

- E1. A DNase-I sensitive site is one that is very easily cleaved by DNase-I compared to most other sites in the chromosomal DNA. In other words, its conformation is very accessible to cleavage. When a gene becomes transcriptionally active, it will become more susceptible to DNase-I digestion because the DNA in this region has been converted from a closed to an open conformation. This will make it easier for DNase-I to gain access to the DNA and cleave it.
- E2. S1 nuclease cuts single-stranded DNA but not double-stranded DNA. If a gene is in an open conformation and cut by DNase-I, it would not hybridize to the probe that is complementary to it. In this case, the single-stranded probe would be chewed up by the S1 nuclease. In contrast, if the gene is in a closed conformation and resistant to DNase-I, the probe will hybridize to the gene and will not be digested with S1 nuclease. Therefore, the ability of S1 nuclease to digest the probe tells you whether or not the gene is being cleaved by DNase-I.
- The precipitation step is needed to separate the DNA fragments from free nucleotides. If the radioactive probe is bound to a complementary DNA strand, it will be protected from degradation by S1 nuclease; it will be found in the pellet. This occurs when the globin gene is in a closed conformation. In contrast, if the radioactive probe does not bind to a complementary DNA strand, it will be digested into free nucleotides; the free nucleotides will remain in the supernatant. This occurs when the globin gene is in an open conformation.
- E3. Certain individuals had defects in globin gene expression even though the structural gene itself was intact. This led researchers to suspect that other regions nearby were important in controlling the chromosomal conformation in a way that would make the globin genes accessible to RNA polymerase and transcription factors.
- E4. These results indicate that the fibroblasts have a maintenance methylase because they can replicate and methylate DNA if it has already been methylated. However, the cells do not express a *de novo* methylase that recognizes this DNA segment, because the DNA in the daughter cells remains unmethylated if the donor DNA was unmethylated.
- E5. If the DNA band is 3,800 bp, it means that the site is unmethylated because it has been cut by *NorI* (and *NorI* cannot cut methylated DNA). If the DNA fragment is 5,300 bp, the DNA is not cut by *NorI*, so we assume that it is methylated. Now let's begin our interpretation of these data with lane 4. When the gene is isolated from root tissue, the DNA is not methylated because it runs at 3,800 bp. This suggests that gene *T* is expressed in root tissue. In the other samples, the DNA runs at 5,300 bp, indicating that the DNA is methylated. The pattern of methylation seen here is consistent with the known function of gene *T*. We would expect it to be expressed in root cells since it functions in the uptake of phosphate from the soil. It would be silenced in the other parts of the plant via methylation.
- E6. Based on these results, there are enhancers that are located in regions A, D, and E. When these enhancers are deleted, the level of transcription is decreased. There also appears to be a silencer in region B, because a deletion of this region increases the rate of transcription. There do not seem to be any response elements in region C, or at least not any that function in muscle cells.
- E7. A. Based on the transformation of kidney cells, a silencer is in region B. Based on the transformation of pancreatic cells, an enhancer is in region A.
- B. The pancreatic cells must not express the repressor protein that binds to the silencer in region B.
- C. The kidney cells must express a repressor protein that binds to region B and represses transcription. Kidney cells express the downstream gene only if the silencer is removed. As mentioned in part B, this repressor is not expressed in pancreatic cells.
- E8. The results indicate that protein X binds to the DNA fragment and retards its mobility (lanes 3 and 4). However, the hormone is not required for DNA binding. Because we already know that the hormone is needed for transactivation, it must play some other role. Perhaps, the hormone activates a signaling pathway that leads to the phosphorylation of the transcription factor, and phosphorylation is necessary for transactivation. This situation would be similar to the CREB protein, which is activated by phosphorylation. The CREB protein can bind to the DNA whether or not it is phosphorylated.
- E9. The gel shown in (a) is correct for ferritin mRNA regulation. The presence and absence of iron does not affect the amount of mRNA, it affects the ability of the mRNA to be translated. The gel shown in (b) is correct for transferrin receptor mRNA. In the absence of iron, the IRP binds to the IRE in the mRNA and stabilizes it, so the mRNA level is high. In the presence of iron, the iron binds to IRP and IRP is released from the IRE, and the transferrin receptor mRNA is degraded.