

Chem 465 Biochemistry II

Multiple choice (4 points apiece):

1. Formation of the ribosomal initiation complex for bacterial protein synthesis does not require:

- A) EF-Tu.
- B) formylmethionyl tRNA^{fMet}.
- C) GTP.
- D) initiation factor 2 (IF-2).
- E) mRNA.

2. Which of the following statements about bacterial mRNA is true?

- A) A ribosome usually initiates translation near the end of the mRNA that is synthesized last.
- B) An mRNA is never degraded but is passed on to the daughter cells at cell division.
- C) During polypeptide synthesis, ribosomes move along the mRNA in the direction 5' - 3'.
- D) Ribosomes cannot initiate internally in a polycistronic transcript.
- E) The codon signaling peptide termination is located in the mRNA near its 5' end.

3. It is possible to convert the Cys that is a part of Cys-tRNA^{Cys} to Ala by a catalytic reduction. If the resulting Ala-tRNA^{Cys} were added to a mixture of (1) ribosomes, (2) all the other tRNAs and amino acids, (3) all of the cofactors and enzymes needed to make protein in vitro, and (4) mRNA for hemoglobin, where in the newly synthesized hemoglobin would the Ala from Ala-tRNA^{Cys} be incorporated?

- A) Nowhere; this is the equivalent of a nonsense mutation
- B) Wherever Ala normally occurs
- C) Wherever Cys normally occurs
- D) Wherever either Ala or Cys normally occurs
- E) Wherever the dipeptide Ala-Cys normally occurs

4. Ubiquitin-mediated protein degradation is a complex process, and many of the signals remain unknown. One known signal involves recognition of amino acids in a processed protein that are either stabilizing (Ala, Gly, Met, Ser, etc.) or destabilizing (Arg, Asp, Leu, Lys, Phe, etc.), and are located at:

- A) a helix-turn-helix motif in the protein.
- B) a lysine-containing target sequence in the protein.
- C) a zinc finger structure in the protein.
- D) the amino-terminus of the protein.
- E) the carboxy-terminus of the protein.

5. The operator region normally can be bound by:

- A) attenuator.
- B) inducer.
- C) mRNA.
- D) repressor.
- E) suppressor tRNA.

6. The DNA binding motif for many prokaryotic regulatory proteins, such as the lac repressor, is:

- A) helix-turn-helix.
- B) homeobox.
- C) homeodomain.
- D) leucine zipper.
- E) zinc finger.

7. Which of the following statements is true of the attenuation mechanism used to regulate the tryptophan biosynthetic operon in *E. coli*?

- A) Attenuation is the only mechanism used to regulate the trp operon.
- B) One of the enzymes in the Trp biosynthetic pathway binds to the mRNA and blocks translation when tryptophan levels are high.
- C) The leader peptide plays a direct role in causing RNA polymerase to attenuate transcription.
- D) Trp codons in the leader peptide gene allow the system to respond to tryptophan levels in the cell.
- E) When tryptophan levels are low, the trp operon transcripts are attenuated (halted) before the operon's structural genes are transcribed.

Essay questions - answer any 5

1. The Wobble base in the anti-codon of a t-RNA can explain how the same t-RNA can bind to a few difference sequences of m-RNA. Make a diagram of a t-RNA showing the 3' end, the 5' end, and which base is the t-RNA is the wobble base. Next say which bases are the wobble bases, and which different bases they pair with on the m-RNA.

Diagram like figure 27-8. mRNA 5' on left, 3' on right. tRNA cloverleaf on top with 5' on right and 3' on left. The wobble base is on the 5' end of the tRNA(1st base in the codon) and this is paired up with the 3' end of the mRNA (3rd base in the codon)

Wobble bases are: U that can pair with A or G on the mRNA

G that can pair with C or U on the mRNA

-or- I that can pair with A, U or C on the mRNA

Note: I was really tempted to give you the entire table of amino acids and codons (Figure 27-7) and have you pick out which redundancies could be explained with the wobble base

2. Describe all the steps involved in translating an m-RNA into a protein in a prokariote, then point out any differences found in eukariotic systems.

I will skip charging the tRNA and focus on just making the protein.

Initiation - Diagram like figure 27-25

- IF-1 and IF-3 bind to 30S subunit of ribosome
- mRNA binds at Shine and Delgarno sequence, and initiation AUG is lined up with P site
- IF-2·GTP brings tRNA^{fmet} to the proper place.
- GTP hydrolyzed to GDP and Pi
- all three IF factors released and 50S ribosome binds

Elongation - Diagram like figure 27-28

- Incoming tRNA·Tu-GTP binds to A site in ribosome
- GTP hydrolyzed to GDP as Tu is released
- (Ts binds to Tu to release GDP
- GTP then binds to release TU and regenerate Ts-GTP)
- Peptide bond is formed from N on incoming AA and the AA at the P site on the ribosome
- The GTP on an EF-G factor is hydrolyzed to translocate the ribosome (move to next codon)

Termination - Diagram like Figure 27-31

- either RF-1 or RF-2 binds to termination codon
- peptide is cleaved from the tRNA in the P site
- RF-3 , EF-G-GTP hydrolysis and RRF (ribosome releasing factor) completely dissociate the ribosome.

Difference in Eukariotic system

In initiation, the mRNA is bound to ribosome with eIF3, eIF4E (at 5' cap), eIF4G, PAB(at poly A tail). At least 9 initiation factors. Does not use Shine and Delgarno sequence, but in stead uses the first AUG from the 5' end

In Elongation, system is very similar, eEF1 α acts like EF-Tu, eEF1 β works like EF-Ts, and eEF2 is analogous to EF-G. The other difference is that the Eukariotic ribosome does not have an E site.

In termination the eukariotic system is a bit simpler, a single eRF recognizes all three termination codons.

3. Describe the process in which a protein like a histone get targeted and end up in the nucleus. How does this compare to a protein like albumin that must be both glycosylated and transported out of the cell.

All nuclear proteins contain an internal signal sequence called the NLS (nuclear Localization Sequence) this sequence usually has 4-8 residues that contains several consecutive R or K residues. This sequence is recognized by α importin, and a complex of the protein, α importin, and β importin docks with a receptor on a nuclear membrane pore and the complex enters the nucleus. The β importin is separated from the complex by Ran GTPase, and the α importin is separated by CAS protein.

A secreted protein like albumin carries a signal sequence at its N-terminus that is removed as the protein is processed. The processing goes like this...(Figure like 27-38) The signal sequence is synthesized first. As this sequence leaves the ribosome, it is recognized by the SRP (Signal Recognition Particle) which binds a GTP and stops the synthetic process. The arrested ribosome with the bound SRP is then attached to the ER by a receptor protein and peptide translocation complex on the surface of the ER membrane. The GTP is hydrolyzed, the SRP and the SRP receptor leave the ribosome/translocation complex, and peptide synthesis on the ribosome starts up again, but now the peptide goes from the ribosome, through the translocation complex, and emerges on the inside of the ER. The signal peptide is removed by a signal peptidase within the ER lumen.

As the protein continues to be synthesized the appropriate residues are glycosylated as shown in a figure like figure 27-39. Here 2 GlcNAc residues are first attached to a dolichol via a di-phosphate linkage (the dolichol is in the membrane and the GlcNAc residues are on the outside of the ER). 5 mannose residues then attached to the GlcNAcs. This complex is then transferred to the inside of the membrane and additional mannose and glucose residues are attached to the growing carbohydrate complex. This entire complex is then added to the appropriate site on the protein and the dolichol recycled.

4. Describe the trp operon in E coli, and the various ways the transcription of this operon is controlled.

Start with a diagram like Figure 28-19.

The trp operon is an operon that regulates the genes that synthesize tryptophan from precursors. There are 5 structural genes in this complex that are all coded on a single mRNA.

The first level of control in this system is standard promoter/operator system: The DNA sequence starts with a promoter, that is then followed by an overlapping operator sequence that can be bound by a trp repressor protein. The trp repressor protein is coded for on a different site, and makes a dimeric protein. When there is excess tryptophan in the cell, the repressor protein binds the trp, and this, in turn, increases the binding of the repressor protein to the operator region so RNA polymerase cannot bind to the promoter site and mRNA for this operator cannot be synthesized.

The second level of control starts when the trp levels are low enough that the repressor protein is released, and the RNA polymerase binds to the promoter to start synthesizing the RNA message. The message contains an attenuator peptide before the rest of the structural protein. As this sequence undergoes transcription to mRNA, the mRNA is also being translated by a ribosome.

The attenuator peptide contains 2 trp residues. If the cell contains enough trp that this peptide can be synthesized, the ribosome quickly advances on the mRNA, and the mRNA sequence ahead of the ribosome folds into a hairpin, that signals the end of the mRNA message, so the polymerase dissociates from the DNA and transcription stops. On the other hand, if the trp levels are really low, the ribosome stalls at the trp residues, and an alternate hairpin loop is formed in the mRNA. This alternate hairpin does not resemble the signal used to stop transcription, so the polymerase transcribes the entire mRNA message, and all the proteins on the message are translated into functional proteins.

5. How is the synthesis of ribosomal protein and r-RNA coordinated in prokaryotes?

Overall control depends on the level of amino acids in the cell through the stringent response. When Amino acid levels are so low that uncharged tRNA are abundant enough to get into a ribosome complex, the stringent response protein (RelA protein) takes ATP and GTP and forms p/ppGpp. This molecule binds to the β factor of RNA polymerase to slow the transcription of some genes and increase the transcription of other messages until things are brought into balance. In particular the p/ppGpp signal halts the synthesis of rRNA, which in turn will shut down the synthesis of r- proteins, so all ribosome production and protein translation stops until amino acids become available again.

R- proteins and rRNA are kept in balance by a second mechanism. There are 52 ribosomal genes coded on at least 20 different operons that contain between 1 and 11 structural genes. In most of these genes the first r-Protein synthesized also binds to the first part of its own mRNA, as well as the rRNA in the ribosome itself. If there is enough rRNA, the rProtein binds to the rRNA and completes the ribosome. If the [rRNA] is low, the rProtein binds to its own message, which blocks translation of the message, so no further rProteins can be synthesized.

6. Describe the control of gene expression in Eukariotes, or at least as much as we had time to get to in lecture this year.

Start with a diagram like figure 28-29.

Control of gene expression in eukariots works through three types of proteins, basal transcription factors, coactivator protein complexes and transcriptional activators.

Transcription activators are proteins that bind to enhancer sequences that are usually 100's or 1000's of base pairs upstream of a gene to be expressed.

Basal transcription factors are the factors that bind to RNA polymerase to start the transcription process. The binding of these factors is very weak, however, and RNA polymerase will not initiate transcription unless the basal factor have been bound by other proteins. These other factors are the coactivator proteins that act as intermediates between the transcription activators and the basal transcription factors.

The process starts with a few transcriptional activators that can bind to DNA strongly enough to displace the histone/nucleosome structure around the enhancer sequence. Once the first activator binds, then other, weaker activators can bind. This triggers the enzymes like HAT's or SWI/SNF to start remodeling the chromatin in this region to expose the DNA for further interactions. Next coactivator proteins like mediator binds, and this allows the basal transcription factors to start assembling RNA polymerase complexes at many sites in the affected region, and RNA synthesis begins.

1: A 2: C 3 : 3: C 4: D 5: D 6: A 7: D