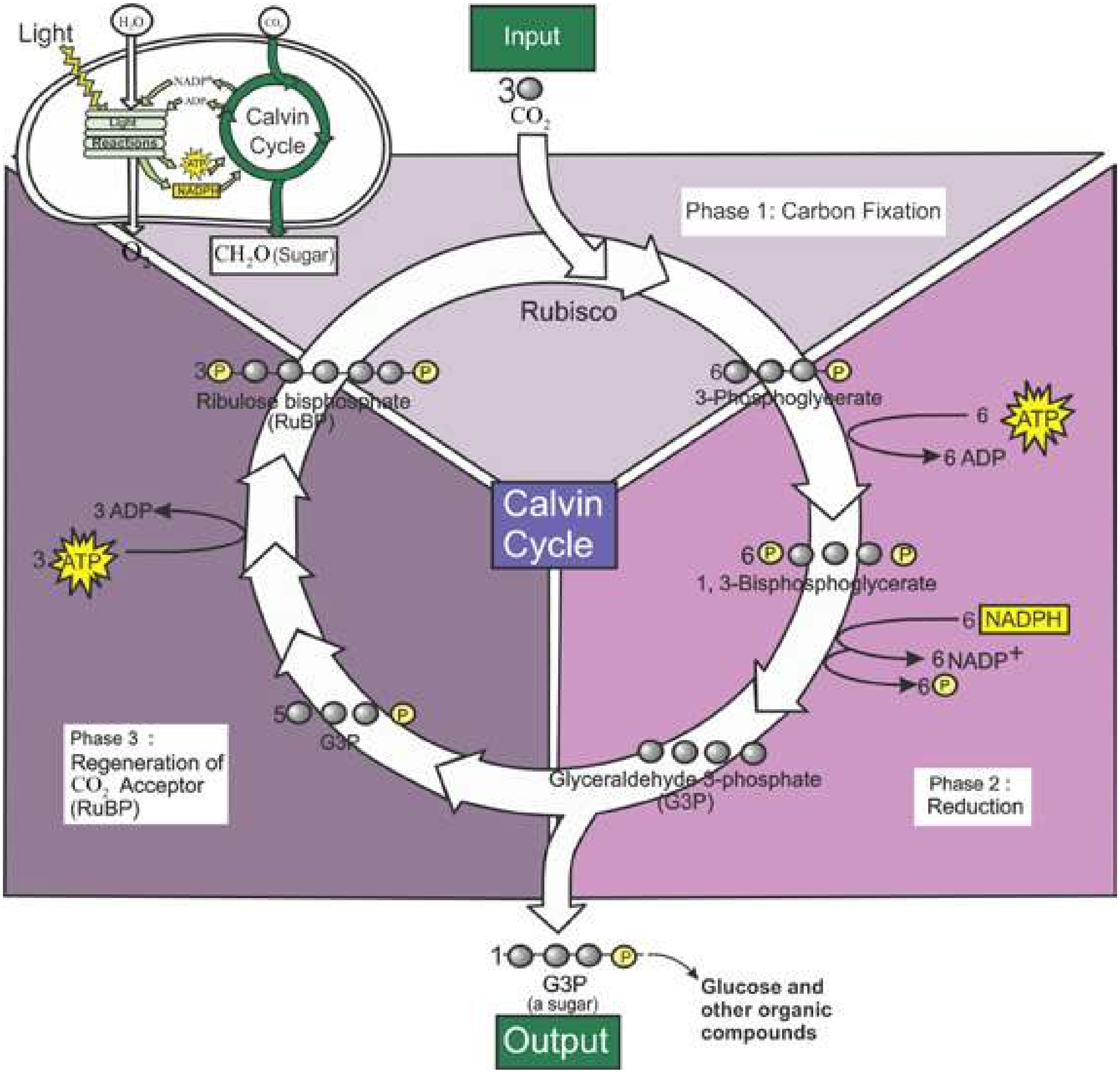


Fig. 11.10: The Calvin cycle occurs in stroma of chloroplast. Carbon is fixed and reduced to sugar.

RESPIRATION

Living organisms need energy to carry on their vital activities. This energy is provided from within the cells by the phenomenon of respiration. Respiration is the universal process by which organisms breakdown complex compounds containing carbon in a way that allows the cells to harvest a maximum of usable energy.

In biology the term respiration is used in two ways. More familiarly the term respiration means the exchange of respiratory gases (CO_2 and O_2) between the organism and its environment. This exchange is called **external respiration**. The cellular respiration is the process by which energy is made available to cells in a step by step breakdown of C-chain molecules in the cells.

Aerobic and Anaerobic Respiration

The most common fuel used by the cell to provide energy by cellular respiration is glucose,. The way glucose is metabolized depends on the availability of oxygen. Prior to entering a mitochondrion, the glucose molecule is split to form two molecules of pyruvic acid. This reaction is called **glycolysis** (glycolysis literally means splitting of sugar), and occurs in the cytosol and is represented by the equation:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{2NAD \;\rightarrow\; 2NADH_2} \underset{\text{Pyruvic acid}}{2C_3H_4O_3} + \text{Energy}$$

This reaction occurs in all the cells and biologists believe that an identical reaction may have occurred in the first cell that was organized on earth.

The next step in cellular respiration varies depending on the type of the cell and the prevailing conditions (Fig. 11.11).

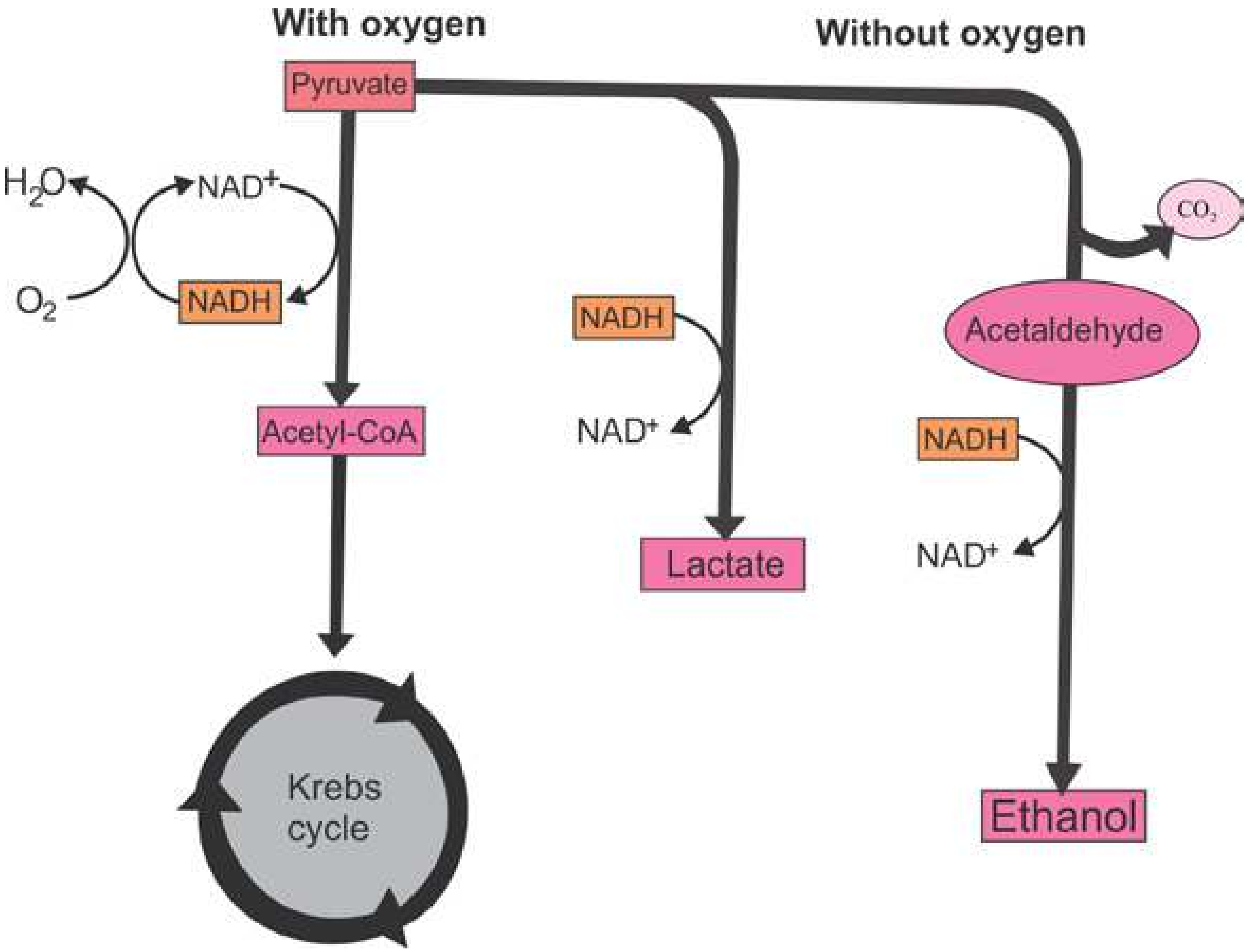


Fig. 11.11 Pyruvate, the end product of glycolysis, follows different catabolic pathways depending on the organism and the metabolic condition.

Cell processes pyruvic acid in three major ways, alcoholic fermentation, lactic acid fermentation and aerobic respiration. The first two reactions occur in the absence of oxygen and are referred to as anaerobic (without oxygen). The complete breakdown of glucose molecule occurs only in the presence of oxygen, i.e. in aerobic respiration. During aerobic respiration glucose is oxidized to CO_2 and water and energy is released.

Anaerobic Respiration

(i) Alcoholic Fermentation: In primitive cells and in some eukaryotic cells such as yeast, pyruvic acid is further broken down by alcoholic fermentation into alcohol (C_2H_5OH) and CO_2.

$$\underset{\text{Pyruvic acid}}{2(C_3H_4O_3)} \xrightarrow{2NADH_2 \;\rightarrow\; 2NAD} \underset{\text{Ethyl Alcohol}}{2(C_2H_5OH)} + 2CO_2$$

(ii) Lactic acid fermentation: In lactic acid fermentation, each pyruvic acid molecule is converted into lactic acid $C_3H_6O_3$ in the absence of oxygen gas:

$$\underset{\text{Pyruvic acid}}{2(C_3H_4O_3)} \xrightarrow{2NADH_2 \;\rightarrow\; 2NAD} \underset{\text{Lactic acid}}{2(C_3H_6O_3)}$$

This form of anaerobic respiration occurs in muscle cells of humans and other animals during extreme physical activities, such as sprinting, when oxygen cannot be transported to the cells as rapidly as it is needed.

Both alcoholic and lactic acid fermentations yield relatively small amounts of energy from glucose molecule. Only about 2% of the energy present within the chemical bonds of glucose is converted into adenosine triphosphate (ATP).

Aerobic respiration (Discussed in detail under cellular respiration).

Role of mitochondria in respiration Mitochondria are large granular or filamentous organelles that are distributed throughout the cytoplasm of animal and plant cells. Each mitochondrion is constructed of an outer enclosing membrane and an inner membrane with elaborate folds or **cristae** that extend into the interior of the organelle.

Mitochondria play a part in cellular respiration by transferring the energy of the organic molecules to the chemical bonds of ATP. A large "battery" of enzymes and coenzymes slowly release energy from the glucose molecules. Thus mitochondria are the **"Power houses"** that produce energy necessary for many cellular functions.

Adenosine triphosphate and its importance Adenosine triphosphate, generally abbreviated 'ATP' is a compound found in every living cell and is one of the essential chemicals of life. It plays the key role in most biological energy transformations.

Conventionally, 'P' stands for the entire phosphate group. The second and the third phosphate represent the so called "high energy" bonds. If these are broken by hydrolysis, far more free energy is released as compared to the other bond in the ATP molecule. The breaking of the terminal phosphate of ATP releases about 7.3 K cal. of energy. The high energy 'P' bond enables the cell to accumulate a great quantity of energy in a very small space and keeps it ready for use as soon as it is needed.

The ATP molecule is used by cells as a source of energy for various functions for example, synthesis of more complex compounds, active transport across the cell membrane, muscular contraction, and nerve conduction, etc.

Biological oxidation The maintenance of living system requires a continual supply of free energy which is ultimately derived from various oxidation reduction reactions. Except for photosynthetic and some bacterial chemosynthetic processes, which are themselves oxidation reduction reactions, all other cells depend ultimately for their supply of free energy on oxidation reactions in respiratory processes. In some cases biological oxidation involves the removal of hydrogen, a reaction catalyzed by the **dehydrogenases** linked to specific coenzymes. Cellular respiration is essentially an oxidation process.

Cellular Respiration

Cellular respiration may be sub-divided into 4 stages:

i. Glycolysis
ii. Pyruvic acid oxidation
iii. Krebs cycle or citric acid cycle
iv. Respiratory chain

Out of these stages the first occurs in the cytosol for which oxygen is not essential, while the other three occur within the mitochondria where the presence of oxygen is essential.

i. **Glycolysis** Glycolysis is the breakdown of glucose upto the formation of pyruvic acid. Glycolysis can take place both in the absence of oxygen (anaerobic condition) or in the presence of oxygen (aerobic condition). In both, the end product of glucose breakdown is **pyruvic acid.** The breakdown of glucose takes place in a series of steps, each catalyzed by a specific enzyme. All these enzymes are found dissolved in the cytosol. In addition to the enzymes, ATP and **coenzyme NAD (nicotinamide adenine dinucleotide)** are also essential.

Glycolysis can be divided into two phases, a preparatory phase and an oxidative phase (Fig. 11.12). In the preparatory phase breakdown of glucose occurs and energy is expended. In the oxidative phase high energy phosphate bonds are formed and energy is stored.

Preparatory phase The first step in glycolysis is the transfer of a phosphate group from ATP to glucose. As a result a molecule of glucose-6 -phosphate is formed. An enzyme catalyzes the conversion of **glucose-6-phosphate** to its isomer, **fructose-6 - phosphate**. At this stage another ATP molecule transfers a second phosphate group. The product is fructose 1,6-bisphosphate. The next step in glycolysis is the enzymatic splitting of fructose 1 ,6 -bisphosphate into two fragments. Each of these molecules contains three carbon atoms. One is called 3 - phospo- glyceraldehyde, 3-PGAL or Glyceraldehyde **3-phosphate (G3P)** while the other is **dihydroxyacetone phosphate.** These two molecules are isomers and in fact, are readily interconverted by yet another enzyme of glycolysis.

Oxidative (payoff) phase The next step in glycolysis is crucial to this process. Two electrons or two hydrogen atoms are removed from the molecule of 3- phosphoglyceraldehyde (PGAL) and transferred to a molecule of NAD. This is of course, an oxidation-reduction reaction, with the PGAL being oxidized and the NAD being reduced. During this reaction, a second phosphate group is donated to the molecule from inorganic phosphate present in the cell. The resulting molecule is called **1,3 Bisphosphoglycerate(BPG)**.

The oxidation of PGAL is an energy yielding process. Thus a "high energy" phosphate bond is created in this molecule. At the very next step in glycolysis this phosphate group is transferred to a molecule of adenosine diphosphate (ADP) converting it into ATP. The end product of this reaction is **3-phospho glycerate (3-PG)**. In the next step 3-PG is converted to **2-Phosphoglycerate (2PG).** From 2PG a molecule of water is removed and the product is **phosphoenol pyruvate (PEP)**. PEP then gives up its 'high energy' phosphate to convert a second molecule of ADP to ATP. The product is pyruvate, pyruvic acid ($C_3H_4O_3$). It is equivalent to half glucose molecule that has been oxidized to the extent of losing two electrons (as hydrogen atoms).

ii. Pyruvic add oxidation: Pyruvic acid (pyruvate), the end product of glycolysis, does not enter the Krebs cycle directly. The pyruvate (3- carbon molecule) is first changed into 2-carbon acetic acid molecule. One carbon is released as CO_2 (decarboxylation). Acetic acid on entering the mitochondrion unites with coenzyme-A (Co A) to form acetyl Co A (active acetate). In addition, more hydrogen atoms are transferred to NAD (Fig. 11.13).

iii. Krebs cyde or citric add cycle: Acetyl CoA now enters a cyclic series of chemical reactions during which oxidation process is completed. This series of reactions is called the Krebs cycle (after the name of the biochemist who discovered it), or the citric acid cycle. The first step in the cycle is the union of acetyl CoA with oxaloacetate to form citrate. In this process, a molecule of CoA is regenerated and one molecule of water is used. Oxaloacetate is a 4-carbon acid. Citrate thus has 6 carbon atoms.

After two steps that simply result in forming an isomer of citrate, isocitrate another NAD- mediated oxidation takes place. This is accompanied by the removal of a molecule of CO_2. The result is α-**ketoglutarate**. It, in turn, undergoes further oxidation (NAD + 2H —> NADH) followed by decarboxylation (CO_2) and addition of a molecule of water. The product then has one carbon atom and one oxygen atom less. It is **succinate**. The conversion of α-ketoglutarate into succinate is accompanied by a free energy change which is utilised in the synthesis of an ATP molecule. The next step in the Krebs cycle is the oxidation of succinate to **fumarate**. Once again, two hydrogen atoms are removed, but this time the oxidizing agent is a coenzyme called **flavin adenine dinucleotide (FAD),** which is reduced to **$FADH_2$**.

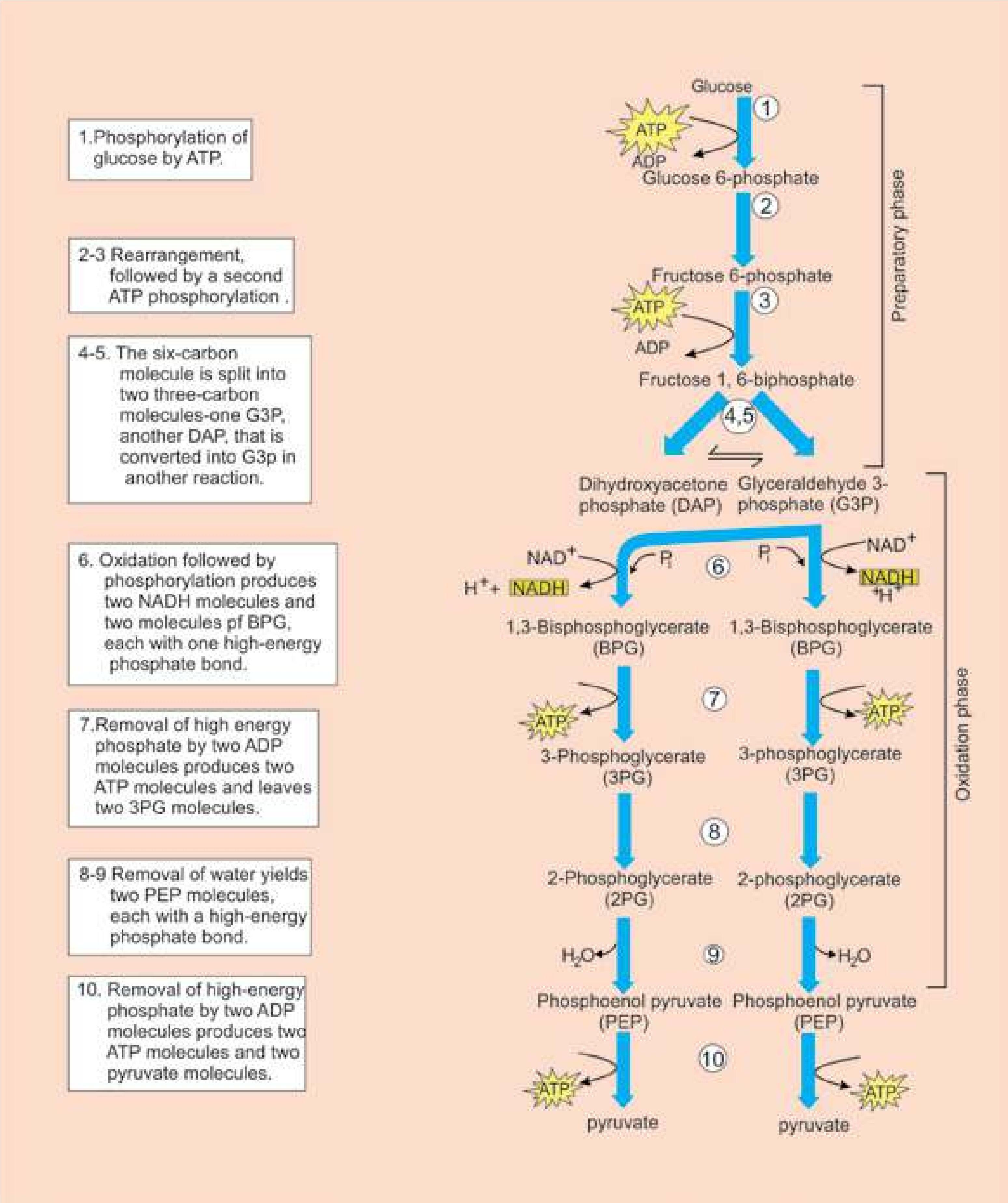


Fig. 11.12 Two phases of glycolysis. All of these reactions take place in the cytosol.

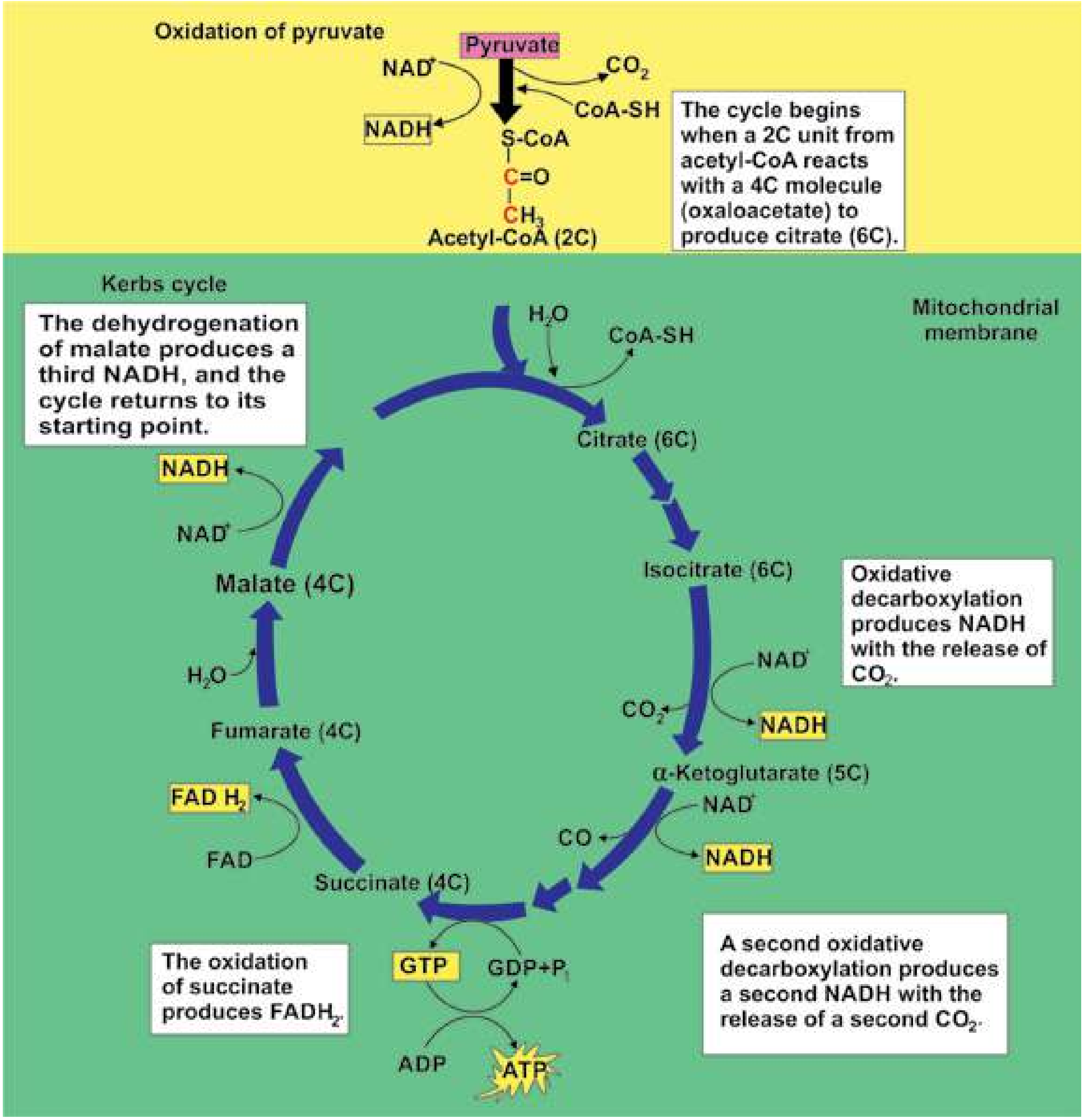


Fig. 11.13 Outline of the Krebs cycle. The brackets give the number of carbon atom in each intermediate of the cycle.

With the addition of another molecule of water, fumarate is converted to **malate**. Another NAD mediated oxidation of malate produces **oxaloacetate,** the original 4-carbon molecule. This completes the cycle. The oxaloacetate may now combine with another molecule of acetyl CoA to enter the cycle and the whole process is repeated (Fig. 11.13).

iv. Respiratory chain: In the Krebs cycle NADH and H^+ are produced from NAD^+. NADH then transfers the hydrogen atom to the respiratory chain (also called **electron transport system**) where electrons are transported in a series of oxidation-reduction steps to react, ultimately, with molecular oxygen. (Fig. 11.14).

The oxidation reduction substances which take part in respiratory chain are:

i. A coenzyme called coenzyme Q

ii. A series of cytochrome enzymes (b,c,a,a_3)

iii. Molecular oxygen (0_2)

Cytochromes are electron transport intermediates containing **haem** of related prosthetic groups, that undergo valency changes of iron atom. Haem is the same iron containing group that is oxygen carrying pigment in haemoglobin. The path of electrons in the respiratory chain appears to be as follows.

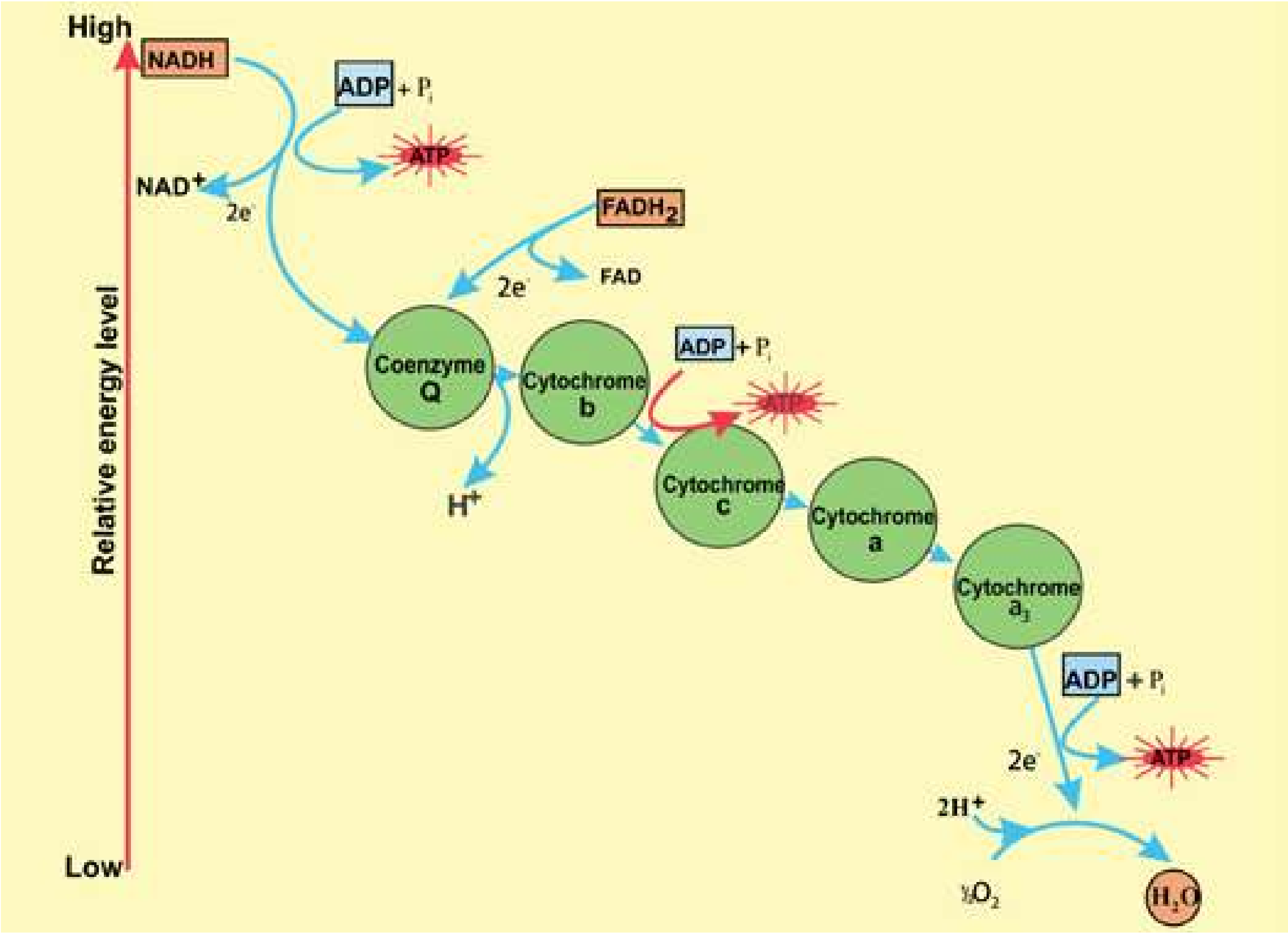


Fig. 11.14 The repiratory electron transport chain and its coupling with oxidative phosphorylation.

NADH is oxidized by **coenzyme Q.** This oxidation yields enough free energy to permit the synthesis of a molecule of ATP from ADP and inorganic phosphate. Coenzyme Q is in turn oxidized by cytochrome b which is then oxidized by cytochrome c. This step also yields enough energy to permit the synthesis of a molecule of ATP. Cytochrome c then reduces a complex of two enzymes called cytochrome a and as (for convenience the complex is referred as cytochrome a). Cytochrome a is oxidized by an atom of oxygen and the electrons arrive at the bottom end of the respiratory chain. Oxygen is the most electronegative substance and the final acceptor of the electrons. A molecule of water is produced. In addition, this final oxidation provides enough energy for the synthesis of a third molecule of ATP.

Oxidative phosphorylation: Synthesis of ATP in the presence of oxygen is called oxidative phosphorylation. Normally, oxidative phosphorylation is coupled with the respiratory chain. As already described ATP is formed in three steps of die respiratory chain (Fig. 11.14). The equation for this process can be expressed as follows:

$$NADH + H^+ + 3ADP + 3Pi + 1/2\ O_2 \longrightarrow NAD^+ + H_2O + 3ATP$$

Where Pi is inorganic phosphate.

The molecular mechanism of oxidative phosphorylation takes place in conjunction with the respiratory chain in the inner membrane of the mitochondrion. Here also, as in photosynthesis, the mechanism involved is chemiosmosis by which electron transport chain is coupled with synthesis of ATP. In this case, however the pumping/movement of protons (H^+) is across the inner membrane of mitochondrion folded into cristae, between matrix of mitochondrion and mitochondrion's intermembrane space. The coupling factors in respiration are also different from those in photosynthesis.

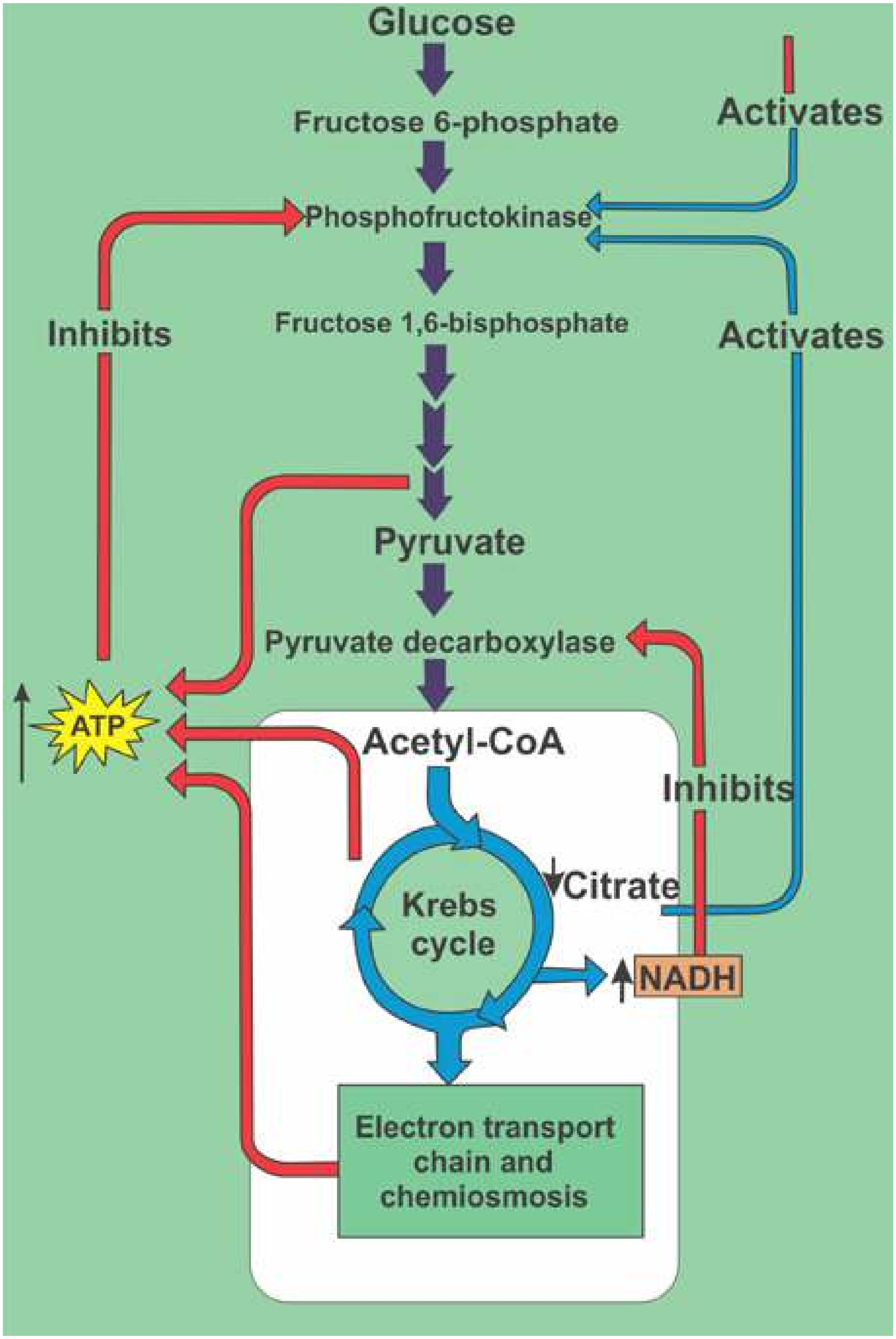


Fig. 11.15 Stages in aerobic respiration. Stage 1: Formation of acetyl-CoA from pyruvate. Stage 2: The Krebs cycle. Stage 3: Respiratory chain and oxidative phosphorylation. Each pair of H atoms entering the respiratory chain as NADH yields 3 ATPs.

EXERCISE

Q1. Write whether the statement is 'true' or 'false' and write the correct statement if it is false.

(i) Hydroponics are the plants grown in water culture.

(ii) Calcium is an essential element for chlorophyll formation.

(iii) Chlorosis means yellowing of leaves due to deficiency of certain essential element of plant nutrition.

Q.2. Short questions.

(i) List four features of a leaf which show that it is able to carry out photosynthesis effectively.

(ii) Summarise the role of water in photosynthesis.

(iii) What are T.W. Engelman and Melvin Calvin famous for?

(iv) What is the difference between an action spectrum and an absorption spectrum?

(V) What is the role of accessory pigments in light absorption?

(vi) When and why is there not net exchange of $C0_2$ and O_2 between the leaves and the atmosphere?

(vii) What is the net production of ATP during glycolysis?

(viii) What is the main difference between photophosphorylation and oxidative phosphorylation?

(ix) What is the location of ETC and chemiosmosis in photosynthesis and cellular respiration?

(X) How did the evolution of photosynthesis affect the metabolic pathway?

(xi) How does absorption spectrum of chlorophyll a differ from that of chlorophyll b?

(xii) Why are the carotenoids usually not obvious in the leaves? They can be seen in the leaves before leaf fall. Why?

(xiii) How is the formation of vitamin A linked with eating of carrot?

Q.3. Extensive questions

(i) Explain the roles of the following in aerobic respiration: (a) NAD+ and FAD (b) oxygen.

(ii) Sum up how much energy (as ATP) is made available to the cell from a single glucose molecule by the operation of glycolysis, the formation of acetyl CoA, the citric acid cycle, and the electron transport chain.

(iii) Trace the fate of hydrogen atoms removed from glucose during glycolysis when oxygen is present in muscle cells; compare this to the fate of hydrogen atoms removed from glucose when the amount of the available oxygen is insufficient to support aerobic respiration.

(iv) Sketch Kreb's cycle and discuss its energy yielding steps.

(v) Describe various steps involved in oxidative break down of glucose to pyruvate.

(vi) Sketch respiratory electron transport chain. Discuss the significance of ETC.

(vii) Compare photosynthesis with respiration in plants.

(viii) Explain the difference between the cyclic and non-cyclic photophosphorylation with the help of Z scheme.

(ix) Give an account of light-independent reactions of photosynthesis.