Polymer Synthesis & Other Dynamic Processes

To assist you in using your time efficiently, the questions are in approximate order of increasing difficulty. **Your tasks are highlighted in bold face.** The total points on the exam are 100. Course points (15) will be determined proportionately.

I. **Relationships** (15 points, 3 each)

Complete the following thoughts with “>”, “<” or “=”.

A. The number of distinct* codons is ____>____ the number of distinct aminoacyl tRNA synthetases.

B. The number of distinct aminoacyl tRNA synthetases is ____<____ the number of distinct tRNA genes.

C. The frequency of unmethylated sites in an actively transcribed gene is ____<____ the frequency of unmethylated sites in a heterochromatic gene.

D. The number of distinct ubiquitin protein ligases in a eukaryotic cell is ____>____ the number of distinct ubiquitin activating enzymes.

E. The size of the RNA polymerase initiation complex is ____>____ the size of the complex following phosphorylation of the CTD of PolII.

*In this question, “distinct” is asking you not for the total number but for the kinds. For example, for distinct fruits in your refrigerator, If you have apples, oranges and kumquats, the number is 3. The number of apples is not important.
II. Matching (16 points)

The following are several ways in which biological systems can handle mistakes made during the synthesis of biological polymers. For each, identify the process(es) that use the described method. Choose among DNA replication (D), transcription (R), translation (P), and viral RNA replication (V). Some methods have multiple correct answers.

This question will be graded as follows. The number of correct answers for all statements will be totaled and multiplied by three. That number will be regarded as your intermediate score unless that number is greater than 16, in which case your intermediate score will be 16. Then, one point will be deducted for each incorrect response.

1. Removing a single polymer unit misincorporated into the growing chain _____D____________.

2. Degrading the entire polymer that contains errors after its synthesis is completed_____R, P__________.

3. Attaching another molecule to the unit to be added to the chain and removing misattached units from that molecule
___________P______________.

4. Correcting mistakes in polymers after completion of synthesis
__________D__________________.

5. Removing a small oligomer containing the misincorporated unit
_____R_____.
III. Matching B (18 points; 3 points each)

The left column contains some techniques that were used to make some of the determinations discussed in this section of the course. Match the technique with the determination in the right column. There is one best answer for each technique.

1. Fluorescence resonance energy transfer ____C____  
A. The precise location of factor binding sites on individual promoters

2. Complementation of activity in extracts _____F___  
B. The half life of specific mRNAs.

3. Location analysis using intergenic arrays ____E____  
C. The comparative movement of the peptidyl moiety and the tRNA 3’ end in peptidyl transfer.

4. Photoaffinity labeling ____D___  
D. The identity of the receptor specific signal molecules

5. DnaseI footprinting _____A____  
E. The totality of binding sites of specific trans-acting factors

6. Engineering genes to have repressible promoters __B__  
F. The classification of proteins required for nucleotide excision repair

IV. Common features (28 points, 4 each)

What are the common features of each of the following sets (be as specific as possible)?

a. PCNA, a translating polyribosome complex, DnaB protein:  *are circular*

b. Gag-pol fusion protein synthesis, synthesis of tau subunit of *E. coli* DNA polymerase III, BYDV replicase synthesis:  *frame-shifting required for synthesis*

c. RuvB, RecBCD, DnaB proteins of *E. coli*  *helicases*

d. RecA protein, SsB protein, Replication protein A (RPA):  *single stranded nucleic acid binding proteins*
e. Internal ribosome entry, BMV replication complex assembly, incorporation of selenocysteine during protein synthesis, iron regulated translation: require a structured RNA element

f. EF2 (aka EF-G), EF1A-aatRNA-GTP ternary complex, eRF1 (Note EF1A is aka EF-Tu): have a molecularly similar shape; or have shapes that can fit in the ribosomal A site

g. RNA polymerase activation and promoter activation: mechanisms of transcription activation in which the bound polymerase is inactive until either an activation of the enzyme or of the DNA occurs.

IV. Short essay (15 points, 7.5 points each)

a. Describe two epigenetic mechanisms that can extend the information potential of the DNA code.

Descriptions of DNA methylation and of histone modification are required.
b. What generalizations can you draw about how the specificity of the central step of translation, the charging of tRNA by aaRS, is determined?

*Basically, the answer is that it is hard to draw generalizations. Relative to the tRNA, the acceptor stem often has specificity determinants, but in some tRNAs determinants include the anticodon loops or other residues. Relative to the aaRS, the two classes of aaRS interact with opposite sides of the tRNA. However, the positions of aaRS residues interacting with the tRNA vary considerably from one aaRS to another.*
V. Result Interpretation (8 points)

The diagram below shows the electrophoretic mobility changes that result when a radioactively labeled 80 bp long fragment of DNA was mixed with increasing concentrations of two proteins. The result with protein A is shown on the left. The result with protein B (same electrophoretic run) is shown on the right. Proteins A and B are of equivalent sizes and have similar net charges. Interpret the patterns relative to how the two proteins bind to DNA.

Both proteins bind to DNA. About five monomers of either are required to saturate the 80 bp of DNA. The binding by protein A is highly cooperative, meaning that when one unit is bound it greatly accelerates the binding of others. Binding by protein B is not appreciably cooperative.