2017 Molecular Techniques STQ MS

2017 / H2 / AJC PRELIM / P2 Q2

1 Recently, scientists discovered the presence of a population of bone-marrow derived stem cells that have the ability to form heart muscles cells when transferred to the heart. The stem cells were removed from the bone marrow and cultured so that they divided by mitosis. It was proposed that these stem cells resembled embryonic stem cells.

(a) (i) Describe **two** similarities between these bone-marrow derived stem cells and embryonic stem cells.

- **Unspecialised** cell with **no specific structure and function**;
- Ability to **self-renew** via **mitosis**
- Ability to **differentiate** into **specialised** cell types under suitable conditions
- Exhibit **pluripotency**: ability to differentiate into **almost any** cell types except cell of extra-embryonic membranal cells

[2]

(ii) Describe how the rate of mitosis is controlled.

- External growth factors serve as ligands that bind to receptors of cell surface membrane;
- fully- activated receptor activates relay protein which activates a series protein kinases in the phosphorylation cascade;
- Cellular response is the switching on genes that code for transcription factors which will bind to promoter of cyclin genes/ genes that promote or slow down cell cycle;
- Increase transcription and translation of proteins that promote or slow down cell division/ M,S, G1 cyclins/CDKs which control checkpoints in cell cycle;
- **G_1 checkpoint** checks that cell size is adequate/ There is sufficient nutrients are available to support daughter cells/ Growth factors (Extracellular signal proteins that stimulate a cell to grow or divide) are present.
- **G_2 checkpoint** checks that cell size is adequate/ DNA replication is complete and successful/ there is no DNA damage.
- **Metaphase (M) checkpoint** checks that chromosomes are under bipolar tension (in other words, properly attached to kinetochore microtubules originating from the two different poles of the cell)/ chromosomes are aligned at the metaphase plate.
- **Cell cycle checkpoints prevent premature progression of the cell cycle**/ E.g. prevent the segregation of chromosomes before DNA replication is completed.
- **Provides time for cell machinery to be repaired should there be any damage/** E.g. To repair incorrectly replicated DNA sequence.
- Tumour suppressor genes code for proteins that preventing the stimulating activity of cellular proto-oncogenes or oncogenes/ activating DNA repairing genes /activating apoptosis (programmed cell death), preventing uncontrolled cell division.

[4]

(iii) State an advantage of using bone marrow derived stem cells rather than heart stem cells for the treatment of heart diseases.

- Idea of bone marrow stem cells are **easier to isolate / extract** by direct aspiration from the bone marrow in the spinal cord

OR

- Idea of isolation/ extraction of bone marrow stem cells are **less less risky**/ may puncture major blood vessels in removing heart stem cells.

[1]

(c) Troponin is a protein that is integral to muscle contraction in heart muscles. **Fig. 2.1** shows part of its DNA sequence. The entire sequence is 63 base pairs.

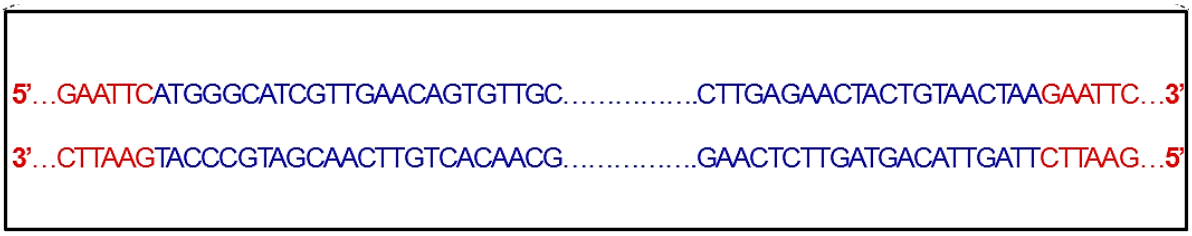

Fig. 2.1

PCR can be used to confirm presence of troponin DNA sequence. The following pair of primers are used.

Primer	Primer sequence
1	5' AATTCATGGGCATCG 3'
2	5' GAATTCTTAGTTACA 3'

(i) In the boxed area in Fig. 2.1, circle and label the DNA sequences where Primers 1 and 2 will anneal. [1]

(ii) Explain how results of gel electrophoresis of the PCR products are able to show that troponin DNA has been successfully amplified.

- If cloning is successful, a band corresponding to a DNA fragment of 63 bp would be observed;

[1]

(iii) Besides the use of PCR, nucleic acid hybridisation can also be used to determine presence of troponin DNA.

Outline how nucleic acid hybridisation can be used to identify troponin DNA.

- Extract DNA from heart cells, add restriction enzyme and perform gel electrophoresis to separate DNA fragments by size and charge/ Heart cells pressed against a special filter paper;
- which is treated with chemical (NaOH) to burst/lyse the cells and denature the **double-stranded** DNA to obtain **single-stranded** DNA on the filter;
- (Solution containing) chromogenic / labelled / radioactive **single-stranded** nucleic acid/DNA probe complementary to (part of) the troponin DNA is added, probe will hybridise/ bind/anneal to troponin DNA sequence if it is present at areas on the filter paper;
- Carry out/perform autoradiography by placing filter paper on a photographic film; [4]

[Total: 13 marks]

2017 / H2 / ACJC PRELIM / P2 Q4 (Mutations Incl)

2 Sickle cell anaemia is a recessive genetic disease caused by a mutation that commonly occurs in the DNA, resulting in hydrophobic valine replacing hydrophilic glutamic acid at the 6th amino acid position of the β chain.

(a) State the type of mutation that commonly occurs to result in sickle cell anaemia. [1]

1. **(single) base-pair substitution;;**

(b) Describe the effects of this change in amino acids on the red blood cells of an individual with the disease. [4]

1. **This results in a change in the tertiary structure/3D conformation of haemoglobin to produce haemoglobin S (HbS) instead of HbA;;**
2. **This decreases the solubility of deoxygenated HbS and at low oxygen concentration, hydrophobic areas of different HbS would stick together;;**
3. **HbS molecules will polymerise and precipitate out of solution to form rigid fibres;;**

4. **the change from HbA to HbS would result in changes to the shape of the red blood cells from circular biconcave shape to become sickle shape;;**

To detect if individuals are afflicted with sickle cell anaemia, restriction fragment length polymorphism (RFLP) analysis can be carried out using gel electrophoresis and Southern Blotting. Restriction enzymes are used to digest the DNA before RFLP analysis and the mutation removes a recognition site of the restriction enzyme *MstII,* as shown in Fig. 4.1. The enzyme's recognition sites on the normal allele and the mutant allele are shown by arrows.

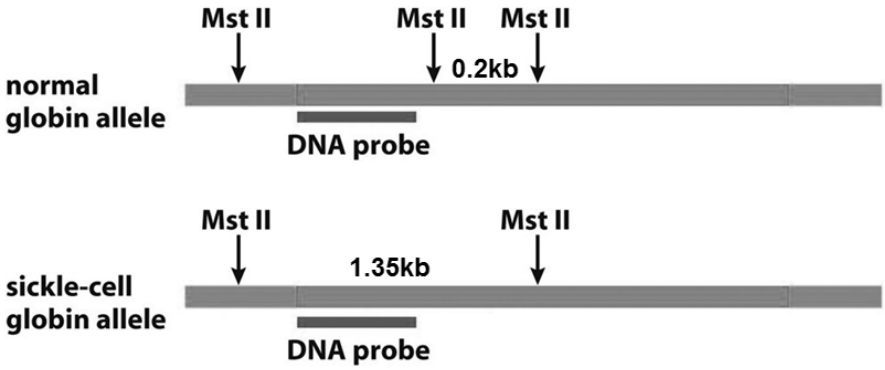

Fig. 4.1

(c) Draw, on Fig. 4.2, the expected band patterns produced by DNA from individuals with sickle cell anaemia. [1]

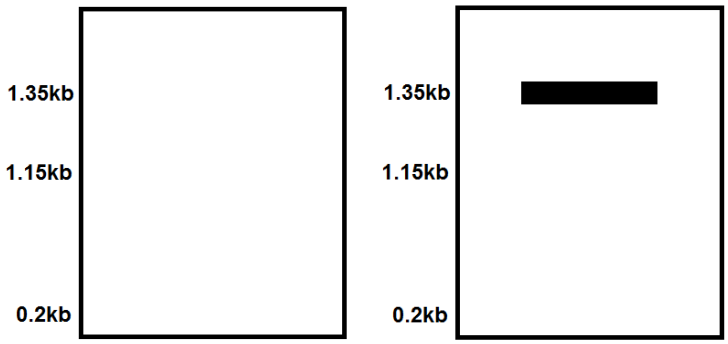

Fig. 4.2

(d) Suggest why it is necessary to carry out Southern Blotting after gel electrophoresis. [2]

1. **After gel electrophoresis is carried out to separate DNA fragments according to size, there might be <u>too many bands to be distinguished individually</u>;;**
2. **Hence, Southern blot is usually carried out to <u>identify the DNA fragment of interest</u>;;**

(e) Outline the role of the DNA probe in Southern Blotting. [1]

1. **The probe is single-stranded to hybridise/complementary base pair with DNA fragments/specific nucleotide sequences on the nitrocellulose paper;;**
2. **The probe is radioactively-labelled so that the DNA fragments will show up as bands after autoradiography/on the photographic X-ray film;;**

[Total: 9]

Question 3

Epidermal growth factor (EGF) is released by cells, and is picked up either by the cell itself or by neighboring cells. It regulates the production of a number of proteins in target cells. Protein produced and its effect depends on the type of target cell.

Fig. 3 shows how EGF regulates 3 genes.

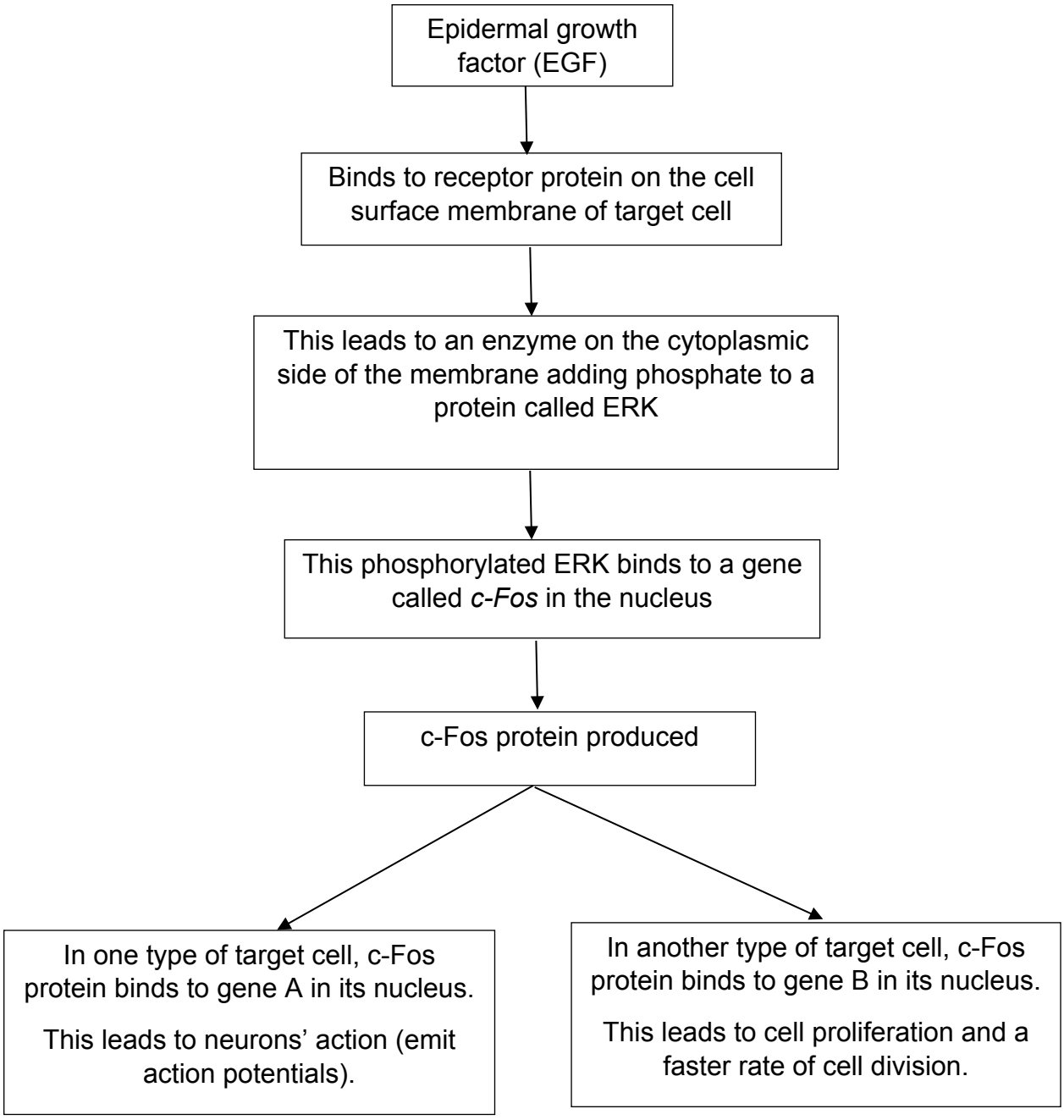

Fig. 3

(a) Name the **two** transcription factors in Fig. 3.

……[1]

1 Phosphorylated ERK ; AND
c-Fos (protein)

(b) The *c-Fos* gene can be a proto-oncogene.

Use the information in Fig. 3 to explain how a mutation of the *c-Fos* gene can result in the formation of a tumour.

……[3]

1 **gain of function mutation** of proto-oncogene to form oncogene
2 [Compulsory point] that code for **abnormal** c-Fos protein [Reject: overexpression] which is constitutively active / degradation-resistant;
3 lead to increased expression of gene B / **form more gene B product**, thus, **over-stimulation of the cell cycle** / cell keeps dividing [only award mark if point 2 is correct]

(c) Gene B has been associated with a significant number of human cancers. Scientists used polymerase chain reaction (PCR) to make multiple copies of gene B extracted from a patient's cancer tissue sample.

The reaction mixture includes the sample of DNA to be copied plus the following ingredients:

- DNA primers
- buffer solution
- heat-stable DNA polymerase (Taq polymerase)
- deoxyribonucleoside triphosphates (deoxyATP, deoxyTTP, deoxyCTP and deoxyGTP)

(i) Suggest why a buffer needs to be present in the reaction mixture.

……[1]

1 to control the pH
/ to stop the polymerase denaturing
/ to optimise pH for polymerase activity

(ii) The deoxyribonucleoside triphosphates that are added to the reaction mixture are the monomers used for making the new DNA strands.

Suggest **one further** reason for adding the deoxyribonucleoside triphosphates to the reaction mixture.

……[1]

1 *Ideas that* it is a source of energy / AW ;
(hydrolysis of the dATP to dAMP and PP release energy which is used in the catalysis of phosphodiester bonds in the polynucleotide chain)

(iii) In the first stage of PCR, the mixture is heated to a temperature of around 90°C to denature the DNA. Suggest why high temperatures are needed to separate the two DNA strands.

……[2]

1 *Idea of* many hydrogen bonds between **complementary** strands together ;
2 Hydrogen bonds break because of increased kinetic energy / vibrations ;

(iv) At the end of several cycles of PCR, many copies of the DNA sample in the reaction mixture will have been made. The DNA samples are then separated out to produce a DNA banding pattern.

State the technique used to separate out the DNA samples **and** describe how this technique works.

……[4]

1 Gel electrophoresis ;

2 (Load 10 µl of sample into the wells in agarose gel ;
Gel electrophoresis conducted at 100V till tracking dye move to ¾ length of gel)
DNA is **negatively-charged** (due to negatively-charged sugar-phosphate backbone) move towards the **positively-charged** electrode

3 through an agarose matrix which acts as a **molecular sieve** ;

4 DNA fragments separated by size ; where shorter DNA fragments move faster [Reject: further] than longer ones;

[Q3: 12 marks]