!!! DISCLAIMER !!!

I took A Levels in 2025, so they were created for the 9816 syllabus. Additionally, this set of notes are **INCOMPLETE** and were personally curated for my own revision, so some topics may be explained in greater detail than others while some sections may be missing or less developed. The explanations are also written in a way that made sense to me, so they may not always match how your school or teacher teaches them.

Please use these notes at your own discretion!! If there are any differences between these notes and your teacher's notes, lectures, or official materials, **listen to them**. Since this was done as a google docs, I have included the link so you can also view the comments and annotations, which may provide additional context. Plus, just a tip, for H3, content is not that important in comparison to your application and evaluative skills, so it's really helpful to work on SBQ/writing practice essays then memorising content!

All the best for your A levels! You got this :D

Contents

 CORE IDEA 1

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THE FLUID MOSAIC MODEL

★ **'fluid':** lipids & proteins can move laterally & some even transversely
★ **'mosaic':** there are proteins randomly embedded in the phospholipid bilayer

DEVELOPMENT OF THE MODEL

TIME	EVIDENCE	CONCLUSION
mid to late 1800s	• cells were viewed as bags of protoplasm – 'protoplasmic theory' • the boundary between cell & env. was thought to be just the outer layer of the cytoplasm ⇒ thought membranes were witchcraft	
Pfeffer (1877)	• studied plant cells and osmotic pressure using semi-permeable membranes • he showed that cell membranes allowed water passage but restricted solute movement	• selective nature of membranes – cell membranes are semi-permeable barriers that regulate exchange between the cell and its environment
Overton (1895)	• found that lipid-soluble molecules entered cells more easily than water-soluble molecules	• suggested that the cell membrane is made of lipid-like substances, likely a thin layer of fats
late 1800s - early 1900s	• starting to accept existence of a thin lipid boundary outside protoplasm • evidence from osmosis & solubility experiments convinced most scientists that the membrane is (1) semi-permeable & (2) have lipophilic character	
Chambers (1922)	• used micro-injection techniques and mechanical manipulation of sea urchin eggs • he showed membranes behaved like physical barriers that could be stretched and deformed	• membranes are physical structures with flexible but resistant properties, not just invisible boundaries
Gorter & Grendel (1925)	• extracted lipids from red blood cells and spread them as a monolayer on a water surface - *langmuir trough experiment* • ⇒ the area covered was twice the surface area of the red blood cells	• proposed that membranes are a lipid bilayer, w hydrophilic heads facing outwards and hydrophobic tails facing inwards
1920s	• G&G's 1925 study gave quantitative evidence of a lipid bilayer ⇒ one of the first structural models of the membrane • but the role of proteins were unclear	

Danielli & Davson (1935)	• proposed a model to explain how membranes could be both thin and selectively permeable • suggested proteins must play a role in stability and permeability	• introduced the 'protein–lipid sandwich' model → a lipid bilayer coated on both sides with protein layers • *explained selective permeability better than a lipid-only model*
1930s - 1950s	• widespread acceptance of D&D's 'protein-lipid sandwich' model • but many viewed the membrane to be 'static' → just coating the cell	
Robertson (1959)	• used electron microscopy with improved staining to visualise membranes • found they appeared as a trilaminar structure ○ *two dark lines separated by a light line*	• proposed the unit membrane hypothesis → all cell membranes share a common trilaminar structure
1960s - 1970s	• w the advent of electron microscopy (1950s), membranes were then visualised as a trilamilar structure • as Robertson saw all membranes were trilaminar → proposed the 'unit membrane model'	
Singer & Nicolson (1972)	• experimental evidence (freeze-fracture electron microscopy, fluidity studies) showed proteins were not fixed layers but dispersed and mobile within the bilayer	• proposed the fluid mosaic model → membranes are a dynamic lipid bilayer with proteins embedded or attached, capable of lateral movement
1970s onwards	• S&N integrated structural, biochemical & mobility evidences ⇒ proposed membranes as fluid lipid bilayer w embedded proteins, moving laterally *(not rigidly coating the surface) - ie. fluid mosaic model*	

PRIONS

★ **DEFINITION:** misfolded, protease-resistant prion proteins that cause infectious neurodegenerative disorders
- ☆ *eg. Creutzfeldt-Jakob Disease (CJD), Kuru, spongiform encephalopathy (mad cow disease)*

★ **CHARACTERISTICS:**
- ☆ **slow-acting** – has a long incubation period
- ☆ **virtually indestructible** – resistant to proteases & not denatured by heating
- ☆ **have some viral attributes but are NOT viruses**
  - ✷ *eg. extremely small size, strain var.*
  - ✷ BUT: resistant to UV radiation
- ☆ **no nucleic acid** – lack DNA, RNA
  - ✷ *protein-only hypothesis*

★ **REPLICATION MECHANISM – TEMPLATED CONVERSIONAL CONFORMATION:**
- ☆ PrP^{Sc} binds to PrP^{c}, triggering conformational conversion → helix 1 unfolds, helices 2 & 3 change into ß sheets
- ☆ several prions aggregate into oligomers then amyloid fibrils → interferes w normal cellular functions & causing tissue damage
  - ✷ this aggregation stabilises the structure, making it resistant to heating/enzymes

PROTISTS

★ **CHARACTERISTICS:**
- ☆ they make up the bulk of eukaryotes
- ☆ usually unicellular
- ☆ diverse: 4 main supergroups – excavata, SAR, archaeplastida, unikonta

★ **BROWN ALGAE:**
- ☆ reproductive cycle – example of **alternation of generations**, where it alternates between haploid & diploid forms
  - ✷ (2n) sporophyte *(the large seaweed form)*, develop into 2n sporangia
  - ✷ 2n sporangia undergoes meiosis produces haploid zoospores (n)
  - ✷ n zoospores grow into haploid gametophytes (n) — small and threadlike
  - ✷ gametophytes produce: sperm (♂) or eggs (♀) via mitosis
  - ✷ fertilisation → forms diploid zygote (2n)
  - ✷ zygote grows into new sporophyte (2n)

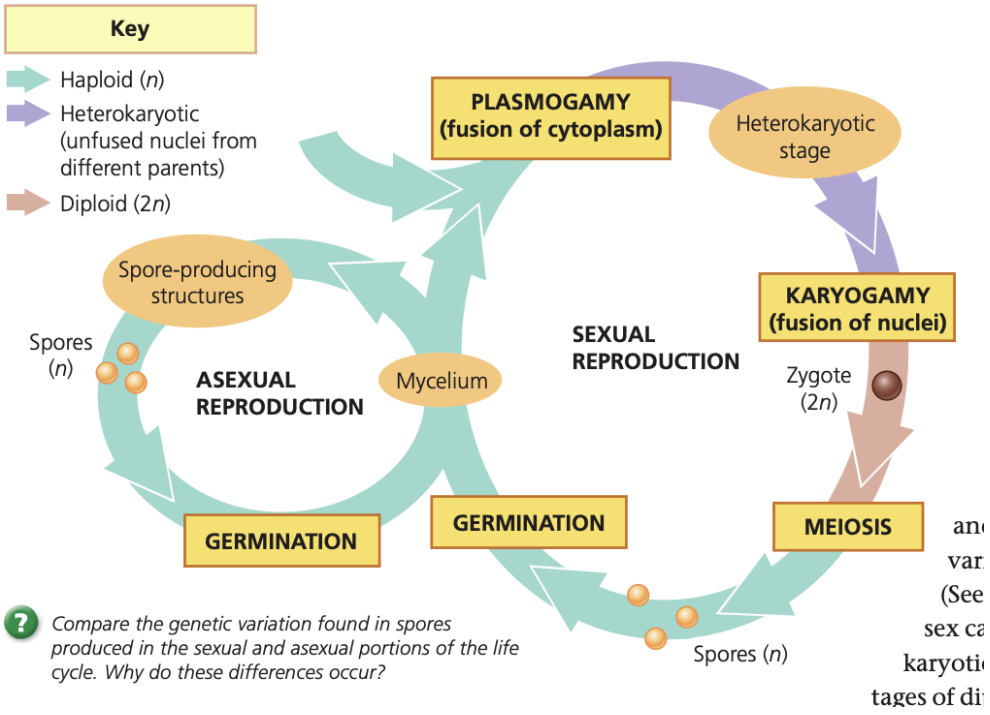
★ **PLASMODIUM:**
- ☆ reproductive cycle – example of **multiple hosts**, where it first infects a mosquito *(definitive host)* then is transferred to humans *(intermediate host)*

FUNGI

★ **CHARACTERISTICS:**

- ☆ **eukaryotes**
- ☆ **heterotrophic** – absorb nutrients from surroundings via external digestion
  - ✶ *secretes hydrolytic enzymes to breakdown complex organic molecules & absorb it*
- ☆ cell wall is made of **chitin** *(not cellulose)* & stores carbohydrates as **glycogen** *(not starch)*
- ☆ can act as:
  - ✶ **decomposers** – recycle dead organic material
  - ✶ **parasites** – absorb nutrients from living hosts
  - ✶ **mutualists** – forms beneficial r/s w plants or algae

★ **GENERAL LIFE-CYCLE:**



YEAST

★ **MORPHOLOGY:**

- ☆ unicellular fungi, w round/oval-shaped cells

★ **REPRODUCTION & LIFE-CYCLE –** reproduce asexually either by budding or fission

- ☆ **budding:**
  - ✶ a small outgrowth forms on the parent cell and enlarges
  - ✶ the nucleus divides and one nucleus migrates to the bud
  - ✶ the bud detaches or forms a pseudohyphae if incomplete

FILAMENTOUS FUNGI

★ **MORPHOLOGY:**
- ☆ composed of **hyphae** – long, threadlike structures
- ☆ hyphae form a **mycelium** – a dense, branched network for nutrient absorption
- ☆ cell walls made of **chitin**
- ☆ specialised hyphae can form **haustoria** – penetrate host cells *(in parasitic or mutualistic fungi)*

★ **REPRODUCTION & LIFE-CYCLE –** reproduce asexually or sexually
- ☆ **<u>asexual reproduction:</u>**
  - ✷ via **mitotic spores** *(eg. conidia)*
  - ✷ spores disperse and germinate into new mycelium
- ☆ **<u>sexual reproduction</u>**:
  - ✷ **plasmogamy** – fusion of cytoplasm from two parent mycelia
  - ✷ **dikaryotic stage (n + n)** – cells with two separate nuclei
  - ✷ **karyogamy** – nuclei fuse to form diploid zygote
  - ✷ **meiosis** – produces haploid spores
  - ✷ spores germinate into new mycelium

CELL DIFFERENTIATION

★ **DEFINITION:** process where a less specialised cell becomes more specialised cell type w distinct morphology & function

★ **MOLECULAR BASIS:**
- ☆ **epigenetic modifications** – control gene accessibility
  - ✷ *eg. DNA methylation, histone acetylation*
- ☆ **transcription factors** – bind to regulatory sequences to activate/repress gene expression

★ **WHY?: division of labour in multicellular organisms**
- ☆ can carry out multiple tasks at once
  - ✷ *eg. Volvox: w/o division of labour, it has to switch between reproduction/locomotion to find food*
- ☆ they can carry out their role more efficiently as ideal structures can be developed with the enzymes needed to carry out chemical reactions associated with the function

★ **CONS:**
- ☆ differentiated cells often lose the ability to make copies of themselves – organisms must retain some unspecialised cells *(stem cells)* to replenish cells when needed

CELL THEORY

★ **TENETS OF CELL THEORY:**
- ☆ cells are the smallest unit of life
- ☆ all organisms are composed of cells
- ☆ all living cells arise from pre-existing cells

ACELLULARITY

★ **DEFINITION:** consisting of no cells

CELL THEORY	VIRUSES	PRIONS
'cells are the smallest unit of life'	**NO** – viruses are acellular	**NO** – prions are proteins, thus also acellular
'all organisms are composed of cells'	**NO** – only consists of nucleic acid in a protein coat	**NO** – consists of amino acids
'all living organisms arise from pre-existing cells'	**NO** – replicate by hijacking a host cell's machinery + challenges what it means to be living	**NO** – propagate by templated conversional conformation

VIRUSES

LIVING	NON-LIVING
have a common hereditary molecule based on one type of nucleic acid	unable to replicate independently outside of their host cells
can pass on genetic characteristics from one viral generation to the next	metabolically inactive outside of host cell
can replicate genetic material within an infected living host cell	don't grow in size
can evolve via antigenic shift and drift	cannot respond to stimulus outside of the cell → no homeostasis
contain viral enzymes that can be used in their reproductive cycles	

PRIONS

★ despite being acellular, they have some living characteristics:
- ☆ able to replicate by themselves via converting PrP^{c} molecules into PrP^{Sc} w/o DNA as a hereditary material
- ☆ prion hypothesis

MULTINUCLEATION

★ **DEFINITION:** condition of having multiple nuclei within a shared cytoplasm, either due to lack of/failure of cytokinesis *(coenocytes)* or cell fusion *(syncytia)*

FUNGI HYPHAE

★ **coenocytic –** formed by mitosis w/o cytokinesis
★ how it challenges cell theory:
- ☆ **they do not have distinct cell boundaries or cell membranes**
  - ✶ the nuclei divide repeatedly within a shared cytoplasm, resulting in multinucleated cells w/o cell walls

ENDOSYMBIOSIS

★ **DEFINITION:** r/s in which 1 organism lives inside the cells of another

EUKARYOTES ENDOSYMBIOTIC ORIGINS

★ **ENDOSYMBIONT THEORY**
- ☆ explains the origin of **mitochondria** and **chloroplasts** in eukaryotic cells
- ☆ these organelles evolved from free-living prokaryotes
  - ✶ mitochondria from aerobic proteobacteria
  - ✶ chloroplasts from cyanobacteria
- ☆ **serial endosymbiosis:**
  - ✶ an ancestral eukaryotic cell engulfed a non-photosynthetic, aerobic prokaryote
  - ✶ this prokaryote became a mutualistic endosymbiont, helping the host generate energy
  - ✶ over time, it evolved into a mitochondrion
  - ✶ later, some eukaryotes engulfed photosynthetic prokaryotes, which became chloroplasts

★ **EVIDENCES SUPPORTING THE THEORY**
- ☆ **double membranes** around mitochondria and chloroplasts
- ☆ they replicate by **binary fission** like bacteria
- ☆ contain **circular DNA** w/o histones
- ☆ have **their own ribosomes** similar in size and structure to prokaryotic ribosomes
- ☆ can **transcribe & translate** their own proteins independently

★ **HOW IT CHALLENGES CELL THEORY**
- ☆ **'all cells arise from pre-existing cells' –** mitochondria and chloroplasts arose through **endocytosis**, not division of a single parent cell

PROTEINS

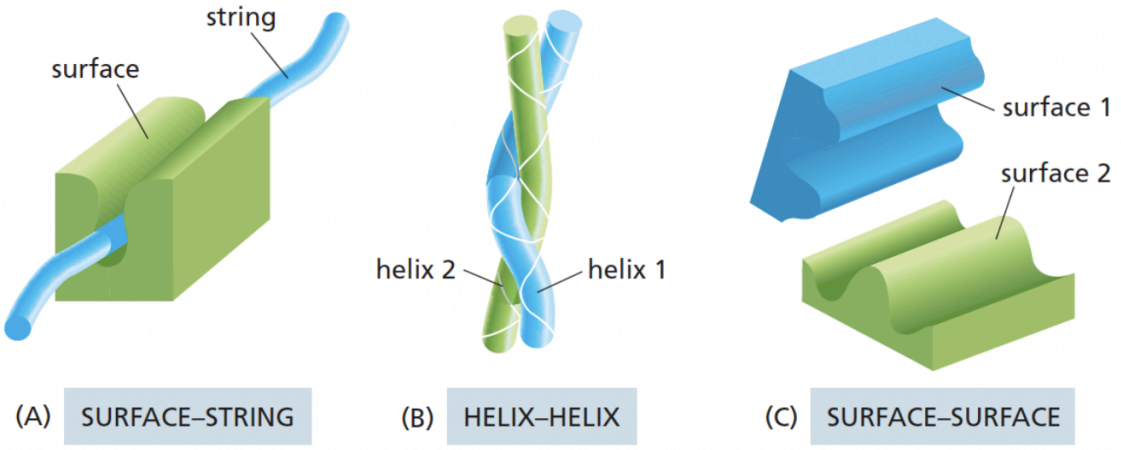
PROTEIN BINDING SITES & SUBUNITS

★ **PROTEIN BINDING SITES**

- ☆ a protein's physical interaction w other molecules determines its biological properties
- ☆ the ability to bind selectively w high affinity depends on **formation of weak non-covalent bonds**
  - ✶ for effective binding, MANY of these bonds form simultaneously
- ☆ only possible if the 3D conformation of the molecules are **complementary** in terms of shape & charge
- ☆ *eg. enzyme-substrate complex, antigen-antibody complex, ligand-receptor complex*

★ **PROTEIN SUBUNITS**

- ☆ **surface-string** – a portion of the surface of 1 protein contacts w an extended loop of a polypeptide chain *(a string)*
  - ✶ *eg. SH2 domain w phosphorylated polypeptide loop*
- ☆ **helix-helix** – 2 α helices coil around e/o
  - ✶ *eg. typically transcription regulatory factors*
- ☆ surface-surface – precise matching of 2 complementary structures, strong due to large no. of weak bonds formed
  - ✶ *eg. ES complex*



DISCUSS THE ADVANTAGES OF MULTI-SUBUNIT PROTEINS OVER SYNTHESIS OF ONE HUGE POLYPEPTIDE CHAIN.

- ★ cooperative regulation
- ★ allosteric regulation
- ★ modular assembly
- ★ error tolerance & repair
- ★ genetic economy
- ★ improved folding & stability
- ★ complex functionality

HAEMOGLOBIN

★ **STRUCTURE**
- ☆ 2 identical α-globin & 2 identical β-globin subunits; each bound to a haem prosthetic group ⇒ $\alpha_2\beta_2$ heterotetramer
- ☆ has 2 conformations
  - ✷ **T-state** *(deoxyhaemoglobin)*
    - ☾ Fe(II) atom sits ~0.6 Å **out of haem plane** due to pyramidal **doming of porphyrin ring** toward His F8 (proximal histidine)
  - ✷ **R-state** *(oxyhaemoglobin)*

★ **INTERACTIONS BETWEEN SUBUNITS**
- ☆ surface-surface interactions between α & β subunits – extensive contact between:
  - ✷ α_1-β_2 *(& its α_2-β_2 symmetry equivalent)* – 35 residues
  - ✷ α_1-β_2 *(& α_2-β_1)* – 19 residues
- ☆ bonds: predominantly hydrophobic, w H-bonds & ion pairs also

★ **BINDING OF OXYGEN**
- ☆ T state → R state
- ☆ when O_2 binds, its electronegativity pulls Fe^{2+} up into the plane of the porphyrin ring → as Fe^{2+} is attached to His F8, His F8 also moves → shifting the entire polypeptide ⇒ tertiary structure changed!
- ☆ as the subunits of Hb are tightly associated, the tertiary structure changes causes shifts in arrangements of all 4 subunits ⇒ quaternary structure changes
  - ✷ 1 αβ dimer rotates ~15º wrt the other αβ dimer ⇒ β subunits closer together, central channel narrowed

IMMUNOLGLOBIN

★ **5 CLASSES** – *dependent on the heavy-chain constant region*
- ☆ IgM, IgD, IgG, IgA, IgE

★ **ANTIGEN BINDING SITE**
- ☆ complementary in shape & charge to epitope of antigen → determines antigen specificity
- ☆ structure
  - ✷ formed by variable regions of heavy & light chains
  - ✷ contains hypervariable loops *(aka CDRs)* ˆ→ forms asymmetric antigen-binding site

PROKARYOTIC RNA POLYMERASE

★ STRUCTURE

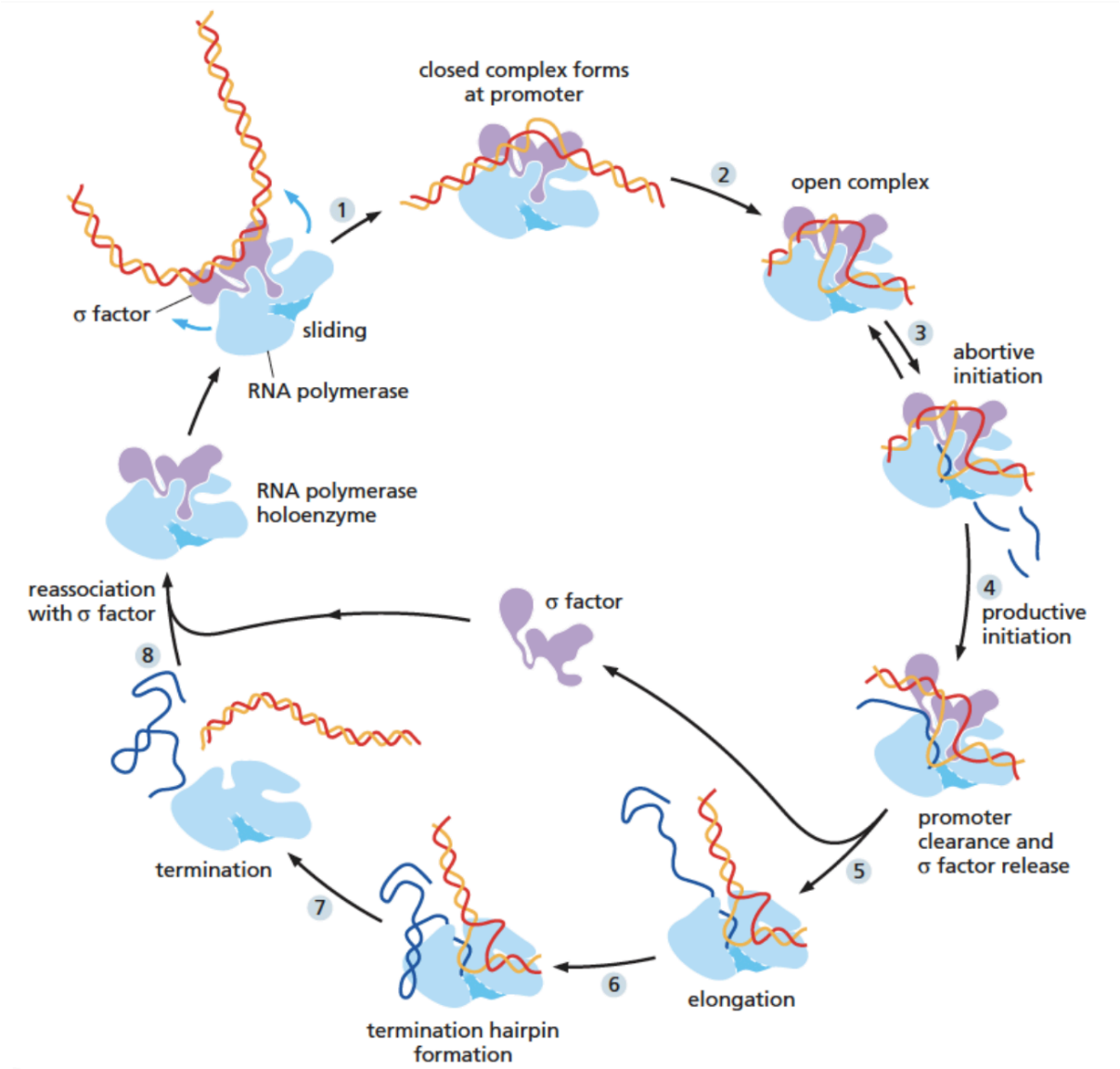
- ☆ metal cofactors (Zn, Mg, etc.) → helps in the process of transcription
- ☆ 5 subunits – 2 α, 1 β, 1 β', 1 ω + sigma (σ) factor
  - ✶ *α + α → α_2 → $\alpha_2\beta$ → $\alpha_2\beta\beta'$ → $\alpha_2\beta\beta'\omega$ (core enzyme)*

★ SUBUNITS

- ☆ **α-subunits (rpoA):** helps in binding RNAP to the promoter
  - ✶ exists as a homodimer (α_2)
  - ✶ 2 domains: αNTD + αCTD
  - ✶ first to dimerise → scaffold for β & β' assembly
- ☆ **β subunit (rpoB)** *– contains the catalytic activity of RNAP*
  - ✶ forms one 'claw' of RNAP → shapes DNA-entry cleft
  - ✶ DPBB domain contributes to active site
  - ✶ provides basic residues to guide incoming NTPs via secondary channel
- ☆ **β′ subunit (rpoC)** – *DNA binding*
  - ✶ other "claw" of RNAP
  - ✶ DPBB domain has Asp triad (DFDGD) → coordinates 2 Mg^{2+} ions
    - ☾ 1 Mg^{2+} = permanently bound *(stabilisation)*
    - ☾ 1 Mg^{2+} = recruited transiently for phosphodiester bond formation
  - ✶ trigger loop → folds to align NTP for catalysis, then resets
  - ✶ bridge helix → stabilises transcription bubble, aids DNA/RNA translocation
  - ✶ lid + rudder motifs → separate RNA from DNA in transcription bubble
- ☆ **ω subunit (rpoZ)**
  - ✶ smallest; not essential for growth
  - ✶ binds β′ DPBB domain; may stabilise folding and catalytic activity
- ☆ **σ (sigma) factor**
  - ✶ directs RNAP to **promoter sequences** and initiates transcription
  - ✶ **functions:**
    - ☾ directs RNAP to the correct promoter
    - ☾ helps unwind DNA at promoter → open complex *(transcription bubble)*
  - ✶ released after >10 nucleotides of RNA are synthesised → RNAP switches to elongation mode
  - ✶ different σ factors recognise different promoter sequences → regulate gene expression under varying condition

★ **TRANSCRIPTION PROCESS**

1. holoenzyme assembly ($\alpha_2\beta\beta'\omega + \sigma$)
2. **initiation**
    a. σ factor locates promoter by sliding
    b. RNAP unzips the DNA at the promoter region
    c. σ factor binds to non-template strand + template strand enters active site of RNAP ⇒ open complex formed
    d. *abortive initiation* – the first few RNA transcripts are short & unproductive ⇒ released
    e. *productive initiation* – once a transcript > 10 nucleotides is produced, it breaks its interactions w promoter + releases σ factor
3. **elongation** – RNAP is highly processive & moves along DNA
4. **termination** – once it reaches a termination sequence, RNA hairpins destabilise RNAP ⇒ RNA released



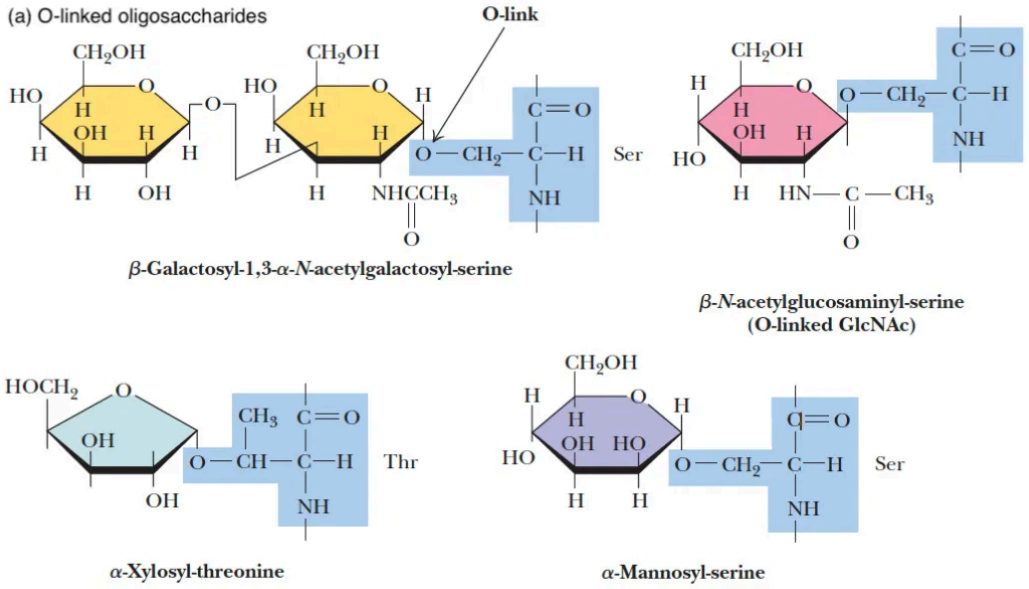
PROTEIN MODIFICATIONS

GLYCOSYLATION

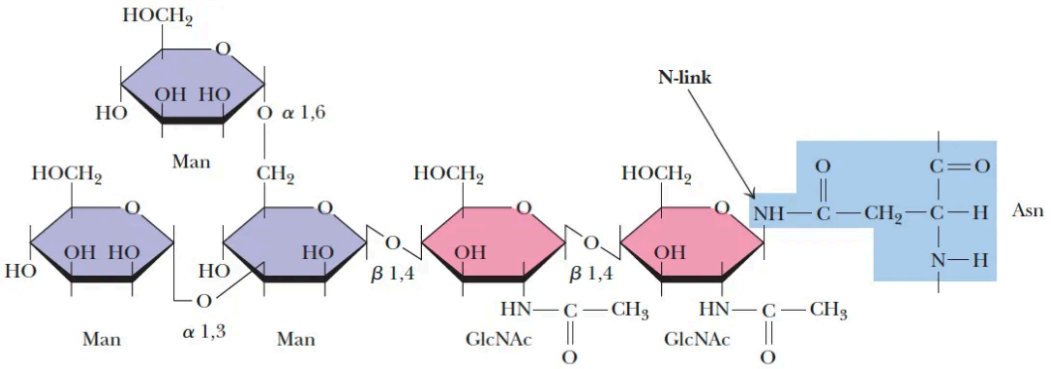
★ **DEFINITIONS**

- ☆ addition of a short carbohydrate chain to proteins to form glycoproteins
- ☆ occurs mainly inside the lumen of rER & golgi apparatus
- ☆ **types of glycosylation** – O-linked, N-linked, C-linked, glypiation, phosphoglycosylation

★ **O-LINKED GLYCOSYLATION** – sugars bind to -OH group of Serine (Ser) or Threonine (Thr) residues via the O-glycosidic bond



★ **N-LINKED GLYCOSYLATION** – oligosaccharide attaches to the -NH_2 group of Asparagine (Asn) residues



- ☆ **function** – facilitates proper protein binding
  - ✶ makes folding intermediates more soluble → prevents aggregation
  - ✶ OR the sequential modifications of the N-linked oligosaccharide establish a **'glyco-code'** that marks the progression of protein folding → this code is used by chaperone proteins *(calnexin & calreticulin)* in the ER to guide protein folding *(see Fig. 1)*

★ **LECTINS**
- ☆ highly specific carbohydrate-binding protein
- ☆ involved in: cell-cell interactions, signalling pathways, & immune responses
- ☆ *eg.*
  - ✶ *calnexins – specific to glucose, is a molecular chaperone in the ER*
  - ✶ *selectins – in cell migration & routing*

★ **SELECTINS & LEUCOCYTE MIGRATION**
- ☆ **selectins**, a class of CAMs, recognise & bind specific oligosaccharides on CSM + mediate attachment between circulating leucocytes/WBCs to endothelial cells CSM
  - ✶ *cell adhesion molecules (CAMs) → glycoproteins on cell surface membrane (CSM) that mediates cell-cell & cell-extracellular (ECM) adhesion*
- ☆ leucocytes always express selectins
- ☆ endothelial cells do so transiently in response to tissue damage

⇒ reciprocal selectin-oligosaccharide interactions between the 2 cells **slow down movement of leucocytes** in the blood → triggers stronger interactions → leucocytes squeeze between endothelial cells & migrate towards infection site

★ **ANTIGEN DETERMINATION**
- ☆ oligosaccharides are antigenic determinants *(see Fig. 2)*
  - ✶ *eg. ABO blood group (O has H antigen; precursor oligosaccharide of A & B antigens)*

★ **PROTEOGLYCANS (PGs) –** *part of ECM*
- ☆ **structure:** aggregation of proteins + glycosaminoglycans (GAGs)
  - ✶ GAGs: unbranched polysaccharides made of repeating disaccharide unit
  - ✶ highly negatively charged ⇒ enable PGs to attract & retain large amounts of H_2O – providing hydration & maintain structural integrity & compressibility
- ☆ **function:** mechanosensors in mechanosensitive tissues ⇒ act as 'shock-absorbers' in the ECM
  - ✶ *resist & distribute mechanical forces exerted on tissues*
- ☆ **aggregans** in cartilage
  - ✶ bottlebrush-like structure – 'bristles' non-covalently bonded to filamentous hyaluronate backbone *(see Fig. 3)*
  - ✶ pressure on cartilage squeezes water away from charged regions of its proteoglycans until electronic repulsion prevents further compression

★ **BACTERIAL PEPTIDOGLYCAN CELL WALL**
- ☆ **structure:** linear chains of alternating N-acetylglucosamine (NAG) & N-acetylmuriamic acid (NAM) w β-1,4-glycosidic bonds
- ☆ neighbouring chains are covalently cross-linked via their tetrapeptide side chains ⇒ provides strength & rigidity to bacterial cell wall
  - ✶ *so they don't swell & lyse in hypertonic solutions*

PHOSPHORYLATION

★ **DEFINITION**
- ☆ transfer a phosphate group from ATP to hydroxyl group of Ser, The, or Tyr residues in proteins, catalysed by protein kinases
- ☆ **dephosphorylation** is by protein phosphates

★ **CHANGE PROTEIN CONFORMATION**
- ☆ phosphate carries 2 negative charges
  - ✶ attracts positively charged AA R groups ⇒ causes major conformational change in protein

⇒ alters binding of ligands → affecting protein activity
- ☆ reversible – can be removed by phosphatases, restoring its original conformation

★ **GLYCOGEN PHOSPHORYLASE**
- ☆ exists in 2 states – R state (active) & T state (inactive)
- ☆ phosphorylation of Ser14 shifts the enzyme from T → R state
- ☆ T-state:
  - ✶ active site is malformed,
  - ✶ surface loops block substrate access
- ☆ R-state – *post-phosphorylation*:
  - ✶ phosphorylation causes Arg569 side chain reoriented to bind the substrate's phosphate ion
  - ✶ surface loop no longer blocks active site

  ⇒ free to catalyse phosphorylation of glycogen

★ **REGULATE PROTEIN-PROTEIN INTERACTIONS**
- ☆ attached phosphate group can:
  - ✶ form **part** of the binding site
  - ✶ OR **mask** a binding site that otherwise holds 2 proteins together ⇒ disrupting protein-protein interactions

★ **SH2 DOMAINS**
- ☆ Src homology 2 (SH2) domains are ~100AAs ⇒ link proteins together, involved in cell signalling pathways
- ☆ **binding specificity** – recognises phosphotyrosine residues within a specific AA sequence of a target protein
- ☆ ***eg. insulin pathway***
  - ✶ *insulin receptor phosphorylates IRS-1 & IRS-2*
  - ✶ *phosphorylated IRS proteins recruit other signalling proteins w their SH2 domains*

PROTEOLYTIC CLEAVAGE

★ **DEFINITION** – breaking of peptide bonds between AAs

★ **PROTEIN ACTIVATION**

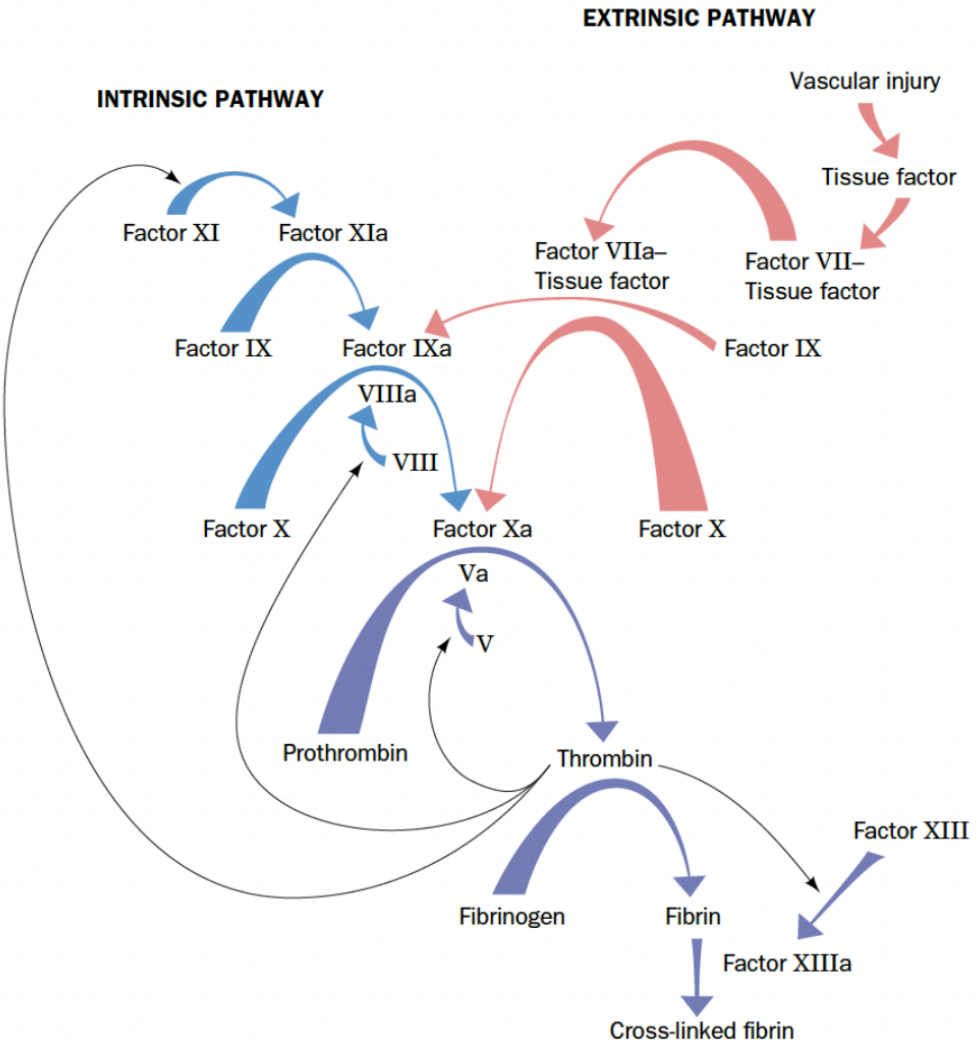
- ☆ proteolytic enzymes are synthesised as inactive precursors – *zymogens* ⇒ proteolytic cleavage changes it to its active state
  - ✶ *prevents digestive enzymes from digesting the tissues that synthesised them*

★ **TRYPSINOGEN TO TRYPSIN**

- ☆ trypsinogen is the zymogen of trypsin
- ☆ **activation**
  - ✶ occurs when trypsinogen enters the duodenum from the pancreas
  - ✶ **enteropeptidase** cleaves Lys15-Ile16 bond of trypsinogen → removes N-terminal hexapeptide
  - ✶ cleavage occurs at a trypsin-sensitive site → small amt. of trypsin produced catalyses trypsinogen activation ⇒ autocatalytic

★ **COAGULATION CASCADE**

- ☆ sequential zymogen activation → causes spike in thrombin activity as factors are sequentially activated by proteolysis of their zymogens



★ CLEAVAGE OF SIGNAL PEPTIDES IN PRE-PROTEINS

- ☆ signal peptides directs proteins to the rER translocon → is recognised by SRPs → guides ribosome to ER membrane *(see Fig. 4)*
- ☆ **cleavage process:**
  - ✶ removal of initial Met residue
  - ✶ signal peptide cleavage by ER signal peptidase
  - ✶ propeptide excision → functional protein formed

★ PROPER PROTEIN ASSEMBLY

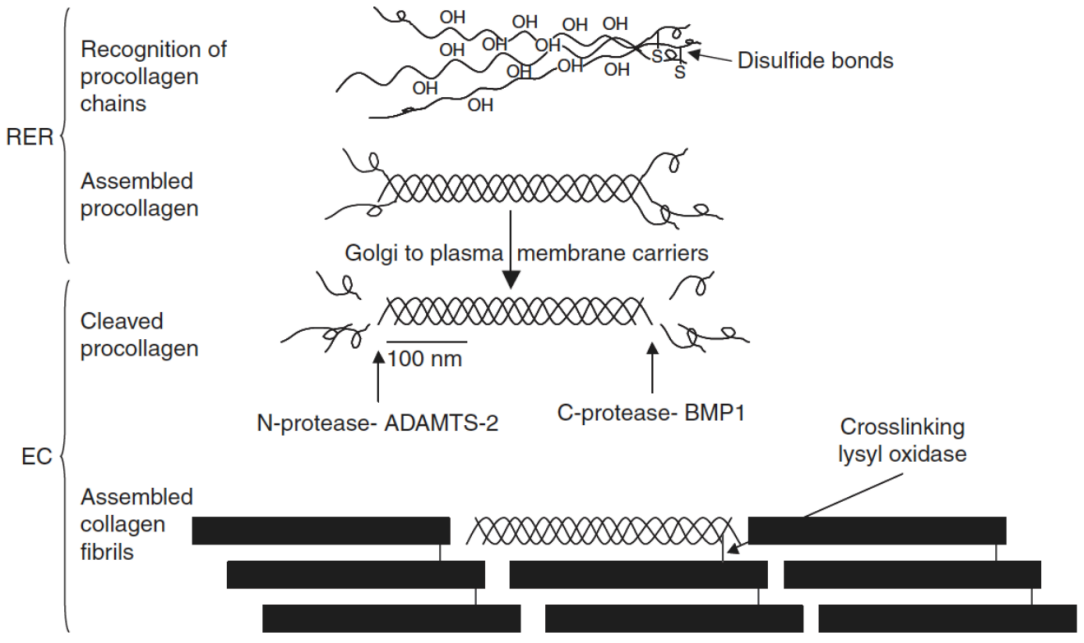
- ☆ propeptides need to be cleaved off from the final protein before assembly
  - ✶ extra sequences added before protein folding, later removed by proteolytic cleavage
  - ✶ **functions:**
    - ☾ helps protein fold properly
    - ☾ keeps protein inactive
    - ☾ guides proteins to the right cellular locations
    - ☾ help in assembly of large proteins

★ INSULIN

- ☆ synthesised as **proinsulin** → cleaved by a proteolytic enzyme after protein has folded into a specific shape *(see Fig. 5)*
- ☆ excision of propeptide sequence of insulin removes necessary assembly information ⇒ prevents spontaneous reassembly after denaturation

★ COLLAGEN

- ☆ synthesised as **propolypeptides** in rER → undergoes hydroxylation & glycosylation
- ☆ 3 modified propolypeptides twist into a triple helix → procollagen formed
  - ✶ *twist starting from C-terminus propeptides stabilised by disulfide bonds*
- ☆ procollagen → golgi apparatus → packaged into secretory vesicles → ECM
- ☆ after procollagen leaves the cell: cleavage of N- & C- termini propeptides ⇒ tropocollagen
- ☆ tropocollagen formed spontaneously assemble into fibrils → extracellular *lysyl oxidase* catalyse cross-linking between collagen



ENZYME REGULATION

ENZYME AMOUNT

★ **GENETIC REGULATION** – *synthesis & degradation*
- ☆ by controlling amt. of enyme → can either activate/terminate metabolic routes
- ☆ degradation: normal turnover of protein OR specific decay mechanisms

ENZYME ACTIVITY

★ **ALLOSTERIC REGULATION**
- ☆ small molecules bind to an allosteric site → can stabilise R (active) or T (inactive) forms of the enzyme
- ☆ often for multi-subunit enzymes
- ☆ **eg. phosphofructokinase (PFK) – regulates glycolysis**
  - ☾ <u>at high energy levels:</u> ATP & citrate binds to allosteric site of PFK → inactivates it ⇒ glycolysis is slowed down
  - ☾ <u>at low energy levels:</u> AMP builds up → AMP binds to PFK, activating it ⇒ glycolysis rate increases

  ⇒ prevents wastage of glucose when not needed
- ☆ **cooperativity** – binding of substrate at allosteric site → conformational change of other subunits → increase activity of catalytic sites
- ☆ **feedback inhibition** – end-product is an inhibitor of an early enzyme
  - ✷ *eg. isoleucine inhibits threonine deaminase*

★ **COVALENT MODIFICATION**
- ☆ reversible covalent bonding of a chemical group
  - ✷ *eg. (de)phosphorylation, acetylation, methylation, ubiquitination*

★ **PROTEOLYTIC CLEAVAGE**
- ☆ enzymes are synthesised as zymogens & activated through proteolytic cleavage
- ☆ IRREVERSIBLE!

★ **COFACTORS**
- ☆ substances that assist enzymes in their function
- ☆ **coenzymes** → binds transiently
  - ✷ *eg. $FAD/FADH_2$ → electron carriers in Succinate Dehydrogenase*
  - ✷ *eg. CoA → carries acetyl group*
- ☆ **prosthetic groups** → tightly bound
  - ✷ *eg. biotin → cofactor for pyruvate carboxylase (carries CO_2)*
  - ✷ *eg. heme group → catalase, peroxidase (carries O_2)*
- ☆ **metal ions**
  - ✷ *eg. Mg^{2+} in kinases → stabilises ATP's negative charges*
  - ✷ *eg. Cu^{2+} in cytochrome c oxidase → electron transfer*

LOCALISATION

★ **COMPARTMENTALISATION**
- ☆ segregates enzymes physically into specific regions or organelles w their substrates/cofactors
- ☆ prevents conflicting pathways from interfering
  - ✷ *eg. lysosomes digestive enzymes (proteases, nucleases, lipases) – work best in acidic pH (not cytosolic pH) + if it were free-floating, it will also denature vital cellular biomolecules*

★ **SCAFFOLDS**
- ☆ protein scaffolds concentrate enzymes & substrates in the same region → minimise time & energy required for molecular diffusion
- ☆ helps amplify & fine-tune cellular responses
  - ✷ ***eg. MAP kinase (MAPK) signalling pathway***
    - ☾ *scaffold protein holds together MAPKKK, MAPKK, & MAPK*
    - ☾ *ensures that once the first kinase is activated → signal is rapidly passed down w/o enzymes diffusing randomly*

ANNEX

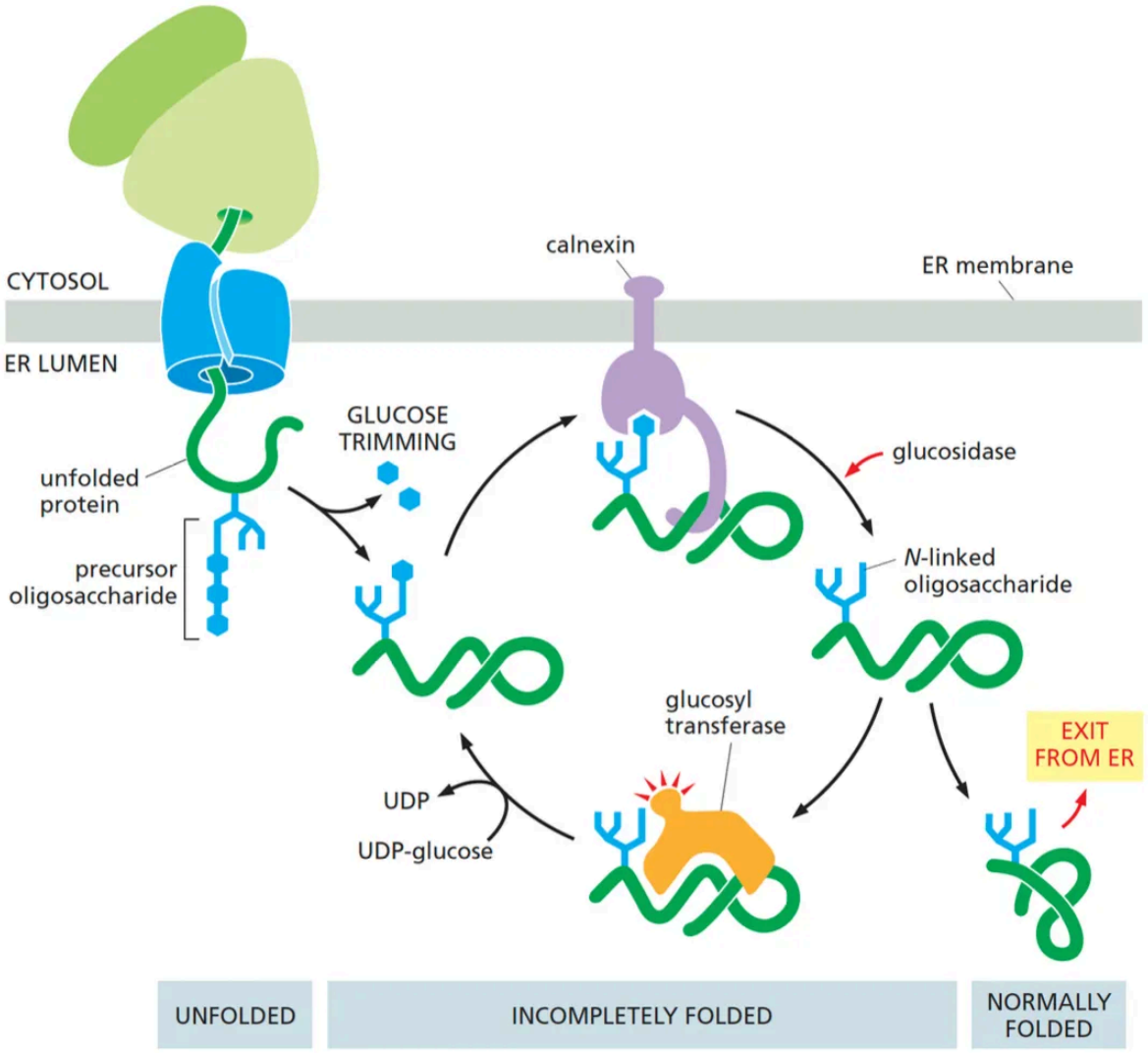
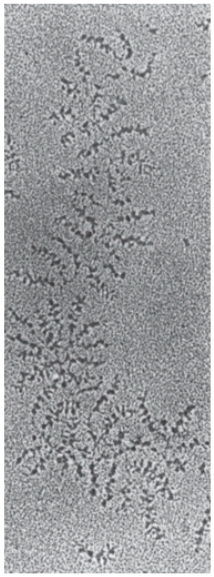


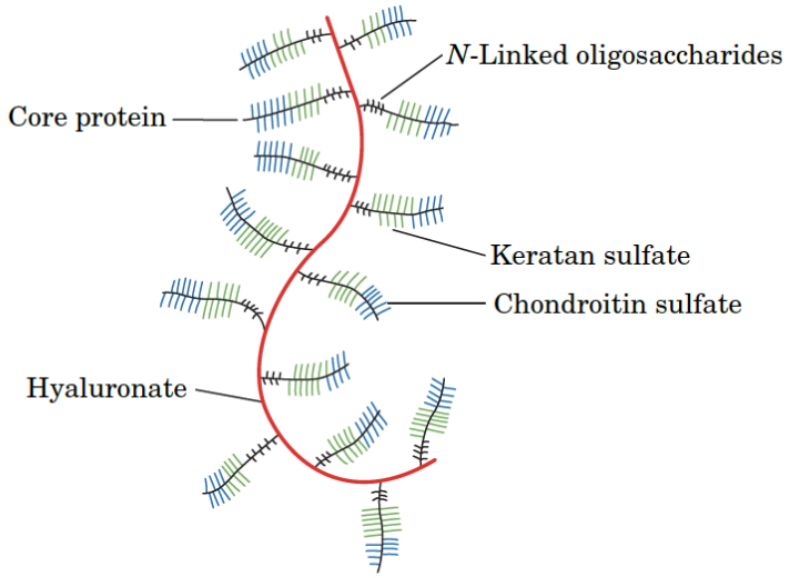
Fig. 1 – Role of N-linked glycosylation in ER protein folding (Alberts, et al., 2022).

Serum from individuals of type	Red blood cells from individuals of type: O	A	B	AB
	Express the carbohydrate structures: R–GlcNAc–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc)	R–GlcNAc–Gal–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc) + R–GlcNAc–Gal–Gal (Fuc)
O — Anti-A and anti-B antibodies	no agglutination	agglutination	agglutination	agglutination
A — Anti-B antibodies	no agglutination	no agglutination	agglutination	agglutination
B — Anti-A antibodies	no agglutination	agglutination	no agglutination	agglutination
AB — No antibodies to A or B	no agglutination	no agglutination	no agglutination	no agglutination

Fig. 2 – Blood types and compatibility for blood transfusion (Murphy & Weaver, 2017). GlcNAc = N-acetylglucosamine, GalNAc = N-acetylgalactosamine, Gal = galactose, Fuc = fucose, R = rest of the molecule which can be protein or lipid



(a) Electron micrograph of aggrecan



(b) Bottlebrush model of the aggrecan

Fig. 3 – Structure of Aggrecan (Voet, Voet, & Pratt, 2016)

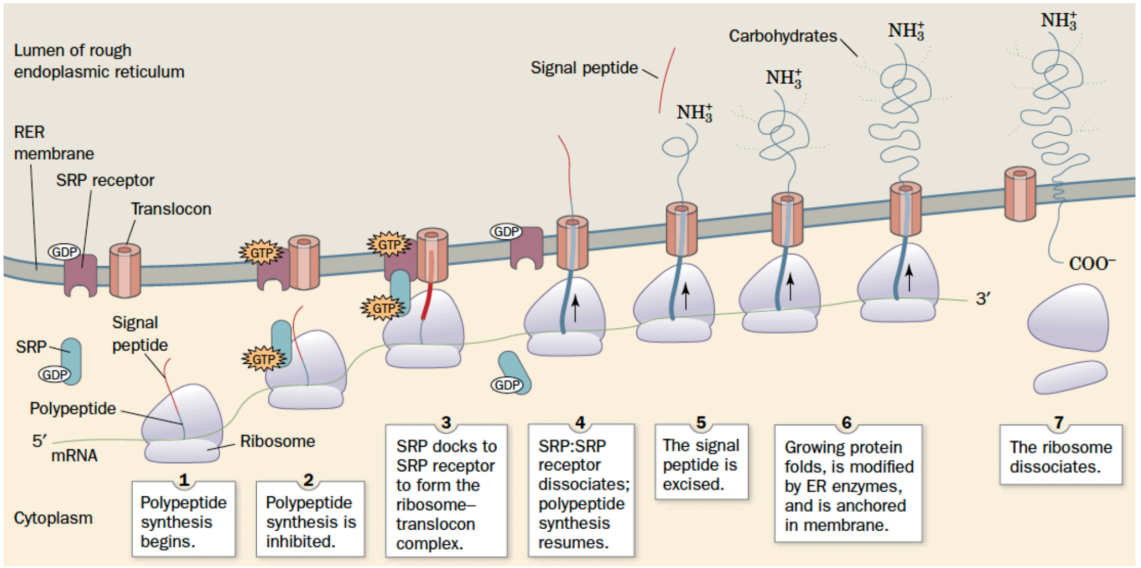


Fig. 4 – Secretory pathway of an integral protein (Voet, Voet, & Pratt, 2016)

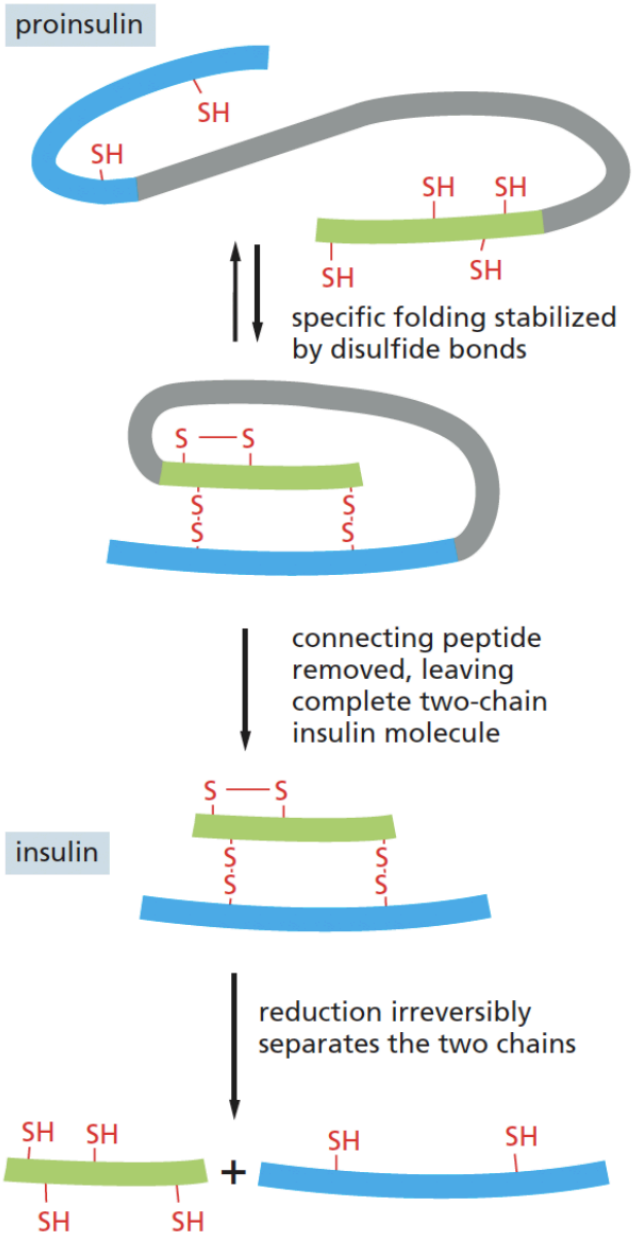


Fig. 5 – Proteolytic cleavage in insulin assembly (Alberts, et al., 2022)

CORE IDEA 2

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STEM CELLS

PROPERTIES

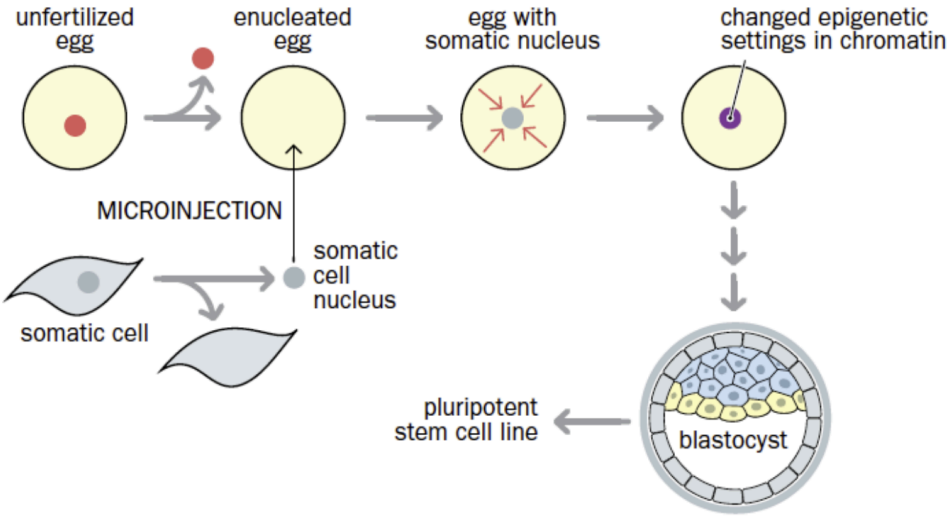
★ **SELF-RENEWAL**:
- ☆ ensures constant pool of stem cells
- ☆ **asymmetric division**: 1 committed cells w a specialised function + 1 stem cell
- ☆ **symmetric division:** 2 committed cells OR 2 stem cells

★ **POTENCY:**

totipotent	**pluripotent**	**multipotent**	***unipotent***
any cell type + extraembryonic tissues	any cell types; except extraembryonic tissues	related cell types of the tissues they reside in	*only 1 cell type*
<5 days after fertilisation	5-7 days after fertilisation	>7 days after fertilisation	*>7 days after fertilisation*
first 4-8 cells & **zygote**	**embryonic stem cells** from the inner cell mass of the blastocyst	adult stem cells, eg. **HSCs** can differentiate into diff. blood cells	***spermatogonia stem cells*** *— only generates sperm cells*

CONVERTING SOMATIC CELL → STEM CELL

★ **SOMATIC CELL NUCLEAR TRANSFER**



★ **INDUCED PLURIPOTENT STEM CELLS (iPSCs)**
- ☆ reprogramming mature, adult stem cells (like skin or blood cells) into an embryonic-like pluripotent state
- ☆ done by introducing specific genes into the cells, known as Yamanaka factors – Oct4, Sox2, Klf4, and c-Myc

GENETIC ENGINEERING

ENZYMES INVOLVED

★ **RESTRICTION ENDONUCLEASES**
- ☆ hydrolyse phosphodiester bonds specific points of the restriction sites → producing fragments w blunt / sticky ends
  - ✶ **blunt**: straight cut across both strands
  - ✶ **sticky**: staggered cut w a ssDNA overhang ⇒ preferred!

★ **REVERSE TRANSCRIPTASE**
- ☆ synthesise cDNA using mRNA as the template to make a dsDNA molecule
- ☆ **steps to synthesise cDNA:**
  - ✶ mRNA containing a 3' polyA tail are mixed w a primer composed of poly-dT primer
  - ✶ reverse transcriptase synthesises cDNA, 5' to 3'
  - ✶ RNaseH partially digests the mRNA template, generating short RNA fragments that are used as primers by DNA pol
  - ✶ DNA pol. synthesises a strand complementary to the one by reverse transcriptase
  - ✶ DNA pol. removes the RNA primers except the one at the 5' end
  - ✶ DNA ligase connects the DNA segments ⇒ producing a continuous DNA strand

★ **DNA LIGASE**
- ☆ catalyse formation of phosphodiester bond between sticky ends

CLONING PROCEDURE

★ **1A: ISOLATION OF VECTOR DNA –** miniprep procedure
- ✶ grow bacteria containing the plasmid in a suitable medium
- ✶ harvest and lyse the bacteria culture
- ✶ pass the bacterial lysate through a filter which binds to DNA
- ✶ wash the filter containing DNA to remove impurities
- ✶ elute the DNA from the filter

★ **1B: ISOLATION OF GOI**
- ☆ **PCR from chromosomal DNA**
  - ✶ used when cloning genes into similar expression systems
- ☆ **RT-PCR of mature mRNA**
  - ✶ used when cloning eukaryotic genes into bacterial cells
  - ✶ mRNA is converted to cDNA by reverse transcriptase using a poly(T) primer
- ☆ **artificial gene synthesis**
  - ✶ requires a known gene sequence
  - ✶ used when sufficient DNA from a natural source is difficult to obtain and gene size is small

★ **2: INSERTION OF GOI INTO VECTOR → RECOMBINANT DNA FORMATION**
- ☆ digest plasmid & GOI w the same restriction enzymes ⇒ complementary sticky ends are formed
- ☆ mix GOI fragments w plasmid & incubate ⇒ annealing of complementary sticky ends
- ☆ DNA ligase is added to catalyse formation of phosphodiester bonds

★ **3: TRANSFORMATION OF RECOMBINANT DNA INTO BACTERIAL HOST CELLS**
- ☆ **chemical transformation w heat shock**
  - ✶ bacterial cells treated w Ca^{2+} ions then incubated w recombinant plasmid
    - ☾ *neutralise negatively charged host cell membrane ⇒ increase permeability; neutralise negatively charged DNA sugar-phosphate backbone ⇒ help DNA adsorb to the competent cell*
  - ✶ brief period of heat shock (42ºC) → place back into ice
    - ☾ *temperature imbalance creates transient pores*
    - ☾ *heating → lipid loss; cooling → protein loss ⇒ increase transformation efficiency*
- ☆ **electroporation**
  - ✶ washed w DI water
    - ☾ *removes proteins & salts that would interfere w electric field during electroporation ⇒ decreasing cell viability + transformation efficiency*
  - ✶ expose competent cells & DNA to a brief pulse of high-voltage electric field w an electroporator
    - ☾ *disturbs the phospholipid bilayer ⇒ induces formation of transient pores*

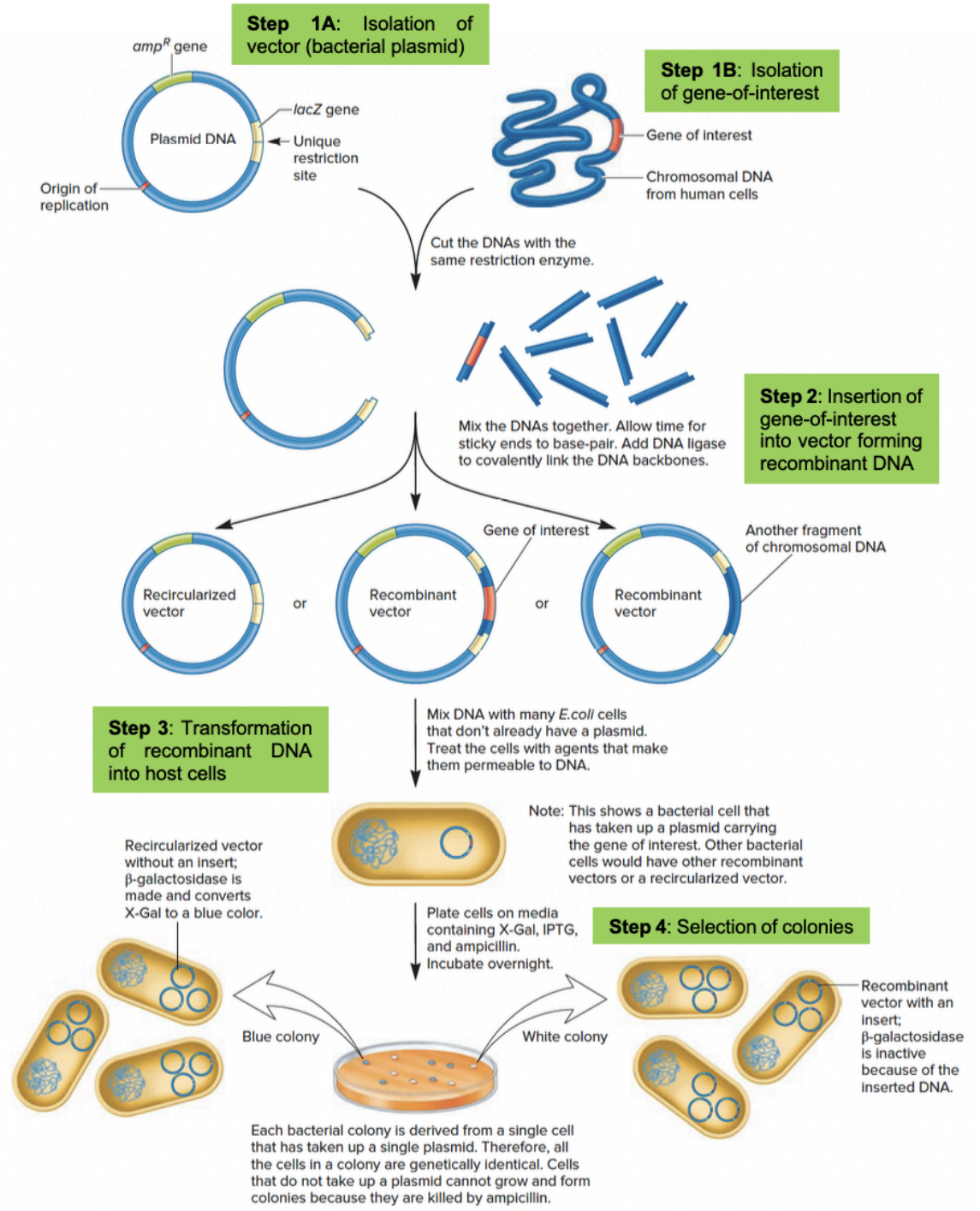
★ **4: SELECTION OF COLONIES**
- ☆ **direct selection** *(of cells that have gained a specific gene survive)*
  - ✶ typically for antibiotic selection – inserted DNA includes an antibiotic resistance marker
  - ✶ ***explain why selection of colonies should not be done immediately after transformation, and suggest what needs to be done before selection of colonies?***
    - ☾ *they need recovery after transformation to repair membranes, establish the plasmid and express the selectable marker*

- ☆ **insertional inactivation selection**
  - ✶ **blue-white screening**
    - ☾ when lacZ is expressed, β-galactidose can break down X-gal into galactidose & an insoluble blue pigment
    - ☾ insertional inactivation of lacZ gene occurs when GOI is inserted into the MCS within lacZ, disrupting the lacZ gene ⇒ select white colonies

- ✷ **replica plating**
  - ☾ transfers colonies from a primary plate to one or more secondary selective plates while preserving their spatial arrangement
  - ☾ primary plate contains antibiotic for which the plasmid's resistance gene is intact; the secondary plate contains an antibiotic whose resistance gene is disrupted by the GOI

☆ **Sanger sequencing – most accurate way!**

- ✷ plasmid DNA is isolated from an overnight culture & sequencing primers specific to the vector are used to read across the entire insert, ensuring the exact sequence is correct
- ✷ typically performed after other selection methods have removed non-recombinants + especially important for large inserts, which have a higher risk of PCR-induced mutations


Step 1A: Isolation of vector (bacterial plasmid)
ampR gene
Plasmid DNA
lacZ gene
Unique restriction site
Origin of replication
Step 1B: Isolation of gene-of-interest
Gene of interest
Chromosomal DNA from human cells
Cut the DNAs with the same restriction enzyme.
Mix the DNAs together. Allow time for sticky ends to base-pair. Add DNA ligase to covalently link the DNA backbones.
Step 2: Insertion of gene-of-interest into vector forming recombinant DNA
Recircularized vector
or
Recombinant vector
Gene of interest
or
Recombinant vector
Another fragment of chromosomal DNA
Step 3: Transformation of recombinant DNA into host cells
Mix DNA with many E.coli cells that don't already have a plasmid. Treat the cells with agents that make them permeable to DNA.
Note: This shows a bacterial cell that has taken up a plasmid carrying the gene of interest. Other bacterial cells would have other recombinant vectors or a recircularized vector.
Recircularized vector without an insert; β-galactosidase is made and converts X-Gal to a blue color.
Plate cells on media containing X-Gal, IPTG, and ampicillin. Incubate overnight.
Step 4: Selection of colonies
Blue colony
White colony
Recombinant vector with an insert; β-galactosidase is inactive because of the inserted DNA.
Each bacterial colony is derived from a single cell that has taken up a single plasmid. Therefore, all the cells in a colony are genetically identical. Cells that do not take up a plasmid cannot grow and form colonies because they are killed by ampicillin.

RIBOZYMES

★ an RNA molecule w intrinsic enzyme-like activity, they can catalyse biochemical reactions in the complete absence of protein cofactors

★ **FEATURES**
- ☆ **single stranded** – fold & base-pair with a complementary region on itself ⇒ forming complex tertiary structure
- ☆ **catalytic ability of 2'- OH group** – can carry out nucleophilic attack
- ☆ **specific** – RNA can form hydrogen bonds via complementary base-pairing with other nucleic acid molecules ⇒ adds specificity to its catalytic activity
- ☆ **divalent cation (Mg^{2+}) attached**
  - ✶ neutralise –ve charge of sugar-phosphate backbone → lower the electrostatic repulsion between phosphate groups ⇒ promote proper folding of RNA into tertiary structures
  - ✶ form the catalytic core
    - ☾ bind to substrates & stabilise the structure of transition state during ES complex formation
    - ☾ involved in acid-base catalysis

★ **LARGE CATALYTIC RNAs**
- ☆ **group I introns**
  - ✶ found within rRNA
  - ✶ P4–P6 domain scaffolds the catalytic P3–P9 core; multiple Mg^{2+} ions
  - ✶ splicing mechanism:
    - ☾ 3'-OH of a guanosine cofactor attacks & cleaves the intron at the 5' splice site → frees the 5' exon
    - ☾ 3′-OH of freed 5′ exon attacks 3′ splice site → ligated exons; intron released
- ☆ **group II introns**
  - ✶ 6 domains (DI – DVI) radiating from a central core (like "spokes of a wheel")
    - ☾ DV forms the centre of the active site which contains a catalytic triad *(AGC or CGC)* & an AY bulge *(where Y can be any pyrimidine)*, → both bind catalytically important Mg^{2+} ions
  - ✶ splicing mechanism:
    - ☾ 2′-OH of branch-site A attacks 5′ splice site → 2′–5′ phosphodiester (lariat)
    - ☾ 3′-OH of 5′ exon attacks 3′ splice site → ligated exons; lariat intron removed
- ☆ **RNase P**
  - ✶ processes the 5' end of tRNA precursors
  - ✶ exists as a ribonucleoprotein complex
  - ✶ requires Mg^{2+} ions to promote proper folding of RNase P RNA and tRNA precursor, binding of RNase P RNA and tRNA precursor, and efficient catalysis

★ **SMALL CATALYTIC RNAs**

- ☆ **hammerhead**
  - ✷ commonly found in the viroids (lack protein coat) and satellite RNAs
  - ✷ 3 helical stems which are variable, and 3 highly conserved single stranded junctions which forms the catalytic core
  - ✷ cleavage occurs after an RUH triplet (arrow), where R is any purine, & H is any nucleotide except guanosine

- ☆ **hairpin**
  - ✷ found in 3 pathogenic, plant, satellite viruses
  - ✷ carries out reversible self-cleavage reaction to process the products of rolling circle genome replication
  - ✷ contains 4 helical stems which radiate from a perfectly base-paired four-way junction

IMPORTANCE TO GENETIC ENGINEERING

★ **AMINOACYLATING RIBOZYMES (ATRibs)**

- ☆ catalyse transfer of AA to the 3’ end of RNA
- ☆ **mechanism**
  - ✷ self-aminoacylation → ATRib attaches an AA to its own 5’-OH end
  - ✷ this AA is transferred from ATRib → 3’ end of a tRNA
- ☆ uses an internal guide seqeunce (IGS) to base pair w donor oglionucleotide ⇒ BUT since all tRNAs share the same CCA-3’ end, ATRibs can only distinguish tRNAs weakly
- ☆ **engineered ATRibs**
  - ✷ recognises both the CCA-3’ end & anticodon loop
  - ✷ mutations in the anti-codon loop allows design of ribozymes tthat selectively charge unique engineered tRNAs
- ☆ **importance to GE**
  - ✷ translation can occur outside a cell, w/o natural enzymes

★ **FLEXIZYMES (Fx)**

- ☆ charge AAs to tRNA via a 3 base pair interaction between the 3’-end of the ribozyme and the 3’-end of the substrate tRNA
- ☆ they recognise the activating group of the AA, NOT the codon ⇒ allow them to load almost ANY AA onto ANY tRNA
- ☆ **importance to GE**
  - ✷ <u>design new genetic codes</u> – as Fx only binds to the activating group, it can charge a tRNA w any AA → can assign unnatural/artificial AAs to any codon

IMPORTANCE OF GENETIC ENGINEERING

CATEGORY	EXAMPLE	GE METHOD	BENEFITS	RISKS / LIMITATIONS
food sustainability – *improving yield*	Bt corn	insert *bacillus thuringiensis* Cry gene	produces insect-specific toxin; reduces pesticide use; increases yield	toxin specific to certain insect orders; public perception issues
	Roundup Ready crops	mutated EPSP synthase gene resistant to glyphosate	allows weed control without harming crops	evolution of glyphosate-resistant 'superweeds'; increased herbicide use & runoff
	DroughtGard® corn	*bacillus subtilis* CSPB gene	improves water-use efficiency and drought tolerance	requires suitable water storage and soil conditions
	Scuba rice	SUB1A gene from indian rice	survives prolonged flooding; improves food security	access to technology may be limited in developing countries
	CRISPR-edited chickens	mutation in chNHE1 receptor	resistance to ALV-J virus; improves poultry health	regulatory and ethical issues
	GM Atlantic salmon	growth hormone cDNA under eel pout's antifreeze protein promoter	growth hormone is actively expressed throughout the year → faster growth rate → faster harvest → higher profit	risk of escape & ecological impact on wild salmon
food sustainability – *better quality*	Golden rice	inserting 3 β-carotene biosynthesis genes under an endosperm-specific promoter → ensure the genes are only expressed in the rice grain	prevents vitamin A deficiency; improves nutrition	acceptance issues; access in target regions

disease treatment	SCID gene therapy	insert functional ADA or IL2RG genes into patient t-lymphocytes via viral vector	restores immune function	some cases of leukemia due to insertion near oncogenes; repeated therapy needed
	sickle cell anaemia treatment	ex vivo CRISPR – edit HSCs to reactivate foetal Hb (BCL11A enhancer)	HSCs will specialise and produce non-Hb^s RBCs + no more need for transfusion dependence	high, high cost / CRISPR-specific: can currently only target single-gene causing diseases
	Cystic Fibrosis therapy	deliver normal CFTR gene to airway cells; CRISPR trials	restores chloride channel function; reduces symptoms	requires repeated delivery; immune response to viral vectors
drug design	Human insulin, growth hormone	rDNA technology in microbes	safe, consistent human protein production	production costs; cold chain needed
	Trastuzumab (Herceptin)	monoclonal antibody production	targeted cancer therapy	expensive; side effects
	HBV vaccine, mRNA Covid-19 vaccines	recombinant antigen production; synthetic mrna	effective prevention; rapid development	cold storage; vaccine hesitancy
	Warfarin pharmacogenomics	identify VKORC1, CYP2C9 variants	personalised dosing; reduces adverse events	requires genetic testing infrastructure
climate change	C4 rice project	insertion of C4 photosynthetic genes *(PEPC, NADP-ME, etc.)* into rice	improves photosynthetic efficiency → higher yields w less fertiliser/water	*too early to tell*

EPIGENETICS

DNA METHYLATION

★ transfer of a methyl group ($–CH_3$) to the 5th carbon of cytosine residue to form 5-methylcytosine (5mC), catalysed DNA methyltransferases (DNMTs)

★ ***de novo* DNMTs**
- ☆ Dnmt3a and Dnmt3b
- ☆ establish a new methylation pattern to unmodified DNA

★ **MAINTENANCE DNMTs**
- ☆ Dnmt1 functions during DNA replication – copy the DNA methylation pattern from the parental DNA strand onto the newly synthesised daughter strand

★ **EFFECT ON TRANSCRIPTION**
- ☆ inhibit the binding of transcription factor(s) to DNA
- ☆ recruits methyl-CpG binding proteins eg. MeCP2 → methyl-CpG binding proteins then recruit histone deacetylases & chromatin remodelling complex ⇒ increase the packing of chromatin
- ☆ DNA methylation in different genomic regions may exert different influences on gene activities based on the underlying genetic sequence
  - ✶ methylation of CpG islands within promoters ⇒ decreases gene expression
  - ✶ methylation of the gene body is associated w higher gene expression
    - ☾ *blocks cryptic promoters inside the gene that RNA pol. may bind to*
    - ☾ *may help prevent the transcription of non-coding regions (introns) within the gene*

HISTONE MODIFICATION

★ each histone has tails *(N-terminal ends)* that stick out from the nucleosome and can be chemically modified *(PTMs)* → changes how tightly DNA is packed, affecting whether genes can be transcribed

★ **WHY HISTONE MODIFICATIONS MATTER** – 'histone code'
- ☆ specific combinations of modifications act as signals to either activate or repress transcription
- ☆ modifications can change chromatin structure and/or protein recruitment *(eg. TFs, chromatin remodelers)*

★ **HISTONE MODIFICATIONS**
- ☆ **acetylation**
  - ✶ **enzymes:**
    - ☾ adding – histone acetyltransferases (HATs)
    - ☾ removing – histone deacetylases (HDACs)
  - ✶ **effect:** neutralises lysine's positive charge → reduces histone–DNA binding → chromatin loosens → transcription can occur
  - ✶ ***eg.*** *H3K9ac, H3K14ac → associated w active transcription*

- ☆ **methylation**
  - ✷ **enzyme:**
    - ☾ adding – histone methyltransferases (HMTs)
    - ☾ removing – histone demethylases (HDMs)
  - ✷ **effect:** activate / repress gene expression → depending on the residue & degree of methylation *(mono-, di-, or tri-methylation)*
  - ✷ ***examples:***
    - ☾ *H3K4me3 – active transcription (found in promoter regions)*
    - ☾ *H3K9me3, H3K27me3 – linked to heterochromatin formation*
    - ☾ *H3K36me3 – associated with actively transcribed gene bodies*

- ☆ **phosphorylation**
  - ✷ **enzyme:**
    - ☾ adding – kinases
    - ☾ removing – phosphatase
  - ✷ **effect:** associated w chromatin remodelling, DNA damage response, & mitotic chromosome condensation
  - ✷ ***eg.*** *H3S10ph promotes gene activation and mitosis*

- ☆ **ubiquitination**
  - ✷ **enzyme:**
    - ☾ adding – ubiquitin ligases
    - ☾ removing – deubitiquinases
  - ✷ **effect:** can signal either transcription activation or repression
  - ✷ ***examples:***
    - ☾ *H2BK123ub – promotes transcription elongation*
    - ☾ *H2AK119ub – associated with gene repression*

CHROMATIN REMODELLING

★ **ATP-dependent chromatin remodelling complexes** use energy from ATP hydrolysis to move, slide, or remove nucleosomes

- ☆ chromatin remodelling makes the genome highly dynamic — can rapidly change which genes are accessible in response to internal or external signals

★ **HOW IT WORKS**

- ☆ ATP hydrolysis provides energy for the remodelling complex to unwind DNA & change how tightly it's wrapped around histones
- ☆ DNA can be moved relative to the histone core → temporarily loosens binding → increases accessibility for transcription factors, replication machinery, or repair enzymes
- ☆ repeated ATP-driven cycles can cause nucleosome sliding – moving DNA along the histone core

★ **REMODELLING ACTIONS**

- ☆ histone exchange: replacing H2A–H2B dimers with variant forms that may alter chromatin properties
- ☆ histone core removal: removing the entire histone octamer → expose naked DNA

- ☆ nucleosome repositioning: shifting nucleosomes to new locations depending on other DNA-bound proteins (can promote or block access)

EPIGENETIC INHERITANCE

★ **DEFINITION –** transmission of patterns of gene expression without altering the DNA sequence

X INACTIVATION – DOSAGE COMPENSATION IN MAMMALS

★ **THE PROBLEM**
- ☆ females have 2 X chromosomes, while males only have 1 ⇒ if both X chromosomes were active, females would produce double the gene products compared to males
- ☆ X-inactivation randomly inactivates one X chromosome in early embryogenesis – 1 X chromosome becomes transcriptionally silent *(Barr body)*

★ **MECHANISM**
- ☆ **initiation:** X-inactivation centre (XIC) on X chromosome produces Xist lncRNA
- ☆ **spreading:** Xist RNA coats the chromosome → recruits PRC2 *(polycomb repressive complex)* → histone methylation & chromosome condensation
- ☆ antisense RNA Tsix protects the active X by silencing Xist on that chromosome

★ **EXAMPLES**
- ☆ **tortoiseshell cats:** random inactivation of X-linked coat-colour alleles → mosaic patterns
  - ✶ most tortoiseshell cats are female → they have the 2 X chromosomes
  - ✶ male tortoiseshell cats are due to non-disjunction
- ☆ **Duchenne muscular dystrophy *(X-linked)*:** female carriers may express some symptoms if the healthy X is inactivated in many cells

GENOMIC IMPRINTING

★ **DEFINITION –** monoallelic & parent-of-origin dependent expression of a subset of genes
- ☆ the copy of a gene an individual inherits from one parent is transcriptionally inactive, while the copy from the other parent is active

★ **MECHANISM**
- ☆ establishment of imprint during gametogenesis
- ☆ maintenance of the imprint during embryogenesis
- ☆ erasure & reestablishment of the imprint in the germ cells

★ **EXAMPLES**
- ☆ **Igf2 (insulin-like growth factor 2)** – only paternal allele is expressed *(see Fig. 1)*
- ☆ **Prader-Willi Syndrome (PWS)**

- ✷ deletion/silencing of paternal allele & maternal allele is normally imprinted
- ✷ symptoms: learning difficulties, short stature, & compulsive eating

☆ **Angelman Syndrome**

- ✷ deletion/silencing of maternal allele & paternal allele is normally imprinted
- ✷ symptoms: learning difficulties, speech problems, seizures, jerky movements, & unusually happy disposition

EPIGENETIC DISEASES

★ **CANCER**

- ☆ normally – there's a balance between oncogenes & tumour suppressors
- ☆ BUT hypermethylation of tumour suppressor promoters → silencing of tumour suppressors
  - ✷ *eg. MLH1 (DNA mismatch repair gene) hypermethylated in colorectal cancer*

★ **ALZHEIMER'S**

- ☆ normally – epigenetic marks regulate genes involved in synaptic function, learning & memory
- ☆ BUT hypomethylation of amyloid precursor protein (APP) & tau-related genes → overexpression → amyloid plaques & neurofibrillary tangles

★ **TYPE 2 DIABETES**

- ☆ methylation of insulin promoter in pancreatic β cells → reduced insulin transcription

ANNEX

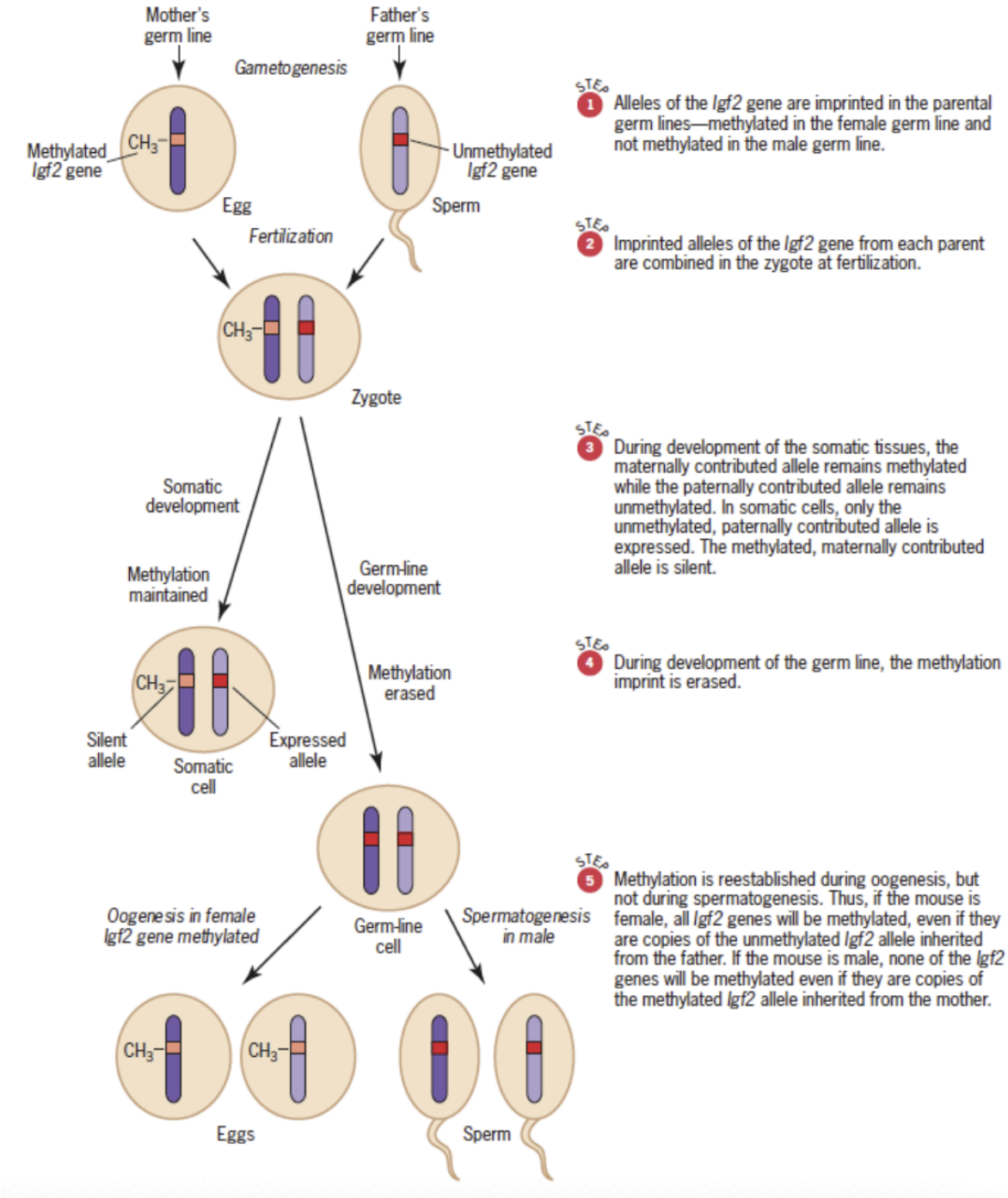


Fig. 1 – Methylation & imprinting of Igf2 gene in mice

X v. Y CHROMOSOMES

Feature	X Chromosome	Y Chromosome
Size	Large (~155 Mb, ~5% of genome)	Very small (~58 Mb, <2% of genome)
Gene content	~1,100 genes	~70–200 genes (depending on individual)
Functions	Carries genes essential for normal cellular function (metabolism, signalling, transcription, etc.) and sex determination	Carries male-specific genes, especially those for sex determination (SRY) and spermatogenesis
Inheritance	Females inherit two (XX), males inherit one (XY)	Passed only from father to son (paternal inheritance)
Dosage compensation	One X in females is inactivated (Barr body) to equalise gene expression between sexes	No inactivation (always active)
Sex-linked diseases	Many X-linked disorders (e.g., haemophilia, Duchenne muscular dystrophy) as males have no “backup” allele	Few Y-linked disorders; traits restricted to males (e.g., some infertility cases)
Recombination	Recombines with Y only in small pseudoautosomal regions (PARs)	Recombines with X only at PARs; most of Y does not recombine → accumulates mutations
Unique genes	Contains genes unrelated to sex (e.g., for brain, immune, and metabolic functions)	Contains SRY (sex-determining region Y) gene → initiates male development; also AZF genes for spermatogenesis
Evolutionary note	Conserved across mammals	Shrinking over evolutionary time (lost most genes, retains male-specific functions)

🪼 CORE IDEA 3

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C3, C4 & CAM PLANTS

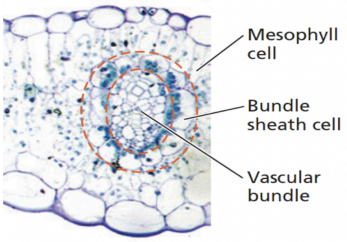
C3 PLANTS

★ **THE PROBLEM –** C3 plants are susceptible to photorespiration under high stress
- ☆ C3 plants use Rubisco for the carbon fixation stage of the Calvin Cycle, but Rubisco can also bind to O_2 instead of CO_2

★ **PHOTORESPIRATION**
- ☆ **wasteful –** it consumes ATP & NADPH, while releasing CO_2 & not producing glucose
- ☆ **increases when temp is high**
  - ✶ Rubisco's affinity for O_2 increases
  - ✶ stomata closes to reduce transpiration, but it causes CO_2 levels to drop & O_2 build up

C4 PLANTS

★ **C4 LEAF STRUCTURE** – kranz anatomy
- ☆ **bundle sheath cells** – main site of Calvin Cycle, w high levels of Rubisco, packed around vascular tissue
- ☆ **mesophyll cells** – contains PEPC, loosely arranged around bundle sheath cells



⇒ creates a diffusion barrier that (1) separates CO_2 uptake in mesophyll from Rubisco activity in bundle sheath cells, and (2) reduces CO_2 leakage back to mesophyll cells

★ **SPATIAL SEPARATION OF INITIAL CARBON FIXATION & CALVIN CYCLE**
- ☆ **<u>mesophyll cells:</u>**
  - ✶ CO_2 is ionised into HCO_3^- which is added to 3C PEP by PEPC → 4C oxaloacetate formed
    - ☾ PEPC has no affinity for O_2 as it reacts w HCO_3^- not CO_2 (g) ⇒ can continue to fix CO_2 even when O_2 conc. is high
  - ✶ oxaloacetate converted to 4C acid *(malate/aspartate)* → transported to bundle sheath cells via plasmodesmata
- ☆ **<u>bundle sheath cells:</u>**
  - ✶ 4C acid is decarboxylated → CO_2 released & pyruvate regenerated
    - ☾ pyruvate is transported back to mesophyll cells → ATP converts pyruvate into PEP
  - ✶ CO_2 released by malate are fixed by Rubisco & enters Calvin Cycle

★ **HIGH OPTIMUM TEMP OF ENZYMES**
- ☆ C4 plants operate efficiently at temperatures above 30°C – *where C3 plants starts photorespiration*
- ☆ PEPC and associated enzymes in the C4 pathway have higher thermal stability and optimum temp
- ☆ enhanced use of cyclic photophosphorylation supports high ATP demand required for regenerating PEP in mesophyll cells, especially when PSII activity is reduced due to heat stress

CAM PLANTS

★ **TEMPORAL SEPARATION OF CARBON FIXATION FROM LIGHT-DEP STAGE**
- ☆ **night-time (cooler)**
  - ✷ stomata opens to reduce water loss
  - ✷ CO_2 is fixed by PEPC into oxaloacetate → 4C → stored in vacuoles
- ☆ **day-time (hotter)**
  - ✷ stomata remains closed to prevent transpiration
  - ✷ malate is decarboxylated → CO_2 released enters Calvin Cycle

⇒ temporal separation avoids water loss while enabling photosynthesis

★ **ADDITIONAL ADAPTATIONS**
- ☆ **thicker cuticles –** reduces evaporation of water from the leaf
- ☆ **sunken stomata –** pits trap moist air, lowering water potential gradient & slowing transpiration
- ☆ **fewer stomata –** fewer openings for water vapour to escape
- ☆ **water-storing tissues in leaves –** increase drought resistance

ALGAE

★ **PYRENOID BASED CO_2 CONCENTRATION MECHANISM (pCCM)**
- ☆ **CO_2 uptake and conversion** – cells take up CO_2 which is converted to HCO_3^-
- ☆ **HCO_3^- transport** – HCO_3^- is transported into the pyrenoid
- ☆ **CO_2 concentration** – within the pyrenoid, HCO_3^- is converted back to CO_2, creating a high local concentration of CO_2 around Rubisco
- ☆ **Rubisco activity** – elevated CO_2 conc ensures that Rubisco primarily binds CO_2 rather than O_2 ⇒ reducing photorespiration and enhancing photosynthetic efficiency
- ☆ **compartmentalisation** – the pyrenoid acts as a diffusion barrier, slowing the escape of CO_2 from the vicinity of Rubisco

★ **REEF-BUILDING CORALS & ZOOXANTHELLAE MUTUALISTIC R/S**
- ☆ **corals provide:**
  - ✷ a protected environment
  - ✷ the coral polyp cells produce CO_2 & H_2O that the zooxanthellae need for photosynthesis
- ☆ **zooxanthellae provide:**
  - ✷ use energy from the sun to turn the CO_2 and H_2O into O_2
  - ✷ help the coral to remove wastes
  - ✷ the building blocks of sugars and proteins, which are the products of photosynthesis
    - ☾ the coral uses these products to make proteins, fats, and carbohydrates, and produce $CaCO_3$ ⇒ leads to coral growth & reproduction

★ **CARBON SEQUESTRATION**
- ☆ they can fix large amt. of CO_2, contributing ~50% of global carbon fixation
- ☆ **fast-growing** – absorb CO_2 rapidly

- ☆ as algae grow in water bodies, when they die, the dead algae sinks, storing carbon long-term as they don't decompose to release CH_4 & CO_2

GLOBAL WARMING MITIGATION

PLANT	STRATEGY	PROS	CONS
C3	direct CO_2 fixation via Rubisco	widespread, major biomass producers in temperate climates	high photorespiration at high stress conditions
C4	spatial separation	highly efficient in hot, sunny climates; low photorespiration	limited to specific ecological niches
CAM	temporal separation	high water-use efficiency; good in arid env	slow growth rates; energy costly to store/release malate
algae	aquatic photosynthesis & carbon pumps	responsible for ~50% of global CO_2 fixation; fast growing	sensitive to temperature changes; causes eutrophication

★ **REEF-BUILDING CORALS**
- ☆ algae *(zooxanthellae)* fix carbon and contribute to reef growth
- ☆ loss of algae *(eg. bleaching)* threatens reef ecosystems and carbon sequestration

★ **CARBON SINKS**
- ☆ algae is considered **blue carbon**, which is better at sequestering carbon

CONTROL & FEEDBACK MECHANISMS

★ **IMPORTANCE**
- ☆ ensures optimal conditions for cellular activity
- ☆ allows organisms to remain independent of environmental fluctuations

HOMEOSTASIS

★ **DEFINITION:** maintenance of a relatively constant internal environment

★ **IMPORTANCE**
- ☆ prevents enzyme denaturation
- ☆ ensures metabolic efficiency
- ☆ maintains optimal conditions for survival

★ **COMPONENTS OF HOMEOSTATIC MECHANISM**
- ☆ **set point –** desired normal range *(eg. 37ºC for body temp)*
- ☆ **stimulus –** change in the env
- ☆ **receptor –** detects stimulus, sends to the control centre
- ☆ **control centre –** sends instructions to effector
- ☆ **effector –** acts to restore set point
- ☆ **feedback**
  - ✷ **negative feedback –** restores to set point *(eg. thermoregulation)*
  - ✷ **positive feedback –** amplifies change *(eg. blood clotting)*

COMMUNICATION SYSTEMS

★ **IMPORTANCE**
- ☆ needed for integrating & coordinating the physiological processes of organisms to maintain homeostasis

★ **TYPES OF COMMUNICATION**
- ☆ **direct contact** – gap junctions or surface molecules
- ☆ **local signalling** – paracrine & synaptic signalling
- ☆ **long-distance signalling** – endocrine (hormonal)

★ **2 MAIN SYSTEMS**
- ☆ **nervous system**
  - ✷ fast, electrical signalling via neurones
  - ✷ used for reflexes, movement & acute regulation
- ☆ **endocrine system**
  - ✷ slower, hormonal signalling via bloodstream
  - ✷ used for long-term processes: growth, metabolism, & reproduction

NERVOUS SYSTEM

★ **MAINTENANCE OF RESTING MEMBRANE POTENTIAL (-70mV)**

- ☆ **Na^+/K^+ pump –** actively transports 2 K^+ into and 3 Na^+ out of the cell
- ☆ **leak channels –** K^+ moves out of & Na^+ into the cell down their conc. gradients
  - ✶ there are very few Na^+ leak channels, and numerous K^+ ⇒ loss of K^+ is greater than gain of Na^+

⇒ more negative inner-membrane env.

ACTION POTENTIAL (AP)

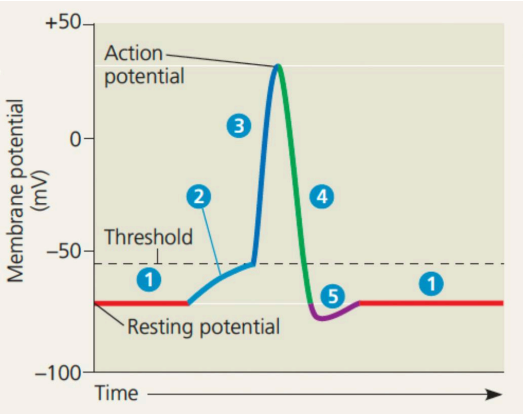
★ **GRADED V. ACTION POTENTIAL**

- ☆ **graded potential** – a **small, localised change** in membrane potential that occurs in response to a stimulus; the change can be:
  - ✶ **depolarisation** – reduction in magnitude of membrane potential
  - ✶ **repolarisation** – increase in magnitude of membrane potential
- ☆ multiple graded potentials summate & reach the threshold potential (-55mV) at the axon hillock ⇒ **AP is triggered**

★ **PHASES OF AN ACTION POTENTIAL**

- ☆ **(1) resting state**
  - ✶ resting membrane potential is maintained
  - ✶ all gated channels are closed
- ☆ **(2) + (3) depolarisation**
  - ✶ a small stimulus opens **ligand-gated Na^+ channels** on the neurone
  - ✶ Na^+ enters the cell, making the inside **less negative**
  - ✶ if this depolarisation brings the membrane potential to **−55 mV** (the threshold):
    - ☾ **VG Na^+ channels open**
    - ☾ more Na^+ rushes in, causing a rapid depolarisation
    - ☾ this is a **positive feedback loop**: more Na^+ entry → more depolarisation → more channels open

  ⇒ AP is triggered
- ☆ **(4) repolarisation**
  - ✶ at +40 mV, VG Na^+ channels close and become inactivated
  - ✶ VG K^+ channels open, increasing K^+ permeability
  - ✶ K^+ efflux makes the inside more negative again ⇒ membrane potential to drop back toward −70 mV
    - ☾ *K^+ efflux is slower than Na^+ influx earlier, because K^+ channels open more slowly*
- ☆ **(5) hyperpolarisation**



- ✷ VG K+ channels remain open after repolarisation ⇒ K+ continues to move out causing membrane potential to become more negative than at rest (-75mV)

★ **REFRACTORY PERIOD**

- ☆ **absolute refractory**
  - ✷ depolarisation - hyperpolarisation
  - ✷ no new AP can occur, regardless of stimulus strength
  - ✷ occurs when VG Na^+ channels are inactivated (from depolarisation to early repolarisation)
- ☆ **relative refractory**
  - ✷ restoration phase
  - ✷ new impulses can be propagated only if stimulus is strong enough to reach threshold
- ☆ **significance**
  - ✷ ensures unidirectional propagation of AP
  - ✷ prevents overlap between signals – keeps them spaced out
  - ✷ limits firing frequency, helping to prevent neurone fatigue

★ **PROPERTIES OF ACTION POTENTIAL**

- ☆ **all or nothing principle –** the threshold potential is the minimum strength a stimulus must reach in order for it to initiate an AP ⇒ a stimulus either evokes a full response or no response at all
  - ✷ it ensures that the signal transmitted is consistent and not weakened by weaker stimuli
- ☆ all APs have a **constant magnitude** (+30mV), regardless of the strength of the stimulus
  - ✷ a stronger stimulus increases the **frequency**, not the magnitude

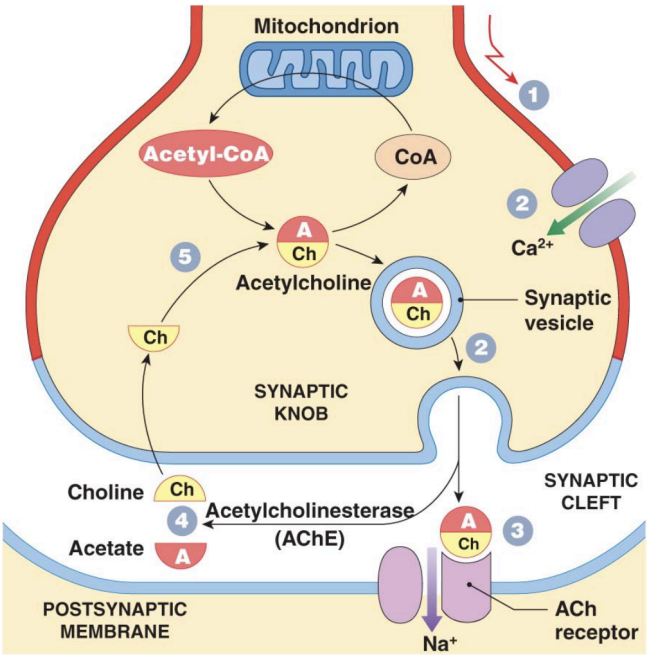
★ **TRANSMISSION OF ACTION POTENTIAL**

- ☆ **propagation of AP**
  - ✷ Na^+ influx at one site causes local depolarisation
  - ✷ this depolarises adjacent regions (backwards & forwards), bringing them to threshold → triggers new APs
  - ✷ AP moves forward only because the region behind is in its refractory period
- ☆ **types of conduction**
  - ✷ **continuous conduction** – unmyelinated neurones
    - ☾ AP moves along the entire membrane
  - ✷ **saltatory conduction** – myelinated neurones
    - ☾ AP jumps from node to node *(nodes of Ranvier)*
    - ☾ faster and more efficient due to insulation from myelin

CHOLINERGIC SYNAPSE

★ STRUCTURE

- ☆ **presynaptic neurone:**
  - ✶ embedded VG Ca^{2+} channels
  - ✶ synaptic knob – contains many mitochondria, synaptic vesicles (filled w NTs like ACh), & microfilaments
- ☆ **synaptic cleft** about 20nm wide
- ☆ **postsynaptic neurone:**
  - ✶ embedded receptors for NTs, which can function as ligand-gated ion channels



★ FUNCTION

- ☆ **transmit information between neurones**
- ☆ **transmit impulses unidirectionally**
  - ✶ NTs are only in synaptic knob, receptors only on postsynaptic membrane
- ☆ **enable integration of signals via summation**
  - ✶ allows neurons to integrate information from multiple sources and make complex decisions about whether to transmit a signal
- ☆ **filter out low-level stimuli**
  - ✶ low NT release = no AP
  - ✶ not enough NT released to depolarise to the threshold
- ☆ **prevent over-stimulation**
  - ✶ fatigue occurs if NT release > synthesis rate as vesicles cannot refill fast enough
  - ✶ acts as a protective mechanism to prevent overexcitation of neurones *(cause seizures)* & damage to effectors

★ SYNAPTIC TRANSMISSION ACROSS CHOLINERGIC SYNAPSE

- ☆ AP reaches synaptic knob → depolarisation
- ☆ VG Ca^{2+} channels open → Ca^{2+} influx
- ☆ Ca^{2+} triggers vesicle fusion with membrane → ACh released via exocytosis (ATP needed)
- ☆ ACh diffuses across cleft → binds to ligand-gated Na^{+} channels on postsynaptic membrane
- ☆ Na^{+} influx → depolarisation → excitatory postsynaptic potential (EPSP)
- ☆ synaptic delay: brief time for NT release + diffusion
- ☆ ACh is hydrolysed by acetylcholinesterase into choline + acetate
- ☆ choline reabsorbed into presynaptic knob and ACh re-synthesised
- ☆ resting potential is restored

★ **GENERATION OF POSTSYNAPTIC POTENTIAL**

- ☆ **postsynaptic potential can be:**
  - ✷ **excitatory postsynaptic potential (EPSP) –** causes the membrane to be more positive (ie. depolarisation) ⇒ more likely to generate AP
  - ✷ **inhibitory postsynaptic potential (IPSP) –** causes the membrane to be less positive (ie. hyperpolarisation) ⇒ less likely to generate AP
- ☆ **summation of postsynaptic potentials –** additive effect for APs to be generated due to buildup of EPSPs (reverse is true for IPSPs):
  - ✷ **temporal summation** – 1 presynaptic neurone sends multiple consecutive impulses ⇒ signals combine to reach the threshold
  - ✷ **spatial summation** – multiple presynaptic neurones simultaneously sends impulses ⇒ signals combine to reach the threshold

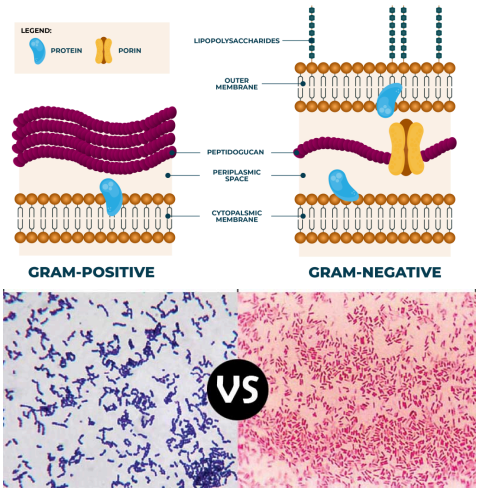
★ **TERMINATION OF NEUROTRANSMITTER SIGNALLING**

- ☆ enzymatic hydrolysis of NT
  - ✷ ACh is hydrolysed by acetylcholinesterase into choline + acetate
- ☆ reuptake of NT by presynaptic neurone

QUORUM SENSING

★ GRAM POSITIVE V. GRAM NEGATIVE BACTERIA

- ☆ **gram-positive**
  - ✶ no outer membrane
  - ✶ thick peptidoglycan cell wall
  - ✶ stain blue-purple
- ☆ **gram-negative**
  - ✶ contain an outer membrane w lipopolysaccharides (LPS)
  - ✶ thin peptidoglycan cell wall between outer & inner membrane
  - ✶ stain red



★ QUORUM SENSING (QS)

- ☆ Bacteria use QS by releasing autoinducers (AIs) to **monitor population density** and **regulate gene expression** ⇒ enabling coordinated colony-wide behaviours like sporulation, bioluminescence, virulence, conjugation, competence, and biofilm formation

★ QS MECHANISM

- ☆ bacteria produce AIs that diffuse out during reproduction
- ☆ as more bacteria grow, AI conc. outside increases, reaching a threshold
- ☆ at threshold, it becomes energetically unfavourable for AIs to leave the cell
- ☆ AIs build up inside and bind receptors, triggering signalling cascades
- ☆ this alters TF activity, often reducing AI production and activating group behaviours

★ QS IN GRAM POSITIVE BACTERIA

- ☆ use peptides (AIPs) as AIs
- ☆ AIPs are processed and secreted via active transport (ABC transporter) through the thick cell wall
- ☆ **at high density:**
  - ✶ AIPs bind to membrane-bound histidine kinase receptors
  - ✶ kinase undergoes autophosphorylation → phosphate passed to response regulator
  - ✶ this activates transcription of QS regulon genes
- ☆ in some cases, AIPs are taken back into the cytoplasm to directly affect TFs

★ QS IN GRAM NEGATIVE BACTERIA

- ☆ use small molecules (eg. AHLs) as AIs
- ☆ AIs freely diffuse across membranes and thin cell wall
- ☆ **at high density:**
  - ✶ AIs bind to cytoplasmic receptors (usually TFs) ⇒ regulate gene expression in the QS regulon
- ☆ in some cases, AIs bind membrane-bound histidine kinase receptors, similar to gram-positive

★ **CHOLERA INFECTION**

- ☆ *Vibrio Cholerae* is a gram-negative bacterium
- ☆ it uses QS to regulate:
  - ✷ **biofilm production** – for protection & nutrient transport
  - ✷ **virulence factors** – for infection & colonisation
    - ☾ *cholera toxin (CTX), toxin coregulated pilus (TCP)*
  - ✷ controlled by LuxO system & changes in cell density:

CONDITION	LOW CELL DENSITY	HIGH CELL DENSITY
AI level	low	high
CqsS receptor activity	acts as kinase	acts as phosphatase
LuxO status	phosphorylated → LuxO-P *(active)*	dephosphorylated → LuxO *(inactive)*
effect on sRNA	activated → produces sRNAs	sRNA production stops
AphA level	increases → turns ON virulence & biofilm genes	decreases
HapR level	decreases → cannot repress genes	increases → represses virulence & biofilm genes
biofilm & virulence	ON → infection & colonisation begins	OFF → conserve energy & avoid immune detection

★ **PROBIOTICS**

- ☆ probiotics produce:
  - ✷ **Quorum Quenching Enzymes (QQE)** – inactivates QS signals *(breakdown AIs like AHL)*
  - ✷ **Quorum Sensing Inhibitors (QSI)** – disrupts QS pathways & reduce QS-controlled functions
- ☆ **purpose** – as an alternative to antibiotics**:**
  - ✷ reduce bacteria pathogenicity
  - ✷ inhibit biofilm production
- ☆ **<u>types of QQEs:</u>**
  - ✷ **class I –** hydrolyses AHLs thus deactivating lactone ring
  - ✷ **class II –** convert carbonyl group to hydroxyl

LACTIC ACID BACTERIA

★ *eg. Lactobacillus, Lactococcus, Streptococcus*

★ they inhibit pathogens by producing:

- ☆ **lactic acid**
  - ✷ lowers pH → acidifies cytoplasm ⇒ inhibits growth
- ☆ **bacteriocins**
  - ✷ pore formation in membrane → ion leakage → energy loss ⇒ death
- ☆ **H_2O_2 *(an OA)***
  - ✷ increases membrane permeability
  - ✷ causes oxidative damage to DNA & proteins

ATMOSPHERIC OXYGEN & EVOLUTION

TIME /bya	EVENT	SIGNIFICANCE
~4.6	formation of Earth	early atmosphere was anoxic, life was anaerobic
~3.5	appearance of cyanobacteria	beginning of oxygenic photosynthesis, slowly releasing O_2
~2.4	Great Oxidation Event (GOE)	O_2 began to accumulate in the atmosphere; reacted w Fe^{2+} to form banded iron
~2.0	rise in aerobic respiration	more efficient ATP production enabled more complex metabolism
~1.5-1.8	origin of eukaryotic cells *(endosymbiosis theory)*	mitochondria allowed sustained aerobic respiration
~0.6-1.0	formation of ozone (O_3) layer	protected organisms from UV; enabled life on land
<0.6	explosion of multicellular life *(eg. Cambrian Explosion)*	high O_2, supported large, energy-demanding organisms

★ **EVIDENCES SUPPORTING THIS**

- ☆ **stromatolites –** fossilised microbial mats formed by cyanobacteria
- ☆ **isotope ratios –** evidence of oxygen-dependent biological processes
- ☆ **banded iron formation –** evidence of oxygen present as O_2 reacted w Fe^{2+} in oceans

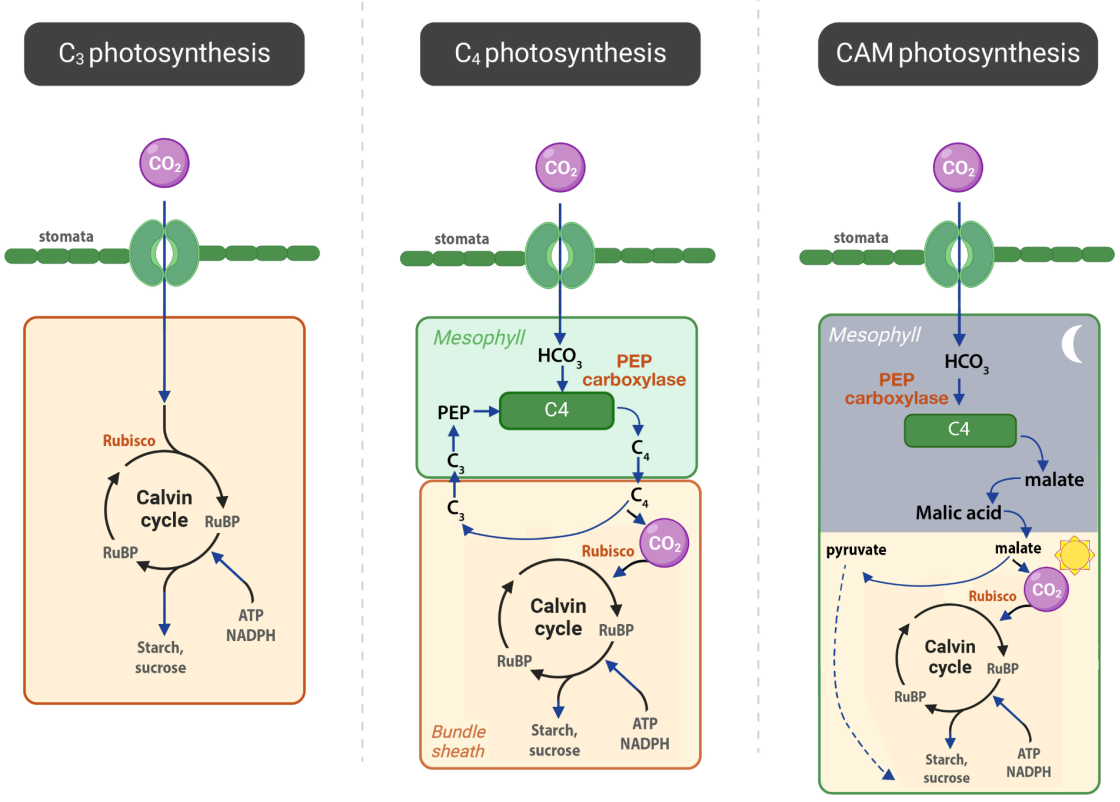
ANNEX

C4 PLANTS

★ **dimorphic chloroplasts:**

- ☆ bundle sheath cells – starch-rich w unstacked thylakoid membranes
- ☆ mesophyll cells – smaller, well-developed grana

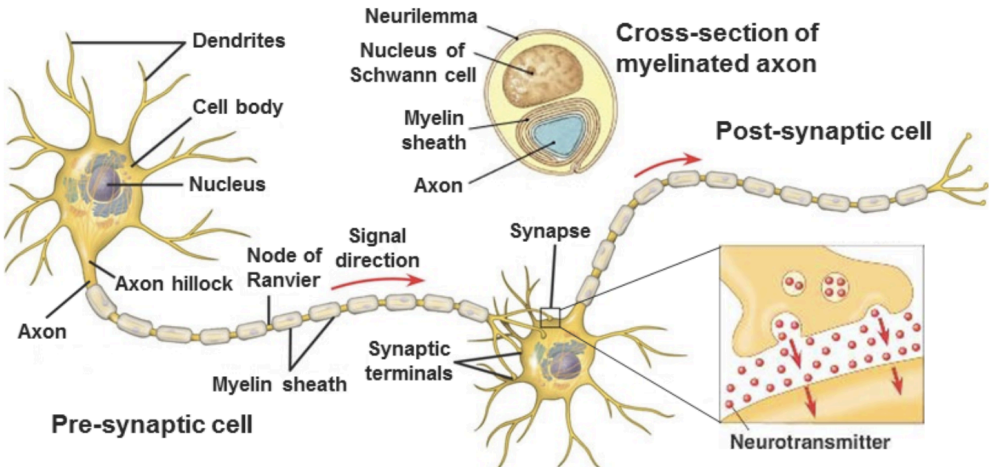
FEATURE	BUNDLE SHEATH	MESOPHYLL
cell wall	thick walls, impermeable to gas	thin walls, permeable to gas
central vacuole	smaller	larger
chloroplast	larger	smaller
main chloroplast enzyme	Rubisco	PEPC
thylakoid membrane	unstacked, no grana	stacked, well-dev grana
starch in chloroplast	abundant	absent
chloroplast arrangement in cell	concentrically arranged	randomly arranged



NERVOUS SYSTEM

General Structure of Neurone:

- **Cell body**: contains most of its organelles.
- **Dendrites**: sensitive branches from the cell body. They receive incoming signals, and transmits signals towards the cell body.
- **Axon**: an elongated extension from the cell body. It carries outgoing signals, and conducts signals away from the cell body towards **axon terminals**.
- **Schwann cells** (a type of glial cells): cover axons, forming a fatty **myelin sheath**. The outermost layer of a Schwann cell surrounding the myelin is called the **neurilemma**. Myelin serves as electrical insulation, increasing the speed of nervous transmission. Small gaps between adjacent Schwann cells are called **nodes of Ranvier**.
- **Synapse**: junction between an axon terminal of a neurone and another neurone or other cell.



★ TYPES OF MEMBRANE CHANNELS

- ☆ leak channels – always open, but its permeability varies
- ☆ gated channels
  - ✶ voltage-gated – open when membrane potential changes
  - ✶ chemically-gated – opens when a ligand binds to it

★ DIFFERENCE IN SPEEDS OF NA & K VG CHANNELS

- ☆ the fast opening of Na^+ channels initiates the action potential
- ☆ the slower opening and closing of K^+ channels allows for the repolarization and after-hyperpolarization that are critical for resetting the neuron and preparing it for the next signal

phase	VG Na^+ channels	VG K^+ channels	na^+/k^+ pump	leak channels
resting	closed	closed	active	always open
graded potential	some open	closed	active	
depolarisation	open	closed	inactive	
repolarisation	inactivated	open	inactive	
hyperpolarisation	inactivated	still open (slow to close)	inactive	
restoration	reset to closed	reset to closed	active again	

QUORUM SENSING

★ **GRAM STAINING**

- ☆ **(1) crystal violet** – primary stain → all bacteria turn purple
- ☆ **(2) iodine** – forms a CV-I complex that fixes the stain inside cells
- ☆ **(3) alcohol/acetone** – decolouriser
  - ✶ dehydrates peptidoglycan and dissolves outer membrane in gram-negative bacteria ⇒ this is the step that differentiates the bacteria
- ☆ **(4) safranin** – counterstain → stains gram-negative bacteria pink/red

	gram positive	**gram negative**
peptidoglycan layer	thick	thin
outer membrane	absent	present
CV-I retention (3)	retained due to thick peptidoglycan wall	lost (becomes colourless)
final colour	purple	red

POSITIVE V. NEGATIVE FEEDBACK

feature	**Positive Feedback**	**Negative Feedback**
definition	process where the output amplifies the initial stimulus, leading to an increased response	process where the output counteracts the initial stimulus, restoring balance
direction of change	reinforces and enhances the original change	reverses and opposes the original change
examples in humans	• oxytocin release during childbirth – *uterine contractions amplify hormone release* • blood clotting cascade – *thrombin activates more clotting factors*	• blood glucose regulation by insulin and glucagon • body temperature regulation (sweating/shivering)
end point	continues until an external factor or endpoint stops it	continues indefinitely to maintain dynamic equilibrium
response to disturbance	small disturbance → exaggerated large response	small disturbance → corrective action to minimise deviation
adaptiveness	for processes that need a fast, irreversible outcome	for homeostasis and long-term survival
energy use	often energy-intensive because it escalates activity	energy-efficient, since it conserves resources by fine-tuning

risk	Can be harmful if not terminated *(eg. cytokine storm in immune response)*	Generally protective, but if it fails → disease *(eg. diabetes)*

🦀 CORE IDEA 4

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SEXUAL SELECTION

★ **DEFINITION –** a form of natural selection in which individuals with certain **inherited traits** have a **higher likelihood** of obtaining mates compared to others of the same sex within a species

★ **WHY SEXUAL REPRODUCTION?**

BENEFITS	COSTS
crossing over in meiosis allows for creation of unique combinations of alleles	**twofold cost of sex** ★ only half the population (females) directly produce offspring ★ each parent only passes 50% of their genes ⇒ breaking up favourable genetic combinations
bring separate beneficial mutations faster than that occurring spontaneously	time & energy to search for a mate
purge delirious mutations	time & energy spent on courtship
accelerate adaptive evolution ★ red queen hypothesis – organisms must continually develop new adaptations *(eg. defences against parasites)* to survive in a changing environment	risk of getting sexually transmitted parasites/diseases

★ **SEXUAL DIMORPHISM**

- ☆ refers to the difference in appearance/behaviour between 2 sexes of the same species
- ☆ affected by **extent of parental care** – the sex that invests less in parental care often experiences stronger sexual selection *(ie. greater sexual dimorphism)*
  - ✷ **<u>female-only parental care</u>** – *eg. peacocks, deers*:
    - ☾ females invest more v. males are free to seek multiple mates
    - ☾ males compete for access to limited females & females are more selective of their mates
    - ☾ ⇒ sexual dimorphism resulting in males are larger, more colourful, aggressive or ornamented
  - ✷ **<u>biparental care</u>** – *eg. songbirds, penguins*:
    - ☾ as both are equally invested in providing parental care ⇒ both will not be able to invest as much energy in pursuing additional mating opportunities
    - ☾ ⇒ results in sexual monomorphism – both look & behave similarly
  - ✷ **<u>male-only parental care</u>** – *eg. phalaropes*:
    - ☾ females compete for access to males → males becomes the limiting resource
    - ☾ ⇒ results in reverse sexual dimorphism where females are the larger, more aggressive & ornamented ones

★ MECHANISMS OF SEXUAL SELECTION

- ☆ **intrasexual selection** – competition within the same sex for access to mates
  - ✷ traits evolve to outcompete members of the same sex, usually involving direct combat or sperm competition
    - ☾ *eg. large antlers in red deer, horns in beetles, specialised damselfly genitalia to remove rival sperm*
  - ✷ other tactics include alternative mating strategies or infanticide *(eg. lions)*
- ☆ **intersexual selection** – mate choice, where one sex selects mates based on specific traits
  - ✷ traits evolve to attract the opposite sex, such as colourful plumage, courtship displays, or complex songs
    - ☾ *eg. female long-tailed widowbirds prefer males with longer tails; peacocks display extravagant tails to attract females*

★ MODELS EXPLAINING FEMALE PREFERENCE

- ☆ **good genes hypothesis** – female preference targets traits indicating high-quality genes
  - ✷ *eg. parasite resistance in sticklebacks*
- ☆ **zahavi's handicap principle** – costly traits are 'honest signals' as only healthy individuals can afford them
  - ✷ *e.g peacock tails*
- ☆ **fisherian runaway hypothesis** – arbitrary preference for a trait & the trait itself becomes genetically linked & exaggerated over generations
  - ✷ both the preference & the trait itself is passed on → positive feedback loop
  - ✷ BUT as these traits become more exaggerated, driven by aesthetic or arbitrary female preference, they can become detrimental to the organisms' survival
  - ✷ *has not been directly observed before*

ADAPTIVE RADIATION

★ **DEFINITION:** a single ancestral species evolves into a wide array of descendant species that differ greatly in their habitat, form, or behaviour
- ☆ **must-know examples** *(check 'example bank' tab for details)*
  - ✷ *Galapagos Finches, Hawaiian Honeycreepers, Hawaiian Silversword Alliance* ⇒ caused by island isolation facilitating allopatric speciation

EFFECT OF MASS EXTINCTIONS

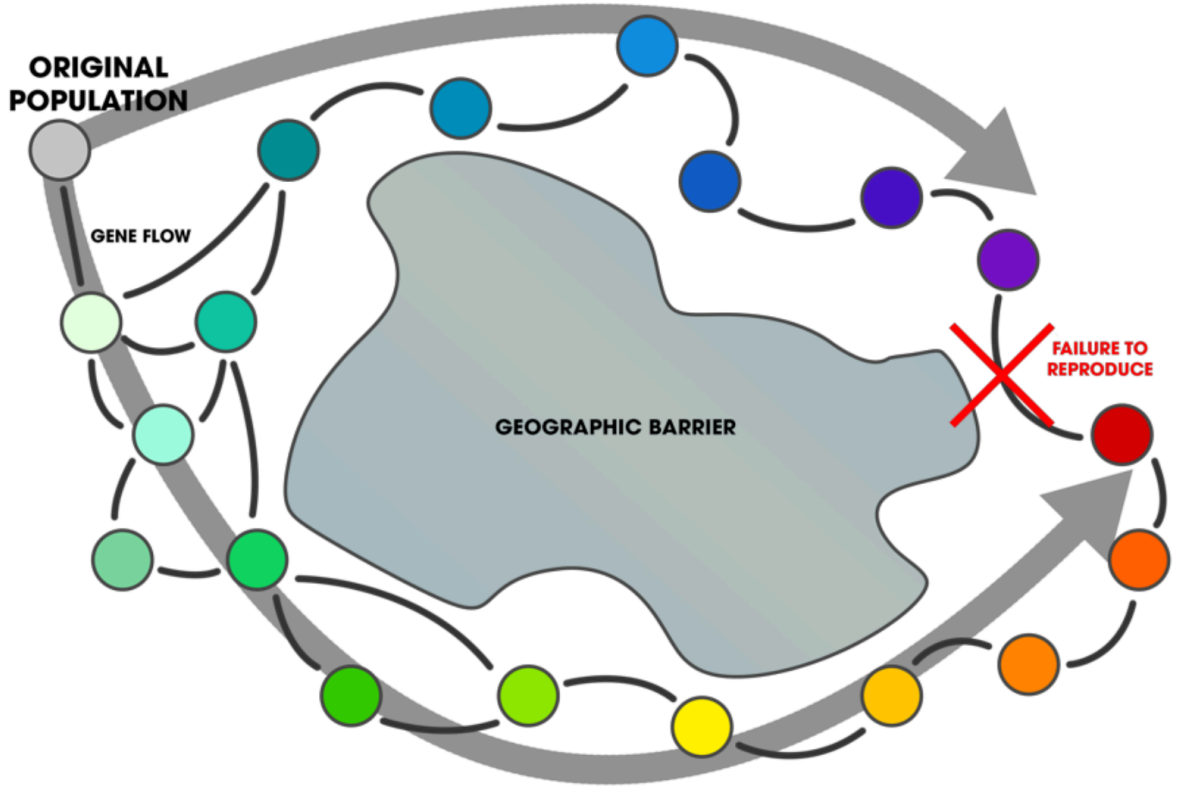
★ the extinction of dominant species frees up ecological niches, allowing surviving lineages to diversify and occupy these newly available niches

★ **MASS EXTINCTION EVENTS**
- ☆ **Ediacaran (541 mya)**
- ☆ **End Cambrian (485 mya)**
- ☆ **End Ordovician (444 mya)** – due to late ordovician glaciation
- ☆ **Late Devonian (360 mya)**
- ☆ **End Permian (250 mya)** – due to flood basalt volcanic eruptions that created the Siberian Traps
- ☆ **End Triassic (200 mya)** – due to extensive volcanic eruptions in the Central Atlantic Magmatic Province (CAMP)
- ☆ **End Cretaceous (65 mya)** – due to asteroid impact in Yucatan, Mexico that killed all the dinosaurs

RING SPECIES

★ **DEFINITION:** species that form a geographic 'ring' of connected populations
- ☆ gene flow occurs between neighbouring populations, but terminal populations are reproductively isolate despite being in contact

★ **key criteria** *(Irwin et al.)*:
- ☆ 2 terminal populations coexist in the same location but don't interbreed
- ☆ a ring of interbreeding populations connect them geographically
- ☆ gradual genetic & phenotypic change occurs around the ring



★ **SIGNIFICANCE**:
- ☆ **shows that speciation can despite gene flow**
  - ✶ challenges the idea that total genetic isolation is always necessary for speciation
  - ✶ parapatric speciation – NOT allopatric / sympatric
- ☆ **demonstrates stepwise evolution & how small changes can lead to reproductive isolation**
  - ✶ present the continuity between evolution within species and between species
- ☆ **highlights importance of geography & gradual divergence**
  - ✶ the physical layout of the environment (like a mountain or body of water) shapes how populations interact and spread
  - ✶ in ring species, gradual geographical spread & isolation-by-distance allow for divergence to occur

POLYPLOIDY, HYBRIDISATION, AND INTROGRESSION

★ **PHYLOGENETIC SPECIES CONCEPT (PSC)** – share a most recent common ancestor and can be distinguished from other such groups; they occupy a single branch on the phylogenetic tree
- ☆ **features of applying PSC**
  - ✶ universally applicable
    - ☾ even for organisms that undergo asexual reproduction – *unlike BSC*
  - ✶ uses DNA or morphology to compare
    - ☾ species are identified based on unique genetic markers (DNA sequences) or physical traits (morphology)
  - ✶ allopatric populations can be different species
    - ☾ only if they show diagnosable differences

HYBRIDISATION

★ **DEFINITION:** process of interbreeding individuals from genetically distinct populations to produce a hybrid ⇒ carry 2 diff alleles of the same gene
- ☆ can occur naturally *(hybrid zones)* OR artificially *(human intervention)*
- ☆ contributes to genetic variation & sometimes adaptive traits
- ☆ BUT complicates phylogenetic trees due to reticulate evolution – *gene flow across lineages*

★ **HYBRID ZONES:** a region in which members of diff species meet & mate, producing at least some offsprings of mix ancestry
- ☆ often form where species ranges overlap due to secondary contact after a period of isolation

★ **OUTCOMES**
- ☆ **reinforcement of genetic barriers**
  - ✶ natural selection strengthens prezygotic barriers to avoid the production of unfit hybrids
  - ✶ promotes reproductive isolation → supports speciation
- ☆ **fusion**
  - ✶ if hybrids are viable & fertile + reproductive barriers are weak, gene flow may be extensive enough that 2 species merge into 1
  - ✶ reverse divergence → speciation collapses
- ☆ **stability**
  - ✶ hybrid zones remain stable over time because hybrids are continuously produced, but don't spread widely due to partial isolation or ecological constraints
  - ✶ maintains separate species despite some gene flow

★ **HYBRID BREAKDOWN:** hybrids are unfit, preventing genetic mixing between species
- ☆ while the F1 generation might survive, the subsequent generations become increasingly weak, sterile, or die early → each generation gets worse until the hybrid line eventually disappears
- ☆ acts as a prezygotic barrier

POLYPLOIDY

★ **DEFINITION:** heritable condition of possessing ≥ 2 complete sets of chromosomes due to mitotic/meiotic failure *(eg. non-disjunction)*

★ **TYPES OF POLYPLOIDY**
- ☆ **autopolyploidy**
  - ✷ all chromosomes are derived from a single species
  - ✷ autopolyploids are homozygous at all loci
  - ✷ can produce fertile offspring through self-pollination or mating w other tetraploids
  - ✷ reproductively isolate from diploid ancestors due to unbalanced triploid offspring
- ☆ **allopolyploidy**
  - ✷ chromosome sets come from 2 diff species *(ie. hybridisation followed by chromosome doubling)*
  - ✷ initial hybrids are usually sterile due to meiotic mismatch
  - ✷ asexual reproduction allows hybrids to persist; subsequent chromosome doubling can restore fertility
  - ✷ allopolyploids can mate w e/o but not w either parent species → new biological species

★ **BIOLOGICAL SIGNIFICANCE**
- ☆ **speciation**
  - ✷ introduces strong postzygotic barrier
  - ✷ leads to instant speciation by BSC, often w/o geographic isolation
- ☆ **phylogeny**
  - ✷ complicates phylogenetic analysis as gene duplication masks true species r/s
  - ✷ reticulate evolution – network-like lineages v. bifurcating trees
  - ✷ molecular data must account for multiple genome donors

INTROGRESSION

★ **DEFINITION:** movement of genes from one species to another by repeated backcrossing of a hybrid & a parental species
- ☆ results in DNA segments from 1 species becoming stably incorporated into another species' genome
- ☆ distinct from incomplete lineage sorting – involves retention of ancestral polymorphism w/o gene flow

★ **MECHANISM**
- ☆ hybridisation produces F1 hybrids
- ☆ F1 hybrids mate w a parental species – backcross
- ☆ repeated backcrossing introduces foreign genome segments into one species
- ☆ these segments may be neutral maladaptive or adaptive
  - ✷ neutral – DNA in low gene density region is broken up by recombination & subject to drift
  - ✷ maladaptive – deleterious regions are selected against & purged
  - ✷ adaptive – beneficial regions increase in frequency due to positive selection

CHROMOSOMAL, GENOME LEVEL

★ **CHROMOSOMAL**
- ☆ breaks the ‘tree of life’ model by creating network-like lineages v. bifurcating trees
- ☆ hybridisation – merges lineages *(eg. allopolyploid sunflowers)*
- ☆ introgression: transfers genes between species *(eg. Neanderthal DNA in humans)*

★ **GENOME**
- ☆ rapid speciation *(eg. hybrid sunflowers Helianthus anomalus)* complicates divergence t

IMPACT ON RECONSTRUCTING PHYLOGENIES

★ **RETICULATE EVOLUTION**
- ☆ breaks the ‘tree of life’ model by creating network-like lineages v. bifurcating trees
- ☆ hybridisation – merges lineages *(eg. allopolyploid sunflowers)*
- ☆ introgression: transfers genes between species *(eg. Neanderthal DNA in humans)*

★ **IMPACT ON SPEED & GENETIC BASIS OF SPECIATION**
- ☆ rapid speciation *(eg. hybrid sunflowers Helianthus anomalus)* complicates divergence timing
- ☆ no ‘speciation clock’ – rates vary (some lineages diverge fast, others slowly)
- ☆ genetic basis: reproductive isolation can involve:
  - ✶ single genes (simple) → easier to trace
  - ✶ many genes (complex) → harder to resolve

★ **DIFFICULTY IN RELYING ON MOLECULAR EVIDENCE**
- ☆ **post-polyploidisation changes:**
  - ✶ gene silencing, epigenetic remodeling, pseudogenes
  - ✶ duplicated genes diverge asymmetrically
- ☆ **signal erosion:**
  - ✶ older introgression becomes hard to detect *(DNA breaks up/mutates)*
  - ✶ recent introgression leaves clearer traces *(long, unbroken DNA segments)*

★ **TLDR**
- ☆ phylogenetics works best for old, isolated species
- ☆ recent divergence/gene flow requires combining morphology + multiple genes
- ☆ reticulation (hybridisation/introgression) breaks the "tree of life" metaphor
- ☆ polyploidy & rapid speciation challenge timeline reconstruction
- ☆ molecular evidence gets fuzzy over time due to genomic changes

ANCESTRY & DIASPORA OF HUMANS (chatGPT)

- ★ introgression left traces of DNA in modern humans, though older events are harder to detect due to recombination and mutation .
- ★ human diaspora involves “Out of Africa” migrations:
  - ☆ first dispersal: ~2 mya (Homo erectus, archaic forms).
  - ☆ second major dispersal: ~100,000 ya (anatomically modern humans).
- ★ genetic diversity is highest in African populations, supporting Africa as the origin of modern humans

EVOLUTION OF *HOMO SAPIENS*

- ★ **named by Linnaeus (1758)**; fossil and DNA evidence show divergence from chimpanzees/bonobos ~6 mya
- ★ early hominins: *Sahelanthropus tchadensis*, *Orrorin tugenensis*
- ★ *Ardipithecus ramidus* (~4.4 mya): bipedal, upright pelvis but still adapted for tree climbing
- ★ later hominins (*Australopithecus*, *Homo habilis*, *Homo erectus*) show trends of **larger brain size, tool use, reduced prognathism**

MODELS FOR HUMAN ORIGIN

- ★ **OUT OF AFRICA (REPLACEMENT) MODEL**
  - ☆ modern humans evolved in Africa and replaced archaic populations outside Africa ~100,000 ya

- ★ **MULTIREGIONAL HYPOTHESIS**
  - ☆ modern humans evolved simultaneously in Africa, Asia, and Europe with continuous gene flow between regions

- ★ **AFRICAN MULTIREGIONALISM MODEL (RECENT)**
  - ☆ *homo sapiens* emerged through admixture of diverse populations within Africa, not from a single group

GENETIC EVIDENCES

- ★ **mtDNA (maternal line):**
  - ☆ small genome (<20 kb), high copy number, no recombination, high mutation rate, useful for tracing lineages
  - ☆ mitochondrial Eve lived in Africa ~100–200 kya; all living humans trace maternal ancestry to her

- ★ **Y Chromosome (paternal line):**
  - ☆ non-recombining region (NRY) passed father → son
  - ☆ mutations accumulate linearly, defining paternal haplogroups
  - ☆ Y-chromosomal Adam is the MRCA of modern male lineages

THE GENOGRAPHIC PROJECT

- ★ launched by National Geographic & IBM (2005)
- ★ used analysis of mtDNA and Y chromosome haplogroups to track human migration routes
- ★ found strong support for Out of Africa, with multiple subsequent dispersals and mixing with archaic humans

SIGNIFICANCE OF BIOMOLECULES

Biomolecules	Biochemical processes through which they are synthesised (Link to H2 Biology)	Significance to understanding of evolution
Carbohydrates		Early organisms relied on simple carbohydrates like monosaccharides for energy. Polysaccharides evolved to support complex life forms by enabling energy storage and structural integrity. Carbohydrates are also needed to synthesise other more complex molecules like nucleic acids.
Lipids	Fatty acids formed by polymerisation of acetyl-CoA by fatty acid synthase. Fatty acids combined with glycerol to form triglyceride / phosphate head to form phospholipid.	Lipid bilayers facilitated the formation of cell membranes, a critical step in the origin of life. Membrane adaptations allowed organisms to inhabit diverse environments.
Proteins		Evolution of enzymes enhanced metabolic efficiency. Structural proteins enabled multicellularity and specialisation.
Nucleic acids		The RNA World Hypothesis suggests that RNA served both as a catalyst and genetic material in early life. DNA later evolved as a more stable genetic repository.

RNA WORLD HYPOTHESIS

★ **RNA CAN PERFORM DUAL ROLES** – it can store genetic info. & catalyse reactions (ribozymes) ⇒ likely the first biomolecule

★ **EVIDENCE FROM TRANSLATION MACHINERY**
- ☆ mRNA, tRNA, ribosome
- ☆ as the ribosome's active site is pure RNA → its a ribozyme ⇒ suggests ancient ribosomes were entirely RNA

★ **DNA BECOMING MORE PREFERED**
- ☆ due to the lack of proofreading in RNA replication, mRNAs coding for enzymes would have high rates of mutations ⇒ selection for templates with lower mutation rates, resulting in a gradual transition to DNA as a template coding for mRNA

- ☆ lower mutation rates of DNA allowed reliable transmission of longer messages, enabling larger genomes coding for more complicated organisms to emerge

CARBOHYDRATES

- ★ **SUPER IMPORTANT** – it's an energy source, provides structural support, & enable cell signalling
  - ☆ simple carbohydrates can form spontaneously under prebiotic conditions & participate in reactions that lead to the synthesis of more complex molecules essential for life ⇒ carbohydrates likely contributed to the emergence of early biochemical pathways and the transition from non-life to life

LIPIDS

- ★ **LIPID WORLD HYPOTHESIS**
  - ☆ **need for compartmentalisation**
    - ✶ Without a membrane, there are no forces/affinities that could keep together the RNA precursors and peptides
  - ☆ **amphipathic nature of lipids**
    - ✶ they can easily form unstable multi-molecular structures like protocell membranes and small micelles
    - ✶ micelles act as simple chemical factories, carrying out reactions similar to those done by complex modern proteins

ANNEX

SEXUAL SELECTION

★ **FEMALES V. MALES**

- ☆ **gamete size & mating needs:**
  - ✷ **females –** fewer, larger gametes which are more energetically costly ⇒ usually only need 1 mating to fertilise since no. of eggs are limited
  - ✷ **males –** many, small mobile gametes which are cheap & plentiful ⇒ can fertilise many females
- ☆ **selection pressure on traits:**
  - ✷ **females –** more matings does NOT increase no. of offspring because all her eggs can usually be fertilised in 1 mating
    - ⋆ traits that increase no. of matings don't improve her fitness
    - ⋆ selection may favour for traits involving **mate choice** or **offspring care**
  - ✷ **males –** more matings = more fertilised eggs = more offspring
    - ⋆ traits that help a male get more mates is favoured – *eg. bright feathers, loud calls, etc…*

What are the similarities and/or differences in key ideas among Good gene hypothesis, Zahavi's handicap principle, and Fisherian runaway hypothesis?

similarities:

- all three are about sexual selection — how mate choice drives evolution.
- all three explain why animals (usually males) evolve exaggerated traits (like big antlers, colorful feathers, loud calls).
- all involve female choice influencing male trait evolution

Feature	Good Genes	Zahavi's Handicap	Fisherian Runaway
Trait indicates genetic quality?	✅ Yes	✅ Yes	❌ Not necessarily (especially later in the process)
Requires costly traits?	⚠️ Not required, but often implied	✅ Yes, essential	❌ No
Female preference is adaptive?	✅ Yes	✅ Yes	⚠️ Initially maybe, but can become non-adaptive
Positive feedback loop?	❌ No	❌ No	✅ Yes
Mechanism of honesty?	Trait correlates with real quality	Costly to fake (handicap)	No honesty mechanism required
Leads to trait exaggeration?	⚠️ Possibly	✅ Often	✅ Strongly

POLYPLOIDY, HYBRIDISATION, AND INTROGRESSION

★ **SPECIES CONCEPTS:**

- ☆ **biological**
  - ✶ a species is a group of organisms that is able to interbreed in nature to produce viable and fertile offspring and are reproductively isolated from other groups
  - ✶ **advantage:** organisms can be interbred to see if they produce fertile, viable offspring
  - ✶ **limitation:** this definition cannot be applied to asexually reproducing organisms and extinct species whose breeding behaviour cannot be observed
  - ✶ **tldr:** interbreeding groups

- ☆ **ecological**
  - ✶ a group of organisms sharing the same ecological niche, which includes both the place where an organism lives and its interactions with the environment
  - ✶ differences between species arise due to the differences in the ecological resources they depend on
  - ✶ **advantage:** every organism has a niche, making this concept widely applicable
  - ✶ **limitation:** unrelated species that occupy similar niches may be mistakenly classified as the same species, despite having different evolutionary origins
  - ✶ **tldr:** based on ecological niche

- ☆ **morphological**
  - ✶ a group of organisms sharing similar body shape, size, and other structural features
  - ✶ **advantage:** can be applied to all organisms, including both sexually and asexually reproducing ones
  - ✶ **limitation:** can be difficult to determine the degree of morphological difference required to classify separate species, and convergent evolution may result in superficially similar but unrelated organisms being classified together
  - ✶ **tldr:** based on physical appearance — ie. body shape/form

- ☆ **genetic**
  - ✶ a group of genetically compatible interbreeding organisms in a natural population that is genetically isolated from other such groups
  - ✶ different genetic species are genetically distinct and evolve independently → behavioural changes or changes in species recognition
  - ✶ **advantage:** genetic data from mitochondrial and nuclear DNA can be unambiguous in deducing evolutionary relationships
  - ✶ **limitation:** technology required to study DNA sequences is relatively expensive
  - ✶ **tldr:** based on genetic similarity

🐟 ET 1: INFECTIOUS DISEASES

Table Of Contents

ADAPTIVE & INNATE IMMUNITY RELATIONSHIP

MUTUALLY EXCLUSIVE

Feature	Innate immunity	Adaptive immunity
Organism	All multicellular organisms	Only in vertebrates
Specificity	Non-specific, recognises pathogen-associated molecular patterns	Highly specific, recognises unique antigens
Receptor diversity	Limited, germline-encoded pattern recognition receptors (PRRs) which recognises pathogen-associated molecular patterns (PAMPs)	Large diversity of immunoglobulins and T cell receptors (TCR), which are generated by somatic recombination, somatic hypermutation and class switching
Activation requirement	Does not require prior exposure to antigen	Requires activation through exposure and recognition of specific antigen
Time of response	Immediate (minutes to hours)	Delayed (hours to days)
Duration of response	Short-lived (days)	Long-lived (weeks to lifetime)
Memory	No immunological memory, response is the same each time	Has immunological memory, faster and stronger response upon re-exposure
Cells types involved	Neutrophils, macrophages, dendritic cells, NK cells, etc	B and T lymphocytes
Mechanisms	Barrier defences, phagocytosis, induced apoptosis, inflammatory response	Cell-mediated (cellular) and antibody-mediated immune (humoral) responses

INTERDEPENDENT

★ **ANTIGEN PRESENTATION AND ACTIVATION OF ADAPTIVE IMMUNITY** – innate immune system provides the first line of defence by recognising pathogens through PAMPs, while adaptive immunity relies on antigen presentation by innate immune cells to activate T cells, which are needed to activate B cells

★ **ADAPTIVE IMMUNE MECHANISMS ENHANCE INNATE FUNCTIONS** – adaptive immunity enhances innate responses, such as antibodies marking pathogens for phagocytosis by macrophages and neutrophils (opsonisation) and infected cells for induced apoptosis by natural killer cells (ADCC), as well as helper T cells that enhance the functions of innate immune cells

★ **CYTOKINE COMMUNICATION** – innate immune cells release cytokines to activate adaptive immune responses, while adaptive immune cells secrete cytokines to enhance the activity of innate cells

★ **MEMORY FORMATION AND RAPID SECONDARY RESPONSE** – adaptive immunity creates immunological memory, enabling faster recruitment and stimulation of innate immune cells upon re-exposure to the same pathogen

★ **PATHOGEN ELIMINATION** – The responses of the innate immune system act early to restrain pathogens while simultaneously helping to initiate the adaptive immune response, which takes a longer time to fully develop a targeted response and eliminate pathogens that evade innate defences

IMMUNOLOGICAL SELF-TOLERANCE

★ V(D)J recombination is random, developing lymphocytes may generate receptors that recognise self-antigens → if these self-reactive B or T cells are not eliminated or inactivated during development, they may react against the body's own tissues

GENERAL MECHANISMS

★ **CENTRAL TOLERANCE** – occurs during lymphocyte development in the primary lymphoid organs
- ☆ strongly self-reactive lymphocytes are either eliminated (clonal deletion) or functionally inactivated (*eg. receptor editing in B cells or development into regulatory T cells in the thymus*)

★ **PERIPHERAL TOLERANCE** – controls or eliminates self-reactive lymphocytes that have escaped central tolerance
- ☆ takes place in the peripheral tissues & secondary lymphoid organs
- ☆ **mechanisms:**
  - ✶ anergy (functional unresponsiveness)
  - ✶ suppression by regulatory T cells
  - ✶ activation-induced cell death

B-LYMPHOCYTES

CENTRAL TOLERANCE (BONE MARROW)

★ **RECEPTOR EDITING** – self-reactive immature B cells undergo further V(D)J recombination in the light chain
- ☆ if the receptor remains autoreactive, rearrangement continues until a non-autoreactive receptor is produced OR no additional light-chain V and J gene segments are available for recombination

★ **CLONAL DELETION** – if receptor editing fails: undergoes apoptosis to eliminate their specific autoreactivity

★ **ANERGY** – immature B cells that binds to soluble self-antigens: inactivated and enter a state of permanent unresponsiveness (anergy), but do not immediately die

★ **CLONAL IGNORANCE** – immature B cells whose antigens are inaccessible to them OR binds to antigens w low affinity → don't receive enough signals to mature normally
- ☆ these B cells are POTENTIALLY self-reactive, however, and are said to be clonally ignorant because their ligand is present but is unable to activate them

PERIPHERAL TOLERANCE (SPLEEN)

★ **CLONAL DELETION** – undergoes apoptosis IMMEDIATELY to eliminate their specific autoreactivity

★ **ANERGY** – B cells that encounter and bind an abundant soluble antigen w/o co-stimulation by T_H cells become unresponsive ⇒ won't receive signals for maturation and long-term survival, & often die within a few days

★ **SUPPRESSION** – T_{regs} inhibit the activation of self-reactive B cells

T-LYMPHOCYTES

CENTRAL TOLERANCE (THYMUS)

★ **POSITIVE SELECTION** – selection for working immature T cells
- ☆ thymocytes whose TCRs cannot bind self-MHC at all receive no survival signal & undergo apoptosis
- ☆ determines fate of thymocytes
  - ✶ if TCR binds MHC class II → $CD4^+$ T cell
  - ✶ if TCR binds MHC class I → $CD8^+$ T cell

★ **NEGATIVE SELECTION** – selection for self-reactive immature T cells
- ☆ thymocytes whose TCRs bind to self-MHC too strongly are eliminated by apoptosis

PERIPHERAL TOLERANCE

★ **CLONAL DELETION** – T cells reacting to self-antigens in the periphery are eliminated by activation-induced cell death

★ **ANERGY** – T cells become inactive if they encounter self-antigens without co-stimulatory signals or with inhibitory signals

★ **SUPPRESSION** – T_{regs} secrete inhibitory cytokines to suppress self-reactive T cells

REGULATORY TOLERANCE

★ **MECHANISMS OF T_{regs}**
- ☆ T_{regs} produce inhibitory cytokines, such as interleukin-10 (IL-10), transforming growth factor-β (TG-Fβ) & IL-35 → inhibit activation of lymphocytes, dendritic cells & macrophages
- ☆ mediate cytolysis via granzymes & perforins
- ☆ bind & consume IL-2, an essential T cell growth factor → starving the other T cells ⇒ cytokine-deprivation-mediated apoptosis
- ☆ express CTLA4 → binds to CD80/CD86 on APCs, inhibiting APCs maturation ⇒ no activation of T cells

HUMAN MICROBIOTA

HEALTH

★ **NUTRITION & METABOLISM**
- ☆ increases energy extraction from food and nutrient harvest
- ☆ metabolises indigestible carbohydrates
  - ✷ *eg. dietary fibre: xyloglucans → bacteroides*
- ☆ synthesises essential vitamins
  - ✷ *eg. folate, vitamin K, biotin, riboflavin (B2), cobalamin (B12), etc.*
- ☆ helps regulate appetite and energy homeostasis

★ **PROTECTION AGAINST PATHOGENS**
- ☆ provides colonisation resistance → blocks pathogen attachment by competitive exclusion
- ☆ produces antimicrobial substances that inhibit invading microbes
- ☆ acts as a physical barrier on mucosal surfaces

★ **DEVELOPMENT OF GUT & IMMUNE SYSTEM**
- ☆ essential for proper development of intestinal mucosa and gut structure
- ☆ stimulates humoral and cellular mucosal immunity
- ☆ microbial signals/metabolites shape innate immune responses

DISEASE

★ **INFECTIOUS DISEASES**
- ☆ dysbiosis increases susceptibility to infection
- ☆ pathogens colonise gut → strong inflammation → bacterial translocation
- ☆ *Clostridium difficile* infection (CDI):
  - ✷ triggered by antibiotic use → loss of normal flora → *C. difficile* overgrowth
  - ✷ effectively treated by faecal microbiota transplantation (FMT)

★ **GASTROINTESTINAL CANCERS**
- ☆ *H. pylori* → chronic inflammation → strong risk factor for gastric cancer

★ **METABOLLIC DISORDERS**
- ☆ gut microbial imbalance contributes to obesity, type 2 diabetes, and cardiovascular disease
- ☆ obese individuals show ↑ *Prevotella*, ↑ *Firmicutes*, ↑ *methanogenic archaea*
- ☆ these microbes harvest carbohydrates more efficiently → excess calories stored as fat

PANDEMIC FACTORS

1. **Sanitation**
    - **Poor sanitation** (e.g. uncollected waste, open defecation) increases the spread of pathogens through contact with contaminated surfaces or human waste.
    - Especially important for **faecal-oral diseases** (e.g. cholera, typhoid).
    - Increases chances of **initial outbreaks** turning into wider epidemics or pandemics.

2. **Water Supply**
    - **Unsafe or limited access to clean drinking water** allows waterborne pathogens to spread easily (e.g. cholera, hepatitis A).
    - Contaminated water is a key route for disease transmission in overcrowded or disaster-affected areas.

3. **Food Safety**
    - **Poor food hygiene and unsafe food storage** can spread bacteria and viruses (e.g. *Salmonella*, *E. coli*).
    - Consumption of **wild animals** or improperly cooked meat can introduce **zoonotic diseases** (e.g. SARS, COVID-19).
    - **Global food supply chains** may help spread contaminated food across borders.

4. **Climate and Environmental Change**
    - Warmer temperatures can **expand the habitats of disease-carrying vectors** (e.g. mosquitoes that transmit Zika, dengue, malaria).
    - Changes in rainfall and temperature can influence **pathogen survival** and **animal migration**, increasing the chance of new host–pathogen interactions.
    - **Extreme weather** (e.g. floods) can damage sanitation infrastructure and increase disease spread.

5. **Large-Scale Movements of People**
    - **Urbanisation**, **refugee crises**, **mass gatherings**, and **international travel** allow diseases to spread quickly between populations and across borders.
    - Overcrowding increases contact rates, particularly in refugee camps or informal settlements.
    - Air travel enables **rapid global transmission** of emerging pathogens.

6. **Evolution of New Strains of Virulent Pathogens**

- Viruses and bacteria **mutate rapidly**, especially RNA viruses like influenza and coronaviruses.
- Genetic changes can lead to:
    - **Antigenic shift or drift** (e.g. in influenza)
    - Increased **transmissibility** or **virulence**
    - The ability to **infect new host species** (zoonosis)
- Emergence of **novel strains** with no pre-existing immunity in the population can trigger a pandemic.

7. **Development of Drug Resistance**

- Overuse or misuse of **antibiotics**, **antivirals**, and **antimalarials** allows resistant strains to survive and spread.
- Pathogens that become **resistant to treatment** (e.g. MDR-TB, resistant gonorrhoea) are harder to control.
- **Limited treatment options** mean outbreaks are more likely to spiral out of control.

ANNEX

Type of tolerance	Mechanism	Site of action
Central tolerance	Deletion Editing	Thymus (T cells) Bone marrow (B cells)
Antigen segregation	Physical barrier to self-antigen access to lymphoid system	Peripheral organs (e.g., thyroid, pancreas)
Peripheral anergy	Cellular inactivation by weak signaling without co-stimulus	Secondary lymphoid tissue
Regulatory T cells	Suppression by cytokines, intercellular signals	Secondary lymphoid tissue and sites of inflammation; multiple tissues in steady state
Functional deviation	Differentiation of regulatory T cells that limit inflammatory cytokine secretion	Secondary lymphoid tissue and sites of inflammation
Activation-induced cell death	Apoptosis	Secondary lymphoid tissue and sites of inflammation

ET 2: CLIMATE CHANGE

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H2 CLIMATE CHANGE

ANTHROPOGENIC GREENHOUSE EFFECT

★ **GHGs & GREENHOUSE EFFECT**

- ☆ **Greenhouse Gases (GHGs) –** gases which absorb infrared radiation, slowing rate at which heat escapes into space; common examples of anthropogenic GHGs:
  - ✶ **CO_2**
    - ☾ cellular respiration in living organisms
    - ☾ human activities → burning of fossil fuels & deforestation
    - ☾ removed via photosynthesis or dissolving in oceans
  - ✶ **CH_4**
    - ☾ anaerobic decay of vegetation in wetlands
    - ☾ human activities → fossil fuel production (natural gas), agriculture (esp rearing ruminants like cows), waste treatment, landfill sites & biomass burning
- ☆ **greenhouse effect –** natural warming of the earth that results when GHGs trap heat from the sun in the atmosphere
  - ✶ due to human activities → increased GHG emissions has led to enhanced greenhouse effect

★ **HUMAN ACTIVITIES THAT INCREASE GHGs**

- ☆ **burning of fossil fuels** – coal, petroleum, and natural gas
  - ✶ coal: production of heat & electricity
  - ✶ crude oil: gasoline & diesel production for transportation
  - ✶ natural gas: electricity & heat production in households & industry
- ☆ **deforestation**
  - ✶ forests – which are carbon sinks – are cleared to make way for agricultural products or other human activities
  - ✶ the subsequent decomposition & burning of biomass releases for GHGs
- ☆ **food choices (consumption of meat)**
  - ✶ enteric fermentation of ruminants digestive process produces large quantities of CH_4 → released via belching or flatulence
  - ✶ manure also releases CH_4 & N_2O during decomposition
  - ✶ large plots of land are deforested to make space for livestock + transportation, etc.

EFFECTS OF CLIMATE CHANGE DUE TO GHG EMISSIONS

★ **MELTING OF POLAR ICE CAPS**

- ☆ due to rising temperatures – ice sheets are melting, more ice calving
- ☆ affects the animals who live on these ice sheets – *polar bears, seals, walruses, penguins, etc.*

★ **ICE-ALBEDO EFFECT**

- ☆ as temp. increases → the darker ocean absorbs more heat, causes more ice to melt, and makes the Earth warmer overall ⇒ positive feedback loop

★ **RISING SEA LEVELS** – due to:
- ☆ added water from melting ice sheets & ice shelves
- ☆ thermal expansion of seawater

★ **HEAT WAVES** – due to high-pressure system in the atm
- ☆ warm air gains heat → rises → high pressure in the atm pushes warm air back down → compressed air becomes hotter → rises AGAIN due to convection currents → pushed BACK down ⇒ heat dome formed
- ☆ **Urban Heating Island (UHI) effect** – higher density of buildings, roads, infrastructure that absorbs & retains heat
  - ✶ made of materials like concrete & asphalt w high thermal mass → absorb heat in the day, release at night ⇒ HOT nights

★ **HEAVY RAINS**
- ☆ increased evaporation rates ⇒ more water vapour than the atm can hold

★ **STRESS ON FRESHWATER SUPPLIES**
- ☆ increased evaporation → more common droughts
- ☆ heavier rainfall
  → increase runoff that exceeds the capacity of reservoirs
  → rainfall brings w them pollutants (fertilisers & petrochemicals)
- ☆ contamination of freshwater areas w saltwater
- ☆ warmer temp. water bodies become a breeding groud for bacteria & viruses

★ **MELTING OF PERMAFROST**
- ☆ releases GHGs into the atmosphere → positive feedback loop w more permafrost defrosting, more GHGs, etc.
- ☆ ancient viruses & bacteria also thaws & are released

EFFECT OF CLIMATE CHANGE ON THE BIOSPHERE

★ **HABITAT ALTERATION**
- ☆ **sea level rise** – threatens coastal habitats like intertidal zones, salt marshes, etc. → habitat loss + coastal erosion
- ☆ **glacier retreat** – affects species adapted to cold environments & alters water availability in downstream habitats
- ☆ **changes in vegetation** – shift distribution of plant species → changes forest compositions, grassland extent & desertification

★ **EFFECTS ON ORGANISMS**
- ☆ **increased stress** – physiological stress from heat waves, droughts & ocean acidification → impacts growth, reproduction & survival
- ☆ **shifts in distribution** – moving polewards to higher elevation, or deeper oceans → affects community composition & ecological interactions
- ☆ **disruption of phenology** – changes in seasonal timing of biological events like flowering time, migration, reproduction, etc. → disrupts ecological interactions & synchronisation w resources

★ **FOOD CHAIN DYNAMICS**

- ☆ **altered primary productivity** – decreases food availability throughout the food chain/web
- ☆ **disruption of trophic interaction** – disrupt predator-prey r/s
- ☆ **loss of keystone species**

★ **NICHE OCCUPATION & COMPETITION**

- ☆ **range shifts** – as species move into new habitats, they may encounter new competitors or predators
- ☆ **invasive species** – climate change can facilitate the spread of invasive species into new regions
- ☆ **resource limitations** – intensifies competition among species, affecting niche partitioning & species co-existence

★ **CORAL REEFS**

- ☆ **corals & zooxanthellae symbiotic r/s**
  - ✶ corals provide raw materials for photosynthesis + protection
  - ✶ zooxanthellae provides nutrition via products of photosynthesis
- ☆ **coral bleaching due to increase in temperature**
  - ✶ zooxanthellae's photosynthetic system gets overwhelmed → starts producing ROS
  - ✶ these ROS causes damage to the cellular structure of corals
  - ✶ thus, corals expel the zooxanthellae ⇒ leaving the white coral skeleton covered by the now colourless tissue layer
- ☆ **decalcification due to ocean acidification**
  - ✶ CO_2 when dissolved forms carbonic acid → reduces the availability of CO_3^{2-} to form its $CaCO_3$ skeleton
  - ✶ if it's too acidic, the corals may begin to dissolve the skeletons themselves

★ **EFFECTS ON PLANTS**

- ☆ **plant distribution** → migration to higher latitudes & altitudes
  - ✶ but as plants are more sedentary → migrate slower & at higher risk of extinction
- ☆ **plant adaptations**
  - ✶ <u>reduced stomatal density & closure</u>
    - ☾ reduction as: due to higher CO_2, less stomata needed to receive eqv CO_2 levels + reduces transpiration, allowing for water conservation
  - ✶ <u>increased leaf thickness</u>
    - ☾ minimise water loss via transpiration & lower SA:VR
  - ✶ <u>decreased leaf production & size</u>
    - ☾ decreases their ability to carry out photosynthesis ⇒ affecting net primary productivity & carbon storage
  - ✶ <u>increased root growth</u>
    - ☾ increased probability of securing rare water resources

★ **EFFECT ON FISHES**
- ☆ **migration**
  - ✶ warmer waters → migrate to higher latitudes or deeper waters
  - ✶ cause ecological disruptions → invade another area & compete w other species over food & other resources
- ☆ **phenology shifts**
  - ✶ change the timings of adult migration & reproduction, age at maturity & age at juvenile migration
  - ✶ rising temp causes mountain snowpack to melt earlier and faster → spring runoff arrives too early. by late summer and autumn, water levels are too low, disrupting migratory species that rely on steady stream flow to move upstream to their spawning sites
  - ✶ migration timing change ⇒ mismatches w optimal environmental conditions & food availability → decreased survival rates & reproductive success

EFFECTS OF CLIMATE CHANGE ON HUMANS

DENGUE

★ **SEROTYPES**
- ☆ 4 antigenic groups – DENV-1 to DENV-2

★ **LIFE CYCLE OF *AEDES AEGYPTI***
- ☆ *Aedes Aegypti* is a mosquito vector that can spread dengue fever
- ☆ **egg stage**
  - ✶ 1-2 days
  - ✶ female mosquitoes lay egg on inner containers w stagnant water
- ☆ **larva stage**
  - ✶ 4-8 days
- ☆ **pupa stage**
  - ✶ 1-3 days, metamorphosis is triggered
- ☆ **adult stage**
  - ✶ males → nectar & fruit juice, don't bite humans; takes around 2 days to develop reproductive organs
  - ✶ females → need blood meals for development of eggs

★ **DEVELOPMENT OF DENGUE**
- ☆ **transmission** → bites of infected female mosquitoes
- ☆ **dengue virulence**
  - ✶ can cause dengue haemorrhagic fever (DHF) & dengue shock syndrome (DSS)
    - ☾ **DHF**: increased vascular permeability & leakage of fluids
    - ☾ **DSS**: more severe, severe plasma leakage → lack of blood circulation → circulatory shock, internal bleeding, multi-organ failure
  - ✶ first infection → minor disease
  - ✶ second infection w a diff serotype → significantly higher change of developing DHF & DSS due to antibody-dependent enhancement (ADE) where antibodies aid the dengue virus in infection immune cells more efficiently

★ **EFFECT OF TEMPERATURE**

- ☆ range expansion of mosquitoes
- ☆ more eggs laid & shorter life cycle → faster developmental rates up to optimum temperature of ~32ºC
- ☆ survival of egg-to-adult & adult lifespan will decrease
- ☆ increase biting rate of mosquitoes up to optimum temperature of 34ºC ⇒ increasing rate of transmission
- ☆ shorten extrinsic incubation period → reduces time between infection & transmission to humans

H3 CLIMATE CHANGE

RESPONDING TO CLIMATE CHANGE

MITIGATION

★ **CARBON SEQUESTRATION –** process of capturing & storing atmospheric CO_2 to mitigate climate change
- ☆ **biological carbon sequestration** – capturing & storing via natural processes, usually photosynthesis
  - ✶ terrestrial carbon sequestration → terrestrial ecosystems (vegetation, soil, etc.)
  - ✶ blue carbon sequestration → coastal & marine ecosystems (mangroves, seagrass beds, salt marshes, etc.)
- ☆ **non-biological carbon sequestration** – carbon capture & storage via physical & chemical reactions

★ **CARBON SEQ POTENTIAL OF DIFFERENT PHOTOSYNTHETIC ORGANISMS**
- ☆ **C3 –** fixes carbon using Rubisco → 3-phosphoglycerate as the first stable product
- ☆ **C4** – PEP carboxylase in mesophyll cells → 4C malate, which is later decarboxylated & refixed by Rubisco
- ☆ **CAM** – PEP carboxylase at night → 4C malate, which is later decarboxylated & refixed by Rubisco in the day
- ☆ **algae** → fix CO_2 w Rubisco in the calvin cycle, w increased efficiency via pCCM

TREE PLANTING

★ trees are natural carbon sinks

★ **REFORESTATION** – planting trees to restore an existing forest impacted by deforestation
- ☆ *China's Loess Plateau Restoration*

★ **AFFORESTATION** – planting trees where there were no trees previously → creating a new forest

★ BUT must be planned carefully → if not more harm > good
- ☆ planting trees in unnatural places like grasslands which already act as carbon sinks
- ☆ cultivating fast growing, non-native trees → monocultures

DEVELOPMENT OF DROUGHT-RESISTANT CROPS

★ **INCREASING PHOTOSYNTHETIC EFFICIENCY**
- ☆ introducing C4 & CAM traits into C3 crops → improve resilience to climate stressors like drought, salinity, & heat
- ☆ challenges: chromosome mismatching & hybrid sterility

ADAPTATION TO CLIMATE CHANGE

- ★ **PHENOTYPE PLASTICITY** – short-term behavioural or physiological adjustments
  - ☆ *on warmer days, snails temporarily elevate heat-shock protein expression to survive extreme temperatures → allows short-term survival during heatwaves*

- ★ **EVOLUTIONARY CHANGES** – genetic changes across generations
  - ☆ *pitcher plant mosquito → its spring emergence is controlled by daylength (photoperiod) and varies with latitude; between 1972 - 1996, populations from Georgia to Maine evolved a shift towards earlier emergence, showing an evolutionary change in plastic sensitivity to photoperiod*

- ★ **PHENOLOGICAL CHANGES** – changes in the timing of the life cycle events
  - ☆ *Pied Flycatchers and Barn Swallows in Europe now arrive 10–14 days earlier on average due to warmer springs*

- ★ **BROADER EFFECTS OF THESE CHANGES**
  - ☆ some species may gain selective advantages as they adapt to the changing climate
  - ☆ range shifts to cooler or more suitable areas would change the local biodiversity
  - ☆ changes in the timing of life cycle events (e.g., migration, reproduction) result in phenological mismatch of interdependent species (e.g., birds arriving after peak insect abundance, flowering before pollinators emerge from hibernation)
  - ☆ this disrupts existing species interactions and interspecies relationships (e.g. predator-prey, host-parasite, and pollinator-plant)
  - ☆ novel interactions may emerge which can increase predation, parasitism, competition for resources, etc.
  - ☆ the loss or change in one trophic level can ripple through the food web
    - ✶ *grey wolves in yellowstone: reintroduction lead to elk populations decreasing → reduces overgrazing → allowed vegetation to recover after years of overgrazing by elk → beaver populations increased due to increased availability of vegetation → wetlands created via beaver dams, etc.*

MISC THEMES

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COMMUNICATION

Communication is fundamental to life, enabling cells, tissues, organisms, and populations to coordinate behaviour, regulate physiology, respond to environmental changes, and ensure survival and reproduction. Biological communication occurs across a spectrum of distances—from **intramolecular signalling** within a single molecule, to **intercellular communication** over short or long distances, and finally to **communication between organisms**, shaping complex behaviours such as mate choice and social organisation. This essay will explore these levels of communication, demonstrating how information flows from molecular to ecological scales and how evolutionary pressures have shaped these signalling systems.

SHORT DISTANCE COMMUNICATION

Intramolecular communication

- intramolecular communication refers to the **transfer of information within the same molecule**, usually a protein.
- Many proteins undergo **allosteric regulation**, where binding of a ligand at one site induces conformational changes elsewhere in the same molecule
- *Example: **Haemoglobin**. Oxygen binding at one subunit increases the affinity of the remaining subunits → cooperative binding.*
- Enzymes may switch between active and inactive states through phosphorylation, producing structural changes that propagate across the molecule to modify activity.
- **Importance:** Intramolecular signalling allows rapid, reversible regulation without requiring external molecules, enabling fine-tuned control of metabolism and cellular responses.

Intermolecular short-distance communication (cell-to-cell)

- Cells communicate with neighbouring cells via:
- (a) Paracrine signalling
    - Short-range diffusion of chemical signals such as:
        - growth factors (e.g., PDGF)
        - cytokines
        - neurotransmitters in synaptic clefts
        - These signals act locally, ensuring that only nearby cells respond
- (b) Autocrine signalling
    - Cells signal to themselves via secreted ligands that bind their own receptors.
    - Example: cancer cells secreting growth factors to stimulate their own division.
- (c) Direct cell-to-cell contact
    - **Gap junctions** in animal cells allow ions and small molecules to pass directly.
    - **Plasmodesmata** in plants permit cytoplasmic continuity across cell walls.
    - **Cell-surface receptors** interact directly during immune signalling (e.g., T-cell receptor binding to antigen–MHC complex).
- **Importance:** Short-distance signalling allows precise spatial control, enabling tissue patterning during embryogenesis, coordinated contraction in cardiac muscle, and tightly regulated immune responses.

LONG DISTANCE COMMUNICATION

Nervous system signalling

- Nervous signalling uses electrical impulses (action potentials) and synaptic chemical transmission.
- Key features
    - **Very fast** (milliseconds).
    - Signals travel along **neurons** to specific target cells.
    - Communication is **highly directional** and **precisely targeted**.
- Process
    a. Action potential triggered.
    b. Depolarisation propagates along axon.
    c. Neurotransmitter released into synaptic cleft.
    d. Receptors on postsynaptic cell trigger a response.
- Examples
    - Reflex arcs (e.g., withdrawal from heat).
    - Coordination of muscle contraction.
    - Sensory perception (vision, hearing, touch).
- **Importance:** Rapid, specific signalling is essential for movement, homeostasis, behaviour, and survival.

Endocrine system signalling

- Endocrine signalling relies on **hormones** transported through the bloodstream.
- Key features
    - **Slow but long-lasting** effects.
    - Acts on **distant tissues**.
    - Hormones have wide-ranging effects depending on the receptors expressed.
- Examples
    - Adrenaline and cortisol controlling stress responses.
    - Insulin regulates blood glucose levels.
    - Thyroid hormones regulate metabolic rate.
- **Importance:** Endocrine communication allows large-scale coordination of physiological processes across the entire organism, enabling homeostasis, growth, reproduction, and metabolic control.

QUORUM SENSING IN BACTERIA

- Quorum sensing is a form of **population-level communication** in prokaryotic cells
- How it works
    - Bacteria produce signalling molecules called **autoinducers**.
    - When cell density is low, autoinducer concentration is low → no response.
    - When population density increases, autoinducers accumulate and bind receptors.
    - This triggers coordinated changes in gene expression across the community.
- Examples
    - *Vibrio fischeri* producing light (bioluminescence) when at high density in squid
    - Biofilm formation in *Pseudomonas*
    - Virulence activation in pathogenic bacteria
- **Importance**: Quorum sensing allows bacteria to behave as a **multicellular collective**, coordinating behaviours that are ineffective at low densities but powerful at high densities.

COMMUNICATION BETWEEN ORGANISMS

Chemical communication (pheromones)

- Used widely in insects and mammals.
- Examples:
  - Trail pheromones in ants.
  - Alarm pheromones in aphids.
  - Territorial marking in mammals.
- These signals allow organisms to coordinate behaviour without direct contact.

Visual and auditory communication

- Examples:
  - Birdsong for territory defence and mate attraction.
  - Colour displays in cuttlefish.
  - Bioluminescence in fireflies.
- These allow long-range but species-specific signalling.

Sexual selection

- Sexual communication often evolves through **sexual selection**, involving:
- Intersexual selection (mate choice)
  - Peahens choosing peacocks with more elaborate tails.
  - Female birds choosing males based on song complexity
  - These signals indicate genetic quality or fitness
- Intrasexual selection (competition)
  - Deer using antlers to signal strength.
  - Territorial calls in frogs.
- **Importance:** Sexual communication drives evolution of exaggerated traits, influences reproductive success, and shapes biodiversity.

Communication systems evolve based on trade-offs:

- **Speed vs duration** (nervous vs endocrine).
- **Energy cost vs precision** (visual displays vs chemical signals).
- **Stability vs flexibility** (protein allostery vs hormonal cascades).
- **Individual vs group benefit** (quorum sensing).

This reflects the varying ecological and evolutionary pressures acting on organisms—from unicellular bacteria to complex animals.

DIVERSITY

Biological diversity, or biodiversity, refers not only to the enormous variety of species on Earth but also to the variation that exists within organisms, between organisms, and across ecosystems. Diversity emerges through genetic mechanisms, developmental processes, ecological interactions, and evolutionary forces. It underpins the resilience of life and enables adaptation in a dynamic environment. This essay explores diversity across multiple biological levels, from molecules to ecosystems, demonstrating how variation is generated, maintained, and selected.

MOLECULAR LEVEL

Genetic diversity

- DNA sequences vary between individuals due to:
    - mutations (point mutations, insertions, deletions)
    - recombination during meiosis
    - transposable elements
    - gene duplication events
- This diversity creates different alleles that influence traits from metabolism to disease resistance.

Protein and biochemical diversity

- Different proteins arise due to:
    - alternative splicing
    - post-translational modifications (phosphorylation, glycosylation)
    - allosteric regulation
- These molecular differences allow cells to respond to environmental signals, produce distinct phenotypes, and carry out specialised functions

Importance: Molecular diversity provides the raw material upon which natural selection acts.

CELLULAR LEVEL

Cellular specialisation

- Eukaryotes exhibit:
    - muscle cells for contraction
    - neurons for communication
    - xylem vessels for water transport
    - phagocytes for immunity
- Specialisation increases efficiency in multicellular organisms.

Signalling diversity

- Cells communicate via:
    - short-distance signals (paracrine, autocrine, gap junctions)
    - long-distance signals (hormonal, neuronal)
- Different cell types express different receptors → producing diverse responses to the same signal.

Importance: Cellular diversity allows division of labour and complex physiological integration.

ORGANISMAL LEVEL

Structural and physiological adaptations

- Examples:
  - thick fur in Arctic mammals vs thin skins in tropical species
  - cactus water-storage tissues
  - counter-current heat exchange in penguins
- Such adaptations arise through natural selection acting on genetic variation.

Behavioural diversity

- Organisms display distinct behaviours:
  - tool use in primates
  - cooperative hunting in wolves
  - pollinator preference in bees
  - anti-predator behaviours in fish and insects
- Behaviour enhances survival and reproductive success.

Importance: Organismal diversity enables exploitation of a wide range of ecological niches.

POPULATION LEVEL

Genetic polymorphism

- Populations contain multiple alleles at many loci.
- Examples:
  - ABO blood groups in humans
  - sickle-cell allele providing malaria resistance

Genetic variation within populations increases resilience to disease and environmental change.

Sexual selection

- Communication between individuals (e.g., calls, colour displays, pheromones) leads to:
  - intersexual selection (mate choice)
  - intrasexual competition (male–male competition)
- These processes drive remarkable diversity, such as:
  - peacock tails
  - bird-of-paradise dances
  - deer antlers

Importance: Population-level diversity enhances adaptability and shapes evolutionary trajectories.

SPECIES LEVEL

- Species diversity encompasses:
  - species richness (number of species)
  - species evenness (relative abundance)
- Drivers:
  - Adaptive radiation (e.g., Darwin's finches)
  - Co-evolution (e.g., flowering plants and pollinators)
  - Geographic isolation (speciation)
  - Ecological specialisation

High species diversity enhances ecosystem stability, productivity, and resilience.

ECOSYSTEM LEVEL

- Ecosystems differ widely:
    - tropical rainforests vs deserts
    - coral reefs vs deep-sea vents
    - temperate grasslands vs mangroves
- Ecosystem diversity arises from variations in:
    - climate
    - soil type
    - salinity
    - light availability
    - community interactions
- These differences support unique communities and energy flow patterns.

Importance: Ecosystem diversity ensures that life persists across a broad range of environmental conditions.

Evaluation

Diversity arises from both random processes (mutation, drift) and non-random processes (selection, sexual selection, co-evolution). Diversity acts as a buffer against extinction, enhances ecosystem functioning, and increases the likelihood that populations can adapt to global change. However, rapid anthropogenic impacts—climate change, habitat destruction, pollution—are causing unprecedented losses of diversity across all levels. Understanding diversity at multiple levels is essential for conservation and sustainable management of natural resources.

COMPLEMENTARITY 1

- Complementarity refers to the principle that two biological molecules, structures, or signals fit together in a specific, precise, and matching way—like a lock and key.
- It ensures specificity, accuracy, and reliability in biological processes.
- It appears at every level of biology: molecular, cellular, organismal, ecological.

MOLECULAR

Base-pairing in nucleic acids

- example:
  - A pairs with T/U (2 hydrogen bonds)
  - C pairs with G (3 hydrogen bonds)
- This complementarity ensures:
  - accurate DNA replication
  - transcription fidelity
  - correct structure of RNA molecules

Protein–ligand complementarity

- Proteins bind to their substrates due to:
  - shape complementarity
  - charge complementarity
  - hydrophobic complementarity
- Examples:
  - enzyme active site matching a substrate
  - receptor binding a hormone
  - antibody recognising an antigen epitope
- This allows specific signalling and regulation.

tRNA–mRNA complementarity

- The anticodon of a tRNA pairs with a complementary codon on mRNA.
- This ensures correct amino acid placement during translation.

CELLULAR

Cell–cell recognition

- Cells communicate using complementary molecules on their surfaces:
  - immune cells recognising MHC–peptide complexes
  - sperm binding to complementary receptors on egg surface during fertilisation
- This ensures specificity during immunity and reproduction.

Hormone–receptor complementarity

- Each hormone fits only its specific receptor:
  - insulin receptor
  - adrenaline receptor
  - thyroid hormone receptor
- This allows precise long-distance communication.

PHYSIOLOGICAL

- Systems work together using complementary roles:
    - nervous system (fast) + endocrine system (slow)
    - sympathetic vs parasympathetic nervous systems
    - osteoblasts (build bone) vs osteoclasts (break down bone)
- Complementarity creates balance (homeostasis).

ECOLOGICAL

Niche complementarity

- Different species use different resources in complementary ways:
    - forest layers (canopy, understory, ground flora)
    - grazing vs browsing herbivores
    - pollinators specialising on different flower shapes
- This increases biodiversity and ecosystem stability.

Mutualistic complementarity

- Two species evolve complementary traits:
    - flowers and their pollinators
    - mycorrhizal fungi and plant roots
    - cleaner fish and host fish
- Complementarity here drives co-evolution.

Complementarity ensures:

- Fidelity (e.g., DNA replication)
- Specificity (e.g., enzyme reactions)
- Efficiency (e.g., signalling pathways)
- Compatibility (e.g., reproduction)
- Coexistence (e.g., niche partitioning)

Without complementarity, biological systems would be chaotic, inaccurate, and unstable.

PERFECT COMPLEMENTARITY (2)

YES PERFECT COMPLIMENTARITY

DNA base pairing (central dogma accuracy)

- DNA replication requires exact base complementarity (A–T, C–G).
- Ensures genetic fidelity → prevents mutations.
- Why perfect? – Errors would change amino acids → harmful mutations → loss of function.

RNA interference (siRNA)

- siRNA binds to mRNA with perfect complementarity.
- RISC slices the mRNA → degradation.
- Why perfect? – Mismatch would prevent slicing and block gene silencing.

Enzyme–substrate complementarity (in the active site core)

- Even though enzymes undergo induced fit, the catalytic residues still require precise orientation.
- The transition state must match perfectly.
- Wrong substrate → no catalysis.
- Why perfect? – Catalysis requires exact positioning of atoms and charges.

tRNA charging

- Aminoacyl-tRNA synthetases match:
  - exact tRNA anticodons
  - specific amino acids
- Why perfect? – A mismatch would place the wrong amino acid → mis-translation.

Protein–DNA binding (transcription factors)

- Transcription factors recognise specific DNA motifs with near-perfect sequence complementarity.
- Why perfect? – Controls gene expression with high precision.

NO PERFECT COMPLIMENTARITY

Induced fit model of enzymes

- An enzyme's active site is not a perfect rigid match.
- Instead:
  - substrate binds loosely → enzyme changes shape → perfect complementarity achieved after binding.
- Why imperfect? – Allows:
  - broader substrate range (promiscuous enzymes)
  - regulation (allosteric control)
  - avoidance of unwanted tight binding to wrong molecules

Wobble base pairing in translation

- The third codon position allows imperfect complementarity:
  - G–U pairs
  - Inosine (I) pairing with A, U, C

- Why imperfect? – Reduces the number of tRNAs needed As one tRNA can read multiple codons.

miRNA regulation (animals)

- miRNAs bind to mRNA with partial complementarity, especially in the 3′UTR.
- Why imperfect? – Allows one miRNA to regulate hundreds of genes → broader control.

Antigen–antibody interactions

- Antibodies bind with shape complementarity, not sequence complementarity.
- Binding is not “perfect”; instead, affinity improves during somatic hypermutation.
- Why imperfect? – Allows the immune system to recognise a huge range of antigens.

Cell–cell interactions (immune synapse)

- Receptors like TCR recognise peptides on MHC with loose but specific complementarity.
- Why imperfect? – T cells need to recognise millions of peptides from a limited receptor set.

Hormone–receptor binding

- Hormones bind receptors via a combination of:
    - charge complementarity
    - hydrophobic complementarity
    - partial shape matching
- Why imperfect? – Allows multiple hormones to modulate the same pathway (e.g., cortisol, aldosterone

CONTROL

MOLECULAR

A. Control of metabolic pathways
- Allosteric regulation (e.g. ATP inhibits phosphofructokinase).
- End-product inhibition.
- Reversible phosphorylation by kinases/phosphatases.
- Compartmentalisation for micro-environment control (mitochondria, chloroplasts).

B. Control of protein structure/function
- Intramolecular signalling → conformational changes.
- Example: haemoglobin cooperative binding.

GENE EXPRESSION

A. Transcriptional control
- Prokaryotic operons (lac operon regulation).
- Eukaryotic transcription factors, enhancers, chromatin remodelling.
- Epigenetic modifications (DNA methylation, histone tails).

B. Post-transcriptional & translational control
- Alternative splicing; miRNAs; mRNA stability.
- Translational control via initiation factors.

C. Post-translational control
- Protein folding, cleavage (e.g. POMC), chemical modification.

Purpose: spatial and temporal regulation of protein synthesis.

CELL CYCLE

A. Checkpoints
- G1 (DNA integrity), G2/M (replication completion), spindle checkpoint.

B. Cyclin–CDK system
- Cyclin accumulation/degradation regulates transitions.

C. Failure of control
- Leads to cancer → importance emphasised.

HOMEOSTASIS IN MULTICELLULAR ORGANISMS

A. Nervous system control
- Fast, specific control using action potentials + synapses.
- Examples: reflex arcs, heart rate control.

B. Endocrine control

- Hormone-mediated, long-lasting responses: insulin, ADH, cortisol.

C. Feedback mechanisms

- Negative feedback for stability (thermoregulation).
- Positive feedback for rapid amplification (oxytocin, blood clotting).

DEVELOPMENT

A. Embryonic pattern formation

- Morphogen gradients (e.g. bicoid, Sonic hedgehog).

B. Cell differentiation

- Gene regulatory networks, master transcription factors.

Importance: ensures correct formation of tissues and organs.

POPULATION & ECOSYSTEMS

A. Population control

- Density-dependent limiting factors: predation, competition, disease.

B. Predator–prey control

- Lotka–Volterra cycles regulating population sizes.

C. Ecosystem-level control

- Control of biogeochemical cycles by microbes and environmental factors.

A. Quorum sensing in bacteria

- Autoinducers, biofilm formation, virulence control.

REDUNDANCY

Redundancy refers to the presence of duplicate, overlapping, or backup components within biological systems. This includes redundancy at the genetic, cellular, organ, physiological, and behavioural levels. Far from being wasteful, redundancy is a fundamental feature that enhances the survivability, robustness, and evolutionary fitness of living organisms. It allows organisms to tolerate damage, buffer against environmental fluctuations, and maintain essential functions even when key components fail. This essay will explore the multifaceted importance of redundancy and evaluate why natural selection favours redundancy even though it incurs metabolic costs.

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1. Genetic Redundancy Ensures Survival Despite Mutations

Organisms carry two copies of most genes (diploidy), and this provides a critical safety net.

- If one allele is damaged by mutation, the second allele can still function.
- Recessive deleterious mutations remain phenotypically silent in heterozygotes, preventing immediate loss of fitness.
- Gene duplication events create paralogous genes with overlapping functions. Even if one copy mutates, the other can maintain the essential function.

Example:

The Hox gene clusters in vertebrates exist in mostly duplicated sets following whole-genome duplications. Mutations in one Hox cluster may not be lethal because the paralogous cluster compensates.

This redundancy is crucial because DNA is constantly exposed to mutagens; without genetic redundancy, mutation load would rapidly accumulate and threaten survival.

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2. Redundancy in the Genetic Code Reduces the Impact of Errors

The genetic code is degenerate:

- Many amino acids are encoded by more than one codon.
- Mutations in the third base of a codon often produce a silent mutation.

This degeneracy protects against the harmful effects of point mutations and ensures translation accuracy despite noise, replication errors, or base damage.

Example:

Glycine is encoded by four different codons (GGU, GGC, GGA, GGG). A mutation in the third base often does not change the amino acid.

Thus, redundancy at the coding level preserves protein function even when sequences mutate.

---

3. Cellular and Chromosomal Redundancy Protects Against Damage

(a) Paired chromosomes

Diploid organisms have two sets of chromosomes, so losing one does not immediately remove entire genetic pathways.

(b) Sister chromatids in DNA repair

During S phase, each chromosome is duplicated, producing two identical sister chromatids. This redundancy is essential for:

- homologous recombination repair of double-strand breaks
- accurate chromosomal segregation

Without sister-chromatid redundancy, error rates during DNA repair and cell division would dramatically increase.

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4. Redundancy in Metabolic Pathways Enables Flexibility

Many metabolic networks have:

- alternative pathways
- backup enzymes
- isozymes (enzymes with different structures but the same function)

This ensures metabolism continues even if one pathway is inhibited.

Example:

Aerobic respiration can shift to anaerobic fermentation when oxygen is absent.

This redundancy allows cells to generate ATP even under stress.

Another example is glycolysis, where multiple enzymes exist in different tissue types (hexokinase vs glucokinase), ensuring glucose utilisation can proceed under varying physiological conditions.

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5. Physiological Redundancy Enhances Survival in Unpredictable Environments

(a) Immune system redundancy

The immune system has:

- Innate and adaptive branches
- Multiple pathogen-recognition receptors
- Overlapping cytokine signalling pathways

This redundancy ensures that if one pathway fails, others compensate.

Example:

Toll-like receptors (TLRs) detect overlapping sets of microbial molecules, so the immune system can still respond even if one TLR is mutated.

(b) Hormonal redundancy

Homeostasis is maintained by multiple hormones working in parallel.

Example:

Blood glucose is regulated by:

- insulin
- glucagon
- adrenaline
- cortisol

If one hormone pathway fails, the others buffer the disturbance.

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6. Organ-Level Redundancy Prevents Catastrophic Failure

Many organisms possess duplicated or partially duplicated organs.

(a) Paired organs

- kidneys
- lungs
- eyes
- testes/ovaries

Loss of one does not eliminate function.

(b) Reserve capacity

Even single organs have substantial redundancy:

- The liver can operate normally with <30% of its mass.
- The heart has collateral blood vessels that re-route blood when arteries are blocked.
- The brain forms alternative neural pathways to compensate after injury (plasticity).

Such redundancy protects organisms from injury, disease, and ageing.

7. Behavioural Redundancy Increases Chances of Success

Animals often have multiple strategies for feeding, mating, defence, or navigation.

Examples:

- Birds use redundant cues for navigation: magnetic fields, stars, the Sun, and landmarks.
- Prey species use a combination of hiding, fleeing, and mobbing predators.
- Humans have redundant communication strategies (speech, gestures, facial expression).

This redundancy ensures survival even if one strategy becomes ineffective.

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8. Redundancy Promotes Evolutionary Innovation

Gene duplication creates extra genetic material that is initially redundant.

One copy maintains the original function while the other accumulates mutations → this can lead to:

- new functions (neofunctionalisation)
- division of labour between copies (subfunctionalisation)

Redundancy therefore acts as the raw material for evolutionary complexity.

Example:

The evolution of vertebrate haemoglobin and myoglobin from a duplicated ancestral globin gene.

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9. Costs and Limitations of Redundancy (Evaluation)

Although redundancy is vital, it has costs:

- higher metabolic demands
- more DNA to replicate
- more tissue to develop and maintain
- potential for harmful overcompensation (e.g., cytokine storms)

If redundancy were too excessive, the organism would waste resources.

However, evolution keeps redundancy at an optimal level: enough to protect against failure but not so much as to be biologically expensive.

GENETIC STABILITY

Genetic stability refers to the ability of an organism to maintain the integrity, accuracy, and reliability of its genetic information across cell divisions and generations. It is essential for survival because it ensures that DNA sequences are transmitted faithfully, allowing normal development, proper cellular function, and accurate inheritance. Despite constant threats from replication errors, environmental damage, and metabolic by-products, living organisms use multiple layers of mechanisms to preserve genomic stability. This essay explores the molecular basis of genetic stability, the cellular systems that maintain it, and why failure of stability has severe consequences.

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1. Structural Features of DNA Contributing to Stability

Genetic stability begins with the chemistry and structure of DNA.

(a) DNA is double-stranded

- The complementary strands provide a template for accurate repair.
- If one strand is damaged, the other guides restoration.

(b) Base pairing ensures specificity

- Hydrogen bonds and correct base geometry (A–T, C–G) ensure accurate replication.
- Any mismatch distorts the helix → easily detected by repair enzymes.

(c) Sugar–phosphate backbone protects the bases

- The backbone faces outward while bases are internal, shielding them from chemical attack.

(d) Thymine replaces uracil

- Cytosine deamination produces uracil.
- DNA repair enzymes recognise uracil as damage because thymine is the “real” base.
- This prevents permanent mutations.
- These structural features create a molecule that is stable yet accessible to repair machinery.

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2. Fidelity of DNA Replication

Accurate DNA replication is a central mechanism ensuring genetic stability.

(a) DNA polymerases have high specificity

- Correct base pairs fit perfectly within the active site (“induced fit”).
- This drastically reduces misincorporation.

(b) Proofreading (3’→5’ exonuclease activity)

- If the polymerase inserts the wrong base, it removes it immediately.
- This lowers the error rate from 1 in 10^5 to 1 in 10^7.

(c) Semi-conservative replication

- Each new DNA molecule contains one original strand, reducing copying errors and allowing mismatch repair to identify the newly synthesised strand.

(d) Replication forks contain multiple quality-control proteins

- Helicase, SSBs, topoisomerases, and sliding clamps maintain fork stability, preventing strand breakage and mutations.

---

3. DNA Repair Mechanisms Maintain Stability

Organisms have developed a wide range of sophisticated DNA repair pathways to handle different types of damage.

(a) Mismatch Repair (MMR)

Fixes replication errors missed by proofreading.

- Proteins detect helix distortions.
- The newly synthesised strand is selectively removed.
- DNA polymerase re-synthesises the region.

Defects in MMR cause microsatellite instability and cancers (e.g., Lynch syndrome).

(b) Base Excision Repair (BER)

Fixes small lesions such as oxidised or deaminated bases.

- Glycosylases remove damaged bases.
- AP endonuclease cuts the backbone.
- DNA polymerase and ligase restore the sequence.

Critical for removing uracil resulting from cytosine deamination.

(c) Nucleotide Excision Repair (NER)

Repairs bulky lesions.

- UV-induced thymine dimers
- Chemical adducts

A short DNA segment surrounding the lesion is excised and resynthesised.

Defects in NER cause xeroderma pigmentosum, demonstrating its importance.

(d) Double-Strand Break Repair

Two pathways maintain chromosomal integrity:

- Homologous recombination (HR) uses sister chromatids as templates, accurately repairing breaks.
- Non-homologous end joining (NHEJ) rejoins broken ends; less accurate but essential when HR is unavailable.

Without these pathways, chromosomal fragmentation and cell death would occur.

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4. Cell Cycle Control and Checkpoints Maintain Genetic Stability

Cell division is tightly regulated to prevent the propagation of damaged DNA.

(a) G1 checkpoint

- Checks DNA integrity before replication begins.
- p53 halts the cell cycle and induces repair or apoptosis if necessary.

(b) S-phase checkpoints

- Monitor replication fork stability.
- If forks stall, ATR/ATM pathways pause replication to prevent DNA breakage.

(c) G2/M checkpoint

- Prevents entry into mitosis until DNA is fully replicated and repaired.

(d) Spindle assembly checkpoint

- Ensures chromosomes are attached correctly to the spindle before separation.
- Prevents nondisjunction and aneuploidy.
- Checkpoints ensure only accurate, fully replicated genomes are passed to daughter cells.

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5. Telomeres and Telomerase Protect Chromosome Ends

Telomeres are repetitive DNA sequences that protect chromosome ends from degradation or fusion.

Functions for genetic stability:

- Prevent chromosome ends from being recognised as double-strand breaks.

- Buffer the loss of nucleotides during DNA replication.
- Ensure accurate segregation during mitosis.

Telomerase extends telomeres in germ cells, stem cells, and some somatic cells, ensuring long-term genomic stability.

When telomeres shorten excessively, cells enter senescence, preventing the propagation of damaged DNA.

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6. Genetic Redundancy Enhances Stability

Diploidy and gene duplication act as biological backup systems.

- If one allele is mutated, the other may still function.
- Polyploid plants often have greater genetic stability due to extra copies of essential genes.
- Duplicated genes allow repair of double-strand breaks through homologous recombination.

Redundancy reduces the likelihood that single mutations become lethal.

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7. Chromatin Structure and Epigenetics Maintain Stability

(a) Packaging DNA into chromatin protects it from damage

- Histones and nucleosomes shield DNA from physical and chemical insult.

(b) Heterochromatin silencing prevents transposon activity

- Transposable elements can destabilise genomes.
- DNA methylation and histone modifications keep them inactive.

(c) Epigenetic marks regulate gene expression accurately

- Errors in epigenetic states can cause inappropriate gene activation or silencing, leading to instability.
- Stable epigenetic inheritance contributes to orderly development.

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8. Consequences of Loss of Genetic Stability (Evaluation)

Genetic instability has severe consequences:

(a) Cancer

- Accumulating mutations activate oncogenes and deactivate tumour suppressors.

- Defective checkpoint control and impaired DNA repair accelerate instability.

(b) Developmental abnormalities

- Mutations disrupt signalling pathways, causing congenital defects.

(c) Ageing

- Telomere shortening, mitochondrial DNA mutations, and accumulated DNA damage contribute to cellular ageing.

(d) Loss of species fitness

- Populations with high genomic instability suffer reduced fertility, increased lethality, and greater susceptibility to environmental change.
- Thus, natural selection strongly favours mechanisms enhancing stability.

Conclusion

Genetic stability is vital for life because it ensures that DNA is replicated faithfully, repaired efficiently, and transmitted reliably across generations. The double-helical structure of DNA, high fidelity replication, proofreading and repair pathways, cell cycle checkpoints, telomere maintenance, chromatin organisation, and genetic redundancy all contribute to maintaining genome integrity. Without these systems, organisms would accumulate harmful mutations, leading to cancer, ageing, or death. Genetic stability is therefore a fundamental requirement for development, evolution, and survival.

DIRECTIONALITY

MOLECULAR

DNA/RNA directionality (5′ → 3′)

What:

- DNA/RNA strands have inherent polarity.
- Polymerases synthesise nucleic acids only **5′→3′**.
- Template read **3′→5′**.

Importance:

- Ensures **accurate replication** and **proofreading**.
- Prevents mutated nucleotides being added unpredictably.
- Gives a universal direction for the “flow of information” → central dogma.

Protein synthesis direction (N-terminus → C-terminus)

What:

- Ribosomes add amino acids only to the C-terminal end.

Importance:

- Guarantees consistent folding pathways
- Ensures that signal peptides (at N-terminus) enter ER first → correct targeting.
- Prevents malformed or partially reversed proteins.

Directional enzyme movement (helicases, polymerases, motor proteins)

What:

- Enzymes act along substrates in fixed directions.

Importance:

- Prevents collisions between enzymes.
- Ensures ordered replication fork movement.
- Makes metabolism predictable and coordinated.

CELLULAR

Cytoskeletal polarity (microtubules + actin)

What:

- Microtubules have + and − ends → kinesin and dynein move in fixed directions.

Importance:

- Ensures directional vesicle trafficking (e.g., neurotransmitter delivery).
- Establishes cell polarity (apical/basal orientation).
- Essential for mitotic spindle function.

Membrane and transport directionality

What:

- Pumps and channels move ions in one direction.
- Asymmetric membranes (inside vs outside leaflet).

Importance:

- Maintains ion gradients vs entropy (e.g. Na^+/K^+ pump).
- Enables nerve impulses, muscle contraction, and nutrient absorption.
- Prevents backflow of materials.

Cell polarity in tissues

What:

- Epithelial cells have distinct apical/basal surfaces.

Importance:

- Coordinates absorption (gut), secretion (glands), and barrier formation.
- Prevents leakage and mixing of compartments.

GENE EXPRESSION

Transcription orientation

What:

- Promoter directs RNA polymerase in only one direction.

Importance:

- Ensures correct gene is transcribed.
- Prevents antisense RNA interfering with translation.
- Allows regulated gene networks.

Translation directionality (ribosome reads 5′ → 3′)

Importance:

- Maintains reading frame accuracy.
- Prevents frameshifts and truncated proteins.
- Allows consistent initiation at AUG.

Protein targeting signals (N-terminal signals)

Importance:

- Ensures proteins reach correct organelles (ER, mitochondria, peroxisomes).
- Prevents toxic mislocalisation.

ANIMAL PHYSIOLOGY

Nervous system directionality

What:

- Impulses travel dendrite → cell body → axon → synapse.

Importance:

- Ensures information flows rapidly and predictably.
- Prevents backward conduction due to refractory period.
- Basis of reflexes, coordination, perception.

Circulatory directionality

What:

- Blood flows in one direction: heart → arteries → capillaries → veins.

Importance:

- Efficient oxygen and nutrient delivery.
- Removal of waste products.
- Prevents mixing of oxygenated/deoxygenated blood.

Digestive system directionality

Importance:

- Peristalsis ensures unidirectional food movement.
- Prevents blockage and microbial overgrowth
- Coordinates enzyme action in sequence.

Endocrine directionality

What:

- Gland → blood → target cells.

Importance:

- Allows regulation of distant tissues (glucose, metabolism).
- Prevents hormones acting on wrong tissues.

PLANTS

Polar auxin transport

What:

- PIN proteins mediate directional auxin export.

Importance:

- Drives tropisms → adaptive growth toward light or gravity.
- Establishes root/shoot axes during development.

Transport in xylem and phloem

Importance:

- Xylem: root → shoot ensures effective water transport.
- Phloem: source → sink ensures efficient sugar distribution for growth, fruiting, storage.

Meristem directionality

Importance:

- Ensures organised plant architecture.
- Correct formation of leaves, flowers, roots.