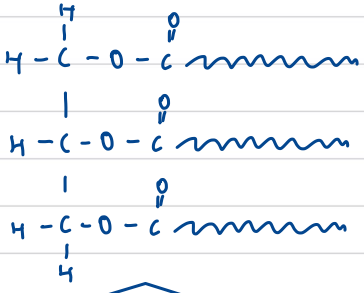
Lipids

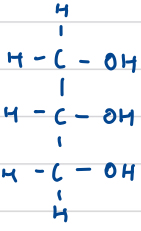
Properties of lipids :

1 g)

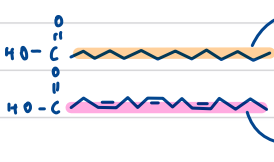
triglycerides



glycerol :



Fatty acids :

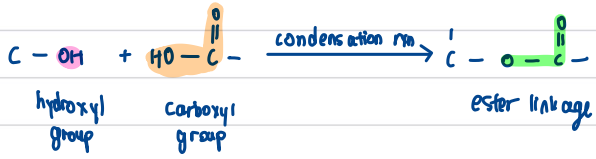


properties of fatty acids (in lipids) :

1. melting point of lipids ↑ w/ hydrocarbon chain lenght
   - as lenght ↑, hydrophobic interacts become more extensive

2. melting point of fats ↓ as ° of unsaturation ↑
   - kinks in f.a. prevents molecules from packing tgh. closely
     ↳ when f.a. are less closely packed, ↓ thermal energy rqd. to overcome hydrophobic interactions

condensation rxn



Relating structure to function:

1. $\frac{C,H}{total} > \frac{O}{total}$; greater no. C atoms / unit mass than carbs ⟶ triglycerides release ↑ amt. of energy
   ∴ triglycerides ≫ carbs in efficiency

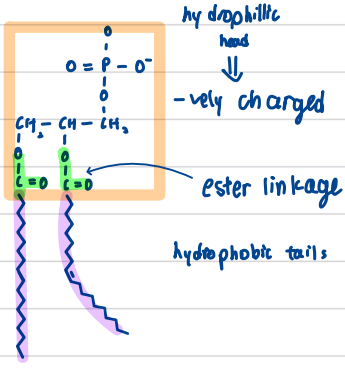
2. Triglycerides are highly reduced (↑↑ hydrogen) ⟶ triglycerides release ↑↑ H_2O during respiration

3. C-H bonds non polar (hydrophobic) ⟶ triglycerides do not affect water potential when stored in large amts.
   ↳ no associated H_2O molecules stored
   ↳ mass kept to a minimum
   ↳ gd thermal insulator

4. Hydrophobic tails → non polar ⟶ triglycerides can slide under pressure (to protect vital organs)
   ↳ weak hydrophobic interactions

5. Triglycerides have < weight than water ⟶ aids buoyancy

Phospholipids



Properties:

- amphipathic
- types of lipid aggregates
  1. micelle
  2. Bilayer
  3. Liposome / vesicle

Glycolipid (relating structure to function)

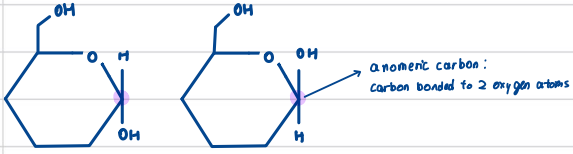
1. A (short) carb chain attached to the glycerol ⟶
   - marker for cell-cell recognition
   - involved in cell-cell adhesion
2. Hydrophobic interactions btw. fatty acid tails ⟶
   - anchor entire glycolipid at cell membrane

Carbo

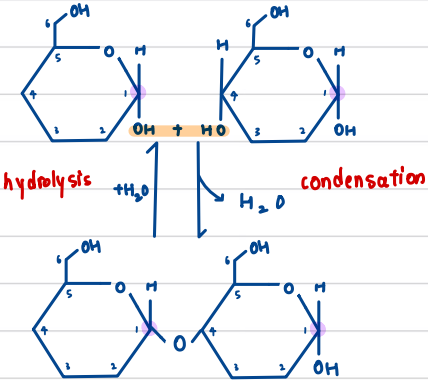
Uses of monosaccharides:

1. Energy sources to produce ATP during cellular respiration
2. Building blocks for synth of di- and poly- saccharides
3. Raw material for synth of other organic molecule

Structure of monomers:



α-glucose β-glucose



Benedict's test:

- uses free carbonyl group in red. sugar to reduce Cu^{2+} to Cu^{+}
- under alkaline conditions, blue $CuSO_4$ is reduced to insoluble red Cu_2O

- non-reducing sugars must undergo acid hydrolysis before it can be detected

starch (α-glucose)

amylose (less)
- α(1,4) bonds
- blue black in KI solution

amylopectin (more)
- α(1,4) bonds
- α(1,6) bonds
- red violet in KI solution

glycogen (α-glucose):
- α(1,4) & α(1,6) bonds
- Red violet in KI solution

Relating structure to function:

Large molecule
- stores large amt. of glucose
- insolube → does not affect water potential

α(1,4) glycosidic bond
→ easily hydrolysed by enzyme (+ lack of cross links)
→ helical coil → compact shape

α(1,6) glycosidic bond
→ highly branched
→ ↑ free ends for ↑ hydrolysis by enzyme
→ ↑ compact structures

+ in starch, anomeric carbon

Structural polysaccharides:

cellulose

- β-glucose ; β(1,4) glycosidic bond

To obtain β(1,4) bonds, alternate monomers are inverted
  - ↳ chains run parallel to each other;
  - ↳ OH groups project outwards
  - ↳ extensive hydrogen bonds btwn. protruding OH allows cross links to be established btwn. chains

structure: cross-linked cellulose chains ⟶ microfibrils ⟶ macrofibrils

Relating structure to function:

1. large molecule ⟶ insoluble

2. β(1,4) glycosidic bonds ⟶ • usually cannot be hydrolysed by enzymes
   ↳ stable

3. • Alt. inverted β glucose units linked by β(1,4) glycoside bonds
   • extensive hydrogen bonds
   • microfibrils → macrofibrils

   } high tensile strenght for structural support

Proteins

Properties of amino acids (colourless & crystilline solids w/ relatively high mp)

1. Ability to form Zwitterions
   ↳ electrically neutral, dipolor ion

2. Ability to act as Buffer
   ↳ amphoteric

Structures of proteins

1. Primary structure (the unique number + linear sequence of a.a.)
   ↳ proteins synth. in vivo via stepwise polymerisation of a.a.
   ↳ (peptide bonds)

2. Secondary structure
   ↳ regulary coiling + folding give rise to repeated patterns

a. α-helix
   ↳ extended spiral spring; stabilised by extensive hydrogen bonds
   ↳ hydrogen bonds // to main axis, ALL C=O and N-H groups are able to participate in hydrogen bonding
   ↳ R-groups rest outside the helix
   ☆ proline and hydroxyproline insert a kink & disrupt the formation of the α helix

b. β-pleated sheet
   ↳ extended zigzag, sheet like conformation
      ↳ stabilised by hydrogen bonds btw. C=O and N-H groups
         • can be intra-chain or interchain (non considered 2° structure)
   ↳ can be antiparallel β-pleated sheet or parallel β-pleated sheet

   • bulky R groups can cause steric hinderance (interfere w/ formation of β-pleated sheet)
   ∴ a.a. residues in β-pleated sheets have small R group

3. Tertiary structure

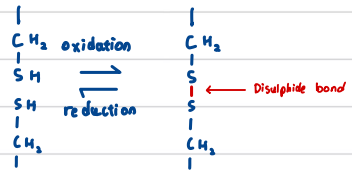
↳ further bending twisting and folding of p.p. chain w/ 2° structures to give an overall 3D conformation

a. Types of bonds

1. ionic bonds
2. hydrogen bonds
3. hydrophobic interactions

} non-covalent interactions (weak)

4. disulfide bonds → covalent bonds (strong)



4. Quaternary structure

↳ the overall protein structure that results from the association of two or more protein chains to form a functional protein

types of bonds: ionic, hydrogen, disulphine bonds, hydrophobic interactions and disulphide bonds

(same as 3° structure)

	Fibrous protein	Globular protein
Shape	elongated; rope-like	compact, spheroidal structure
a.a. sequence	repetitive	non-repetitive
a.a. variety	small, specific variety	wide variety
variation	small variation	never varies
lenght	may vary	always identical
stability	stability	relatively unstable
function	structural	metabolic
solubility	insoluble	more soluble

Haemoglobin (globular protein)

2° structure : 8 × α-helices (stabilised by hydrogen bonds)

3° structure : • 2 α-chains ; 2 β-chains
• 2 identical dimers ; 2(αβ)

↳ hydrophillic a.a residues at surface, hydrophobic a.a. residues at interior } soluble in aq. medium

↳ formation of hydrophobic cleft w/ haem prosthetic grp to bind
• 4 chains total ; 1 O_2 / prosthetic grp. (4 O_2 / molecule total)

Haem group : structure : • Fe^{2+} grp in a polyphyrin ring
• binds reversibly w/ O_2

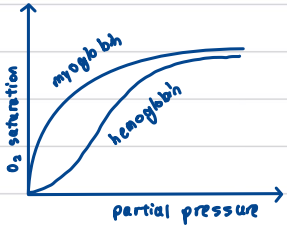
4° structure : • subunits in each dimer held w/ hydrophobic interactions
• form globular molecule held w/ non-covalent interactions

Relating structure to function

- when Fe^{2+} in 1st haemg. subunit binds to 1 molecule of O_2, a strain is created on other haemoglobin subunits previously obscured haem groups revealed
- remaining subunits change 3D conformation to bind to O_2 more readily
- remaining subunits' affinities for O_2 ↑.

Subunit cooperativity:

- O_2 is loaded onto haemog. in lungs where partial pressure is high
  - ↑ O_2 bound to haemgl subunits
- O_2 is unloaded from haemgl. where partial pressure is low
  - ↓ O_2 bound to haemgl. subunits



* As myoglobin has a higher affinity binding to O_2 than haemoglobin, it loses O_2 efficiently
  ∴ haemgl. is a more efficient O_2 carrier than myoglobin

Collagen

1° structure: repeating tripeptide sequence of Glycine – X – Y
- X is often proline
- Y is often hydroxyproline

2° structure: collagen helix (left handed twist; 3 residues / turn)
- ↳ regulary repeated structure ★

(proline residues prevent α-helix from forming)

4° structure: 3 parallel α-chain wind around each other w/ a right-handed; rope like twist (right handed triple helix) to form tropocollagen
- well packed, triple helix
- small R group of Glycine passing through center of helix
- residues in X and Y position w/ bulky R-groups located outside triple helix
- proline stabilises collagen helix

→ high tensile strenght

- tropocollagen is held tight by extensive network of hydrogen bonds
  - ↳ betwn. N-H grp of Gly reside and C=O grp in diff α-chains
  - ↳ OH grp of hydroxy proline / - lysie also have interchain H bonding
- covalent cross links also contribute to high tensile strenghts

tropocollagen ⟶ collagen fibril ⟶ collagen fibre

⇓

arranged in staggered manner for greater tensile strenght

Denaturation

Involves the disruption of 2°, 3° and 4° structure

Conditions:

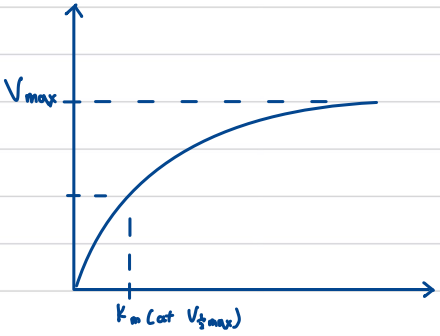
- Heat ⟶ excessive heat ↑ vibrations of atoms
  - disruption of all non-covalent bonds
- change in pH ⟶ Changes charges in acidic / basic R groups
  - disruption of ionic & hydrogen bonds
- organic solvents ⟶ transfer from aq. medium to organic solvent
  - disrupts hydrophobic interactions at core of globular proteins
  - protein turns inside out ; hydrophobic & hydrophilic regions change place
- urea defergents ⟶ addition of chemicals disrupt ionic and hydrogen bonds

Enzymes

Enzyme kinetics

V_{max} → max. rate at which enzymes can perform the reaction

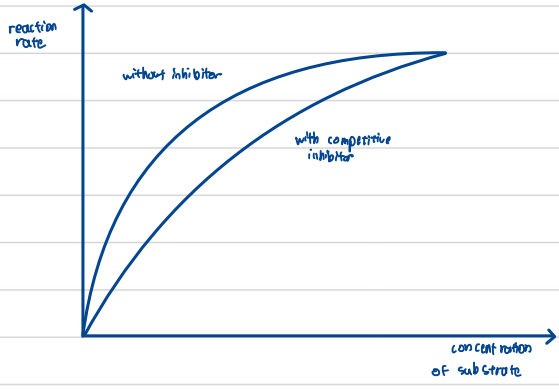
K_m (michaelis constant) → the affinity of the enzyme for its substrate



Inhibition

Competitive inhibition:

- structurally similar to substrate molecule and compete w/ substrate for binding w/ active site
- inhibitor remains bound to active site and prevents substrate from binding to active site



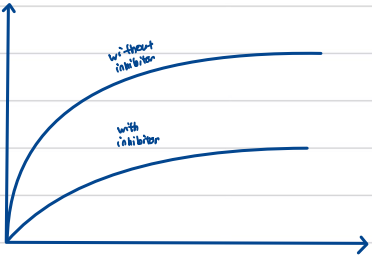
Explain:

- ↑ in substrate concentration reduces effect of inhibition
- as chance for substrate is able to outcompete the inhibitor,
- rate of rxn and final amt. of product is the same

Non-competitive inhibition:

- inhibitor has no structural resemblance to substrate
- binds to site on enzyme other than the active site
  - ↳ binding alters 3D conformation of enzyme molecule and active site

∴ no e-s complexes formed



Explain

- binding of inhibitor causes a change in the 3D conformation of active site
  - ↳ prevents substrate from binding
- as certain proportion of enzymes are inactive, Vmax lowered

∴ ↑ in substrate concentration has no effect on inhibition

(as Km remains unchanged, final amt. of products formed is the same)

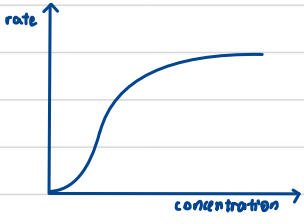
Allosteric regulation (multiple binding sites)

Allosteric activation:

when activator binds, it stabilises active form of enzyme and ↑ affinity of enzyme

Allosteric regulation:

When inhibitor binds, it stabilises inactive form of enzyme and ↓ affinity of enzyme



Subunit cooperativity:

1. binding of one substrate to an active site of a multimeric enzyme triggers favourable conformal change in active sites of all other subunits
2. one substrate primes enzyme to accept additional substrate molecule

reversible inhibition ⟶ weak hydrogen bonds

irreversible inhibition ⟶ strong covalent bonds

advantages of metabolic pathways

1. biochem rxns can proceed w/out accumulation of products / products used in subsequent rxns

2. rxns may be modified in small steps

3. each step catalyzed by specific enzyme provides a point for control

4. permits high conc. of molecules so biochem rxns can proceed rapidly

5. multi-merit complex allows for sequence of rxns, ↑ efficiency

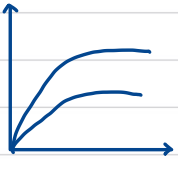
End product inhibition ⟶ when end products accumulate, products may act as inhibitors on enzymes controlling preceding steps

via 1. altering conformation of enzyme

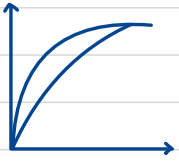
2. block entry of substrate into active site

Sample graphs:

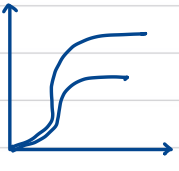
single binding site, NCI



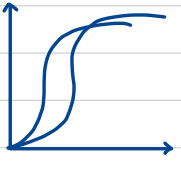
single binding site, CI



multiple sites, NCI



multiple sites, CI



Standard explanations

1) At low substrate concentration : proportionate increase as

- not all active sites are occupied, rate limited by the concentration of substrate
- as fewer successful collisions between correctly-oriented substrate with active site of enzyme, rate of enzyme substrate complex formation is limited — rate of product formation is limited

2) At high substrate concentration :

- rate is limited by number of available active sites
- As all active sites are fully saturated, any further increase in substrate conc. will not result in an increase

3) At low enzyme concentration : proportional increase as enzyme is limiting factor

- increase in enzyme concentration provides more active sites.
- As frequency of effective collisions is higher, rate of enzyme-substrate complex formation is higher — rate of product formation is higher
- faster rate of reaction

4) At high enzyme concentration : no further increase, graph plateaus

- no more ESC can form

1) As T increases to optimum temp,

- at low temp, enzymes are inactivated
- increasing temperature increases KE — frequency of effective collisions between substrates and active sites increase
- rate of formation of ES-complex increase — rate of reaction increases ; highest at optimum T

2) As T increases beyond optimum temp

- decrease in the rate of reaction as thermal agitation disrupts the H bonds, ionic bonds that stabilise the specific 3D conformation of the protein molecule
- loss in specific 3D conformation of active site causes enzyme to not be complementary to shape of the substrate
- frequency of effective collusions decrease — ES complex formation rate decrease — rate of product formation decreases

1) Changes in pH affect changes the ionic charge of the acidic and basic R groups — disrupts the ionic bonds/hydrogen bonds that maintain the 3D conformation of the enzyme, denaturing it

- structural aa residues denatured — no longer complementary — no ESC formed
- Binding aa residues at active sites denatured — substrate cannot be held in correct orientation
- Catalytic aa resides

Cell structure

Cell theory :

1) All living organisms are composed of one or more cells

2) The cell is the most basic unit of structure in all organisms

3) All cells come from pre-existing cells

Advantages to having membranous organelles

1) allows maintenance of characteristic difference — compartmentalisation provides diff local environments for which incompatible processes can occur simultaneously

2) internal membranes increase membrane surface area — enables embedding of enzymes and proteins, providing optimal enzyme concentration for reactions to occur

DNA Replication

Why form complementary base pairs?

1. Steric restrictions (must always pair a purine w/ a pyrimidine)
2. Hydrogen bond factors (A = T, C ≡ G)

Significance:

As DNA is only stable with comp. base. pairing, it is necessary in DNA replication

Structural features that stabilise DNA

1. extensive hydrogen bonds (btw. bases)
2. hydrophobic interactions (btw. stacked base pairs)
3. Only sugar-phosphate backbone is exposed
4. Nitrogenous bases being inside double-helix
5. Euk only: DNA winds around histones

Specific, complementary base pairing results in invariant base sequencing

Semi conservative DNA replication:

a. DNA strands unwind and separate
   - ↳ hydrogen bonds broken
b. Each DNA strand acts as a template for complementary strand
c. Nucleotides undergo complementary base pairing
d. DNA polymerase join nucleotides at sugar phosphate backbone

Conservative model: Parental DNA molecules remain intact

Dispersive model: mixture of old and new DNA

Meselson - Stahl Experiment

Mechanism of DNA replication

Origin of replication

- A specific sequence which is A-T rich
  ↳ only 2 hyd. bonds btw A and T ∴ easier to disrupt

A1. initiator proteins bind to oriR sequence, forming a replication 'bubble'.

A2. replication fork is formed, where replication fork moves away from oriR bidirectionally

B1. Topoisomerases create transient single stranded nick and unwind parental DNA molecule

B2. Helicase unwinds DNA helix and separates parental DNA strands

B3. ssb proteins stabilise unwound parental DNA strands → form templates
(single-stranded binding protein)

C. Primase begins RNA primer synthesis

i. portion of parental DNA is used as a template with complementary base sequence

ii. primase joins nucleotides

iii. primer provides free 3' OH end that DNA polymerases can extend

iv. DNA polymerases then replaces RNA primer w/ dNTPs

D1: DNA pol. III synth leading strand continuously towards replication fork

D2: DNA pol. III synths Okazaki fragments against overall direction of replication fork.

D3: DNA pol. I replaces RNA primer from Okazaki fragments with dNTPs

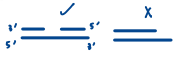
D4: DNA ligase catalyses formation of covalent bond btw. 5' and 3' end of Okazaki fragments

End replication problem

↳ as DNA poly. is incapable of completely replicating ends of linear chromosomes, resulting in shortening of telomeres

∵ As final RNA primer is removed and there is no upstream strand to fill in, there is a gap.

∴ DNA strand is shortened

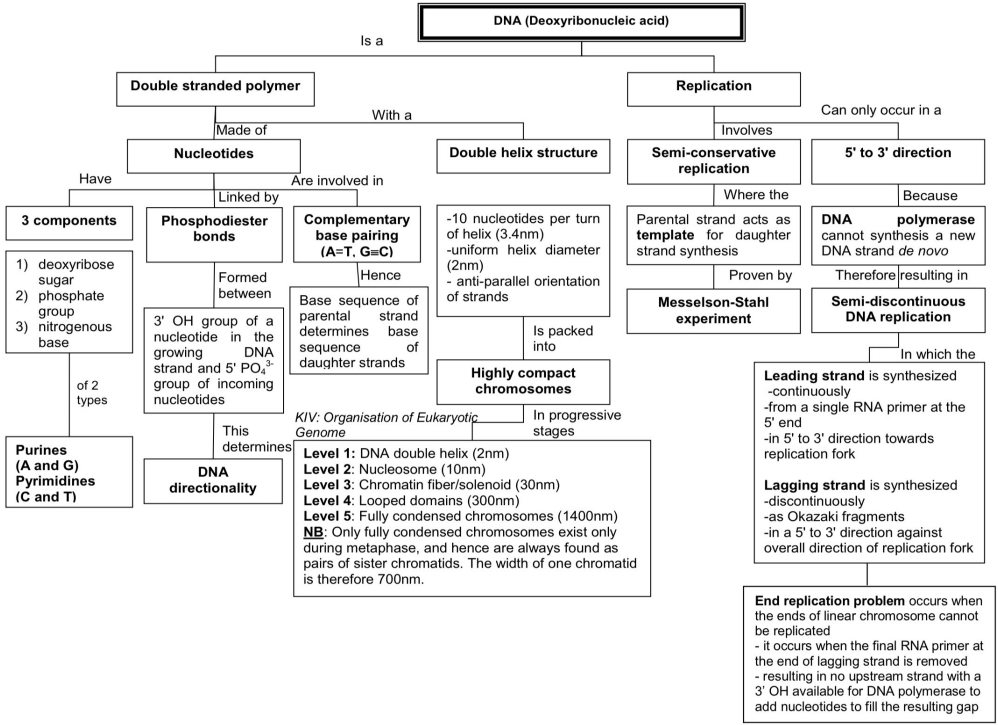


(c) Explain how the end replication problem arises. [3]

1 DNA polymerase **only extends in a 5' → 3' direction** ;
2 unable to fill gap after the **last RNA primer is removed** at end of lagging strand / 5' end of daughter strand ;
3 as **no upstream 3' OH** to which DNA polymerase can add nucleotides ;
4 resulting in **shorter daughter strand compared to the template strand / 3' overhang on the parental strand** ;

Explain the reason for the production of Okazaki fragments:

1. DNA strands are antiparallel
2. DNA polymerase can only add new nucleotides to the free 3' OH end
3. Growing DNA strand can only elongate in the 5' → 3' direction


DNA (Deoxyribonucleic acid)
Is a
Double stranded polymer
With a
Made of
Nucleotides
Have
3 components
1) deoxyribose sugar
2) phosphate group
3) nitrogenous base
of 2 types
Purines (A and G)
Pyrimidines (C and T)
Linked by
Phosphodiester bonds
Formed between
3' OH group of a nucleotide in the growing DNA strand and 5' PO_4^{3-} group of incoming nucleotides
This determines
DNA directionality
Are involved in
Complementary base pairing (A=T, G≡C)
Hence
Base sequence of parental strand determines base sequence of daughter strands
Double helix structure
-10 nucleotides per turn of helix (3.4nm)
-uniform helix diameter (2nm)
- anti-parallel orientation of strands
Is packed into
Highly compact chromosomes
In progressive stages
KIV: Organisation of Eukaryotic Genome
Level 1: DNA double helix (2nm)
Level 2: Nucleosome (10nm)
Level 3: Chromatin fiber/solenoid (30nm)
Level 4: Looped domains (300nm)
Level 5: Fully condensed chromosomes (1400nm)
NB: Only fully condensed chromosomes exist only during metaphase, and hence are always found as pairs of sister chromatids. The width of one chromatid is therefore 700nm.
Replication
Involves
Semi-conservative replication
Where the
Parental strand acts as template for daughter strand synthesis
Proven by
Messelson-Stahl experiment
Can only occur in a
5' to 3' direction
Because
DNA polymerase cannot synthesis a new DNA strand de novo
Therefore resulting in
Semi-discontinuous DNA replication
In which the
Leading strand is synthesized
-continuously
-from a single RNA primer at the 5' end
-in 5' to 3' direction towards replication fork
Lagging strand is synthesized
-discontinuously
-as Okazaki fragments
-in a 5' to 3' direction against overall direction of replication fork
End replication problem occurs when the ends of linear chromosome cannot be replicated
- it occurs when the final RNA primer at the end of lagging strand is removed
- resulting in no upstream strand with a 3' OH available for DNA polymerase to add nucleotides to fill the resulting gap

Expression of eukaryote DNA

transcription : DNA ⟶ RNA RNA is intermediary

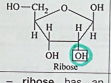
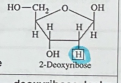
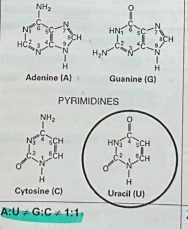
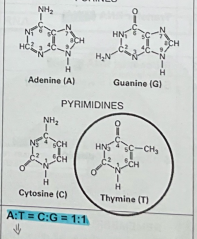
translation : RNA ⟶ polypeptides

Similarities btwn. RNA and DNA:

- Both are polynucleotides
- both have sugar-phosphate backbone joined by phosphodiester bond
- both have 3 nitrogenous bases
- both are determined by complementary base pairing w/ a template
- both formed via condensation rxn where water molecule is removed

Differences:

2.2 Differences between RNA & DNA

	Characteristic	RNA	DNA
1	Molecular mass	Smaller (MW 20 kDa to 2000 kDa)	Larger (MW 100 kDa to 150000 kDa)
2	No. of polynucleotide chain(s)	One	Two (double stranded)
3	Secondary structure	Almost always a single-stranded helical molecule, which can be folded into a complex tertiary structure	Always a double-stranded helical molecule
4	Monomers	Ribonucleotides	Deoxyribonucleotides
5	Pentose sugar	 Ribose	 Deoxyribose
6	Chemical stability	Less stable – ribose has an additional reactive 2' OH group	More stable – deoxyribose lacks 2' OH group
7	Nitrogenous bases	Adenine (A), Guanine (G), Cytosine (C) and Uracil (U) → RNA 	Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) 
8	Ratio of bases, i.e. ratio of purines to pyrimidines	A:U ≠ G:C ≠ 1:1 (as single stranded)	A:T = C:G = 1:1 (Chargaff's rule?)

	Characteristic	RNA	DNA
9	Basic forms	Several different kinds and sizes of RNA, each with its own function: 1. messenger RNA (mRNA) 2. transfer RNA (tRNA) 3. ribosomal RNA (rRNA) 4. small nuclear RNA (snRNA) 5. small interfering RNA (siRNA)	Only one basic form
10	Location	Synthesised in the nucleus but found throughout the cell	Found almost exclusively in the nucleus (and) with exceptions of mitochondria and chloroplasts
11	Amount per cell	Amount varies from cell to cell and, within a cell according to metabolic activity	Amount is constant for all somatic cells of a species

Stages of Transcription

A: initiation

step 1
- General transcription factors assembled along promoter, TFIID binds to TATA box
- Promoter + GTF + RNA polymerase form transcription initiation complex
  (GTF mediates binding of RNA polymerase to promoter)

step 2
- Binding of RNA poly. causes DNA double helix to unwind and separate
  - ↳ hydrogen bonds disrupted
  - ↳ transcription bubble created

step 3
- one exposed DNA acts as template for complementary base pairing to direct assembly of ribonucleotides
  - ↳ RNA poly. catalyses formation of phosphodiester bonds

Activators bind to promoter, facilitating the binding of gTF & RNA polymerase
⇒ stable TIC
⇒ ↑ rate of transcription

B: Elongation

Step 1
- As RNA polymerase moves along template DNA, double helix transiently unwinds

step 2
- Ribonucleotides form complementary base pairs w/ template
  - ↳ free 5'-phosphate group added to free 3'-hydroxyl group of RNA chain
    - ↳ via formation of phosphodiester bonds by RNA poly. (synth in 5'→3' direction)

Step 3
- RNA poly. reanneals unwound DNA +
  proofreads RNA to remove incorrectly inserted ribonucleotides

C: Termination

- RNA poly transcribes termination sequence (polyadnylation signal)
- RNA chain released some nucleotides downstream + RNA poly dissociates
- at cleavage site, poly A tail added

Explain role of RNA poly in the synth of mRNA:
1. Unwind ds DNA to expose DNA to synth of mRNA
2. Assembly of ribonucleotides, which form CBP w/ template to form mRNA
3. RNA poly cat. formation of phosphodiester bonds btwn free ribonucleotides to form sugar phosphate backbone

post transcriptional modification

1. Addition of 5' methyl guanosine cap

Functions of 5' cap:

a. protects mRNA from degration by hydrolytic enzymes

b. defines 5' end of mRNA → for recruitment of small ribosomal subunit for translation

c. distinguishes mRNA from other types of RNA

2. RNA splicing (alternative splicing)

- Exons (protein coding sequences) kept
- Introns (non-coding sequences) removed * uses ATP
- carried out by spliceosomes (made of snRNPs and proteins)

3. Addition of 3' poly A tail

Functions of poly A tail:

a. protects mRNA from degration → makes mRNA ↑stable

b. required to facilitate export of mRNA out of nucleus

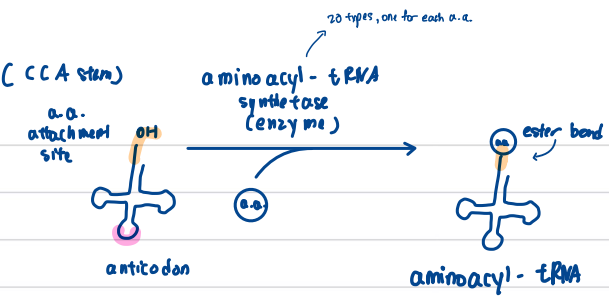
Features of genetic code (TUND)

1. is a triplet code
2. is almost universal
3. is continuous and non-overlapping (read in reading frames of 3)
4. is degenerate but unambiguous
   ↳ each a.a. is coded by ≥1 diff codons except AUG and UGG
5. wobble base phenomenon
   ↳ single tRNA recog. ≥2 degen. codes

tRNA

2° structure : 2d cloverleaf

3° structure : 3d L-shaped structure



1. serves as an adaptor molecule in translation
2. used to bring specific a.a. in sequence corresponding to the sequence of codons in mRNA

it acts as an adaptor due to

1. anticodon ability to determine specific a.a. attached to CCA stem
2. anticodon able to form complementary base pair w/ mRNA codon

How a.a is activated

- a specific type of aminoacyl-tRNA synthetase recognises the specific anticodon on tRNA + a specific a.a.
- synthetase enzyme attaches a specific a.a. to the CCA stem of tRNA via ester linkage forming aminoacyl-tRNA
  - ↳ active site of synthetase must be complementary to 3D conformation of a.a. + specific anticodon sequence of tRNA

rRNA

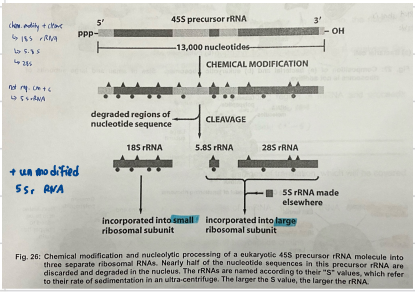


Fig. 26: Chemical modification and nucleolytic processing of a eukaryotic 45S precursor rRNA molecule into three separate ribosomal RNAs. Nearly half of the nucleotide sequences in this precursor rRNA are discarded and degraded in the nucleus. The rRNAs are named according to their "S" values, which refer to their rate of sedimentation in an ultra-centrifuge. The larger the S value, the larger the rRNA.

role:

1. forms the core of the ribosome
   - ↳ main constituent of A and P site
   - ↳ main constituent of large & small subunits
2. rRNA in large subunit has peptidyl transferase
   - ↳ catalyses formation of peptide bonds btw. a.a.

Ribosome:

- large ribosomal subunit and small ribosomal subunits (protein
- rRNA

How synth?:

- rRNA genes (in nucleolus) ⟶ rRNA
- two protein subunits enter nucleolus through nuclear pores
- (complete ribosomal subunit exits nuclear pore

small subunit:
- contains mRNA binding site

large binding site:
- has 3 binding sites for tRNA (A, P, E)

role of ribosomes:
1. provides an env. for specific recognition btw. codon of mRNA and anticodon of mRNA
2. holds tRNA and mRNA in close proximity ⟹ positions a.a. for addition to p.p. chain
3. large subunit has peptidyl transferase activity

ribosome translates in 5′ ⟶ 3′ direction

Translation factors:
- initiation factor
- elongation factor
- release factor

* uses GTP as energy source

Transcription mechanism

1A: Eukaryotic Initiation Factors (eIFs) bind to small subunit and positions initiator tRNA (carrying methionine) to P site

1B:
- small subunit binds to mRNA (by recog. of 5′ cap)
- moves 5′ ⟶ 3′ in search of AUG

1C:
- anticodon of initiator tRNA associates w/ start codon on mRNA thru c.b.p.
- eIFs dissociates; large subunit binds, → translation initiator complex forms

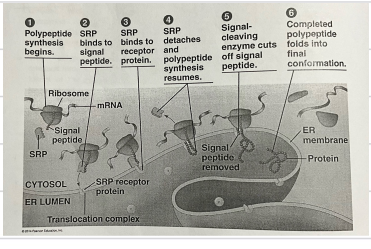
2A: · 2nd aminoacyl-tRNA binds @ A site via c.b.p (held via h. bonds)
- tRNAs brought by elongation factor (via hydrolysis of GTP)

2B: · peptidyl transferase catalyses formation of peptide bond btw. a.a.
- a.a. chain transfered to A site
- deacylated tRNA (no a.a) @ P site, new peptidyl-tRNA @ A site

2C: · ribosome translocated one codon in 5' → 3' direction (GTP used)
↳ deacylated tRNA moved to E site, peptidyl-tRNA moved to P site to accommodate new a.a.

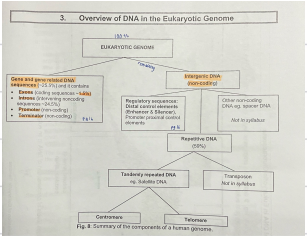
2A to 2C repeated until stop codon enters A site [A → P → E]

3: · when stop codon enters A site, release factor cause
- addition of water molecule instead of a.a
↳ frees carboxyl ends by hydrolysis
- ribosome releases mRNA then separates



Post translation modification of polypeptides
1. Attach to biochem functional group
↳ Glycosylation (form glycoproteins)
↳ Reverse phosphorylation (signal transduction)
2. making structural changes
↳ (eg. form disulphide bonds)
3. removing sequence of a.a.
4. Attaching it to ubiquitin
↳ which marks proteins for proteolysis

Org. of euk. genome



Complexity

1. more complex org. have larger genomes
2. no correlation btw. bio complexity and size + no. of genes
3. gene density pro. > euk.
4. gene density of eukaryotes less complex > more complex

Packing of euk. genome

1. Nucleosomes packed around octamer of histone proteins
   - histones (+) attract sugar-phosphate backbone (−)
   - histones assemble into octamer
   - double stranded DNA wound around nucleosome core, forming beads-on-a-string look

2. Multiple nucleosomes packed to produce 10-nm chromatin fibre (nucleosome fibre)
   - DNA further coiled to produce solenoid (30-nm chromatin fibre)
   - coiling involves histone H1 and linker DNA

3. scaffold proteins involved in coiling to form looped domains
   - looped domains then form chromatin
   - particular genes always end up in same space (highly specific & precise)

Describe the packing of DNA in euk chromosomes

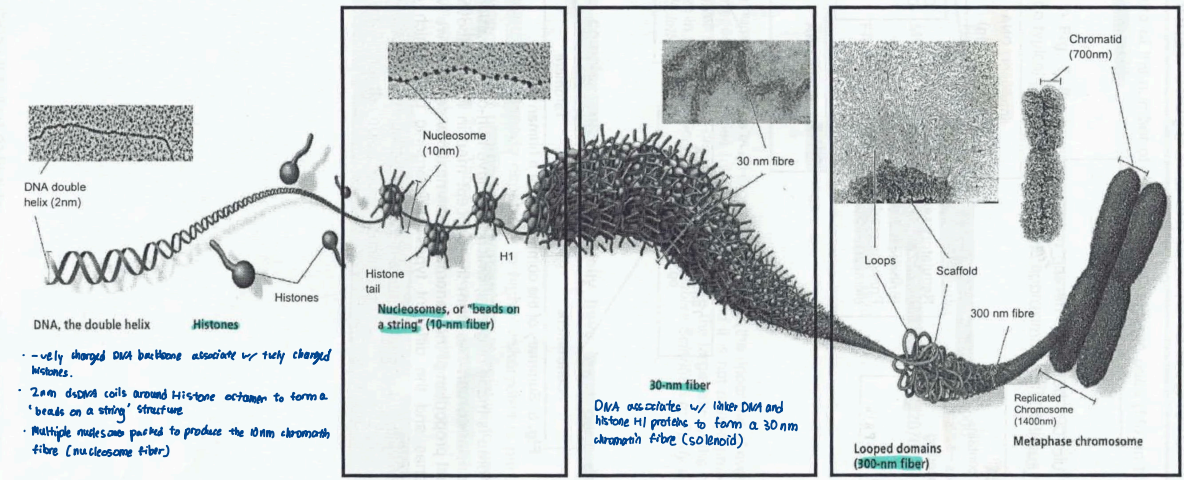
- DNA wraps around histone octamer to form a nucleosome. Nucleosomes linked together to form beads on a string structure
- Histone H1 and linker DNA involved in the further coiling of a nucleosome to form a 30 nm - solenoid structure
- Non-histone chromosomal proteins form a scaffold which is involved in condensing the 30nm chromatin fiber into 300nm looped domains. Looped domains coil and fold to produce 700 nm metaphase chromosome

LO 2d

3.1 Packing of DNA in Eukaryotic Chromosome

DNA is packaged in an orderly and systematic manner within a cell. **Fig. 9** depicts the progressive levels of DNA coiling and folding of a double stranded DNA molecule which leads up to the formation of chromosomes in eukaryotes.

★ Particular genes always end up in the same place, indicating that packing steps are highly specific and precise



- -vely charged DNA backbone associate w/ +vely charged histones.
- 2nm dsDNA coils around Histone octamer to form a 'beads on a string' structure
- Multiple nucleosome packed to produce the 10nm chromatin fibre (nucleosome fiber)

First level of condensation

DNA associates w/ linker DNA and histone H1 protein to form a 30 nm chromatin fibre (solenoid)

Second level of condensation

- Scaffold protein associate w/ 300 nm chromatin fibers, forming a 10nm looped domain.
- Further coiling & folding of looped domain forms a 700nm chromatin

Third level of condensation

Role of condensation:

① To organise and pack DNA into structures that facilitate their segregation onto daughter nuclei

② Ensures DNA molecules will not be entangled and break during separation of anaphase

Fig. 9: Figure depicting the various levels of condensation of DNA in an eukaryotic cell.

Organisation of euk. gene

1. protein-coding genes (exons)
2. transcription unit
3. non-coding DNA regulatory sequences
   a. promotor
   b. control elements
      i. promotor-proximal element
      ii. distal elements
   c. untranslated regions (UTR)
      i. 5' UTR (starts/ends one nucleotide before/after start codons)
      ii. 3' UTR (starts after stop codon; contains DNA seq. needed for termination)

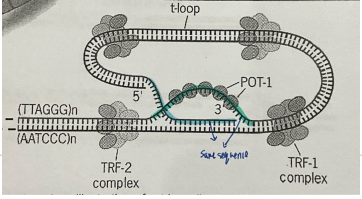
org. of euk. DNA:

1. repetitive DNA (seq. present in multiple copies in genome)
2. tandemly repeated DNA: satellite DNA (short seq. repeated many times in tandem in localised area of genome)
   ↳ reg. @ centromeres
   ↳ minisatellites @ telomeres

Comparisons between Centromere and Telomere (Fig. 17)

	centromere	telomere
1. Region found on chromosome	The **centromere** is the region where two **sister chromatids** are **joined** in a **replicated chromosome** during cell division	The **telomere** is located at the two **physical ends** or tips of a linear eukaryotic chromosome.
2. Form of DNA packaging	**Heterochromatin**	
3. Nature of DNA sequence	Consists of **tandem repetitive, non-coding satellite DNA.**	
4. Category of satellite DNA	Regular satellite	Minisatellite

★ condensation prevents transcription factors & RNA polymerase from gaining access to the promoter of a specific gene



Telomeres:

a specialised nucleoprotein composed of telomeric DNA bound by specific proteins
- ↳ has many many tandem repeats
- ↳ has a 3' single-stranded overhang which forms a hairpin loop (telomere loop)

Function:

1. protects 3' and 5' ends of linear chromosomes from degradation by cellular exonucleases + prevent it from being recognised as damaged DNA
2. maintain stability by preventing chromosome tips from fusing to other chromosomes
3. prevent loss of stability (genes) due to end replication problem
   - ↳ ensures DNA replication w/out loss of impt. coding sequences
4. regulates replicative cell senescence — as cell reaches Hayflick limit and begins senescence

Replicative cell senescence:

the period in which a cell withdraws permanently from the cell cycle after reaching the Hayflick limit when it has dividing too many times

- when cells reach Hayflick limit, it triggers apoptosis
  - ∴ regulates cell's life span

How telomerase maintains telomere lenght (in stem cells)

1. telomerase's RNA template binds complementarily to 3' overhang
2. it extends 3' overhang via complementary base pairing
3. by maintaining no. of repeats, senescence is delayed enabling cell to replicate indefinitely

centromere:

- satellite DNA
- short, AT-rich, repeated thousands of times in tandem
- centromere DNA bound by centromere-specific histones that form specialised nucleosomes

Functions:

1. sister chromatid cadherin ⟶ sister chromatids join
2. kinetochore formation ⟶ is site of kinetochore assembly
3. proper chromosome segregation ⟶ essential for correct segregation of daughter chromosome

Histone modification: [chromatin level]

- chem grp in histone tails can alter tightness of DNA winding around histones
  ∴ alter ease of transcription initiation

histone acetylation (upregulate DNA transcription)

1. +vely charged lysine residues can be acetylated by HATs (histone acetyl transferases)
2. +ve charges are neutralised, affinity of histone complex for DNA ↓
3. ∴ chromatin becomes less compact
   ↳ promotes binding of RNA polymerases to promoter / formation of TIC / Binding of TF to promote

histone deacetylation (downregulate DNA)

1. histone deacetylases (HDACs) catalyses deacetylation of residues
2. lysine residue regain +ve charge, affinity of histone complex for DNA ↑
3. ∴ chromatin becomes more compact
   ↳ prevents binding of RNA polymerase to the promoter / formation of TIC / Binding of TF to promote

DNA methylation:

represses gene expression thr:

- DNA methyltransferase adding methyl groups to CpG islands in promoter regions,
  - ↳ change in 3D conformation of DNA, prevents binding of transcription factors + RNA polymerase to promoter
  - ↳ stimulates deacetylation, ↓ transcription

IMPORTANT:
Read page 419 to 420 on Chemical Modification & Epigenetics in Campbell Biology 11/E

Gene Accessibility			
Process	DNA methylation	Histone modification	
		Deacetylation	Acetylation
Site	CpG islands in promoter regions	Histone tails	
Enzyme	DNA methyltransferases	Histone deacetylases (HDACs)	Histone acetyltransferases (HATs)
Mechanism	• Changes the 3D conformation of DNA ➢ Prevents binding of transcription factors and RNA polymerase to the promoter • Stimulate deacetylation	• Deacetylation of acetylated lysine residues in the histone tails • Increase in affinity of the histone complex for the DNA molecule • Chromatin becomes more compact	• Acetylation of positively-charged lysine residues in the histone tails • Reduction in the affinity of the histone complex for the DNA molecule • Chromatin becomes less compact
Outcome	Down-regulate transcription	Down-regulate transcription	Up-regulate transcription

Control elements:

1. Promoter (TATA box)
   - determines start-point of transcription
   - point of assembly of GTF & RNA poly.
2. Proximal control elements
   - binding sites for GTF
   - essential for eff. transcription
3. Distal control elements
   - enhancers and silencers
     - ↳ ↑ transcription rate
     - ↳ ↓ transcription rate

Transcription factors
- recog + bind to enhancers/silencers
- contain:
  1. DNA binding domain
  2. protein binding domains

Events leading to initiation of transcription:

1. Activators bind to enhancers
2. GTFs bind to promoter + mediate binding to RNA poly
   ⇒ forming transcription initiation complex
3. DNA-bending protein causes looping of DNA
4. Activators interact w/ mediator protein + facilitate interaction of activator w/ GTF & RNApoly.
   ↳ ↑ recruitment of GTF & RNA poly. → form stable transcription initiation complex
   ↳ proper positioning of transcription initiation complex
   ∴ rate of transcription ↑

Repressor proteins (how:)

a) competitive DNA binding
b) masking activation surface
c) blocks assembly of GTF
d) recruits chromatin remodelling complex
e) attracts histone deacetylase to promoter
f) attracts histone methyl trans ferase

1. mRNA splicing
   ↳ cleavage causes simultaneous ligation of exons, resulting in a lariat-like structure
   ↳ spliceosomes can bind

2. alt. splicing
   ↳ produces diff mature mRNA, generating diff proteins

Mutations

Types of gene / point mutations:

a. nucleotide substitutions

b. nucleotide insertion / deletions

   i. in multiples of 3

   ii. not in multiples of 3

mutations result from:

1. Gene / point mutation
2. chromosomal aberrations

"Types of mutation":

- addition
- deletion
- base substitution

consequences / how the mutation affects gene product:

1. missense mutation
   - ↳ diff a.a. coded for ⇒ loss in 3d conformation
2. nonsense mutations
   - ↳ stop codon coded for
     - ↳ premature termination ⇒ loss in 3d conformation / non functional protein
3. silent mutation
   - ↳ same a.a. inserted ⇒ no change in 3d conformation
     - ∵ degeneracy in genetic code
4. neutral mutation
   - ↳ a.a. with similar chemical properties to original a.a. substituted
     - ⇒ no detectable change in function of protein
5. frameshift mutation (extensive missense mutation)
   - ↳ all codons subsequent to insertion / deletion site are changed

Sickle-cell Anaemia

Mutation in the β-globin gene:

- Substitution of thymine ⟶ adenine
- 6th a.a. residue glutamate (hydrophilic) ⟶ valine (hydrophobic)
  - ↳ creates hydrophobic spot on outside of Hb protein which sticks to hydrophobic region of adjacent Hb β-chain

∴ mutant Hb units stick to each other when $[O_2]$ low
- ↳ eg. in capillaries & veins
- ↳ polymerise & form fibre-like structures within RBC

Causing:
- RBCs lose normal morphology and become sickle-shaped
  - ↳ less able to move through capillaries & block blood flow
- fragile and easily destroyed

Causes of gene mutations:

Spontaneous mutations:
1. errors in DNA replication and repair eg. errors in DNA polymerase, wrongly-added codon, ribosome errors
2. DNA slippage

Induced mutations (deliberate application of mutagens)

Chromosomal abberation:

Change in structure / no. of chromosomes

change in structure:

i) Deletion (chromosome breaks in ≥1 places)

↳ genotype altered

↳ fatal if deletion affects same gene loci on both homologous chromosome

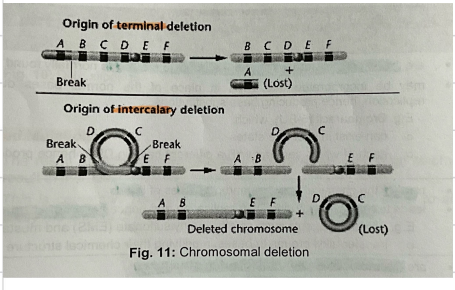
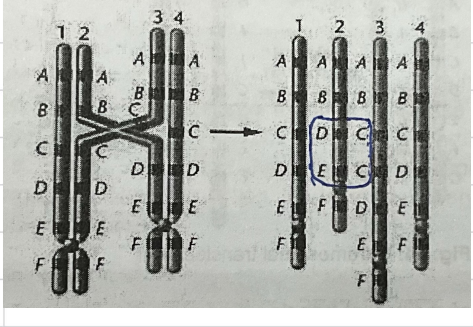


Fig. 11: Chromosomal deletion

ii) Duplication (unequal crossing over)



iii) Inversion (rearranges linear sequence)

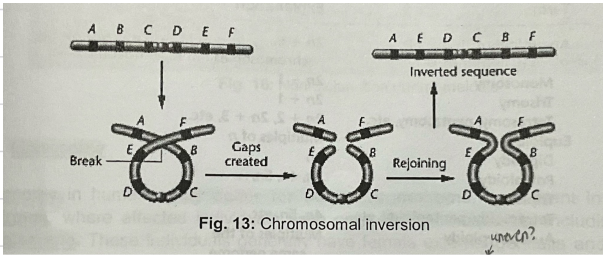


Fig. 13: Chromosomal inversion

uneven?

iv) translocation (movement of chromosomal segment to new location in genome

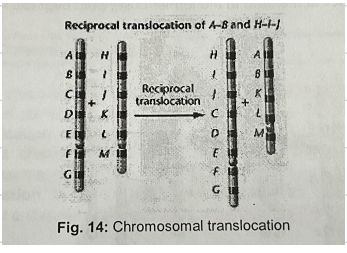


Fig. 14: Chromosomal translocation

Reciprocal translocation:
exchange btw. 2 non-homologous chromosomes

change of chromosomal no.

1. Aneuploidy : organism loses ≥1 chromosomes, but not complete set
   ↳ non disjunction → failure to divide
   - monosomy (only X chromosome) : X
   - trisomy XXY

2. Polyploidy : ≥2 multiple of haploid chromosome set
   Origins:
   1. Addition of 1 extra set of chromosomes of the same species (autoploidy)
      ↳ due to failure of chromosomes to segregate during meiosis
      ↳ can be lab produced by crossing w/ tetrapoly ploids

   2. Combination of chromosome sets of diff species (allopolyploidy)
      ↳ hybridisation of closely related species

Molecular techiques

Polymerase Chain Reaction (PCR)

Start needed:

1. DNA template
2. PCR primers
3. Free dNTPs (deoxyribonucleoside triphosphates)
4. Thermostable Taq DNA polymerase
5. PCR reaction buffer

PCR cycle:

① Denaturation of DNA template
- ↳ 95°C for 30s
- hydrogen bonds in helix break ⟶ become single-stranded

② Annealing of primers
- ↳ 54°C for 1 min; presene of large excess of DNA primers
- anneal specifically w/ 3' end of ss DNA template via h. bonds

③ Extension of primers
- ↳ ~72°C for 2 min (opt. temp of Taq DNA poly)
- prime DNA synth using dNTP in 5'→3' direction

Impt Features:
- chain reaction ⟶ newly synth DNA strands are templates for new DNA
- highly specific ⟶ only sequence of interest is amplified
- All DNA molecules are exact copies of gene of interest
- no. of DNA molecules ↑ exponentially

Explain why RNA cannot act as a template fort PCR amplification:
1. RNA single stranded
2. Taq polymerases need double-stranded template (two strands neded for both primers to bind to gene of interest
3. Conformation of active site of Taq polymerase complementary to DNA but not RNA
4. Rna is unstable and will degrade upon heating

practical applications of PCR:

1. amplification (large no. of copies of DNA sequences in a short time)
2. specifically amplifies section of DNA btwn two primers

advantages:

1. sensitivity (can amplify minute amts of DNA)
2. speed and ease of use (rapid and can be easily automated)
3. Robustness (permits amplification of DNA from badly degraded material)

limitations

(eg. wrong primer used)

1. risk of contamination (as PCR is extremely sensitive, contamination of non-template nucleic acid may cause non-target sequences to be amplified)
2. Infidelity of DNA replication in vitro (Taq polymerases lack 3' to 5' exonuclease activity)
3. Short size and limiting amounts of PCR products (can only amplify sequences up to a few thousand base-pairs)
4. Need for target DNA sequence information (some prior sequence information is needed)

(for primer to bind to)

Gel Electrophoresis:

By application of a direct current through a semi-solid gel material, DNA molecules are separated by rate of movement and thus lenght

shorter DNA fragments are less impeded by the pores than longer ones, and size fractionated into discrete bands

practical applications of GelE:

1. separate DNA fragments according to size
2. determine ~molecular weight of DNA fragments
3. isolate/purify indiv. DNA fragments
4. determine if a PCR experiment is successful

Practical steps of GelE:

1. agarose powder is mixed with a buffer solution (to maintain stability), then poured into a gel tray with a gel comb (to create wells in the gel) and allowed to cool and solidify.
2. gel tray placed in electrophoresis chamber (allows DC electric current flow through gel). Gel comb is removed.
3. DNA samples mixed with loading dye (makes DNA visible). One well is reserved for molecular weight marker
4. DC power supply is connected; negatively charged DNA moves from -ve electrode to +ve electrode
5. DNA molecules stained with DNA-binding dye (with methylene blue; ethidum bromide; radioactively labelled)

Nucleic acid hybridisation: (Includes Southern Blot, Northern Blot etc)

- the process by which two complementary, single stranded nucleic acid chains base pair and reform a double stranded helix

1. DNA denaturation:

- double helix is separated into two single stranded strands (disrupt h. bonds)
- heated to 100ºC; high ph ≥13; low salt concentration

2. DNA renaturation

- permit hydrogen bonds between c.b.p to re-establish, anneal and re-form double helix
- prolonged period at lower temp of 65ºC

application:

to detect specific DNA and RNA base using ss nucleic acid probes of known sequences

advantages:

- highly sensitive : complementary sequences as low as 1 mol. per cell can be detected
- highly selective : probe only hybridises to nucleic acid molecules carrying all/part of the complementary sequence

practical applications of nucleic acid hybridisation:

1. to detect, characterise and quantify specific nucleotide sequences/genes
2. to locate particular genes of interest in cells, tissues and organisms
3. to study gene expression and changes in gene expression profiles
4. to screen libraries to identify colonies carrying DNA insert of interest
5. to compare nucleotide sequences in phylogeny stides

Southern blotting:

- after GelE separates DNA sequences,
- replica is made by transferring DNA in gel onto a membrane made of nitrocellulose/nylon
- DNA denatured by exposing it to alkaline denaturing conditions
- membrane incubated in solution containing labelled ss dna/rna probe
- DNA-probe hybrids are located by autoradiography/ chemical means
- size determined by reference to the molecular weight markers

practical steps of SB:

1. DNA molecules separated on basis of size by GelE
2. gel placed on paper wick (absorbing buffer solution from a reservoir)
3. nitrocellulose/nylon membrane binds nucleic acids
4. capillary action draws (alkaline) buffer solution through gel and it separates DNA molecule

5. nitrocellulose membrane containing DNA incubated in a sealed bag with a buffered salt solution containing radioactively labelled DNA/RNA probes, which hybridises with gene of interest
6. detection of bound probe — membrane removed and washed thoroughly
7. autoradiography used to show DNA which shows up as bands on autoradiograph

Southern blot : DNA

Northern blot : RNA

Western blot : protein

Cell signalling

ligand - receptor signalling:

- highly specific, as ligand can only bind to a specific complementary site on receptor to form a ligand-receptor complex
  - ↳ receptor protein undergoes a conformal change which activates the receptor

Signal transduction: process where a target cell converts an extracellular signal into an intracellular signal that results in a specific cellular response.

- each protein alters conformation of proteins downstream (phosphorylation)
  - ↳ triggers phosphorylation cascade

Cellular response:

- cytoplasmic response
- nuclear response

G-protein linked receptor (GPLR)

structure:

1 pp chain ⟶ 7 α-helices ⟶
- Hydrophobic interactions btw α-helices result in a barrel-shape conformation
- disulfide loops stabilise protein.

Relating structure to function

- hydrophilic aa residues: interhelical → soluble in aq medium for interaction w/ water soluble ligand (glucagon)
  loops & N & C termini
- hydrophobic interactions btwn hydrophobic a.a. residues in 7 transmembrane α-helixes & hydrophobic f.a. tails of phospholipids in membrane bilayer → enables membrane-embedded domain to be stabilised & embedded within bilayer
- specific a.a. @ signal-binding site (extracellular) → enables specific 3D conformation for interaction w/ specific ligand
- specific a.a @ G-protein interaction site → 3D conformation to bind and activate G-protein
- binding of GPLR causes conformal change, → enables GPLR to initiate signal transduction pathways allowing it to interact w/ GPLR

★ To initiate signal-binding pathways

Signal reception

1. ligand binds to GPLR and causes a change in receptor conformation, activating GPLR

Signal transduction

2. GPLR ↑ affinity for G-protein ⇒ GPLR binds to inactive G-protein,
   ∴ GTP displaces GDP bound to G-protein (activating G-protein)
3. G-protein dissociates from GPLR; diffuses along membrane.
4. activated G-protein binds to target protein, altering target protein activity
   ↳ initiates signal transduction, causing:
   - production of cAMP OR
   - production of inositol triphosphate (IP_3) and release of Ca^{2+}

   } second messengers

Cellular response: triggers cellular response

↳ intrinsic GTPase activity of G-protein hydrolyses bound GTP → GDP, so G-protein becomes inactive again

↳ signal molecule dissociates from G-protein

↳ inactive G-protein leaves enzyme, allowing it to return to original state.

cyclic adenoside monophesphate (cAMP)

1. Activated G-protein activates adenyl cyclase, catalysing synth of a lot of cAMP.
   ↳ boost conc. 20-fold
2. activates protein kinase A (serine / tyrosine kinase), which then phosphorylates other proteins.

- cAMP molecules do not last long as phosphodiesterase converts cAMP → AMP

Second messengers (Ca^{2+} & IP_3)

Low conc. of Ca^{2+} maintained by

1) Ca ATPase → sequester Ca^{2+} into ER lumen
   → actively pump Ca^{2+} from cytosol into extracellular fluid
2) Sodium calcium exchangers → export Ca^{2+} w facilitated diffusion of Na^+ into cytosol
3) Mitochon Ca^{2+} pups → move Ca^{2+} into mitochon.

Receptor Tyrosine kinases:

in each RTK polypeptides:

- extracellular signal-binding site
- α-helix spanning membrane
- intracellular tail containing multiple tyrosines + tirosine kinase domains

How RTK:

Signal reception:

① signal molecule binds to RTK, resulting in signal aggregation (→ associate closely) and dimerisation (→ two subunits form a dimer)

Y+Y → Y̲Y̲

② dimerisation leads to activation of tyrosine kinase activity
   ↳ autophosphorylation / cross phosphorylation

③ each tyrosine kinase domain adds phosphate from ATP to a tyrosine on its own / other subunit's tail
   ↳ RTK fully activated

Signal transduction:

④ activated RTK binds cytoplasmic relay proteins, altering activity
  ↳ each protein recog and binds to specific phosphorylated tyrosine
  ↳ bound protein becomes activated, undergoing conformal change

⑤ each activated relay protein triggers a transduction pathway, initiating signal cascade

Cellular response: last activated molecule triggers a cellular response

Signal amplification: the process of amplifying signal strength as signal is relayed through a transduction

- at each step, no. of activated protein >> than preceding step
- small no. of ligands needed to trigger response
- response is large

Possible due to:

- presence of multiple steps in transduction pathway
- persistance of proteins in pathway in active form long enough to process alot of substrate.

Regulation points:

- protein phosphatase activity ⟶ catalyses inactivation of proteins
- intrinsic GTPase activity ⟶ GTPase rapidly catalyses hydrolysis of bound GTP to GDP, inactivating G-protein
- phosphodiesterase activity ⟶ catalyses conversion of cAMP to AMP, ↓ conc. cAMP

Insulin (RTK) & Glucagon (GPLR)

Insulin (RTK)

when blood glucose level
> 90mg/100mL of blood

- activated by binding of Insulin
- Downstream activated protein leads to activation of glucagon synthetase
- movement of cytoplasmic vesicles containing GLUT-4 glucose transporters (secretory vesicles) towards cell membrane
  - ↳ fuses to cell membrane

- Restore to set point of [90mg/100mL]

Glucagon (GLPR)

when blood glucose level
< 90mg/100mL of blood

- binding of glucagon activates adenylyl cyclase
  - ↳ activated form catalyses synth. of large amts of cAMP. Protein kinase A activated
- active protein kinase A phosphorylates glycogen phosphorylase kinase, activating it
  - ↳ Also activated by Ca^{2+} released
- prot. kin. A also phosphorylates glycogen synthetase
  - ↳ inhibits its catalytic activity, prevents conversion of gluc → glyc.
- active glyc. phosphorylase kinase phosphorylates glycogen phosphorylase
  - ↳ active glyc. phosphorylase stimulates glycogenolysis

- Restore to set point of [90mg/100mL]

Sex. reproduction & cell cycle

Human reproductive cells:

haploid → maintain const. no. of chromosomes upon fusion + prevent chromosome doubling

Terms & Def:

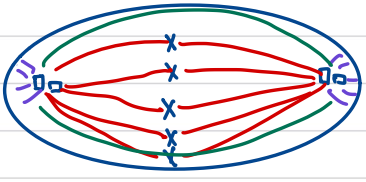
1. Chromatin
   → in a less condensed state during interphase

2. Chromosomes
   → the condensed form of chromatin
      • scaffolding proteins aid in condensation of chromosomes
   → genes located @ gene loci

3. Homologous chromosomes:
   a) structurally similar: same size, shape, centromere position, sequence of gene loci
   b) not genetically identical: diff. alleles of same gene loci

Types of microtubules

1. polar
2. astral
3. kinetochore



Phases of mitosis:

G_1 phase (Gap 1)

- cells ↑ in size and acquire ATP
- intensive cellular gene expression + synth of appropriate organells & proteins

S phase (synth.)

- undergoes semi-conservative DNA rep.
- histone proteins syth. & associate w/ DNA molecules
- DNA remains fully extended and uncoiled
- centrioles replicate + mitotic spindles form

G_2 phase (G_2 phase)

- cell undergoes second growth + ATP acquisition
- cells ↑ in size + acquire ATP

Prophase:
- Chromatin becomes more tightly coiled, condenses into discrete chromosomes
- Nuclear envelope disintegrates, nucleolus disappears
- centriole pairs migrate to opposite poles of the cell
- spindle fibers that form in G_2 continue to develop

Metaphase:
- Chromosomes migrate and align singly at metaphase plate
- kinetochore microtubules attach to kinetochores of centrioles
- centriole pairs positioned at opposite poles of the cell
- Maximum shortening & thickening of chromosomes

Anaphase :
- Centromeres divide & sister chromatids separate
- spindle fibres shorten & daughter chromosomes move centromere first to opposite poles of the cell (V shape pattern)
- polar microtubules slide past each other, elongating the cell

Telophase :
- nuclear envelope & nucleolus reform
- Chromosomes decondense into chromatin
- microtubules disassemble

Cytokinesis :
- formation of a cleavage furrow due to a contractile ring of microfilaments
- In plant cells, cell plate is formed at metaphase plate.
  - ↳ vesicles from Golgi body move to metaphase plate where they fuse, depositing materials for a cell wall.

Significance of mitosis:

1) Genetic stability
- Produces two cells w/ same no. of chromosomes (equal distribution)
- semi-conservative DNA rep. produces genetically identical daug
- As no genetic variation, genetic stability preserved

2) Growth, repair regen.
- growth req. genetically identical cells to carry out same fxn
- damaged cells replaced by genetically identical cells
- mitosis allows for regen of missing parts

3) Asexual reproduction
- prod. genetically identical offspring, ensuring favourable traits from gen to gen

Stages of meiosis (diff)

Prophase I ——— Crossing over ⇒ creates genetic diversity

- Homologous chromosomes pair up to form a bivalent (synapsis)
- Chiasma formed btw. homologous chromosomes, enabling exchange of alleles

Metaphase I ——— Independant assortment

- bivalents randomly align @ metaphase plate

* chromosomes decondense into chromatin during telophase I, and recondense into chromosomes during prophase II

Stem cells

Unique features of stem cells

① Unspecialised
- ↳ no tissue-specific functions.
- ↳ can give rise to specialised cells

② Capable of dividing & renewing for long periods of time (clonogenic)
- ↳ can replicate many times (proliferation)

③ Can give rise to specialised cells
- ↳ called differentiation (triggered by internal + external signals)

Types of stem cells:

A. Totipotent stem cells (zygote) — eg. zygotic stem cells
- can give rise to all cell types that make up an organism
  - ↳ any cell in the adult body
  - ↳ any cell of the extra-embryonic membrane
- can form whole organism

B. Pluripotent stem cells (embryo) — eg. inner cell mass of the blastocyst
- ↳ give rise to three germ layers of the body (mesoderm, ectoderm, endoderm)
- ↳ cannot form extra-embryonic membrane
- ↳ from inner mass of human embryos + fetal tissue from gonads

C. Multipotent stem cells (adult) — eg. haematopoietic cell
- ↳ can only differentiate into limited amt. of cell types
- ↳ more specialised than toti-/pluri-potent stem cells

significance of mitotic stem cell division (asymmetrical division):

① preserves pool of undifferentiated cells

② steadily produces stream of differentiating cells.

Location of embryonic stem cells:

1. Embryonic stem cells (ESCs) → inner cell mass of blastocyst
2. Embryonic Germ cells → isolated from gonad precursors

Defining properties of embryonic stem cells:

1. Capable of undergoing an unlimited no. of symmetrical divisions w/o differentiating
2. maintains full diploid no. of chromosomes
3. can differentiate into cells that form 3 primary germ layers of embryo
4. can develop into all fetal tissues
5. Clonogenic
6. easy to obtain pure + can be cultivated in large no.

Defining properties of adult stem cells

a. capable of long-term self renewal
b. can give rise to fully differentiated cells ↓
   1. mature phenotypes 2. fully integrated into tissue 3. capable of specialised fxn.
c. clonogenic

Blood stem cells → Hematopoietic stem cells (HSC)

- relatively accessible
- can be grown in culture

} relatively well-studied

1. lymphoid lineage (B lymphocytes & T lymphocytes)
2. Myeloid lineage (rest of WBC)
3. Erythroid lineage (RBC + megakaryocytes → platelets)

Similarities of totipotent, pluripotent, multipotent stem cells

1. Unspecialised
2. Capable of long-term self-renewal via mitosis
3. Can give rise to specialised cells
4. Capable of differentiating into >1 cell lineage
5. Can express telomerase genes to produce telomerase to maintain lengths of telomerase
6. Can respond to signals / signalling molecules to give rise to differentiate into specialised cells
7. Clonogenic

Characteristics	totipotent stem cells	pluripotent stem cells	multipotent stem cells
1 potency	can specialise to any cell size	can specialise to be nearly every type of cell	can specialise to be many types of cells
	give rise to all/any types that form a whole organism	give rise to cells that develop from the three germ layers OR cannot form extra-embryonic membranes	differentiate into a limited number of cell types
2 specialised cells derived from stem cells	give rise to any cell type in the adult body AND any cell of the extra-embryonic membranes	give rise to any cell type in the adult body	give rise to a specific cell type in the adult body
3 types of stem cells	consists only of zygotic stem cells	consists only of embryonic stem cells	consists of adult stem cells / specific e.g. blood stem cells
4 presence of stem cells	present at the earliest stage of embryonic development/ before blastocyst stage	present after blastocyst stage	presents during adulthood
5 located in	in the zygote	in the inner cell mass of developing embryo	adult tissues / organs
6 function of stem cells	mainly involved in growth	mainly involved in growth	maintain and repair tissues / replace cells that die because of injury or disease

The use of stem cells for research and medical applications has potential benefits and risks. Discuss why and how societies should regulate this technology

Max 6 from 'Why socieites should regulate this technology'

1. Ethical implications in research and medical applications for human ESCs

any two from A1 - A3: Potential benefits:

A1 ability to **create ESC** by somatic nuclear transfer (SCNT) to **produce cells for therapy**

A2 able to be created fom the cells of patients suffering from rare, complex diseases, creating a **vast resource** available for research

any four from B1-B8: Potential risks

B1 **three main sources of human ESCs**; most controversial is using **spare embryos**

B2 if stem cell therapies become routine, there will be a **decrease repect for human lifee, ref. to respect for person**

B3 **beginning of slippery slope**, dehumanising scenarios from embryo farms, cloned babies etc

B4 encourages society to **tolerate the loss of life to save a life**

B5 usage of SCNT may lead to misguided individuals to **create a cloned person**

B6 increased use of ESCs might result in **exploitation of women** to donate their eggs

B7 therapeutic/medical applications may only be available to those who can afford etc

max five from 'How societies should regulate this technology'

C1 **increase research on induced pluripotent stem cells (iPSCs)** instead of ESCs for medical and research applications

C2 benefits of iPSCs: **iPSCs can provide a source of patient-specific specialised cells that will not be rejected** by the patients' body

C3 : **avoids ethical issues**

C4 **regulation of accessibility** of iPSCs (and ESCs) based therapies

C5 requires **well-developed healthcare system**

C6 requires **laws and regulations** to be created

C7 **establishing a bioethics council**

C8-9 **informed consent; public education;**

Cancer

1. High rate of cell division
2. Genome instability & Mutations
3. Replicative Immortality
   - telomerase genomes reactivated → evade apoptosis
4. Loss of anchorage dependence
5. Lack of contact inhibition
6. Inducing angiogenesis
   ↳ formation of new blood vessels stimulated by tumor
   ↳ done by expressing angiogenesis-activating protein genes
   ↳ allows ↑ blood flow to tumor
7. Metastasis
   ↳ establish new secondary tumor @ distant sites from primary tumor; transported by circulatory system

How dysregulation of check points can occur

- mutation in CDK gene that codes for synth. of CDK / enzymes that break down CDK
- change in specific 3d conformation of cyclin → hyperactive / overproduction of cyclin
- more CDK complexes formed / CDK continuously bound to cyclin.

Tumour suppressor gene (eg. p53)

functions

1. inhibits cell cycle if DNA is damaged
2. trigger repair mechanisms
3. Initiate apoptosis
4. Maintain cell division

☆ Loss of function mutation → both copies of gene must be mutated so no functional protein produced

(normal) (cancer)
proto-onco gene → oncogene

Gain in function mutation → · promotes excessive cell division
· only 1 allele needs to be mutated.

ras protein: GTP mutation causes 3d conformation to change,
GTP remains bonded to ras protein
↳ constant cell signalling even w/o presence of growth protein
↳ hyperactive ras protein.

Acc. of mutation: several independent mutations in cancer-critical genes in a single-cell lineage

Angiogenes:
· tumour cell releases angio-genesis proteins that attract endothelial cells
· secrete matrix metalloproteinases (MMPs) (proteases)
↳ break down blood vessel walls and components of extracellular matrix,
allowing endothelial cells to be organized into new networks of blood vessels.

Causes of cancer:
a) Cigarette & tobacco smoking → polycyclic aromatic hydrocarbons (PAHs) form an adduct causing DNA damage
b) Exposure to chem. carcinogens → heterocyclic amines (HCAs) and PAHs bind to DNA and cause mistakes in DNA synth.
c) Radiation / UV radiation exposure → free radicals cause double stranded breaks, leading to chromosomal rearrangements / deletions.
UV-B radiations damage DNA
d) Age → acc. of mutations.
e) genetic predisposition.
f) Loss of immunity
g) Viral infections.

Causes of cancer

Lifestyle & Diet
- Cigarette and tobacco smoking
  - Polycyclic aromatic hydrocarbons (**PAHs**) bind to DNA for **form an adduct**, causing **DNA damage**
- Exposure to chemical carinogens
  - Heterocyclic amines (**HCAs**) and **PAHs** can form when meats are cooked using high temp methods; HCAs and PAHs **bind to DNA and cause mistakes in DNA sythesis**

Radiation Exposure
- Ionising radiation
  - **Free radicals** produce **double-stranded breaks in DNA**, leading to **chromosomal rearrangements and deletions**
- UV radiation
  - **UV-B radiations are DNA damaging**

Age
- **Accumulation of mutations increases with age**

Genetic Predisposition
- Inheriting an oncogene/mutant allele of a tumour-suppressor gene are more likely to get ancer types like colon and breast cancer

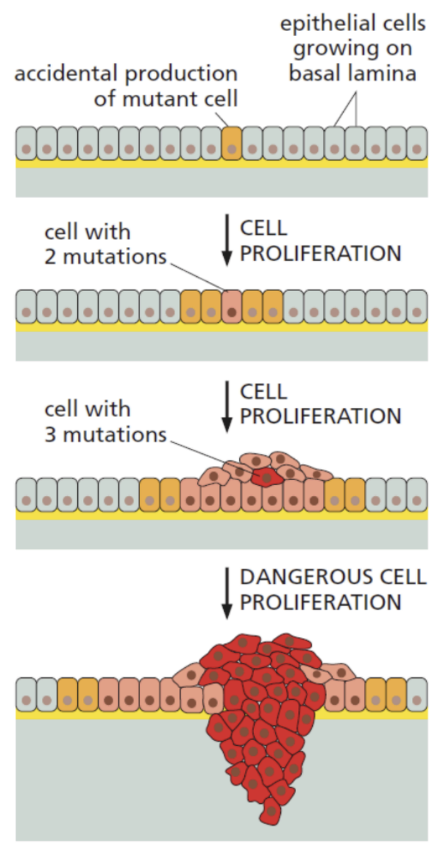
Molecular basis of cancer

Multi-step model of cancer progression

The accumulation of mutations in a single cell lineage

1) Accumulation of mutations
- Genesis of a cancer**requires gradual accumulation of several independent mutations in cancer-critical genes in a**__single__**cell lineage**

Cancer critical genes
- Proto-oncogenes
- Tumour supressor genes
- Telomerase gene
- Angiogenesis-activated protein gene



2) Activation of telomerase
- Telomerase genes are activated; telomerase is produced and**maintains telomere lengths**so cancer cells can**divide indefinitely**
- **Evade apoptosis**

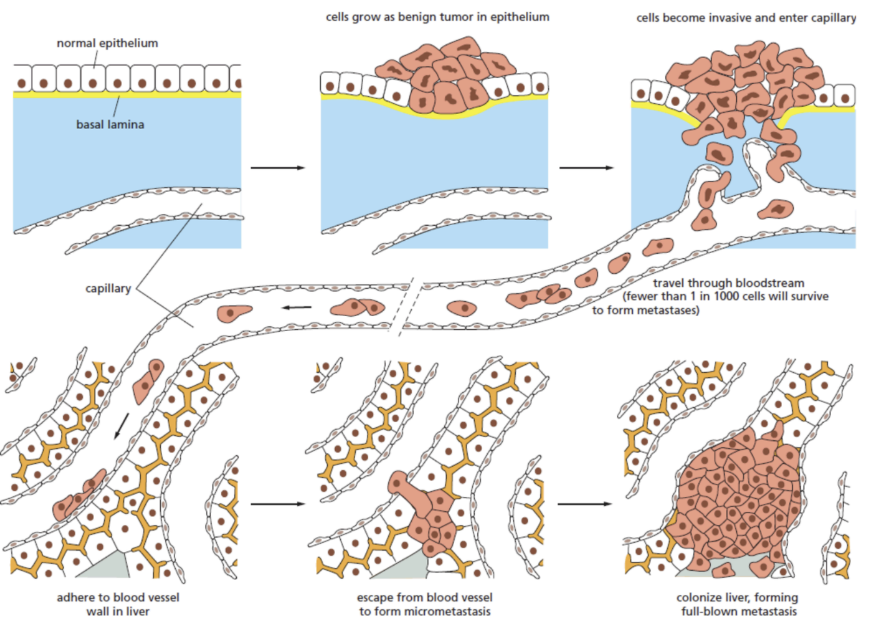
3) Angiogenesis

Growing tumour stimulates **formation of new blood vessels (angiogenesis)/vascularisation**

1) Tumour cell releases **angiogenesis-activating** proteins that **attract endothelial cells**
2) Endothelial cells secrete proteases - **matrix metalloproteinases (MMPs)**
3) MMPs break down components of extracellular matrix (ECM), allowing endothelial cells to become **organised into new networkds of blood vessels**

4) Metastasis

1) Cancer cells **invade surrounding tissues** and penetrate through walls of **lymphatic** and **blood vessels**, gaining access to bloodstream
2) Cancer cells **transported by circulatory system**
3) Cancer cells leave bloostream and **establish new secondary tumours at distant sites from primary tumour**



Cell cycle checkpoints (not LO)

Role of Cyclins and Cyclin-depedent protein kinase in cell cycle
- **Cdks phosphorylate substrates** through transferring of phosphate groups from ATP
- **Cyclins activate Cdks by binding to them**, forming **cyclin-Cdk complexes (maturation-promoting factors); promote mitosis**

How dysregulation of checkpoints may occur:

- Mutation in Cdk gene that codes for synthesis of Cdk/enzymes which break down Cdk
- Change in specific 3D conformation of cyclin --> results in hyperactive/overproduction of cyclin
- More Cdk complexes formed/Cdk being countinually bound to cyclin

In normal tissues, rate of cell division and rate of cell loss are kept in balance so no net accumulation of new cells ocurs

In cancer cells, __**rate of cell division far exceeds the rate of cell loss**__

__**M Checkpoint -- assessment of mitosis:**__
- Occurs at metaphase
- check for successful formation and attachment of spindle fibers to kinetochores of chormosomes
- Ensres successful separationof DNA to two daughter cells
- If spindle fibers not formed/attached adequately, mitosis is arrested

__**G1 Checkpoint -- assessment of cell growth:**__
- __Checks for - Checks for__ - Checks for **presence of growth factors** (proteins) required to stimulate cell division
- Checks for **DNA damage and cell size**
- Checks if **nutrients are sufficient**
- Apoptosis occurs if DNA is damaged and cannot be repaired

__**G2 checkpoint -- assesnent of DNA replication**__
- **Checks if DNA is successfully replicated w/o damage** - apoptosis if there is irreperable damage

Important characteristic of cancer cells

1. High rate of cell division	• Cell devides/profilerates in absence of**growth factors**
2. Genome instability & mutation	• Increased rate of__**accumulation of mutations**__at chromosomal and gene level
3. Replicative immortality	• In normal somatic cells, telomerase genes are not expressed (can only divide a limited amt of times • __**Telomerase genes are reactivated**__**incancer/tumour cells**, where telomerase is produced and__**maintains telomere lengths**__so cancer cells divide indefinitelyTelomerase enables cancer cells to__**evade apoptosis**__(programmed cell death)
4. Loss of anchorage dependance	• Cancer cells are able to proliferate without needing to attach to a surface(__**anchorage-independent growth**__), unlike normal somatic cells
5. Lack of contact inhibition and density-dependent inhibition	• Normal cells display**contact inhibition**while cancer cells don't • Normal cells proliferate until they make contact w neighbouring cells • Cancer cells continue to divide after making contact w neighbours, growing over adjacent cells in disordered, multilayered patterns
6. Inducing Angiogenisis	• __**Angiogenesis**__→ **the formation of new blood vessels** • To grow larger, tumours**stimulates the formation of new blood vessels**, which**supply nutrients and oxygen**and**remove toxic waste products** • Tumour cell expresses**angiogenesis-activating protein genes**to produce**angiogenesis activating proteinss**
7. Metasisis	__**Metastasis**__ → Process where primary tumour cells invade local tissues and blood vessels, and **establish secondary tumours** (metastases) at **distant sites** 1. Cancer cells**invade surrounding tissues**and penetrate walls of**lymphatic**and**blood vessels**, gaining access to bloodstream 2. Cancer cells**transported by circulatory system**throughout body 3. Cancer cells**establish new secondary tumours at distant sites from primary tumour**
8. Avoiding immune destruction	• Some tunour cells can**evade immune destruction**by disabling components of immune system

Mutation in genes controlling cell cycle

Tumour suppressor gene (inhibits uncontrolled cell division)
- {D} Abolish protein function
- **Both copies (recessive) of the tunour suppressor genes must be mutated**so that**no functional gene product can be produced**

Functions of gene proteins:
1) Take part in **cell signaling pathways** to **inhibit cell cycle**
2) **Halt cell division if DNA is damaged**
3) **Trigger DNA repair mechanisms, preventing cells from accumulating DNA damage**
4) **Initiate apoptosis** (if DNA damage cannot be repaired)
5) **Maintain cell adhesion**

P53 gene

A transcription factor that binds to DNA to trigger transcription of genes involved in cell cycle inhibition
- it itself does not directly repair DNA

- **Activates DNA repair proteins** when DNA has sustained damage
- **Arrests cell growth** by holding cell cycle at the G1 regulation point (Ensures damaged DNA is not replicated)
  - **Activated p53 protein** binds to specific DNA control elements and **promotes transcription** for the relevant genes eg. **p21** gene
  - **p21 protein stops the cell cycle** by **binding to proteins** that are **involved in cell cycle progression** such as Cdks
- **Initiates apoptosis**

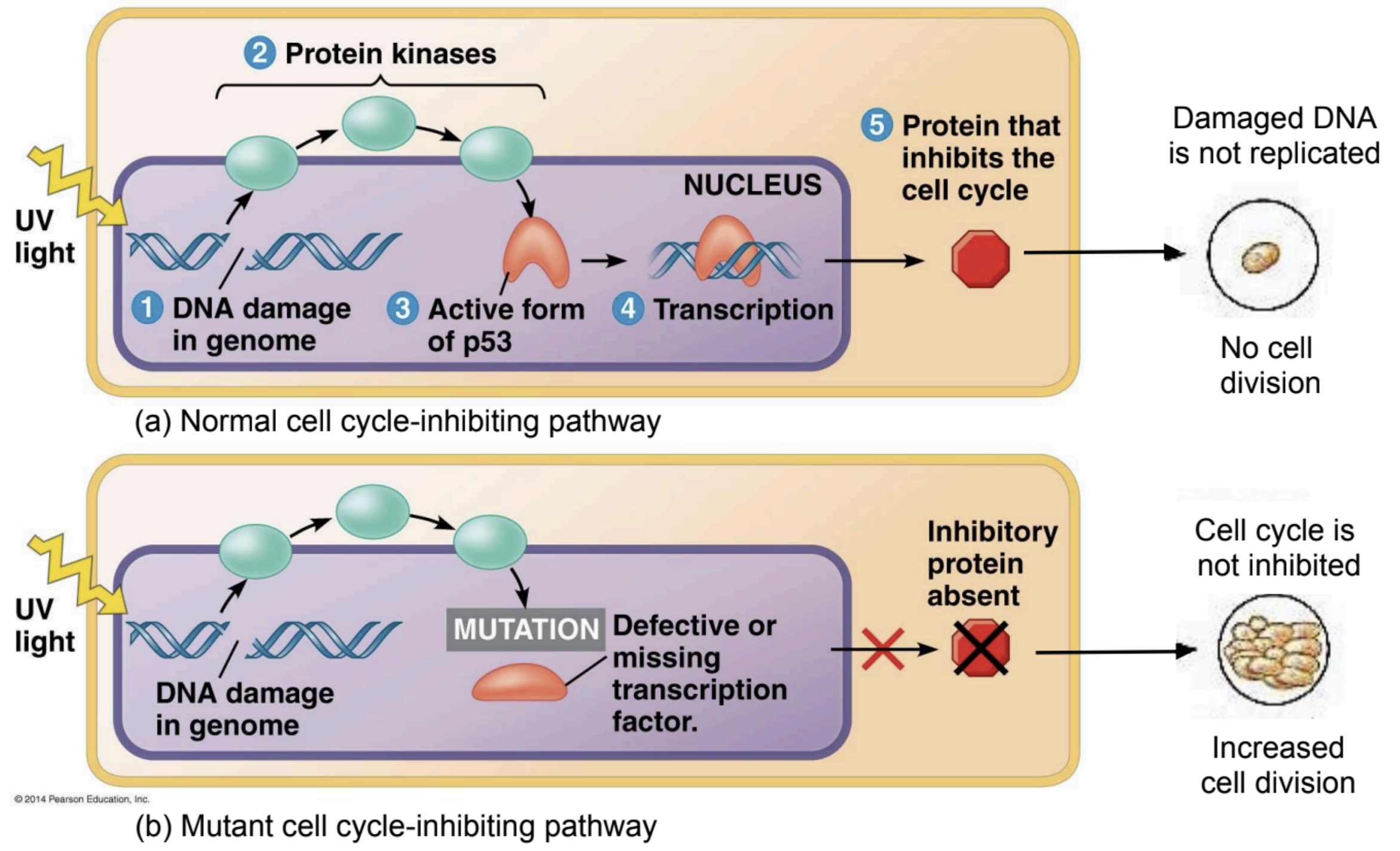


Fig. 11: Effect of normal p53 proteins and mutated form of p53 proteins on cell cycle.

Proto-oncogene (stimulates normal cell division)

Function of gene products:
1) Growth factors (GFs)
2) Growth factor receptors
3) Protein kinases
4) Inhibitors of apoptosis
5) Transcription factors

Proto-oncogenes can **undergo gain of function mutation** and be converted to **oncogenes**; **promotes excessive cell division**

Only 1 copy needs to be mutated (dominant); leads to proteins being **over-expressed**

ras protein (a GPLR)

Activated ras protein relays signals from a growth factor receptor to a series of protein kinases.
Last protein kinase of signal transduction pathway **activates transcription** of genes encoding proteins that **stimulate cell division**

Activated ras protein relays signals from a growth factor receptor to a series of protein kinases.
Last protein kinase of signal transduction pathway **activates transcription** of genes encoding proteins that **stimulate cell division**

GIF mutation **changes 3D conformation of ras protein**; GTP remains bonded to protein as a **ras-GTP complex**

Ras protein is in **constant"activate" state** even in **absence of growth factor (hyperactive protein)**

increase in cell signalling, transcriptiong and stimulationof cell cycle

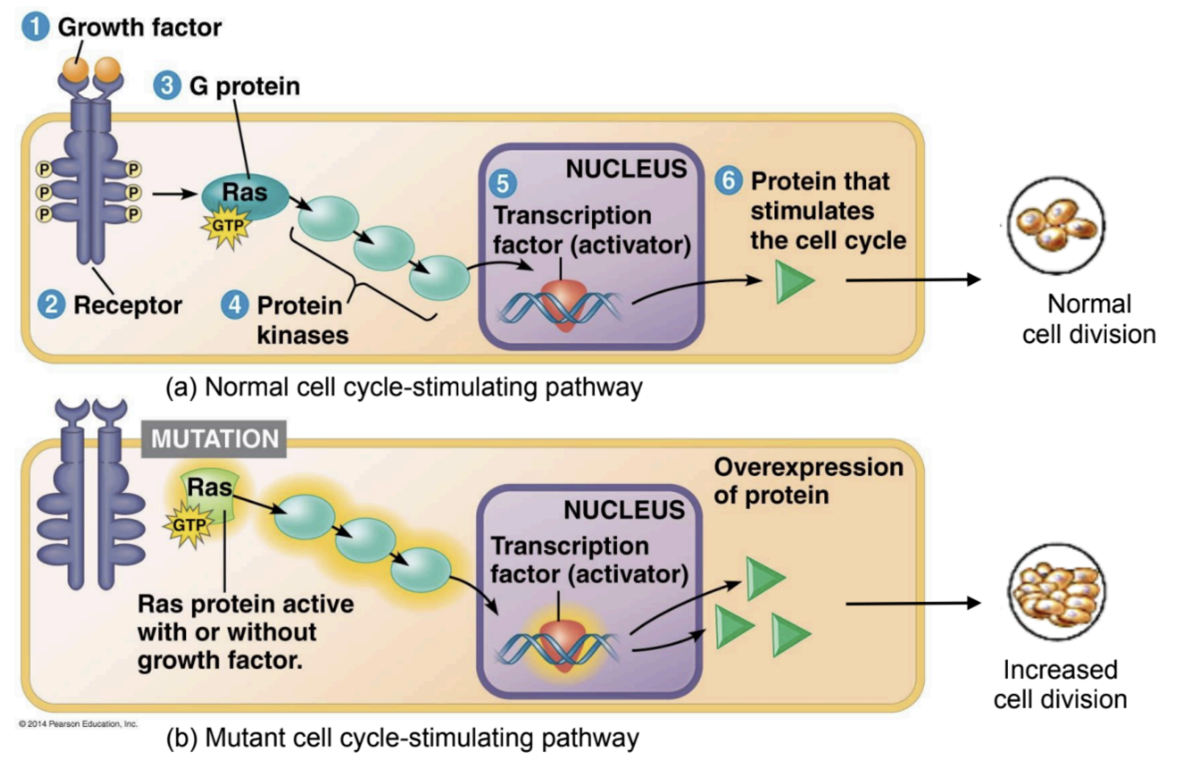


Fig. 13: Effect of normal ras proteins and mutated form of ras proteins on cell cycle.

Inheritance

Heredity • Transmission of genetic characteristics from one gen to the other + effects

Variation • The recognizable differences btw. individuals of same species + btw. parents and offsprings

1. **Gene**
   - A unit of inheritance located at a particular locus of a chromosome
   - A specific DNA nucleotide sequence which codes for RNA / polypeptide

2. **Locus**
   - a specific location of a gene on a chromosome

3. **Allele**
   - An alt. form of a gene at a particular theory
     - ↳ resp. for determining contrasting theory
     - ↳ alleles ⟶ same character, w. unique DNA nucleotide sequence ⟶ diff phenotypes
     - ↳ occurs in pairs

4. **Genotype**
   - genetic makeup / allelic composition of an organism

5. **Phenotype**
   - physical manifestation of a genetic trait ← from a specific genotype + interaction w/ env.

6. **Wild-type**
   - The most common allele / phenotype in nature

7. **Homozygous**
   - alleles of a gene pair in diploid condition are identical
   - organism : homozygote → true / pure breeding

8. **Heterozygous**
   - alleles of a gene pair are diff.
   - organism : heterozygote

9. Dominant allele
- Produces effects in both homozygous + heterozygous condition
- masks influence of recessive alleles

10. Recessive Alleles
- produce effects only in homozygous condition

11. True breeding
- organism gives rise to all offspring of same phenotype

12. Carriers
- Organism inherited recessive allele for genetic trait/mutation
- does not display that trait / show symptoms.

First law of segregation
- During formation of gametes, paired alleles segregate randomly so each gamete recieves one or another with equal likelihood

Second law of independant assortment
- A pair of traits segregate independently of another pair during gamete formation. Diff traits get equal opportunity to occur together

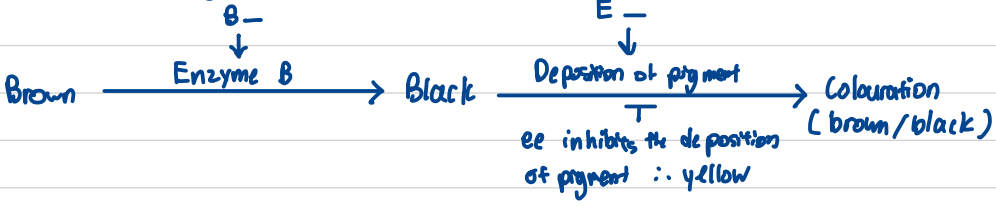
Monohybrid cross → 3 : 1

Non-epistatic gene interactions: Two independently assorting genes may interact to influence a single character (eg. comb shape in chicken)

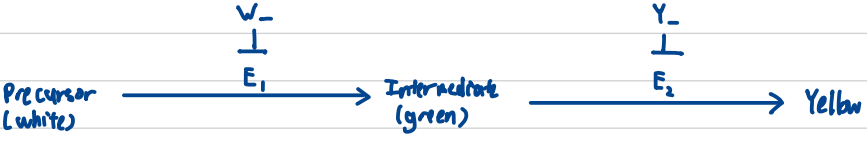
- dHD x dHR = genetic ratio of 9 : 3 : 3 : 1

Epistatic gene interactions — Expression of an allele of one gene inhibits the expression of allele of a different gene

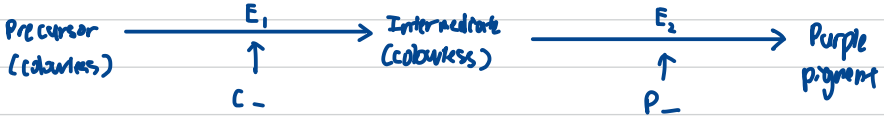
- Recessive epistasis — two recessive alleles at the gene locus will suppress inhibit the effect of either allele of the hypostatic gene at a different locus
  - dHD x dHR = genetic ratio of 9 : 3 : 4



- Dominant epistasis — one dominant allele at the epistatic gene locus will suppress the effect of both allele of the hypostatic gene at a different locus
  - dHD x dHR = genetic ratio of 12 : 3 : 1



- Duplicate recessive epistasis — two recessive alleles at either of the two gene loci suppress/inhibit the effect of the dominant allele at the other locus
  - dHD x dHR = genetic ratio of 9 : 7



Variation — discontinuous and continuous

	Discontinuous variation	Continuous variation
Observable phenotypes	Discrete phenotypic classes observed Intermediates are not observed	Range of phenotypes observed Intermediates are observed
No. of genes controlling phenotypic variation	Variation controlled by a single/a few gene(s)	Variations controlled by multiple genes — polygenic inheritance Genes can act in an additive manner, combined effect produce infinite no. of varieties
Effect of environment on phenotype	Little or no environmental effect	Phenotypes modified by the cumulative effect of varying environmental factors

Opportunities for genetic variation arise via:

1. Crossing over between non-sister chromatids of homologous chromosomes during prophase I of meiosis
2. Independant assortment of bivalents along the metaphase plate during metaphase I of meiosis
3. Random fertilisation

Gene linkage

Can be detected by performing a test cross.

- If genes are on diff chromosomes, 4 diff phenotypes, ratio 1:1:1:1
- If genes are completely linked, 2 (parental) phenotypes, no recombinant phenotypes, 1:1
- If genes are incompletely linked, 4 diff phenotypes, two parental, two recombinant, no fixed ratio but larger percentage of parental phenotypes and smaller percentage of recombinant phenotypes

Coupling: two dominant alleles on one chromosome, two recessive on other chromosome

$$\frac{A\ B}{a\ b}$$

Repulsion: dominant allele is linked with a recessive on one chromosome

$$\frac{A\ b}{a\ B}$$

(c) Explain how it is then possible to obtain the observed numbers in the 100 progeny from the series of crosses between the F_1 offspring and brown, chinchilla rabbits. [3]

any three from:

1 **both genes** B/b and H/h are **incompletely linked ;**
2 **crossing over** occurs between the linked genes **during prophase I,** results in mutual **exchange of segments of non-sister chromatids ;**
3 **in F_1 offspring**, where the **linked genes** are in a **repulsion arrangement / AW ;**
4 resulting in **production of recombinant gametes,** with both **dominant alleles B and H,** and thus **recombinant phenotype** of **black, full colour in progeny** as minority ;

OR

resulting in **production of recombinant gametes,** with both **recessive alleles b and h,** and thus **recombinant phenotype** of **brown, chinchilla in progeny** as minority ;

(d) Suggest how researchers can use similar breeding experiments with many different pairs of characters to map the position of genes on the chromosomes of rabbits. [3]

any two from:

1 **recombinant frequency (RF) / cross-over value (COV)** may be **determined** via numerous breeding experiments**, by calculating number of individuals showing recombinant phenotypes / total number of offspring x 100% ;**
2 **distance between genes** can therefore be **determined by / RF / COV / proportion of recombinants** OR a **COV of 1% represents** a relative distance of **1 centimorgan** (**cM**) **/ map unit on** the **chromosome ;**
3 as the **chance for crossing over** occurring **between two linked genes** is **proportional to the distance between them / reduced** if they are located **close to each other / ORA ;**
4 **AVP: e.g.** if the **expected phenotypic ratio** is obtained, there is **no linkage present ;**

and

5 * thus **by analyzing** the **relative distance between different pairs of genes,** the **exact order / position of genes** on a chromosome can be **determined** and used to generate a **chromosomal map ;**

*** Note:** MP5 is compulsory for full credit.

Explain why there is a greater number than expected of parental phenotypes

1. Genes are linked on the same chromosome
2. crossing over between genes on homologous chromosomes to produce recombinants does not occur frequently
3. Other parent only passes on gametes of genotype bb.

Photosynthesis

3a) Identify components of chloroplasts and mitochondria in drawings, photomicrographs and electron micrographs

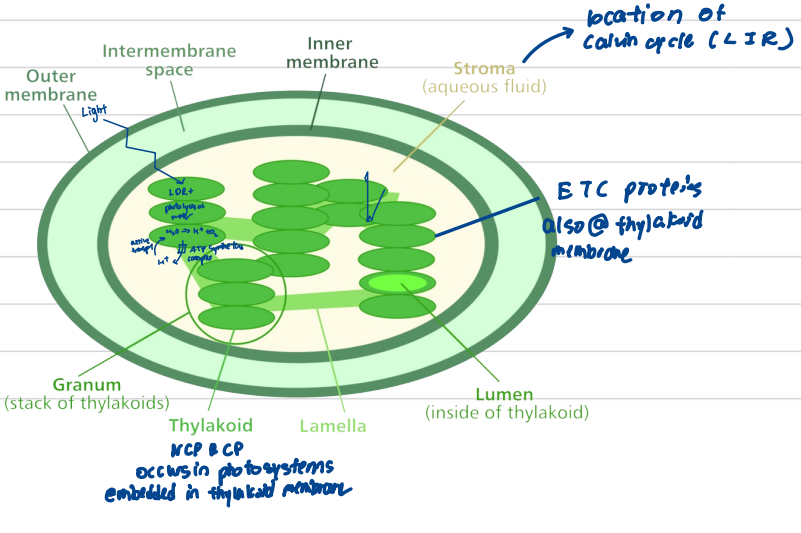
(i) With reference to Fig. 7.1, explain how structure **A** is related to its function. [3]
(Components **A** are the thylakoid sacs)

1. many ***thylakoid*** sacs are stacked up to form a granum to increase surface area for:
2. the thylakoid membranes have photosystems containing photosynthetic pigments embedded on the surface for light absorption;
3. the thylakoid membranes have electron carriers/electron transport chain embedded on the surface to pump protons/create a proton gradient between the thylakoid space and the stroma;
4. thylakoid membranes have ***ATP synthase*** embedded on the surface for chemiosmosis and ATP synthesis;
5. thylakoid membranes have ***NADP reductase*** to reduce NADP;
6. Hydrophobic core of phospholipid bilayer is impermeable to protons thus allowing a high concentration of protons inside the thylakoid lumen/space;

Structural features of chloroplast:

Table 1: Structural Features of the Chloroplast

Structure	Description
1. Size and shape	• Lens-shaped (in plants). • About 5 – 10 μm in length and 4 – 7 μm in width.
2. Chloroplast envelope	• Made up of a **double membrane**. • The outer membrane is selectively permeable to some solutes. • The inner membrane is highly permeable. Substances pass through with the aid of transporters.
3. Stroma	• A gel-like matrix enclosed by the **chloroplast envelope**. • Contains circular DNA, 70S ribosomes, starch granules, oil droplets and enzymes involved in the Calvin cycle.
4. Thylakoids	• A third membrane system within the stroma consisting of flattened sacs or pouches. • **Photosynthetic pigments and electrons carriers are embedded** within the membrane. • The space enclosed within the thylakoid is known as the **thylakoid lumen** or **thylakoid space**. • This compartmentalisation allows chemiosmosis to take place and for ATP to be produced by photophosphorylation.
5. Granum (plural: grana)	• A stack of thylakoids • This **increases the surface area** and the amount of pigments available for the light-dependent reaction of photosynthesis. • Connecting the grana are flattened tubular thylakoids known as intergranal lamellae (singular: lamella). These lamellae connect the thylakoid compartments into a single, continuous compartment within the stroma.



(b) Explain the absorption and action spectra of photosynthetic pigments

Photosynthetic pigments

1) Chlorophyll

↳ hydrophilic porphyrin ring : flat, hydrophilic head which contains Mg^{2+} atom

↳ hydrophobic hydrocarbon tail : projects into thylakoid membrane to embed chlorophyll

a) Chlorophyll a [absorbs blue and red light] → major pigment which participates in LDR

b) Chlorophyll b (accessory pigment) → × LDR

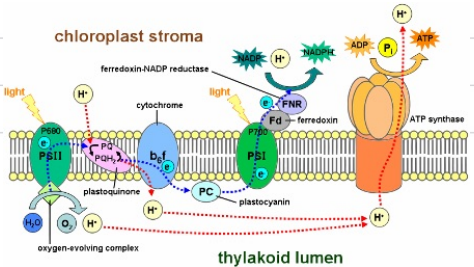
Carotenoids (accessory pigments) [absorb blue - violet light]

- broadens spectrum of light for photosynthesis
- photoprotection.

c) w.r.t chloroplast structure, describe and explain how light energy is harnessed and converted into chemical energy during LDR

Function of thylakoid membrane

1) Electron carriers embedded into thylakoid membrane enable e^- transport to occur, flow of e^- releases energy to pump H^+ from stroma into thylakoid space.
2) Thylakoid membrane is impermeable to H^+ and enables a proton gradient to be generated across the membrane.
3) ATP synthetase complex embedded in thylakoid membrane enable ATP synthesis by chemiosmosis
4) Stacking of thylakoids provide a large surface area and ↑ amount of photosynthetic pigment available for light absorption.



Photoactivation of a chlorophyll

- When chlorophyll absorbs a photon of light, one electron in the chlorophyll gets excited to a higher level
- Energy is transferred to a neighbouring chlorophyll molecule through resonance energy transfer
- Energy displaces an electron from the pair of special chlorophyll a molecules (P680/P700) at reaction centre of PSII or PSI and is accepted by an electron acceptor.

LOCS

4. PHOTOSYNTHESIS: A THREE STAGE PROCESS

- Photosynthesis is essentially a process of **energy transduction** where light **energy** is converted into **chemical energy**.
- Photosynthesis has three main stages (**Fig. 13**):

(1) **Light Harvesting Stage**
- Light energy is **captured** by the plant using a **mixture of pigments** including chlorophyll.

(2) **Light-dependent / Light Reaction**
- Light energy is harnessed to excite and displace an electron from chlorophyll.
- **Light energy is converted to chemical energy** through a **flow of electrons** that is **coupled** to **ATP synthesis**.
- **NADPH** is produced.
- **Photolysis of water** – light is involved in the splitting of water into hydrogen ions and oxygen.

The first two stages **require light** and occur in the **thylakoid membranes** of chloroplasts.

(3) **Light-Independent Reaction**
- **Chemical energy** of **ATP and NADPH** (produced from the light-dependent stage) is **used** in the reduction of carbon dioxide and hence, the **manufacture of sugars**.

The third stage **does not require light** and takes place in the **stroma** of chloroplasts.

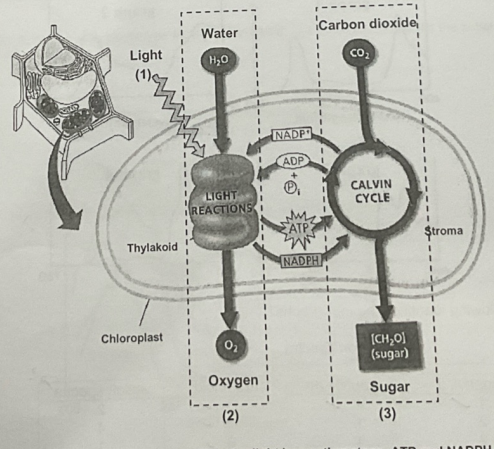


Fig. 13: Photosynthesis as a three-stage process. (1) is the light harvesting stage. ATP and NADPH from the (2) lig

Light dependant reaction (Production of ATP and reduced NADP)

Non-cyclic phosphorylation

1) A photon of light strikes a pigment molecule in a chloroplast, and the energy is transferred via resonance energy transfer until it reaches the P680 (in PSII) molecule in the reaction center of PSII. It excites in electron in P680 to a higher energy state
2) This electron is captured by the primary electron acceptor and passed from PSII to PSI via an electron transfer chain.
3) The photolysis of water produces an electron, a proton and O2 gas. The electron replaces the lost electron from PSII.
4) As the electron flows unidirectionally down the ETC, it drops to lower energy levels. This energy is used to pump H+ from the stroma into the thylakoid space, creating a proton gradient
5) This proton gradient drives ATP synthesis by diffusing into the ATP synthase complex embedded in the thylakoid membrane.
6) Meanwhile, another photon of light strikes a pigment molecule in PSI. The energy is transferred via resonance energy transfer until it excites a P700 molecule in the reaction center of PSI, which causes it to lose an electron.
7) The electron from PSII travels down the ETC until it reaches PSI where it replenishes the lost electron
8) The excited electron is passed down a second ETC through ferrodoxin, where NADP reductase transfers electrons from Fd to NADP, forming reduced NADP

Cyclic photophosphorylation:

1) A photon of light strikes the light harvesting complex (LHC) and is passed on to P700 via resonance energy transfer. This causes an electron in P700 to be excited and be picked up by the primary electron acceptor in the reaction center.
2) The energised electrons are passed to ferrodoxin (Fd) where they are cycled back to cytochome (into the first electron transfer chain) back to PSI.
3) As these electrons are passed down the ETC, enough energy is released to synthesize ATP from ADP

Ways cyclic phosphorylation and non-cyclic phosphorylation differ

1. Non-cyclic phosphorylation(NCP) involves both the PSI and PSII system, while cyclic phosphorylation(CP) only involves the PSI system/p700
2. In NCP, first electron donor is water while in CP first electron donor is P700 in PSI
3. In NCP, photophosphorylation of water occurs while in CP it does not
4. In NCP, through the flow of electrons via the electron transfer chain. the electron does not return to the same molecule while in CP, electron returns to the same molecule
5. In NCP, final electron acceptor is NADP while in CP, the final electron accptor is P700 in PSI
6. In NCP, NADP reductase is required in the process while in CP, it is not
7. In NCP, products of NCP are ATP & NADPH while in CP, products of CP are ATP only
8. In NCP, O2 is produced as a byproduct while in CP, it is not

"Describe how plants convert light energy to chemical energy"

① Light/photon strikes pigment molecule/chlorophyll in light-harvesting complex (LHC) of PSI/II

② Resonance energy transfer (→ transfer of energy; ✗) from one pigment molecule to another within the LHC

③ Electron in special chlorophyll a / P680 molecule becoming photoexcited / raised to a higher energy level *award once only for PSI and PSII

④ Electron captured by primary electron acceptor in reaction center

⑤ Photolysis of water, to replenish electron deficit from reaction center of PSII
R: hydrolysis

⑥ e^- passing through electron transport chain (ETC), from PSII to PSI

⑦ free energy released used to pump protons, against a concentration gradient / via active transport

⑧ from stroma into thylakoid space

⑨ diffusion of H^+, down proton gradient, through ATP synthase complex

⑩ driving ATP synthesis / phosphorylation of ADP

⑪ via chemiosmosis

⑫ e^- from PSII replenishing e^- deficit in PSI

⑬ excited e^- from PSI passed down a 2nd ETC to ferrodoxin (Fd), which passes e^- to ...

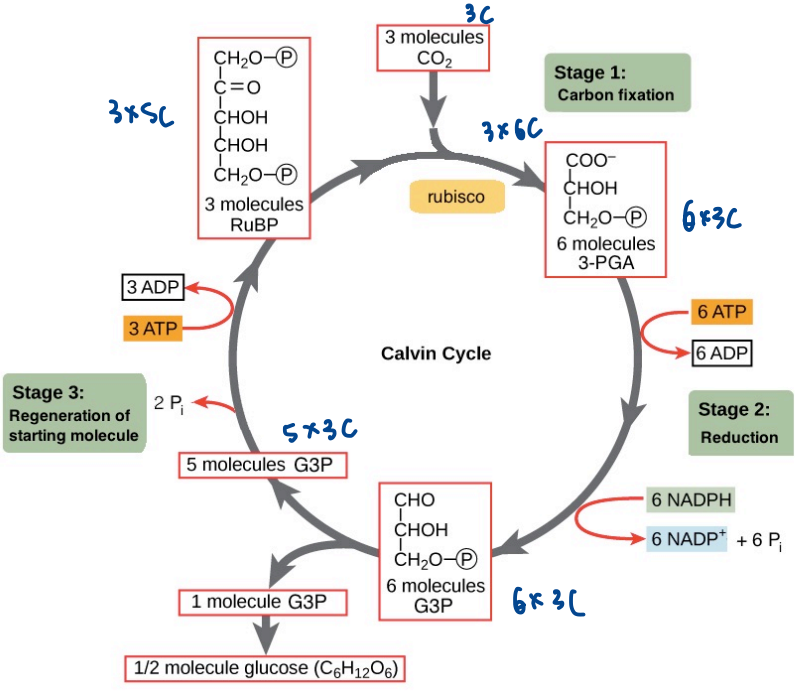
⑭ reduction of final electron acceptor $NADP^+$ to NADPH

⑮ phosphorylation of GP/PGA to 1,3-BPG using ATP

⑯ reduction of 1,3-BPG to G3P/TP using NADPH

d) Outline the 3 phases of the Calvin cycle in C3 plants : i) CO_2 fixation, ii) PGA reduction and iii) RuBP regenerate, indicating the roles of rubisco, ATP & NADPH in these processes that ultimately allow synth. of sugar

Light independent reaction (Calvin cycle) @ stroma



- Occurs in the stroma of chloroplasts
- Reduce CO2 using ATP (energy source) and NADPH (reducing agent)

1) Carbon fixation

- CO_2 diffuses through stomata into cytoplasm of mesophyll cells and into the chloroplasts, where it combines with a 5C acceptor ribulose bisphosphate (RuBP) to form an unstable 6C intermediate. (reaction catalysed by ribulose bisphosphate carboxylase oxygenase - rubisco)
- Unstable 6C intermediate breaks down spontaneously into phosphogyceric acid (PGA)/ glycerate-3-phosphate (GP)

2) Reduction of PGA/GP

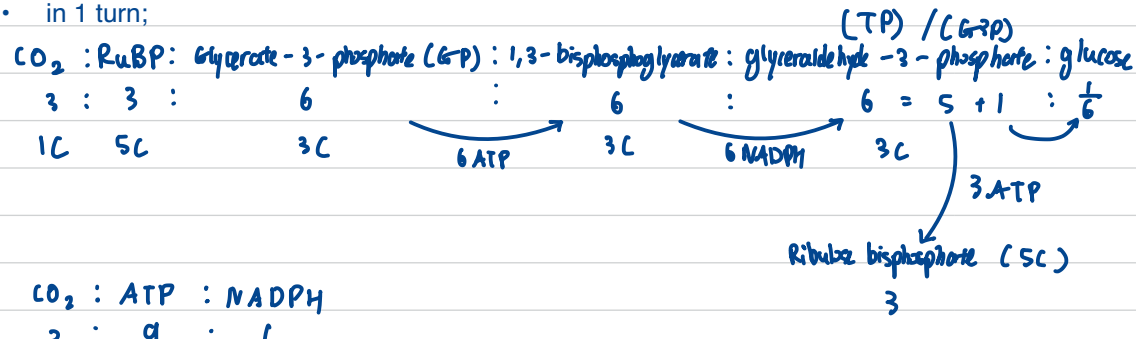
- Each molecule of PGA is phosphorylated by ATP, forming 1,3 - bisphosphoglycerate
- A pair of e from NADPH further reduces 1,3 - bisphosphoglycerate into glyceraldehyde-3-phosphate (G3P) / triose phosphate (TP). Energy for this step comes from ATP

3) Regeneration of CO_2 acceptor (RuBP)

- For every 3 CO_2, 3 RuBP are invested and 6 TP are formed
- Only one molecule of TP is considered a net gain; other 5 molecules of TP are used to regenerate the 3 molecules of RuBP used in step 1 (3 ATP used)
- RuBP is generated

Product synthesis and sugar formation:

- 2 TP are used to synth 1 hexose sugar. One molecule of hexose sugar requires 2 turns of the Calvin cycle
- in 1 turn;



Fate of photosynthetic products

- PGA and TP are intermediates in glycolysis and do not accumulate
  → used in the synthesis of all other forms of carbon-containing substances.

↳ TP ⇒ hexose sugar (glucose & sucrose) : respiratory substrates
  ↳ excess glucose converted into starch granules

↳ TP ⇒ lipids : PGA → acetyl $\xrightarrow{+\text{coenzyme A}}$ acetyl CoA → fatty acids
  : TP → glycerol $\xrightarrow{+\text{F.A.}}$ fatty acids

↳ PGA & TP + N : amino acids → nucleotides

e) Discuss limiting factors in photosynthesis & carry out investigation

Light intensity:

- not normally a major limiting factor
- compensation point — no NET gaseous exchange; exists at low light-intensity

Sun and shade plants:

- shade plants have a lower rate of respiration; have fewer cells (req. less energy) — have a compensation point at a lower light intensity compared to sun plants
- sun plants have a higher level of respiration + higher rate of photosynthesis

Chlorophyll concentration - caused by:

- diseases, mineral deficiency, normal ageing process, lack of light

Water — plants close their stomata in response to less water, which prevents access of carbon dioxide

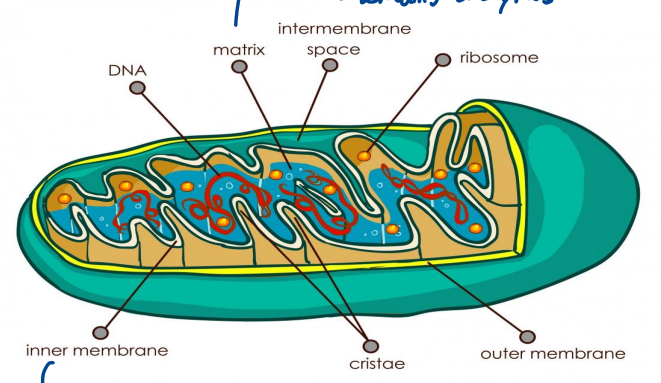
Specific inhibitors:

- DCMU, cyanide

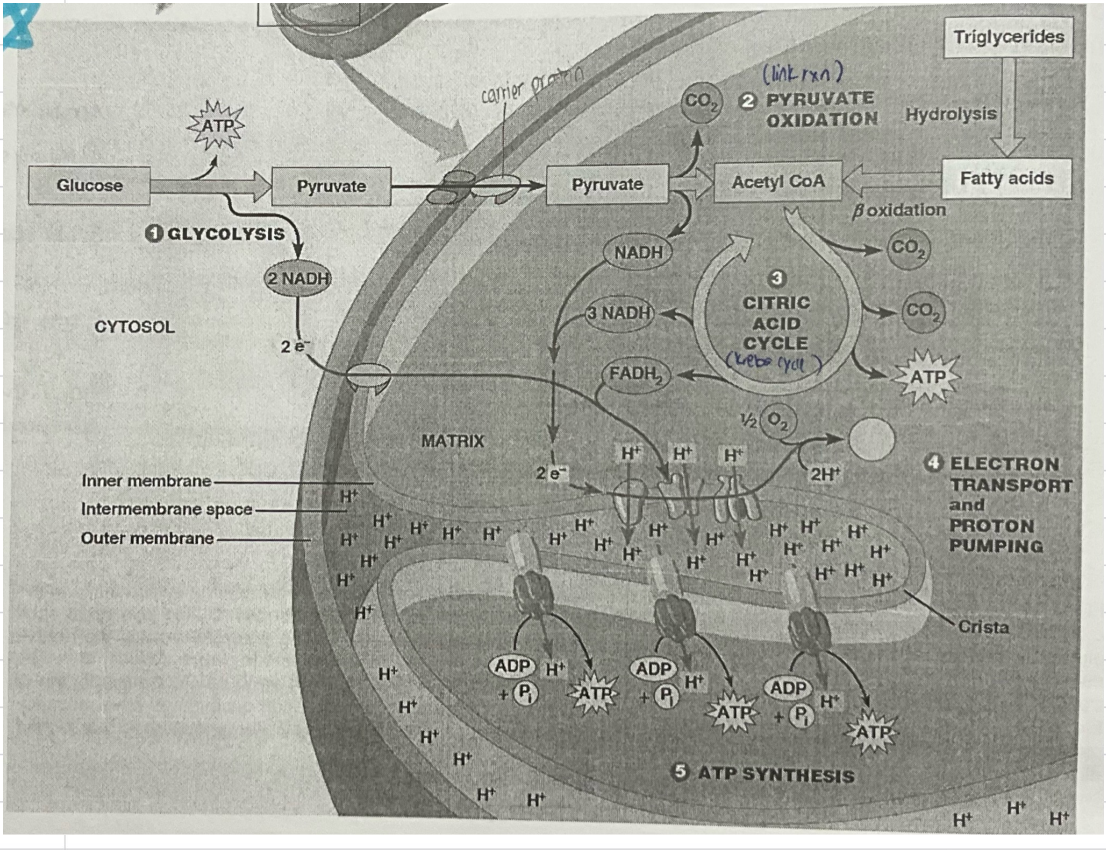
Cellular Respiration

a) Identify components of chloroplasts and mitochondria in drawings, photomicrographs and electronmicrographs

↗ Compartment enclosed by inner membrane
- Site of Link reaction & Krebs cycle
- contains enzymes



↓
- selectively permeable membrane
- highly folded to form cristea
  ↳ ↑ surface area for embedding ETC & ATP synthase complexes
- not permeable to NADH
- contains protein transporters
- contains ETC & ATP synthase complexes
- permeable to pyruvate
- site of oxidative phosphorylation


Triglycerides
carrier protein
(link rxn)
CO_2
2 PYRUVATE OXIDATION
Hydrolysis
ATP
Glucose
Pyruvate
Pyruvate
Acetyl CoA
Fatty acids
β oxidation
1 GLYCOLYSIS
NADH
CO_2
2 NADH
3 NADH
3 CITRIC ACID CYCLE (Krebs cycle)
CO_2
CYTOSOL
2 e⁻
$FADH_2$
ATP
½ O_2
MATRIX
H^+
2 e⁻
2H^+
4 ELECTRON TRANSPORT and PROTON PUMPING
Inner membrane
Intermembrane space
Outer membrane
Crista
ADP + P_i
ATP
5 ATP SYNTHESIS

f) Outline the process of glycolysis, highlighting location, raw materials used and products formed (knowledge of details of intermediates & conservation not req.)

Ten-step reaction sequence (In cytosol)

$C_6H_{12}O_6 + 2ATP + 2NAD \longrightarrow 2C_3H_4O_3 + 2ATP + 2NADH + 2H^+$

(glucose) — useful molecules

Energy-investment Phase

Glucose (6C)

ATP → ADP — Hexokinase

ATP → ADP — Phosphofructokinase (PFK) ⇒ rate limiting step

Fructose-1,6-bisphosphate (6C)

cleavage

2 [glyceraldehyde-3-phosphate] (3C)

drive ATP production by oxidative phosphorylation at inner mitochondrial membrane

2 NAD → 2 NADH ⇒ each contains 2 energized e⁻

Energy-Payoff Phase

2 [1,3,-bisphosphoglycerate (BPG)]

2ADP → 2ATP (--> H_2O)

2[3-phosphoglycerate (3PG)]

2ADP → 2ATP — pyruvate kinase

2[pyruvate]

2 ATP payoff

Aerobic respiration

Glycolysis

↓

link rxn

↓

Krebs cycle

↓

Oxidative phosphorylation

Importance of glycolysis:
vital source of energy through production of net 2 molecules of ATP by substrate-level phosphorylation

1. Glycolysis is the only catabolic process that does not require O_2.
2. In the presence of O_2, is required for the Link reaction to occur
   NADH and RAD supply energised e for ATP production

Supplies cells w essential biosynthetic precursors

- liver carries out glycolysis to provide precursors for fats, cholesterol, bile acids etc
- In a well-fed animal, pyruvate is converted to fats (fat biosynthesis)

Regulation of Glycolysis (PFK)

Phosphofructokinase is an allosteric enzyme

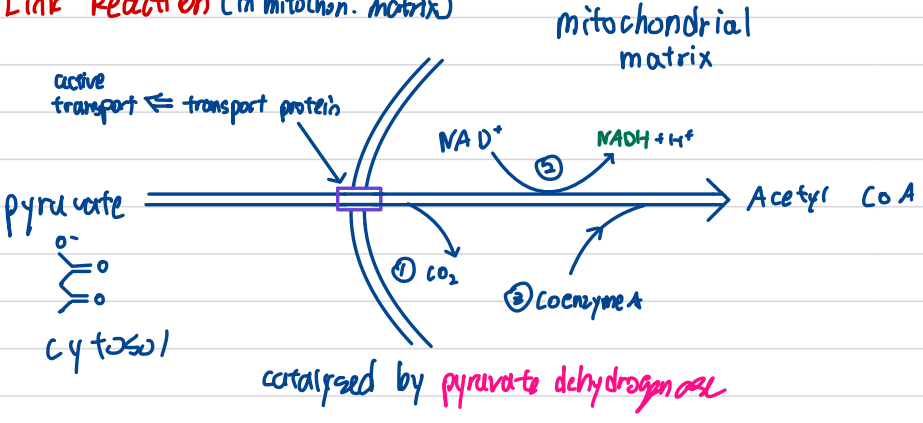
1. As [ATP] ↑, it acts as an allosteric inhibitor by binding to PFK and ↓ rate of glycolysis

2. Stimulated by AMP (derived from ADP)
   ↳ enzyme re-activated as cellular work converts ATP → ADP + AMP faster than ATP is being regen.

3. When [citrate] ↑ in mitochondria, some enters cytosol & inhibits PFK
   ↳ sync. rates of glycolysis & Krebs cycle

f) Outline the process of the link reaction and Krebs cycle, highlighting the location, raw materials used and the products formed (in terms of dehydrogenation and decarboxylation)

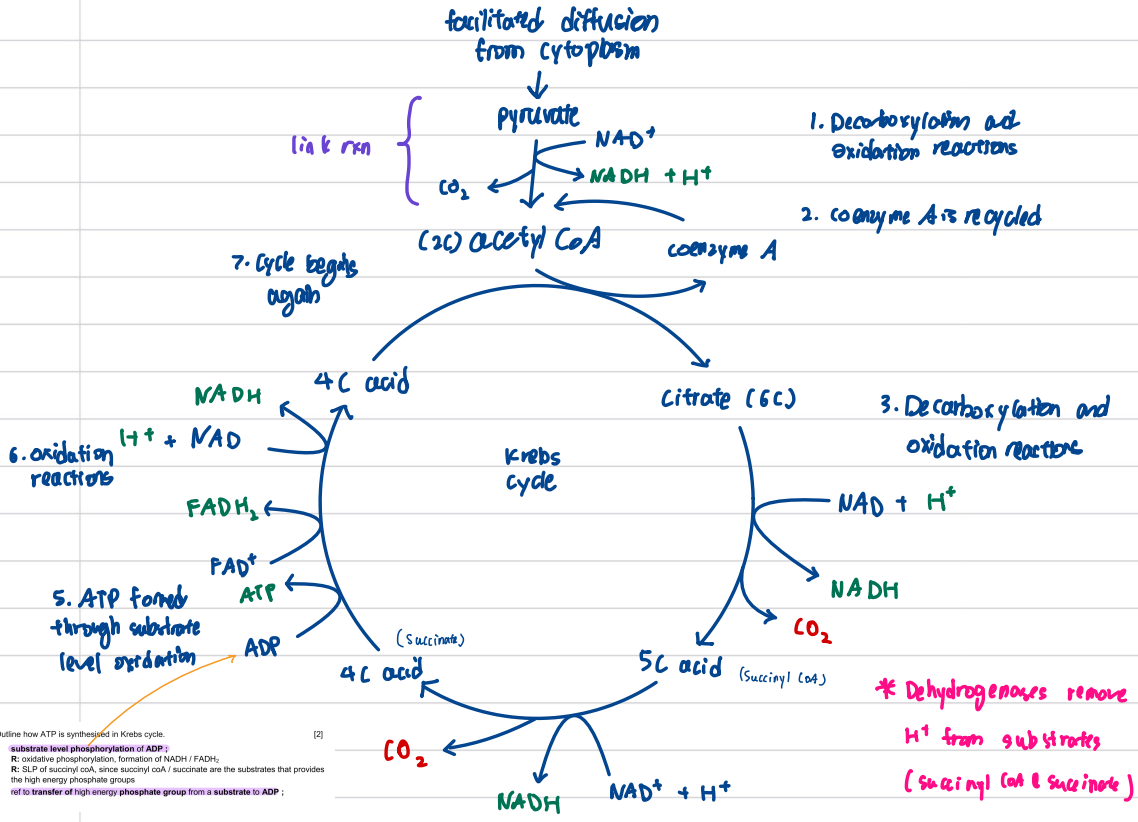
Link Reaction (in mitochon. matrix)



Oxidative decarboxylation Per glucose molecule:

$$2\ \text{Pyruvate} + 2\text{NAD} + 2\ \text{CoA} \longrightarrow 2\ \text{Acetyl CoA} + 2\text{NADH} + 2CO_2$$

Krebs cycle (citric acid cycle) @ mitochondrial matrix



(c) Outline how ATP is synthesised in Krebs cycle. [2]

1 **substrate level phosphorylation of ADP ;**
R: oxidative phosphorylation, formation of NADH / $FADH_2$
R: SLP of succinyl coA, since succinyl coA / succinate are the substrates that provides the high energy phosphate groups

2 ref to **transfer of** high energy **phosphate group** from a **substrate** to **ADP** ;

4. Decarboxylation and oxidation reaction

Per glucose molecule:

	CO_2	ATP	NADH	$FADH_2$	
Glycolysis	-	2	2	-	1NADH → 3ATP
Link rxn	2	-	2	-	1 $FADH_2$ → 2ATP
Krebs cycle (2 turns)	4	2	6	2	
Total :	6	4	10	2	

∴ max ATP → 36/38 molecules / glucose

h) Outline the process of oxidative phosphorylation including the role of O_2 and ETC in aerobic respiration

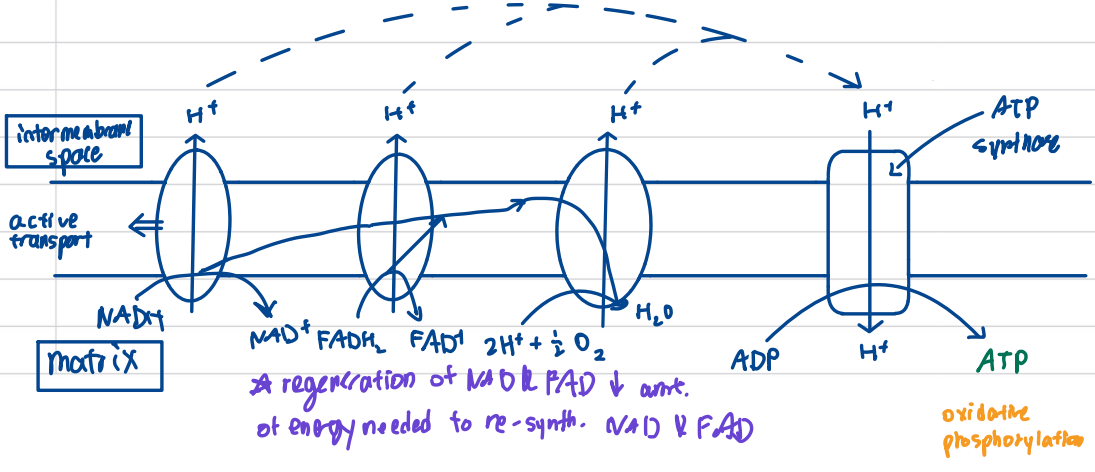
Electron Transport Chain (ETC)

↳ occurs in inner mitochondrial membrane, highly folded to form cristae

- ETC → a sequence of electron carriers
- undergo temp. red. and ox. as e^- (from NADH & $FADH_2$) are passed to final electron acceptor, O_2

- No ATP directly generated
- energy from e^- carried by $FADH_2$ & NADH used to pump protons across inner mitochon membrane into intermembrane space, generating a proton gradient (vs ETC in photosynthesis where free e is released)
- flow of H^+ through ATP synthase complex, allows for ATP synth by oxidative phosphorylation

★ Each subsequent complex in the ETC has a higher affinity for e^- than its predecessor (↑ e^- affinity), which ensures a one-way (unidirectional) transport of e^- down the ETC through the reversible reduction and oxidation of a seq. of e^- carriers,



Chemiosmosis:

pumping gens proton gradient w/:

1. Conc. gradient of H^+
2. Electrical gradient across membrane

} Build up of H^+ conc. in intermembrane space causes H^+ to diffuse back into matrix, → proton motive force

ATP synthase complex: couples the exergonic passage of H^+ w/ endergonic phosphorylation of ADP
↳ spins to catalyze ATP production

ATP yield:

NADH	: H^+	: ATP
1	: 10	: 3

$FADH_2$	: H^+	: ATP
1	: 6	: 2

Comparing substrate level phosphorylation and oxidative phosphorylation

	Substrate – level phosphorylation	Oxidative phosphorylation
process	• The enzymatic endergonic phosphorylation of ADP → exergonic dephosphorylation of organic substrate	enzymatic • endergonic phosphorylation of ADP to form ATP by ATP synthase → exergonic electron transport to final e^- acceptor, O_2 • exergonic passage of protons along a proton gradient
occurence	• during glycolysis in cytoplasm • during krebs cycle in mitochondrial matrix	• During ETC in inner mitochon. membrane
ATP production	• produces only a small amount of ATP	• Produces ~ 90% of ATP in respiration

Respiratory poison / inhibitors:

1. poisons that block electron flow (down the ETC)
   - eg. carbon monoxide, cyanide, Hydrogen sulphide
   - ATP production is completely inhibited

2. Poison that inhibit ATP synthase
   - eg. oligomycin — prevents the influx of protons through ATP synthase
   - although proton gradient ↑, its potential energy cannot be tapped to make ATP

3. Poisons that make the inner mitochon. membrane leaky to protons
   - eg. 2,4 - dinitrophenol (uncoupling agents) — carry protons across the inner mitochon membrane
   - Proton gradient dissipates ; no ATP can be formed

i) Explain the production of a small yield of ATP from anaerobic respiration in yeast and mammalian tissue

In an absence of oxygen, no further oxidation of pyruvate occurs, no acetyl CoA is formed and no additional ATP can be generated

Anaerobic respiration

- O_2 independent rxn
- Occurs in cytosol
- organic molecules (pyruvate) used as final e^- acceptor for regen. of NAD

	Lactic Acid Fermentation	Alcoholic Fermentation
Equation	NADH + pyruvate → NAD + lactate	NADH + pyruvate → NAD + ethanol + CO_2
Steps involved	• Pyruvate is reduced directly by NADH to form lactate, w/ no release of CO_2	① CO_2 released from pyruvate → acetaldehyde ② Acetaldehyde + NADH → ethanol + NAD^+
Occurance	• In animals & certain fungi, bacteria In animals, • Short term: satisfies greater priority of regen. NAD • long term: lactic acid is toxic & must ultimately be removed	In fungi eg. yeast plant tissues
Products	Lactic acid, NAD	Ethanol, NAD

Aerobic respiration
max./glucose = 38/36

Anaerobic respiration
max./glucose = 2

↓
Depending on the shuttle system used (to move NADH produced during glycolysis, either 2 or 3 ATP molecules would be produced per NADH.

QUESTION 5

(a) Describe the features of the processes of aerobic respiration that allow energy from a glucose molecule to be harnessed. [15]

any fourteen from:

A1 ref. to aerobic respiration being the process in which **glucose** is completely **oxidised ;**
A2 ref. to yield total of **36 / 38 ATP ;**

A3 * ref. to **enzyme-catalysed reactions ;**
A4 ref. to, glucose / pyruvate / citrate / α-ketoglutarate, being the **substrates ;**
A5 ref. to **decarboxylases** catalysing **removal of carbon dioxide** from, pyruvate during link reaction / citrate during Krebs cycle / α-ketoglutarate during Krebs cycle ;
A6 ref. to **dehydrogenases** catalysing **removal of hydrogen atoms** from, PGAL / TP during glycolysis / citrate during Krebs cycle / α-ketoglutarate during Krebs cycle ;
R: ref. to substrate being 'broken down' or 'converted' instead of being decarboxylated or oxidised

A7 * ref. to **redox reactions ;**
A8 ref. to substrates such as **PGAL / pyruvate / citrate / α-ketoglutarate** being **oxidised** by **removal of hydrogen atoms ;**
A9 ref. to coenzymes such as **NAD / FAD** being **reduced** by **accepting hydrogen atoms** from substrates ;
R: substrates, e.g. glucose / PGAL / pyruvate / citrate are reduced – substrates are oxidised and coenzymes are reduced!

A10 * ref. to **substrate-level phosphorylation ;**
A11 ref. to endergonic **phosphorylation of ADP**
A12 being coupled to exergonic **dephosphorylation of an organic substrate ;**
I: '*formation of ATP from ADP*' without reference to substate as the source of phosphate

A13 * ref. to **oxidative phosphorylation ;**
A14 ref. to reduced coenzymes / NADH and $FADH_2$ **transferring electrons down the electron transport chain ;**
A15 ref. to electron transport occurring **across** the **inner mitochondrial membrane ;**
A16 ref. to **final electron acceptor, oxygen, being reduced to water ;**
A17 ref. to **energy released** from the electrons flowing through the ETC being used to power the **pumping of H^+ ions** across the inner mitochondrial membrane, to establish an **proton gradient ;**
A18 ref. to **diffusion of hydrogen ions** through the **ATP synthase complex ;**
A19 ref. to idea of **phosphorylation of ADP with P_i to ATP ;**
I: '*formation of ATP from ADP*' without reference to inorganic phosphate
A20 ref. to **3 ATP** per **NADH** and **2 ATP** per **$FADH_2$;**

A21 ref. to **glycolysis** occurring in **cytosol / cytoplasm ;**
A22 **(a)** ref. to **phosphorylation of glucose ;**
(b) ref. to one molecule of **glucose** being converted to **2 molecules of pyruvate**, with the generation of **2 net ATP** and **2 NADH** during glycolysis ;
R: merger of glucose activation with oxidation of glyceraldehyde-3-phosphate – these are separate steps in glycolysis

A23 ref. to **link reaction / Krebs cycle** occurring in **mitochondrial matrix** in the presence of oxygen ;
A24 ref. to one molecule of **pyruvate** being oxidatively decarboxylated into **acetyl CoA** and **1 NADH** during link reaction ;
R: tally of products without consideration of stoichiometry, i.e. wrt per glucose or per pyruvate
A25 ref. to one molecule of **acetyl CoA** combining with a **4C molecule / oxaloacetate** to form a 6C intermediate / citrate during Krebs cycle ;
A26 ref. to **regeneration of oxaloacetate ;**
A27 ref. to **oxidative decarboxylation** to **release** 2 carbons as **CO_2 ;**
A28 ref. to substrate-level phosphorylation to generate **1 ATP per acetyl CoA ;**
R: tally of products without consideration of stoichiometry, i.e. wrt per glucose or per acetyl coA
A29 ref. to dehydrogenation to produce **3 NADH** and **1 $FADH_2$ per acetyl CoA ;**
R: tally of products without consideration of stoichiometry, i.e. wrt per glucose or per acetyl coA

** compulsory for award of full marks*

QWC: Good spread of knowledge communicated without ambiguity to include (1) *accurate description of key features* of aerobic respiration (* points must be present), and (2) responses that are structured appropriately using paragraphing for separate features / stages of the process.

Comments: Hardly any students flagged out key features of aerobic respiration and organised their responses as such. Nearly all students spammed details of the four stages of aerobic respiration and the mark for QWC was not awarded. Most students maxed out the marks for content simply because there were twice the number of mark points. There is a pressing need to work on organisation of responses beyond basic paragraphing.
Content-wise, many responses lacked resolution. Generic terms such as *broken down, converted* were used to refer to key biochemical processes. Many students were penalised for merging two separate biochemical processes (e.g. oxidation of substrate with substrate level phosphorylation). Some students went the opposite way and referred to substrates being reduced in process of aerobic respiration, when substates should have been oxidised.

(b) Discuss the significance of membranes in aerobic respiration. [10]

M1 *ref. to **hydrophobic core** of cell surface membrane forms an effective **barrier** to the movement of polar / charged solutes such as **phosphorylated glucose** out of the cell ;
A: impermeable to H^+ in relation to MP11
M2 ref. to **phosphorylated glucose** being **retained in cytoplasm** for **glycolysis** to continue ;

M3 ref. to **glucose transporters (GLUT)** on **cell surface membrane** allowing **for facilitated diffusion of glucose** into the cell ;
M4 ref. to **transporter proteins** on **outer mitochondrial membrane / inner mitochondrial membrane** allowing **for facilitated diffusion of pyruvate** into mitochondrial matrix ;
A: ref. to shuttle system for NADH

M5 *ref. to **inner mitochondrial membrane, being highly folded / forming cristae ;**
M6 ref. to idea of **increasing surface area** for **embedding electron transport chain / ATP synthase ;**
A: attach many enzymes to increase concentration of enzymes for higher rates
M7 ref. to **electron transport chain** allowing for **movement of electrons down energy levels ;**

M8 ref. to **proton pumps** that **pump H^+ from mitochondrial matrix into intermembrane space ;**
M9 ref. to **ATP synthase** catalysing **phosphorylation of ADP with P_i to ATP ;**

M10 *ref. to **inner mitochondrial membrane,** being **selectively permeable ;**
M11 ref. to **formation of proton gradient ;**

M12 *ref. to **compartmentalisation ;**
M13 ref. to allowing for **optimal conditions** for biochemical reactions ;
M14 ref. to allowing **incompatible reactions** to **occur simultaneously ;**

** compulsory for award of full marks*

QWC: Good spread of knowledge communicated without ambiguity to include (1) *linking of structural features* of membrane *with a particular process* (* points must be present) (2) responses that are structured appropriately using paragraphing.

Comments: Good responses appropriately linked structural features of membranes (e.g. cell surface membrane, OMM, IMM) with how the feature *facilitated a particular step* in aerobic respiration.
Low scoring scripts simply repeated spamming of process of oxidation phosphorylation in part (a) without reference to *how* membranes (i.e. which aspect of membrane? What does it do?) facilitated the processes. Conversely, other low scoring scripts spammed fluid mosaic model of membrane without explicitly relating to the process of aerobic respiration.

[Total: 25]

Evolution

Biological evolution [D] — **descent with modification through the mechanism of natural selection**

Microevolution [D] — **Small-scale evolutionary change within the species level; caused by changes in allele type/genotypic frequencies that occur in a population over a few generations**

Macroevolution [D] — **large-scale evolutionary events over geological times; descent of different species from shared ancestors over many generations**

Essential features of Darwin-Wallance theory of evolution

1. Organisms have great potential to reproduce — large numbers of offspring
2. Environmental restrictions/Constancy in numbers — most populations are able to maintain relatively constant numbers
3. Struggle for existence / survival — competition is inevitable
4. Variation within a population — no two sexually produced offspring are identical
5. Survival of the fittest by Natural Selection
6. Differential reproduction leading to reproductive success — those that survive to breed are likely to produce offspring similar to themselves
7. Formation of new species — over many generations, the proportion of individuals possessing the advantageous traits increases, whereas the proportion of those lacking the characteristics decreases, leading to evolution of the population

NATURAL SELECTION [D] — the process where **the environment or nature selects for** well-adapted individuals with **inherited traits that are best suited to the local environment**

Adaptation — an evolutionary modification that improves the chances of survival and reproductive success in a given environment

Species name : Homo sapiens

Evidence for Darwin's theory of evolution

1) Homologies & Divergent evolution

- homologous structures — show evidence of shared ancestry
- early embryonic development
- vestigial structures
- Molecular/biochemical homologies — similar nucleotide sequences in DNA/RNA and aa sequence in proteins

2) Fossil evidence — fossil records

- Progressive changes in the structures of organisms/increase in complexity in structures of fossilised organisms in younger rocks than older rocks

— Shows Descent with modification through changes in homologous structures due to environmental selection pressures

3) Biogeography

— Evidence to support evolutionary deductions based on homology:

— Closely related species sharing similar characteristics found in same environment

- Island biogeography
  - Most island species are closely realted to species from the nearest mainland or neighbouring island ; islands tend to have larger no. of endemic species
- Continent biogeography (analogies and convergent evolution)

—— Shows descent with modification from a common mainland ancestor

4) Direct observations of evolutionary change

Convergent evolution and analogy — where species from different evolutionary branches come to resemble each other if they have similar ecological roles and same environmental selection pressures which shaped similar adaptations

Natural selection [D]:

1. The process by which certain individuals that are better adapted to an environment survive to reproduce (i.e. differential survival and reproduction)
2. which increases the frequency of favourable alleles in the gene pool, and the resultant population becomes adapted to its particular environment

Genetic drift — the random change of allele and genotype frequencies, as a result of chance alone, can differ from generation in a small gene pool

Effects: 1) Genetic drift is significant in **small populations**

2) Genetic drift causes random change of **allele frequencies**

3) Genetic drift can lead to **loss of genetic variation within populations** and creates genetic divergence between populations

4) Can cause **harmful alleles to become fixed**

Explain how natural selection could have caused the relative allele frequency

① existance of (phenotypic) variation
② Who has selective advantage ⟶ natural selection due to (selection pressure)
③ ... survive to sexual maturity to produce VFO
④ survive to pass favourable alleles to offspring
⑤ ↑ allele freq. for C allele)

eg. 1. Mice w/ light fur are less easily seen by predators and are less at a selective advantage
2. Light fur mice survive to reproductive age and pass on favourable alleles
3. ↑ freq. of allele
4. 95% of population has allele C.

Species concepts:

Genetic species concept — A **genetically distinct** group of natural populations that share a common gene pool

Limitation: common gene pool and common karoytypes may change due to:

- directional selection
- interbreeding bwtn two different species

Biological species concept — SPECIES: a **population/group of populations whose members have the potential to interbreed in nature and produce viable, fertile offspring**

REPRODUCTIVE ISOLATING MEASURES

Advantages & limitations of the biological species concept

Advantage: The focus on reproductive barriers and how speciation occurs

Limitations:

1. No way to evaluate the reproductive viability of fossils
2. Does not apply to species that reproduce asexually / self-fertilising species
3. Designated as the absence of gene flow — does not account for the formation of rare hybrids between different species
4. Some invididuals of the same species rarely interbreed

Phylogenic species concept [D] — The smallest group of individuals that share a common ancestor

- as they descent from a common ancestor, they have a shared and unique evolutionary history
- Done by comparing the morphological characteristic/molecular sequences such as DNA sequences with those of other organisms

Advantages:

- ability to distinguish groups of individuals that are sufficiently different to be considered separate species
- Reveals the existence of “sibling species"

Limitations

- difficulty in determining the degree of difference required to determine separate species

4.1.4 MORPHOLOGICAL SPECIES CONCEPT (SELF-DIRECTED LEARNING)

Morphological species concept

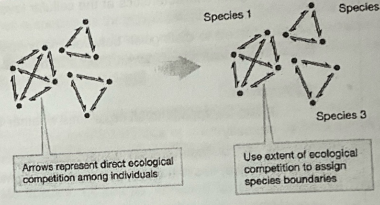
- It characterizes a species by **body shape and other structural features.** Organisms are classified as the same species if **their anatomical traits appear to be very similar.** Likewise, microorganisms can be classified according to morphological characteristics at the cellular level.
- We are forced to distinguish between many species in this way because there is **little or no information about their mating capabilities.**
- ***Advantages:***
  - It can be applied to all organisms whether they are reproducing asexually or sexually.
  - It can be useful even without information on the extent of gene flow. In practice, this is how scientists distinguish most species.
- ***Limitations:***
  - It may be difficult to decide how many morphological characters to consider when characterizing individuals.
  - It is difficult to analyze quantitative traits that vary in a continuous way among members of the same species. Researchers often disagree about how much morphological difference is necessary to separate different species.
  - Members of the same species sometimes look very different and conversely, members of different species sometimes look remarkably similar to each other. For example, **Figure 4.8(a)** shows two different frogs of the species *Dendrobates tinctorius,* commonly called the dyeing poison frog. This species exists as many different-coloured morphs, which are individuals of the same species that have noticeably dissimilar appearances. In another example, in **Figure 4.8(b)** shows two different species of frog, the Northern leopard frog *(Rana pipiens)* and the Southern leopard frog *(Rana utricularia)* look similar.

4.1.5 ECOLOGICAL SPECIES CONCEPT (SELF-DIRECTED LEARNING)

Ecological species concept

Ecological niche *is the unique set of habitat resources that a species requires, as well as its influence on the environment and other species. For example, two species of amphibians might be similar in appearance but differ in the foods they eat or in their ability to tolerate dry conditions. Within their own niche, members of a given species compete with one another for survival. If two organisms are very similar, their needs will overlap, which results in competition. Such competing organisms are likely to be of the same species.*

- It views a species **in terms of its ecological niche** that is within its native environment, focusing on **unique adaptations to particular roles in a biological community.**



- Species from different evolutionary branches may come to resemble one another if they have the same ecological niche – result of **convergent evolution.** Species which are distributed over a wide geographical range or have occupied well-separated geographical habitats for a long period of time may show considerable phenotypic differences – result of **divergent evolution.**
- ***Advantages:***
  - Unlike the biological species concept, the ecological species concept can accommodate asexually reproducing as well as sexually reproducing species.
  - According to this concept, species are formed because evolutionary mechanisms control how each type of species uses resources. This species concept is particularly useful in distinguishing between bacterial species that reproduce asexually. Bacterial cells of the same species are likely to use the same types of resources (sugars, vitamins, etc.) and grow under the same types of conditions (temperature, pH, etc.).
- ***Limitation:***
  - It does not take into account the species' morphology and reproductive

Mechanism of speciation

speciation — the evolution/origin of species; occurs whenever the inherited characteristics of a population or of a species change over a period of time

STAGE 1: Single ancestral population

STAGE 2: Barrier developes (geographical isolation & ecological isolation)

- members can still interbreed if brought together

STAGE 3: Differentiation due to different selection pressures

- Gene flow between geographically isolated populations is interrupted

- the different environment delivers **different selective pressures**, leading to the **genetic distinctiveness of each separate population. Due to natural selection and changes in the gene pool, each population becomes more adapted to its own environment**

STAGE 4: Barrier disappears (reproductive isolation)

- when different populations come into contact, whether they reform and become a single species or if they remain different species depends on the time where RIMs have sufficiently accumulated
- **when accumulation of sufficient reproductive isolating mechanisms (RIMs), adaptations and genetic diversity** forms, the two populations are **reproductively isolated/genetically incompatible and have become two different**

RIMs

(a) **PREZYGOTIC BARRIERS** ("before the zygote") **impede mating between species or hinder the fertilization** of eggs if members of different species attempt to mate. Such mechanisms are those that **prevent** formation of zygotes by blocking fertilization.

Prezygotic Mechanisms

Species 1 Species 2

Types of Prezygotic Barriers

- **HABITAT ISOLATION** (includes **geographic isolation**): Species may **occupy different habitats**, so they never come in contact with each other.
- **TEMPORAL ISOLATION**: Species have **different mating or flowering seasons or times of day, or become sexually mature at different times of the year**. Different flowering times in plants may mean that cross-pollination is impossible. These are both examples of seasonal isolation.
- **BEHAVIOURAL ISOLATION**: **Sexual attraction between males and females of different animal species is limited due to differences in behaviour or physiology.** Before copulation can take place, many animals undergo elaborate courtship behaviour. This behaviour is often stimulated by the colour and markings on members of the opposite sex, the call of a mate or particular actions of a partner. Small differences in any of these may prevent mating. The song of a bird or the call of a frog must be exact if it is to elicit the appropriate breeding response from the opposite sex. The timing of courtship behaviour and gamete production is also important. If the breeding season of two groups does not coincide, they cannot breed.

Attempted mating

- **PHYSIOLOGICAL ISOLATION**:
  - ***Mechanical isolation***: Morphological features such as size and incompatible genitalia may prevent two members of different species from interbreeding.
  - ***Gametic isolation***: Gametic transfer takes place, but the gametes fail to unite with each other. This can occur because the male and female gametes fail to attract, because they are unable to fuse, or because the male gametes are inviable in the female reproductive tract of another species. In plants, the pollen of one species usually cannot germinate a pollen tube after landing on plant tissue of another species to fertilize the egg cells of that species.

(b) If a sperm cell from one species does overcome prezygotic barriers and fertilizes an ovum from another species, **POSTZYGOTIC BARRIERS** ("after the zygote") often prevent the hybrid zygote from developing into a viable, fertile adult. Postzygotic barriers occur when prezygotic ones are overcome. They are mechanisms that create sterile hybrids.

Types of Postzygotic Barriers

- **PHYSIOLOGICAL ISOLATION**:
  - ***Hybrid inviability***: The egg of one species is fertilized by the sperm from another species, but the fertilized egg fails to develop past the early embryonic stages.
  - ***Hybrid sterility***: The interspecies hybrid survives, but it is sterile. For example, the mule, which is produced from a cross between a male donkey *(Equus asinus)* and a female horse *(Equus caballus)*, is sterile.

- **Hybrid breakdown**: The F_1 interspecies hybrid is viable and fertile, but succeeding generations (F_2, etc.) become increasingly inviable. This is usually due to the formation of less fit genotypes by genetic recombination.

Interspecies hybrid

Allopatric speciation

— where a new species if formed when one population becomes geopgraphically deparated from the rest of the species and evolves by natural selection and/or genetic drift

- any physical barrier can block gene flow
- Different selection pressures accentuate divergence caused by genetic drift

Sympatric speciation

— where a new species evolves within the same geographic region as the parental species OR geographically overlapping populations

- Can occur very rapidly and in a short time
  - POLYPLOIDY
    - Autopolyploidy — Non-disjunction of chromosomes lead to the formation of diploid gametes (4n) through meiosis. If self fertilisation occurs, fertile tetraploid offspring is produced [instantaneous speciation event]
    - Allopolyploidy — Fusion of haploid gametes from two different species results in sterile offspring being produced (due to odd number of chromosomes in each gamete). If subsequent errors (eg. non-disjunction) occurs to produce chromosomal duplications, a fertile tetraploid hybrid species can exist)
  - ECOLOGICAL ISOLATION
    - when different areas of the same landmass have different ecological niches (think an island with both a forest and a desert)

*However, if a population of allopolyploids becomes established, it can either :

1) be less well adapted compared to to parents and go extinct
2) Assume an ecological niche and co-exist w parental species
3) be more well adapted than parental species and replace it

Macro-evolution

— large-scale phenotypic changes in a population that result in a formation of a new species

Adaptive radiation

— evolutionary diversitifcation of many related species from one or a few ancestral species in a relatively short period of time

Due to

A) Ecological opportunities — availability of new or novel types of resources.

- As a colonising species will encounter no competitors, they can rapidly diversify which leads to efficient use of the available resources.
- Succeeding generations diversify into new species,leading to rapid speciation

B) Evolutionary novelties through morphological innovation

- Environment selects for members with a key morphological trait which allows descendants to exploit resources (usually from modifications of pre-existing structures)

Phylogeny

Biological classification — act of systematically arranging organisms into groups based on particular shared characteristics (mainly morphology)

Phylogeny — organisation of species according to particular characteristic which takes into consideration the evolutionary relationship between species

- Phylogenetic tree — visual representation of a phylogeny to illustrate lineages and their evolutionary relationships

Reconstructing phylogeny using molecular homologies:

- The greater the degree of homology / similarity in the primary sequences of macromolecules between two species, the more closely related the two species are considered to be
- Number of differences may reflect thow much time has passed since the groups branched/diverged from a common ancestor

Multiple Sequence alignment

align comparable sequences from the species being studied

- If they are closely related, they only differ at one/two sites
- If distantly related, different bases at many sites and may have different lenghts (as insertions and deletions accumulate over long periods of time)
- May not be aligned cleanly, making comparison difficult

Amino acid sequences

- Compare structure (aa sequence) of homologous proteins— variable residues

DNA extraction

↓

DNA sequencing

↓

multiple sequence alignment

↓

genetic diff :

> difference nucleotide seq, less closely related

< difference ---- ----, more closely related

+ Sanger Sequencing

(ii) State **one** reason why classification **Y** is a better representation of evolutionary relationships than classification **X**. [1]

any one from:

1 **genome sequence variation**, have a **clear genetic basis**, that is **easy to interpret ;**
2 All the fish **possess nucleic acids** hence, **molecular data can be collected for comparison ;**
3 **molecular evidence,** in the form of the genome sequences of the fishes is **unambiguous ;**
4 genome sequences are **precise, accurate and quantifiable,** which **facilitates the objective assessment of evolutionary relationships / open to statistical analysis ;**
5 fishes have **certain molecular traits in common** (such as ribosomal RNA sequences), offering **a valid basis for comparison among all organisms ;**
6 **morphological characteristics more subjective / ambiguous / may be due to convergent evolution ;**

ADVANTAGES OF MOLECULAR METHODS IN THE STUDY OF EVOLUTIONARY RELATIONSHIPS (SELF-DIRECTED LEARNING)

1. **Molecular data is genetic.** Anatomical, behavioural, and physiological traits often have a genetic basis, but the relationship between the underlying genes and the trait may be complex. Nucleic acid and amino acid sequence variation has a clear genetic basis that is easy to interpret.

2. **Molecular methods can be used with all organisms.** All living organisms possess nucleic acids and proteins, and so molecular data can be collected from any organism.

3. **Molecular methods can be applied to a huge amount of genetic variation.** An enormous amount of data can be accessed by molecular methods. The human genome, for example, contains more than 3 billion base pairs of DNA, which constitutes a large pool of information about our evolution.

4. **The molecular approach helps us to understand phylogenetic relationships that cannot be determined by non-molecular methods such as comparative anatomy.** All organisms have certain molecular traits in common, such as *ribosomal RNA sequences* and the amino acid sequences of some fundamental proteins. These molecules offer a valid basis for comparison among all organisms. For example, evolutionary relationships between angiosperms were traditionally assessed by comparing floral anatomy, whereas evolutionary relationships of bacteria were determined by their nutritional and staining properties. Due to the lack of such common structural characteristics between plants and bacteria, evaluating their relatedness was difficult in the past.

5. **Molecular data is quantifiable.** Nucleic acid and amino acid sequence data is precise, accurate, and easy to quantify, which facilitates the *objective assessment* of evolutionary relationships.

6. **Molecular data often provides information about the process of evolution.** For example, the study of DNA sequences revealed that one type of insecticide resistance in mosquitoes probably arose from a single mutation that subsequently spread throughout the world.

7. **The database of molecular information is large and growing.** The large database of DNA and amino acid sequences can be used for making comprehensive evolutionary comparisons between many groups of organisms.

8. **Molecular methods allow us to reconstruct phylogenies among groups of present-day prokaryotes and other microorganisms for which we have no fossil record at all.** Molecular biology has helped to extend systematics to evolutionary relationships far above and below the species level, ranging from the major branches of the tree of life to its finest branches. Still, the findings are often inconclusive, as in cases of several taxa diverging at nearly the same time in the distant past. The differences may be apparent, but not the order of their appearance.

9. **Different genes evolve at different rates, even in the same evolutionary lineage. As a result, molecular trees can represent short or long periods of time, depending on what genes are used.** For example, the DNA that codes for ribosomal RNA (rRNA) changes relatively slowly, and so comparisons of DNA sequences in these genes are useful for investigating relationships between taxa that diverged hundreds of millions of years ago. Studies of rRNA sequences indicate, for example, that fungi are more closely related to animals than to green plants. In contrast, mitochondrial DNA (mtDNA) evolves relatively rapidly and can be used to explore recent evolutionary events. One research team has traced the relationships among Native American groups through their mtDNA sequences. The molecular findings corroborate other evidence that the Pima of Arizona, the Maya of Mexico, and the Yanomami of Venezuela are closely related, probably descending from the first of three waves of immigrants that crossed the Bering land bridge from Asia to the Americas about *13,000* years ago.

"suggest how knowledge of genetic diversity ....."

Evidence used to establish phylogenetic relationships:

1. Fossil record
2. Amino acid sequences
3. DNA base sequences
4. behavioral traits
5. morphological traits

Why less? :

1. The energy in ethanol is permanently unavailable to yeast as 1 carbon has been lost as CO_2 and cannot be regen to pyruvate (inefficient)
2. Lactic acid can be reconverted to pyruvate for use in the Krebs cycle

Genetics of Viruses

I(f) Discuss how viruses challenges the cell theory and concepts of what is considered living

Viruses lack enzymes for most metabolic processes, as well as machinery for protein synthesis.

- Depends on host cells for a.a. and nucleotides, ribosomes, ATP

Arguments for Viruses being living organisms

1. Viruses can reproduce (can only reproduce in the intracellular state)
2. viruses are able to direct metabolic processes (direct them when existing in a virus state — intracellular)
3. Viral genomes can evolve
   - Viruses vary greatly in their structural and genetic complexity
   - Viruses evolve with their host and acquire their metabolic and translational genes from the host cells (genetic recombination)

Arguments for viruses being non-living organisms

1. Viruses are not cells (no protoplasm or organelles)
2. Viruses lack some of the characteristsic of living organisms
   - unable to carry out metabolic processes
   - do not require nutrition + unable to grow nor excrete
   - unable to synth own ATP
   - unable to respond to stimuli

How do viruses challenge the cell theory

Cells are the smallest unit of life

- Viruses lack the necessary moleuclar machinery; however they contain the genetic material to reproduce and can evolve

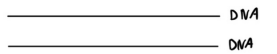
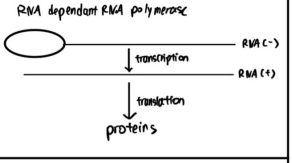
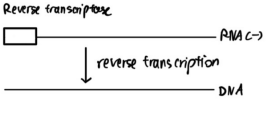
All cells come from pre-existing cells

- viruses rely on host cells to provide energy and materials needed to replicate genome + synthesis proteins — cannot replicate until they have entered a host cell

All living organisms are composed of cells

- viruses are acellular and do not have protoplasm or organelles (metabolically inert)

1 (e) Describe the structural components of viruses, including enveloped viruses and bacteriophages, and interpret drawings and photographs of them

Group	Type of virus	Illustration of viral genetic material
I	Double-stranded DNA viruses eg. T4 and lambda phages	
V	(-) Sense single-stranded RNA viruses Genome must be **converted to (+) sense RNA by RNA-dependant RNA polymerase** before translation eg. Influenza viruses	
VI	Single-stranded RNA -Reverse transcriptase (RT) viruses Makes use of **reverse transcriptase** which is an **RNA-dependant DNA polymerase, to produce DNA from the initial viral RNA genome** eg. HIV	

Parts of a virus

Regulatory proteins ⟶ regulate action of host genes
Structural proteins ⟶ eg. capsid protein

Capsid ⟶ protein coat which encloses viral genome

Envelope ⟶ • derived from host cell by budding, contains host cell surface membrane (phospholipid bilayer + viral proteins)

Enzymes: Lysozyme ⟶ makes hole in bacterial cell wall that allows for viral nucleic acid to enter. + case host cell to lyse and release virus

Neuraminidase ⟶ breaks glycosidic bonds + glycolipidids, aids in liberation of virus

General reproductive cycle of viruses

1. adsorption (attaches to host cell by specific binding of its glycoproteins to host cell receptors
2. penetration
3. Synthesis and replication (of viral proteins and replication of viral nucleic acid)
4. Assembly
5. Release

Bacteriophages : lytic & lysogenic cycle (lambda, T4 phage)

T4 phage (virulent phage)

1. Genome : linear double-stranded DNA
2. Capsid : capsomeres surrounds nucleic acid, contained in head of the phage
3. Head containing DNA of the virus

   Tail
   - consisting of tail sheath, multiple tail fibres and a base plate

   Tail fibres
   - allows phage to adsorb onto surface of the bacterial cell by binding to specific receptor site found on cell surface
   - Enables base plate to come into contact with the surface of the cell; triggers conformal change such that the central tube is pushed through the bacterial wall
4. Base plate
   - comes into contact with the host cell surface and undergoes a conformal change to allow DNA to be extruded from the head, through the central tube and into the host cell

Reproductive cycle — lytic cycle — virulent phage

Stage 1 : Adsorption

- multiple tail fibres of the T4 phage attach to specific receptor sites on the surface of a bacterial host cell such as E. coli
- base plate settles on host cell surface

Step 2 : Penetration

- conformal changes occur in the tail, causing it to contract and tube pierces the bacterial cell wall and cell membrane
- T4 uses lysozymes to hydrolyse peptidoglycan
- DNA is extruded from the head, through the tail tube into the host cell
- capsid is left on the outside of the bacterial cell wall

Step 3 : Synthesis and replication

- after DNA is injected, synthesis of host DNA, RNA and proteins is halted. Host machinery is taken over by virus for:

(A) T4 phage DNA is replicated by host DNA polymerase

- host DNA is degraded into nucleotides, providing raw materials for phage DNA replication

(B) T4 phage mRNAs are synth by host RNA polymerase via transcription

- phage mRNAs are synthesised by host cell ribosomes, tRNAs and translation factors into viral proteins and enzymes

Step 4 : Assembly

- Viral proteins are assembled to form phage heads, tails and tail fibres, diff components are assembled into complete bacteriophage

Stage 5 : Release

- T4 phage lyse the host cell through lysozyme, which digests bacterial cell wall
- Water enters the cell by osmosis, causing the cell to swell and burst

Lambda phage — temperate phage

1. linear double-stranded DNA
2. Capsid : Capsomeres surrounds the nucleic acid, contained in the head of the phage
3. Head contains the DNA of the virus
   - the 5'-terminus of each DNA strand is a single-stranded tail of 12 nucleotides long; important in prophage formation
4. A single tail fibre enables phage to adsorb on to the surface of the bacterial cell by binding to specific recpetor site on cell surface

Reproductive cycle — lambda cycle — termperate phage

Step 1 : Adsorption

- single tail fibres attach to specific receptor sites on the surface of the bacterial host cell
- base plate settles down on host cell surface

Step 2 : Penetration

- DNA extruded from the head, through the tail tube and injected into the host cell passing through both the bacterial cell wall and cell membrane,
- Capsid is left on the outside of the bacterial cell wall

Step 2A : *Prophage formation*

- lambda phage DNA circularises and inserts itself into a specific site on the bacterial chromosome (prophage insertion site) — genetic recombination; does not cause the loss of host DNA
- viral DNA is replicated along with chromosomes each time the host cell divides, and is passed on to generations of host daughter cells
  - a single infected host cell can give rise to a large population of bacteria carrying viral DNA in prophage form

Step 2B

- when there is an environmental trigger, viruses switch from lysogenic to lytic cycle
- causes lambda phage genome to be excised from bacterial chromosome and give rise to new active phages

Influenza virus

Genome : eight different segments of negative sense ssRNA

(-) sense strand RNA must be converted to complementary (+) sense RNA before being used for translation of viral proteins

Capsid : nucleoprotein (NP) associated with with the viral nucleic acid to form nucleocapsid

Viral envelope : phospholipid bilayer obtained from host upon budding

Surface Glycoproteins:

- Haemmagglutin (HA) - HA binds to sialic acid containing receptors
  - attaches virus to host cell membrane
- Neuraminidase (NA) - hydrolyses muscus allowing virus to enter cells of the respiratory tract
  - facilitates budding by cleaving sialic acid containing receptors
- Protein envelope - matrix proteins forms second layer of envelope, enclosing the nucleocapsid
- - M1 - monomers of matrix protein
  - M2 - acts as ion channel to lower/maintain pH of endosome in host cell
- Enzymes - PB1, PB2, PA. To form RNA-dependant RNA polymerase (replicase)

NS1 - regulates viral replication mechanisms and cellular signalling pathways

Reproductive cycle of influenza virus

Step 1 : Adsorption

- Haemmaglutinin (HA) molecules on viral membrane bind to sialic-acidic containing receptors on host cell membrane

Step 2 : Penetration

- virus taken in by receptor mediated endocytosis, forming an endosome
- fusion of endosome with acidic lysosome lowers pH of vesicle, triggering conformal changes in the HA protein which causes viral envelope and endosome membranes to fuse, releasing the eight viral segments of the influenza genome into host cell cytoplasm

Step 3 : synthesis of viral components

- Viral replicase (RNA-dependant RNA polymerase) copies (-) sense RNA template into (+) sense RNAs used for:

(A) Viral nucleic Acid syth

- +ve sense RNAs used as templates for synth of full-lenght (-) sense strand viral RNA by viral replicase
- new (-) sense viral RNAs can be packaged into new viral particles

(B) Viral protein synthesis

- (+) sense RNAs used as mRNAs which are translated in cytoplasm by host cell synth machinery
- viral transmembrane proteins synth by host cell machinery are incorporated into the host cell membrane via vesicle which fuses with host cell membrane

Step 4 : assembly of new virion

- assembly is complete when eight (-) sense viral RNAs associate with NP and enzymes eg. viral replicase are packaged
- glycoprotein studded membranes are acquired during release of virus

Step 5 : Release

- virus is released from host cell by budding, acquiring lipid bilayer containing HA, NA and M2
- HA on viral envelope and sialic-acid containing cellular receptors results in new viral particle being attached to the host cell
- NA cleaves sialic acid residues on cellular receptor that binds newly formed virions to the cell

*host cell lyses when phospholipid bilayer is depleted through excess budding

peaks @ 48 hrs

HIV

Genome : two identical ssRNA ;

- ssRNA is converted to DNA for integration into host genome.
- it is then used for transcription of viral mRNA which is translated into viral proteins

Capsid : capsid surrounds nucleic acid

Viral envelope : phospholipid bilayer obtained from host upon budding

Surface glycoproteins : gp120 - binds to CD4 receptors on macrophages and T helper cells

gp41 - aids in fusion of the HIV envelope and the host cell membrane

Protein coat : matrix protein forms the 2nd layer of the protein envelope, enclosing the capsid

Enzymes :

- reverse transcriptase - 2 molecules each associate with 1 RNA molecule to reverse transcribe viral RNA into DNA
- Integrase - facilitates integration of dsDNA into host cell's genome
- Protease - cleaves viral polypeptide into functional proteins during viral maturation

Reproductive cycle of HIV

Step 1 : adsorption

- glycoprotein gp 120 on HIV binds to CD4 receptor on T helper cells

Step 2 : Penetration

- upon binding to CD4, gp 120 undergoes a conformal change, allowing it to bind to a co-receptor (CXCCR4) on the surface of T helper cells (and CCR5 on macrophages)
- gp41 pulls virus closer to the host cell. co-receptor (CXCCR4 / CCR5) facilitates entry of gp120-CD4 complex through host cell membrane
- HIV envelop fuses with host cell membrane, releasing viral contents into cell

Step 3 : Synthesis of viral components

- reverse transcribes viral RNA into complementary DNA strand. RNA strand is broken down, and the remaining DNA is replicated to produce dsDNA
- DNA passes through nuclear pore and enters the nucleus
- integrase catalyses the integration of viral DNA into the genetic material of the host
- newly integrated DNA is a provirus — (latent phase)

When stimulated by an immune response :

(A) nucleic acid synthesis

- when host cell recieves a signal, proviral DNA is transcribed by host RNA polymerase into new viral RNA

(B) Viral protein synthesis

- proviral DNA is transcribed into viral mRNA, which is translated into long chain of HIV proteins which is later cleaved
- viral surface glycoproteins are incorporated into the host cell membrane via vesicles

Step 4 : Assembly of new virions

- copies of HIV proteins and viral RNA genome assemble near the host cell membrane
- assembly occurs when 2 ssRNA molecules associated with reverse transcriptase and enzymes (eg. integrate and proteins) are surrounded by assembled capsid

Step 5 : Release

- acquision of glycoprotein studded membrane envelope occurs during release of virus
- immature HIV buds off
- HIV protease cleaves single long chain of HIV proteins into smaller functional proteins, forming a mature HIV particle

Pathogenesis of HIV (LO)

- Infected macrophages lose their ability to ingest and kill foreign microbes
- HIV attacks Th cells w primary CD4 receptor and macrophages and dendritic cells (requires the presence of a co-receptor CCR5 or CXCR4)
- Reverse transcriptase allows for ssRNA to be generated, and intergrase facilitates the integration of viral DNA into host chromosome
- HIV replicates predominantly in activated T cells
- HIV can establish latent infection in T cells and remain invisible to cytotoxic T cells
- Viral particles bud off an infected cell over time

Development of symptoms

- Cell mediated immunity is lost, body becomes susceptible to opportunistic bacteria

I. Acute phase (Primary HIV infection)

- within 2 - 4 weeks : flu-like symptoms (eg. fever, swollen glands, sore throat etc
- active replication to infect as many Th cells, virus particles in bloodstream is the highest
- Depletion of Th cell population (through excessive budding)
- Depletion to viral set point, after set point Th cells begins to increase
- High risk of transmitting HIV due to high concentration in blood stream

2. Chronic phase (Clinical latency) — asymptomatic HIV infection

- integration of viral DNA into host genome allows infected Th to evade immune system
- HIV virus continues to reproduce at very low levels
- Macrophages act as major reservoirs for the virus
- Eventually, viral load will begin to rise and Th cell count declines

3. Crisis phase (AIDs, symptomatic)

- immune system badly damages, vulnerable to opportunistic bacteria
- Destruction of WBC population leads to immune-compromised / state of immune deficiency
- HIV is considered to progress to AIDs when Th cells falls below 200 cells / cubic millimetre of blood

4. Transmission to other organisms

- asymptomatic host with latent phase passes virus to others through sex or blood transfusions

Mechanism for variation in viral genome:

a) mutation — no proofreading mechanisms (of RNA) in host cell causes RNA viruses to experience a much higher rate of mutations + reverse transcriptase has very low fidelity, causes antigenic drift

b) Recombination — viruses undergo recombination with genome of another strain, resulting in a new combination of alleles

c) Reassortment — host cell may be infected with two viral strains — introduces two sets of genetic material into host genome. During formation, this results in different segments of viral genome being packaged into progeny virus. Reasults in sudden, drastic change in viral genome (antigenic shift)

Antigenic shift — a sudden change in the antigenicity of a virus owning to reassortment of the segmented virus genome with another genome of a different antigentic type

- occurs due to the reassortment of RNA from different strains (eg. new combinations of NA and HA)

Antigenic drift — the gradual accumulation of minor mutations in the genes of influenza that results in altered antigenicity (small changes which produce viruses which are closely related to each other and usually share the same antigenic properties)

- RNA strands lack a complementary strand, polymerases cannot perform proofreading
- viral polymerases are also prone to errors and will introduce mutations during replication
- results in production of surface proteins w different 3D conformations

Antigenic shift: sudden & drastic change in the antigenicity of a virus due to a reassortment of a viral genome with another genome of different antigenic type
→ causes diff translation & transcription products of HA / NA

Antigenic drift: Gradual accumulation of minor mutations that results in altered antigenicity

...resulting in only a

Table showing differences between antigenic shift and antigenic drift

	Antigenic Shift	Antigenic Drift
Number of viruses	**Two or more** viral strains are involved	Only **one** viral strain is involved
Mechanism for change	**Reshuffling of genome** between different strains Results in **dramatic alternation** of type of hemagglutinin or neuraminidase on progeny virus	**Accumulation of point mutations** in the gene of the surface antigen Results in **minor alteration** of 3D conformation of hemagglutinin or neuraminidase on progeny virus
Nature of change	Abrupt and **major change** in genome of virus	Gradual accumulation of **minor point-mutations** in genome of virus
Rate of occurrence	**Occasionally occurs** to give rise to pandemics	**Regularly occurring** to give rise to seasonal epidemics
Effect of host immunity	Population has **no immunity** to novel combination of surface proteins No drugs or vaccines present to treat virus	A proportion of the population may still have **pre-existing immunity** to the modified surface proteins Anti-viral drugs and seasonal vaccines available to treat virus
Cross-species transmission	May result in a progeny virus which can **infect a new species**	Virus only **infects individuals** of the **same species**

Table 1: Differences between antigenic shift and antigenic drift.

Genetics of Bacteria

Cell ultra structure

All bacterial cells are prokaryotic and lack a true nucleus and membrane-bound organelles

Cell surface structures

- (peptidoglycan) cell wall : glycan comprises of a linear polymer of alternating monosaccharide subunits — N-acetylglucosamine and N-acetylmuramic acid
  - "peptido" portion is a short string of a.a. that serves to cross-link adjacent polysaccharide strands, forming network with high tensile strength

Gram-positive bacteria :

- have thick, multi-layered peptidoglycan cell walls that are exterior to the membrane
- teichoic acids are major cell surface antigens
- retain crystal violet dye

Gram-negative bacteria :

- two membranes — outer membrane and inner (cytoplasmic) membrane.
  - peptidoglycan layer is located between the two membranes (periplasmic space)
- peptidoglycan layer is thin
- outer membrane has presence of various embedded lipopolysaccarides
  - polysaccaride portion is antigenic
  - lipid portion is toxic to humans and animals
- must be counterstained with red dye safranin

3. Cell membrane 4. Flagellum 5. Pilus

6. Ribosomes

Prokaryotic genome

- single, circular, dsDNA that contains essential genes
- DNA is associated with +vely charged histone-like proteins that aid in supercoiling
- Genes are grouped into operons where multiple genes come under the control of the same promoter, and same regulatory elements
- Prokaryotic genes lack introns (due to no nucleus, no clear separation btwn transcription and translation)

Plasmids : small, circular ds extrachromosomal DNA

- contains beneficial genes which confer protective traits such as antibiotic resistance, toxin synthesis and enzyme production

Binary fission — transmisison of genetic material from parent to offspring

1. Bacterial chromosome attached to plasma membrane before DNA replication
2. DNA replication begins are single origin of replication, replication bubble is formed and two DNA strands separate.
   - each used as template for synthesis of daughter strand through semi-conservative DNA replication. Replication bubble grows bi-directionally
3. each circular DNA is attached to cell membrane as cell grows
4. cell elongates, causing two chromosomes to be moved apart
5. septal ring directs assembly of septum
   - extends as cell membrane grows, adding peptidoglycan
6. forms new septum, split cell by cytokinesis, gives rise to two genetically identical daughter cells

- no genetic variation created

Horizontal gene transfer (genetic recombination)

Transformation : recipient cell uptakes naked DNA

- donor bacterial cell lyses and releases DNA into surrounding environment
- only competent bacterial cells (w competence factors) can uptake DNA

☆ requires competency factors ⇒ proteins which can bind to naked DNA

1. Donor bacterial cell lyses and released naked DNA fragments (donor DNA)
2. competent recipient cell takes up DNA fragments via competence factor
3. Homologous recombination
4. homologous segment is incorporated into the recipient cell's chromosome to form a recombinant cell

can be induced artificially through

- heat shock
- electroporation

Generalised Transduction

during the reproduction of virulent phages (lytic cycle), new virions may contain a random fragment of the bacterial genome

- occurs due to accidental incorporation of a random fragment of DNA from its first host cell
- when defective phage infects second host cell, donor genes are integrated into the recipient cell's genome by homologous recombination

generalised transduction — as each portion of the bacterial genome has the same probability of being transferred

1. virulent phage injects its DNA into its first host cell, degrading host cell genome
2. phage uses host DNA replication machinery to synth more phage DNA, and host gene expression machinery to sytnh more phage proteins
3. occasionally, piece of first host's degraded DNA is accidentally packaged within phage capsid during assembly of the lytic cycle (defective phage). Defective phages are released into env when bacterium is lysed
4. defective phage contains first host cell's DNA fragments may infect a second host cell
5. Donor DNA is incorporated into second host cell"s genome by homologous recombination (recombinnt cell)

Specialised Transduction (Temperate phage)

Only genes near the prophage insertion site on the host (donor chromosome have a high probability of being transferred

1. genome of the temperate phage integrates into chromosome @ prophage insertion site
2. Upon induction, phage genome is excised from host cell chromosome. Phage DNA sometimes takes a small region of the bacterial DNA that was adjacent to the prophage insertion site
3. new phages contain part of the first host cell"s DNA
4. host bacterium is lysed, releasing phages.
5. donor DNA Is incorporated into the second cell's genome

(i) Prophage insertion (if DNA contains genes required to enter lysogenic cycle

(ii) homologous recombination (if segment of phage DNA does not contain genes rep to enter lysogenic cycle

Conjugation:

1. F+ donor cell uses a sex pilus to attach to F- recipient cell (direct contact)
2. temp. cytoplasmic mating bridge formed
3. sugar-phosphate backbone of one strand of the F plasmid is nicked by an endonuclease. ssDNA moves to F- recipient cell through the cytoplasmic DNA
4. Each parental strand becomes a template for the DNA sysnthesis of a complementary daughter strand by semi-conservative replication. DNA ligase catalyses synthesis of a phosphodiester bond to close gap
5. Cells move apart and sex pilus breaks, forming two bacterial cells that are both F+

3 differences between prokaryotes and eukaryotes:

1. Degree of compaction of chromosomes — prokaryote chromosome less compact compared to eukaryote chromosomes
2. presence of operons — prokaryotic genes are grouped into a cluster under the control of one promoter while eukaryotic genes have a promotor for each gene
3. Presence of nuclear membrane — absent in eukaryotes

*State two ways in which a plasmid such as the F plasmid differs from the bacterial chromosome

- The F plasmid contains genes which are selectively useful, while the bacterial chromosome contains genes essential for the bacterium's survival
- The F plasmid is much smaller than the bacterial chromosome

Structure	Eukaryotes	Prokaryotes
Promoter structure	contains TATA box — binding site for general transcription factor(eg. TFIID) — facilitates binding of RNA polymerase into a transcription initiation complex and transcription start site	RNA polymerase recognition site (-35 bp recognition sequence) + Pribnow box(RNA polymerase binding site) — (35bp and 10 bp upstream) -
Coding region	Presence of introns and exons — introns are excised by exonucleases	Not interrupted by introns
5' - UTR	after transcription, 5'-methylguanosine cap is added on mRNA	When transcribed, gives rise to the Shine-Dalgarno sequence required for ribosome binding
3'- UTR	after transcription, enzyme catalysed addition of the 3'- poly a tail on mRNA	sequences immediately following the stop codon + terminator sequence to dislodge the RNA polymerase from the template DNA
Stage 1: initiation	GIFs assembled along the promoter + TFIID binds to TATA box (forms a TIC) RNA poly. binding causes DNA double helix to unwind and 2 strands to separate - hydrogen bonds disrupted - transcription bubble created Forms phosphodiester bonds between ribonucleotides	RNA polymerase binds to the promoter in the presence of a <u>sigma factor @ -35bp recognition site</u> RNA transitently unwinds DNA to form a transcription bubble
Stage 2: elongation	RNA poly. reads the DNA template strand in a 3' to 5', RNA poly moves down the template strand RNA added in the 5' to 3' direction ssmRNA formed, DNA upstream are re-wound (& reannealed) Euk. RNA poly have proofreading abilities, pro. RNA poly do not	
Stage 3: termination	Transcription continues until after the RNA poly. transcribes a termination sequence(polyadenylation signal sequence) transcription continues until	occurs when core RNA polymerase dissociates from the template DNA (not usually tested) a. Intrinsic termination(rho-inde

	around 10 to 35bp downstream of the polyadenylation sequence. - cleavage site triggers the release of the RNA and dissociation of the RNA polymerase from the DNA - cleavage site is also site of addition of poly A tail	pendent transcription termination) i. utilises a terminator sequence (GC-rich followed by 4 or more U residues) ii. GC rich region forms a hairpin loop structure via complementary base pairing — causes dissociation) b. rho-dependant termination i. uses a termination factor (rho factor) which binds at rho recognition site and moves along the mRNA towards RNA poly ii. When p factor reaches the RNA poly, it destabilised the mRNA-DNA hybrid

Operon

1. Promoter for structural genes — provides a site for RNA polymerase to bind to and initiate transcription
2. Operator — regulates the rate of transcription of the structural gene by binding to repressor protein
3. Structural genes — regulatory genes that lie outside of the transcription unit formed by the operon ; codes for a protein/RNA molecule that forms part of a cellular structure or have an enzymatic function

structural gene ⟶ gene coding for a pp that is a fxnal cellular protein

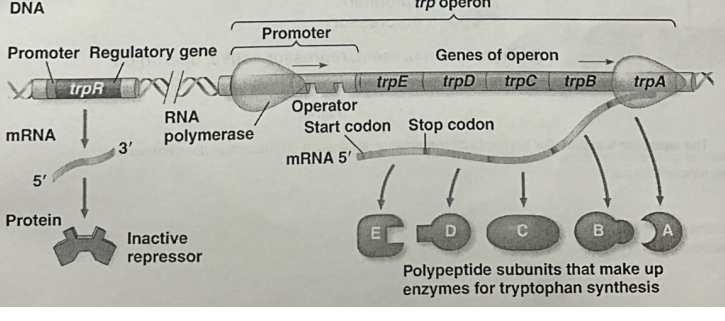
regulatory gene ⟶ gene coding for a pp which functions as a regulatory protein

trp operon (repressible operon)

The *trp* operon is under the control of a **repressor protein** coded for by the *trp R* gene.

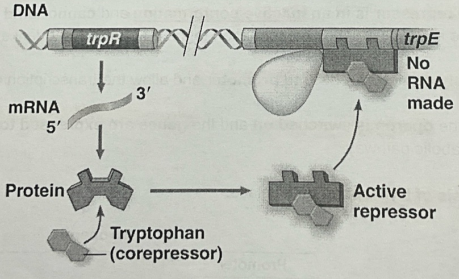
(a) In the **absence** of tryptophan (**Fig. 45**), → anabolic pathway

- The ***trp* repressor is in an inactive conformation** and **cannot bind** to the **operator** as it does not have a complementary conformation to the *trp* operator sequence.
- **RNA polymerase can bind to promoter** and allow the transcription of *trp* operon genes.
- Hence the **operon is switched on** and the **genes are expressed to give enzymes** involved in the metabolic pathway.
- Function ': **Synthesis of tryptophan** takes place.



(b) In the **presence** of excess tryptophan (**Fig. 46**),

- Tryptophan functions as a **corepressor**.
- It **binds to the *trp* repressor**, changing it to **its active conformation** which can now **bind** to the **operator** as it is complementary in conformation to the *trp* operator sequence.
- **RNA polymerase is blocked from binding to the promoter** and **transcription cannot take place.**
- Hence the **operon is switched off** and the **genes are not expressed to give enzymes** involved in the metabolic pathway.
- **No synthesis** of tryptophan takes place.



(b) Tryptophan present, repressor active, operon off

Iac Operon (Inducible)

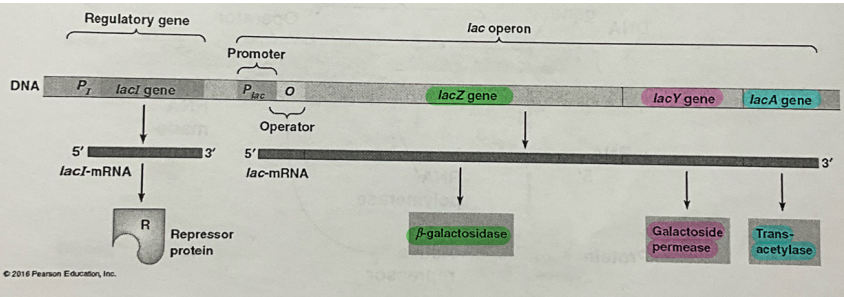


Fig. 47: The *lac* operon, an example of an operon in *E. coli*

Two of the products of the *lac* operon, galactoside permease and β-galactosidase, allow the bacterium to take up lactose and metabolize it to give the component monosaccharides glucose & galactose respectively. The function of the third operon product (transacetylase) in lactose metabolism remains unclear.

The lac operon is under **dual control – negative control by the *lac* repressor** (influenced by presence / absence of lactose) and **positive control by the catabolite activator protein (CAP)** (influenced by presence / absence of glucose which will be discussed in the next sub-sections.

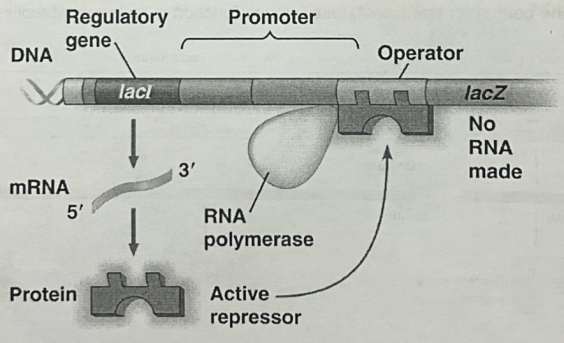
dual control
(−) Iac repressor → presence/absence of lactose
(+) activator (CAP) → presence/absence of glucose

Fully operated by presence of lactose + absence of glucose

* catabolic pathway

(a) In the **absence** of lactose (**Fig. 48**), (Negative Regulation)

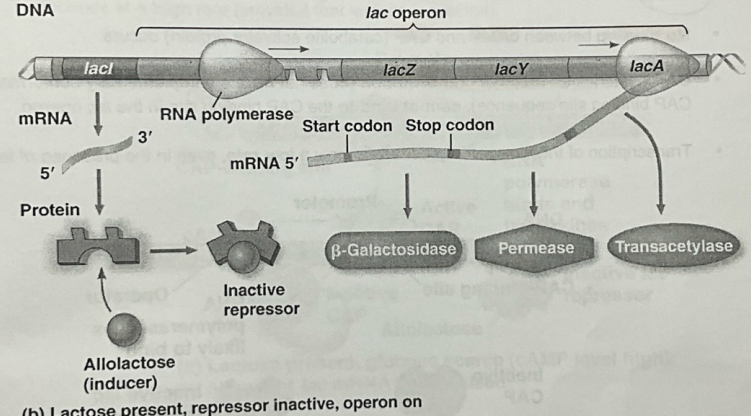
- The *lac* repressor protein is in an **active conformation** and **binds** to the **operator sequence** of the **operon** as it is complementary in conformation to the *lac* operator sequence.
- **RNA polymerase is prevented from binding to the promoter** and transcription cannot take place.
- Hence the **operon is switched off** and **hydrolysis of lactose cannot occur** as **β-galactosidase is not produced.**



(a) Lactose absent, repressor active, operon off

(b) In the **presence** of lactose (**Fig. 49**),

- **Allolactose**, an isomer of lactose, is formed in small amounts from lactose that enters the cell. It acts as an **inducer** by **binding to the *lac* repressor**, switching it to its **inactive conformation**. The **inactive repressor cannot bind to the operator** as it does not have a complementary conformation to the *lac* operator sequence.
- **RNA polymerase can bind to promoter** and **allow transcription of *lac* operon genes.**
- Hence the **operon is switched on and hydrolysis of lactose occurs.**



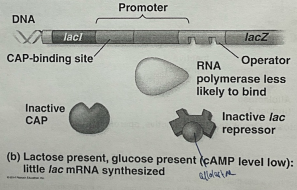
(b) Lactose present, repressor inactive, operon on

3.4.3.2 Positive Regulation of *lac* Operon – Catabolite Activator Protein

Bacteria preferentially utilize glucose over lactose, and will not utilize lactose for energy production until all glucose in the environment is completely used up. Hence, bacterial cells would only require the enzymes for lactose breakdown if **glucose is absent, in addition to lactose being present**. This mechanism is known as **catabolite repression**. The positive regulation mechanism utilizes the **catabolite activator protein (CAP)** to greatly **increase production** of β-galactosidase in the absence of glucose.

(a) In the **presence** of glucose (**Fig. 50**),

- ↓ **cAMP** (cyclic adenosine monophosphate, a signal molecule) **concentration falls**.
- **No binding** between **cAMP** and **CAP** (catabolite activator protein) occurs.
- **CAP** assumes an **inactive conformation** (does not have a complementary conformation to the CAP binding site sequence), **cannot bind to** the CAP binding site in the *lac* operon.
- Transcription of the *lac* operon proceeds at only a **low rate**, even in the presence of lactose.

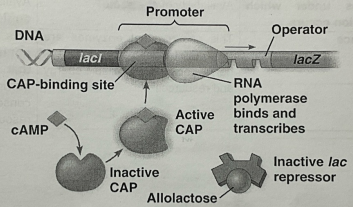


(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

Fig. 50

(b) In the **absence** of glucose (**Fig. 51**),

- **cAMP** (cyclic adenosine monophosphate, a signal molecule) **concentration increases.**
- **Binding** occurs between **cAMP** and **CAP** (catabolite activator protein).
- **CAP** assumes an **active conformation**, **binding to CAP binding site** (a short DNA sequence **upstream of the promoter**) as it is complementary in conformation to the *lac* operator sequence.
- **Binding of RNA polymerase** to promoter is **enhanced** and transcription of the lac operon proceeds at a **high** rate (provided that lactose is present).



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

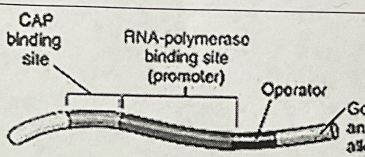
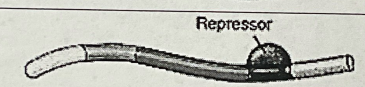
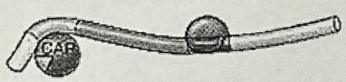
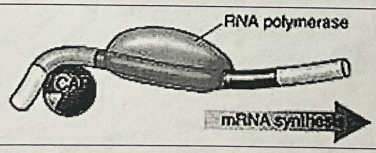
3.4.4 Comparison of Inducible and Repressible Operons

	Inducible Operon	Repressible Operon
Type of metabolic pathway	Catabolic	Anabolic
Usual condition of operon	Switched off	Switched on
Example	*lac* operon	*trp* operon
Usual state of repressor protein	**Active** conformation → **binds** to operator → **prevents RNA polymerase** from **binding to promoter**	**Inactive** conformation → **does not bind** to operator → **RNA polymerase can bind** to **promoter**
Molecule bound to repressor protein	**Inducer** (allolactose in the case of *lac* operon)	**Co-repressor** (tryptophan in the case of *trp* operon)
Conditions under which transcription occurs	Availability of **substrate**	Availability of **end product in sufficient quantity**
Significance	This ensures that **enzymes are only synthesized when needed,** hence conserving available energy and resources	This ensures that **resources are not used to synthesize products that are already present in sufficient quantity,** hence conserving available energy and resources

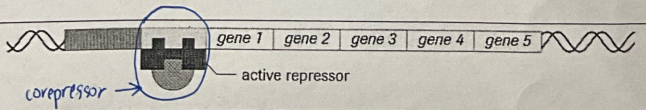
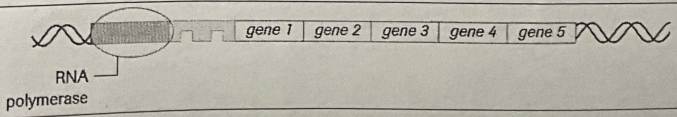
Concept Check 2

Complete the tables by filling **(+/ –)** in the correct boxes accordingly.

***lac* Operon**

Glucose	CAP binding	Lactose	Lac repressor binding		Transcription of operon
✓	×	✓	×		no
✓	×	×	✓		no
×	✓	×	✓		no
×	✓	✓	×		yes

***trp* Operon** repressible, C→control

Tryptophan	Trp repressor binding		Transcription of operon
+	+		no
−	−		yes

ETA: Infectious diseases

major fns of immune cells
1. recognises and removes abnormal 'self' cells
2. removes dead/damaged cells and old RBCs
3. protects the body from disease-causing pathogens

Failure to perform normal fns:
1. Incorrect responses (eg. Type I diabetes mellitus Cancer immune disorders)
2. Overactive response (eg. Allergies)
3. lack of response (eg. AIDS)

Innate immunity

① Non-specific external barriers
- limits the entry of microorganisms into the body

a) Physical – epidermis, mucol epithelium, cilia in respiratory tag
b) Chemical & env. – pH, microcidal action of secreted molecules
eg. antimicrobial peptides, lysozymes, RNases, DNases
c) Biological — commensal microbes

② Innate inflammatory response
- blocks microbial invasions, destroys microbes, controls & eradicates infections
- always present in healthy individuals.
- combats microbes immediately.
- instructs adaptive immune system to respond to diff microbes + clear damaged tissues
- always immediately available → lasting immunity
- phylogenetically older

Functions of innate immunity cells

Phagocytes : recruited to sites of infection, recog. & ingest microbes for intracellular killing

Neutrophils → motile phagocytic leukocytes which ingest, kill and digest microbial pathogens

monocytes & macrophages
- During inflammatory rxns, monocytes enter extravascular tissues and differentiate into macrophages
- Actively sample environment by phagocytes, scavengers to remove extracellular debris

Dendritic cells →
- act as APCs to activate T_H, T_c and Bcells by presenting antigens to adaptive lymphocyte populations
- actively engulf cells & particles by phagocytosis
  - ↳ bridge btwn innate & adaptive immunity

Defensive proteins:

a) cytokines → secreted proteins that function as mediators of immune & inflammatory reactions.

b) complement system
1: destroys pathogen through punching holes in cell membrane
2: enhance phagocytosis — attracts macrophages & neutrophils to site
3: stimulation of inflammation — causes vasodialation

APCs : B cells, dendritic cells, macrophages

Inflammatory response

- tissue reaction that delivers mediators of host defense, circulating cells & proteins
- causes: a) ↑ in local blood flow
  b) exudation of plasma proteins
  c) triggering of nerve endings

② Phagocytosis

③ Fever ⟶ combats infection by:

a. ↑ activity of phagocytic leukocytes while ↓ bacterial reproduction
b. iron deficiency hampers bacterial multiplication
c. causes adaptive immune cells to multiply more rapidly
d. stimulates virus infected cells to produce interferon, travels to other cells & ↑ resistance to viral attack.
e. stim NK cells that destroy virus infected body cells.

Adaptive immunity

Antibodies ⟶ bind specifically to toxins to neutralize & eliminate microbes & toxins

stops microbes from gaining access to & colonizing host cells & tissue
prevents infections from being established.

B cells:

a) small no. of B cells w/ receptors complementary to antigen are stimulated to divide by mitosis **clonal selection**

b) small clone of cells divides repeatedly by mitosis to produce large no. of identical B cells **clonal expansion**

c) some activated B cells become plasma cells. **Monoclonal**: as all antibodies produced have the same antigenic specificity

d) other B cells become memory cells → remain circulating in body for long time

T-lymphocytes: only recog. peptide fragments of protein antigens that are bound to specialized peptide display MHC mol. on APCs.

(HIV) effector $CD4^+$ T cells (T_H): release cytokines that stim. app. B cells to divide, develop into plasma cells & secrete antibodies

effector $CD8^+$ T cells (T_C): attach to surface of infected cells & secrete toxic substances

How plasma cells form from a plasma cell

1. B cell uses B cell receptors to trigger receptor-mediated endocytosis of antigen
2. Lysosome hydrolyses antigens, epitopes presented on MHC-peptide complex at cell surface membrane
3. APC engulfs, processes & presents antigen in similar way to activate naive T cell, forming T_H cell
4. T_H cell uses T cell receptor to bind to epitope presented by specific B cell; secrete cytokines to activate B cell
5. Activated B cell undergoes clonal expansion forming many identical daughter cells which differentiate into plasma cells

Steps of the adaptive immune system

1. (something) evades first two lines of defense and enters body

2. Macrophage engulfs foreign material and digests it into smaller pieces through phagocytosis.

3. macrophage (APC) transports some digested pieces to cell membrane where they bind to MHC self-markers on membrane

   macrophage presents antigen fragment to T_H cell, binding w/ CD_4^+ receptor
   macrophage also secretes cytokines which activate other immune cells.

4. T_H cells secrete cytokines which activates (immune cells). T_H cells bind to B cells w/ complementary antigens, promote their differentiation into plasma cells & memory B cells.

5. naive B cells / T cells undergo clonal selection and clonal expansion.

6. Activated B cells (plasma cells) secrete antibodies which:

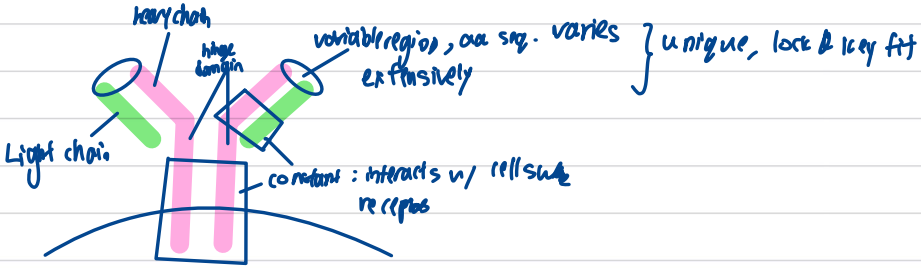
   a) Neutralise proteins on surface of virus to prevent infection of host cell / bind to toxins released in bodily fluids
   b) Opsonisation → bind to antigens & present a readily recognised structure for macrophages / neutrophils; promote phagocytosis
   c) Activation of complement system → work w proteins of complement system (eg. form pore on membrane of cell)

Cytotoxic T cells can & destroy cells that display MHC class
↳ releases cytotoxic pore-forming molecules (perforins) + granzymes (induces apoptosis)

⑦ Primary immune response → slow Secondary immune response → fast

Epitope → the small accessible portion of an antigen that binds to antigen receptor

BCR (eg. Ig G)



Key points: · basic - two L chains, two H chains
· covalently linked by disulfide bridges → unique generation

Function: Besides activating complement, tail region of an Ig G molecule binds to specific receptors on macrophages and neutrophil (Fc receptor)

antibody repertoire ⟶ total number of antibody specificities

Somatic recombination:

- The process of DNA recombination by which genes encoding variable regions of antigen receptors are formed during lymphocyte development
- where DNA sequences are brought together by enzymatic deletion of intervening sequences and rejoined.

2° diversification

Somatic hypermutation

- Extensive mutation in V-region DNA sequence produces variant immunoglobins, some which bind antigen w/ higher affinity leading to affinity maturation

Class switching

- One heavy-chain constant region gene is replaced w/ another or a diff isotype.
- switch from production of antibodies of IgM to produce IgG, IgA, IgE occurs during adaptive immune response
- As intervening DNA is lost, B cell cannot "switch back" to an isotype that has been deleted.

TB transmission

TB transmitted of droplet contact transmission and airborne transmission of fine droplets of respiratory mucus

Evasion of phagocytosis:

- alveolar macrophages engulf the bacteria and pathogen is enclosed in endosome/ phagosome
- endosome fuses with lysosomes. TB modifies the endosomal compartment such that lysosomal fusion cannot occur
- pathogens can remain in the endosomal compartment within macrophages and replicate

Pathogen multiplies within macrophages:

- Specific infection sites (tubercles) may form, consisting of a central core contaiing TB bacteria, enlarged infected macrophages and an outer wall made of fibroblasts, lymphocytes and neutrophils
- Latent phase — no symptoms, and infection is not contagious

Active stage:

- if immunity is weakened, tubercle may expand and eventually rupture, causing damage to lung tissue and function
- symptoms of chest pain, cough, contagious
- TB bacteria may disseminate and cause secondary infections in lymph nodes, bones and gut

Antibiotics : Bacteriostatic —— inhibit growth; does not kill
Bactericidal —— kill bacteria; no lysis
Bacteriolytic —— kill cells by lysis

Penecillin

- contains a β-lactam ring

- binds and blocks transpeptidase that form cross-links between NAM residues in cell wall synth.
- in absence of transpeptidation, continued activity of autolysins weakens peptidoglycan
- weakened cell wall cannot withstand the pressure potential exerted on it by cell contents & bursts

ETB: Climate change

Explain how human activities have contributed to climate change :

increased emission of greenhouse gases through :

- burning of fossil fuels linked to increasing energy usage
- clearing of forests
- food choices (increasing consumption of meat)

How climate change affects

- plant distribution (vertical and latitude)
- plant adaptations (morphology and physiology)

Consequences to the global food supply from climate change

How temperature change impacts insects :

- increased metabolism and narrow temperature tolerance of insect
- outline the life cycle of the Aedes mosquito + development of viral dengue disease
- How global warming causes the spread of mosquito-borne infectious diseases (including malaria and dengue) beyond the topics

Sample question : Describe and explain the effect of climate change on the distribution of rain forest vegetation in the tropics as shown in fig. 9.1

Explain :

1) Rain forest vegetation will only grow in temp range of 21 - 27 C
2) Lower temperatures at higher altitude
3) As temperature increases the distribution of rainforest vegetation moves upwards

Organism has a specific habitable range — as temperature increases, temperature of the environment increases above the habitable range. Hence, proteins in organisms become denatured, organism dies, loss of biodiversity due to species extinction

Time management :

P1 1m → 2 min

P2 1m → 1.2 min → 10m = 12min

5m = 6 min

P3 1m → 1.6 min → 25m → 40 min

10 m : 16 min

15 m : 24 min