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(Family Name, First Name) (Student No.)

FACULTY OF SCIENCE

FINAL EXAMINATION

BIOCHEMISTRY 507-454A

NUCLEIC ACIDS

Version 1

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

Examiners: Dr. G. Shore (Coordinator)
Dr. J. Pelletier
Dr. M. Tremblay
Dr. N. Sonenberg
Dr. M. Park
Dr. C. Stanners

Monday, December 20th, 1999

14:00 hrs. - 17:00 hrs.

Answer all multiple-choice questions by blackening the corresponding number on the answer sheet. 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.

NOTE THE FOLLOWING FURTHER INSTRUCTIONS:

(l) Do not fold or bend the answer sheets.

(2) Blacken one space and only one for each question. If more than one space is blackened, the machine will reject your sheet.

(3) 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.

(4) The mark values are given beside each question.

(5) This exam comprises 14 pages (does not include cover page, just the number of pages with questions).

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

Biochemistry 507-454A (Dr. Sonenberg=s Section)

SECTION I

Select the one which is best in each case, and mark the right answer on the computer score sheet.

1. DEAD box proteins function in splicing of pre-mRNAs possibly:
(1 pt)

a) as kinases that phosphorylate splicing factors
b) as part of snRNPs (U1,U2...)
c) as helicases that destabilize secondary structure
d) by dephosphorylating splicing factors
e) by recognizing the 5N cap structure

2. Self-splicing of tetrahymena rRNA depends upon:
(1 pt)

a) a lariat intermediate
b) an exogenous guanosine
c) hydrolysis of ATP
d) hydrolysis of GTP
e) conserved consensus sequences

3. Nuclear tRNA splicing
(1 pt)

a) requires spliceosome formation
b) involves an ATP-dependent endonuclease
c) occurs in trans
d) depends upon lariat formation
5) requires the cap structure

4. 2,2,7-trimethylguanosine is found at the 5' end of
(1 pt)

a) eukaryotic mRNAs
b) U1 snRNA
c) U3 snRNA
d) 5S RNA
e) all small nuclear RNAs

5. U2-U6 pairing is postulated to be involved in
(1 pt)

a) formation of the catalytic center
b) recognition of the 3' splice site
1) modification of RNA structure
d) dissociation of U2-U4 pairing
5) juxtaposition of the donor and acceptor splice sites

6. Thalassemia is caused (in some cases) by inappropriate splicing of
(1 pt)

a) U2 snRNA
b) plasminogen mRNA
3) immunoglobulin mRNA

4) globin mRNA
5) thalassemin mRNA

Biochemistry 507-454A

SECTION I- CONT.

Select the one which is best in each case, and mark the right answer on the computer score sheet.

7. Amylase mRNA is spliced differentially
(1 pt)

1) during development
2) in a tissue specific manner
3) in a species specific manner
4) to produce different proteins
5) in a sex-dependent manner

Biochemistry 507-454A

SECTION II

For each of the statements below one or more are correct. Decide which answer(s) or completion(s) are correct and mark the correct answer on the computer score sheet:

1) if only a, b and c are correct
1) if only a and c are correct
2) if only b and d are correct
3) if only d is correct
4) if all are correct

8. Splicing of most nuclear pre-mRNAs requires:
(1 pt)
a) GU and AG at the 5N and 3N ends of introns
b) ATP
c) U4 and U6
d) A polypyrimidine track upstream of the 3' splice site

9. U2 snRNP
(1 pt)
a) interacts with U6
b) recognizes the branch site
c) contains one RNA and several proteins
d) is inactive when bound to U4

10. SR proteins
(1 pt)
a) are involved in mitochondrial splicing
b) are components of the small nuclear RNPs
3) bind the 2,2,7-trimethylguanosine cap
4) contain serine/arginine-rich domains

11. Several forms of differential splicing have been reported. These include:
(1 pt)
a) optional exons
b) optional introns
c) mutually exclusive exons
d) internal splice sites

12. The branch site sequence is
(1 pt)
a) required in all splicing systems
b) 100% conserved throughout evolution
c) critical for tRNA splicing
d) functions in the formation of the lariat structure

SECTION II - CONTINUED

For each of the statements below one or more are correct. Decide which answer(s) or completion(s) are correct and mark the correct answer on the computer score sheet:

1. if only a, b and c are correct
2. if only a and c are correct
3. if only b and d are correct
4. if only d is correct
5. if all are correct

13. The U12 splicing system of some nuclear pre-mRNAs requires:
(1 pt)

1) AU and AC at the 5' and 3' splice junctions
b) U4 ATAC and U6 ATAC snRNAs
c) GTP
4) U11 RNP

14. U3 and U1 RNAs have what in common?
(1 pt)

1) they have no secondary structure
b) they are components of small nuclear RNPs
c) they participate in splicing
4) they have nuclear functions

Biochemistry 507-454A (Dr. Park=s section)

SECTION III

Each multiple choice question is 2.0 points.

15. Which statement is incorrect.

a) The concept that there are genes capable of causing cancer was based largely on studies carried out with transplantable tumors in animals.
b) Retrovirally transduced oncogenes differ from their mammalian counterpart in that they lack the genomic structure found with most functional mammalian genes.
c) Proto-oncogenes are highly conserved in evolution and their products are important regulators of cell growth and differentiation.
d) The causative agent in the majority of human tumors was not found to be a retrovirus.
e) None of the above.

16. Which statement is incorrect

Activation of oncogenes by retroviruses-

a) Can occur by retroviral transduction of the cellular proto-oncogene.
b) Can occur by integration of the retroviral provirus upstream or downstream from the cellular proto-oncogene
c) Always requires point mutations in the proto-oncogene.
d) Results in inappropriate expression of the proto-oncogene product.
e) None of the above.

17. Which combination of statement(s) regarding the mechanism of activation of proto-oncogenes are incorrect.

1. Chromosome translocations always result in the fusion of proto-oncogenes with proteins that alter the activity of the proto-oncogene product.
2. Increasing oncogene dosage through overexpression is effective only when accompanied by mutations in the proto-oncogene

3. Chemical carcinogenes can result in specific nucleotide base substitutions resulting in specific amino acid alterations.
4. Chemical carcinogens only activate Ras oncogenes.
5. Oncogenic alterations in proto-oncogene products act in a dominant manner.

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

Biochemistry 507-454A

SECTION III CONT.

18. In a single tumor there are frequently multiple genetic changes that involve activation of oncogenes and inactivation of tumor suppressor genes. Which of the following statements about tumor suppressor genes are incorrect.

a) Tumor suppressor genes were initially identified at the genomic level as a loss of heterozygosity in tumor DNA when compared with normal DNA from the same patient.
b) Tumor suppressor genes have been cloned by a process of reverse genetics.
c) Tumor suppressor genes when altered act in a recessive manner.
d) Tumor suppressor genes are not altered in sporadic cancers.
e) None of the above.

19. Which combinations of statements are incorrect about the Rb tumor suppressor gene.

1. Rb is only altered in retinoblastoma tumors
2. Phosphorylated Rb forms complexes with E2F type transcription factors.
3. Mutations in Rb in human tumors result in the constitutive association of Rb with E2F type transcription factors.
4. Mutations in Rb in human tumors involve deletion or mutation of the E2F binding domains.

a) 1 and 2
b) 2 and 3
c) 2, 3 and 4
d) 1,2, and 3
e) 1 and 4

Biochemistry 507-454A (Dr. Shore=s Section)

SECTION IV

Mark the <u>best</u> answer as correct (2.0 points each)

20. Which of the following might conceivably be regulated by transcription?

    a) Fas ligand
    b) Bax
    c) caspase-9
    4) a and b

21. Bcl-2 is transcriptionally up-regulated in certain cancers, including follicular B cell lymphoma. The mechanism involves

    1) induced translocation of a transcriptional activator into the nucleus
    2) cleavage and activation of a transcriptional activator by caspase-3
    3) cleavage and inactivation of a transcriptional repressor by caspase-3
    4) loss of functional p53
    5) chromosomal translocation

22. In cell lines derived from a FADD-minus mouse, the cells are resistant to killing by Fas ligand, but are sensitive to killing by the oncogene, QRT. From this, it can be concluded

    1) QRT does not function by regulating Fas ligand
    2) QRT does not activate caspase-8
    3) QRT activates caspase-8 by a FADD- independent mechanism
    4) QRT activates a close relative of caspase-8, caspase-10
    5) none of the above can be concluded

23. The transcriptional factor, NF-?B, is activated by certain death signals, such as TNF, during tumor surveillance, leading to inappropriate cell survival in certain contexts

1) This makes NF-?B an ideal target for an anti-cancer drug because the drug will re-instate apoptosis..
2) NF-?B is an unlikely target for cancer therapy because it regulates other essential cell functions.
3) NF-?B requires p53 for regulation of cell survival, but p53 function is lost in many types of cancer.
4) NF-?B promotes cell survival, but it also can be proapoptotic in neuronal cells under certain conditions. Thus, a drug against NF-?B may work in cancer treatment but the patient would be brain dead
5) none of the above statements are correct

24. Fas ligand is often a more potent inducer of apoptosis than TNF. Why?

1) Fas ligand does not influence NF-?B
2) Fas ligand triggers a more potent caspase cascade than does TNF.
3) Fas ligand triggers dephosphorylation of the NF-?B/I?B complex
4) Fas ligand triggers phosphorylation of the NF-?B/I?B complex
5) Fas ligand activates RIP

Biochemistry 507-454A

SECTION IV-CONT.

Mark the <u>best</u> answer as correct (2.0 points each)

25. The role of the scaffold protein, IKK? in the IKK complex is to

1) recruit IKK_a and $IKK_ß$
2) phosphorylate I?B
3) link IKK_a and $IKK_ß$ to upstream kinases
4) inhibit NF-?B binding to IKK
5) inhibit NF-?B interaction with I?B

26. A new therapeutic molecule has been designed that prevents NF-?B from protecting cells agains Atricept@, an anticancer drug that induces apoptosis. Which of the following mechanisms of action for this new therapeutic molecule would explain its effect.

1) it inhibits I?B kinase (IKK)
2) it activates NF-?B - inducing kinase (NIK)
3) it prevents interaction between NF-?B and I?B
4) it blocks NF-?B phosphatase
5) none of the above explain the mechanism of action of the therapeutic molecule

27. In childhood acute lymphocytic leukemia (ALL), a specific chromosomal DNA translocation event results in the production of a fusion protein between E2A and HLF. The consequences of this is (are)

1) The protein fusion changes the DNA recognition properties of HLF

2) the protein fusion changes the transactivation properties of HLF
3) the protein fusion creates an activator of apoptosis
4) a and c are correct
5) b and c are correct

28. The ability of p53 to respond to genotoxic stress is influenced by

1) phosphorylation of p53
b) dephosphorylation of p53
3) enhanced binding of Mdm-2 to p53
d) a and c are correct
5) b and c are correct

29. When wild type p53 is introduced into the human colorectal cancer cell line, DLD-1, the cells quickly die by apoptosis. Several gene products are induced, as determined by SAGE. These include

1) caspases
2) Bax
3) mitochondrial permeability transition pore
4) oxidoreductase enzymes
5) all of the above

Biochemistry 507-454A (Dr. Tremblay=s Section)

SECTION V

NAME_____STUDENT NO._____

1. Mouse genetics is an important aspect of medical research. Please answer the following two questions.
(10 points)

1) Identify three stages where mouse embryos can be manipulated. For each of these stages describe in two to three phrases the techniques employed at these stages.

B) Identify at least three conditions required in order to construct an optimal gene targeting vector.

Biochemistry 507-454A (Dr. Stanner=s Section)

SECTION VI
NAME:_____STUDENT NO._____

2. **(6 points)** You have cloned a gene from human cells that you believe is required for normal cellular proliferation. How could you prove directly that the gene possesses this function?

3. **(5 points)** Could a cosmid be used as an expression vector for cDNAs obtained from animal cells? Justify your answer.

Biochemistry 507-454A (Dr. Stanner=s Section)

SECTION VI CONT.
NAME:_____STUDENT NO._____

4. **(6 points)** How long would an oligonucleotide have to be to be unique, i.e., not occur randomly, in a virus genome consisting of 50 genes coding for proteins of an average length of 500 amino acids each? Similarly, how long (i.e., how many amino acids) would a peptide have to be to be unique in the total amino acid sequence coded for by the virus?

5. **(12 points)** (a) You discover an extended human family in the jungles of the West Island in which all members over several generations contract lung cancer by the age of 40. Assuming that mutation in one gene is the determining factor in the development of this cancer, give one method for cloning the gene in question.

(More space on next page)

(b) give a feasible molecular function for the gene
(c) give a feasible description of the nature of the mutations in the gene.

Biochemistry 507-454A (Dr. Stanner=s Section)

SECTION VI CONT.
NAME:_____STUDENT NO._____

6. **(7 points)** Applying radioactive mRNA from a patient=s breast tumour to an array of random genomic DNA clones on a microchip yields a different pattern of hot spots than application to the same chip of mRNA from normal breast cells and different patterns for breast tumours from each patient. How would you interpret these results and use them to identify important genes for the development of breast cancer?

Biochemistry 507-454A (Dr. Pelletier=s Section)

SECTION VI I
NAME:_____STUDENT NO._____

7.