AQA Biology A-level

Topic 8: The control of gene expression

Notes

Mutations

Mutations are changes in the sequence of nucleotides in DNA molecules. Types of mutations include:

- **Insertion/deletion mutations** - where one or more nucleotide pairs are inserted or deleted from the sequence. This type of mutation alters the sequence of nucleotides after the insertion/deletion point known as a **frameshift**.
- **Duplication** - one or more bases are repeated and therefore produces a **frameshift**.
- **Inversion** - a group of bases become separated from the DNA sequence and then rejoin at the same position but in the **reverse order**. This therefore affects the amino acid that is produced.
- **Translocation** - a group of bases become separated from the DNA sequence on one chromosome and are inserted into the DNA sequence on another chromosome. This can often lead to significant effects on the **phenotype**.

Cause of mutations

Gene mutations can arise spontaneously during DNA replication, and can be caused by mutagenic agents that affect DNA, causes of gene mutations are:

1. **Chemical mutagens** - these include alcohol, benzene and substances in asbestos and is tar in tobacco.
2. **Ionising radiation** - alpha and beta, but also UV and X-ray.
3. **Spontaneous errors** in DNA replication

Mutations can either have neutral effects where the mutation causes no change to the organism, for example in a case where the mutation occurs in a non-coding region of DNA or is a silent mutation. A mutation can also be neutral when a change in tertiary structure of the protein has no effect on the organism.

Some mutations are **beneficial**, for instance, humans developed trichromatic vision through a mutation. Harmful mutations include a mutation in the CFTR protein which causes cystic fibrosis.

Stem cells

Stem cells are undifferentiated cells which can keep dividing to give rise to other cell types. Types of stem cells include **pluripotent cells** that are able to **give rise to many types of specialised cells** apart from embryonic cells and **totipotent cells** which can **give rise to all types of specialised cells** including embryonic cells.

Totipotent stem cells that are able to differentiate into any type of cell found in body and into extra embryonic cells such as those in the placenta. These cells are found in the embryo at an early stage called the blastomere . These stem cells are sometimes called **embryonic stem cells**.

The totipotent cells in the embryo are **initially unspecialised** however when they become specialised they **differentiate** to form tissues which make up the foetus. The cause of this is a change in gene expression where some genes are selectivity switched on and others switched off.

There are a variety of different types of stem cells and are named according to their ability to differentiate. They are summarised below:

1. **Totipotent** - can form any type of cells in the body plus extra embryonic cells.
2. **Pluripotent** - these cells can form any cell type in the body, however cannot form extra embryonic cells. They are also found in the early stages of an embryo. These are often used in replacing damaged tissues in human disorders.
3. **Multipotent** - can differentiate into other cells types but are more limited e.g. the cells in the bone marrow and umbilical cord.
4. **Unipotent** - these cells can only differentiate into one type of cell.

Pluripotent cells also have a number of different uses in **repairing damaged tissue**, these are shown in the table.

Type of cell	Disease that could be treated
Heart muscle cells	Heart damage, for example, as a result of a heart attack
Skeletal muscle cells	Muscular dystrophy
β cells of the pancreas	Type 1 diabetes
Nerve cells	Parkinson's disease, multiple sclerosis, strokes, Alzheimer's disease, paralysis due to spinal injury
Blood cells	Leukaemia, inherited blood diseases
Skin cells	Burns and wounds
Bone cells	Osteoporosis
Cartilage cells	Osteoarthritis
Retina cells of the eye	Macular degeneration

Pluripotent stem cells can also be created from unipotent stem cells and are therefore known as **induced pluripotent stem cells (iPS)**.

Regulation of transcription and translation

Control by oestrogen

The hormone **oestrogen** has the ability to **alter transcription** through altering molecules called **transcription factors**. These are molecules that bind to a specific site on DNA to begin the process of transcription. The action of oestrogen in controlling transcription is described below:

1. The **lipid soluble** nature of oestrogen means that is can freely **diffuse** across the cell membrane where is binds to a **receptor molecule on a transcription factor**.
2. The binding alters the shape of the **DNA binding site** on the transcription factor and makes it able to bind to the DNA.
3. The transcription factor therefore enters the **nucleus via the nuclear pore** where it binds to DNA. This stimulates the transcription of the gene that makes up the DNA.

Small interfering RNA

Small interfering RNA (siRNA) also called **silencing RNA** is used for short term switching off of genes. The **siRNA** binds to a complementary sequence of mRNA. As mRNA is usually single stranded and the cell therefore detects the double stranded form on mRNA and views it as **abnormal**. Therefore the mRNA is broken down by **enzymes preventing translation**.

Epigenetic changes

- **Epigenetics** involves heritable changes in gene function, without changes to the base sequence of DNA. It shows that environmental factors can make changes to the function of genes which can be inherited.
- **DNA methylation** is a process by which **methyl groups** are added to DNA. Methylation **modifies the function of the DNA**, typically acting to **suppress gene transcription**. DNA methylation alters the expression of genes in cells as they divide and become specialised. The change is permanent and prevents the cell from converting back into a stem cell or a different cell type. The methylation is through the addition of a **CH_3 chemical group** to **cytosine bases**, which both **prevents binding of transcriptional factors to DNA and stimulates decreased acetylation of histones**.
- **DNA acetylation** also changes DNA structure. Histones are **positively charged proteins** closely associated with DNA, which is **negatively charged**. **Decreased acetylation** of histones increases their positive change, so they bind **DNA more tightly**. When this happens, **transcriptional factors can no longer access the DNA**, so the gene is switched off.

Gene expression and cancer

Cancer can arise as a result of mutation. **Uncontrolled cell division** in cancer leads to the formation of a **tumour**. There are two types of tumours, **benign** which do not tend to cause much harm apart from light mechanical damage caused by pressing against blood vessels or other cells. **Benign tumours** grow slowly and do not spread, whereas **malignant tumours** grow rapidly and can spread to the neighbouring cells via **metastasis** (through the blood stream or lymphatic system) thus causing damage by disrupting the running of important processes. Malignant tumours are difficult to treat in comparison to benign tumours.

The following play an important role in cancer:

- **Proto-oncogenes** – stimulate cells to divide by producing proteins that stimulate cell division, allow the checkpoints of the cell cycle to be passed, and can cause cancer if mutated.
- **Oncogenes** - these are formed from **mutated proto-oncogenes** and result are permanently switched on resulting in **cell division that is uncontrolled**. It does this by permanently **activating a cell surface receptor** or coding for a **growth factor**.

- **Tumour suppressor genes** – control cell division, cause the cell cycle to stop when damage is detected. They also play a role in the programming of **apoptosis** (cell death). When these are switched off the cell cycle becomes unregulated.

- **Abnormal methylation of tumour suppressor genes and oncogenes** - increased methylation also called **hyper-methylation** plays an important role in **controlling tumour suppressor genes and oncogenes**. The hyper-methylation of a tumour suppressor gene called **BRAC1** can lead to breast cancer.

- **Increased oestrogen concentrations** can be linked to breast cancer development. These elevated levels are found in fatty tissues called adipose tissue in the breast of post-menopausal women. **Oestrogen** binds to the **transcription factor** which activates the genes promoting cell division, leading to tumour formation.

Genome projects

Sequencing projects have read the genomes of a wide range of organisms, including humans. Determining the genome of simpler organisms allows the sequences of the proteins that derive from the **genetic code** of the organism to be determined. This may have many applications, including the **identification of potential antigens for use in vaccine production**. In more complex organisms, the presence of **non-coding DNA** and of regulatory genes means that knowledge of the genome cannot easily be translated into the **proteome**. The **proteome** is **all the proteins that the genome can code for**. However due to **selective gene expression** not all of these proteins will be found in every cell in the body.

Gene sequencing allows for **genome-wide comparisons** between **individuals and between species.** Comparing genomes between species is significant as it allows **evolutionary relationships** between species to be determined, and it is also beneficial to **medical research.** Comparing genomes of individuals enables differences to be identified which can then be used for **development of personalised medicine** tailored to a particular genome, as well as in studies of **human diseases.**

Apart from allowing genome-wide comparisons to be made, gene sequencing has allowed for the **sequences of amino acids in polypeptides to be predicted** and has allowed for the **development of synthetic biology.**

The **Human Genome Project** is an international scientific project which has successfully determined the sequence of bases of a human genome. Potential applications include: **screening for mutated sequences, carriers and pre-implantation screening** as well as screening for disorders such as **Huntington's disease** before the symptoms appear. However, there are many **ethical concerns** regarding the Human Genome Project, such as people being discriminated against as well as regarding the misuse and ownership of the genetic information.

Recombinant DNA technology

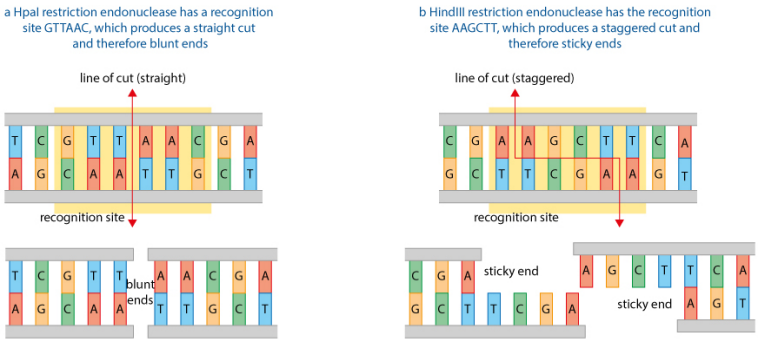
Recombinant DNA technology involves many ways of manipulating DNA, these processes are all detailed below.

Using reverse transcriptase to make DNA

The enzyme **reverse transcriptase** is a enzyme that is found in only some viruses and bacteria and **catalyses** the formation of a **double strand of DNA from a single strand on RNA**. This allows us to make working versions of DNA that **act as genes**, by extracting mRNA from cells where that gene is being expressed.

Using restriction endonucleases to cut DNA fragments

Restriction endonucleases are enzymes, extracted from bacteria, that cut DNA at specific sequences, usually six base pairs in length. The most useful restriction endonucleases are those that make staggered cuts, as they leave **sticky ends** on the DNA. The digram shows the difference between sticky ends and blunt ends.



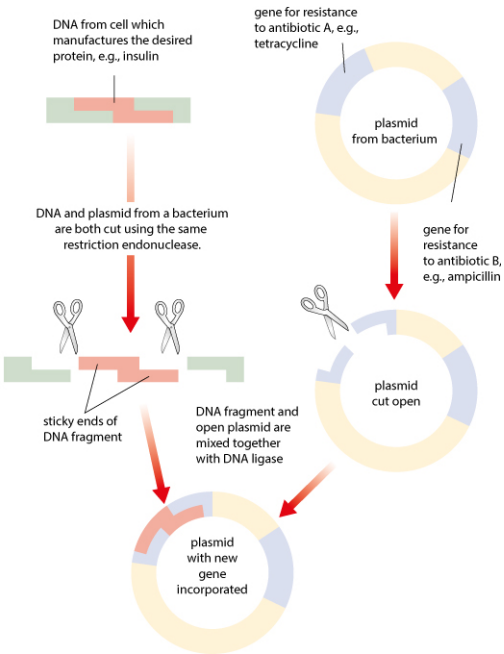
Sticky ends are important because if the **same restriction endonuclease** is used to cut **two DNA fragments** then the ends will be **complementary**. This allows then to attach together before stronger covalent bonds form.

In-vivo gene cloning

If a DNA fragment was placed in a cell it would be digested by enzymes and therefore a vector is used to insert DNA into cells. They are the plasmids from bacterial cells that naturally occur.

Isolated DNA fragments can be placed in plasmids in a following way:

- Plasmid and gene are cut with the same restriction enzyme to create **complementary ends sticky ends**. This means that they inserted DNA and vector are complementary and can be joined.
- The fragments are **incubated with the plasmids.** If a plasmid takes up the insert, base pairing takes place between the complementary ends which are then **sealed with the use of DNA ligase which forms phosphodiester linkages.**
- **A recombinant DNA** molecule is created



In the formation of **transgenic microorganisms, electroporation** is used to stimulate bacterial cells to take up plasmids. Electroporation facilitates the process by **increasing the permeability of bacterial membranes thus increasing the chance of success**. This is achieved via the use of **calcium salts and rapid temperature change from 0 to 40 degrees.**

Gene markers

In order to check whether the DNA has been taken up by the bacteria **gene markers** are used. There are different types of gene markers, these are **antibiotic restraint genes**, **fluorescent markers** and **enzyme markers**. These **genes are incorporated into the plasmid** so that those who have the plasmid can be separated from the bacteria that do not.

They are also used to determine whether the desired DNA has enter the plasmid, as the marker gene will become inactivated.

Gene technologies

DNA profiling is a forensic technique used to **identify individuals by characteristics of their DNA.** It can also be used to **determine genetic relationships** between organisms. One example is PCR.

Polymerase chain reaction known as PCR is used to amplify DNA by making millions of copies of a given DNA sample. It occurs as following:

1) A reaction mixture is set up by mixing the **DNA sample, primers, free nucleotides and DNA polymerase** which is the enzyme involved in creating new DNA strands.
2) The mixture is then **heated to 95 degrees** to break the hydrogen bonds and to separate the two strands.
3) The mixture is then **cooled to a temperature between 50-65 degrees** depending on the type of primers used, so that they can bind (anneal) to the strands.
4) Temperature is increased to about **70 degrees** as this is the temperature DNA polymerase works at. The DNA polymerase is called **Taq polymerase** and is from bacteria that live in hot springs.
5) **DNA polymerase** creates a copy of the sample by **complementary base pairing using the free nucleotides.**
6) **This cycle is repeated around 30 times** and gives rise to an amount of DNA sufficient to create a DNA profile.

In-vivo and in-vitro cloning

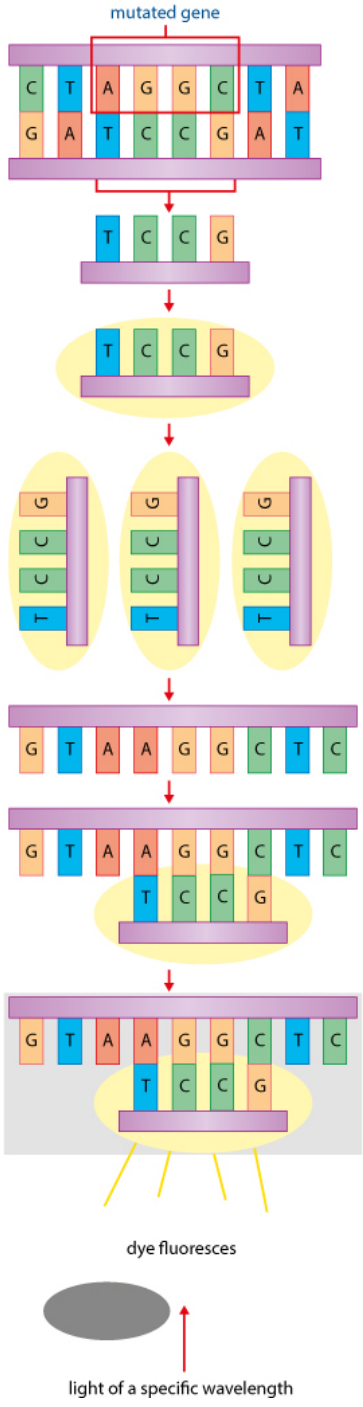
In-vitro - this gene cloning can be done with **PCR**. This is **fast, automated and reliable** once conditions are established. This **does not require living cells** and can have problems such as **contamination** and **errors**.

In-vivo - gene cloning that can be done using **recombinant plasmids** in bacteria. This is **accurate and useful** as the gene is placed in cells where it can be **expressed**. The disadvantage though is it is very **time consuming** and **requires monitoring of cell growth**.

DNA probes

A **DNA probe** is a **short, single stranded DNA molecule** that is designed to complementary to a sequence to be detected. DNA probes are made in smaller quantities and then **amplified using PCR**. The DNA labelling of the fragments either uses **radioactive isotopes** or a **fluorescent dye** which glows under certain wavelengths of light.

DNA probes can be used in order to detect heritable conditions of health risks. The diagrams shows the process.



1 The sequence of nucleotides on the mutated gene is determined by DNA sequencing. Genetic libraries now store the DNA sequences of many of the genes responsible for common genetic diseases.

2 Fragment of DNA with complementary bases to the mutant allele of the gene is produced.

3 DNA probe is formed by fluorescently labelling the DNA fragment.

4 PCR techniques are used to produce multiple copies of the DNA probe.

5 Probe is added to single-stranded DNA fragments from the person being screened.

6 If the donor has the mutated gene, some donor DNA fragments will have a base sequence that is complementary to the probe and the probe will bind to its complementary bases on the donor DNA.

7 These DNA fragments will now be labelled with the probe and can be distinguished from the rest of the DNA fragments.

8 If complementary fragments are present, the DNA probe will be taken up and the dye will fluoresce – this is detected by a special microscope. If complementary fragments are not present, the DNA probe will not fluoresce.

Genetic fingerprinting

Genetic fingerprinting is a technique that can detect differences in people DNA. It uses **variable number tandem repeats (VNTRs)** which are short repeating sequences or bases. The probability of two individuals having **identical VNTRs** is **extremely low** therefore VNTR analysis can be used in **genetic fingerprinting**.

Gel electrophoresis is a process used to **separate the DNA fragments and proteins according to their size using an electric current**. It occurs as shown in the digram on the right.

Genetic fingerprinting can be used in the fields of forensic science, medical diagnosis as well as animal and plant breeding. It is carried out as follows as shown in the diagram below.

Figure S-2: Gel Electrophoresis