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BIOLOGY 401 **EXAM 1** **Summer 1999**

----Write your name on each answer sheet.
----Read the questions carefully.
----Your exam will be graded for accuracy, logic and organization. 3 points will be reserved for judging general writing skills and legibility. Diagrams will not be graded.

PART A. (20 points) Choose one.

Consider the underlined statements, then decide the best experimental evidence that would prove the statement is true. You may describe experiments that have actually been done or your own experiment. Compose an essay that discusses parts a) through d) in order. Try to stay within the sentence limit for each section.

(a) What particular process and/or specific molecule must be assayed? (1 sentence)
(b) Describe an experiment that would allow you to best accomplish part (a). Be sure to present the logic of the approach. Assume that you have clones for the genes and antibodies for the proteins of interest. (3-5 sentences)
(c) What are the necessary controls for your experiment? (2 sentences)
(d) What specific results do you expect to obtain? (2 sentences)

Question A-1. Our study of early frog development has suggested that the Wnt gene is activated in the dorsal cells of the Organizer region but not in ventral cells on the opposite side of the embryo. Describe an experiment to show that changes in chromatin structure occur within the promoter region of the Wnt gene before transcription starts.

Question A-2. The even-skipped (eve) gene of Drosophila is a member of the pair-rule class of segmentation genes. The eve protein is expressed in seven stripes across the embryo with each stripe under the control of a unique set of regulatory proteins. The eve stripe 3 module contains multiple regulatory elements within the transcription control region.

Question A-3. In cells transformed by the v-src oncogene, B-catenin becomes phosphorylated at tyrosine residues in the carboxy-terminal region (downstream of the armadillo repeat region). Cadherins are expressed in these cells but are non-functional. Phosphorylation of B-catenin maybe relevant to alterations in cell adhesion and you attempt to isolate the novel kinase responsible for this modification. This protien:protein interaction is very stable.

PART B. SHORT ANSWER (20 points)

1) (a) 5'----------(A T G)----------3'} Gene +3
(b) 3'----------(T A C)----------5'

(c) 5'----------(A U G)----------3'} mRNA

A) Fill in the blank. Which species of nucleic acid (a,b,c) is the coding strand of the DNA a ; the antisense strand of the DNA c ; and the template for the mRNA b .
B) Fill in the above diagram. Complete the nucleotide sequence of the gene according to the sequence given in the mRNA.

2) In general, when a message is not alternatively spliced, RNA polymerase II transcription is temporally coupled to splicing. Why is this functionally significant?

It makes for a more efficient process, that splicing can occur during transcription not just afterwards. +1

3) Define morphogen and explain why the Bicoid protein fits the definition of a morphogen? +2

morphogen is a transcription factor distributed throughout an early embryo through a gradient. Bicoid is transcription factor that is found most abundantly in the central area of an embryo but distributed by a decreasing gradient to the dorsal 6

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4) It is estimated that the human genome contains sufficient DNA to code for approximately 50,000 different proteins. But it is also estimated that human lymphocytes can produce more than 100,000 different proteins. Explain this discrepancy.

lymphocytes also interact with the viruses and bacterium and can produce proteins found in them

5) With respect to gene regulation, what do you think might happen if the gene encoding.....
A) A helix-loop-helix transcription factor underwent a mutation that caused an arginine to proline change in the DNA binding domain?

+1/5 This transcription factor most likely could not do its job of gene regulation properly, because it would no longer be able to bind to DNA.

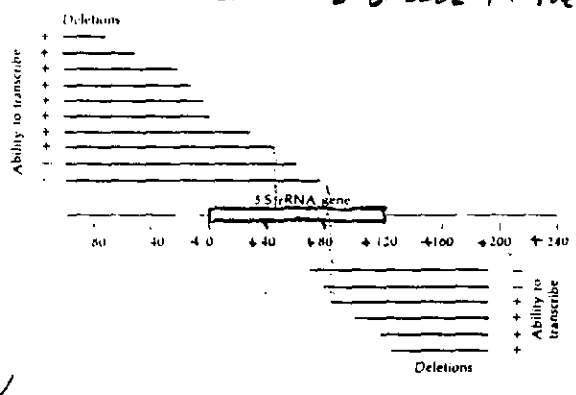
B) A leucine zipper transcription factor underwent a mutation that caused a leucine to arginine change in the dimerization domain?

+1/5 It could still bind to DNA, but possibly would have trouble interacting with other proteins (transcription factors).

6) Your research has uncovered a frog mutant that produces embryos which by the 2-cell stage have translated all of their available XTcf-3 (do not consider Lef1 in this question). Furthermore, you have discovered that this mutant XTcf-3, once localized, can not shuttle out of the nucleus. Nevertheless, independent CAT assays suggest that the mutant XTcf-3 can still bind B-catenin and activate transcription. You inject an mRNA that codes for a B-catenin mutant (shown below) at the 4-cell stage. Do you expect to see a duplicated axis in these embryos? Explain why or why not?

NH_2-pro-pro-lys-lys-lys-arg-lys-val-B-catenin-COOH No. I do not expect to see a duplicated axis, while XTcf-3 is not able to leave the Nucleus, B-catenin is not able to enter the nucleus unless it binds to XTcf-3 in the cytoplasm. Since XTcf-3 is stuck in the Nucleus, B-catenin will remain in the cytoplasm.

7)



Analysis of the effects of various deletions upon the transcription of the 5S-rRNA gene are shown above. Deletion mutants that are transcribed are marked with a (+). Based on these data where is the putative control region that regulates transcription of the 5S-rRNA gene. Explain your reasoning.

the control region is not in the regions deleted. The regions deleted that showed affect of no transcription were deletions in the gene that would not allow for proper translation.

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8) How do gradients establish polarity during early *Drosophila* development?

By the different levels of the products of morphogens and how areas of the cell interpret this by combinatorial control

9) 5'------- 7-methyl-GAUGAACUGUUAGAAUAAAGCUAGCCUGAAAAAAAAAAAAAAA -------3'

Write out the sequence for the coding strand of the DNA using the above message.

~~CTACTTGTCAATCTTATTTCGATCGGGACTTTTTTTTTTTTTTT~~

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10). Specific comments/ Suggestions for improvements in the course. (1 point)

More specific examples of short answer questions on exam

PART C. TRUE/FALSE. If false correct the statement to be true. (6 points)

T Engrailed (en) is a member of the pair-rule gene class in Drosophila.

F The starting material for a cDNA library is ~~Genomic DNA~~ mRNA

T GAP genes are active after cellularization takes place in the embryo.

F Injection of ~~Zest-white 3 (Zw-3)~~ Lef-1 will induce axis duplication in frogs.

F Single copy genes (encoding mRNA) ~~tend to~~ occur ~~in clusters~~ once on the chromosome.

F Homeodomain proteins contain a zinc-finger motif that contacts the ~~major~~ minor grove through an α-helix.

+.5

6.5

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A-1 chromatin from the organizer of Xenopus embryos will be treated to a limited digestion by DNase 1 and the DNA of actively transcribed genes will be the DNA digested. Chromatin is taken from the dorsal cells of the organizer and also from ventral cells of the Xenopus embryo. Dorsal cells from the organizer and ventral cells will be subjected to a limited DNase 1 digestion and then a high salt concentration will be added to remove proteins (Histones) associated to the DNA. The DNA of these cells will further be treated to denature them and thus single stranded. Radioactively labeled cDNA probes for Wnt will be added. Dorsal cells of the organizer and ventral cells that have not been subjected to DNase digestion will also be tested to see if they hybridize the radioactively labeled cDNA. The predicted results is that the DNA of the cells from the Dorsal side of the organizer after limited DNase 1 digestion will not be able to hybridize with the Wnt cDNA, but ventral cells subjected to digestion will and also the Dorsal and ventral cells not subjected to digestion. DNA active in transcription is unwound from the histones and thus sensitive to DNase 1. The Wnt gene is actively transcribed in Dorsal cells of the organizer, thus the DNA for the Wnt Gene is digested and the cDNA probe not able to bind. Changes of chromatin structure around the promoter of the Wnt dod occur.

Good!

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