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LEARNING MATERIALS

Subject: Chemistry / Medicine

Unit: Applied Biochemistry

Theme: Science

Topic 1: Applied Biochemistry

Level: 3

Recommended reading:

Recommended reading for this topic is listed in the back pages of these learning materials.

Further resources:

Further resources to deepen your learning on specific aspects of this topic are listed throughout these learning materials.

If you see this icon you will be able to listen to how the word is pronounced

Read and make notes:

Making notes turns reading into studying. Notes should be brief, clear and helpful.

To help your understanding:

- **rewrite or summarise what you have learned (avoid copying what you have read);**
- **note down any questions your studying has raised, either to ask your tutor, or to develop into further research.**

To help you to remember:

- **sum things up to improve long term memory;**
- **write things down to aid motor memory;**
- **use colour, images or patterns to help visual memory.**

To help you in your assessments:

- **record where you will find information (e.g. page numbers);**

- **keep your notes organised and easy to navigate;**
- **highlight where further reading and research are needed, including any recommended or further reading;**
- **use your notes to help you get started on your TAQs.**

Read the Specification:

So that you will know the criteria you will be assessed on please read the unit specification in my courses on the Materials page.

Subject: **Chemistry / Medicine**
Unit: **Applied Biochemistry**
Topic 1: **Applied Biochemistry**
Level: **3**
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Introduction

In this topic:

In this topic, we are going to start by exploring cellular metabolism. We will focus on specific anabolic and catabolic reactions that occur intracellularly (within cells), and extracellularly (outside of cells). To understand some of these reactions it will be necessary for us to consider the structure and function of biomolecules that include; Nucleic acids, Proteins, Carbohydrates and Lipids. We will address how these complex biomolecules are formed from their component parts via (anabolic) condensation reactions, and how they are broken down via (catabolic) hydrolysis reactions. By looking at a range of biochemical tests that can be performed in a laboratory, we will discover how it is possible to determine the presence of different biomolecules in solution.

We will then go on to focus on the structure and function of enzymes. These are globular proteins that are vital as the biological catalysts that control the rate of all anabolic and catabolic reactions. Once we have gained an understanding of the structural characteristics that make different enzymes extremely specific in catalysing chemical reaction, we will then explore the factors that affect their metabolic activity (with a focus on the effects of: temperature, pH, enzyme and substrate concentration and inhibitors). By looking at data trends related to the effects that some of these factors have on the rate of enzyme-controlled reactions, we will be able to apply our understanding of enzyme structure and function. This will help us gain further insight into the importance of maintaining optimal conditions for enzymes within the body.

Building on our understanding of the structure and function nucleic acids; Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA), we will complete the unit by exploring some of the medical applications of genetic engineering, with a focus on recombinant DNA technology and Polymerase Chain Reaction (PCR). To understand the importance of these biochemical techniques from a medical perspective, it will be necessary to look in detail at the laboratory-based procedures that have been developed to obtain evidence for diagnostic analysis.

Subject: **Chemistry / Medicine**
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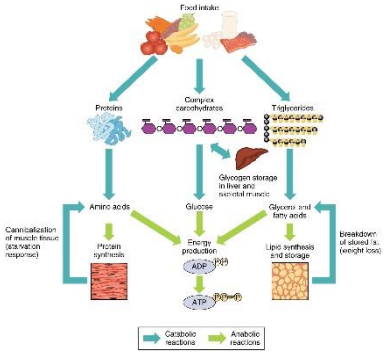
LEARNING MATERIALS

1. Anabolic and catabolic reactions



Ready, steady, go!

In this section, we will define anabolic and catabolic reactions, and briefly explain what they are.

In biochemistry when we refer to the term **metabolism** we are looking at all the chemical reactions that take place within an organism.

These reactions:

1. extract useable sources of energy from nutrient biomolecules (such as proteins, carbohydrates, and lipids) and
2. either involve the synthesise of larger biomolecules from monomers (e.g. DNA from nucleotides, formation of glycogen from glucose and proteins from amino acids), or the breakdown of larger biomolecules to smaller ones (e.g. Glycogen to glucose, triglycerides to glycerol and fatty acids, and glucose to ATP energy during aerobic respiration).

Metabolism is often divided into **catabolic** and **anabolic** reactions

- Catabolic reactions or catabolism release energy through the breakdown of large biomolecules (as occurs during digestion), and
- Anabolism reactions are energy-utilizing reactions that result in the synthesis or combination of large biomolecules so that they can be used either used immediately by the cell, used to start another reaction or stored.

Anabolic and catabolic reactions take place simultaneously in cells throughout the body, so that at any given moment, some biomolecules are being synthesized while others are being broken down.

Keyword/s:

Substrate molecule is a molecule on which an enzyme reacts. Enzymes have particular shapes which allows substrate molecules to fit into them in what is classed as an active site

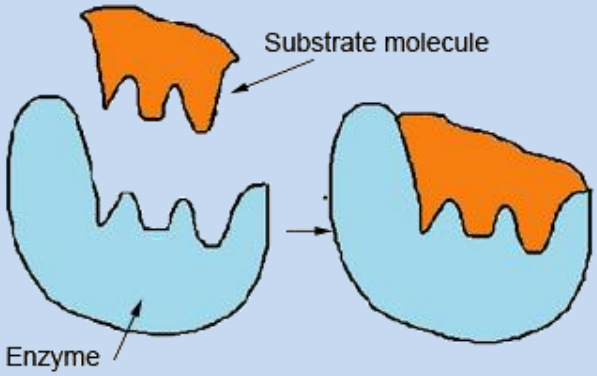


Figure. 1 Substrate Molecule

An **anabolic** reaction (as illustrated in **Figure. 2**) occurs when two substrate molecules are combined. If the substrate is two chemicals that the enzyme joins together in an anabolic reaction, then the active sites are next to each other so that when the product is released the molecules will be released joined together by a chemical bond. For example; a peptide bond formed between two amino acids to create a dipeptide molecule via a condensation reaction.

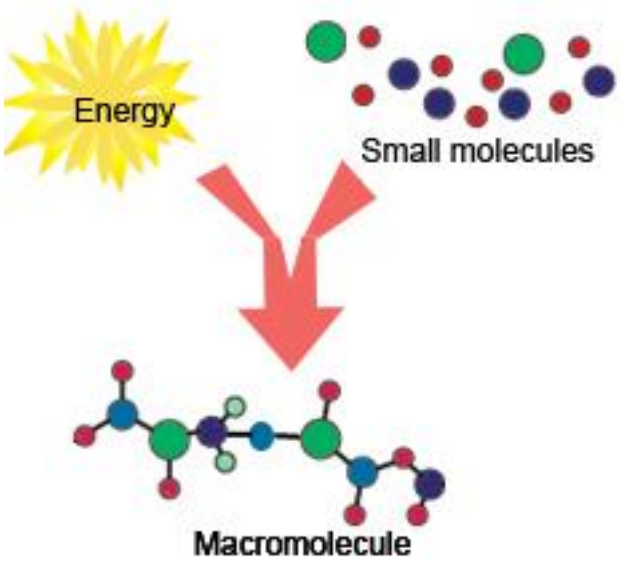


Figure 2 : An anabolic reaction

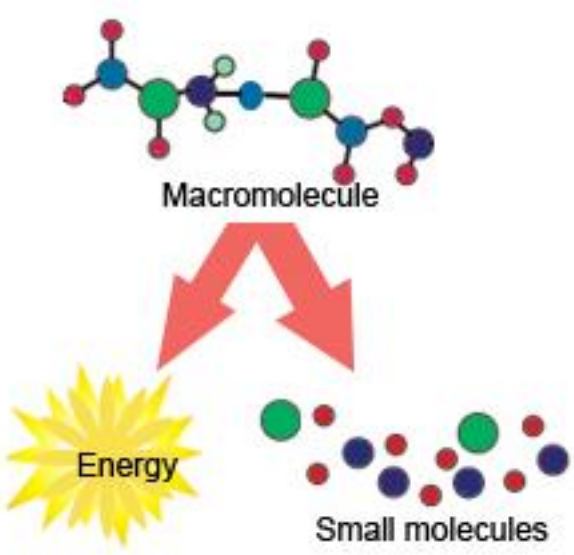


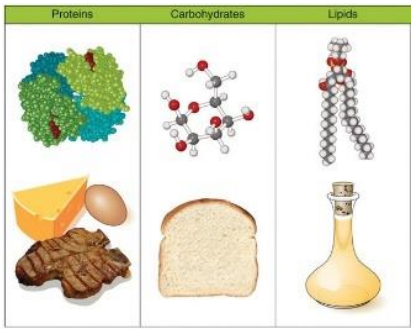
Figure 3 A catabolic reaction

A **catabolic** reaction (as illustrated in **Figure. 3**) is the breakdown of complex substances into simpler ones. In enzymes, the molecules being split are bonded together at the same site and released when separated. For example; the hydrolysis of a triglyceride to form glycerol and free fatty acids.

In a nutshell:

In this section, we looked very briefly at the definitions for catabolic and anabolic reactions, we are now going to look at how these work in more depth and the reactions that take place so that these molecules can be either combined or broken down to produce energy. To do this we are going to discuss large biomolecules before looking in more depth at enzymes.

2. Biomolecules



Ready, steady, go!

In this section, we will look in detail at three different classes of Biomolecules. We will discuss carbohydrates and the monosaccharides that make them up, lipids (Triglycerides, phospholipids and steroids) and proteins. We will also explore the chemical reaction that is needed to create a bond between two Biomolecules, which is referred to as a condensation reaction. The chemical reaction that is needed to break a bond between to a-joined Biomolecules (referred to as a Hydrolysis reaction), will also be explored.

Keyword/s:

monomers	Macromolecules (very large molecules) are made up of lots of smaller molecules which are termed monomers. Monomers are able to bond in long chains
dimers	A dimer is a macromolecule that is made up of monomers that are chemically bonded
polymers	Many monomers make up a polymer and there therefore a macromolecule made up of long chains of monomers

2.1 Carbohydrates

Carbohydrates are important biomolecules in biology; the majority of them are known as energy producing molecules. In fact, they are singly the most efficient form of fuel for metabolism but they can also play other roles within the body, such as carrying information for cell to cell communication.

Carbohydrates are made up of three elements: Carbon (C), Hydrogen (H) and Oxygen (O).

They are found in one of **three forms**:

- Monosaccharides (Carbohydrate monomers);
- Disaccharides (Carbohydrate dimers);
- Polysaccharides (Carbohydrate polymers).

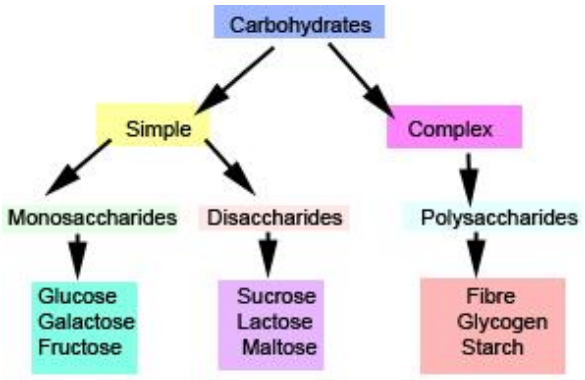


Figure 4 Carbohydrate sub-classes

Keyword/s:

Cellular Respiration

A series of metabolic processes that take place within a cell in which biochemical energy is harvested from organic substance (e.g. glucose) and stored as energy carriers (ATP) for use in energy-requiring activities of the cell. http://www.biology-online.org/dictionary/Cellular_respiration

2.1.1 Monosaccharides

Monosaccharides usually consist of 3 to 9 carbon atoms that are bound to hydroxyl groups (an oxygen atom bound to a hydrogen atom) forming, in effect, one sugar group. Most of the time we focus concentrate on a group called **hexoses** which have six carbon atoms.

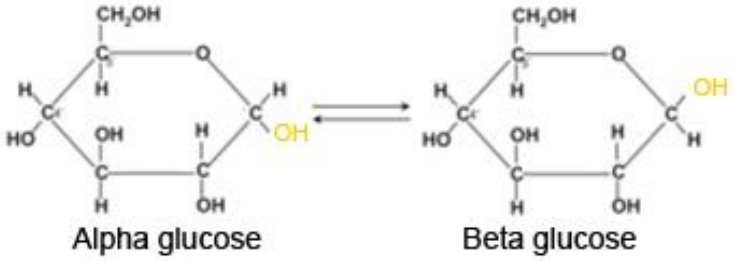


Figure 5 Two isomers of glucose

The main monosaccharide utilised by the body is **alpha-glucose** (**Figure 5**, which is fundamental to the production of energy in the form of Adenosine triphosphate (**ATP**) within the cell, during the process of **Cellular Respiration**.

Some organisms (such as plants) also utilise another form of glucose called **beta-glucose** (as shown in **Figure 5**). They use this slightly different form of glucose to synthesise the cellulose (a type of **polysaccharide**) that makes up a plant cell wall. This is only different to alpha-glucose due to the alternative position of the hydroxyl (OH) group on the carbon to the right of the lone oxygen atom in the ring structure (in yellow in **Figure.5**).

Both forms of glucose are said to be '**isomers**' because they possess the same chemical formula ($C_6H_{12}O_6$), but have a different structural arrangement. An additional hexose sugar that is an isomer of glucose is called fructose and as you can see in **Figure. 6**, there is a striking difference between alpha-glucose and fructose, even though they both have the same chemical formula.

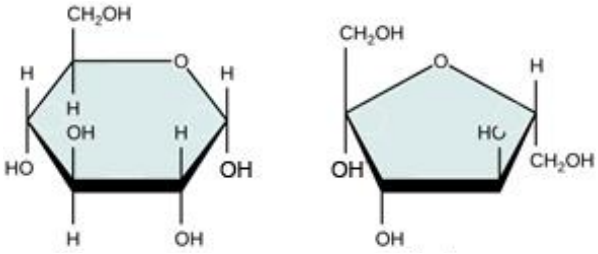


Figure 6 Comparison between the structure of glucose and fructose

Further resources:

TP & Respiration: Crash Course Biology #7

2.1.2 Disaccharides

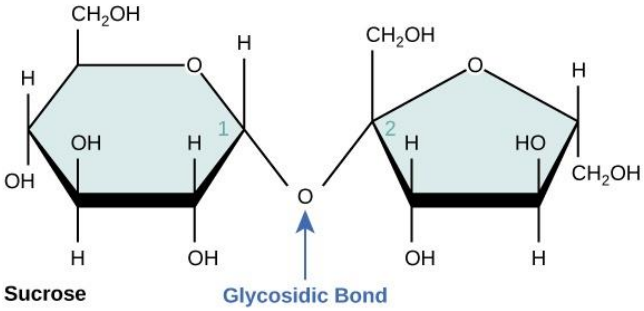


Figure 7 Sucrose molecule formed from a glucose and a fructose molecule

Disaccharides are two monosaccharides that have bonded together at an oxygen atom in a **condensation reaction.** Some examples of disaccharides are:

Sucrose: formed from glucose and fructose (F**igure. 7**)

Lactose: formed from glucose and galactose

Maltose: formed from glucose and glucose

2.1.3 Condensation Reactions

A **condensation reaction** is a chemical process where two molecules are bonded together to make larger more complex biomolecules, with the loss of a molecule of water. It forms the basis for the synthesis of all complex macromolecules such as the carbohydrate polymers; glycogen, starch and cellulose.

In all biochemical condensation reactions, one molecule with a free -H atom will be linked to another molecule with a free -OH group, forming H_2O, with both molecules connected via bonds to an oxygen (-O-).

A useful example is the formation of the disaccharide **maltose** from the joining of two alpha-glucose monosaccharides. In the illustration of the condensation reaction (shown in **Figure. 8**) notice that when two glucose molecules are aligned side-by-side there is a free hydroxyl (-OH) group in both molecules that are in close proximity to each other (one

attached to carbon-4 in the right-sided glucose molecule and the other attached to carbon-1 in the glucose to its left.

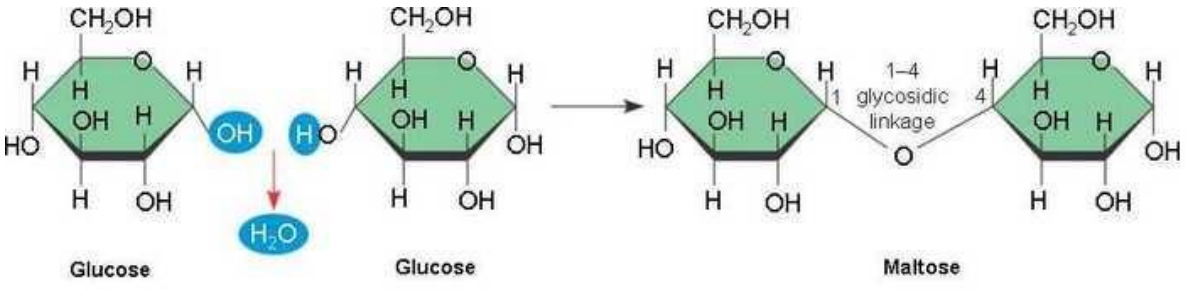


Figure 8 Formation of maltose via a condensation reaction

In the condensation reaction, the -OH on one glucose will combine with the Hydrogen in the adjacent (-OH) to form a water (H_2O) molecule. Upon completion, the water molecule is released and the two glucose molecules are now bonded together via a **glycosidic linkage**. The resulting disaccharide contains a -O- bridge between the two monosaccharide units. These links can be extended many times, resulting in the production of a polysaccharide.

Because the glycosidic link now holding the two glucose molecules together is positioned between carbon-1 (in one of the monosaccharides) and C-4 in the other. This bond is sometimes referred to as a α-1,4-glycosidic link.

Further resources:

Organic reactions: Condensation

2.1.4 Hydrolysis Reactions

Hydrolysis reactions typically involve the breaking of a bond between two biomolecules with the addition of a molecule of water. The digestive enzyme **Maltase** catalyses the hydrolysis of maltose to form two molecules of α-glucose. This enzyme is located within the phospholipid bilayer of the epithelial cells that line the villi of the small intestines. The word 'Hydrolysis' means water-splitting and so it stands to reason that water is an essential participant in this reaction as it is used to add a -OH group and a -H either side of the glycosidic bond. Basically, the water molecule replaces the one that was released in the condensation reaction (as shown in **Figure. 9**), displacing the glycosidic bond and creating two separated glucose monosaccharides. This reaction is shown in **Figure.8**

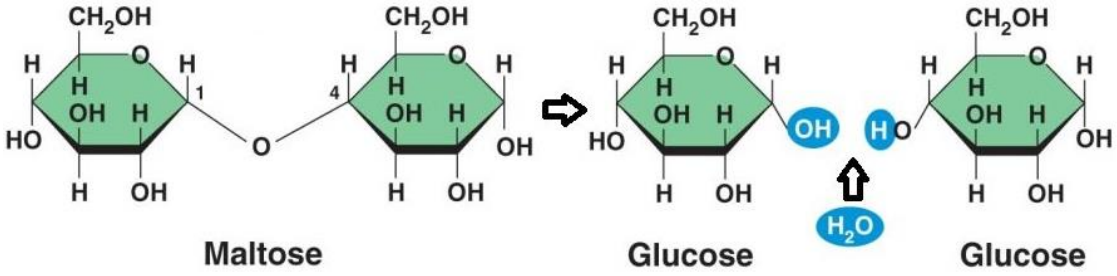


Figure 9 Formation of two molecules of glucose via a hydrolysis reaction

The **chemical equations** for both the condensation and hydrolysis reactions are as follows;

Formation of maltose by a condensation reaction:

$$C_6H_{12}O_6 + C_6H_{12}O_6 \longrightarrow C_{12}H_{22}O_{11} + H_20$$

Formation of glucose monomers by a hydrolysis reaction:

$$C_{12}H_{22}O_{11} \xrightarrow[\text{Maltase}]{H_20} C_6H_{12}O_6 + C_6H_{12}O_6$$

Further resources:

Hydrolysis Reactions by Holden Chemistry, SAT supplement: hydrolysis reactions

2.1.5 Polysaccharides

Keyword/s:

Facilitated diffusion	Facilitated diffusion is a form of facilitated transport involving the passive movement of molecules along their concentration gradient, guided by the presence of another molecule – usually an integral membrane protein forming a pore or channel. Source: https://biologydictionary.net/facilitated-diffusion/

Many saccharide groups can be bonded together via successive condensation reactions, creating chains or branching arrangements to form polysaccharides such as **starch** and **glycogen**. As mentioned, **monosaccharides** are the chemicals used by the cell for the creation of energy. However, storing a large amount of these in a cell would seriously impair the osmotic balance of that cell and could eventually cause cell death.

Polysaccharides are much more useful as a form of storage as they are insoluble in water and so cannot influence this osmotic balance in the same way that monosaccharides or disaccharides might. Complex storage compound such as **starch** (which is stored in the roots of plants) and used as a source of nutrition by humans, can be hydrolysed by amylase enzymes that are secreted into the mouth from salivary glands, to form the disaccharide maltose. Maltose is then further hydrolysed by maltase enzymes to form glucose within the small intestine. This monosaccharide can them be transported through epithelial cells lining the villi via **facilitated diffusion** and active transport, before being absorbed into the blood stream to be transported to respiring cells (along with oxygen) to create cellular energy (ATP). The polysaccharide **glycogen** (also composed of pure glucose) is stored in muscle and liver cells to act as a glucose (and thus energy) reserve. It is formed by successive condensation reactions between cytoplasmic glucose molecules that are not needed straight away as a source of fuel for cellular respiration. It is arranged in chains with extensive branching (as is shown in **Figure. 10**). Starch, in contrast is highly branched.

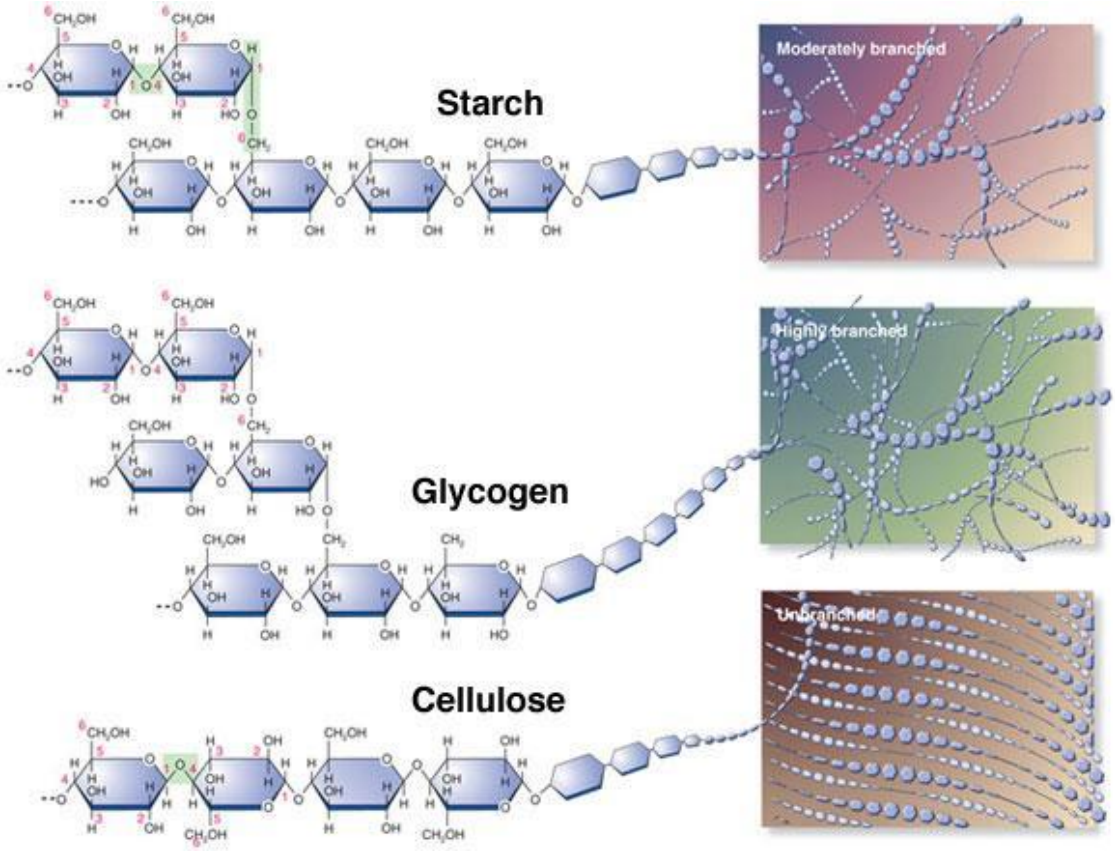


Figure 10 Structure of polysaccharides

2.1.6 Biological functions of carbohydrates

Carbohydrates have many functions within the body. These include:

- **Energy production and storage:** We have already discussed how polysaccharides, (such as starch and glycogen) can be formed in order to store energy and then broken down when that energy is needed for cellular respiration. The main carbohydrate that the body utilises for this process is glucose.
- **Structure:** Cellulose, for example, has long chains which help form the tough protective wall around plants cells.
- **Cell recognition and signalling:** At a cell membrane, carbohydrates can attach to proteins to form glycoproteins, or to phospholipids to form glycolipids. These play a role in cell-to-cell interaction.
- **Building blocks for nucleic acids (DNA and RNA):** Both DNA and RNA possess a backbone that is composed of a pentose (5-carbon) deoxyribose / ribose sugar and a phosphate, this will be discussed in more detail later on in the topic.

SAQ 1:

1. **Explain the difference between a disaccharide and a polysaccharide.**
2. **What biomolecule is required as a reactant in a Hydrolysis reaction, and forms s by-product in a condensation reaction?**

Further resources:

ScientificPsychic.com: Carbohydrates – Chemical Structure (Advanced)

RSC.org - Chemistry for Biologists: Carbohydrates

Kimball's Biology Pages: Carbohydrates

2.2 Lipids

Lipids are a group of molecules made up of carbon, hydrogen and oxygen similarly to carbohydrates. We will look at three different types of lipids:

- Fats (Triglycerides);
- Phospholipids;
- Steroids.

2.2.1 Fats (Triglycerides)

Fats (or **triglycerides**) consist of a glycerol molecule with three fatty acid chains attached to it (see ***Figure. 11***).

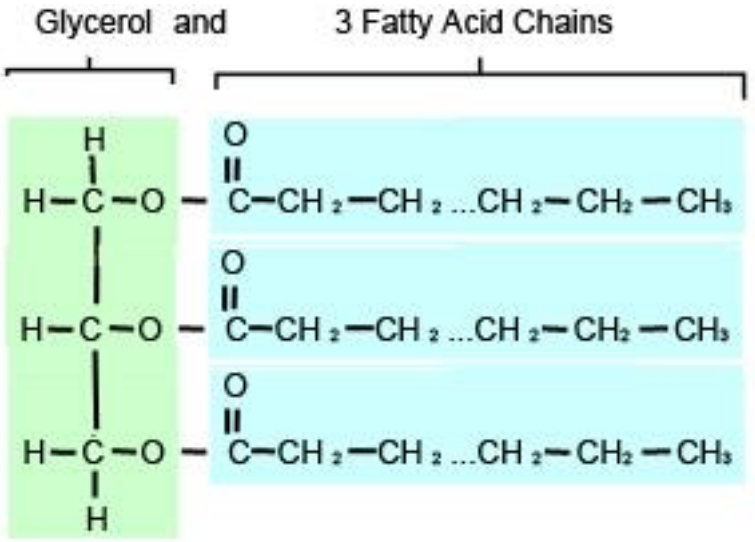


Figure 11 Triglyceride made up of a glycerol molecule and 3 attached fatty acid chains

In the body, these substances play a major role in the storage of energy. In fact, fatty acids can be stored in great amounts by the body and when there is a surplus the body puts fatty acids aside by producing fat cells. A fat cell consists of a large droplet of lipids with a coating of the cell cytoplasm and nucleus. Sometimes, this is also referred to as **adipose tissue** and is built up on the exterior of the body since adipose tissue makes for a good thermal insulator.

In places within the body, triglycerides also makes for effective shock absorbers due to the flexibility of the tissue. In cases where the fat reserves are depleted or a person has very little fatty reserves, such an individual becomes much more susceptible to hypothermia as well as bruising and damage from impacts due to a lack of that protective layer.

In cellular metabolism, the involvement of **triglycerides** is a little more complicated. We have already discussed the importance of the carbohydrate **glycogen** as a means of storing energy (in liver and muscle cells), and the monosaccharide glucose as the main direct fuel source for ATP energy production utilised by respiring cells. However, glycogen is only a 'short term' storage compound; and once the available free glucose molecules within a cell has been **catabolised**, the glycogen stores can very quickly and easily be hydrolysed (and glucose mobilised) by the processes already mentioned. Very often, when exercising, it is the glycogen store within the muscles that is being consumed. In fact, marathon runners call the point where the glycogen is used up as 'hitting the wall',

which is characterised by physical fatigue that can be so profound as to make it difficult to move.

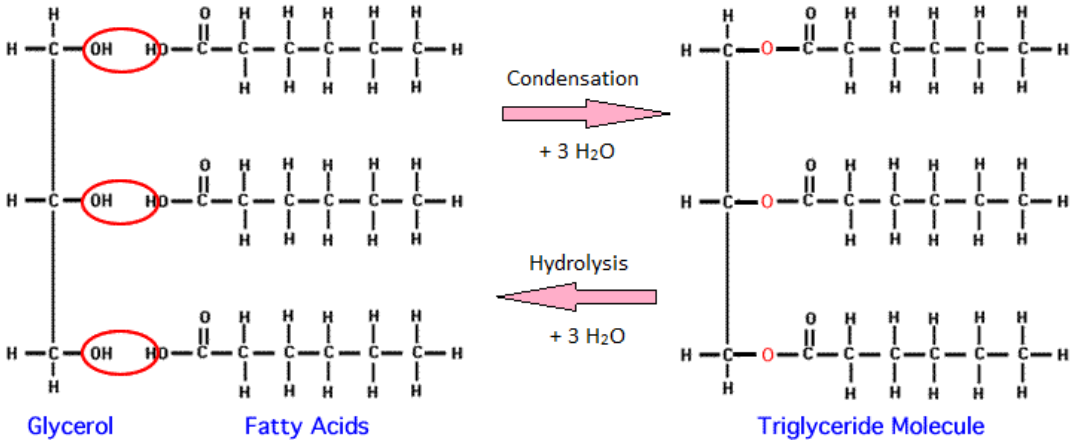


Figure 12 Hydrolysis reactions of lipase

Triglycerides (specifically their **Fatty acids**), are a form of energy storage designed for the **long term**, and this is partly because the process of converting fatty acids into a useable energy form is much more difficult that hydrolysing glycogen to release molecules of glucose; it is much more complicated.

The enzyme **Lipase** must be mobilised by the release of either adrenaline (epinephrine) or glucagon, which then breaks the triglyceride reserves down into fatty acids and glycerol which can't be easily transported into the blood stream. This enzyme catalyses in a series of hydrolysis reactions (as is shown in **Figure. 12**).

The protein **albumin** has to bind to fatty acids to transport them to the required location while released glycerol is absorbed at the liver. Finally, in the liver, glycerol is turned into glucose while, within other tissues, the citric acid (Krebs) cycle transforms the fatty acids into glucose within the mitochondrial matrix.

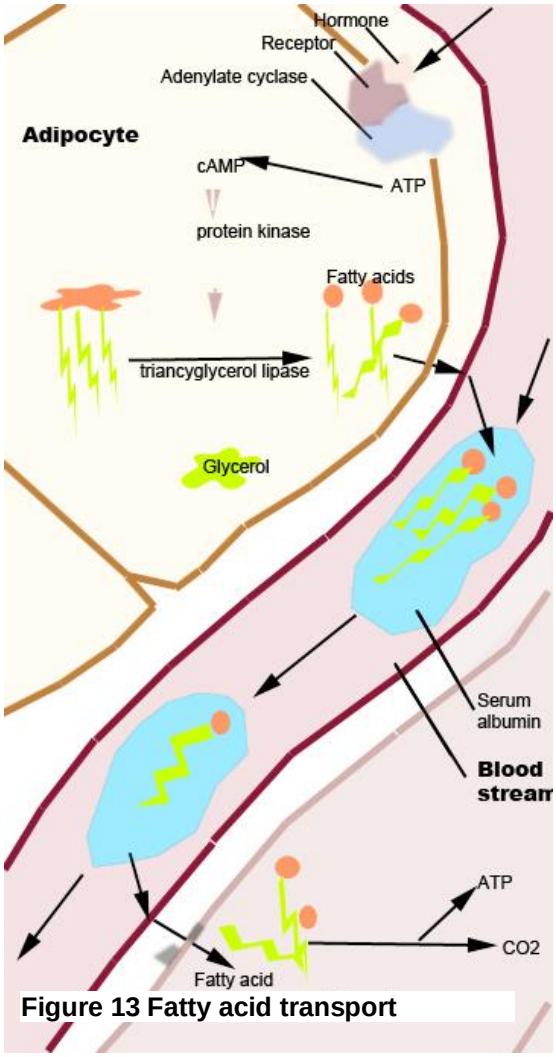


Figure 13 Fatty acid transport

The process of turning stored triglycerides into energy involves the liberation from the adipose tissues, the transport and the final breakdown into usable energy, meaning that fat requires several major processes to reclaim the chemical energy within. (**Figure. 13**) If we compare that energy to money, glucose would be the money within a person's pocket, very easily obtainable and usable without needing to get to it from an external source.

Glycogen would be best thought of as the money within the person's bank account, easy to obtain still but not always immediately available. Fatty acids would in contrast, be the equivalent of applying for a monetary loan, it can be a long process but the money borrowed (once processed and deposited into the bank) can be substantial. In other words, despite being a highly concentrated source of spendable energy for cells, fatty acid catabolism would less effective as readily available fuel for energy production, due to the effort required to liberate them.

2.2.2 Phospholipids

A Phospholipid is one sub-class of lipids that has an additional **phosphate group** located in the hydrophilic head region, while there are also two (instead of three) fatty acid chains that are hydrophobic. Between these is a glycerol group which acts as a bridge between the two ends of the molecule. Often these are shown as a sphere for the hydrophilic end and two tails for the hydrophobic fatty acid chains (see ***Figure. 14***).

These carry out an important role, as mentioned in the Topic 1 (Introduction to Cell Biology) hydrophilic Phosphate containing heads of phospholipids, when present in water are prone to making spheres of a single lipid layer called a **micelle**, the hydrophobic end with the two tails facing away from the water while the hydrophilic end faces into the water.

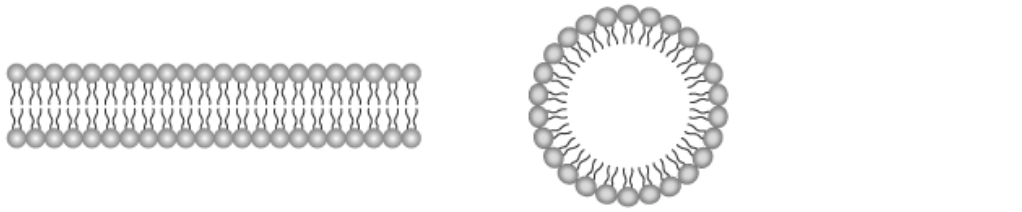


Figure 14 Structure of a phospholipid; and (to the left) arrangement of phospholipids as a Micelle and a bilayer

With enough phospholipids, a double layer with the **hydrophobic** ends shielded from the water by the **hydrophilic** end is formed. This makes the phospholipid the major constituent of a cell membrane, often called a phospholipid bilayer.

2.2.3 Steroids

Steroids are a type of lipid consisting of four fused carbon rings (see ***Figure. 15***). Cholesterol is a steroid found in humans, known for its negatively involvement with cardiac dysfunction and heart disease.

However, cholesterol plays an important part when it comes to the functioning of the body and even the cell. At the cellular level, cholesterol is a component of the cell membrane and imparts flexibility to the cell membrane maintaining the desired fluidity of the phospholipid bilayer. Cholesterol also acts as a precursor molecule to materials such as the sex hormones and vitamin D, among others.

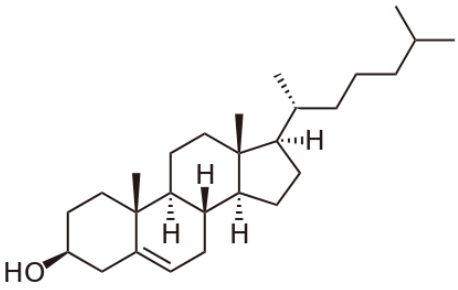


Figure 15 Cholesterol, an example of a steroid molecule with four fused carbon rings

Steroids are also split during the process of digestion and the liver secretes them in the form of bile acids which emulsify fats to allow lipase to digest them.

SAQ 2:

1. **Describe the differences in the structure of a Triglyceride and a phospholipid.**

Further resources:

BBC Bitesize: Biological Molecules- Lipids

Clermont College - Biology Course Information: Lipids - Fats, Oils, Waxes, etc.

2.3 Proteins

Proteins are one of the more complex and vastly varied structures within biochemistry. While lipids and carbohydrates are taken into the body from food that comprises our diet, proteins are the biomolecules that we can manufacture with any level of complexity. They are very complex indeed. With roles in transport (**haemoglobin** and membrane carrier proteins); as catalysts (enzymes); structural support (keratin and collagen); chemical communication (hormones); and immune protection (antibodies), they are incredibly diverse and fundamental to our survival on a physiological and biochemical level.

Like DNA, proteins are best thought of as long chains of single units, this time called **amino acids**. All amino acids possess an amino (NH_2) group to one side of a central carbon and a carboxylic acid group (COOH) to the other side of this carbon.

The chemical composition of an amino acid is usually written as **$H_2NCHRCOOH$**, but can be presented in its structural form (as shown in **Figure.16**).

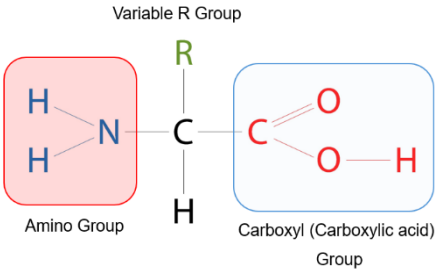


Figure 16 Generalised amino acid structure

In addition to a common hydrogen atom bonded to the central carbon, each amino acid possesses a variable (R) group. This is (a specific) side-chain that differs from amino acid to amino acid. As such, the composition of this R group determines the name of each amino acid and importantly, its properties. See **Figure. 17** and **18** respectively to view the different R-groups for the simplest amino acid (**glycine**) and a much more complex one for **phenylalanine**.

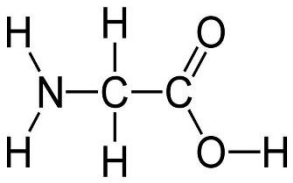


Figure 17 Structure of Glycine

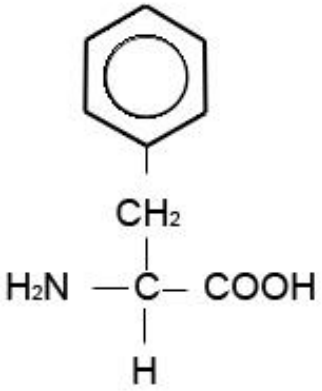


Figure 18 Structure of phenylalanine

The position of the amino (NH_2) group and carboxylic (COOH) group are of importance, since they link and are the points of attachment that bond amino acids together, first creating a dipeptide (double amino acid) and then gradually a polypeptide. The bond formed between two amino acids is called a **peptide bond**.

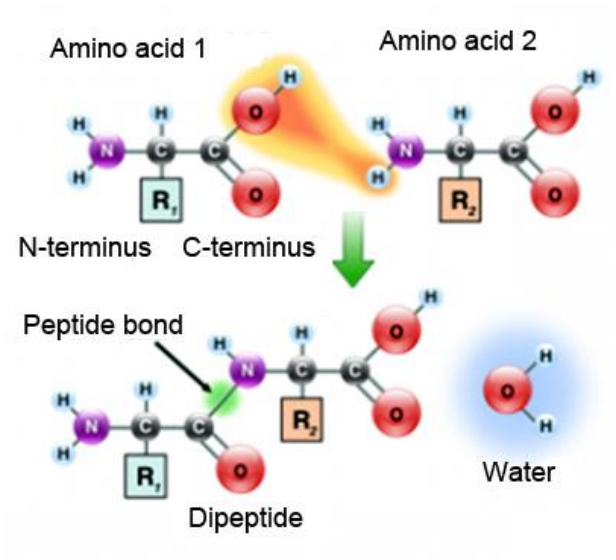


Figure. 19 illustrates the condensation reaction that converts two separate amino acids into a **dipeptide**. Notice that the position where the condensation reaction occurs is between the hydroxyl (-OH) group in the **carboxylic acid** region in **amino acid 1** and a (-H) from the amino ($-NH_2$) region of **amino acid.2**. This results in the release of a water molecule and the peptide bond that hold the two amino acids together is located between the remaining nitrogen and carbon atom.

Figure 19 Dipeptide formation via a condensation reaction

Proteins have a structure hierarchy ranging from primary structure to quaternary structure (see ***Figure. 20***).

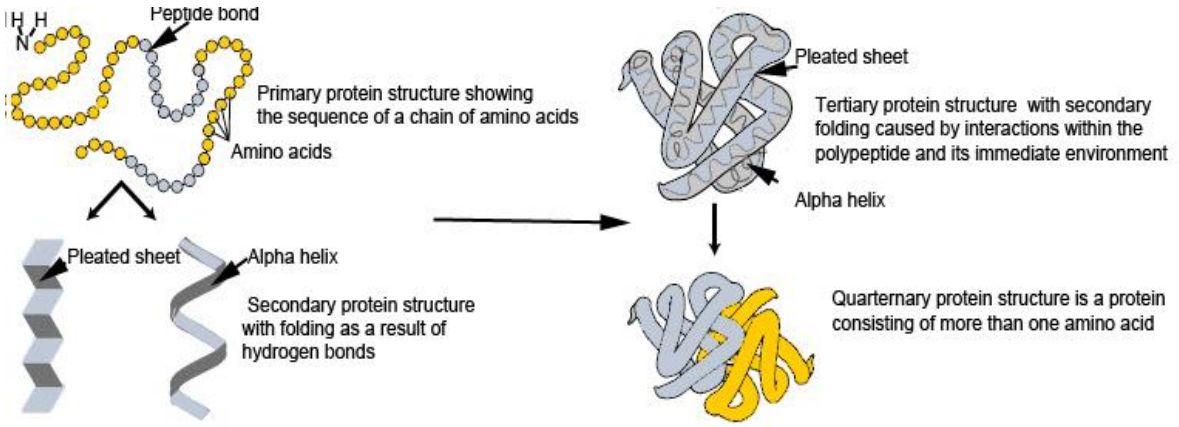


Figure. 20 Structure hierarchy of proteins

- **Primary structure:** The primary structure of the protein refers to the precise sequence of amino acids in a polypeptide chain. Despite the extensive range of different proteins in the human body, there are only 20 different amino acids. These are bonded together like beads on a string to form each one of these complex structures. The types of amino acids and the order they are in bonded together determines the primary structure and ultimately the shape and properties of a protein.
- **Secondary structure:** This is the next level up and accounts for the initial folding of the polypeptide chain (primary structure) into repeating structures.

The main structures that were identified by the ground-breaking study of Pauling and Corey (1951) were the **alpha helix** and **beta pleated sheet**.

The **alpha-helix** (as illustrated in F**igure. 21**) is formed by interchain hydrogen bonds, or the bonds between the hydrogen atoms at the side of chains, drawing those chains together when they turn back around, since each of the individual peptides turn when they are connected onto the last one, so the secondary structure can be like a spiral staircase with the atoms at the side of the backbone being pressed together to form the helix.

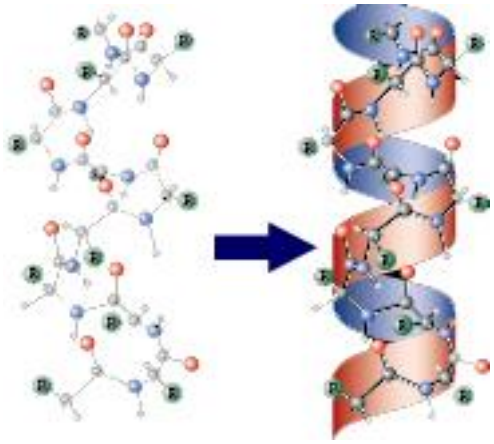


Figure 21 Structure of an alpha-helix

In the **beta pleated sheet**, however, are formed by polypeptide chains that are less helical but fold back on themselves, pressing the oxygen and hydrogen bonds together again in the same way that a piece of cloth can be formed from a single thread going back and forth (as illustrated in **Figure. 22**). The hydrogen bonds between the hydrogen and oxygen atoms form the support framework that prevents the entire structure unwinding. One important factor is that the polypeptide chains can turn sharply in any direction, which allows the formation of reversing turns and loops. This is particularly interesting when some of these structures are used within support proteins, one example being helical coiled coils, which is a complex way of saying that the helix formed by amino acids also turns around another helix to form a super helix.

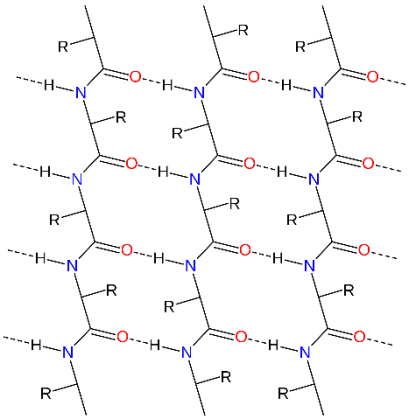


Figure 22 Structure of a Beta-pleated sheet

- **Tertiary structure:** The tertiary structure (as illustrated in **Figure. 23**) is the three-dimensional shape of a single major polypeptide chain. Generally, these shapes are without any form of symmetry. The shape is purely determined by the amino acid sequences and how the different amino acids cross link either with other agents or with other amino acids they are close to within the structure. An enzyme is a good example of a protein that is functional in the tertiary conformation. In addition to hydrogen bonds, the tertiary structure of a protein is further stabilised with hydrophobic interactions and ionic bonds

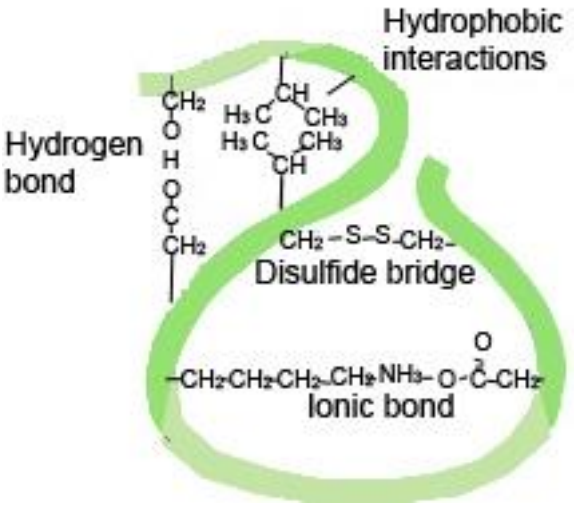


Figure 23 The Tertiary Structure of a Protein

- **Quaternary structure:** Some proteins might well merely have a tertiary structure with one major polypeptide chain, such as enzymes, but other proteins, such as haemoglobin, are made up of more than one major polypeptide chain. With haemoglobin there are two alpha-chains and two beta-chains (as illustrated in **Figure. 25**). The simplest forms of these are called 'dimers' which just means two polypeptide chains while others can have over a hundred units making up a complete quaternary structure.

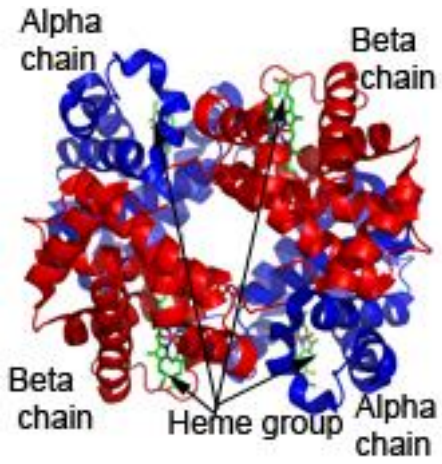


Figure 24 The Quaternary structure of Haemoglobin

Some substances, when added to a protein, break or change these bonds and, therefore, change the nature of the protein. In almost all circumstances, this will stop the protein carrying out its role, demonstrating that the structure and shape of the protein is instrumental to its biological function. This is referred to as **denaturing** a protein, which can be confused with 'killing' the protein because proteins are not alive so, therefore, cannot be killed. Instead, a protein can be denatured by chemicals or even by temperature. This process is particularly interesting since, once denatured, some proteins are capable of rearranging themselves and regaining function while some changes are permanent, meaning that once the protein is denatured it is no longer able to function.

Due to the huge range in structural arrangement and properties of different proteins, they have a large number of functions within organisms. Some of these include:

- **Biochemical reactions:** One subset of proteins called **enzymes** catalyse reactions, in some cases, speeding the reactions up enormously. While many of these reactions will occur without the presence of proteins, enzymes speed up such things as the uptake

of oxygen and the breakdown of nutrients in digestion. These will be discussed more later.

- **Physical structure:** Protein is a major factor in the production of hair, fingernails and collagen, as well as being support structures for a broad range of tissue types, especially in muscle.
- **Immunology:** The immune system produces proteins known as **antibodies** which are involved in recognition of alien microbes. Normally, the antibody is made with a unique composition for each type of pathogen identified by the immune system and if they find that pathogen, they will attach to it, which is a signal for the immune system to begin the destruction of that pathogen.
- **Receptor proteins:** These proteins are within the membrane of certain cells and are there to signal a change in the cell when a chemical messenger binds to that protein. Similar proteins to these can even be found in the nuclear envelope to signal changes within the nucleus.
- **Membrane transport:** As discussed in Topic 1 Introduction to Biology there are other proteins which also remain within the cell membrane and these can form gateways (channels) for ions to diffuse in or out of the cell, while carrier proteins form conveyors that move ions against the concentration gradient.
- **Movement:** Proteins, such as actin or myosin, can form fibres that lengthen or shorten depending on stimulation. These fibres are the foundation of muscles and without these protein structures, movement of organisms larger than a few cells would be all but impossible.
- **Signalling:** Just as proteins can be found to inform cells when a chemical messenger has been sent, there are several proteins which are released from cells to signal a change in behaviour in other cells as well.
- **Storage:** Certain proteins can also be used to store chemicals that are needed by the organism in case there is likely to be a shortfall. Strictly speaking, haemoglobin could be called a storage protein since it stores oxygen to be released elsewhere within the blood stream.

SAQ 3:

1. **Describe the structure of an amino acid.**
2. **Proteins have a structural hierarchy, list the four structures that make up this hierarchy.**

Further resources:

ALevelNotes.com: Protein structure

YouTube - RCSBProteinDataBank: What is a Protein?

2.4 Reagent Tests Used to Identify Biomolecules

There are several chemical tests available for the identification of the major types of organic biomolecules found in a food sample. Typically, these tests are used to determine the makeup of an unknown material. For instance, a dietician may be interested in identifying the nutrient composition in an unknown food sample. As the tests are carried out, the dietician will also use known solutions containing biomolecules, or controls, for comparison. During the experiment the dietician will compare the experimental results of the unknown food sample with the control solutions response to that same procedure. Controls are important because they reveal the specificity of a particular test. For example, if water and a glucose solution react similarly in a particular test, the test cannot distinguish water from glucose. But if the glucose solution reacts differently from distilled water, the test can distinguish water from glucose. In this instance, the distilled water is a negative control for the test, and a known glucose solution is a positive control.

There are a number of different tests available for biological molecules. Some of these tests include;

1. using **Benedict's reagent** to test for the presence of a reducing sugar such as glucose,
2. the use of **iodine solution** to test for the presence of starch in solution,
3. using the **Biuret reagent** to test for the presence of protein
4. using **ethanol** to determine the presence of lipids in solution.

1. Testing for the presence of a reducing sugar (glucose, fructose) in solution.

Many monosaccharides such as glucose and fructose are reducing sugars, meaning that they possess free aldehyde (-CHO) or ketone (-C=0) groups that reduce weak oxidising agents such as the copper in Benedict's reagent. Benedict's reagent contains cupric (copper) ion complexes with citrate (a salt of citric acid) in alkaline solution. Benedict's test identifies reducing sugars based on their ability to reduce the cupric (Cu^{2+}) ions to cuprous oxide at basic (high) pH. Cuprous oxide is green to reddish orange.

Procedure- Benedict's test for reducing sugars

Using a glass pipette, transfer 2 cm^3 of the unknown solution to be tested to a boiling test tube. Add 2 cm^3 of (blue) Benedict's reagent to the same boiling test tube and stir the mixture using a glass rod. Place the test tube into a boiling water-bath for three minutes and observe the colour change during this time.

A green solution indicates a small amount of reducing sugars, and a reddish orange solution indicates an abundance of reducing sugars. Non-reducing sugars, such as sucrose produce no change in colour (as such the solution remains blue).

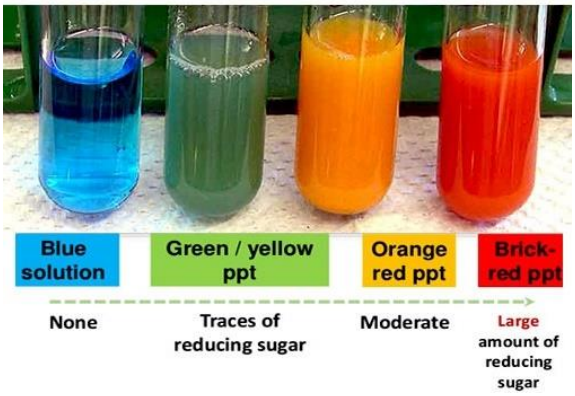


Figure 25 Interpreting the Benedict's test

Things to do:

- **Watch the video testing for simple sugars with Benedict's solution**

2. Testing for the presence of starch in solution

Staining by iodine (iodine-potassium iodide, I_2KI) distinguishes starch from monosaccharides, disaccharides, and other polysaccharides. The basis for this test is that starch is a coiled polymer of glucose and iodine interacts with these coiled molecules and becomes bluish black. Iodine does not react with carbohydrates that are not coiled and remains yellowish brown.

Procedure- the iodine test for starch

Using a glass pipette, transfer 2 cm^3 of the unknown solution to be tested to a boiling test tube. Add three to five drops of iodine to the test tube and mix. A bluish-black colour is a positive test for starch, and a yellowish-brown colour (i.e., no colour change) is a negative test for starch.

Figure 26 Looking for the presence of starch in a solution

Things to do:

- **Watch the video testing for the presence of starch in solution**

3. Testing for Proteins using Biuret solution

Proteins are remarkably versatile structural molecules found in all life-forms. Proteins are composed of amino acids each of which has an amino group ($-NH_2$), and a carboxyl group (-COOH). The amino group on one amino acid is linked to the carboxyl group on an adjacent amino acid by a peptide bond. The peptide bond forms as a result of a condensation reaction. This bond is the site of action for the Biuret test for protein. Biuret reagent is a 1% solution of $CuS0_4$ (copper sulphate). In this test, a copper ion (Cu_2^+) complexes with the peptide bond producing a violet colour.

Procedure- The Biuret Test for Protein

Add 2 cm^3 of the unknown solution to a boiling test tube and then add 2 cm^3 of 2.5% sodium hydroxide (NaOH). Add three drops of Biuret reagent and mix.

The violet colour is a positive test for the presence of protein; the intensity of colour relates to the number of peptide bonds that react. A Cu_2^+ must combine with at least four to six peptide bonds to produce a colour; therefore, free amino acids and very short chains do not react positively. However, free amino acids and very short chains may result in a pinkish colour. Long-chain polypeptides (proteins) have many peptide bonds and produce a positive reaction.

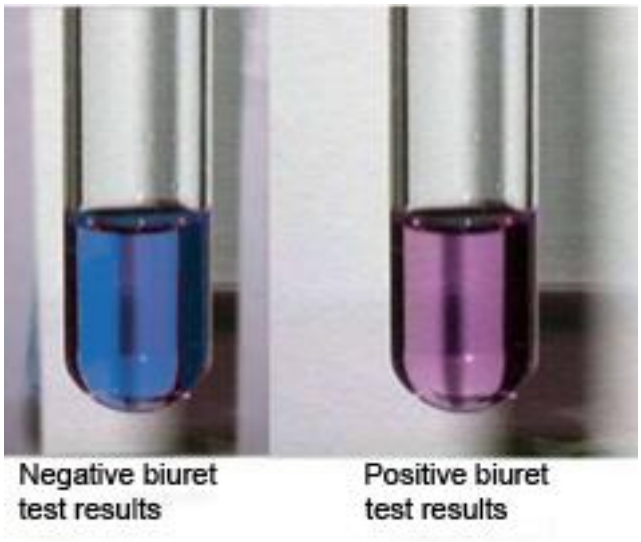


Figure 27 The biurets test for proteins

Things to do:

- **Watch the video testing for the presence of protein in solution**

4. Emulsion (ethanol) test for Lipids

Lipids include a variety of molecules that dissolve in solvents such as ethanol but not in polar solvents such as water. The primary component of fat or lipid is the fatty acid. When it occurs alone it is called a free fatty acid. This molecule is composed of an even number of carbon atoms terminated in a carboxyl group (- COOH). This test is done to show the presence of lipids in a substance. The substance is first dissolved in ethanol. This solution is then dissolved in water. If lipids are present in the mixture, it will precipitate and form an emulsion.

Procedure- The Emulsion test for lipids

Add 2 cm^3 of the unknown solution to a boiling test tube and then add 2 cm^3 of ethanol and shake well. Allow the mixture to settle in a boiling test tube rack. Then transfer any clear liquid into a boiling test tube containing 2 cm^3 of distilled water.

A milky-white emulsion indicates that the mixture contains lipid, but if the mixture remains clear, there are no lipids present in the sample.

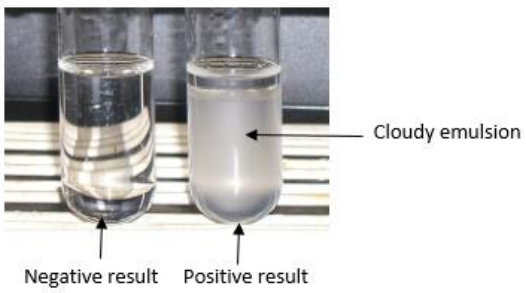


Figure 28 Emulsion test for lipids

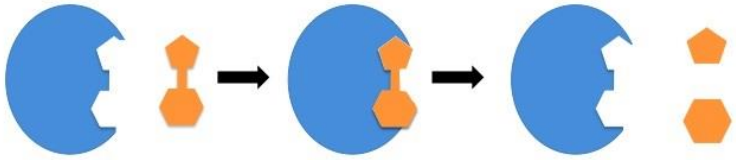
Things to do:

- **Watch the video testing for the presence of lipids in solution**

In a nutshell:

In this section, we discussed the structure and function of three different macromolecules and considered biochemical tests that can be used to distinguish between them based of their structural properties. We examined carbohydrates and their role in metabolism. We then explored three different types of lipids, including triglycerides, phospholipids and steroids. Finally, we looked at proteins, their structural hierarchy and some of the biological roles that proteins can undertake.

3. Enzymes



Ready, steady, go!

In this section, we will examine enzymes and some of their features including specificity, catalytic power, active sites and energy conversion. We will also look at the effects of temperature and pH on enzymes. Finally, we will discuss inhibition of enzymes.

Enzymes are a group of globular proteins that serve as biological **catalysts**, in that they are able to **speed up the rate** of both intracellular and extracellular chemical reactions throughout the body, without being used up during the reaction. As such, enzymes can be reused over and over again. Enzymes are enormously important proteins, and without them all chemical reactions would be too slow to sustain life. In fact, in the entire genome of an individual (all the genes contained in DNA), about 25 percent encodes for the production of enzymes. In this section we are going to look at some of the important features of enzymes including:

- specificity;
- their role in anabolic and catabolic reactions;
- catalytic power;
- active sites;
- energy conversion;
- influence of temperature and pH;
- inhibition.

SAQ 4:

1. **What do enzymes do?**

3.1 Enzyme Specificity

The shape of an enzyme, and more specifically the shape of the indentation in its tertiary structure called the enzymes **active site**, is specific to certain reactant chemicals, these chemicals are called the **substrate**. Minor variations in chemical arrangement of a substrate molecule can prevent it from binding to the active site. As such only when the shape of the active site of an enzyme is complementary to the shape of its substrate will an **enzyme-substrate complex** be formed that results in the production of a specific **product**.

In fact, it is the specificity of an enzyme to a particular substrate that led to the **lock and key** model (as illustrated in **Figure. 29**). This model addresses enzyme-substrate specificity by suggesting that the substrate can be likened to a specific key that fits into the lock (or active site) of the enzyme. Other keys that are like the substrate either do not fit into the lock or may jam that lock (referred to as inhibitors) so that the correct key (substrate) is unable to fit into the lock.

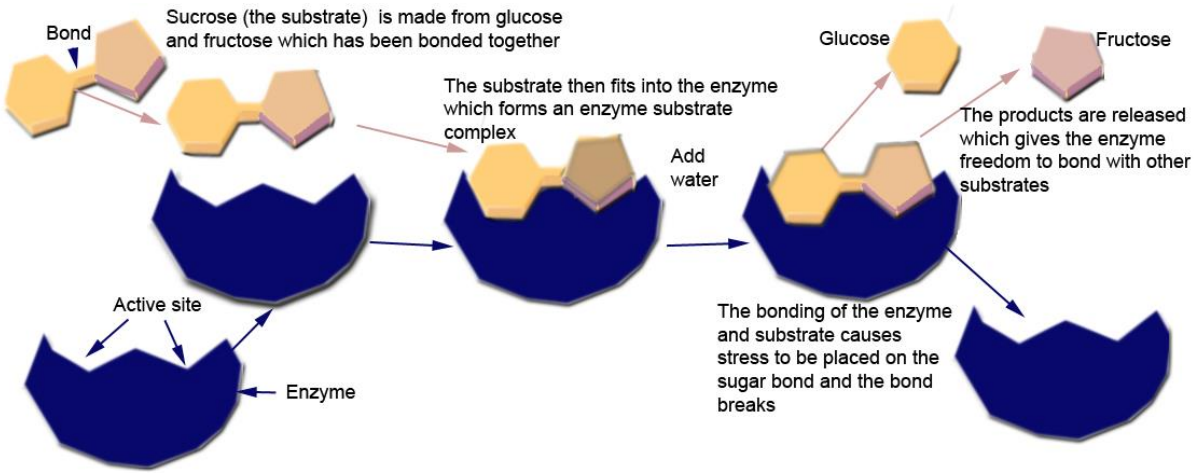


Figure 29 The lock and key hypothesis for enzyme specificity

The lock and key model is useful in highlighting enzyme specificity but it is not indicative with the way in which an enzyme interacts with a substrate biologically. A more recently proposed **induced fit theory** follows the principles of the lock and key model but it is more representative of how enzymes and substrates interact at the cellular level.

The induced fit theory (as illustrated in **Figure. 30**) suggests that the enzyme changes shape in the presence of the substrate to allow the active site to become complementary to the fit of the substrate. As such, the substrate must be similar in shape to the active site of a specific enzyme, but not identical. Once the enzyme-substrate complex has turned into the **enzyme-product complex**, the product is no longer close to the shape of the active site and it is therefore released. Upon releasing this product, the active site returns to its original form.

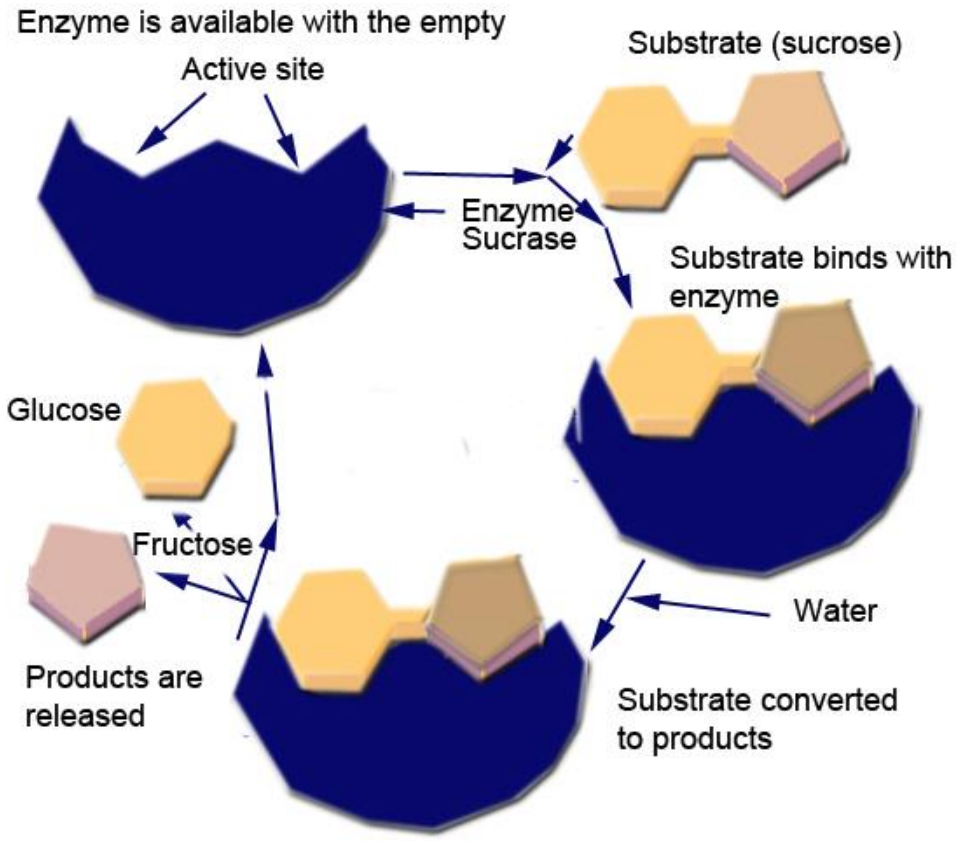


Figure 30 The induced fit theory for enzyme specificity

SAQ 5:

1. **What is the lock and key model?**
2. **How does the induced fit theory differ from the lock and key theory?**

3.2 Catalytic power

Keyword/s:

Catalyst	A catalyst is a substance that speeds up a chemical reaction, but is not consumed by the reaction; hence a catalyst can be recovered chemically unchanged at the end of the reaction it has been used to speed up, or catalyse. Source: https://www.chemicool.com/definition/catalyst.html

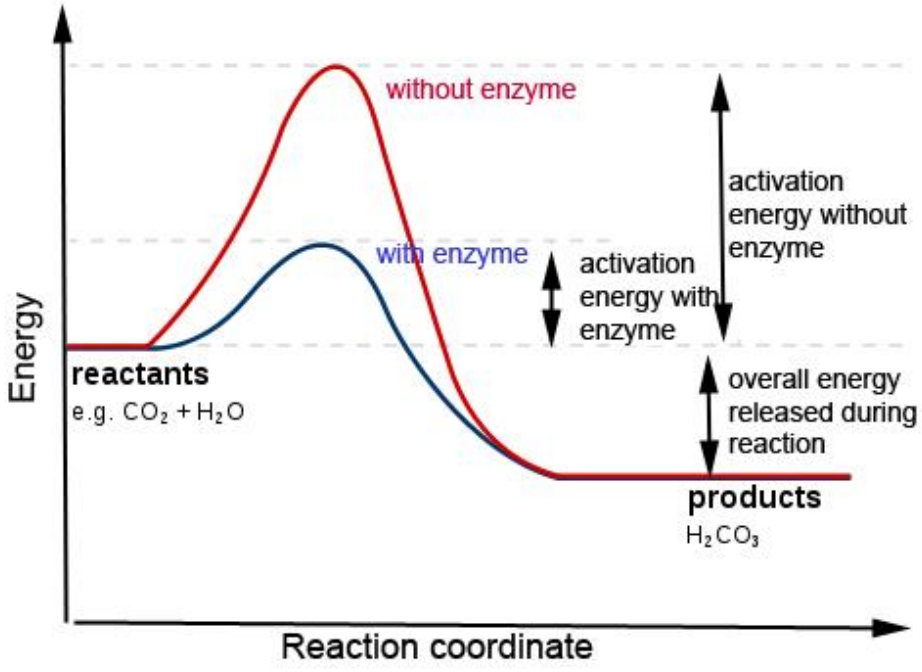


Figure 31 Activation energy with and without an enzyme

As Biological catalysts, enzymes increase the rate of chemical reactions. They do this by reducing the activation energy of the chemical reaction which means that in the presence of an enzyme less energy is required to complete a reaction (as is illustrated in **Figure. 31**).

This is the case for enzymes such as carbonic anhydrase which hydrate carbon dioxide for transfer from the blood to the interior of the alveoli. If the rate of reactions is quicker and require less energy, then additional energy is made available for other cellular tasks.

3.3 Active site

Keyword/s:

Biosynthesis	the formation of chemical compounds by a living organism. Source: http://www.dictionary.com/browse/biosynthesis
Phosphorylate	to introduce the phosphoryl group into (an organic compound). Source: http://www.dictionary.com/browse/phosphorylate?s=t

As mentioned previously, the shape of a protein is fundamental to its function. This is even more important in the case of enzymes as the precise arrangement of amino acids during their biosynthesis determines their globular arrangement and vitally the shape of the active site. This is shown in **Figure. 32** (where the substrate binds for the reaction to take place).

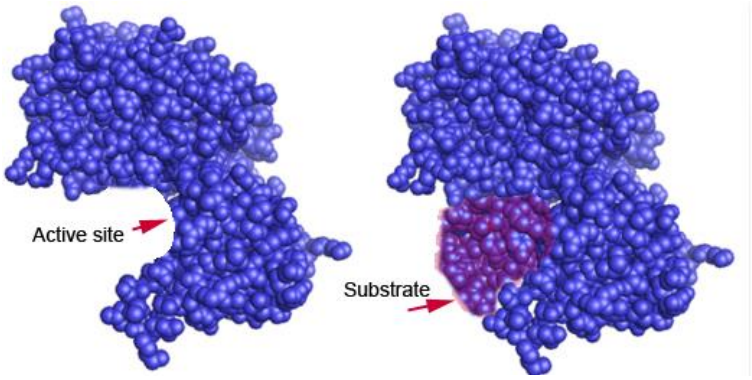


Figure 32 The active site of an enzyme

This presence of the active site means that only one set of the substrate can bond with the enzyme molecule at once, however, this site can be cleared and reoccupied thousands of times a second.

3.4 Energy conversion

The conversion of energy from one form to another is fundamental to the operation of an organism. Some examples of these conversions include; plant photosynthesis, which takes light energy and transforms it into chemical energy, and ATP synthase enzymes, which phosphorylate, adenosine diphosphate (ADP) into adenosine triphosphate (ATP). This can be used by other enzymes involved in anabolic reactions such as protein and glycogen synthesis. The reaction that converts ADP to ATP is illustrated in (**Figure. 33**)

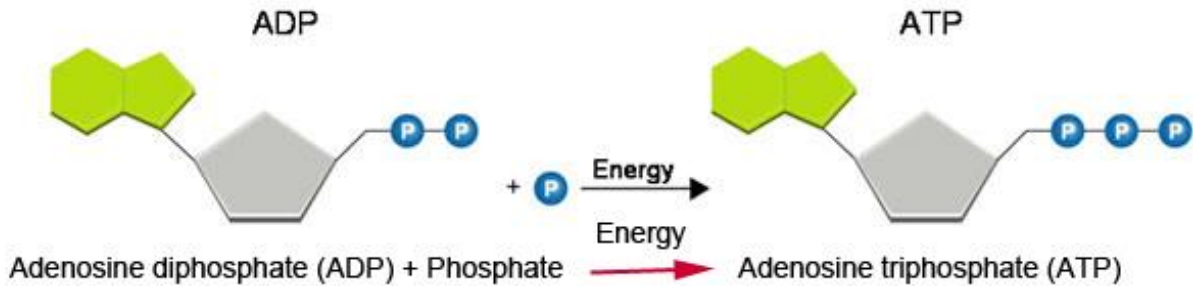


Figure 33 From ADP to ATP

The energy produced from these conversions is a key source of energy throughout the organism, making energy transformation vital to the cell and the organism as a whole.

3.5 Factors affecting enzyme activity

There are a number of factors that can alter the rate of an enzyme controlled reaction. The three major factors are:

- temperature,
- pH and

- inhibitors.

Every enzyme has an **optimum** temperature and pH, or a temperature and pH that the enzyme is most effective as a biological catalyst at.

For instance, in the stomach, the enzyme pepsin work best at a very low pH and at body temperature. If those enzymes are removed from that environment and placed in one with a different pH, such as the small intestine (which has a pH of 8), the catalytic action of the enzyme will sharply decrease. The same would occur if the enzyme were warmed up past body temperature. As such, specific enzymes function over a very narrow pH and at **37°C**. Beyond these parameters their ability to catalyse reactions at the correct rate to sustain life would be seriously compromised.

3.5.1 The effects of temperature on enzyme activity

Keyword/s:

Kinetic Energy	Kinetic energy is the energy associated with the movement of objects. Although there are many forms of kinetic energy, this type of energy is often associated with the movement of larger objects. For example, thermal energy exists because of the movement of atoms or molecules, thus thermal energy is a variation of kinetic energy. However, most of the time, kinetic energy refers to the energy associated with the movement of larger objects. Therefore, if an object is not moving, it is said to have zero kinetic energy. The kinetic energy of an object depends on both its mass and velocity, with its velocity playing a much greater role. Source: http://www.softschools.com/examples/science/kinetic_energy_examples/4/
Denatured	When an enzyme becomes denatured, it has become modified by breaking some of this links in the structure. Denaturing can occur because of heat or from chemical reactions that have rendered the enzyme inactive. As enzymes are not alive they cannot be killed instead we say that they have been denatured. Source: https://www.britannica.com/science/denaturation

Enzymes are extremely sensitive to fluctuations in temperature and the human body relies on its ability to regulate alterations in peripheral temperature to ensure the core temperature remains constant.

At low temperatures (as evident in the line graph: **Figure. 34**) enzyme controlled reactions will be slow and may not happen at all. This is because reactant molecules (enzymes and their substrates) move about very slowly at low temperatures (as they have low kinetic energy). As the temperature increases, so too does the kinetic energy.

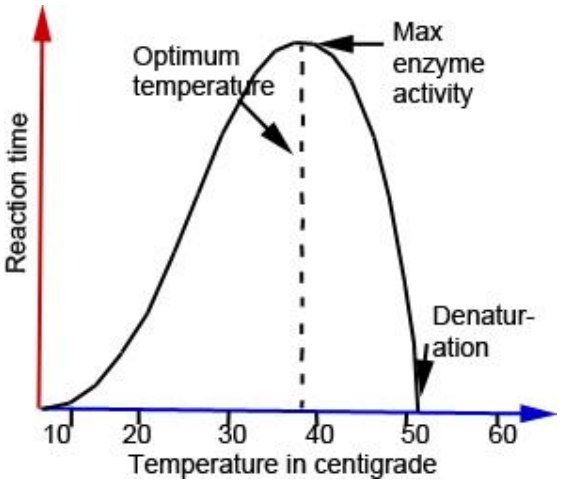


Figure 34 : A line graph showing the effects of temperature on enzyme activity.

Enzyme and substrate molecules move around much faster and collide with each other more frequently. As a result, there is an increased likelihood that substrate molecules will bump into and then bind with the enzymes active site, creating increasingly more enzyme-substrate complexes. Therefore, as temperature increases there will be a greater chance of substrate being converted into product, and as such there will be a greater rate of reaction. In Humans, the rate of reaction is fastest at around **37°C**. This is the **optimum temperature** for an enzyme controlled reaction. Beyond this temperature the rate of reaction decreases rapidly. This is because enzymes are globular proteins and their three-dimensional shape is held in place by chemical bonds between the R-groups of amino acids. Some of these bonds (hydrogen bonds) are weak and tend to break at high temperatures as enzymes and substrate molecules collide with greater force. These changes to the shape of an enzyme will also alter the shape of the active site and as a result the substrate will no longer be able to fit into it. These changes are irreparable and the enzyme is said to be **denatured**. The rate of an enzyme controlled reaction therefore decreases when the temperature is increased beyond the optimum. The higher the temperature increases beyond an enzyme's optimum the more enzymes will be denatured and the slower the rate of reaction will be. At a temperature of between 50°C and 60°C all enzymes will be denatured and the reaction will not take place at all.

3.5.1 Concept of Q_{10} (the temperature Co-efficient)

In order to determine the effect that a rise in temperature by 10°C will have on the rate of an enzyme catalysed reaction we can use a simple equation known as the **temperature Co-efficient** (or **Q_{10}**).

$$Q_{10} = \frac{\text{Rate of reaction at T°C (+ 10°C)}}{\text{Rate of reaction at T°C}}$$

T°C in this equation is a temperature of your choice e.g. **20°C**

Therefore, if the temperature that you choose is **20°C** then this can be worked into the equation as follows;

$$Q_{10} = \frac{\text{Rate of reaction at } \mathbf{20°C} \text{ (+ 10°C)}}{\text{Rate of reaction at } \mathbf{20°C}}$$

Taking into consideration the (+ 10°C) part of the equation, it can be shortened to;

$$Q_{10} = \frac{\text{Rate of reaction at } \mathbf{30°C} \text{ (20°C + 10°C)}}{\text{Rate of reaction at } \mathbf{20°C}}$$

If an investigation into the effects of temperature on lipase activity (an enzyme that catalyses the hydrolysis of triglycerides into glycerol and free fatty acids) resulted in the following rate of activity values;

Temperature (°C)	**10**	**20**	**30**	**40**	**50**	**60**
Rate of reaction **1 /T (x 10^{-4})**	4	**7**	**14**	30	3	0

What would the Q_{10} value be according to the above simplified equation?

All that we need to do is insert the rate of reaction values at the two temperatures highlighted into the equation. The rate of reaction at 30°C according to the Table is **14 x 10^{-4}**, and the rate of reaction at the chosen temperature of 20°C is **7 x 10^{-4}**. Therefore;

$$Q_{10} = \frac{14 \times 10^{-4}}{7 \times 10^{-4}} \qquad Q_{10} = 2$$

What does this value actually allow us to conclude?

A Q_{10} value of **2** simply means that for a 10°C rise in temperature (between 20°C and 30°C), the rate of reaction catalysed by the enzyme lipase has **doubled**. A Q_{10} value of **3** would indicate that for a 10°C rise in temperature the rate of reaction has **tripled**, while a Q_{10} value of **0.5** would suggest that for a 10°C rise in temperature, the rate of reaction has **halved**.

3.5.1 The effects of pH on enzyme activity

Just as high temperature can cause irreversible changes to the three-dimensional shape of an enzyme so too can pH.

pH is a measure of how acidic or alkaline a solution is. A solution with a pH below 7 is acidic and one with a pH above 7 is alkaline. Most protein molecules including enzymes have a stable three-dimensional structure at around pH7 (neutral). If the pH falls or rises beyond the optimum then the hydrogen bonds and ionic bonds between the R-groups of amino acids in a proteins structure will break. Enzymes will become increasingly denatured and less able to catalyse a reaction as pH conditions further deviate beyond the enzymes optimum pH (as is shown in **Figure. 35**).

In addition, extreme changes in pH can cause enzymes to completely unravel and become tangled around each other forming an insoluble clump. As such pH conditions either side of an enzymes optimum can cause the coagulation of proteins

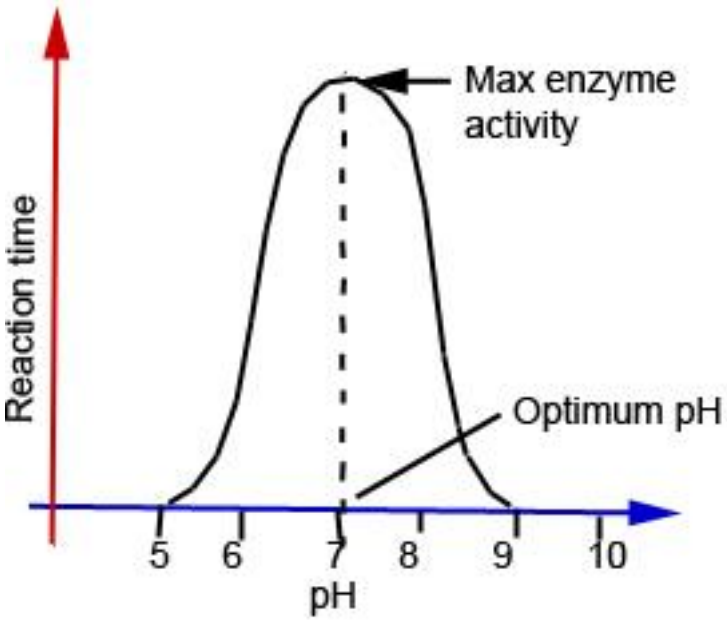


Figure 35 A line graph showing the effects of pH on enzyme activity.

3.5.2 Inhibition

So, what happens if something changes the shape of the active site?

Well, as we have discussed, this can happen in the induced fit theory of enzyme action where the enzyme changes shape to accommodate the substrate. However, in most cases, the changing of shape can stop the enzyme from working entirely. In fact, certain molecules, when added to the enzyme, are said to embed themselves in the enzyme structure, not at the active site. That embedding changes the shape of the active site enough to stop the enzyme from working.

This is called **inhibition** of the enzyme and the embedding of a competitor into the protein and not the active site is called **non-competitive** inhibition (which is illustrated in **Figure. 36**). This is because the molecule does not stop the substrate from binding with the enzyme but instead deforms the active site enough to prevent the enzyme from operating fully.

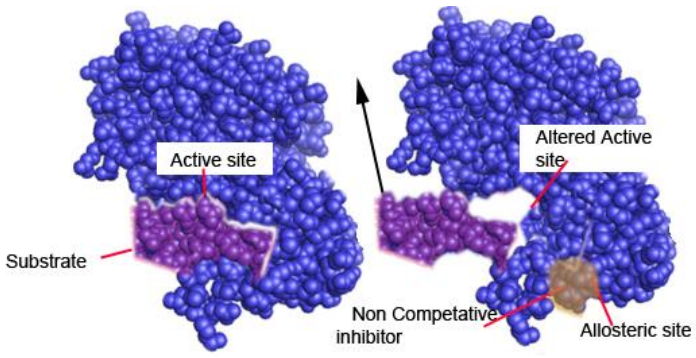


Figure 36 The effects of a non-competitive inhibitor on enzyme activity.

Non-competitive inhibition can also just decrease the efficiency of the enzyme by reducing the amount of the active site that is in contact with the substrate, therefore slowing the turnover of the enzyme.

Competitive inhibition works differently, in that the inhibitor is very similar in shape to the normal substrate of the enzyme and binds more strongly with it. (**Figure. 37**)

This means that the enzyme binds to the inhibitor in the same way as would the substrate but the molecule then remains in place and the enzyme does not release it. The substrate is therefore physically prevented from bonding to the active site with the inhibitor in place. Often, an increase in concentration of the substrate can lessen the effect of this kind of inhibition because, the normal substrate is able to out-compete the inhibitor.

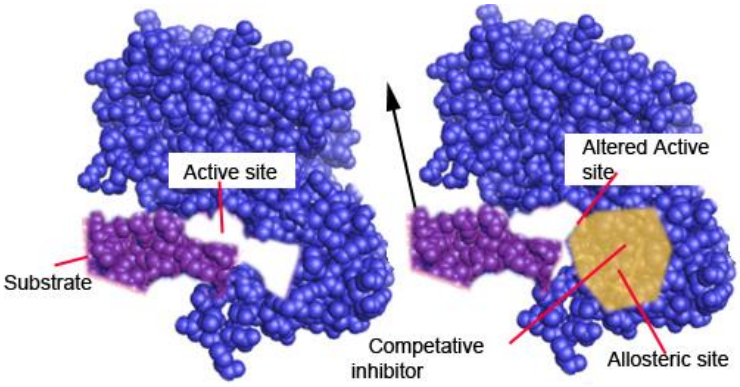


Figure 37 The effects of a competitive inhibitor on enzyme activity.

SAQ 6:

1. **List 3 factors that will have an effect on the rate of enzyme catalysed reactions.**
2. **What is the optimum temperature for enzyme activity in the Human Body?**
3. **Describe the effect that a temperature of 20°C would have on the rate of an enzyme-controlled reaction.**

Further resources:

ChemGuide.co.uk: Proteins as enzymes

KhanAcademy.org: Enzymes and activation energy (Advanced)

In a nutshell:

In this section, we discussed the structure of enzymes and their role as biological catalysts. We also looked at some theories of action of the enzymes, namely lock and key and induced fit. We also explored the influence of pH, temperature and inhibitors on the action of enzymes.

4. Nucleic acids

Ready, steady, go!

In this section, we will discuss the structure of the nucleic acids DNA and RNA. We will also look at how DNA is replicated semi-conservatively and, finally, we will examine the organisation of DNA within the nuclei of cells.

The term **nucleic acid** refers to Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA), both of which play a vital role in the expression of genetic information that is inherited from one generation to another. The genetic information that is required to create every protein that the body needs to function is contained within the nucleus of almost every specialised Human cell. We will start by looking at the structure of these nucleic acids.

4.1 Structure of DNA Heading

Keyword/s:

homologous pairs	those chromosomes that come one from the male and one from the female via reproduction
haemoglobin	is the oxygen-carrying pigment in the red blood cells
chromatid	is one copy of a newly copied chromosome which is still joined to the original copy by a single centromere which is the part of the chromosome that links chromatids

Deoxyribonucleic acid is held within the nucleus of almost every cell and forms long chains that are arranged in a double helix. Erythrocytes (or red blood cells) are one exception because these specialised cells lose their nucleus once matured, which maximises the space so that it can carry haemoglobin. As a result, they lose the ability to make proteins and are unable to reproduce.

DNA can be likened to an intricate computer programme, in that the entire contents provides information (in the form of a genetic code) for every trait that an organism possesses, such as an individual's blood group, eye colour and gender. The entire genetic blueprint of an individual is contained within the DNA molecule - and that means, that if a medical condition is the result of an error in the genetic code, then the condition is likely to be passed on from generation to generation.

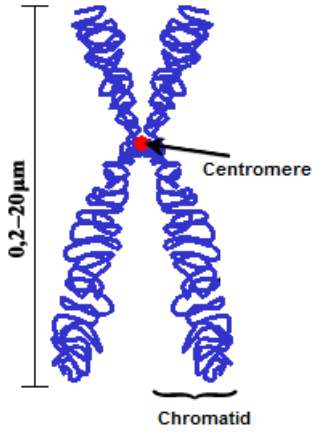


Figure 38: Diagram of a chromosome

DNA is found in homologous pairs of **chromosomes**; there are 23 such pairs in almost every human body cell. Each of these 23 chromosome pairs is made up of two identical chromatids connected by a **centromere** (see **Figure. 38**). Though there are 46 of these chromatids, there are only really 23 that are individual, each of them having a duplicate.

Different animals and plants have differing number of chromosomes and, though it seems counterintuitive, size and complexity of an organism has no connection with the number of chromosomes it possesses, although the total number of base pairs does correlate with complexity.

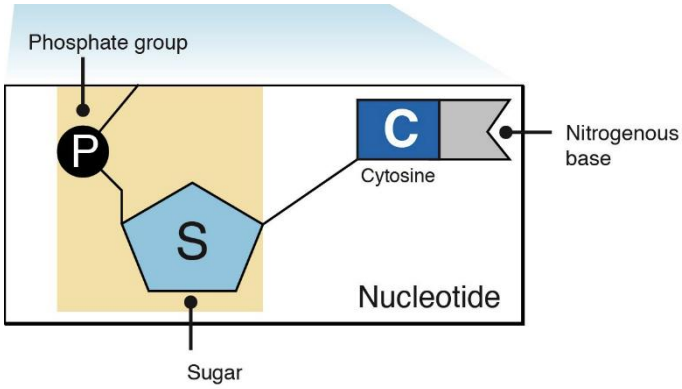


Figure 39 Nucleotide structure consisting of a phosphate group, a ribose sugar and a base.

At its most basic, DNA is a polymer (long chain) of sub-units called **nucleotides.** These nucleotides are made up of a phosphate, a sugar (deoxyribose) and a base (see ***Figure.39***). The phosphate and sugar are always the same and bond together to form the **sugar-phosphate backbone**. The base unit, however, can differ.

The nucleotides are arranged in two chains which spiral to gether into a **double helix** (see ***Figure. 40***).

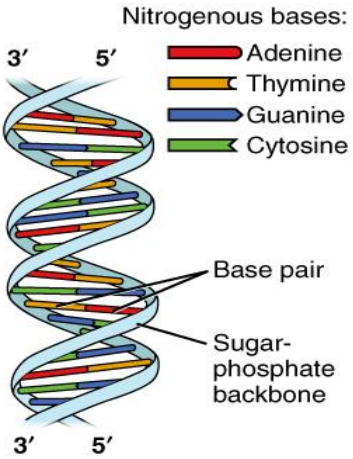


Figure 40 Nucleotide bases between two sugar-phosphate backbones

SAQ 7:

1. **What is contained within our DNA?**
2. **What are the three components that make up a DNA nucleotide?**

4.1.1 Base units

There are only four nitrogenous base units found in DNA. These are  **Guanine**, **Cytosine**, **Adenine** and **Thymine**. These four units pair up to make only two combinations of **base pairs,** this is because Guanine is always paired with Cytosine and Adenine always pairs with Thymine. Therefore the information for all of the proteins and characteristics within a person is stored within the sequence of these chemicals.

The order of these base units makes up the code for specific characteristics in the body, such as eye colour or hair length. Therefore, our body's DNA uses just 4 letters (the 4 nucleotide bases) to code for millions of different proteins that dictate human characteristics.

A very small section of a chromosome might be represented as;

A T C G C T G C C A
T A G C G A C G G T

The sequence of bases in a small section of a DNA strand holds the genetic instructions that are required to create a specific protein and this portion of DNA is called a **gene**.

Each ribose sugar molecule in the DNA strand binds to one particular nucleotide base. Remember, each molecule of DNA is made up of 2 nucleotide strands cross-linked together. Each nucleotide base in the DNA strand will cross-link (via hydrogen bonds) with a nucleotide base in a second strand of DNA forming the famous double helix structure (as illustrated **F*igure.41***).

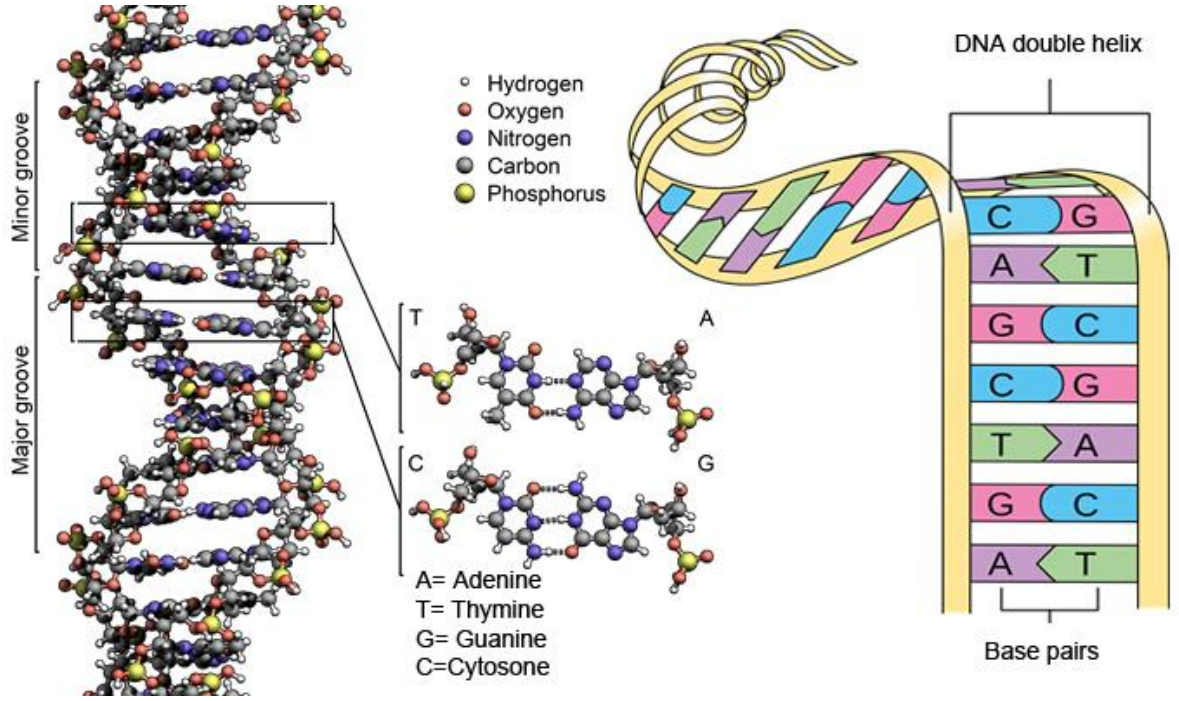
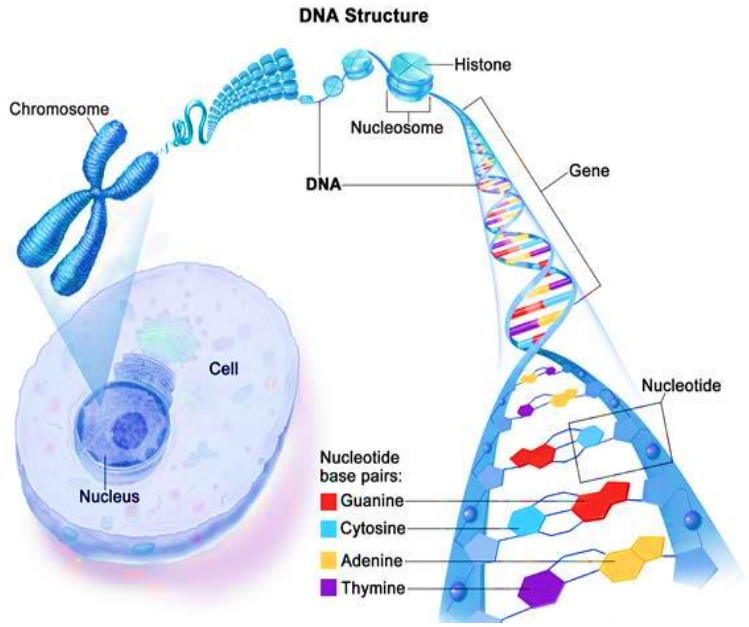


Figure 41 : Watson and Crick's DNA double helix

The Double helix

In 1953, James Watson, Francis Crick and Rosalind Franklin discovered that DNA is arranged in a double helix (**see Figure.42**).



The specific base-pairing of DNA aids in replication of the double helix when more genetic material is needed (such as during cell division). When DNA replicates, the two strands unzip from each other (as the hydrogen bonds to connect the paired bases are broken and enzymes add new bases to each separated strand, thus forming two new identical DNA strands. Adenine always base pairs with thymine, while cytosine base pairs with guanine.

Figure 42 From Chromosome to DNA to a gene.

A **chromosome** is a relatively large collection of DNA that contains many **genes** and the support proteins needed to control these genes. Chromosomes are formed by the super-coiling of a DNA double helix, aided by proteins called **histones.** A **nucleosome** is a sub-unit of chromatin that consists a segment of DNA that is wrapped around an octamer of histones proteins (see **Figure. 42**). A chromosome is therefore composed of many nucleosomes (and as such is a combination of DNA and associated histones). It is these histones which ensure that an extremely long stretch of DNA can fit within the limited space available in the nucleus of a cell.

SAQ 8:

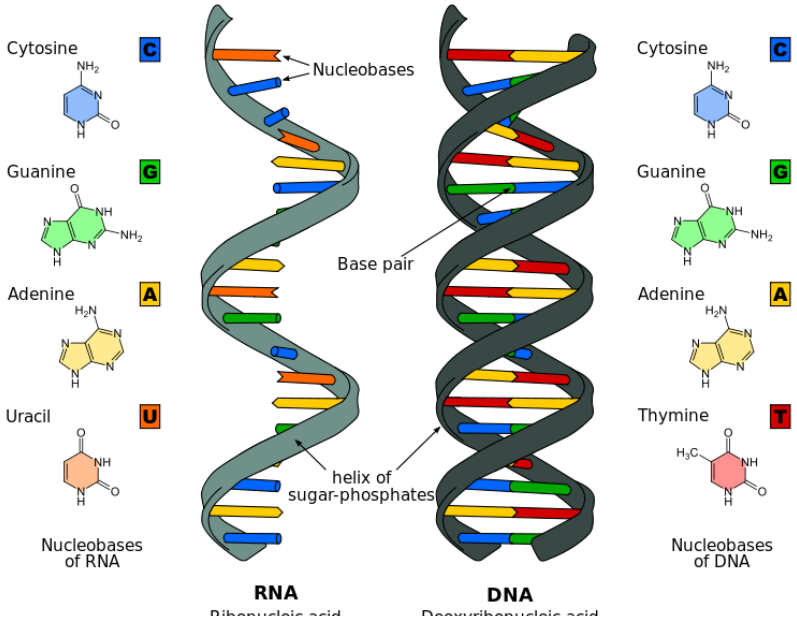
1. **What is the difference between chromosomes and genes?**

Further resources:

- **ChemGuide.co.uk: DNA - Structure**
- **Genetics Home Reference: What is DNA?**
- **YouTube: DNA structure**
- **You Tube The Structure of DNA**
- **You Tube MITx Bio The Structure of DNA**

4.2 Structure of RNA

This second type of nucleic acid is ribonucleic acid (RNA). RNA differs from DNA in a number of ways.



The first difference is that RNA is single-stranded, unlike DNA with consists of a double helix. Also, the sugar units in RNA are ribose as compared to DNA's deoxyribose, which means that in RNA, the ribose sugar units possess one more oxygen atom than is present in the deoxyribose sugar units of DNA.

Figure 43 Comparison between DNA and RNA structure

Due to this slight structural difference, RNA does not possess the nucleotide base thymine. Instead, RNA contains the nucleotide base **Uracil** (U) in place of it. Similar to DNA, a RNA strand does contain the other three bases: (Adenine, Cytosine and Guanine). ***Figure. 43*** shows a comparison of DNA and RNA.

There are **three** main types of RNA:

- **Messenger RNA (mRNA)**: mRNA is the functional molecule connected with DNA. The structure of mRNA is similar to that of DNA but is usually much shorter and also single-stranded. It functions as a transcript formed from a specific gene sequence in DNA by the enzyme RNA polymerase. This mRNA is therefore the template from which proteins are synthesised on cytoplasmic ribosomes. It is formed during a process called **transcription** that occurs in the nucleus, but unlike DNA, the mRNA transcript leaves the nucleus and binds to ribosomes in the cytoplasm to initiate **translation** of the genetic code into a specific sequence of amino acids, forming a specific polypeptide. The polypeptide is then modified to create a functional protein.

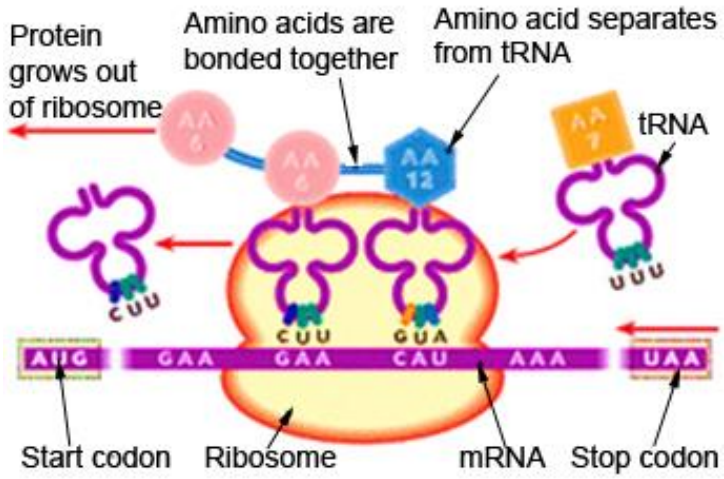


Figure 44 The roles of mRNA and tRNA in translation

- **Transfer RNA (tRNA):** This is relatively small and there is one version of tRNA for each of the 20 different amino acids that go into making proteins. tRNA transports the amino acids to the ribosomes for successive building of a polypeptide (protein) chain, using the mRNA strand as a template (with a sequence of three bases termed a **codon** in mRNA, being complementary to the sequence of three bases or an **anti-codon** in tRNA). See ***Figure. 44***.

- **Ribosomal RNA (rRNA):** rRNA acts more as a catalyst for the production of protein and is one of the major components of the ribosome.

Further resources:

Exploring Life's Origins: What is RNA?

Nature.com: Chemical Structure of RNA (Advanced)

SAQ 9:

1. **Name the three types of RNA.**

4.3 DNA replication

During cell division (Mitosis and Meiosis) more DNA is needed and the molecule is designed to facilitate this replication, forming two complete double helixes from one in a process called **semi-conservative replication**.

4.3.1 DNA directionality

Before we discuss the process of DNA replication it is necessary to understand that the DNA molecule has directionality, with a 5' (prime) end a 3' (prime) end. These ends are determined by which of the carbon atoms within the **deoxyribose** molecule the next **phosphate** is attached to (as shown in **Figure 45**).

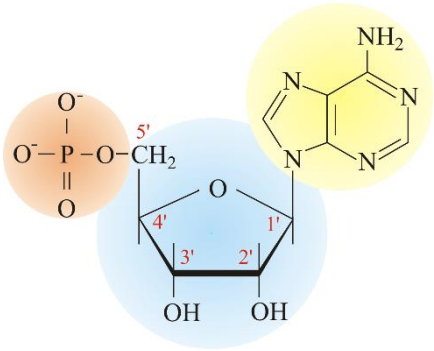


Figure 45 Deoxyribose sugar with numbered carbons

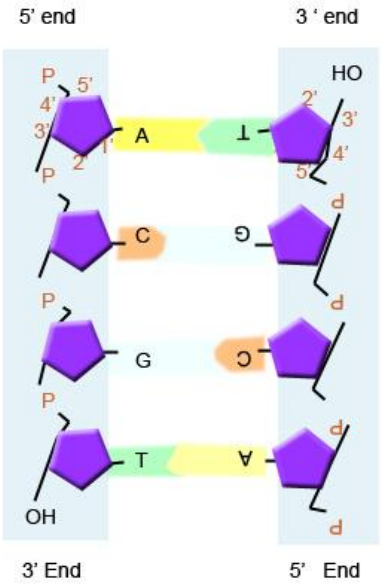


Figure 46 Anti-parallel DNA strands

If you look at ***Figure. 45*** you can see that the deoxyribose sugar has 5 carbons. The 3' carbon of one deoxyribose is connected to the 5' of the next carbon by the phosphate. Because of this linking, one strand of DNA will have a spare 5' carbon at one end (where there is an OH attached), and with the opposite strand, a spare 3' carbon at the other (again where you see the OH).

This concept is illustrated in **Figure. 46**. This is important to remember, since the process of manufacturing more DNA can only occur in **one direction**.

4.3.2 Process of DNA replication

The process of DNA replication involves a range of enzymes performing specific roles in order to unzip the DNA strand, add new bases to the two exposed strands, and then rezip the two new DNA strands to reform a double helix arrangement. ***Figure. 47*** shows the action of enzymes, splitting one double helix to create two.

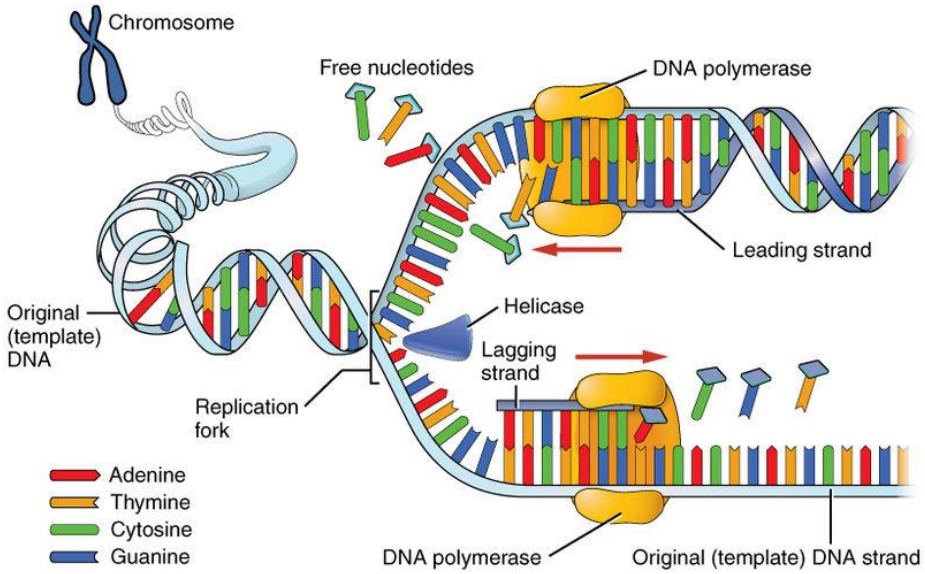


Figure 47 The process of DNA replication

First, the enzyme **DNA topoisomerase** breaks one strand of the DNA, allowing it to unravel.

The DNA is then unzipped, turning the double helix into a **replication fork** (the whole molecule is never completely uncoiled). The double helix of DNA means that the different strands contain the full information of the genome of the individual organism. The enzyme **DNA helicase** breaks the hydrogen bonds between base pairs, and the strands start to separate.

A small primer made up of 5 base pairs of RNA attaches to where the 5' end would be, opposite the 3' end of the other strand, and this forms a basis for the enzyme **DNA polymerase** to start the **replication** process. It attaches to the 3' end just on the end of the primer and starts adding the complimentary base pair for each. So, if it finds a thymine, it adds an adenine and if it finds a guanine, it adds a cytosine, building a new strand of DNA. Some of the DNA polymerases have a specific function, which means that errors are checked or 'proofread' by another part of the enzyme, and then changed if the base has been added in error. This reduces the chance of an error in the base sequence resulting in a gene mutation. If the DNA has been damaged at some point, the DNA polymerase often has the ability to repair any damage, making the process not only rapid but accurate at the same time.

The other strand goes through a similar but slightly different process. Instead of occurring in a single chain, the primers attach toward the 3' end of the molecule, but the polymerases attach in fragments called **Okazaki fragments,** several thousand base pairs in length, heading towards the 3' end, making up the other strand in pieces rather than a long and single piece.

Another enzyme called **DNA ligase** then joins the ends of these fragments together to form another DNA helix.

Further resources:

NobelPrize.org: Replication

YouTube - yourgenome: DNA Replication - 3D

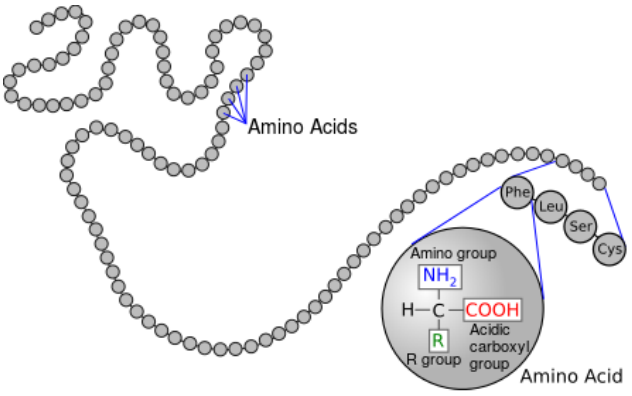
SAQ 10:

1. **Look at the websites and your notes. What is a replication fork? What is its purpose?**

In a nutshell:

In this section, we have looked at the basic structure of DNA and RNA, including the sugar-phosphate backbone and the base units. We have briefly examined the role of histones and supercoiling in the organisation of DNA within the nucleus of a cell. Finally, we discussed DNA replication.

5. Protein synthesis



Ready, steady, go!

In this section, we will examine the process of protein synthesis, focusing on the stages of transcription and translation.

Proteins are long chains composed of many amino acids joined together by peptide bonds. Protein synthesis is a two-part process that involves both nucleic acids that we have already looked at: **DNA** and **RNA**. We will explore the two parts of this process, **transcription** and **translation**, in more detail.

5.1 Transcription

Transcription occurs when the two DNA strands in the region of a specific gene that codes for a specific protein unzip from each other. Similar to the way in which DNA replicates itself, a single strand of **messenger RNA** (mRNA) is then made by pairing up mRNA bases with the exposed DNA nucleotide bases. This involves the use of an enzyme called RNA polymerase. This process is illustrated in **Figure.48**.

The completed mRNA molecule detaches from the DNA strand and moves out of the nucleus through a nuclear pore, and enters into the cytoplasm of the cell.

Further resources:

NCBI Bookshelf: An Introduction to Genetic Analysis, 8th edition - Transcription and RNA Polymerase

YouTube - NDSU Virtual Cell: Transcription

5.2 Translation

In the cytoplasm, ribosomes now attach themselves to the mRNA. Look back at *Topic 1: Introduction to Cell Biology* if you need to recap on the biological function of ribosomes and where they are found. In the ribosome, the mRNA code is translated into a transfer RNA (tRNA) code which, in turn, is transferred into a sequence of amino acids that form a protein. (**Figure. 49**)

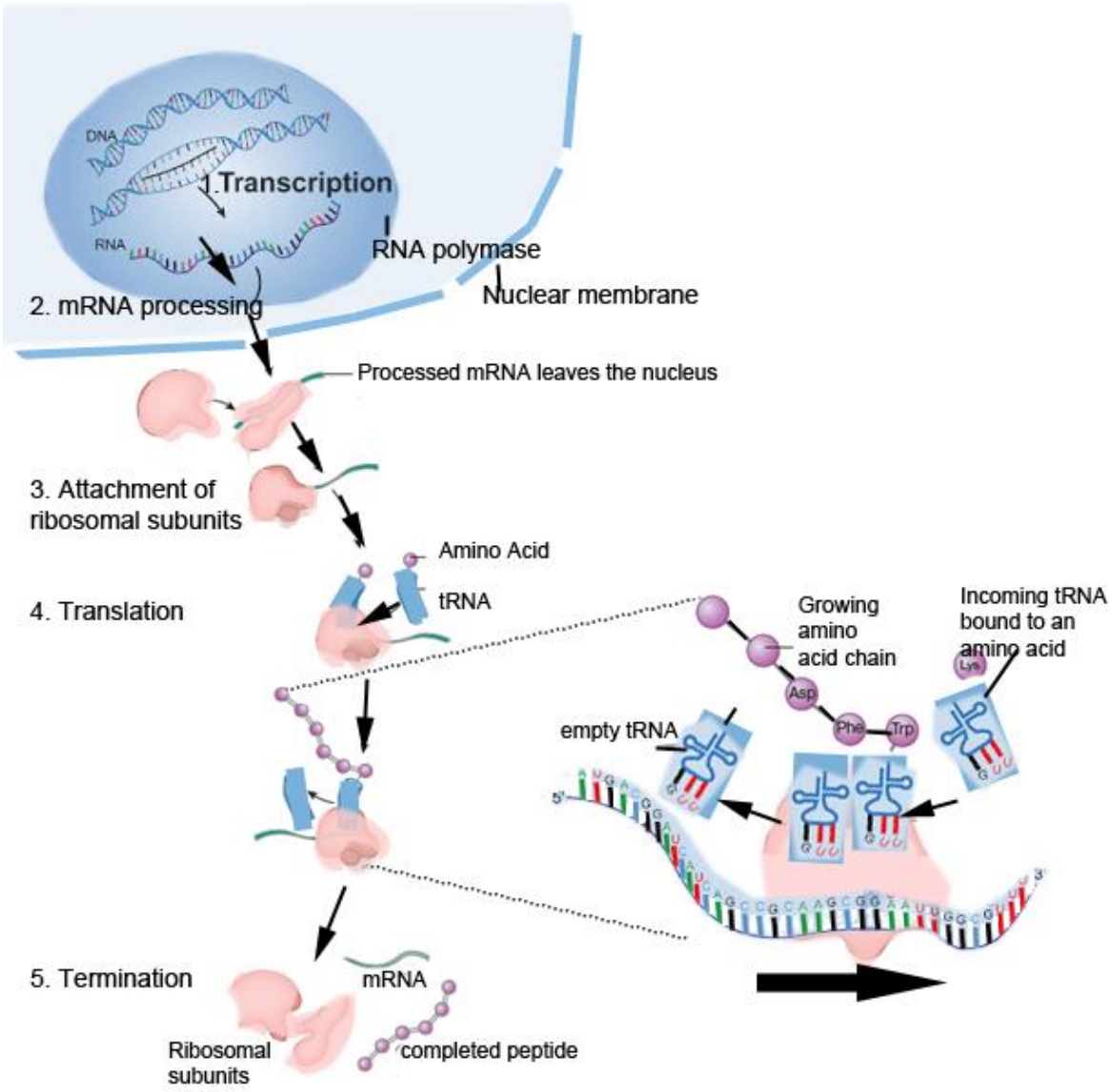


Figure 48 Transcription and Translation for an animated explanation of Transcription and Translation please see https://www.thinglink.com/scene/613961966762852354 for a closer look at the transcription and translation site.

In this process, each set of 3 mRNA bases (called a **codon**) will pair with a complimentary tRNA base triplet (called an **anticodon**). Each tRNA is specific to one of 20 different amino acid. As tRNAs are added to the sequence, amino acids are linked together by **peptide bonds**, eventually forming a polypeptide chain. The tRNA then detached from its

amino acid and will go on to then bind with another of the same amino acid to be used again. Proteins normally consist of hundreds or thousands of amino acids and during protein synthesis, this Biomolecule is methodically constructed, one amino acid after the other, until a particular triplet of bases is reached called a **stop-codon**, this signals the ribosome to stop the construction of the protein. Termination of protein synthesis is illustrated in (**Figure.49)**

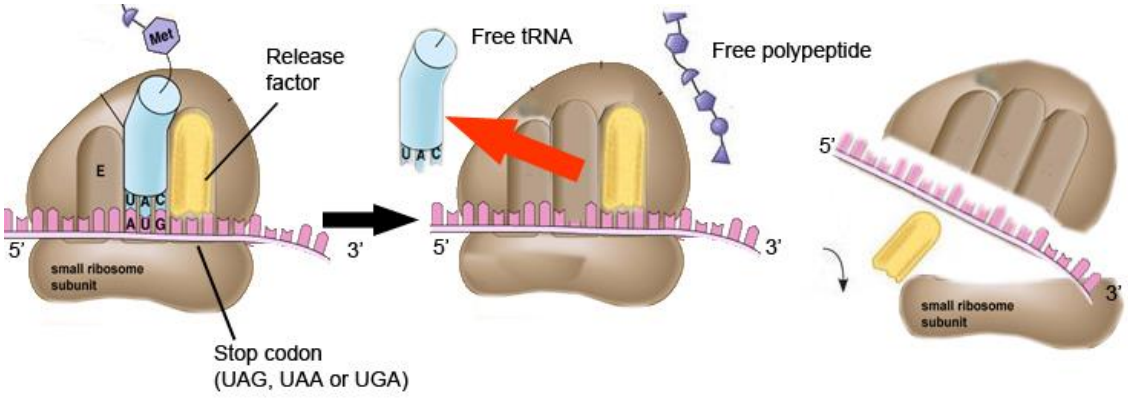


Figure 49 Termination of Protein Synthesis

After the processes of transcription and translation are complete, we are left with a protein that consists of the precise chain of amino acids as dictated by the genetic code originally held with the DNA gene.

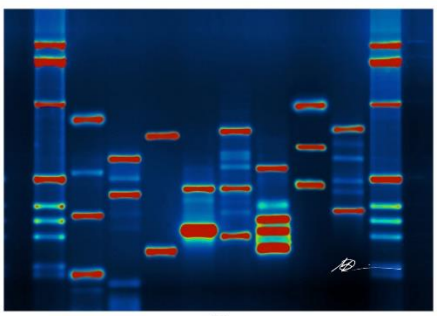
Further resources:

- **Nature.com: Nucleic Acids to Amino Acids - DNA Specifies Protein**
- **Nature.com: Transcription and Translation**
- **Transcribe and Translate a Gene Activity**
- **YouTube - CoolScience Videos: DNA Replication**

In a nutshell:

In this section, we examined the role of DNA and RNA in transcription, as well as the role of ribosomes along with mRNA and tRNA in translation.

6. Basic Principles of gene cloning and DNA analysis



Ready, steady, go!

In this section, we will examine laboratory-based procedures used to clone Human genes in Bacterial cells using vectors and also to amplify DNA in a process known as Polymerase Chain Reaction (PCR). We will then examine some of the medical applications of genetic engineering.

Genetic engineering is now used very widely for a variety of purposes ranging from manufacturing individual gene products such as human insulin to improving the yield of crop plants.

A number of different techniques are used but they usually involve the following stages (as illustrated in **Figure.50**):

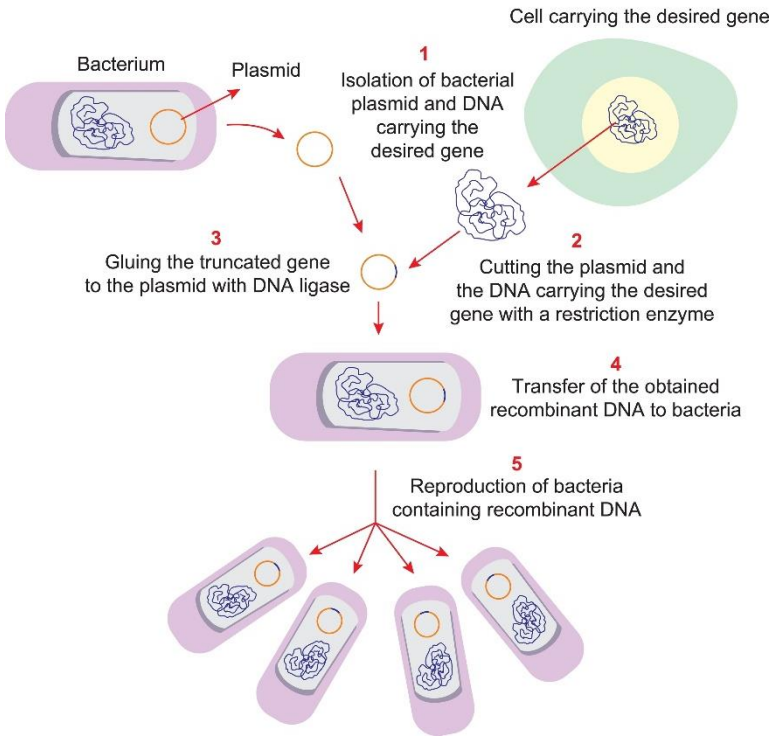


Figure 50 Stages of gene cloning.

- The isolation of the gene required to produce the product,
- Insertion of this foreign gene into the DNA of a host cell by using a suitable DNA carrier called a vector,
- Checking to find the host cells which contain the new gene,
- Multiplying or cloning the organism containing the new gene to produce large numbers of genetically identical cells or organisms for commercial use

The toolkit

In order to isolate individual genes and stick or splice them into other pieces of DNA, we need a suitable tool kit. This involves three different types of enzyme:

- **Restriction endonucleases** or restriction enzymes: In the early 1970s, researchers discovered restriction endonucleases in bacterial cells. These enzymes are now known to be part of the natural defence system of bacteria against bacterial viruses. They cut the virus DNA into small fragments and stop the infection process. There are many different types of restriction endonuclease, each one cutting the DNA at a specific nucleotide sequence. Some cut the DNA straight across. Others produce a staggered cut like that shown in **Figure. 52**. Each cut piece of DNA will have a sequence of unpaired bases called “sticky ends”. These are very important as they can join with complementary sticky ends on other pieces of DNA.

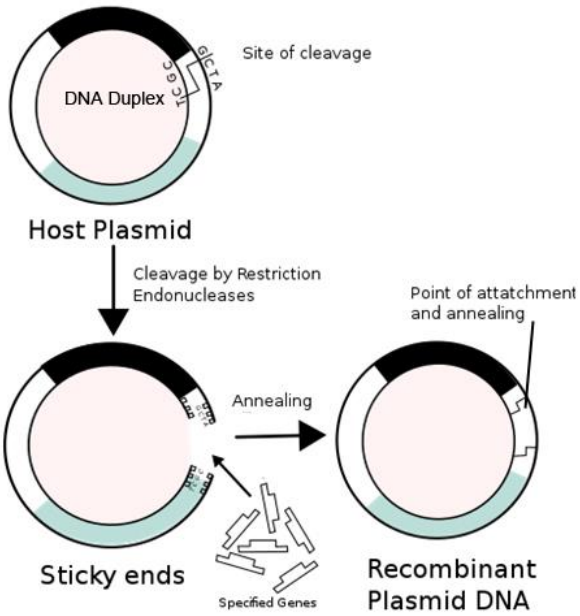
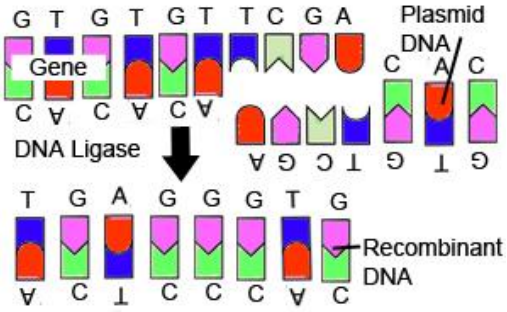


Figure 51 Sticky end formation

- **Ligase:** An enzyme which is able to join two pieces of DNA. In genetic engineering, ligase enzymes are used to join DNA from two different sources to make recombinant DNA.
- **Reverse transcriptase:** Transcription is the production of mRNA from DNA. Reverse transcriptase is therefore an enzyme which catalyses the opposite reaction. It enables a single chain of DNA to be made from the corresponding mRNA molecule. This single chain of DNA, called complementary DNA or cDNA, can then be used to make a double chain. From the mRNA in the cytoplasm of a cell we can produce a copy of the gene from which it was transcribed.

The sequence of nucleotide bases on a DNA molecule makes up the genetic code. Each triplet of three bases codes for a particular amino acid. Using this code, a cell can produce any one of the many proteins that it requires. One of the many remarkable features of the genetic code is that it is universal; the three bases CAC, for example, will code for the amino acid valine whether it is in a bacterial cell or a cell from a human liver. A gene taken

from one organism should then be able to produce exactly the same product if it is introduced into another organism. This is the basis of genetic engineering, the production of new characteristics by the insertion of a gene from one organism into another.



A gene can be joined to another piece of DNA with complementary sticky ends to form recombinant DNA (as illustrated in **Figure. 52**).

Figure 52 Formation of Recombinant DNA

6.1 Isolating the Gene

The starting point for genetic engineering involves isolating the required gene. This may be done in a number of different ways:

The DNA of an organism may be cut up using restriction enzymes. The resulting fragments will be of different size and therefore have different electrical charges. This means that they can be separated from each other by means of a technique called **electrophoresis**. Some of the fragments will contain the gene we want. Obviously, this technique is much easier in organisms such as viruses which only contain a small amount of DNA.

- Insulin is a protein. It is a hormone produced in large quantities in the b-cells of the Islets of Langerhans in the pancreas. Obviously, much of the mRNA produced by these cells should code for insulin. If we isolate mRNA from appropriate cells and incubate it with reverse transcriptase, we can produce a copy of the insulin gene.
- Since we know the sequence of bases which codes for each amino acid, we can work backwards to produce an artificial gene. What we need to do is to analyse the sequence of amino acids which make up the protein in which we are interested. We can then use a so-called 'gene machine' to assemble an artificial gene which will carry the correct code.

6.2 Producing recombinant DNA

Keyword/s:

Genetic vectors are vehicles for delivering foreign DNA into recipient cells. *Vectors* can replicate autonomously and typically include features to facilitate the manipulation of DNA as well as a *genetic* marker for their selective recognition. The most common *vectors* are DNA plasmids, viruses and artificial chromosomes.

	Source: https://www.nature.com/subjects/genetic-vectors
Recombinant DNA	Recombinant DNA, which is often shortened to rDNA, is an artificially made DNA strand that is formed by the combination of two or more gene sequences. This new combination may or may not occur naturally, but is engineered specifically for a purpose to be used in one of the many applications of recombinant DNA. Source: https://www.news-medical.net/life-sciences/What-is-Recombinant-DNA.aspx

The next problem that researchers face is to insert the required gene into the DNA of the host cell. Unfortunately, this cannot usually be done directly, so use has to be made of a vector. This is often a **plasmid** - a small, circular piece of DNA found in bacterial cells. Once such commonly used plasmid is called **pBR322**. Firstly, plasmids are isolated. They are then cut open with restriction enzymes and the new gene inserted with the aid of ligases. We now have recombinant DNA; DNA from different organisms joined in a single molecule. The plasmid is then put into the host organism. (**Figure. 53**)

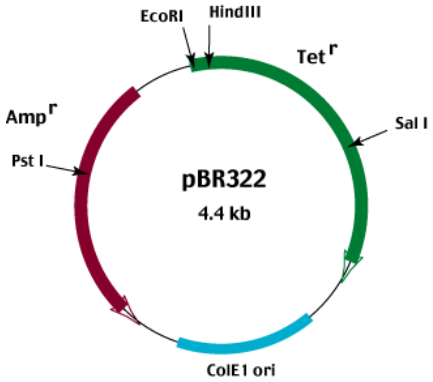


Figure 53 The plasmid pBR322

6.3 Checking the host cells

It is by no means certain that treating the plasmids in this way will ensure that they all take up the new gene. In practice, very few of them do and we are left with a mixture of plasmids, most of which do not contain this gene. There is another problem as well. If we incubate plasmids and bacterial cells, only some of the cells will take up the plasmids. We need a way to identify the few bacteria which contain the recombinant DNA. Plasmids carry genetic information of their own and the ones used in genetic engineering usually contain DNA which codes for resistance to particular antibiotics. We know that the plasmid we are interested in, the one containing the new gene, is resistant to certain antibiotics. By growing the bacteria on a medium containing these antibiotics, we can identify the ones we want.

So why is the fact that pBR322 contains antibiotic resistance genes is very important in the next part of the procedure.

It is likely that only a few of the bacteria have actually taken up the recombinant plasmid. These few bacteria have to be identified and separated from the majority of bacteria that do not contain the recombinant plasmid. Treatment with **antibiotics** can be used to achieve this.

pBR322 contains a gene that makes any bacteria containing it resistant to **ampicillin** and **tetracycline**. There is a chance however that the plasmid picked up by the bacteria does not contain the desired Human gene. Instead, it may have just reformed from its own sticky ends. However, these unmodified plasmids can be identified.

How does the position of the recombinant gene within pBR322 aid in the identification of bacteria that have taken up the recombinant plasmid?

The BamH1 restriction or cutting site is in the middle of the gene for resistance to the antibiotic tetracycline. If the human gene had been successfully inserted, the tetracycline gene would be inactive because the inserted gene will have split it in two. Any bacteria that have taken up the recombinant plasmid as opposed to the ones that did not pick up the human gene, will not be resistant to tetracycline. All bacteria that have taken up the pBR322 plasmid regardless of its recombinant nature will be resistant to ampicillin.

Figure.54 illustrates the procedure for identifying and culturing bacterial colonies that possess the recombinant plasmid containing the human gene.

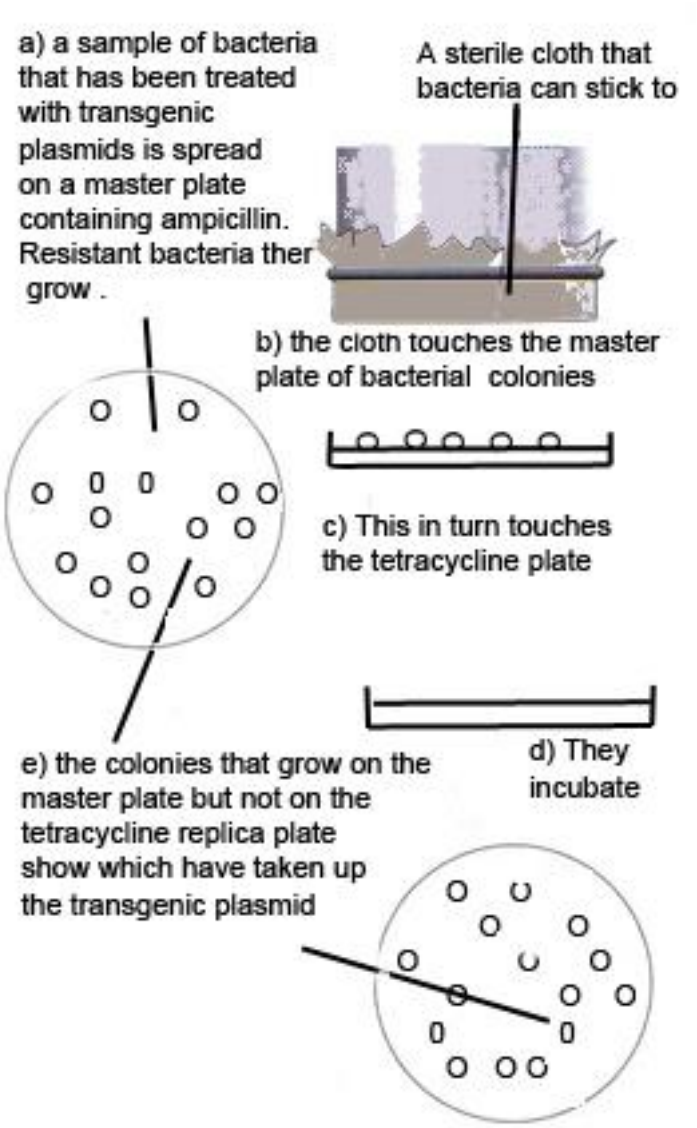


Figure 54 : Identifying recombinant bacteria

The bacteria are therefore first grown on agar plates containing ampicillin. All bacteria that have not taken up a pBR322 plasmid will be unable to grow. Small samples from the surviving colonies of bacteria are then transferred onto agar plates containing tetracycline, in a way that keeps the colonies in exactly the same position on the new plate as they were found on the ampicillin containing plate. This is called **replica plating**. Those bacterial colonies that do not grow must contain the recombinant plasmid, while those that do grow will contain plasmids that do not contain the human gene.

Therefore, any colonies of bacteria that grow on the ampicillin-containing plates, but do not grow on the tetracycline containing plates are almost certain to have taken up the recombinant plasmid and therefore contain the Human gene.

6.4 Producing the recombinant human gene.

The bacterial colonies identified as possessing the recombinant plasmid are picked off the original ampicillin-containing plate. They are cultured and allowed to multiply. This means that many copies of the bacteria and hence many copies of the recombinant plasmid are made. This clones the Human gene.

Transgenic E. coli are nowadays cultured in large fermenters on a commercial scale. The bacteria follow the genetic code on the human DNA that they contain, synthesising the human protein in vast quantities. The human protein is then extracted from the bacteria and purified (**Figure. 55**).

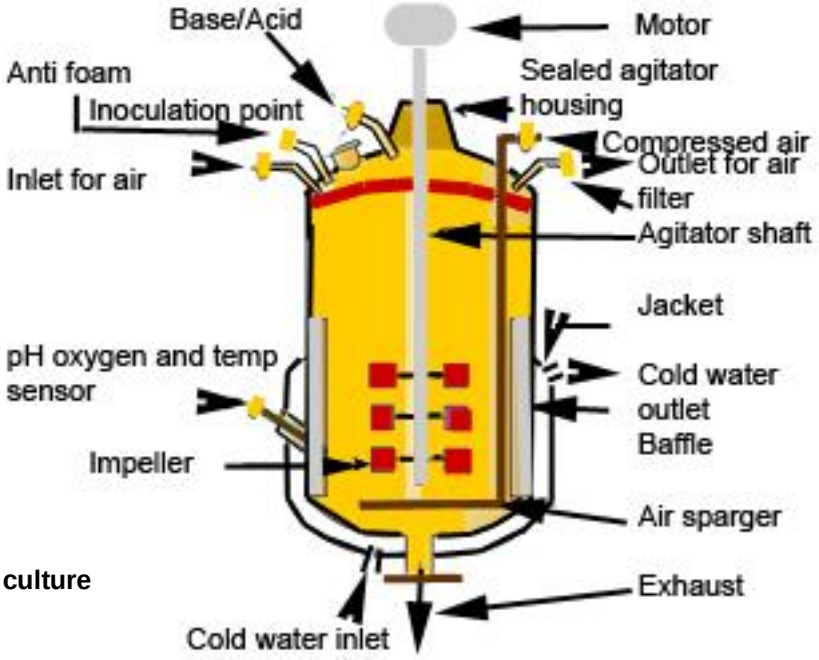


Figure 55 Fermenter used to culture recombinant bacteria.

6.5 Applications of Recombinant DNA Technology

Making products from genetically modified organisms

- Human insulin

It has been estimated that there are approximately 60 million people in the world who have diabetes and who require daily injections of insulin. At one time, this insulin was obtained from the pancreases of pigs and cattle. This only produced limited supplies and, in addition, insulin from these sources could produce an allergic reaction since it was a foreign protein. Genetically-engineered human insulin is cheaper to produce and less likely to produce allergic reactions.

- Bovine somatotrophin (BST)

This is a hormone produced in very small quantities in the pituitary gland of a cow. It stimulates growth of young animals. If given to older cows, it stimulates milk production by increasing the cow's appetite and by diverting more of the food intake towards milk production. Several countries have used genetically-engineered BST to increase milk production.

- Interferons

Interferons are naturally occurring chemicals produced in very small quantities when the cells of a mammal are attacked by viruses. Interferons can be used to treat certain types

of cancer. Only with the development of genetic engineering techniques, however, has it become possible for enough interferon to be produced for medical use.

SAQ 11:

1. **Briefly describe the function of the following enzymes in gene cloning;**
   i. **Reverse Transcriptase;**
   ii. **Ligase.**
2. **Summarise the key stages involved in genetic engineering.**

6.6 Polymerase Chain Reaction (PCR)

DNA replication can be made to occur artificially and repeatedly in a laboratory process called polymerase chain reaction (PCR). PCR is a very accurate process which enables many identical copies to be made from a single strand of DNA. To start the process short strands of DNA called primers are needed. These provide the starting sequence for DNA replication. The following are also required; the double stranded DNA molecule to be replicated, free nucleotides (A, T, G and C) and the enzyme DNA polymerase.

This process which is carried out in an automated PCR machine is illustrated in (**Figure.56)** below.

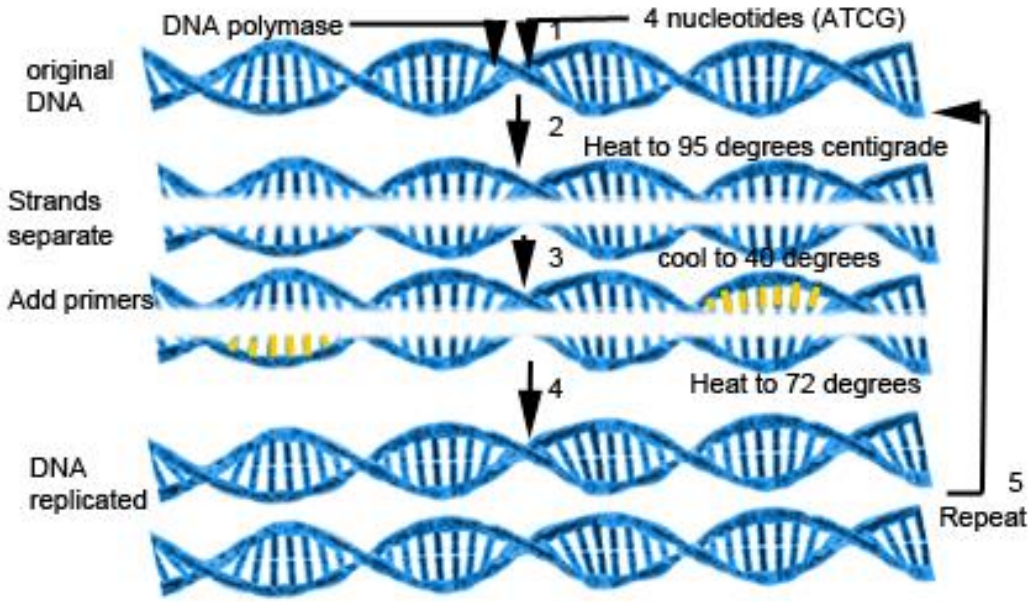


Figure 56 The five stages of polymerase chain reaction

The diagram shows the events that occur during PCR, because this is a cyclic process many identical copies of the original DNA strand can be produced, very quickly**.**

The Stages of PCR

1) **Adding the ingredients**: To start with a sample of the DNA to be amplified, a mixture containing the four nucleotides required to form a copy of the DNA, and the enzyme DNA polymerase that joins the nucleotides together, are added to PCR machine.
2) **Strand separation**: The double-stranded DNA is split into two single-stranded templates by heating it to 95ºC for about 30 seconds. This breaks the hydrogen bonds between complementary base pairs that hold the double-stranded DNA together.
3) **Binding of the primers**: The mixture is suddenly cooled to around 37ºC for a further 30 seconds. This allows DNA primers that are around 20 base pairs in length to bind or anneal to their complementary sequence on the now separated DNA strands.
4) **DNA synthesis**: The mixture is heated to 72ºC and kept at this temperature for 60-120 seconds. This is the optimum temperature for the DNA polymerase enzyme. The polymerase used in PCR is extracted from a thermophilic bacterium that grows naturally in hot springs. The DNA polymerase uses the free nucleotides present in the mixture to build new strands starting from the primers. Each original DNA molecule has now been replicated to form two molecules.
5) **Repeat cycling**: The mixture is heated again to 95ºC to separate the newly formed DNA strands. The cycle of cooling and heating is repeated. The number of copies of the original DNA doubles with each cycle. After 25 cycles, more than a million copies of the DNA will have been made.

6.6.1 The Uses of PCR

1) Rapid amplification of tiny fragments of DNA using PCR enabled several techniques such as southern or northern blot hybridization even when the amount of sample material available was very small.
2) Study of gene expression patterns is another common application of PCR, where in cells or tissues are analysed in different stages to check for expression of a specific gene. PCR can be used here to quantitate the level of gene expression.
3) PCR also assists techniques like DNA sequencing using which segments of DNA from an area of interest can be easily amplified to study genetic mutations and their consequences.
4) The Human Genome Project used PCR to indicate the presence of a specific genome segment in a particular clone. This enabled mapping of the clones and collating results from several laboratories.
5) Advanced variants of the PCR technique have been found to be useful in chromosomal analysis techniques that can help in early detection of genetic birth defects in children.
6) PCR augments the traditional method of DNA cloning by amplifying tiny DNA segments for introduction into a vector. By altering the PCR protocol, site-directed or general mutations can be achieved in the DNA fragment of interest.

Further resources:

Southern and Northern blotting hybridization

SAQ 12:

List the 5 key stages in Polymerase Chain Reaction (PCR).

6.7 Agarose Gel Electrophoresis

Agarose Gel Electrophoresis is a technique often used by scientists to separate molecules such as DNA and proteins. The material being separated is placed into a gel-like platform called agarose. Agarose is an organic substance derived from seaweed and when used in the lab, it has a similar consistency to a thick jelly. As such, it is prepared in a similar way.

The agarose is a fine powder that is mixed with water and a buffer solution. The mixture is heated to its boiling point. This allows the agarose to dissolve fully and disperse evenly. The solution is then poured into a casting mould. The liquid takes the shape of the tray and is allowed to set for a period of twenty to thirty minutes or until it solidifies.

In order to separate molecules, there must be tiny slits made in the agarose gel by placing a “comb” into the mixture before it solidifies. The resulting wells (as illustrated in ***Figure. 57***) can be filled with the particular substances to be separated. The substances are separated based on their overall charge and size.

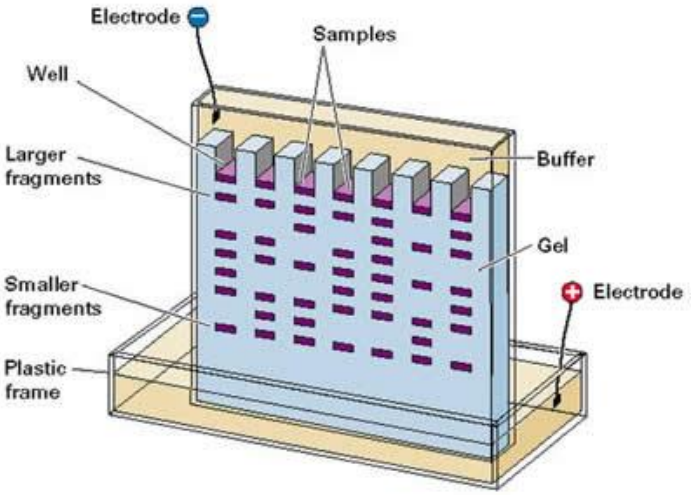


Figure 57 Electrophoresis apparatus

The resulting gel is a matrix of agarose with tiny holes between each agarose molecule. A direct current (DC) is passed through the gel. Positively charged molecules migrate towards the negative end of the current while the negatively charged molecules travel towards the positive end of the current.

The gel itself, acts as an obstacle course for the substances as they migrate to the different ends. Smaller molecules move faster through the holes between the agarose molecules. The larger the molecule the more time it takes for it to travel. At the end of the experiment the gel has various bands of a separated substance

One of the advantages of gel electrophoresis is that scientists can separate several samples side by side so they can compare them. The comparison of separated DNA molecules is the basic method behind the *DNA fingerprints* that forensic scientists use to compare samples from crime scenes with those of suspects.

Scientists conduct gel electrophoresis by inserting molecules such as DNA into the *wells* within agarose gel. They then place the gel in a box called an *electrophoresis chamber* that's filled with a salty, electricity-conducting buffer solution.

The DNA molecules, which have a negative charge, move toward the gel box's positive electrode because opposite charges attract. When the scientists run an electrical current through the gel, the gel becomes like a racetrack for the DNA molecules as they try to get to the positively charged end of the box.

When the power is turned off, all the DNA molecules stop where they are in the gel, and the scientists stain them. The stain sticks to the DNA, creating stripes called *bands.* Each band represents a collection of DNA molecules that are the same size and stopped in the same place in the gel (as illustrated in ***Figure.58***).

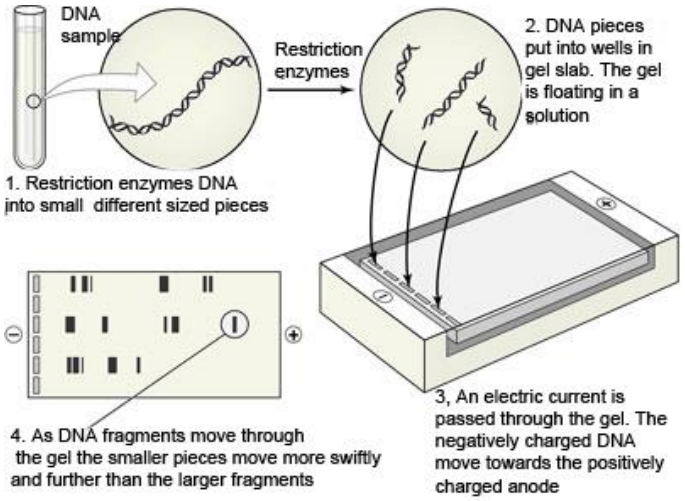


Figure 58 Illustrated method for separating DNA using gel electrophoresis.

In order to work with the information from the gel more easily, scientists can make an identical copy of the gel by transferring the DNA molecules to a thin sheet of nylon or nitrocellulose, a strong but flexible material that binds to DNA. This procedure is called making a *blot* of the gel (this is illustrated in ***Figure. 59*** below). A blot on a thin, flexible material can be handled, whereas the original slab of gel can crack and break.

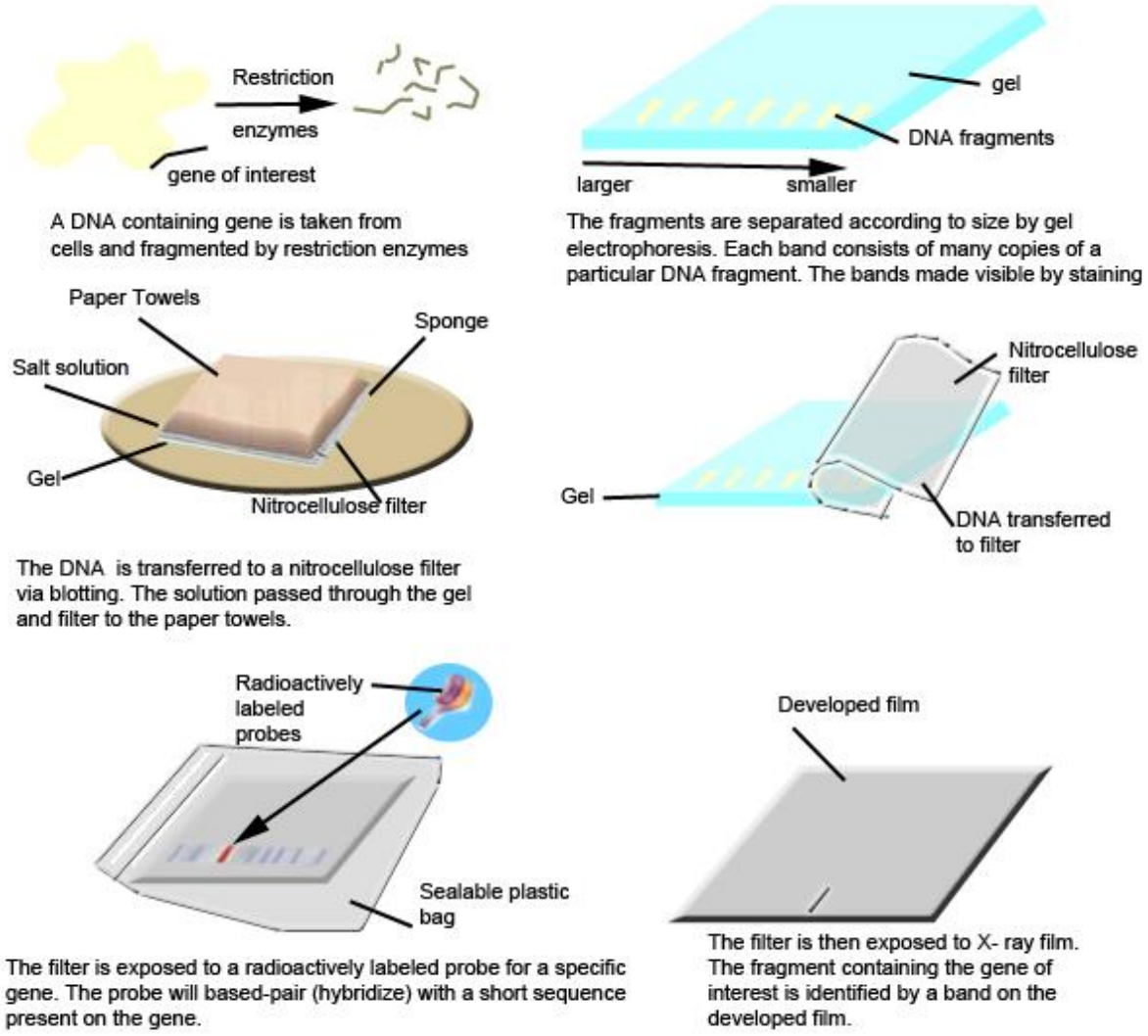


Figure 59 Southern Blotting analysis of DNA.

6.8 Applications of gel electrophoresis

Gel electrophoresis is widely used in the molecular biology and biochemistry labs in areas such as forensic science, conservational biology, and medicine.

Some key applications of the technique are listed below:

- In the separation of DNA fragments for DNA fingerprinting to investigate crime scenes.
- To analyse results of polymerase chain reaction.
- To analyse genes associated with a particular illness.
- In DNA profiling for taxonomy studies to distinguish different species.
- In paternity testing using DNA fingerprinting.

- In the study of structure and function of proteins.
- In the analysis of antibiotic resistance.
- In blotting techniques for analysis of macromolecule.
- In the study of evolutionary relationships by analysing genetic similarity among populations or species.

Let's recap this topic:

In this topic, we have looked at some of the important macromolecules in organisms, such as nucleic acids, carbohydrates, lipids and proteins. We also investigated the structure and functions of biological catalysts (enzymes) and examined the effects that temperature, pH and inhibition will have on enzyme-controlled reactions. We completed the topic by addressing recombinant DNA technology, PCR and gel electrophoresis, considering medical applications related to these methods.

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Section.33: From ADP to ATP

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Figure. 39: Nucleotide structure consisting of a phosphate group, a ribose sugar and a base. Courtesy of the National Human Genome Research Institute

Figure 40: Nucleotide bases between two sugar-phosphate backbones Creative Commons Attribution 3.0 Unported license.https://commons.wikimedia.org/wiki/User:

Figure. 41: Watson and Crick's DNA double helix Zephyris

Figure. 42: From Chromosome to DNA to a gene. The National Cancer Institute https://www.cancer.gov/publications/dictionaries/genetics-dictionary?cdrid=46470

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Figure. 44: The roles of mRNA and tRNA in translation.

Figure. 45: Deoxyribose sugar with numbered carbons

Figure. 46: Anti-parallel DNA strands:

Figure. 47: The Process of DNA Replication

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Figure.57. **Electrophoresis apparatus**

Figure. 58. **Illustrated method for separating DNA using gel electrophoresis.**

Figure. 59. **Southern Blotting analysis of DNA. DistanceLearningCentre.com Graphic**