# Topic 8 – The control of gene expression

## Key words:

**Mutagenic agent** – an agent such as radiation of carcinogens that increase the rate of mutations in DNA.

**Frame shift** - gene mutations change all the base triplets downstream of the mutation.

**Degenerate** – more than one triplet code per amino acid.

**Universal** – DNA all organisms codes for the same amino acids.

**Transcription factors** – proteins that more from the cytoplasm to the nucleus that bind to the promoter region of a gene to inhibit or promote translation.

**Epigenetics** – Heritable changes to gene function that’s does not change DNA bases.

**Genome** - The complete set of genes in a cell or organism.

**Proteome** - The full range of proteins that a cell can produce.

**Recombinant DNA** - DNA from multiple organisms.

**Transformed cells** – Cells that have taken up the plasmid.

**Vector** – A DNA sequence that can transport foreign genetic material.

**Plasmid** – A circular piece of DNA.

**Marker** – A gene that gives the bacteria a characteristic (normally either antibiotic resistance or florescence).

**Probe** – A short section of single stranded DNA with either a radioactive of florescent label that will bind to a known sequence.

**Oligonucleotide** – Short sections of DNA.

**Primer** – Short single stands of DNA complimentary to the start of the gene that prevents the strands from annealing and allows DNA polymerase to bind.

**Annealing** – Bind together.

***In vitro*** – Made outside a living organism.

***In vivo*** – Made inside a living organism.

**VNTRs** –Variable numbers of tandem repeats – ATCGATCG Vs ATCGATCGATCGATCG

**Somatic cells** – Any cell in the body that are not gametes.

**Germ line cells** – Gametes (sperm and egg cells)

**Sticky ends** – single stranded DNA at the end of the DNA fragment.

**Metastasize** - break off and travel around the body in the blood stream.

## Enzymes you need to know

**DNA polymerase** – forms phosphodiester bonds, through a condensation reaction, between adjacent DNA nucleotides.

**Ligase** – anneals sticky ends by using ATP for form the phosphodiester bond.

**Reverse transcriptase** – uses mRNA as a template to join free complimentary DNA nucleotides forming a strand of cDNA.

**Restriction endonuclease** – hydrolyses phosphodiester bonds in DNA at palindromic sequences to leave sticky ends.

**Thermostable DNA polymerase** – DNA polymerase that is not denatured by heat.

1. The mutations are **random** but are increased by mutagenic agents.
2. There are different types of mutations
   1. **Addition** – adding an extra nucleotide – causes frame shift.
   2. **Deletion** – misses a nucleotide – causes frame shift.
   3. **Substitution** – a nucleotide is swapped for an incorrect one. Could be silent dur to the degenerate code of the DNA.
   4. **Inversion** – a section is reversed.
   5. **Duplication** – a section is repeated.
3. Some gene mutations change only one triplet code. Due to the **degenerate** nature of the genetic code not all mutations result in a change in amino acid – this is called a **silent** mutation.
4. Some gene mutations change all the base triplets downstream of the mutation – this is called **frame shift**.
5. Mutations can also be cause by **base analogues** (changing the base for something other than a nucleotide), **base substitutions** (non-complimentary base pairing) and **structural changes** (UV radiation changing the double helix structure).
6. **Stem cells** are undifferentiated cells that need to be able to divide by **mitosis** and need to be able to **differentiate** to become specialised.
7. There are different types of stem cells
   1. **Totipotent** – found in embryos, they can only form very first cells in an early embryo, umbilical cord and placenta.
   2. **Pluripotent** (can be used to treat human disorders) – can divide into all types of cells and are found in the embryo.
   3. **Multipotent** – found in somatic cells (adults) can only form a small number of cells.
   4. **Unipotent** e.g.– cardiomyocytes can only differentiate in to one type of cell.
   5. **Induced pluripotent stem cells** are produced from adult somatic (body) cells.
      1. These are created by **removing** all transcription factors from the somatic cell.
      2. Then **adding** the transcription factors back into the somatic cell to turn it into a pluripotent cell.
8. **Transcription factors** are proteins that move from the cytoplasm into the nucleus. The bind to the promotor region of the DNA and either inhibit transcription by preventing RNA polymerase binding or initiate transcription by helping RNA polymerase binding.
9. **Oestrogen** is a lipid soluble hormone:
   1. It diffuses through the phospholipid membrane.
   2. Inside the cell there is a transcription factor with an inhibitor attached.
   3. Oestrogen binds to the transcriptions factor
   4. This causes the inhibitor to become unattached
   5. The **oestrogen-oestrogen receptor complex** can then move from the cytoplasm into he nucleus, bind to the promotor region of a gene and initiate transcription.
10. **Epigenetics** are heritable changes in gene function without changes to the DNA sequence.
    1. **Methylation** attaches onto **cytosines on DNA**
    2. **Acetylation** attaches onto **histones.**
11. **More methylation** leads to **decreased transcription**
12. **Less acetylation** means histones move closer together, so **transcription is decreased.**
13. There are two genes that control the cell cycle: **proto-oncogenes** increase the rate of cell division. **Tumour suppressor** genes decrease the rate of cell division.
14. In cancerous cells
    1. Proto-oncogenes become **oncogenes** – have **decreased methylation** and **increased acetylation of histones**, this means they are over expressed.
    2. **Tumour suppressor genes**- have in**creased methylation** and **decreased acetylation** of histones, this means they are not expressed.
    3. **Increased level of Oestrogen** can stimulate certain cells to divide therefore there is an **increased risk of cancer.**
15. Benign tumours, grow slowly, well define capsule, they are not invasive and do not metastasize (break off)
16. Malignant cancer, grow rapidly, not encapsulated, invasive, poorly differentiate and can metastasize (break off and spread and form secondary tumours).
17. **RNAi** – (interfering RNA) are short strands of single stranded RNA that stops translation occurring. There are two types of RNAi:
    1. **siRNA** (small interfering RNA)
       1. siRNA starts off as double stranded comes from outside of the cell.
       2. siRNA binds to extra proteins and **becomes single stranded**
       3. Forms a RISK complex
       4. The **RISK** complex with the siRNA binds to complimentary mRNA
       5. The mRNA **is hydrolysed**
    2. **miRNA** (micro RNA)
       1. siRNA starts off as double stranded comes from outside of the cell.
       2. siRNA binds to extra proteins and **becomes single stranded**
       3. Forms a RISK complex
       4. The **RISK** complex with the siRNA binds to complimentary mRNA
       5. The mRNA **is hydrolysed**
18. **Genome** – the complete set of genes in a cell or organism
    1. Lots of genomes have now been sequenced.
    2. It is hard to take the genome of complex organisms and work out the proteins that can be made because of the introns (non-coding sequences).
19. **Proteome** - the full range of proteins that a cell can produce.
    1. We can sequence the proteomes of pathogens to work out the proteins that will be made and therefore the antigens on the cell surface.
    2. If scientists identify the antigens, they can then potentially create a vaccine.
20. **Recombinant organisms (**DNA from multiple organisms) can be made because DNA is **universal**.
21. Genes can be isolated in 3 different ways:
    1. Using **reverse transcriptase**
       1. This start with **mRNA** 
          1. This is an advantage because there are more than 2 copies, it isn’t in the nucleus therefore is easier to obtain and doesn’t contain introns so therefore the gene can be inserted into prokaryotes.
       2. Reverse transcriptase adds free complimentary DNA nucleotides to the strand of mRNA.
       3. This makes a strand of **cDNA** (complimentary DNA)
    2. Using **restriction endonuclease**
       1. This starts with DNA
       2. Restriction endonuclease cuts the DNA at **palindromic sequences** (the same forwards as backwards)
       3. It leaves behind **sticky ends** (single stranded pieces of DNA at the end of the gene).
    3. Using **a gene machine**
       1. This uses a machine where you can program in a specific sequence.
       2. The machine makes short stands of DNA at a time called **oligonucleotides.**
       3. The oligonucleotides are **then pieced together** to form a DNA fragment.
22. We can amplify (make more) DNA by using **PCR** (polymerase chain reaction).
    1. Fist we **heat** the DNA to 95oC – this is to break the hydrogen bonds between the complementary bases on the double stranded DNA.
    2. Secondly, we cool the DNA to 55oC so the **primers can annea**l (bind)
       1. Primers are short sections od DNA that bind to the starts of the DNA (we need 2 primers per piece of DNA because they need to be complimentary to either end). We use primers for 2 reasons:
          1. To enable DNA polymerase to bind
          2. To stop the 2 strands from annealing.
    3. Lastly the DNA is heated up to 75oC so the **thermostable DNA polymerase** can add free complimentary nucleotides to the template strand.
       1. Thermostable DNA polymerase is used so the enzyme is not denatured every time the DNA is heated to 95oC to break the hydrogen bonds.
    4. Repeat.
23. PCR is an *in* ***vitro*** technique
24. Fragments can also be amplified ***in vivo*** (inside a living organism)
    1. **Promoter** and a **terminator** sequence are added to the DNA fragment
    2. Using the **same restriction endonuclease** cut the ends of the DNA fragment and the vector (to produce complementary sticky ends).
    3. Use **a ligase** to bind the DNA fragment into the vector.
    4. **Transform** (put the vector into the host cell) by using heat shock.
25. We can use markers inside the plasmids to identify cells that have successful been transformed with the vector and the vector has successfully had the gene incorporated.
    1. Inside the plasmid is an **antibiotic resistant gene (first marker**). The bacterium is first grown on agar containing antibiotics. Only the bacteria that have been transformed with the plasmid (that includes that antibiotic resistance gene) will survive.
    2. There is also a **gene that produces a florescent** protein with in the plasmid (a second marker). The DNA fragment is intentionally inserted into the centre of the florescence gene – this causes the florescent gene to become non-functional.
       1. If the bacterium has been transformed (taken up the vector) but the gene fragment hasn’t been inserted, then the bacterium will glow.
       2. If the bacterium has been transformed and the gene fragment has been inserted in the vector the bacterium will not glow under UV light.
26. We can also use the genes in gene therapy. We can use a gene that is making the correct protein and insert them into cells.
    1. **Germ line therapy** – is inserting the correct gene into the sperm or the egg cell so that when the cells divide by mitosis call cells will have the correct gene.
    2. **Somatic cell gene therapy** – you insert the correct gene into adult body cells. However, this is only inserted into one cell at a time and you need to be careful about where in the genome of the cell is the gene inserted. It could be under or over express or not inserted into the nucleus at all.
27. **DNA probes** are short sections of single stranded DNA with either
    1. A florescent tag – that can be detected by UV light
    2. A radioactive tag – that can be detected on x-ray film.
28. DNA need to be single stranded for DNA probes to bind.
29. We can use **DNA probes to locate specific** known sequences. For example, to find out if a person has a heritable condition, how the have reacted to a drug or if the have genes that might cause a risk to health.
30. If we know the know a patient has the known genetic sequences, then we can use this information to provide genetic counselling (giving guidance to parents considering the risk of giving a genetic disorder to a future child) and personalised medicine.
31. To analyse DNA samples, we can separate short sections out using **gel electrophoresis**.
    1. First, we use **PCR** to amplify the sample of DNA
    2. Then you cut up the DNA into fragments using res**triction endonuclease.**
    3. You load the sample into wells on a gel sheet next to the negative electrode.
    4. An **electrical current** is supplied to the gel
    5. The DNA fragments **will move towards the positive electrode** as DNA is negatively charged.
    6. The s**horter** the **fragment** the further it will travel along the well.
    7. The DNA is then put on a nylon membrane
    8. The DNA is then stained with a DNA binding dye so each of the bands can be seen. When placed under a UV light you can see the bands of DNA.
32. We can use DNA fragments with known lengths called a **DNA ladder** to compare the unknown samples to.
33. We can use gel electrophoresis to analyse **genetic fingerprints.**
34. Most of the genome between members of the same species is the same but there are regions called **VNTRs** (variable number of tandem repeats) that vary massively between individuals. The probability of 2 individuals having the same VNTRs is low.
35. To analyse if two samples are form the same individual or a related individual you should do the following:
    1. Use **PCR** to amplify the sample
    2. Cut the sample up into fragments using a **restriction endonuclease**
    3. Separate the fragments out using **get electrophoresis**
    4. Compare the marks on the gel (if the are next to each other they are the same length fragments).
36. You can use genetic fingerprinting to
    1. **Identify a potential suspect** to DNA from a crime scene
    2. **Medical diagnosis** (there has been a link between some diseases and lengths of VNTRs)
    3. **Animal and plant breeding** – you don’t want to breed two animals or plants that are closely related as there is a higher risk of inbreeding and therefore genetic disease.