Name:

2 points

Chem 465
Biochemistry II

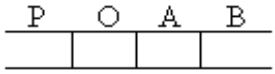
Multiple choice (4 points apiece):

1. Which of the following statements about tRNA molecules is false?
   A) A, C, G, and U are the only bases present in the molecule.
   B) Although composed of a single strand of RNA, each molecule contains several short, double-helical regions.
   C) Any given tRNA will accept only one specific amino acid.
   D) The amino acid attachment is always to an A nucleotide at the 3' end of the molecule.
   E) There is at least one tRNA for each of the 20 amino acids.

2. The enzyme that attaches an amino acid to a tRNA (aminoacyl-tRNA synthetase):
   A) always recognizes only one specific tRNA.
   B) attaches a specific amino acid to any available tRNA species.
   C) attaches the amino acid at the 5' end of the tRNA.
   D) catalyzes formation of an ester bond.
   E) splits ATP to ADP + Pi.

3. Posttranslational glycosylation of proteins is inhibited specifically by:
   A) chloramphenicol.
   B) cycloheximide.
   C) puromycin.
   D) streptomycin.
   E) tunicamycin.

4. The diagram below represents a hypothetical operon in the bacterium *E. coli.* The operon consists of two structural genes (A and B) that code for the enzymes A-ase and B-ase, respectively, and also includes P (promoter) and O (operator) regions as shown.



When a certain compound (X) is added to the growth medium of *E. coli,* the separate enzymes A-ase and B-ase are both synthesized at a 50-fold higher rate than in the absence of X (which has a molecular weight of about 200). Which one of the following statements is true of such an operon?
   A) Adding X to the growth medium causes a repressor protein to be released from the O region.
   B) Adding X to the growth medium causes a repressor protein to bind tightly to the O region.
   C) Synthesis of the mRNA from this operon is not changed by the addition of compound X.
   D) The mRNA copied from this operon will be covalently linked to a short piece of DNA at the 5′ end.
   E) Two mRNA molecules are made from this operon, one from gene A the other from gene B.

5. Consider the lac operon of E. coli. When there is neither glucose nor lactose in the growth medium:

A) CRP protein binds to the lac operator.
B) CRP protein displaces the Lac repressor from the lac promoter.
C) repressor is bound to the lac operator.
D) RNA polymerase binds lac promoter and transcribes the lac operon.
E) the operon is fully induced.

6. Attenuation in the trp operon of E. coli:

A) can adjust transcription of the structural genes upwards when tryptophan is present.
B) can fine-tune the transcription of the operon in response to small changes in Trp availability.
C) is a mechanism for inhibiting translation of existing (complete) trp mRNAs.
D) results from the binding of the Trp repressor to the operator.
E) results from the presence of short leader peptides at the 5' end of each structural gene.

7. Which one of the following statements about eukaryotic gene regulation is correct?

A) Large polycistronic transcripts are common.
B) Most regulation is positive, involving activators rather than repressors.
C) Transcription and translation are mechanistically coupled.
D) Transcription does not involve promoters.
E) Transcription occurs without major changes in chromosomal organization.

Longer questions. 14 points each. Do five:

8. Discuss how RNA editing can be done to mRNA to change the protein that is synthesized from a given mRNA.

Translational frameshifting
- Ribosome 'hiccup' to change frame of message being read from RNA
- Best example gag-pol in Rous Sarcoma Virus
  - Gag has a UAG termination
  - ~ 5% of time does a -1 frameshift
  - No longer reads temination and makes gag-pol
  - Then pol is made from large precursor by proteolytic clevage
- In some retrovirus even do frameshift for gag-pol-env

Addition, deletion, or alteration of mRNA
- most common in mitochondrial or chloroplast genomes
- used gRNA or guide RNA (encoded in organelle)

Alteration
- deamination
  - A to I I interpreted as G in Translation
    - Carried out by ADARs
    - ADAR prevalent in Primates
      - Occures in Alu elements
      - Transposons
      - About 10% of genome
      - But in introns and outside of ends of exons

Problems in ADAR tied to ALS and epilepsy

C to U

Carried out by *APOBEC* family of proteins

Best known example apolipoproteinB

513,000 mass protein made in liver

250,000 mass made in intestine

APOBEC found only in intestine makes CCA(Gln)

Into UAA (termination)

9. Describe, in detail, the many steps associated with initiation, elongation and termination of protein synthesis in a eukaryotic system.

Figures like 27-27 - 27-31 modified for eukaryotic system are helpful.

Initiation

12 initiation factors eIF1A like IF-1, eIF3 like IF3

eIF1,eIF1A and eIF3 bind to 40S subunit bind first

Creation of 43 S preinitiation complex

charged initiator Met bound with eIF2 and GTP bind to P site

Also eIF5B·GTP, eIF5

mRNA binds to eIF4F complex

complex - eIF4E binds to 5' cap

eIF4A - ATPase helicase

eIF4G - linker protein between eIF3 and eIF4E and polyA binding protein(PAB) so loop of RNA

Now mRNA linked to 43S complex to make 48S complex

complex now scans RNA starting at 5' cap until finds AUG

Now bind 60S ribosome binds and most factors released with 2 GTP→2GDP

Elongation

eEF1α like EF-Tu

eEF1βγ like EF-Ts

eEF2 like eEF-G

eEF1α binds GTP and aminoacyl-t-RNA

Binds to A site

GTP hydrolyzed and AA placed in position to add to peptide chain

And eEF1α·GDP released

eEF1α·GDP binds with eEF1βγ to release GDP

GTP binds to eEF1α to release eEF1βγ

Amino end of AA in A site attacks bond between AA and tRNA in P site

form peptide linkage with peptide now on A site

eEF2·GTP now enters A site

As GTP hydrolyses, ribosome moves so peptide-tRNA is now on P site

eEF2·GDP released

Termination

single releasing factor eRF recognizes all three termination codons

Binds at termination codon

makes ribosome hydrolyze off link between peptide and tRNA

complex falls apart

10. Describe the signal used to target a protein to the nucleus of a cell, then tell me about the system that transports proteins into the nucleus

Figure like 27-42 helps
Nuclear localization Sequence (NLS) can be anywhere in protein typically 4-8 AA with several Arg or Lys in a row, not removed from protein

protein in cytosol has α + β importin bind at NLS site
complex transported through nuclear pore
β importin released by Ran-GTP complex
α importin released by Ran-GTP + CAS
both of the above complexes are transported out of nucleus
Ran-GTP hydrolyzed to GDP to release importins for reuse in cytosol
Ran-GDP binds NTF2 and reenters nucleus through pore
Nuclear transport factor 2?
Ran-GEF removes GDP and replaces with GTP
NTF2 book does not say much about NTF2 or CAS recyclin

11. As a protein person I usually focus on how proteins are used to regulate gene expression. However there are now many examples of how RNA can be used to regulate gene expression. Tell me about the different ways that RNA is used to regulate gene expression.

Separate RNA binds to mRNA to affect expression 'in trans'
mRNA itself has built in regulator 'in cis'

Example 'in trans'
RNA for σ^S - gene called *rpoS*
turns on set of genes used when e coli is out of nutrients and needs to go into stationary phase
Normally mRNA is transcribed but not translated because large hairpin upstream of coding region inhibits ribosome binding
Two different RNA's (DsrA, RprA) can bind to hairpin to allow translation
Yet another small RNA, called OxyS is induced under oxidative stress
Also bind to rpoS mRNA to prevent traspaltion
All three of these small RNA's require protein Hfq, and RNA chaperone that facilitates RNA-RNA pairing
Similar systems seen in Eukariotes

Example 'in cis'
Aptamer, RNA that can bind other small molecules built into mRNA
also called riboswitch
when binds small molecule makes hairpins in RNA that prevent translation
Seen in several systems where translated mRNA is tied to either synthesis or transport of the small molecules that binds to aptamer. Thus if cell has high level, proteins needed to transport or make mole of that molecules are blocked.
Riboswitch seen in many organisms for TPP.

You Could also describe transcriptional attenuation, since this is a structure of the mRNA that regulates its own translation.

12. Let's assume that Dr. Bergmann discovers a unique assembling micro-organism in the underground lab that has a new amino acid in it. The synthesis of this amino acid relies on 4 genes that are linked together in an operon. This operon is turned on in the presence of glutamine. It is partially turned off in the presence of arginine, but it is completely turned off in the presence the amino acid itself. Describe to me a possible model for the control of the expression of this operon.

What I was looking for was a standard operon model with Glutamine binding to a repressor protein to release repression, Arginine binding to an activator protein to enhance expression and the amino acid itself using transcriptional attenuation to turn off entirely, but you could make several different models to fit my description.

13. Describe the steps involved gene expression in a Eukaryotic cell. Make sure you include transcriptional activators, architectural regulators, chromatin modification and remodeling proteins, coactivators and basal transcription factors in your description.

A figure like 28-29 is a good starts
The book warns that the exact order of some of these steps may vary, but let's give it a shot .
Step 1. Bind a strong transcriptional activator that can find its target sequence even when bound up in chromatin. As one activator binds, others can bind around it displacing nucleosomes and opening up DNA structure. These activators interact with HAT's, SWI/SNF to further remodel the chromatin structure in the activator region. At this point the HMG proteins also help in remodeling the chromatin and bending the DNA around.

Next the activators bind the large Mediator complex (or other similar complexes). The mediator complex then provides a scaffold to begin assembling TBP, TFIID, TFIIF and finally grab RNA Pol II by its CTD and create a pre-initiation complex that can find the TATA box and the Inr sequence and start transcribing the DNA into RNA.

1 A 2 D 3E 4A 5C 6B 7B