- C1. A constitutive gene is unregulated, which means that its expression level is relatively constant. The expression of a regulated gene varies under different conditions. In bacteria, the regulation of genes oftentimes occurs at the level of transcription by combinations of regulatory proteins and small effector molecules. In addition, gene expression can be regulated at the level of translation or the function of a protein can be regulated after translation is completed.
- C2. In bacteria, gene regulation greatly enhances the efficiency of cell growth. It takes a lot of energy to transcribe and translate genes. Therefore, a cell is much more efficient and better at competing in its environment if it expresses genes only when the gene product is needed. For example, a bacterium will express only the genes that are necessary for lactose metabolism when a bacterium is exposed to lactose. When the environment is missing lactose, these genes are turned off. Similarly, when tryptophan levels are high within the cytoplasm, the genes that are required for tryptophan biosynthesis are repressed.
- C3. In this case, an activator protein and inhibitor molecule are involved. The binding of the inhibitor molecule to the activator protein would prevent it from binding to the DNA and thereby inhibit its ability to activate transcription.
- C4. A. Regulatory protein
  - B. Effector molecule
  - C. DNA segment
  - D. Effector molecule
  - E. Regulatory protein
  - F. DNA segment
  - G. Effector molecule
- C5. Choices B and C are correct. In both of these cases, the presence of the small effector molecule will turn off transcription. In contrast, the presence of an inducer turns on transcription.
- C6. A *cis*-mutation is within a genetic regulatory sequence, such as an operator site, that affects the binding of a genetic regulatory protein. A *cis*-mutation affects only the adjacent genes that the genetic regulatory sequence controls. A *trans*-mutation is usually in a gene that encodes a genetic regulatory protein. A *trans*-mutation can be complemented in a merozygote experiment by the introduction of a normal gene that encodes the regulatory protein.
- C7. The term *enzyme adaptation* means that a particular enzyme is made only when a cell is exposed to the substrate for that enzyme. It occurs because the gene that encodes the enzyme that is involved in the metabolism of the substrate is expressed only when the cells have been exposed to the substrate.
- C8. A. No transcription would take place. The lac operon could not be expressed.

B. No regulation would take place. The operon would be continuously turned on.

- C. The rest of the operon would function normally but none of the transacetylase would be made.
- C9. It would be impossible to turn the *lac* operon on even in the presence of lactose because the repressor protein would remain bound to the operator site.
- C10. Diauxic growth refers to the phenomenon whereby a cell first uses up one type of sugar (e.g., glucose) before it begins to metabolize a second sugar (e.g., lactose). In this case, it is caused by gene regulation. When a bacterial cell is exposed to both sugars, the uptake of glucose causes the cAMP levels in the cell to fall. When this occurs, the catabolite activator protein is removed from the *lac* operon so that it is not able to be (maximally) activated.
- C11.A. Cis-effect. It would affect only the genes that are in the adjacent operon.
  - B. Trans-effect. This is a mutation that affects a protein that can move throughout the cell.
  - C. Trans-effect. This is a mutation that affects a protein that can move throughout the cell.
  - D. Cis-effect. It would affect only the genes that are in the adjacent operon.
- C12. A mutation that prevented the *lac* repressor from binding to the operator would make the *lac* operon constitutive only in the absence of glucose. However, this mutation would not be entirely constitutive because transcription would be inhibited in the presence of glucose. The disadvantage of constitutive expression of the *lac* operon is that the bacterial cell would waste a lot of energy transcribing the genes and translating the mRNA when lactose was not present.

- C13. AraC protein binds to the *araI*, *araO*<sub>1</sub>, and *araO*<sub>2</sub> operator sites. The binding of the AraC protein to the *araO*<sub>1</sub> and *araO*<sub>2</sub> sites inhibits the transcription of the *araC* gene. Similarly, the AraC proteins bound at *araO*<sub>2</sub> and *araI* repress the *ara* operon. The AraC proteins at *araO*<sub>2</sub> and *araI* can bind to each other by causing a loop in the DNA. This DNA loop prevents RNA polymerase from transcribing the *ara* operon. In the absence of arabinose, the *ara* operon is turned off. When cells are exposed to arabinose, however, arabinose binds to the AraC protein and breaks the interaction between the AraC proteins at the *araO*<sub>2</sub> and *araI* sites and thereby breaks the DNA loop. In addition, a second AraC protein binds at the *araI* site. This AraC dimer at the *araI* operator site activates transcription.
- C14. A. Without  $araO_2$  the repression of the *ara* operon could not occur. The operon would be constitutively expressed at high levels because AraC protein could still activate transcription of the *ara* operon by binding to *araI*. The presence of arabinose would have no effect. Note: The binding of arabinose to AraC is not needed to form an AraC dimer at *araI*. The dimer is able to form because the loop has been broken. This point may be figured out if you notice that an AraC dimer is bound to  $araO_1$  in the presence and absence of arabinose (see Figure 14.12).
  - B. Without *araO*<sub>1</sub>, the AraC protein would be overexpressed. It would probably require more arabinose to alleviate repression. In addition, activation might be higher because there would be more AraC protein available.
  - C. Without *araI*, transcription of the *ara* operon cannot be activated. You might get a very low level of constitutive transcription.
  - D. Without *araO*<sub>2</sub> the repression of the *ara* operon could not occur. However, without *araI*, transcription of the *ara* operon cannot be activated. You might get a very low level of constitutive transcription.
- C15. Attenuation means that transcription is ended before it has reached the end of an operon. Since it causes an end to transcription, it is a form of transcriptional regulation even though the translation of the *trpL* region plays a key role in the attenuation mechanism.
- C16.A. Attenuation will not occur because loop 2-3 will form.
  - B. Attenuation will occur because 2-3 cannot form, so 3-4 will form.
  - C. Attenuation will not occur because 3-4 cannot form.
  - D. Attenuation will not occur because 3-4 cannot form.
- C17. A defective tryptophanyl-tRNA synthetase would make attenuation less likely. This is because the bacterial cell would have a lower amount of charged tRNA<sup>trp.</sup> Therefore, it would be more likely for the ribosome to stall at the tryptophan codons found within the *trpL* gene, even if the concentration of tryptophan amino acids in the cell was high. When the ribosome stalls at these tryptophan codons, this prevents attenuation.
- C18. The addition of Gs and Cs into the U-rich sequence would prevent attenuation. The U-rich sequence promotes the dissociation of the mRNA from the DNA, when the terminator stem-loop forms. This causes RNA polymerase to dissociate from the DNA and thereby causes transcriptional termination. The UGGUUGUC sequence would probably not dissociate because of the Gs and Cs. Remember that GC base pairs have three hydrogen bonds and are more stable than AU base pairs, which only have two hydrogen bonds.
- C19.If you look very carefully at the RNA sequence in Figure 14.14, you will notice that a UAA codon is found just past region 2. Therefore, in this mutant strain, the UGA stop codon at the end of region 1 could be read by the mutant tRNA<sup>gly</sup> and then the ribosome would stop at the UAA codon that is found just past region 2. If the ribosome paused here, it would probably cover up a portion of region 3, and therefore the terminator 3–4 stem-loop would not form. According to this scenario, attenuation could not occur. However, we should also keep in mind the issue of timing. The ribosome would have to be really close to RNA polymerase to prevent attenuation in this nonsense suppressor strain. It is possible that the 3–4 stem-loop might form before the ribosome reaches the UAA stop codon that is just past region 2. Therefore, attenuation might occur anyway because the 3–4 stem-loop might form before the ribosome reaches the UAA stop codon.
- C20. It takes a lot of cellular energy to translate mRNA into a protein. A cell wastes less energy if it prevents the initiation of translation rather than a later stage such as elongation or termination.
- C21. Antisense RNA is RNA that is complementary to a functional RNA such as mRNA. The binding of antisense RNA to mRNA inhibits translation.

- C22. One mechanism is that histidine could act as corepressor that shuts down the transcription of the histidine synthetase gene. A second mechanism would be that histidine could act as an inhibitor via feedback inhibition. A third possibility is that histidine inhibits the ability of the mRNA encoding histidine synthetase to be translated. Perhaps it induces a gene that encodes an antisense RNA. If the amount of histidine synthetase protein was identical in the presence and absence of extracellular histidine, a feedback inhibition mechanism is favored, because this affects only the activity of the histidine synthetase enzyme, not the amount of the enzyme. The other two mechanisms would diminish the amount of this protein.
- C23.*lac* operon: The binding of allolactose causes a conformational change in the repressor protein and removes it from the operator site.
  - *ara* operon: The binding of arabinose to AraC breaks the looping interaction and leads to the activation of the *ara* operon.
  - *trp* operon: The binding of tryptophan to the *trp* repressor causes it to bind to the operator site and inhibits transcription.
- C24. The two proteins are similar in that both bind to a segment of DNA and repress transcription. They are different in three ways. (1) They recognize different effector molecules (i.e., the *lac* repressor recognizes allolactose and the *trp* repressor recognizes tryptophan. (2) Allolactose causes the *lac* repressor to release from the operator, while tryptophan causes the *trp* repressor to bind to its operator. (3) The sequences of the operator sites that these two proteins recognize are different from each other. Otherwise, the *lac* repressor could bind to the *trp* operator and the *trp* repressor could bind to the *lac* operator.
- C25. A. Antisense RNA or a translational repressor would shut down protein synthesis the fastest. A transcriptional repressor would also shut down the synthesis of mRNA, so it would eventually shut down protein synthesis once all of the preexisting mRNA had been degraded. Feedback inhibition would have no effect on protein synthesis.
  - B. Only a transcriptional repressor protein would shut down the synthesis of mRNA.
  - C. Feedback inhibition is the fastest way to shut down the function of a protein. Antisense RNA and transcriptional repressors eventually prevent protein function once all of the preexisting mRNA and protein have been degraded.
- C26. In the lytic cycle, the virus directs the bacterial cell to make more virus particles until eventually the cell lyses and releases them. In the lysogenic cycle, the viral genome is incorporated into the host cell's genome as a prophage. It remains there in a dormant state until some stimulus causes it to erupt into the lytic cycle.
- C27.A. The lysogenic cycle would occur because cro protein is necessary to initiate the lytic cycle.
  - B. The lytic cycle would occur because cI encodes the  $\lambda$  repressor, which prevents the lytic cycle.
  - C. The lytic cycle would occur because cII protein is necessary to initiate the lysogenic cycle.
  - D. Both cycles could try to initiate but the lysogenic cycle would fail because it would be unable to integrate into the host chromosome.
  - E. Neither cycle could occur.
- C28. The  $O_R$  region contains three operator sites and two promoters.  $P_{RM}$  and  $P_R$  transcribe in opposite directions. The  $\lambda$  repressor will first bind to  $O_{R1}$  and then  $O_{R2}$ . The binding of the  $\lambda$  repressor to  $O_{R1}$  and  $O_{R2}$  inhibits transcription from  $P_R$  and thereby switches off the lytic cycle. Early in the lysogenic cycle, the  $\lambda$  repressor protein concentration may become so high that it will occupy  $O_{R3}$ . Later, when the  $\lambda$  repressor concentration begins to drop, it will first be removed from  $O_{R3}$ . This allows transcription from  $P_{RM}$  and maintains the lysogenic cycle. By comparison, the cro protein has its highest affinity for  $O_{R3}$ , and so it binds there first. This blocks transcription from  $P_{RM}$  and thereby switches off the lysogenic cycle. The cro protein has a similar affinity for  $O_{R2}$  and  $O_{R1}$ , and so it may occupy either of these sites next. It will bind to both  $O_{R2}$  and  $O_{R1}$ . This turns down the expression from  $P_R$ , which is not needed in the later stages of the lytic cycle.
- C29.  $P_{RE}$  is activated by the cII-cIII complex. However, later in the lysogenic cycle the amount of the cII-cIII complex falls. This would prevent further synthesis of the  $\lambda$  repressor. However, the  $\lambda$  repressor can activate its own transcription from  $P_{RM}$ . This will maintain the lysogenic cycle.
- C30. It would first increase the amount of cro protein so that the lytic cycle would be favored.
- C31. A cell that has a  $\lambda$  prophage is making a significant amount of the  $\lambda$  repressor. If another phage infects the cell, the  $\lambda$  repressor inhibits transcription from  $P_R$  and  $P_L$  and thereby inhibits the early steps that are required for either the lytic or lysogenic cycles.

- C32. Neither cycle could be followed. As shown in Figure 14.19, N protein is needed to make a longer transcript from  $P_{\rm L}$  for the lysogenic cycle and also to make a longer transcript from  $P_{\rm R}$  for the lytic cycle.
- C33. A genetic switch is a DNA sequence that governs a choice between two genetic pathways. The segment of DNA usually contains several operator sites that are recognized by two or more transcriptional regulatory proteins. A genetic switch is similar to a simple operator in that the binding of proteins to the DNA is the underlying event that controls transcription. The main difference between a genetic switch and simple operator is the degree of complexity. A simple operator usually controls a single gene or a single operon, while a genetic switch often has multiple operator sites and controls the choice between two (or more) promoters.
- C34. If the  $F^-$  strain is lysogenic for phage  $\lambda$ , the  $\lambda$  repressor is already being made in that cell. If the  $F^-$  strain receives genetic material from an *Hfr* strain, you would not expect it to have an effect on the lysogenic cycle, which is already established in the  $F^-$  cell. However, if the *Hfr* strain is lysogenic for  $\lambda$  and the  $F^-$  strain is not, the *Hfr* strain could transfer the integrated 1 DNA (i.e., the prophage) to the  $F^-$  strain. The cytoplasm of the  $F^-$  strain would not contain any  $\lambda$  repressor. Therefore, this  $\lambda$  DNA could choose between the lytic and lysogenic cycle. If it follows the lytic cycle, the  $F^-$  recipient bacterium will lyse.