

Tampines Meridian Junior College

JC1 H2/9744 Biology 2023

Core Idea 3A

3. Transformation of Energy – Photosynthesis & Cellular Respiration

Practices of Science

Nature of Scientific Knowledge | Science Inquiry Skills | Science sand Society



(A) Infectious Diseases

(B) Impact of Climate Change on Animals and Plants

| | | SYLLABUS OVERVIEW | | |
|-----|--|--|--|--|
| No. | Overarching Idea | Topics | | |
| 1 | Core Idea 1 | Cell – The Basic Unit of Life | | |
| 2 | of Life | Biomolecules of Life and Cellular Transport | | |
| 3 | Core Idea 3 Energy and Equilibrium | Transformation of Energy – Photosynthesis and Cellular Respiration | | |
| 4 | | Genetics and Inheritance (I) – The Cell Cycle | | |
| 5 | | Genetics and Inheritance (II) – DNA Replication and Gene Expression | | |
| 6 | | Genetics and Inheritance (III) – DNA Mutations and their Consequences | | |
| 7 | | Genetics and Inheritance (IV) – Molecular Techniques in DNA Analysis | | |
| 8 | Core Idea 2 Genetics and Inheritance | Genetics and Inheritance (V) – Organization of Genome & Control of Gene Expression in Eukaryotes [Includes Core Idea 1D: Stem Cells] | | |
| 9 | | Genetics and Inheritance (VI) – Organization and Inheritance of Viral Genomes | | |
| 10 | | Genetics and Inheritance (VII) – Organization of Genome & Control of Gene Expression in Prokaryotes | | |
| 11 | | Genetics and Inheritance (VIII) - Inheritance | | |
| 12 | Core Idea 3 Energy and Equilibrium | Communication and Equilibrium in Multicellular Organisms | | |
| 13 | Core Idea 4 Biological Evolution | Biological Evolution | | |
| 14 | Extension Topic A Infectious Diseases | Immunity and Infectious Diseases | | |
| 15 | Extension Topic B Impact of Climate Change on Animals & Plants | Climate Change – Causes and Impacts on Animals and Plants | | |

TOPIC SYNOPSIS

This core idea describes how energy is obtained, transformed and utilised in biological systems. The following questions should help you frame you learning:

• How do organisms obtain and use energy in order to live, grow and survive?

Energy is needed to drive biochemical processes in organisms

To maintain life-sustaining processes, organisms require materials and energy from their environment. Nearly all energy that sustains life ultimately comes from the sun. Plants and other photosynthetic organisms make use of sunlight to synthesise carbohydrates from carbon dioxide and water during the process of photosynthesis. Light energy from the sun is converted into chemical energy in the form of carbohydrates. This chemical energy may be used to form plant matter or subsequently released to fuel activities within the plants.

All other organisms depend on autotrophs for energy, either directly, by feeding on autotrophs such as plants; or indirectly, as energy is passed along food chains from one organism to the next. Food provides a source of carbohydrates which are broken down to release energy to phosphorylate ADP to ATP during aerobic respiration. Anaerobic respiration follows a different and less efficient chemical pathway to provide ATP. ATP obtained from respiration is used to drive various essential cellular processes.

In eukaryotes, photosynthesis and respiration occur in membrane-bound organelles. Many steps in photosynthesis and respiration are controlled by enzymes sequestered in these organelles and therefore are also limited by similar factors that will affect enzymatic reactions.

LEARNING OUTCOMES

Core Idea 3A: Transformation of Energy between the Environment and Organisms

Plants and other photosynthetic organisms use sunlight to synthesise carbohydrates from carbon dioxide and water during the process of photosynthesis. The light-dependent (cyclic and non-cyclic photophosphorylation) and light-independent stages of photosynthesis facilitate the conversion of light energy to chemical energy in the form of carbohydrates. Carbohydrates produced from photosynthesis can be assembled into macromolecules or broken down subsequently to fuel activities within the plants. Carbon fixation occurs during the light-independent stage and the Calvin cycle ultimately results in the synthesis of sugars in plants.

As heterotrophs consume plant matter, energy from the plants is transferred to them. Chemical processes occur during aerobic respiration whereby carbohydrates are broken down to release energy to phosphorylate ADP to ATP during aerobic respiration. The energy is transferred between interacting molecules through the four stages of aerobic respiration when oxygen is present. In the absence of oxygen, fermentation occurs with the release of fewer ATP molecules and the formation of either lactate or ethanol depending on the cell type.

Candidates should be able to:

- a) Identify components of chloroplasts and mitochondria in drawings, photomicrographs and electronmicrographs.
- **b)** Explain the absorption and action spectra of photosynthetic pigments.
- c) With reference to the chloroplast structure, describe and explain how light energy is harnessed and converted into chemical energy during the light dependent reactions of photosynthesis. Outline the three phases of the Calvin cycle in C3 plants: (i) CO2 fixation (ii) PGA reduction and (iii) ribulose bisphosphate (RuBP) regeneration, indicating the roles of rubisco, ATP and reduced NADP in these processes that ultimately allow synthesis of sugars.
- **d)** Discuss limiting factors in photosynthesis and carry out investigations on the effect of limiting factors such as temperature, light intensity and carbon dioxide concentration on the rate of photosynthesis.
- e) Outline the process of glycolysis, highlighting the location, raw materials used and products formed. (Details of the intermediate compounds and isomerisation are not required).

- f) Outline the processes of the link reaction and Krebs cycle highlighting the location, raw materials used and products formed (in terms of dehydrogenation and decarboxylation).
- **g)** Outline the process of oxidative phosphorylation including the roles of oxygen and the electron transport chain (ETC) in aerobic respiration. (Names of complexes in the ETC are not required).
- **h)** Explain the production of a small yield of ATP from respiration in anaerobic conditions in yeast and in mammalian muscle tissue.
- i) Explain the significance of the formation of ethanol in yeast and lactate in mammals in the regeneration of NAD.
- **j)** Investigate the effect of factors such as substrate concentration, type of substrate and temperature on the rate of respiration.
- **k)** Outline chemiosmosis in photosynthesis and respiration (Names of complexes in the ETC are not required).

LECTURE OUTLINE

1. Introduction: Overview on the transformation of energy

- 1.1 The need for energy
- 1.2 The role of ATP

2. Photosynthesis

- 2.1 Overview of Photosynthesis
- 2.2 Chloroplast structure in relation to function
- 2.3 Light Dependent Reactions of Photosynthesis
 - 2.3.1 Roles of Thylakoid Membrane proteins
 - 2.3.2 Light-Dependent reactions: Photoactivation of Chlorophyll
 - 2.3.3 Light-Dependent reactions: Photophosphorylation
- 2.4 Light Independent Reactions of Photosynthesis (Calvin Cycle)
- 2.5 Relationship between the Light Dependent and Light Independent Reactions
- 2.6 Factors affecting rate of Photosynthesis
- 2.7 Relationship between Photosynthesis and Respiration in plants

3. Cellular Respiration

- 3.1 The big picture
- 3.2 ATP as the product of cellular respiration
- 3.3 Mitochondrial structure in relation to function
- 3.4 Cellular Respiration Overview
- 3.5 Aerobic Respiration
 - 3.5.1 Glycolysis
 - 3.5.2 Link Reaction
 - 3.5.3 Krebs Cycle
 - 3.5.4 Electron Transport Chain and Oxidative Phosphorylation
- 3.6 Anaerobic Respiration
 - 3.6.1 Production of ethanol in plants and yeast
 - 3.6.2 Production of lactic acid in animal cells

4. Comparisons

- 4.1 Aerobic VS Anaerobic Respiration
- 4.2 Photophosphorylation VS Oxidative Phosphorylation
- 4.3 Lactate Fermentation VS Alcoholic Fermentation
- 4.4 Krebs Cycle VS Calvin Cycle

REFERENCES

- 1. Advanced Biology Principles & Applications by Clegg & Mackean. (1st Edition: 242 269, 318 323)
- 2. Biology by Campbell and Reece. (10th Edition: 184 205, 162 –183)
- 3. Biological Science 1 by Green, Stout and Taylor. (3rd Edition: 196 213, 223 226, 264 272)

Web-links And Animations

Photosynthesis:



Respiration:

| The overview of cellular | An explanation of feedback | How glycolysis works |
|-----------------------------------|--------------------------------------|------------------------------|
| respiration (excellent animation) | inhibition in a multi-step reactions | |
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1. Introduction: Overview on the transformation of energy

1.1 The need for energy

- Living organisms are able to exist because of a continual input of energy.
 - Most of the energy is used for cellular metabolism, growth and reproduction.
 - Remainder of the energy is lost as heat.
- The primary source of energy for most living organisms is **light** from the sun.
 - <u>Autotrophs</u> (producers: green plants and photosynthetic bacteria) carry out <u>photosynthesis</u>. It is a process where **light energy** from the sun is used to produce organic molecules (e.g. carbohydrates such as glucose) from inorganic raw materials (e.g. carbon dioxide and water). Oxygen is released as a waste product (Fig. 1.1).

The chemical equation for photosynthesis is:

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

More specifically, the light energy captured is first converted to **chemical energy** in the form of **ATP and NADPH** (more details will be covered in Section 2.3), which are then used for the formation of organic molecules (more details will be covered in Section 2.4).

 <u>Heterotrophs</u> (consumers) obtain food by eating plants or other animals that feed on plants. These organic food molecules in turn undergo multiple catabolic reactions via <u>respiration</u> to convert the stored chemical energy to ATP, which is needed for metabolic functions (Fig. 1.1).



Fig. 1.1: Photosynthesis converts light energy into chemical energy, while respiration converts the stored chemical energy into ATP.

1.2 The role of ATP

a) **ATP** is an **Energy Carrier** in the Cells of Living Organisms

- The energy carrier molecule used by most living organisms is known as <u>Adenosine</u> <u>Triphosphate</u> (ATP).
 - Adenosine Triphosphate (ATP) = Ribose sugar + Adenine nitrogenous base + 3 phosphate groups (Fig. 1.2).
 - Adenosine Diphosphate (ADP) = Ribose sugar + Adenine nitrogenous base + 2 phosphate groups (Fig. 1.2).
 - Adenosine Monophosphate (AMP) = Ribose sugar + Adenine nitrogenous base + 1 phosphate group.
- ATP is used as a convenient and versatile store of energy to drive a variety of cellular reactions.
 - Energy from light or glucose is stored when adenosine diphosphate (ADP) is phosphorylated (i.e. addition of an inorganic phosphate, P_i) to form ATP.
 - When energy is required, **ATP** is <u>hydrolysed</u> to form **ADP** and inorganic phosphate (P_i). Hydrolysis of one mole of **ATP** yields **30.6** kJ of energy (Fig. 1.2).



Fig. 1.2: Hydrolysis of ATP to ADP and Pi releases energy.

b) The energy released through the hydrolysis of ATP is used for many purposes. Example:

(i) Movement of organelles within the cells:

- > Movement of ER vesicles from the Endoplasmic Reticulum to the Golgi Apparatus.
- Movement of secretory vesicles from the Golgi Apparatus to the cell surface membrane.
- Movement of centrioles to the opposite poles of the spindle axis during nuclear division (Topic: Cell cycle).
- Movement of chromosomes to the metaphase plate during nuclear division (Topic: Cell cycle).

(ii) Active transport of substances across membranes:

- Proton pump on lysosomal membrane pumps protons (H⁺ ions) from cytosol into lysosome, against the concentration gradient.
- > Endocytosis, Exocytosis, Pinocytosis and Phagocytosis at the cell surface membrane.

(iii) Formation of chemical bonds

- > Amino acid activation during translation (Topic: Gene expression).
- Formation of deoxyribonucleoside triphosphates for DNA replication (Topic: DNA replication).
- ATP is converted to cyclic adenosine monophosphate (cAMP) by adenylyl cyclase. cAMP binds and activates protein kinases responsible for phosphorylating other proteins (*Topic: Cell signalling*).

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Building Links – Significance and Role of ATP

The conversion analogy

If you have foreign currency (eg. Japanese yen), can you spend it in a coffeeshop in Singapore?

Can living organisms use sunlight/photons directly as an energy source?

What is the advantage of using ATP as an energy carrier?

2.1 Overview of Photosynthesis

Key Concepts:

- Chloroplasts are sites where photosynthesis occurs.
- Photosynthesis consists of light-dependent reactions that occur on the thylakoid membrane, and light-independent reactions (Calvin cycle) that occur in the stroma of chloroplasts.
- The light dependent and light independent reactions are linked by their products.
- Photosynthesis (Greek: photos = light) converts <u>light energy</u> to <u>chemical energy</u>. The light energy is absorbed by photosynthetic pigments (e.g. chlorophyll).
- Chloroplasts are sites where photosynthesis occurs.
- Two sets of reactions are involved (Fig. 2.1). These are:
 - i. Light-dependent reactions
 - light energy is required
 - > occurs on the **thylakoid membrane**
 - ii. Light-independent reactions / Calvin Cycle
 - light energy is not directly required for the Cycle to take place
 - occurs in the stroma

The light-dependent reactions and the light-independent reactions (Calvin cycle) are linked by their products.



Fig. 2.1: Overview of Photosynthesis.

Light-dependent reactions occur on the thylakoid membrane, while the light-independent reactions (Calvin cycle) occur in the stroma.

2.2 Chloroplast structure in relation to function

• The leaf (Fig. 2.2) is the main photosynthetic structure of a plant, although stems and sepals may also photosynthesize.



Fig. 2.2: Leaves are the major organs of photosynthesis in plants. Chloroplasts are the site of photosynthesis and they are found mainly in the mesophyll cells of leaves.

- **<u>Chloroplasts</u>** (Fig. 2.2 and 2.3) are found mainly in mesophyll cells of leaves.
- These chloroplasts are discoid in shape and circular in surface view with a size of 3-5μm and have a composition of 40%-60% proteins, 25%-35%, lipids, 5%-10% chlorophyll and 12% DNA.
- Each chloroplast has **two membranes** (outer membrane and inner membrane), enclosing a central aqueous space known as the <u>stroma</u> (Fig. 2.4 and 2.5).
- In the stroma are a series of membranous sacs called the <u>thylakoid membranes</u>. (Hence there are three membranes in the structure of chloroplasts) (Fig. 2.4 and 2.5).
- The thylakoid membranes have an internal aqueous space, known as the thylakoid space.
- Thylakoids may form stacks called grana (singular: granum) (Fig. 2.4 and 2.5).



Fig. 2.3: Electron micrograph of plant cell with chloroplasts (left) and of a chloroplast (right).



Fig. 2.5: Structure of a chloroplast in diagrammatic form (left) and in electron micrograph (right).

• The structure of the chloroplast is required for the process of photosynthesis (Table 1).

| Structure | Description | Structure in relation to function | |
|--|--|--|--|
| 1) Chloroplast envelope | Consist of outer membrane and inner membrane . | • Serves to separate the organelle from the cell; allows compartmentalisation and specialisation of the chloroplast for photosynthesis. | |
| | | A site where light-dependent reactions occur. | |
| 2) Thylakoid | (a) Grana Many flattened stacks of coin-like fluid filled sacs, 600nm in diameter. | • Thylakoid membrane has a large surface area for attachment of multiple photosystems, electron carriers (proteins), proton pumps and stalked particles (ATP synthase). This increases rate of photosynthesis. | |
| within the chloroplast envelope) | (Granum =1 flattened stack) | Photosystems and electron carriers are closely located and arranged in sequential order (e.g. for passage of electrons down the chain of electron carriers of progressively lower energy levels, to release energy for active transport of H⁺ into thylakoid | |
| | (b) Intergranal Iamellae : Sheet like with a narrow space in between. | space). This increases efficiency of photophosphorylation. | |
| | | • The phospholipid bilayer is impermeable to protons , thus allowing the accumulation of protons in the thylakoid space to create a proton gradient . | |
| | Contains circular DNA | • Carry genes that code for electron carriers (which are proteins) and enzymes needed for light independent reactions. | |
| | Contains 70S ribosomes | Allow chloroplast to carry out protein synthesis independently of the cell. | |
| 3) Stroma | Gel-like medium that contains soluble enzymes, organic acids, sugars and lipids. Also contains varying concentrations of ATP /ADP, NADPH/NADP. | A site where <u>light-independent reactions</u> occur. Closely associated with thylakoid membranes, such that the products of light-dependent reactions can be channelled to the light-independent reactions (and vice versa). Large area for the temporary storage of starch. | |
| | | | |

Table 1: The structure of the chloroplast in relation to its function.

2.3 Light-Dependent Reactions of Photosynthesis

Key Concepts:

- Light-dependent reactions occur on the thylakoid membranes.
- Light dependent reactions involve photoactivation followed by photophosphorylation.
- Light-dependent reactions convert light energy to chemical energy (in the form of ATP and NADPH) for the light-independent reactions. Oxygen is released as a by-product.
- Light-dependent reactions are directly dependent on light Intensity.
- It is a series of reactions that take place on the <u>thylakoid membranes</u> of chloroplasts. It involves membrane proteins on the thylakoid membrane (Section 2.3.1)
- Light-dependent reactions involve:
 - **Photoactivation of chlorophyll** (Section 2.3.2), followed by
 - Photophosphorylation (Cyclic or Non cyclic) (Section 2.3.3)
- Purpose of light-dependent reactions:
 - Synthesis of ATP and NADPH for light-independent reactions.
 - **Oxygen** is released as a by-product.

2.3.1 Roles of Thylakoid Membrane proteins

- The thylakoid membrane consists of membrane proteins (Fig. 2.6) as follows:
 - A. Photosystems (PSII and PSI)
 - B. Electron carriers (Note: names of electron carriers are not required)
 - **C.** Proton pumps (Cytochrome complex)
 - D. NADP reductase (enzyme)
 - E. Stalked particles (ATP synthase)



Fig. 2.6: Overview of the structure of the thylakoid membrane, with its associated membrane proteins.



A. Photosystems

Key Concepts:

- Photosynthetic pigments are organised into photosystems. A photosystem consists of lightharvesting complexes surrounding the reaction centre.
- There are two types of photosystems PSI and PSII.

• Photosynthetic pigments

1. Chlorophyll

Examples: Chlorophyll a and Chlorophyll b

Absorbs mainly red and blue-violet light. Reflects green light (which explains why most leaves appear green to our eyes).

Chlorophyll a is the **most abundant photosynthetic pigment** and is of universal occurrence in all photosynthetic plants.

Functions of chlorophyll:

- Chlorophyll a is known as the primary pigment
 - the only pigment that can act directly to convert light energy to chemical energy.
- Chlorophyll b is an accessory pigment.
 - It absorbs light energy and transfers it to chlorophyll *a*. It does not convert light energy to chemical energy.

2. Carotenoids

Functions of carotenoids:

- Yellow, orange, red and brown pigments that strongly absorb in the blue-violet range of light act as accessory pigments.
 - They can absorb wavelengths of light that chlorophyll *a* cannot, thus broaden the spectrum of colours from which the plant can obtain energy to drive photosynthesis.

• A photosystem consists of **light harvesting complexes** surrounding the **reaction centre** (Fig. 2.7).



Fig. 2.7: Side View of a Photosystem (diagrammatic representation), comprising the (I) light harvesting complex and (II) reaction centre.

I. Light harvesting complex

 Consists of various photosynthetic pigments bound to proteins. These are the <u>accessory</u> <u>pigments</u> that absorb red and blue-violet wavelengths of light.

Examples:

- Chlorophylls
 - > Absorb red (650-680nm) and blue-violet (440-500nm) wavelength of light.
 - E.g. chlorophyll b.
- Carotenoids
 - Absorb <u>blue-violet</u> (440-500nm) wavelength of light.
 - > E.g. β carotene and xanthophyll.
- II. <u>Reaction Centre</u> complex
 - Consists of an association of proteins holding together:
 - (i) a pair of <u>special chlorophyll a molecules</u> (a₆₈₀ or a₇₀₀), which are the <u>primary</u> <u>pigments</u>. They are able to use harvested light energy to excite one of their electrons to a higher energy level, and then transfer them to the primary electron acceptor.

special chlorophyll a \rightarrow special chlorophyll a⁺ + e

(ii) a <u>primary electron acceptor</u>, which is able to **capture excited electrons** from special chlorophyll a and becomes **reduced**.

- There are 2 types of photosystems:
 - I. Photosystem I (PS I)
 - Consists of accessory pigments (chlorophyll and carotenoids) surrounding primary pigment, chlorophyll a₇₀₀.
 - a₇₀₀ absorbs photons of light at the wavelength of 700nm (Fig. 2.8) hence the reaction centre of PS I is known as <u>P700</u>.
 - II. Photosystem II (PS II)
 - Consists of **accessory pigments** (chlorophyll and carotenoids) surrounding primary pigment, **chlorophyll a**₆₈₀.
 - a₆₈₀ absorbs photons of light at the wavelength of 680nm (Fig. 2.8), hence the reaction centre of PS II is known as <u>P680</u>.
- The <u>absorption spectrum</u> is a graph that shows the relative amount of lights absorbed at different wavelengths by different photosynthetic pigments.



Fig. 2.8: The absorption spectrum.

Sunlight consists of a spectrum of light (a rainbow of colours from red to violet). Each colour has a different wavelength, and not all colours are equally absorbed by photosynthetic pigments.

Building Links – Why do plants have both chlorophylls and carotenoids as photosynthetic pigments, in addition to special chlorophyll a?

B. Electron carriers

- Electron carriers are molecules that can undergo many redox reactions. They receive electrons and pass them on to other molecules.
- An electron transport chains (ETC) can be found on the thylakoid membrane of chloroplasts.
 - It is a sequence of electron carrier molecules (membrane proteins) of progressively lower energy levels.
 - It functions to transport electrons through a series of redox reactions, from higher to lower energy levels.
 - As electrons move from one electron carrier to the next, energy is released.
- The ETC between PSII and PSI is made up of the electron carrier plastiquinone (pg), a cytochrome complex, and a protein called plastocyanin (Pc). The ETC between PSI and NADP reductase is made up of the protein ferredoxin (Fd) (Fig. 2.9). (Note: Names of electron carriers are not required)
- C. Proton Pumps (Cytochrome complex)
- Harness energy released from electron transport chain is used to pump protons (H⁺) from stroma into thylakoid space against a concentration gradient, via active transport (Fig. 2.9).
- This creates a **proton gradient** across the thylakoid membrane that will subsequently **drive** ATP synthesis (more details in section 2.3.3 on Chemiosmosis).
 - Thylakoid space: High proton (H⁺) concentration
 - Stroma: Low proton (H⁺) concentration



Fig. 2.9: The electron transport chains (ETC) present on the thylakoid membrane consists of a sequence of electron carriers of progressively lower energy levels. As electrons move from one carrier to the next, energy is released. The proton pumps harness the energy released from the ETC to pump protons from the stroma into the thylakoid space, via active transport. This creates a proton gradient across the thylakoid membrane.

Building Links – Protons (H⁺ ions) are actively pumped from the stroma into the thylakoid space to create a proton gradient. Why is it possible for H⁺ ions to accumulate within the thylakoid space? Biology Unit, Tampines Meridian JC

D. NADP Reductase

A membrane-bound <u>enzyme</u> that catalyses the <u>formation of reduced NADP/NADPH</u> from NADP⁺:

NADP⁺ + 2e⁻ + 2H⁺ \longrightarrow reduced NADP / NADPH + H⁺

• Reduced NADP produced is used as a **reducing agent** in the **light-independent reactions** (Calvin cycle).

NADP/NADPH (FYI: NADPH - Reduced nicotinamide adenine dinucleotide phosphate) NADP/NADPH is a molecule that can undergo many redox reactions. • It acts as a **coenzyme** required for **redox reactions** occurring during photosynthesis. Recall topic on Enzymes: Coenzymes are small, organic, non-protein and vitamin-derived 0 molecules that are loosely associated with the enzyme during reactions. It exists in two forms: an oxidised form and a reduced form (Fig. 2.10). • The light-dependent reactions require the oxidised form (NADP / NADP⁺). They are at a 0 lower energy level and function as hydrogen/electron acceptors. The light-independent reactions require the reduced form (NADPH / reduced NADP). 0 They are at a higher energy level and function as **electron carriers/donors**. oxidized form NADPH reduced form NADP* nicotinamide ring IBOS ADENINE ADENINE this phosphate group is missing in NAD[®] and NADH (See: respiration topic) Fig. 2.10: In photosynthesis, NADP+ accepts electrons from special chlorophyll a and becomes reduced, forming NADPH. NADPH then transfers the electrons to molecules in the Calvin cycle, thereby reducing that molecule and itself reoxidized to NADP+.

D. Stalked particles (ATP Synthase)

- <u>Enzyme</u> that catalyses the <u>synthesis of ATP</u> using the proton gradient across the thylakoid membrane.
- ATP produced is used in the light-independent reactions (Calvin cycle).
- Stalked particles (ATP synthase) (Fig. 2.11) are protein complexes comprising :
 - (1) F₀ transmembrane hydrophilic proton channel embedded in thylakoid membrane
 Allows the <u>facilitated diffusion of protons (H⁺)</u> down its concentration gradient <u>from</u> thylakoid space to stroma.
 - (2) F1 ATP synthase head portion facing the stroma
 - \circ F₁ ATP synthase head portion rotates as H⁺ ions passes through the hydrophilic channel.
 - It harnesses the <u>proton-motive force</u> (electrical potential energy) to <u>synthesise ATP</u> from ADP and inorganic phosphate (Pi).



Fig. 2.11: ATP Synthase harnesses the proton motive force to synthesise ATP from ADP and inorganic phosphate (Pi).

2.3.2 Light-Dependent Reactions: Photoactivation of Chlorophyll

Key Concept:

- Photoactivation of chlorophyll uses the light energy absorbed by accessory pigments to excite electrons from special chlorophyll a molecules.
- Light energy absorbed by the different <u>accessory pigments</u> (chlorophyll and carotenoids) is passed from one pigment to another until it reaches the pair of <u>special chlorophyll a</u> <u>molecules</u> in the reaction centres <u>P680</u> (Photosystem II) and <u>P700</u> (Photosystem I).
- Light energy excites an electron of each special chlorophyll a molecule to a higher energy level, leaving the chlorophyll a molecule positively charged. The excited electrons are then captured by the primary electron acceptor (Fig. 2.12).





Fig. 2.12: Photoactivation of chlorophyll on the Thylakoid membrane.

2.3.3 Light-Dependent Reactions: Photophosphorylation

Key Concepts:

- Photophosphorylation uses the high energy electrons released to synthesise ATP.
- There are two types of photophosphorylation non-cyclic (involves both PSII and PSI)
- and cyclic photophosphorylation (involves only PSI).
- It is the <u>synthesis of ATP</u> from ADP and inorganic phosphate (P_i) using <u>light energy</u> in photosynthesis via <u>chemiosmosis</u>.
- There are two types of photophosphorylation that can occur at the thylakoid membrane:
 - a) Non-cyclic photophosphorylation and
 - b) Cyclic photophosphorylation
- Both types of photophosphorylation occur in plants. They are alternative pathways for electron transfer during the process of photosynthesis. Photophosphorylation can switch between the non-cyclic and cyclic pathway, depending the needs of the plant.

FYI: For example, when concentration of carbon dioxide in leaf in the light becomes very low, fixation of carbon dioxide during light-independent reaction comes to a standstill. As a result, reduced NADP accumulates in the stroma and NADP disappears. Without a continuous supply of NADP, photosystems II and I are no longer able to operate together. In this situation, cyclic phosphorylation occurs to form ATP.

a) Non-cyclic photophosphorylation

- Non-cyclic photophosphorylation involves both photosystem II (PS II) and photosystem I (PS I) in a 'Z scheme' of electron flow.
- Process of non-cyclic photophosphorylation (the numbered points correspond with the numbers on Fig. 2.14):
 - 1. **Photoactivation** occurs first (Fig. 2.13):
 - a. Light energy is absorbed by the <u>accessory pigments</u> of both photosystems, <u>PS II</u> <u>and PS I.</u>
 - b. This light energy is passed via accessory pigments to **special chlorophyll a** molecules in the respective **reaction centres (P680 and P700).**
 - c. Excited electrons are emitted from the primary pigments of both reaction centres and transferred to the primary electron acceptors.



Fig. 2.13: Non-cyclic photophosphorylation on the thylakoid membrane involves both PSII and PSI.

- These electrons absorbed by primary electron acceptors are passed along a chain of electron carriers and proton pumps in the <u>electron transport chain</u> of progressively lower energy levels through redox reactions.
- The energy released during this process is used by <u>proton pumps</u> to transport protons (H⁺ ions) from the stroma into the thylakoid space against a concentration gradient via <u>active transport</u>.

This creates a **proton gradient** across the thylakoid membrane, where the concentration of H^+ is higher in the thylakoid space than the stroma.

- Protons (H⁺ ions) move <u>from thylakoid space back into the stroma</u> through the <u>ATP</u> <u>synthase</u> (stalked particle), via <u>facilitated diffusion</u> down a concentration gradient. (Fig. 2.14)
 - a. The ATP synthase harnesses the <u>proton-motive force</u> to <u>generate ATP</u> from ADP and P_i .

- b. The process where the proton gradient created is used to drive ATP synthesis (from ADP and P_i) is known as <u>chemiosmosis</u>.
- c. ATP generated during non-cyclic photophosphorylation is used in the lightindependent reactions (Calvin Cycle).
- 5. Ultimately, the **electrons** flow down the electron transport chain from PSII to PSI, and then **combine with NADP+ and H+** from the stroma to **form NADPH.** NADPH will be used in **light-independent reactions.**

This reaction is catalysed by **NADP reductase. NADP**⁺ is the **final electron acceptor**.

2H⁺ + 2e⁻ + NADP → reduced NADP (NADPH) + H⁺

- 6. <u>PS I</u> receives replacement of electrons from <u>PS II</u>.
- <u>PS II</u> receives a replacement of electrons from <u>photolysis of water</u> which produces electrons, H+ and oxygen (as a by-product). Hence, <u>water</u> is the <u>electron donor</u> for noncyclic photophosphorylation.



Fig. 2.14: Non cyclic photophosphorylation and chemiosmosis via ATP synthase.

Quick Revision – Non-cyclic Photophosphorylation

| Location | |
|--|---------------------------------|
| Source of Energy | |
| Source of Electrons/Electron Donor | |
| Final Electron Acceptor | |
| Energy Conversion | energy toenergy |
| In what direction were the protons pumped to | From the to the |
| generate proton gradient for chemiosmosis? | (against a concentration |
| | gradient) |
| In what direction did the protons flow via | From theto the |
| facilitated diffusion during chemiosmosis? | (down a concentration gradient) |
| Enzyme(s) involved | |
| Final product(s) | |

b) Cyclic Photophosphorylation (Fig. 2.15)



Fig. 2.15: Cyclic Photophosphorylation at the Thylakoid membrane involves only PSI.

- Involves **photosystem I (PS I)** only. This type of photophosphorylation is called 'cyclic' because the electrons are returned to the photosystem from which they originated. They are 'recycled' rather than used to form reduced NADP, but ATP is still generated.
- Process of cyclic photophosphorylation (the numbered points correspond with the numbers on Fig. 2.15):
 - 1. **Photoactivation** occurs:
 - a. Light energy is absorbed by accessory pigments of PS I.
 - b. This light energy is passed via accessory pigments to **<u>special chlorophyll a</u>** molecules in the reaction centre, **P700.**

- c. Excited electrons are emitted from <u>special chlorophyll a</u> (electron donor) and transferred to the <u>primary electron acceptors</u>.
- The excited electrons are then passed along a chain of electron carriers and proton pumps in the <u>electron transport chain</u> of progressively lower energy levels via a series of redox reactions. Eventually they are passed back to <u>special chlorophyll a</u> (<u>final</u> <u>electron acceptor</u>) in reaction centre P700.

The energy released from the passage of electrons is used to transport protons (H⁺ ions) from the stroma into the thylakoid space against a concentration gradient, via <u>active transport</u>.

This creates a **proton gradient** across the thylakoid membrane, where the concentration of H^+ is higher in the thylakoid space than the stroma.

- 3. Protons (H⁺ ions) move <u>from thylakoid space back into the stroma</u> via the <u>ATP</u> <u>synthase</u> (stalked particle), via <u>facilitated diffusion</u> down a concentration gradient.
 - a. <u>ATP synthase</u> harnesses the <u>proton-motive force</u> to <u>generate ATP</u> from ADP and P_i.
 - b. The process where the proton gradient created is used to drive ATP synthesis (from ADP and P_i) is known as <u>chemiosmosis</u>.
 - c. ATP generated during cyclic photophosphorylation is also used in the lightindependent reactions (Calvin cycle).

| Quick Revision – Cyclic Photophosphorylation | |
|--|--|
| Lange Care | |

| Location | |
|---|---|
| Source of Energy | |
| Source of Electrons/Electron Donor | |
| Final Electron Acceptor | |
| Energy Conversion | energy toenergy |
| In what direction were the protons pumped during | From theto |
| to generate proton gradient for chemiosmosis? | the(against a concentration |
| | |
| | gradient) |
| In what direction did the protons flow via | gradient) From the to the |
| In what direction did the protons flow via facilitated diffusion during chemiosmosis? | gradient) From theto the(down a concentration gradient) |
| In what direction did the protons flow via facilitated diffusion during chemiosmosis? Enzyme(s) involved | gradient) From theto the(down a concentration gradient) |

c) Non-Cyclic Photophosphorylation vs Cyclic Photophosphorylation (Fig. 2.16)

Animation on Non-Cyclic vs Cyclic Photophosphorylation and ATP synthesis: http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter10/animations.html

| Level of comparison | Non-Cyclic Photophosphorylation | Cyclic Photophosphorylation |
|-------------------------|---------------------------------------|-----------------------------|
| Photosystem(s) | PSII and PSI | |
| involved | | |
| Enzyme(s) involved | ATP synthase and NADP | |
| | reductase | |
| Electron donor | Water | |
| | | |
| Flow of electrons | Electrons move forward from | |
| | special chl a in PSII, to special chl | |
| | a in PSI, and finally to NADP | |
| Does replacement of | Involves photolysis of water to | |
| electrons involve | replace the electrons lost in PSII | |
| photolysis of water? | | |
| Final Electron Acceptor | NADP | |
| | | |
| Product(s) formed | ATP, reduced NADP and oxygen | |
| | | |



(b)



Fig. 2.16: Generation of proton gradient in (a) non-cyclic photophosphorylation as compared to that of (b) cyclic photophosphorylation.

2.4 Light-Independent Reactions of Photosynthesis (Calvin Cycle)

Key Concepts:

- Light-independent reactions (Calvin Cycle) occur in the stroma.
- Light-independent reactions use ATP and NADPH from the light-dependent reaction to synthesize carbohydrates.
- The first carbohydrate synthesized is glyceraldehyde-3-phosphate / triose phosphate.



| į | • 3-phosphoglycerate (PGA) (Fig. 2.18) is also known as glycerate-3-phosphate (GP) |
|---|---|
| į | (Fig. 2.17). |
| | Glyceraldehyde-3-phosphate (GALP) (Fig. 2.18) is also known as triose phosphate (TP) (Fig. 2.17). |
| B | biology Unit, Tampines Meridian JC Page 28 of 62 |

- The light-independent reactions (Calvin Cycle) occurs in the stroma of the chloroplast.
- The process utilises <u>ATP</u> and <u>NADPH</u> from the <u>light-dependent reactions</u>. Hence, it is **dependent on the rate of light dependent reactions** (but not *directly* dependent on light intensity).
- The rate of the Calvin Cycle is directly dependent on:
 - I. **Carbon dioxide concentration** as the Calvin cycle fixes carbon dioxide from the atmosphere to form organic molecules.
 - II. **Temperature -** as it is catalysed by enzymes.
- Occurs in 3 main stages (Fig. 2.17 and 2.18):

1. Carbon dioxide fixation

- One molecule of carbon dioxide combines with a 5C ribulose bisphosphate (RuBP) molecule to give a 6-carbon intermediate. Process is catalysed by enzyme <u>RUBISCO</u> (FYI: ribulose bisphosphate carboxylase oxygenase the most abundant plant enzyme in the world).
- This 6C intermediate is unstable, hence it will break down to give two molecules of 3C glycerate-3-phosphate (GP) / 3-phosphoglycerate (PGA).

2. PGA reduction

- Each molecule of PGA/GP receives 1 additional phosphate group from an ATP molecule (from the light-dependent reactions) to form glycerate-1,3-bisphosphate / 1,3-bisphosphoglycerate (Fig. 2.18).
- Each molecule of glycerate-1,3-bisphosphate is reduced by NADPH from the lightdependent reactions (ie. NADPH provides reducing power and becomes oxidised).
 Glycerate-1,3-bisphosphate also loses a phosphate group to form glyceraldehyde-3phosphate (GALP) / triose phosphate (TP) (3C sugar with phosphate) (Fig. 2.18).
- \circ **GALP/TP** is the **first carbohydrate** product of photosynthesis (hence, this pathway of carbon fixation is called the C₃ pathway).
- NADP, ADP and P_i are recycled at the thylakoid membrane for the light-dependent reactions.

3. <u>Ribulose bisphosphate (RuBP) regeneration</u>

- A small proportion of GALP/TP produced exits the Calvin Cycle, and is used as the starting material for metabolic pathways that synthesize organic compounds (e.g. glucose) (Fig. 2.19).
- Majority of the GALP/TP produced remain in the Calvin Cycle, and is used to regenerate RuBP. ATP from the light dependent reactions provides energy and phosphate for the rearrangement of carbon atoms between sugar phosphates to regenerate a 5C RuBP from the 3C sugar molecules.
- ADP and P_i are recycled at thylakoid membrane for light-dependent reactions.



Fig 2.19: Product synthesis phase of photosynthesis -

Using GP/PGA and TP/GALP from Calvin Cycle to synthesize organic compounds.

Fate of triose phosphate that exits Calvin cycle:



L.....

Quick Revision – Calvin Cycle

| Location | |
|--------------------|--|
| Source of Energy | |
| Energy Conversion | energy (in the form of ATP and NADPH) is converted into energy (in the form of organic molecules/glucose) |
| Main stages(s) | |
| Role(s) of RuBP | (Stage 1) RuBP combines with to form unstable to form unstable It is the substrate for |
| Role(s) of ATP | (Stage 2) ATP supplies energy/phosphate to to form (Stage 3) ATP supplies energy and phosphate to regenerate from |
| Role(s) of NADPH | (Stage 2) NADPH donatesto, itself being oxidised to NADP ⁺ . Hence it1,3- bisphosphoglycerate to form |
| Enzyme(s) involved | |
| Product(s) formed | |

2.5 Relationship between Light-Dependent & Light-Independent Reactions



Fig. 2.20a: Relationship between light-dependent and light-independent reactions. They are linked by their products.

- <u>NADPH</u> and <u>ATP</u> formed on the thylakoid membrane from <u>light-dependent reactions</u> are used for the Calvin Cycle, which takes place in the stroma (Fig. 2.20a and b).
- <u>NADP⁺</u>, <u>ADP</u> and <u>P</u>_i from the <u>Calvin Cycle/ light-independent reactions</u> in the stroma are recycled for use in light-dependent reactions in the thylakoid membrane (Fig. 2.20a and b).

***IMPORTANT:**

ATP produced during photophosphorylation in the chloroplast is <u>not</u> used by other cellular processes except Calvin cycle.

ATP produced at the mitochondria in respiration, however, can be used by other cellular processes e.g active transport.

.....

Building Links: Explain why a poison that inhibits an enzyme of the Calvin Cycle will also inhibit the light-dependent reaction (and hence decreases the overall rate of photosynthesis)?



Fig. 2.20b: The relationship between photophosphorylation at the thylakoid membrane and the Calvin Cycle in the stroma.

2.6 Factors Affecting Rate of Photosynthesis

- Factors affecting rate of photosynthesis are:
 - i. Light (Colour and Intensity)
 - ii. Temperature
 - iii. Carbon Dioxide Concentration
 - iv. Oxygen Concentration
 - v. Water Content
- Recall: When a biochemical reaction is affected by more than one factor, the rate is limited by the factor that is nearest to its minimum value (i.e. the factor with the least supply will limit the rate of reaction). This factor is known as the <u>limiting factor</u>.
- Example of how to use terms:
 - Low carbon dioxide concentration (0.03%) is a **limiting factor** to the Calvin Cycle.
 - If the carbon dioxide concentration is increased to 3.0%, carbon dioxide is **no longer a limiting factor** and **other factors** (e.g. light intensity) are limiting.
- To investigate the effects of **limiting factors** on the rate of photosynthesis, **measurable factors** (dependent variable) should be used to represent the rate.
 - E.g. To investigate the effect of temperature, the rate of photosynthesis can be determined by the measuring the **rate of production of oxygen gas** (Fig. 2.21).



Fig. 2.21: Apparatus for measuring the rate of oxygen evolved by a water plant during photosynthesis.

- i. Light
- Colour of Light
 - The <u>absorption spectrum</u> of chlorophyll (Fig. 2.22a) shows that the <u>absorption of light</u> is the highest at the wavelengths of red and blue-violet lights and lowest at the wavelength of green light.
 - The <u>action spectrum</u> (Fig. 2.22b) shows that the <u>rate of photosynthesis</u> is highest at wavelengths of **red and blue-violet light**, while the rate of photosynthesis is **lowest** at wavelengths of green light.
 - The action spectrum corresponds closely to the absorption spectrum of chlorophyll. This is because the absorbance of light affects <u>light-dependent reactions</u>. As more light is absorbed, more energy is available for photoactivation and photophosphorylation, hence increasing the overall rate of photosynthesis.



Fig. 2.22: The (a) absorption spectrum and (b) action spectrum. The rate of photosynthesis corresponds closely to the absorption of light.

- Light intensity (Fig. 2.23 a and b)
 - At low light intensities:
 - Light intensity is a <u>limiting factor</u>. Hence, the rate of photosynthesis increases linearly with increasing light intensity.
 - The rate of cyclic and non-cyclic photophosphorylation in the <u>light-dependent</u> reactions will be low. Hence, the rate of production of ATP and NADPH will also be too slow to allow the Calvin Cycle to proceed at its maximum rate.
 - As light intensity increases:
 - the rate of cyclic and non-cyclic photophosphorylation increases. This increases the production of ATP and NADPH for Calvin Cycle to take place, hence increasing the overall rate of photosynthesis.
 - At high light intensities:
 - Light saturation may occur, and increasing the light intensity will not increase the rate of photosynthesis (Fig. 2.23a).
 - Very high light intensities may bleach chlorophyll and decrease the rate of photosynthesis (Fig. 2.23b).



Fig. 2.23a: Effect of light intensity on rate of photosynthesis.



Fig. 2.23b: Effect of light intensity on rate of photosynthesis in a plant that is not adapted for high light intensities.

ii. Temperature (Fig. 2.24)

- Photosynthesis is affected by temperature because reactions of the <u>Calvin Cycle</u> are catalyzed by <u>enzymes</u>.
- When the temperature is below the optimum, for each **rise of 10°C** (relate to the Q₁₀ temperature coefficient of enzymatic reactions), the **rate of enzymatic activities doubles**, causing a doubling in the rate of photosynthesis.
- When the temperature is above the optimum, enzymes start to **denature**, causing the photosynthetic rate to decrease.



Fig. 2.24: Effect of temperature on photosynthesis.

iii. Carbon dioxide concentration (Fig. 2.25)

- At low carbon dioxide concentrations (<0.04%) [Note: atmospheric carbon dioxide concentration is 0.035%], carbon dioxide concentration is a <u>limiting factor</u> to <u>Calvin Cycle</u>. Hence, rate of photosynthesis increases linearly with increasing carbon dioxide concentrations.
- At high carbon dioxide concentrations, <u>carbon dioxide saturation</u> occurs as <u>all</u> RUBISCO active sites are occupied (carbon dioxide fixation is a rate-determining step).





iv. Oxygen concentration

- Atmospheric oxygen concentration is approximately 21%. The current concentration does not limit photosynthesis.
- Increasing oxygen concentration decreases rate of photosynthesis as oxygen outcompetes carbon dioxide to bind to the active site of RUBISCO (Ribulose Bisphosphate Carboxylase Oxygenase).
- This prevents carbon dioxide from binding with RUBISCO hence leading to a decrease in the rate of Calvin Cycle, resulting in the lowering of the rate of photosynthesis. This phenomenon is known as photorespiration.

v. Water content

- Low amounts of water will be a <u>limiting factor</u> due to the closure of the stomata which limits carbon dioxide uptake for photosynthesis.
- Decreased carbon dioxide uptake leads to a **decrease in rate of Calvin Cycle**, hence lowering the rate of photosynthesis. [Note: It is <u>NOT</u> due to having insufficient water for photolysis.]

2.7 Relationship between Photosynthesis and Respiration in Plants

Key Concepts:

- In plants, oxygen from photosynthesis is used for respiration, and CO₂ from respiration is used for photosynthesis.
- Compensation point is the light intensity at which the rate of photosynthesis is equal to the rate of respiration. At compensation point, there is no gaseous exchange between the plant and its environment.
- In plants, oxygen from photosynthesis is used for respiration, and CO₂ from respiration is used for photosynthesis (Fig. 2.26).
- The <u>light intensity</u> at which the rate of photosynthesis is equal to the rate of respiration is known as the <u>compensation point</u> (Fig. 2.27).
 - The compensation point is reached at quite **low light intensity**, usually at sunrise and sunset.
 - At compensation point, all the CO₂ produced during respiration is used for photosynthesis, and all the O₂ produced during photosynthesis is used for respiration. Thus, there is no gaseous exchange (O₂ and CO₂) between the plant and its environment.



Fig. 2.27: Graph showing the Compensation point, where there is no net loss or gain in oxygen.

3. Cellular Respiration

3.1 The Big Picture

- As mentioned previously, living organisms are able to exist because of a continual **input** of **energy**.
- **Heterotrophs** require food/nutrition as they cannot synthesise carbohydrates the way plants and photosynthetic organisms are able to.
- Food needs to be broken down before organic molecules can enter into respiration (Fig. 3.1).



Fig. 3.1: Breakdown of food molecules.

Food molecules are broken down in three stages (Fig. 3.1):

Stage 1: Digestion (*the focus of 'O' Level syllabus*)

- The enzymatic breakdown of food molecules in intestine or in cellular lysosomes
- Large polymeric molecules of food are hydrolysed into monomeric subunits
 - > Protein \rightarrow Amino Acids
 - > Polysaccharides \rightarrow Sugars
 - \succ Fats \rightarrow Fatty Acids + Glycerol
- Amino acids, glucose, fatty acids are absorbed into the blood and delivered to all cells in the body. When absorbed by cells and enter the cytosol, the next stage begins.

Stage 2 and 3: Cellular Respiration

Key Concept:

Cellular respiration is a process in which **organic molecules** are <u>oxidised</u> in stages to release chemical potential energy for the **synthesis of ATP.** ATP is needed for cellular activities such as **metabolic reactions**, **active transport** of substances and **mechanical work**.

- Oxidising glucose to carbon dioxide and water releases a relatively large amount of energy in one step. In cells, the energy contained in glucose is released in a **stepwise manner** through a large number of reactions, in a process called **respiration**.
- Two types of cellular respiration can occur in a cell (Fig. 3.2)
 - Aerobic respiration
 - Anaerobic respiration



Fig. 3.2: Two types of respiration – aerobic respiration and anaerobic respiration.

(A) Aerobic Respiration

- Occurs when O₂ is available, and comprises four main processes:
 - > Glycolysis
 - Link Reaction
 - Krebs Cycle
 - Oxidative phosphorylation
- A limited amount of ATP is synthesized in glycolysis and the Krebs cycle via a process called **substrate-level phosphorylation**.

- However, these two processes function mainly to supply electrons to drive oxidative phosphorylation. This is the process which accounts for most of the ATP made during aerobic respiration.
- Aerobic respiration involves the following types of biochemical reactions:
 - Decarboxylation removal of -COOH, -COO⁻ or CO₂
 - > **Oxidation** removal of hydrogen atoms or electron
 - > Dehydrogenation transfer of hydrogen atoms from a donor to an acceptor
 - (Also an oxidation reaction)
 - Phosphorylation addition of phosphate group to a molecule
- Since respiration involves the transfer of hydrogen / electrons from one molecule to another, hydrogen (or electron) acceptors and donors are involved.
- There are 2 molecules (coenzymes) involved as proton and electron acceptors:
 - (1) NAD⁺ (nicotinamide adenine dinucleotide) (Fig 3.3)



(2) FAD (flavin adenine dinucleotide)

 $FAD^+ + 2H^+ + 2e^-$ FADH₂ (oxidised form) (reduced form)

 Hence, NAD⁺ and FAD function as electron and proton acceptors, which transfer electrons from substrates and then pass the electrons to the electron transport chain to drive ATP synthesis via oxidative phosphorylation.



Fig. 3.3: Structure of NAD+, an important electron acceptor in cells. (<u>Note</u>: NAD+ is lacking the phosphate group present in NADP+)

• **38 ATP molecules** can be formed from the **complete oxidation** of **one glucose molecule during aerobic respiration**.

(B) Anaerobic Respiration

- \circ Occurs when O₂ is unavailable, and comprises two main processes:
 - Glycolysis
 - Anaerobic Pathway to lactate (in animals) or ethanol (in plants)
- The sites of cellular respiration in the cell (Fig. 3.4) are summarised in Table 3.

Cytosol of cytoplasm



Fig. 3.4: Sites of cellular respiration in a cell.

| Stage | Site |
|---------------------------|------|
| Glycolysis | |
| Link Reaction | |
| Krebs Cycle | |
| Oxidative Phosphorylation | |
| Anaerobic Pathways | |

Table 3: Summary of the sites of cellular respiration.

3.2 ATP as the Product of Cellular Respiration

- Significance and role of ATP as the product of respiration:
 - $\circ~$ The hydrolysis of ATP to ADP and inorganic phosphate (P_i) yields 30.6 kJ mol^-1 of free energy

 $ATP + H_2O \xrightarrow{hydrolysis} ADP + P_i + energy$

- This energy released from hydrolysis of ATP is used to drive biochemical processes in the cell.
- Examples of processes driven by energy released from hydrolysis of ATP include:
 - anabolic reactions (e.g. synthesis of proteins)
 - > active transport
 - maintenance of body temperature
 - > phosphorylation in the initial stages of glycolysis
- Hence, ATP is known as the source of "cellular energy".
- ADP and inorganic phosphate can be converted back to ATP by a condensation reaction. This requires the input of 30.6 kJ of energy per mole of ATP formed. The addition of a phosphate to ADP is also known as phosphorylation.

30.6 kJ + ADP + P_i $\xrightarrow{condensation}$ ATP + H₂O

3.3 Mitochondrion structure in relation to function

Key Concept:

The mitochondria is the site of <u>aerobic</u> respiration where majority of the ATP is produced.

- **Mitochondria** (Fig. 3.5) are present in **all eukaryotic cells**, though number per cell varies considerably, depending on type of organism and nature of the cell (few to 1000 in liver cells)
- Mitochondria are able to **change shape** and move by **cytoplasmic streaming** to areas where the need for ATP is greater.
- Mitochondria, like chloroplasts, are double membrane-bound structures that are **prokaryotic** in origin.
- Dimensions (variable):
 - Length: 1.5-10µm
 - Width: 0.25-1.00µm
 - Diameter: less than 1µm



Fig. 3.5: Electron micrograph of mitochondrion

• The structure of the mitochondrion is adapted for respiration (Table 4).

| Structure | Description | How structure is related to function | |
|----------------|---|--|--|
| | Consist of 2 membranes | Separate the organelle from the cell; allows compartmentalisation & specialization. | |
| | enclosing the intermembrane space. | Both membranes are impermeable to H⁺. Allows accumulation of high H⁺ concentration in the intermembrane space to create a proton gradient. | |
| | (a) Outer mitochondrial membrane | Channel protein called porin for inward movement of substances ≤5KDa such as pyruvate and O₂ | |
| ۵. | (b) Intermembrane space | Accumulation of high H⁺ concentration to create a proton gradient across the inner mitochondrial membrane. | |
| n envelope | (c) Inner mitochondrial membrane, highly folded into cristae . (singular: crista) | Provides a large surface area for attachment of (i) Transport proteins (ii) Electron carriers and proton pumps in sequential order (iii) Stalked particles with ATP synthase | |
| ndrio | | (i) Transport proteins for passage of metabolites in & out of cell | |
| ocho | | Mitochondrial pyruvate carrier to actively transport pyruvate into the mitochondrial matrix | |
| Mit | | (ii) Electron Carriers and Proton pumps in sequential order | |
| | | Passage of electrons along the chain of electron carriers of progressively lower energy levels release energy for active transport of H⁺ into intermembrane space. | |
| | | (iii) Stalked Particles with ATP synthase | |
| | | Protein complex with hydrophilic channel for diffusion of H⁺ down its concentration gradient (from intermembrane space to the matrix). Energy released is used by associated ATP synthase to synthesize ATP from ADP and Pi. | |
| Irial | Circular DNA | • Circular DNA codes for electron carriers and mitochondrial enzymes needed for link reaction, Krebs cycle and oxidative phosphorylation. | |
| shond atrix | 70S ribosome and tRNA | • Allow mitochondria to carry out protein synthesis independently of the cell. | |
| Mitoc | Gel-like matrix contains soluble enzymes, organic acids and lipids | Site where Link Reaction and Krebs cycle occur. Lies next to inner mitochondrial membrane such that NADH & FADH₂ of link reaction and Krebs Cycle can be channeled to oxidative phosphorylation | |

Table 4: The structure of the mitochondrion in relation to its function.

3.4 Cellular Respiration Overview

Key Concept:

Aerobic respiration occurs in the presence of oxygen.

Anaerobic respiration occurs when there is a shortage or lack of oxygen.

- Respiration can be **aerobic** (Fig. 3.6) or **anaerobic** (Fig. 3.7).
- Glycolysis occurs in both processes.



Fig. 3.6: Overview of Aerobic Respiration.



Fig. 3.7: Overview of Anaerobic Respiration.

3.5 Aerobic Respiration

Key Concept:

Four major enzyme-catalysed processes are involved in the **oxidation of glucose** to release energy for ATP synthesis:

- 1. Glycolysis (Section 3.5.1)
- 2. Link Reaction (Section 3.5.2)
- 3. Krebs Cycle (Section 3.5.3)
- 4. Oxidative phosphorylation (Section 3.5.4)

3.5.1 Glycolysis

- Glucose taken into the cell is constantly channelled into **glycolysis**. Hence, a steep glucose gradient is maintained between the inside and outside of cell.
- Glycolysis occurs in the cytosol and is the common initial pathway for both aerobic and anaerobic respiration.
- This process takes place in ten steps and each step is catalyzed by specific enzyme that breaks down a glucose molecule (6C) into 2 molecules of pyruvate (3C) (Fig. 3.8).
- The ten steps are categorized into four types of reactions processes, namely (Fig. 3.8):
 - 1. Phosphorylation of hexose
 - 2. Splitting / lysis of 6C sugar bisphosphate
 - 3. Oxidation/ Dehydrogenation of triose phosphate
 - 4. Substrate Level Phosphorylation of 3C sugar bisphosphate





Process of Glycolysis (Fig. 3.8):

1. Phosphorylation of Hexose

- **Glucose** is **phosphorylated** by 1 molecule of **ATP** to give **glucose-6-phosphate** (6C). This is to make glucose more reactive.
- Glucose-6-phosphate (6C) is **isomerized** to give **fructose-6-phosphate** (6C)
- Fructose-6-phosphate (6C) is further phosphorylated by 1 molecule of ATP to give fructose-1,6-bisphosphate (6C)

2. Splitting / Lysis of 6C sugar bisphosphate

- The 6C fructose-1,6-bisphosphate **splits** into 2 molecules of **triose phosphate** (3C) (dihydroxyacetone phosphate and glyceraldehyde-3-phosphate).
- Dihydroxyacetone phosphate is converted to glyceraldehyde-3-phosphate.

3. Oxidation/ Dehydrogenation

- Oxidation / dehydrogenation of glyceraldehyde-3-phosphate (GALP) by NAD⁺ forms 1,3bisphosphoglycerate.
- NAD⁺ are **reduced** to NADH (reduced NAD). As 1 glucose molecule gives 2 GALP molecules, 2 molecules of NADH are generated per molecule of glucose.
- The NADH produced by glycolysis are channelled to the **electron transport chain** (see section 3.5.4)

4. Substrate Level Phosphorylation (Fig. 3.9)

- Substrate level phosphorylation is the formation of ATP by a direct enzymatic transfer of a high-energy phosphate group to ADP from an organic substrate (Fig. 3.8).
- 1,3-bisphosphoglycerate undergoes a series of steps to form the final product, pyruvate. In the process, 2 molecules of ATP are generated via substrate level phosphorylation. Thus, 4 molecules of ATP are generated per molecule of glucose



Fig. 3.9: Substrate level phosphorylation

• Products of Glycolysis:

| 1 glucose molecule \rightarrow net | 2 ATP | \rightarrow | used to drive cellular processes |
|---|------------|---------------|---|
| | 2 NADH | \rightarrow | channelled to oxidative phosphorylation |
| | 2 pyruvate | \rightarrow | enters Link reaction, converted to acetyl CoA |

3.5.2 Link Reaction

- Occurs in the **mitochondrial matrix**.
- Pyruvate from glycolysis is actively transported from the cytoplasm into the mitochondrial matrix by the **mitochondrial pyruvate carrier** (Fig. 3.10).



Fig. 3.10: The Link reaction. *OM:* Outer membrane. *IM:* Inner membrane. *IMS:* Inter-membrane space

- In the matrix, pyruvate undergoes oxidative decarboxylation, where pyruvate is oxidised to acetyl CoA by NAD⁺ with the removal of CO₂. This reaction catalysed by pyruvate dehydrogenase (Fig. 3.10).
- The sequence of the Link reaction (Fig. 3.10):
 - 1. CO₂ is removed from the substrate molecule.
 - 2. Protons and electrons are transferred to NAD⁺, forming reduced NAD (NADH).
 - 3. The remaining 2C compound combines with coenzyme A (CoA) to give acetyl CoA.
- Products of link reaction:

| 1 glucose \rightarrow 2 pyruvate \rightarrow | 2 CO ₂ | \rightarrow diffuse out of cell |
|--|---|---|
| | 2 NADH | \rightarrow channelled to oxidative phosphorylation |
| | 2 acetyl CoA \rightarrow enters Krebs cycle | |

3.5.3 Krebs Cycle

- Also known as the Tricarboxylic Acid Cycle or Citric Acid Cycle.
- Krebs cycle is a closed pathway of enzyme-catalysed reactions, which occurs in the mitochondrial matrix.
- The Krebs cycle, which takes place in 8 steps, is the complete oxidation of acetyl CoA to CO₂ and H₂O with the release of energy (Fig. 3.10).
- Importance of the Krebs Cycle:
 - 1. It is the pathway where **glucose**, in the form of acetyl CoA, is **completely oxidised** to give CO_2 and H_2O with the **release of energy** in the form of **NADH and FADH**₂.
 - 2. The Krebs Cycle **provides reducing power** for the electron transport chain, in the form of **reduced NAD (NADH)** and **reduced FAD (FADH**₂). Energy from the transfer of electrons is used to set up a proton gradient for oxidative phosphorylation to synthesise ATP.
 - 3. It is the pathway by which all carbon atoms from carbohydrates, lipids and amino acids are oxidised. When glucose is in short supply, **amino acids** and **fatty acids** are channelled into Krebs cycle to be oxidised for ATP production
 - 4. In the Krebs Cycle, carbohydrate intermediates can be converted to amino acids or fatty acids.



Fig. 3.11: Krebs Cycle: Note the reactions where NADH, FADH₂, ATP and CO₂ are produced. Note to students: You are **<u>REQUIRED</u>** to know the **names** of the intermediates.

- Three major types of reactions occur in the following sequence:
 - Oxidative decarboxylation
 - Substrate-level phosphorylation
 - > Dehydrogenation / Oxidation
- Steps in the Krebs Cycle (Fig. 3.11):
 - 1. (2C) acetyl CoA is taken up by (4C) oxaloacetate to form (6C) citrate. (Oxaloactetate is the acceptor of acetyl CoA.)
 - 2. (6C) citrate undergoes isomerisation to form (6C) isocitrate.
 - (6C) isocitrate undergoes oxidative decarboxylation to produce (5C) α-ketoglutarate, with the removal of CO₂. NAD+ is the oxidising agent. Hydrogen atoms are removed from isocitrate and added to NAD+, forming reduced NAD.
 - 4. (5C) α-ketoglutarate is undergoes further oxidative decarboxylation to form (4C) succinyl CoA with the production of reduced NAD and CO₂.
 - 5. (4C) Succinyl CoA is converted to (4C) succinate. ATP is produced by substrate-level phosphorylation.
 - 6. (4C) Succinate is oxidised to (4C) fumarate by coenzyme FAD. Reduced FAD is formed.
 - 7. (4C) Fumarate is converted to malate (4C) with the addition of one water molecule.
 - (4C) Malate is oxidised to oxaloacetate (4C) by NAD+ with the release of 2 hydrogen atoms to form reduced NAD (NADH).
 Oxaloacetate is regenerated to take up another acetyl-CoA.
- Products of Krebs cycle per glucose molecule (after two turns of the cycle):

1 glucose \rightarrow 2 acetyl CoA \rightarrow 6 NADH \rightarrow 2 FADH₂

- \rightarrow channelled to oxidative phosphorylation
- \rightarrow channelled to oxidative phosphorylation \rightarrow formed by substrate level phosphorylation
- $\begin{array}{ccc} \rightarrow & 2 \text{ ATP} & \rightarrow \\ \rightarrow & 4 \text{ CO}_2 & \rightarrow \end{array}$
 - \rightarrow diffuse out of cell
- \rightarrow 2 oxaloacetate \rightarrow regenerated and combine with acetyl CoA

3.5.4 Electron Transport Chain and Oxidative Phosphorylation

- The oxidation of glucose via glycolysis and the Krebs cycle yields a net total of 4 ATP molecules by substrate-level phosphorylation. The **majority of the ATP molecules** are subsequently formed via a process called **oxidative phosphorylation**.
- Oxidative phosphorylation produces (90%) 34 out of 38 ATP per glucose molecule oxidised.
- Definition of oxidative phosphorylation:

"The NADH and FADH₂ (formed in glycolysis, conversion of pyruvate to acetyl CoA and the Krebs cycle) transfer their electrons to O₂ through a series of electron carriers of progressively lower energy levels, present on the inner mitochondrial membrane. The energy released from this electron transfer is used to create a proton gradient across the inner mitochondrial membrane. The electrical potential energy of this proton gradient is used for the synthesis of ATP."

The Electron Transport Chain

- Mitochondrial Hydrogen Region Ion Movement Intermembrane Space 3 ATP Inner Production Membrane ADP 2H,0 ADH. Matrix 2 NADH FAD 1 Electron Transport 2 NAD⁺
- The electron transport chain consists of a series of electron carriers located on the **inner mitochondrial membrane** (Fig. 3.12).

Fig. 3.12: The electron transport chain. Electrons from NADH and FADH₂ enter the ETC and are transferred by a series of electron transport proteins of progressively lower energy levels. The energy released from the passage of electrons is used to pump protons from the matrix into the intermembrane space, generating a proton gradient across the inner membrane.

- Most of these are proteins, with prosthetic groups that shift between **reduced** and **oxidised** states as they accept and donate electrons.
- The electron carriers are of progressively lower energy levels. Electrons are transferred from NADH or FADH₂ to the series of carriers and finally to O₂ by a series of redox reactions. (Fig. 3.12)
- The final electron acceptor is O₂, which is then reduced to H₂O (Fig. 3.13).

The energy released from this transfer of electrons is used to actively transport protons (H+ ions) from the matrix of mitochondrion into the intermembrane space.



Fig. 3.13: Changes in energy levels of electron carriers during transfer of electrons. Note: you are <u>NOT REQUIRED</u> to know the names of the electron carriers.

Stalked Particles / ATP Synthase

- The stalked particles / ATP synthase (Fig. 3.13) on the inner mitochondrial membrane are protein complexes comprising of
 - Fo Transmembrane hydrophilic channel
 - embedded within the inner mitochondrial membrane for diffusion of H⁺ down its concentration gradient from intermembrane space to mitochondrial matrix
 - **F1 ATP synthase head portion**
 - faces the mitochondrial matrix.
 - \succ binds ADP and P_i.
 - \succ The head portion rotates as H⁺ ions passes through the hydrophilic channel.
 - > The electrical potential energy released from diffusion of H^+ is used to synthesise ATP.



Fig. 3.14: The stalked particle (ATP synthase). **Note:** Details of the ATP synthase structure are <u>NOT REQUIRED</u>.

Oxidative Phosphorylation

- In oxidative phosphorylation, electron transport is coupled to ATP synthesis by chemiosmosis (Fig. 3.15).
- The process is as follows:
 - By a series of redox reactions, electrons are transferred from NADH or FADH₂ to electron carriers of **progressively lower energy levels**, finally to O₂ to form water.
 - The energy released from the passage of electrons is used for active transport of H⁺ from the mitochondrial matrix into intermembrane space via proton pumps.
 - This creates **proton gradient** (H⁺ concentration gradient) across the inner mitochondrial membrane.
 - When H⁺ ions diffuse back into the matrix (chemiosmosis) through ATP synthase, down its concentration gradient by facilitated diffusion, energy is released to generate ATP from ADP & P_i.



Fig. 3.15: Electron transport and chemiosmosis

- Oxidation of one molecule of NADH yields 3 ATP whereas oxidation of one molecule of FADH₂ yields 2 ATP by oxidative phosphorylation.
- Importance of oxidative phosphorylation:
 - To produce **ATP**
 - To regenerate NAD⁺ and FAD (for the Krebs cycle and Link reaction)
- Summary of the number of ATP formed per glucose molecule:

| Per glucose | From Glycolysis | From Link reaction | From Krebs cycle | Total |
|--|-----------------|-----------------------|------------------|-------|
| NADH | | | | |
| FADH ₂ | | | | |
| Total ATP formed by substrate level phosphorylation | | | | |
| Total ATP formed by oxidative phosphorylation from NADH or FADH ₂ | | | | |
| Total ATP produced per molecule of glucose | | | 38 | |

3.6 Anaerobic Respiration

Key Concept:

When there is a shortage or absence of oxygen, only glycolysis synthesises ATP.

- When there is a shortage or absence of oxygen,
 - Micro-organisms e.g. yeast and bacteria can respire anaerobically.
 - o Skeletal muscles of animals also respire anaerobically during vigorous activity.
- In anaerobic respiration, only glycolysis takes place to give 2 pyruvate and 2 ATP per glucose molecule.
- This is because Krebs cycle and oxidative phosphorylation cannot operate in the absence of oxygen due to the absence of oxygen as the final electron acceptor which causes the electron transport chain to stop operating.
- Hence, NAD⁺ and FAD cannot be regenerated for Krebs cycle and link reaction to proceed.
- Glycolysis can still continue to produce ATP by substrate-level phosphorylation because
 - o pyruvate (for mammals, Fig. 3.16 left) or
 - o ethanal/acetaldehyde (for yeasts, Fig. 3.16 right)

acts as an alternative hydrogen acceptor to **regenerate NAD**⁺ for **oxidation** (Fig. 3.8, step 6) to occur.

Key Concept:

Under anaerobic conditions, fermentation occurs to regenerate NAD+ for glycolysis to occur.





3.6.1 Production of ethanol in plants and yeast

- Also known as alcoholic fermentation.
- From glycolysis, one molecule of glucose \rightarrow 2 pyruvate + 2H₂O + 2ATP + 2 reduced NAD.
- Purpose: To regenerate the pool of NAD+ in the cytoplasm so that ATP can be continuously synthesized by glycolysis. The availability of ATP allows cellular processes to continue for a limited time.
- Pyruvate (3C) undergoes decarboxylation forming ethanal (2C) and carbon dioxide (Fig. 3.17).
- Reduced NAD from glycolysis donates 2H atoms to ethanal and reduces it to ethanol by alcohol dehydrogenase. NAD+ is regenerated.
- This reaction regenerates **NAD+** for glycolysis to continue with ATP production even though O₂ is not available as a final acceptor of electrons.



Fig. 3.17: Production of ethanol.

3.6.2 Production of lactic acid in animal cells

- Also known as lactate fermentation.
- From glycolysis, one molecule of Glucose \rightarrow 2 pyruvate + 2H₂O + 2ATP + 2 reduced NAD.
- Lactate fermentation is the conversion of pyruvate to lactate / lactic acid, catalysed by **lactate** dehydrogenase (Fig. 3.18).
- Purpose: To regenerate the pool of NAD⁺ in the cytoplasm so that ATP can be continuously synthesized by glycolysis
- This occurs so that cellular activities can still continue in the absence of oxygen.



Fig. 3.18: Production of lactate.

- When energy demand is great and oxygen supply is limited, anaerobic respiration is used in addition to aerobic respiration, to power muscle. As a result, lactic acid builds up in the muscle and blood.
- The muscle is said to be incurring an oxygen debt, which causes muscular pain, fatigue and cramps.
- When muscular activity slows down and aerobic condition returns, lactate is removed from the muscle and carried by the blood to the liver. In the liver cells, lactate is converted back to pyruvate through a pathway that is the reverse of glycolysis.
- The liver oxidises 20% of the incoming lactate to carbon dioxide and water. The remainder of the lactate is converted to **glycogen** by the liver.

4.1 Aerobic VS Anaerobic Respiration

| Feature | Aerobic Respiration | Anaerobic Respiration |
|---|--|-----------------------|
| Site of specific reactions | Cytosol of cell: Mitochondrial matrix: Inner mitochondrial membrane: | Cytosol of cell |
| Occur when | Oxygen is available | Oxygen is unavailable |
| Processes involved? | Glycolysis, Krebs cycle and Oxidative Phosphorylation | Only glycolysis |
| What is the proton / electron acceptor? | Oxygen | Pyruvate |

4.2 Photophosphorylation VS Oxidative Phosphorylation

| Feature | Photophosphorylation | Oxidative phosphorylation |
|--|----------------------|---------------------------|
| Occurs in which organelle? | | |
| Precise location in the organelle where it occurs? | | |
| Protons are concentrated at? | | |
| Energy conversion | | |
| Role of coenzymes? | | |
| Sources of electrons? | | |
| Roles of O ₂ ? | | |
| Roles of water? | | |

4.3 Alcoholic Fermentation VS Lactate Fermentation

| Feature | Alcoholic Fermentation | Lactate Fermentation | |
|--------------|--|---------------------------------|--|
| | 2-step process | 1-step process | |
| Process | Pyruvate \rightarrow ethanolPyruvate \rightarrow lactate | | |
| | Decarboxylation and reduction | Reduction only | |
| Draduata | Ethanol | Lactate | |
| Products | CO ₂ is produced | CO ₂ is not produced | |
| Enzyme | Ethanol/alcohol dehydrogenase | Lactate dehydrogenase | |
| Significance | Both regenerates NAD+ so that glycolysis can continue to produce ATP | | |

4.4 Krebs Cycle VS Calvin Cycle

| Feature | Calvin cycle | Krebs cycle |
|------------------------|-----------------------------------|--|
| Function | To synthesis 3C sugars for | to reduce NAD ⁺ and FAD for |
| | synthesis of other sugars and | production of ATP by oxidative |
| | organic compounds. | phosphorylation at the ETC |
| Site | Stroma of chloroplast | Matrix of mitochondria |
| Chemical processes | Reduction and Carboxylation | Oxidation and Decarboxylation |
| | Anabolic process – building up of | Catabolic process – breaking |
| | organic molecules with higher C | down of organic intermediates |
| | numbers and energy content, via | (derived from glucose) to produce |
| | reduction processes | carbon dioxide, via series of |
| | | oxidation processes |
| Substrate required for | Carbon dioxide | Acetyl coenzyme A |
| cycle | | |
| | | |
| OR | Carbon dioxide, RuBP, ATP, | Acetyl coenzyme A, oxaloacetate |
| Initial reactants | NADPH | |
| Hydrogon/alastron | | NAD+ FAD |
| | NADP | NAD [*] , FAD |
| camer(3) | | |
| Fate of Carbon | Expended: | Liberated/released; |
| dioxide | 6 used, for synthesis of per | 2 per cycle (from 1 pyruvate) |
| | glucose molecule. | |
| ATP | Expended: | Synthesised; |
| | 1 per reduction of 1 GP to 1 TP | 1 per cycle (from 1 pyruvate) |
| Molecule | Ribulose bisphosphate | Oxaloacetate |
| regenerated at the | | |
| end of cycle | | |
| Enzymes involved | Carboxylases | Dehydrogenases, decarboxylases |
| Useful products | Triose phosphate | Reduced NAD, FADH ₂ & ATP |

| Requirement for oxygen | Does not require oxygen | Requires oxygen for cycle to continue |
|---------------------------|--------------------------------------|---------------------------------------|
| Requirement for light | Requires light for cycle to continue | Does not require light |
| Nature of Process | Anabolic | Catabolic |

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