Name:

2 points

Chem 465
Biochemistry II

Multiple choice (4 points apiece):

1. Assuming that the average amino acid residue contributes 110 to the peptide molecular weight, what will be the minimum length of the mRNA encoding a protein of molecular weight 50,000?
A) 133 nucleotides
B) 460 nucleotides
C) 1,400 nucleotides
D) 5,000 nucleotides
E) A minimum length cannot be determined from the data given.

2. Which of the following statements about aminoacyl-tRNA synthetases is *false*?
A) Some of the enzymes have an editing/proofreading capability.
B) The enzyme attaches an amino acid to the 3′ end of a tRNA.
C) The enzyme splits ATP to AMP + PP_i.
D) The enzyme will use any tRNA species, but is highly specific for a given amino acid.
E) There is a different synthetase for every amino acid.

3. Which one of the following statements about the elongation phase of protein synthesis is true?
A) At least five high-energy phosphoryl groups are expended for each peptide bond formed.
B) During elongation, incoming aminoacylated tRNAs are first bound in the P site.
C) Elongation factor EF-Tu facilitates translocation.
D) Peptidyl transferase catalyzes the attack of the carboxyl group of the incoming amino acid on an ester linkage in the nascent polypeptide.
E) Peptidyl transferase is a ribozyme.

4. The signal sequences that direct proteins to the nucleus are:
A) always at the amino terminus of the targeted protein.
B) cleaved after the protein arrives in the nucleus.
C) glycosyl moieties containing mannose 6-phosphate residues.
D) not located at the ends of the peptide, but in its interior.
E) the same as those that direct certain proteins to lysosomes.

5. Protein amino acid side chains can hydrogen bond in the major groove of DNA, and discriminate between each of the four possible base pairs. In which one of the following groups of amino acids can all three members potentially be used in such DNA-protein recognition?
A) Ala, Asn, Glu
B) Arg, Gln, Leu
C) Asn, Gln, Trp
D) Asn, Glu, Lys
E) Glu, Lys, Pro

6. The binding of CRP (cAMP receptor protein of *E. coli*) to DNA in the *lac* operon:
A) assists RNA polymerase binding to the *lac* promoter.
B) is inhibited by a high level of cAMP.
C) occurs in the *lac* repressor region.
D) occurs only when glucose is present in the growth medium.
E) prevents repressor from binding to the *lac* operator.

7. Which one of the following types of eukaryotic regulatory proteins interact with enhancers?

A) Basal transcription factors
B) Coactivators
C) Repressors
D) TATA-binding proteins
E) Transactivators

Essay questions - answer any 5

1. Describe the process by which amino acids are attached to t-RNA's. In your description address the following points: A.) What enzymes are involved, B) what are the energy costs of this process, C.)Are there any proofreading activities, and D.) What are the structural queues used by the enzymes to find the right t-RNA's

Amino acids are attached to their appropriate t-RNA by aminoacyl-tRNA synthases.

Except for methione there is generally one synthase for each amino acid, and the synthase is specific for that amino acid, but it can accept any of the appropriate t-RNA's. In this reaction 1 ATP is degraded to AMP + PPi, so this is equivalent to 2 ATP's worth of energy. Proofreading is accomplished by a separate active site on the synthase that acts to hydrolyze any incorrect AA-tRNA pairs. In recognizing the correct t-RNA the synthase does not use the anticodon, but instead looks for other structural cues along the surface of the t-RNA molecule

2. What is ubiquitin? What is its role in protein degradation and in the nucleus?

Ubiquitin is a 79 residue peptide that is essentially identical in organisms as different as yeast and humans. In the cytosol it is covalently linked to the ε amino groups of lysines to target a protein for destruction. In the nucleus is seems to have a very different role. Here ubiquitin is used to modify the lysines in the lysine rich N-terminal domain or histones, and this modification makes the DNA bound to these nucleosomes more transcriptionally active.

3.Define the following terms:

proteosome - enzyme complex designed to degrade and destroy proteins

nuclear localization sequence - a sequence of amino acids within a protein that targets the protein to be placed into the nucleus

Shine-Dalgarno sequence - Sequence on prokaryotic mRNA just a few bases upstream from the initiation AUG that is complimentary to a sequence on the 16S rRNA that properly aligns the mRNA in the ribosome.

peptidyl transferase The ribozyme activity in a ribosome that transfers an amino acid on a tRNA onto a growing peptide chain.

wobble base - The third base in a codon which does not have to match exactly with its compliment in a tRNA molecule.

clathrin - A protein that makes cage-like structure in the cell surface as part of the endocytosis

repressor - In prokaryotes a protein that binds to a region of DNA to prevent RNA polymerase from transcribing DNA to RNA

activator - A prokaryotes a protein that binds to a region of DNA to enhance the transcription of a gene by RNA polymerase

enhancer - In eukaryotes, a DNA region 100's to 1000's of base pares upstream from an initiator sequence that helps regulate the expression of the gene

coactivator - In eukaryotes a protein that binds to both transactivators and RNA polymerase II complex to help regulate the expression of a gene

basal activation factor - In eukaryotes the basic factors of the RNA polymerase II complex that are needed to establish an initiation complex

DNA-binding transactivator - In eukaryotes the proteins that bind to enhancer regions that help regulate the expression of a gene

4. Chapter 28 is all about proteins that bind to DNA. Tell me about the different ways that proteins are made to interact with DNA.

There are three major structural motifs for DNA binding domains, the Helix-turn-helix, the zinc finger, and the homeodomain.

In the helix-turn-helix motif there are two short (7-9 residue) helices separated by a beta turn, where one of the helixes can lie in the major groove of the DNA an interact with the bases of the DNA. This binding region is too small to be a true domain, and is always a part of a larger DNA binding domain

In the zinc finger 30 amino acids are held in a helix-elongated loop that is held together by a Zn2+ ion that is coordinately bonded to the protein either through 4 cystines, or two cystines and to histidines.

The homeodomain is a 60 residue domain found in eukaryotes that was first discovered in the homeotic genes that regulate body pattern in fruit flies, but has now been recognized in a wide variety of organisms including fruit flies. The DNA binding part of this domain is probably similar to the helix-turn-helix motif.

5. In general terms compare and contrast control of gene expression in prokaryotes and eukaryotes.

Prokaryotes

Nonrestrictive transcriptional ground state (RNA polymerase has access to all promoters an can initiate transcrpition unless specifically inhibited.

Related genes clustered into an operon so can be turned on and off at one point

No chromatin structure to deal with during transcription

Most frequent control mechanism via repression of transcription, although some activation is observed

activator/repressors bind to DNA to directly effect RNA polymerase.

Translational control achieved by early termination of translation of RNA

Eukaryotes

Restrictive transcriptional ground state - RNA polymerase does not have access to most promoters and cannot initiate transcription unless specifically activated.

Related gene spread over different chormosomes so have to be turned off and off independently.

Chormatin structure must be altered before a gene can be transcribed

Most frequent control is via multiple activation events, although some repression is observed

Activators or presessors that bind to DNA must interact through coactivators before they have their effect on RNA polymerase

Translational control via proteins or RNA that bind to mRNA to inhibit ribosomal function.

6. The chemical mechanisms used to avoid errors in protein synthesis are different from those used during DNA synthesis. DNA polymerase used a 3'→5' exonuclease proofreading activity to remove mispaired nucleotides from the growing chain. There is no analogous proofreading activity in the ribosomes so there is no proofreading activity in protein synthesis. Tell me why a proofreading activity on a ribosome would not be practical.

Suppose an incorrect amino acid-tRNA is brought into the ribosome at the A site to be incorporated into the growing peptide. To incorporate this incorrect amino acid into the growing chain, the peptide chain must first be transferred from the tRNA in the A site to the tRNA with the wrong amino acid in the A site. At this point the only chemical connection between the growing peptide chain and the ribosome is through the incorrect AA and it's tRNA to the mRNA. Thus if you were to perform a proofreading event at this step to remove the incorrect amino acid, you would have to sever the connection between the rest of the peptide and mRNA on the ribosome and this would completely halt protein synthesis.

1. Ans: C
2. Ans: D
3. Ans: E
4. Ans: D
5. Ans: D
6. Ans: A
7. Ans: E