MCDB 3500
Exam #2
Fall, 2004

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

Name KEY

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 BENDS THE PROMOTOR REGION & 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, TFIIB & POSSIBLY A.
IT HAS A PHOSPHORYLATED CTD.
IT HAS RELEASED TFIIE & 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 RNAP & DISRUPTS ELONGATION COMPLEX W/ HELICASE ACTIVITY

Initiator

(SOMEWHAT) THE CONSERVED SEQUENCE AT THE PT OF EUK RNA POL II INITIATION.

Gel shift

DETECTION OF PROTEIN-NA COMPLEXES BY CHANGE IN GEL MOBILITY OF A LABELED NA.

-35 box

THE 5' CONSERVED SEQUENCE IN A BACTERIAL RNAP PROMOTER, CONTACTED BY σ.

TFIID

THE TBP CONTAINING BASAL EUK RNA POL II FACTOR THAT ALSO CONTAINS TAF's

Anti-TRAP

TRAP BINDING PROTEIN; REGULATES TRP 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'

CICI box

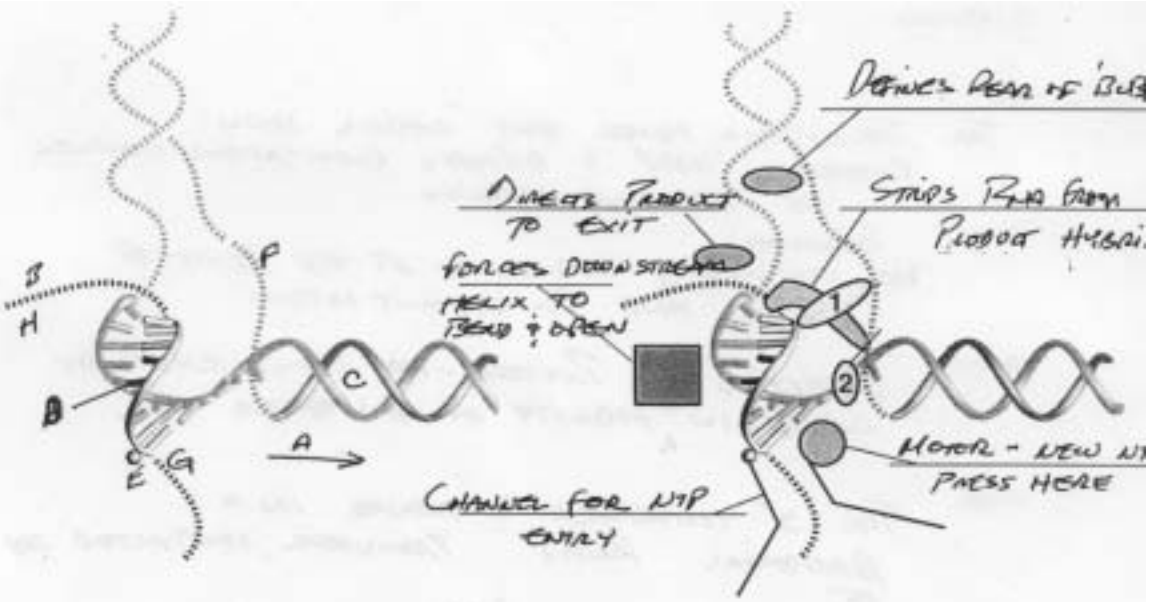
TATA BOX W/ T=C, A=I

Silencer

A NEGATIVE REGULATORY ELEMENT IN A EUK RNA POL 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 TOP OF DIAGRAM)

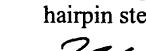
4. On the horizontal line across the page below, representing the Trp operon of E coli, draw arrows pointing to:

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 REPRESSOR DISSOCIATES & RIBS STALL IN LEADER
EXPRESSION ↑ TO MAXIMUM.

b) The leader peptide is joined to the first biosynthetic enzyme by deletion of the DNA between?
BECAUSE THIS REMOVES THE ATTENUATOR, 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?
BECAUSE this  SEQUENCE IS MISSING, ANTI-TERMINATOR HELIX ALWAYS FORMS, SO ATTENUATOR IS LOCKED 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?
COULD SAY:
a) NOTHING - SEQ'S 1 & 2 ARMOUR STILL FIND EACH OTHER & REPRESSION/ATTENUATION STILL WORKS. OR
b) BECAUSE THE ADDED SEQS PREVENT PROPER 1-2 INTERACTION, THIS IS JUST 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 O, ALEX

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

HOW IS THE DNASE DIGESTION of CODED 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 RELATION BETWEEN THE DNA-BINDING BY SEVERAL PROTEINS?

Transplantation and inversion do not remove the effect.

WHAT ARE THE DNA MECHANICS / TYPICAL PROPERTIES OF ENHANCERS?