

NATIONAL JUNIOR COLLEGE, SINGAPORE  
Senior High 2  
Preliminary Examination  
Higher 1

CANDIDATE  
NAME

BIOLOGY  
CLASS

2bi2\_\_\_\_ / 2IPbi2\_\_

REGISTRATION NUMBER

## BIOLOGY

Paper 2

**8875/02**

**25 August 2017**

**2 hours**

Additional Materials: Answer Paper

### READ THESE INSTRUCTIONS FIRST

Write your name and Biology class on all the work you hand in.  
Write in dark blue or black pen.  
You may use an HB pencil for any diagrams or graphs.  
Do not use staples, paper clips, glue or correction fluid.

#### Section A

Answer **all** the questions.

#### Section B

Answer **one** question.

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

You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.

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

For Examiner's Use	
<b>Section A</b>	(Total: 40)
<b>1</b>	/ 12
<b>2</b>	/ 12
<b>3</b>	/ 7
<b>4</b>	/ 9
<b>Section B</b>	(Total: 20)
<b>5 or 6</b>	/ 20
<b>Total</b>	<b>/ 100</b>

This document consists of **15** printed pages.

## Section A

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

- 1 Fig. 1.1 shows the main steps involved in the synthesis of preproinsulin to insulin in the pancreatic  $\beta$ -cell. The preproinsulin is synthesised into the lumen of organelle **A** as proinsulin.

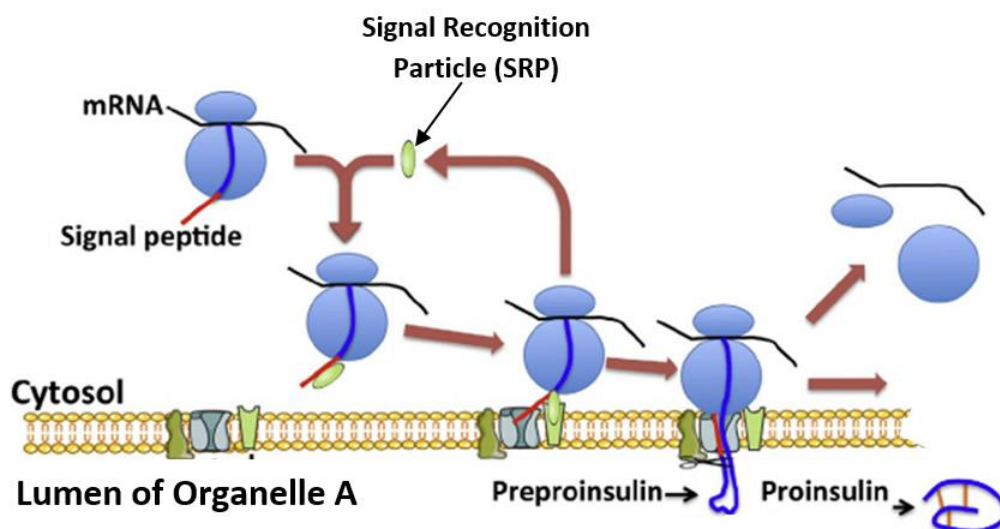


Fig. 1.1

The proinsulin is then be transported to organelle **B** where it is further processed to form insulin.

Fig. 1.2 shows the conversion of proinsulin to insulin in organelle **B**.

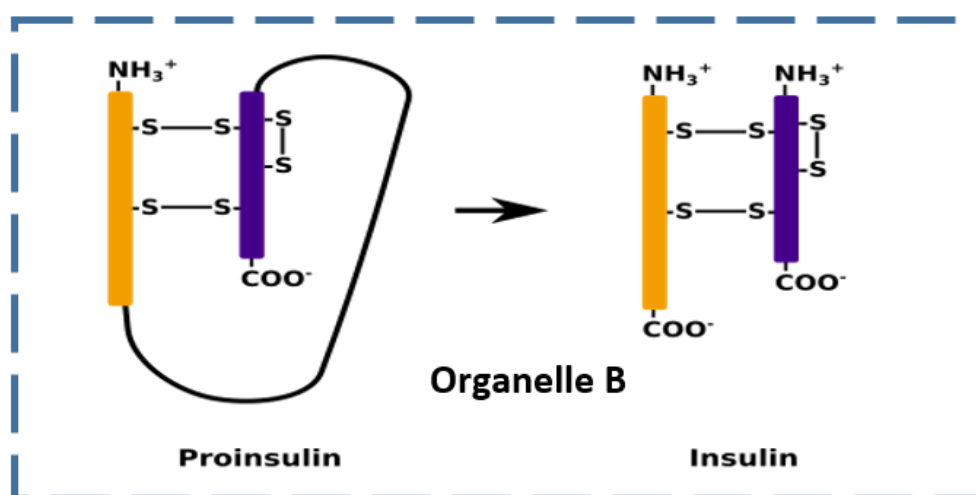


Fig. 1.2

- (a) Name the organelles labelled A and B.

Organelle A: **Rough Endoplasmic reticulum (Must spell in FULL)**

Organelle B: **Golgi apparatus / Golgi body**

[1]

- (b) State the role of rRNA in insulin protein synthesis

1. rRNA along with ribosomal proteins forms the structural component of ribosome (large and small sub-unit)
2. rRNA is responsible for catalytic function of ribosome in the formation of peptide bond between amino acids (found at the large sub-unit)
3. rRNA in the small ribosomal subunit binds to 5' end of mRNA sequence during protein translation
4. rRNA at the A site binds to the amino-acyl tRNA while the rRNA at the P site binds to the peptidyl-tRNA

**OWTTE**

**\* Any 2 of the above**

[2]

**Examiner's Comments:**

*Most students were able to get this part of this question right. It is crucial to use the key words when describing the catalytic sites found in the large ribosomal sub-unit. It was common to see among weaker students describing the process of protein synthesis without making reference to the role of rRNA.*

- (c) Insulin is released by pancreatic  $\beta$ -cell. Outline the route taken by proinsulin.

1. From Rough ER, a transport vesicle takes the Proinsulin to the Golgi apparatus (GA).
2. After chemical modification and packaging, a secretory vesicle pinched/buds off from GA.
3. The transport of secretory vesicles (aided by microtubules-the cytoskeletal elements) in the cytoplasm, until they fuse to the plasma membrane.
4. Secretory vesicle fuses with the cell surface membrane before releasing insulin by exocytosis.

**\* 3 points to get 2 marks. Pt 4 is crucial to talk about.**

[2]

**Examiner's comments:**

*Most students obtained partial mark for this question. In order to get full mark, they will need to mention that secretory vesicles are involved in releasing the insulin via exocytosis outside the pancreatic  $\beta$ -cell.*

Fig. 1.3 shows the structure of small sections of DNA and messenger RNA (mRNA) in the nucleus of pancreatic  $\beta$ -cell during transcription of the gene coding for insulin.

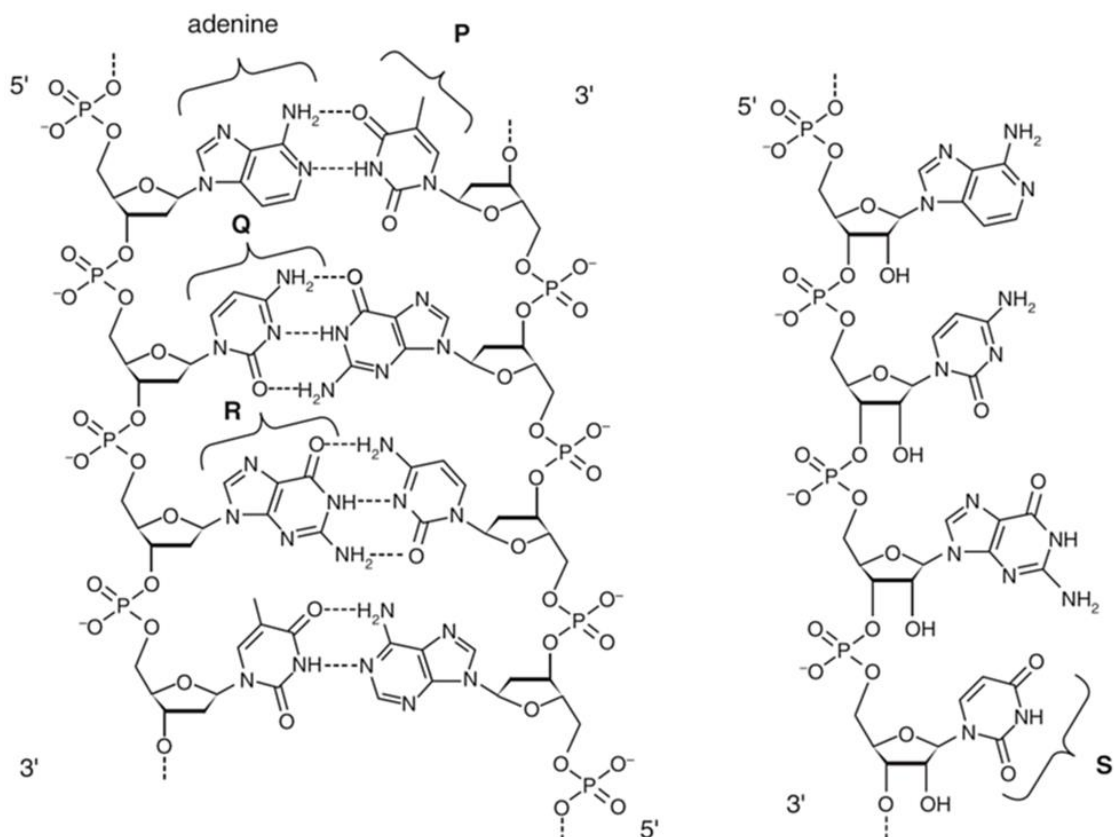


Fig. 1.3

(d) Name the bases **P** to **S**.

**P: Thymine , Q: Cytosine , R: Guanine , S: Uracil**

[2]

(all 4 correct – 2 marks, 2-3 correct – 1 mark)

(e) Describe how messenger RNA coding for insulin is synthesised in pancreatic  $\beta$ -cell.

1. **RNA polymerase recognises and binds to the promoter of the gene causing the DNA double helix to unwind / uncoil and separate.**
2. **One of the two DNA strands serves as template strand.**
3. **Free ribonucleoside triphosphates formed complementary base pairing (A,T,G,C) with ribonucleotides on the template DNA strand.**
4. **RNA polymerase catalyses formation of phosphodiester bonds between ribonucleotides through condensation reaction.**
5. **Transcription proceeds until after the RNA polymerase transcribes a termination sequence.**

[3]

- (f) Explain why gene mutations do not always produce mutated insulin protein whereas mutations of the splicing sites involved in RNA splicing will produce mutated insulin.

**Why gene mutations do not always produce mutated insulin: [1]**

1. Gene mutations that involve substitution may result in the same amino acid being coded for and due to the Degenerate code/ same amino acid can be coded for by different codons.
2. Gene mutation could occur at the intro region instead of exons.

**Why mutations at RNA Splicing sites will produce mutated insulin: [1]**

Mutation at the Splice site will affect the binding of spliceosome, and will affect the removal of introns & exons, hence giving rise to a mutated protein with loss of function.

**Example of the kind of mutations at RNA splicing sites and the outcome:**

An example of mutation at RNA splicing sites <i>(any one)</i>	Effect of such mutation (i.e. production of mutated collagen) <i>(any one)</i>
Different combinations of exons being produced	Different primary sequences of amino acids resulting in different protein (mutated protein)
An exon is lost / wrong excision of exons	Large number of bases and hence amino acids is lost/ as above
Introns not removed by spliceosome	Introns translated and became additional amino acids, this will lead to change the protein structure

[2]

***Examiner's comments:***

*Most students were able to get this question right. Do note that incorrect splicing (due to mutation at the splice sites) do not need to frame shift mutation.*

[Total: 12]

- 2 Clover is an important crop plant grown as food for sheep and cattle. It is a leguminous plant and its root nodules contain nitrogen-fixing bacteria.

Some clover plants can produce hydrogen cyanide when their tissues are damaged. This is a poisonous compound which will prevent herbivores such as slugs from feeding on the plant. Cyanide is also poisonous to the plants that produce them. Those plants that can produce cyanide are called cyanogenic; those that cannot are called acyanogenic.

Fig. 2.1 shows how the production of cyanide in a species of European clover is genetically controlled.

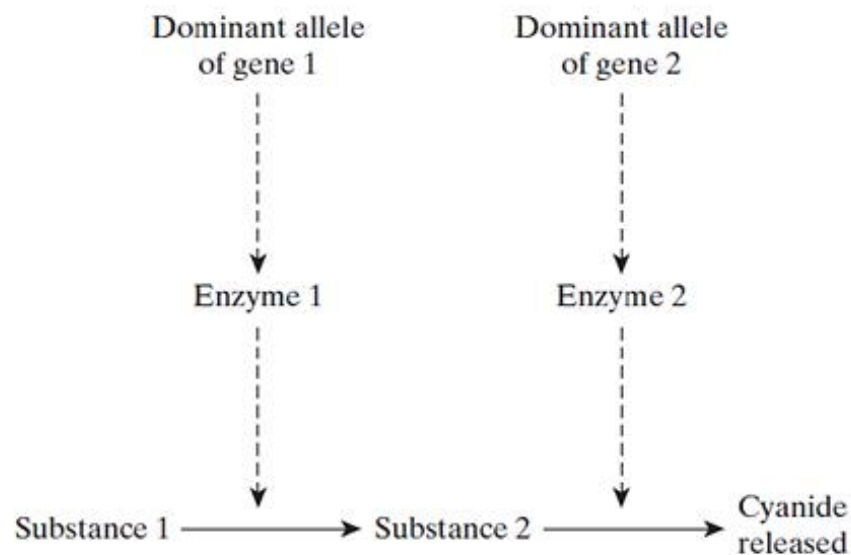


Fig. 2.1

- (a) Distinguish between gene and alleles.

- **Gene – sequence of bases that codes for a polypeptide/product**
- **Alleles –alternative forms of a gene / Have slightly different base sequences**
  - **Occupies the same gene loci**
  - **Codes for different products**

[2]

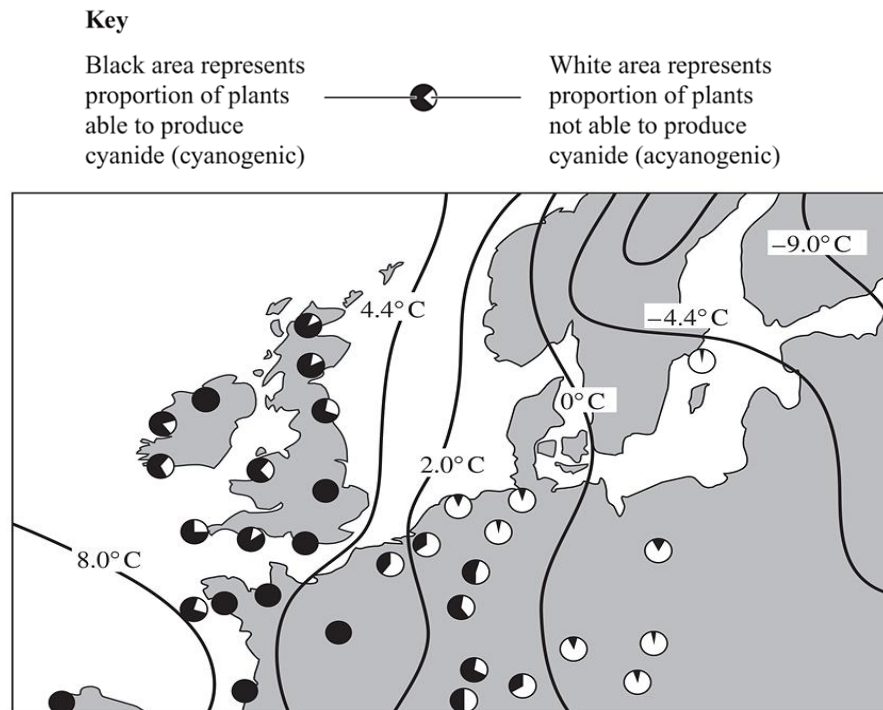
**Examiner's Comments:**

*Students were not able to recall the full definition. Only partial definitions were given by students.*

- (b)** Using appropriate symbols, show by means of a genetic diagram, the different genotypes and phenotypes obtained when two plants that are heterozygous at both the gene loci are crossed.

When the leaves of cyanogenic plants are damaged by slugs, or exposed to low temperatures, membranes within the cells are broken. This causes the release of the enzymes that control the reactions that produce cyanide.

Fig. 2.2 shows the proportions of cyanogenic and acyanogenic plants in clover populations in different parts of Europe and the mean minimum winter temperatures. It also shows isotherms, which are lines joining places with the same mean January temperature. Slugs are not usually active at temperatures below 0°C.



**Fig. 2.2**

(c) Explain how different proportions of cyanogenic and acyanogenic plants may have evolved in populations in different parts of Europe.

- Genetic variations/variation in gene/allele(s) are present in populations for cyanide production among these plants; [impt key point]
- Different environments (due to temp differences) have different selection pressure;
  - Cold areas, plants are acyanogenic: Selective advantage is not to produce cyanide releasing cyanide will kill itself! (Slugs are also not active at 0°C)
  - Warmer areas, plants are cyanogenic: Slugs present as selective pressure; cyanide production will kill slugs!
- At colder/below 0°C areas, cyanogenic plants die while non-cyanogenic survive; non-cyanogenic allele/gene passed on more often; [4]



- At Warmer areas, cyanogenic plants at selective advantage, because of less herbivore feeding; so cyanogenic have a higher probability of surviving and reproducing to pass on the advantageous cyanogenic allele/gene.
- The proportion of each population with advantageous alleles, altering allele frequencies. Proportion of the population exhibiting favourable traits will increase.

**Examiner's Comments:**

*This question was generally well answered. It is important to mention that the presence of genetic variation already existed in the population so that different proportion of cyanogenic and acyanogenic plants could have evolved is crucial here. This was often left out by many students. Similarly, the use of keywords such as "selective pressure, selective advantage" were also left out.*

(d) Explain using an example, how homology supports Darwin's theory of natural selection.

- **Homology / similarity in characteristics resulting from shared/common ancestry even though they may have different functions.**
  - E.g. Flipper of dolphin, forelimb of human, wings of bat, etc.

**Example of various types of homology:**

- **Analogical structures with similar functions but based on vastly different structures, and organisms do not share common ancestry.**
  - E.g. Fish fin and dolphin flipper.
- **Anatomical homology sharing common ancestry in aspect of morphology in form and structure.**
- **Embryological homology common ancestry based on similarity of developmental pathways (anatomical characteristics in embryos).**
  - e.g. (comparative embryology) - all vertebrate embryos have gill pouches on sides of throat.
- **Molecular homology common ancestry in molecular (DNA, amino acid sequences) makeup of related species.**
  - Ref. to amino acids sequences of cytochrome C / haemoglobin;
- **Conclusion: *Such homology* in very different organisms suggests a possible common ancestor**
- **Only descent with modification results due to differential survival and reproduction of organisms based on their environment with different selection pressure→ natural selection**

[2]

**Examiner's Comments:**

*Generally well answered. Students need to draw the connection between homology (with examples) and how that supports Darwin's theory of natural selection.*

[Total: 12]

**Note and other information (For your reference)**

- **Biogeography** – study of past and present distribution of individual species/ entire communities.
  - e.g. island biogeography & sugar gliders vs. flying squirrels.
  - many species are endemic to islands, most island species closely related to species from neighbouring islands / island hopping is the rise of new species when populations spread out.
  - species tend to be closely related to other species from same area than to others with same way of life in other areas / sugar gliders (Australia), flying squirrels (North America) are species with similar characteristics due to environmental factors but are not closely related (convergent evolution/ analogy).
  - By studying distribution of organisms/how they disperse, this determines if they are homologous and traces evolutionary pathway.
- **Fossil Records** – support evolution by showing succession of organisms shown within layers.
- **Deeper stratum (Rocks)** – older organism, transitional forms, also show depopulation / immigrations/ mass extinctions, but are often incomplete.

- 3 (a) Human newborns and hibernating mammals contain large amounts of brown adipose tissue ('body fat').

Fig. 3.1 shows the electron micrograph of a brown adipocyte. Brown adipocytes are characterised by presence of numerous vacuoles and organelle X throughout the cell.

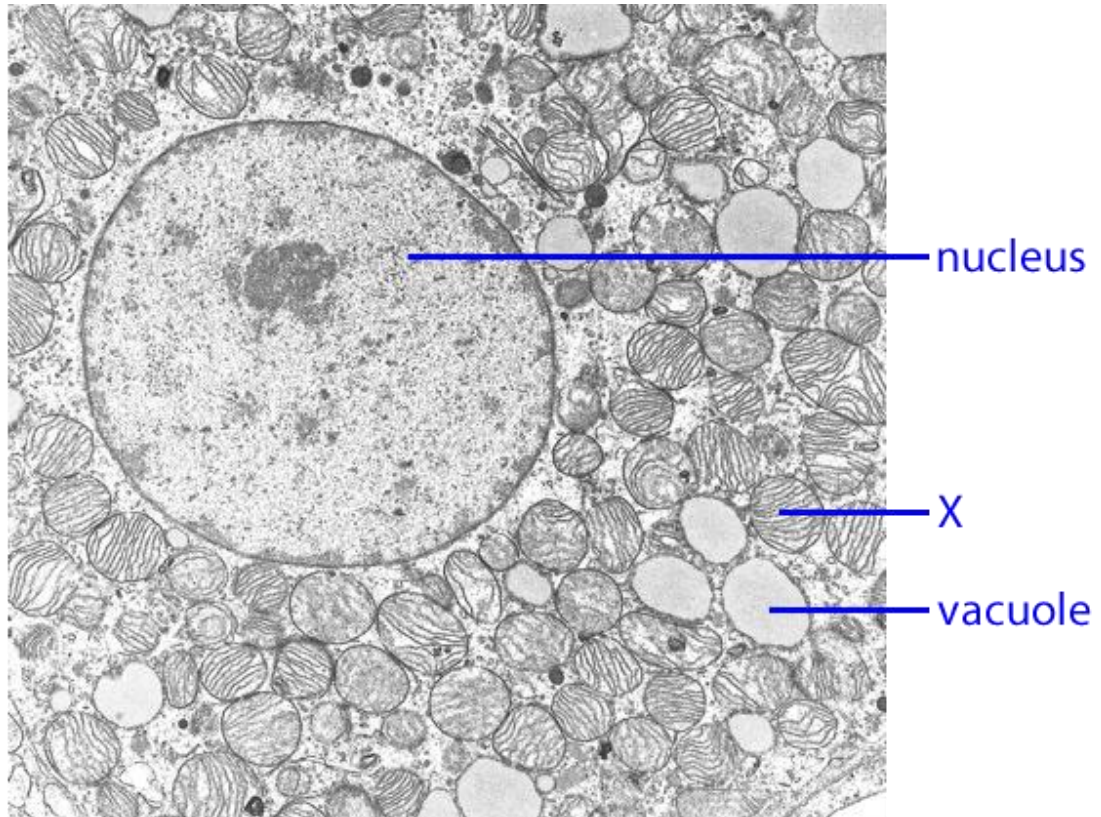


Fig. 3.1

- (i) Identify organelle X.

**mitochondrion**

[1]

**Examiner's comments:**

Majority of the students wrote "mitochondria" as the answer even when the line in Fig. 6.1 clearly pointed to one mitochondrion only. Students were not penalized this time. They should pay attention to singular / plural forms of naming when answering such questions next time.

- (ii) Suggest the role of the numerous vacuoles found in brown adipocytes.

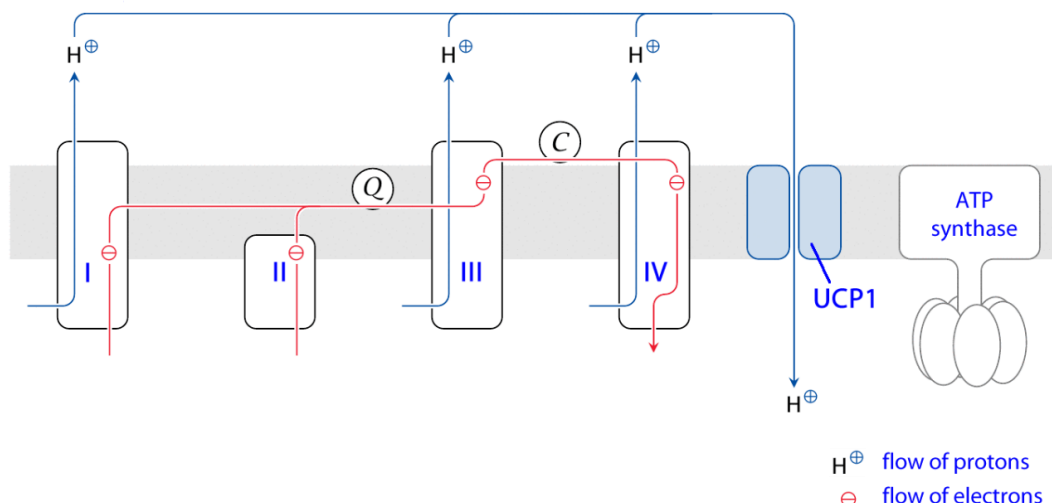
**They store lipids / triglycerides / fats.**

[1]

**Examiner's comments:**

Students who were mindful that the question context is about brown adipocytes were more likely to make a correct guess. Wrong answers were plentiful and varied.

- (b) Fig. 3.2 shows the schematic representation of a series of protein complexes found on the inner membrane of organelle X.



**Fig. 3.2**

- (i) Oxygen is required to sustain the process illustrated in Fig. 3.2.

With reference to the Fig. 3.2, describe the role played by oxygen.

**Oxygen serves as the final electron acceptor, receiving electrons from complex IV to form water.**

[1]

**Examiner's comments:**

*Majority of the students were familiar with the role of oxygen in aerobic respiration. However, many did not get the mark as they failed to make explicit reference (e.g. quote "IV") to Fig. 6.2 and only described in general terms.*

- (ii) Brown adipocytes contain a unique protein, UCP1, which is not found in organelle X in any other cell type.

Evaluate the impact of UCP1 on the normal functioning of the process illustrated in Fig. 3.2 and suggest the physiological significance of brown adipose tissue.

- 1. As UCP1 allows protons to leak back into the matrix without passing through the ATP synthase, no ATP will be synthesized from the NADH and  $\text{FADH}_2$ .**
- 2. The energy released from the spontaneous flow of protons through UCP1 is lost as heat, which helps to keep the organisms warm.**

[2]

**Examiner's comments:**

*Many students could get Point 1, but failed to suggest the correct physiological significance of brown adipose tissue (i.e. Point 2). No ATP synthesis does not mean that the respiratory substrates would be reserved for use during hibernation. So long as oxygen is present, the first three stages of aerobic respiration will still occur to produce NADH and FADH<sub>2</sub> for electron transport to occur at the inner mitochondrial membrane. The lipids in brown adipose tissue will still be broken down, not for ATP synthesis but for non-shivering thermogenesis (heat production), which is important for regulating body temperature of human newborns and hibernating mammals.*

- (c) In other cell types, NADH and FADH<sub>2</sub> are used to drive ATP synthesis by ATP synthase.

Using relevant information from Fig. 3.2, suggest and explain why more ATP is produced from NADH.

1. NADH and FADH<sub>2</sub> donates electrons to complex I and II respectively, the energy released from transfer of electrons through the complexes is used to pump protons across the inner membrane.
2. Because NADH started with Complex I, it had more chances to pumps more protons across the gradient, which powers the ATP synthase and gives us 3 ATP per molecule of NADH, while FADH<sub>2</sub> produces 2 ATP during the ETC because it gives up its electron to complex II, bypassing complex I.

[2]

[Total: 7]

4 Fig. 4.1 shows the life cycle of a water flea.

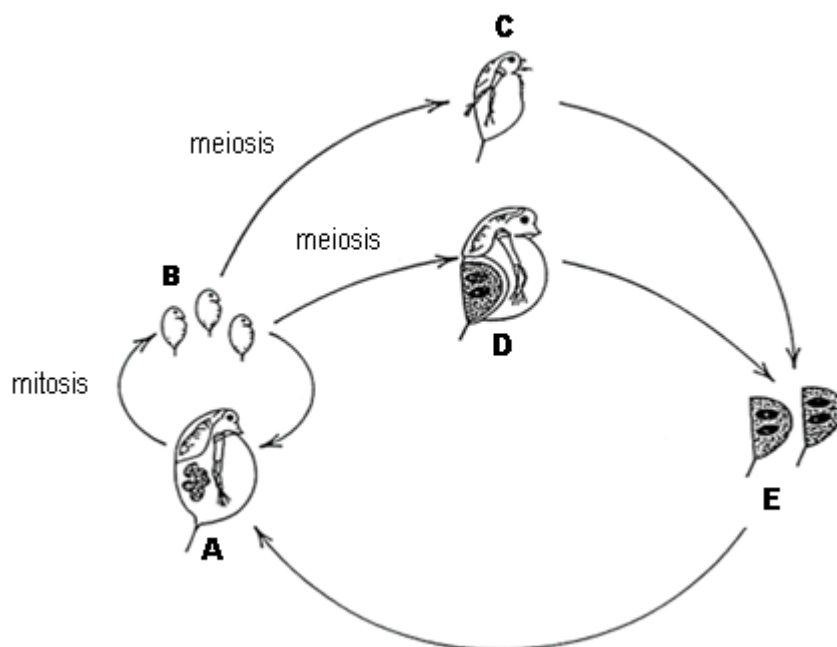


Fig. 4.1

In favourable conditions, all the animals in a population are females (**A**). These females produce eggs by mitosis, which develop into young females (**B**) without being fertilized. In unfavourable conditions, eggs produced by meiosis develop directly without fertilization into either males (**C**) or females (**D**). The eggs produced by the females (**D**) are fertilized by the sperms from the males (**C**), then released in protective egg cases (**E**) which enable them to survive unfavourable conditions. When favourable conditions return, these eggs develop back into females (**A**).

- (a) The females at stage **A** of the life cycle have 18 chromosomes.

Complete the table to show the number of chromosomes at the other stages of the life cycle.

stage of life cycle	chromosome number
<b>A</b>	18
<b>B</b>	18
<b>C</b>	9
<b>D</b>	9
<b>E</b>	18

[1]

**Examiner's comments:**

Weaker students gave all kinds of answers. They should read the paragraph below Fig. 3.1 very carefully before filling up the table.

(b) Explain why the eggs from **D** and the sperms from **C** must be produced by mitosis.

1. Since **C** and **D** (developed from unfertilised eggs from **B**) are haploid, mitosis ensures that the haploid chromosome number is preserved / the eggs and sperms are haploid.
2. Thus, when the haploid sperm and haploid egg fuse, the original diploid chromosome number is restored.

[2]

**Examiner's comments:**

*On the whole, the question was interpreted correctly by majority of the students. To score Point 1, it should be clear in the answer that both **C** and **D** are haploid to begin with. It is irrelevant to state the general definition or roles of mitosis. Some students showed critical thought by explaining that it is not possible for a haploid cell to undergo meiosis as there is only one copy of each type of chromosome (pairing of homologous chromosomes is not possible). Weaker students mistook "the eggs from **D** and the sperms from **C**" as "the eggs produced by meiosis in **B** that developed into **C** and **D**" and wrote answers that went off tangent.*

(c) Explain why females **A**, developed from fertilized eggs **E**, are genetically different from each other.

(Any 3)

1. **C** and **D** developed from eggs that are produced by meiosis in **B**.
2. Crossing over between non-sister chromatids of homologous chromosomes at prophase 1 of meiosis
3. Independent assortment of homologous chromosomes at metaphase 1 of meiosis
4. Independent assortment of non-identical chromatids at metaphase 2 of meiosis
5. Random mating between **C** and **D**
6. Mutations can occur at any time.

[3]

**Examiner's comments:**

*Students who did well knew that this question was about how genetic variation could be produced in water fleas. Weaker students mainly lost marks due to careless reading of the paragraph below Fig. 3.1 or incorrect use of key words related to meiosis. Some students misinterpreted the question as why **A** is genetically different from **E**. They failed to realize that **A** and **E** should be genetically identical since each fertilized egg **E** would develop into a female **A** (just like how your first cell developed into the present multicellular you). They should be explaining why females **A** are genetically different from each other. It should also be noted that random fertilization or random fusion of gametes from **C** and **D** would not produce further genetic variation in this case as the gametes from **C** and **D** are produced by mitosis.*

- (d) Give an example of a favourable condition in which females will develop from eggs formed via mitosis.

(Any 1)

- Presence of water in a previously dry pond
- Reasonably high temperature (~20°C)
- Abundant food source
- Lack of competition
- Stable environment
- Few or no predators
- Appropriate photoperiod
- Water of optimal pH
- Suitable salinity
- (any other valid point)

[1]

**Examiner's comments:**

*This question was generally well done with "abundant food source" and "few or no predators" as the most common answers.*

- (e) The eggs of the water flea are produced by stem cells in the ovary.

Explain what makes a stem cell unique from a normal adult cell in the water flea.

(Any 2)

1. capable of indefinite self-renewal
2. unspecialised / undifferentiated
3. can give rise to specialised cells through differentiation

[2]

**Examiner's comments:**

*Most students are familiar with the unique properties of a stem cell. However, some students wrote that "stem cell could give rise to any type of cells" without realizing that only totipotent stem cells can do that. Do note that this question requires unique properties that apply to ALL types of stem cells.*

[Total: 9]



## Section B

Answer EITHER 5 OR 6.

Write your answers on the separate answer paper provided.

Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.

Your answers must be in continuous prose, where appropriate.

Your answers must be set out in sections **(a)**, **(b)** etc., as indicated in the question.

- 5 (a) Describe how the molecular structure of phospholipids is related to their function in the plasma membrane. [6]

(Any 6)

- [1F1] Each phospholipid consists of a phosphate group, a glycerol backbone and two fatty acid chains.
- [1F2] Each phospholipid is amphipathic / contains both a hydrophilic region and a hydrophobic region within the same molecule.
- [1F3] Hydrophilic phosphate heads are on the outside of the bilayer, in contact with the surrounding aqueous medium.
- [1F4] Hydrophobic fatty acid chains point towards the interior of the bilayer, away from the surrounding aqueous medium.
- [1F] Major component of the plasma membrane / Form a bilayer
- [2F] Selectively permeable to solutes due to presence of hydrophobic core in the bilayer
- [3F] Determine the fluidity of membrane
- [3F1] The more unsaturated fatty acid chains are, the more fluid the membrane is.
- [3F2] Kinks in unsaturated fatty acid chains prevent close packing of the phospholipids and decrease the interaction between adjacent fatty acid chains.
- [3F3] Phospholipids with shorter fatty acid chains are more fluid.
- [3F4] Shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another.
- [4F] Some types of phospholipid can be split to produce products that function as second messengers in signal transduction.

**Examiner's comments:**

*Students who did well kept their answers to the scope of the structure-function relationship of phospholipids. Some students went off tangent by writing about the structure-function relationship of plasma membrane and even the role of cholesterol in regulating membrane fluidity.*

- (b) Explain the mode of action of enzymes in terms of specificity and activation energy. [8]

**Specificity:**

- [S1] catalyse only one particular reaction
- [S2] act on molecules that have specific functional groups / act on particular type of chemical bonds / act on a particular stereo or optical isomer
- [S3] lock and key hypothesis
- [S4] enzyme's active site is the "lock" while substrate is the "key"
- [S5] shape of substrates must be complementary to that of the enzyme active site

OR

- [S6] induced fit hypothesis
- [S7] substrates enter the active site, induces a conformational change in the enzyme, substrates fit more snugly into the active site
- [S8] shape of the active site of enzyme may not be exactly complementary to that of substrate

**Activation Energy:**

- [E1] definition: the energy barrier that has to be overcome before a reaction can take place to form products
- [E2] enzymes lower the activation energy
- [E3] substrate molecules bind to the enzyme molecule at the active site to form enzyme-substrate complexes
- [E4] the enzyme molecule holds the different substrate molecules in an arrangement that forces them closer together in the correct orientation
- [E5] the proximity of the substrates within the enzyme-substrate complex greatly increases the probability of a reaction occurring
- [E6] certain bonds in the substrate molecule may be placed under physical stress
- [E7] the R-group of amino acid residues at the active site can change the charge on the substrate which will increase the reactivity of the substrate

**Examiner's comments:**

*Insufficient elaboration and inappropriate phrasing are the two key reasons why students lost marks for this question.*

- (c) Explain the effects of competitive and non-competitive inhibitors on the rate of enzymatic activity. [6]

**(Max 3m for Competitive Inhibitors)**

- [C1] inhibitor is similar in shape / structure with the substrate
- [C2] competes for and binds at / occupies the active site of the enzyme
- [C3] thus blocking the substrate from binding with the enzyme
- [C4] number of enzyme-substrate complexes formed per unit time decreases
- [C5] effect of inhibition can be overcome by increasing substrate concentration
- [C6]  $V_{\max}$  of uninhibited reaction can be reached

**(Max 3m for Non-competitive Inhibitors)**

- [N1] inhibitor has no structural similarity to the substrate
- [N2] does not compete with substrate for binding to active site
- [N3] binds to a site away from the active site on enzyme
- [N4] Enzyme is still able to bind with the substrate at the active site but catalysis is unable to take place.
- [N5] effect of inhibition cannot be overcome by increasing substrate concentration
- [N6]  $V_{\max}$  of uninhibited reaction can only be reached with an increase in enzyme concentration while keeping inhibitor concentration constant.

***Examiner's comments:***

*Students could score high or even full marks for this question if not for careless mix-up of where each type of inhibitor binds to.*

[Total: 20]

- 6 (a) Describe the polymerase chain reaction and explain the advantages and limitations of this procedure. [8]

### Procedure of PCR [4]

#### Purpose of PCR:

- PCR allows for the amplification of a specified segment of DNA in vitro.
- 5 Components required for PCR are Template DNA, primers (DNA in nature), Taq polymerase, dNTPs (Nucleotides- A;T;G;C) and buffer

#### Procedure of PCR [4]

1. There are 3 main steps in PCR: Denaturation, Primer annealing and Extension step
2. Denaturation Step: [1]
  - Heat treatment (up to 95°C) to break the hydrogen bonds holding double stranded DNA together to form 2 separate strands of DNA
  - Each strand will act as a template for the synthesis of its complementary strand (daughter strand)
3. Primer annealing Step: [1]
  - subsequent cooling of DNA (55 °C - 64°C) in the presence of excess DNA primers allows their specific attachment to their complementary DNA.
  - Two types of DNA primers are used, the forward and reverse primers which will bind to 3' end of the target DNA sequence to be amplified.
4. Elongation or Extension step: [1]
  - Taq polymerase performs synthesis of the complementary DNA strand at up to 72°C, the optimal temperature of this enzyme
  - Taq polymerase catalyzes the synthesis of the new complementary stand by addition of free deoxynucleotides to the 3' end of the primer
  - The primers provide free 3'OH for DNA polymerase to add new dNTPs to elongate the newly synthesised strand.
5. Repeat the 3 steps all over again for another 25-30 cycles: Each denaturation – hybridization - synthesis cycle results in a doubling in number of the DNA sequence being replicated

#### Advantages [2]

1. The amount of desired sequences increase exponentially, e.g. n cycles will yield 2<sup>n</sup> strands of target DNA.
2. Fast and efficient way to amplify. This technique is also fully automated within the thermocycler, thus it is a convenient way to amplify DNA.
3. PCR is highly sensitive such that a target sequence can be amplified even when a minute amount of DNA source is available
4. The large amount of desired DNA sequence can be used in clinical diagnosis e.g. genetic screening of cystic fibrosis and early detection of HIV
5. Amplified DNA samples can be used for forensic analysis, archaeology and palaeontology

**Limitations [2]**

1. Not so accurate: Taq polymerase lacks 3' to 5' proofreading ability and this makes it impossible for the polymerase to check if the base inserted is the correct one
2. Success of PCR requires knowledge of the sequences flanking the target region to be amplified. If the flanking sequences of a gene of interest are unknown, no proper primers can be synthesized, and PCR cannot be used.
3. Limited length of DNA fragments which can be amplified. DNA fragments to be amplified are limited to about 3kb. (3000 base pairs)

**Examiner's Comments:**

*This question was well answered. Students were aware of the main stages involved in PCR. Some minor details such as the purpose of the respective steps were left out in weaker students.*

- (b) Explain the significance of genetic engineering in improving the quality and yield of crop plants and animals and also in solving the demand for food in the world (e.g. Bt corn, golden rice and GM salmon). [6]

**Define genetic engineering** - the application of recombinant DNA technology to introduce genetic material/ foreign genes in order to alter the hereditary traits/ genetic makeup of a cell, organism, or population [1]

**State one example from both Quality & Yield + explain how it is significance. [5 marks]**

**Improved yield e.g. Bt corn [2]**

- The gene of interest is derived from the soil bacterium, *Bacillus thuringiensis*. The bacteria produces a protein called Bt toxin (Cry proteins) that kills European corn borer.
- This gene codes for Cry proteins is inserted into corn plant to form BT corn.
- This Cry protein acts as insect stomach poisons that must be eaten to kill the insect. Once eaten, the insect's own digestive enzymes activate the toxic form of the protein. The Cry proteins bind to specific receptors on the intestinal lining and rupture the cells. Within hours, the gut wall breaks down and normal gut bacteria invade the body cavity where they multiply and cause sepsis and subsequent death of the organism within 2 or 3 days.
- Genetically modified Bt maize has revolutionized pest control and many farmers have benefited financially.
- As this toxin is lethal to the pest but harmless to other animals, this Bt corn allows farmers to control pest infestations in order to reduce crop losses.
- Growers create Bt corn as an alternative to spraying insecticides for control corn borer.

### Improved quality e.g. golden rice [2]

- Vitamin A deficiency is the leading cause of preventable blindness in children.
- Rice grain, which serves as a food staple for much of the world do not contain vitamin A naturally.
- It was discovered that geranyl geranyl diphosphate (GGPP) found in rice seed can be a precursor to carotenoid production. Beta-carotene and other carotenes (the red, yellow, and orange pigments found in carrots and other vegetables) are natural precursors (inactive form) of vitamin A.
- Thus it is possible to genetically engineer a new breed of rice variety, golden rice which can express the enzymes necessary for the conversion of GGPP to beta-carotene.
- To engineer golden rice, genes coding for phytoene synthase (isolated from plant) and phytoene desaturase (isolated from bacteria) must be introduced into the rice plant cells. These enzyme-coding genes catalyze the biosynthesis of beta-carotene from precursor GGPP in the endosperm (edible part of the grain)
- A bacterium, *Agrobacterium tumefaciens*, containing a Ti plasmid, was used to introduce all the enzyme-coding genes necessary for the complete biochemical pathway for beta-carotene production. OR another way of introducing DNA into plant cells is through DNA coated particles that are literally shot through the cell wall using a modified gun. This is commonly referred to as the use of a 'gene gun'.

### Improved yield e.g. GM salmon [2]

- Recombinant DNA composed of an antifreeze promoter from an ocean pout and a growth hormone gene from a Pacific Chinook salmon is synthesized.
- Fusing of a strong gene promoter such as the ocean pout antifreeze promoter leads to enhancement in the expression of the gene construct.
- The recombinant DNA is then introduced into fertilized eggs of Atlantic salmon. Subsequent selection and breeding led to development of the genetically modified salmon.
- Due to the year-round production of growth hormone (due to the antifreeze promoter), this allows for continuous feeding and growth of the GM salmon.
- The GM salmon is able to grow quicker in size while feeding more efficiently (less feed is consumed to reach a larger size).

### **Examiner's Comments:**

*This question was generally well answered. Students generally knew what the examples were. The stronger candidates were able elaborate and provide the details for each examples.*

- (c) Describe the natural functions of restriction enzymes and explain how they can be used in the process of gene cloning. [6]

**Natural functions of Res [1]**

1. REs are naturally found in and isolated from bacteria and its natural function is to provide a protective mechanism to help bacteria resist attack from bacteriophages
2. REs work by recognizing specific sequences in the phage DNA and cleaving those sequences thereby degrading the incoming viral DNA
3. thus preventing / restricting the bacteriophage from succeeding in causing a full-blown bacterial infection

**How REs can be used in gene cloning [5]**

1. REs each binds to a double-stranded DNA molecule at a specific DNA sequence / restriction site and makes a double-stranded cut at or near that sequence [1]

**Choice of RE for extracting Gene of interest / Cut a vector [2]**

2. A chosen RE is used to cleave the gene donor / chromosome / cDNA library to isolate the gene of interest
3. The same RE is used to cleave the cloning vector / plasmid making the plasmid DNA linear to facilitate subsequent insertion of the excised gene of interest into plasmid

**Types of Ends Created [2]**

4. Usually, the chosen RE will cleave the DNA in a staggered manner resulting in short single-stranded overhangs / sticky / cohesive ends at each end of the molecule
5. sticky-ended fragments of gene of interest are incubated with complementary sticky-ended linearized vector fragments under conditions that favour annealing so as to facilitate the formation of a recombinant plasmid by DNA ligase
6. If the chosen RE make a cut in the middle of the recognition sequence resulting in blunt / flush ends. For blunt ends, adaptors will have to be ligated by DNA ligase to the ends of gene of interest and plasmid fragments to generate sticky-ended fragments

**Examiner's comments:**

*This question is generally well answered. The natural functions of restriction enzymes were often left out among the students.*

[Total: 20]