



**TEMASEK JUNIOR COLLEGE**  
**Preliminary Examinations 2016**  
**Higher 1**

CANDIDATE  
NAME

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CENTRE  
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**BIOLOGY**

**8875/02**

Core Paper

**Wednesday, 31 August 2016**

Paper 2

**2 hours**

Additional Materials: Writing Paper

**READ THESE INSTRUCTIONS FIRST**

Write your Centre number, index number and name on all the work you hand in.

Write in dark blue or black pen on both sides of the paper.

You may use a soft pencil for any diagrams, graphs or rough working.

Do not use staples, paper clips, highlighters, glue or correction fluid.

DO **NOT** WRITE IN ANY BARCODES.

Section A

Answer **all** 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 any working or if you do not use appropriate units.

At the end of the examination, fasten the writing papers securely and hand up separately.

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

For Examiner's Use	
Q1	/ 10
Q2	/ 10
Q3	/ 10
Q4	/ 10
Essay	/ 20
Total	/ 60

1 Fig. 1.1 shows the cell cycle.

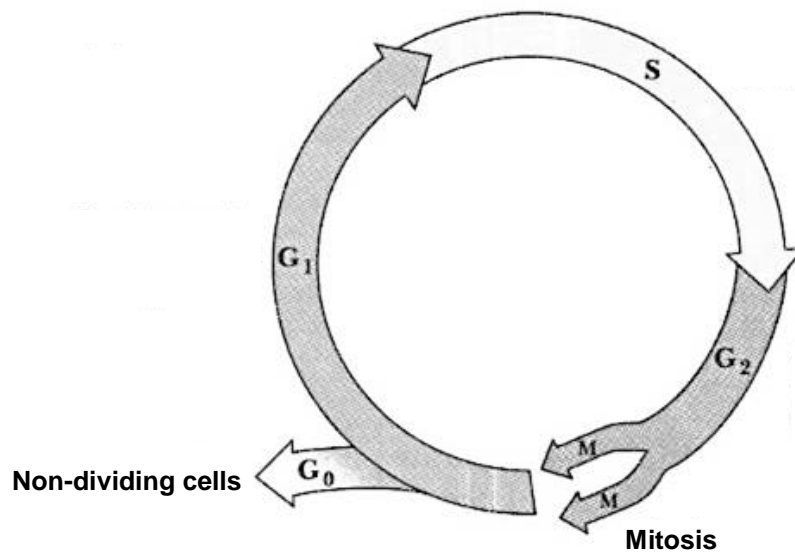


Fig. 1.1

(a) Describe the events that occur during **S** and **G<sub>2</sub>** phases. [2]

1. **S (synthesis) phase:** Replication of DNA occurs. This doubles the DNA content of the cell
2. **G<sub>2</sub> (second "gap") phase:** Synthesis of proteins – These proteins include histones, ribosomal proteins and proteins which later make up the microtubules of the spindle.  
OR  
Formation of new organelles (e.g. mitochondria, centrioles, chloroplasts, ribosomes).

(b) Mitosis, a form of nuclear division, occurs after the **G<sub>2</sub>** phase. This process produces genetically identical cells.

Explain the need for the production of genetically identical cells. [2]

1. Genetically identical cells contain the same number and type of chromosomes.

Mitosis is needed to produce genetically identical cells

2. during the growth and development of a multicellular organism, e.g. in the development of a fertilized egg into an adult human being.
3. And during the replacement of worn-out parts of the body.
4. Mitosis is the basis of asexual reproduction, where parents and offspring are genetically identical.

Sometimes, errors during cell division can lead to genetically non-identical cells, causing the production of polyploid cells. Polyploidy is common in plants, and one such example is the tetraploid (4n) snapdragons.

(c) State what is meant by *polyploidy*. [1]

Cells with one or more extra sets of chromosomes [3n, 4n etc.].

(d) Explain how a tetraploid cell may be formed due to errors during mitosis. [4]

1. DNA replication occurs prior to mitosis,
2. hence the DNA content is doubled in the parental cell.
3. However, during metaphase
4. spindle fibers fail to attach to chromatids / spindle fibers are not formed / spindle fibers are not stretched across the cell properly
5. Thus, during anaphase, centromeres divide,
6. But sister chromatids are not pulled to opposite poles
7. Cytokinesis fails to occur.
8. Thus, there is double the number of chromosomes in the daughter cell.

Triploid plants, such as bananas, are sterile as they do not produce gametes, and are hence seedless.

(e) Suggest how farmers can grow bananas without seeds.

Stem cuttings / plant cloning / micropropagation / plant tissue culture

[Total: 10]

2 Fig. 2.1 is a diagram showing the process of translation in a cell.

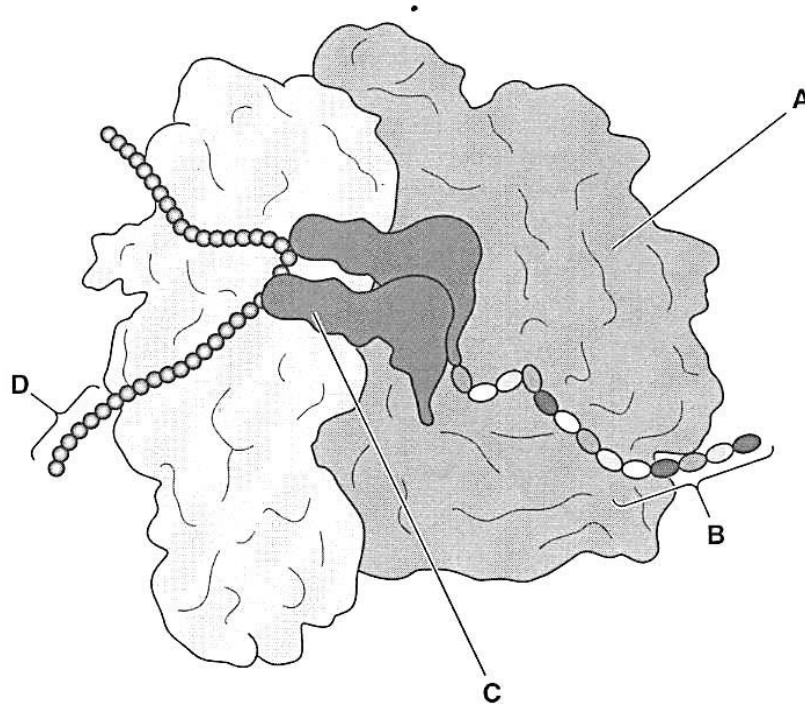


Fig. 2.1

(a) Identify A, B and C. [3]

A – large (60S) subunit of ribosome

B – polypeptide chain / chain of amino acids / amino acid residues

C – tRNA

(b) Briefly describe how the amino acids are joined in the correct order during translation. [4]

1. The first amino acyl-tRNA complex with the anticodon UAC binds to the start codon (AUG) on mRNA. This complex is positioned at the “P” site of the ribosome.
2. tRNA with the anticodon UAC always carries the amino acid methionine. Methionine is always the first amino acid in a polypeptide chain.
3. The second amino acyl-tRNA complex binds to mRNA. Its anticodon is complementary to the second codon on mRNA. The complex is held at the “A” site of the ribosome.
4. Formation of a peptide bond between the first and the second amino acids then occurs, utilising energy from hydrolysis of GTP (a molecule similar to ATP). This is catalysed by the peptidyl-transferase (RNA enzyme) which is present on the large subunit of the ribosome.
5. Once the peptide bond is formed, the ribosome moves along the mRNA to the next codon. / 5' to 3' direction / that the ribosome “reads” the mRNA in the 5' to 3' direction.
6. The first tRNA, now at the “E” site, is then released into the cytoplasm for it to be recycled. It can be attached again to their respective amino acids.

7. The second amino acyl-tRNA complex thus moves from the “A” site to the “P” site, leaving the “A” site empty for the next amino acyl-tRNA complex, with anticodon complementary to the third codon along the mRNA.
8. (Idea : each tRNA carries a specific amino acid)

Note that polypeptide chain is synthesized from the amino to carboxyl end. The process is repeated until the ribosome reaches the “stop” codon on the mRNA.

Parasitic infections affect about one billion people. Scientists are exploring new ; parasitic drugs that target the translation process in parasites. One such target is aminoacyl-tRNA synthetases.

(c) Explain how drugs targeting aminoacyl-tRNA synthetases are effective. [2]

1. Drug inhibits aminoacyl-tRNA synthetases. (OWTTE)
2. Aminoacyl-tRNA synthetases cannot ensure the correct / specific amino acid is joined to a specific tRNA. / Therefore cannot form the specific aminoacyl tRNA complexes.
3. It is unable to catalyse formation of ester / covalent bond between tRNA and amino acid with expenditure of ATP.
4. Therefore cannot form functional protein in the parasite.

(d) Suggest **one** way how the drugs act on aminoacyl-tRNA synthetases. [1]

1. Bind to anticodon binding site to block binding to tRNA
2. Bind to the amino acid binding site to block binding of amino acid
3. Bind to an allosteric site to change shape of active site.
4. Bind to ATP binding site to block the binding to ATP

[Total: 10]

- 3 Rats and mice are common pests. Warfarin was developed as a poison to control rats; was very effective when first used in 1950. Resistance to warfarin was first reported in E rats in 1958 and is now extremely common. Warfarin resistance in rats is determined single gene with two alleles,  $W^S$  and  $W^R$ , which show codominance.

The  $W^S$  allele confers susceptibility. In areas where warfarin is used to kill rats, those that homozygous for this allele would not survive.

The  $W^R$  allele confers resistance. Rats that are homozygous for this allele are resistant to warfarin but have a very high demand for vitamin K and would rarely survive.

Rats which are heterozygotes are also resistant to warfarin and yet have a lower demand for vitamin K for full health.

(a) Explain what is meant by *codominance*. [1]

- A pattern of inheritance in which both alleles are expressed and contribute equally to the phenotype of the heterozygote.

(b) Rats homozygous for the resistance allele rarely survive. Describe the role of natural selection in maintaining the relatively high **frequency of the  $W^R$  allele** in the population after **1950**. [3]

1. After 1950, warfarin was used as pesticide, and acted as a selection pressure.
2. Heterozygous rats were selective advantage.
3. These rats were resistant to warfarin but required a lower amount of vitamin K to survive.
4. These survived to reproductive age to produce viable offspring and
5. pass their favourable allele /  $W^R$  allele to their offspring.
6. More rats in the population with the favourable allele /  $W^R$  allele

- (c) Draw a genetic diagram to show the offspring of a cross between rats which survive well in the presence of warfarin and shortage of vitamin K.

Parental phenotype:                      Rats resistant to warfarin with low demand of vitamin K                      ×                      Rats resistant to warfarin with low demand of vitamin K

Parental genotype:                       $W^R W^S$                       ×                       $W^R W^S$

Parental gametes:                       $W^R$                        $W^S$                       ×                       $W^R$                        $W^S$

Punnett Square:

	$W^R$	$W^S$
$W^R$	$W^R W^R$ Rats resistant to warfarin with high demand of vitamin K	$W^R W^S$ Rats resistant to warfarin with low demand of vitamin K
$W^S$	$W^R W^S$ Rats resistant to warfarin with low demand of vitamin K	$W^S W^S$ Rats susceptible to warfarin

Offspring genotype:                       $W^R W^R$                        $W^R W^S$                        $W^S W^S$

Offspring phenotype:                      Rats resistant to warfarin with high demand of vitamin K                      Rats resistant to warfarin with low demand of vitamin K                      Rats susceptible to warfarin

Phenotypic ratio of offspring:                      1                      :                      2                      :                      1

[4]

In humans, death from conditions where blood clots form inside blood vessels occur frequently in adults over the age of 50. These conditions may be treated successfully with warfarin. However, some people possess a dominant allele which gives resistance to warfarin and blood clots cannot be prevented.

- (d) Explain why the frequency of this allele does **not** decrease in the human population even though warfarin is commonly used to treat blood clots. [2]

1. This is because the clotting condition usually occurred in adults over the age of 50, which is past the reproductive age.

2. The dominant allele / allele for warfarin resistance would have been passed to the next generation

**B:** Even if the allele confers any selective disadvantage to individuals, Therefore the allele is not subjected to natural selection.

[Total: 10]

- 4 GM crops are genetically modified crops that have had specific changes introduced into their DNA. This gives the GM crops unique traits that are not found naturally.

Table 4.1 shows the use of agricultural land devoted to the cultivation of GM crops, from year 1996 till 2006.

**Table 4.1**

Year	Global area of genetically engineered crops (million hectares)		
	Trait		Total Land Use
	Herbicide-tolerant (HT)	Insect-resistant (IR)	
1996	0.6	0.4	1.1
1997	6.9	1.1	8.1
1998	19.8	4.5	24.4
1999	28.1	7.7	35.9
2000	32.7	8.9	41.7
2001	40.6	10.1	50.8
2002	44.2	8.3	53.0
2003	49.7	7.5	58.1
2004	58.6	5.2	65.1
2005	67.7	2.3	71.7
2006	79.1	1.3	82.5

- (a) With reference to Table 4.1, compare the trends shown by the global area of herbicide-tolerant and insect-resistant crops over the years. [2]

1. From 1996 to 2001, there is an increase in the cultivation of both herbicide-tolerant (HT) and insect-resistant (IR)crops;
2. increasing from 0.6 million hectares in 1996 to 40.6 million hectares in 2001 for HT crops and increasing from 0.4 million hectares in 1996, to 10.1 million hectares for IR crops in 2001;



3. From 2001 to 2006, there was an increase in cultivation of HT crops, but a decrease in cultivation of IR crops;
4. increasing from 40.6 million hectares in 2001 to 79.1 million hectares in 2006 for HT crops but decreasing from 10.1 million hectares in 2001, to 1.3 million hectares for IR crops in 2006

(b) Discuss the social and ethical issues concerning the growing of

(i) herbicide-tolerant crops; [2]

Effect of herbicide-tolerant crops on biodiversity or environment

1. Crops genetically-engineered to withstand high environmental herbicide concentrations may grow very quickly and establish themselves as weeds/ superweeds, outcompeting the species in the wild and presenting a threat to food chain and biodiversity/ OWTTE
2. Cross-pollination among closely-related species spread the gene that confers herbicide resistant, thus changing the gene pool and presenting a threat to biodiversity/ OWTTE

Effect of herbicide-tolerant crop on human health and safety

3. There is a potential of health risks, eg. allergy caused by (foreign) proteins present in herbicide-tolerant crop or its pollen, as there lacks comprehensive and conclusive studies that prove consumption is indeed safe.
4. The growing of herbicide-tolerant crops also means that farmers can now use herbicides liberally / at large concentrations. Herbicides can contaminate ground water / have a negative effect on crops / cause allergies in humans if consumed.

Reference to social injustice/ exploitation by the rich (applicable for IR crops)

5. The GM technology is largely controlled by the private sector, as its sustainability is dependent on its profitability (i.e. profit driven). Only large companies with the resources and capability to harness this technology will stand to benefit from it.

(ii) insect-resistant crops. [2] – produce toxins

Effect of insect-resistant crop on human health and safety

1. There is a potential of health risks, eg. allergy caused by (foreign) proteins present in insect-resistant crop or its pollen, as there lacks comprehensive and conclusive studies that prove consumption is indeed safe.

Effect of insect-resistant crops on biodiversity or environment

2. Leakage of toxin found in insect-resistant crops (eg. Bt delta endotoxin in Bt corn) into the soil can have a negative impact on the soil ecosystem / or the toxin kills non-pest such as useful pollinators.

Reference to social injustice/ exploitation by the rich (applicable for IR crops)

3. The GM technology is largely controlled by the private sector, as its sustainability is dependent on its profitability (i.e. profit driven). Only large companies with the resources and capability to harness this technology will stand to benefit from it.

A type of herbicide-tolerant soybean was grown under light of varying wavelengths. Fig. 4.1 shows the correlation of the absorption spectrum with the action spectrum (rate of photosynthesis) in the plant.

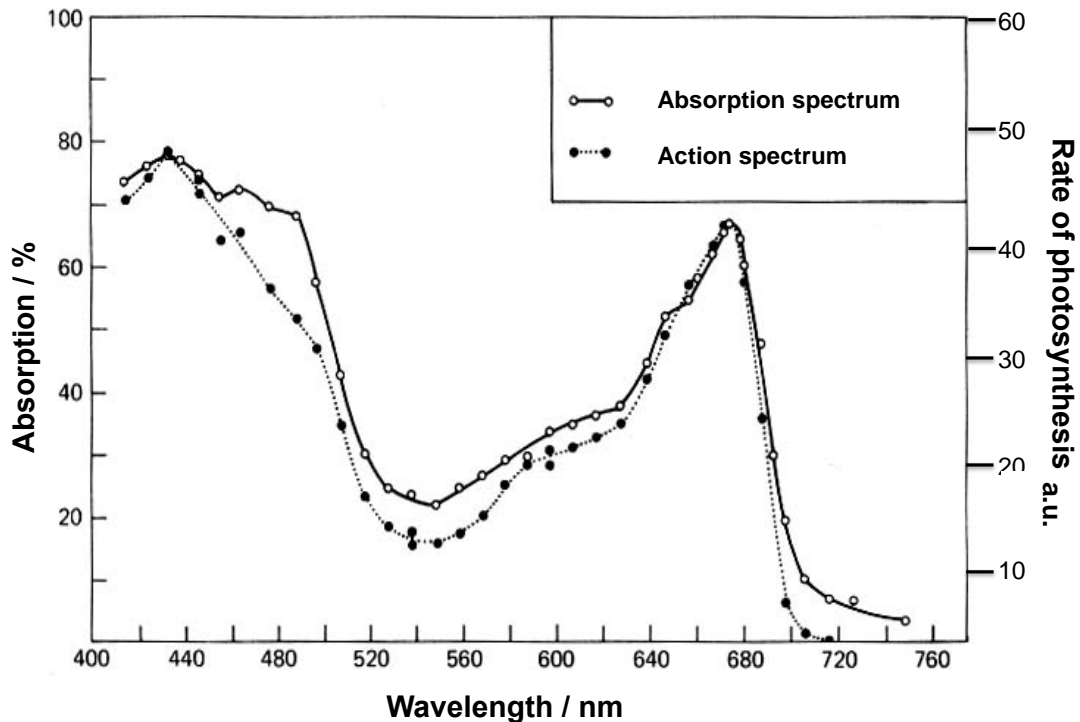


Fig. 4.1

(c) With reference to Fig. 4.1, state the wavelengths of light that are most effective in bringing about photosynthesis. Explain your answer. [2]

1. At 420-440nm and 660 - 680nm (accept range of values)
2. There is a close relationship between the rate of photosynthesis and the absorption of light.
3. There is highest absorption of light at about 70% for 660 - 680nm which corresponds to maximum rate of photosynthesis at 42 a.u.
4. And 80% for 420-440nm which corresponds to maximum rate of photosynthesis at 45-48 a.u.

- (d) A chemical compound, added to a chloroplast extract of the herbicide-tolerant soybean leaves, **preferentially binds to free electrons displaced from P700 only**.

With reference to the light dependent reactions, briefly explain the effect of this inhibitor on the production of ATP and reduced NADP at optimal photosynthesis conditions. [2]

1. Disruption of electron transport chain leading to NADP<sup>+</sup>;
2. Electron cannot be passed on to NADP<sup>+</sup> and reduced NADP cannot form;
3. Electron transport chain from P680 / PSII to P700 / PSI continues to function normally to replenish the electron lost from P700 / PSI;
4. ATP synthesis by non-cyclic photophosphorylation proceeds normally;

[Total: 10]

## SECTION B

## Answer one question.

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) Explain how the structure of the cell surface membrane facilitates bulk transport.  
[9]
1. The basic structure of the cell surface membrane is a phospholipid bilayer.
  2. The hydrophilic phosphate heads of the phospholipids are in contact with the aqueous environment on either side of the membrane.
  3. The hydrocarbon tails are shielded away from the aqueous environment and create a hydrophobic interior.
  4. The fluidity of the membrane allows for bulk transport to occur. This is increased by
    5. more unsaturated the fatty acid tails which form kinks in the fatty acid tails,
    6. and presence of cholesterol
    7. that disturb the close packing of phospholipids.
  8. Bulk transport mechanisms involve packaging in vesicles
  9. and this requires energy/ a form of active transport.
  10. Endocytosis is the process whereby the cells take in biological molecules and particulate matter (Accept: reference to Phagocytosis, pinocytosis)
  11. by forming new vesicles from the plasma membrane.
  12. The plasma membrane extends outwards, forming extensions around the particles.
  13. The plasma membrane can also invaginate (sinks inwards) to form a flask-like depression around the particles to engulf the particles.
  14. Exocytosis is the process whereby the cells release/ secrete certain biological molecules
  15. by the fusion of vesicles with the plasma membrane.
  16. When the vesicle membrane comes into contact with the plasma membrane, specific proteins rearrange the lipid molecules of the two bilayers so that the two membranes fuse and the contents of the vesicles are released out of the cell./ The vesicle membrane now becomes part of the plasma membrane.
  17. Membrane proteins / Proteins embedded in the cell membrane function as receptors, where specific ligands can bind.
  18. This allows for receptor-mediated endocytosis, which enables bulk quantities of specific substances to be taken in, to take place.

(b) Describe the roles of membrane-bound proteins in a eukaryotic cell. [5]

(any 5, include valid examples)

	Membrane-bound Protein	Role(s)
1.	Transport proteins:	<p>(one mark for each type of transport protein)</p> <p>a. carrier proteins e.g GLUT (Glucose Transporter) transports only glucose and rejects fructose, a structural isomer</p> <p>b. channel proteins e.g aquaporin or hydrophilic channel on stalked particle for proton pass through</p> <p>c. ion pumps for active transport e.g. Na<sup>+</sup>-K<sup>+</sup> pump, H<sup>+</sup> pump</p>
2.	Glycoproteins	<p>(any one role)</p> <p>a. cell-cell recognition: <u>is the ability of a cell to determine if other cells it encounters are alike or different from itself.</u></p> <p>b. receptors, e.g. receptor mediated endocytosis, GPCR may have a <u>binding site</u> that binds to a chemical messenger (eg a hormone). This allows an <u>external signal</u> to trigger or initiate reactions within the cell.</p> <p>c. cell adhesion: Membrane proteins of adjacent cells may be attached together in various <u>kinds of intercellular junctions.</u></p> <p>d. (Membrane protein) attachment to cytoskeleton, important in <u>maintaining cell shape</u></p>
3.	Enzymes	Catalyse a series of coordinated reactions in a sequential manner, e.g. adenylyl cyclase
4.	Electron carriers along ETC	<p>Electrons are <u>transferred from electron donors to electron acceptors</u></p> <p>(Accept: NADH or FADH<sub>2</sub> to O<sub>2</sub>; PSII to NADP<sup>+</sup>; PSI to PSI) by a <u>series of redox reactions</u> / undergo <u>redox reactions, chemiosmosis-ATP synthesis.</u></p>
5.	ATP synthase/ stalked particle	<u>Catalyze the formation of ATP from ADP and Pi in the matrix of mitochondria/ stroma of chloroplast.</u>
6.	NADP <sup>+</sup> reductase	<u>Reduce NADP<sup>+</sup> to NADPH for carbon reduction in Calvin Cycle</u>

- (c) Suggest why plant cells mainly store carbohydrates and animal cells mainly store lipids. [6]

1. Carbohydrates, such as starch, serve as a major fuel store in plant cells. Lipids, such as triglycerides, act as long-term energy store in animal cells. [1]

Why animal cells store lipids [max 4]

2. Animal cells mainly store lipids (eg. triglycerides) because triglycerides yield more chemical energy per gram (about 9 kcal), as compared to carbohydrates (about 4 kcal). This is useful for animals where locomotion requires mass to be kept to a minimum.
3. Triglycerides also release twice as much metabolic water as compared to carbohydrates when oxidized in respiration. Metabolic water is particularly important to desert animals such as camels.
4. Triglycerides are good thermal insulators, but carbohydrates are not. It prevents excessive heat loss. This is important for mammals, which live in cold climates (e.g. polar bear) and aquatic mammals (e.g. whales).
5. Triglycerides can be used for protecting delicate organs (e.g. kidneys) but carbohydrates cannot. Hence this is important for animals to protect their organs from shock or damage.
6. Aquatic mammals (eg. whales) also store lipids (triglycerides) because lipids are less dense than water, hence providing buoyancy.

Why plant cells store carbohydrates / starch [max 1]

7. Starch is a large molecule made up of many □ glucose units. Therefore insoluble in water and is prevented from diffusing out of cells. Thus, starch can be stored in large amounts without having any great effect on water potential of cells.
8. Can be folded into compact shapes, hence large amounts can be stored within a fixed volume. Starch molecules accumulate to form starch grains in chloroplasts.

- 6 (a) Explain how and why eukaryotic pre-mRNA is processed by splicing. [5]

### RNA splicing

#### [Why]

1. The introns / NON-CODING SEQUENCES are cut out/ REMOVED from the pre-mRNA
2. and the exons are joined together to form an mRNA molecule with a continuous coding sequence.
3. Splicing helps to regulate the export of mRNA from the nucleus to the cytoplasm.
4. It also helps in regulating gene expression

#### [How]

#### Process

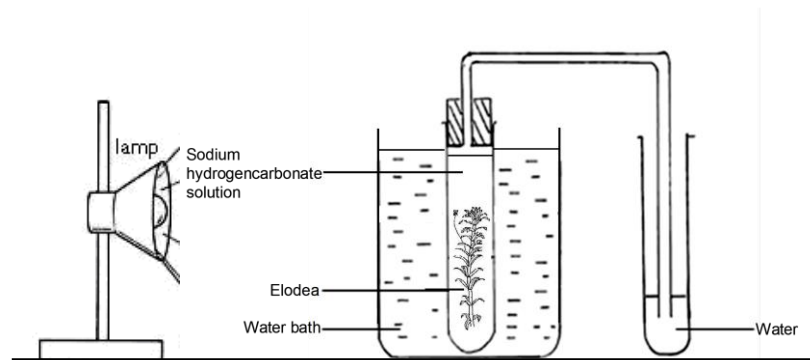
5. Splice sites are found at the ends of the introns
6. RNA called small nuclear RNA (snRNA) base pair with the splice sites
7. Allowing proteins called small nuclear ribonucleoproteins (snRNPs) to recognise and bind the splice sites
8. Several snRNPs join with additional proteins to form an even larger assembly called a spliceosome
9. The spliceosome interacts with the splice sites at the ends of an intron it cuts at specific points / cuts at splice sites / catalyse hydrolysis of phosphodiester bond to release the intron then immediately joins together the two exons that flanked the intron
10. The components of the spliceosome dissociate and the mature mRNA (containing only exons) is released



(b) Compare the Krebs cycle and the Calvin cycle. [5]

	Calvin cycle	Krebs cycle
site	It occurs in the stroma of chloroplast	It occurs in the matrix of mitochondrion
electron / hydrogen carriers	The electron carriers are NADPH (donor) and NADP <sup>+</sup> (acceptor).	The electron carriers are NADH and FADH <sub>2</sub> (donor) and NAD <sup>+</sup> and FAD (acceptor).
CO <sub>2</sub>	Carbon dioxide is fixed by RuBP carboxylase.	Carbon dioxide is released during oxidative decarboxylation
ATP	ATP is utilised for 1. phosphorylation of <u>3-phosphoglycerate to 1,3-bisphosphoglycerate</u> OR Reduction of <u>3-phosphoglycerate to glyceraldehyde 3-phosphate</u> , 2. regeneration of RuBP.	ATP is synthesized by substrate level phosphorylation.
Starting point	RuBP (the starting point) was regenerated	Oxalacetate (the starting point) was regenerated
Overall process	Overall, it is an anabolic process OR Occurs during photosynthesis	Overall, it is a catabolic process  Occurs during Cellular Respiration

- (c) Describe how you would carry out an investigation to determine the effect of temperature on the rate of photosynthesis. [10]



1. Aquatic plants, e.g. *Elodea*, are to be used. Place all plant material in the dark overnight before use.
2. Set up a water bath at 30°C as shown in the diagram above.
3. Place 30 cm<sup>3</sup> sodium hydrogen carbonate into the boiling tube. Use a syringe to add 1 cm<sup>3</sup> of pH 7.0 buffer.
4. Place a piece of *Elodea* into the boiling tube. Cover the boiling tube with a rubber bung attached to a delivery tube. Place this boiling tube into the water bath prepared in step 1.
5. Place the free end of the delivery tube into a test tube half-filled with water. / Attach a gas syringe to the free end of the delivery tube.
6. Place a bench lamp at least 10 cm from the boiling tube
7. To prevent warming up of the water, place a piece of plastic between the light source and the beaker and switch on the lamp.
8. Start the stopwatch and allow 3 min for the plant to adapt to the conditions provided before taking readings.
9. When the bubbles are produced at a regular rate, count the number of bubbles evolved in the next 5 min. /Collect the gas for the next 5 min.  
Note and record the number of bubbles /volume of gas evolved in 5 min in a table.
10. To ensure reliability of results, repeat steps 3 to 9 to obtain a total of three readings (triplicates) at this temperature using the same piece of *Elodea* in fresh samples of the existing sodium hydrogen carbonate solution.
11. To show that the bubbles that are formed are due to photosynthesis taking place in the *Elodea* and not other factors, a control is set up.
12. Steps 3 to 10 are performed with the same setup, but boiling tube that contains the plant is wrapped in aluminium foil. The control is subjected to the same environmental factors as that for the experiment.  
**Accept: absence of water plant**
13. Repeat steps 3 to 11, varying the temperature of the water bath, at 20, 40, 50, 60, 70 and 80°C. {at least temperature settings, at regular intervals}{marked as point "12"}
14. To ensure reproducibility of data, repeat the entire experiment (steps 1 to 12) twice, using freshly prepared solutions, and another *Elodea* plant of same size and batch.
15. Apart from the temperature of the water bath, all other factors that can affect the rate of photosynthesis should be kept constant as far as possible.
16. pH of the reaction mixture
  - It affects the conformation of the proteins / enzymes involved in photosynthesis.
  - pH is kept constant through the use of a pH 7.0 buffer, and monitored using a pH sensor.
17. Chlorophyll concentration

- Amount of photosynthetic pigments affects the absorption of light energy for photosynthesis.
- Use the same piece of *Elodea* throughout the experiment and a different piece of *Elodea* of the same size and the same number of leaves when repeating the entire experiment. *[not necessary, if chlorophyll concentration is indicated in Materials]*

18. Duration of reaction

- It affects the amount of oxygen formed.
- A digital stopwatch is used to ensure that the time of reaction is measured accurately.

19. Light Intensity

- This affects the amount of light energy absorbed by photosynthetic pigments.
- Ensure that same bench lamp is used at a fixed distance from the plant.

20. CO<sub>2</sub> concentration

- It affects the rate of carbon fixation and hence rate of photosynthesis.
- Use fresh sodium hydrogen carbonate solution from the same stock for each reading to ensure that the initial CO<sub>2</sub> concentration is kept constant.

[Total: 20]