

Section A

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

- 1 Fig. 1.1 is an electron micrograph of a liver cell.

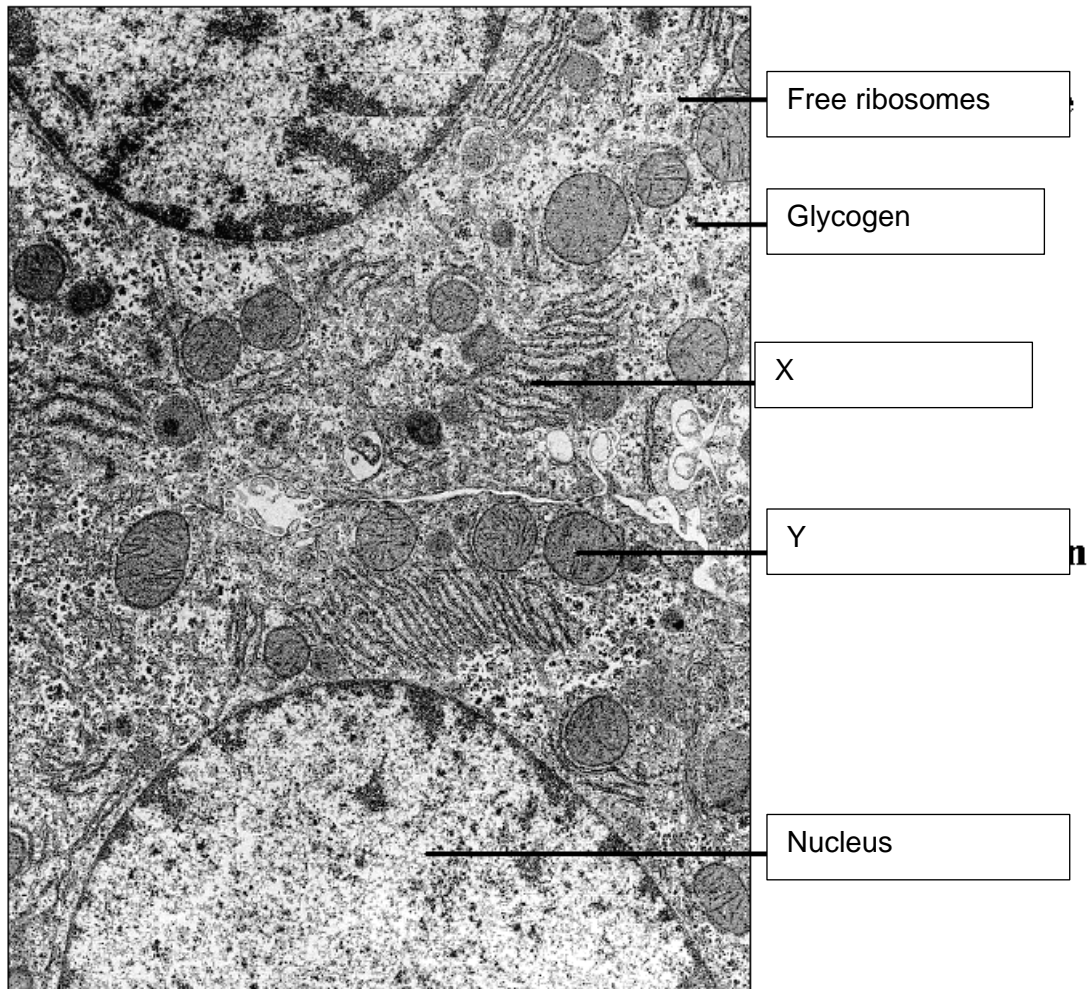


Fig. 1.1

- (a) Identify the structures labelled **X** and **Y**.
Rough Endoplasmic Reticulum

Mitochondrion

[2]

- (b) Explain for the high concentration of structure **Y** in the liver cell.

- **Adenosine triphosphate is synthesised** via respiration for the various metabolic activities performed in the liver in the presence of oxygen.
- This suggests that a lot of ATP is required for the proper functioning of the liver cell e.g. detoxification.

Growth hormone released from the anterior lobe of the pituitary binds to receptors on the surface of liver cells which stimulates the synthesis and release of Insulin-like Growth Factor 1 (IGF-1) from them. IGF-1 is a protein with 70 amino acids.

Many cells have receptors for IGF-1, especially cells in the bone marrow and in the cartilaginous growing regions of the long bones.

Binding of IGF-1 to cells with receptors for it stimulates them to move from G₁ of the cell cycle to S phase and on to mitosis.

(c) (i) Outline how mRNA of IGF-1 is used to synthesise IGF-1 and is secreted out of the liver cell.

- mRNA is used as a template for translation of IGF-1. Initiation begins when the ribosomes attached to the rough endoplasmic reticulum (RER) synthesize the IGF-1 polypeptide via translation and passes it into the lumen of the RER for further modification.
- IGF-1 starts to fold into its tertiary structure and is packaged into an ER vesicle which buds off from the ER and fuses with the cis face of the Golgi apparatus.
- IGF-1 is sorted and modified by enzymes and packaged into a secretory vesicle which buds off from the trans face of the golgi apparatus and moved to the cell membrane to fuse; IGF-1 is released outside the cell via exocytosis.

[3]

(ii) Explain one main event occurring during G₁ phase.

1. Increase in cell size with intensive cellular synthesis to form new organelles e.g. ER, Golgi apparatus, mitochondria, lysosomes, vacuoles and vesicles → make new organelles for another new cell.
2. Nucleolus actively synthesises ribosomal materials like rRNA, mRNA and tRNA → rRNA to form ribosomes → level controls the G₁-S phase transition/ to form more proteins
3. Synthesis of protein at the ribosomes → enzymes for DNA replication /make proteins for cell growth to reach a correct biological size for mitosis to proceed.

[max 1]

- 2 The light micrographs labelled **A** to **F** in Fig. 4.1 show different stages of the mitotic cell cycle in a living cell from the lung epithelium of a newt.

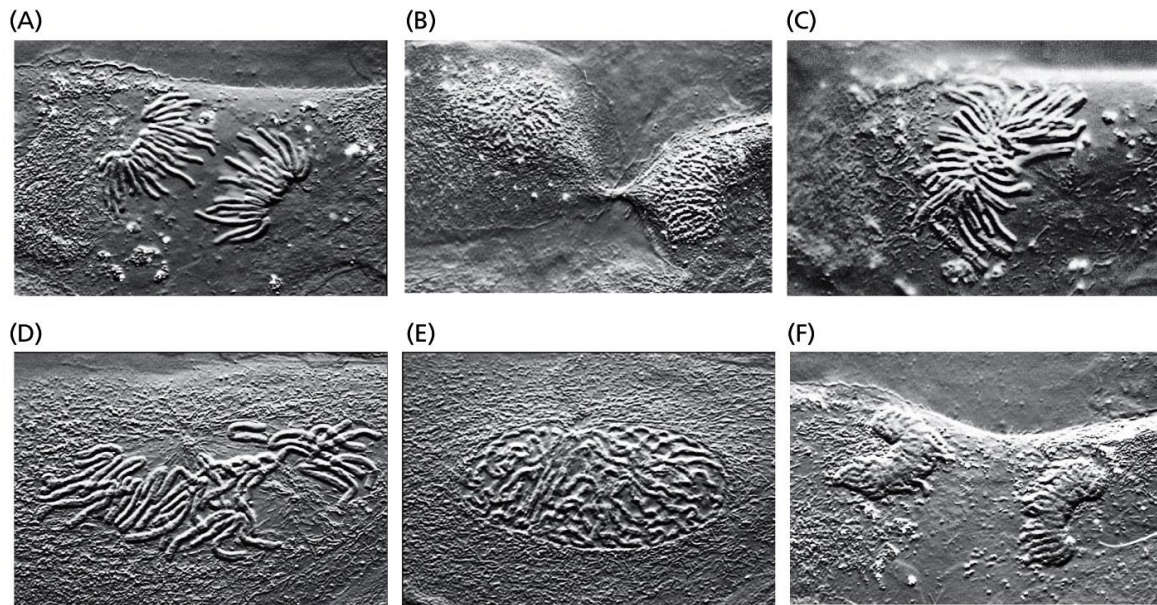


Figure 17-14 MBoC5: The Problems Book (© Garland Science 2008)

Fig. 4.1

- (a) (i) List the stages shown in **A** to **F** in the order of progression through the cell cycle. [1]
- **E → D → C → A → F → B**
- (ii) With reference to the photomicrograph, identify and explain what is happening in the cell during Stage **A**. [2]
- **Stage A is anaphase**
 - where centromeres divide and sister chromatids are separated as they move towards the opposite poles of the cell;

The NF1 gene contains 8454 base pairs and codes for a protein called neurofibromin. Neurofibromin regulates the action of the Ras protein, which promotes cell division. Mutant forms of NF1 produce a protein that cannot regulate Ras properly.

People with mutations in the NF1 gene develop neurofibromatosis type 1, a disease of the nervous system that affects 1 in 3500 people worldwide. Several different mutations result in neurofibromatosis. Some of these mutations involve the RNA transcript.

- (b) Discuss how a mutation may arise in the NF1 gene, and the effect this mutation could have on the control of cell division. [3]

- An **insertion / deletion** of a single base within the NF1 gene can lead to a **frameshift mutation** / will shift the reading frame of the gene;
- Translation of the resultant mRNA produced will give rise to a polypeptide chain that folds into a different three-dimensional conformation;
- resulting in a non-functional neurofibromin that is unable to regulate the Ras protein, leading to dysregulation of cell division;

OR

- A **substitution in the 1st or 2nd base** of the DNA triplet code in NF1 gene can lead to a different codon found on the resulting mRNA;
- A different amino acid with a R group having a different property compared to the original amino acid, will be present in the polypeptide formed during translation which will then fold into a different / wrong three-dimensional conformation due to different R group interactions;
- resulting in a non-functional neurofibromin that is unable to regulate the Ras protein, leading to dysregulation of cell division;

Edeine is an antibiotic that inhibits protein synthesis but has no effect on either DNA synthesis or RNA synthesis. When added to the cell extract of an immature red blood cell, edeine stops protein synthesis after a short lag, as shown in Fig. 4.2. By contrast, cycloheximide, which is also an inhibitor, stops protein synthesis immediately. Protein synthesis is measured via radioactivity in haemoglobin.

Analysis of the edeine-inhibited cell extract showed that no polyribosomes remained at the time protein synthesis had stopped. Instead, all the globin mRNA were found associated with small ribosomal subunit and initiator tRNA.

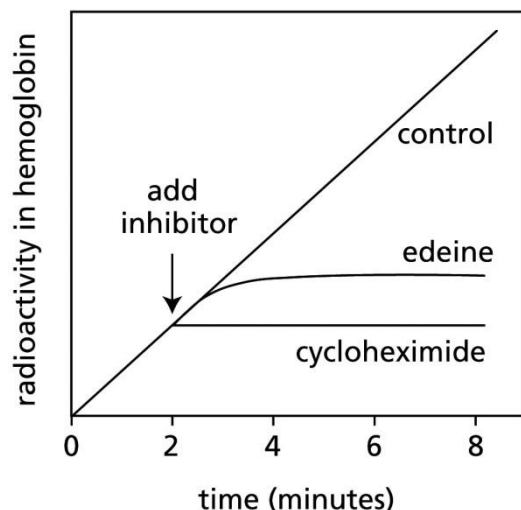


Figure 6-28 MBoCS: The Problems Book (© Garland Science 2008)

Fig. 4.2

(c) (i) Explain how protein synthesis is measured via radioactivity in haemoglobin. [2]

- Incorporating radioactive amino acids to form globin chains that make up haemoglobin;
- Higher radioactivity (in haemoglobin) higher production of protein;

(ii) Describe how edeine inhibits protein synthesis. [2]

- Inhibits initiation of protein synthesis;
- By blocking/ preventing the joining of the large ribosomal subunit to the small subunit/ mRNA/ initiator tRNA complex;

(iii) Explain why there is a lag between the addition of edeine and cessation of protein synthesis. [2]

- (Inhibits initiation) but has no effect on elongation;
- Ribosomes that have started making new polypeptide is free to complete it;
- Lag time is equal to the time to complete the synthesis of the polypeptide chain;

[Total: 12]

3 Fig. 3.1 and Fig. 3.2 show the structure of a part of glycogen and the structure of haemoglobin respectively.

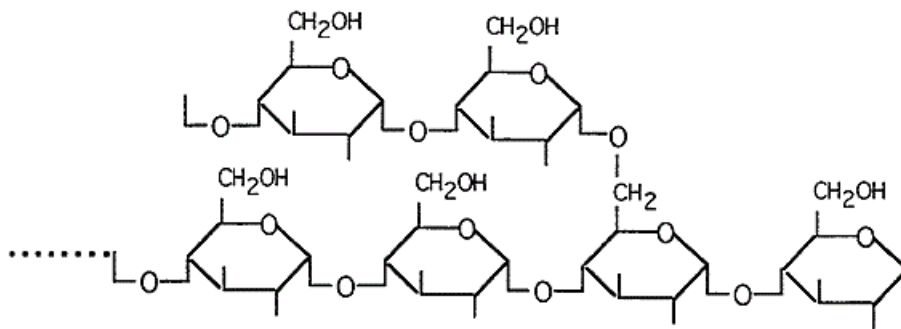


Fig. 3.1

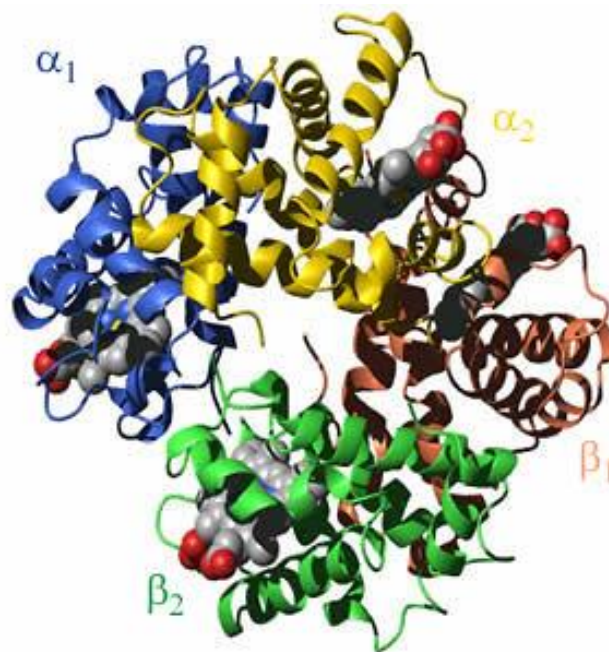


Fig.3.2

With reference to Fig. 3.1 and Fig. 3.2,

- (a) state one similarity and one difference in the structure of glycogen and haemoglobin.
[2]

Similarity:

Both are polymers formed through condensation reaction.

Difference (Any 1):

- Type of monomers – glucose vs amino acids
- Type of bond formed between adjacent monomers: glycosidic bond vs peptide bonds

AVP

Fig. 3.2 shows some steps involved in glycolysis and the Krebs cycle. Some ATP is made directly. Hydrogen is also released and this can result in the production of more ATP.

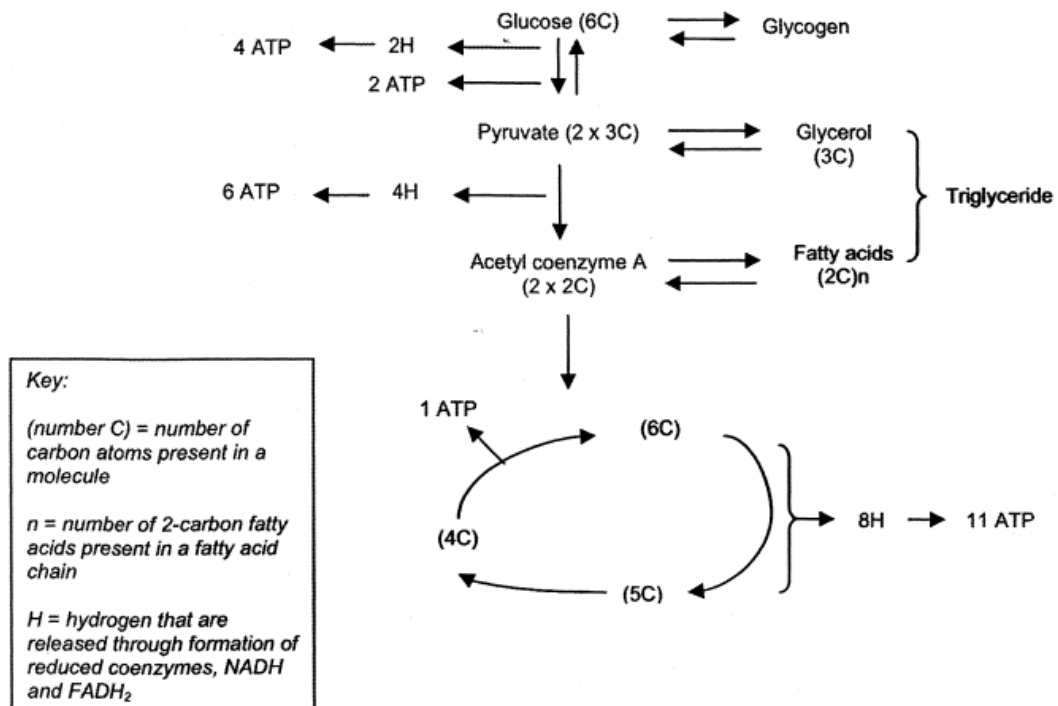


Fig.3.3

(b) Describe how the hydrogen released during glycolysis and the Krebs cycle results in the production of ATP.

- Hydrogen released during glycolysis is used to **reduce coenzymes NAD⁺ and FAD⁺**. The NADH and FADH₂ will be reoxidised through transfer of their electrons and protons to the electron transport chain at the inner mitochondrion membrane;
- Protons are pumped from the mitochondrion matrix into the intermembrane space**, generating an **electrochemical gradient**;
- This electrochemical gradient contains potential energy and the **re-entry of the protons into the mitochondrial matrix** provides the **energy to generate ATP at the ATP synthase**;
- This process by which ATP is formed as electrons are transferred from NADH and FADH₂ to oxygen via a series of electron carriers is called **oxidative phosphorylation**;

[4]

Similar to glycogen, triglycerides can be respired. The first step is to hydrolyse each triglyceride molecule. Each fatty acid formed is broken down into acetyl CoA molecules. The acetyl CoA molecules then enter the Krebs cycle.

(c) Using the information from Fig. 3.3, suggest why fatty acids can only be respired under aerobic conditions. [2]

- Fatty acids enter the mitochondria directly to be respired under aerobic conditions, by being converted to acetyl coA so that it can enter the Krebs cycle. This bypasses the process of glycolysis in the cytosol.

OR

- Glycerol is converted directly into pyruvate in the cytosol. Pyruvate formed then enters the mitochondria to be respired under aerobic conditions, by first undergoing the link reaction where oxidative decarboxylation occurs to form acetyl CoA which then enters Krebs cycle. This bypasses glycolysis.

AND

In order to be completely oxidised, oxygen is needed as the final electron acceptor in the process of oxidative phosphorylation in order for electron carriers and reduced coenzymes to be reoxidised.

[Total: 8]

4 Plasmids are circular pieces of extrachromosomal DNA, found in many types of bacteria.

pUC19 is a plasmid that is frequently used for gene cloning. As shown in Fig. 4.1, the pUC19 plasmid has:

- an origin of replication (ori);
- a *lac Z* gene with its promoter. The *lac Z* gene codes for the α peptide fragment of the enzyme β -galactosidase;
- a short length of DNA called the 'Multiple Cloning Site' (MCS) which contains the recognition sites of 3 different restriction enzymes;
- a gene conferring resistance to the antibiotic ampicillin (amp^R).

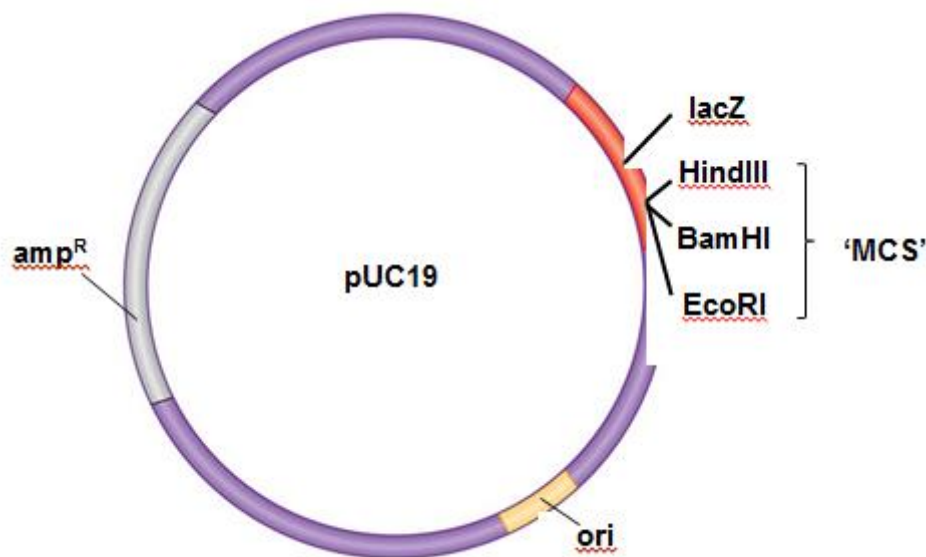


Fig. 4.1

(a) Explain the purpose of the ori and amp^R gene on pUC19. [4]

- Ori allows circular double-stranded pUC19 plasmid DNA, carrying the gene to be cloned to be replicated independently of the nucleoid;
- So that numerous copies of recombinant pUC 19 plasmid can be passed down to genetically identical daughter cells;
- The amp^R gene present on the pUC 19 plasmid acts as a selectable marker*;
- and enables the selection for transformed bacterial cells containing the plasmid (recombinant & reannealed plasmids) when grown in a medium containing the antibiotic ampicillin;

Recombinant plasmids were created by cloning the gene coding for human insulin chain B into the pUC19 plasmid.

Table 4.1 shows the composition of two different nutrient media.

Table 4.1

medium	X	Y
composition	nutrient agar+ampicilin	nutrient agar with lactose + Compound S + ampicilin

Competent *Escherischa coli* (*E.coli*) were transformed with recombinant plasmids and grown on medium **X**.

Bacterial colonies growing on medium **X** were replica plated onto medium **Y**. Fig 4.2 shows the growth of the resultant bacterial colonies on different media.

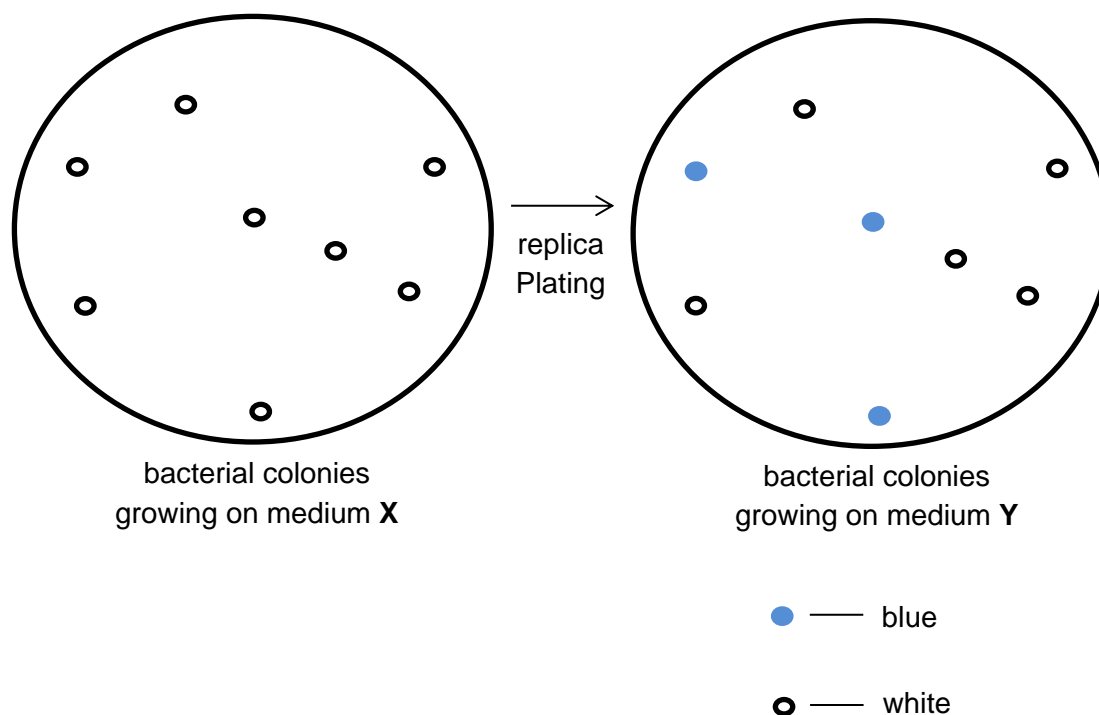


Fig. 4.2

(b) Describe how the human insulin chain B gene can be derived. [2]

- The **ampR** gene present on the **pUC 19** plasmid acts as a **selectable marker***;
- and enables the selection for transformed bacterial cells containing the plasmid (recombinant & reannealed plasmids) when grown in a medium containing the antibiotic ampicilin;

(c) With reference to Table 4.1 and Fig. 4.2, identify the compound **S**. [1]

- **Compound S is X-gal;**

(d) Explain why compound **S** was used in medium **Y**. [2]

- **Compound S used to test for the presence of functional Lac Z gene;;**
- **Presence of Lac Z will produce the functional enzyme that will hydrolyse X gal to give a blue colouration.**

(e) Explain how the recombinant bacteria are distinguished from the others. [3]

- **Recombinant Bacteria will have insertional inactivation of the Lac Z gene, due to the insertion of the gene of interest;;**
- **This would result in no functional B galactosidase and hence the colonies appear white**
- **The non recombinant bacteria will appear blue due to intact lac Z gene;;**

[Total: 12]

Essay

5 (a) Compare and contrast the process of replication and translation. [7]

Feature	DNA replication	Translation
Definition	Transfer of genetic information from one DNA sequence to another DNA sequence	Transfer of genetic information from RNA sequence to amino acid sequence
Location	Nucleus	Free ribosomes in cytosol or bound ribosomes at rough endoplasmic reticulum (<i>Reject: ribosome</i>)
Template	Each parental single-stranded DNA	mRNA (<i>Reject: RNA</i>)
Monomers	Deoxyribonucleoside triphosphate (dNTP)	Amino acids; attached to tRNAs or aminoacyl tRNAs
End product	Two Double-stranded DNA molecules (<i>Reject: polynucleotide</i>)	Polypeptide (<i>Reject: protein</i>)
Adaptor	No need for adaptor	tRNA serves as an adaptor; to carry the specific amino acid as dictated by codon on mRNA to the growing peptide chain
	thus there is a direct association / base pairing between template and monomers	No direct association
Ribosome	Not required	Requires ribosomes
Start site	Origin of replication (<i>Reject: replication bubble</i>)	Start codon (AUG)
Priming of strand synthesis	Each new strand synthesis begins with RNA primers	Each polypeptide chain synthesis starts with initiator tRNA attached to methionine / No priming is needed for every new polypeptide synthesised
Base pairing at the template	Specific complementary base pairing between the template and a nucleotide / nucleotide on template is read one at a time	Base pairing between codon of mRNA and anticodon of tRNA / nucleotide on mRNA read 3 at a time

Feature	DNA replication	Translation
Direction of reading the template	3' to 5'	5' to 3'
Type of bond between monomers	Phosphodiester bond	Peptide bond
Enzyme catalyzing polymerization	DNA polymerase	peptidyl transferase (Reject: ribosome)
Direction of growth of product	DNA grows in 5' to 3' direction	Polypeptide grows in N to C terminal direction
End site of synthesis	Until the entire DNA / all chromosomes are duplicated (Reject: end of the DNA strand)	At stop codon (UAA, UAG or UGA)
Energy required	ATP	GTP
	for synthesis of every strand, there is a continuous and discontinuous synthesis	synthesis of every polypeptide is continuous
Reading of template	Entire template is read until the whole genome is replicated	Translate the coding region of the template
Involvement of helicase	Helicase will unwind the dsDNA	Not needed as mRNA is single-stranded
Cell cycle phase of event	S phase of interphase	G1 and G2 phases of interphase
Conservation of template	Parental DNA template becomes part of the newly synthesised DNA molecule (semi-conservative replication)	mRNA template remains intact after synthesis of every polypeptide (conservative)
Amount of end product	2 double-stranded DNA molecules for every round of replication	Many polypeptides for translation of mRNA because many ribosomes can translate one mRNA at the same time by forming polysomes
Duration of event	Longer time is required	Shorter time is required
Fate of template after event	DNA template is conserved after replication	mRNA template is degraded after the desired amount of polypeptides is produced

Similarities (at least 1 must be written to gain full credit):

- Both processes occur in interphase of the eukaryotic cell cycle
- Both processes involve complementary base pairing of nucleotides in order for the products to be formed.

(b) Explain the steps in gel electrophoresis [7]

Agarose gel electrophoresis Is used for **resolving or separating DNA molecules** on the basis of **different molecular lengths or masses**

1. The gel is inserted into the electrophoresis chamber, and covered with an **electrophoretic buffer** which provide ions to support conductivity.
2. A **loading buffer with a tracking / marker dye** added to the DNA samples before loading into the sample wells. This serves to allow tracking of DNA that is colourless and allows visual monitoring of how far the electrophoresis has proceed.
3. The loading buffer contains glycerol, a dense liquid which helps the DNA sample to sink to bottom of well as denser.
4. Because of its phosphate groups, DNA is **negatively charged** at physiological / neutral pH. When electric current applied across the gel, opposite charges attract, DNA molecules must travel to reach the **positive electrode (anode)**.
5. The agarose comprises of a complex network of pores act like a sieve that separates molecules by molecular size → hence the migration distance is **inversely proportional** to the molecular size of a DNA fragment.
6. **DNA fragments (not visible in gel) are visualized by staining with ethidium bromide.** This fluorescent dye **intercalates** (penetrates) between the bases and when exposed to ultraviolet light, DNA fragments will appear as **discrete bands** on the gel.
7. **DNA markers** are loaded. The DNA markers serve as “ladders” of **fragments of known sizes** which is used to determine the size of DNA molecule studied.

(c) Discuss the ethical and social implications of genetically modified crop plants. [7]

Ethical implications [max 3]:

1. Ref. to tampering of nature via mixing of genes among species with named example (e.g. Bt corn/ Golden rice);;
2. Ref. to violation of natural organism's intrinsic values and negative response from naturalists;;
3. Ref. to animal genes in plants and response from religious groups/groups with dietary restrictions;;

Social implications: [max 4]

(a) Threat to human safety (max 1)

4. Ref. to transgenic food causing allergies with named examples (e.g. Bt toxin in Bt corn/genes in Golden rice coming from several organisms);;

5. Ref. to long term unexpected/negative effect of transgenic food on human health with named examples;;
6. Ref. to use of antibiotic resistance genes as selectable markers in vectors used for transforming plants and concerns in making bacterial species in human gut more resistant;;

(b) Threat to safety of the environment (max 1)

7. Ref. to cross-pollination/unintended transfer of transgenes from GM crops/crop-to-weed hybridization with named example (e.g. soy crop plants with herbicide-resistance);;
8. Ref. to GM crops establishing as weeds/weeds becoming 'superweeds' and thus are invasive/reduces biodiversity/disrupts ecological balance;;
9. Ref. to GM crops being toxic to non-target organisms with named example (e.g. Bt toxin affecting larvae of monarch butterflies);;
10. Ref. to reduced numbers/extinction of non-target organism/disruption of food chain/disruption of ecological balance;;

(c) Issues pertaining to Access and Intellectual Property (max 1)

11. Ref. to why research companies seek patents (e.g. make profits/protect results of research);;
12. Ref. to impact of patents (e.g. increase in price of seeds/ domination of world food production by few companies);;
13. Ref. to biopiracy and how developed countries exploit the resources of developing countries;;

(d) Labelling is not mandatory in some countries (max 1)

14. Ref to need for labelling GM food (e.g. religious/medical/dietary concerns);;
15. Ref. to varying standards in labelling transgenic food with named examples (e.g. labelling threshold is 1% for Brazil and 5% for Japan/ labelling not mandatory in US);;
16. Ref. to difficulties in maintaining standard of labelling due to pollen drift with named example (e.g. unauthorized corn in Mexico)

(e) other social implications (max 1)

17. Ref. to advances being skewed to interests of developed countries/benefiting only rich countries;;
18. Ref. to dominance of world food production by developed countries / increasing dependence of developing countries on industrialized nations;;
19. Ref. to impact on international trade with named example (e.g. Europe being more hesitant in accepting GM products compared to US);;

Question 6**(a) Compare and contrast between Starch and Cellulose [6]**

Characters	Starch	Cellulose
Monomer	α -glucose	β -glucose
Type of bond between monomers	1,4 glycosidic bond (amylose) + 1,4 and 1,6 glycosidic bond (amylopectin)	1,4 glycosidic bond
Nature of chain	Amylose is coiled unbranched Amylopectin is long branched chains, some coiling	Straight, long unbranched chains form H-bonds, with adjacent chains
Occurrence	In plants	In plants
Function	Carbohydrate energy store	Structural
General form	Globular	Fibrous

(b) Describe how the structure of the membrane affects the movement of substances into and out of a cell.[7]

<http://www.sumanasinc.com/webcontent/animations/content/diffusion.html>

- 1 Membrane is selectively permeable ;
- 2 Due to phospholipid bilayer ;
- 3 Hydrophilic phosphate heads face the aqueous environment on both sides ;
- 4 Hydrophobic fatty acid tails face inwards, unable to interact with aqueous environment ;

Non-polar substances

- 5 Hydrophobic / non-polar substances diffuse across phospholipid bilayer directly ;
- 6 Able to interact with the hydrophobic core / tails of fatty acid chains ;

Polar substances

- 7 Polar molecules and ions are unable to pass through phospholipid bilayer directly ;

- 8 Require the presence of integral proteins on membrane ;
- 9 Ref facilitated diffusion ; Facilitated diffusion is a modified form of diffusion in which the particles are allowed through the membrane by specific protein molecules.
- 10 Channel proteins ;Allows selective ions and polar molecules to pass through water-filled pore ;
- 11 Channel protein forms a hydrophilic tunnel in the membrane, so water-soluble substances (e.g. ions) can pass through it.
- 12 The channels are selective, allowing only certain ions to pass through. Channel proteins are able to transport in either direction depending on the concentration gradient.
- 13 Carrier proteins ;
- 14 Carrier proteins transport substances that are insoluble in lipid across the membrane. The carrier protein can exist in two conformation:
 - the binding sites open to the outside of the cell
 - the same binding sites open to the inside of the cell
- 15 Selective binding and transport of polar molecules and ions across cell membrane ; When the diffusing substance binds to the binding sites, it triggers a conformational change and the substance is deposited on the other side of the membrane
- 16 Active transport ; Active transport is the movement of ions or molecules across a membrane from a region of lower concentration to a region of higher concentration (i.e. against a concentration gradient) by means of specific transport proteins and with the expenditure of energy. The energy is usually from the hydrolysis of ATP.
- 17 A **sodium-potassium pump** actively pumps Na^+ out and pumps K^+ into the cell.
- 18 Certain particles are either too big to pass through the small pores in the plasma membrane or too hydrophilic to diffuse through the phospholipid bilayer.
- 19 These particles move into a cell by a process called endocytosis and out of a cell by a process called exocytosis.
 - Both endocytosis and exocytosis require energy and are therefore active processes
- 20 Involves formation of vesicle which encloses molecule to be transported ;

- 6 (c) Discuss how bacteria evolve through natural selection and the implication in antibiotic resistance. [7]

How antibiotic resistance in bacteria arises:

1. Due to spontaneous mutation, there is existing **variation*** in the population of bacteria with antibiotic resistant strains and non-resistant strains;
 2. (idea of horizontal gene transfer) The allele for antibiotic resistance can also be acquired through **transformation***
- (1m = need to state the process + elaborate process) [2m max]
3. When exposed to antibiotic, antibiotic act as **selection pressure*** to select against/kill those non-resistant bacteria;
 4. while those bacteria with antibiotic resistance allele are selected for/best adapted and survive;
 5. and pass on the allele for antibiotic resistance to subsequent generations of bacteria cells during binary fission thus most of the bacteria are resistant

- to antibiotic (idea of vertical gene transfer);
6. allele frequency changes as a result of natural selection;

Implication in antibiotic resistance:

7. Over many generations, bacteria cells evolve by natural selection and the bacteria become resistant to the antibiotic rendering that **antibiotic ineffective**;
8. Thus, other types / different combinations of antibiotics have to be used;
9. possibility of developing **multiple antibiotic resistance**;

END OF PAPER