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*In compliance with the Dartmouth honor principle, all the work you hand in on this exam is to be your own. Please remember to be **precise** in your wording – scientific descriptions rely on accurate use of specific terms. Also, try to keep your answers **concise**. If you can say something briefly there is no need to create a lengthy answer just to fill up space. Use the space provided and the point values for each question as indicators of the amount of detail your answer should contain. If you really need extra space for an answer, turn the page over and continue your answer on the back of the same piece of paper. To facilitate grading, we separate the exam by page; if your answer is on a different sheet of paper we will not see it. **Please put your name on each page now.***

*If something is not clear to you, please ask me during the exam. That is why I stay in the room. Good luck. - Prof. Gross*

1. In both prokaryotes and eukaryotes we have discussed the need for coordinated gene regulation - turning genes on and off together.

a) Explain why this is an important capability for living systems to have. **2 points**

*Gene products often act together to carry out their jobs (e.g. ribosomes, subunits in DNA polymerases, enzymes in a metabolic pathway, etc.). It is more efficient to be able to control these coordinated gene activities as a group to ensure that they are all synthesized at the same level.*

b) How are genes coordinately regulated in prokaryotes? **2 points**

*Prokaryotic genes are typically coordinately regulated through operons. The gene products are encoded on a polycistronic RNA that is responsible for making all the related products.*

c) How are genes coordinately regulated in eukaryotes? **2 points**

*Coordinately regulated eukaryotic genes share common transcription factor binding motifs. Thus, if a transcription factor is active in a cell it will simultaneously turn on all of these genes.*

2. In prokaryotic DNA, it is typical to have an organization of negatively controlled operons as promoter-operator-gene. Sometimes the promoter and operator can overlap. However, one never finds the order to be operator-promoter-gene. Explain why this last organization is not found. **4 points**

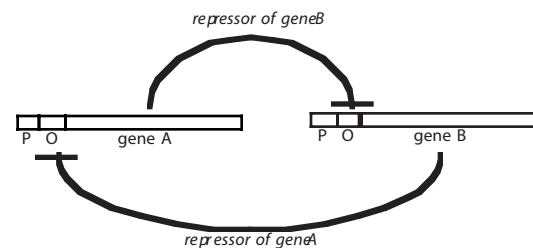
*When a repressor binds to an operator region it functions by preventing an RNA polymerase from transcribing the gene it is serving. If the operator is upstream of the promoter, the RNA polymerase will still be able to bind and start transcription even if there is a repressor bound to the operator site.*

3. In prokaryotes, positive control is mediated by activators, while negative control is mediated by repressors. The Lac operon uses both kinds of control. Explain how each kind of control works in the Lac operon and how this control allows the cell to respond specifically to different conditions. You can use a labelled diagram. **6 points**

*negative control: Lac repressor binds to DNA to prevent transcription. If lactose is present it binds to the repressor and causes it to not bind to the operator regions, thus allowing the synthesis of lactose metabolizing gene products. Therefore, the cell will only make lactose metabolizing enzymes when there is lactose present.*

*positive control: when the activator protein is complexed with cAMP (special small molecule in the book) it binds to an activator site near the lac promoter to stimulate transcription. If cAMP is not available, the activator protein cannot bind to the activation site, thereby only allowing a trickle of transcription to occur. The level of cAMP is inversely related to the level of glucose available (more glucose, less cAMP). This allows the cell to utilize its preferred carbon source, glucose, if both lactose and glucose are available.*

4. Consider the situation in the figure. There are two genes present that each synthesize a repressor to inhibit the other gene.
- a. Describe the state of balance of these two gene products in the cell. Is this a stable situation? **4 points**



*Each gene will be transcribed at approximately the same rate since each gene synthesizes an inhibitor of the other. This situation is not very stable since any small change in the rate of synthesis of either gene A or gene B product will dramatically affect the balance.*

- b. The host cell comes in contact with a toxin that interacts with the product of gene A to render it unable to interact with the operator for gene B. Describe how this affects the state of balance of the gene products A and B. (**2 points**) Explain if this will be a stable state even if the toxin is removed. (**2 points**)

*If gene B is not repressed it will make lots of product, which in turn will repress gene A. Since gene A cannot make any more repressor for gene B, gene B will be on while gene A will be off. This is a stable situation since small changes in expression of gene A or gene B will not change the general system behavior.*

5. Explain how T4 phage makes the switches from early to middle to late gene expression. You may use a labelled diagram. **4 points**

*Early genes are transcribed using a promoter that is recognized by the bacterial holoenzyme (RNA polymerase core + host sigma factor). One of the products of the early genes is a middle specific sigma factor; while another product of the early genes is a protein that interferes with the host sigma factor. This serves to stop initiation at the early promoter (no host sigma is available) and start initiation at the middle promoter (core + middle sigma). Switching from middle to late works in a similar manner. One product of the middle genes is a late specific sigma; another product blocks the middle sigma. This causes a switch to late genes.*

6. RNA polymerase II does not recognize a specific termination site at which it will stop transcribing the DNA. How does it lead to the production of a specific sized RNA product? **3 points**

*RNA polymerase II reads past the end of the coding region and keeps on going. One of the sequences it transcribes serves as a site for an enzyme that recognizes a site on the growing RNA and cuts the RNA at that specific location. This produces a specific sized RNA product. (ie - RNA site specific cleavage produces a specific RNA)*

7. Describe three steps in the processing of pre-mRNAs to mRNAs in the nucleus of eukaryotic cells. **6 points**

*capping at 5 end: puts a specific structure at this location*

*splicing: removes introns and joins exons together*

*polyadenylation at 3 end: puts a string of As at the 3 end*

8. How do transcription factors so precisely regulate the expression of different genes in different cells under different conditions? **5 points**

*Different genes have specific collections of TF binding sites by which they are regulated. The level of activation of a gene will depend on which TFs are present in a cell (and on their concentrations) and on which specific sequences reside in front of a gene. If the gene has sites for binding many of the present TFs it will be transcribed at a high level. Fewer binding sites and TFs will lead to a lower level of transcription. As the conditions in (and around) a cell change, levels of different TFs change. This allows for very fine level of control of transcription for each gene.*

9. What are enhancers and how are they thought to work? **4 points**

*An enhancer is a region of DNA that contains binding sites for a number of transcription factors. An enhancer is distinct from a promoter in that it can reside in almost any position with respect to a gene (up to 20kb away) and can be in either orientation. It can affect multiple genes that are nearby.*

10. How was the ability to splice RNA (the appearance of introns) thought to have provided an evolutionary advantage to primitive eukaryotic organisms? **5 points**

*Primitive organisms are thought to have had small genes, each with their own promoter. The termination of transcriptions was not that efficient. Sometimes transcripts started in one gene and ran into the next. If a cell developed a way to splice out the intervening RNA, it could create proteins with new capabilities combining the functionality of the two original genes. The new capability could provide the cell with an advantage. This would all be possible without deleting or rearranging any DNA - meaning that if it did not work out to be an advantage, there was no permanent loss to the cell. On the other hand, rearranging DNA to create the new protein would be an irreversible process since the cell would have lost some critical DNA.*

11. How is it possible for some intron mutations to alter the protein product of a gene? **2 points**

*A mutation in an intron can alter the way an RNA is spliced (e.g. not removing an intron) which can cause the production of a different protein product.*

12. What are pseudogenes? **2 points**

*A pseudogene is a remnant of what used to be a functional gene. Typically, it is the result of a mutation, deletion or insertion that either changed the protein function or changed the promoter of the gene.*

13. The globin gene family contains a number of genes that are very similar. The subtle differences between family members are thought to have coincided with the development of placental mammals. Explain how this works. **4 points**

*In mammals, the embryo needs to get its oxygen from the mother's bloodstream. This means that the embryonic hemoglobin must have a higher affinity for oxygen than the mother's hemoglobin. Some of the globin genes (e.g. delta and zeta) fulfill this role and provide the ability for an embryo to develop inside the protective environment of the mother. All non-mammalian species have their young develop outside the mother, where they are not as protected.*

14. Explain how PCR has led to a major improvement in forensic capability. **4 points**

*PCR provides the ability to amplify any DNA sequence - making available a lot of material to work with compared to the scarce amount of starting material. At crime scenes, this is particularly important because evidence is hard to come by. PCR can amplify trace amounts of DNA to aid in identification.*

15. Explain how DNA microarray technology is leading to the development of individualized treatments for different kinds of cancers. **5 points**

*This technology is capable of examining the gene expression profiles for any cell. Each type of cancer has its own unique pattern of gene expressions. By knowing the exact type of cancer from the expression profile in the tumor cell, it is possible to choose the most effective treatment.*

16. What is meant by the term "cDNA library?" **3 points**

*A cDNA library is a collection of cloned DNAs representing the mRNA population of a specific cell type. The library is usually in a plasmid vector.*

17. Bacterial cells have restriction enzymes yet they do not digest their own DNA. How is this possible? **3 points**

*They also contain a modification enzyme that recognizes the same site as the restriction enzyme. This enzyme modifies the DNA so that it is not cut by the restriction enzyme.*

18. Describe chromosome walking. You may use a labelled diagram. **4 points**

*Chromosome walking involves using a piece of genomic DNA of interest to screen a phage library for adjacent fragments of DNA. This is accomplished by using a fragment of the probe DNA (e.g. the 3' end) to screen the library for overlapping phage clones. These new clones are then used in the same manner to screen the library for additional adjacent fragments of DNA.*

19. You have developed a gene therapy approach to curing cystic fibrosis in mice. You can deliver the "correct" gene into all cells in the mouse, yet you find that the delivery of this gene has no effect on the mouse's symptoms - there is no improvement. Give one reason why this might be the case. **4 points** (continue on back if necessary)

*A number of reasons are possible. Here are some of them: the gene is not transcribed, the bad gene product is still made and interferes with the good gene product, the gene is inserted into a heterochromatic region and is unavailable to be transcribed.*

20. In the last video we saw, PGD was discussed in the case of Molly Nash, a young girl who was dying from a genetic disorder, but could be saved by a bone marrow transplant. There was not compatible donor, however, so the parents wanted to have another child that could serve as a donor for Molly. Is it ethical to select an embryo for implantation based on its ability to serve as a donor to save the life of an older sibling? Please explain your thinking. **9 points** (continue on other side if needed)

21. A theme addressed by a number of our speakers was supporting people with disabilities. Given that the government has a limited budget and must make choices in what programs to support, how should choices be made among education, support for our elderly, support for people with disabilities, social security funding, and support for other endeavors (such as foreign policy and defense programs)? **9 points** (continue on other side if needed)