Lecture 1H. Transcription: the prokaryotic model; transcription initiation.

Study questions.

1. The sequence of part of an mRNA is:
   CGAGUUAGCCUUGACCGUACGUGCCGAU

   What is the sequence of the non-template strand of the corresponding DNA (written in the conventional 5' to 3' direction)?

   CGAGTTAGCCTTGACCGTACGTGCCGAT

2. Is the -10 box an example of a consensus sequence or a cis-acting sequence or both?

   BOTH.

3. Does *E. coli* core RNA polymerase bind to DNA?

   YES. The enzyme does bind to DNA, but not specifically -- it needs the sigma factor to recognize the promoter sequence.

4. DNA and RNA synthesis are enzymatically very similar processes, but not identical. Which statement is not true regarding RNA synthesis?

   a) RNA synthesis requires a free hydroxyl end for chain elongation.
   b) RNA polymerase utilizes UTP instead of TTP.
   c) DNA synthesis requires a preexisting primer whereas RNA polymerase does not.
   d) **RNA synthesis proceeds in the 3' to 5' direction**.
   e) Gene promoters are to transcription what origins are to DNA replication.

5. When growing *E. coli* are subjected to a rapid increase in temperature, a new and characteristic set of genes is expressed. Explain how this alteration in gene expression occurs.

   Upon heat shock, a new sigma factor is produced that can interact with the core RNA polymerase to recognize and transcribe a set of genes that will be used to protect the cell in this enivronmental situation

Lecture 2H. Transcription: elongation and termination.

Study questions

1. Which of the following are components or structures that lie within the transcription bubble formed by *E. coli* RNA polymerase

   a) **The polymerization site of the enzyme.**
   b) The sigma subunit.
   c) Approximately 17 nucleotides of the coding strand in single stranded form.
   d) Approximately 12 nucleotides of the 5' end of the elongating RNA strand.
   e) **An RNA/DNA hybrid.**

2. Name two main differences between a rho dependent terminator and a factor independent terminator.

   (1) The rho dependent terminator utilizes a protein as a transacting factor to facilitate the termination reaction. The sequence to which the rho factors binds in the RNA is not well understood.

   (2) The factor-dependent terminator is a cis-acting sequence that is made up of a well defined RNA secondary structure; a hairpin stucure followed by a run (about 5) of U residues.

3. Explain how a mutation might give rise to an *E. coli* strain that is resistant to the antibiotic rifampicin.

   The antibiotic rifampicin binds to the β-subunit of the RNA polymerase core enzyme. Mutations in the gene that codes for this b-subunit may arise that inhibit rifampicin binding.

Lecture 3H. Gene regulation in prokaryotes: the operon.

Study questions.

1. A deletion of the operator region of the Lac operon would result in constitutive expression of β-galactosidease. Explain. Name another mutation that would have the same effect.

   A mutation in the gene that codes for the repressor protein may also result in a constituative phenotype. For example: A mutation that affects synthesis of the protein (i.e. limits the amount of transcription) or a mutation that reduces the ability of the repressor to bind to DNA would be two examples of a type of mutation in the lacI gene.

2. Why is it significant that the promoter and the operator contain overlapping sequences in the Lac operon?

   Because the binding of the repressor at the operator is mutally exclusive to binding of RNA polymerase to the promoter.

3. What is the difference between induction and derepression in gene regulation?

   Omit this - we did not discuss de-repression

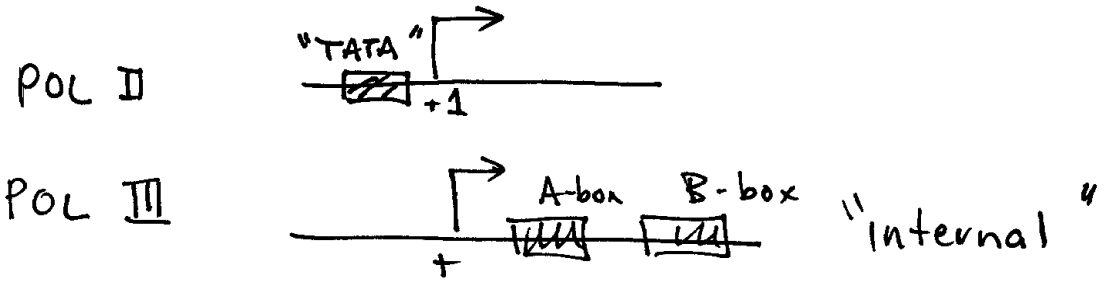
4. Would regulation by attenuation as described by the trp operon be a viable mechanism for gene expression eukaryotes? Explain.

   NO! Attenuation is dependent on the coupling of transcription and translation -- i.e. you observe attentuation when the ribosome stalls on the nascent RNA due to environmental circumstance (e.g. limited trp). As eukaryotes have their ribosomes physically separated from the DNA -- i.e. in the cytoplasm as oppose to the nucleus, attenuation would not be a viable mechanism for gene expression.

Lecture 4H. Transcription in eukaryotes.

Study questions.

1. Draw a diagram comparing the RNA polymerase II and III promoters, and highlighting their differences.



2. Assembly of the RNA polymerase II initiation complex in eukaryotes requires a much larger number of proteins than is needed for the initiation complex in prokaryotes. What answer best explains the reason for this difference?

   a) **Cell-specific regulation in eukaryotes requires that transcriptional initiation be tightly controlled and multi-subunit protein complexes facilitate this**.
   b) Eukaryotes have a larger number of DNA binding proteins than prokaryotes.
   c) Eukaryotes have more genes than prokaryotes.
   d) Eukaryotic promoters contain a TATA box for TBP binding and prokaryotes contain -35 and a -10 region to which RNA polymerase binds.

3. What structural feature of the largest subunit of RNA polymerase II is important for the transition from closed to open complex formation? Why?

   The CTD (C-terminal domain). A heptapeptide (7 amino acids) repeated 54 time in the human protein, that is rich in Ser and Thr residues, and are phosphorylated upon the transition from the closed to the open complex.

4. Which RNA polymerase transcribes tRNA? 5S RNA? hnRNA?

   tRNA - RNA polymerase III
   5S RNA - RNA polymerase III
   hnRNA - RNA polymerase II

Lecture 5H. Gene regulation in eukaryotes.

Study questions.

1. If α-globin genes are on chromosome 16 and the β-globin gene is on chromosome 11, how is it that they are transcribed coordinately?

   They have regulatory sequences (response elements) in their promoter region that allow RNA polymerase II to activate transcription in a coordinated fashion (e.g. in a tissue specific manner)

2. We've considered the modular nature of the promoter, now explain why many transcription factors also have modular structures (i.e., different domains).

   Because many transcription factor operate by binding to DNA as well as by binding to other proteins or to RNA polymerase itself. The structural domains to achieve these activities are distinct, but may be present in a single polypeptide For example -- a leucine zipper motif, coupled with a helix turn helix motif, or the bZIP domain described in your text book.

3. If we define the pol II basal promoter, as the site to which RNA polymerase II and its accessory proteins bind to the DNA, why is an enhancer not necessarily part of the promoter? Is this also true for a response element?

   The enhancer (cis-acting signal) may be very far (i.e. >10 Kbp) away from the site to which RNA polymerase binds -- yet it will still be able to bind to transcription factors that influence RNA polymerase because it causes the DNA to "loop" around. The net result is that the enhancer is brought close to the promoter spatially, but is not physically close to it. A response element usually does not have to induce looping because it is already situated close to the core promoter.

4. Name two differences between actively transcribed DNA and non-transcribed DNA.

   Nucleosome positioning
   DNAse one sensitivity (discussed in text book)

Lecture 6H. RNA processing.

Study questions.

1. List three roles for the 5' cap structure.

   (1) Influences the stability (or half life) of the RNA by protecting the RNA from 5' to 3' exonucleolytic enzymes

   (2) Participates in the selection of mRNA for translation due to interaction with eIF4E.

   (3) Involved in nucleo-cytoplasmic transport

2. Splicing of pre-mRNAs occurs in a two step process -- what are the two steps?

   First step: Cleavage of the 5' splice site, and lariat formation by a 2'5' phosphate linkage to branch point Adenosine

   Second step: Cleavage at the 3' splice site, exon lagation, and lariat intermediate released

3. Explain why the presence of introns increases the coding capacity of eukaryotic DNA.

   Because it allows for different combinations of exons sequences to be chosen for RNA processing and ultimately end up in the mature mRNAs. The net effect would result in different coding regions and therefore different proteins synthesized all from the same gene.

4. Which of the following are important sequence elements in the splicing reactions that produce eukaryotic mRNAs?

   a) Exon sequences located between 20 and 50 nucleotides from the 5' splice site.
   b) Exon sequences located between 20 and 50 nucleotides from the 3' splice site.
   c) Intron sequences located between 20 and 50 nucleotides from the 5' splice site.
   d) **Intron sequences located between 20 and 50 nucleotides from the 3' splice site.**
   e) Intron sequences at the 5' splice site.
   f) Intron sequences at the 3' splice site.

5) List three functions for the poly(A) tail.

(1) Influences the stability (or half life) of the RNA by protecting the RNA from 3' to 5' exonucleolytic enzymes

(2) Participates in the selection of mRNA for translation

(3) Involved in nucleo-cytoplasmic transport

Lecture 7H. Protein synthesis: the ribosome and tRNA.

Study questions.

1. Draw the cloverleaf structure of tRNA and identify the regions containing the anticodon and the amino acid attachment site.

2. Which of the following statements about functional tRNAs are correct?

   a) **They contain many modified nucleosides**.
   b) About half of their nucleotides are in base-paired helical regions.
   c) They contain fewer than 100 ribonucleosides. They consist of two helical stems that are joined by loops to form a U-shaped structure.
   d) They have a terminal AAC sequence at their amino acid accepting end.

3. AUG and UAG specify start and stop codons in protein synthesis, respectively. Which open reading frame(s) in the mRNA shown below would encode a short polypeptide?
   5' - UUAUGAAUGUACCGUGGUAGUU - 3'

   a) Reading frame 1.
   b) Reading frame 2.
   c) **Reading frame 3.**
   d) Reading frames 1 and 3.
   e) Reading frames 2 and 3.

4. Explain how some codons are recognized by more than one anticodon, that is, how they interact with more than one species of aminoacyl-tRNAs. List the base-pairing interactions allowed by the wobble hypothesis.

   A given amino acid may have more than codon, e.g. proline:

   CCU, CCC, CCA, CCG. The tRNA for proline could have a NGG in the anti-codon region, where by N = Inosine or G. These two nucleotides (Inosine and Guanine) can base pair with U, C, A in the case of inosine, and A and U in the case of guanine. Thus if the anitcodon sequence were IGG, this tRNA could recognize 3 of the 4 codons for proline.

Lecture 8H. Protein synthesis II: peptide bond formation.

Study questions.

1. The methionine codon AUG functions to initiate a polypeptide chain and to direct methionine incorporation into the internal positions in a protein. By what mechanisms are the AUG start codons selected?

   In eukaryotes an initiation factor (eIF4f) binds to the cap and helps direct the mRNA to the 40S ribosome. Once bound to the mRNA, the 40S subunit "scans" to mRNA for the first AUG, and the tRNA -met (I) binds to the AUG by anti-codon interactions in the P site of the ribosome

   In prokaryotes, as the mRNA does not contain a cap the mechanism for recognizing the first AUG is different. IN this case, a sequence present in the mRNA - call the Shine-Delgarno sequence (or Ribosome binding site) base-pairs with a complementary sequence in the 16S rRNA. this interaction orients the ribosome, so that the first AUG downstream of the ribosome binding site is recognized by the f-met tRNA.

2. You wish to express a cloned eukaryotic cDNA in bacteria. What type of sequence must you add in order for the mRNA to be translated on prokaryotic ribosomes?

   As a eukaryotic gene would not have a Shine-Delgarno sequence as part of its mRNA, you would have to modify the gene to contain this special sequence.

3. What effect would puromycin have on the translation of this mRNA? What if you were expressing the protein in mammalian cells?

   The puromycin would inhibit the translation of the mRNA, and it wouldn't matter if I expressed it in a eukaryotic cell, puromycin inhibits both euks and proks.

4. Which of the following statements are true? Explain.

During protein synthesis:

more than one ribosome can be bound to the mRNA at one time.
the carboxyl terminus of a polypeptide chain is synthesized before the amino terminus.
two high-energy bonds are ultimately used for the synthesis of an aminoacyl-tRNA.
formation of the peptide bond is coupled directly to GTP hydrolysis.

5. Be sure not to confuse trancriptional termination with translational termination. What would be the functional homologue of the stop codon for prokaryotic transcription termination?

A rho independent terminator