M. Sc. Bio-Technology Part I Examination, 2018

Molecular Biology

Paper : MSBT-1/3

Time : Four hours | Full Marks : 100

GROUP - A

Answer Question No. ***1***

1. Answer ***all*** questions. 10×1=10

   a) Mention the types of chemical bonds that are possible between two macromolecules.

   b) What is the approximate molecular weight of a protein (assume that it consists of a single polypeptide chain), which is encoded by 2481 base pair long gene ?

   c) Give an example of transcription-translation coupled gene regulation in *E. coli*.

   d) Show qualitatively how the UV-ratiation survival curves of wild-type, *rec* mutant, and *uvr* mutants *E. coli* would look like.

   e) give an example of pairs of amino acid side chains that can interact with each other through an ionic bond at neutral pH.

f) Predict the likely effect of substituting one or more of the conserved cysteines or histidines in a Cys_2His_2 zinc finger with alanine. Explain your answer.

g) Why is a Holliday crossover resloved in one way only in the cell ?

h) What is "Wobble Hypothesis" ? Briefly mention the structural flexibility around the anticodon of the tRNA molecule, which accounts for the wobble rule.

i) What makes the Chi site a recombination hot-spot in *E. coli* ?

j) What are the candidates involved in positive and negative regulation of *LacZ* gene in *E. coli* ?

19. The errors in DNA replications arising from the mistakes made by the polymerases are removed by the polymerases themselves. But the repair polymerases often fail to do that. Explain. 10

i) What does the length of the C=N bond in the peptide linkage indicate about its strength and its bond order (i.e., whether it is single, double, or triple) ?

ii) What do the observations of Pauling and Corey tell us about the ease of rotation about the C=N peptide bond ? 2+2

b) Although an overall structural monotony is displayed by the double-stranded DNA, sequence-specific binding of DNA binding proteins relies on the distinct micro-viration observed at the specific segments of the double helix. Which additional structural parameters define these micro variations ? 4

c) What is the net charge of histidine at pH 1, 4, 8, and 12 ? For each pH, will histidine migrate toward the anode (+) or cathode (–) when placed in an electric field ? 2

4. a) Histones are proteins found in eukaryotic cell nuclei, tightly bound to DNA, which has many phosphate groups. The pI of histones is very high, about 10.8 Predict, which amino acid residues must be present in relatively large numbers in histones ? How these residues contribute to the strong binding of histones to DNA ? 3

13. a) What is microRNAs (miRNA) ? Mention the underlying principle how miRNAs contribute to regulation of gene expression in eukaryotes ? What are the ways these small RNAs impact the final level of protein in the cell under a given sets of condition. 4

b) Cite one example where pre-mRNA splicing plays a role in the regulation of gene in eukaryotic system. 2

c) What do mean by “euchromatin” and “heterochromatin”? Explain the underlying principle by which a specific segment of chromosome can be converted from euchromatin to heterochromatin and vice versa. 2

d) What is “insulator” ? Explain how an insulator element can influence the expression of surrounding genes ? 2

PART - II

(Answer ***any 2*** questions)

6. a) What is Okazaki fragment ? Why these fragments are generated during DNA replication ? Using necessary diagrams and flowchart, briefly describe the experiment (long description is not necessary), which Okazaki designed to detect the existence of these smaller fragments. Why was it important to use a short exposure to the radioactive label rather than a longer one in this experiment ? 4

   b) Why telemerase is not required in *E. coli* cells. Justify your answer. 1

   c) Describe the role of a DNA helicase at a replication fork. 2

   d) The *E. coli* chromosome contains 4,639,221 bp.

      i) How many turns of the double helix must be unwound during replication of the *E. coli* chromosome ?

      ii) How long would it take to replicate the E. coli chromosome at 37^{o}C if two replication forks proceeded from one origin ? Assume replication occurs at a rate of 1,000 bp/s. Under some conditions *E. coli* cells can divide every 20 min. How might this be possible ? 1+2

PART - III

(Answer ***any 2*** questions)

10. a) Describe one experiment/evidence that supports the statement that an rRNA and not a protein component of the ribosome catalyzes the peptidyl transferase reaction. 4

    b) Describe the significance of the sequence 5'-CCA-3'' at the 3' terminus of every tRNA. 2

    c) Explain where the energy comes from for peptide bond formation. 2

    d) Explain what do you mean by "peptide anticodon" ? What is the functional role of the peptide anticodon in the translation ? 2

11. a) Describe the different mechanisms used to ensure translation accuracy, beginning with the charging of the tRNA and continuing up to the transfer of the amino acid to the growing polypeptide chain. 4

    b) Explain the cellular advantage for the codon 5'-AAG-3' to code lysine and the codon 5'-AGG-3' to code for arginine. 2

    c) Shown below is a portion of a wild-type DNA sequence that encodes the last amino acids of a protein that is 270

7. a) Some *E. coli* conditional mutants contain defective DNA ligase. When these mutants are exposed to ^{3}H-labeled thymine and the DNA produced is sedimented on an alkaline sucrose density gradient, two radioactive bands appear. One corresponds to a high molecular weight fraction, the other to a low molecular weight fraction. Explain the results. 3

   b) What is mRNA editing ? Among the various kinds of RNA editing reactions, mention just one fundamental reaction. Which enzyme plays a vital role in this process and briefly describe its function. 3

   c) What do mean by "Exon Shuffling". Mention the role of this process in generating huge amount of diversity observed in the structures of present day proteins. 4

8. a) What is TBP ? Which basic feature of TBP helps to recruit other factors to the eukaryotic promoters in sequential manner ? Briefly explain why additional factors such as activators and modifiers are required to activate transcription in eukaryotic promoters. 4

   b) What steps in the eukaryotic transcription cycle are stimulated by phosphorylation of the carboxyl terminal (CTD) of the large subunit of RNA polymerase II and beyond ? 4

   c) What purposes do capping and poly-A tail addition serve for eukaryotic mRNAs ? 2

9. a) Describe the product of a splicing reaction in which the spliceosome recognizes a "pseudo" 3'-splice site within the intron instead of the actual 3'-splice site. The "pseudo" site is slightly upstream of the actual 3'-splice site. How could this alter the protein product after translation ? 3

   b) Compare the self-splicing reactions carried out by group I and group II introns. Which is most similar to the reaction carried out by the spliceosome on pre-mRNAs ? 4

   c) How the transcription termination in eukaryotes are accomplished. Explain the role of the phosphorylation of c-terminal domain (CTD) in this process. 3

amino acids long. The first three bolded base pairs indicate the frame and include the coding region.

5'-**GCT**AAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3'

3'-**CGA**TTCATAACGAGTTCTAATCCTACTATTTATTGACC-5'

i) Which strand is the template strand for transcription of this gene ? Briefly explain how you know it. 1

ii) An insertion of one base pair causes the protein to decrease in length by seven amino acids. With respect to the sequence given above, where does this insertion occur ? 3

12. a) Briefly describe how many regulators play functional role in accomplishing the regulation of GAL genes in yeast Saccharomyces cerevisiae ? Describe how the regulatory circuitry works in absence and presence of glucose and galactose in the medium. 4

b) Other than the level of transcription, what other types of regulation for gene expression occurs in eukaryotic cells. 2

c) Mention how many domains/motifs are found in eukaryotic transcription activators ? Which of these motifs among various transcriptional activators share common structural features and which motif displays structural diversity ? 4

b) Under appropriate conditions, hemoglobin dissociates into its four subunits. The isolated α - subunit binds oxygen, but the O_2-saturation curve is hyperbolic rather than sigmoid. In addition, the binding of oxygen to the isolated α -subunit is not affected by the presence of H^+ or CO_2. What do these observations indicate about the source of the co-operativity in hemoglobin ? 3

c) Hairpins may be formed at self-complementary sequences in the single strand of either RNA or DNA. How is the helical structure of a long and fully base-paired (except at the end) hairpin in RNA different from that of a similar hairpin in DNA ? 2

d) Which protein domain(s) recognize the acetylated as well as methylated histone amino-terminal tails ? 2

5. a) Mention why the genomic DNA in the eukaryotic cell is condensed into chromatin ? 2

b) What is nucleosome remodeling complex ? Explain how a remodeling complex alter the chromatin structure and how that alteration of chromatin structure alter the expression of a given gene ? Give two example of such nucleosome remodeler. 6

c) Briefly mention the forces involved in facilitating the transition from 10 nm chromatin fiber to 30 nm chromatin fiber. 2

GROUP - B

(Answer ***any three***)

14. Describe one DNA repair system that you consider as highly energy demanding. Explain why you consider the process highly energy demanding. 10

15. There are two organisms, one possessing and one without the error prone DNA repair system. Which one could possibly evolve faster and why ? Which one among them is more likely to develop cancer if exposed to DNA damaging agents ? In a RecA deletion E. coli, all the SOS proteins are getting over expressed upon UV irradiation of the cells. How could this be possible ? 10

16. How do spontaneous mutations arise in a chromosome ? What makes the known TLS polymerases in eukaryotic cells to increase the mutation loads in chromosomes moderately ? 10

17. There are TLS DNA polymerases in Yeast, Human and Bacterial cells. Name some of them and discuss which of these organisms are likely to accumulate more mutations during error prone repair. 10

18. In E.coli, each of the Holliday crossovers is resolved in one way only. Discuss the activities of proteins that make this possible. 10

PART - I

(Answer ***any 2*** questions)

2. a) What is activation energy of a chemical reaction ? Explain why the pathway of a given biochemical reaction has to bypass the activation energy barrier. 3

   b) Explain why nucleoside triphosphates rather than nucleoside di-phosphates are used in DNA synthesis. 2

   c) Define polar and nonpolar molcules in terms of dipole moments. Does van der Waals interaction occur between polar molecules ? 2

   d) What do you mean by “coupling” of biochemical reactions. Explain how does a cell benefit from such coupling. 3

3. a) In x-ray studies of crystalline peptides, Linus Pauling and Robert Corey found that the C-N bond in the peptide link is intermediate in length (1.32 Å) between a typical C-N single bond (1.49 Å) and a C = N double bond (1.27 Å). They also found that the peptide bond is planar (all four atoms attached to the C-N group are located in the same plane) and that the two -carbon atoms attached to the C-N are always trans to each other (on opposite sides of the peptide bond).