HWA CHONG INSTITUTION (COLLEGE SECTION)
2015 JC2 H3 BIOLOGY
PRELIMINARY EXAMINTIONS PAPER 1 MARK SCHEME

Question 1

(a) Explain the role of signal peptide in IL-6 receptor. [2]

1. Ref to signal recognition particle (SRP) binding to signal peptide to target protein synthesis in endoplasmic reticulum (ER) and sorting in Golgi apparatus (GA)
2. IL-6 functions on the cell membrane

(b) Describe the change in binding forces between IL-6R and Tocilizumab at pH 6.0 and pH 7.4. [3]

1. Ref to binding via hydrogen bonds, ionic bonds and hydrophobic interactions at pH 7.4
2. R-groups of antigen-binding sites of anti-IL6-R antibodies and IL6-R epitopes may be protonated at pH 6.0
3. The hydrogen bonds and ionic bonds are disrupted resulting in dissociation

(c) Suggest the region(s) and identity of amino acid(s) that were modified in Tocilizumab to enable its recycling for repeated actions in plasma. [2]

1. hypervariable regions
2. histidine

(d) Suggest one benefit of engineering pH dependency into the interactions of therapeutic antibodies with their targets. [1]

1. It allows the antibody drug to be delivered less frequently
2. It allows the antibody drug to be delivered at lower doses
3. It reduces overall cost of treatment

Question 2

(a) Describe one protein-protein interaction between
(i) the subunits of a named protein [2]

1. Coiled-coiled interaction between α helix of α keratin
2. Ref to two α helices supercoil
3. Ref to interdigitation of side chains

OR

4. Surface-surface interaction between subunits of haemoglobin
5. Ref to matching of surface between subunits of haemoglobin
6. Ref to bonds between these surfaces

(ii) named proteins in a complex [2]

1. Surface-surface interaction between the three enzymes of pyruvate dehydrogenase;;
2. Ref to name of enzymes
3. Ref to bonds

(b) Explain the significance of pyruvate dehydrogenase as a protein complex. [4]

1. Ref to prevention of rate limitation of enzyme activity
2. Ref to spatial organization of functional enzymes to a reaction sequence
3. Ref to substrate channeling occurs in PDC

Question 3

(a) Suggest how the modifications act to keep myogenin non-functional. [2]

1 Reference to Id blocking the binding site for DNA / changing the conformation of the binding site for DNA
2 Reference to phosphorylation making the DNA-binding domain negatively-charged and hence repelling DNA

(b) Explain the use of an *in vivo* system to identify the interaction partners of myogenin. [4]

1 Reference to GAL4 transcription factor comprising DNA-binding domain (DBD) and activation domain (AD)
2 Reference to bait plasmid and prey plasmid
3 Transform the bait plasmid and the prey plasmids into yeast strains and allow the yeast strains to mate
4 If a prey plasmid encodes an interaction partner of myogenin, the bait and the prey protein interact to serve as an transcription activator. Expression of *lacZ* reporter gene occurs and the yeast colony turns blue

(c) Name an interaction partner of myogenin that cannot be identified by the system described in **(a)**. [1]

DNA

(d) Describe how proteins from muscle cell samples can be prepared for SDS-PAGE. [3]

1 Reference to physical means and chemical means.
2 Determine protein concentration. Add 100 µg of total protein for each time point to a microcentrifuge tube.
3 Add 20 µL sample buffer with SDS to each tube. Heat the samples at 100 °C for 5 min.

(e) Explain why actin was included in the Western blot analysis. [2]

1 Reference to actin serving as a loading control with constant intensity of actin bands observed
2 This shows that different intensities of myogenin bands is due to differential gene expression

Question 4

(a) Explain how mass spectrometry can be used to study the phosphorylation of IRF-3. You may sketch a graph to illustrate your answer. *(Hint: Enzyme alkaline phosphatase catalyses dephosphorylation. HPO_3 = 80 Da)* [4]

1 Correct axes, e.g., relative abundance / intensity and mass-to-charge ratio (m/e)
2 Ref to *in vitro* treatment with and without phosphatase
3 The two base peaks differing by 80 Da on the x-axis (m/e)
4 Ref to MS separates fragments based on size in an electric filed

(b) Describe how co-immunoprecipitation (co-IP) can be used to detect the interaction partner(s) of IRF-3 and suggest an advantage of such an *in vitro* system. [3]

1 Ref to preparing appropriate protein lysates and incubation with anti-IRF-3 antibodies conjugated to beads
2 Ref to washing, elution and suitable detection method
3 Co-IP may detect interactions between more than two partners / Ref to endogenous proteins / native state

(c)(i) Suggest an experiment with a proper control to verify this hypothesis. [4]

1 Ref to cloning of GFP tag in frame to create *IRF-3-GFP* fusion gene
2 Ref to including mutant IRF3 (control) that cannot be phosphorylated vs wild-type IRF3
3 Fluorescent microscopy
4 To visualise living cells for nuclear translocation
Accept: ref to create *IRF-7-GFP / RFP* fusion gene
Accept: ref to fluorescence resonance energy transfer (FRET)

(c)(ii) Suggest a potential problem in the approach you have suggested. [1]

1 Overexpression may lead to interaction that does not exist under physiological conditions
2 GFP-tag may hinder the nuclear translocation of the fusion protein

Question 5

(a) Explain what is meant by a *protein family*. [2]

1 Ref. to group of evolutionary related proteins
2 That share common domains/motifs/sequences

(b) Explain how FAD^+ facilitate the function of SDHA. [2]

1 FAD+ acts as a co-enzyme of SDHA
2 Binding of a FAD^+ cofactor oxidizes SDHA, producing reduced FADH2

(c) Describe the structure of SDHA and the function of the FAD domain. [4]

1 SDHA is a hydrophilic flavoprotein that extend into the matrix
2 The presence of a FAD domain allows SDHA to be covalently attached to a FAD cofactor
3 FAD allows for the directing of electrons from succinate to FAD

(d) Explain how SDHA can be a substrate of SIRT3. [2]

1 Ref to 13 acetylated sites of SDHA pointing to active site of SIRT3
2 SIRT3 will deacetylate the 13 acetylated sites of SDHA

Question 6

(a) Describe how ultracentrifugation can be used to determine the molecular weight of haemoglobin. [3]

1 Formation of gradient

2 Principle of analytical ultracentrifugation
3 Use of globular proteins as standards

(b) Explain how the number of haemoglobin subunits can be determined. [3]

1 Determination of mass of entire haemoglobin
2 Determine mass of 1 subunit
3 Determine quotient to obtain number of subunits

(c) Distinguish between velocity sedimentation and equilibrium sedimentation. [4]

1 Reference to formation of density gradient
2 Reference to principle
3 Reference to basis of separation
4 Reference to time taken for separation

Question 7

(a) Discuss the importance of hydrogen bonds in proteins besides in the protein structures. [3]

1 Ref to enzyme catalysis
2 Ref to ligand-receptor binding
3 Ref to antibody-antigen binding
4 AVP

(b) Explain how unique amino acid sequences in proteins are specified by genes. [3]

1 Genes contain unique base sequences
2 Genes are transcribed to mRNA and mRNA codons are read in non-overleaping triplets
3 Specific codon sequences specify the particular amino acids to be incorporated, when mRNA acts as a template for translation in synthesizing the unique sequence of polypeptide chain

(c) Outline the principle of nuclear magnetic resonance (NMR) and x-ray crystallography in protein structure determination. [4]

NMR

1 Ref to nuclear spin of proton (^{1}H)/ nitrogen-15 (^{15}N) / carbon-13 (^{13}C) in an electromagnetic filed
2 The different nuclear spins allow us to calculate the chemical shifts of functional groups / to investigate the 3 dimensional interaction of distant elements for structural determination

X-ray

3 Crystalline structures diffract X-ray, whose wavelength is about the diameter of an atom into a regular pattern
4 Fourier transformation allows data interpretation and construction of an electron density map to visualize the 3 dimensional structure of protein

Question 8

(a) Discuss the mechanism of enzyme action. [4]

1. Ref to increase the local concentration of substrate molecules

2. Ref to a series of intermediate states of substrate
3. Ref to unstable transition state
4. Ref to activation energy

(b)(i) catalytic triad [3]

1. Catalytic conserved catalytic triad formed by Ser-195, His-57 and Asp-102 hydrolyses peptide bonds in target proteins
2. Ref to positioning of residues that helps catalysis

(b)(ii) hydrophobic and hydrophilic cleft [3]

1.
2. Hydrophobic clef possesses neighbouring parts of a polypeptide chain that may interact to restricts the access of water molecules
3. Allowing the ligand to bind by displacing water from protein

4. In hydrophilic clef, the different clustering of neighboring polar amino acid side chains can alter their reactivity
5. Clustering of negatively charged side chains allows for greatly increased affinity for a positively charged ion

Question 9

(a) Discuss the structural changes of HA in facilitating the release of the influenza viral genome into the cytoplasm. [6]

1. HA molecule is a homotrimer
2. Ref to HA 1 and HA2 subunits and how they are held together
3. Ref to HA1 subunit, consisting of a globular domain forming the receptor binding site
4. Ref to HA2 subunit at the stem region consisting of the hydrophobic/ fusion peptide
5. which is buried within the molecule

6. Fusion of the endosome with an acidic lysosome lowers the pH of the vesicle, resulting in conformational change of HA
7. three HA1 globular domains separate from one another but remained tethered to the HA2 subunits by the disulphide bonds
8. fusion peptides are exposed and inserted into the endosomal membrane
9. triggering the fusion of viral and endosomal membranes.

(b) Explain the necessity for annual vaccine updates and the challenges behind developing vaccines. [4]

1. Ref to mutations of HA and NA due to poor proofreading ability of the viral RNA polymerase
2. inability of antibodies to neutralise the antigens leading to antigen drift

3. different viruses from different host species coinfecting a single host cell
4. reassortment of genome segments
5. Leading to a new subtype

Question 10

(a) Suggest the features that make *C. elegans* a model organism in the study of the genetics of development. [4]

1 Reference to small number of cells
2 Reference to transparency
3 Reference to rapid growth
4 Reference to ability to self

(b) Describe the mechanism of RNAi. [6]

1 Long dsRNA triggers RNAi
2 Dicer processes dsRNAs into 21-23 nucleotide short interfering RNA (siRNA) with 2-nt 3' overhangs
3 The siRNAs are incorporated into the RNA-inducing silencing complex (RISC)
4 Argonaute (Ago) protein in RISC cleaves and discards the passenger (sense) strand of the siRNA duplex
5 Guide (antisense) strand of the siRNA guides RISC to its homologous mRNA
6 This results in endonucleolytic cleavage of the target mRNA

(c) Discuss the advantages and disadvantages of using mTn over RNAi in genetics studies. [4]

Advantages
1 Multipurpose minitransposon (mTn) may give absolutely no gene expression (knockout) compare to partial gene expression in RNAi (knockdown), and therefore the conclusions can be made easily
2 The mutant phenotype generated by mTn can be stably maintained / passed on to future generations
3 AVP

Disadvantages
1 More time-consuming to generate the mutants in the mTn mutant screen than RNAi
2 mTn rely on random mutagenesis, and therefore no specific design of sequence to be knocked out if only one particular gene is to be studied
3 mTn is unable to study the genes crucial for development or survival of organism
4 AVP

(d) Explain how bioinformatics can analyse disrupted gene sequences generated by mTn technology in a large scale mutant screen. [4]

1 Sequencing of the mutant genome to obtain DNA sequences
2 Ref to BLAST / local sequence alignment of the mutant genome with a database (of the organism)
3 Ref to statistical significance
4 To find gene that is disrupted corresponding with the mutant phenotype

(e) Suggest how you would verify if the mTn or RNAi has worked. [2]

1 quantitative real-time PCR;;
2 western blot
3 rescue experiment