

Topic 1

DNA and proteins

Key

∴ = therefore

↑ = increase/high

↓ = decrease/low

aa = amino acid

BG = blood glucose

Conc = concentration

Euk = eukaryotes

GF = growth factor

GOI = gene of interest

H = hydrogen

H⁺ = hydrogen ion

HC = high concentration

LC = low concentration

No. = number

NT = neurotransmitter

p. = page

ROR = rate of reaction

SA = Surface Area

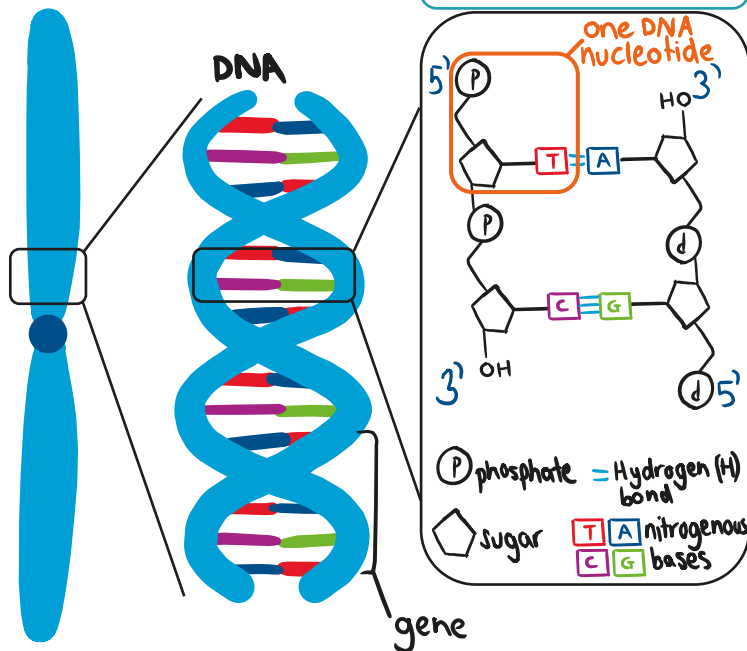
Vol = Volume

Refer to the most recent Stage 2 Biology Subject Outline:

<https://www.sace.sa.edu.au/web/biology>

DNA (Deoxyribonucleic acid)

Chromosome



DNA

- Helical double-stranded molecule
- Runs in a 5' → 3' direction
- **Weak** H bonds between complementary bases (A=T, T=A, C=G, G=C) → easily broken and reformed
- Genes are segments of DNA that code for polypeptides

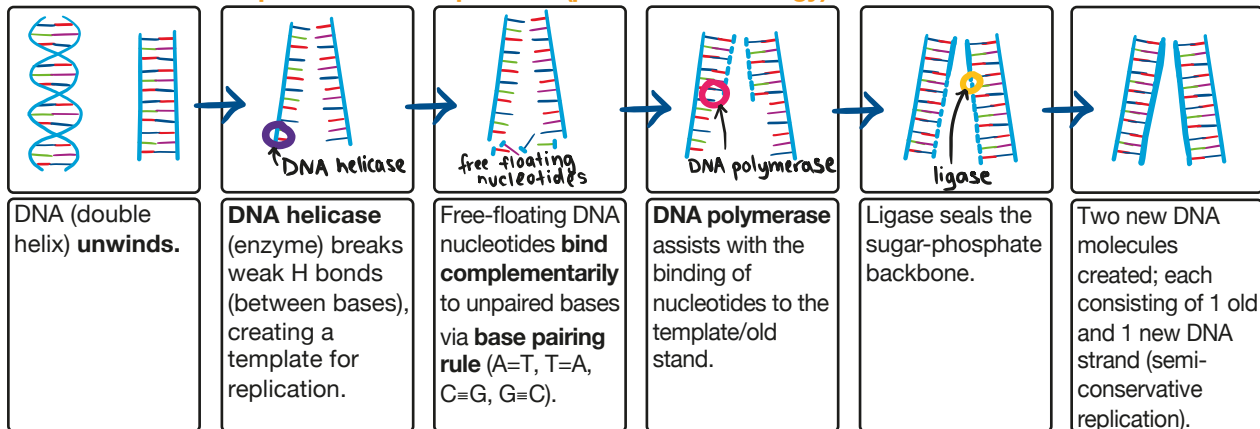
DNA related terms

- **Locus** of a gene → location of gene on a chromosome
- **Chromatin** → long and thin tangled strands of DNA (when cell is not dividing)
- **Chromatids** → condensed, shorter, thicker strands of DNA (during cell division)
- **Exon** → expressed/coding region of gene
- **Intron** → interrupting/non-coding region of gene

How can chromosomes be distinguished from each other?

- Different size/shape/genes/banding pattern

Semi-conservative replication/DNA replication (process uses energy)



Prokaryotic DNA vs Eukaryotic DNA

Prokaryote DNA (including mitochondria and chloroplasts)

Circular
None (unbound)

Eukaryote DNA

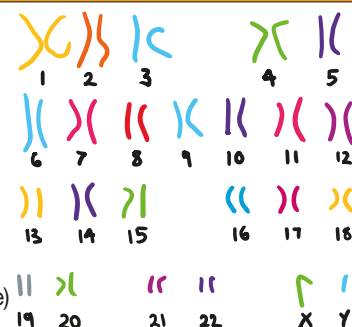
Linear
Histone proteins bound to DNA

Cytosol
No introns

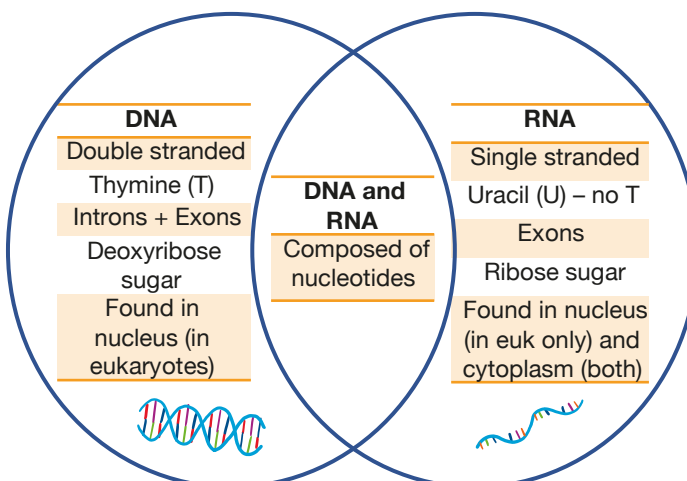
Nucleus
Introns + Exons

Human karyotype consists of:

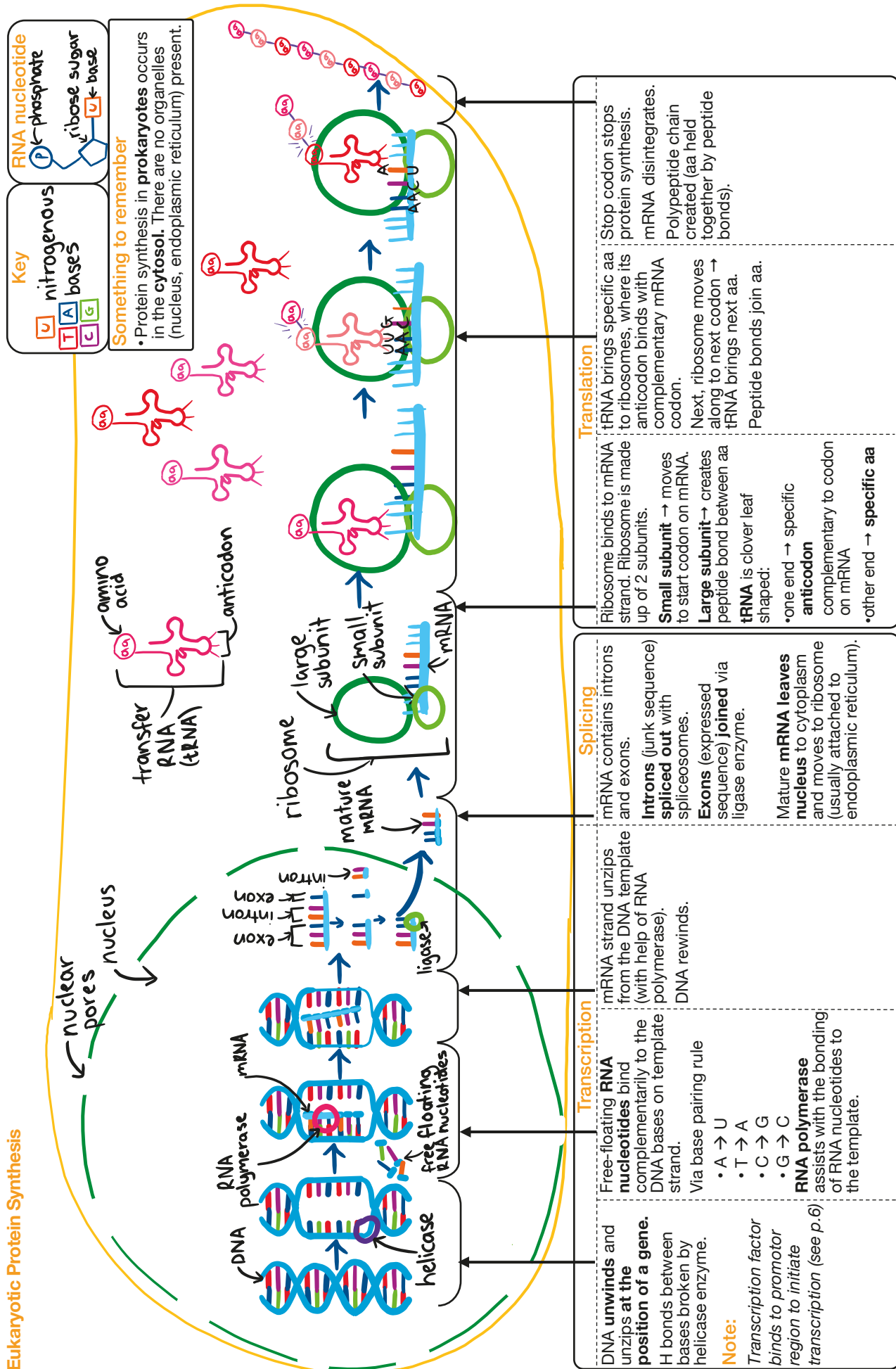
- 22 pairs of homologous chromosomes
- Overall 46 chromosomes
- 1 pair of sex chromosomes (XX=female; XY=male)



Similarities and differences between DNA and RNA



Eukaryotic Protein Synthesis



Genes and Proteins

A gene consists of a unique:

- Number, type, sequence of **DNA** bases/ Nucleotides
 - This determines number, type, sequence of mRNA nucleotides
 - This determines number, type, sequence of **amino acids (aa)**
 - This determines the **structure** of the polypeptide
 - Which determines the **function** of the polypeptide/protein

Flow of genetic information:

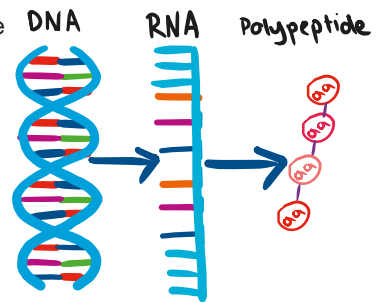
• DNA → RNA → polypeptide

Transcription:

- mRNA makes copy of gene (DNA)

Translation:

- mRNA is translated into aa sequence of a polypeptide



Protein synthesis important notes:

- 3 mRNA bases = 1 codon = 1 amino acid (aa)
- There are 20 aa
- 64 possible codons → several codons can code for same aa
- **rRNA** = ribosomal RNA (ribosome contains rRNA)
- **mRNA** = messenger RNA
- **tRNA** = transfer RNA

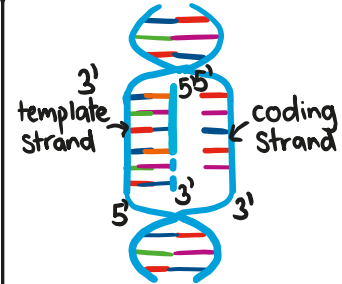
Template and coding strands:

Template strand = strand used to transcribe the mRNA

- Template is complementary to mRNA strand

Coding strand = Contains exact same sequence of nucleotides in mRNA (except T)

- Not used as template for transcription



mRNA Codon and Amino Acid Table

		<u>Second base</u>				
<u>First base</u>	U	C	A	G	<u>Third base</u>	
U	Phe	Ser	Tyr	Cys	U	
	Phe	Ser	Tyr	Cys	C	
	Leu	Ser	Stop	Stop	A	
	Leu	Ser	Stop	Trp	G	
C	Leu	Pro	His	Arg	U	
	Leu	Pro	His	Arg	C	
	Leu	Pro	Gln	Arg	A	
	Leu	Pro	Gln	Arg	G	
A	Ile	Thr	Asn	Ser	U	
	Ile	Thr	Asn	Ser	C	
	Ile	Thr	Lys	Arg	A	
	Met	Thr	Lys	Arg	G	
G	Val	Ala	Asp	Gly	U	
	Val	Ala	Asp	Gly	C	
	Val	Ala	Glu	Gly	A	
	Val	Ala	Glu	Gly	G	

Examples of proteins with specific shape

Enzymes (end in ...ase)

Antibodies

Hormones

Receptor

Four Levels of Protein Structure

Primary structure

- Linear sequence of aa in a chain held linked by peptide bonds
- There is a specific number, type, and sequence of aa.

Secondary structure

- Alpha helices and beta pleated sheets
- Coiling/folding of polypeptide chains (due to H bonds)
- E.g. fibrous (such as keratin) and globular proteins

Tertiary structure

- 3D structure
- Forms due to interactions (attractions/repulsions) between aa
- Number, type, sequence of aa determines the shape of protein
- This determines function

Quaternary structure

- 2 or more polypeptide chains lock together
- E.g. DNA polymerase, haemoglobin

What are enzymes?

Enzymes

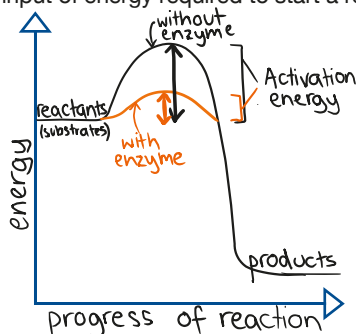
- Globular proteins
- Have a specific shape
 - \therefore Have specific active site shape
- They are reused

Definitions:

- \uparrow rate of reaction
- Speed up chemical reactions
- Provide an alternative pathway with a lower activation energy

Activation energy

- Initial input of energy required to start a reaction



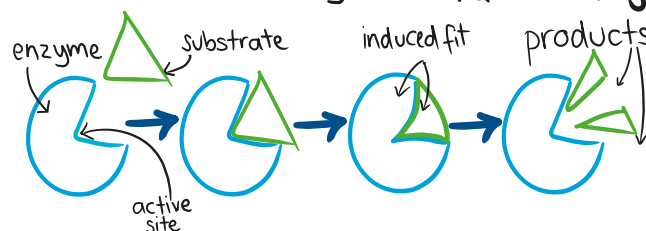
How do enzymes increase ROR or enzymes lower activation energy and speed up reactions by:

- Bringing substrate molecules together in **correct orientation**, favouring the reaction
 - Without the enzyme, it is unlikely substrates will randomly collide
- Enzyme strains and **distorts** covalent (strong) **bonds** in the substrate (induced fit)
 - **Strained bonds** can break more easily (also called the transition state)

Induced fit model/enzyme substrate complex:

- Substrate binds complementary to active site of enzyme (in correct orientation)
- Shape of both substrate and enzyme change to get a closer fit (induced fit)
- Bonds of substrate are strained (transition state)
- Product(s) is released and enzyme returns to original shape
- Enzyme is reused \rightarrow this is why a small number of are needed to catalyse many substrate(s)

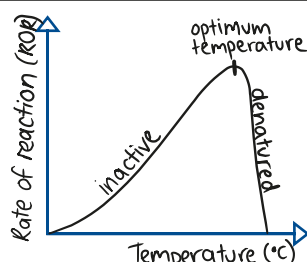
Normal reaction: Enzyme-Substrate Binding



Factors affecting enzyme activity

Temperature

- Optimum temperature for enzyme activity varies
- In the human body, it is 37°C



High temperature (irreversible)

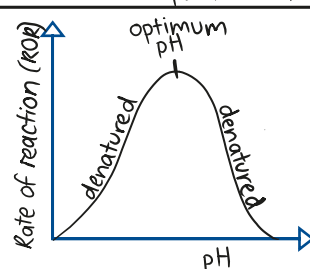
- Enzyme **denatures**
 - \therefore H bonds holding 3D shape destroyed
 - Shape of protein changes \rightarrow active site changed
 - Change in function \therefore substrate cannot bind

Low temperature (reversible)

- Enzyme is **less active/inactive**
 - Not enough kinetic energy for enzyme to collide with substrate
 - Low rate of reaction
 - Collisions \downarrow
 - Once temp \uparrow , enzyme gains kinetic energy, ROR \uparrow

pH

- Different enzymes have different optimum pH ranges

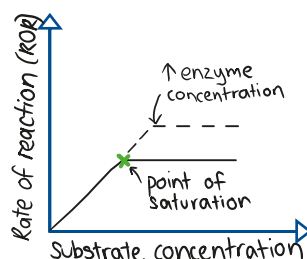


Outside optimum pH

- Out of optimum range \rightarrow denatures
- Change in charges on the aa (by adding or removing hydrogen ions (H⁺))
- Changes shape of enzyme \rightarrow changes active site
- Changes function \therefore substrate cannot bind
- Irreversible

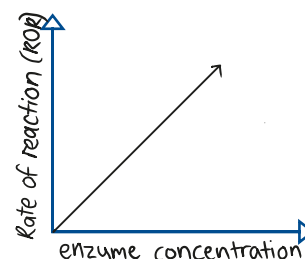
Substrate concentration

- As substrate concentration \uparrow , so does the ROR, up to a point
- Plateaus as all enzyme active sites are full
- \uparrow ROR by \uparrow concentration of enzymes



Enzyme concentration

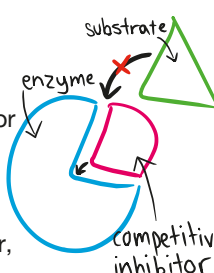
- As \uparrow enzyme concentration, \uparrow ROR
- More enzymes = more collisions
- This \uparrow in ROR is only up to a point. Other factors may limit ROR (such as substrate).



Inhibitors

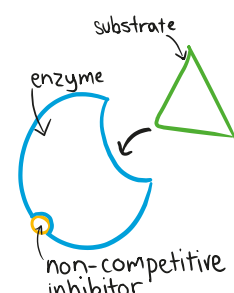
Steric/Competitive inhibitors

- e.g. cyanide, CO
- Competitive inhibitor has a similar shape to substrate
- Inhibitor competes with substrate for the active site of enzyme
- Prevents substrate from binding
- Most are reversible and can detach
- The more concentrated the inhibitor, the greater the chance it will bind



Allosteric/Non-competitive inhibitors

- Binds to place on enzyme other than the active site
- Changes shape of enzyme \therefore active site changes
- \therefore substrate cannot bind (enzyme is unable to function)
- Generally irreversible



Epigenetics

What is an epigenetic modification (epi = over)?

- DNA modification that DOES NOT change DNA sequence
- BUT can affect gene activity/expression

Epigenetic changes can:

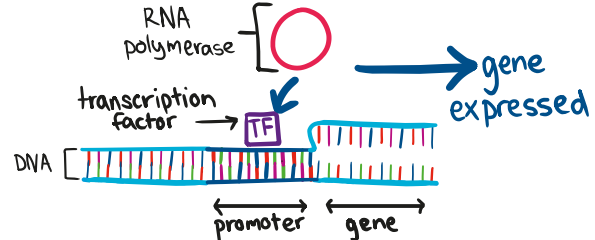
- Determine whether genes are turned off/on
- ∴ only required proteins are produced

Transcription factors

- Proteins that help switch on/off genes
- Activators ↑ transcription
- Repressors ↓ transcription

Transcription factors as activators:

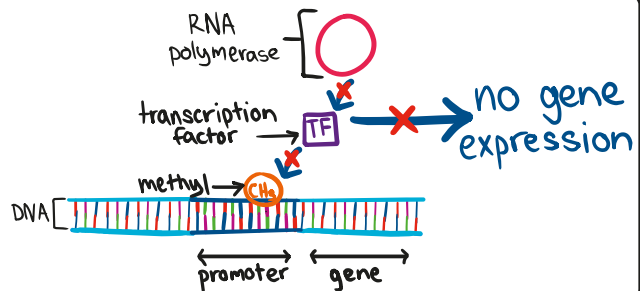
- Initiate transcription
- Transcription factors bind specifically to the (CpG site of) **promotor** region (upstream of the gene)
- Recruit other factors
- Recruit RNA polymerase for transcription



Methylation

Common epigenetic modification: Methylation

- CH₃ (methyl) group attached cytosine nucleotide on the promotor region of the gene (at a CpG site)
- ∴ Transcription factor cannot bind
- ∴ Gene is switched off/silenced
- ∴ Gene is NOT expressed
- ∴ No protein



Epigenetic changes, such as methylation, are involved in:

- Cell differentiation
- Phenotypical expression of somatic (body) cells
- Cancer

Cell differentiation

- Cell **differentiation** is the process by which **cells specialise**
 - Stem cells become specialised cells
 - All stem cells contain a full set of chromosomes (and genes)
 - Each stem cell becomes a specific cell type due to different genes being switched on and off
 - This is due to **methylation**
- E.g nerve, muscle, connective, epithelial

Phenotypical expression of somatic (body) cells

- Epigenetic changes (methylation) in genes can be caused by environmental factors. These are random
- This contributes to the phenotype of the individual
- E.g. identical twins or clones → have the same DNA → different environments → could develop different methylation patterns throughout life → different genes expressed

Methylation of somatic cells is mitotically heritable

- Somatic cell division (mitosis) will produce identical daughter cells
- These cells inherit the same gene methylation pattern
- This ensures identical cells continue to produce the same proteins with the same functions

DNA methylation and cancer

- All cells contain genes which control cell division e.g. tumour suppressor genes, (proto)oncogenes
- If tumour suppressor gene, which normally stops cell division, is **methyated**
- Gene will NOT be expressed
- Gene switched off/silenced
- ∴ uncontrollable cell division = **cancer**

Summary comparison of gene expression

Gene "switched on"	Gene "switched off"
Active	Silent
Unmethylated gene	Methylated gene
Acetylated histones (DNA loosely packed)	Deacetylated histones (DNA tightly packed)
Expressed	Not expressed

Mutations

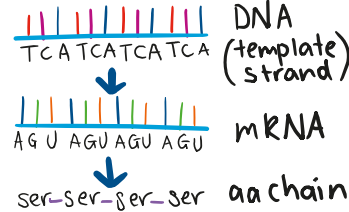
Mutation → change in nucleotide sequence

- Generally permanent change in the DNA
- Can be passed to future generations if it occurs in gametes (germ line cells)

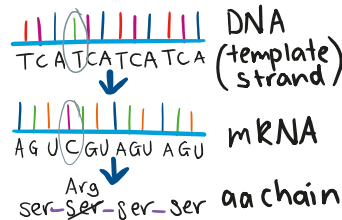
Mutagen → factor that ↑ rate of mutation



Without mutation



Base substitution



Base insertion/deletion

- Alters frame of reading (frame shift)
- Every codon after the insertion/deletion changes
- ∴ changes respective aa sequence
- Most serious mutation generally



Mutation rate can ↑ by:

- Ionizing radiation



- Mutagenic chemicals



- Viruses



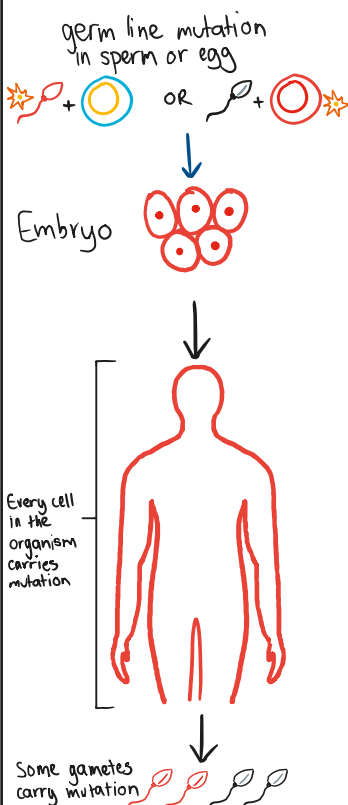
Mutations:

A **change** in the number, type or sequence of DNA bases/nucleotides:

- Changes the number, type, sequence of mRNA nucleotides
- This changes number, type, sequence of aa
- This changes the **structure/shape** of the polypeptide
- Which changes the **function** of the polypeptide/protein

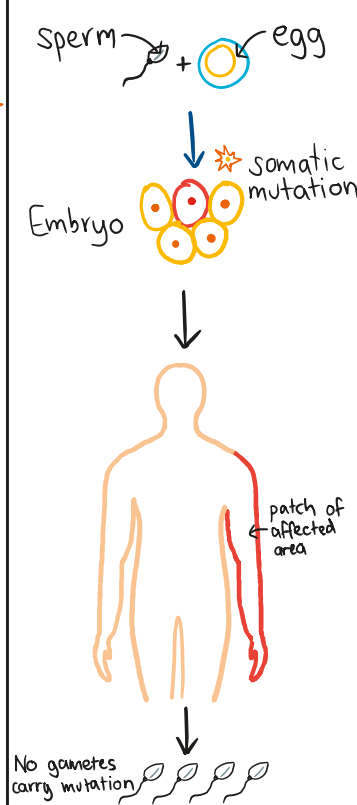
Germ line mutation

- In sex cells (gametes)
- Hereditary
- Passed on to children



Somatic mutation

- Localised in organism or tissue or location (somatic cell = body cell)
- **Not** passed on to children



Mutations in genes and chromosomes can result from:

- Errors in DNA replication
- Errors in cell division
- Damage by physical or chemical factors in environment

Phenotypic expression

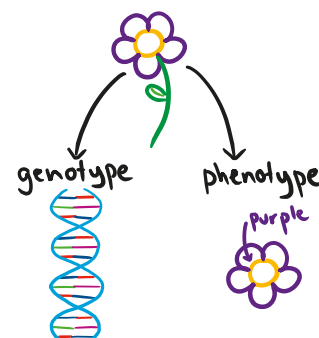
- Relates to physical, biochemical, physiological characteristics

Phenotype

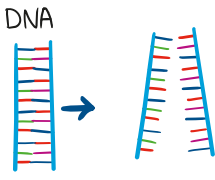
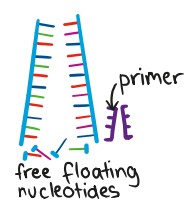
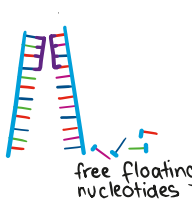
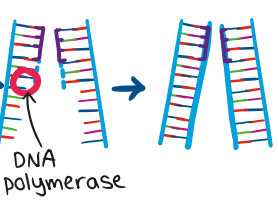
- Genotypes + environmental influences
- **Phenotype** = physical appearance

Genotype

- Genetic makeup of an organism

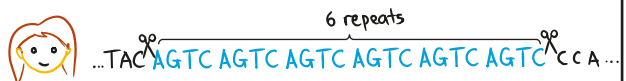


PCR (polymerase chain reaction) → amplify DNA/Make copies of DNA

			
Heat DNA (to 90°C) • Heat breaks the weak H bonds holding bases together	Add • Primers – short single stranded DNA sequence complementary to DNA • DNA polymerase – from bacteria which live in hot springs (have a higher optimum temperature) • DNA nucleotides	Cool (to 40°C) • Allows primers to anneal (prevents DNA strands from reannealing)	Heat (to 72°C → optimum temperature for DNA polymerase) • Allows DNA polymerase to extend the primer • Nucleotides bind complementarily to DNA via base pairing rule • Cycle repeated 30 times

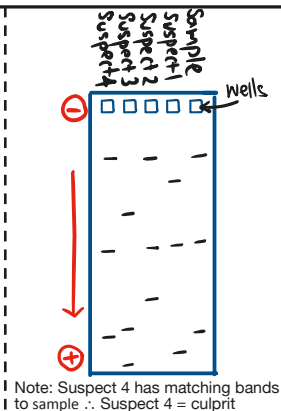
DNA profiling identifies the unique genetic makeup of individuals**Forensic DNA profiling**

1. PCR (to amplify DNA)
2. Select and use short tandem repeats (OR >13 unique regions)
 - These contain highly repetitive, non-coding (introns) sequences → short tandem repeats (STRs)
3. Specific restriction enzyme is used to cut STRs (or 13 regions)
 - This produces a **unique number of restriction fragments** of varied sizes
4. Those fragments are radioactively labelled
5. These fragments are run on gel electrophoresis
6. Unique DNA fingerprint/profile produced by every individual made up of a distinct number of bands of different sizes

**Gel electrophoresis**

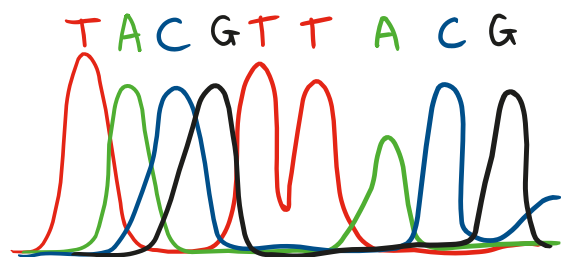
Separates DNA fragments according to their size

1. Place fragments into wells on gel
(Note: A different well is used for each suspect DNA)
2. Place charge across gel from negative (-) to positive (+)
3. DNA fragments move towards + end because **DNA is negatively charged**
4. Smaller fragments move furthest (due to less friction)



DNA sequencing – method used to determine the base sequence of DNA (e.g. a gene)

Electropherogram – is a plot of DNA sequences

**Discuss ethical, economic, cultural issues related to collection of genetic information****Ethical**

- Life insurances can discriminate based on what genetic disorders individuals can develop
- Employee discrimination
- Test only requires a hair follicle → could be carried out without consent of individuals
- Privacy and confidentiality
- Framing other suspects (forensics)
- Who controls and monitors these databases?

Cultural

- Different cultures and religions may be against the collection and retainment of genetic information
- Can't assume all cultures will interpret genetic information same way
- Predicting medical conditions/disorders can be unnatural
- Collecting samples may be invasive
- Some ethnicities can be stigmatized/victimized because genetic condition more prevalent amongst them

Economical

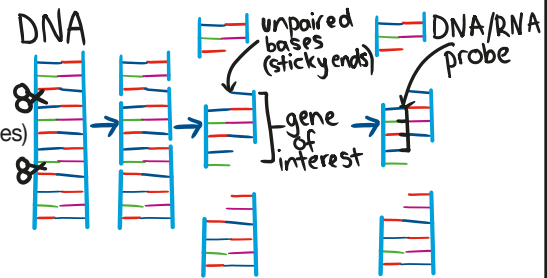
- Cost to store and access databases
- Genetic information could be sold to make a profit (also ethical)
- Collection of genetic information can help in screening for diseases. Therefore, they can be detected earlier, reducing the risk of disease and reducing the future economical burden

Recombinant DNA technology/genetic engineering/transgenic organisms

→ to produce desirable characteristics; could have undesirable effects

How gene of interest can be selected (probe) and removed (restriction enzymes)?

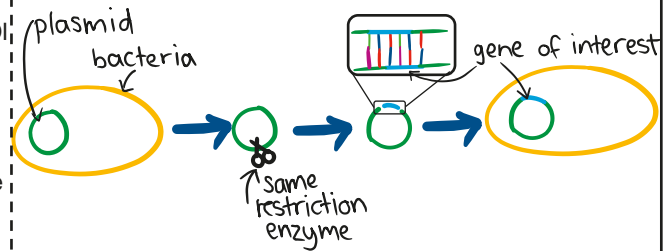
- Gene of interest (GOI) is located (via DNA sequencing)
- **Specific** restriction enzymes cut around gene of interest
- **Restriction fragments** are created – these have sticky ends (unpaired bases)
- DNA fragments are heated to separate the strands
- Radioactively/fluorescently labelled, single-stranded DNA/RNA **probe** is able to complementarily bind to the exposed gene of interest when cooled
- Autoradiograph/UV light is used to isolate the labelled gene of interest



Gene transfer

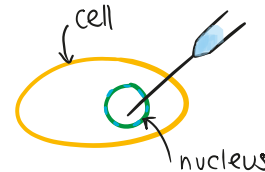
Ti plasmid (bacteria can infect plant)

- Plasmid is removed from bacterial cell
- Plasmid cut with the **same** restriction enzyme used to cut GOI
- Creates complementary sticky ends
- Plasmid and gene of interest (GOI) bind complementarily and ligase helps glue/seal the backbone
- Recombinant plasmid inserted back into bacteria (via **electroporation** – electrical charge is applied, disrupting the cell membrane and allowing the introduction of the plasmid)
- Bacteria infects target cell and inserts Ti plasmid with GOI



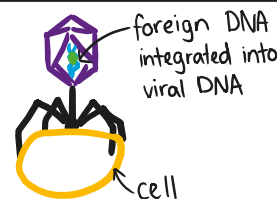
Microinjection

- Gene of interest delivered via very fine needle directly to pronucleus of very first cell
- Must be inserted into first cell (fertilized egg cell) → so that all cells contain gene of interest and can express it
- E.g. mainly used in animal cells



Viral vector

- Gene inserted (via electroporation) into a viral capsule
- Virus used to infect cells
- Carries foreign gene (GOI) along with its own DNA into target cell
- Side note: harmful genes can be removed from virus



CRISPR (clustered regularly interspaced short palindromic repeats)

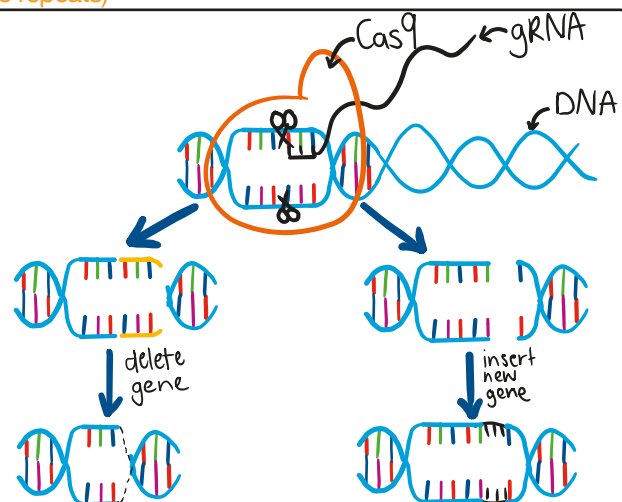
- Genome editing tool
- Fast, cheap, more accurate
- Edit parts of DNA by removing, adding or altering sections of DNA

How does it work?

System has 2 key molecules that introduce change/mutation:

1. **Enzyme Cas9** → Molecular scissors that cut across both DNA strands
2. **Guide RNA (gRNA)** → Predesigned RNA sequence complementarily binds to part of the DNA

- gRNA guides Cas9 to correct part to cut both DNA strands
- Cell recognises DNA is damaged and tries to repair it
- Here genes can be introduced or mutations can be removed
- Scientist can use DNA repair machinery to introduce changes to 1 or more genes in genome of cell of interest



Discuss design of specific proteins + their uses

Computer analysis can determine the folding of known proteins with particular functions. Using this knowledge, proteins can be designed and manufactured.

- Determine the desired protein shape required
- Determine the aa sequence that will create this shape
- Determine the DNA sequence that will create this aa sequence
- Incorporate constructed DNA into bacteria and use bacteria to produce the protein

Uses

- Create ion protein channels to deliver drugs to cells
- Create more stable enzymes with wider tolerance limits to be used for the production of material, food, fuel, and medicine
- Creating synthetic peptide vaccines that are easier to store and less likely to have side effects
- Create synthetic pharmaceutical proteins that would be more effective than natural protein drugs

Topic 4 Test: Evolution

1. Reproductive isolating mechanisms can be broadly separated into two categories, prezygotic and postzygotic.

Which of the following correctly identifies the reproductive isolating mechanism?

	Type of isolation	Pre or postzygotic	Explanation
(a)	Mechanical isolation	postzygotic	Anatomical differences
(b)	Temporal isolation	prezygotic	Isolated by different temperatures (e.g hot and cold)
(c)	Hybrid sterility	Postzygotic	Unable to produce fertile offspring
(d)	Gamete isolation	Postzygotic	Fertilisation does not occur, despite transfer

2. A species is defined as individuals that can interbreed successfully to produce fertile offspring. However, now there are other criteria that are used to define species. These do **not** include:
- (a) morphological similarity
 - (b) biochemical similarity
 - (c) biodiversity
 - (d) sharing a common gene pool.
3. A large gene pool results in:
- (a) Increased mutations
 - (b) Biodiversity
 - (c) Lower chance of survival
 - (d) Greater genetic variability
4. Examples of convergent evolution include the similar characteristic of wings / flying in insects, birds, bats.
- Therefore, these organisms must have:
- (a) Recent common ancestors
 - (b) Same selective pressures
 - (c) Homologous structures
 - (d) Similar selective pressures
5. Allopatric speciation does **not** involve:
- (a) Natural selection
 - (b) Physical barrier
 - (c) Reproductive isolation
 - (d) The formation of one species from two
6. Honeycreeper birds in Hawaii underwent adaptive radiation. Adaptive radiation is a type of:
- (a) Convergent evolution
 - (b) Divergent evolution
 - (c) Reproductive isolation
 - (d) Genetic drift
7. Genetic drift is defined as a:
- (a) Change in the number of alleles
 - (b) Change in the type of alleles
 - (c) Change in the frequency of alleles
 - (d) Change in the gene pool

8. In a population of bacteria, the following genotypes exist amongst the population:

A^E , A^E no streptomycin resistance

A^E , A^e carrier of streptomycin resistance gene

A^e , A^e streptomycin resistant

Prior to exposure to the antibiotic streptomycin, this population had a high frequency of A^E genes and a very low frequency of A^e genes in the gene pool.

Following exposure to streptomycin over a number of generations the gene pool changed significantly.

Which of the following would occur following exposure of the bacteria to the antibiotic streptomycin?

- The frequency of the allele A^E would remain high
 - Bacteria with genotypes A^E , A^e would be selected for, surviving and reproducing at a rapid rate.
 - Bacteria with 2 alleles for streptomycin resistance would survive the selective pressure.
 - The antibiotic streptomycin would change the alleles of the bacteria with genotypes A^E , A^E and A^E , A^e to become resistant.
9. DNA-DNA hybridization is a technique used to obtain evidence of the relatedness of different species.

The following table shows the % similarity between 4 different species of sea turtle based on DNA-DNA hybridization evidence.

Percentage (%) similarity in DNA between species of Sea Turtles (1-4)

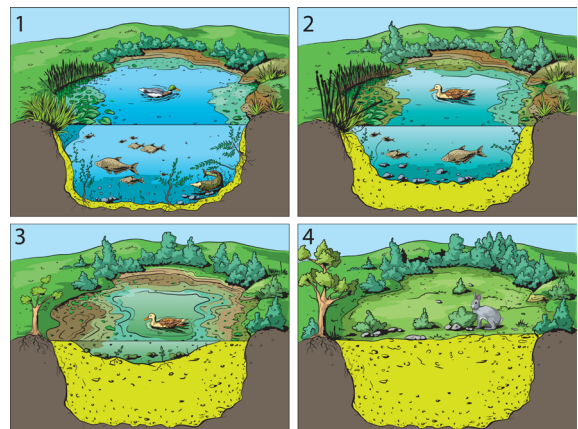
	1	2	3	4
1	100			
2	58	100		
3	54	46	100	
4	56	31	23	100

Based on the information above which of the following is true:

- The temperature required to separate DNA hybrid between species 1 and 4 would be greater than the temperature required to separate DNA hybrid between species 1 and 2.
 - Species 2 and 3 are likely to share a more recent common ancestor than species 2 and 4.
 - Hybrid DNA between species 4 and 3 would have more base pairs than hybrid DNA between species 4 and 2.
 - The highest temperature required to separate hybrid strands would be between species 3 and 4 and the lowest temperature between species 1 and 2.
10. The following image shows the change in a lake community as a creek bed dries up over time.

Which of the following is **incorrect** regarding the processes that occur throughout this change?

- Throughout this process, the biodiversity of the organisms will change.
- New colonising species of plants will establish themselves early as the creek bed dries up.
- Throughout the process there will be no change to the characteristics of the soil.
- Some organisms may lose their habitat as a result of this change.



11. Describe the evidence that exists to show that prokaryotic cells existed before eukaryotic cells

12. Mitochondrial DNA (mtDNA) is often referred to as ancient DNA and is being used currently to trace individuals' heritage. Minor mutations in mitochondrial DNA are passed on through generations from mother to child. These mitochondrial DNA mutations are markers which can reveal ancestry.

(a) How does the presence of DNA in the mitochondria provide evidence for endosymbiosis?

(2)

(b) What other evidence exists to show the ancestry of most existing eukaryotic cells involved endosymbiotic events?

(2)

(c) The first simple cells may have used RNA and ribozymes. What roles did RNA and ribozymes play in those first cells?

(2)

13. Molecular biological techniques have been used to establish relationships between species.

DNA/DNA hybridization is one such method.

Below is a table showing the temperature needed to separate hybrid DNA strands between 4 different species.

	Species A	Species B	Species C	Species D
Species A	100°C	95°C	71°C	82°C
Species B		100°C	72°C	84°C
Species C			100°C	88°C
Species D				100°C

(a) Draw a phylogenetic tree showing the relationship between these 4 species based on the information above.

(4)

(b) Describe the technique of DNA-DNA hybridisation?

(4)

(c) Which 2 species are most closely related? Explain based on DNA-DNA hybridisation results.

(4)

(d) Name one other molecular biological technique that can be used to determine relatedness between species A, B, C and D (1)

14. In the late 1950s, a new penicillin antibiotic Methicillin was introduced to treat *Staphylococcus Aureus* (*S. aureus*) infections. Shortly after the introduction of this antibiotic, scientists isolated strains of *S. aureus* bacteria that were resistant to methicillin (MRSA). Methicillin resistant *S. aureus* (MRSA) infections have since been increasingly transmitted in hospitals and aged care centres.

(a) Explain the role of methicillin in the formation of resistant strains of *S. aureus*.

(4)

(b) Other factors can cause changes to gene pool other than the mechanism explained above.

List another of these factors which could cause evolutionary changes.

(1)

15. In a review on antimicrobial resistance, in 2015 British Prime Minister David Cameron endorsed efforts to understand the health and economic consequences of antimicrobial resistance. Three reports were published in this review, which revealed alarming findings. More than 500,000 people were dying due to antibiotic resistance annually. The reports estimated that if this issue wasn't tackled, 10 million people could die as a result of antibiotic resistance and the economy would lose 100 billion dollars. Furthermore, the rapid emergence and spread of antibiotic resistance will increase the likelihood of death after an accident, chemotherapy, childbirth and surgery. Excessive, unnecessary and uncontrolled applications of antibiotics in agriculture, livestock and among humans are the prime culprits behind the emergence of antibiotic-resistant superbugs. In a perspective in *PLOS Biology*, scientists call for the end of nonmedical use of antibiotics, such as use in agriculture and livestock. "The global crisis of antibiotic resistance has reached a point where, if action is not taken, human medicine will enter a post antibiotic world and simple injuries could once again be life threatening," said Meek and colleagues.

Source: Adapted from: Zohorul Islam, M. (2019). *An overview of #antibioticresistance after the first World Antibiotic Awareness week* | PLOS ECR Community. [online] The Student Blog. Available at: <https://blogs.plos.org/thestudentblog/2015/11/24/antibioticawareness/>

Read the article above and explain which SHE key concepts emerge from it.

(4)