

**Biochemistry and Molecular Biology 400**  
**Third Examination**  
 Fall 2001  
 November 09, 2001

Instructor: Hardison

This examination has 20 questions worth a total of 100 points. All are multiple choice, and each is worth 5 points. Please answer these on the enclosed answer sheet. **BE SURE TO WRITE YOUR NAME, STUDENT NUMBER AND TEST FORM ON THE ANSWER SHEET AND ENCODE YOUR NUMBER! PLEASE TURN IN YOUR ANSWER SHEET.**

***THIS IS FORM A; with answers.***

Some questions request you to choose all the correct statements from a list. Partial credit is given for choosing **some** of the correct answers, **maximal** credit is given for choosing **all** the correct statements, but **no** credit is given for a choice that includes **any incorrect** statements. **Pick only one option per question.**

The Genetic Code

		Position in Codon								
1st	2nd								3rd	
	U			C			A			G
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U	
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C	
	UUA	Leu	UCA	Ser	UAA	Term	UGA	Term	A	
	UUG	Leu	UCG	Ser	UAG	Term	UGG	Trp	G	
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U	
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C	
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A	
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G	
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U	
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C	
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A	
	AUG*	Met	ACG	Thr	AAG	Lys	AGG	Arg	G	
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U	
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C	
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A	
	GUG*	Val	GCG	Ala	GAG	Glu	GGG	Gly	G	

\* Sometimes used as initiator codons.

1. Which of the following statements about the subunits of *E. coli* RNA polymerase are true?

- (1) Dimerization of the  $\beta$  subunits is the initial step in assembly of the polymerase.
- (2) The  $\beta$  subunit confers specificity for a promoter.
- (3) The  $\beta'$  subunit blocks abortive initiation of transcription.
- (4) The antibiotic rifampicin binds to the  $\beta$  subunit to inhibit initiation of transcription.

Correct statements are:

- a. 1, 2, 3, 4      b. 1, 2, 4      c. 2, 3      d. 1, 2      e. 2

**b. is correct, 3 pt for d, 2 pt for e.; 1, 2 and 4 are correct**

2. Which statements concerning the C-terminal domain (CTD) of the large subunit of eukaryotic RNA polymerase II are correct?

- (1) The CTD is highly phosphorylated in an elongating RNA polymerase II.
- (2) The *SRB* genes were discovered as suppressors of mutations in the CTD.
- (3) Some of the enzymes required for polyadenylation of the mRNA are associated with the CTD.
- (4) A function analogous to that of the CTD is found on the  $\beta'$  subunit of *E. coli* RNA polymerase.

Correct statements are:

- a. 1      b. 1, 2      c. 1, 4      d. 1, 2, 3      e. 1, 2, 3, 4

**d is correct, 3 pts for b, 2 pt for a. Although the large subunit of Pol II is homologous to the  $\beta'$  subunit of *E. coli* polymerase, the repeating structure of the CTD is not found in the *E. coli* enzyme, which is not phosphorylated. Also, the shift from an initiating to an elongating enzyme is accompanied by/caused by the dissociation of  $\sigma$ , not by a change in  $\beta'$ .**

3. Which of the following statements are true?

- (1) One stage in transcription initiation is a shift from a closed to open complex.
- (2) The closed complex has the RNA polymerase encircling the DNA template (i.e. forming a ring around it).
- (3) The closed to open transition **does not** require ATP hydrolysis at promoters transcribed by RNA polymerase from *E. coli*.
- (4) The closed to open transition **does** require ATP hydrolysis at promoters transcribed by eukaryotic RNA polymerase II with its general transcription initiation factors.

- a. 1      b. 1, 2      c. 1, 3      d. 2, 4      e. 1, 3, 4

**e. is correct; 3 pts for c, 2 pts for a; 1, 3, 4**

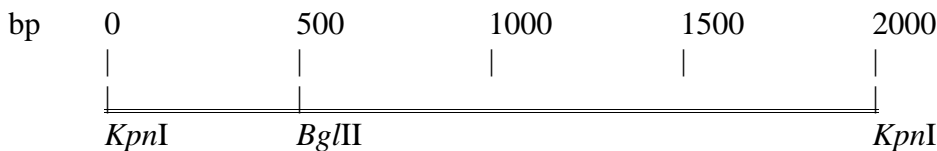
4. Which of these statements about proteins involved in transcription initiation in eukaryotes are correct?

- (1) TBP binds in the narrow groove of DNA containing a sequence related to TATA.
- (2) TFIIF contains a protein kinase and a helicase.
- (3) A mediator complex containing SRB and other proteins is needed to respond to activators.
- (4) TFIIB, SL1 and TFIID contain TBP.
- (5) TFIID is needed to recognize the core (or basal) promoter of RNA polymerases I, II and III.

- a. 1, 2, 3      b. 3, 4      c. 1, 3, 5      d. 1, 2, 3, 4      e. 1, 2, 3, 4, 5

**d. is correct; 3 pts for a, 2 pts for b; 1, 2, 3, 4**

5. An exon of a gene called *GENEY* is contained within the 2000 bp *KpnI* fragment shown in the map below; this exon contains a *BglII* site.



The DNA was cleaved at the *BglII* site (located 500 bp from the left *KpnI* site) and **labeled at the 3' ends**. The *KpnI* to *BglII* fragments (500 and 1500 bp) were isolated, hybridized to *GENEY* RNA and digested with the single-strand specific nuclease S1. Part of the 500 bp probe was protected by RNA, generating a labeled fragment of 200 nucleotides, but the 1500 bp probe was not protected by RNA. When the *BglII* site was **labeled at the 5' ends**, hybridization of the 1500 bp *KpnI* to *BglII* fragment to *GENEY* RNA protected a 300 nucleotide fragment from digestion with S1, but the 500 bp fragment was not protected. What do you conclude from these data?

- a. *GENEY* is transcribed from right to left, and has an exon extending from positions 800 to 300.
- b. *GENEY* is transcribed from right to left, and has an exon extending from positions 700 to 200.
- c. *GENEY* is transcribed from right to left, and has an exon extending from positions 1200 to 200.
- d. *GENEY* is transcribed from left to right, and has an exon extending from positions 200 to 1200.
- e. *GENEY* is transcribed from left to right, and has an exon extending from positions 300 to 800.

**a. is correct.**

6. A PCR-based procedure called rapid amplification of 5' cDNA ends (abbreviated 5' RACE) was used to find the 5' end of a gene called *GENEX*. The particular procedure used takes advantage of the fact that reverse transcriptase adds 3 to 5 C's at the 3' end of cDNAs that extend to the 5' end of the mRNA. Oligonucleotide 1, with the sequence

5' TGGTAACAACGCAGAGTACGCGGGGG

was annealed to the 3' end of the cDNA, and reverse transcriptase further extended the cDNA to make the complement of this oligonucleotide. This extended cDNA was used as the template for PCR, with oligonucleotide 1 as one of the primers. The other primer, oligonucleotide 2, has the sequence of the template strand for part of the first protein-coding exon of *GENEX*; this corresponds to positions 6600 to 6624 in the genomic DNA sequence of the nontemplate strand of this region. The PCR product for 5' RACE was 200 bp long. The sequence of the 5' RACE product was determined; starting at the end corresponding to the 5' end of the mRNA, it reads:

5' to 3' left to right:

TGGTAACAACGCAGAGTACGCGGGGGAGGCCTCTCCCTGACTTTGCAGCCCAACCTGGGAAGCCTGTGAGCAGGAAT  
GGGGAAGAGGAAATGTTGAGCCACATTGAAGGCGCCTCATTAATGGGAGGGAGAGCAG...

This aligns with the genomic DNA sequence shown below (nontemplate strand).

5' to 3' left to right:

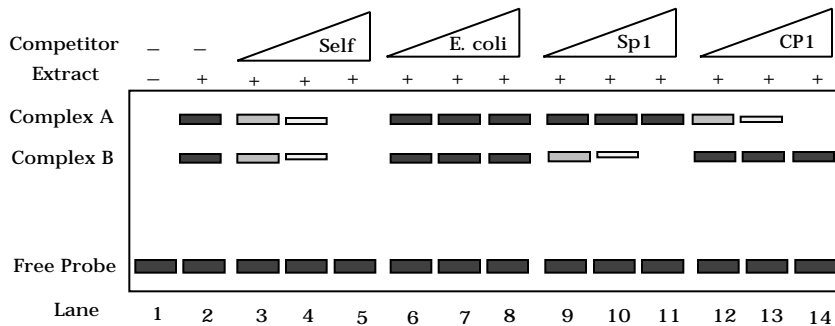
1010	1020	1030	1040	1050	1060	1070
ACCGAGATAAAGCAGGTGATGGGCTTCTCAGGCCTCTCCCTGACTTTGCAGCCCAACCTGGGAAGCCTGA						
1080	1090	1100	1110	1120	1130	1140
TGAGCAGGAATGGGGAAGAGGAAATGTTGAGCCACATTGAAGGCGTTGCAGATAGAAATATCCCATCAGC						

Where is the 5' end of *GENEX* in the genomic DNA sequence?

- a. position 6424
- b. position 6800
- c. position 1030
- d. position 1115
- e. position 6824

**c is correct, 1030**

7. An electrophoretic mobility shift assay was used to test for the ability of a short duplex DNA fragment (the probe) to bind to nuclear proteins, e.g. from liver cells. The DNA fragment was radioactively labeled, mixed with an extract containing the nuclear proteins, and run on a non-denaturing polyacrylamide gel. Lanes 1 below shows the free probe and lane 2 shows the probe plus liver nuclear extract. Further tests of specificity are shown in the competition lanes, in which the labeled probe was mixed with an increasing excess of other, unlabeled DNA before mixing with the nuclear proteins to test for binding. Competitor DNAs included the unlabeled probe (self competition, lanes 3-5; the triangle above the lanes indicates that an increasing amount of competitor is used in successive lanes), a completely different DNA (sheared *E. coli* DNA) as a nonspecific competitor (lanes 6-8), and two different duplex oligonucleotides, one containing the binding site for Sp1 (lanes 9-11) and the other containing the binding site for the protein CP1. Thinner, less densely filled boxes denote bands of less intensity than the darker, thicker bands.



What do these data tell you?

- a. No proteins bind specifically to this DNA probe.
- b. *E. coli* proteins bind specifically to this DNA probe.
- c. A protein whose binding site is similar to that of Sp1 binds to the probe to form complex A.
- d. A protein whose binding site is similar to that of CP1 binds to the probe to form complex A.
- e. Heterodimers of Sp1 and CP1 bind to the probe to generate complexes A and B.

**d is correct.**

8. Which proteins induce hydrolytic cleavage of short RNA fragments as part of the process of overcoming transcriptional pausing?

- (1) *E. coli* GreA and GreB
- (2) Eukaryotic P-TEFb
- (3) Eukaryotic TFIIS
- (4) Eukaryotic DRB
- (5) Eukaryotic TFIIH

- a. None of the above
- b. 2, 3, 5
- c. 1, 3
- d. 2, 4
- e. 1

**c is correct, 2 pt for e**

9. Which statements about polyadenylation of eukaryotic mRNAs are true?

- (1) Polyadenylation occurs at the site at which transcription terminates.
- (2) The sequence AAUAAA in the RNA is a key determinant of the position of polyadenylation.
- (3) Proteins CPSF, CFI and CFII are needed for specific cleavage prior to polyadenylation.
- (4) Poly(A) polymerase is a template-directed RNA polymerase.
- (5) Some factors needed for polyadenylation are bound to the CTD of RNA polymerase II during elongation.

Choose all the correct statements.

- a. 2, 3, 5                      b. 1, 4                      c. 1, 2, 3                      d. 2, 5                      e. 2

**a is correct, 3 pts for d, 2 pt for e; 2, 3, 5**

10. Which of the following statements about processing of pre-tRNAs in *E. coli* is/are correct?

- (1) None of the bases are modified covalently after transcription.
- (2) Processing of the pre-tRNA is coupled with translation.
- (3) RNase P cleaves to generate the 5' end.
- (4) The 3' end is formed by cleavage by an endonuclease followed by an exonuclease.

- a. 1                      b. 3, 4                      c. 2, 3                      d. 1, 4                      e. 3

**b is correct, 2 pts for e; 3, 4**

11. In an investigation of the mechanism of splicing of precursor RNA for a novel gene, you discovered that :

- splicing of the precursor RNA could be carried out in a cell-free system,
- splicing of the precursor RNA did **NOT** require the addition of nuclear proteins or RNAs (other than the precursor RNA),
- splicing requires ATP. Neither ADP nor AMP would substitute for ATP, nor would any other nucleotide.

What do you conclude about this novel splicing reaction?

- a. It is self-splicing and **does** proceed by a phosphoester transfer mechanism.
- b. It is self-splicing but does **not** proceed by a phosphoester transfer mechanism.
- c. It is **not** self-splicing and does **not** proceed by a phosphoester transfer mechanism.
- d. It is **not** self-splicing but **does** proceed by a phosphoester transfer mechanism.

**b. is correct.**

12. Which of the following statements about RNA splicing mechanisms (including pre-tRNA, pre-rRNA, and pre-mRNA) elucidated to date are correct?

- (1) All splicing reactions proceed by a phosphoester transfer mechanism.
- (2) The individual reactions in phosphoester transfers are reversible.
- (3) Assembly of a spliceosome for splicing of pre-mRNA requires the cleavage of high-energy bonds from ATP.
- (4) The initiating nucleophile for splicing of Group I introns (including the intron of pre-rRNA from *Tetrahymena*) is the 2' hydroxyl of an internal guanine nucleotide.
- (5) The initiating nucleophile for splicing of nuclear pre-mRNA is the 2' hydroxyl of an internal adenine nucleotide.

a. 2, 3, 5      b. 1, 2, 4      c. 3, 5      d. 1, 2, 3, 4, 5      e. 1, 3, 5

**a is correct, 3 pts for c**

13. What strategy would allow you to design an artificial gene using the Group I intron from *Tetrahymena* pre-rRNA to replace a mutated segment of an mRNA in mammalian cells? The following list has possible steps in constructing a modified Group I intron that when expressed after transfection into mammalian cells should correct the defect in the mutant mRNA.

- (1) Change the internal guide sequence of the Group I intron so that it is **complementary** to a sequence **5'** to the altered nucleotide in the mutant mRNA.
- (2) Change the internal guide sequence of the Group I intron so that it is **identical** to a sequence **5'** to the altered nucleotide in the mutant mRNA.
- (3) Change the internal guide sequence of the Group I intron so that it is **identical** to a sequence **3'** to the altered nucleotide in the mutant mRNA.
- (4) **Eliminate** the guanine nucleotide-binding site at position 414 of the Group I intron.
- (5) Link the wild-type version of the part of the gene that is altered in the mutant **downstream** of the Group I intron.

Correct choices are:

a. 3      b. 5      c. 1, 4, 5      d. 2, 4      e. 1, 5

**e is correct, 2 pts for b; 1, 5**

**14.** Which of the following statements about splicing of the precursors to mRNA in eukaryotes are correct?

- (1) Introns in RNA have the dinucleotides **GC** at their 5' end and **AU** at their 3' end.
- (2) The U1 snRNP binds at the 5' splice junction.
- (3) Assembly of the spliceosome requires ATP hydrolysis.
- (4) A U2:U6 snRNA heteroduplex catalyzes a phosphoester transfer reaction involving the A at the branch.

a. 1

b. 2, 3

c. 2, 3, 4

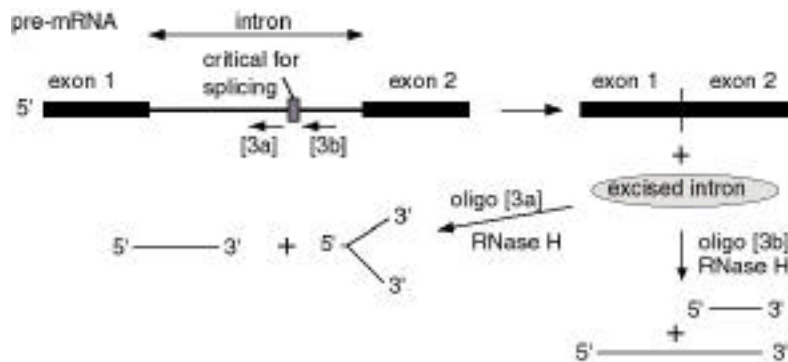
d. 1, 2

e. 1, 2, 3, 4

**c. is correct, 3 pts for b; Statements 2, 3, 4 are correct.**

15. Imagine that you are examining a **NOVEL** splicing mechanism in a cell-free system by incubating a radioactively labeled precursor to mRNA containing two exons and an intron with a nuclear extract, ATP, GTP and appropriate buffers. You make the following observations.

- [1] The products of the reaction are the two exons spliced together and an excised intron, but this excised intron does not move as a linear molecule during gel electrophoresis.
- [2] Mutagenesis studies show that an internal region of the intron is critical for splicing.
- [3] The structure of the nonlinear, excised intron was studied by annealing it with short oligodeoxyribonucleotides followed by RNase H treatment, which will cleave any RNA that is in a heteroduplex with DNA.
- [3a] When an oligodeoxyribonucleotide complementary to the intron sequence just **5'** to the critical region is used, the excised intron is broken into a linear RNA and an almost linear RNA. This latter species has two arms extending from the critical region, each with a 3' end, and a very short 5' tail at the critical region.
- [3b] When an oligodeoxyribonucleotide complementary to the intron sequence just **3'** to the critical region is used, the excised intron is broken into a two linear RNAs, one long and one short.



What do you conclude about the excised intron?

- a. The intron was duplicated in the process of excision.
- b. The excised intron has a circle and a linear tail, i.e. it is a lariat.
- c. The excised intron is a simple circle.
- d. The excised intron is an X-shaped molecule
- e. The excised intron is a Y-shaped molecule.

**e. is correct**

**16.** A codon for leucine can be converted to codons for either serine (Ser), valine (Val) or methionine (Met) by a single nucleotide substitution (a different nucleotide substitution for each amino acid replacement). What is the codon for leucine?

- a. CUG                      b. CUU                      c. UUA                      d. UUG                      e. CUC

**d. is correct**

**17.** Allowing for "wobble" between the 3rd position of the codon and the 1st position of the anticodon, what is the minimum number of tRNAs required to recognize codons for arginine (Arg) in the usual genetic code? (See cover sheet of the exam.)

- a. two  
b. three  
c. four  
d. six

**b. is correct. Anticodon 3' GCI can pair with codons 5' CGU/C/A, anticodon 3' GCC can pair with codon 5' CGG, and anticodon 3' UCU can pair with codons 5' AGR.**

**18.** Which statements about charging of tRNAs (adding an amino acid) are correct?.

- (1) One aminoacyl-tRNA synthetase catalyzes addition of all amino acids to their correct tRNAs.
- (2) Dimeric aminoacyl-tRNA synthetases have one subunit for recognizing the anticodon of a tRNA and another subunit for catalyzing addition of the amino acid.
- (3) The reaction requires binding but not hydrolysis of ATP.
- (4) The reaction proceeds in two steps.
- (5) Aminoacyl-tRNA synthetases have a proofreading function that removes amino acids that should not be linked to a particular tRNA.

- a. 1, 4                      b. 2, 3                      c. 1, 3, 5                      d. 2, 4                      e. 4, 5

**e. is correct; Correct statements are 4, 5.**

**19.** Which of the following statements about the translation initiation in *E. coli* are correct?

- (1) IF-3 plus GTP brings methionyl-tRNA to a ribosomal subunit.
- (2) Formylmethionyl-tRNA<sub>f</sub><sup>Met</sup> is brought to a ribosomal subunit in a complex with IF-2 in a reaction requiring GTP.
- (3) Formylmethionyl-tRNA<sub>f</sub><sup>Met</sup> binds to the A site on the large ribosomal subunit.
- (4) GTP hydrolysis is needed to remove the initiation factors from the ribosomal subunit.

a. 1, 3      b. 2, 4      c. 2, 3, 4      d. 1, 4      e. 2

**b. is correct, 2 pts for e. Statements 2 and 4 are correct.**

**20.** Which of these processes is/are catalyzed by RNA?

- (1) Cleavage by RNase P to form the 5' end of tRNA.
- (2) Remove Group I introns from precursor RNAs.
- (3) Phosphoester transfer in splicing of nuclear pre-mRNA.
- (4) Form the peptide bond between peptidyl-tRNA and aminoacyl-tRNA on the ribosome.
- (5) Add a polyA tail to the 3' end of mRNA.

a. 1, 2      b. 3, 4      c. 1, 2, 3, 4      d. 2, 3, 5      e. 1, 2, 3, 4, 5

**c. is correct, 2 pts for a, b.**

**Correct choices are 1, 2, 3, 4.**