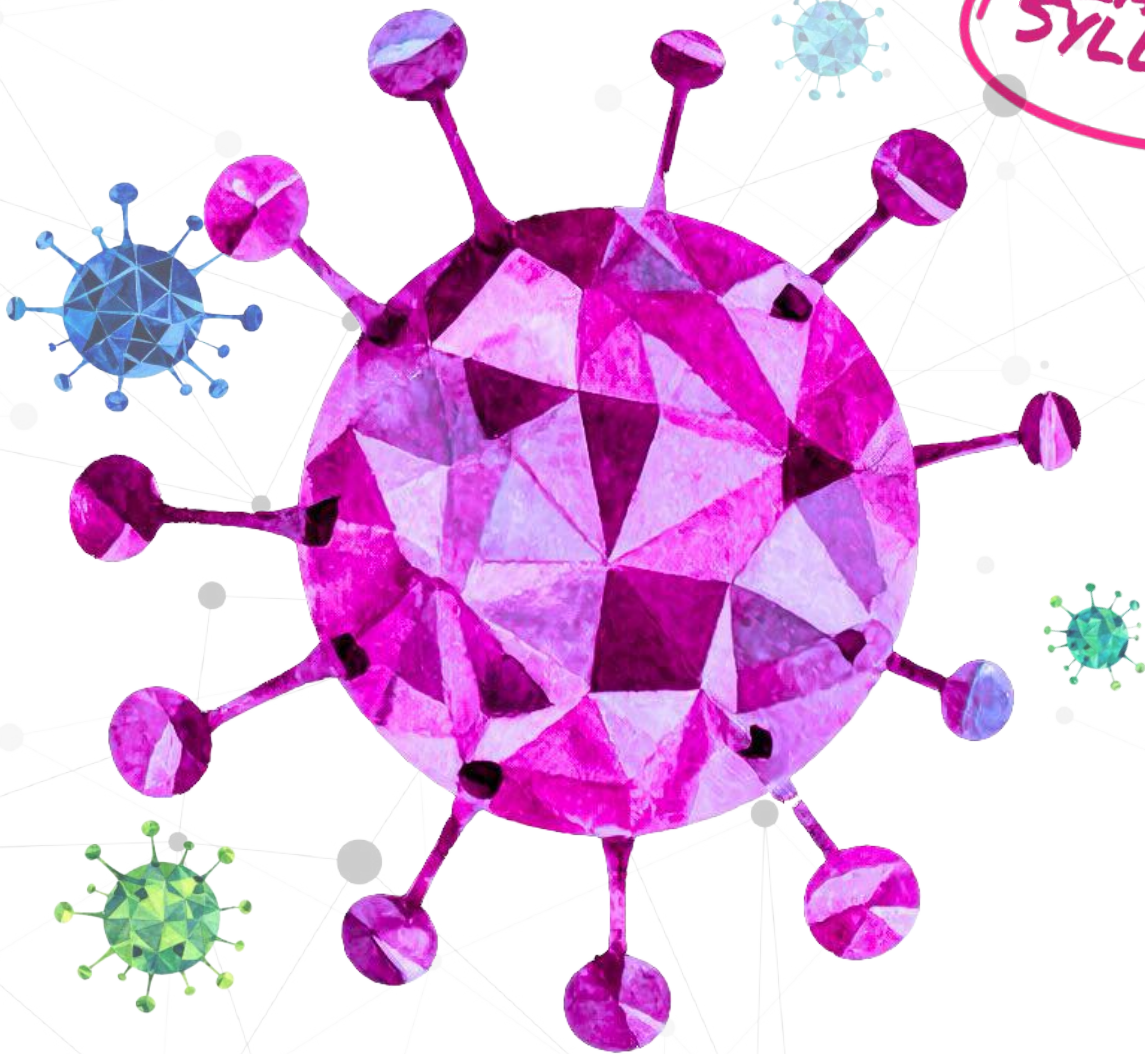


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Biology

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Part of syllabus

UNIT 4

Dna Profiling

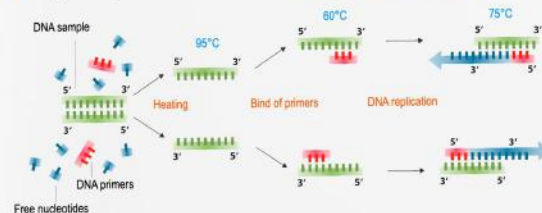
13

13.1 DNA Profiles

- DNA profile is a fingerprint of an organism's unique DNA
- As you have seen in AS biology, exons (genes that code for proteins) are only 2% of the human DNA
- Introns occupy a very large portion of the human genome
- What makes someone's DNA so unique are repetitive DNA sequences found within the introns
- The bases of the DNA sequence and the number of times this sequence is repeated are always different
- Scientists have used the pattern of the DNA repeats to make a unique DNA profile for every individual
- The more closely related two individuals are, the more likely it is that similarities will be apparent in their DNA patterns. DNA profiles have a wide range of applications, including:
 1. To identify criminals in (forensics)
 2. To determine relationships between people such as paternity tests
 3. To prevent inbreeding in animals or plants in conservation programs
 4. To classify organisms (organisms with more similar DNA profiles have a more recent common ancestor)

13.2 Amplification Of DNA By PCR

- To produce a DNA profile, DNA is first extracted from cells found in blood or saliva samples
- The samples may contain a very small amount of DNA to produce a valid DNA profile
- PCR (polymerase chain reaction) technique is used to make millions of copies (amplification) of specific regions of the DNA sample. PCR has several stages and is repeated to make more copies
 1. The reaction mixture of PCR is first set up in a buffer solution, it contains all the following:
 - a. The extracted DNA sample
 - b. Free nucleotides (A, T, C, G)
 - c. Primers: two short pieces of DNA complementary to the bases at the start and the end of the DNA segment to be copied; primers are needed for DNA polymerase to bind DNA to each DNA strand
 - d. Taq polymerase: a heat-resistant version of DNA polymerase taken from bacteria is used
 2. The DNA mixture is heated to 95 °C to break the hydrogen bonds between the two strands of DNA
 3. The mixture is then cooled to between 50 and 65 °C so that the primers can bind to the DNA strands
 4. The reaction mixture is heated again to 75 °C (optimum temperature for DNA polymerase)
 5. The DNA polymerase lines up free DNA nucleotides alongside each template strand, forming two new complementary strands of the DNA fragments that are supposed to be copied
 6. The cycle starts again, with heating and cooling, which doubles the number of DNA strands



Important Definition

Mark scheme **points**

Practical details

Key facts in **green**

Key terms in **pink**

01

PHOTOSYNTHESIS



09

ECOLOGY

10

SAMPLING
TECHNIQUES



11

SUCCESSION

12

GLOBAL
WARMING

14

SOLVING
GLOBAL
WARMING

UNIT 4

15

TEMPERATURE
AND
DEVELOPMENT



16

EVOLUTION

17

DNA
PROFILING

19

FORENSICS



20

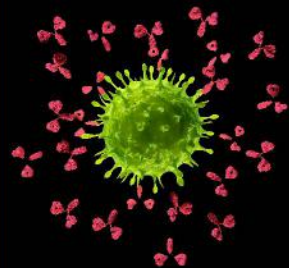
NON-
SPECIFIC
IMMUNITY

22

SPECIFIC
IMMUNITY

24

CELLULAR
IMMUNE
RESPONSE

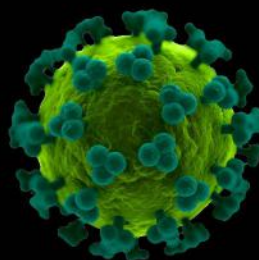


26

VIRUSES

29

BACTERIA



30

MEASURING
BACTERIAL
GROWTH

33

ANTIBIOTICS

36

RESPIRATION

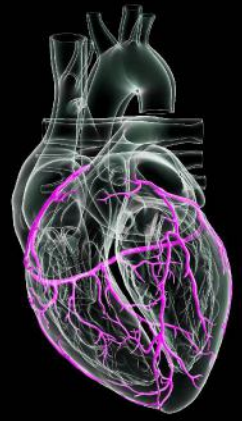


42

MUSCLES

43

THE HEART
MUSCLE



UNITS 5 + 6

47

REGULATION OF
HEART RATE

48

REGULATION
OF BREATHING
RATE



49

CARDIAC
OUTPUT

50

ANIMAL
HORMONES

51

HOMEOSTASIS

52

THE KIDNEY

56

THE
NERVOUS
SYSTEM

59

THE
SYNAPSE

61

HABITUATION

62

THE EYE

64

REFLEX
ACTIONS

65

COORDINATION
IN PLANTS

67

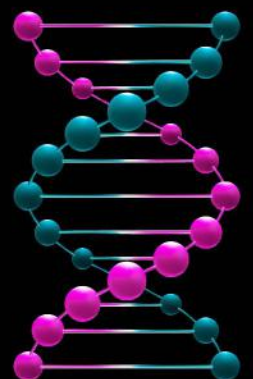
THE HUMAN
BRAIN

69

BRAIN
DISEASE

70

GENE
TECHNOLOGY

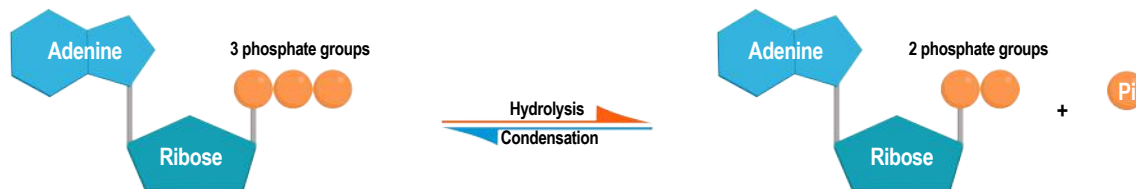


1.1 Essential Definitions

- ☞ **Phosphorylation**: adding phosphate to a molecule; for example, ADP is phosphorylated to ATP
- ☞ **Photophosphorylation**: adding phosphate to a molecule **using energy from light**
- ☞ **Photolysis**: the splitting of a water molecule using light energy into an oxygen atom and two protons
- ☞ **Hydrolysis**: the splitting of a molecule using water; for example, ATP is hydrolysed to ADP (see below)
- ✳ **Redox reactions**: reactions that involve oxidation and reduction
- ✳ **Reduction** is the gain of electrons, loss of oxygen or gain of hydrogen
- ✳ **Oxidation** is the loss of electrons, gain of oxygen or loss of hydrogen
- ✳ Oxidation of one molecule always involves the reduction of another molecule

1.2 About ATP

- ✳ ATP (adenosine triphosphate) is the energy currency for cells in all organisms
- ✳ ATP is made up of three parts: ① **Nitrogen base (adenosine)** ② **Sugar (ribose)** ③ **Three phosphate groups**
- ✳ The third phosphate bond can be broken by a hydrolysis reaction. This is catalysed by the enzyme **ATPase**
- ✳ The result of this reaction is ADP (adenosine diphosphate), inorganic phosphate group (**Pi**) and energy
- ✳ **Energy from ATP is used for biological processes** in the cell such as active transport or protein synthesis
- ✳ The breakdown of ATP into ADP and phosphate is a **reversible reaction**, ATP can be made again from ADP and Pi using the same enzyme (ATPase). This reaction is known as phosphorylation, which is a condensation reaction that requires a supply of energy
- ✳ The energy for the phosphorylation of ADP to ATP can come from respiration
- ✳ In photosynthesis, the energy for phosphorylation comes from light, this is known as photophosphorylation



1.3 Overview Of Photosynthesis

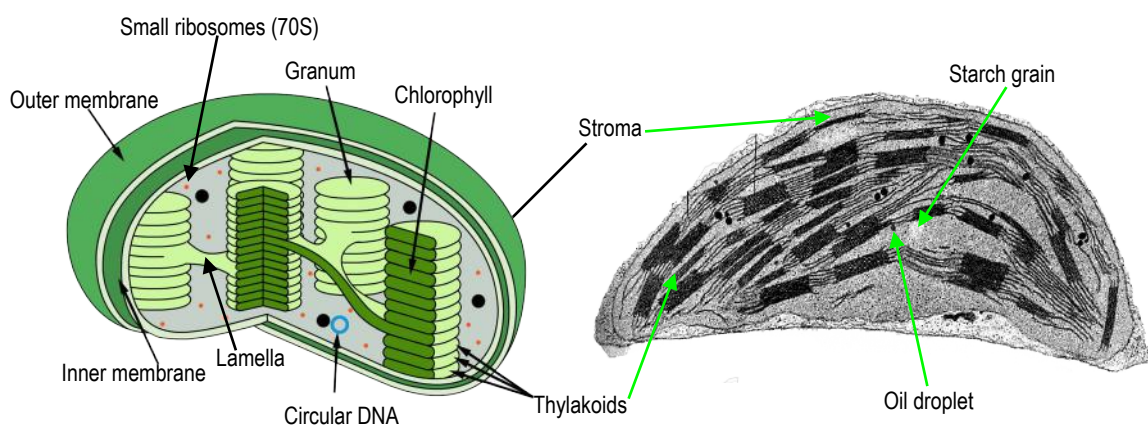
- ✳ Photosynthesis is the process where **light energy is used to break the bonds in water molecules**
- ✳ The hydrogen from the breakdown of water combined with carbon dioxide to form glucose
- ✳ Oxygen is released into the atmosphere as a waste product of this process



- ✳ **Glucose** in plants can be **stored as starch or respired** to release energy
- ✳ Glucose can also be **used to make other biological molecules** such as amino acids or lipids
- ✳ Animals (heterotrophs) acquire their energy by feeding on plants (autotrophs) or other animals
- ✳ Organisms require energy for processes such as active transport, protein synthesis and cell division
- ✳ In animals, energy is also needed for the maintenance of body temperature and muscle contraction
- ✳ The entire series of reactions of photosynthesis are **controlled by enzymes**
- ✳ Factors that affect enzyme activity such as temperature or pH do also affect photosynthesis reactions
- ✳ **Coenzyme** is a molecule that aids the function of an enzyme
- ✳ Coenzymes work by **transferring a chemical group from one molecule to another**
- ✳ **NADP** is a coenzyme that is used in photosynthesis
- ✳ NADP works by **transferring hydrogen atoms** from one molecule to another (which involves redox reactions)
- ✳ NADP exists in a reduced form called NADPH (or simply **reduced NADP**) which can be oxidised to NADP

2.1 The Chloroplast

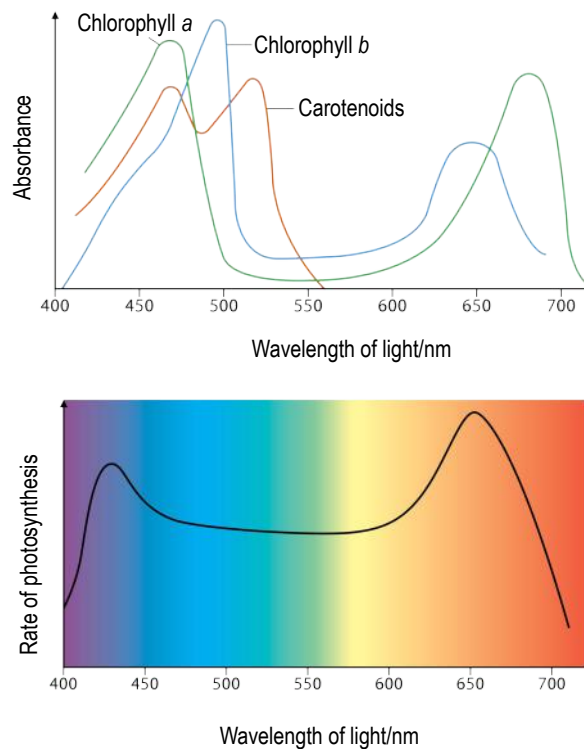
- * The chloroplast contains circular DNA that codes for enzymes needed for photosynthesis
- * Replication of DNA in the chloroplast is independent of the replication of the DNA of the plant cell
- * Chloroplasts have a double membrane (outer and inner membranes) called the chloroplast envelope
- * The chloroplast envelope keeps the reactants for photosynthesis close to their reaction sites
- * Chloroplasts also contain inner membranes called thylakoids (fluid-filled sacs)
- * These membranes separate different sites of the chloroplasts into separate compartments
- * This allows different reactions of photosynthesis to occur separately
- * The thylakoid sac is made up of a membrane where electron carriers and enzymes are found
- * The thylakoid membrane encloses a lumen called the thylakoid space
- * The thylakoids have a large surface area to allow as much light energy to be absorbed as possible
- * Light energy is absorbed by the green pigment chlorophyll and other pigments (see next section)
- * The pigments are arranged on the thylakoids in the best possible position for capturing light energy
- * Thylakoids are stacked up in the chloroplast into structures called grana (singular is granum)
- * The grana are linked by intergranal membranes called lamellae (singular is lamella)
- * The lamellae act as a skeleton inside the chloroplast, maintaining a distance between the grana so that they receive the maximum amounts of light energy
- * Lots of ATPase enzyme molecules are present in the thylakoid membranes to produce ATP
- * The membrane layers are surrounded by a cytoplasm-like substance called the stroma
- * Stroma is where enzymes needed for the making of glucose are found
- * Thylakoid membranes contain photosynthetic pigments (chlorophyll a, chlorophyll b and carotene)
- * These pigments are attached to proteins to form what is known as photosystems
- * There are two types of photosystems known as photosystem I (PSI) and photosystem II (PSII)
- * Each system contains a different combination of chlorophyll pigments and therefore absorbs light in a slightly different area of the spectrum which helps to maximise the absorbance of light energy



2.2 The Action Spectrum

- * Chlorophyll is not a single molecule. It is a mixture of closely related pigments including: ① Chlorophyll a (found in the highest amounts in most plants) ② Chlorophyll b ③ Carotenoids
- * Each of the pigments absorbs and captures light at particular areas of the light spectrum
- * These pigments reflect the wavelength that they don't absorb, this is why plants appear to be green
- * As a result, much more of the light energy falling on the leaf can be used by the plant
- * Absorption spectrum is the different amounts of light of different wavelengths that a pigment absorbs
- * Action spectrum is the rate of photosynthesis at different wavelengths of light

- * Action spectra help to determine which wavelength is mostly used by plants in photosynthesis
- * The action spectrum of different pigments can be determined by exposing a water plant to different wavelengths of light and calculating the volume of oxygen produced per unit of time (page: 14)
- * When exposing a solution of chlorophyll a to ultraviolet light, red fluorescence is observed
- * The ultraviolet light is absorbed and electrons from chlorophyll are excited (move to higher energy levels)
- * In plants, these electrons move along electron carriers and the energy from light is used to make ATP
- * In isolated pigments in solutions, energy is transferred to the surroundings in the form of light and that explains the observed red fluorescence



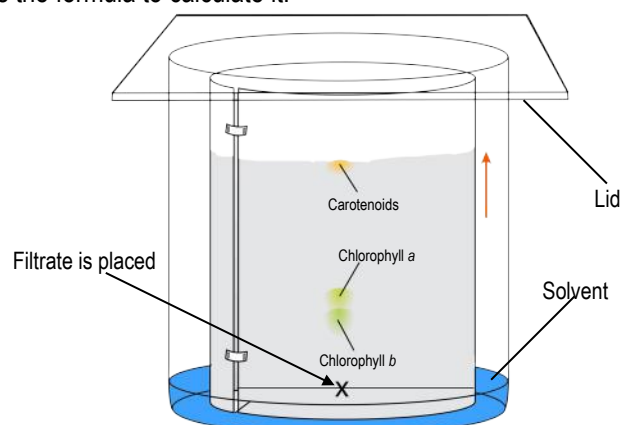
The action spectrum shows higher rate of photosynthesis when light absorbance was high



2.3 Separation Of Photosynthetic Pigments By Chromatography

- * The different pigments of the chloroplast can be extracted by first grinding up a leaf with propanone (acetone)
- * The mixture is then filtered, and the filtrate is applied to a chromatography paper
- * Chromatography is used to identify species since photosynthetic organisms have different types of pigments
- * Pigments travel on the paper depending on their solubility, the more soluble pigment travels further
- * To identify each pigment, R_f values are calculated and compared to the values of the same solvent
- * It is important to compare R_f values using the same solvent because these differ in different solvents
- * The R_f value is always between 0 and 1, and this is the formula to calculate it:

$$R_f \text{ value} = \frac{\text{Distance travelled by the pigment}}{\text{Distance travelled by the solvent}}$$



3.1 The Electron Transport Chain

- ✧ The electron transport chain is a series of proteins and photosystems found in the thylakoid membrane
- ✧ The transfer of electrons along this chain involves a redox reaction (loss and gain of electrons)
- ✧ The chain couples this electron transfer with the transfer of protons (H^+ ions) across a membrane from the stroma into the thylakoid space (the space enclosed by the thylakoid membrane)
- ✧ This builds up a proton gradient between the thylakoid space and the stroma
- ✧ The flow of protons back into the stroma (from the thylakoid space) through the enzyme ATPase channel provides the energy needed for the phosphorylation of ADP to ATP
- ✧ NADP is the final electron acceptor in the electron transport chain where it becomes reduced NADP

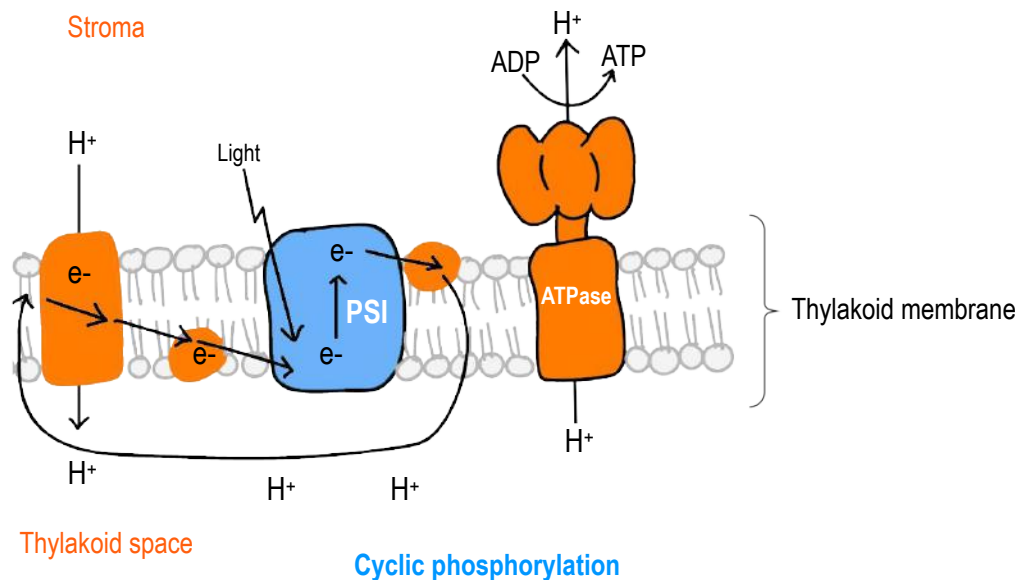
3.2 The Light-Dependent Reactions Of Photosynthesis

- ✧ This is one of the two stages of photosynthesis, it takes place on the thylakoid membranes
- ✧ In the light-dependent reaction, the light energy absorbed by the photosystems is used for the following:
 1. Making reduced NADP from NADP
 2. Photolysis: the splitting of water into protons (H^+ ions), electrons and oxygen (a by-product)
 3. Photophosphorylation (making ATP from ADP and inorganic phosphate) using light energy
- ✧ When a photon of light hits a chlorophyll molecule, the energy is transferred to the electrons of the chlorophyll molecule. The electrons are excited and are raised to higher energy levels
- ✧ The excited electron is collected by the electron transport chain in the thylakoid membrane
- ✧ The flow of electrons along the electron transport chain eventually leads to the phosphorylation of ADP
- ✧ The flow of electrons in the thylakoid membrane leading to phosphorylation can take two types: cyclic phosphorylation and non-cyclic:

Phosphorylation	Cyclic	Non-cyclic
Photosystems involved	Photosystem I only	Both photosystems
Oxygen	No oxygen is produced	Oxygen is produced
Reduced NADP	No reduced NADP is made	Reduced NADP is made
Amount of ATP made	Less	More
Role of ATP	Supplies energy for the plant cell	Needed for light Calvin cycle

3.3 Cyclic Phosphorylation

- ✧ Cyclic phosphorylation occurs when there is plenty of NADPH and little ATP in the plant cell
- ✧ The following events take place in cyclic phosphorylation leading to the making of ATP:
 1. Light first hits a chlorophyll molecule in PSI (see the diagram at the top of the next page)
 2. The light excites an electron in the chlorophyll molecule
 3. A light-excited electron leaves the chlorophyll molecule
 4. The electron is collected by an electron acceptor and transferred directly along an electron transport chain
 5. As the electron moves along the electron transport chain, it loses energy
 6. The energy from the flow of electrons along the electron transport is used to move protons from the stroma to the thylakoid space where these protons accumulate
 7. Protons then flow through the ATPase channel (also known as the stalked particle) back to the stroma
 8. The movement of protons through ATPase provides energy for the phosphorylation of ADP into ATP
 9. The electron returns to the chlorophyll molecule in PSI, where it can then be excited in the same way again



3.4 Non-Cyclic Phosphorylation

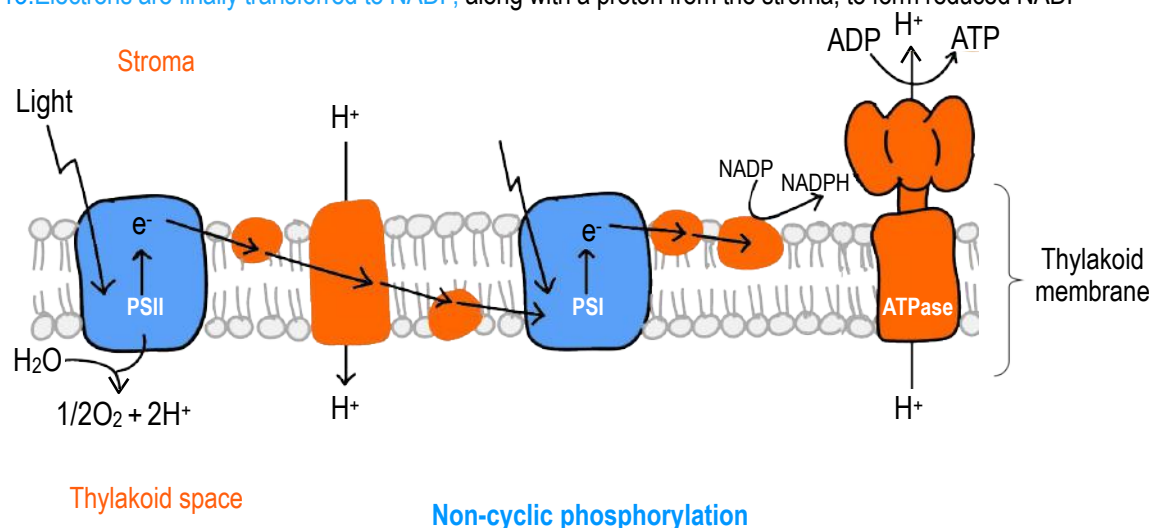
✧ Plant cells can shift from cyclic to non-cyclic phosphorylation based on the amounts of ATP and NADPH

✧ Cyclic phosphorylation occurs when there is a high demand for ATP within the cell

✧ Non-cyclic phosphorylation provides both ATP and NADPH needed for the light-independent reactions

✧ The following events take place during non-cyclic phosphorylation:

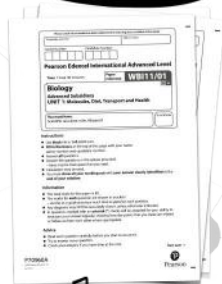
1. Light energy is absorbed by PSII
2. The light energy excites an electron in the chlorophyll molecule
3. The excited electron moves along the electron transport chain towards PSI
4. The excited electron loses energy as they move along the electron transport chain
5. This energy is used to transport protons into the thylakoids' space
6. This forms a proton gradient across the thylakoid membrane (higher H^+ in the thylakoid than stroma)
7. Meanwhile, photolysis takes place, where light energy splits water into protons (H^+), electrons and oxygen
8. The electrons from photolysis replace the electrons that have left photosystem II
9. Protons move down their concentration gradient, into the stroma, via the enzyme ATPase
10. The energy from the proton movement combines ADP and inorganic phosphate (P_i) to form ATP
11. Chemiosmosis is the movement of protons down their concentration gradient through ATPase to generate ATP
12. The light energy absorbed by photosystem I moves electrons once again to a higher energy level
13. Electrons are finally transferred to NADP, along with a proton from the stroma, to form reduced NADP



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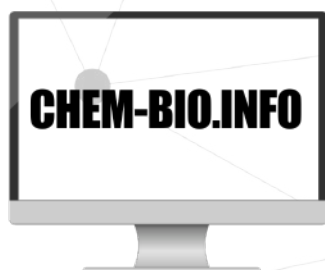
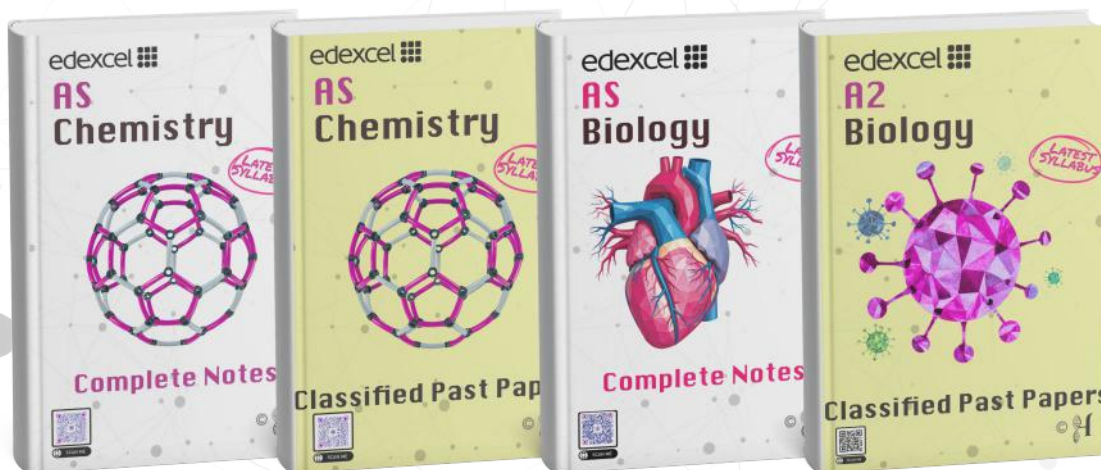


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