EUNOIA JUNIOR COLLEGE
JC1 Promotional Examinations 2020
General Certificate of Education Advanced Level
Higher 2

CANDIDATE NAME	**ANSWER KEY**							
CIVICS GROUP	**2**	**0**	**-**			REGISTRATION NUMBER		

H2 Biology

9744/02

Paper 2
Structured Questions & Free Response Questions

02 October 2020
2 hours

Candidates are to answer questions in **Section A in this question booklet**.
Candidates are to answer questions in **Section B in the answer booklet** provided.

Additional Materials: 8-page Answer Booklet

READ THESE INSTRUCTIONS FIRST

Write your name, civics group and registration number on all the work you hand in.

There are **two** sections in this paper, Section A and Section B.
You are advised to plan your time appropriately to complete both Sections.

Answer **all** questions.
Write in dark blue or black pen on both sides of the paper.
You may use an HB pencil for any diagrams or graphs.
Do not use paper clips, highlighters, glue, or correction tape/fluid.

The use of an approved scientific calculator is expected, where appropriate.

The number of marks is given in brackets [ ] at the end of each question or part question.

For Examiner's Use	
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Total	/80

This document consists of **16** printed pages and **1** blank page.

Section A

Answer **all** the questions in this section.

1 Emperor penguins are the only species of penguin that breed during the Antarctic winter and they may stand still for multiple days to incubate their eggs. In order to survive in freezing temperatures, the feet of emperor penguins contain a high percentage of unsaturated fats.

(a) Explain the significance of unsaturated ~~fatty acids~~ (fatty acid tails) in cell membranes. [2]

1. Presence of **C=C bonds** causes **kinks** in unsaturated fatty acid tails [R: unsaturated fats] of phospholipids [R: kinks in membrane];
2. This **prevents close packing of phospholipids** at **low temperatures**, reducing hydrophobic interactions between fatty acid tails, thus maintaining membrane fluidity [R: increasing fluidity] OR preventing cell membranes [R: unsaturated fatty acid tails] from freezing/solidifying;

Teacher's comments:

- Please take note of the edit in the question. Due to the errata, marks were still awarded if students wrote fatty acids instead of fatty acid tails. However, note that fatty acids are different from fatty acid tails. Fatty acids are long-chained carboxylic acids, consisting of a long hydrocarbon chain and a carboxyl group. Fatty acid tails are fatty acids that have formed ester bonds with glycerol in phospholipids (or triglycerides).
- Fats were rejected as fats are esters of fatty acids but the specific type of lipids in cell membranes is phospholipids.
- Quite a large number of students mentioned the presence of kinks but did not state that they are caused by C=C bonds, which are only present in unsaturated fatty acid tails, but absent in saturated fatty acid tails.
- Unsaturated fatty acid tails only confer higher membrane fluidity in comparison to saturated fatty acid tails at the same temperature. At lower temperatures, the kinetic energy of phospholipids decreases and phospholipids are more closely packed together due to stronger hydrophobic interactions. Presence of unsaturated fatty acid tails in phospholipids helps to MAINTAIN membrane fluidity by reducing the extent at which the membrane loses its fluidity, but not increasing the fluidity.

(b) Fig. 1.1 shows the structure of a lipoprotein in the blood of emperor penguins. Lipoproteins transport fats from the liver to other tissues via the bloodstream. The membrane proteins of lipoproteins allow for fats to be deposited to the target tissue.

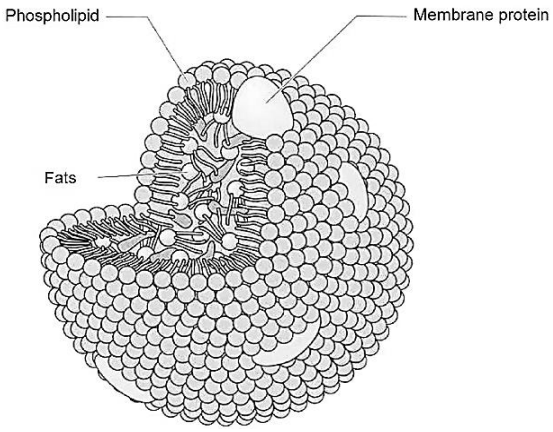


Fig. 1.1

With reference to Fig 1.1, describe how the structure of lipoproteins allows for fats to be transported from the liver to a specific tissue via the bloodstream. [3]

1. Phospholipids form a single layer / monolayer [R: bilayer] to serve as boundary between blood and (hydrophobic) fats;
2. (Non-polar) **hydrophobic hydrocarbon / fatty acid tails** of phospholipids face **inwards** and interact with hydrophobic fats in the lipoprotein core OR exclude water;
3. (Charged) **hydrophilic phosphate heads** of phospholipids face **outwards** and interact with (polar) water molecules in blood / aqueous environment in blood, allowing the lipoprotein to be soluble in blood;
4. Membrane protein recognises and binds to receptors of target cells [R: target tissue] via **complementary conformation and charge**;

Points 1-3 (max 2)

Teacher's comments:

- This application question is generally not so well done. Question requires student to make the conceptual link between **structure** and **function** of different components of lipoprotein (phospholipids and membrane protein).
- Some students wrongly identified the phospholipid monolayer as bilayer, which suggests lack of / incorrect interpretation of Fig 1.1.
- To obtain points 2 and/or 3, students must address the **orientation** of the hydrophilic phosphate heads and/or hydrophobic fatty acid tails, AND suggest **what they interact with** respectively, based on the information given in the question.
- Lipoprotein is a macromolecular complex of lipids and proteins. It is NOT a cell, so it is wrong to refer the phospholipid monolayer as cell membrane, or suggest that membrane protein is involved in cell-cell recognition.
- Membrane protein of lipoprotein, also known as apolipoprotein (FYI), is **NOT** transport protein (channel protein / carrier protein) / enzyme / receptor protein. It serves as a ligand to bind to lipoprotein receptors on cell surface membrane.
- With reference to Fig 1.1, fats inside the lipoprotein have 3 tails, which differ from the surrounding phospholipids with 2 tails. The fats are triglycerides (glycerol attached to 3 fatty acid chains).
- Fats (triglycerides) are non-polar and hydrophobic, **NOT** polar and hydrophilic.

(c) Explain how the structure of haemoglobin allows it to transport oxygen efficiently in red blood cells. [3]

1. **[Binding of 4 oxygen molecules]** Haemoglobin has **4 subunits** (2 α-globin subunits and 2 β-globin subunits), each containing a polypeptide chain (globin) and a haem group [A: ref. to Fe^{2+} ion in haem group], which allows one haemoglobin to carry up to **4 oxygen molecules** at the same time;
2. **[Reversible binding of oxygen]** **Fe^{2+} ion** of the **haem** group binds **reversibly** to oxygen;
3. **[Cooperative binding of oxygen]** Weak intermolecular bonds (hydrogen bonds, ionic bonds, hydrophobic interactions) between the 4 subunits allow for cooperative binding of oxygen when binding of one oxygen molecule to one subunit induces a **conformational change** in the other 3 subunits, increasing their affinity for oxygen;

   [A: unloading of one oxygen molecule from one subunit induces conformational change in other 3 subunits, reducing their affinity for oxygen]

4. **[Solubility]** Polar/charged, **hydrophilic R groups** of amino acids project outwards while non-polar, **hydrophobic R groups** of amino acids are pointed towards the interior, shielded away from the aqueous environment. This makes haemoglobin **soluble** in the cytoplasm of red blood cells for efficient transport of oxygen;
5. **[Compactness]** Its **globular** structure makes it **compact** so that more haemoglobin can be packed into red blood cells (RBCs) for more efficient oxygen transport;

Teacher's comments:

- Students who are clear about the structure of haemoglobin generally do well for this question.
- Many students confuse haemoglobin with red blood cell. Haemoglobin does NOT have a biconcave shape. It is a globular protein which is found in red blood cell.
- The prosthetic (non-protein) component in a subunit is the haem group. Some answers seem to wrongly suggest that they are referring to different things.
- One haemoglobin does not only have one haem group. Each subunit consists of a haem group (and globin chain). Hence, haemoglobin, which is made up of 4 subunits, has 4 haem groups.
- Haemoglobin transports oxygen molecules, NOT oxygen atoms. Students should spell oxygen in full, instead of O_2.
- For point 3 on cooperative binding of oxygen, students need to describe the effect of oxygen binding to 1 subunit on the other 3 subunits.
- For point 4, students also need to address the orientation of hydrophobic R groups of amino acids. If hydrophobic R groups are also projected outwards, it will lead to polymerisation of haemoglobin.

[Total: 8 marks]

2 Beta-glucosidase is an enzyme that converts cellobiose to glucose during cellulose hydrolysis. Samples of beta-glucosidase were extracted from two species of bacteria, *Agrobacterium tumefaciens* (enzyme **A**) and *Thermotoga maritima* (enzyme **T**)

Fig. 2.1 shows the results of an experiment that investigated the effect of temperature between 0°C and 100°C, on the activity of enzyme **A** and enzyme **T**.

L represents the lowest temperature at which activity of each enzyme was detected. H represents the highest temperature at which activity of each enzyme was detected.

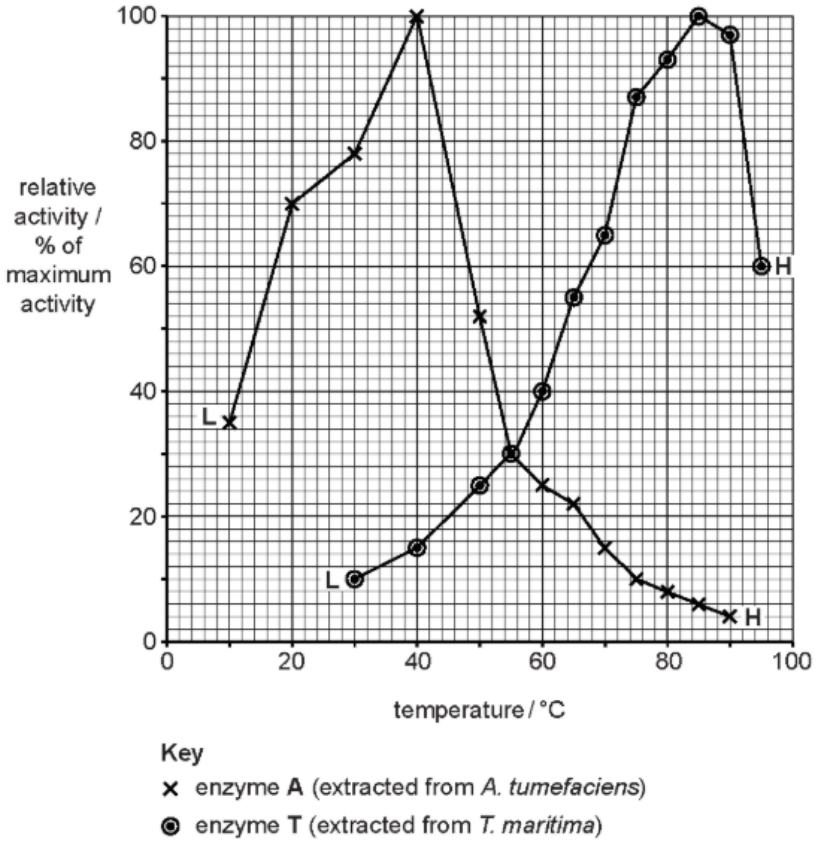


Fig. 2.1

(a) With reference to Fig. 2.1, describe the differences in the relative activities of enzymes **A** and **T**. [3]

1. **[Optimal temperature]** Enzyme A has a lower optimal temperature of **40°C** (with **100% of maximum activity**) while enzyme T has a higher optimal temperature of **85°C** with (**100% maximum activity**);
2. **[Lowest temperature]** At the lowest temperature (L) of **10°C**, enzyme A has a higher relative activity at **35% of maximum activity** while at the lowest temperature (L) of **30°C** , enzyme T has a lower relative activity at **10% of maximum activity**;
3. **[Highest temperature]** At the highest temperature (H) of **90°C**, enzyme A has a lower relative activity at **4% of maximum activity** while at the highest temperature (H) of **95°C**, enzyme T has a higher relative activity at **60% maximum activity**;
4. **[Working range of temperature]** Enzyme A has a wider working range of temperature from **10°C to 90°C** while T has a smaller working range of temperature from **30°C to 95°C**;
5. AVP

Teacher's comments:

- This question is poorly done, due to the following reasons:
- **LACK OF COMPARISON:** Many students merely described and quoted data but did not describe the **differences** using comparative terms, which would result in an overall deduction of 1 mark.
- **INCOMPLETE QUOTING OF DATA:** Many students did not quote data from either x-axis (temperature / °C) or y-axis (relative activity / %).
- As enzymes A and T have different relative activity at different L and H, students should quote the respective values of relative activity and temperature, and compare the relative activity.
- Students need to read the graph more carefully. Optimal temperature of enzyme T is 85°C, not 84°C or 86°C. H for enzyme T is 95°C, not 94°C or 96°C.
- **WRONG SEGMENTATION OF GRAPH:** Many students have different incorrect ways to segment the graph (e.g. 10°C–40°C, 40°C–85°C, 85°C–95°C). The correct way would be to split the graph into 3 parts – before, at & beyond optimal temperature (for each enzyme).
- For comparison of specific points of the graph, only relative activity at significant points like optimal temperature, L and H should be compared.
- Enzyme A and T do not only denature at temperatures at/higher than 90°C and 95°C respectively. Beyond optimal temperature, denaturation has already occurred, causing a decrease in relative activity. Before and at 90°C and 95°C, there is still presence of enzyme activity. However, beyond 90°C and 95°C, there is no activity detected, due to denaturation of all enzymes.

(b) Explain why an increase in temperature affects the activity of beta-glucosidase. [3]

1. As temperature increases to the optimal temperature, the **kinetic energy** of beta-glucosidase and cellobiose **increases**;
2. This **increases** the **frequency of effective collisions** between beta-glucosidase and cellobiose, which results in **higher rate of formation of enzyme-substrate complex**, increasing rate of reaction (enzyme activity);
3. Beyond the optimal temperature, the rate of reaction (enzyme activity) decreases rapidly as **hydrogen bonds, ionic bonds and hydrophobic interactions** [I: disulfide bonds] **between R groups of amino acids** are **disrupted**,
4. thus **changing the 3D conformation and charge of active site** and **denaturing** beta-glucosidase;

Teacher's comments:

- Many students stopped at explaining the increase in relative activity before optimal temperature and did not explain the decrease in relative activity beyond optimal temperature, limiting them to a maximum of 2 marks.
- Similar to Paper 4, for rate of enzymatic reaction, students should refer to increased/decreased **frequency/rate** of effective collisions & formation of enzyme-substrate complexes, as more/less effective collisions & formation of enzyme-substrate complexes occur **per unit time**.
- Make it a good practice to address the given context of the question by being specific about the enzyme (beta-glucosidase) and substrate (cellobiose).
- Many students only mention either enzyme or substrate having an increase in kinetic energy. Any molecules will experience increase in kinetic energy at higher temperatures.
- Requirements of effective collisions include 1) energy levels of enzyme & substrate need to be equal to or greater than the activation energy, and 2) correct orientation of both enzyme and substrate.
- For point 3, students are required to provide examples of weak intramolecular bonds/interactions in the enzyme. Bonds/interactions should be indicated clearly, instead of shortening to "hydrogen and ionic bonds".

(c) Suggest why enzyme **T** has a higher optimal temperature as compared to enzyme **A**. [2]

1. Enzyme T has a higher proportion of cysteine residues enabling the formation of **more disulfide bonds** than in enzyme A; [R: ionic bonds / hydrogen bonds / hydrophobic interactions]
2. Disulfide bonds are **strong** covalent bonds which are difficult to be broken by high temperatures, and hence the enzyme is **not denatured** / **conformation of active site** remains **intact/unchanged** at high temperatures.

Teacher's comments:

- This question is poorly done although it is highly similar to a question in the enzyme tutorial.
- Many students misinterpreted the question as "why enzyme T needs to have a higher optimal temperature" and wrongly suggested that it is because *T. maritima* is found in environment(s) with higher temperatures. The question is **NOT** asking for the importance of higher optimal temperature. The question requires students to identify the difference between enzyme T and enzyme A that **causes** the former to be more thermostable than the latter.
- Reference to ionic bonds, hydrogen bonds and hydrophobic interactions are rejected as they are weak bonds that would break at high temperatures.
- Students need to link bond stability to structural stability in maintaining 3D conformation of active site for functional/high enzyme activity at higher temperatures.

[Total: 8 marks]

3 Fig. 3.1 shows the gene expression of a ribosomal protein, rS2.

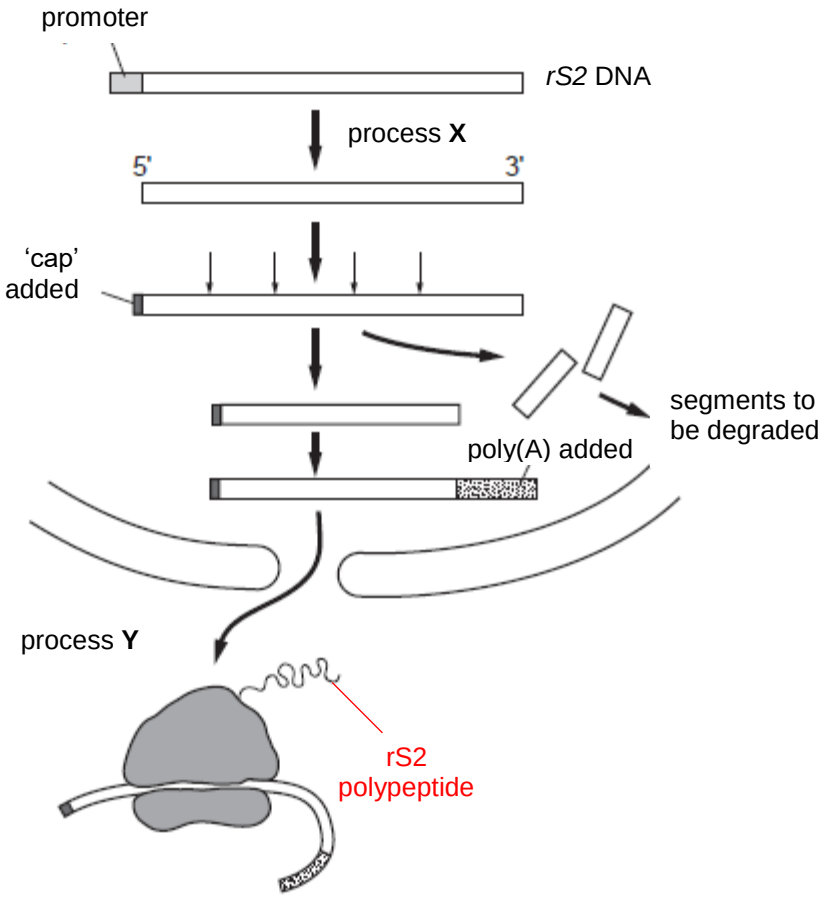


Fig. 3.1

(a) (i) List three differences between process **X** and process **Y**. [3]

1. **[Location]** Process X occurs in **nucleus** while process Y occurs at **ribosomes** in cytosol or bound to RER; [R: just cytoplasm or RER]
2. **[Template]** Process X uses **DNA template strand** [R: DNA] as template while process Y uses **mRNA strand** as template;
3. **[Product]** Process X produces **mRNA, tRNA and rRNA** [R: RNA] while process Y produces **polypeptide**;
4. **[Enzyme]** **RNA polymerase** catalyses the formation of phosphodiester bond between ribonucleotides in process X while **peptidyl transferase** (in ribosome) catalyses the formation of peptide bond between amino acids; [R: ribosome]
5. **[Bond + Monomer]** **Phosphodiester bond** is formed between adjacent **ribonucleotides** in process X while **peptide bond** is formed between adjacent **amino acids**;
6. **[Direction of reading template strand]** The template DNA strand is read in 3' to 5' direction in process X while mRNA is read in 5' to 3' direction in process Y;
7. **[Direction in which product is synthesised]** mRNA is synthesised in 5' to 3' direction while polypeptide is synthesised from N-terminus to C-terminus;
8. AVP

Any **3** comparisons; 1m each
R: presence/absence type of comparison

Teacher's comments:

- This question is generally well done. Most students correctly identify process X to be transcription, NOT addition of 5' cap, splicing or polyadenylation (post-transcriptional modification), and process Y to be translation.
- Students should do point to point comparison and avoid writing more than 1 factor of comparison in one sentence.
- Point 1 (location): Students need to state the specific organelle at which both processes occur at.
- Point 2 (template): DNA is a molecule and it consists of two DNA strands. Only one strand, specifically the DNA template strand, is used as template in transcription.
- Point 3 (product): Students are to identify the different types of RNA that can be synthesised during transcription.
- Point 4 (enzyme): Ribosome is not an enzyme, it is an organelle. The enzyme found within ribosome is peptidyl transferase.
- Point 5 (bond + monomer): When stating the bond formed, you are expected to state the monomers involved in bond formation for a complete answer.
- In transcription / translation (process), DNA template strand / mRNA strand (template) is used as a template to produce mRNA / polypeptide (product). The template is NOT converted to the product.

(ii) Describe how ribosomal subunits are assembled after the rS2 polypeptide is synthesised. [3]

After translation:

1. The synthesised rS2 polypeptide **folds** into its specific **3D conformation** in the cytosol;
2. rS2 protein is transported into the **nucleolus** [R: nucleus] via the nuclear pores;
3. where it **associates** with **ribosomal RNA (rRNA)** to form **small and large ribosomal subunits**;

Teacher's comments:

- It was stated in the question that rS2 is a ribosomal protein. Recognising that ribosomal proteins are part of a ribosomal subunit would help students to interpret the question correctly.
- rS2 polypeptide is synthesised by free ribosomes in cytoplasm (as shown in Fig 3.1), NOT bound ribosomes. It folds into its specific 3D configuration in cytoplasm, NOT in rough endoplasmic reticulum. Protein synthesis involving the endomembrane system serves 3 purposes – secretion, embedding proteins in cell membranes or packaging into organelles (formation of lysosome). Ribosomal protein remains inside the cell for formation of ribosome.

Alternative answer (in light of error in Fig. 3.1):

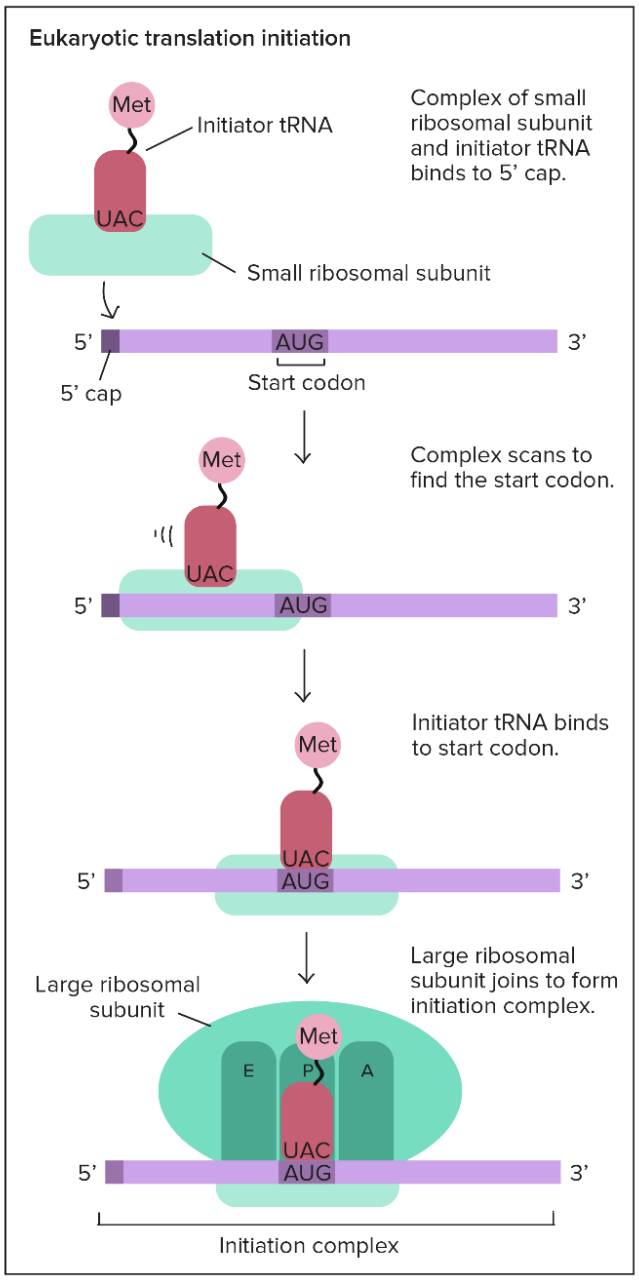
(ii) Describe how ribosomal subunits are assembled after the rS2 **mRNA** is synthesised. [3]

Initiation of translation, after post-transcriptional modification:

1. Small ribosomal subunit, *eukaryotic translation initiation factors* and initiator tRNA form a complex;
2. The complex formed recognises and **binds to 5' end** (or 5' cap) **of mRNA**;
3. then scans the mRNA to locate the **start codon, AUG**;
4. **Binding of the large ribosomal subunit** to the complex completes the translation initiation complex.

If not mentioned in point 1, small ribosomal subunit and initiator tRNA must minimally be mentioned in point 2 and point 3 respectively.

- **Small ribosomal subunit** recognises and **binds to 5' end of mRNA**.
- Small ribosomal subunit scans the mRNA to locate the **start codon, AUG**, which the **initiator tRNA** (or **methionine-tRNA complex**) binds to.



(b) Fig. 3.2 shows a section of the rS2 DNA template strand.

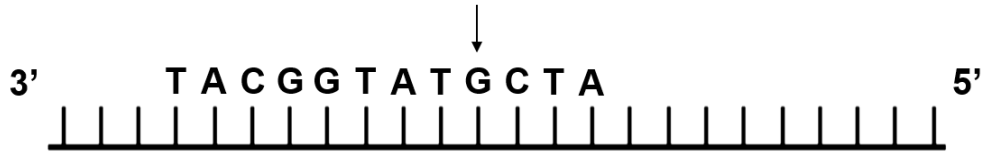


Fig. 3.2

(i) State the corresponding base sequence of the mRNA strand. [1]

5' A U G C C A U A C G A U 3' (or 3' U A G C A U A C C G U A 5')

(Must state directionality to be awarded the mark)

(ii) The nucleotide indicated by the arrow in Fig. 3.2 undergoes a substitution mutation to C.

Describe the effect of this mutation on the function of rS2 protein. [3]

1. mRNA codon is changed from **UAC** to **UAG**, which is a **stop codon**;
2. leading to a **nonsense mutation** OR
   where **translation** is **prematurely terminated**;
3. The polypeptide formed is **truncated**, thus resulting in **non-functional** rS2 protein;

Teacher's comments:

- Students who got part (i) wrong usually did not indicate the correct directionality or the correct complementary base pairs (U instead of T in mRNA).
- For part (ii), students should make it a good practice to state what has **changed** when mutation occurs. What is the original mRNA codon / amino acid? What is the new mRNA codon / amino acid (if any)?
- Students need to be specific to the given **context** of the question. Although it is a change in the 3rd base of a codon, it is not a silent mutation as UAC is changed to UAG, which is a stop codon that does not code for an amino acid. Missense mutation is also wrong as UAG does not code for a (different) amino acid.
- Translation (synthesis of polypeptide) is the process that is terminated prematurely at the new stop codon in mRNA, NOT transcription. In eukaryotes, termination of transcription occurs at the terminator sequence in DNA. Introduction of new stop codon will not cause mRNA to be truncated.
- The idea of **premature** termination of translation needs to be conveyed as without introduction of new stop codon, translation will also terminate downstream of the original mRNA strand at a stop codon.

[Total: 10 marks]

4 Several members of an endangered white rhinoceros species were found to be infertile.

Scientists found that their germline cells, which give rise to gametes, were not able to undergo meiosis properly.

(a) Explain how meiosis gives rise to haploid gametes. [2]

1. Germline cells undergo a reduction division during **meiosis I**, which halves the number of chromosomes per cell;
2. Because **homologous chromosomes** are **separated** and pulled to opposite poles during **anaphase I**;

Teacher's comments:

- Majority of students who did not gain full credit for this question had the misconception that the reduction division occurs in meiosis II, when in fact it is meiosis I.
- A small number of students gave irrelevant answers due to misunderstanding the term 'haploid'. Ploidy refers to the **number of chromosomes** per cell, and NOT the amount of DNA per cell.
- When describing any process of cell division, always remember to state which phase (PMAT). If meiosis is involved, students must also state **I or II**.

(b) During nuclear division, CenH3 is a protein that associates the kinetochore protein complex with centromeric DNA. Scientists observed that infertile white rhinoceroses had a high frequency of mutation in the *CenH3* gene.

Suggest the consequences of this loss-of-function mutation on meiosis. [3]

1. **Kinetochore proteins** and subsequently **kinetochore microtubules/spindle fibres** would be **unable to attach** to the **centromeres** of each chromosome;
2. **Bivalents / pairs of homologous chromosomes** would not be aligned at the **equator / metaphase plate** during **metaphase I**;
3. Cell would be **unable to pass the M checkpoint** of the cell cycle;
4. **Non-disjunction** of **homologous chromosomes** occurs during **anaphase I**
   [A: homologous chromosomes fail to separate during anaphase I];
5. Thus resulting in additional or missing chromosomes (**aneuploidy**) or additional or missing sets of chromosomes (**polyploidy**)
   OR no viable gametes are formed;

Teacher's comments:

- This question was generally well done, with most students able to gain at least partial credit.
- Credit was also given if students discussed relevant events in meiosis II for points 2 and 4.
- Once again, students lost credit for not naming which phase of meiosis they were describing, or not stating **I or II**.
- Simply stating that a 'non-disjunction event' would occur is not specific enough; a better description would be to describe exactly where the non-disjunction would occur.

(c) To counteract the problem of infertility, scientists attempted to induce the expression of normal *CenH3* gene in white rhinoceroses.

However, amplification and overexpression of the *CenH3* gene resulted in prostate cancer.

Explain the effects of **two** other genes that are overexpressed in the development of cancer. [4]

Any two pairs (1+2 / 3+4 / 5+6):

1. Overexpression of **proto-oncogenes/oncogenes** causes overproduction of **cell cycle-promoting proteins / growth factors** (OWTTE);
2. resulting in **excessive cell division/proliferation** to form a tumour; [R: uncontrolled]

3. Overexpression of genes coding for **angiogenic factors** stimulates **growth** of new **blood vessels** within the tumour;
4. To supply cancer cells with **oxygen and nutrients** for growth;

5. Upregulation of genes coding for **extracellular proteases** which **degrade the extracellular matrix**;
6. Resulting in the formation of a **malignant tumour / metastatic cancer** capable of spreading to other parts of the body to establish secondary tumours;

[R: activation of gene encoding telomerase]

Teacher's comments:

- A large majority of students named **telomerase** as one of the genes that would be overexpressed. However, this is not accurate since somatic cells do not normally express the telomerase gene, and hence is an example of activation, and not overexpression.
- In point 2, overexpression of oncogenes only promotes abnormally fast cell division, but does not result in 'uncontrolled cell division' yet. In order for the cell cycle to become dysregulated, **tumour suppressor genes** must first undergo a loss-of-function mutation; therefore, only when both types of genes are mutated will the tumour cell undergo uncontrolled cell division.
- A significant number of students named "genes coding for angiogenesis" and/or "genes coding for metastasis". However, angiogenesis and metastasis are processes, which genes cannot code for.
- In point 5, only genes coding for extracellular proteases will be upregulated (i.e. overexpressed). Genes coding for cell-cell adhesion proteins will be **downregulated** instead, since tumour cells need to escape from the cell layer to metastasise.

[Total: 9 marks]

5 The human *GDAP1* gene codes for the protein GDAP1. Mutations in the coding region of *GDAP1* have been identified as a major cause of a neurodegenerative condition known as Charcot–Marie–Tooth disease (CMT).

It was discovered that the *GDAP1* gene promoter is transcriptionally regulated by YY1.

Fig. 5.1 below shows the 245-bp fragment of the promoter where RNA polymerase binds to initiate transcription after YY1 has bound. The YY1 binding site, -4/+5 region (i.e. nucleotide sequence from -4 to +5) is found within the *GDAP1* promoter.

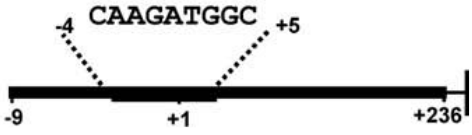


Fig. 5.1

(a) State the type of transcription factor that YY1 is classified as. [1]

1. **General** transcription factor;

Teacher's comments:

- Most students correctly identified the transcription factor that binds to the *GDAP1* gene promoter.

(b) In eukaryotes, modification of histones lead to changes in frequencies of gene expression.

Explain how histone acetyltransferase affects the gene expression of *GDAP1*. [4]

1. Histone acetyltransferase adds an **acetyl group** (to lysine residues) **of histone** proteins & **removes/neutralises** the **positive charge** of histones;
2. This **reduces the electrostatic interactions** between histones and negatively charged DNA, thus resulting in DNA being **less tightly wound around histones**;
3. *GDAP1* **promoter** is **more accessible** to **general transcription factors and RNA polymerase**, hence allowing transcription to occur / increasing gene expression of *GDAP1*;

Teacher's comments:

- Most students were able to interpret this question correctly to gain partial or full credit.
- Those who did not gain full credit frequently missed out important points in the full explanation, such as DNA being less tightly wound around histone proteins (point 2) or increased accessibility of the promoter to general transcription factors (point 3).
- A small number of students also failed to link their explanation back to the question by stating the overall effect that histone acetylation has on gene expression: to increase or promote expression.

(c) Fig. 5.2 shows three deletion constructs which were created at the upstream region of the human GDAP1 gene promoter. The relative transcriptional activities associated with each of the 3 constructs of the promoter were also measured.

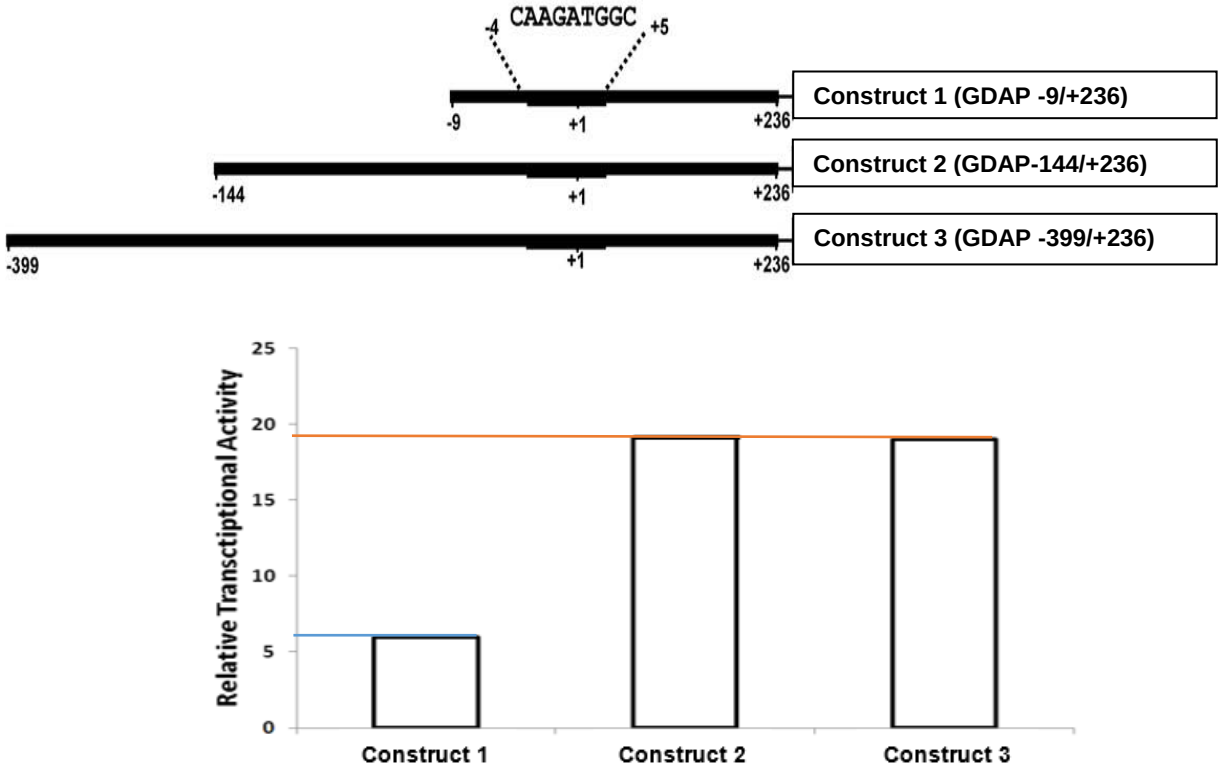


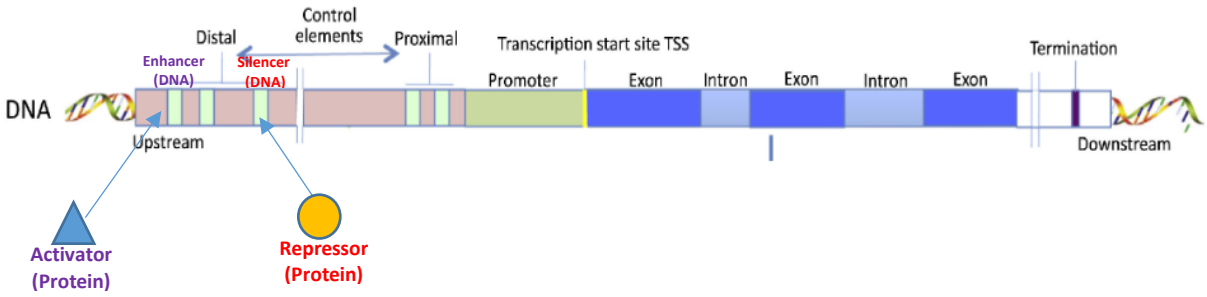
Fig. 5.2

(i) Identify the type and location of the regulatory sequence found in constructs 2 and 3. [1]

1. **Enhancer** sequence between **-9 to -144** region;

Teacher's Comments:
A majority of students were not able to gain credit for this question. The question involves understanding of control of transcription in eukaryotic genes.

Constructs 1, 2 & 3 are contain the GADP gene sequence and the differing portions of the upstream nucleotide sequences as shown in Fig. 5.2. A typical gene sequence is made up of the promoter (proximal control element), exons/intron sequences & the terminator sequence. Regulatory sequences such as enhancer or silencer sequences (distal control elements) may be present further upstream or downstream from the gene. Activators bind to enhancers and Repressors bind to silencers respectively. These help to increase or decrease rates of transcription.



A number of students were able to identify that the deleted segment of construct 1 contained an enhancer sequence. This is due to the fact that the transcriptional rate for construct 1 is much lower than it is for constructs 2 & 3. However, the location was not stated correctly (-9 to -144 bp).

(ii) Explain the differences in the relative transcriptional activities of the three constructs as described in **(c)(i)**. [4]

1. Construct 1 had **6 units** of transcriptional activity (quote data). In constructs 2 and 3, the relative transcriptional activity was **19 units** / There was a **13 unit increase as compared to construct 1** (quote data);
2. **Binding of activators to the enhancer sequences** in constructs 2 & 3,
3. caused **the bending of DNA/ recruitment of chromatin remodelling complexes/ histone acetyltransferases/ DNA demethylases**
4. which **promoted assembly of/stabilized the transcription initiation complex.** Hence, the rates of transcription were higher;

Teacher's Comments:
Another poorly attempted question. As explained in (i), the difference in the transcriptional activities is due to the absence of the enhancer DNA sequence in construct 1.

Description of data: A number of students quoted incorrect data for the relative transcriptional activities. Please do draw proper lines to indicate the correct readings when quoting data.

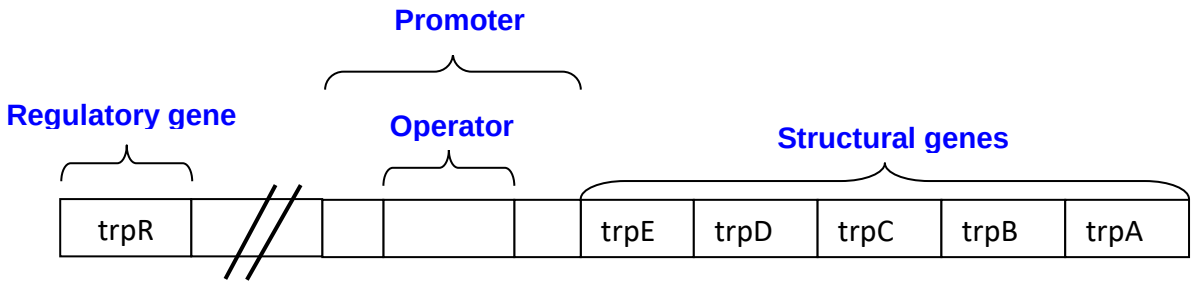
Explanation of observations: A majority of students also missed out on points 2, 3 & 4 to explain how the binding of activators to enhancer helped to increase transcription rates.

[Total: 9 marks]

6 In bacteria, the production of the amino acid tryptophan is catalysed by five specific enzymes encoded by specific genes *trpE, trpD, trpC, trpB and trpA* respectively.

The *trp* operon is transcriptionally regulated by a repressor protein, **R**, encoded by the *trpR* gene.

(a) Draw a simple diagram to show the organisation of the *trp* operon and the regulatory gene associated with it. [2]



Reject: if operator not within promoter
Reject: if the structural genes and regulatory gene are not in order of sequence; all or none

Teacher's Comments:
This is a factual recall type of question. Common misconceptions include:

1. Operator site is adjacent to promoter site and the sites overlap with each other – This is incorrect and is applicable to the lac operon instead.
2. trp R gene is beside the promoter site. – This is incorrect as the regulatory gene (trp R) is located further upstream from the promoter. The double lines indicate that the sequences is far away from the trp operon.
3. Some of the trp structural genes are missing.

(b) Table 6.1 below indicates the activity levels of the functional enzymes E, D, C, B and A in wild type bacterial cells in the presence and absence of tryptophan (Trp).

Table 6.1

	activity level of enzymes/units	
enzyme	Trp absent	Trp present
E	700	0
D	700	0
C	700	0
B	700	0
A	700	0

A group of Eunoians managed to obtain several bacterial mutants. Each mutant is the result of a single base-pair substitution in a single component of the *trp* operon. The activity levels of the functional enzymes **E**, **D**, **C**, **B** and **A** in the bacterial cells having these individual mutations are shown in Table 6.2.

Table 6.2

	activity level of enzymes/units					
	mutant 1		**mutant 2**		**mutant 3**	
enzymes	Trp absent	Trp present	Trp absent	Trp present	Trp absent	Trp present
E	700	700	700	0	0	0
D	700	700	0	0	0	0
C	700	700	700	0	0	0
B	700	700	700	0	0	0
A	700	700	700	0	0	0

(i) Using the information provided, state and explain which mutant has a phenotype that is consistent with a loss-of-function mutation in the *trpR* gene. [3]

1. Mutant 1 [Must state];
2. Loss of function mutation results in **non-functional/absence of repressor protein**;
3. tryptophan-repressor protein complex **cannot bind to operator site**;
4. This results in **constitutive expression of *trp* genes** as shown by **700 units of enzyme activity in the presence of tryptophan**

Teacher's Comments:
Most students were able to identify that the loss of function mutation had occurred in Mutant 1. Many students did not state that the repressor protein binds to the operator site. Some had missed out on quoting the data to support their explanation.

(ii) The phenotype of mutant 3 is caused by a mutation in the *trpR* gene. Explain how this mutation would affect the structure and function of the repressor protein. [3]

1. Mutation causes a **change in amino acid** / Missense mutation in the *trpR* gene;
2. which causes the protein to **fold into the same conformation as the active repressor as it would be when bound to tryptophan**;
3. This results in the repressor being **permanently bound to operator / binds to operator even in absence of tryptophan**;

Teacher's Comments:
Point 1 was missed out in most answers. It is important to state the effect of mutation of the polypeptide sequence before going on to explain how the protein would fold in a different manner.

A few students had confused the functional Lac repressor (which is synthesised in its active form) with the functional Trp repressor (which is synthesised in its inactive form). Furthermore, the repressor protein binds to the operator site. The operator site was often confused with the promoter site.

The term 'active site' was also used to describe the repressors' DNA binding site in some answers. This is incorrect. Active site is a specific term used to describe the site in an enzyme to which substrates bind. The repressor protein is not an enzyme.

[Total: 8 marks]

7 The rabies virus is classified in the same group as the influenza virus. Fig. 7.1 shows a simplified reproductive cycle of the rabies virus.

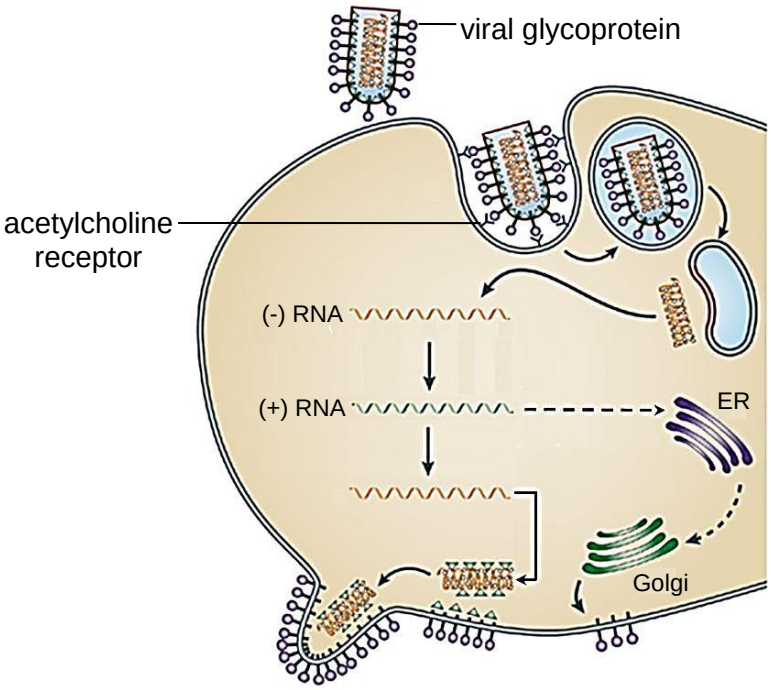


Fig. 7.1

(a) Using the information given in Fig. 7.1, explain the role of a named viral enzyme in the rabies reproductive cycle. [3]

1. Viral **RNA-dependent RNA polymerase**;
2. Uses the **negative strand RNA/ viral RNA genome** as a **template** to transcribe/synthesise the **complementary positive strand RNA / viral mRNA**;
3. which are used **for translation to synthesise viral proteins** by host cell **ribosomes**;
4. and also used as **templates for replication to form new copies of viral ssRNA genome**;

Teacher's Comments:
Many students were able to identify the viral enzyme to be RNA dependent RNA polymerase. Some students incorrectly mentioned terms such as 'haemagglutinin' & 'neuraminidase'. First error is that haemagglutinin is not an enzyme, it is a glycoprotein. Second error is both of these proteins are not suggested to be existent in the rabies virus based on Fig. 7.1. The replication and synthesis of the (+) RNA and (-) RNA indicates the presence of the RNA dependent RNA polymerase.

Another common misconception which was seen was based on the site of translation of the viral mRNA. Many students did not mention the host cell ribosomes as the site of translation and has plainly stated it to be the Endoplasmic Reticulum instead. Please be specific.

(b) Since late 2019, the spread of the Covid-19 virus (SARS-CoV-2) has caused a global pandemic resulting in more than 900,000 human deaths.

Fig. 7.2 shows conversations between different individuals who have been observing the developments surrounding this pandemic.

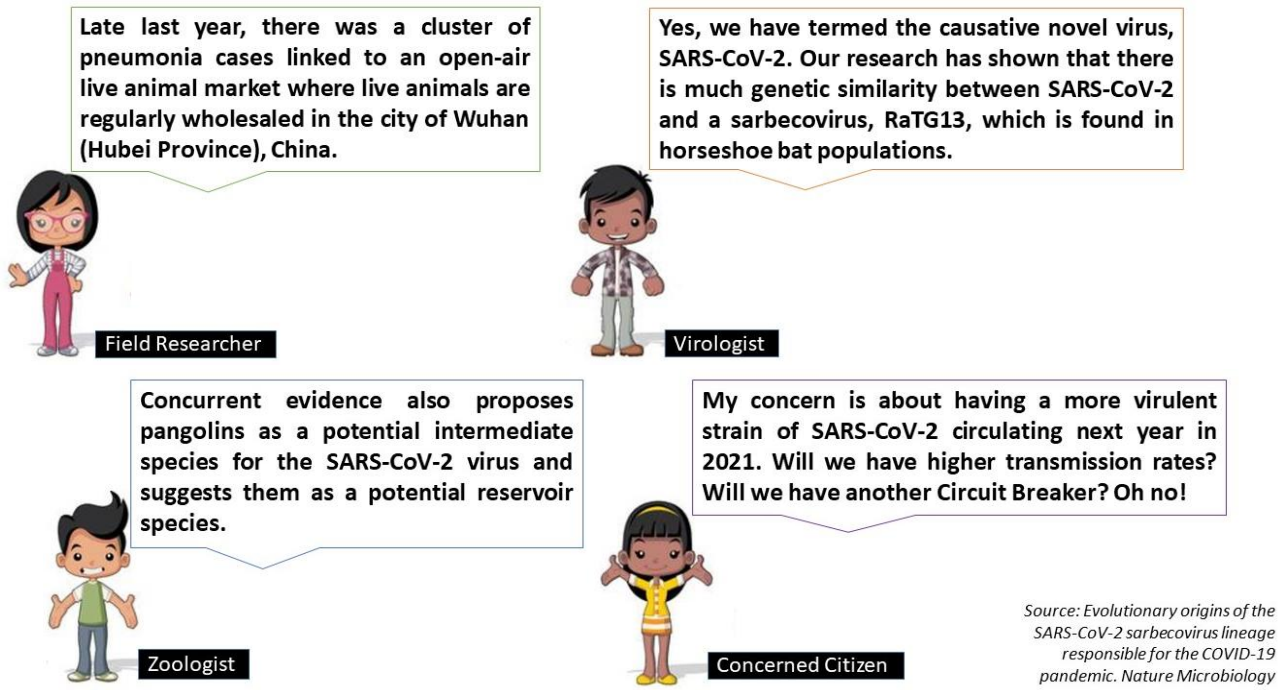


Fig. 7.2

(i) Using information from Fig. 7.2, suggest the type of viral evolution that SARS-CoV-2 had undergone to 'jump' to human populations in 2019. Explain your answer. [2]

1. **Antigenic shift**;
2. The SARS-CoV-2 virus could have been **originally found in the horseshoe bats populations** and it **crossed the species barrier** to infect and spread amongst humans;
3. Alternatively, pangolins could have acted as an intermediary species & the **virus could have initially crossed from the horseshoe bats to pangolins and eventually humans**.

Teacher's Comments:
Many students were able to identify type of evolution as 'Antigenic Shift' but were not able to explain it based on the information in Fig. 7.2.

Antigenic shift is said to have occurred when:
1. There is genetic recombination of viral genome segments following co-infection of a host cell by different viral subtypes (e.g. H5N1 & H2N3). The resultant viral progeny is classified as a new subtype (e.g. H2N1) as it carries a different combination of viral genomic segments as compared to the original viruses which infected the host cell.
2. There is evidence of 'crossing the species barrier' as the virus infects a new species of animals as it jumps from its current host species population.

Based on the information in Fig. 7.2, point 2 is more apparent as should be used as the supporting reason with the examples stated.

(ii) Based on your knowledge of viral evolution, comment on the validity of the statement of having '*a more virulent strain of SARS-CoV-2 in 2021*' by the concerned citizen. [3]

1. There could be **accumulation of mutations,** in the viral genome, over the year, as a result of **antigenic drift**;
2. This could lead to **changes in conformation and charge of surface glycoproteins/viral proteins/antigens** of the virus.

Either point 3 or 4 or 5 (to score full credit):

3. Hence, her statement is valid as the new viral proteins/antigens would lead to **more efficient attachment of the virus to host cells and higher transmission rates**.
4. **Immune cells are not able to recognise the new surface antigens** and hence it leads to **higher transmission rates**.
5. Hence, her statement may not be valid as the new viral proteins/antigens would lead to **less efficient attachment of the virus to host cells and lower transmission rates**.

Teacher's Comments:
Many students were able to identify type of evolution as 'Antigenic Drift'. Some answers were vague as they did not refer to antigenic drift occurring due to an accumulation of mutations over time. The point on change in 3D conformation of viral glycoproteins/antigens was also missing in a number of responses.

[Total: 8 marks]

Section B

Answer **all** the questions in this section.

Begin each part question on a fresh page.

8(a) Compare the structure and reproductive cycle of HIV and lambda phage. [12]

Similarities in reproductive cycle [max 3m]

	Feature of comparison	HIV	Lambda phage
R1	Integration of viral DNA into host cell genome	HIV and lambda phage both **integrate/incorporate viral DNA** into host cell chromosome/genetic material, forming a **provirus** and **prophage**, respectively	
R2	Viral enzyme that catalyses integration	Both HIV and lambda phage require **integrase** for integration of viral DNA into host cell genome/chromosome	
R3	Latent period after integration	Once integrated, provirus/prophage enters **latent stage** / **remains latent** for long period of time	
R4	Enzyme involved in formation of viral mRNA	**Host cell RNA polymerase** transcribes provirus and phage DNA to form viral mRNA	
R5	Location of translation of viral mRNA	Viral mRNA is translated in host cell **cytoplasm**	
R6	Organelle for translation of viral mRNA	**Host cell ribosomes** for translation of viral mRNA to synthesise **viral proteins**	

Structural differences [max 3m]

	Feature of comparison	HIV	Lambda phage
S1	Genome	**2** (positive sense) **single-stranded RNA** molecules	**1 double-stranded DNA** molecule
S2	Viral enzymes	**Reverse transcriptase, integrase, protease** [R: if any one missing]	Phage **lysozyme**
S3a	Capsid shape	**Conical**	**Icosahedral**
S3b	Virus structure	**Spherical**	**Complex** – icosahedral capsid head + tail + tail fibre
S4	Proteins on virus surface	Glycoproteins **gp120** and **gp41** embedded in viral envelope [R: wrong numbers]	**Contractile sheath** and **tail fibres** attached to capsid head OR no glycoproteins
S5	Viral envelope	Presence of **viral envelope** (made of a phospholipid bilayer derived from the host cell surface membrane)	**No viral envelope** present / naked virus

Differences in reproductive cycle [max 7m]

	Feature of comparison	HIV	Lambda phage
R6	Attachment of virus to host cell	Viral **gp120 binds** to **CD4 receptors** on **T helper cells** [not T4 helper cells]	Phage **tail fibres bind** to **complementary receptor sites** on bacterial **cell wall**
R7	Entry of virus into host cell	HIV enters via **fusion** of **viral envelope** with host **cell surface membrane**, releasing capsid into host cell	**Contractile sheath** of tail **contracts** and drives a hollow tube through bacterial cell wall to **inject genome** into host cell
R8	Release of viral genome	Entire **capsid enters host cell** and is **degraded to release viral RNA / genome**	**Phage DNA** is **released directly** into bacterial cell cytoplasm without capsid
R9	Reverse transcription	Viral RNA must undergo **reverse transcription** to form viral DNA before integration	Phage DNA **does not undergo reverse transcription**
R10	Induction / excision of viral DNA	Upon activation, provirus is **not excised** from host cell chromosome	Upon activation, **prophage / phage DNA** is **excised** from host cell chromosome
R11[a]	Enzyme involved in replication of viral genome	**Host cell RNA polymerase** transcribes provirus to viral mRNA, which is the HIV genome	**Host cell DNA polymerase** replicates phage DNA genome

R11[b]	Location of replication of viral genome	Replication of viral RNA genome occurs in **nucleus** [A: location of transcription]	Replication of phage DNA occurs in host cell **cytoplasm/nucleoid** [A: location of transcription]
R12	Location of assembly of new viruses	Assembly of RNA genome and polyproteins occurs at **inner surface of host cell surface membrane**	New phages are assembled in **host cell cytoplasm** where phage DNA is packaged into capsid and attached to tail
R13	Timing of maturation	HIV virions are only fully mature **after budding / release from host cell**	Lambda phages are fully mature **once assembly of new phages** is complete
R14	Formation of functional viral proteins	Some viral proteins are **translated / synthesised** as **polyproteins** which need to be **cleaved by HIV protease** to form functional viral proteins	Viral proteins are **translated / synthesised individually** as functional proteins OR Viral proteins are not synthesised as polyproteins
R15	Release of virus	HIV **buds off** from host cell surface membrane / HIV is released via **budding**	Host bacterium cell is **lysed** and phages are released / released via **lysis** of host cell
R16	Breakdown of cell wall by lysozyme	**Lysozyme not required** since host (animal) cells do not have cell wall	Host bacterium cell wall is **digested by phage lysozyme** before lysis of cell membrane

Quality of Written Communication (QWC) [1m]

Point for point comparison demonstrated throughout

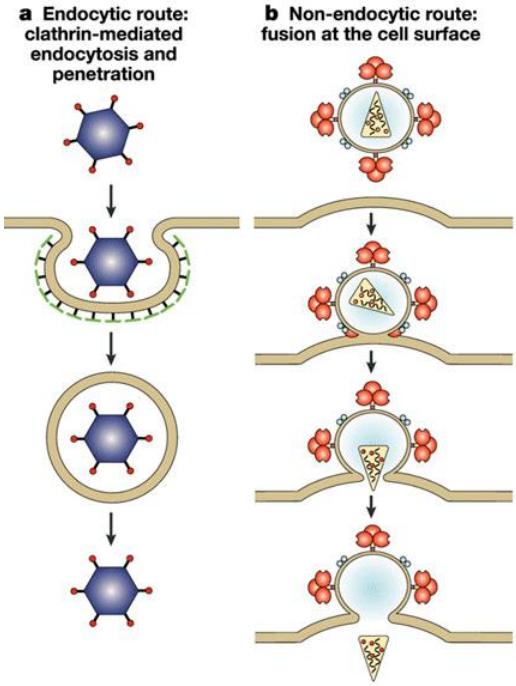
Answer must be well-structured:

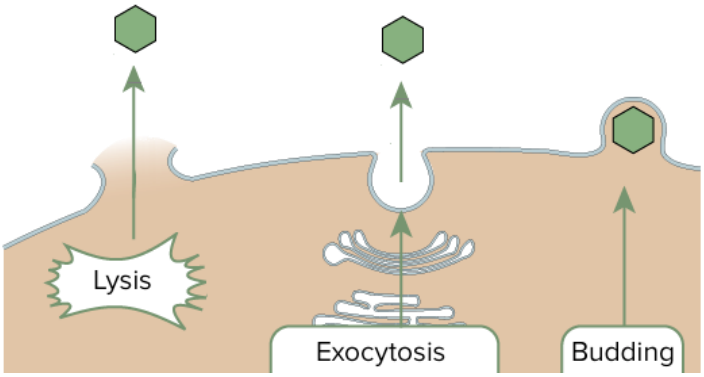
- similarities and differences for viral structure are listed, followed by similarities and differences for reproductive cycle OR similarities and differences grouped together

Teacher's comments:

- Most students correctly interpreted the "compare" command word in the question stem, and gave both similarities and differences.
- Some students, however, failed to give point-for-point comparisons. Writing an entire paragraph about HIV followed by another paragraph about lambda phage does not demonstrate any ability to answer the question. Moreover, if the examiner has to sieve out the comparison from an overly long sentence, then the answer has failed to succinctly capture the essential point of comparison.
- When making comparisons, it is always preferable to distinguish features across both viruses than to make simple absent/present comparisons.
- Statements applicable to all viruses were not accepted: "both viruses have their genome contained within a capsid" or "viral capsid is made of protein subunits called capsomeres".
- General statements not specific to structure or reproductive cycle were also not accepted: "HIV is an animal virus while lambda phage is a bacteriophage" or " both viruses follow the attachment, penetration, replication, maturation, and release stages in their reproductive cycle".
- The terms "host cell machinery" and "macromolecule synthesising machinery" are too vague to score any credit; it is far better to specify the precise enzyme or organelle involved in the process:
  - ✓ host cell DNA polymerase (for phage DNA replication),
  - ✓ host cell RNA polymerase (for viral mRNA synthesis),
  - ✓ host cell ribosomes (for viral protein synthesis), etc.

- **Common misconceptions** that students wrote in their answers include:
  - × "HIV uses reverse transcriptase to replicate/synthesise its genome" → reverse transcriptase only synthesises double stranded DNA using the viral mRNA as a template; it is actually host cell RNA polymerase that synthesises more copies of viral mRNA that serves as the HIV genome.
  - × "HIV undergoes the lytic/lysogenic cycle" → these terms are only for bacteriophages.
  - × "HIV enters host cell via endocytosis" and "HIV exits via exocytosis/evagination" → HIV enters host cells via fusion of the viral envelope with host cell surface membrane (see diagram below, on left), and exits via budding (on right); the term 'evagination' is not a process, but simply refers to the host cell surface membrane bulging outward during the budding process.





(b) Explain how DNA-binding proteins control gene expression in prokaryotes. [8]

Sigma factors and RNA polymerase

1. **The sigma factor and RNA polymerase holoenzyme;**
2. **recognize and bind to both the critical elements (-10 & -35 sequences) at the promoter site to initiate transcription of genes;**
3. **As different sigma factors recognise and bind to different gene promoters,**
4. **their availabilities determine which genes can be transcribed.**

In operons,

Repressor

5. **Repressor proteins bind to the operator site of the operon via their DNA binding site;**
6. **and block RNA polymerase from binding to the promoter and prevent transcription of structural genes;**
7. **(only for lac operon) Lac repressor is inactivated by the binding of inducer allolactose;**
8. **(only for trp operon) Trp repressor is activated by the binding of co-repressor, tryptophan**

Catabolite Activator Protein (CAP) (only for lac operon)

9. **DNA binding site of Activator proteins e.g. Catabolite Activator Protein (CAP);**
10. **Bind to the CAP binding site of promoter to upregulate transcription ;**
11. **Activators are regulated by the other molecules, e.g. cAMP;**
12. **This regulates the number of polycistronic mRNA which are transcribed and, in turn, regulate gene expression.**

Genomic level of control:

13. **Prokaryotic chromosomes associate with histone-like proteins and cause them to become condensed/more compact;**
14. **This regulates the accessibility of promoter sites of genes to RNA polymerase and sigma factors, hence regulating transcriptional rates.**

Teacher's Comments:

A number of students did not manage their time to complete/attempt this question during the examination. Hence, answers were usually incomplete or short. Please do exercise better time management for further assessments.

Misconceptions which were picked out in answers:

1. Enhancers and silencers are DNA sequences found in eukaryotes and not in prokaryotes (as asked in this question).
2. General transcription factors bind to eukaryotic promoters. Sigma factors are proteins which bind to prokaryotic promoters and recruit RNA polymerase to the promoter sequence.
3. The activity of the Trp protein was usually confused with that of the Lac repressor.
4. Question is focusing on transcription level of control. Hence, do not confuse it with translation/post-translational levels of control.