Student Name:_____Student Number:

BCH/BIO 3170
Molecular Biology – Final Exam - December 2006
Dr. Odette Laneuville
Dr. Alain Stintzi
Dr. Martin Pelchat

Part 1. Multiple choice questions (25 at 2 pts each):

Dr. Laneuville #1 to 10 - 2 pts each:	/20
Dr. Stintzi #11 to 13 – 2 pts each	/6
Dr. Pelchat #14 to 25 – 2 pts each	/24

Part 2. Essay questions

Dr. Laneuville #1 and 2 - 10 pts each	/20
Dr. Laneuville #3 – 4 pts	/4
Dr. Stintzi #4 – 2 pts	/2
Dr. Pelchat #5 and 6 - 10 pts each	/20
Dr. Pelchat #7 – 4 pts	/4
Total:	**/100**

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

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

Write your answers to the multiple choice questions (#1 to 25) on the red bubble sheet. For the multiple choice questions, select the best answer and only one answer.

Write your answers to essay questions directly on the Questionnaire.

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

Student Name:_____Student Number:

The Genetic Code

Student Name:_____Student Number:

1. Which position(s) in a purine ring of a nucleotide inserted in the DNA double helix have the potential to form hydrogen bonds but are not involved in Watson-Crick pairing?
Answer:A

A. N-3 and N-7
B. N-1
C. N-7
D. N-3 and N-1
E. N-3

2. Which is incorrect concerning the effects of tautomerism on base pairing at the center of the DNA double helix?
Answer:E

A. the enol form of thymine base-pair with guanine.
B. the imino tautomer of adenine base-pair with cytosine.
C. the imino tautomer of adenine base-pair with guanine.
D. the enol form of guanine pairs with thymine.
E. none of the above

3. How many GTP are necessary to synthesize two linear peptides of 11 amino acids each using an *in vitro* prokaryotic system? Assume the tRNAs provided are already loaded with their proper amino acids. All 3 steps of the protein synthesis reaction must be completed.
Answer:B

A. 46
B. 44
C. 23
D. 22
E. 0

4. Identify the DNA sequence on the DNA matrix strand which when transcribed produces a sequence capable of perfectly hybridizing to the anticodon GUC.
Answer:D

A. GAT
B. CAG
C. CTG
D. ATC
E. GAU

5. Identify the anticodon matching the amino acid Serine.
Answer:C

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

6. What is the role of the eukaryotic elongation factor Ts (eEF-Ts is also called eEF1B)?
Answer:B

A. it binds the aminoacyl-tRNA.
B. it increases the off-rate of GDP from EF-Tu (also called EF1A).
C. it reduces the affinity of the tRNA for the peptidyl site on the ribosome.
D. it favours the formation of EF-Tu-GDP.
E. it catalyses the translocation of the ribosome along the mRNA.

7. What is the role of releasing factor-3 (RF-3) in protein synthesis?
Answer:A

A. it carries the energy necessary to the termination step of the protein synthesis reaction.
B. it catalyses the hydrolysis reaction leading to the release of the peptide from the tRNA.
C. it recognizes the stop codons UAA or UGA on mRNAs.
D. it recognizes the stop codons UAA or UAG on mRNAs.
E. it dismantles the translational machinery at the end of the translation reaction.

8. Identify a situation likely to be observed in someone with low levels of free iron in their blood circulation.
Answer:C

A. ferritin mRNA is bound to ribosomes.
B. the transferring receptor mRNA is degraded.
C. the IRP (iron regulatory protein) is bound to the ferritin mRNA.
D. ferritin mRNA is highly translated.
E. transferring receptor mRNA is not bound to the IRP.

9. Identify the incorrect answer concerning the regulation of gene expression by DNA methylation.
Answer:B

A. the methyl group is added to carbon 5 of cytosine residues.
B. efficient repression does not require the methylated C binding proteins.
C. access to promoter is blocked due to the altered structure of chromatin induced the recruitment of chromatin remodeling factors and due to histone deacetylases.
D. the transcription apparatus block the access of DNA methyltransferases to the CpG sites.
E. the regulated gene is off but leaky in absence of methylation and of the regulatory proteins of the transcription apparatus.

10. Identify the correct answer concerning Hsp 60 proteins and the mechanisms of protein folding?
Answer:D

A. Hsp 60 are necessary for the formation of the molten globule.
B. Hsp 60 takes over when spontaneous protein folding fails.
C. Hsp 60 does not require ATP to fold proteins.
D. Hsp 60 binds to the protein GroES cap.
E. Hsp 60 has protease activity.

11. Which protein is observed exclusively in association with prokaryotic DNA replication?:
Answer:C

A. **Helicase**
B. **Primase**
C. **DNA polymerase I**
D. **Sliding clamp**
E. **SSB proteins**

12. Which of the following statement about DNA microarray is false?:
Answer:B

A. **They are constituted of PCR products printed onto a glass slide.**
B. **They are constituted of proteins printed onto a wafer.**
C. **They are constituted of short oligonucleotides synthesized in situ.**
D. **They are constituted of oligonucleotides printed onto a glass slide.**
E. **None of the above answers**

13. Which of the following statement about DNA microarray experiment and analysis is true?:
Answer:E

- **A. Direct labeling of cDNA during reverse transcription allows even incorporation of the 2 fluor-dNTPs (Cy3-dNTP and Cy5-dNTP).**
- **B. Oligonucleotides exhibiting internal secondary structures are specifically selected to be printed onto a glass slide to construct the microarray.**
- **C. One DNA microarray slide contains a maximum of 200 spots.**
- **D. Indirect labeling of cDNA consists in incorporating aminoallyl-dUTPs after the reverse transcription step using a polynucleotide kinase.**
- E. **Hierarchical clustering analysis is a statistical method that can be used to assign genes to groups.**

14. When lactose is absent, the *lac* repressor is:
Answer: C

a) inactive and can bind a site on the DNA upstream of the lactose operon
b) inactive and cannot bind a site on the DNA upstream of the lactose operon
c) active and can bind a site on the DNA upstream of the lactose operon
d) active and cannot bind a site on the DNA upstream of the lactose operon
e) inactive and can bind a site on the mRNA upstream of the lactose operon

15) In the classical model of transcription regulation described by Jacob et Monod, a repressor protein binds to:
Answer: C

a) an enhancer
b) a AUG sequence
c) an operator
d) a ribosome binding site
e) the -10 and -35 boxes

16) All these elements contribute to the binding of *E. coli* RNA polymerase to a promoter, **EXCEPT**:
Answer: A

a) the rho factor
b) the -10 consensus sequence
c) the -35 consensus sequence
d) the sigma subunit
e) the DNA structure

17) What happen when *E. coli* is in a medium low in glucose ?
Answer: A

a) The level of cAMP rises, cAMP binds CAP, the cAMP-CAP complex binds to a site on DNA, which increases transcription.
b) The level of cAMP rises, cAMP binds CAP, the cAMP-CAP complex binds to a site on DNA, which inhibits transcription.

c) The level of cAMP rises, cAMP binds CAP, the cAMP-CAP complex cannot bind to a site on DNA, which increases transcription.
d) The level of cAMP decreases, CAP is free, the CAP protein binds to a site on DNA, which increases transcription.
e) The level of cAMP decreases, CAP is free, the CAP protein binds to a site on DNA, which inhibits transcription.

18) Which one of those sugars is preferentially used by *E. coli*?
Answer : C
a) lactose
b) galactose
c) glucose
d) sucrose
e) boutonrose

19) The expression of the *trp* operon in *E. coli* is regulated in part by the level of the amino acid tryptophan, without the implication of regulatory proteins. This type of regulation is called :
Answer: A
a) Attenuation
b) Repression
c) Alternative splicing
d) Antitermination
e) Non-sense suppresion

20) If a DNA fragment, including the *trp* promoter (including all: promoter, regulatory sequences, etc.) up to the end of the *trp* operon of *E. coli*, could be inserted as a transgene in the yeast genome, you will observed:
Answer:D
a) No changes in the expression of the *trp* operon when compared to the same operon in *E. coli*.
b) The loss of activation of the *trp* operon by lactose.
c) A greater inhibition of the *trp* operon by tryptophan
d) A lesser inhibition of the *trp* operon by tryptophan
e) The yeast will become blue

21) You have an *E. coli* strain with the following genotype: *lac*I+ ,*lac*P+,*lac*O-,*lac*Z+ (*lac*I = represser; *lac*P = promoter ; *lac*O = operator ; *lac*Z = β-galactosidase). What could be the expression of β-galactosidase ?
Answer: A
a) Constitutive
b) Always repressed
c) Inducible by lactose
d) Repressed by lactose
e) None of the above

Student Name:_____Student Number:

22) Which one of these enzymes involved in transcriptional regulation does NOT bind DNA?

Answer: E

a) RNA polymerase
b) CAP
c) Lamda repressor
d) Sigma subunit of RNAP polymerase
e) Adenylcyclase

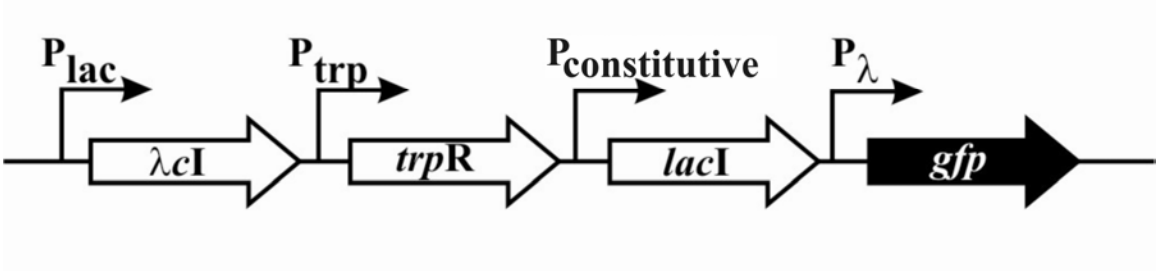
23) The molecule acting as **inducer** for the *trp* operon is:

Answer: E

a) tryptophan
b) lactose
c) λ*cro*
d) β-galactosidase
e) None of the above

24) You have discovered the following genetic circuit. All the promoters indicated include, in addition to the promoter sequences, **only** the corresponding operators.

Answer: D



Indicate what happens to the level of expression of **each gene** when tryptophan and IPTG are present in the medium (increase [▲], decrease [▼] or no change [—]):

a) λ*c*I ▲, *trp*R ▲, *lac*I ▲, *gfp* ▲
b) λ*c*I ▼, *trp*R ▼, *lac*I ▼, *gfp* ▼
c) λ*c*I ▲, *trp*R ▼, *lac*I ▼, *gfp* ▼
d) λ*c*I ▲, *trp*R ▼, *lac*I —, *gfp* ▼
e) None of the above

25) A riboswitch is :

Answer: E

a) A regulatory protein that can bind a small metabolite
b) A DNA sequence that can bind a small metabolite and regulate gene expression.
c) A DNA sequence that can bind a protein.
d) An RNA sequence that can bind a protein.
e) None of the above.

Student Name:_____Student Number:

Student Name:_____Student Number:

1. Explain the mechanism used by the cell to globally reduce protein synthesis. Give the name and role of key protein(s) involved, the step of the reaction of protein synthesis targeted and complete mechanism.
Dr. Laneuville (10 points)

Key protein involved is eIF-2 (eukaryotic initiation factor 2) (2 marks)
eIF-2 is involved at the step of initiation of protein synthesis (1 mark) and catalyzes the binding of the initiation Met-tRNA onto the small ribosomal subunit (1 mark).

eIF-2 must be bound to GTP in order to be active (1 mark)
exchange of GDP for GTP on eIF-2 is catalyzed by eIF-2B (1 mark)

to reduce protein synthesis globally, the eIF-2 kinase will phosphorylate the eIF-2-GDP complex (1 mark) and the phosphorylated eIF-2-GDP complex will bind to eIF-2B (1 mark) but no GDP to GTP exchange occurs (1 mark) and eIf-2B is not available to regenerate the active eIF-2 (1 mark).

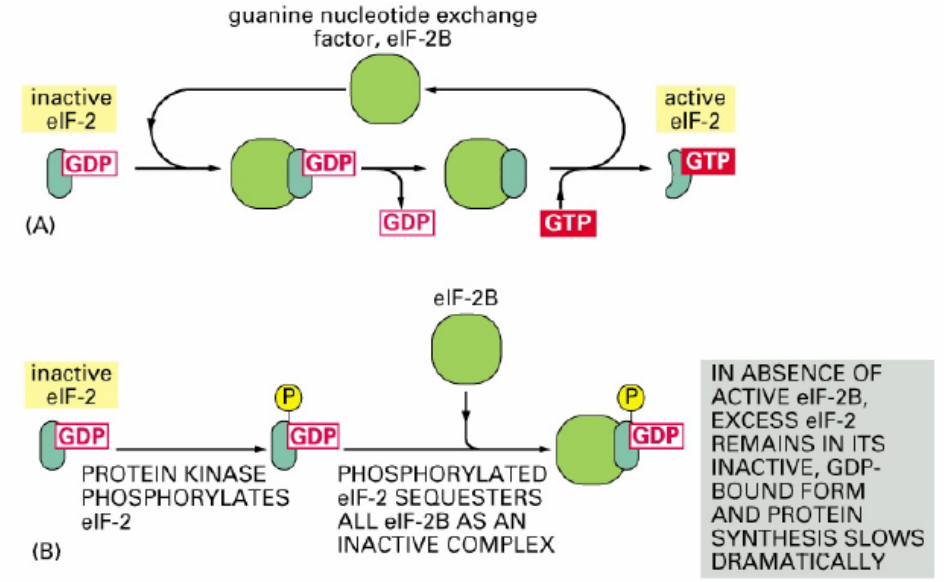


Figure 7–101. Molecular Biology of the Cell, 4th Edition.

2. You are provided with an unusual helix-turn-helix regulatory DNA binding protein. The protein has three alpha helical domains of which one contains a leucine zipper. At the N-terminus, the leucine zipper is found. The C-terminal region of the protein is rich in arginines. The alpha helical region in the mid-section of the protein has no significant features.

A. **Draw the detailed structure of the HTH protein and identify all component motifs, helices, domains and ends. (2.5 marks)**
B. **What function can you ascribe to each of the 3 helical regions? Why? (2.5 marks)**
C. **What would happen to the DNA binding properties of the HTH protein if the C-terminal helix was to be truncated? Why? (2.5 marks)**
D. **Can you explain how DNA binding protein such as this one can establish sequence-specific contacts with double-stranded DNA without disrupting the hydrogen bonds between base-pairs? (2.5 marks)**

A. From N-terminus, draw an alpha helix with leucine every 8 to to residues. Then a turn and comes the second alpha helix. After the second alpha helix come a turn and finally the alpha helix number 3 at the C-terminus.

1 mark for indicating C-terminal and N-terminal ends.
0.5 marks for drawing the 3 helices in the right order from N-terminus to C-terminus.
0.5 mark for indicating two turns
0.5 mark for adding leucine residues every 8-10 amino acids in helix #1 at N-terminus

B. First, at N-terminus, helix #1 is involved in protein interaction such dimerization through the leucine zipper. (1 mark)
Second, at the C-terminus, helix #3 is involved in DNA binding and specifically recognizes a response element; a specific sequence. (1 mark)
Third, helix #2 together with helix #3 form a classic helix-turn-helix architecture important for DNA binding. (0.5 mark)

C. Truncated protein would likely lose its DNA binding capacity (1 mark). Truncated protein could dimerize with a regulatory DNA binding protein containing 3 helices (0.5 mark) and preventing the last one to be active due to the lost of DNA binding activity. (1 mark)

D. Arginine residues in helix 3 at the C-terminus form hydrogen bonds (1 mark) with guanine residues of the DNA double helix (1 mark). Keto group on C6 and N7 of guanine could form hydrogen bonds with ariginine residues of DNA binding protein. (0.5 mark)

Student Name:_____Student Number:

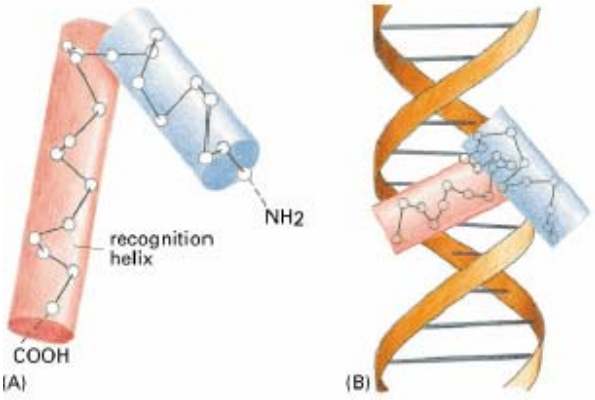


Figure 7–13. Molecular Biology of the Cell, 4th Edition.

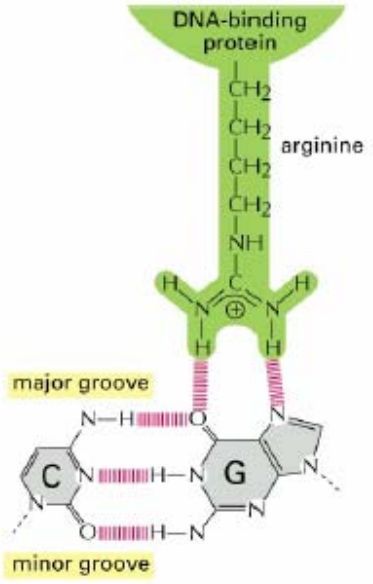


Figure 7–27. Molecular Biology of the Cell, 4th Edition.

3. Name 4 possible mechanisms used by regulatory DNA binding proteins to reduce transcriptional activity of a gene in order to regulate gene expression. (4 marks)

Four of these five possibilities:

1. **Competition between activators and repressors for overlapping DNA binding sites.**
2. **Activator and repressor bind to DNA and their protein binding sites interact to block the protein binding site of the activator.**
3. **repressor interacts with the assembling of the nucleation complex necessary for the transcriptional reaction.**
4. **repressor recruits remodeling complex to favor the formation of nucleosomes.**
5. **repressor attracts a histone deacetylase to favor the formation of nucleosomes.**

Student Name:_____Student Number:

4. (Dr. Stintzi 2 marks) You are interested in using DNA microarrays to study bacterial gene expression in response to iron restriction. To conduct this experiment, you collect mRNA from bacteria grown in iron rich and iron restricted medium. You reverse transcribe the RNAs and you label the cDNA originating from the bacteria grown in the iron-restricted medium with the red fluorescent tag and the cDNA originating from the bacteria grown in iron rich medium with the green fluorescent tag. You mix the two labeled cDNAs and hybridize them on a microarray.
Explain what each of the different colored spots on the microarray tells you about relative gene expression levels:

Red:
Yellow:
Green:

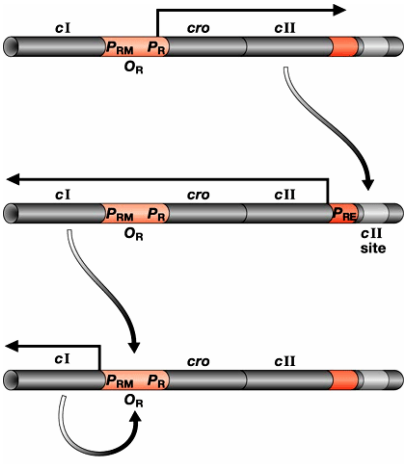
Answer:
Red: the gene expression is up-regulated in response to iron restriction (or down-regulated by the presence of iron in the growth medium as compare to iron-restricted medium) – 0.5 point
Yellow: the gene is equally expressed under iron-restricted and iron rich conditions. - 1 point.
Green: the gene expression is down-regulated in response to iron restriction (or up-regulated by the presence of iron in the growth medium as compare to iron-restricted medium) – 0.5 point.

5) Explain what happens (**from the start up to recovery**) when *E. coli* is put in a medium with a very low amount of amino acids (i.e. stringent response) (10 marks).

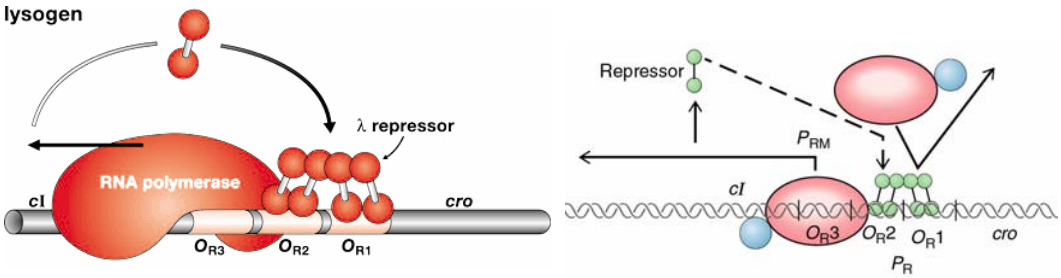
1. **uncharged tRNA are found in site A of ribosome (2 marks)**
2. **RelA releases uncharged tRNA from site A (2 marks)**
3. **ppGpp and/or pppGpp is produced during this release (2 marks)**
4. **ppGpp is a broad spectrum inhibitor (2 marks)**
    - **Transcription initiation for rRNA operons is inhibited at promoter level**
    - **Transcription elongation of most genes is reduced by inducing RNAP pausing**
5. **Recovery : degradation of (p)ppGpp by *Spo*T (2 marks)**

Student Name:_____Student Number:

6) Describe the steps leading to the formation of the lysogen state (prophage) after infection of a bacterial cell by the lambda phage (10 marks).



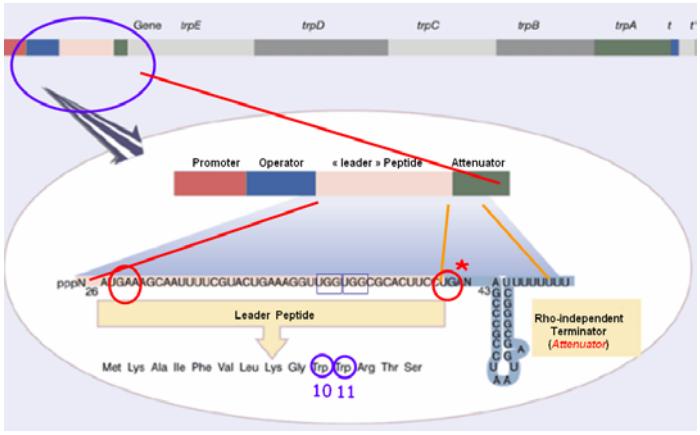
1. **Transcription is initiated at the *cro* promoter (1 mark)**
2. ***cro* and *c*II are initially synthesized (2 marks)**
3. ***c*II is an activator of P_{RE} (i.e. a promoter going in the reverse direction) (1 mark)**
4. **Transcription from P_{RE} produces *c*I (1 mark)**

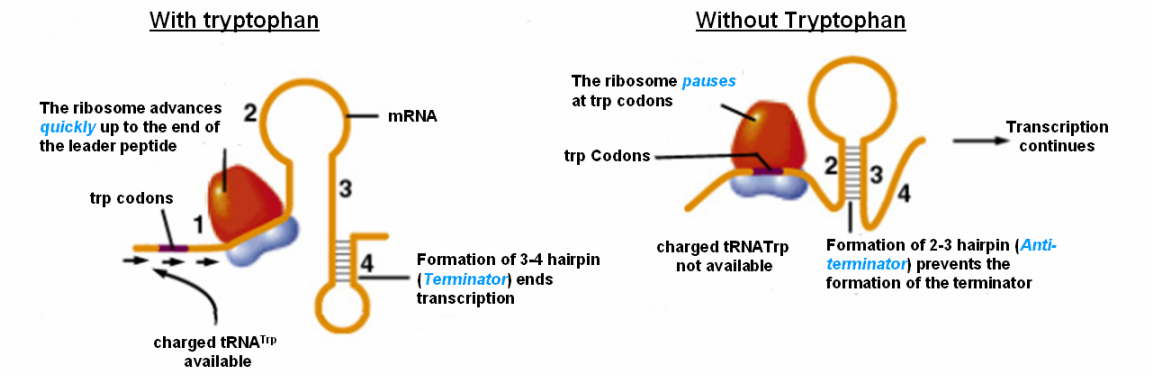


5. ***c*I is a repressor protein (1 mark), which activates its own synthesis (1 mark) and turns off the synthesis of the Cro protein (1 mark) by binding to 2 operators (i.e. OR1 and OR2) (1 mark)**
6. ***c*I can then initiate and maintain its transcription (1 mark)**

Student Name:_____Student Number:

7) It was observed that in the absence of the *trp* repressor, the synthesis of the *trp* operon mRNA is still partially blocked by tryptophan. Describe the mechanism used to control this operon in the absence of the *trp* repressor (4 marks).





1. **A small coding sequence upstream of the trp operon contains 2 trp codons (1 mark)**
2. **When cellular tryptophan is limiting, the ribosome *pauses* at these trp codons (1 mark)**
3. **The capacity of ribosome to read those codons regulates the choice between stem-loops (*terminator* (1 mark) or *antiterminator* (1 mark))**