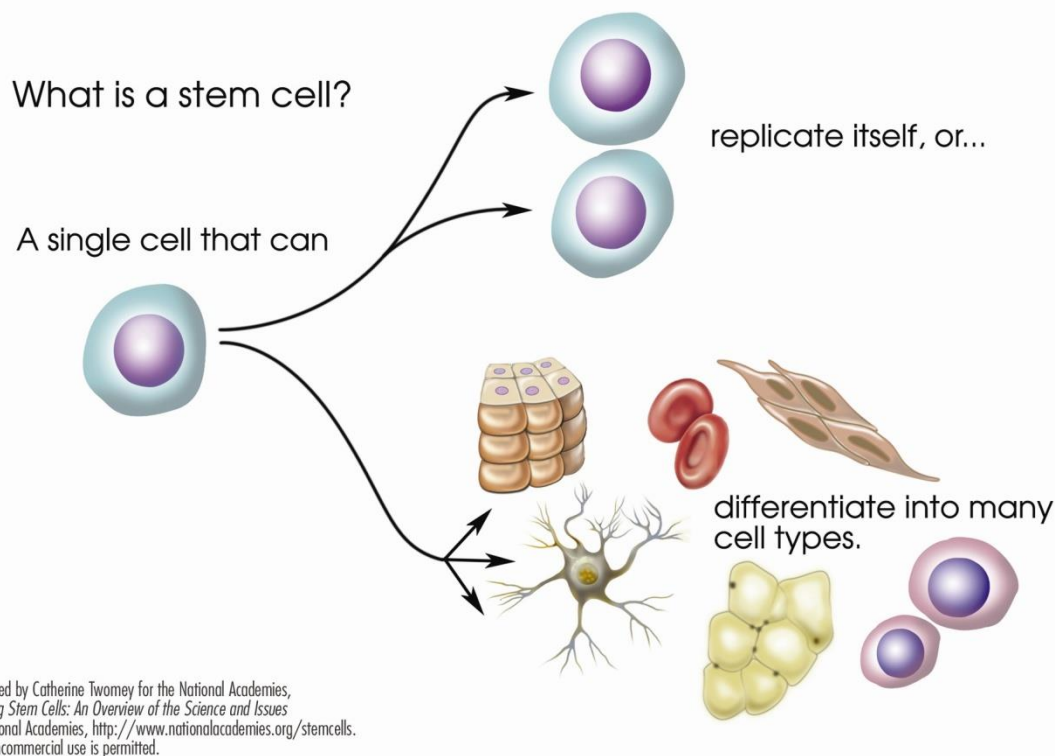


STEM CELLS, DIFFERENTIATION & CANCER CELLS

Stem cells are undifferentiated biological cells, that can differentiate into specialized cells and can divide (through mitosis) to produce more stem cells. There are two types - embryonic stem cells and tissue (adult) stem cells.

→ What are stem cells? Be able to describe both types.



Embryonic stem cells come from 4-5 day old embryos. These stem cells are pluripotent, that is, they can differentiate into all cell types. There are various ethical considerations when using these types of stem cell - including how they were created, how they are used, and ultimately, the destruction of the embryo to harness the stem cells.

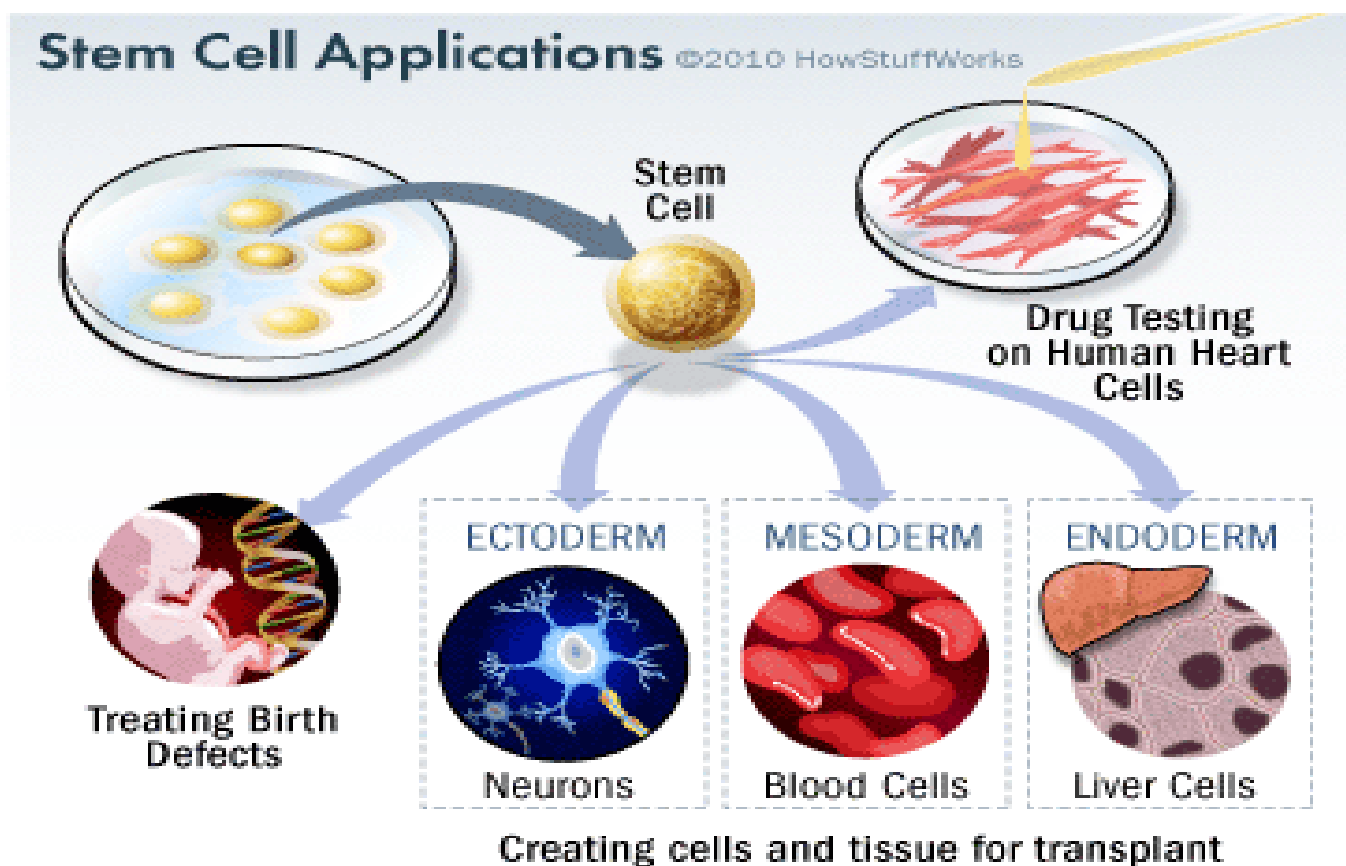
→ Discuss the ethical considerations that must be taken into account when using embryonic stem cells for research.

Embryonic stem cells could be used to treat or cure a variety of conditions including Parkinson's disease, juvenile diabetes and spinal cord injuries etc.

Tissue (adult) stem cells are multipotent - they can only differentiate into limited cell types found in that tissue e.g. bone marrow. Bone marrow cells can differentiate and become specialised to form the red and white blood cells of the immune system. Tissue stem cells can be used in the treatment of diseases such as leukaemia, where a bone marrow transplant is often used.

→ Describe the differences between embryonic and tissue stem cells.

→ Give examples of some conditions or diseases that may be treated using stem cells in the future.



Occasionally, cells can grow and divide uncontrollably, forming a mass of cells known as a tumour. This type of cell growth can lead to cancer, either benign (non cancerous) or malignant (cancerous).

Benign tumours are discrete groups of abnormal cells, but are localised in one place and are surrounded by otherwise healthy, normal tissue. Common examples include warts, and benign tumours can often be easily removed.

Malignant tumours are when the surface cells break away from the original group, enter the circulatory system and spread through the body. This can often mean secondary tumours can develop in many areas of the body. This is known as metastasis.

→ What is a) cancer b) a tumour?

→ Describe the difference between malignant and benign melanomas.

→ Explain why some women with breast cancer can also develop cancers of other areas of the body e.g. lymph nodes or the brain.

Most cancers occur due to mutations in the genes controlling cell division. As the mutation increase in number, uncontrolled division occurs, and cannot be controlled by normal mechanisms.

→ Name some common cancers and describe why these types are most common.

In the differentiation of cells, specialisation occurs through gene expression. This simply means that genes can be turned on or off, giving rise to specialised cell types.

→ Using a named example, describe the specialised features of a cell in a human tissue

In a simple example of gene expression, the lac operon on *E.Coli* uses three genes - the regulator, the operator and the structural gene to produce an enzyme. This model can explain how genes can become switched on and off, and how genes can be expressed to produce certain proteins. This process also links into how metabolic pathways operate and are controlled.

→ Describe how the enzyme  $\beta$ -galactosidase is produced in a) the presence of lactose sugar and b) what happens when lactose sugar is absent?

## DNA & DNA REPLICATION

DNA is deoxyribonucleic acid. It is a double stranded molecule, that twists to form a double helix. DNA is made up of many nucleotides. A nucleotide consists of deoxyribose sugar, a phosphate group and an organic nitrogenous base. There are four bases present in DNA.

- Name the four bases present in DNA.
- Draw and label the structure of a DNA nucleotide

Often in past paper questions, you will be asked to work out the proportion of bases present in DNA. For example:

- A DNA molecule consists of 4000 nucleotides, of which 20% contain the base adenine. How many of the nucleotides in this DNA molecule will contain guanine?

DNA is tightly coiled in chromosomes, and is found wrapped around protein molecules called histones.

The 3' end of DNA is found on the deoxyribose sugar, whereas the 5' end is at the phosphate group. The DNA strand grows from the 3' end (as new nucleotides are added). This forms two strands running in antiparallel directions: The left hand strand (leading strand) and right hand strand (lagging strand).

- Draw the structure of the 5' to 3' double stranded DNA molecule running in antiparallel directions.

- State where new nucleotides are added in a DNA molecule.

DNA replication is a semi conservative process.

- Explain what is meant by the term semi-conservative replication.

DNA replication is controlled by two enzymes.

→ Name the two enzymes used in DNA replication, and describe their function.

DNA replication begins with the DNA molecule unwinding, the H bonds breaking between base pairs, and the two strands of the molecule separate 'unzip'.

After unwinding and unzipping, new nucleotides base pair with the template DNA strand in a replication fork.

→ What is a primer?

→ Compare replication of DNA on the leading strand, to the lagging strand. Why does this occur?

→ What other compounds are required for DNA replication to occur?

→ Explain why the accurate replication of DNA is so very important.

## RNA

RNA is another type of nucleic acid - ribonucleic acid. It has a very similar structure to DNA in that it is made up of nucleotides, containing a sugar, a phosphate group and organic nitrogenous bases. There are key differences however, as RNA is a single stranded molecule.

→ Name the four bases present in RNA.

→ Draw and label the structure of a RNA nucleotide

## PROTEIN SYNTHESIS

The structure, shape and function of a protein depends on the sequence of amino acids coded for by our DNA.

To allow the code to be made into a protein or polypeptide, the DNA code is first changed into messenger RNA (mRNA), which will leave the nucleus. This process is called transcription. A region of DNA, called a promoter, initiates transcription.

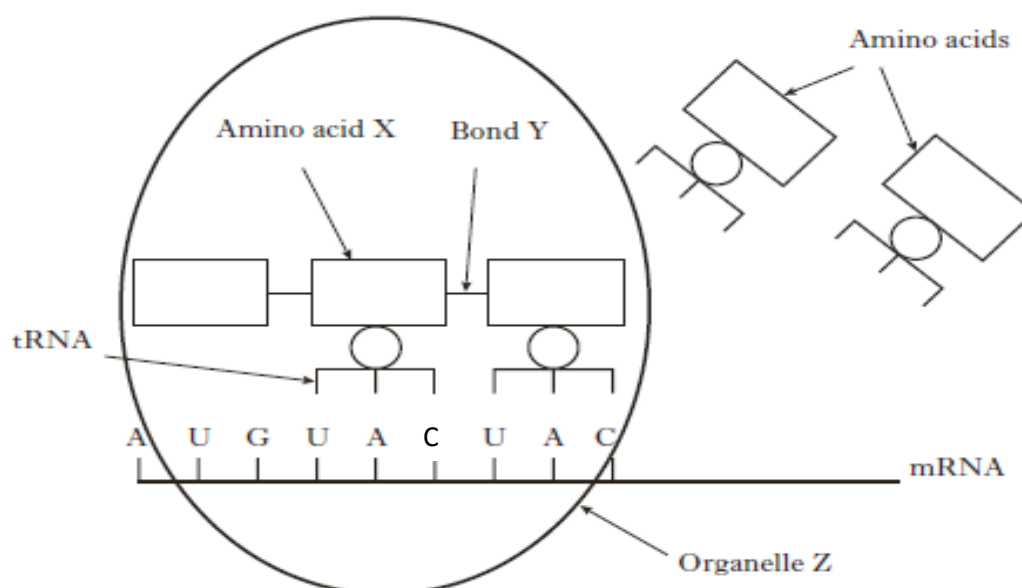
RNA polymerase is the enzyme used to add RNA nucleotides to the 3' end of the DNA strand. Due to base pairing, a complementary copy of DNA is taken by the mRNA molecule. mRNA will stop being synthesised when a terminator sequence of DNA is reached. The section of mRNA formed is known as the primary transcript.

The extra information transcribed from DNA contains coding regions called exons, as well as non coding regions called introns.

Introns are cut out of the primary transcript, and exons are spliced (stuck) together. The finished mRNA then passes out through the nuclear pore into the cytoplasm, ready for translation.

mRNA meets transfer RNA (tRNA) at the ribosome, in the cytoplasm. tRNA molecules carry specific amino acids to the ribosome on an attachment site. Translation of the mRNA into amino acids to form a protein is called translation.

Anticodons on tRNA are complementary to specific codons on mRNA.



→ What is a) a codon b) an anticodon?

→ From the previous diagram, name: (i) organelle Z

(ii) the sequence of bases on anticodon X

(iii) bond Y

→ Write down the sequence of DNA that coded for the mRNA sequence above.

The mRNA codon AUG is a start codon (and also codes for the amino acid methionine). Translation will continue until a code stops the process. These codes are called stop codons. mRNA codons UAA, UAG and UGA are all stop codons.

→ What would happen if the second mRNA codon in the sequence shown was UAG rather than UAC?

→ Why does the codon AUG initiate the process?

Ribosomes are composed of rRNA (ribosomal RNA) and proteins.

Once translation is complete, proteins often need to be modified before they can fully function. Fully completed proteins need to be folded, coiled, cleaved or a functional group added. Proteins can then be secreted out of the cell.

→ Give an example of a group that can be added to a protein molecule.

Proteins are made up of four basic elements:

Carbon (C)

Oxygen (O)

Hydrogen (H)

Nitrogen (N)

Often, proteins have the additional element, sulphur (S), present.

There are 20 amino acids. Amino acids join to form proteins. Different proteins have different amino acid sequences.

Proteins have a 3D shape. This shape is due to various bonds that form between amino acids, and between elements.

→ Name the bonds which can form in proteins.

## MUTATIONS

A mutation is a change to an organisms DNA, and can be either a gene mutation, or a chromosome mutation. A change to a gene or a chromosome may result in a protein that does not function properly, or the protein may not even function at all.

Single gene mutations involve the change to one or more nucleotides (and therefore one or more amino acids). There are two types of gene mutation, point mutations and frameshift mutations.

→ What is the difference between a point and a frameshift mutation?

There are four examples of point mutations: substitution, missense, nonsense and splice site mutations.

→ Describe each of these gene mutations, and give an example of a genetic condition linked to each type.

There are two examples of frameshift mutations: insertion and deletion.

→ Describe each of these gene mutations, and give an example of a genetic condition linked to each type.

In chromosome mutations, sections of chromosomes are broken, and then joined together again, forming a new sequence or a new number of genes.

For chromosome mutations, there are three main types: deletion, insertion and translocation.

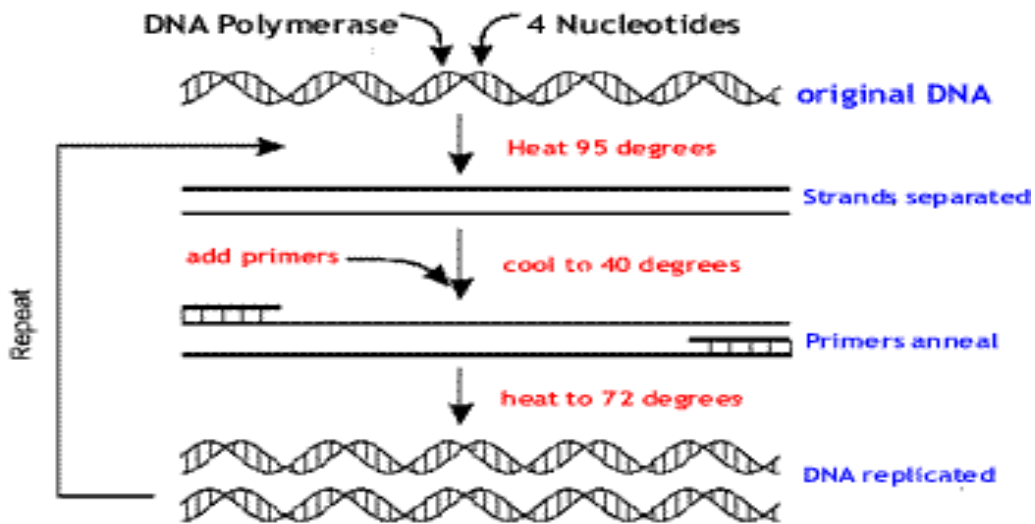
→ Describe each of these chromosome mutations, and give an example of a genetic condition linked to each type.



## GENOMICS

PCR is a type of technology which amplifies DNA.

→ What is meant by the amplification of DNA?



There are several stages involved in PCR. Firstly, a primer is used to identify a specific target sequence of DNA.

→ What is a primer?

DNA is then heated to separate the strands. Cooling allows primers to bind to target sequences. Heat tolerant DNA polymerase then adds nucleotides to the primers on the 3' end of DNA, replicating the region of DNA.

→ Why is heat tolerant DNA polymerase used?

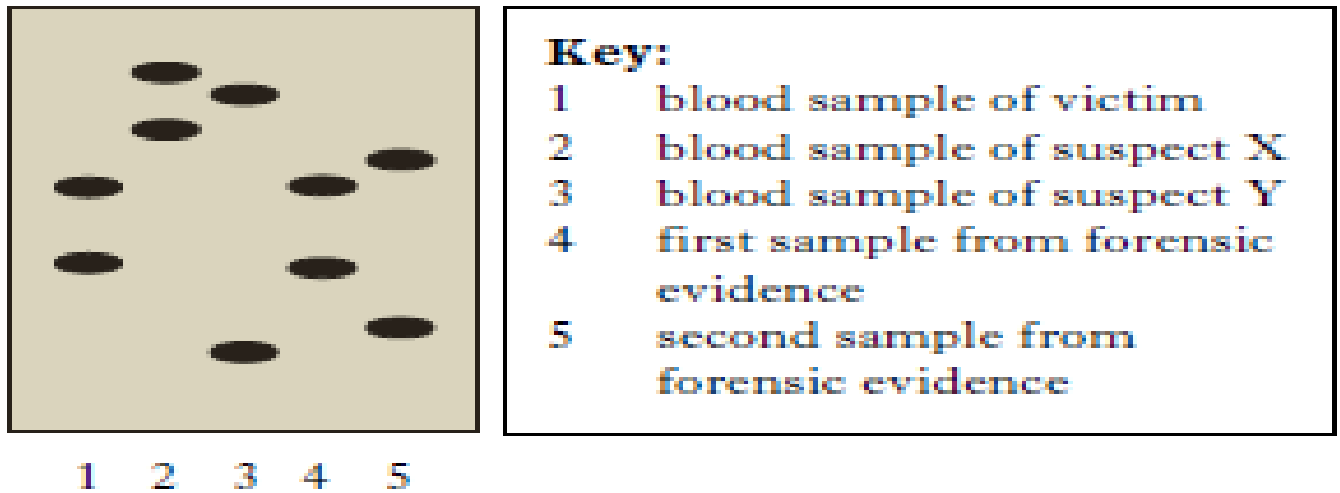
Repeated cycles of heating and cooling amplify this region of DNA.

In other types of DNA technology, probes are used.

→ What is the difference between a primer, and a probe? What type of DNA technology are probes involved in?

DNA can be used in a wide range of medical or forensic and cases.

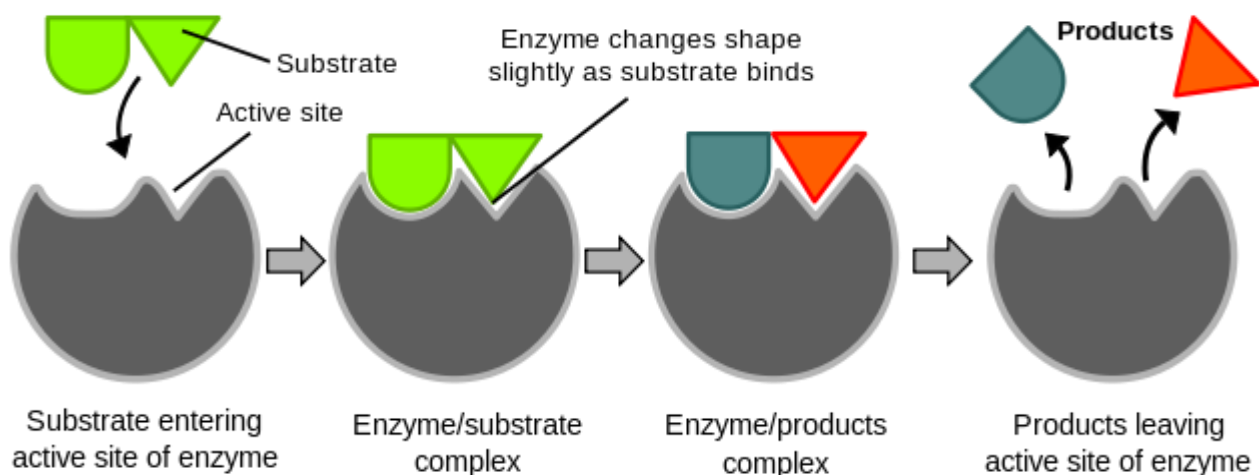
→ Give some examples of the use of DNA technology in these types of cases



→ What could be concluded from the above DNA samples?

## ENZYMES

Enzymes are biological catalysts. Catalysts speed up the rate of a chemical reaction. Catalysts take part in reactions but remain unchanged at the end of the reaction. Catalysts lower the activation energy required for a chemical reaction to take place.



As enzymes are made of protein, they have a particular 3D shape, and all enzymes have specific active sites that allow them to act on only one type of substance.

Enzymes have an affinity to their substrate molecule. As the two combine, the shape of the active site changes slightly - this is known as induced fit. This allows the active site and substrate to fit together very closely, and the chemical reaction takes place.

When a substrate attaches to the active site on the enzyme, the active site orientates or lines up the molecules. This causes induced fit - the enzyme changes shape slightly so that the active site fits better. This lowers the activation energy required for the reaction to occur. Products are released as they have a low affinity for, or attraction to the active site.

Chemicals known as inhibitors can slow down the rate of an enzyme reaction, or can even stop it completely. There are three types of inhibition - competitive, non-competitive and feedback inhibition.

→ Describe competitive and non-competitive inhibition, using diagrams where appropriate.

Temperature, pH and increasing substrate concentration can all affect enzyme activity.

→ Describe the effect of increasing substrate concentration on the activity of an enzyme

Enzymes can have two forms - active and inactive - where the key difference is the shape of the enzyme. When a regulatory molecule binds to a non active site, the active form of the enzyme will be produced, simply due to a change in shape. Inactive forms will not affect the cells, glands or tissues that are producing them, as they are only activated when necessary.

→ Give an example of an enzyme which has an active and an inactive form, and describe why this occurs.

## METABOLIC PATHWAYS

In many reactions, one chemical is changed to another, then to another via a series of enzyme controlled steps. This forms a metabolic pathway. Each enzyme is coded for by a particular gene, and as long as the enzyme proteins are correctly functioning, the pathway proceeds. Many metabolic pathways also contain alternative routes. This usually occurs when there is a plentiful supply of one metabolite in a pathway.

→ Give an example of an alternative route in glycolysis.

End product inhibition takes place with enzymes in a metabolic pathway (feedback inhibition). As the concentration of the final product increases, some of these molecules bind to enzyme 1 in the pathway, slowing down the reaction, and regulating the process. Similarly, if the concentration of the final product decreases, less molecules of the first enzyme are affected, increasing the rate of the reaction. This is how a metabolic pathway are be regulated.

→ Give an example of feedback inhibition in the citric acid cycle

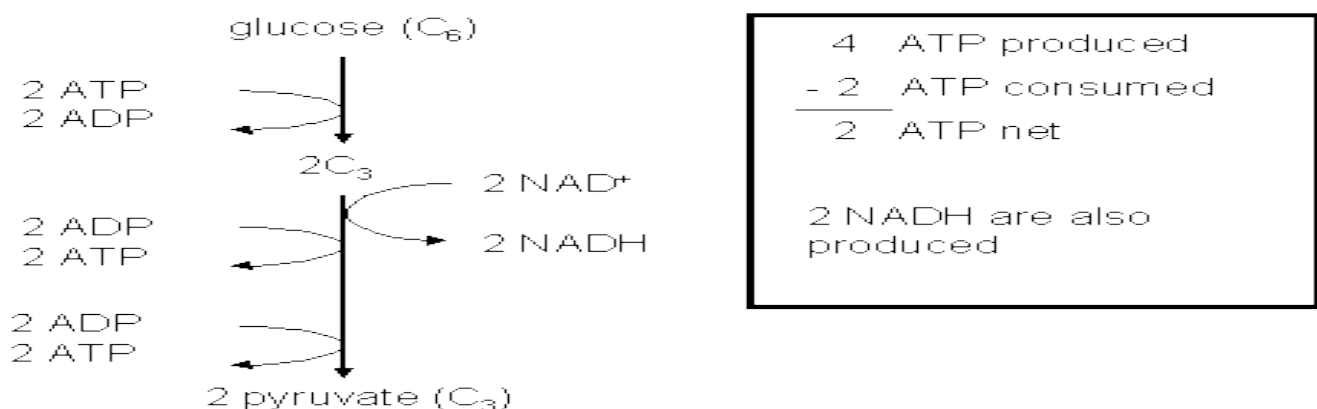
## RESPIRATION

To release energy, ATP is broken down into ADP + phosphate (P). To build up energy, ADP + phosphate (P) is built up into ATP. To produce ATP, H<sup>+</sup> ions are pumped across the membrane of the mitochondrion. The movement of H<sup>+</sup> ions from a HC to a LC across the membrane rotate ATP synthase molecule, producing ATP from ADP and P.

In glycolysis, glucose (a 6C compound) is broken down into pyruvate. Glycolysis is a series of enzyme controlled steps, and occurs in two main stages:

Stage 1 - 2ATP are used per glucose molecule in an energy investment phase. Dehydrogenase enzymes release H<sup>+</sup> ions, which are then held onto by NAD (a carrier molecule)

Stage 2- 2 ATP are produced in the energy payoff phase. This means that a net gain of 2ATP is produced per glucose molecule in glycolysis.



Respiration can only progress through the remainder of glycolysis by the action of the enzyme phosphofruktokinase.

This enzyme functions as a key regulatory point, and can slow down glycolysis if too much ATP is being produced. A high concentration of citrate can also inhibit this enzyme, slowing down glycolysis. If ATP levels or citrate levels drop, the enzyme is no longer inhibited, and glycolysis speeds up once more. This ensures that ATP is only made when needed, compounds do not needlessly build up and resources are conserved.

Only when oxygen is present (aerobic) can this next stage of respiration occur.

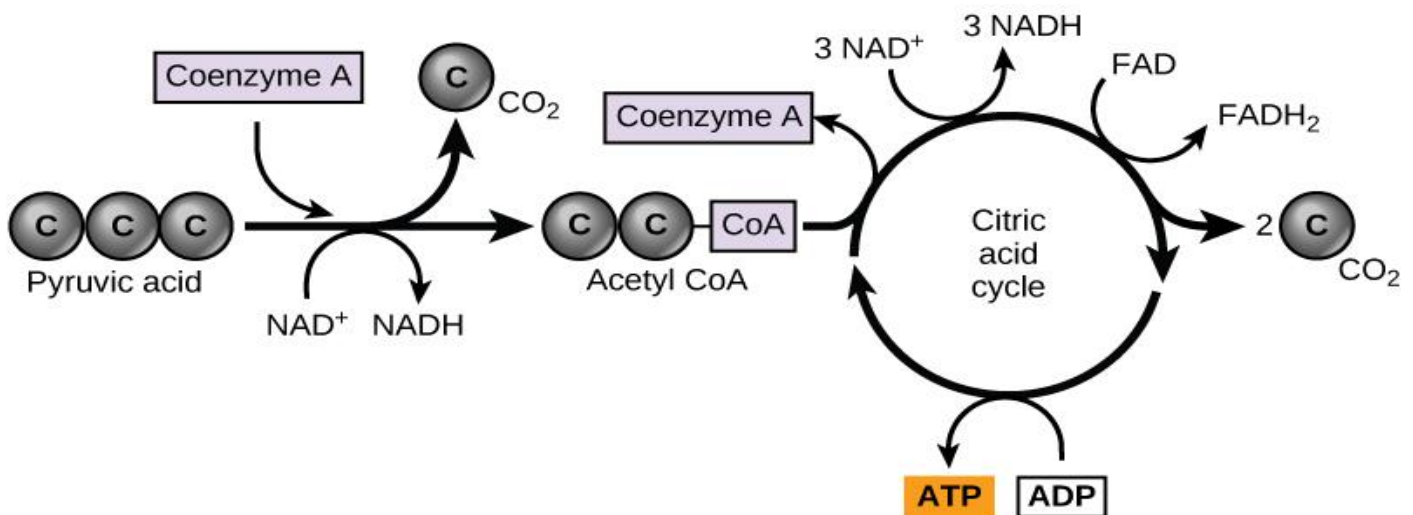
In the next stage of respiration, pyruvate is broken down into an acetyl group, and carbon dioxide is released. Acetyl combines with a coenzyme A molecule to form acetyl coenzyme A (acetyl coA).

H<sup>+</sup> ions are also released, and become bound to NAD forming NADH<sub>2</sub>.

Each molecule of acetyl coA (a 2C compound) combines with oxaloacetate (a 4C compound) to form citrate (6C) and enter the citric acid cycle.

At each step where compounds are changed into other compounds, H<sup>+</sup> ions are released, and held onto by NAD (forming NADH<sub>2</sub>). Similar reactions occur with another coenzyme molecule called FAD (H<sup>+</sup> ions join to FAD forming FADH<sub>2</sub>).

2 ATP is made during the citric acid cycle, and more carbon dioxide is also released.



The electron transport chain occurs on the folded inner membrane of the mitochondrion known as the cristae. It is made up of a group of protein molecules, and the high energy electrons (from NAD and FAD), move along the transport chain, releasing energy. This flow of H<sup>+</sup> ions cycles ATP synthase, producing ATP.

- How much ATP is produced from a) each of the stages and b) from one molecule of glucose
- What is the final hydrogen acceptor?

## ANAEROBIC RESPIRATION

During exercise muscles do not generate enough oxygen for aerobic respiration, especially the electron transport chain. Pyruvate is converted to lactic acid, involving the transfer of hydrogen from NADH produced during glycolysis. This regenerates the NAD needed to maintain ATP production by glycolysis. Lactic acid builds up in muscles causing fatigue - an oxygen debt. When exercise stops lactic acid is converted back into pyruvate.

Skeletal muscles are made up of both slow twitch and fast twitch muscle fibres.



Slow twitch muscle fibres contract slowly, but maintain contractions for a long time. They rely on aerobic respiration to produce ATP, and therefore contain many mitochondria, as well as a good blood supply and high quantities of myoglobin. Their main energy source is from fats.

Fast twitch muscle fibres contract quickly but cannot maintain contractions for a long time. They generate their ATP through glycolysis, and conversely, have less mitochondria and a lower blood supply. They use glycogen and creatine phosphate for fuel.

→ Compare and contrast slow twitch and fast twitch muscle fibres.

→ What is creatine phosphate?

→ Give examples of activities which use a) slow twitch muscle fibres b) fast twitch muscle fibres.

Other respiratory substrates, other than glucose, can be used to produce ATP. Both starch and glycogen (A storage carbohydrate in the liver) are made up of chains of glucose molecules. These carbohydrates can be broken down during respiration into glucose very quickly, to generate ATP. Maltose and sucrose can also be converted in a similar way.

Fats are broken down into fatty acids and glycerol. Glycerol is converted to a glycolysis intermediate, and fatty acids are changed into molecules that enter the pathway as acetyl coA. Proteins are broken down into amino acids. This breakdown releases urea, and amino acids enter the pathway in a similar way as glycerol, as intermediate metabolites.

→ Under what conditions would a) fats and b) proteins be used to generate ATP?