Department of Life and Consumer Sciences

Molecular Biology – BMI2604

Semester code: 02

Assignment 02

Due Date: 21St September 2018

Unique assignment number: 893083

INSTRUCTIONS

1) Type your assignment on a computer. You may print on ordinary white paper and not necessarily the Unisa typing paper provided. Please use 1,5 spacing and Arial or a similar font of 11 or 12 pitch. Leave a line open between questions. If you are not able to type your assignment on a computer, use a black or blue pen and please write neatly.

2) If you want to submit a hard copy of this assignment, use the assignment cover and envelope provided. When stapling your answers inside the cover, staple only in the top left-hand corner.

3) Your student number is the number just below your address. This number must be filled in on the assignment cover and must also be quoted in all correspondence with the university.

4) Answer all questions as briefly and clearly as possible in your own words.

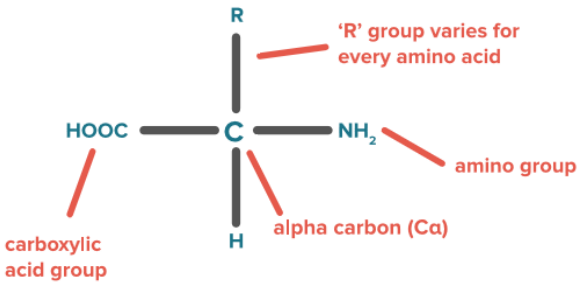
5) Number your answers correctly.

Question 1 **[20]**

1.1 Which DNA purine forms three H bonds with its partner in the other DNA strand? Which forms two H bond? **(2)**

- Adenine (purine) and thymine (pyrimidine) form two hydrogen bonds.
- Guanine (purine) and cytosine (pyrimidine) form three hydrogen bonds.

1.2 Draw the general structure of an amino acid and give the correct names for the four groups attached to the alpha-carbon of all amino acids **(5)**



1.3 Name and briefly discuss three important features of the genetic code **(9)**

Codons are triplets that are read sequentially, the genetic code is degenerate and non-random, the genetic code was systematically deciphered. The sequence of the nucleotides in the mRNA molecule is read in consecutive groups of three. RNA is a linear polymer of four different nucleotides, so there are 4x4x4=64 possible combinations of the three nucleotides: the triplets' AAA, AUA, AUG and so on. However, only 20 different amino acids are commonly found in protein (61 codons code for 20 amino acids). Three codons do not code for any amino acids. They function as stop codons to end protein synthesis. These are UAG, UGA, and UAA. Each codon codes for only one amino acid, hence, it is unambiguous and specific. Some amino acids are coded for by more than one codon, hence the genetic code is degenerate. Most of this degeneracy involves the third nucleotide of a codon.

1.4 Use a rough diagram to compare the structures of a protein α-helix and an antiparallel β-sheet. (4)

See page 116 of your prescribed textbook.

Alberts, B, Johnson, A, Lewis, J, Morgan, D, Raff, M, Roberts, K & Walter, P. 2015. Molecular Biology of the Cell. 6th edition. New York: Garland Science. ISBN: 978-0-8153-4464-3.

QUESTION 2 **[20]**

Outline steps involved in DNA synthesis at the replication fork. How do DNA polymerase correct their mistakes.

At the replication fork, a multienzyme complex that contains the DNA polymerase synthesizes DNA of both new daughter strands. Initially, the simplest mechanisms of DNA replication seemed to be the continuous growth of both new strands, nucleotide by nucleotide, at the replication fork as it moves from one end of a DNA molecule to the other. However, due to antiparallel orientation of the two DNA strands in the double helix, this mechanism would require one daughter strand to polymerize in the 5'-to-3' direction and the other in the 3'-to-5' direction. Such replication fork will require two distinct types of DNA polymerase enzymes. However, the DNA polymerase at the replication forks can synthesize only in the 5'-to-3' direction. Special proteins help to open up the DNA double helix in front of the replication fork. For DNA synthesis to proceed the following enzymes which are varied in their functions are involved. DNA Helicase opens up the DNA at the replication fork. It could unwind the DNA double helix by moving in the 5'-to-3' direction along one strand or in the 3'-to-5' along the other. Single-strand binding proteins (helix stabilizing proteins) coat the DNA around the replication fork to prevent rewinding of the DNA. Binds tightly and cooperatively to exposed single-strand DNA without covering the bases, which therefore remain available as templates. These proteins are unable to open a long DNA helix directly, but they aid helicases by stabilizing the unwound, single-strand conformation. Through cooperative binding, they coat and straighten out the regions of single strand DNA, which occur routinely in the lagging strand template, thereby preventing the formation of the short hairpin helices that readily form in single strand DNA. If not removed, these hairpin helices can impede the DNA synthesis catalysed by DNA polymerase. The proteins at replication fork cooperate to form a replication machine. Topoisomerase works at the region ahead of the replication fork to prevent supercoiling. Primase synthesizes RNA primers complementary to the DNA strand. DNA polymerase III extends the primers, adding on to the 3' end, to make the bulk of the new DNA. RNA primers are removed and replaced with DNA by DNA polymerase I. The gaps between DNA fragments are sealed by DNA ligase. DNA polymerases are the enzymes that build DNA in cells. During DNA replication, most DNA polymerases can monitor their work with each base added by the process called proofreading. If the polymerase detects that there's incorrectly paired nucleotide has been added, it will remove and replace the Mismatch repair. Many errors are corrected by proofreading, but a few slip through. Mismatch repair happens right after new DNA has been made, and its job is to remove and replace mis-paired bases (ones that were not fixed during proofreading). Mismatch repair can also detect and correct small insertions and deletions that happen when the polymerases "slips," losing its footing on the template nucleotide right away, before continuing with DNA synthesis.

QUESTION 3 [20]

3.1 Describe the process of cloning a DNA fragment into the *Pst*I site of the vector pBR322. How would you screen for clones that contain an insert? (10)

DNA cloning is the process of making multiple, identical copies of a particular piece of DNA. In a typical DNA cloning procedure, the gene or other DNA fragment of interest is first inserted into a circular piece of DNA called a plasmid. The insertion is done using restriction enzymes such as P*stI* that recognize and cut both strands of a DNA molecule at specific restriction site, the very same restriction enzyme is used to cut recombinant plasmid (vector pBR322) the gene of interest is then inserted into plasmid (vector pBR322) produces a molecule of recombinant DNA, or DNA assembled out of fragments from multiple sources. The recombinant plasmid (vector pBR322) is introduced into bacterial cells/bacterium. The bacteria are then grown in a media containing antibiotics to identify bacterial cells carrying the plasmid with the gene of interest since they will survive because they have a resistance gene. Thus, plasmids and other DNA can be introduced into bacteria, such as the harmless *E. coli* used in the laboratories, in a process called transformation. During transformation, specially prepared bacterial cells are given a shock (such as high temperature) that encourages them to take up foreign DNA. A plasmid typically contains an antibiotic resistance gene, which allows bacteria to survive in the presence of a specific antibiotic. Thus, bacteria that took up the plasmid can be selected on nutrient plates containing the antibiotic. Bacteria without a plasmid will die, while bacteria carrying a plasmid can live and reproduce. Each surviving bacterium will give rise to a small, dot-like group, or colony, of identical bacteria that all carry the same plasmid.

3.2 Define the following:

3.2.1 Coding strands: the DNA strand with the same sequence as the transcribed mRNA (given U in RNA and T in DNA) and containing the linear array of codons which interact with anticodons of tRNA during translation to give the primary sequence of a protein.

3.2.2 Reading frame: the grouping of three successive bases in a sequence of DNA that constitutes the codons for the amino acids encoded by the DNA.

3.2.3 Posttranscriptional modification: is the process in eukaryotic cells where primary transcript RNA is converted into mature RNA.

3.2 4 Coding region: is that portion of a gene's DNA or RNA that codes for protein. The region usually begins at the 5' end by a start codon and ends at the 3' end with a stop codon.

3.2.5 Promoter: is a region of DNA that initiates transcription of a particular gene. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the sense strand).

(5x2=10)

QUESTION 4 **[20]**

Discuss control of transcription initiation by regulatory proteins.

control of transcription initiation—is the most important mechanism for determining whether or not most genes are expressed and how much of the encoded mRNAs, and consequently proteins, are produced. The term gene expression commonly refers to the entire process whereby the information encoded in a particular gene is decoded into a particular protein. Theoretically, regulation at any one of the various steps in this process could lead to differential gene expression in different cell types or developmental stages or in response to external conditions. Synthesis of mRNA requires that an RNA polymerase initiate transcription, polymerize ribonucleoside triphosphates complementary to the DNA coding strand, and then terminate transcription.

This can be discussed focusing in bacteria; or transcription in eukaryotic cells which is controlled by proteins that bind to specific regulatory sequences and modulate the activity of RNA polymerase.

QUESTION 5 **[20]**

Explain the morphological changes in apoptosis. **(8)**

The onset of apoptosis is characterised by shrinkage of the cell and the nucleus as well as condensation of nuclear chromatin into sharply delineated masses that become marginated against the nuclear membranes. Later on, the nucleus progressively condenses and breaks up (karyorrhexis). The cell detaches from the surrounding tissue and its outlines become convoluted and form extensions. The condensation starts peripherally along the nuclear membrane, forming a crescent or ring like structure. During later stages of apoptosis, the nucleus further condenses, and finally it breaks up inside a cell with an intact cell membrane. Again, the cells shrink, and finally the blebs separate, forming apoptotic bodies densely packed with cellular organelles and nuclear fragments that are engulfed by phagocytosis of surrounding cells.

Discuss features of the eukaryotic cell cycle. **(12)**

The division cycle of most cells consists of four coordinated processes: cell growth, DNA replication, distribution of the duplicated chromosomes to daughter cells, and cell division. To allow time for growth, most cell cycles have gap phases: - G1 phase between M phase and S phase. G2 phase between S phase and mitosis. Thus, the eukaryotic cell cycle consists of four discrete phases including G1, S, G2 and M. G1, S and G2 are called interphase. The two gap phases are simple time delays to allow cell growth. This two gap phases they provide time for the cell to monitor the internal and external environment to ensure that conditions are suitable are suitable and preparations are complete before the cell commits itself to the major upheavals of S phase and mitosis. Although cell growth is usually a continuous process, DNA is synthesized during only one phase of the cell cycle, and the replicated chromosomes are then distributed

to daughter nuclei by a complex series of events preceding cell division. Progression between these stages of the cell cycle is controlled by a conserved regulatory apparatus, which not only coordinates the different events of the cell cycle but also links the cell cycle with extracellular signals that control cell proliferation.

Total marks= 100