BCH / BIO 3170 **Molecular Biology** Fall 2003

Answers for Midterm Exam (Thursday, October 16, 2003)

1. (i) Adenine and Guanine

(ii) You get one point for knowing the correct ring structure of a purine and a pyrimidine, and a second point for knowing the carbon and nitrogen atoms in the ring.

(iii) DNA contains thymine rather than uracil because it is less similar to cytosine, and it contains deoxyribose rather than ribose because the lack of the 2' hydroxyl makes it less reactive and more stable.

2 (i) 5' T T A G A A T T T A G C G C C C T A 3'

(ii) Met Gly Ala Lys Phe Stop

(iii) There is special tRNA, the selenocysteine tRNA, that is first charged with a serine amino acid by a special aminoacyl-tRNA synthetase. This tRNA does not contain one of the serine anticodons, but rather than anticodon 5'UCA3' that matches the codon 5'UGA3' - which is normally a stop codon. Next, the attached serine is enzymatically converted to selenocysteine (see Figure 6-77 in textbook) and, because of a change in mRNA secondary structure, the UGA codon is used to decode the selenocysteine tRNA rather than being used as a stop codon.

3. (i) When nuclei are gently lysed, most of the chromatin is seen as a 30 nanometer chromatin fiber. These fibers represents the first level of chromosome packing beyond the nucleosome structure. The structure is illustrated in Figures 4-29 and 4-30. The nucleosomes within the fibers are thought to be held together by the action of histone H1, and perhaps by the action of the "tails" of the histones that lie within the nucleosomes (see Figure 4-32).

(ii) In addition to their possible role within the 30nm fiber, the histone tails play an important role in the regulation of which proteins bind to chromatin. For instance, under acetylated histone tails form a substrate for the binding of Sir proteins (see Figure 4-47).

(iii) Through their binding of the histones within chromatin, the Sir proteins increase the degree of chromatin packing and give rise to heterochromatin. They are also thought to have a role in ensuring that the heterochromatic state is passed through the

DNA replication / cell division process. They do this by attracting additional Sir proteins to the heterochromatin regions of newly replicated DNA (see Figure 4-48).

4. (i) primase is the RNA polymerase enzyme that synthesize the RNA primer during DNA replication. Many primers need to be made on the lagging strand.

(ii) helicase is the multi-subunit enzyme that opens up the double stranded DNA ahead of the replication fork during DNA replication.

(iii) topoisomerase I is the enzyme that makes single stranded nicks in the DNA in order to allow the DNA molecule to swivel on the other strand and relieve superhelical tension (not to be confused with topoisomerase II that makes double stranded cuts in DNA).

(iv) telomerase is the enzyme that recognizes specific sequences at the ends of linear chromosomes and extends them using an RNA bound to the enzyme as a template. This enables DNA replication on the lagging strand to be completed (because it provides a template for the RNA primers).

(v) DNA ligase is an enzyme that joins two single stranded DNA molecules, end to end. Ligases are used to join Okazaki fragments during DNA replication, and also in many other situations - such as during DNA repair and recombination.

5. (i) These are the two most frequent forms of DNA damage, and both are due to hydrolytic attack (see Figure 5-46). Removal of the amine from cytosine changes it to uracil but it remains attached to the DNA backbone. Depurination results from the attack of the bond that binds adenine or guanine to the backbone and it results in the removal of the base.

(ii) There are a total of eight strands, because there are two chromosomes, each having been replicated into a pair of chromatids, and each chromatid containing a double-stranded DNA molecule.

(iii) When the DNA mismatch repair enzymes "correct" one strand of the heteroduplex to match its partner strand, we see gene conversion in the resulting gametes. (see Figures 5-65 and 5-66).

6. (i) This statement is true. Along the chromosome, some genes are transcribed using one strand as template, while other genes are transcribed from the opposite strand. The direction of transcription depends on the strand being transcribed (see Figure 6-14).

(ii) General transcription factors are required for the transcription of all (or almost all) eukaryotic protein-coding genes, while activator proteins are more specialized because they bind to gene-specific enhancer sequences. Thus the general factors are a necessary, but not sufficient requirement for the expression of many genes (see Figure 6-19).

(iii) The eukaryotic transcription factor TFIIH has several functions. Two of the best characterized functions are a helicase activity that helps to unwind the DNA template during transcription, and a protein kinase activity that phosphorylates the "tail" of the RNA polymerase, thus liberating the polymerase from many of the general transcription factors. (You were required to describe only one of the functions to answer the question).

7. (i) The branch point sequences are recognized by a special protein factor and snRNA. Then a specific adenine nucleotide within the branch point sequence attacks the 5' splice site and the 5' cut end of the intron becomes attached to this Adenine to form the intron lariat structure (see Figure 6-26).

(ii) Polyadenylation of eukaryotic mRNAs involves: recognition of the polyadenylation signal (AAUAAA) in the 3' non-coding region of the mRNA; cleavage of the RNA; addition of A nucleotides by the Poly-A polymerase; and addition of poly-A binding proteins.

(iii) Basically the number of tRNAs (and consequently the number of corresponding anticodons) is much greater than the number of amino acids, but much smaller than the number of codons - yet all of the 61 codons are translated into twenty amino acids. The fact that different, synonymous codons are translated to give the same amino acid explains why we see more anticodons (and codons) than amino acids. The fact that there are fewer anticodons (47) than codons (61) is explained by the wobble hypothesis, whereby one anticodon can pair with more than a single codon.