

**PART FOUR: GENE REGULATION
ANSWERS**

Answers to questions from Chapter 15 on Positive and negative control of the *lac* operon

15.1 *lacI*, *lacZ*, *lacY*, *lacA*

15.2 The *lac* operon is negatively regulated by a repressor, the product of the *lacI* gene (additional positive aspects of *lac* regulation result from action of cAMP-CAP). The *lac* repressor binds to a specific DNA sequence called the operator (*lacO*) and prevents efficient initiation of transcription by RNA polymerase from the promoter (*lacP*). An inducer (allolactose or an analog) binds to the repressor and prevents its binding to the operator, thereby releasing the repression and allowing transcription of the *lac* operon.

- a) Most mutations in the operator, the binding site for repressor, lead to lower affinity for the repressor and hence less binding. Thus these mutations allow continued transcription (and thus expression) of the *lac* operon even in the absence of inducer; this is referred to as constitutive expression.
- b) Mutations in the repressor that prevent its binding to the operator will lead to constitutive expression (no repression in the absence of inducer). Mutations that prevent binding of the inducer without affecting the ability to bind to the operator lead to a non-inducible phenotype.
- c) The *lac* promoter is not a particularly strong promoter, and mutations have been obtained that either increase or decrease its efficiency of initiating transcription. Base substitutions that make the promoter sequence more similar to the consensus generate a stronger promoter (promoter "up" mutations) whereas those that make the promoter less similar to the consensus generate a weaker promoter (promoter "down" mutations). An "up" mutation would make the *lac* operon no longer dependent on the positive regulation by the cAMP-CAP complex (when the operon is induced). A "down" mutation would not allow expression even in the de-repressed state (presence of inducer) and hence would show a non-inducible phenotype.

- 15.3**
- a) Choice (c) is correct. O^C causes constitutive expression of genes in *cis*, but may not give full expression without an inducer.
 - b) Choice (c) is correct. R^- causes depression, but B^- gives no B activity.
 - c) Choice (b) is correct. O^C causes constitutive expression in *cis*.
 - d) Choice (a) is correct. R^+ is dominant in *trans*.

15.4 The catabolite regulated operons are examples of positive regulation. Glucose is the preferred carbon source for many bacteria, and the operons for metabolism of other sugars, such as lactose and arabinose, are less active when glucose is available (even in the presence of inducer). The [cAMP] is low when glucose is available. As glucose is depleted, the [cAMP] increases, and cAMP forms a complex with the catabolite activator protein (CAP). The

cAMP-CAP complex binds to a specific site near the promoter for the *lac* operon and the *ara* operon, and in both cases cAMP-CAP increases the efficiency of transcription from the promoter under induced conditions.

At least three different explanations can be offered for the observed loss of activity upon purification.

- (i) Using the example of cAMP-CAP, perhaps the fungal RNA polymerase is active on its promoter only in the presence of an activator protein. The efficiency of initiation by the polymerase may be low for the purified polymerase, but high in the presence of the activator.
- (ii) Some critical subunit of the RNA polymerase is not tightly associated with the rest of the polymerase and is dissociated during purification.
- (iii) If the assay for polymerase activity during the purification scheme is specific initiation of transcription from the promoter, then the decline in activity may reflect a loss of specific initiation but not a decline in nonspecific transcription. In this case, a specificity factor, such as the sigma subunit of the *E. coli* RNA polymerase, may be lost at one step of the purification.

15.5	a)	Lyase activity	Arginase activity
	Strain 2	<u>_(A⁻)_constitutive_____</u>	<u>_____constitutive_____</u>
	Strain 3	<u>_(B⁻)_defective_____</u>	<u>_____inducible_____</u>
	Strain 4	<u>_(C⁻)_constitutive_____</u>	<u>_____constitutive_____</u>
	Strain 5	<u>_(D⁻)_inducible_____</u>	<u>_____defective_____</u>
	Strain 6	<u>_(D⁻/B⁻)_inducible_____</u>	<u>_____inducible_____</u>

(Note that D⁻ and B⁻ complement in *trans*.)

- b) *citB* encodes argininosuccinate lyase (or lyase)
citD encodes arginase
- c) Strain 7 is inducible, so *citA*⁺ is dominant to *citA*⁻ in *trans*.
Strain 8 is constitutive, and Strain 9 is constitutive for arginase.
Therefore, *citC*⁻ (the mutant) is dominant to *citC*⁺ (when *citC*⁻ is in *cis* to wild-type structural genes).
- d) *citA* encodes a *trans*-acting regulatory protein, e.g., a repressor.
citC is a *cis*-acting regulatory site, e.g. an operator.

15.6

	ALA synthetase	ALA dehydrase
Strain 2	___constitutive___	___constitutive___
Strain 3	___nonfunctional___	___repressible___
Strain 4	___repressible___	___nonfunctional___
Strain 5	___constitutive___	___constitutive___
Strain 6	___repressible___	___repressible___

b)

porA Strain **7 or 9** is **constitutive for the A⁻, B⁺, C⁺ and D⁺ chromosome**, which shows that **mutant *porA* is dominant to wt, when A⁻ is in cis to B⁺, C⁺.**

porB Strain **6** is **repressible**, which shows that **wild-type *porB* is dominant to mutant (or mutant is recessive to wild-type).**

porC Strain **6 (or 9 or 8)** is **repressible (for ALA dehydrase)**, which shows that **wild-type *porC* is dominant to mutant (or mutant is recessive to wild-type).**

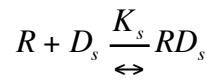
porD Strain **8 (or 9)** is **repressible**, which shows that **mutant *porD* is recessive to wild-type (or wild-type is dominant to mutant).**

For strain 9, note that chromosome A⁺B⁻C⁺D⁻ is repressible when D⁺ is provided on the other chromosomes.

- c) One concludes that *porA* is an operator, *porB* encodes ALA synthetase, *porC* encodes ALA dehydrase, and *porD* encodes an apo-repressor (it forms a repressor when heme binds).
- d) This operon is under negative control, since *porD*⁻ gives full activity of the synthetase and the dehydrase.

Answers to questions from Chapter 16, on Transcriptional regulation by effects on RNA polymerase

16.1



$$K_s = \frac{[RD_s]}{[R][D_s]}$$

$$\frac{[RD_s]}{[D_s]} = K_s [R]$$

$$\text{slope} = K_s = \frac{60}{1 \times 10^{-11} M} = 60 \times 10^{11} M$$

$$K = 6 \times 10^{12} M^{-1}$$

In practice, it is difficult to measure [R], [RDS] and [DS] simultaneously, so a more complicated procedure is used. But this problem illustrates the general idea.

- 16.2.** The [TBP] = 1/Ks when the ratio of bound to free probe = 1. From a plot of the data, one obtains [TBP] = 0.47 x 10⁻⁹ M when bound/free = 1. Thus Ks = 1/ 0.47 x 10⁻⁹ M ,
or Ks = 2 x 10⁹ M⁻¹.

You can also use the slope of this plot to get Ks = 2 x 10⁹ M⁻¹

- 16.3** The *lac* repressor dissociates from the operator and rebinds to nonspecific sites on the DNA.
- 16.4** By binding to an operator centered on the sequence at +11 and holding the complex between RNA polymerase and the promoter in the closed conformation.
- 16.5** . The most accurate value of ΔG for binding of AP1 to this duplex oligonucleotide is -11 kcal/mole. The minimum in error is the maximum in accuracy.
- 16.6** The most accurate measure of the equilibrium constant, Ks, is 1.17 x 10⁸ M⁻¹. Use the equation ΔG = -RT ln Ks.

- 16.7** The value for k_f in either the presence or absence of the repressor is 2 per sec. The graph shows that in both conditions, the y-intercept is 0.50 sec, which is $1/k_f$.
- 16.8** The value for K_B is $1.0 \times 10^8 \text{ M}^{-1}$ without repressor and $1.25 \times 10^7 \text{ M}^{-1}$ with repressor.

The forward rate constant does not change, but binding constant is decreased, consistent with a promoter occlusion model for the repressor. The graph shows an increase in slope with no change in the y-intercept in the presence of the repressor, so this means that the binding constant decreased. Note that d is the only option with reasonable binding constants that showed this decrease. The binding constants were calculated from the slopes of $0.5 \times 10^{-8} \text{ sec M}$ in the absence of repressor, $4.0 \times 10^{-8} \text{ sec M}$ in the presence of repressor, and $k_f = 2 \text{ sec}^{-1}$ (previous problem).

Answers to questions from Chapter 17 on Transcriptional regulation in lambda

- 17.1** Lysogeny results from the integration of a λ prophage under conditions where the expression of all the λ genes except cI are repressed. The λ repressor, or CI protein, will bind to the leftward and rightward operators of λ to prevent transcription from P_L and P_R , hence blocking the expression of the genes required for lytic infection.
- Bacteria that are lysogenic for λ are already producing the CI protein, or repressor. Subsequent infection by another λ phage results in the immediate binding of the λ repressor to the leftward and rightward operators of the incoming phage, thereby preventing transcription of any of its genes, including those required for lytic infection. The newly introduced phage DNA also cannot integrate (no expression of *int* and *xis*) and it is eventually degraded.
- 17.2** Statements 1, 2, and 3 are correct.
- 17.3** The two phage would complement to form turbid plaques. The λ phage that are mutated in the *cII* gene (and are wild-type for the *cI* gene) will be found in the lysogen.
- 17.4** Since the λ repressor is present in 100 times the molar concentration of a single operator (and thus operator binding can use only one of the 100 repressor dimers), the distribution of the repressor to nonspecific sites will predominate in terms of determining the fraction of repressor molecules that are not bound to DNA. Since each base pair in the E. coli genome is the beginning of a nonspecific binding site for λ repressor, there are 4.2×10^6

nonspecific sites per cell. This means that $[D_{\text{tot}}] = [\text{total concentration of nonspecific sites}] = 7 \times 10^{-3} \text{ M}$. This is much greater than the concentration of repressor or Cro, and can be treated as a constant.

From the equation

$$K_{\text{ns,r}} = \frac{[\text{RD}]}{[\text{R}][\text{D}]} = 10^5 \text{ M}^{-1}$$

one can calculate $\frac{[\text{R}]}{[\text{RD}]} = \frac{1}{K_{\text{ns}} \times [\text{D}]} = \frac{1}{(1 \times 10^5 \text{ M}^{-1})(7 \times 10^{-3} \text{ M})}$

$$\frac{[\text{R}]}{[\text{RD}]} = 0.14 \times 10^{-2} = 0.0014$$

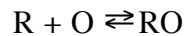
This can be expressed as the fraction of total repressor molecules as follows:

$$[\text{R}] = 0.0014 [\text{RD}]$$

$$\frac{[\text{R}]}{[\text{R}_{\text{tot}}]} = \frac{[\text{R}]}{[\text{R}] + [\text{RD}]} = \frac{0.0014 [\text{RD}]}{(0.0014 + 1)[\text{RD}]} = \frac{0.0014}{1.0014} = 0.0014$$

This means that only 1.4 in 1000 repressor dimers is not bound to DNA at an operator or at a nonspecific site. Even though the affinity of λ repressor for its operator or for nonspecific DNA is less than seen for the *lac* repressor, and the amount of λ repressor is about 10 times higher, the λ repressor is still distributed between specific and nonspecific sites. Very little is not bound to DNA.

17.5. The relevant reaction is



$$K_{\text{s,r}} = \frac{[\text{RO}]}{[\text{R}][\text{O}]} = 10^{11} \text{ M}^{-1}$$

$$K_{\text{ns,r}} = \frac{[\text{RD}]}{[\text{R}][\text{D}]} = 10^5 \text{ M}^{-1}$$

The specificity, which is the ratio of $K_{\text{s,r}}$ to $K_{\text{ns,r}}$ can be used to calculate the ratio of free to bound operator.

$$\text{specificity} = \frac{K_{\text{s}}}{K_{\text{ns}}} = \frac{[\text{RO}]}{[\text{O}]} \times \frac{[\text{D}]}{[\text{RD}]}$$

$$\frac{[\text{O}]}{[\text{RO}]} = \frac{K_{\text{ns}}}{K_{\text{s}}} \times \frac{[\text{D}]}{[\text{RD}]} = \frac{K_{\text{ns}}}{K_{\text{s}}} \times \frac{[\text{D}]}{[\text{R}_{\text{tot}}] - [\text{O}_{\text{tot}}]}$$

since $[RD] = [R_{tot}] - [RO] - [R] = [R_{tot}] - [O_{tot}]$, given that the concentration of free R is negligible (see 4.35) and $[RO] = [O_{tot}]$ when operator sites are saturated.

$$[R_{tot}] = 100 \text{ molecules/cell} = 17 \times 10^{-8} \text{ M}$$

$$[O_{tot}] = 1 \text{ site/cell} = 0.17 \times 10^{-8} \text{ M}$$

Thus:

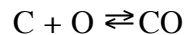
$$\frac{[O]}{[RO]} = \frac{10^5 \text{ M}^{-1}}{10^{11} \text{ M}^{-1}} \times \frac{7 \times 10^{-3} \text{ M}}{17 \times 10^{-8} - 0.17 \times 10^{-8}}$$

$$\frac{[O]}{[RO]} = 0.0416$$

$$\frac{[O]}{[O_{tot}]} = \frac{0.0416}{1.0416} = 0.04$$

Thus 4% of the operator sites are free, and 96% are bound by repressor.

17.6. The relevant equations are



$$K_{s,c} = \frac{[CO]}{[C][O]} = 10^{10} \text{ M}^{-1}$$

$$K_{ns,c} = \frac{[CD]}{[C][D]} = 10^5 \text{ M}^{-1}$$

and the analysis is the same as in 4.19, except that $K_{s,c} = 10^{10} \text{ M}^{-1}$. Using the equation for specificity as in 4.19, one can calculate that

$$\frac{[O]}{[CO]} = 0.416$$

$$\frac{[O]}{[O_{tot}]} = \frac{0.416}{1.416} = 0.29$$

Thus about 30% of the operator sites would not be bound by Cro, considerably more than the 4% of the sites that would not be bound by λ repressor.

17.7. Dividing eqn 2 by eqn 5, you obtain

$$\frac{K_s \text{ for repressor}}{K_s \text{ for Cro}} = \frac{\frac{[RO]}{[R][O]}}{\frac{[CO]}{[C][O]}} = \frac{[RO]}{[CO]} \times \frac{[C]}{[R]}$$

As calculated in 4.18, the concentration of free R and free C is very small, and are equal for equal concentrations of R and C.

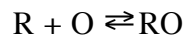
$$[R] = [C] = (0.0014)(17 \times 10^{-8} \text{ M}) = 2.4 \times 10^{-10} \text{ M}$$

Thus the

$$\frac{[RO]}{[CO]} = \frac{10^{11} \text{ M}^{-1}}{10^{10} \text{ M}^{-1}} = 10$$

or there is 10 times more repressor bound to operator than is Cro bound to the operator. The distribution of λ repressor and Cro to nonspecific sites was used in this treatment to calculate the same low concentration for free repressor and Cro (problem 4.35).

17.8



$$K_{s,r} = \frac{[RO]}{[R][O]}$$

$$K_{s,r} = 10^{11} \text{ M}^{-1} \text{ without cooperativity}$$

$$K_{s,r} = 3 \times 10^{12} \text{ M}^{-1} \text{ with cooperativity}$$

$$[R] = \frac{[RO]}{[O]} \times \frac{1}{K_s}$$

$$\text{Without cooperativity, } [R] = \frac{99}{1 \times 10^{11} \text{ M}^{-1}} = 9.9 \times 10^{-10} \text{ M}$$

$$\text{With cooperativity, } [R] = \frac{99}{3 \times 10^{12} \text{ M}^{-1}} = 3.3 \times 10^{-11} \text{ M}$$

Thus it takes 30 times more repressor to fill 99% of the operator sites in the absence of cooperativity. The cooperativity at the operator sites means that less repressor is needed to fill the sites. The sigmoidal shape of the binding curve for cooperative interactions also means that a smaller decrease in [R] will lead to dissociation of repressor from a greater fraction of operators, giving a more dramatic response to a lowering of [R].

Answers to questions from Chapter 18: Regulation after initiation of transcription

18.1 Statements 1, 2, and 4 are correct.

18.2 In lysogeny, transcription of *int* is from the p_{int} promoter, close to the gene. There is no *nut* site in this transcript, hence transcription from p_{int} is not susceptible to N-mediated antitermination. Thus the transcript terminates at t_{int} and the secondary structures that target RNases to a transcript are not formed. Thus the *int* transcript is stable, and Int protein is made during lysogeny.

18.3 See Figures 4.4.8 and 4.4.9 for diagrams of the secondary structures under these conditions.

The ribosome will progress to the *trp* codons in the leader and either stall (low *trp*) or continue to a termination codon (high *trp*). Stalling of the ribosome at *trp* codons (A situation) prevents formation of the termination loop between regions 3 and 4 of the mRNA. Progress to the UGA terminator allows the 3-4 loop to form (B situation) and thus terminate transcription.

18.4 Statements 1, 4, and 5 are correct.

18.5 The *trp* operon is subject to regulation both by repression and by attenuation. Attenuation depends on the tight coupling between transcription and translation in bacteria. When the [Trp] is high, translation of the *trp* leader is completed and the ribosome blocks sequence 2. This allows the transcribed sequences 3 and 4 to form the stem-loop attenuator structure. Formation of the 3:4 loop, which resembles a rho-independent transcription terminator, results in termination of transcription the *trp* operon before the structural genes (EDCBA) are transcribed, and the enzymes for Trp biosynthesis are not produced. When the [Trp] is low, translation of the *trp* leader is stalled at two Trp codons. In this position, the ribosome does not cover sequence 2. Sequence 2 can now base-pair with sequence 3 in an alternative secondary structure. Formation of the 2:3 stem-loop precludes formation of the 3:4 attenuator loop, and transcription proceeds on through the *trp*EDCBA genes. Thus when the bacteria has a low [Trp], the biosynthetic genes are expressed and more Trp is synthesized.

- a) Increasing the distance between sequence 1 (encoding the *trp* leader peptide) and sequence 2 will decrease attenuation under conditions of high [Trp]. In this situation, the ribosome, after completing translation of the leader, will not cover sequence 2. Hence the 2:3 stem-loop can form, preventing formation of the 3:4 stem loop and thereby losing the normal attenuation with a greater distance between sequences 1 and 2.
- b) A large increase in the distance between sequences 2 and 3 could disfavor their formation of a stem-loop and hence formation of the

- 3:4 attenuator structure. Thus when the [Trp] was low, even though the ribosome has stalled, the 2:3 loop would not form, allowing the 3:4 attenuator structure to form with the result of a decrease in trp operon expression (due to attenuation) even in low [Trp].
- c) Since sequence 4 is required to form the 3:4 attenuator stem-loop, in its absence no attenuation would be observed.

Answers to questions from Chapter 19. Regulation of eukaryotic genes

- 19.1** The discrete DNA binding domains of transcriptional regulatory proteins form specific complexes with defined sequences of DNA. Their affinity for these defined sequences is about 10^5 to 10^6 greater than their affinity for other sequences.

Using the example of the *lac* repressor, the binding site (operator) is 22 base pairs (bp) long. Ten molecules of the *lac* repressor are sufficient to keep this operator in a bound state even in the context of 4.6×10^6 bp of nonspecific DNA (the rest of the *E. coli* genome). This amounts to finding 1 specific site in a sea of 4.6×10^6 bp, since you can consider each nucleotide pair to be the beginning of a nonspecific binding site. The human repressor has an even harder job - it must find its specific site within the 3×10^9 nonspecific sites (the haploid genome size). Thus the ratio of nonspecific to specific sites is 652 times greater in the human cell (3×10^9 divided by 4.6×10^6). Extrapolating from the *lac* repressor information, we estimate that 6520 molecules of repressor will be needed per cell (10 molecules per cell x 652).

- 19.2** If the radius of the nuclear sphere is 5 microns (μm), then the volume of that sphere is 5×10^{-13} L. (Recall that the volume of a sphere is $(4/3)\pi r^3$). The concentration of specific sites is about 6.6×10^{-12} M and the concentration of nonspecific sites is about 6.6×10^{-3} M.
- 19.3.** The fraction of OBF1 not bound to DNA is 0.0015. This is calculated from equation 3, using the $[\text{Dns}] = 6.6 \times 10^{-3}$ M and $K_{\text{ns}} = 10^5 \text{ M}^{-1}$.
- 19.4.** From the equation for specificity, or the ratio of equations 2 and 3, one can calculate that $[\text{PDs}]/[\text{Ds}] = 9$ when $[\text{Ptot}] = 60 \text{ nM}$. For a nuclear volume of 5×10^{-13} L, this requires 18,060 (or about 18,000) molecules of OBF1.
- 19.5.** *Deletion* of the region between -250 and -200 causes an *increase* in expression of the reporter in adipocytes, but no effect on expression in melanocytes. Thus it has a *negative* effect in adipocytes.

- 19.6.** *Deletion* of the region between -200 and -150 has no effect on expression in melanocytes or adipocytes.
- 19.7.** *Deletion* of the region between -150 and -100 causes an *decrease* in expression of the reporter in adipocytes and melanocytes. Thus it has a *positive* effect in both types of cells.
- 19.8.** The formation of complex A is specific to the sequence between -150 and -100, as shown by the loss of signal in the self-competition but lack of competition by the *E. coli* DNA. The protein forming complex A is not Sp1, since the binding site for Sp1 did not compete (lanes 12-14). A candidate for the protein forming complex A is AP1 or its relatives; the binding site for AP1 competes as well as the self DNA (lanes 9-11).
- 19.9.** Since binding site for AP1 competes as well as the self DNA (lanes 9-11), then you would expect to find a sequence similar to this binding site in the -150 to -100 fragment, and that it would be bound specifically by a protein. The binding site for AP1 is TGASTCA.
- 19.10.** All of the DNA fragments compete equally well for both complexes (B and C), thus these complexes are not specific for any particular DNA sequence.
- 19.11.** Since this region contains a site needed for negative regulation in adipocytes, mutation so that the site is no longer functional should allow expression in adipose tissue, i.e. ectopic expression due to loss of function of a tissue-specific, *cis*-acting negative regulator.
- 19.12.** Transcriptional activators have at least two domains which frequently function separately: the DNA-binding domain and the activation domain. The DNA-binding domain is required for the sequence-specific binding of the protein to DNA; two familiar structural motifs found in different proteins are a helix-turn-helix and a Zn-finger. A different portion of the protein is responsible for activation; this domain may make direct contact with the RNA polymerase or it may facilitate the action of co-activators or other proteins that stimulate transcription. Three different activation domains have been identified: acidic, proline-rich and glutamine-rich, and their mechanisms of action are the subject of much current research.

Our biochemist has done part of a domain-swap experiment - she has the activation domain of GAL4 fused to the DNA binding domain of the λ repressor. This new hybrid will no longer recognize the *GAL4* binding site in DNA (called UASG), since that DNA binding domain is no longer present. However, replacement of UASG with the λ operator (binding site for the λ repressor) in the *GAL* operon should allow the λ repressor-*GAL4* hybrid protein to function as a transcriptional activator of *GAL* genes in yeast.

4.40 Cys²-His² zinc fingers.

19.14. A leucine zipper.

19.15 (ASC) The activity of transcription factors can be controlled by their differential synthesis, resulting in the presence or absence of specific factors in specific cell types. The activity transcription factors already present within cells can be controlled by the presence or absence of inhibitor (as with NF κ B) and by the activation of pre-existing factors. An example of the last mechanism is the protein-protein interaction that forms an active complex of MyoD with E12. Another mechanism for activating pre-existing factors is covalent modification, such as phosphorylation. In at least some cells, AP1 is activated by phosphorylation.

19.16 (ASC)

- a) The data in table 4.49 show that insertions in four different regions of the receptor coding sequence cause decreased induction. This finding suggests that the glucocorticoid receptor has four separate functional domains. These four domains correspond to the following insertions: domain 1 = insertion D, E, and F; domain 2 = insertion I; domain 3 = insertion K, L, M, and N; and domain 4 = insertion Q, R, and S.
- b) Only insertions at Q, R, and S produce receptor proteins with decreased steroid-binding ability. Therefore, the region of the protein corresponding to these insertions is the steroid-binding domain.
- c) The data indicate that insertions in three domains block induction without affecting steroid binding. An EMSA-type assay could be used to determine whether changes in any of these three functional domains affect DNA binding of dexamethasone-complexed receptor. Alternatively, the DNA-binding domain could be inferred by comparing the sequence of glucocorticoid receptor with that of known DNA-binding domains in other steroid hormone receptors.

Answers to questions from Chapter 20. Regulation by changes in chromatin structure

- 20.1.** 6 kb from the right end of the restriction fragment, which is about 1 kb 5' to the start site for transcription.
- 20.2.** The DNase hypersensitive site could mark the position of an enhancer located 1 kb 5' to the start site of transcription. The hypersensitive site is too far 5' to the start site for transcription to be a candidate for a promoter. Alternatively, it could be a locus control region, a matrix binding site, a silencer, a replication origin, or other potential regulatory sites.
- 20.3.** Gcn5p aids in the activation of target genes by interacting with other transcriptional activators which bind to specific DNA sequences.
- 20.4.** histone acetyltransferase
- 20.5.** chromatin remodeling/ activation
- 4.55.** Several functions have been assigned to the locus control region, including to abilities to:
- Generate an open, active domain of chromatin.
 - Insulate from negative effects of adjacent sequences (negative position effects).
 - Enhance transcription of genes within the domain in a developmentally regulated manner.
- 20.7.**
- a) The cytoplasm of uninduced cells and nucleus of induced cells.
 - b) The PRE-binding activity is in the nucleus of induced cells.
 - c) It must be phosphorylated and interact with something in the nucleus in order to bind the PRE site.
 - d) Binding requires phosphorylated NFL2 plus UBF3.
 - e) The protein kinase is in the induced cell cytoplasm.
 - f) After exposure of lung cells to pulmonin, NFL2 in the cytoplasm is phosphorylated by a kinase that is activated by the pulmonin treatment. Phospho-NFL2 translocates to the nucleus where it binds UBF3 and subsequently the heterodimer binds to the PRE.