14.1 (POB) Methionine Has Only One Codon.

Methionine is one of the two amino acids having only one codon. Yet the single codon for methionine can specify both the initiating residue and interior Met residues of polypeptides synthesized by *E. coli*. Explain exactly how this is possible.

14.2 Are the following statements concerning aminoacyl-tRNA synthetase true or false?

a) Two distinct classes of the enzymes have been defined that are not very related to each other.

b) The enzymes scan previously-synthesized aminoacyl-tRNAs and cleave off any amino acids that are linked to the incorrect tRNA.

c) Proofreading can occur at the formation of either the aminoacyl-adenylate intermediate (in some synthetases) or at the aminoacyl-tRNA (in other synthetases) to insure that the correct amino acid is attached to a given tRNA.

d) The product of the reaction has a high-energy ester bond between the carboxyl of an amino acid and a hydroxyl on the terminal ribose of the tRNA.

14.3 A preparation of ribosomes in the process of synthesizing the polypeptide insulin was incubated in the presence of all 20 radiolabeled amino acids, tRNA's, aminoacyl-tRNA synthetases and other components required for protein synthesis. All the amino acids have the same specific radioactivity (counts per minute per nanomole of amino acid). It takes ten minutes to synthesize a complete insulin chain (from initiation to termination) in this system. After incubation for 1 minute, the completed insulin chains were cleaved with trypsin and the radioactivity of the fragments determined.

a) Which tryptic fragment has the highest specific activity?
b) In the same system described above, the insulin polypeptide chains still attached to the ribosomes after ten minutes were isolated, cleaved with trypsin, and the specific activity of each tryptic peptide determined. Which peptide has the highest specific activity?

14.4 Which component of the protein synthesis machinery of *E. coli* carries out the function listed for each statement.

a) Translocation of the peptidyl-tRNA from the A site to the P site of the ribosome.

b) Binding of f-Met-tRNA to the mRNA on the small ribosomal subunit.

c) Recognition of the termination codons UAG and UAA.

d) Holds the initiator AUG in register for formation of the initiation complex (via base pairing).

14.5 a) In the initiation of translation in *E. coli*, which ribosomal subunit does the mRNA initially bind to?

b) What nucleotide sequences in the mRNA are required to direct the mRNA to the initial binding site on the ribosome?

c) What other factors are required to form an initiation complex?

14.6 What steps in the activation of amino acids and elongation of a polypeptide chain require hydrolysis of high energy phosphate bonds? What enzymes catalyze these steps or which protein factors are required?

14.7 (POB) Maintaining the Fidelity of Protein Synthesis

The chemical mechanisms used to avoid errors in protein synthesis are different from those used during DNA replication. DNA polymerases utilize a 3' → 5' exonuclease proofreading activity to remove mispaired nucleotides incorrectly inserted into a growing DNA strand. There is no analogous proofreading function on ribosomes; and, in fact, the identity of amino acids attached to incoming tRNAs and added to the growing polypeptide is never checked. A proofreading step that hydrolyzed the last peptide bond formed when an incorrect amino acid was inserted into a growing polypeptide (analogous to the proofreading step of DNA polymerases) would actually be chemically impractical. Why? (Hint: Consider how the link between the growing polypeptide and the mRNA is maintained during the elongation phase of protein synthesis.)

14.8 (POB) Expressing a Cloned Gene.

You have isolated a plant gene that encodes a protein in which you are interested. What are the sequences or sites that you will need to get this gene transcribed, translated, and regulated in *E. coli*?

14.9 The three codons AUU, AUC, and AUA encode isoleucine. They correspond to "hybrid" between a codon family (4 codons) and a codon pair (2 codons). The single codon AUG encodes methionine. Given the prevalence of codon pairs and families for other amino acids, what are hypotheses for how this situation for isoleucine and methionine could have evolved?

14.10 Use the following processes to answer parts a-c:
1. synthesis of aminoacyl-tRNA from an amino acid and tRNA.
2. binding of aminoacyl-tRNA to the ribosome for elongation.
3. formation of the peptide bond between peptidyl-tRNA and aminoacyl-tRNA on the ribosome.
4. translocation of peptidyl-tRNA from the A site to the P site on the ribosome.
5. assembly of a spliceosome for removal of introns from nuclear pre-mRNA.
6. removal of introns from nuclear pre-mRNA after assembly of a spliceosome.
7. synthesis of a 5' cap on eukaryotic mRNA.

(a) Which of the above processes require ATP?
(b) Which of the above processes require GTP?
(c) For which of the above processes is there evidence that RNA is used as a catalyst?