Student name: _____ Student number: _____

BCH/BIO 3170
Molecular Biology – second mid-term November 2009
Dr. Odette Laneuville

Part 1. Multiple Choices Questions (15 at 2 pts each):
/30

Part 2. Essay Questions – Short (6 at 5 pts each):
/30

Part 3. Essay Questions – Long (4 at 10 pts each):
/40

Total: /100

Instructions:
WRITE YOUR NAME and STUDENT NUMBER on the bubble sheet and on the Questionnaire.

Absolutely no books, handouts, notes or calculators are allowed.

Write your answers to the multiple choice questions (#1 to 15) on the red bubble sheet. For the multiple choice questions, select the best answer and only one answer. For the essay questions, please limit your answer to the space provided.

Write your answers to essay questions (short and long) directly on the Questionnaire

At the end of the examination period, you must return the bubble sheet and the Questionnaire.

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The Genetic Code

Fig.1 The genetic code

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

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Part 1. Multiple Choices Questions (15 at 2 pts each):

1. Select the TRUE statement concerning the sigma subunit of the RNA polymerase of prokaryotes.
ANSWER: D

A) Sigma increases the dissociation constant of RNA pol for all DNA sequences.
B) Sigma reduces the association constant of RNA pol for all DNA sequences.
C) Sigma reduces the dissociation constant of RNA pol for non promoter DNA sequences.
D) Sigma increases the dissociation constant of RNA pol for non promoter DNA sequences.
E) Sigma increases the association constant of RNA pol for non promoter DNA sequences.

2. Identify the TRUE statement concerning the state of transcription of the lactose operon when bacteria are growing in a media without lactose and rich in glucose.
ANSWER: B

A) The repressor is not bound to the operator and CAP protein is bound to the CAP site.
B) The repressor is bound to the operator and CAP protein is not bound to the CAP site.
C) The repressor is not bound to the operator and CAP protein is not bound to the CAP site.
D) The repressor is bound to the operator and CAP protein is bound to the CAP site.
E) RNA pol is transcribing the lactose operon.

3. Where is the attenuator sequence localized in the tryptophan operon?
ANSWER: E

A) Between the promoter and leader sequences.
B) Immediately before the *trpA* gene.
C) Between the operator and leader sequences.
D) Between the promoter and operator sequences.
E) None of the above.

4. **How is the binding of the repressor to the lactose operon regulated?**
ANSWER: B

A) The repressor is inactive in absence of allolactose.
B) Allolactose prevents the binding of the repressor to the operator.
C) Allolactose induces conformation changes to the repressor allowing binding of the repressor to DNA.
D) Allolactose acts as a co-repressor.
E) None of the above.

5. Identify the DNA sequence on the coding strand (sense strand) that is transcribed into this RNA sequence: CCGUUAAGU.
ANSWER: C

A) GGCAATTCA
B) TGAATTGCC
C) CCGTTAAGT
D) ACTTAACGG
E) None of the above.

6. Which of these factors does not contribute to the formation of complexes between promoter sequences and RNA pol?
ANSWER: E

A) Activating proteins.
B) Conformation changes of RNA pol induced by repressive proteins.
C) The sequence TATAAT localized 10 nucleotides before the transcription start site.
D) A distance of 15 to 19 nucleotides between the sequences TATAAT and TTGACA.
E) None of the above.

7. How does glucose repress the catabolism of lactose?
ANSWER: B

A) By preventing the binding of the repressor to the promoter.
B) By reducing the concentrations of intracellular cAMP.
C) By promoting the binding between CAP and DNA.
D) By increasing the concentrations of intracellular cAMP.
E) None of the above.

8. Identify the situation corresponding to the 2nd phase (delayed – early) of the lytic cycle of lambda phage:
ANSWER: A

A) This phase is mostly regulated by the mechanism of anti-termination.
B) Transcript R5 is produced.
C) The expression of cI is repressed.
D) Transcript L1 is generated from the promoter p_L.
E) Genes encoding for glycoproteins R and S are transcribed.

9. The genetic code is said to be degenerated because:
ANSWER: A

A) The same amino acid can be encoded by many different codons.
B) Mitochondria and chloroplasts, for example, use a different code.
C) In many cases, the nucleotide in 5' of triplets of nucleotides encoding for the same amino acid is different.
D) Answers a, B and C are correct.
E) None of the above.

10. Identify the anti-codon corresponding to the amino acid serine (Ser).
ANSWER: A

A) GCU
B) UCI
C) UCA
D) AGU
E) UCG

11. Select the CORRECT statement concerning protein synthesis?
ANSWER: D

A) Ribosomes are responsible for protein synthesis in the nucleus.
B) For prokaryotes, but not for eukaryotes, many ribosomes can translate simultaneously the same RNA strand.
C) The formation of the peptidic bond will induce conformational changes of the ribosome and triggers the translocation of the 2 tRNA from sites E and P towards sites P and A.
D) Immediately after a protein domain emerges from the ribosome, a secondary structure is formed that may contain alpha helices and beta sheets.
E) None of the above.

12. Identify the RNA sequence transcribed from the DNA matrix strand and that can hybridize with the anti-codon IUU.
ANSWER: C

A) TTT
B) CTT
C) GTT
D) TTC
E) TTG

13. What is the role of the factor EF-G ?
ANSWER: B

A) Catalyses the hydrolysis of the peptide from the tRNA when the translation machinery runs into a STOP codon.
B) Catalyzes the translocation of the ribosome to the next codon onto the mRNA.
C) Is part of the protein complex that allows the circularization of eukaryotic mRNA.
D) Recycles the ribosomal sub-units at the end of the protein synthesis reaction.
E) Facilitates the binding of the small ribosomal sub-unit to the mRNA during the step of initiation of translation.

14. Which of the following reactions associated to protein synthesis requires the hydrolysis of GTP into GDP?
ANSWER: E

A) Transpeptidation.
B) Binding of the factor RF-1 to a STOP codon.
C) Hydrolysis of the peptide attached to the tRNA after the addition of the last amino acid.
D) Circularization of the eukaryotic mRNA.
E) Binding of the factor RF3 to the A site of the ribosome.

15. What is the role of the factor EF-Ts involved in the reaction of protein synthesis?
ANSWER: D

A) Hydrolysis of GTP into GDP.
B) Catalyze the translocation of the ribosome to the next codon.
C) Reduce the affinity of the EF-Tu for GDP.
D) Remove GDP from the factor EF-Tu.
E) None of the above.

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Part 2. Essay Questions – Short (6 at 5 pts each):

1. During the transcription reaction, where does the energy necessary to move the enzyme RNA polymerase on the DNA matrix strand come from? (5 points)

ANSWER: From the hydrolysis of phosphates beta and gamma located on the free nucleotide (2 points) used as a substrate in the reaction and from the hydrolysis of pyrophosphate (1 point) into 2 inorganic phosphates (1 point) catalyzed by the enzyme pyrophosphatase (1 point).

2. What is the name of the DNA sequence to which the repressor of the lactose operon binds? How is the binding of the repressor of the lactose operon to DNA controlled when lactose is present? (5 points)

ANSWER:

Operator (1 point)
Lactose will generate allolactose, the ligand of the repressor (1 point), and the complex repressor - allolactose will not bind to the operator (2 points) allowing for the operon to be transcribed (1 point).

3. Operon lactose is under a positive control to ensure maximal transcription of the lactose operon. Name the ligand of the activating protein CAP (catabolic activating protein). Does glucose increase or decrease the concentration of the CAP ligand? (5 points)

ANSWER:
cAMP (2 points)
Glucose decreases levels of cAMP (3 points)

4. Cro protein binds to the 3 sites of the repressor O_R of lambda phage. What is the affinity of Cro for the 3 sites? Is the binding of Cro to the O_R sites cooperative or not? (5 points)

ANSWER:
$o_{R3} > o_{R2} = o_{R1}$ (non cooperative binding). (2 points for $o_{R3} > o_{R2}$, 1 point for $o_{R2} = o_{R1}$, 2 points for non-cooperative binding)

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5. What is the sequence in amino acids corresponding to the following mRNA of prokaryotic origin:
GUAAUGGCUUAUCGGUUGAGGAGGGUACCUUCAUGAACCCCGCC? (5 points)

ANSWER:

Met-Asn-Pro-Ala (1 point for each amino acid and 1 point for the order)
2 points if you have used the wrong initiation codon; the initiation eukaryotic codon.

6. How many peptidic bonds are present in a linear peptide of 8 amino acids? (5 points)

ANSWER:
7

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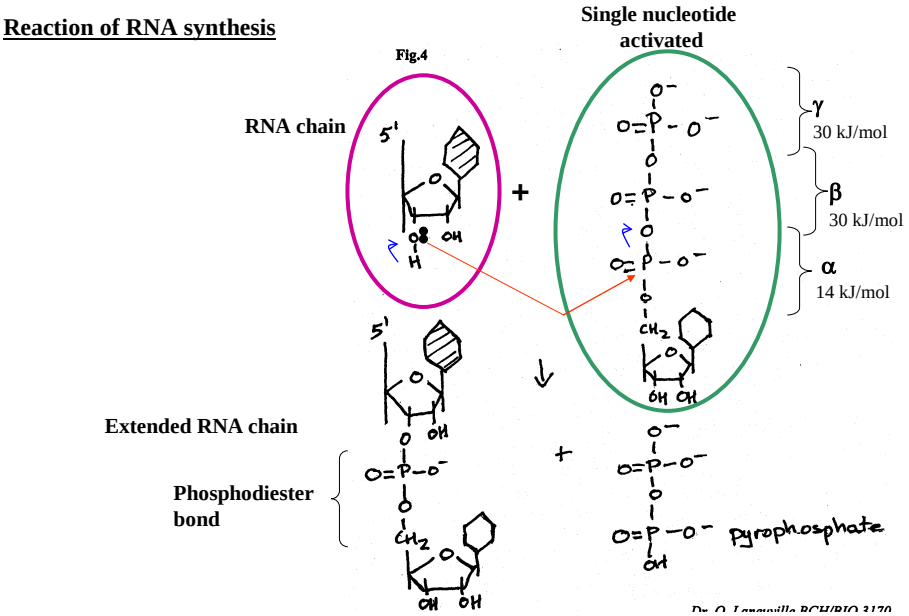
Part 3. Essay Questions – Long (4 at 10 points each):

1. The reaction of mRNA synthesis is catalyzed by the enzyme RNA polymerase. (10 points)

A. **Illustrate the nucleophilic attack that takes place during this reaction. Your answer must include the structure of all the substrates and products and important atoms involved in the reaction. (6 points)**
B. **Why the free nucleotides added to the mRNA chain are in the triphosphate form and not in the monophosphate form? (4 points)**

ANSWER:
A.



1 point for the Oxygen on C3 of the RNA chain, 1 point for the structure of the NTP, 1 point for the phosphodiester link, 1 point for the pyrophosphate, 2 points for the arrow showing the pair of free electron on oxygen attached to C3 is attacking the phosphate alpha of the free nucleotide.

B. Triphosphate: delta G of the reaction is negative and therefore favorable and pyrophosphate is formed. The hydrolysis of pyrophosphate catalyzed by the enzyme pyrophosphatase will generate 2 inorganic phosphate and will drive the reaction towards the formation of products; elongation of the RNA chain. (2 points)

Monophosphate: delta G of the reaction is positive and not favorable. No formation of pyrophosphate and therefore the reaction is not driven towards the formation of products; elongation of the RNA chain. (2 points)

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2. The amino acid tryptophan (trp) regulates the two mechanisms of regulation of expression of the trp operon. Name the two mechanisms and explain how the regulation occurs when there is very low levels of the amino acid trp in the extracellular milieu. Your answer must include an illustration of the trp operon and the sequences of the control region. (10 points)

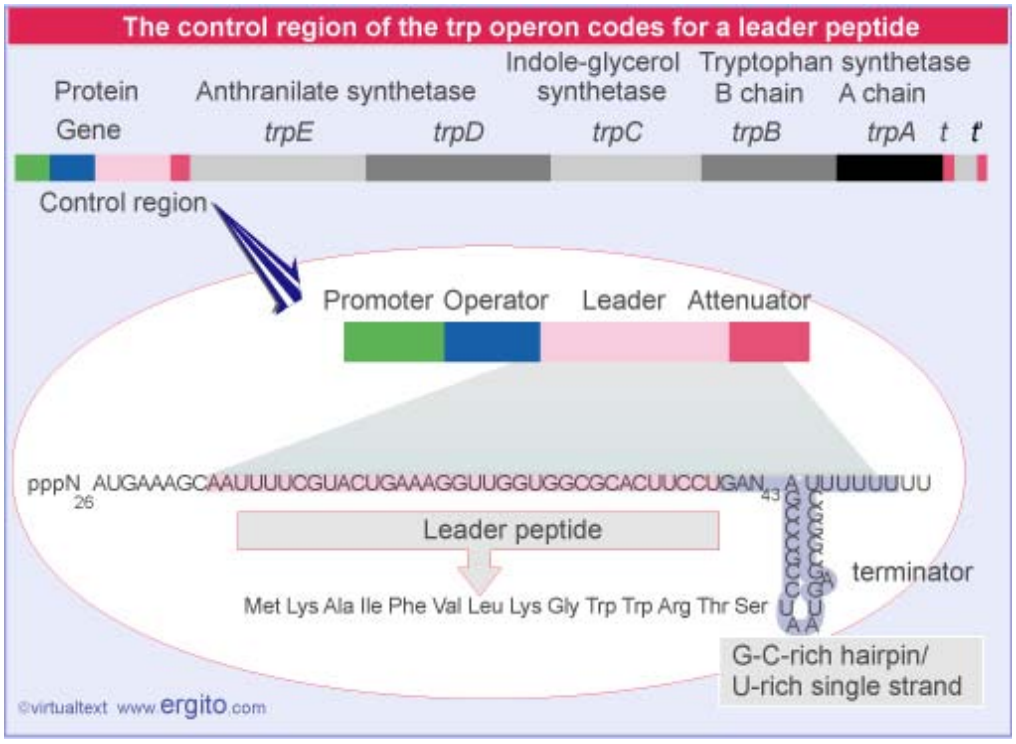
ANSWER:
1. The two mechanisms are repression and attenuation. (1 point)

2. When the amino acid trp is absent in the milieu, the repressor does not bind to the operator and the trp operon is transcribed. (1 point)

3. The attenuator provides a barrier to transcription into the structural genes (1 point). Transcription of the trp operon can terminate after the attenuator before the structural genes (1 point). Since this is a prokaryotic system, the reactions of transcription and of translation occur simultaneously for the same transcript and this is necessary for the attenuation to occur. (1 point)

Region 1 of the leader RNA includes 2 consecutive codons for trp. Ribosomes make a pause over region 1 of the leader mRNA where the 2 consecutive codons for trp are located (1 point). This allows region 2 of the mRNA to hybridize with region 3 (1 point). Therefore region 3 of the mRNA is not available to hybridize with region 4 and there is no formation of a stop signal for the RNA pol; the trp operon is transcribed (1 point).

Structure of the trp operon (top illustration only): (2 points)



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3. Explain how cI repressor of phage lambda can simultaneously stimulate the transcription of its own gene and repress the expression of other genes. (10 points)

ANSWER:
2 points for the structure of the region including: P_{RM}, o_{R1} , o_{R2} , o_{R3} **and** P_R

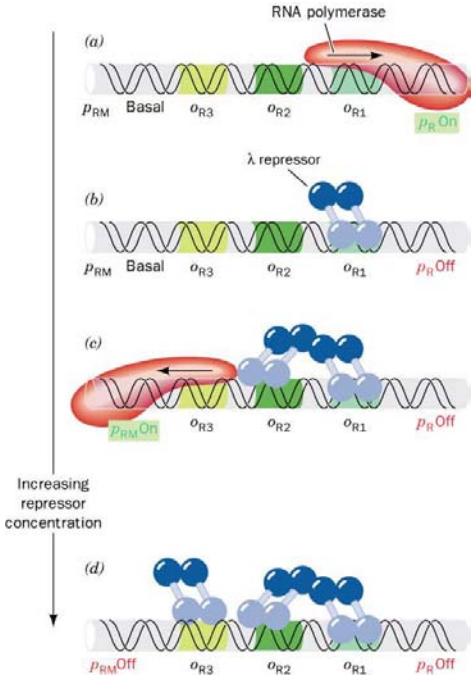
(2 points) By controlling the binding to the O_R (Operator Right) sites. The affinity of cI for O_R:
$o_{R1} > o_{R2} > o_{R3}$

a. Absence of repressor: RNA pol initiates Transcription at high level from p_R and at basal level at p_{RM}.

b. Repressor increases: affinity of cI for o_R: $o_{R1} > o_{R2} > o_{R3}$ binding to o_{R1} first and transcription from p_R is off and therefore expression of all genes other than cI is repressed. (3 points)

c. Repressor increases higher and binding to o_{R1} and to o_{R2} (cooperative binding). Stimulates transcription from p_{RM} and therefore CI gene is expressed. (3 points).

d. Very high concentration of repressor all o_R sites are occupied and transcription from both p_R and p_{RM} is off.



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4. The reaction of the loading of an amino acid onto a tRNA is not favorable energetically. Explain what renders the reaction favorable and favors the formation of the product amino acyl tRNA. What confers the specificity to the reaction? (10 points)

ANSWER:

1. The reaction is:
amino acid + tRNA +ATP $\longrightarrow$ aminoacyl-tRNA + AMP + PPi

2. the activation of the amino acid with ATP renders the reaction of the loading of the amino acid onto the tRNA favorable energetically (2 points). The products formed are: the complex aminoacyl-AMP and pyrophosphate (2 points).

amino acid + ATP $\longrightarrow$ aminoacyl-AMP + PPi

3. The complex aminoacyl-AMP is loaded onto the tRNA (1 point):

aminoacyl-AMP + tRNA $\longrightarrow$ aminoacyl-tRNA + AMP

4. The reaction is driven towards the formation of products: amino acyl tRNA because the enzyme pyrophosphatase hydrolyses pyrophosphate into 2 inorganic phosphates; LeChatelier principle (2 points).

5. **The aminoacyl-tRNA synthase (or synthetase):** confers the specificity to the reaction (2 points) and matches the anti-codon on the tRNA with the corresponding amino acid according to the genetic code (1 point).