VICTORIA JUNIOR COLLEGE
BIOLOGY DEPARTMENT
JC2 PRELIMINARY EXAMINATIONS 2015
Higher 3

CANDIDATE NAME

CLASS | | | | | | INDEX NUMBER | |

BIOLOGY 9815/01

28 September 2015

Additional Materials: Answer Paper **2 hours 30 minutes**

READ THESE INSTRUCTIONS FIRST

Write your CLASS/ INDEX no. and name on all the work you hand in.
Write in dark blue or blue pen.
You may use a soft pencil for any diagrams, graphs or rough working.
Do not use any staples, paper clips, highlighters, glue or correction fluid.

Section A
Answer **all** questions.

Section B
Answer **three** out of **four** questions.

Section C
Answer the question.

At the end of the examinations,
1. Fasten all your work securely;
2. Circle the number of the section B question you have answered in the grid opposite.

For Examiner's Use	
Section A	
1	
2	
3	
4	
5	
Section B	
6	
7	
8	
9	
Section C	
10	
Total	

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

Section A

Answer all questions in this section.

1 (a) **Fig.1** shows GDP in the binding site of a G protein.

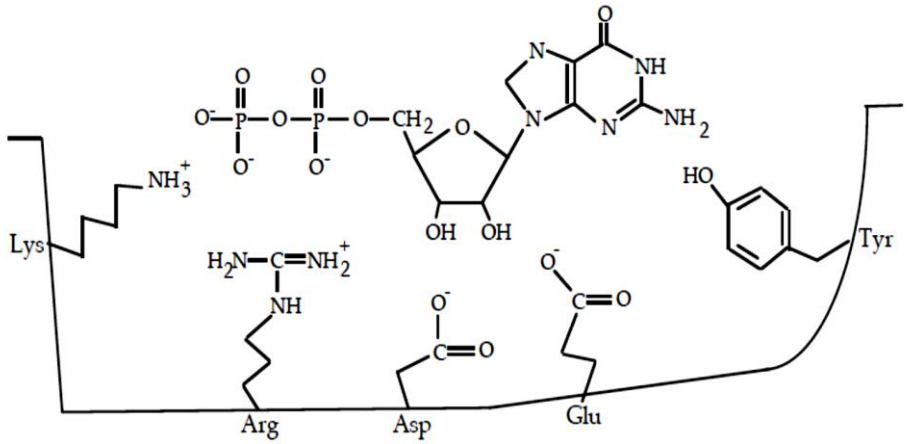

Fig. 1

(i) With reference to **Fig. 1**, describe the types of interactions between GDP and the amino acids that make up the binding site. **[3]**

(ii) A mutation results in the change of the amino acid from Arg to Asp at the binding site. A similar change occurs within the protein core of G protein.

Discuss the difference, if any, the effect of these mutations on the function/ activity of the protein. **[4]**

(b) A **protein family** is a group of proteins that have descended from a common ancestor. Members of the same family typically have similar three-dimensional structures, functions and significant sequence similarity especially in the binding or catalytic sites.

Many protein families belong to a protein superfamily which is the largest grouping (clade) of proteins for which common ancestry can be inferred. Members belonging to the same superfamily share structural similarity but not necessarily sequence similarity.

(i) Explain how it is possible for two proteins to share structural similarity without sequence similarity. **[2]**

(ii) On the other hand, proteins with similar primary sequence can differ in their 3-dimensional configuration.

Explain how this is possible. **[2]**

[Total: 11]

2 The antibody immunoglobulin G (IgG) is a type of globulin protein that is found in blood or other bodily fluids of vertebrates, and is used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. It is produced by a type of white blood cell called a B cell.

Fig. 2.1A is a schematic diagram of IgG while **Fig. 2.1B** shows the ribbon model of the same IgG molecule.

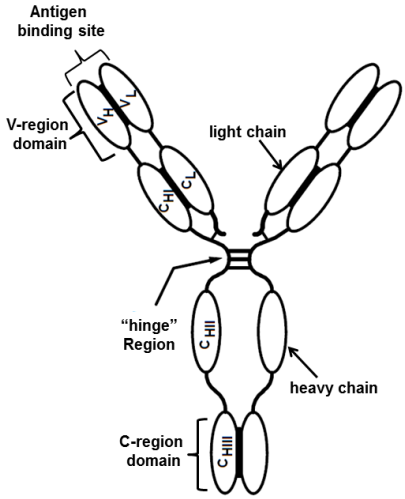

Fig. 2.1A

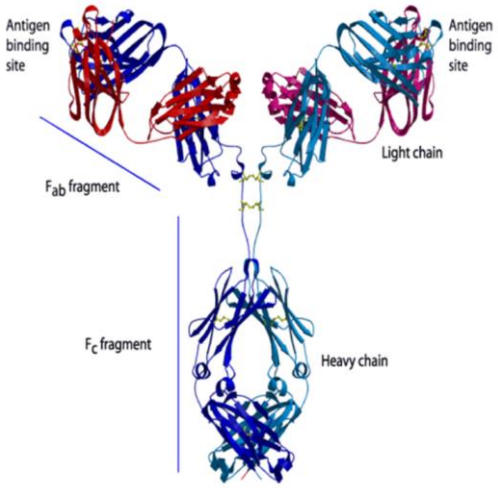

Fig. 2.1B

(a) With reference to **Fig. 2.1**, explain the term "domain". **[2]**

(b) Account for how the four IgG domains at the antigen-binding sites of the IgG molecule are different from the rest of the IgG domains, with reference to the function of the IgG. **[3]**

(c) The IgG molecule can be broken down into smaller fragments by proteolytic enzymes such as papain, and pepsin. Treatment with papain yields two kinds of fragments, a pair of F(ab) fragments, and one Fc fragment while treatment with pepsin yields a single fragment, the $F(ab')_2$ but degrades the Fc region.

Indicate, on **Fig. 2.1B**, the site(s) where papain and pepsin are likely to act on IgG. **[1]**

(d) Predict the antigen-binding and agglutination capabilities of the resulting fragments that result from separate treatment by these enzymes. **[2]**

(e) Papain is found as a component of extracellular latex of *Carica papaya* and is synthesized as an inactive precursor that becomes active in response to wounding of the plant. It is synthesised as a precursor protein called prepropapain that undergoes two cleavage steps, an initial cleavage of the amino acid signal sequence followed by further cleavage of the proregion, to produce the mature peptide.

Explain the importance of the signal sequence to the function of papain. **[2]**

[Total: 10]

3 Large-scale transposon insertional mutagenesis in mice has made significant advances through the development of an efficient *PiggyBac* (PB) transposition system. Two transgenic lines are required in this system. One is a PBase-expressing transgenic line that carries the gene for transposase and a reporter gene for coat colour. The other line carries a non-autonomous PB transposon.

Mating the two transgenic lines produces offspring that can be identified visually by coat colour for subsequent mutant screens. Additionally, the PB system is versatile since the transposon can be modified for different applications.

(a) With reference to the PB system, describe how transposition works and how it can be controlled. **[3]**

(b) Outline how disrupted genes can be identified. **[2]**

(c) The availability of complete genome sequences from humans and model organisms has allowed us to exploit various reverse genetic approaches.

Outline how RNAi can be used in mutant screens. **[3]**

(d) Describe one advantage and one disadvantage each of (i) RNAi and (ii) insertional mutagenesis. **[2]**

[Total: 10]

4 Human lactase belongs to the family of glycosyl hydrolases. This enzyme enables infants to break down lactose, the main sugar in milk. The gene that codes for this enzyme is expressed in the small intestine of humans and other mammals, and remains active throughout the lives of people who are lactose tolerant. On the other hand, it is turned off after infancy in people who are lactose intolerant.

These adults who consume significant amounts of lactose in dairy products may experience symptoms that include abdominal bloating and cramps, flatulence, diarrhoea, nausea or vomiting. To manage lactose intolerance, these individuals often avoid lactose-rich food. However, there may be occasions where lactose avoidance is not possible.

Imagine you are a scientist in a pharmaceutical company who wants to produce lactase in tablet form to help these lactose-intolerant individuals.

(a) (i) Outline how the molecular weight of a purified polypeptide of lactase can be determined by mass spectrometry. Assume ions formed in a mass spectrometer only have a single charge. **[2]**

(ii) **Fig. 4.1** shows the processing and localization of human lactase, while **Fig. 4.2** shows three distinct peaks in the hypothetical mass spectrum of various translational products of the lactase gene.

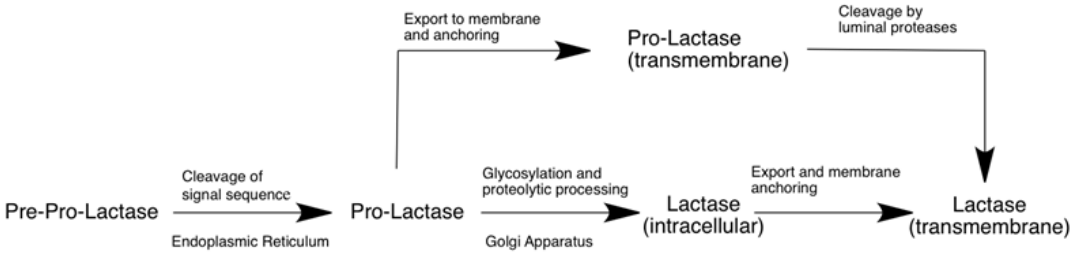

Fig. 4.1

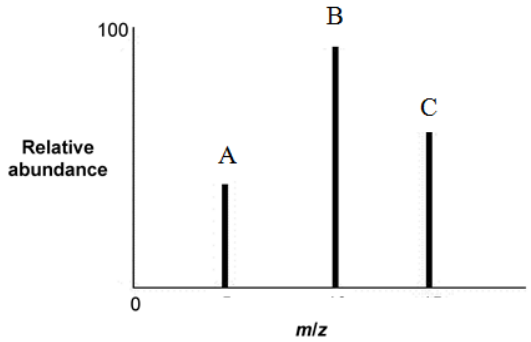

Fig. 4.2

With reference to **Fig. 4.1** and **4.2**, identify which peak (**A**, **B**, or **C**) was likely to be the molecular weight of lactase. Explain your answer. **[2]**

(b) (i) Explain how ultracentrifugation and a specific column chromatography may be employed to obtain a fraction from the cell extract containing the 160 kDa enzyme. **[2]**

(ii) The quantity of lactase present in the cell fraction can be detected by incubation with its substrate lactose and performing the Benedict's test followed by comparing to a colour standard.

State another method that can detect and quantify the enzyme accurately. **[2]**

(c) Describe one similarity and one difference between the specific column chromatography and SDS PAGE in the separation of proteins. **[2]**

[Total: 10]

5 The electron transport chain of the mitochondria is organised into four complexes. Complex III is known as coenzyme Q reductase. It consists of two b-type cytochromes and one c-type cytochrome (**Fig. 5.1**).

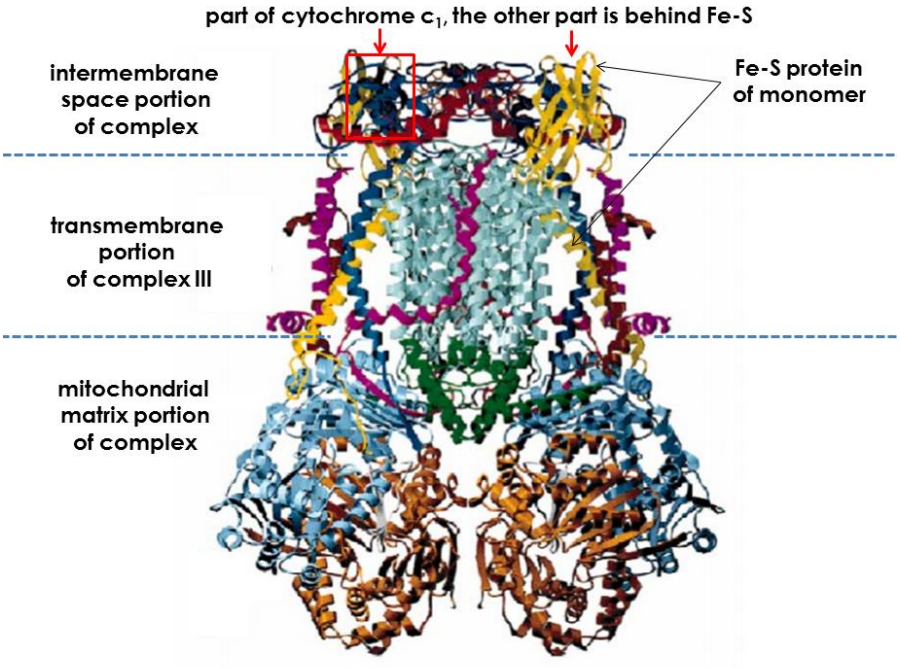

Fig. 5.1
(adapted from https://www.tamu.edu/faculty/bmiles/lectures/electrontrans.pdf)

In a deficiency in the mitochondrial respiratory chain, the level of core proteins (Fe-S protein) and subunit VI of complex III were greatly diminished in the patient's mitochondria. Cytochrome c_1 polypeptide was found at normal levels but was sensitive to proteolysis by trypsin. These results show that complex III is not assembled in the patient's mitochondria.

(a) Explain the role of complex III in the respiratory electron transport chain. **[2]**

(b) Describe the advantage of complex III being a multi-enzyme complex. **[3]**

(c) Describe the role of iron-sulfur in the Fe-S protein. **[2]**

(d) State a post-translational modification and suggest how it can prevent cytochrome c_1 polypeptide from undergoing proteolysis by trypsin. **[2]**

[Total: 9]

Section B

Answer 3 out of the 4 questions in this section.

6 *c-Myc* codes for the transcription factor c-Myc, a nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. A mutated version of *Myc* is found in many cancers, which leads to constitutive (continuous) gene expression. Overexpression of the oncoprotein leads to the unregulated expression of many target genes, some of which are involved in cell proliferation. The result is the formation of cancer.

(a) Briefly discuss one possible benefit and one possible limitation to studying the nucleotide sequences of the *Myc* as compared to studying the amino acid sequence of the protein. **[2]**

(b) A student purified the protein from a bacterial expression system. After precipitating the abundant protein out of solution, Instead of resuspending the purified protein in protein storage buffer, he added an excess of dilute 0.1M HCl to Myc protein.

Explain what would happen to the structure of the protein. **[2]**

(c) **Fig. 6** shows the structure of the Myc protein. In this particular instance, it is shown to be dimerized with its partner Max, another transcription factor.

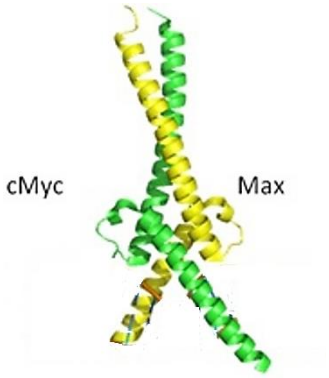

Fig. 6

Account for at least two domains that should be present on Myc protein so that it can carry out its function. **[2]**

(d) *c-Myc* protein is believed to regulate expression of 15% of all genes through binding enhancer sequences and by facilitating chromatin remodelling.

Explain how Myc protein is able to regulate gene expression

(i) by binding to enhancer sequences. **[1]**

(ii) by facilitating chromatin remodelling. **[3]**

[Total: 10]

7 **(a)** (i) Outline how Edman degradation may be employed to determine the amino acid sequence of a protein. **[2]**

(ii) Imagine you are a scientist who has previously isolated the 160 kDa human lactase enzyme from a cell extract. There are 1927 amino acids in the human lactase enzyme.

Explain why this Edman degradation sequencing technique is unlikely to be the method of choice for the sequencing of the protein and state a method of choice.

[1]

(b) (i) You have obtained the amino acid sequence of lactase. Comment on the significance of this sequence if you were to make use of yeast or bacterial cells for mass production of the protein. **[2]**

(ii) Assuming that no antibody is available that binds specifically to your protein of interest, describe how the protein can be extracted from the host cells in a purified form using a specific column chromatography. **[5]**

[Total: 10]

8 In high throughput analysis of gene function, genes were obtained from a library of open ORFs that code for proteins with a variety of functions (kinases, transcription factors and cell surface receptors). The genes were amplified by PCR and then placed into Gateway destination N- and C-terminal GFP fusion vectors. These constructs were introduced into HEK293T cells and GFP fluorescence was visualized by confocal microscopy.

All C-terminal fusion proteins localized to cellular compartments in accordance with previous studies and bioinformatic predictions, while less than half of the N-terminal fusion proteins localized correctly.

(a) (i) What conclusions can be drawn from these observations about tagging at N-terminal and C-terminals protein tagging? **[1]**

(ii) N-terminal signal peptides are found in the N-terminal GFP fusion proteins that did not localise correctly. Suggest a cause for the incorrect localisation. **[1]**

(b) Other than fluorescent tags, state another way of tagging proteins that can be used for tracking their movement.

Describe one advantage and one disadvantage of each type of protein tags. **[3]**

To study protein localization in budding yeast, Erin O'Shea and co-researchers created a GFP-tagged yeast strain collection and database. They reported new information on 70% yeast proteins whose subcellular localisations were previously unknown, providing a better understanding of the processes of transcriptional co-regulation, as well as protein interactions that occur within organelles in eukaryotic cells.

Fig. 8.1A illustrates how they constructed the GFP-tagged library. **Fig. 8.1B** shows distinct subcellular localization of some of these yeast proteins.

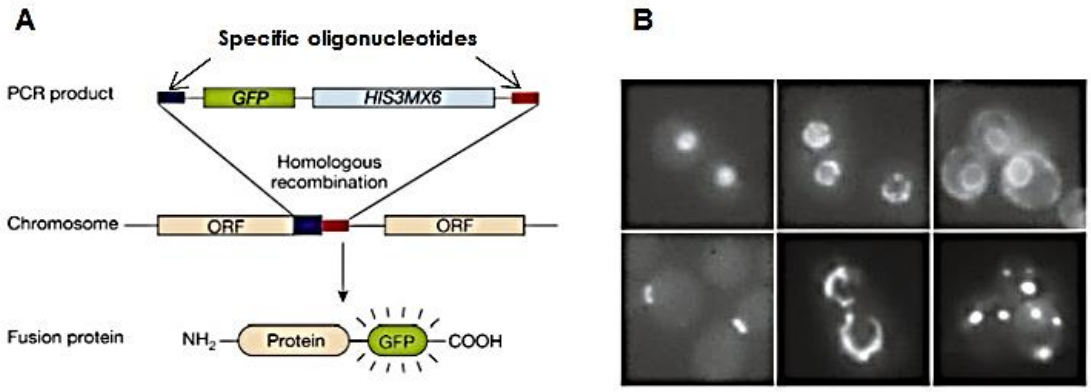

Fig. 8.1 (A) tagging of an ORF in its chromosomal location and **(B)** subcellular localisations of different GFP-tagged proteins (top panel from left: nucleus, nuclear periphery, endoplasmic reticulum; bottom panel from left: bud neck, mitochondrion, lipid globules).

(c) Yeast strains can be mated. Use this information and **Fig. 8.1** to outline the major steps of an experiment to investigate if two proteins colocalize. **[3]**

(d) Aberrant protein localization is a prominent feature of many human diseases. Aberrant folding of proteins with important cellular roles can cause diseases due to loss of the protein function or result in proteins that retain intrinsic function yet cease to function normally due to mislocalization.

State the name(s) of experiment(s) that you will use to confirm the second outcome.

[2]

[Total: 10]

9 Phosphorylation of protein kinases (PKs) plays a central role in the signal transduction of cells.

(a) Describe the type of protein-protein binding that allows a protein kinase to recognize the proteins that it will phosphorylate. **[2]**

(b) Explain the effects of phosphorylation of a named protein kinase of your choice. **[4]**

(c) Previous studies have shown that Mg^{2+} ions are required at the active site of PKs. Explain the role of Mg^{2+} ion in the function of PK. **[4]**

[Total: 10]

Section C

Answer the question in this section.

10 **(a)** A team of researchers have recently isolated microbial organisms (termed extremophiles) from regions on earth with extreme conditions. They hope that these microbial genomes will contain novel genes that allow these microbes to adapt to life in inhospitable environments. These genes are of interest in biotechnology as the high stability of their protein products can be exploited commercially in industrial processes.

You have received the sequenced genomes of 6 such microbial isolates. Outline the major bioinformatics steps you will take to investigate (i) if these genomes contain potentially novel genes and (ii) the potential functions of these genes. **[8]**

(b) Briefly describe the significance of protein glycosylation. Using your knowledge of analytical techniques, Suggest and outline one method that you would use to detect and quantify glycosylated proteins. You can choose an antibody-based method or mass spectrometry. **[6]**

(c) Proteins exert their biological functions within the spatiotemporal context of an intact cell. A protein needs to be at the right place at the right time to gain access to appropriate molecular interaction partners.

A cDNA library from metastatic human breast cancer tissue is available to you. One of these cDNA clones codes for Molecule **C**, which is hypothesised to be a major component of a complex pathway that promotes formation of new blood vessels in metastatic tumours.

Describe an *in vivo* experiment that can be performed to identify all potential interacting partners of Molecule **C** in this pathway. **[6]**

[Total: 20]