SYLLABUS 9816 (2021)

LEARNING OUTCOMES

1: The Cell and Biomolecules of Life

(a) Describe how the fluid mosaic model of the cell has developed to the current understanding

(b) Describe the basic characteristics of

(i) Prions

(ii) Fungi (including yeasts and filamentous fungi)

(iii) Protoctista (including algae)

(c) Explain the following terms and discuss the extent to which each conforms to the cell theory

(i) Acellularity (including prions and viruses)

(ii) Multinucleation (including hyphae of filamentous fungi)

(iii) Endosymbiosis (including endosymbiotic origin of eukaryotes)

(d) Justify the need for cell differentiation in multicellular organisms

(e) Describe protein binding sites and protein subunits in producing large protein and glycoprotein molecules (including haemoglobin, immunoglobulin and prokaryotic RNA polymerase)

(f) Explain, with examples, how protein modification (including cleavage, phosphorylation and glycosylation) confer new capabilities

(g) Discuss and explain why proteins are able to recognise and bind to highly diverse molecules, with reference to the properties and shapes of their surfaces and clefts that allow highly complementary interactions

(h) Discuss how a living cell regulates thousands of enzymes

2: Genetics and Inheritance

(a) Discuss how mature cells can be returned to a stem cell state

(b) Explain that genetic engineering involves the insertion of a gene, obtained either by synthesis or by extraction from an organism, into another organism (of the same or different species), such that the receiving organism expresses the gene product

(c) Explain the roles of restriction endonucleases, reverse transcriptase and ligases in genetic engineering

(d) Outline the procedures for cloning a eukaryotic gene in a bacterial plasmid and describe the properties of plasmids that allow them to be used as DNA cloning vectors

(e) Explain how eukaryotic genes are cloned using *E. coli* cells to produce eukaryotic proteins

(f) Explain the structure and roles of ribozymes and their potential role in genetic engineering (including novel peptide synthesis and modifications)

(g) Evaluate the significance of genetic engineering to the world and humanity (including food sustainability for a rapidly growing population, disease treatment and drug design)

(h) Explain that epigenetics is a process that affects the expression of specific genes, without involving a change in DNA sequence

(i) Discuss how epigenetics has contributed to the study of genetics and heredity

3: Energy and Equilibrium

(a) Explain how the anatomy and physiology of the leaves of C4 plants, such as maize and sorghum, are adapted for high rates of carbon fixation at high temperatures in terms of

(i) The spatial separation of initial carbon fixation from the light-dependent stage (biochemical details of the C4 pathway are required in outline only)

DO NOT MONETISE MY SHIT

(ii) The high optimum temperatures of the enzymes involved

(b) Discuss and compare the importance in mitigating global warming of photosynthetic carbon fixation by C3 plants, C4 plants, CAM plants and algae, including those in reef-building corals

(c) Explain how the physiology of the leaves of CAM plants is adapted to allow photosynthesis while minimising water loss by transpiration, in terms of (i) The temporal separation of initial carbon fixation from the light-dependent stage (biochemical details of the CAM pathway are required in outline only) (ii) Stomatal opening during the night

(d) Explain the changes in atmospheric oxygen concentration during the evolution of life on Earth and evaluate the importance of these changes to evolution

(e) Describe and explain the transmission of an action potential along a myelinated neurone (the importance of Na^+ and K^+ions in the impulse transmission should be emphasised)

(f) Describe the structure of a cholinergic synapse and explain how it functions, including the role of Ca^{2+}ions

(g) Explain that quorum sensing is a system of signalling processes that respond to changes in population density in bacteria

(h) Explain the need for control in organised systems and explain the principles of homeostasis in terms of receptors, effectors and negative feedback

(i) Explain the need for different communication systems within organisms

4: Biological Evolution

(a) Explain, with examples, sexual selection and its significance for evolution (b) Explain, with examples, the evolutionary concepts of adaptive radiation and ring species

(c) Discuss the contributions of polyploidy, hybridisation and introgression in evolution and their implications for reconstructing phylogenies

(d) Explain the significance to living organisms of biomolecules, including carbohydrates, lipids, proteins and nucleic acids, and the biochemical processes through which they are synthesised

(e) Discuss the contributions of mitochondrial DNA and the Y-chromosomal Adam (the Genographic Project) to trace and support the ancestry and diaspora of humans

A: Infectious Diseases

(a) Explain why specific (adaptive) and non-specific (innate) immunity can be both mutually exclusive and interdependent in the protection against pathogens (b) Explain how immunological self-tolerance ensures that B lymphocytes and T lymphocytes do not normally attack host cells that are functioning correctly (c) Explain why the human microbiota is important for our health

(d) Explain the factors affecting the probability that a pandemic will occur, including sanitation, water supply, food, climate, large-scale movements of people, evolution of new strains of virulent pathogens and development of drug resistance

B: Impact of Climate Change on Animals and Plants

(a) Discuss how humans are responding to mitigate climate change, including biological measures (such as tree planting and developing drought-resistant varieties of crops) and lifestyle changes (such as reducing use of cars and consumption of meat)

(b) Discuss, with examples, how animal and plant species can adjust and adapt to climate change, and the possible consequences of climate change for ecosystems and the organisms within them in the longer term

DO NOT MONETISE MY SHIT

LO1(a): Describe how the fluid mosaic model of the cell has developed to the current understanding.

Evidence	Conclusion
1807: Link demonstrated that pigments from one cell did not pass into neighboring cells unless the cell walls were broken 1812: Treviranus, Moldenhawer and Dutrochet managed to separate the cells from the plant tissue	Cells are not simply a cavity, but unconnected independent structures bounded by separate cell walls
1895: Overton immersed cells in solutions of over 500 different substances at the same concentration. He noticed that solutions of ether-soluble (apolar) molecules did not result in the shrinking of cells, contrary to solutions of water-soluble (polar) substances. He concluded that apolar molecules entered the cells with less difficulty than polar substances, and he showed that this was irrespective of their molecular size	Cells have semi-permeable membranes
1922: Chambers applied a water-soluble cytolysogenic solution in different parts of the cell. He could apply the hydrophilic cytolysogenic substance on the surface of starfish eggs without damaging them. When a small amount was injected in the interior of the cell, cytolysis was observed	The nature of the cell surface is chemically different from the rest of the protoplasm, supporting the existence of the cell membrane
1925: When Gorter and Grendel compared the surface covered by the lipids to the estimated sum of cell surfaces, they found a 2:1 ratio	Cells are surrounded by a lipid bilayer
1941: Dean postulated that the agent of ion movement against the gradient could result from the activity of some “pump” located in the muscle fiber membrane 1946: Conway found that the energy necessary to extrude all the $Na^+$from the muscle was larger than the actual available energy in the resting muscle	Na^+/K^+ asymmetries across the membranes were the result of active transport using ion pumps
1971: Singer and Nicolson presented the fluid mosaic model based on (1) the permeability and transport studies that predicted enzyme-like transmembrane proteins (2) the apparent lack of lipids to make up complete bilayers, pointing to the participation of proteins in the membrane plane (3) electron microscopy pictures, including freeze-etching studies that suggested the presence of proteins within the membranes (4) the stability of artificial lipid bilayers that supported them as suitable and sufficient components to make up structures similar in the biological membranes (5) the favorable conformations predicted for the membrane proteins	Fluid mosaic model of the cell membrane

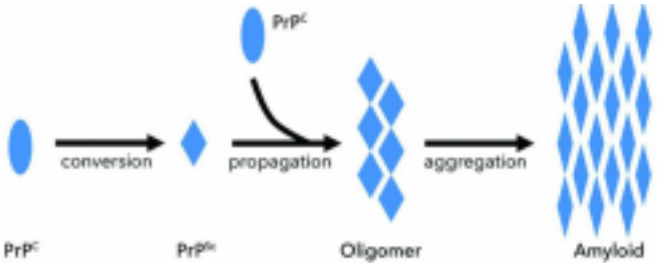
DO NOT MONETISE MY SHIT

LO1(b): Describe the basic characteristics of prions, fungi (including yeasts and filamentous fungi) and protoctista (including algae).

Prions

- Definition : misfolded protease-resistant prion protein that cause infectious neurodegenerative disorders affecting animals and humans
    - Prion diseases characterised by long incubation periods, characteristic spongiform changes associated with neuronal loss, failure to induce inflammatory response, rapidly progressive and ALWAYS fatal
- Able to transmit biological information through propagation of alternative protein folding, can expand phenotypic diversity without changes in the genome
    - Can exist as normal protein or the pathological isoform → pathological isoform is the one that cause diseases
        - Pathological isoform forms from a conformational change whereby the alpha helical content of the normal protein diminishes and the beta sheet content increases → changes in biochemical properties (eg protease resistance, solubility and ability to form larger order aggregates)
    - Can transmit biological information in the absence of a nucleic acid
        - Non Mendelian genetic element can be transmitted cytoplasmic transference in absence of nucleic acids
        - Prion phenotype can be reverse by protein denaturation, but can also arise again spontaneously at a low frequency
        - Requires the chromosomal gene encoding the normal prion protein
        - Overproduction of normal prion increase the chance of spontaneous occurrence of the prion form
        - Prions can exist in a normal soluble, protease sensitive state as well as an insoluble protease resistant state with a high tendency to form a beta sheet rich fibrillar aggregates
        - Prion forming domain is modular and transferrable
- Significant characteristics
    1. Protein molecule
    2. Alternatively folded variant of cellular prion protein (normal prion protein is a GPI anchored protein)
    3. Contain predominantly beta pleated sheets with h bonds present within each sheet
    4. Insoluble
    5. Ability to aggregate into oligomers, amorphous aggregates, amyloid fibrils and 2d crystals (repetition of a specific protein in beta pleated sheets conformation
    6. Resistant to degradation by protease due to aggregation that stabilises the structure
        a. enzymes are unable to bind to proteins due to changed 3D conformation, hence not complementary / specific to active site
        b. enzymes are unable to access the binding site of proteins due to the formation of aggregates
        c. Hence not recognised / accessed by enzymes which attach ubiquitin to these proteins
        d. cannot be hydrolysed by proteasomes / proteases into short peptides / amino acids
    7. Can bind to normal prion protein and change their conformation to add on to their aggregate to form more infectious prions
    8. The amyloidogenic PrP will aggregate, with the fibrils / proteins layered one over the other as a continuous stack of β-sheets

DO NOT MONETISE MY SHIT



- Misfolded proteins can bind to normal proteins and convert them into the misfolded form

Prion hypothesis/protein only hypothesis

- States that a single protein is the sole component of the infectious agent responsible for prion diseases
    - Protein can behave like a living microorganism to infect an individual
    - Survive metabolic clearance
    - Self replicate in the body
    - Reach the target organ to induce a cascade of neurodegenerative damage
- Evolution of prion
    - Dna-less evolution
    - Infectious protein occurring in different strains
    - When propagated in tissue culture cells, cloned prion populations become diverse by mutational events, can undergo selective amplification

- Examples of prion diseases:
- Bovine spongiform encephalopathy (BSE) in cattle (commonly known as "mad cow disease")
- Chronic wasting disease (CWD) in deer
- Creutzfeldt-Jakob disease (CJD) in humans
- All known prion diseases in mammals affect the structure of the brain or other neural tissue; all are progressive, have no known effective treatment and are always fatal

Types of prion proteins

- The physiological function of the PrP^C protein remains poorly understood - It has 209 amino acids (in humans), one disulfide bond, a molecular mass of 35–36 kDa and a mainly alpha-helical structure
- Several topological forms exist; one cell surface form anchored via glycolipid and two transmembrane forms
- PrP^C binds Cu^{2+}ions with high affinity. The significance of this finding is not clear, but it is presumed to relate to PrP structure or function
- PrP^C has been reported to play important roles in cell-cell adhesion and intracellular signaling in vivo, and may therefore be involved in cell-cell communication in the brain

PrP^{res}

- The name given to any isoform of PrP^C which is structurally altered and converted into a misfolded proteinase K-resistant form in vitro

PrP^{Sc}

- The infectious isoform of PrP, known as PrP^{Sc}, or simply the prion, is able to convert normal PrP^C proteins into the infectious isoform by changing their conformation; this, in turn, alters the way the proteins interconnect
- PrP^{Sc} always causes prion disease
- Although the exact 3D structure of $PrP^{Sc}$is not known, it has a higher proportion of β-sheet structure in place of the normal α-helix structure

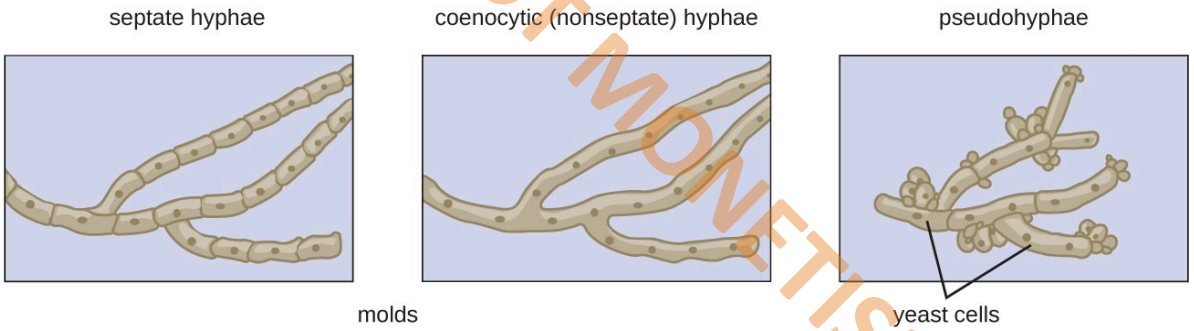
DO NOT MONETISE MY SHIT

Fungi

- Definition : eukaryotic, heterotrophs, grow by growing multicellular filaments
- Absorb nutrients from environment outside body by
    - Secreting hydrolytic enzymes into their surroundings that break complex molecules into smaller organic compounds
    - Use enzymes to penetrate the walls of cells so that they can absorb the nutrients from inside the cells
- Classes of fungi
    - Decomposers : break down and absorb nutrients from nonliving organic material
        - Serve to keep ecosystems stocked with inorganic nutrients essential for plant growth (carbon, nitrogen and other elements)
    - Parasites : absorb nutrients from cells of living hosts
        - Can attack food crop and produce compounds that are toxic to humans
    - Pathogenic : spread disease in plants and animals
    - Mutualistic : absorb nutrients from host but will reciprocate with actions that will benefit the host
        - Endophytes → fungi plant mutualism, increasing host plant tolerance of heat, drought heavy metals and making toxins that deter herbivores
        - Lichens → photosynthetic microorganism and a fungi, photosynthetic provide organic nutrients and fix nitrogen, fungi provide suitable environment for growth
        - arbuscular mycorrhizal fungi soil microorganisms able to form mutualistic symbiosis with most terrestrial plants
            - Ectomycorrhizal fungi → form sheath of hyphae over surface of the root, grow into extracellular space of the root cortex
            - Arbuscular mycorrhizal fungi → extend arbuscule through the root cell into tubes formed by invagination (but it is still mutual benefitting)
- Benefits
    - Decomposers and recyclers of organic matter
    - For human consumption
    - Yeasts for production of alcoholic beverages → anaerobic conditions
    - For medicine, eg compounds extracted from ergots reduce high blood pressure, stop maternal bleeding after child birth
    - Antibiotic that can treat bacterial infections

DO NOT MONETISE MY SHIT

- Body structure
    - Multicellular filaments
        - Network of hyphae → tubular cell walls surrounding the plasma membrane and cytoplasm, with chitin cell walls
        - Chitin cell walls can enhance feeding by absorption (also found in hard shelled animals)
            - Septate → divided into cells by cross walls, have pores large enough to allow ribosomes, mitochondria, and nuclei to flow from cell to cell
            - Challenge the cell theory
            - Coenocytic → lack septate and walls, consist of continuous cytoplasmic mass having many nuclei (multinucleated)
            - Pseudohyphae → incomplete budding, no cytoplasmic connection between cells (multicellular yeast cells stuck together, similar to a colony)
        - Hyphae will form interwoven mass (mycelium) → to maximise surface area to volume ratio
            - Grows rapidly, extending the tips of hyphae (focusing on adding hyphal length and overall absorptive surface area)
    - Single cells (as yeasts)

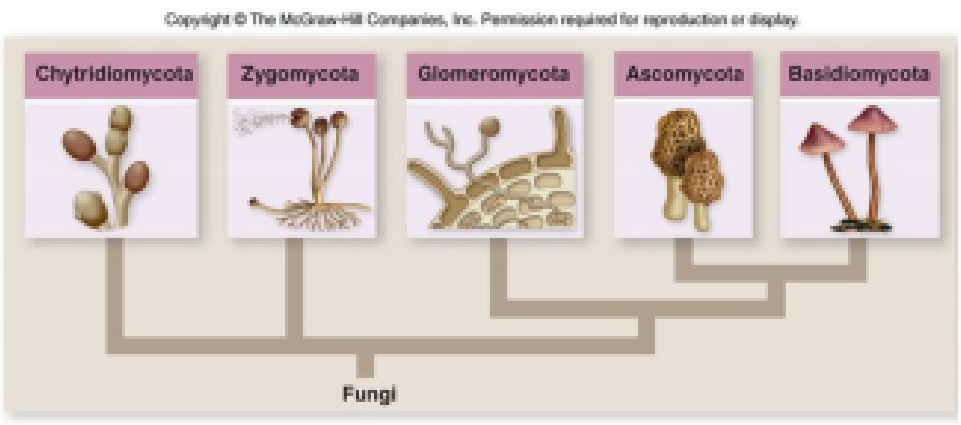


DO NOT MONETISE MY SHIT

- Reproduction methods
    - Can undergo sexual or asexual reproduction
    - Sexual reproduction
        - Release pheromones → if recognise and bind to different hyphae, hyphae of 2 fungi will extend towards the source of pheromones
        - Undergo compatibility test, to prevent in-breeding depression → contributes to genetic variation
            - Inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding, or breeding of related individuals
        - Plasmogamy occur, no fusion of nuclei, mycelium is dikaryotic
        - Karyogamy occur, fusion of nuclei, producing diploid cells
    - Asexual reproduction
        - Growing as filamentous fungi that produce haploid spores by mitosis

DO NOT MONETISE MY SHIT

Types of fungi



Chytrids

- Most primitive
- Most are unicellular, some multicellular with hyphae without septa - Reproduce asexually and sexually
- Gametes are the only fungal cells known to have flagella

Zygomycetes e.g. bread mould

- No septa in hyphae
- Reproduce asexually or sexually if in close proximity
- This phylum is no longer recognised as it was not believed to be truly monophyletic

Glomeromycetes

- Most members form biotrophic relationships with plants, interacting with root cells to supply soil minerals to the plant and the plant supplies carbon source and energy - Coenocytic hyphae, reproduce asexually

Ascomycetes e.g. yeast, cordyceps

- Septa in hyphae
- Reproduce asexually and sexually
- Some are dimorphic
- When spores gain entrance to a warm blooded animal, they germinate into yeast cells and reproduce by budding
- When they leave the host they return to sporulating hyphal state

Basidiomycetes (mushrooms)

- Septa in hyphae
- Asexual reproduction is unusual, sexual reproduction by spores to produce fruiting bodies

Fungal evolution

- Coenocytic hyphae are more primitive; septal hyphae diverged from common ancestor
- Advantage is that nutrients can move rapidly from regions of uptake to regions of growth
- Some fungi produce hyphae with unique adaptations for capture of prey e.g. nematodes

DO NOT MONETISE MY SHIT

Protoctista or Protista

- Definition : diverse, mostly unicellular group of eukaryotes that is not a plant, animal or fungi
    - In domain eukarya
    - Have a nucleus and membrane enclosed organelles such as mitochondria and golgi apparatus
    - Have well developed cytoskeleton that extends throughout the cell
- most protists are unicellular → can perform same essential functions as other eukaryotes but they do so via subcellular organelles
- Can be colonial → no cell specialisation
    - formation of a colony of the same type of cells via cell nuclear division
    - Aggregation of cells with slightly different genome (not via cell and nuclear division)
- Can be multicellular species with cell specialisation involved

- Key roles in ecological communities
    - Formation of symbiotic associations →
        - can be food providing symbiotic partners if animals that build coral reef
    - Can be parasitic → cause devastating effects on species
    - Serve as important producers, all other organisms in the community depend on them for food directly or indirectly
        - majority much of the world photosynthesis is contributed by photosynthetic protists together with plants
        - Form the foundation of food webs

DO NOT MONETISE MY SHIT

LO1(c): Explain the following terms and discuss the extent to which each conforms to the cell theory - acellularity (including prions and viruses), multinucleation (including hyphae of filamentous fungi), endosymbiosis (including endosymbiotic origin of eukaryotes).

Acellularity

3 tenets of cell theory

- All organisms are composed of one or more cells
- The cell is the basic unit of life in all living things
- All cells are produced by the division of pre-existing cells

Viruses

- Considered living because
    - Have a common hereditary molecule based on one type of nucleic acid
    - Can pass on genetic characteristics from one viral generation to the next
    - Can replicate genetic material within an infected living host cell
    - Contain viral enzymes that can be used in their reproductive cycles
    - Can evolve via antigenic shift and drift
- Not considered living because
    - Unable to replicate independently outside a host cell
    - Metabolically inactive outside host cell
    - Acellular (hence challenge the cell theory)
    - Do not grow in size, cannot undergo homeostasis
    - Cannot respond to stimuli outside a host cell
- Hence considered obligate parasites

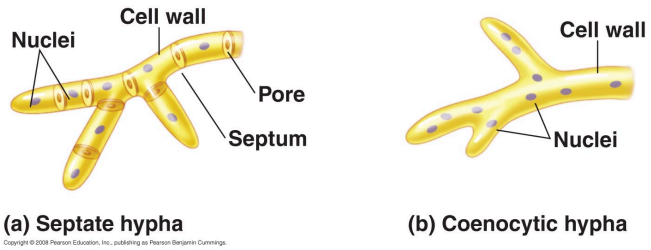
Prions

- Prions are acellular but it has living characteristics
    - They are able to replicate themselves by converting normal prion proteins into prions without DNA as a hereditary material
    - Prion hypothesis

DO NOT MONETISE MY SHIT

Multinucleation

- One cell contains multiple nuclei
- Eukaryotic cells that have more than nucleus in one cell, sharing common cytoplasm
- Can occur via syncytia (cell fusion) or coenocytes (nuclear division without cytokinesis)
    - Fungi is almost entirely multicellular, a single fungal syncytium can harbor thousands or millions of mobile and potentially genotypically different nuclei → not small basic form of life as the structure are not strictly made up of individual cells



(a) Septate hypha (b) Coenocytic hypha

Copyright © 2008 Pearson Education, Inc., publishing as Pearson Benjamin Cummings.

How does Acetabularia, the giant algal cell that can grow up to 10cm in length and has three distinct body parts, disturb the cell theory?

- Cell theory states that organisms are made of cells. Acetabularia is a whole organism and it is larger than a cell. This disturbs the cell theory because it seems to break the rule that all organisms are made up of cells by itself not being made up of cells

How does the multinucleated cytoplasm of fungal hyphae and skeletal muscles bring the cell theory into question?

- The cell theory states that cells are the smallest unit of life and that organisms are made of cells. Both skeletal muscle cells and fungal hyphae are made from elongated cytoplasm with more than one nucleus. These structures are a problem for cell theory as they are not strictly made of cells

Endosymbiosis theory

- Origins of protists is endosymbiosis
    - One organism lives inside the cells or cells of another organism
    - Proof : mitochondria and plastics are derived from prokaryotes that were engulfed by the ancestors of early eukaryotic cells
        - Host cell engulfing a bacterium, bacterium would end up becoming an organelle → mitochondria and plastids
- Primary endosymbiosis
    - Larger cell engulf archean or close relative of archean (mitochondria) or proteobacterium which is an aerobic prokaryote → form a symbiotic association
    - Ancient heterotrophic eukaryote engulf chloroplast (probably cyanobacterium) and becomes an autotroph
- Secondary endosymbiosis
    - Secondary endosymbiosis occurs when a eukaryotic cell engulfs and absorbs another eukaryotic cell
    - Unicellular eukaryotes aggregate to form colonies and eventually cell specialisation occur to form a multicellular eukaryote
- Support
    - Mitochondria and chloroplast are bounded by double membrane
        - Membrane enclosing chloroplast and mitochondria are both derived from ancestral bacteria, phagosomal membrane disappeared
        - Mitochondria and chloroplast contain 70s ribosomes, circular dna

Mitochondria and chloroplasts are autonomous organelles that grow and reproduce within cells

DO NOT MONETISE MY SHIT

LO1(d): Justify the need for cell differentiation in multicellular organisms.

Unicellular Organisms

- Carry out all functions of life (metabolism, reproduction, sensitivity, homeostasis, excretion, nutrition, growth)
- Structure usually more complex than multicellular organisms
- As size increases, surface area to volume ratio decreases → surface area for exchange will no longer be optimal → when a cell reaches its optimal size it stops growing/divides into two

Multicellular Organisms

- When cells in multicellular organisms divide they become part of tissues → organs → system
- Differentiation is necessary for specialisation of cells to make up a complex organism

Cell Differentiation

- A process during development where a cell changes from a less specialised cell to a more specialised cell type
- All cells of an organism share an identical genome
- The expression of different genes within a cell by the presence of molecular signals will cause it to differentiate
- Different cell types form due to differential gene expression in cells - E.g. each olfactory receptor cell expresses one gene to make one type of receptor to detect one type of odorant

Benefits

- Division of labour: differentiated cells in tissues can carry out their role more efficiently
- Ideal structures can be developed with the enzymes needed to carry out chemical reactions associated with the function
- Gives rise to systems with functions beyond what any single cell type can accomplish

Costs

- Differentiated cells often lose the ability to make copies of themselves - Organisms must retain some unspecialised cells (stem cells) to replenish cells when needed

DO NOT MONETISE MY SHIT

“There is no evolutionary advantage in being multicellular.” Discuss this view.	
Meaning of evolutionary advantage: - More likely to survive - More likely to pass on genes to the next generation - More abundant - More long-lasting - More diverse	
No advantage	Advantage
Unicellular organisms can also have specialised organelles for specialised functions	Differentiation - specialised cells for specialised functions, division of labour
Unicellular organisms evolved first, suggesting that	Structural diversity, complexity and variety in appearance and behaviour
Can reproduce quickly via asexual reproduction in favourable conditions	For the same size, there is greater surface area for exchange (e.g. fungi)
Use less energy for metabolic functions	Homeostasis - can regulate internal environment better and be more resistant to changes in the external environment
More ubiquitous and found in a variety of extreme habitats that multicellular organisms cannot survive	More mobile - can move away from unfavourable environments towards more suitable environments
Unicellular organisms are also able to form symbiotic relationships with other organisms, becoming more successful e.g. lichen	All multicellular organisms are eukaryotes, as they are symbiotic unions of previously separate cells - this suggests that symbiotic unions are more successful than prokaryotes
- Consideration of acellularity:	
- Evolutionary dogma: fitness to survive increases with natural selection, therefore more recently evolved life forms should be most successful - This is a flawed argument because natural selection operates on all species all the time - Therefore current life forms have equal status in terms of success	

DO NOT MONETISE MY SHIT

Mark sheme by cambridge
1. specified range of multicellular organisms to include animals, plants, many fungi, some protoctists, e.g. some algae
2. specified range of acellular / unicellular organisms to include prokaryotes / bacteria, some protoctists / fungi, e.g. yeasts
prokaryotes evolved, first / about 3500 million years ago
3. discussion of meaning of evolutionary advantage / more likely to survive / more successful / abundant / long-lasting / diverse
4. could argue that prokaryotes / unicells are more successful
5. (perhaps) greater biomass than eukaryotes / multicellular organisms
greater numbers / more ubiquitous / AW
6. still present and successful (after 3500 million years)
7. (perhaps) more likely to survive natural disasters / survive in wider range/ extremes of physical conditions
8. great diversity of types of metabolism amongst prokaryotes / unicells
9. some prokaryotes can both photosynthesise and fix nitrogen / ref unicells forming symbioses with fungi as lichens and their even greater success in these associations
10. all multicellular organisms are eukaryotes
11. ref eukaryotic cells being symbiotic unions of previously separate cells / endosymbiosis
12. (perhaps) suggesting symbiotic unions superior to prokaryotes
ref structural diversity of multicellular organisms / complexity / variety of behaviour
13. ref to advantages of division of labour between organs / specialised cells

14. ref to the greater potential of division of labour / specialisation
15. discussion with respect to evolution
16. evolutionary dogma is that fitness to survive increases with natural selection
17. therefore most recently evolved life forms should be superior
18. this is a flawed argument because natural selection operates on all species all the time
19. therefore current life forms have equal status in terms of success / can only
20. judge on basis of future possibilities
21. could consider further the particular example of humans
humans have more control over environment than any other organism
22. they are a product of an evolutionary trend towards greater complexity
23. perhaps control over environment may be greater evolutionary advantage than adaptation to change

DO NOT MONETISE MY SHIT

LO1(e): Describe protein binding sites and protein subunits in producing large protein and glycoprotein molecules (including haemoglobin, immunoglobulin and prokaryotic RNA polymerase).

Proteins and their basic characteristics

- Shape of protein is determined by folding of protein (r group interactions) which is in turn determined by the specific amino acid sequence
- Steric limitations on the bond angles in the polypeptide chain, h bonds, electrostatic forces of attractions, van der waals attractions and hydrophobic interactions will have constraint on the protein folding
    - Most proteins exist in the stable state, because protein with an unpredictably variable structure and biochemical activity is unlikely to be beneficial to the cell that contains it and would naturally be eliminated
- Molecular chaperones an assist in protein folding, for the most energetically favourable folding pathway
    - Specific 3d conformation → which is the one that minimise its free energy which is the most stable state
- Protein domains
    - A substructure produced by any part of a polypeptide chain that can fold independently into a compact stable structure (distinct from the primary, secondary, tertiary and quaternary structure)
    - Conserved part of a given protein sequence and tertiary structure that can evolve function and exist independently of the protein chain
    - Distinct functional or structural units, hence conserved, help to contribute to the overall role of a protein

    - Proteins are composed of a series of protein domains → different regions of the polypeptide chain fold independently to form compact structures
        - Believed to have originated from the accidental joining of the dna sequences that encode each domain that create a new gene
    - Domain shuffling : by the insertion of a sequence from one gene into another by recombination
        - Allows for many large proteins to be evolved through the joining of preexisting domains in different and new combinations

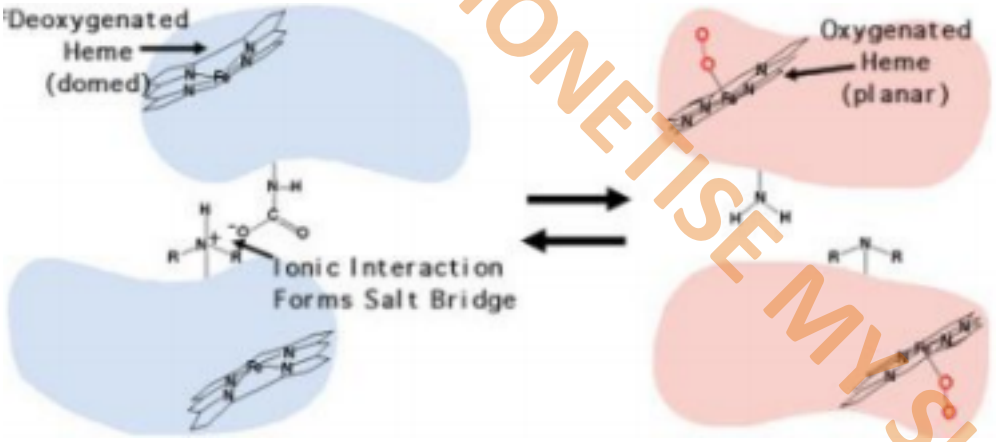
DO NOT MONETISE MY SHIT

Haemoglobin

- Globular protein that transports oxygen in blood and found in red blood cells of vertebrates
- Acts as a ph buffer to maintain ph at optimum level of blood
- Structure of haemoglobin
    - Primary consist of 2 alpha chains and 2 beta chains
    - Secondary consists of each part of chain folded into alpha helices, stabilised by h bonds
    - Tertiary structure is each chain folded into globular structure stabilised by h2id
    - Quaternary structure consist of 4 chains assemble to form a protein
    - Each polypeptide subunit contains haem group attached to an iron ion, haem group is a prosthetic group (non protein component of haemoglobin that is required for function)
        - O2 will bind to the fe2+ as ligands (under transition metals of h2 chem)
        - Binding of 1 o2 ti haem group of one subunit will cause the tertiary structure to change that cause other subunits to have increased affinity for oxygen
    - Haem group lies in a hydrophobic cleft lined with amino acid residues with hydrophobic r groups to form hydrophobic interactions
    - Most of the amino acid residues that face the aqueous medium is hydrophilic so that it is soluble and suitable as a transport agent in blood

DO NOT MONETISE MY SHIT

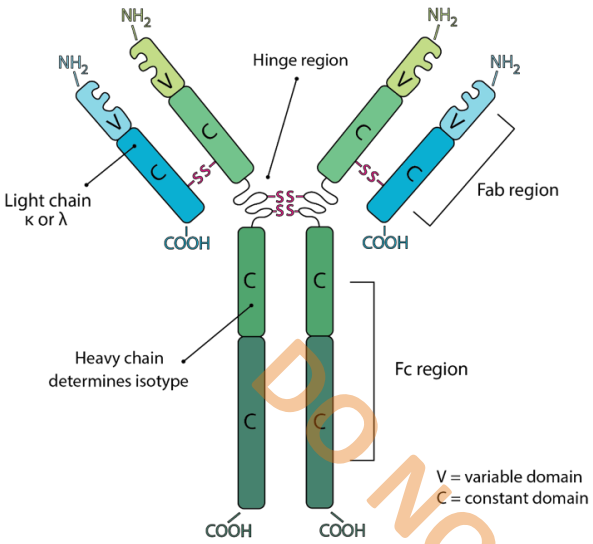
- Extensive bonding between α and β subunits
- α1-β1 interface and α2-β2 interface involve 35 residues each
- α1-β2 and α2-β1 interface involve 19 residues each
- Mostly hydrophobic interactions, with many hydrogen and some ionic bonds
- Limited interaction between α1-α2 and β1-β2 due to the solvent-filled channel between them
- Cooperative binding
    - haemoglobin consist of 4 globin subunits (2 and 2), each associated with its own heme group
    - In deoxyhemoglobin, the ferrous ion lies outside the plane of the porphyrin ring because iron, in this form, is slightly too large to fit into the well-defined hole within the porphyrin ring
    - The binding of oxygen molecule at the sixth coordination site of the ferrous ion rearranges the electrons within the iron so that the ion becomes effectively smaller, allowing it to move into the plane of the porphyrin ring
    - When the ferrous ion moves into the plane of the porphyrin ring, the histidine residue to the ferrous ion moves with it.
    - This histidine residue is part of an α helix, which also moves. The carboxyl terminal end of this α helix lies in the interface between the two αβ dimers, allowing changes to occur in the other subunits
    - The salt bridges between the subunits that are involved in stabilising the T state are broken, allowing for conformational changes to occur for the transition to R state



DO NOT MONETISE MY SHIT

Antibodies (h2)

- Produced by plasma cells, derived from b cells → have tight selective binding, binds to antigen for opsonization, agglutination and neutralisation
    - Different antibodies generate enormous diversity of antigen binding site by changing length and amino acid sequence without altering the basic protein structure

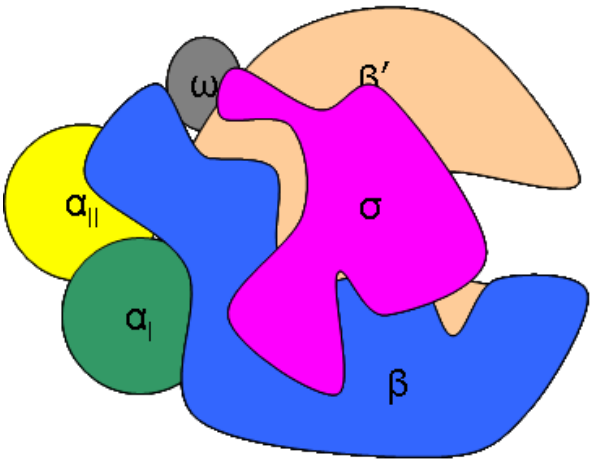


    - Consist of 2 identical heavy chain and 2 identical light chains
    - Globular proteins, allow to maintain solubility
    - Light chain : either lambda or kappa, never one of each
        - 1 variable and 1 constant domain
    - Heavy chain
        - 1 variable domain and 3 constant domains
    - Variable domain of 1 antibody differ greatly in amino acid sequence and the 3d conformation of another variable domain of another antibody
    - Variable domains form the antigen binding site → combination of the heavy and light chain variable domains → specific 3d information that determines the final antigen specificity
- 2 heavy chain are linked together by disulfide bonds
- 2 regions : fragment crystallisable and fragment antigen binding → connected by the hinge region which is flexible and allow independent movement of the 2 Fab arms
    - Allows the antibody to bind to antigens at different angles and at sites some distance apart
    - Allow for the antibody to interact with antibody binding proteins that mediate immune effector mechanisms
    - Fc region of the antibody determines the effector function and the class of the antibody (there are 5 classes of antibody)

DO NOT MONETISE MY SHIT

Prokaryotic RNA Polymerase

- Catalyse the synthesis of mrna trna and rrna



    - Have 5 subunits (β′,β,α′,α″,ω)
    - B′ subunit : largest subunit, contain part of the active centre responsible for rna synthesis
        - Bind to dna template strand
        - Active site
    - B subunit : second largest subunit, contains the rest of the active centre responsible for rna synthesis
        - Bind to ribonucleoside triphosphate
    - A′,α″ subunits : third largest subunit and present in 2 copies, N terminal domain contains determinants for assembly for RNAP, C terminal domain contain determinants for interaction with promoter dna
        - CTD interact with promoter
        - NTD assembly of RNAP
- ω subunit : smallest subunit, facilitates assembly of RNAP and stabilises the assembled RNAP
- σ subunit (not part of the core enzyme)
    - Reduce affinity of RNAP for nonspecific dna while increasing specificity for promoters, for correct transcription initiation
    - Involved in transcription initiation
    - Confer transcriptional specificity to allow polymerase to begin synthesizing mrna from the initiation site
    - Conserved basic residues in region provide critical contact with dna phosphate backbone and play a role in separating dna strands
    - σ factors : recognise promoter sequence, act as transcription factors for RNAP

DO NOT MONETISE MY SHIT

LO1(f): Explain, with examples, how protein modification (including cleavage, phosphorylation and glycosylation) confer new capabilities.

- Signal sequences target protein to different subcellular localisations - Contain N-terminal signal sequences that allow these proteins to bind to receptor proteins and be fed into the translocation channel, after which signal peptide is removed by signal peptidase e.g. signal recognition particle for transport into RER

Cleavage :

- Cutting of polypeptides into smaller active final products → can expose active sites for the formation of es complexes
- Trypsinogen → trypsin
- Protease of proteins in the complement system synthesized as proenzymes that will only be enzymatically active after cleavage
- Advantage : ready stock of protein that would be activated when needed, prevent breaking down of useful substances within the host cell
-

Formation of Disulphide Bonds (RER)

- Occurs exclusively in RER
- Disulphide bonds can be formed sequentially while the polypeptide is being synthesised
- E.g. IgG light chain contains 2 disulphide bonds. The first and second cysteines closest to the N-terminus form a disulphide bond before the third cysteine is added, automatically ensuring the correct pairing of cysteines
- Disulphide bonds that do not occur sequentially have to be rearranged - Protein disulphide isomerase (PDI) enables rearrangement

Proper Folding (RER)

- Presence of ER chaperone proteins to prevent misfolding and help proteins fold into their proper conformation
- Peptidyl-prolyl isomerases accelerate rotation about peptidyl-prolyl bonds in unfolded segments of polypeptides

Glycosylation (RER)

- Glycosidic bond links a carbohydrate to
- Side chain of asparagine (N-linked)
- Side chain of serine or threonine (O-linked)
- ER glycosidases remove the terminal glucose monomers → after 2 of 3 glucose are removed, calnexin and calreticulin, ER chaperone proteins, bind to oligosaccharides on incompletely folded proteins and retain them in the ER
- ER glucosyl transferase adds glucose to oligosaccharides, but only to those attached to unfolded proteins

DO NOT MONETISE MY SHIT

Further Modifications (Golgi)

- Modifications to oligosaccharides/carbohydrate components of glycoproteins - Different modifications target the protein for secretion, insertion in the membrane, or to lysosomes

Proteolytic Cleavage of Proproteins (secretory vesicles)

- Endoproteases are enzymes involved in catalysing specific proteolytic cleavages to convert proproteins to functional proteins
- E.g. proinsulin to insulin, chymotrypsinogen to chymotrypsin in small intestine Phosphorylation
- Catalysed by protein kinases

Phosphorylation

- Reversibly alter activity of enzyme, activate or deactivate regulatory proteins
- Can activate phosphorylation cascade → facilitate signal transduction in cell signalling
- P53 protein → phosphorylated at multiple residues to increase activity as a transcription factor

DO NOT MONETISE MY SHIT

Ubiquitination

- Tagged by ubiquitin for targeting to the proteasome for hydrolysis - Effective means of controlling protein activity because
- Uses ATP which is easily accessible as it is the cellular energy currency - Low cost requirement (only 1 ATP)
- Adds 2 negative charges to protein → changes interactions → changes conformation

Assembly into Multimeric Proteins

- Multimeric proteins: a group of two or more polypeptide chains
- Homomeric proteins: all polypeptide chains are identical
- Heteromeric proteins: not all polypeptide chains are identical
- Proper assembly is important as disordered assembly leads to aggregation - Obligate vs non-obligate protein complexes
- Obligate: the subunits of a multimeric protein complex cannot function individually and must be associate with other subunits to function
- Non-obligate: the subunits of a multimeric protein complex can function individually and do not need to associate with other subunits
- Transient vs permanent protein complexes
    - Transient: Form and break down quickly. Though transient by nature, transient interactions are very important for cell biology: human interactome is enriched in such interactions, these interactions are the dominating players of gene regulation and signal transduction
    - Permanent: Have a relatively long half-life

DO NOT MONETISE MY SHIT

LO1(g): Discuss and explain why proteins are able to recognise and bind to highly diverse molecules, with reference to the properties and shapes of their surfaces and clefts that allow highly complementary interactions.

Protein subunit: 1 polypeptide within a larger protein that is made up of multiple polypeptides
Protein domain: distinct functional/structural units within a protein that are able to fold independently into a compact, stable structure. Usually responsible for a specific function or interaction which contributes towards the overall role of the protein
Protein family: a group of proteins that share an evolutionary origin, reflected by related functions and similarity in sequence or structure

Protein Evolution

- Homologous molecules: molecules that are derived from a common ancestor e.g. cytochrome c
- Paralogues: homologues present within 1 species and often differ in their detailed biochemical functions
- Orthologues: present in different species and have very similar or identical functions

Cytochrome C

- Single polypeptides of 104-112 amino acid residues
- Same amino acid appears in all species at 23 positions
- Most of the remaining positions have chemically similar residues
- Only 8 positions accommodate 6 or more different residues
- Evolutionarily conserved protein

Information Provided by Sequence Comparison

- Invariant position: same residue found at a particular position
- Residues essential for protein function
- Conservatively substituted positions: can accommodate residues with similar characteristics
- Residues less significant
- Hypervariable positions: can tolerate many different amino acid residues - Residues have little specific function

Tertiary Structure More Conserved than Primary Structure

- Mutations affect the primary structure which has an impact on the tertiary structure, thereby affecting function of a protein
- Tertiary structure is more closely associated with function than primary structure → more evolutionarily conserved

- E.g. globin family: leghaemoglobin, myoglobin, haemoglobin α, haemoglobin β

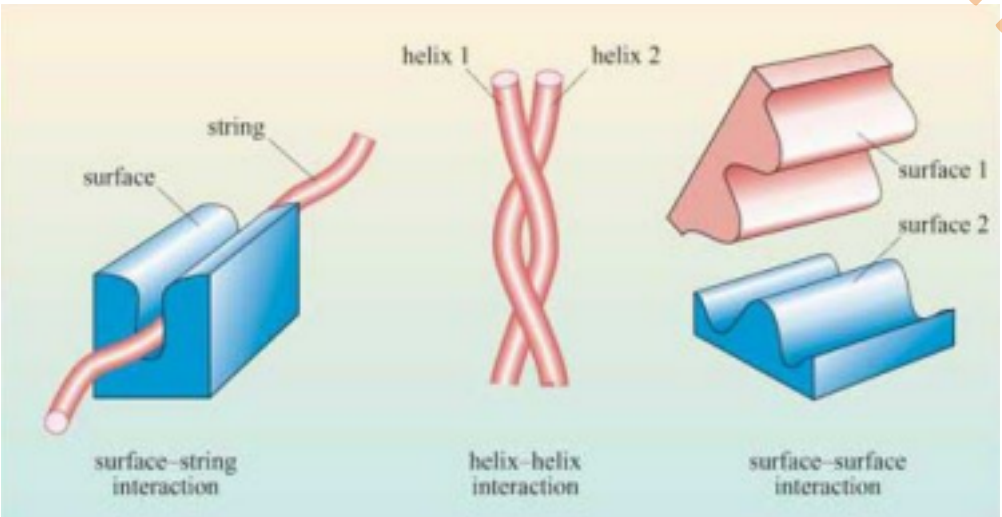
DO NOT MONETISE MY SHIT

How Proteins Interact with Other Molecules

- Each protein consist of precise sequence of amino acid (20 naturally occurring amino acids) that allow it to fold up into a particular 3d shape or conformation (energetically favourable conformation)
- Binding specificity arise from the type of shape, type of bond formed and the type of interaction
    - Comparing carbs and lipids, carbs and lipids are unable to interact and bind with highly diverse molecules due to the same sequence of monomers, and therefore have same 3d conformation which only allow for recognition and binding to specific molecules
- All protein bind to other molecules, the ability of a protein to bind selectively with high affinity to another molecule depends on the set of weak non covalent bonds (h2id)

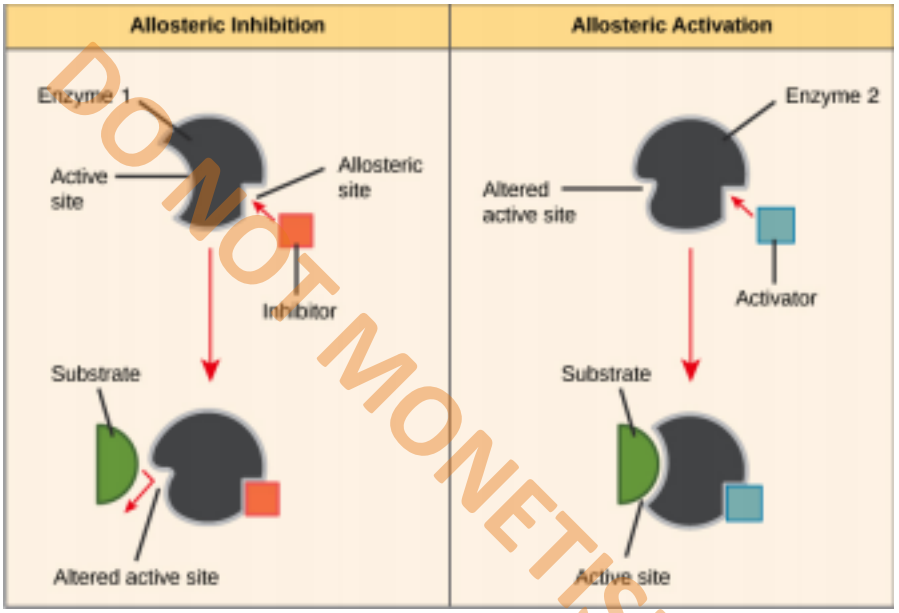
- Formation of weak non-covalent bonds: hydrogen and ionic bonds, hydrophobic interactions
- Since bonds are weak, many bonds need to be formed simultaneously for effective binding
- Complementary conformation, charge, polarity, hydrophobicity
- Surface conformation of a protein
    - R group of neighbouring amino acid will interact to enhance the chemical reactivity
        - Hydrophobic a.a clustered can restrict access of water molecules to the protein ligand binding site, because water can form h bonds readily that compete with ligands for the binding sites → energetically unfavourable for an individual water molecule to break away from the water water hydrogen bonded network to bind to the protein binding site
        - Clustering of neighbouring polar amino acids can increase affinity
            - When there is concentration of negatively charged side chains against their mutual repulsion, will have increased affinity for a positively charged ion
- Protein binding → via complementary shape
    - Surface string → recognition of a phosphorylated polypeptide loop within a protein
    - Helix-helix → found in several families of gene regulatory proteins
    - Surface-surface → most common way for proteins to interact (the interaction taught in h2)

DO NOT MONETISE MY SHIT

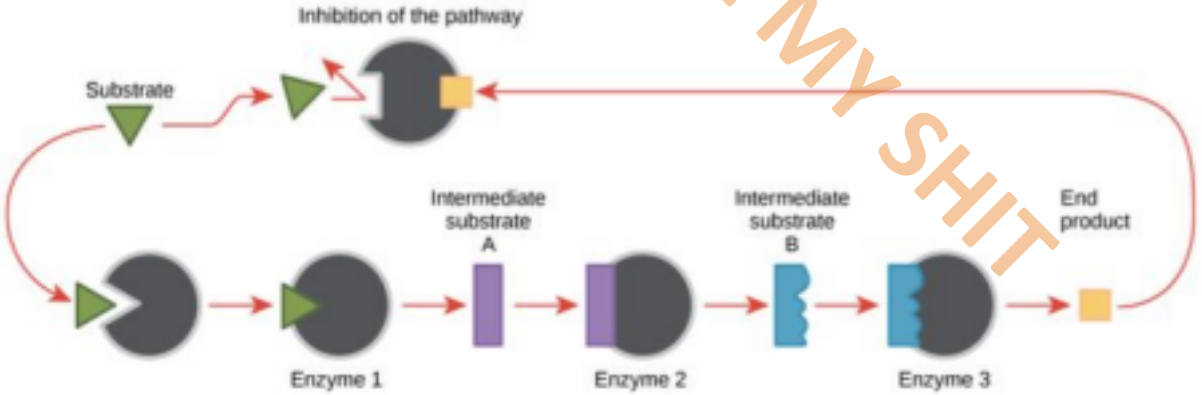


LO1(h): Discuss how a living cell regulates thousands of enzymes.

- Availability of enzymes (synthesis, degradation)
- Compartmentalisation (correct conditions, increased concentration for reaction) - Controlling amount of enzyme activity
- Post-translational modification → functional/active enzymes
- Proteolytic cleavage, phosphorylation
- Inhibition/allosteric regulation
- An allosteric inhibitor binds to the allosteric site of the enzyme and alters the conformation of its active site → enzyme is unable to bind to its specific substrate → enzyme is unable to perform its catalytic activity and is now inactive
- An allosteric activator binds to the allosteric site of the enzyme and alters the conformation of its active site → enzyme is now able to bind to its specific substrate → enzyme is able to perform its catalytic activity and is now active



- Feedback inhibition



DO NOT MONETISE MY SHIT

Sample essay:

Q: How a living cell regulate thousands of enzymes

- Level/amount/concentration of enzymes can be increased/decreased through increasing /decreasing rate of gene transcription;
- Specific transcription factors / activators / repressors can bind to control elements/
- enhancers /silencers sequences;
- Ref.to controlling / stabilising / destabilising the rate of formation of transcription initiation complex on the promoter / RNA polymerase and general transcription factors binding to promoter to form transcription initiation complex;
- Specific transcription factors can be activated by cell signalling pathways;
- Signal ligands bind to receptor proteins on cell surface membrane;
- Ref. to signal transduction pathways are activated within cell cytoplasm / phosphorylation cascade leading to activation of specific transcription factors / phosphorylation cascade directly leading to activation of enzymes when they are phosphorylated; (e.g. MAP kinase, a key enzyme in many cell signaling pathways, can be activated by phosphorylation)
- Level/amount/concentration of enzymes can be increased / decreased through increasing /decreasing mRNA stability;
- The longer the poly-A tail, the longer half-life of mRNA / longer the time taken for the poly-A tail to be shortened to critical length resulting complete breakdown of mRNA;
- More stable mRNA leads to increased rate translation to produce more enzymes;
- Level/amount/concentration of enzymes can be increased / decreased through increasing / decreasing cellular mRNA levels;
- Increasing/decreasing the rate of mRNA export out of nucleus;
- Increasing/decreasing rate of splicing / alternative splicing / addition of poly A tail / 5' cap;
- Increased/ decreased binding of proteins to 5' cap and poly A tail to transport it out of nucleus for translation in cytoplasm;
- Alternative splicing of primary mRNA leads to different mRNA sequences, resulting in different enzymes (proteins) produced;
- Level/amount/concentration of enzymes can be increased/decreased through increasing / decreasing gene translation;
- Translational repressor proteins bind to mRNA;
- Preventing the binding of small and large ribosomal subunits to mRNA at 5'UTR;
- Preventing the formation of translation initiation complex (award only once);
- Activation/inactivation of translation initiation factors by dephosphorylation / phosphorylation;
- Promoting/preventing the formation of translation initiation complex (award only once);

DO NOT MONETISE MY SHIT

- Level/amount/concentration of enzymes can be decreased through protein degradation;
- Through ubiquitination and hydrolysis / degradation via proteasome;
- Ref.to end-products of metabolic pathways acting as non-competitive inhibitors/end-
- product inhibition/feedback inhibition;
- Non-competitive inhibitor bind to allosteric site of enzymes
- Causing conformational change/change in 3D configuration/tertiary structure of enzymes (award only once);
- Active site shape no longer complementary to substrate shape (award only once);
- Allosteric activator can bind to allosteric site of enzymes;
- Causing conformational change / change in 3D configuration/ tertiary structure of enzymes (award only once); more complementary in shape (award only once); higher affinity to substrate;
- Ref.to proenzymes have extra amino acids as part bf the polypeptide chain located with the active site
- Shape of active site no longer complementary to shape of substrate (award only once);
- Extra amino acids can be removed by cleavage/peptide bond hydrolysis,therefore enzyme become activated;
- Ref.to allosteric enzymes may have multiple subunits/more than one subunit;
- Cooperativity:Binding of substrate at one subunit active site causes conformational change such that the active site at another subunit can bind substrate with higher affinity;
- Ref.to cell compartmentalisation through membrane-bound organelles, creating optimal pH for enzyme activity;
- Optimal pH can allow contact/catalytic residues to have correct charge tó bind substrate / catalyse the reaction;
- Optimal pH also allow acidic and basic amino acid residues R - groups to have correct charge to form R-group interactions such as ionic bonds and hydrogen bonds;
- Enzyme has correct 3D conformation/configuration / tertiary structure (award only once);
- Active site shape complementary to substrate shape and, can bind substrate (award only once);
- Ref to formation of multi-enzyme complex: groups of enzymes required for several metabolic pathway steps are assembled into a multi-enzyme complex.
- This arrangement facilitates the sequence of reactions, with the product from the first enzyme becoming the substrate for an adjacent enzyme in the complex. This goes on till the end product is released from the complex.

DO NOT MONETISE MY SHIT

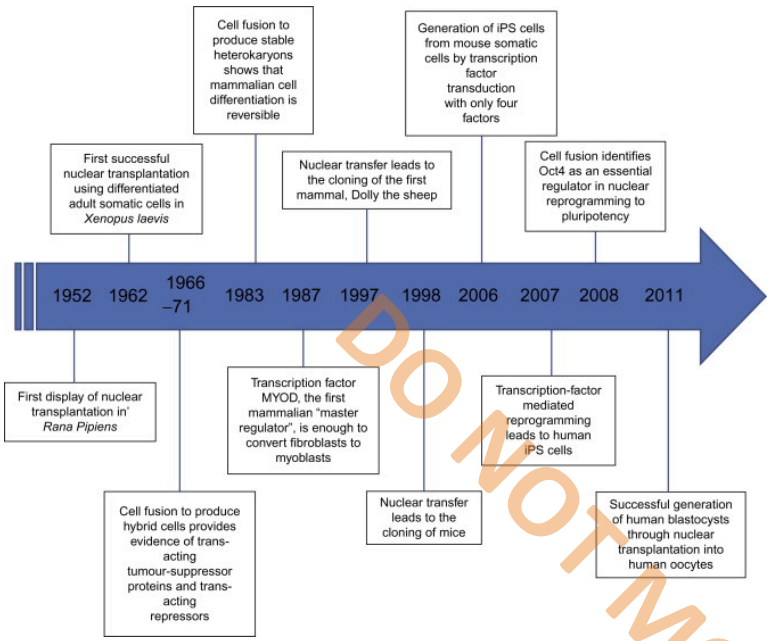
- Enzymes can be phosphorylated/dephosphorylated to activate/inactivate the enzymes;
- specific amino acid sequence at a region of the protein;
- Complementary in shape to the active site of the protein kinase activating the enzyme,
- Sequence contains tyrosine/serine/threonine;
- Enzyme has correct 3D conformation/configuration/tertiary structure (award only once);
- Active site shape complementary to substrate shape and can bind substrate (award only once);
- Chymotrypsin, a digestive protease, is synthesised in the inactive form Chymotrypsinogen and subsequently cleaved to produce the active form. Only Chymotrypsin can catalyse the hydrolysis of polypeptides .

DO NOT MONETISE MY SHIT

LO2(a): Discuss how mature cells can be returned to a stem cell state.

Stem cells :

- Unspecialised cells that can divide without liit as needed and in the presence of specific growth factors can differentiate into specialised cells
- Can be totipotent, pluripotent, multipotent and unipotent



Initially it was thought that cell differentiation was an irreversible process

In 1960s it was clear that program of development relied on differential gene expression (through switching off potency genes and switching on genes that encode for proteins with specific functions and structure)

1. In 1952 Briggs and KIng performed experiment in frogs
    - Transplanted blastula nuclei into enucleated eggs and produced viable tadpoles
    - But when repeated with endoderm cells that are more differentiated than blastula, there were limited results
2. In 1962, Gurdon had higher success → yielded fertile adult frogs with differentiated brush border intestinal nuclei (which are somatic cells)
    - Saw as evidence that cell differentiation did not involve permanent changes to the genome
    - Steps to SCNT
        - Extract nucleus of the differentiated mature cell
        - Remove nucleus from the egg oocyte and obtain an enucleated oocyte
        - Insert the nucleus of the differentiated cell into the enucleated oocyte
        - Cells will divide to form 2 cell, 4 cell, blastocyst and this give rise to the different germ layers and eventually the entire organism
    - Significance : realisation that the nucleus of a mature specialised cell can be returned to an immature pluripotent state , and that the egg cytoplasm was able to reshape the epigenetic environment that allow the entire program of development to be reset
3. 1996, ian wilmut and keith campbell were the first to obtain a normal fertile adult mammal (dolly the sheep) by transplanting nuclei from cells of an adult
4. In 2006, shinya yamanaka discovered that intact mature cells can be reprorammed to become immature stem cells by introducing a few genes that reprogram the cells to pluripotent stem cells
    a. Genes express 4 transcription factors → OSKM or yamanaka factors
    b. The iPS cells were not completely identical to the ES cells and their ability to contribute to tissues in chimera were limited with no germline transmission

DO NOT MONETISE MY SHIT

Significance of discovery of SCNT and iPSC

- Solved the controversy over the destruction of embryos associated with the use of ESC in researc
- ESC are generated from somatic cells of one's body and there is no risk of immunorejection of autologous cells
- Disease modelling
    - Differentiate and obtain many cells from a diseased individual
    - Possibility of efficiently making disease specific stem cells for understanding disease mechanisms
- Regenerative medicine
    - Generation of damaged and injured tissues with the help of iPSCS
    - Overcome the problem of scarcity of donors and increasing need for organs
- Drug discovery
    - Use of animals is limited in the sense that they are unable to replicate the exact human psychological conditions and related phenotypic attributieons and hence use of stem cells that arise from humans can replicate exactly without the need to use human subjects

Concerns of using iPSC

- Health risks
    - c-Myc gene used to reprogram somatic cells has a possibility to be activated to become oncogenic by a retrovirus -> increase chances of cancer
- Individuals might misuse the knowledge of altering the human genome for unethical purposes
    - e.g. human cloning, human-animal genetic admixtures, etc. -> threaten our understanding and protection of the human identity
    - Sperm cells that are induced from iPSCs will result in complicated genetic relationships
    - Children born from sperm cells induced from iPSCs will suffer from serious health problems
- Inefficient
    - Certain non-dividing cell types' reprogramming rate to iPSCs is very low (less than 0.02%) -> need to pursue a quantitative assessment of the final quality of cells and screen for any genetic or epigenetic alterations during the reprogramming process.
- Genetic privacy
    - iPSCs would contain the genetic information of the donor -> donor's privacy might be compromised

DO NOT MONETISE MY SHIT

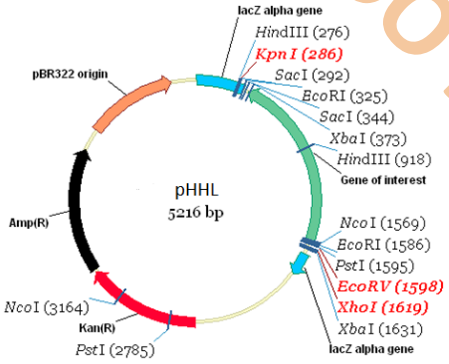
LO2(b): Explain that genetic engineering involves the insertion of a gene, obtained either by synthesis or by extraction from an organism, into another organism (of the same or different species), such that the receiving organism expresses the gene product

Genetic engineering

- Use of techniques to cut and join together genetic materia and introduce the reeulting hybrid dna into an organism to form new combinations of heritable genetic material in the receiving organism
- Involves insertion of a gene from a newly synthesized or from another organism into a receiving organism, and the transgenic organism must be able to express the gene product

Gene cloning (recombinant dna technology)

- Makes it possible to identify and isolate a single gene in the genome and produce large quantities in the form of cloned dna molecules
    - Steps
        1. Isolate gene of interest
            - Using reverse trascriptase, synthesizing gene artiically, or shotgun approach
            - Reverse transcriptase : isolate the mrna from specialised cells that express gene of interest, and use reverse transcriptase to synthesize the ssDNA, add alkali to digest the mrna and the ssDNA is made double stranded using dna polymerase → dna does not contain introns because mature mrna is used
            - Gene sequencing : only can happen if the nucleotide sequence of gene of interest is known, and the gene is relatively small → gene is synthesized by artificially combining nucleotides in the right order and recombined with an appropriate vector and introduced into a suitable host
            - Shotgun approach : isolating dna containing gene of interest and randomly fragmenting it using restriction enzyme and cutting plasmid using same restriction enzyme → introduce into bacteria and identify gene of interest by gene probing



2. Isolate bacteria plasmid (vector
    - Useful marker genes are ampR for antibiotic resistance and lacZ that encodes for beta galactosidase
3. Insert gene of interest into plasmid dna
    - Restriction enzyme to cut gene of interest and plasmid to produce sticky ends, and after the complementary ends anneal, dna ligase is added to form a recombinant vector
4. Introduction of recombinant dna into host cells
    - Transformation, add calcium ions (because calcium ions are positively charged and they will bind to the negatively charged dna and bring to the negatively charged glycoproteins
    - Brief shock and pores appear transiently and allow the dna molecule to enter the cell
    - Bacteria cells can be
        - Containing gene of interest only
        - Containing plasmids only
        - Containing recombinant plasmid (good)

DO NOT MONETISE MY SHIT

5. Screening for colonies and select/expand the positive clones to exponentially replicate copies of desired dna
    - Blue white screening method
        - Used when the plasmid vector contains one antibiotic resistance genes and the lacZ genes as the marker genes
        - Put the bacteria on a nutrient medium containing ampicilin and x gal
        - Ampicilin ensures that only plasmid containing cells grow → selection for transformed cells with plasmid
        - X gal is hydrolysed by beta galactosidase and a blue product is yielded → shows that the blue colonies are bacteria with non recombinant plasmid
        - Bacteria that successfully taken in recombinant plasmid will form white colonies
    - Replica plating method
        - Both marker genes code for antibiotic resistance
        - Nonrecombinant plasmid is resistant to both tetracycline and ampicillin
        - Recombinant plasmid is only resistant to ampicilin → not able to grow on tet containing agar
        - Bacteria plated onto ampicilin to select for transformed cells
        - Bacteria that can grow on ampicilin are replica plated into medium containing tetracycline → only bacteria containing nonrecombinant plasmid will grow
6. Probing
    - Using nucleic acid hybridization (h2)

Issues with cloning eukaryotic genes using bacteria to produce proteins and how to overcome them

- Gene expression in eukaryotic and prokaryotic cells are different and bacteria rna polymerase cannot recohnise eukaryotic promoters
    - Expression vector can be used → contains the prerequisite prokaryotic promoter upstream of restriction site → bacteria host will recognise bacterial promoter and express foreign gene
- Lack of post transcriptional modifications
    - Presence of introns in eukaryotic genes → prevent correct expression of the gene as abcteria do not have rna splicing machinery
        - Use mature mrna for reverse transcription to generate gene of interest without introns
        - Make artificial eukaryotic genes
- Lack of post translational modicications
    - Do not have the relevant enzymes present → mechanisms for folding into 3 and 4 structures may be unabvailable
        - Use eukaryotic cells such as yeast instead of bacteria as they can post translational modification, have quick generation time and have plasmids

DO NOT MONETISE MY SHIT

LO2(c): Explain the roles of restriction endonucleases, reverse transcriptase and ligases in genetic engineering.

Components in recombinant dna technology

- Dna containing gene of interest : obtained through artificial synthesis or extracted from an organism
- Restriction enzymes : naturally produced by bacteria as a defense mechanism against viral ifections
    - Function is to cut up dna molecules into fragments and restricts growth of foreign dna in bacteria host
        - Bacteria can protect its own dna from restriction enzymes by methylation of the restriction sites
    - Role in cloning :
        - Can recognise and bind to specific restriction site and catalyse the hydrolysis of phosphodiester bonds → has active site specificity because different restriction enzymes recognise different restriction sites
        - Restrictions that leave sticky ends are good because the dna stransg with sticky ends can anneal to complementary sticky ends cut by the same enzyme by forming h bonds
        - Blunt ends do not have dna sticking out, not very good because overhangs are preferred for h bonds to form and this facilitates the process of ligation by dna ligase
- Dna ligase
    - Catalyse formation of phosphodiester bond between adjacent nucleotides in dna molecule
- Vector (uaually a bacterial plasmid)
    - Restriction fragments cannot enter the host cell directly, has to be joined to a vector → function as a carrier dna molecule that transfers and replicate inserted dna fragments to the receiving organism
    - To insert the dna into vector, both vector and gene of interest have to be cut with the same restriction enzyme → complementary sticky ends to anneal → formation of recombinant vector (vector that contain gene of interest)
    - Why plasmid are usually used
        - Dna molecules with a known structure and can be easily isolated from bacteria cells due to high copy number
        - Have several recognition sequence so that a variety of restriction enzymes can be used for cloning
        - Have selectable marker genes to allow for selection of cells that
            1. Have taken up the vector
            2. Have taken up the recombinant dna molecules

DO NOT MONETISE MY SHIT

LO2(d): Outline the procedures for cloning a eukaryotic gene in a bacterial plasmid and describe the properties of plasmids that allow them to be used as DNA cloning vectors.

Plasmids

- Are circular extra-chromosomal DNA molecules in bacteria
- Are capable of replicating independently of the bacterial chromosome
- The plasmid must be able to replicate within the host cell so that during cloning, copies of the recombinant DNA molecule can be produced and passed to daughter cells as the cell divides
- Have a single origin of replication
- The origin of replication is a DNA sequence of 50-100 bp, where host cell enzymes (i.e. DNA polymerase) bind to initiate replication
- Contain genetic markers
    - A genetic marker is usually a gene conferring readily selectable phenotypic traits on bacterial cells which allows for
- Selection of host cells that have taken up the vector molecule
- Identification of host cells which have taken up the recombinant (as opposed to non-recombinant) vector DNA molecules
- Examples of genetic markers include
    - Genes coding for resistance to antibiotics
    - Genes coding for the enzyme β-galactosidase
    - ampR marker gene and lac Z gene are examples of marker genes (found commonly in pUC18)
- Range in size (2 to several hundred kb) and in copy number (0 to a few hundred) - A useful cloning vehicle should be present in the cell in multiple copies (30-40 per host cell) so that large quantities of the recombinant DNA molecule can be obtained from each cell

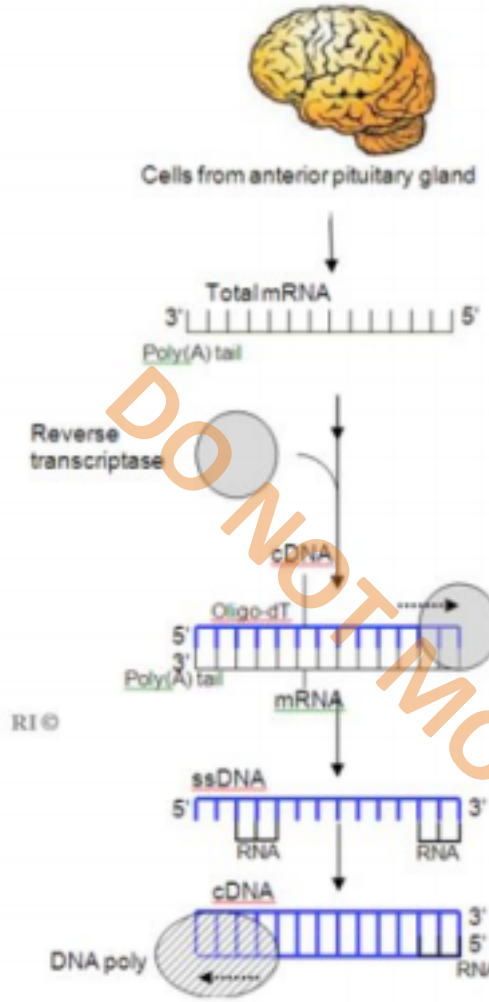
Note:

- Plasmids are not limited to bacteria. Saccharomyces cerevisiae (yeast) contain plasmids too
- Plasmids used in research have been engineered to include additional selectable markers, promoters etc
- Plasmids confer some advantageous features to bacteria and hence continue to be found in many of them e.g. plasmids may contain genes that make the bacterial cell they are found in resistance to some antibiotics e.g. the F_1 plasmids ensures that conjugation can occur between bacterial cells

DO NOT MONETISE MY SHIT

LO2(e): Explain how eukaryotic genes are cloned using *E. coli* cells to produce eukaryotic proteins.

Step 1: Isolation of mRNA and preparation of cDNA



DNA source
Tissue from a human organ (e.g. the anterior pituitary gland) provides human cells that produce the protein (e.g. hGH).

hGH mRNA will be found in large quantities in the pool of total mRNA because the gene is transcriptionally active in this tissue.

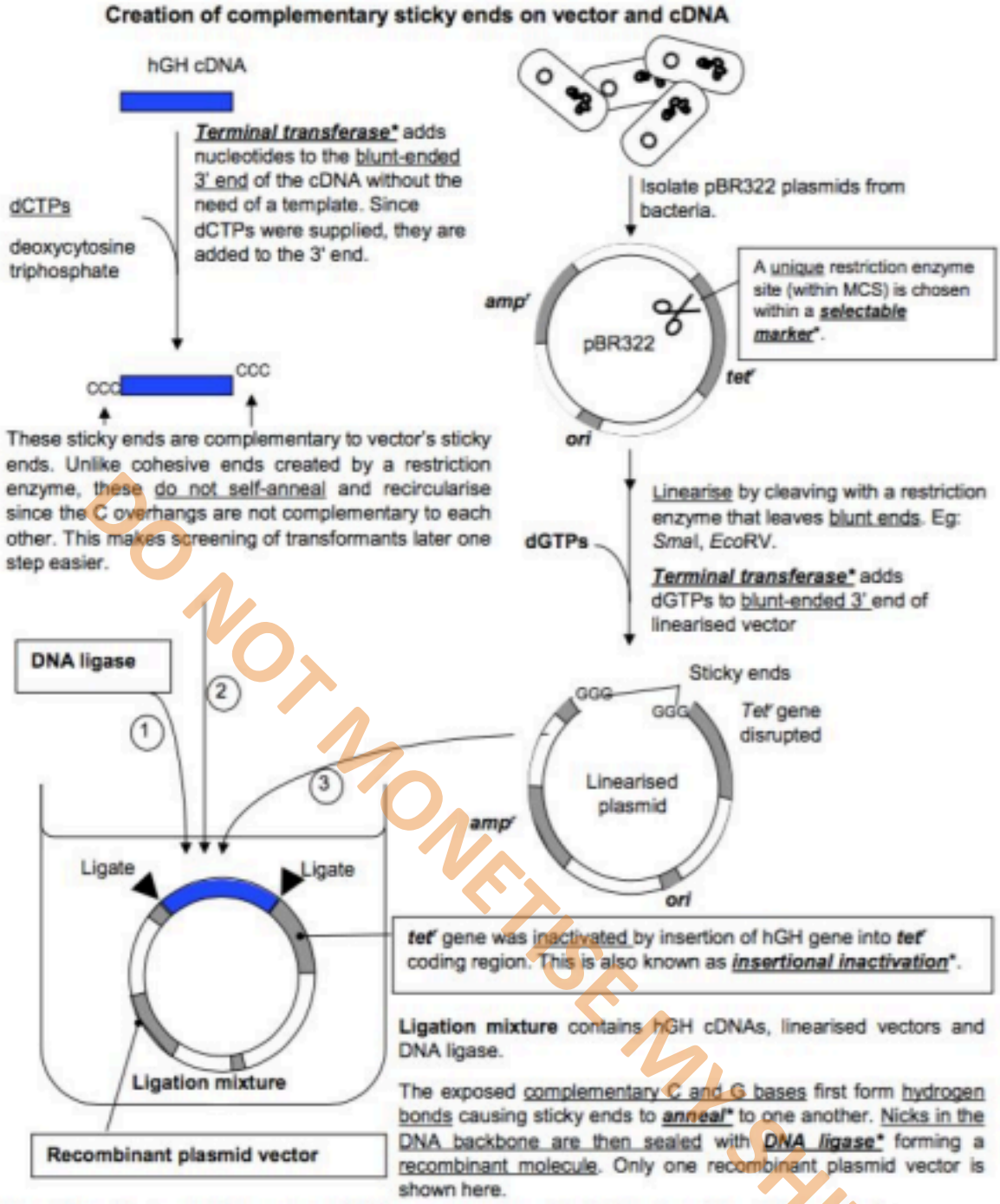
Free oligo-dT serve as primers by annealing to the 3' poly (A) tail of each mRNA template. Reverse transcriptase synthesizes a cDNA strand using mature mRNA as a template. An RNA-DNA hybrid molecule is formed

Treat RNA-DNA hybrid with alkali or RNase to partially break down mRNA leaving **single-stranded DNA** (ssDNA). The remaining mRNA fragments act as primers for DNA polymerase.

DNA polymerase synthesizes 2nd DNA strand. Another DNA polymerase removes RNA primers & replaces the gaps with dNTPs. The resultant double stranded cDNA can be cloned into a vector.

DO NOT MONETISE MY SHIT

Step 2: Digestion, Recombination and Ligation



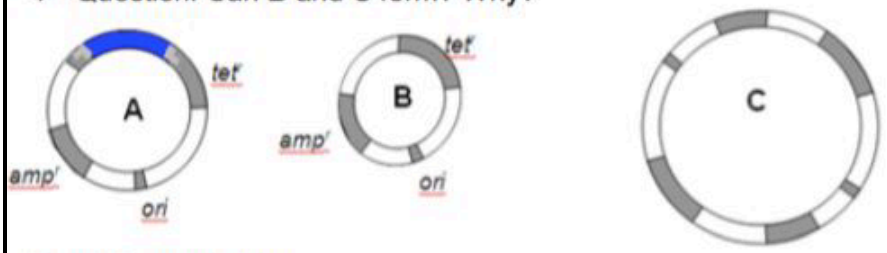
Note: Alternatively use the same restriction enzyme to cut both the plasmid and the gene of interest (from genomic DNA) thus producing ***complementary sticky ends****. Mix the 2 types of DNA to allow them to ***anneal**** through base-pairing of the sticky ends by forming hydrogen

DO NOT MONETISE MY SHIT

bonds and add ***DNA ligase**** to seal the nick by forming a phosphodiester bond between the 2 DNA fragments.

Step 3: Transformation

❖ Question: Can **B** and **C** form? Why?



B and C cannot form.

(B = plasmid that reanneals and recircularises; C = 2 cut plasmids which anneal)

Since terminal transferase is used to produce overhangs. The overhangs found on both ends of the molecule are identical and not complementary and hence will not anneal.

B and C forms when the restriction enzyme used produces complementary sticky ends.

Step 4a: Screening (Part 1)

Overview

- Screening identifies the transformed host cells with the recombinant DNA molecule.
- Selection for the correct clone is carried out at two sequential steps:

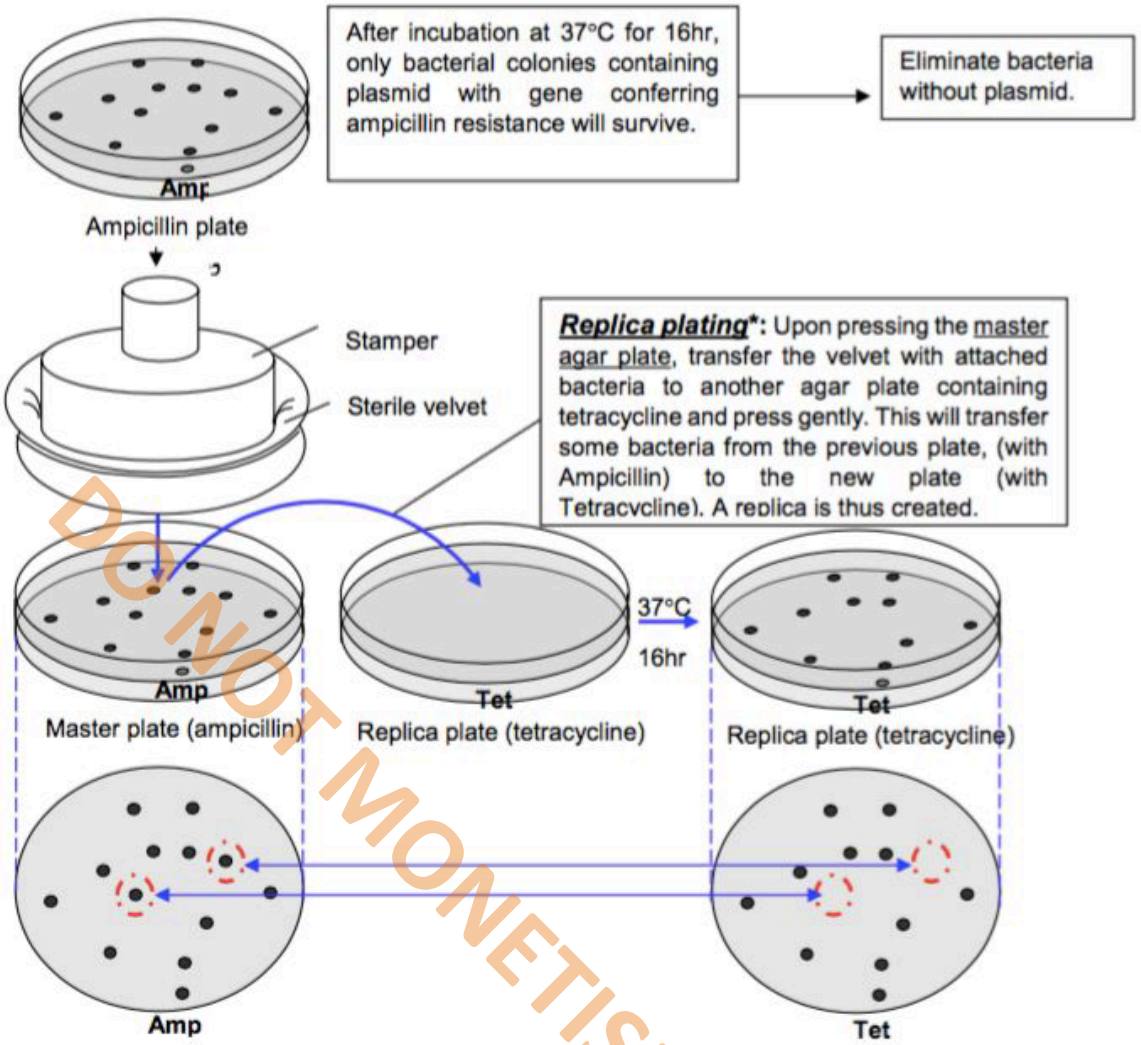
  Step 1: selection for bacteria transformed with plasmid vector DNA
  (i.e. to distinguish between (i) transformed, and (ii) non-transformed bacteria)

  Step 2: selection for presence of recombinant plasmid vector DNA.
  (i.e. to distinguish between bacteria cells transformed with (i) reannealed plasmids, and (ii) recombinant plasmids).

- **Screening process**
  - After transformation, the cells are all incubated in a suitable growth medium, allowing all transformed cells to express the first selectable marker (i.e. gene that codes for proteins resulting in antibiotic-resistance).
  - The cells are then plated on agar plates containing a selective agent, i.e. ***ampicillin****. At this stage, all cells that are able to grow on the plate must have taken up exogenous DNA but it is still unknown at this point, which cells have taken up the gene of interest.

DO NOT MONETISE MY SHIT

Step 4b: Screening (Part 2)



- Bacteria transformed with non-recombinant plasmid will be resistant to both tetracycline and ampicillin as the genes coding for resistance to both are intact.
- Bacteria transformed with recombinant plasmid will be resistant to ampicillin but susceptible to tetracycline as the gene coding for ampicillin resistance is intact but the gene coding for tetracycline resistance has been ***insertionally inactivated****.

DO NOT MONETISE MY SHIT

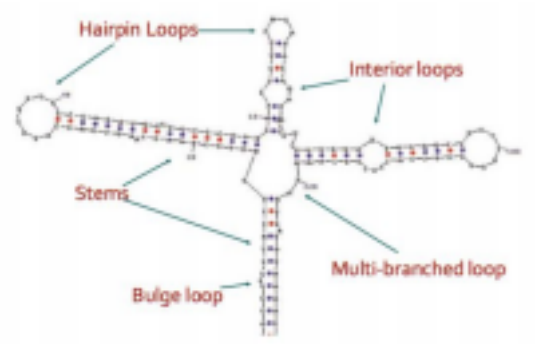
LO2(f): Explain the structure and roles of ribozymes and their potential role in genetic engineering (including novel peptide synthesis and modifications).

Ribozymes: RNA molecules with catalytic activity. They are often found to catalyse cleavage of either its own or other RNAs.

- Occur in plant, bacteria, virus, lower eukaryotes, have one in human
- Catalytic rna separated into large catalytic rna and small catalytic rna
- All rna except rnaseP catalyse reactions that modify themselves and cannot be considered true enzymes or catalysts
- Have an absolute requirement for divalent cations for catalysis → usually mg2+ for
    - Proper folding of rna
    - Forming the catalytic core of the ribozyme

RNA world hypothesis

- Chains of free floating RNA in the world in the early stages of its formation may have been the first things to replicate and evolve
- Without enough free nucleotides, RNA strands fold and can catalyse reactions → ribozyme



Molecules that could have participated in the formation of early life

- Proteins: amino acid diversity, catalysis
- DNA: stability and storage
- RNA: diversity, storage and catalysis

Roles

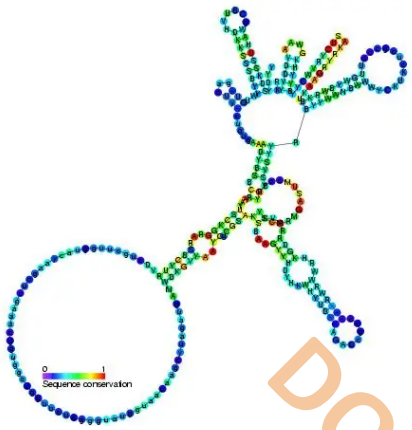
- Molecular scissors to cleave precursor RNA chains
- Molecular staplers that ligate two RNA molecules together
- They increase reaction rates by up to 10^{11}-fold (still about 10^{3}less than protein enzymes)
- Large catalytic RNAs use the cations for a general two-metal-ion reaction mechanism as their mode of action for catalysis
    - Mg^{2+} can neutralise negative charge of RNA backbone when ribozymes bind to RNA
    - Mg^{2+} can be used as a catalytic cofactor as hydrated form can act as a general base during acid-base catalysis
    - Mg^{2+} can speed up catalysis by stabilising transition state charge during enzyme-substrate complex formation

DO NOT MONETISE MY SHIT

Characteristics

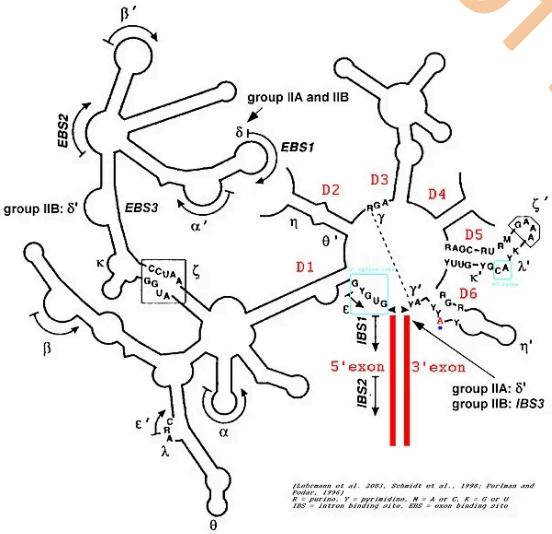
Large catalytic rna

- Group 1, group 2, RNase P
- Fold to adopt a secondary structure
- Ability for self splicing, by cleavage and ligation of phosphodiester bonds
- Group 1 introns



Consist of 9 paired segments surrounding a catalytic core
Abundant in plant and fungal mitochondria
Not found in higher eukaryotes

- Group 2 introns



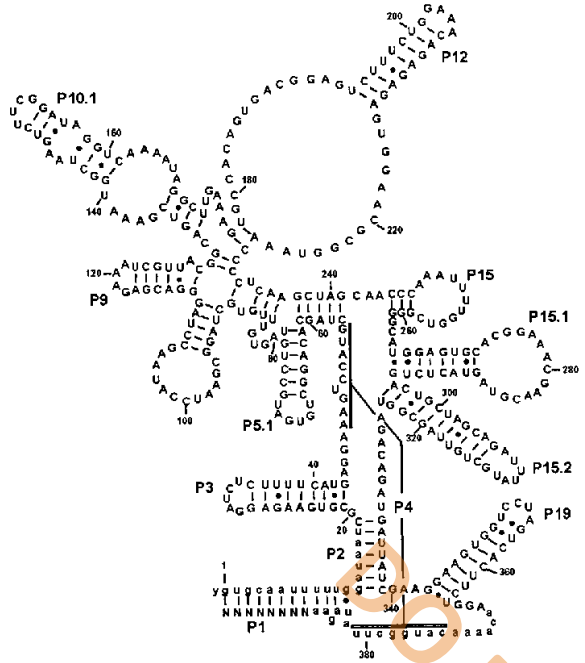
6 helical domains radiating as spokes from a central wheel

- Except for domains 1 and 5, the domains can be modified or deleted and ctaalytic activity will still be retained

Less widely distributed
Not found in higher eukaryotes

DO NOT MONETISE MY SHIT

- RNase P



Made up of m1 rna and c5 protein

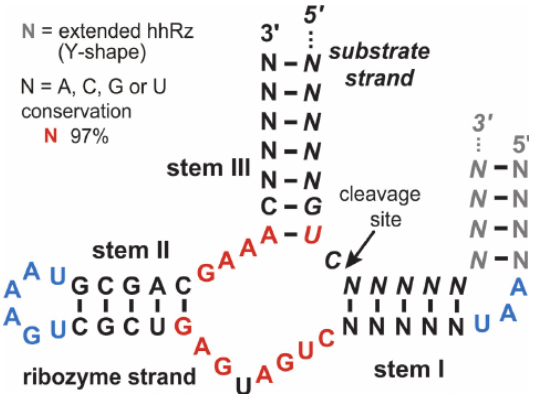
- C5 protein : enhance the substrate binding affinity and the catalytic rate → by increasing the metal ion affinity in the active site

Has a substrate specificity domain and a catalytic domain

Rna from bacterial rnase p retain catalytic activity in the absence of the protein subunit

Small catalytic rna

- Include the hammerhead and hairpin, hep d and vsrna
- Ability for self cleavage of a particular phosphodiester bond
- hammer head



Found in viriods and satellite rna

- Viriods are small infectious pathogens of flowering plant, composed of solely short strand of naked circular ssRNA, no protein coding
- Satellite rna are non coding rna and have been shown to alter interaction of their helper virus with their hosts (parasites of parasites)

3 base paired helices separated by short linkers of conserved sequences

Conserved uridine turn links stems 1 to stem 2 and usually contains CUGA

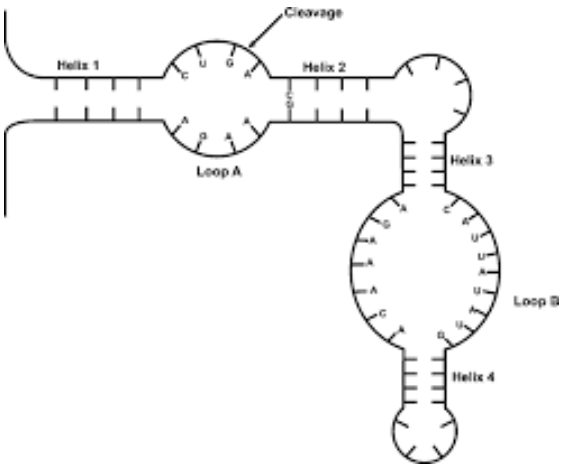
Stem 2 and 3 linked by GAAA

Some full length hammerhead riboxyme contain additional sequences that permit additional tertiary contacts to form

Tertiary interactions stablise the active formation of the ribozyme

DO NOT MONETISE MY SHIT

- Hairpin



Found in pathogenic plant satellite virus
Fold into a secondary structure that have 2 domains (loop a and loop b)
Loop a contains substrate and the primary substrate recognition region of the ribozyme
Loop b is larger and contains the primary catalytic region of the ribozym
2 domains are covalently joined via phosphodiester linkage that connects helix 2 and helix 3 → domains must interact with one another for catalysis to occur

DO NOT MONETISE MY SHIT

Properties of rna

- Single stranded → region of rna molecule may base pair with complimentary region elsewhere in the same molecule
- Bases in rna contain functional group
- Ability of rna to form h bonds with other nucleic acid molecules

Roles of ribozymes

- Act as molecular scissors or molecular staplers
- Linkage of amino acids into proteins is catalysed by rna (peptidyl transferrase)
- snRNP → splicing
- Self splicing rna have ribozyme built into their own intron → responsible for removal of the intorn in which it is found
- Small catalytic rna
    - Used to downregulate cellular and viral gene expression
    - Interfere with the expression of undesirable gene products → advantageous for the therapy of dominant negative genetic disorders where product of the mutant allele interferes with the normal functions
        - Eg marfan syndrome → reduce extrallular deposition of fibrillin protein → downregulate the production of mutant protein and restore the normal fibrillin 1 function
    - Trans-cleaving ribozyme against the HIV
        - Ribozymes recognise base sequence → high specificity
        - But virus may mutate and the specificity may not be as high since the eukaryotic genome is large and there may be other sequence that the ribozyme recognise that is not the viral seuqence
- Large catalytic rna
    - Interfere with the expression of faulty genes by repairing the mutant rna
    - Transsplicing → exons from 2 primary rna transcripts are join end to end and ligated → generate a single rna transcript from multiple separate premrna
        - Less likely to induce a host immune response and easily incorporated into the gene therapy vectors
    - Eg myotoinc dystrophy : ribozyme bind upstream of mutation area and replace 3’ end with rild type rna sequence → repair
- Advantage
    - Revised gene is still present in the natural regulatory environment
    - Ideally suited to dominant genetic diseases where it is difficult to abolish the function of the mutant allele completely
        - Low sequence specificity arise from
    - Powerful tools for rna repair

DO NOT MONETISE MY SHIT

LO2(g): Evaluate the significance of genetic engineering to the world and humanity (including food sustainability for a rapidly growing population, disease treatment and drug design).

Genetic engineering significance to the world and humanity

- Genetic engineering is a sign of progress and an opportunity to better the lives of humans and animals in various possible applications through
    - Food sustainability for the rapidly growing population
    - Disease treatment
    - Drug design

Genetically modified organisms

- In the past, farmers save seeds from the best looking plants to replant the following years → but it is time intensive and the gene assortment is a random process and hence there is a need to wait many years to produce the desired variety
- With recombinant DNA technology → possible to produce crop or animal with desirable qualities by replacing the genes and designing plants with the most desirable traits
    - Increase yield
    - Higher quality such as increased nutritional content

GM crops

- Transgenic plants can be produced via
    - Agrobacterium tumefaciens mediated transformation → have the Ti plasmid that allows for the formation of recombinant plasmid and can infect plant cells → foreign genes expressed in the plants
    - Gene gun aka microprojectile bombardment → dna coated tungsten/gold particles shooted into plant cells
    - Electroplating → short burst of electricity to introduce dna into the plant cells
- Higher crop resistance → to increase yield of plants, GM helped to increase plants resistance to pests and pathogens, hence reducing crop loss and increase profits + make the plants grow faster
    - Roundup herbicide resistant plants (herbicide resistance)
        - Roundup herbicide contains glyphosate and it kills plants by inhibiting action of EPSP synthase → function to synthesize tryptophan, tyrosine and phenylalanine required for protein synthesis in plants
        - Roundup herbicide resistant plants have the EPSP synthase gene recloned to an active promoter → increase concentration of the EPSP synthase protein in the plant
        - Roundup Ready plants carry the gene coding for a glyphosate-insensitive form of EPSP synthase, obtained from Agrobacterium sp. strain CP4
    - Bt corn (pest resistance)
        - Bacillus thuringiensis (Bt) is a soil bacterium that contains a crystalline protein (Cry protein) → when in gut, the protein breaks down to release a toxin (delta-endotoxin) → create pores in the intestinal lining resulting in ionic imbalance and paralysis of the digestive system resulting in insect death
        - However does not deter sap suckers like aphids
        - Reduces non specific harming of other insect species as it only kill the larvae of a common pest
        - Can also reduce cost time and labour to spray or purchase pesticides
- Higher yield → change growth rate of many plants and animals -> increased growth rate lead to increase in yield andgreater profit
    - Low mow grass → grass that grow at a slower pace

DO NOT MONETISE MY SHIT

- Higher quality → can be in the form of delayed fruit ripening or increasing nutritional value
    - Flavr savr tomato (delayed fruit ripening)
        - Normal tomatoes are not long lasting due to the production of polygalacturonase in the mature fruit that promote tissue softening
        - Inhibiting the action of polygalacturonase retards the process and keep the fruit firm and hene prevent the proess of fruit ripening and keeping the fruit firm
        - Inserted additional copy of PG encoding gene in antisense orientation; reduces translation of endogenous PG mRNA.
            - Antisense RNA binds to the polygalaturonase mRNA, preventing it from being expressed → inhinit the production of polygalacturonase
    - Golden rice (increased nutritional value)
        - In many less developed countries there is the issue of vitamin a deficiency → leads to blindness and affects up to 70% of children aged 1-4 years old in southeast asia
        - Rice grains make geranylgeranyl diphosphate (GGDP) in the immature rice endosperm → GGDP is located at the beginning of the provitamin a synthetic pathway but the rice lacks the enzymes necessary to convert GGDP into provitamin A
        - With genetic engineering → 2 genes that codes for the necessary enzymes are introduced via the agrobacterium mediated gene transfer → the rice will have the necessary genes which can produce the enzymes necessary to convert GGDP into vitamin A
            - One of the genes come from daffodil → gives it its distinct yellow colour
            - Other gene comes from erwinia uredovora

DO NOT MONETISE MY SHIT

GM animals

- Process of getting a genetically modified animals/trangenic animals
    - Eggs cells are surgically removed from a female and fertilised in vitro
    - Desired gene is cloned and injected directly into the nuclei of the fertilised egg by DNA microinjection
    - The dna is microinjected into the male pronucleus → integration into the chromosomal dna
- To obtain animals with higher nutrition
    - Cows are very important to humans because they provide food resources (meat and milk) and other by-products such as leather
        - Genetic engineering will enhance the quality of food resources from cows for human consumption
    - Cows produce large amounts of proteins naturally in their milk
        - Transgenic cows can produce therapeutic proteins in the milk from their mammary glands that can be used to treat human diseases (biopharming)
    - First transgenic cow rosie in 1997 produced human protein enriched milk at 2.4g per litre → more nutritionally balanced product than natural bovine milk and can be given to babies or elderly with special nutritional or digestive needs
- Increased growth and yield
    - Salmon only produce growth hormones in the light and their growth is limited by the summer months
    - With genetic engineering by addition of a promoter sequence to the growth hormone gene → produce the hormone all year round including winter and the salmon can grow maximum size 3-6 times faster than their wild counterparts → more meat available

DO NOT MONETISE MY SHIT

Disease treatment

- Aims at correcting defective genes which have caused the genetic disease by genetically modifying certain cells
- Prevent people from contracting the potentially deadly diseases → eliminate or correct the passing of disease genes

Gene therapy

- Introduction of a normal gene to a genome of an individual carrying a single defective gene → alleviate disease by genetically modifying the cells → normal gene enable synthesis of the functional gene product and restore normal phenotype
- Somatic cell gene therapy
    - Normal gene introduced into the somatic cells and only those cells are genetically modified → limited to one individual and it is non heritable
    - Safeguards the danger of transmitting the negative effects of the therapy to future generations → children can still have the disease
- Germline gene therapy
    - Normal gene introduced into the germ cells and the genetic alteration will be passed onto the offspring
    - However it is not recommended as it has the potential of introducing new inheritable mutation that affects the descendants

Delivery systems

- Can be done through viral or non viral vectors
- Vectors should be
    - Easy for large scale production so that the high vector concentration increases the chances of infection of cells
    - Safe to use such that it does not cause any immune response in the host
    - High tissue specificity → contain certain receptors that are complementary to certain cells
    - Does not mutate easily and allow insertion of a large size gene
- Viral vectors → rna viruses (retroviruses) or dna virus (adenovirus, adeno associated virus and herpes simplex virus)
    - Retrovirus
        - Possess reverse transcriptase and double stranded dna are integrated into the host cell chromosomes
        - Removal of viral genes prevent virus to unergo productive infection and hence there is no replucation and assembly of new virions
    - Adeno associated virus
        - Non enveloped virus with a ssDNA
        - Integrate dna into a known specific site at chromosome 19

DO NOT MONETISE MY SHIT

- Non viral vectors
    - Allow for simple large scale production
    - Low host immunogenicity
    - However the efficiency of gene transfer is low and there is minimal long term expression of the gene
    - Liposomes
        - Vesicles composed of synthetic lipid bilayers → dna packed in vitro and dna transferred in vivo
    - Dna injection
        - Using a syringe and needle → inject into specific target cells
        - simple and safe but poor efficiency of gene transfer and unstable in most tissues

Severe combined immunodeficiency (SCID)

- Inherited disrder which both the T and B lymphocytes of the immune system are impaired → unable to produce antibodies
- Individual with SCID is vulnerable to infectious diseases
- Have autosomal inheritance or X linked inheritance
- X linked recessive
    - Defective gene carried on the x chromosome
    - Mutation in the interlukin 2 receptor gamma gene → produce a potent which is component of IL receptor → preventing proper development of T lymphocytes → no cell mediated immunity
- Autosomal recessive
    - Mutation in the ADA gene found in chromosome 20 → lack of functional enzyme adenosine deaminase (ADA deficiency) → no purine metabolism, and allowing deoxyadenosine and dATP to accumulate in the cells which are toxic to immature lymphocytes → no proper development of both T and B lymphocytes
    - Can also be mutation in the JAK3 gene on chromosome 19
- Treatment options
    - Intravenous injections of adenosine deaminase to patients with ADA deficiency
    - Provide haemotopoetic stem cell transplantation derived from the bone marrow from a donor → supply of normal stem cells
    - Ex vivo gene therapy using retroviral delivery system
        - Normal ADA gene is identified and cloned by cutting out normal gene from dna with the appropriate restriction enzymes
        - RNA copy of the human ADA gene is inserted into modified inactivated retrovirus vector → introduced via ex vivo method
        - Blood stem cells are extracted from bone marrow and the isolated cells are infected with the vector → ADA gene is integrated into chromosomal DNA of blood stem cells
        - Treated stem cells are transplanted back into patient → ADA gene is expressed, producing the functional ADA protein

DO NOT MONETISE MY SHIT

Cystic fibrosis (CF)

- Autosomal recessive inherited disorder that affect the lungs, digestive tract and other organs → caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7
- The CFTR gene encodes for the CFTR protein needed to form chloride channels found on epithelial cells in lungs, sweat glands, pancreas and other tissues → functions in chloride ion transport → help to regulate the movement of water in tissues that is necessary for the production of mucus, digestive juices and sweat
- Most common mutation of CFTR gene is the deletion of 3 nucleotides → loss of phenylalanine → change tertiary structure → nonfunctional CFTR protein
- Cannot regulate movement of chloride ions and water across membranes → sticky mucus and resulting in
    - Blockage of respiratory tracts in lungs → breathing difficulties
    - Blockage of pancreatic duct → reduced secretion of pancreatic enzymes
    - Blockage of sperm ducts → infertility
    - Environment for the breeding of bacteria → infection and inflammation of lungs
    - Poor absorption of nutrients from the intestines → malnutrition and poor growth
- Gene therapy involves using viral or nonviral vector to introduce a normal functioning CFTR allele into patients

DO NOT MONETISE MY SHIT

Drug design

- Improve the drugs available on the marketplace by making them more effective and safer → create safer and more effective versions of conventionally produced pharmaceuticals
- Create new pharmaceuticals
- Produce substances identical to conventionally made pharmaceuticals that are more cost effective
- Can be categorieed under
    - Human protein replacements (hormones, clotting factors etc)
    - antibiotics/anticancer
    - Vaccines (HPB vaccine)
        - Vaccine for hepb virus is produced by expressiving the HBV surface antigen using yeast expression system → immunogenic hence effective

DO NOT MONETISE MY SHIT

LO2(h): Explain that epigenetics is a process that affects the expression of specific genes, without involving a change in DNA sequence.

Epigenetics

Epigenetics

- Any process that alters gene activity and expression without changing the dna sequence → modifications that can be transmitted to daughter cells
- Epigenome → comprises of teh complex modifications associated with the genomic DNA → integrates the information encoded in the genome with all the molecular and chemical cues of cellular, extracellular and environmental origin
- Dna methylation
    - Addition of methyl groups to dna and it is specific → occurs at CpG sites (cgcgcgcgc)
    - Transcription of most protein promoters coding genes in mammals are initiated at promoters at CG sites
    - CpG sites are methylated by DNA methylases
        - De novo methylation → methylation of previously unmethylated sequences
            - Maintenance methylation → methylation of hemi methylated sequences
- Histone modification
    - Histone acetylation
        - By histone acetyltransferases → add acetyl groups to lysine residues
    - Histone methylation
- Rna associated silencing
    - Turning off genes in the form of antisense transcripts, noncoding rna or rna interference
    - RNA that do not encode proteins e.g. siRNA, miRNA, lncRNA
    - Mode of action (small interfering RNA, microRNA):
        - Multi-protein complex, RNA-induced silencing complex, incorporates ssRNA fragment to recognise target mRNA → recruit histone methyltransferases to form heterochromatin
    - Mode of action (long non-coding RNA):
        - Guide ncRNAs: recruit/reject epigenetic regulators (chromatin modifying complexes, chromatin remodelling complexes)
    - Architect ncRNAs: modify chromatin conformation
    - Enhancer ncRNAs: enhancer-like functions

DO NOT MONETISE MY SHIT

(h) Explain that epigenetics is a process that affects the expression of specific genes, without involving a change in DNA sequence.

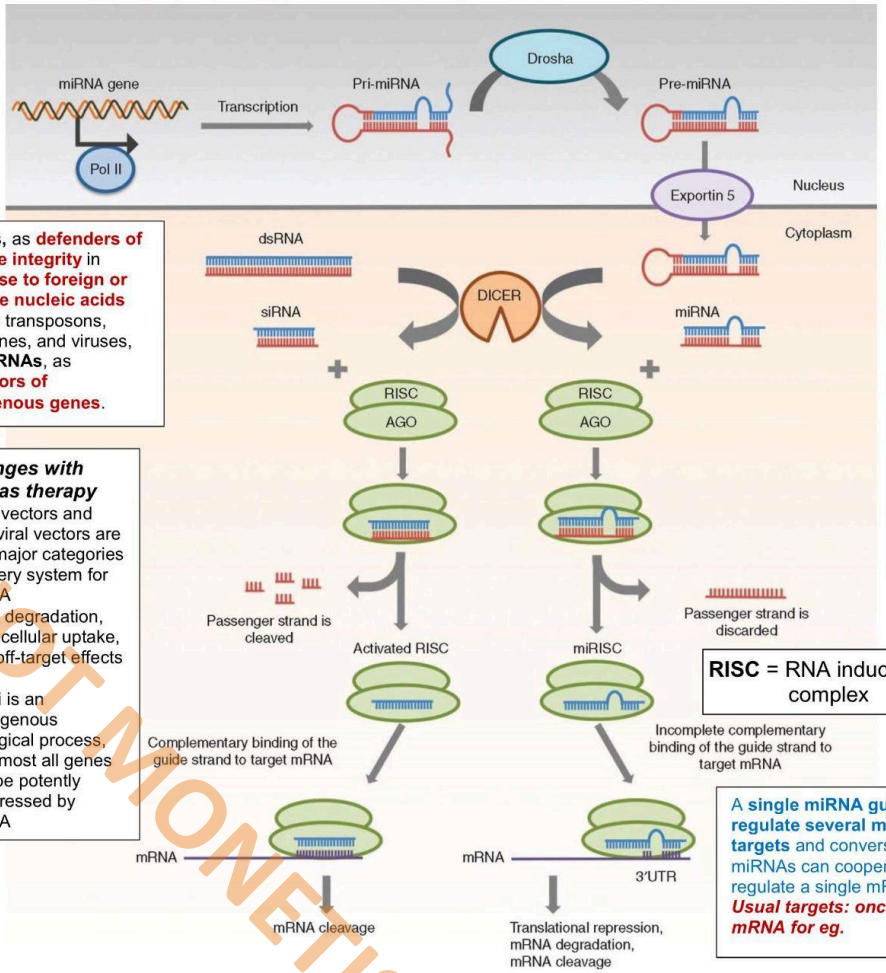


Fig 42: Gene silencing mechanisms of siRNA and miRNA.

siRNA: dsRNA (either transcribed or artificially introduced) is processed by Dicer into siRNA which is loaded into the RISC. AGO2 **(argonaute protein)**, which is a component of RISC, cleaves the passenger strand of siRNA. The guide strand then guides the active RISC to the target mRNA. The full complementary binding between the guide strand of siRNA and the target mRNA leads to the cleavage of mRNA.
miRNA: Transcription of miRNA gene is carried out by RNA polymerase II in the nucleus to give pri-miRNA, which is then cleaved by Drosha to form pre-miRNA. The pre-miRNA is transported by Exportin 5 to the cytoplasm where it is processed by Dicer into miRNA. The miRNA is loaded into the RISC where the passenger strand is discarded, and the miRISC is guided by the remaining guide strand to the target mRNA through partially complementary binding. The target mRNA is inhibited via translational repression, degradation or cleavage.

DO NOT MONETISE/SHIT

LO2(i): Discuss how epigenetics has contributed to the study of genetics and heredity.

Epigenetics and the environment

- Cell's epigenome is dynamic and can be affected by genetic and environmental factors
- can epigenetic modifications can be reversible → genome is flexible to respond to environmental changes such as nutrition, stress, toxicity, exercise and drugs
- Nutritional components
    - Folate plays an important role in methylation → influence methionine production by homocysteine remethylation
    - Folate defect or shortage → enhance colorectal carcinogenesis through hypomethylation of genomic dna
- Stress
    - People with post traumatic stress disorder or experience abuse exhibit different levels of dna methylation and gene expressions
- Arsenic exposure
    - Result in global dna alterations and gene promoter methylation levels, histone acetylation, histone phosphorylation and miRNA expressions → epigenetic dysregulation and carcinogenesis
- Type 2 diabetes
    - Hypermethylation in several genes : peroxisome proliferator activated receptor gamma and coactivater 1 alpha

Epigenetic factor

- Can act through direct or indirect mechanism
- Type 1 direct effect
    - Epigenetic factor directly alters the state of epigenetic enzymes (DNMT, HDAC, HAT, HMT) by
        - Binding to them and preventing them from carrying out their normal function
        - Upregulating them
    - Results in aberrant recruitment of epigenetic tags to promoters and enhancers on a genome wide scale
- Type 2
    - Epigenetic factor cause a change in biochemical process that results in an altered availability of a substance, intermediate, by product or any other metabolite participating in the biochemical pathway used to make epigenetic tags
    - Can also influence cell signalling pathways that leads to altered expression of growth factors, receptors and ion channels → alter transcription factor activity at gene promoters

DO NOT MONETISE MY SHIT

Epigenetics across human lifespan

- Total reprogramming is necessary soon after conception → epigenetic clean state
    - Important because eggs and sperms develop form specialised cells with stable gene expression profiles and are marked with epigenetic tags that must be erased before the new organism can grow into a healthy embryo
    - Occurs as global dna demethylation followed by remethylation to reprogramme the maternal and paternal genomes
- As fertilised egg develops, signals receives cause steady changes in gene expression patterns → each signal activate some genes and inactivate others
- Genomic imprinting
    - When one copy of a gene is silenced due to its parental origin
        - If paternal allele is expressed, maternal allele is silences, vice versa
    - For a small minority of genes, the epigenetic tags avoid being erased during reprogramming and pass unchanged from parents to offspring
    - Imprinted genes keep their epigenetic tags → begin with the process of developments with epigenetic tags in place
    - Improper imprinting results in individual having 2 active copies or 2 inactive copies leading to severe developmental abnormalities, cancer and other problem
    - Prader-willi syndrome and angelman syndrome → deletion in the same part of chromosome syndrome
        - Angelman syndrome → disabilities, seizures and speech deficits as well as motor oddities
            - Deletion involves 4 million base pair deletion includes a gene involved in the ubiquition pathway
        - PWS patients suffer from feeding problems and insatiable appetite ad obsession with food as well as delayed motor skills
            - Deletion of genes in the same region including SNRPN involved in mrna splicing

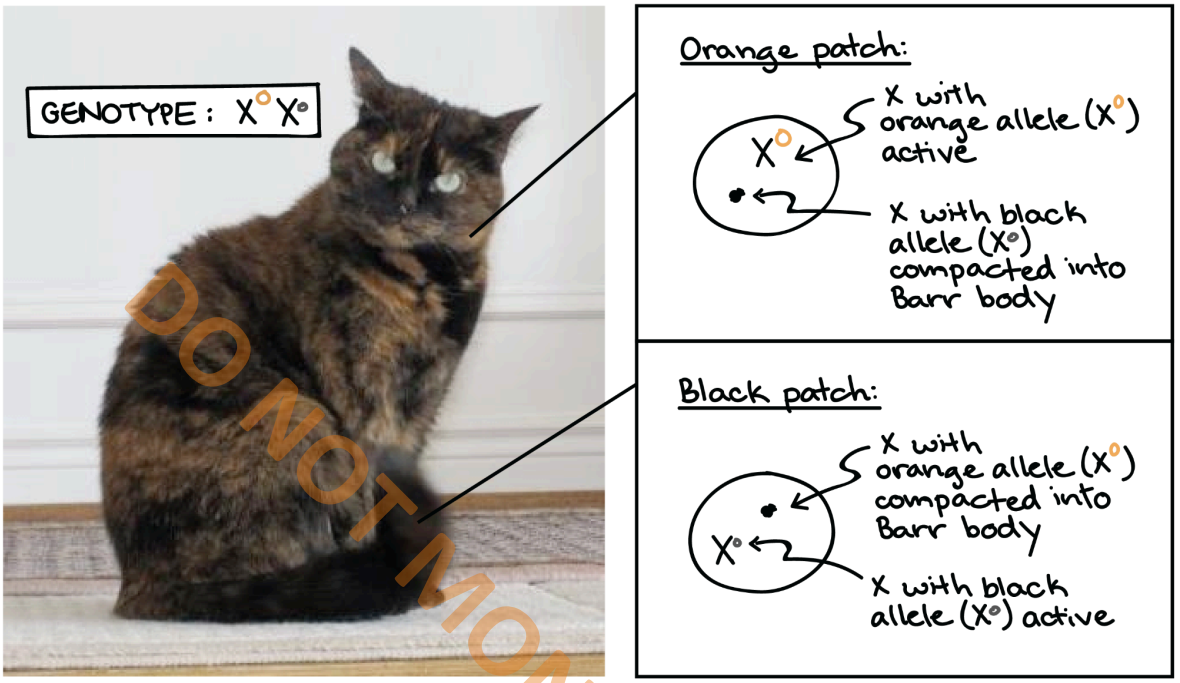
Understanding of genetics and inheritance

- Goes against the idea that inheritance happens only through the dna code that pass from parents to offspring
- Parents experiences in the form of epigenetic tags can be passed down to future generations
- The unsuccessful attempts in cloning animals probably stems from the epigenetics issue → donor nucleus is from a differentiated cell with epigenetic tags → but the epigenetic erasing is faulty, delayed and incomplete

DO NOT MONETISE MY SHIT

X chromosome inactivation

- Females carry 2 x chromosome → seme to have 2 doses of the products of x linked genes → x chromosome inactivation is a dosage compensation that corrects the disparity
- One of the 2 x chromosome in somatic cells become transcriptionally inactive early in development → inactivated at random and after inactivation the same x chromosome remains inactive throughout all further cell divisions in the life of that individual
    - Calico cats



DO NOT MONETISE MY SHIT

epigenetics and disease

- Epigenetic changes can be responsible for some disease states
- Disruption of systems that contribute to epigenetic alterations cause abnormal activation or silencing of genes

Disease	Symptom	Aetiology
ATR-X syndrome	Intellectual disabilities, α-thalassaemia	Mutations in *ATRX* gene, hypomethylation of certain repeat and satellite sequences
Fragile X syndrome	Chromosome instability, intellectual disabilities	Expansion and methylation of CGG repeat in *FMR1* 5′ UTR, promoter methylation
ICF syndrome	Chromosome instability, immunodeficiency	*DNMT3b* mutations, DNA hypomethylation
Angelman's syndrome	Intellectual disabilities	Deregulation of one or more imprinted genes at 15q11–13 (maternal)
Prader–Willi syndrome	Obesity, intellectual disabilities	Deregulation of one or more imprinted genes at 15q11–13 (paternal)
BWS	Organ overgrowth	Deregulation of one or more imprinted genes at 11p15.5 (e.g. *IGF2*)
Rett syndrome	Intellectual disabilities	*MeCP2* mutations
α-Thalassaemia (one case)	Anaemia	Methylation of α2-globin CpG island, deletion of *HBA1* and *HBQ1*
Various cancers	Microsatellite instability	*De novo* methylation of *MLH1*
	Disruption of Rb, p53 pathway, uncontrolled proliferation	*De novo* methylation of various gene promoters
	Disruption of SWI–SNF chromatin remodelling complex	Mutations in *SNF5*, *BRG1*, *BRM*
	Overexpression of IGF2, silencing of *CDKN1C*	Loss of imprinting
Leukaemia	Disturbed haematopoiesis	Chromosomal translocations involving HATs and HMTs
Rubinstein–Taybi syndrome	Intellectual disabilities	Mutation in CREB-binding protein (histone acetylation)
Coffin–Lowry syndrome	Intellectual disabilities	Mutation in *Rsk-2* (histone phosphorylation)

- Loss if dna methylation can cause abnormally high gene activation by altering the arrangement of chromatin
- Hypermethylation of CpG islands can cause tumours by shutting off tumour suppressor genes
- Epigenetic changes can cause mutations → hypermethylation if the promoter of MGMT causes the number of G to A mutations to increase
- Hypermethylation lead to instability of microsatelites
    - Microsatellites are repeats of dinucleotide CA
    - Too much methylation of the dna gene repair gene MLH1 make the microsatellite unstable → linked to cancers such as colorectal, ovarian and gastric cancers

Mental retardation

- Fragile x syndrome
    - Most frequently inherited mental disability → more affected in malles since males only have 1 x chromosome and 1 fragile x will impact them more severely
    - Patients have intellectual disability, delayed verbal development and autistic like behaviour
    - Caused by the abnormality in the fragile x mental retardation gene (FMR1) → individuals with the fragile x have over 200 repeats of the CGG in FMR1 gene while normal individuals only have 6 to 50 repeats
        - Cause the CpG islands at the promoter region of the FMR1 gene to be methylated when they are normally not → stop producing the fragile X mental retardation protein that is important for connection between neurones

DO NOT MONETISE MY SHIT

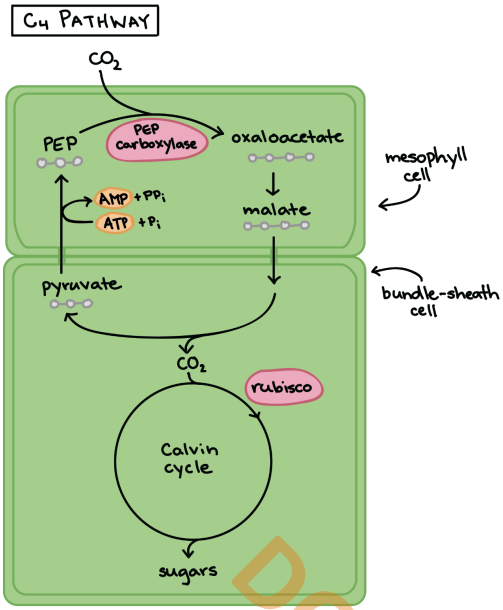
LO3(a): Explain how the anatomy and physiology of the leaves of C4 plants, such as maize and sorghum, are adapted for high rates of carbon fixation at high temperatures in terms of the spatial separation of initial carbon fixation from the light-dependent stage (biochemical details of the C4 pathway are required in outline only), and the high optimum temperatures of the enzymes involved.

C3 plants : issue of photorespiration

- Majority of plants are c3 plants (learnt in h2 bio) → light dependent stage and calvin cycle
- Photorespiration reduce the efficiency of the calvin cycle → results in no ATP, no NADPH and no resulting sugars
- occurs because RuBP oxygenase-carboxylase (Rubisco) is an enzyme that can also
- use oxygen (O2) as a substrate instead of just carbon dioxide (CO2).
- This happens when two conditions are met in a hot, dry climate:
    - High temperature
        - Rubisco has a higher affinity for O2 when temperature increases.
        - At mild temperatures, Rubisco's affinity for CO2 is about 8080 times higher than its affinity for O2.
        - At high temperatures, however, Rubisco has a higher affinity for O2.
    - low CO2: O2 ratio
        - When a plant has its stomata opened, CO2 diffuses in while O2 and water vapour diffuse out. Thus photorespiration is minimized due to high CO2: O2 ratio.
        - However, when a plant closes its stomata—for instance, to reduce water loss by transpiration, photorespiration increases due to the lower ratio of CO2 to O2
        - If Rubisco uses CO2 as substrate, it produces 3-phosphoglycerate/ glycerate-3-phosphate (GP), a 3-carbon sugar. GP is then used to make G3P which then leaves the Calvin cycle to form glucose
        - However, if Rubisco uses O2 as substrate, it produces 2-phosphoglycolate, a 2-carbon sugar. This product cannot be used in the Calvin cycle
        - The carbon is oxidized, which is the reverse of photosynthesis—the reduction of carbon to carbohydrate.
    - Requires for the salvage pathway to take place (in the peroxisome) → 2 phosphoglycolate converted to PGA, results in energy wasting
    - Rubisco is therefore inefficient
        - Enzyme activity and specificity is inversely related
        - Rubisco evolved before the first GOE, hence mechanism not constrained to o2
        - After second GOE, fubisco have to discriminate between co2 and o2, hence either slower and more specific catalyst or faster and less specific catalyst

DO NOT MONETISE MY SHIT

C4 plants (spatial separation of steps, kranz anatomy)



Can overcome the issue of photorespiration

- Phosphoenolpyruvate carboxylase (PEP carboxylase) converts co2 to oxaloaetate in the mesophyll cell → PEP carboxylase has a higher affinity with co2 compared to rubisco, and o2 is a poor substrate → hence oxygenation of rubp does not occur
- NADPH reduces oxaloacetate to malate and transported to bundle sheath cells
- Malic enzyme cleave malate to pyruvate and co2 and generate NADPH → calvin cycle can occur
- Pyruvate transported to mesophyll cell and rephosphorylated
- PEP carboxylase concentrated in the mesophyll cells that are the most exposed to the atmosphere
- Calvin cycle involving rubisco take place in bundle sheath cells that are located in the inner part of the leaf

Limitations:

- Consumes 2 atp equivalents to deliver a co2 to rubisco but it is not a problem because atp can be synthesized through photophosphorylation

c3	c4
Carbon fixation and calvin cycle occur in mesophyll cell in presence of o2	Carbon fixed in mesophyll cells → transported to bundle sheath cells where calvin cycle occur without o2
Bundle sheath cells do not contain chloroplat	Bundle sheath cells contain chloroplasts
Co2 acceptor is rubisco	Co2 acceptor is PEP carboxylase
First stable compound is PGA	First stable compound is oxaloacetate
Optimum temperature of 20-25 → predominant in temperate regions	Optimum temperature is 35-44 → found in tropical climate since they operate more efficiently at high temperature and high light intensities
Phosphorespiratory loss is high, hence adapted to environment with more co2	Photorespiration does not take place, adapted to environment with more o2
No kranz anatomy	Have kranz anatomy

DO NOT MONETISE MY SHIT

LO3(b): Discuss and compare the importance in mitigating global warming of photosynthetic carbon fixation by C3 plants, C4 plants, CAM plants and algae, including those in reef-building corals.

Expected Effects of Global Warming

- Increase in temperature as a result of increased carbon dioxide
- Uneven precipitation

C3 and C4

- Generally, higher CO_2 concentrations increase photosynthetic rate as it decreases the rate of photorespiration due to O_2 as a competitive inhibitor
- C4 is a better sequester of carbon than C3 at the same CO_2 concentration but C3 is theoretically better at higher concentrations

Use of Microalgae in Aqueous Environment

- They are able to sequester carbon and close the carbon loop of decomposition into the air by acting as food for low-carbon-footprint herbivorous fish.
- High carbon capture rate: less need to spend energy on combating gravity, hence it can be used as a better food source
- Algae capture carbon in the form of bicarbonate and concentrate them in the chloroplast, where it is dehydrated to form algal biomass
- Pyrenoids of chloroplast play a great role in inhibiting photorespiration by increasing carbon dioxide concentration around RuBisCO beyond that is possible by equilibrium by air, reducing the competitive inhibition of oxygen on carbon fixation by RuBisCO
- $CO_2$is converted to biomass which can be processed further with carbon capture and sequestration technology
- Permanent burial of total fresh biomass
- Permanent burial of algal lipids
- Soil amendment with algal biochar

What makes algae more useful compared to other kinds of plants

- Ability to capture CO_2in non gaseous form as bicarbonate underwater compared to atmospheric CO_2 above ground in other plant types
- Actively prevents photorespiration by active pumping of bicarbonate into cells

Algae in mitigating climate change

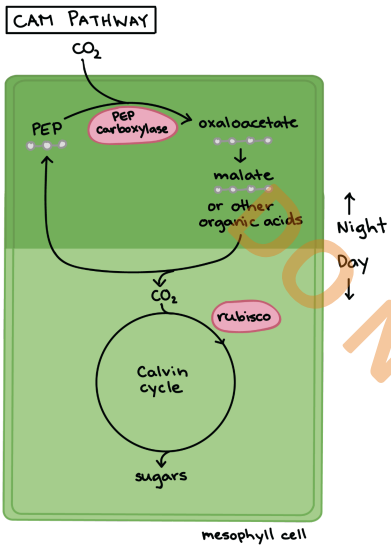
- Algae are simple plants that can range from microscopic (microalgae) to large seaweeds (macroalgae). Marine phytoplankton are mainly composed of microalgae known as dinoflagellates and diatoms. Microalgae include cyanobacteria and green, brown, and red algae.
- Due to their very high productivity, marine algae offer great potential for carbon sequestration. Marine free-living algae account for almost 50% of the world's carbon biogenic fixation, even though accounting for less than 1% of the photosynthetic biomass on Earth. This is possible because on average, the entire global population of phytoplankton is replaced on average every two to six days.
- Overall, the world’s phytoplankton incorporate yearly around 45–50 Pg C into their cells making them as important in modifying the planet’s cycle of carbon and carbon dioxide as all the world’s land plants combined. Per unit area, the productivity of the oceans is approximately one third of that found on land plants.
- On Earth, half of the carbon captured by photosynthetic organisms is linked to phytoplankton activity. This carbon captured at the sea is known as “blue carbon”.

DO NOT MONETISE MY SHIT

LQ3(c): Explain how the physiology of the leaves of CAM plants is adapted to allow photosynthesis while minimising water loss by transpiration, in terms of the temporal separation of initial carbon fixation from the light-dependent stage (biochemical details of the CAM pathway are required in outline only), and stomatal opening during the night.

CAM plants

- Use both c3 and c4 pathways, but use temporal separation of steps instead of spatial separation of steps



- As CO2 diffuses in, it combines with phosphoenolpyruvate (PEP) in the cytoplasm of the mesophyll cells, forming oxaloacetic acid. The oxaloacetic acid is then reduced to malate, which accumulates in the cell vacuoles.
- This is the C4 pathway, run in the dark of night with its product, malate, migrating only to the vacuole of the cell in whose cytoplasm it was made.
- As day arrives and temperature rises, the stomata close once more, and photosynthesis commences. The malate is now transported back into the cytoplasm and is decarboxylated to pyruvate and CO2.
- The CO2 enters the chloroplast and is picked up in the Calvin cycle. Glyceraldehyde-3-phosphate (G3P) and then sucrose or starch are produced.

Other adaptations

- Thick, reduced leaves with low surface area to volume ratio - Thick cuticle
- Sunken stomata
- high degree of succulence maintained by highly vacuolated cells → large central cell sap vacuoles are important for effective nocturnal storage of organic acids

DO NOT MONETISE MY SHIT

LO3(d): Explain the changes in atmospheric oxygen concentration during the evolution of life on Earth and evaluate the importance of these changes to evolution.

We tend to think through anthropocentric viewpoints

- Oxygen is actually toxic - oxidation kills slowly

4.5bya: Earth finished forming

- At first, O_2 was only present in CO_2 and H_2O (no atmospheric O_2)
- Volcanic gases produced mostly H_2O and CO_2, minor gases H_2, HCl, SO_2 - Some free O_2 produced by dissociation of H_2O in upper atmosphere from UV radiation

3.9bya: life emerges

- Microscopic fossils of prokaryotic cells have been identified in 3.5byo rocks - Rocks 3.9byo contain organic matter rich in C_{12}(marker of life)
- Likely extremophile bacteria → thermal vents at the bottom of the oceans - 2.8-3.0bya: oxygenic photosynthesis begins
- Photosynthetic microbes
    - Oldest organisms that could produce chlorophyll were cyanobacteria - First cyanobacteria appeared ~3.5bya and were obligate anaerobes - Cyanobacteria that evolved oxygenic photosynthesis could exploit new niches - harvest abundant energy
    - Still exist: stromatolites
    - Led to rise in oxygen

2.5-2.8bya: Great Oxidation Event

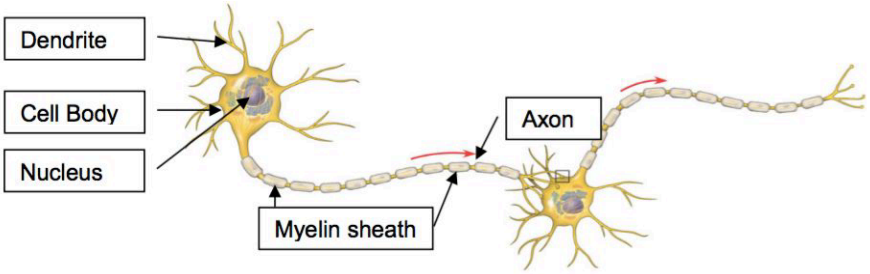
- At first most of the oxygen was absorbed through oxidation of surface iron and decomposition
- Later, as population of cyanobacteria grew, oxygen sinks became saturated - Free oxygen accumulates in the atmosphere
- Oxygen concentration surpassed that of CO_2 and methane
- Toxic to most of the life forms that existed then, favoured organisms which were adapted to utilise oxygen
- Oxidative stress from oxygen and production of reactive oxygen species - Adaptation to the presence of oxygen in the atmosphere was a driving force for the evolution of organisms
- Killed most of the anaerobic life (except some: anaerobes)
- Oxidised methane to carbon dioxide and water
- Less greenhouse gases → less heat trapped → first glaciation episode - Snowball Earth
- Freed by volcanic activity → release of GHGs that warmed the Earth and melted the ice → sea absorbed radiation → positive feedback

Endosymbiosis

- Aerobic bacteria + cyanobacteria + archaea = eukaryotes
- Evolution
    - Oxygen levels increase → aerobic organisms developed → use up more free oxygen and produce more carbon dioxide → more photosynthesis → more oxygen produced → oxygen levels become high enough to support more complex life → produce more oxygen → even more evolution of aerobic organisms

DO NOT MONETISE MY SHIT

LO3(e): Describe and explain the transmission of an action potential along a myelinated neurone (the importance of Na^+ and K^+ ions in the impulse transmission should be emphasised).



Neurone: cell specialised for transmitting chemical and electrical signals from one location in the body to another

Cell body

- Contains the nucleus
- Where metabolic activities of the neurone are carried out (e.g. synthesis of membrane, neurotransmitter)
- Abundance of RER, Golgi Apparatus, mitochondria

Dendrites

- Highly branched projections of a neurone that receive the electrochemical stimulation from other neurones/the external environment, and conduct it towards the cell body

Axon

- A long single projection of a nerve cell (neurone) that extends from the cell body and conducts impulses away from the cell body
- Fluid in the axon is known as axoplasm
- It is capable of propagating electrical impulse known as action potential - Eventually branches out to terminate at swollen regions known as synaptic knobs/terminals

Myelin sheath

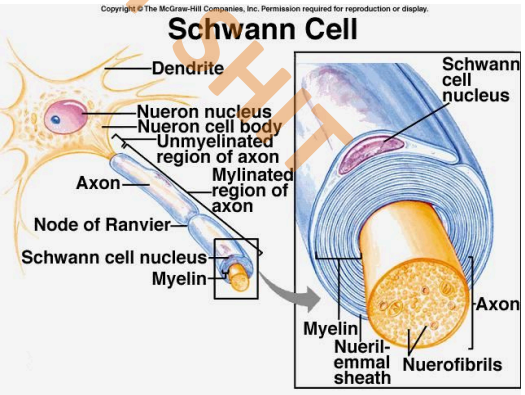
- The axon may be myelinated i.e. has a myelin sheath
- Myelin sheath is formed by Schwann cells that wrap around and enclose the axon in many layers of their plasma membranes
- Myelin sheath contains high lipid content, hence provide insulation, preventing exchange of ions across the cell membrane along the axon

Nodes of Ranvier

- Between the myelinated regions, are exposed regions/gaps along the axon, where myelin sheath is absent

Schwann cell

- wrap axons in many layers of myelin (electrically insulating material)



DO NOT MONETISE MY SHIT

Membrane potential:

- A difference in electrical charge (voltage) across the plasma membrane of a cell - All cells have a membrane potential. However, only neurones have the unique ability to generate and propagate electrical signals/nerve impulse through the movement of charged ions
- A nerve impulse is the movement of an action potential along an axon in response to stimuli

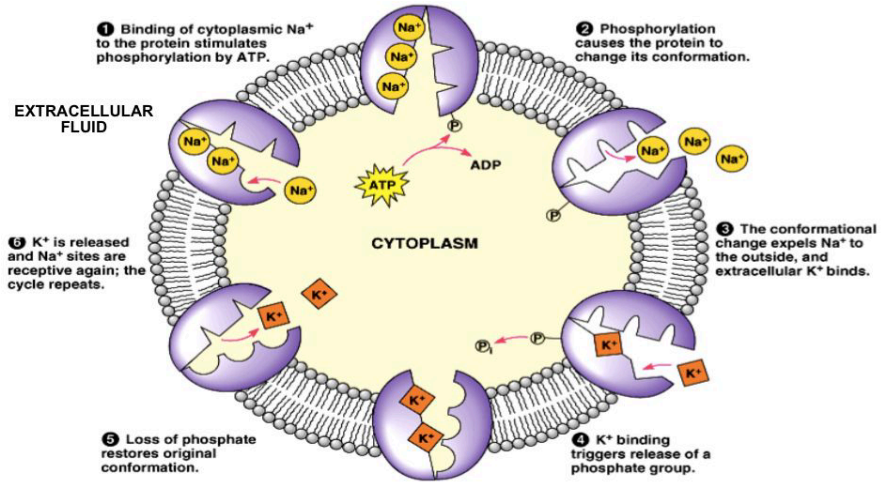
Resting membrane potential:

- When a neurone is not stimulated/not transmitting a nerve impulse, it maintains a resting membrane potential of -70mV
- Cytoplasmic side of the membrane is negative with respect to the extracellular side of the cell
- Cytoplasmic side is -70mV relative to the extracellular side of the cell - Membrane is said to be polarised due to unequal distribution of charged ions - The cytoplasm in the neurone has high concentrations of K^+ and organic anions while low in concentrations of Na^+ and Cl^-
- Extracellular fluid has lower concentrations of K^+ and organic anions and higher concentrations of Na^+ and Cl^-
- These differences in charge and ion concentration across the membrane are known as electrochemical gradients

DO NOT MONETISE MY SHIT

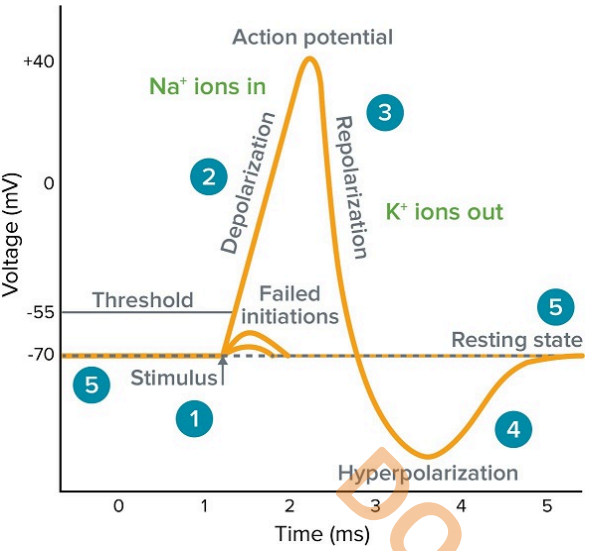
<u>How the resting membrane potential is maintained</u>

- There are K^+ and Na^+ leak channels on the membrane
- Ion channels allow ions to diffuse down their electrochemical gradients back and forth across the membrane
- There are more K^+leak channels than Na^+leak channels
- So the number of K^+leaving the cell is greater than the number of Na^+ entering it via facilitated diffusion
- This contributed to the negative electrical charge inside the cell
- Over time, the ionic gradients will dissipate but the Na^+-K^+ pump uses energy from ATP to pump 3 Na^+ out for every 2 K^+ pumped into the cell via active transport - This helps to maintain the concentration of relatively high Na^+ outside and high K^+inside the cell
- As the pump moves 3 Na^+ out for every 2 K^+ pumped in, it causes a net transfer of positive charge out of the cell and this contributes to the negative resting membrane potential
- Large negatively charged organic anions such as proteins and nucleic acids within the cell. They are called fixed anions
- They are contained within and cannot diffuse out of the cell because of their size
- They contribute to the negative electrical charge inside the cell



Generation of an action potential

- Massive change in the membrane potential → occurs when the membrane potential increases to a threshold value at -50mV



Consists of depolarisation, repolarisation and hyperpolarisation

- Depolarisation : membrane potential increase from resting potential → less negative than at resting potential
  - Local voltage gated na+ ion channels open → increased permeability of neurone membrane to na+
  - Na+ diffuse down electrochemical gradient into the neurone, neurone becomes less negative
  - Once threshold potential of -50mV is reached, all na+ channels open, causing further depolarisation that results in increase in voltage to +40mV
  - Threshold must be reached if not the action potential will not be triggered → all or nothing law
  - At subthreshold, the membrane will return to resting potential without triggering of an action potential
  - Amplitude of action potential is constant and does not depend on strength of stimulus, hence it is either a full response or no response
- Graded potential : any change in membrane potential, can be action potential or not action potential (localised change in the potential difference across the neurone plasma membrane
- Repolarisation : membrane potential returns towards the resting potential of -70mV
  - Permeability of neurone plasma to na+ decreases as the na+ channels close
  - Membrane permeability to k+ ion increase as the voltage gated k+ ion channel open and k+ will diffuse out down the electrochemical gradient, neurone become more negative relative to outside the cell
  - Membrane potential decreases from +40mV to the resting potential of -70mV

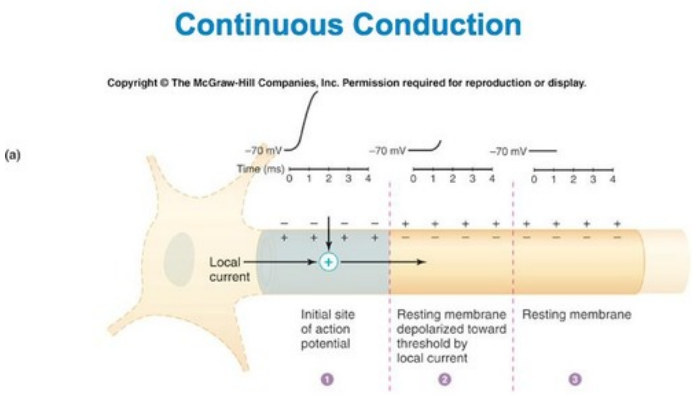
DO NOT MONETISE MY SHIT

- Hyperpolarisation : further decrease from the resting potential to about -85mV
    - This is due to the voltage gated k+ channels closing more slowly relative to the na+ voltage gated ion channels after the repolarisation
    - K+ will continue to leave the axon even after the membrane potential returns to the resting potential level
    - Results in a refractory period : sets a limit on the maximum frequency at which action potentials can be generated and prevents fatigue of neurones and ensure that the nerve impulse travel in one direction
        - Absolute refractory period : coincides with depolarisation and repolarisation phases of the action potential
        - Relative refractory period : coincides with the hyperpolarisation phase of action potential → will require a stronger than normal stimulus to generate another action potential
    - As voltage gated k+ ion eventually close, the membrane potential will return to the resting potential (maintained by various processes mentioned)

DO NOT MONETISE MY SHIT

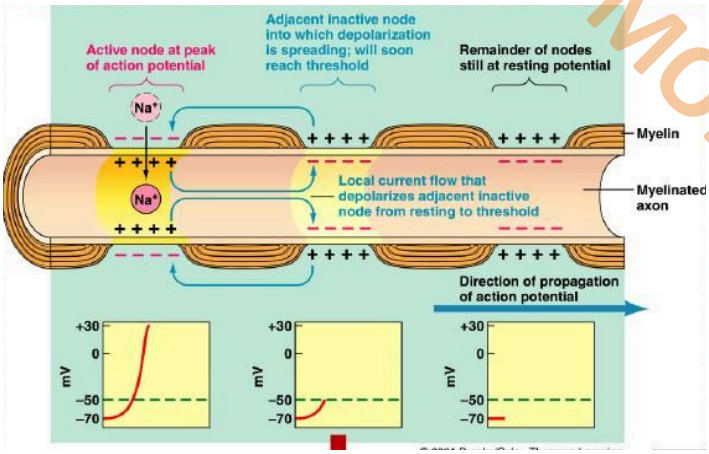
Transmission of an action potential

- Nerve impulse refers to a series of action potentials sweeping down an axon, can be done via continuous conduction along the non-myelinated neurone and by saltatory conduction along the myelinated neurone



Continuous conduction :

- Local voltage gated na+ channel open allowing for na+ influx → depolarisation of the membrane → -50mV → further depolarisation as all the remaining voltage gated na+ channel open
- depolarisation spreads to the portion of the membrane ahead of the membrane potential → all voltage gated na+ ion channel open in that particular region and action potential triggered
- The influx of na+ can allow for it to travel backward and forward of the axon, but behind the travelling zone of depolarisation caused by the na+ inflow there is a zone of repolarisation caused by k+ outflow and hence the voltage gated na+ ion channel remain inactivated since it is in the refractory period → prevent multiple generation of the action potential
- Ensures that the action potential can only travel in one direction
- Action potentials generated at the nodes of ranvier (unmyelinated regions)



- During saltatory conduction, na+ will diffuse within the axoplasm to the next node of ranvier to stimulate the voltage gated na+ ion channels → as the opening and closing of ion channels is a time consuming process, hence only limited to the nodes of ranvier
- Increases the speed of transmission of action potentials along the axon
- Conserve energy as depolarisation only occur at the nodes

DO NOT MONETISE MY SHIT

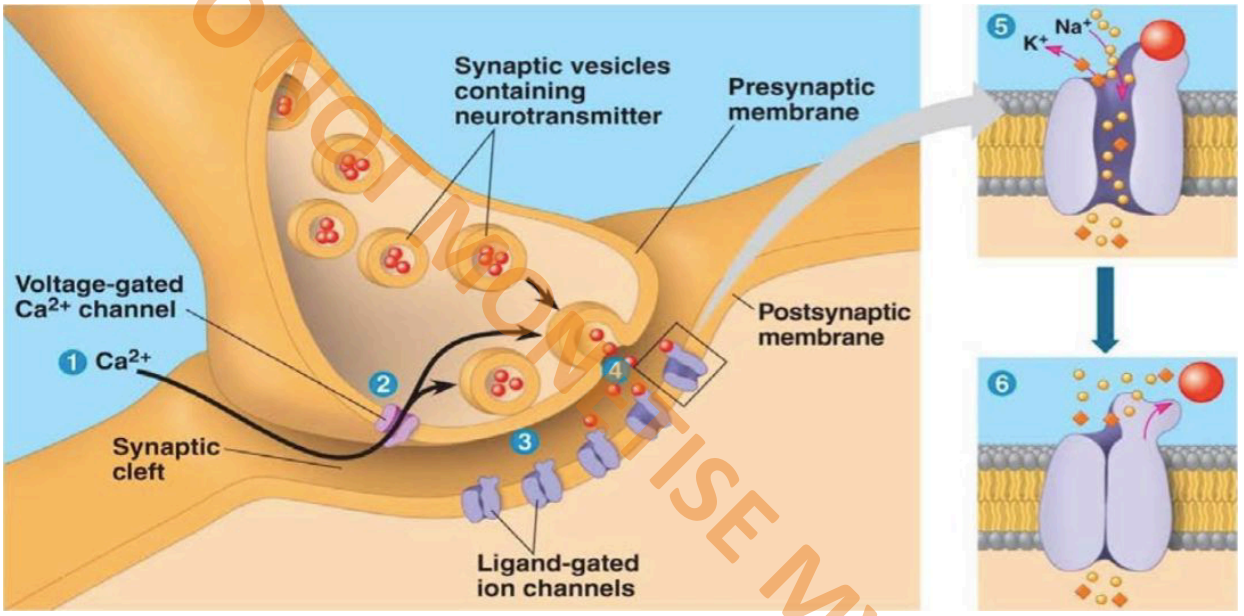
LO3(f): Describe the structure of a cholinergic synapse and explain how it functions, including the role of Ca^{2+} ions.

Synapses

- Action potentials eventually reach the end of the axon (axon terminals). The axon terminals form junctions with dendrites of other neurones, muscle cells or gland cells. These junctions are called synapses

Synapses consist of

- A presynaptic terminal/knob: an expansion of the fine axon terminal of the presynaptic neurone
- Contains synaptic vesicles, mitochondria and microfilament
- Synaptic vesicle contains neurotransmitters (e.g. acetylcholine)
- A postsynaptic knob/membrane of the target cell that contains specific ligand-gated ion channels to which the neurotransmitters bind, enabling the transmission of a signal across the synapse
- The synaptic cleft, a space between the presynaptic and postsynaptic endings of an average width of 20nm



DO NOT MONETISE MY SHIT

Transmission of synaptic impulse

1. When an action potential arrives it depolarises the pre-synaptic membrane
2. The depolarisation opens voltage-gated calcium channels, triggering an influx of Ca^{2+}
3. The elevated Ca^{2+} concentration causes synaptic vesicles to fuse with presynaptic membrane
4. This releases neurotransmitters (e.g. acteylcholine) into the synaptic cleft
5. The neurotransmitters diffuse across the synaptic cleft
6. The neurotransmitter binds reversibly to the ligand binding site portion of ligand-gated Na+ ion channels in the postsynaptic membrane, opening the channels 7. Na^{+} diffuses through the channels
8. The influx of Na^{+} into the postsynaptic neurone results in the local depolarisation of the postsynaptic neurone and if the threshold potential is reached, an action potential will be generated in the postsynaptic neurone
9. The neurotransmitter is released from the receptors and the channels close
    a. Acetylcholine is degraded by enzyme acetylcholinesterase to acetic acid and choline
    b. The choline is taken back into presynaptic terminal where it is combined with acteyl CoA to reform acetylcholine

Role of Ca^{2+} in the transmission of impulse across the synapse

- There is low Ca^{2+} concentration in the presynaptic terminal compared to the extracellular fluid
- An action potential arriving at the synaptic terminal depolarises the presynaptic membrane, causing Ca^{2+} voltage-gated channels to open, resulting in the influx of Ca^{2+} into the synaptic terminal
- The sudden rise in Ca^{2+} concentration stimulates synaptic vesicles containing neurotransmitters such as acetylcholine to fuse with the presynaptic membrane - This releases neurotransmitters into the synaptic cleft by exocytosis - The Ca^{2+} ions are rapidly removed from the cytoplasm by active transport out of the cell

DO NOT MONETISE MY SHIT

Excitation and inhibition of nerve impulse

- Excitation :
    - depolarisation that leads to excitation of neurone is called excitatory postsynaptic potential (EPSP)
    - If EPSP reach threshold of -50mV, then an action potential is triggered
- Inhibition
    - Neurotransmitters can also inhibit impulse transmission in the postsynaptic neurone by preventing depolarisation or causing hyperpolarisation
        - Decrease in permeability of postsynaptic membrane to na+
        - Increase the permeability of postsynaptic membrane to k+ and cl= ions
        - Hyperpolarisation occur → inhibitory postsynaptic potential (IPSP)
- Postsynaptic neurone may receive impulses from many excitatory and inhibitory presynaptic neurones → convergence occurs as the postsynaptic neurone is able to summate the EPSP and IPSP from all the presynaptic neurones (temporal and spatial summation)
    - Hence many EPSP that give an additive effect are required to produce sufficient depolarisation to reach threshold potential to trigger an action potential on the postsynaptic membrane
- Temporal summation
    - When 2 EPSP occur so closely in time due to repeated stimulation of the presynaptic neuron at high frequency → postsynaptic membrane has not returned to the resting potential
    - Hence the 2 EPSP will add together
        - Enables weak background stimuli to be filtered out before they reach the brain, only changes in the intensity of the stimuli is significant to the nervous system (higher intensity EPSP will cross the threshold and become an action potential)
- Spatial summation
    - EPSP produced nearly simultaneously by different presynaptic neurones on the same postsynaptic neurone can add together
        - Enables the synapse to act as a centre for the integration of stimuli from a variety of sources → production of a coordinated response
    - Example :
        - Presynaptic neurone a release a neurotransmitter that results in an EPSP formed in the postsynaptic neurone
        - Presynaptic neurone b release another neurotransmitter that results in an IPSP formed in the postsynaptic neurone
        - Spatial summation occurs and if the resultant graded potential reaches a threshold potential than an action potential is generated and travels down the postsynaptic neurone → membrane depolarisation occurs

DO NOT MONETISE MY SHIT

- Unidirectionality achieved by
    - Synaptic vesicles containing neurotransmitters are only present in the presynaptic neurone terminals → only presynaptic membrane can discharge neurotransmitters
    - Voltage gated ca2+ ion channels are only found in the presynaptic membrane, hence only depolarisation of the presynaptic membrane will lead to the release of neurotransmitters from the synaptic vesicles
    - Ligand gated na+ ion channel receptors are restricted to postsynaptic membrane and absent in presynaptic membrane hence neurotransmitter can only move from presynaptic knob to bind to the receptors on the postsynaptic membrane across the synaptic cleft

DO NOT MONETISE MY SHIT

Characteristics	Graded potential	Action potential
origin	Mainly in dendrites and cell bodies as epsp and ipsp	Arise at trigger zones and propagate along axons (nodes of ranvier)
Types of channel	Chemical, mechanical or light	Voltage gated ion channel
conduction	Not propagated, localised and permit communication over a few mm	Propagated and permit communication over long distances
amplitude	Depends on the strength of stimulus does not follow all or nothing law	Follows the all or nothing law, from -70mV to +40mV
Duration	Long, ranges from milliseconds to minutes	Shorter, ranging to 0.5 to 2 milliseconds
Polarity	May be hyperpolarizing due to ipsp or depolarising due to epsp	Consist of depolarising, depolarising then return to resting to membrane potential
Refractory period	No, hence can exhibit temporal and spatial summation	Yes, hence no summation

Nervous control	Hormonal control
Electrical and chemical transmission (nerve impulse and chemical across synapse)	Chemical transmission of hormones through blood system
Rapid transmission and response	Slower transmission and relatively slow acting except for adrenaline
Short term changes	Long term changes except adrenalin
Specific process, through nerve cells	Pathway is not specifics as blood circulates around whole body but target is specific
localised	Widespread response

DO NOT MONETISE MY SHIT

feature	Neurone	synapse
Nature of message	Propagation of electrical impulse	Chemical transmission as neurotransmitters
Neurones involved	Action potential travels along one neurone	3 adjacent neurones
direction	unidirectional	
ipsp/epsp	Can be both	
depolarisation	Na+ influx increase membrane permeability to na+	Excitatory neurotransmitter leads to na+ influx in postsynaptic membrane
delay	Time taken for movement of ions across axon	Synaptic delay → time for release of neurotransmitters and diffusion
response	All or nothing	Summation (temporal or spatial)
recovery	Recovery to resting potential by repolarisation and hyperpolarisation	Recovery to resting potential by acetylcholinesterase to hydrolyse acetylcholine
Refractory period	Absolute, relative	No, but an experience fatigue
speed	Affected by diameter of axon, temperature, and presence of myelin	drugs
Type of gates	Voltage gated ion channels	Presynaptic : voltage gated ion channels Postsynaptic : chemically gated ion channels

DO NOT MONETISE MY SHIT

LO3(g): Explain that quorum sensing is a system of signalling processes that respond to changes in population density in bacteria.

What Quorum Sensing Is

- can process that enable bacteria to communicate using secreted autoinducers (signalling molecules)
    - Can occur between bacteria species and between bacterial and eukaryotic hosts
- Enable the population of a bacteria to regulate gene expression collectively (synchronisation) → control behaviour on a community wide scale
    - Monitor their self population density by measuring the concentration of autoinducers so that it can reach the optimum density
    - Increase in population density → increase in concentration of external autoinducer → due to the individual organisms producing and secreting the autoinducers to the external environment
    - These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation and biofilm formation

Three basic principles:

1. Production of AIs
2. AIs detected by receptors
3. Activation of gene expression necessary for cooperative behaviours and activation of AI production

DO NOT MONETISE MY SHIT

Need for QS

- As environmental conditions often change rapidly, bacteria need to respond quickly in order to survive
- Enables bacteria to communicate to coordinate gene expression to coordinate the behaviour of the entire community
- It is important for pathogenic bacteria during infection of a host to coordinate their virulence in order to be able to establish a successful infection
    - Regulating bioluminescence, virulence gene expression, biofilm production and antibiotic resistance
    - Functions integral for survival are mediated by quorum sensing, viewed as group dependent or group beneficial
    - Enable bacteria to respond rapidly to changes in the environment
    1. Biofilm formation
        a. Cell disposition and attachment involves
            i. Free floating cells collide with a suitable surface that has been preconditioned with deposits of environmental proteins and other molecules
    2. Colonization
        a. Cell to cell signalling occurs result in expression of biofilm specific genes
        b. Dna secreted by cells can be taken up by others that result in expression of new genes
    3. Maturation
        a. Extracellular polymeric substance fully encase the cells to form a complex dynamic community
    4. Detachment
        a. Individual cells or pieces of biofilm released into the nevironmet as a form of active dispersal, and could be triggered by environment factors
- Advantages
    - Provides protection from harmful conditions or substances → 1000x more resistant to antibiotics compared to free floating cell
    - Continual access to fresh food
    - Cell growth in microbial populations as they can easily benefit from cell cell communication and genetic exchange

DO NOT MONETISE MY SHIT

Mode of Action

luxI/R system

- Used by gram negative bacteria
- Involve the use of acylated homoserine lactones (AHLs) produced by luxI synthase
    - After production, the autoinducer diffuse out of the cell → external AHL concentration is equivalent to internal AHL concentration, increasing proportionally with increasing cell density
- After threshold AHL concentration is achieved, AHL will bind to the cognate luxR receptor and the AHL-luxR complex will bind to promoter DNA elements and downstream target gene transcription

- Each species of gram negative bacteria produces a unique AHL and only members of the same species recognise and respond to it and the detection and alteration of downstream gene expression is mediated by the cognate luxR protein
- The AHL-luxR interactions are specific, limited cross talk in mixed populations

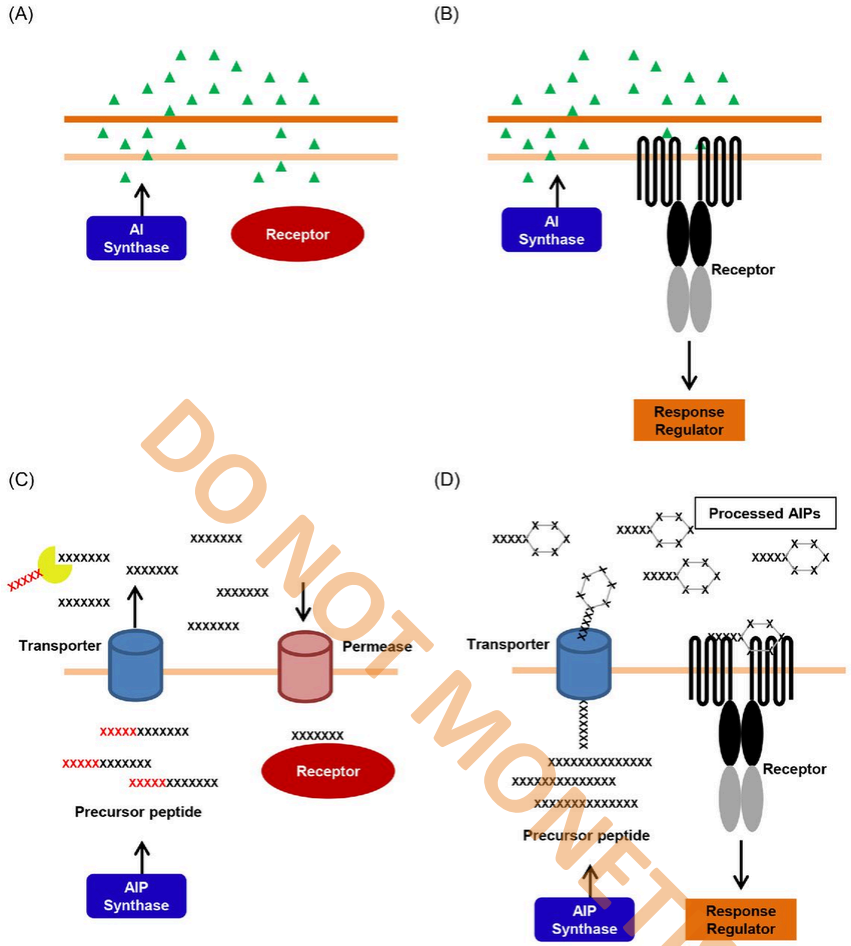
Oligopeptide autoinducer

- For gram positive bacteria, since gram positive bacteria does not use AHL mediated quorum sensing
- Use of oligopeptide autoinducers (AIP) → synthesize and transport to the environment
    - Synthesize AIP that are post translationally modified that sometimes contain unusual side chains that are secreted
    - Presence of cell surface oligopeptide transporters to facilitate AIP secretion into extracellular environment
- Detection of AIP mediated by 2 component sensory transduction system → lead to ATP driven phosphorylation of response regulator protein that binds to promoter DNA and regulates the transcription of quorum sensing regulated genes

DO NOT MONETISE MY SHIT

Hybrid systems

- Function by a conserved phosphorylation cascade involving sensor kinase and response regulator pair
- Fial component is usually a DNA binding protein that alters target gene expression
    - Use of phosphorylation cascade to relay information



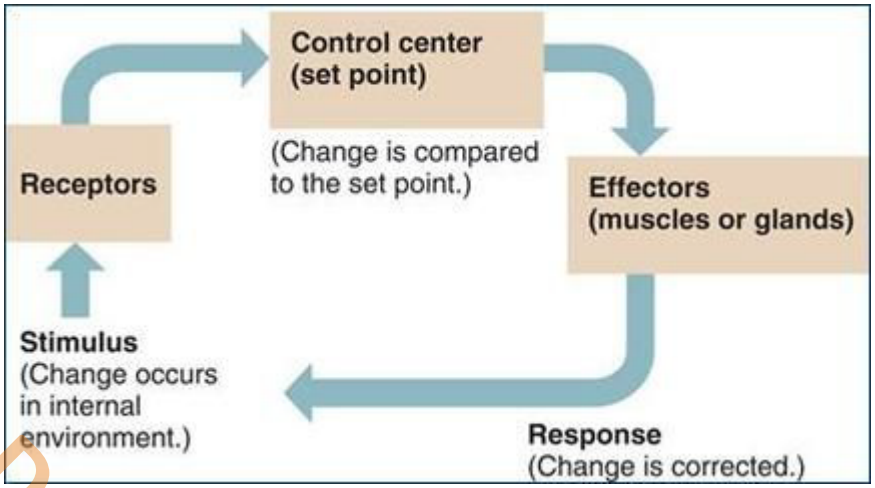
(A) Gram negative one component system
(B) Gram negative two component system
(C) Gram positive one component system
(D) Gram positive two component system

How to suppress quorum sensing to kill bacteria

- Inhibition of bindning of autoinduer to receptor
- Suppressing of quorum sensing system in gram positive organisms
- Suppression of autoinducer synthesis
- Degradation of autoinducers

DO NOT MONETISE MY SHIT

LO3(h): Explain the need for control in organised systems and explain the principles of homeostasis in terms of receptors, effectors and negative feedback.



DO NOT MONETISE MY SHIT

LO3(i): Explain the need for different communication systems within organisms.

In multicellular organisms where different parts of the body perform different functions, it is important to transmit information between these body parts so that the bodily functions can be well-coordinated and carried out efficiently.

The nervous system and endocrine system detect stimuli in the external or internal environment to evoke the appropriate responses.

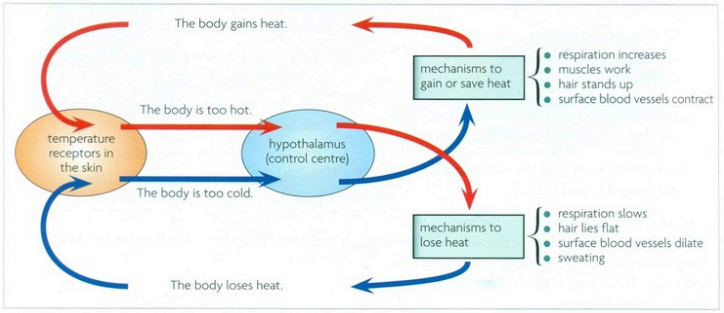
The fast speed at which the nerve impulse can be generated and transmitted makes it an ideal system for rapid detection and reaction, especially towards a dangerous situation.

The transmission of information via hormones by the endocrine system is relatively slower to ensure a constant internal environment.

DO NOT MONETISE MY SHIT

Hormonal control

- Homeostasis aims to maintain a constant internal environment
- Homeostatic control system in negative feedback consists of a
    - Receptor : detects change or deviation from set point, relay information to the control centre
    - Regulator : process information received from receptor → send signals to the effectors to initiate appropriate response to correct the deviation



    - Effector : carry out the process initiated by the regulator and bring about changes to return the system to the set point
    - Negative feedback mechanism : deviation from the set point triggers corrective mechanism to counteract deviation and restore the system to the set point → resist change and associated with increasing the stability of systems
- Positive feedback loop
    - Do not contribute to homeostasis
    - Disturbance increases the disturbances → leading to unstable situation and extreme states

DO NOT MONETISE MY SHIT

Classes of hormones

- 3 classes : amine hormone, peptide hormone and steroid hormone
    - Steroid hormone can pass through the plasma membrane and form a complex with the receptor and the complex acts as a transcription factor to regulate transcription
    - Non steroid hormones are too big to pass through the plasma membrane and binds to the membrane bound receptors that results in signal transduction and cellular responses

Endocrine systems

- Ductless glands that contains secretory cells → secrete hormones directly into the blood and are richly supplied with network of blood capillaries
    - Hormones are chemical messengers that are protein or steroid in nature
        - Produced in minute quantities and effective in small concentrations
        - Released into the blood and transported to target organs → elicit specific biological responses
        - Only affect cell with specific receptors
        - Destroyed in the liver after serving its function
    - Insulin and glucagon
        - Pancreas contains islet of langerhans, alpha cells secrete glucagon, beta cells secrete insulin via exocytosis
        - Secretion of enzymes controlled by negative feedback mechanisms, triggered by fluctuations in the blood glucose concentration
        - Receptors in the islets of langerhans detect the change in blood glucose concentration → hormones secreted into the bloodstream and transported to target cells (liver cells, muscle cells, adipose tissues) where they exert their effect

DO NOT MONETISE MY SHIT

- Blood glucose concentration
    - Normal level of glucose in the blood is 90 mg/100cm3
    - Increase in blood glucose beyond set point cause hyperglycemia -> insulin decrease blood glucose level
        - Beta cells in the islet of langerhans of the pancreas will detect the rise in blood glucose level
        - Beta cells respond by secreting insulin into the blood
        - Insulin secreted acts on almost all type of body cells
            - Bind to receptor tyrosine kinase
            - Increase permeability of cell membrane to glucose → increased uptake of glucose into cells
            - Increased rate of glucose utilisation in respiration and hence higher atp formation
            - Activates intracellular cell signalling resulting in higher glycogenesis lower glycogenolysis, lower gluconeogenesis and increased rate of protein and fat synthesis
        - Corrective mechanism cause the blood glucose concentration to fall back to the set point and to prevent the glucose level from further falling, there is feedback of information to the beta cells and insulin production is switched off and insulin is destroyed in the liver
    - Fall in blood glucose concentration causes hypoglycemia → glucagon will increase blood glucose level
        - Alpha cells detect the fall in blood glucose level
        - Alpha cells respond by secreting glucagon into the blood
        - Glucagon affects mainly liver cells to increase blood glucose levels
            - Glucagon binding to the GPCR that increases glycogenolysis, increased gluconeogenesis and using of fats as the main fuel in respiration
        - Corrective mechanism results in the increase of glucose to the set point and to prevent blood glucose concentration from further increasing there is feedback of information to the alpha cells and glucagon production in the alpha cells are switched off and glucagon is destroyed in the liver
- Diabetes
    - High blood sugar due to body not producing enough insulin (type 1) or cells do not respond well to the insulin that is produced (type 2)
        - Type 1: failure to produce insulin
            - Treatment : injection of insulin
        - Type 2: insulin resistance, reduced responsiveness in the target cells due to change in the insulin receptors
            - Treatment : management of glucose level by dietary control and exercise
    - Implications of diabetes
        - Cardiovascular diseases
        - Kidney damage
        - Nerve damage
        - blindness

DO NOT MONETISE MY SHIT

LO4(a): Explain, with examples, sexual selection and its significance for evolution.

4 Components of Natural Selection

- Variation
- Competition
- Adaptation
- Selection

Sexual Selection

- Process through which males and females attempt to maximise their chances of reproductive success

<u>Intra-sexual selection</u>

Outcompeting other members of the same sex to determine mating success

- Favours traits enhancing the ability to intimidate, deter or defeat rivals

Competition within one sex, with the winner gaining access to the opposite sex

- Organisms, especially makes will compete to establish dominance to secure a territory or member of the opposite sex for mating and breeding
    - Can be in the form of vigorous fighting, courtship display and sperm competition
    - Sperm competition is the competitive process between spermatozoa of two or more different males to fertilize the same egg during sexual reproduction. Competition can occur when females have multiple potential mating partners
- Selects for males that have anatomical and behavioural traits that allows it to get mates
- Results in sexual dimorphism (where the females and the males of the same species look different)
    - This may have evolved due to the advantage that the structures evolved will give in intrasexual conflicts (evolution of horns, antlers and large canine teeth

<u>Inter-sexual selection</u>

Individuals of one sex are choosy in selecting their mates from the other sex - In many species, the female invest heavily in the offspring → need to be picky because a wrong choice for a female is much more detrimental than for a male - Bateman's principle: the sex which invests the most in offspring production will become a limiting resource over which the other sex competes

- Individuals of one sex will advertise themselves and there is a choosing of mates (usually females choosing the males)
- Bateman's principle: males under sexual selection to optimise offspring quantity while female will optimise sperm quality
    - Females can only have limited number of pregnancies in their lifetime, while sperms are cheaper to produce than eggs
- For species in which the males provide care to the offspring, females will choose the male that can provide the best care to the offspring (with a better parent, the offspring number will increase and the quality of growth of offspring will also increase)
- For species in which the males do not provide any care to the offspring, females will choose males with the best territories that will maximise the reproductive success

DO NOT MONETISE MY SHIT

- Females will also get to choose the male that is the healthiest and oldest (better ability to acquire more food and resist parasites and disease)
    - Living for a long time indicates a good genetic makeup, and hence females will prefer to mate with these individuals so that the offspring will receive the good genes (good genes hypothesis)
    - Females will also want their offspring to look attractive, hence they will choose attractive males to increase chances of their offspring finding a mate → positive feedback loop (fisherian runaway hypothesis)
    - Healthy males are also chosen because they are deemed to be disease free, and hence there would be no diseases passed from the males to the females or the offspring during mating
    - Females might also prefer mates with certain handicaps that are detrimental to survival because it shows that their mates are genetically superior such that they are able to survive even with such a handicap → offspring will receive high quality genes (handicap hypothesis)
    - The female sensory system can also be stimulated by certain stimulus (colours, sounds etc), and they will select for males that can stimulate the sensory system (sensory exploitation hypothesis)
- All these would lead to the evolution of a the species to increase the mating chance and quality of offspring
    - Males will evolve such that they can fit into the standards of the female expectation

<u>Sexual dimorphism</u>

Differences between male and female of the same species

- Male is larger in most mammals and some birds, female is usually larger in insects, reptiles
- Female's choice causes more deleterious characteristic development in males - Anagenesis can occur due to sexual selection: changes that occur within a species that continues to interbreed over time
- Sensory exploitation as a result: harmful characteristics evolve in males to attract females
- Guppies with patterns that contrast backgrounds are chosen by females but increases the chances of predation
- Marine iguanas are chosen for size, but they starve to death during El Nino years when food is scarce which favours the smaller lizards

DO NOT MONETISE MY SHIT

LO4(b) Explain, with examples, the evolutionary concepts of adaptive radiation and ring species.

Adaptive Radiation

A small number of ancestral species diversifies rapidly into a larger number of descendant species

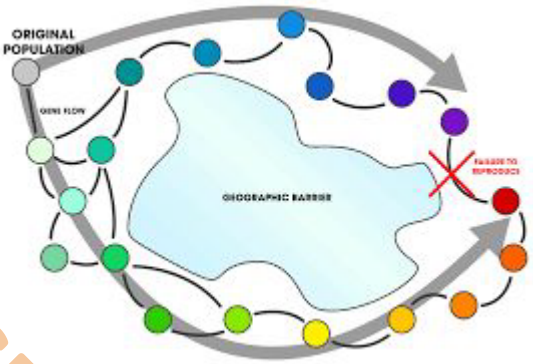
- Initially similar populations become genetically different from one another
- A small number of ancestral species diversifies rapidly into a larger number of descendant species
    - Population disperse into separate geographical location or ecological niches → exposed to unique sets of selective pressure → each subspecies adapt to its unique environment radiating away from other species and eventually become distinct species

- E.g. Adonis lizards, finches on Galapagos

Factors promoting radiation:

- No competitors, available niches
- Extinction of competitors e.g. mammals after dinosaurs
- Environmental change
- New, superior adaptation

Ring Species



Two reproductively isolated populations that live in the same region, connected by a geographic ring of populations that can interbreed

- E.g. Ensatin California salamander
- Descendants moved → new mutations → speciation
- At most points, only the subpopulations can interbreed
- At the meeting point, there are two species and they cannot interbreed
- Connected by a continuous set of intermediate populations

Evidence for evolution

- Common ancestor diverges gradually to generate 2 species

Extremes exist side by side, observable in nature

- Variation can be extensive enough to produce a new species

<u>Problems</u>

- In the real world, may not be a simple ring
- May not be perfectly continuous
- May not have hybridisation and interbreeding at all sites
- May not have extreme species in exactly the same region

- Does not fit comfortably with the standard definition of a species due to
    - There is a continuous gene flow between neighbouring subspecies comprising of a single gene pool
    - Even though the place where the 2 ends of a species population overlap, there is no gene flow between them, and are unable to interbreed feely

DO NOT MONETISE MY SHIT

LO4(c): Discuss the contributions of polyploidy, hybridisation and introgression in evolution and their implications for reconstructing phylogenies.

Polyploidy

Refers to the nondisjunction during cell division (either meiosis or mitosis), resulting in an extra set of chromosomes

Arise by:

- Meiotic or mitotic failures, and fusion of unreduced (2n) gametes
- Mutation of whole set of genomes (rare)

Fertility

- Odd number of chromosome sets → sterile
- Have an unpaired chromosome of each type → cannot produce viable gametes
- Even number of chromosome sets → fertile
- Can produce balanced gametes if multiple sets of chromosomes pair during meiosis
- E.g. plains viscacha rat: first known tetraploid mammal with 2n=102, possibly due to errors in mitosis or meiosis within ancestor's reproductive organs - Relatively common in plants (30-70% of angiosperms)

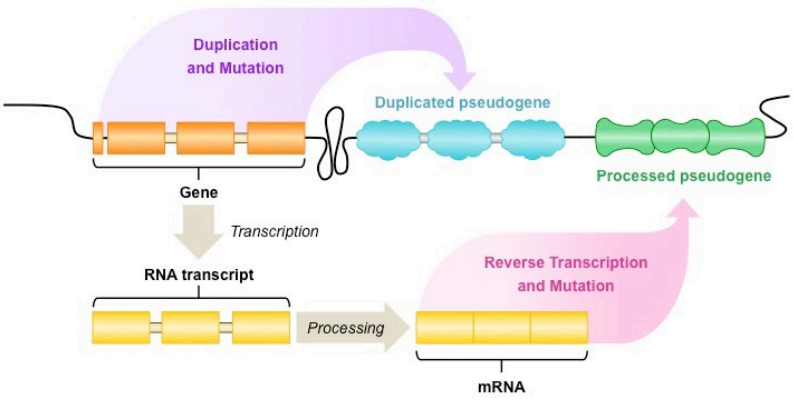
- Autopolyploid : more than 2 chromosome sets that are all derived from a single species → resulting in a tetraploid
    a. The tetraploid can produce fertile and viable offspring via self-pollination or mating with other tetraploids
    b. Generate reproductive isolation without any geographic separation
- Allopolyploid
    - 2 different species interbreed and produce a hybrid offspring
    - Most of them are sterile because there is no pairing of homologous chromosome during prophase 1 of meiosis as the chromosomes are non-homologous
    - Hybrid may be able to propagate itself asexually

DO NOT MONETISE MY SHIT

<table>
<tr><th>Advantages in plants</th><th>Disadvantages in plants</th></tr>
<tr><td rowspan="2">Hybrid vigour:
- Shielded from deleterious effects of recessive mutations
- Protective effect amplified when small, isolated populations inbreed - Greater variation if environmental conditions change</td><td>Double space occupied by nucleus - may disrupt interactions</td></tr>
<tr><td rowspan="2">May interfere with mitosis and meiosis: prone to aneuploidy</td></tr>
<tr><td>Asexual reproduction: can reproduce in the absence of mates</td><td rowspan="2">May change gene expression, epigenetic regulation (epigenetic instability)</td></tr>
</table>

Implications of polyploidy

- Modification of the polyploidy genome
    - Gene silencing : because increase in gene number would result in a uniform increase in gene expression, hence there is gene silencing,, but there is also dosage effects that amplify the expression level



    - Formation of pseudogenes : inheritable genetic elements that are similar to functional genes but are nonfunctional as they do not encode for proteins (resulting in lower gene expression)
    - Divergence of duplicated genes: showing asymmetric evolution in amino acid sequence → different proteins being synthesized and as a result can perform more functions

Evolutionary potential:

- Altered characters in polyploid such as drought tolerance of pathogen resistance → selective advantage
- Decreased gene flow between polyploid and old parental species → reproductive isolation → speciation

DO NOT MONETISE MY SHIT

Hybridisation

Species with incomplete reproductive barriers come into contact with one another, and when the members of the different species meet and mate, it produces offspring of mixed ancestry

- Genetic hybrid: two different alleles of same gene
- Structural hybrid: fusion of gametes where at least 1 has mutations - Intraspecific hybrid: from different subspecies
- Interspecific hybrid: from different species

Occurrence

- Many in nature and agriculture e.g. bananas, coffee, peanuts, dahlias, roses, wheat - Occurs in hybrid zones (places where habitats overlap)
- Factors that promote hybridisation: habitat modification to bring species into same geographical area - can be natural or artificial

Fertility

- Usually sterile due to different number of chromosomes
- Differences in chromosome structure prevent pairing and segregation during meiosis → no viable gametes
- Other barriers e.g. morphological, physiological

Fitness

- Can be lower than either parent, intermediate, or higher (hybrid vigour, common in plants)

Hybrid zones over time

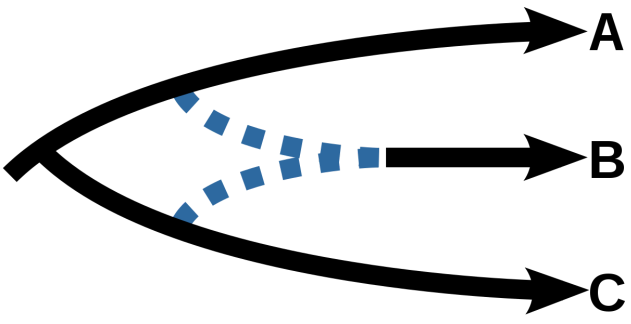
- Reinforcement of reproductive barriers
    - Since hybrids are less fit than members of the parent species, natural selection taking place will strengthen the prezygotic barriers and this reduce the formation of unfit hybrid individuals
- Weakening of reproductive barriers through fusion of species
    - When there is a high level of gene flow, the reproductive barriers will be extremely weakened and this reverses the speciation process
    - The hybridizing species will fuse into a single species
- Continued formation of hybrid individuals
    - Could be due to the fact that hybrids are able to survive and reproduce better than members of either parent species
    - However there are also cases where the hybrids are selected against, and yet they continue to form which is an unexpected result

DO NOT MONETISE MY SHIT

Reticulate evolution

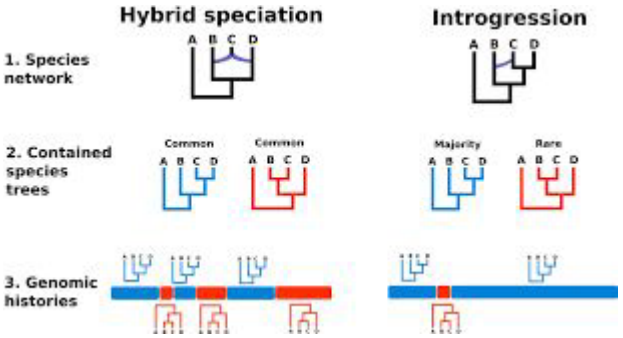
Process which genomes from different species fuse to generate a new hybrid species lineage

- Could result in the transfer of genes coding for adaptive traits
- The 3 hybrid species produced will inhabit extreme environments relative to the parental species



Introgression

Gene flow from parental species to hybrid population



- Repeated backcrossing of a hybrid to the parent
- Does not produce an independent lineage unlike hybridization events

<u>Outcome</u>

- Depends on degree of interfertility with parents
- Often, they interbreed to some extent for a number of generations - Hybrid population builds up a complex mixture of genes from two parental species - Eventually, hybrid population becomes reproductively isolated → speciation

Implications of Polyploidy, Hybridisation, Introgression

- Too many variants
- Difficult to confirm classification
- Spontaneous DNA mutations
- Genetic variations do not occur evenly along gene or chromosome - Taxa which hybridise may become combined into a single species - Difficult to confirm classification
- Hybrids between a protected species and non-protected relative
    - Difficult to confirm protection status
    - Finding a mate
    - Polyploids may be infertile
- New species becomes a pest
    - *Anopheles gambiae* complex: several mosquito populations
- Could help save species suffering from genetic bottlenecks

DO NOT MONETISE MY SHIT

LO4(d): Explain the significance to living organisms of biomolecules, including carbohydrates, lipids, proteins and nucleic acids, and the biochemical processes through which they are synthesised.

DNA

- Made up of 4 nucleotides: ATGC
- Nucleotide analogues: ddNTP, AZT
- Natural modified nucleotide bases: adenine can be deaminated to hypoxanthine which is similar in structure to guanine which leads to errors in DNA replication - Natural non-canonical bases: inosine, thiouridine, queuosine, wyosine Organisation of DNA
- Linear in eukaryotes vs circular in prokaryotes

Advantage: easier for transcription and replication of large genome, less torsional strain Disadvantage: end replication problem (overcome by telomerase) - Gene expression in eukaryotes involves post-transcriptional, post-translational - Operons in prokaryotes

- Non-coding DNA and introns in eukaryotes
- Introns allow for alternative splicing to produce multiple gene products from one gene
- Introns protect highly expressed eukaryotic genes from genetic mutation - R-loop structures are genotoxic: introns reduce R-loop occurrence Diploidy and Meiosis
- Sexual reproduction
- Genetic variation
- Raw material for natural selection
- Homologous recombination allows DNA repair

RNA

- 3-base codons, 20 amino acids
- Variations to genetic code exist
- Non-standard amino acids substituted for standard stop codons
- Acetohalobium arabaticum can expand its genetic code from 20 to 21 amino acids under different conditions of growth
- CUG codes for serine rather than leucine in yeast
- GUG and UUG are start codons in yeast
- Variations occur in some eukaryotic mitochondrial DNA, plastid DNA, ciliate nuclear DNA

Proteins

- Proteins shared by many organisms
- Cytochrome proteins in organisms that undergo cellular respiration - Histone proteins in all eukaryotes
- Polymerases
- Ribosomes

Carbohydrates

- Cellulose in plants
- Chitin in fungi
- Peptidoglycan in bacteria
- Chemoautotrophs use CO_2 as a carbon source with inorganic energy sources like hydrogen sulphide, ferrous iron and ammonia e.g. bacteria living in deep sea vents

DO NOT MONETISE MY SHIT

DNA vs RNA:

- DNA acts as a molecule that stores information in a double helix (which is more stable compared to single stranded RNA) → prevent degradation from exonucleases since it is being protected
- RNA can also act as a molecule that stores information
- RNA direct the synthesis of a protein with a specific sequence of amino acids using the sequence of bases found on itself (look at h2 : oceg + protein synthesis)
- DNA is thought to have evolved from RNA\

Features + roles of RNA:

- Short, single stranded molecule
- Can fold upon itself
- Many functions (info storage, ribozymes etc)

mRNA:

- Base sequence complementary to the non-template strand of DNA, synthesized via transcription of DNA (RNA polymerase…)
- In eukaryotes, the mrna is monocistronic, in prokaryotes the mrna is polycistronic (due to multiple start and stop codons,, trp,lac operon)
- Sequence of mrna determines the primary structure of the protein (type number sequence of the amino acid in the polypeptide chain, which in turn determines the folding and eventually the tertiary/quaternary structure of the protein)

tRNA:

- Mobile carrier of amino acid (during amino acid activation, the tRNA will be attached to an amino acid via a covalent ester bond -- look at h2 : protein synthesis)
- Anticodon of trna complementary to the codon of the mria, during translation trna assist in converting triplet base sequence of the mrna into specific amino acid sequence

rRNA:

- Comes together with large and small ribosomal subunit to form a ribosome
    - Brings the trna and mrna in close proximity to synthesize polypeptide chains
- Have peptidyl transferase (specifically the rrna in the large ribosomal unit), catalyse the formation of peptide bond between 2 amino acid during translation

DO NOT MONETISE MY SHIT

Features + roles of DNA:

- Large molecule, can store genetic information and an be compacted (pre translational modifications in oceg) to be protected, fit into the nucleus, control gene expression
- Double helix, relatively stable due to the h bonds formed between the bases
- Have template strands that can be used to synthesize a complementary set of nucleotides (semi conservative replication)

RNA is superior in the past:

- rna is a simpler molecule compared to dna hence it is preferred the origin of life began in a primordial soup (aqueous solution of organic molecules),, easier for rna to evolve from the soup
    - Given that rna is shorter single stranded and self replicating (it is enough to carry life, hence it is significant)
    - Many functions can be performed too such as catalytic activity and info storage (which is something dna cannot do, and since the functions are essential for life to carry on, rna would be the most suitable molecule to be evolved first)
- But rna also has its cons, it is not stable, hence dna is thought to be evolved from rna as a means of preserving genetic information and protecting it from exonucleases and other derogatory activities.(look at central dogma of the molecular biology)

RNA has multiple functions:

- In the past simple cells would need a molecule that can:
    - Store information
    - Store instructions to form a polypeptide or replicate the information
- Hence rna, functioning as both genes and enzymes can perform autocatalysis
    - Due to the single stranded molecule that can fold nto highly elaborate structures with a specific 3d conformation (from complementary base pairing)

DO NOT MONETISE MY SHIT

LO4(e): Discuss the contributions of mitochondrial DNA and the Y-chromosomal Adam (the Genographic Project) to trace and support the ancestry and diaspora of humans.

Search for the human ancestor

- Both genders pass along one unchanged database during sexual reproduction
- Women: mitochondrial DNA
- Men: Y chromosome

Mitochondrial DNA (mtDNA)

- 20kb, fewer than 40 genes
- Introns absent
- Nuclear genes needed to maintain mitochondrial chromosome function - Circular, multiple copies

Reasons for choosing mtDNA

- Only comes from mother, does not change very much from generation to generation - No crossing over like autosomes
- Sperm mitochondrial DNA is discarded after fertilisation
- Mutations do occur but not very often (<1 per 100 people)

How to compare mtDNA sequences

1. Reversing mutated DNA segments to ancestral condition
    - Original or ancestral DNA sequence is likely determined by comparing human DNA sequences with those of a closely related species
2. Compare mtDNA from different populations
    - The greater the sequence similarity, the more closely related they are - Can try to find how closely related all human beings were in the past Where to study within mitochondria
    - Hypervariable region
    - Mutations are likely and rapid enough to establish a molecular clock - Mutations can be grouped as haplogroups, which tend to be geographically restricted → can be used to genetically distinguish populations
    - Coding region
    - Nucleotide differences especially in single nucleotide polymorphisms and short tandem repeats

Difficulties of mitochondrial ancestry studies

- Difficult to pinpoint to one single ancestral point of origin
- Lack of DNA data
- Lack of human populations
- Difficult to extract DNA

Findings

- Support Recent African Origin model: most likely from East Africa - Humans who lived elsewhere at the time were completely replaced by her descendants, modern humans migrating out of Africa
- Mitochondrial Eve was not the only woman alive at the time, just the only one who can trace descent to everyone alive now
- Other women either left no living descendants or are only related to a smaller subset of people alive today
- Identity of Eve can change, earlier Eves can also be defined accordingly e.g. one who is an ancestor to both modern humans and Neanderthals

DO NOT MONETISE MY SHIT

Y-Chromosomal Adam

Why study Y chromosome

- During crossing over, Y chromosome does not recombine with X chromosome - Transferred intact from father to son
- Allows geneticists to trace patrilineal descent
- However, can recombine with X chromosome at tips of Y chromosome - New mutation about once every 125 years

Problems of studying Y chromosome for evolution

- Low mutation rate
- 60 million bp, lower mutation rate than mitochondria with 40kb
- Slows down identification of polymorphisms

Where to study in Y chromosome

- Single nucleotide polymorphisms
- But not as common as short tandem repeats
- Short tandem repeats
- Suitable because they exhibit different numbers of repeats, thus differ in length of DNA sequence at this location

Findings

- Y chromosome is shared by all men currently alive
- Lived between 160,000 and 300,000 years ago
- Probably lived in Africa
- Reason for discrepancy in timing between Adam and Eve
    - Samples of men and women were from different ethnic backgrounds - Polygamy reduces the number of males that pass on their Y chromosomes → skewing estimate due to different populations studied

Misconception of Y-chromosomal Adam

- Y-chromosomal Adam and mitochondrial Eve most likely never met - There were many other men and women living at the time
- Human genome comes from thousands of other ancestors

DO NOT MONETISE MY SHIT

LOA(a): Explain why specific (adaptive) and non-specific (innate) immunity can be both mutually exclusive and interdependent in the protection against pathogens.

Innate Immunity

Innate immunity

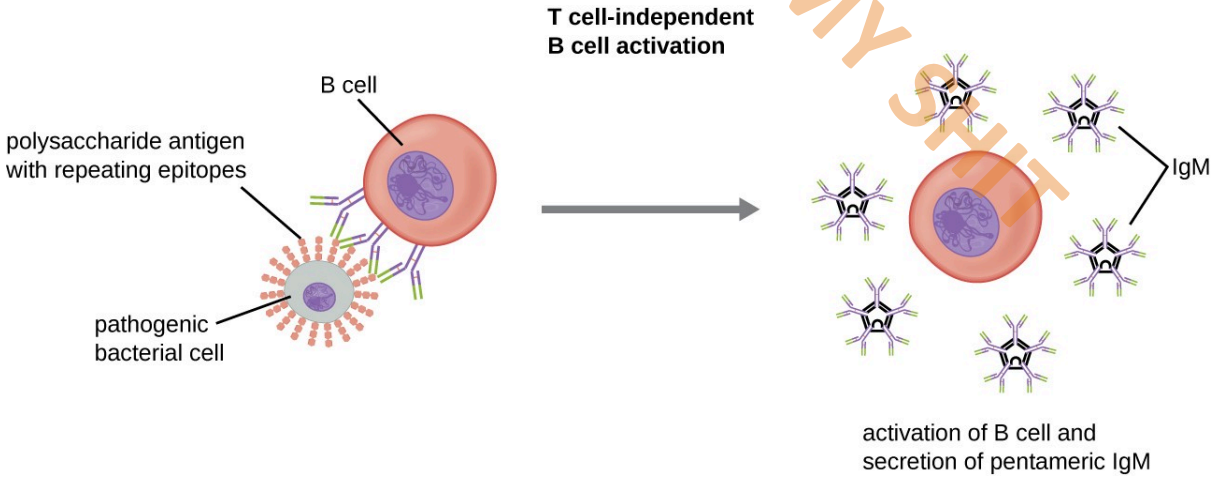
- First response
- Non specific
- Rapid
- Has no immunological memory

Adaptive immunity

- Antigen dependent and antigen specific
- Immunological memory
- Slow on first exposure, more rapid and efficient response on subsequent responses to the same antigen

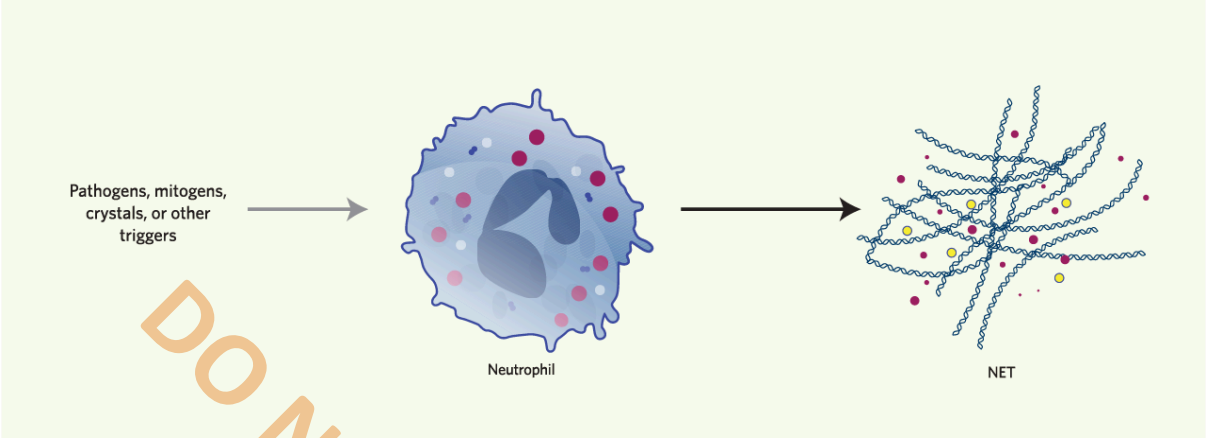
Innate and adaptive being mutually exclusive (2 events that cannot happen simultaneously) because :

- The cells involved in innate and adaptive are distinct and separate
    - B and t lymphocytes (in adaptive imminity) are not involved in the innate immunity (cytotoxic, helper, plasma)
    - Cells of the innate immune system perform specific yet different functions and there are different mechanisms involved to eradicate pathogens (phagocytosis etc)
- Adaptive immune system can be activated without cells of innate immunity
    - H2 talks about t cell dependent activation
    - However certain antigens can directly activate b cells without need of cytokines from helper t cells → no need for processing and antigen presentation to cd4 t cells (t cell independent activation of b cells)
        - Involves cross linkage of b cell receptors by repetitive nonprotein antigen epitopes → production of IgM by plasma cells, no production of memory b cells



DO NOT MONETISE MY SHIT

- Both types of immunity are capable of eradicating pathogens on their own
    - Innate immune system consists of phagocytes, neutrophils, complement system, dendritic cells etc
        - Neutrophils can undergo phagocytosis AND extruding neutrophil extracellular traps (NETs) that are net like structures composed of chromatin fibres and antimicrobial proteins → immobilise and trap pathogens before killing them



DO NOT MONETISE MY SHIT

- Complement system is a set of soluble plasma proteins → attack extracellular forms of pathogens to destroy them
    - Proteins are proteases that are activated by proteolytic cleavage (enzymatic hydrolysis of a peptide bond in a peptide or protein substrateenzymatic hydrolysis of a peptide bond in a peptide or protein substrate)
    - Complement cascade can be activated on the surface of the pathogen → trigger proteolytic cleavage → activation of complement proteins → associate together to form a membrane attack complex → pore formation that disrupt the membrane of the pathogen → cell lysis and death of the pathogen
    - Complement proteins can also opsonise pathogens that lead to destrictuon via phagocytosis (h2 bio antibody)

DO NOT MONETISE MY SHIT

Classical pathway

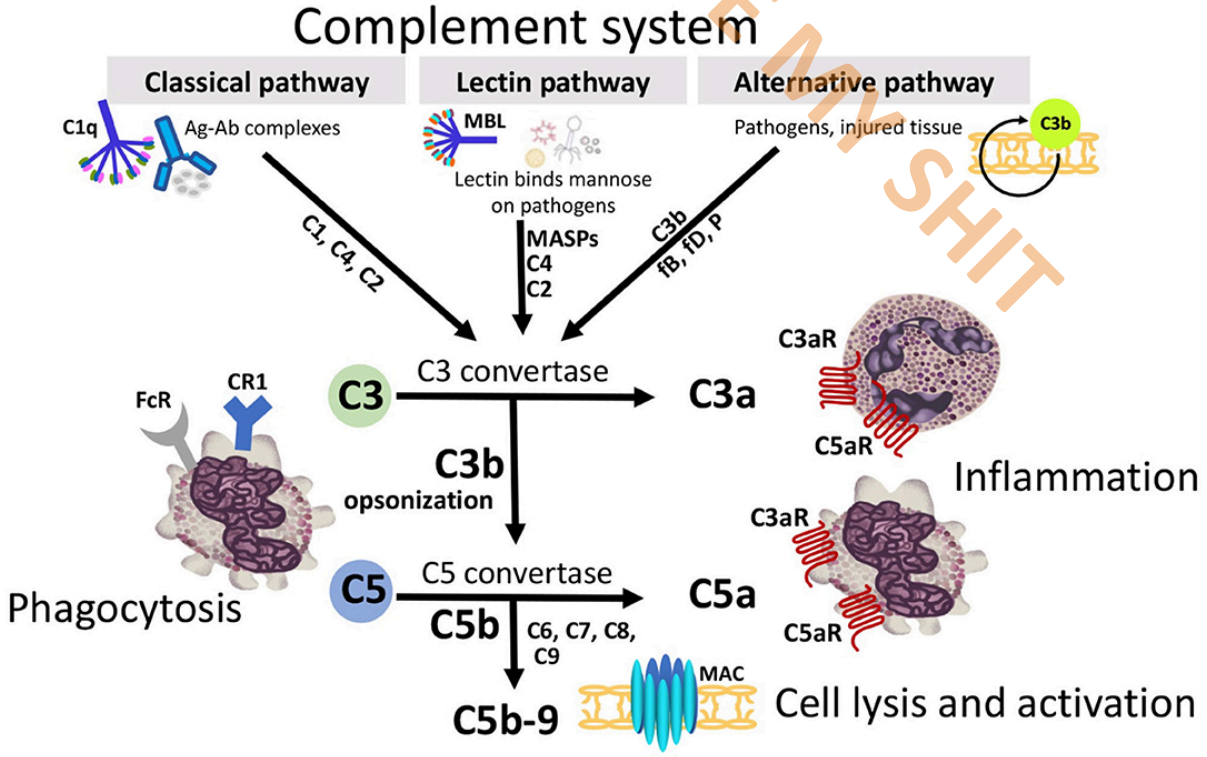
- Involves C1, C2, C4
- Triggered by antibody-antigen complexes binding to C1, which has 3 subcomponents C1q, C1r and C1s
- Pathway forms a C3 convertase, C4b2a, which splits C3 into two fragments

Lectin pathway

- Activated by binding of mannose-binding lectin (MBL) to mannose residues on pathogen surface
- This activates MBL-associated serine proteases, MASP-1 and MASP-2 - MASPs activate C4 and C2 to form the C3 convertase, C4b2a

Alternative pathway

- Involves B, D, H and I
- They interact with each other and with C3b to form a C3 convertase, C3bBb, which activates more C3
- Hence the pathway is sometimes called “the amplification loop”
- Activation of the loop is promoted in the presence of bacterial and fungal cell walls but is inhibited by molecules on the surface of normal mammalian cells

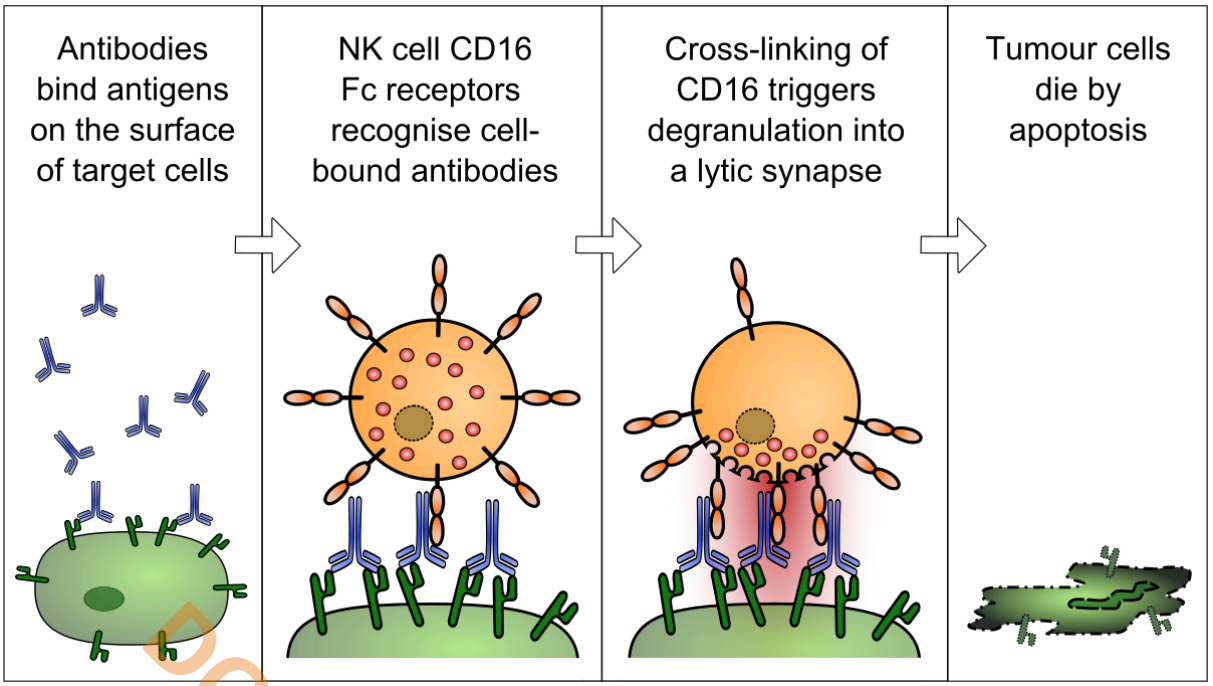


- Natural killer cells are effector lymphocytes → have an action similar to cytotoxic t cell
    - Cytotoxic because they play a major role in killing of tumour cells and viris infected cells
    - Do not need activation unlike cytotoxic t cells → faster in response compared to cytotoxic t cells
    - Ability to recognise non-self cells due to bacteria/tumour cell lacking MHC class 1 (cytotoxic t cells recognise MHC class 1)
        - MHC class 1 present in healthy cells → when NK cell binds to the MHC class 1 of a healthy cell, it acts as a inhibitory receptor and no action is taken
        - When NK cell binds to tumour cell, there is lack of inhibitory receptor → action taken
    - Action involves secreting granules containing perforin and granzymes that induce apoptosis in target cells

Innate and adaptive are interdependent (both systems being dependent on one another)

- Activation of adaptive immunity is dependent on innate immune cells
    - Antigen presentation via antigen presenting cells is necessary for the activation of naive cd4 and cd8 t cells
    - Recruitment and activation of adaptive immune cells requires cytokines and chemokines produced by antigen presenting cells or helper t cells that are activated by apcs
- Innate immunity requires adaptive immune system to enhance the effectiveness of pathogen emoval mechanisms
    - Innate is non-specific → may not effectively remove or kill pathogens → adaptive allows for antigen specific humoral and cell mediated immunity
    - Adaptive allows for immunological memory to mount a faster, stronger and more effective secondary immune response
- Certain adaptive immune response depend on innate immune response to eradicate pathogen completely
    - Innate immune cells are present and readily available → have the role of mounting an immediate response to bind to and eradicate pathogens before adaptive immune cells
    - Antibody opsonisation (secreted by plasma cells) → lead to clearance of pathogen with binding and activation of phagocytes → more efficient response
    - Antibody-dependent cellular toxicity → pathogen bound antibodies have to activate NK cells by binding to fc receptors on nk cells

DO NOT MONETISE MY SHIT



DO NOT MONETISE MY SHIT

LOA(b): Explain how immunological self-tolerance ensures that B lymphocytes and T lymphocytes do not normally attack host cells that are functioning correctly.

Self tolerance : a state of unresponsiveness of the immune system to specific antigens → ensure that the immune system of an individual does not mount an immune response against normal tissues of the body → self reactive lymphocytes are identified and deleted by the immune system

Lymphoid and lymphocytes

- Lymphoid organs :
    - Bone marrow
    - Thymus
- Peripheral organs :
    - Lymph nodes
    - Spleen
    - Mucosal lymphoid tissues
- In peripheral lymphoid organs, mature naive lymphocytes (after selection, somatic recombinations) are maintained and its the location where adaptive immune responses are initiated
- Lymphocyte will leave lymphoid tissue and recirculate via lymph and blood and continually entering lymphoid tissues until encounter antigen or when it dies
    - When it meets its antigen → stop recirculating, proliferate, differentiate, effector cells (h2)

General knowledge of central tolerance

- Induce tolerance of lymphocytes to self antigens developing in the thymus and bone marrow by removing self reactive lymphocytes in the organs
    - Self antigens → sustained and high concentration in cells and tissues
    - pathogens/foreign particles → concentration of antigens increasing suddenly, rapidly and exponentially
- Recognituion of self antigen by an immature lymphocyte → negative signal → death of the lymphocyte or inactivation

General knowledge on peripheral tolerance

- Induced in mature lymphocyte repertoire once cells have left the main lymphoid organs → important for antigens encountered outside the thymus and bone marrow
- Ensure that self reactive cells that escape the central tolerance do not cause an autoimmune disease → act as a second checkpoint
    - Can be achieved via inducing apoptosis, anergy or suppression of self reactive b and t cells

Immune privileged sites : sites that are less subject to immune response

- Endothelial cells cause a very tight junction in the blood brain barrier to prevent unnecessary response

Self antigens in those sites are not presented for testing by the central and peripheral tolerance → hence can trigger immune response if blood brain barrier is broken

DO NOT MONETISE MY SHIT

Self tolerance of b lymphocytes

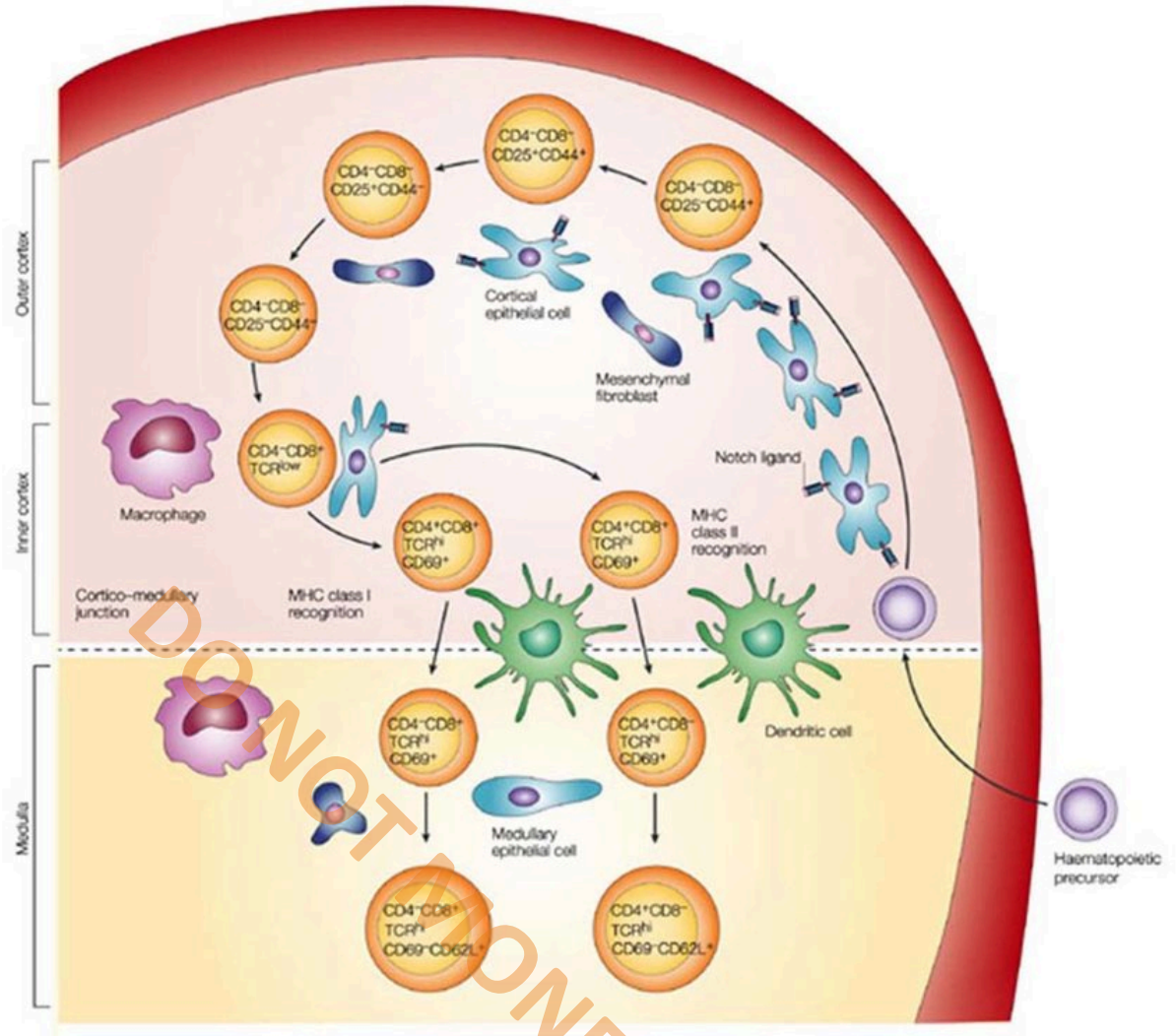
- Central tolerance
  - During development of b cell in bone marrow, once a rearranged light cain (achieved via somatic recombination) has paired witha heavy chain → IgM is expressed on the cell surface → preB cell becomes an immature b cell
  - If self reactive (ie strong cross linking/high affinity binding to antigen in the bone marrow that can lead to a strong response) :
    - Cell death by apoptosis resulting in clonal deletion (if there is strong binding to the self antigens)
    - Receptor editing to produce a new receptor → further gene rearrangements that replace the autoreactive receptor(weaker binding to the self antigens)
      - Light chain gene rearrangement → deleting the self reactive light chain gene and replacing with another sequence until
      - Not autoreactive → continue normal development
      - Autoreactive → rearrangement continues until non autoreactive receptors OR light chain V and J genes are exhausted → apoptosis and die :(
  - If no strong reactivity to self antigens :
    - Allowed to mature, leave the marrow and are carried to the venous blood supply to the spleen
- Peripheral tolerance
  - Mainly for self reactive b cells that escape autoreactivity test in central lymphoid organs and proceed to mature
  - Self reactive cells detected can either
    - Induction of permanent state of unresponsiveness, anergy to antigen → weaker cross linking to self antigens
      - Anergic b cells can migrate to peripheral lymphoid organs but cannot be activated even with help from antigen specific t cells → die quickly due to lack of surviving signals from t cells
    - State of immunological ignorance → not a permanent measure, the self reactive b cell may still respond under certain circumstances and usually this is deployed when antigen concentration is low
      - Have affinity for a self antigen but do not sense and respond to it (can be low conc or weak affinity)
      - Ignorant b cells are held in check by lack of t cell help
      - Can still be activated during certain conditions (inflammation) or when there is a unusually high conc of self antigens
- Central and peripheral tolerance are not perfect
  - Significance : balance within the immune system between purging all self reactivity and maintain ability to respond to pathogens
    - If elimination of self reactive cells is too efficient → receptor repertoire is limited, cannot recognise a wide variety of pathogens (b cells that bind weakly to self antigen may bind strongly to a pathogen)
    - B cells with potential self reactivity are kept in check by the absence of t helper cells

DO NOT MONETISE MY SHIT

Self tolerance of t lymphocytes

- Central tolerance
    - When progenitor cells (cells that has the tendency to differentiate, not yet differentiated) first enter the thymus from the bond marrow → lack surface molecules (receptors) characteristic of mature t cells and their receptor genes are not yet rearranged
    - Interactions with the thymic stroma → initial phase of differentiation followed by cell proliferation → expression of the first cell surface molecules specifi of t cells
        - At the end of the phase, the thymocytes bear distinctive markers of the t cell lineage BUT do not express the cell surface markers that define mature t cells (cd3 t cell receptor complex and co receptors of cd4/cd8) → known as double negative thymocytes due to the absence of cd4 and cd8 → then develop into immature positive thymocytes
    - Positive selection :
        - Occur in the thymic cortical epithelial cells that express both MHC class 2 and MHC class 1 molecules on their surface (within the cortical stroma of the thymus)
        - Selects for t cells that binds to the MHC
            - Cells that are not selected for : when their receptors cannot recognise self-peptide: MHC molecular complexes and they die in the thymus
            - Cells that are selected for : double positive cells that recognise self peptide MHC complexes → mature, and express high levels of t cell receptor → mature and lose one of the coreceptor and becoming either cd4 or cd8
    - Negative selection
        - After positive selection, newly matured single positive t cells migrate from the cortex to the medulla → undergo negative selection
        - Medulla have APCs such as dendritic cells that express co stimulatory molecules, specialised medullary epithelial cells present peripheral antigens
            - T cells whose receptors recognise self peptide MHC complexes too strongly → apoptosis and die
        - Negative selection involves interactions with ubiquitous self antigens and tissue restricted self antigens → tissue specific proteins are expressed by certain stromal cells in the thymic medulla → genative selection can apply even to proteins that are outside the thymus
            - Controlled by autoimmune regulator gene, expressed in medullary stromal cells
    - Affinity hypothesis
        - Low affinity interactions between t cell receptors and self peptide MHC complexes on cortical epithelial cells (positive selection) rescue the cell from death from neglect → allowed to mature
        - High affinity interactions between t cell receptors and self peptide MHC complexes induce apoptosis → negative selection and they die
        - 2% of cells survive dual screening
        - Intermediate affinity binding → become a regulatory t cell → immunosuppressive and suppress or downregulate induction and proliferation of effector t cells

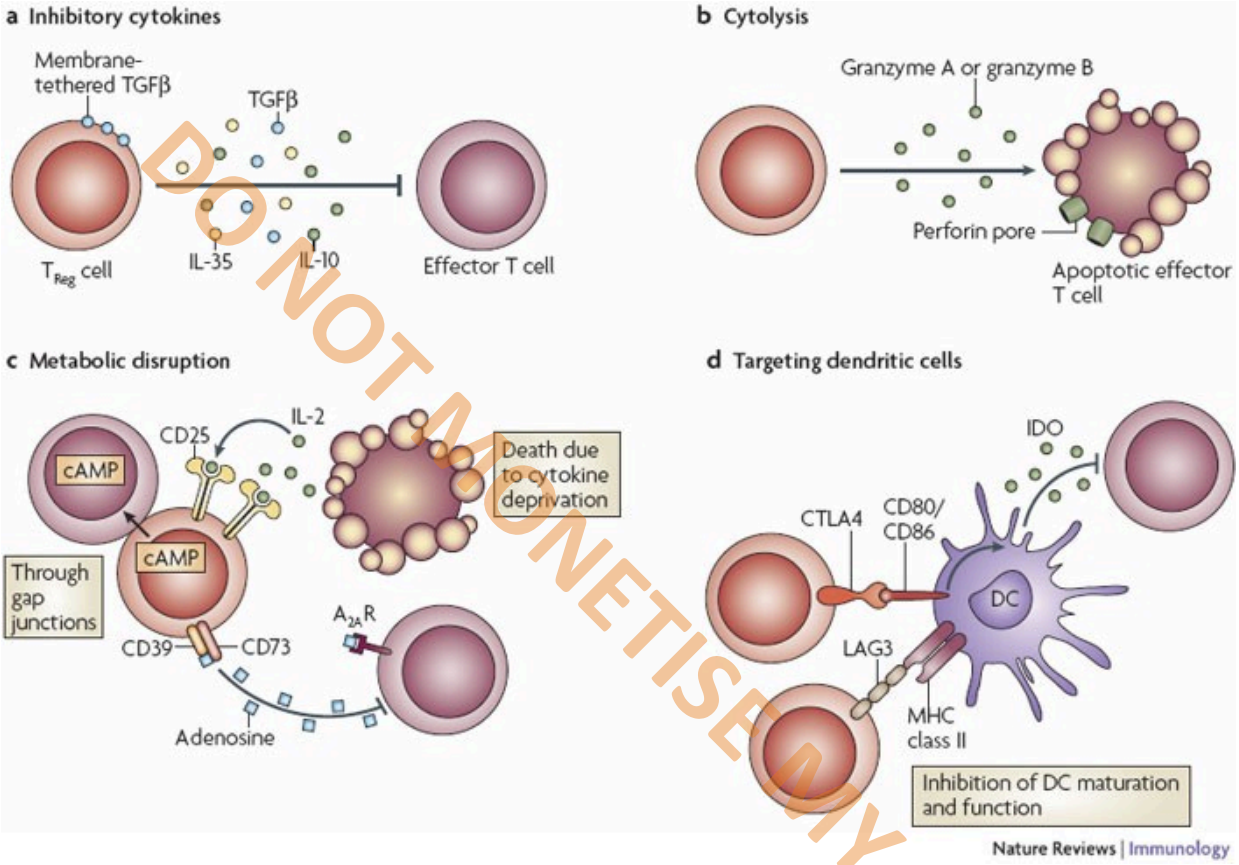
DO NOT MONETISE MY SHIT



DO NOT MONETISE MY SHIT

Regulatory t cells

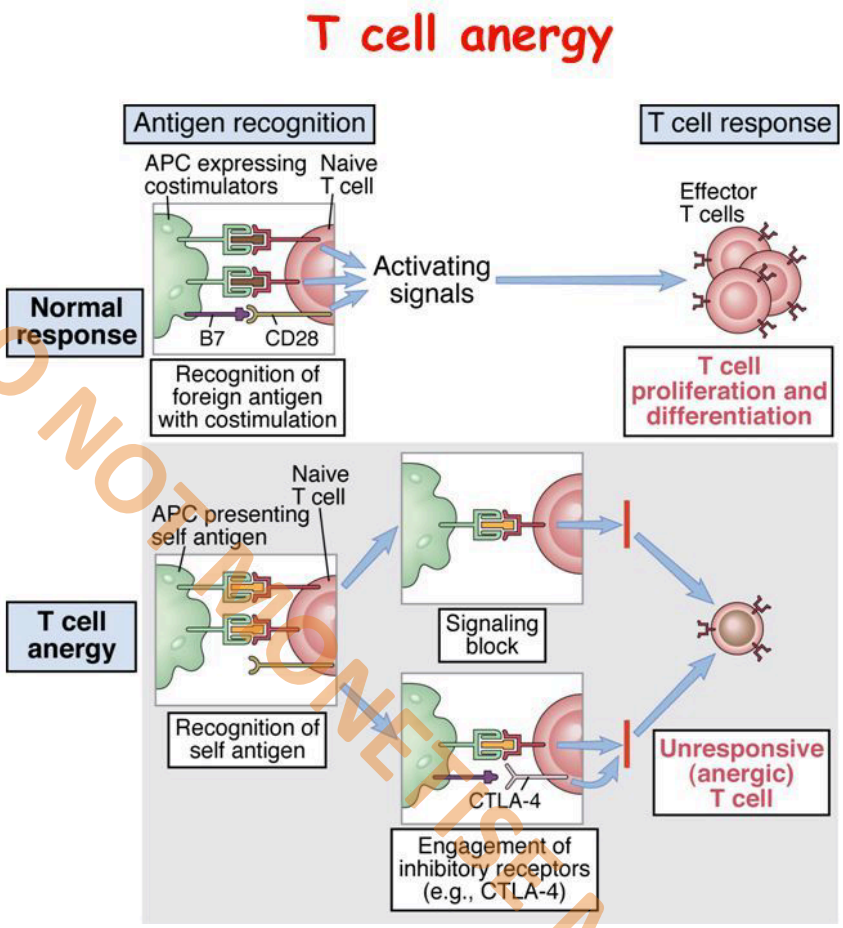
- Intermediate affinities
- Suppression mechanisms
    - Bind to costimulatory receptors on the surface of APC that are needed to bind to naive t cells → decreasing APC activity and prevent activation of naive t cells as signals from APC to naive cells are weak
    - Secretion of inhibitory cytokines such as IL10 and TGFbeta → suppresses the activity of effector cells and APC
    - Direct cytotoxic effect through production of perforin and granzyme and induction of apoptosis in effector t cells
    - Metabolic disruption by competing with effetctor t cells for cytokines (IL2) which is responsible for proliferation of effector t cells



DO NOT MONETISE MY SHIT

Peripheral tolerance

- Since not all self antigens are expressed in central lymphoid organs → newly emigrated self reactive lymphocytes that encounter their self antigen for the first time must be eliminated or inactivated
- Can be
    - Eliminated (clonal deletion) → occurs after brief period of activation and cell division (activation induced cell death)
    - Rendered anergic



DO NOT MONETISE MY SHIT

Immune system necessary to ensure survival of all organisms

- In multicellular organisms → specialised immune cells that are capable of recognition, phagocytosis and killing of foreign cells and removing their own altered and nonfunctional cells caused by cancer, infection, damage and senescence
- There are also humoral factors such as complement cascade (that enhance the ability of antibodies and phagocytotic cells to clear microbes and damaged cells from all organisms) → cooperate with cellular immunity in fighting infection and maintaining homeostasis
- Adaptive immunity → diversification of immune receptors and on immunological memory in each individual

Immune system not necessary to ensure survival of all organisms

- Unicellular organisms have rich arsenals of mechanisms such as
    - Restriction endomucleases
    - Antimicrobial peptides
    - RNA interferences

LOA(c): Explain why the human microbiota is important for our health.

microbiota

- Microorganisms can form commensal, mutualistic, parasitic or pathogeni relationships with their hosts → some good some not good
- Microbiota → microbial community in the host
    - Considered an essential organ → carrying approximately 150x more genes compared to the human genome
- Benefits of human microbiota
    - Increase in nutrient acquisition
        - Has potential to increase energy extraction from food, increase nutrient harvest and alter appetite signalling
        - Provide humans with unique and specific enzymes and biochemical pathways
        - Help in metabolism of undigested carbohydrates and the biosynthesis of vitamins
        - Eg : digestion of certain foods that cannot be digested by the stomach and small intestine → specific bacteroides can digest xyloglucans (dietary fibres that are found in vegetables)
        - Eg : can also digest non-digestible fibres such as fructooligosaccharides and oligosaccharides → lactobacillus and bifidobacterium
        - Eg : Produce vitamin K, vitamin B(B2 and B12) i.e. Escherichia coli
        - Eg : Synthesize essential and nonessential amino acids i.e. Clostridia, Proteobacteria, and Bacillus-Lactobacillus-Streptococcus group
        - Eg : Produce secondary bile acids i.e. Clostridia
        - Eg : Digest the healthy sugars in breast milk that are important for babies' growth
- Protection against pathogenic infections
    - Provides a physical barrier → protecting host against foreign pathogens through competitive exclusion and the production of antimicrobial substances
    - Fully colonising the space and compete and adhere to attachment sites on intestinal epithelial
    - Compete for available nutrients
    - Production of antimicrobial substances (e.g. bacteriocins) Development of intestinal mucosa - As the gut flora gets established, the intestinal epithelium and the intestinal mucosal barrier that it secretes – develop as well, in a way that is tolerant to, and even supportive of, commensalistic microorganisms and also provides a barrier to pathogenic ones
    - Microbial imbalance → changes in microbiota → dysregulation of host homeostasis and increased susceptibility to infectious diseases as growth of opportunistic pathogens may occur

DO NOT MONETISE MY SHIT

- Maturation of the immune system of the host
    - In critical window of life, colonisation of mammalian host mucosal surface → maturation of immune system of the host
    - Pattern recognition receptors (PRRs) and toll like receptors (TLRs) recognise microbial associated antigens during infection → elicit a protective immune response
        - But the ligands for the PRR are not exclusive to pathogens and are produced by commensal microbiota
    - Influences the development and maturation of immune cells and the lymphoid tissue
    - Intestinal commensal microorganisms also provide signals that foster normal immune system development and influence the ensuing immune responses. Example gut microbiota interacts with the immune system, providing signals to promote the maturation of regulatory antigen-presenting cells and regulatory T cells (Tregs), which play a crucial role in the development of immunological tolerance
    - Gut microbiota produce pathogen-associated molecular patterns (PAMPs) and metabolic by-products such as short-chain fatty acids (SCFAs) SCFAs stimulate a rapid increase in the production of innate immune cells like neutrophils, basophils and eosinophils.
    - Gut flora can also regulate the production of antibodies by the immune system. One function of this regulation is to cause B cells to class switch to IgA.
    - Bifidobacteria may also help control the immune system and help the gut wall stay intact in infants, which can help prevent infections
    - Development of a gut microbiota in infancy are closely related to the development of the immune system – affecting one's food allergy
- Gut brain axis
    - The gut-brain axis is a set of communication pathways between the gut and brain occurring largely through the actions of the gut microbiome. Gut flora also release molecules that can directly activate the vagus nerve
    - Microbes can shape our food choices to ensure their own survival. Some metabolites, the small by products of microbial digestion, can make us feel hungry, full or crave certain foods.
    - The gut flora can produce a range of neuroactive molecules, such as acetylcholine, histamine, melatonin, and serotonin, which are essential for regulating peristalsis and sensation in the gut.
    - Serotonin is an antidepressant neurotransmitter
    - Gut microbiota dysbiosis may increase the translocation of gut bacteria provoking an immune response that can lead to the release of inflammatory cytokines and the activation of the vagus nerve and spinal afferent neurons Autism spectrum disorder (ASD) correlated with an altered gut microbiota
    - A number of studies have shown that people with various psychological disorders have different species of bacteria in their guts, compared to healthy people. This suggests that the gut microbiome may affect brain health

DO NOT MONETISE MY SHIT

Hygiene hypothesis

- Increased exposure to microbes (aka less hygienic circumstances) confer
    - Immunity to those pathogenic microbes
    - Less immune dysregulation
- Right amount of exposure to germs build body's library of known disease → teach immune system to remembe harmful microbes

Dysbiosis

- Definition - Microbial imbalance or maladaptation on or inside the body disrupts the symbiotic relationship between host and microbes
- loss of biodiversity in microbiota due to water sanitation, cesarean sections, use of antibiotics in preterm newborns, increased hygiene etc
- dysregulation of the gut flora has been correlated with a host of inflammatory and autoimmune conditions

Proposed mechanism how dysbiosis leads to disease:

- Overgrowth of pathogenic bacteria + inhibition of protective bacteria
- Translocation of bacteria and bacteria products
- Immune activation and pro inflammatory cytokine production
- Chronic infection leads to tissue destruction and complications

DO NOT MONETISE MY SHIT

Infectious disease as a result of dysbiosis

Clostridium difficile infection

- Disruption of the gut flora allows competing organisms like Clostridium difficile to become established
- Symptoms:
  - Diarrhea, abdominal pain, fever
- Due to overgrowth of the bacteria Clostridium difficile CDI develops due to:
  - Antibiotics administered → disturb intestinal mucosa homeostasis
  - Fecal bacterial biodiversity reduced
  - Decrease resistance against toxin-producing C. difficile → progression of CDI
  - Other example: Irritable bowel syndrome with details

Obesity

- Gut microbiota plays a part in energy harvest, storage and expenditure
- Obesity, which is associated with gut microbiota dysbiosis and altered metabolic pathways
- Germ-free (GF) mice are protected against obesity. Transfer of gut microbes from obese mice to GF mice results in dramatic increase in body fat content and insulin resistance.
- Composition of gut microbiota is shown to differ in lean and obese individuals.
- Mechanisms on how the gut microbiota could contribute to obesity and metabolic diseases include:
  - improved energy extraction from diet by the conversion of dietary fibre to SCFA
  - increased intestinal permeability for bacterial lipopolysaccharides (LPS) in response to the consumption of high-fat diets resulting in an elevated systemic LPS level and low-grade inflammation.

Allergies, asthma, and diabetes mellitus

- Colonization of intestines with bacteria and the development of a gut microbiota in infancy are closely related to the development of the immune system
- Individuals with food allergy have different gut microbiomes compared to healthy control subjects.
- Imbalances in the gut microbial ecosystem precede the development of food allergy. The “hygiene hypothesis,” which states that less exposure to parasites and microbes leads to an overactive immune system.
- SCFAs are involved in immune signalling that prevents the triggering of asthma. Babies born via c-section have a less diverse gut microbiota than those born vaginally--those babies get microbes from their mother during birth. C-section babies may be missing out on vital bacteria that make them less likely to develop allergies and asthma and later in life have long-term health consequences, increasing your risk for obesity and type 2 diabetes as an adult.
- Allergy, asthma, and diabetes mellitus are autoimmune and inflammatory disorders of unknown cause, but have been linked to imbalances in the gut flora and its relationship with the host

DO NOT MONETISE MY SHIT

Benefits of understanding microbiota

- Health monitoring by analysing blood microbiome
    - Collection of blood plasma → identify icrobiome with the collection of bacteria and its products → assessment of health status
    - Assay that detects and sequences plasma cell free DNA used to monitor for infection and rejection in lung transplant recipients
    - Bifidobacterium (good bactiera)/enterobacteriaceae (bas bacteria) (B/E) ratio indicated the microbial colonisation resistance of the bowel
- Prediction and early diagnosis of diseases
    - When B/E ratio is higher than 1 → healthy microbiome
    - When B/E ratio is far below 1 → patients with cirrhosis and with avian influenza H7N9 infections
- Health intervention by manipulating microbiota
    - Identification of prebiotics (non digestible food that promotes growth of beneficial microorganisms in the intestine such as probiotics), probiotics and synbiotics (mixture of prebiotic and probiotics which selectively promotes growth→ gut health
- Targeting microbiome for treatment of diseases
    - Microbiome based diagnosis and treatment strategies can be implemented → investigation of prevention and treating of diseases by tragetting the microbiome

DO NOT MONETISE MY SHIT

LOA(d): Explain the factors affecting the probability that a pandemic will occur, including sanitation, water supply, food, climate, large-scale movements of people, evolution of new strains of virulent pathogens and development of drug resistance.

- Epidemic → occurrence in a community or region of cases of an illness clearly in excess of normal expectancy
- Pandemic → an epidemic occurring over a wide area, crossing international boundaries and usually affecting a large number of people
    - Identified by their geographic scale rather than the severity of the illness
    - Key features of a pandemic :
        - Wide geographic extension → transregional (2 adjacent regions), interregional (2 non adjacent regions) and global
        - Disease movement → transmission of disease can be traced from place to place, and there is high attack rates, explosive spread
        - Immunity of population, novelty of virus → hen large population is not vaccinated, population is susceptible to the disease
        - Infectiousness → more infectious has multiple means of transmission, and can be contagious from person to person
        - Severity → fatal/non fatal, but moer often pandemi is associated with fatal diseases
- Endemic → global but maintained at a baseline level
    - The illness doesnt die out, but the number of infeted people do not increase exponentially too

List of Notable Pandemics

Name	Time period	Region	Fatalities	Cause
Plague of Athens	430-426 BC	Greece	75-100 thousand	Typhoid
Antonine Plague	165-180	Italy	5 million	Smallpox
Plague of Justinian	541-750	Byzantine	Uncertain (millions)	Bubonic plague
Black Death	1331-1353	Eurasia	75 million	
Third Plague Pandemic	1855-1927	Worldwide	>12 million	
Spanish Influenza	1918-1920	Worldwide	50-100 million	Influenza
HIV/AIDS	1983-	Worldwide	30 million as of 2009	HIV
COVID-19	2019-	Worldwide	8,312 as of 19/3/2020	SARS-CoV-2

Deadly Diseases

- Cholera
- Influenza
- Typhus
- Ebola
- MERS
- Dengue
- Malaria

DO NOT MONETISE MY SHIT

Koch’s Postulates

Koch's postulates are four criteria designed to establish a causative relationship between a microbe and a disease

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms
    a. Exceptions in the case of asymptomatic carriers of cholera and typhoid
2. The microorganism must be isolated from a diseased organism and grown in pure culture
3. The cultured microorganism should cause disease when introduced into a healthy organism
    a. Exceptions in the case of latent-stage tuberculosis
4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent

DO NOT MONETISE MY SHIT

Causes of Pandemics

Factors that escalates an epidemic to a pandemic

- Sanitation
    - Poor sanitation and poor hygiene lead to diarrhoeal diseases
    - Urban solid waste disposal → improper handling increase the proliferation of disease carrying vectors such as rodents and insects
- Water supply
    - Safe reliable affordable and accessible water supply is essential for maintenance of good health
    - Poor water supply → impacts health by causing acute infectious diarrhoea, repeat or chronic diarrhoea episodes and nondiarrhoeal disease that can arise from arsenic and fluoride
    - Lack of water → indirect cause of disease via malnutrition
        - Kenya study : without access to irrigation there is lower energy intace and higher chronic malnutrition in children
    - Affect health by limiting productivity and maintenace of personal hygiene → disease since water enable sanitation facilities to be kept clean
    - Factors that determine whether water supply can maintain good health
        - Quality of the water → free of pathogens and chemical constituents that can give rise to diarrhoeal and nondiarrhoeal disease
        - Quantity of the water → distance of carry involved (when water have to be transported from place to place) and the wealth of the people
        - Access to water → physical barrier or socioeconomic/cultural barriers → some lower socioeconomic classes may be denied access to water sources through cost or culture
- Food
    - Consumption of infected animals cause for the spread of virus to humans
        - Bats, monkeys are carriers of the ebola virus → diet of many people in africa
        - Poultry are carriers of bird flu → human consume them
    - System of marketing live birds and practices of home slaughtering, defeathering and eviscreating create opportunities for extensive human exposure to potentially contaminated parts of poultry → spread of influenza virus
    - Avian influenza survive in contaminated raw poultry meat and can spread through marketing and the distribution of contaminated food products such as fresh or frozen meat

DO NOT MONETISE MY SHIT

- Climate change
    - Climate change and changes in weather can drive some animals and insect vectors carrying viruses to different areas, spreading disease to people
    - Deforestation will reduce the species geographic barrier and species are brought in closer proximity making it easier to spread diseases from species to species
        - El nino in 1992 → higher than average rainfall → more plants and increase in local rodent population and spreading hantavirus to residents
- Large scale movements of people
    - Globalisation
        - As more people populate the planet → greater possibility that someone will encounter a virus that will spread to others → increase risk of transmission that result in the rapid spread of the disease
        - Greater distances travelled → allow for virus to spread more rapidly over greater distances more quickly
            - People are more internationally mobile and are more likely to live in cities that are densely populated
        - Communication levels → increase the panic and potential infected people will attempt to travel to escape the disease and spread the virus with them
    - Older times
        - Spread of the virus beyond port cities was further facilitated by local transport networks, predominately railways
        - Major transformation of global transportation, with ships being replaced by the faster and more widely used air travel
        - Close proximity that facilitated the transmission of virus infection e.g. high population density in military camps, with their high population density
- Evolution of new strains of virulent pathogens
    - Pathogens can evolve via antigenic drift and antigenic shift → allow them to expand their host range or increase their ease of transmission between humans due to the higher affinity of pathogen for host cell receptors
        - If a subtype arise that gain ability to spread between people → pandemic
        - After a while humans will develop some immunity and this will result in epidemics/endemics
- Host susceptibility to the disease (influenza and viral infections)
    - Certain individuals (e.g. young adults) lack prior exposure to influenza strains, no pre-existing humoral immunity contributed to the high attack rate and rapid spread of the virus
    - Mild symptoms upon infection, symptoms are undetected by the infected people and they continue to interact with others and unknowingly transmitted the virus

DO NOT MONETISE MW SHIT

- Development of drug resistance
    - Bacteria can develop resistance against antibiotic treatment → existing drugs are unable to treat and control the spread of the infection
        - TB → multidrug resistant tuberculosis is a prevalent issue
- Knowledge about viruses and pathogens
    - Lack of knowledge about the influenza virus and etiological (cause of disease) agent/conditions
    - Lack of technology to screen viral genome of human and animal virus isolates for the presence of mutations that increase human adaptation and/or virulence
    - Lack of development of effective treatment/drugs to treat symptoms that facilitated the spread of the disease
    - Lack of clinical diagnosis/risk assessment of the infection that prevents early treatment of symptoms
- Vaccines
    - No available vaccines to prevention infection in individuals
    - No herd immunity that can reduce risk of transmission

DO NOT MONETISE MY SHIT

Comparison of pathogens

- Pathogens include bacteria, fungi, prions, protists → can cause diseases in humans etc
- Nature of a pathogen that influence the possibility of a pandemic
    - For viruses → some have high virulency → ease of spreading diseases
        - Antigenic drift due to
            - High replication rate
            - Lack of proofreading mechanism in viral enzyme for replication
            - Single strand genome means there is no dna repair → changes in dna lead to changes in mrna codon and eventually conformation of the antigens and no longer recognised by the immune system → ability to infect humans since it can evaude the immune system of the host
        - Antigenic shift
            - Especially for viruses with multiple gene segments it allows for genetic reassortment that results in a new strain of virus
    - Ease of transmission of the pathogen
        - Prions → unlikely to result in a pandemi due to the need to consume human meat for it to be transmitted
        - For airborne or waterborne diseases such as influenza and tuberculosis → easy to transmit since there are common areas that allow for transmission (shared water/airspaces)
        - However for viruses that spread through sharing of bodily fluids (HIV) it is difficult to transmit since it require sexual intercourse/exchange of bodily fluids and it can be controlled if there are checks and education in place
    - Incubation time/latency (period where the pathogen is asympomatic)
        - Current understanding of pathogen required → diagnostic kits availability, treatment and ease of delivery of treatment
    - Evolution of superbugs for bacteria (a human intervention)
        - Antibiotics and vaccines act as selection pressures → constant use of antibiotics is inevitably selecting for antibiotic resistant bacteria → evolution of antibiotic/drug resistant bacteria strains
        - Especially with horizontal transfer (transformation, conjugation, transduction) → pass on resistance genes
            - Candida auris is a super fungus !
            - Methicillin-resistant Staphylococcus aureus (MRSA) is a group of Gram-positive bacteria that are genetically distinct from other strains of Staphylococcus aureus

DO NOT MONETISE MY SHIT

How to slow the spread of disease

- Vaccination
- Diagnostic kits
- Isolation and quarantine
- Good personal hygiene
- Avoiding crowded spaces

LOB(a) CLT : Discuss how humans are responding to mitigate climate change, including biological measures (such as tree planting and developing drought resistant varieties of crops) and lifestyle changes (such as reducing use of cars and consumption of meat).

Impact of Climate Change

- Physical system: melting at the poles → glacial regression, snow melting, thawing of permafrost, flooding, droughts, coastal erosion, sea level rise, extreme natural Phenomena
- Biological system: death and displacement of flora and fauna
- Human system: destruction of food production, disease, death, loss of livelihood, migration of climate refugees
- Interrelationships: negative consequences interact and increase in magnitude

Mitigation of Climate Change

The process of reducing emissions or enhancing sinks of GHGs

IPCC: Intergovernmental Panel on Climate Change

- Summarise past climate actions, write policy recommendations to mitigate climate Change
- Aim: maintain warming at below 2°C

Possible Mitigation Responses

Decarbonising: reducing the carbon intensity of electricity generation

- Global CO 2 emissions from the energy supply sector in 2040-2070 should decline by ≥90% compared to 2010
- Replacing coal-fired power plants with efficient natural gas combined-cycle power Plants
- Shifting to renewable energy like solar, wind, hydroelectric, tidal, geothermal

Bioenergy:

- Issues: sustainability of practices, efficiency of bioenergy systems

Geoengineering: set of methods and technologies operating on a large scale that aim to deliberately alter the climate system in order to alleviate the impacts of climate change

- CO 2 removal: e.g. sub-sea geologic storage, carbon sinks

Efficiency enhancements and behavioural changes:

- Use energy-efficient appliances, switch off appliances when not in use, running business on renewables, setting thermostat to 24-26°C in summer or 18-20°C in winter, avoid using one-use items or products with excessive packaging, travel on foot/bicycle/public transport, eat less meat, waste less food, purchase locally made products to lower carbon miles
- Information programmes followed by economic instruments, regulatory approaches and voluntary actions
- Waste reduction → reuse → recycling → energy recovery

DO NOT MONETISE MY SHIT

LOB(b) CLT: Discuss, with examples, how animal and plant species can adjust and adapt to climate change, and the possible consequences of climate change for the ecosystem and the organisms within them in the longer term.

Effect of Climate Change on Physiology of Plants and Animals

Plants :

Under drought conditions and/or high temperatures, plants tend to experience water stress, and undergo physiological and morphological changes:

- Reduction of water content and turgor → cell expansion slows down or ceases
- Alteration in level of photosynthetic pigment (reduction or no pigmentation)
- Rapid decrease in the amount of Rubisco → lower amounts of carbon fixation
- Increased ratio of roots to shoots to access more water while decreasing

transpiration rate:

- Number of stomata per leaf decreases to reduce transpiration; stomatal closure

Effects on plants:

- Photosynthetic rates decrease → plant growth retarded → many plants dehydrate and die
- High temperatures reduce pollen viability
- Deviation from optimal temperature disrupts flowering process and affects seed production

Plants native to dry/hot environments have characteristics to reduce loss of water:

- Morphological: small surface area to volume ratio of leaves, thick waxy cuticle and epidermis, stomata only present on bottom surface of leaves, sunken stomata surrounded by hair to reduce transpiration, some plants have leaves that can curl up when it is dry and unfurl when it is wet
- Physiological: close stomata for most of the day → reduces [CO_2] in the cell → Rubisco fixes O_2, competitive inhibitor, instead of CO_2 → rate of photosynthesis decreases
- C4 plants: carbon fixation and Calvin cycle takes place in different cells. CO_2 is converted to a 4C compound by an enzyme that does not bind O_2. The 4C compound is sequestered to a deeper tissue layer where less O_2 is present. CO_2 can then be released and enter the Calvin cycle without competition from O_2
- CAM plants: carbon fixation occurs at night. CO_2 is converted to a 4C compound which releases CO_2 for Calvin cycle in the day. This allows stomata to remain closed during the day

Animals :

Change in body size of animals:

- Body size becomes smaller in response to general warming → larger surface area to volume ratio → efficient heat dissipation

Effect of change in temperature on insect metabolism:

- Body temperature fluctuates with environmental temperature
- Temperature governs metabolism through its effect on rates of enzyme-catalysed **biochemical reactions**
- Rates of somatic growth and ontogenetic development increase with increasing temperature → time to maturity is shorter at high temperatures
- Thermal safety margins (TSM) increase sharply with latitude - small amounts of warming in the tropics will approach near-lethal temperatures, likely decreasing performance, while small amount of warming in temperature regions may enhance fitness as current temperature is below thermal optimum (TSM: difference between organism's thermal optimum and current climate temperature)

DO NOT MONETISE MY SHIT