Chapter 12 Discussion Questions

1. Explain how an activator can recognize and bind to a specific sequence of DNA without breaking the hydrogen bonds that hold double stranded DNA together. What type of molecular non-covalent interactions (hydrogen bonds, ionic bonds, hydrophobic interactions) would allow a protein to distinguish a GC base pair from an AT base pair. Explain your answer using the information in slide 6.

2. Sketch the structure of a zinc finger. What is the role of zinc in this structure? What component of the structure is most responsible for determining the DNA sequence binding specificity of a zinc finger containing activator?

3. Most regulatory proteins (activators or repressors) form dimers before they can bind. What are the advantages of dimerization of these proteins? Why do heterodimers bind different DNA sequences than homodimers? How might dimerization be used to regulate gene expression?

4. Some gene regulatory proteins bind to DNA and cause the DNA to bend at a sharp angle. Some of these protein promote transcription even though they do not contact the RNA Polymerase, GTF or any other regulatory protein. What is a plausible explanation for how these DNA bending proteins influence gene expression?

5. The yeast GAL4 activator binds to UAS_{gal} elements upstream of the genes for galactose metabolism and increasing transcription of these genes. If GAL4 is not expressed in yeast cells, the galactose metabolism genes cannot be expressed. When GAL4 is normally expressed, this can lead to maximum expression of the galactose metabolism genes. However when GAL4 is over expressed, the galactose genes are turned off. How does too much GAL4 squelch expression of these genes? Would you expect over expression of GAL4 to affect genes without the UAS_{gal} element? Explain your answer.

6. Deletion analysis was conducted to determine the DNA binding domain of the yeast repressor alpha 2. The figure below outlines the results of that test. The column on the left list which amino acids were deleted from the protein. The column on the right indicates the ability to bind its DNA target. Based on these results, which amino acids are important for binding? Describe an assay that could have been used to measure binding. Explain how you might use a chimeric protein experiment to determine if the repression domain of this protein is different from the DNA binding domain.



7. Why might complex enhancers be more advantageous than a series of simple enhancers?

8. What is the distinction between an architectural protein and a true activator?

9. Explain how architectural proteins can be involved in gene repression?

10. The figure below included the results of an experiment that demonstrate the acidic activator domain of VP16 interacts directly with TFIID in vitro. Explain how Stringer isolated proteins that interact with VP16's activating domain. How did he demonstrate that TFIID was among these proteins? A 536 nt RNA was detected in lanes a, b and c. Why was this RNA expected to be present in these lanes? Is it significant that more RNA is apparent in lane C than in lane A and B? Why does it make sense that more RNA was detected in lance C than in lane F? How did he determine that TFIID was the only protein to interact with VP16 in his experiment.



11. Classic enhancers typically function in a distance and orientation independent fashion. However elements such as enhancers can mask the distance independent behavior. For example consider the gene. A enhancer was inserted into three separate regions of the gene (at -200, -300 and -400bp) and the levels of transcription were assays (right hand column). How might insulator elements explain these patterns of expression? Does this experiment suggest where the insulator elements might be?



12. Explain how Ptashne altered the structure of the mediator protein GAL11 to demonstrate that the one way that activators promote transcription is to anchor mediator to a gene. Repressors may also anchor mediator to a gene, however their effect is to decrease expression. How does the interaction of repressors and activators with mediator differ?

13. Greene's experiment on activator mechanisms might suggest that the GAL4 activating domain interacts directly with TFIIB. Design an in vitro experiment to test this hypothesis. What results would support the hypothesis and what would refute.