S1.Describe how the tight packing of chromatin in a closed conformation may prevent gene transcription.

Answer: There are several possible ways that the tight packing of chromatin physically inhibits transcription. First, it may prevent transcription factors and/or RNA polymerase from binding to the major groove of the DNA. Second, it may prevent RNA polymerase from forming an open complex, which is necessary to begin transcription. Third, it could prevent looping in the DNA, which may be necessary to activate transcription.

S2.   What are the two alternative ways that IRP can affect gene expression at the RNA level?

Answer: The ferritin mRNA has an IRE in its 5′-UTR. When IRP binds to this IRE, it inhibits the translation of the ferritin mRNA. This decreases the amount of ferritin protein, which is not needed when iron levels are low. However, when iron is abundant in the cytosol, the iron binds directly to IRP and prevents it from binding to the IRE. This allows the ferritin mRNA to be translated, producing more ferritin protein. IREs are also located in the transferrin receptor mRNA in the 3′-UTR. When IRP binds to these IREs, it increases the stability of the mRNA. This leads to an increase in the amount of transferrin receptor mRNA within the cell when the cytosolic levels of iron are very low. Under these conditions, more transferrin receptor is made to promote the uptake of iron, which is in short supply. When the iron is found in abundance within the cytosol, IRP is removed from the mRNA, and the mRNA becomes rapidly degraded. This leads to a decrease in the amount of the transferrin receptor.

S3.   Eukaryotic response elements are often orientation independent and can function in a variety of locations. Explain the meaning of this statement.

Answer: Orientation independence means that the response element can function in the forward or reverse direction. In addition, response elements can function at a variety of locations that may be upstream or downstream from the core promoter. DNA looping is thought to bring the regulatory transcription factors bound at the response elements and the general transcription factors bound at the core promoter in close proximity with one another.

S4.   To gain a molecular understanding of how the glucocorticoid receptor works, geneticists have attempted to "dissect" the protein to identify smaller domains that play specific functional roles. Using recombinant DNA techniques described in chapter 18, particular segments in the coding region of the glucocorticoid receptor gene can be removed. The altered gene can then be expressed in a living cell to see if functional aspects of the receptor have been changed or lost. For example, the removal of the portion of the gene encoding the carboxyl terminal half of the protein causes a loss of glucocorticoid binding. These results indicate that the carboxyl terminal portion contains a domain that functions as a glucocorticoid-binding site. The figure shown here illustrates the locations of several functional domains within the glucocorticoid receptor relative to the entire amino acid sequence.


Based on your understanding of the mechanism of glucocorticoid receptor function described in figure 15.6, explain the functional roles of the different domains in the glucocorticoid receptor.

Answer: The hormone-binding domain is located in the carboxyl terminal half of the protein. This part of the protein also contains the region that is necessary for HSP90 binding and receptor dimerization. A nuclear localization sequence (NLS) is located near the center of the protein. After hormone binding, the NLS is exposed on the surface of the protein and allows it to be targeted to the nucleus. The DNA-binding domain, which contains zinc fingers, is also centrally located in the primary amino acid sequence. Zinc fingers promote DNA binding to the major groove. Finally, two separate regions of the protein, one in the amino terminal half and one in the carboxyl terminal half, are necessary for the transactivation of RNA polymerase. If these domains are removed from the receptor, it can still bind to the DNA but it cannot activate transcription.

S5. A common approach to identify genetic sequences that play a role in the transcriptional regulation of a gene is the strategy that is sometimes called **promoter bashing.** This approach requires gene cloning methods, which are described in chapter 18. A clone is obtained that has the coding region for a structural gene as well as the region that is upstream from the core promoter. This upstream region is likely to contain genetic regulatory elements such as enhancers and silencers. The diagram shown here depicts a cloned DNA region that contains the upstream region, the core promoter, and the coding sequence for a protein that is expressed in human liver cells. The upstream region may be several thousand base pairs in length.

Upstream region Core promoter Coding sequence of liver-specific gene
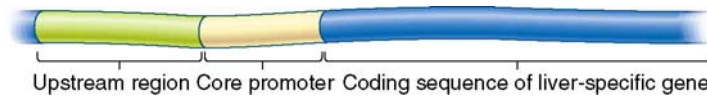
To determine if promoter bashing has an effect on transcription, it is helpful to have an easy way to measure the level of gene expression. One way to accomplish this is to swap the coding sequence of the gene of interest with the coding sequence of another gene. For example, the coding sequence of the *lacZ* gene, which encodes β-galactosidase, is frequently swapped because it is easy to measure the activity of β-galactosidase because there is an assay for its enzymatic activity. The *lacZ* gene is called a "reporter gene" because it is easy to measure its activity. As shown here, the coding sequence of the *lacZ* gene has been swapped with the coding sequence of the liver-specific gene. In this new genetic construct, the expression and transcriptional regulation of the *lacZ* gene is under the control of the core promoter and upstream region of the liver-specific gene.

Upstream region Core promoter *lacZ* gene coding sequence
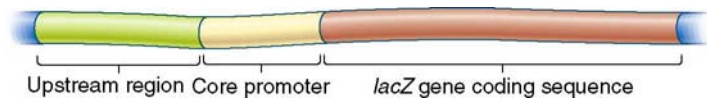
Now comes the "bashing" part of the experiment. Different segments of the upstream region are deleted and then the DNA is transformed into living cells. In this case, the researcher would probably transform the DNA into liver cells, because those are the cells where the gene is normally expressed. The last step is to measure the β-galactosidase activity in the transformed liver cells.

In the diagram shown here, the upstream region and the core promoter have been divided into five regions, labeled A–E. One of these regions was deleted (i.e., bashed out), and then the rest of the DNA segment was transformed into liver cells.

Upstream region Core promoter *lacZ* gene
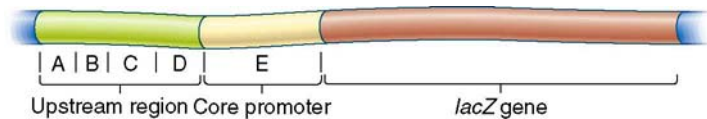
The data shown here are from this experiment.

Region Deleted	Percentage of* β-Galactosidase Activity
None	100
A	100
B	330
C	100
D	5
E	<1

*The amount of β-galactosidase activity in the cells carrying an undeleted upstream and promoter region was assigned a value of 100%. The amounts of activity in the cells carrying a deletion were expressed relative to this 100% value.

Explain what these results mean.

Answer: The amount of *β*-galactosidase activity found in liver cells that do not carry a deletion reflects the amount of expression under normal circumstances. If the core promoter (region E) is deleted, very little expression is observed. This is expected because a core promoter is needed for transcription. If enhancers are deleted, the activity should be less than 100%. It appears that one or more enhancers are found in region D. If a silencer is deleted, the activity should be above 100%. From the data shown it appears that one or more silencers are found in region B. Finally, if a deletion has no effect, there may not be any response elements there. This was observed for regions A and C.

Note: The deletion of an enhancer will have an effect on *β*-galactosidase activity only if the cell is expressing the regulatory transcription factor that binds to the enhancer and transactivates transcription. Likewise, the deletion of a silencer will have an effect only if the cell is expressing the repressor protein that binds to the silencer and inhibits transcription. In the problem described, the liver cells must be expressing the activators and repressors that recognize the response elements found in regions B and D, respectively.