MCDB 3500
Exam #2
Fall, 2004

75 minutes, closed everything; be concise, don’t bog down!

Name ________________________________
ID ________________________________

Pseudonym ____________________________________ (If you plan a decision on whether to take the final, we need a pseudonym beside which to post your four-exam grade. If you have not yet given us one, please put it here.)

Q 1 (20 points) __________
Q 2 (20) __________
Q 3 (20) __________
Q 4 (20) __________
Q 5 (20) __________

Total (100) __________
Grade __________
1. Short answers: the following questions can be answered in a few sentences, or less.

Transcription employs a “bubble” to read the DNA information. However, is enough information available to copy DNA information into RNA without “bubbling” or separating the W/C strands?

*There is enough info in the major groove to read the sequence, potentially.*

Through what mechanism does the cyclic AMP-binding protein, CAP, alter transcription frequency?

*It binds the promoter region *and* touches RNA polymerase.*

Explain how you can determine the nucleotide sequence for which a sequence-specific DNA binding protein has its affinity.

*Footprint alongside sequencing lanes, both with the same end label.*

What things distinguish an RNA pol II molecule which has cleared its promoter from one that has not yet done so?

*It has dropped TBP, TFII B & possibly A.*
*It has a phosphorylated CTD.*
*It has released TFII E & H.*

How are pauses in the movement of RNA polymerase used in transcription?

*They are part of the termination mechanism.*
*(They are used to indicate sites for repair or proofreading, not discussed yet.)*
2. Definitions:

Rho

The termination factor that 'chases down' bacterial RNA polymerase II and disrupts elongation complex by helicase activity.

Initiator

Somehow initiates the conserved sequence at the point of a eukaryotic RNA polymerase II initiation.

Gel shift

Detection of protein-DNA complexes by change in gel mobility of a labeled DNA.

-35 box

The 5' conserved sequence in a bacterial RNA polymerase promoter, contacted by σ.

TFIID

The TBP-containing eukaryotic RNA polymerase II factor that also contains TAF's.

Anti-TRAP

TRAP-binding protein; regulates TRAP metabolism by binding TRAP protein in bacteria.

Linker scan

Replacement of sections of a DNA sequence to test function by changing sequence to a 'linker'.

CIC1 box

TATA box with T=C, A=I

Silencer

A negative regulatory element in a eukaryotic RNA polymerase II promoter.

Riboswitch

An RNA structure (usu. in 5' region) that regulates a message by changing structure when a metabolite is bound.
3. Here are drawings of the active site of RNA pol II.

Fill in the blanks with a phrase about the function of the indicated structure, and mark the polarity of all nucleic acid strands on one side or the other.

Draw an arrow that points to:

A] the direction of polymerase motion.
B] the RNA exit channel
C] a B-form helix
D] an A-form helix
E] the site where ribonucleotides are added
F] the non-template strand
G] site of proofreading ribonuclease
H] area where CTD (C-Terminal Domain) is found
I] holding the bubble fixed and looking outward, which helix is rotating counter-clockwise? **Downstream (AT C)**
J] looking outward from a fixed bubble, which helix is rotating clockwise? **Upstream (AT T of Diagram)**
4. On the horizontal line across the page below, representing the Trp operon of E coli, draw arrows pointing to:

A] the promoter
B] the operator
C] the repressor gene
D] the leader peptide
E] the genes for biosynthetic enzymes
F] the attenuator / terminator

What will happen to the regulation of the Trp operon if:

a) Trp disappears from the environment?
   BECAUSE \textit{REPRESSOR DISSOCIATES} \& \underline{\textit{RIBS STAY IN LOW EXPRESSION \uparrow TO MAXIMUM}}.

b) The leader peptide is joined to the first biosynthetic enzyme by deletion of the DNA between?
   BECAUSE \textit{THIS REMOVES THE ATTENUATOR}, \underline{\textit{TRP BIOSYNTHESIS IS NOW REGULATED BY REPRESSION ONLY}}.

c) The 5' sequence of the 4 sequences making up the anti-terminator and terminator hairpin stems is deleted?
   \underline{\textit{BECAUSE THIS SEQUENCE IS MISSING}}, ANTI-TERMINATOR HELIX ALWAYS FORMS, SO ATTENUATOR \underline{\textit{IS LEFT ON FULL ON BUT STILL COULD HAVE FULL-RANGE REPRESSION}}.

d) A large new tract of DNA is inserted between the end of the leader peptide and the attenuator?
   \underline{\textit{COULD SAY:}}
   a) \underline{\textit{NOTHING- SEQ'S 1 \& 2 ABOVE STILL FIND EACH OTHER \& REPRESSION STILL WORKS.}} \underline{\textit{ATTENUATION}}
   b) \underline{\textit{BECAUSE THE ADDED SEQ'S PREVENT PARTS 1-2 INTERACTION, THIS IS TOOT LIKE QUESTION C}}}
5. For each of the following answers, supply a precisely matching, one sentence question:

Four domains, two of which interact with DNA.

**What is the substructure of the protein α, Alex?**

You would see alternating regions of enhanced and decreased susceptibility to DNase.

**How is the DNase digestion of coerco DNA altered?**

That information is in the minor groove of a B-form helix.

**Where is a structure indicating that either an A-T or a G-C pair exists?**

I’d do a gel shift experiment.

**What would be a good way of asking about the relationship between the DNA-binding by several proteins?**

Transplantation and inversion do not remove the effect.

**What are the DNA mechanics/typical profile of enhancers?**