0628

STUDENT NAME: (last name, first name) STUDENT NO.

McGill UNIVERSITY

BIOCHEMISTRY 507-312B, MACROMOLECULES

FINAL EXAMINATION

VERSION NO. 1

PLEASE MARK YOUR STUDENT NO. AND VERSION NO.
ON YOUR COMPUTER SHEET,
NOW.

Examiner: Dr. A. Nepveu

DATE: Tuesday, April 17, 2001
9:00 a.m.- 12:00 p.m.

Associate Examiners: Dr. M. Parniak
Dr. N. Sonenberg
Dr. M. Park
Dr. G. Shore
Dr. A. Herscovics

This examination consists of 43 questions and a total of 11 pages including the cover page: 40 multiple choice questions worth 2.25 points each (90/40), 2 essay questions worth 2.5 points each and 1 question worth 5 points.. Answer all multiple choice questions, questions 1 to 40, by blackening the corresponding number on the answer card. Your line must connect the brackets on either side of the number. If you change your mind, erase the mark completely, and then enter your new choice. Answer questions 41, 42 and 43 on page 11 and make sure to write your student number on page 11.

NOTE THE FOLLOWING INSTRUCTIONS:

1. Write your name, student # and version # on the computer sheet, now.
2. Calculators, notes, dictionaries and textbooks are not allowed.
3. Do not fold or bend the answer card.. Blacken one space and only one for each question. If more than one space is blackened, the machine will reject your card.
4. Answer all questions, guessing if necessary. No marks lost for wrong answers.
5. If you find it necessary to challenge a question, indicate the question challenged on the front page. Put your comments in the margin adjacent to the question.
6. All examination papers must be returned with your name and student number on the front page and last page.

WARNING

The Examination Security Monitor Program detects pairs of students with unusually similar answer patterns on multiple-choice examinations. Data generated by this program can be used as admissible evidence, either to initiate or corroborate an investigation or a charge of under the Code of Student Conduct and Disciplinary Procedures.

DO NOT TURN THIS PAGE UNTIL YOU ARE TOLD TO DO SO

STUDENT NO.

Section I - M. Parniak, N. Sonenberg and A. Nepveu
Decide which is the best answer and blacken the corresponding brackets.

1. Consider the following statements: (i) pyrophosphate is an important substrate in purine biosynthesis; (ii) the enzyme thymidylate synthase converts deoxyuridine diphosphate (dUDP) to thymidine diphosphate (TDP); (iii) the enzyme ribonucleotide reductase converts nucleoside triphosphates (NTPs) to the corresponding deoxynucleoside triphosphates. Which of the following best describes these statements:
   a. only statements (i) and (iii) are correct
   b. only statements (ii) and (iii) are correct
   c. only statements (i) and (ii) are correct
   d. all statements are correct
   e. none of the statements is correct

2. Methotrexate is folate analog that is commonly used in the treatment of cancer. This compound functions by:
   a. preventing the binding of 5,10-methylenetetrahydrofolate to the enzyme thymidylate synthase
   b. binding to the enzyme dihydrofolate reductase and preventing the reduction of dihydrofolate to tetrahydrofolate
   c. interfering with the ribonucleotide reductase catalyzed conversion of ribonucleotides to deoxyribonucleotides, thereby preventing DNA synthesis (and cell proliferation)
   d. reducing the intracellular pools of ribonucleoside triphosphates, thereby interfering with messenger RNA synthesis

3. Which of the following statements is false?
   a. deoxyribonucleotides are formed by the reduction of the corresponding ribonucleoside diphosphate
   b. cytosine, adenine and guanine are bases found in RNA
   c. cytosine, adenine and guanine are bases found in DNA
   d. thymidylate kinase catalyzes the conversion of TMP to TDP
   e. none of the above
   f. all of the above

4. In the presence of ATP, which of the following will be substrates for ribonucleotide reductase
   a. CDP and UDP
   b. CDP and GDP
   c. GDP only
   d. ADP only
   e. All ribonucleotides are used equally

5. Which of the following is correct in terms of oxidation states of tetrahydrofolate (THF)?
   a. N^5-methyl THF < N^5,N^{10}-methenyl THF < N^{10}-formyl THF
   b. N^5-formyl THF = N^{10}-formyl THF < N^5,N^{10}-methylene THF
   c. Dihydrofolate < tetrahydrofolate = N^5,N^{10}-methylene THF
   d. N^5-methyl THF < N^5,N^{10}-methylene THF < N^{10}-formyl THF
   e. All forms of THF have the same oxidation state

6. The *Drosophila* nanos protein regulates the translation of hunchback mRNA by

   (A) Binding to the 3'UTR of hunchback mRNA.
   (B) Binding the cap structure of hunchback mRNA.
   (C) Suppression of translation through binding to the 5'UTR of hunchback mRNA.
   (D) Stimulation of translation through binding to he 5'UTR of hunchback mRNA.
   (E) Correct localization of hunchback mRNA.

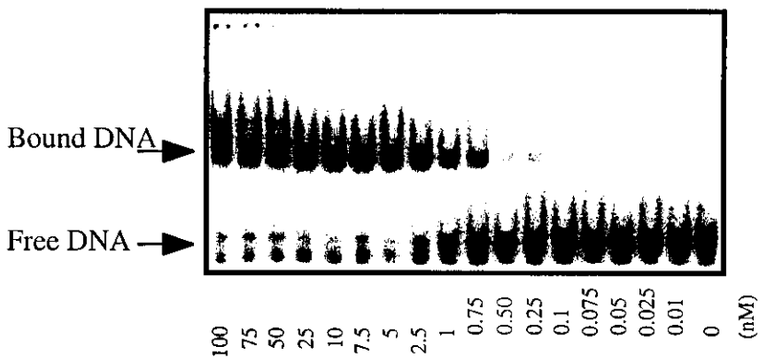
STUDENT NO.

Section I - continued
Decide which is the best answer and blacken the corresponding brackets.

7. Which of following statements is incorrect: Translation initiation on cricket paralysis virus mRNA occurs:

(A) Without Met-tRNA.
(B) Without eIF2
(C) In the absence of AUG
(D) In the absence of 40S ribosomes
(E) Without eIF4E

8. To estimate the DNA binding affinity of a transcription factor to its consensus binding site, EMSA was performed using a fixed amount of DNA (0.1 nM) and a wide range of protein concentrations. What is your estimation of the dissociation constant?



(A) 0.01 nM.
(B) 0.1 nM.
(C) 0.5 nM.
(D) 1 nM.
(E) 10 nM.

STUDENT NO. _____

Section II - N. Sonenberg and A. Nepveu

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

9. Growth factors can stimulate translation initiation by

(A) phosphorylation of 4E-BP1 -(PHAS-I).
(B) Enhancing capping.
(C) Activation of signalling pathways.
(D) Acetylation.

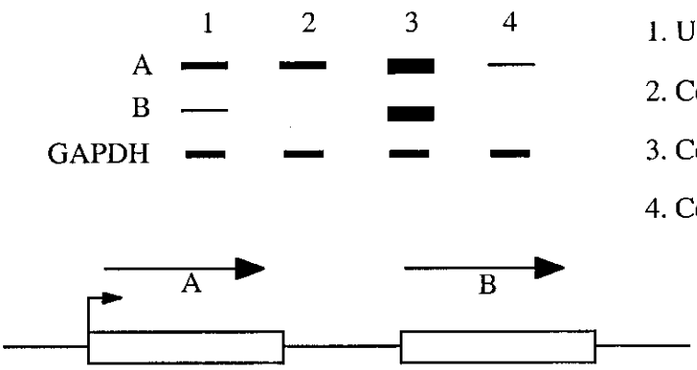
10. IRES function of poliovirus (a picornavirus) absolutely requires:

(A) eIF4E.
(B) cap structure.
(C) Poly A binding protein.
(D) eIF2

11. GCN2 is a:

(A) eIF2 kinase.
(B) phylogenetically conserved (conserved through evolution).
(C) stimulates translation of yeast GCN4 mRNA.
(D) binds to deacylated tRNA.

12. Interpretation of a nuclear run-on transcription assay. Indicate which conclusions can be drawn from the results of this experiment.



1. Untreated Cells
2. Cells treated with drug X
3. Cells treated with drug Y
4. Cells treated with drug Z

(A) The GAPDH control serves to establish that there is an equal level of radiolabeling in each sample.
(B) Drug X causes a complete block to elongation of transcription.
(C) Drug Z reduces transcription initiation.
(D) Drug Y stimulates transcription initiation but not transcription elongation.

STUDENT NO. _____

SECTION II - Continued

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

13. Attenuation within the tryptophan operon *in vivo* could be affected one way or another by the following manipulations. Assuming that tryptophan is present in low amount whereas all other amino acids are present in high amounts in the bacteria, indicate which of the following manipulations will cause an increase in readthrough transcription (less attenuation).

(A) deletion of the entire leader region between the transcription start site and the trpE gene
(B) replacement of all the tryptophan codons with alanine codons in the leader coding region.
(C) deletion of the sequence "1" in the leader region.
(D) introducing mutations that decreases the stability of the 3:4 hairpin.

14. Regarding rho-dependent termination:

(A) rho is incapable of unwinding the RNA.
(B) the polarity of some nonsense mutations may be linked to their effect on rho-dependent termination.
(C) rho binds to RNA that contain a rho consensus binding site.
(D) rho functions as a hexamer.

15. Regarding the process of antitermination in bacteria,

(A) antitermination during late transcription of bacteriophage lambda requires the action of the bacterial proteins NusA, NusG, NusB and S10.
(B) antitermination during delayed early transcription of bacteriophage lambda requires the action of the viral protein, N.
(C) antitermination functions better on rho-independent than rho-dependent termination sites.
(D) the NusA protein functions alternatively as a termination or an antitermination factor.

16. In the case of imprinted genes,

(A) the pattern of methylation may change in the germ cells.
(B) the maternally inherited allele is never expressed.
(C) maintenance of the imprint pattern in somatic cells requires the action of the cytosine methyl-transferase (CMT).
(D) deletion of the silent allele results in the homozygous null phenotype.

17. Regarding DNA methylation,

(A) DNA methylation was proposed to play an important role in the control of gene expression in *Drosophila melanogaster.*
(B) The incorporation of 5-aza-2'-deoxycytidine (5azaC) in the DNA may ultimately increase the mutation rate.
(C) The promoter region of most house-keeping genes is highly methylated.
(D) 5-aza-2'-deoxycytidine (5azaC) can be incorporated in the DNA but cannot be methylated.

STUDENT NO. _____

SECTION II - Continued

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

18. Regarding DNA methylation,
(A) In the "min" mice system, inhibition of DNA methylation led to a reduction in tumor formation.
(B) In tumor cells, certain CpG-rich islands can be hypermethylated even when the overall rate of methylation is decreased.
(C) DNA methylation can be used as a marker to determine whether the BRCA1 gene is inactivated in breast tumors.
(D) The observed rate of point mutations in mammalian cells is equal for the A, C, G and T nucleotides.

19. Regarding DNA methylation,

(A) Repetitive DNA sequences are generally unmethylated in the adult mouse.
(B) The level of methylation in the genome of mammals changes drastically before and after the time of implantation.
(C) Cells deficient for the DNA methyltransferase 1 exhibit a lower rate of mutations.
(D) DNA methylation has an impact on the rate of recombination.

20. Transcription in eukaryotes: indicate which of the following statements are correct.

(A) TFIIB can bind to RNA polymerase II and to TFIIE.
(B) TFIIF can affect transcription initiation and elongation.
(C) The Gal4 activation domain can recruit both TFIIB and TFIIH.
(D) The interaction between TFIIB and RNAPII is critical in determining the position of the transcription start site.

21. Transcription in eukaryotes: indicate which of the following statements are correct.

(A) TFIIH is essential for promoter clearance.
(B) One of the kinases that phosphorylate RNA polymerase II is a cyclin-dependent kinase.
(C) The helicases present in the TFIIH complex can also play a role in DNA repair.
(D) The function of TFIIE is modulated by TFIIH.

22. Transcription in eukaryotes: indicate which of the following statements are correct.

(A) The Gal4 activation domain functions when fused to any DNA binding domain.
(B) The reporter plasmid must encode for a protein whose concentration or activity can be easily measured.
(C) In the yeast two-hybrid system, the activation domain and the DNA binding domain are present in separate proteins.
(D) A transcriptional repressor must necessarily contain a DNA binding domain.

23. Transcription in eukaryotes: indicate which of the following statements are correct.

(A) Repression of a methylated gene may involve the recruitment of a histone deacetylase.
(B) DNA methylation is required for efficient mating-type switching in yeast.
(C) DNA methylation may cause the stable repression of a gene.
(D) The presence of methylated DNA sequences within a gene invariably correlates with lack of expression.

STUDENT NO. _____

SECTION II - Continued

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

24. Regarding histone acetylation:

(A) Although histone acetyl-transferases are found in organisms as distantly related as yeast and man, they exhibit sequence conservation within their catalytic domains.
(B) Histone acetyl-transferases act only on lysine residues.
(C) Histone acetyl-transferases are believed to mediate global as well as local chromatin alterations.
(D) Histone acetylation increases sensitivity to DNase 1.

25. Nuclear receptors.

(A) The ligand-binding domain can bind alternatively to a co-repressor or a co-activator complex.
(B) Co-repressor complexes prefer to interact with a ligand binding domain that is bound to its ligand.
(C) Nuclear receptors bind to DNA as homo- or hetero-dimers.
(D) The ligand is usually a hydrophilic molecule.

26. Transcriptional repressors.

(A) Quenching occurs when a repressor counteracts the action of an activator that is bound far away from it.
(B) Active repression requires that the repressor protein contains both a DNA binding domain and an active repression domain.
(C) All transcriptional repressors bind to DNA.
(D) Active repression requires that the repressor binds to DNA and interacts with at least one protein.

27. Silencing

(A) Position effect variegation refers to the fact that a given gene is expressed in some cells but not in others within the same tissue.
(B) Specifically modified histones H3 and H4 are found at silenced loci.
(C) Genes that are located closed to a telomere may be subject to position effect variegation.
(D) Telomeric silencing involves the association between the Rap1 protein and Sir3 or Sir4 proteins.

28. Chromatin structure and gene regulation.

(A) The extent to which transcription can be activated by a transcriptional activator *in vitro* can approach the level of activation observed *in vivo* provided that chromatin DNA is used as a template for transcription.
(B) In the context of chromatin, antirepression refers to the process by which the chromatin DNA template is made accessible to transcription factors.
(C) The method that measures the sensitivity of a given locus to DNase 1 has revealed a link between gene expression and the state of the chromatin.
(D) The DNase sensitivity of the globin locus is constant among various cell types.

STUDENT NO.

SECTION II - Continued

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

29. Acetyltransferases and chromatin remodeling complexes.

(A) All chromatin remodeling complexes contain an ATPase activity.
(B) The process of transcription elongation results in the progressive acetylation of histones within a gene.
(C) Histones are acetylated within their amino-terminal tails.
(D) Acetylation of histones results in the loss of a negative charge.

30. Chromatin structure and gene regulation.

(A) Inclusion of a locus control region within a transgene ensures that the transgene will be expressed independently of its site of integration.
(B) In transgenic mice, the locus control region confers tissue-specific gene expression and copy number dependent gene expression.
(C) A functional globin locus control region induces DNA replication of the globin gene cluster early during S phase in erythroid cells.
(D) At the molecular level, the consequence of the Hispanic deletion is to remove most of the globin locus control region.

31. More complicated types of genetic switches can be constructed by combining positive and negative controls. Indicate which of the following statements are correct:

(A) Operator-constitutive mutations are cis-dominant.
(B) The catabolite activator protein (CAP) enables bacteria to use alternative carbon sources in the presence of glucose.
(C) A mutation within the coding sequence for the lactose repressor may be trans-dominant.
(D) Following addition of lactose to the medium, the lactose repressor protein binds to the operator of the lactose operon.

32. Regarding co-activators

(A) The "Mediator" is a co-activator complex that is required to support activated transcription *in vitro*.
(B) Some mutations within SRB genes can suppress the phenotype caused by the truncation of the RNA pol B carboxy-terminal domain.
(C) Co-activator complexes include SWI/SNF, SAGA, SRB/MED and the TAFs.
(D) Co-activator complexes exhibit specific DNA binding activity.

STUDENT NO.

SECTION III - M. Park and A. Hescovics

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the corresponding brackets.

33. From the 3 D structure of a serine/threonine kinase (A kinase) and a tyrosine kinase (Insulin receptor). Which of the following are true.

(A) The 3D structure of a serine/threonine kinase is similar to that of a tyrosine kinase.
(B) In the inactive conformation the kinases are bound to AMP
(C) Activation of the kinase does not require a conformational change
(D) Activation of the kinase is stabilised by phosphorylation of a Tyrosine, Serine or Threonine residue that lies in the activation loop.

1. A, B and C
2. C and D
3. A
4. A and D
5. A, B and D

34. You label your protein in vivo with radioactive phosphate [^{32} P] and you believe that your protein is phosphorylated on **only** serine or threonine residues. How would you prove this and identify which serine or threonine is phosphorylated (you know the sequence of your protein and you have more than one serine or threonine in your protein).

(A) Subject your protein to partial alkali hydrolysis and separate amino acids by chromatography.
(B) Subject your protein to high temperatures and separate amino acids by chromatography
(C) Subject your protein to acid hydrolysis and separate amino acids by chromatography.
(D) Cleave your protein with a protease, separate peptides and sequence radioactive peptides.
(E) Separate proteins by SDS polyacrylamide gel electrophoresis and subject to acid treatment

1. A and D
2. A, B and E
3. C and D
4. B and D
5. A, D and E

35. Which of the following are true for CRE binding protein (CREB).

(A) Phosphorylation of CREB on its DNA binding domain is required for recruitment of a histone deacetylase, to the complex.
(B) Phosphorylation of CREB causes it to translocate from the cytoplasm to the nucleus.
(C) Phosphorylation of CREB stabilizes its association with DNA
(D) Phosphorylation of CREB on its DNA binding domain stimulates transcription

1. A and D
2. B and D
3. A and C
4. C and D
5. none of the above

STUDENT NO.

SECTION III - continued

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the corresponding brackets.

36. Targeting of lysosomal enzymes to the lysosomes is mediated by:

(A) protein phosphorylation.
(B) P-type lectins.
(C) sialic acid on oligosaccharides.
(D) mannose 6-phosphate on $Man_8GlcNAc_2$.

1. A
2. B
3. B and C
4. D
5. B and D

37. Glycoprotein folding is facilitated by:

(A) glycosyltransferases in the Golgi.
(B) binding to calreticulin.
(C) interaction with C-type lectins.
(D) the presence of O-linked oligosaccharides .

1. A
2. B
3. B and C
4. D
5. B and D

38. Indicate which form of mannose can act as a direct donor in glycosylation:

(A) UDP-mannose.
(B) mannose-1-phosphate.
(C) GDP-mannose.
(D) Dolichol phosphate mannose.

1. A
2. B
3. C
4. D
5. C and D

39. Glycosaminoglycans have the following structural characteristics

(A) they contain sialic acid.
(B) they always contain sulfate.
(C) they always contain N-acetylhexosamines.
(D) when attached to protein, the glycopeptide linkage region is variable.

1. A
2. B
3. C
4. D
5. C and D

STUDENT NO.

SECTION III - continued

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the corresponding brackets.

40. Lipid intermediates containing dolichol are required

(A) for O-linked oligosaccharide synthesis.
(B) for transfer of oligosaccharide to nascent polypeptide chains.
(C) for all glycosylation reactions occurring in the endoplasmic reticulum.
(D) for the synthesis of the GPI anchors in the endoplasmic reticulum.

1. A
2. B
3. B and C
4. D
5. B and D

SECTION IV - G. Shore

Give a clear and concise answer to each question.

Questions 41 and 42
You have identified a new resident protein of the Golgi membrane, GP24, which has a single predicted transmembrane segment.

41. You determined that the COOH terminus of the protein faces the cytoplasm. Describe 2 experiments that you conducted to arrive at this conclusion. **(2.5 pts.)**

42. Describe experiments to identify the Golgi retention signal in the GP24 polypeptide. **(2.5 pts.)**

43. Name 8 genes in which mutations might be expected to be found in cancer cells. **(5 pts.)**