Prokaryotic Gene Regulation

(CHAPTER 14- Brooker Text)

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BIO 184
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Gene Regulation

- Constitutive Genes = unregulated
  essentially constant levels of expression (often required)

- Regulation can occur at:
  – Transcription (regulatory proteins; attenuation)
  – Translation (repressors; antisense RNA)
  – Posttranslational (feedback inhibition)

Transcriptional regulation:

- Repressors → Bind to DNA and inhibit transcription (confers Negative Control)
- Activators → Bind to DNA and increase transcription (confers Positive control)

- Effector molecules bind to regulatory proteins and not to DNA directly (either increase or inhibit transcription)

  - Inducers increase transcription by either:
    - ➢Bind activators and cause them to bind to DNA
    - ➢Bind repressors and prevent them from binding to DNA

  - Inhibitors of transcription (2 types)
    - ➢Corepressors bind to repressors and cause them to bind to DNA
    - ➢Inhibitors bind to activators and prevent them from binding to DNA

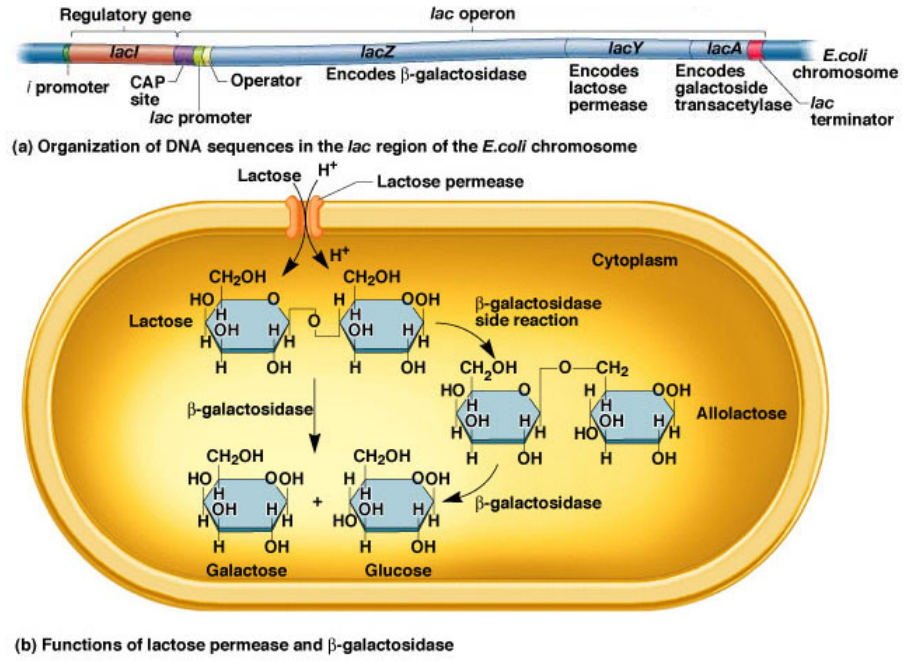


Figure 14.3

The *lac* Operon Is Regulated By a Repressor Protein

- The *lac* operon can be transcriptionally regulated
  - 1. By a repressor protein
  - 2. By an activator protein
- The first method is an inducible, negative control mechanism
  - It involves the *lac* repressor protein
  - The *inducer* is allolactose
    - It binds to the lac repressor and inactivates it

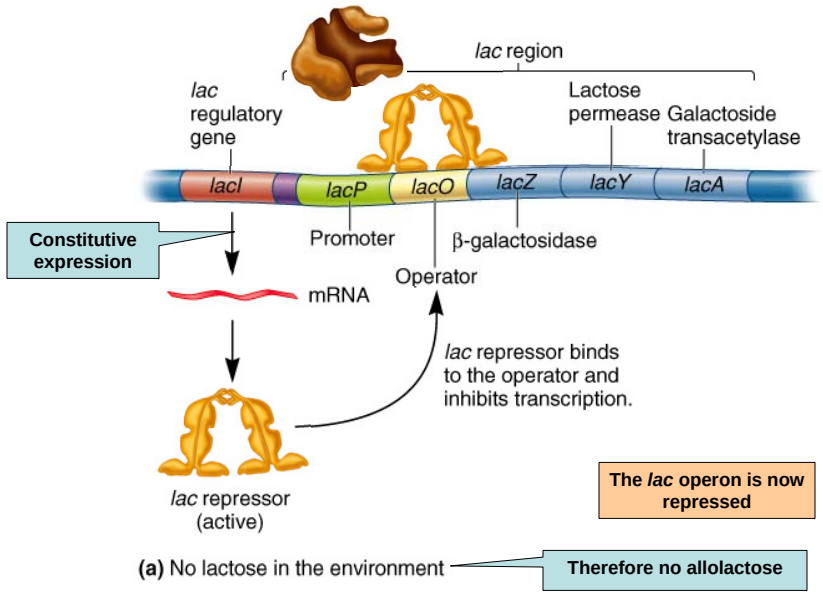
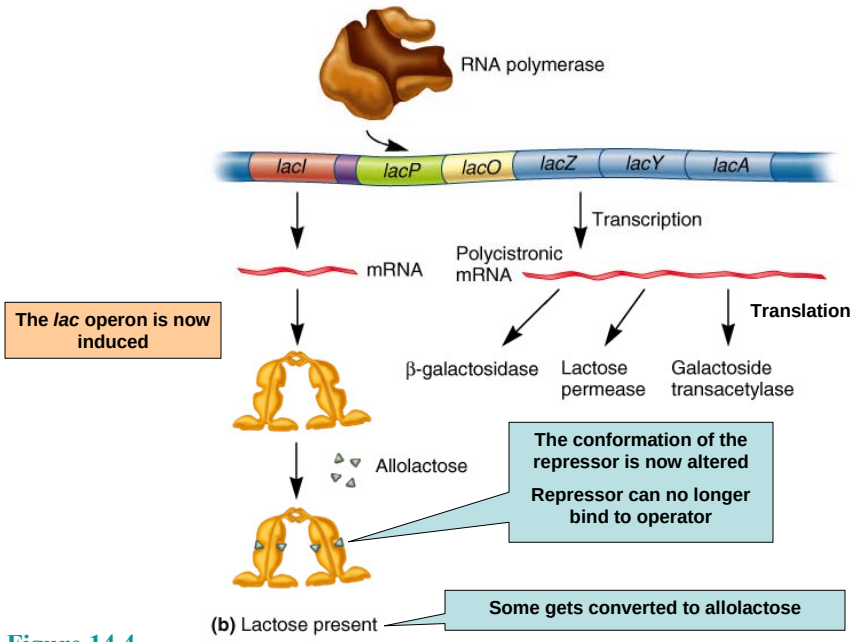


Figure 14.4



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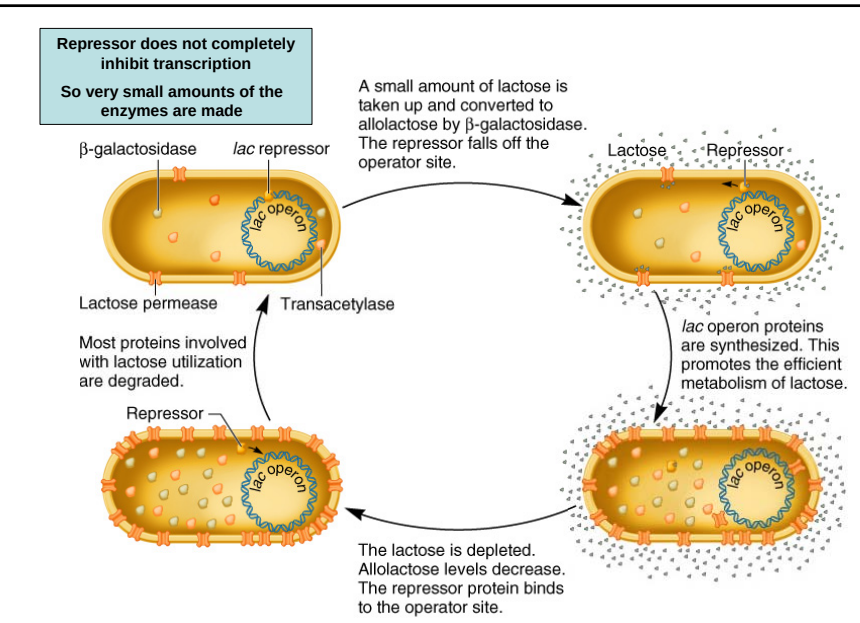


Figure 14.5 The cycle of *lac* operon induction and repression

- The interaction between regulatory proteins and DNA sequences have led to two definitions

  – 1. *Trans*-effect
    - Genetic regulation that can occur even though DNA segments are not physically adjacent
    - Mediated by genes that encode regulatory proteins

  Mutants: $lacI^-$ = repressor not made
  $lacI^s$= Super repressor; inducer can't bind and thus repressor remains bound to operator

2. *Cis*-effect or *cis*-acting element

   ➢ DNA sequence must be adjacent to regulating gene

   ➢ Mediated by sequences that bind regulatory proteins

Mutants: $lacO^c$ = Repressor can't recognize and bind to mutant operator
$lacP^-$ = Promoter is non-functional

The *lac* Operon Is Also Regulated By an Activator Protein

- catabolite repression
- When exposed to both lactose and glucose
  - *E. coli* uses glucose first, and catabolite repression prevents the use of lactose
  - When glucose is depleted, catabolite repression is alleviated, and the *lac* operon is expressed
- The sequential use of two sugars by a bacterium is termed diauxic growth

- Regulation involves a small molecule, cyclic AMP (cAMP)
  - produced from ATP via the enzyme adenylyl cyclase
  - cAMP binds an activator protein known as the Catabolite Activator Protein (CAP)
- cAMP-CAP complex is an example of genetic regulation that is inducible and under positive control
  - The cAMP-CAP complex binds to the CAP site near the *lac* promoter and increases transcription
- In the presence of glucose, the enzyme adenylyl cyclase is inhibited
  - This **decreases the levels of cAMP** in the cell
  - Therefore, cAMP is no longer available to bind CAP
  - And **Transcription rate decreases**

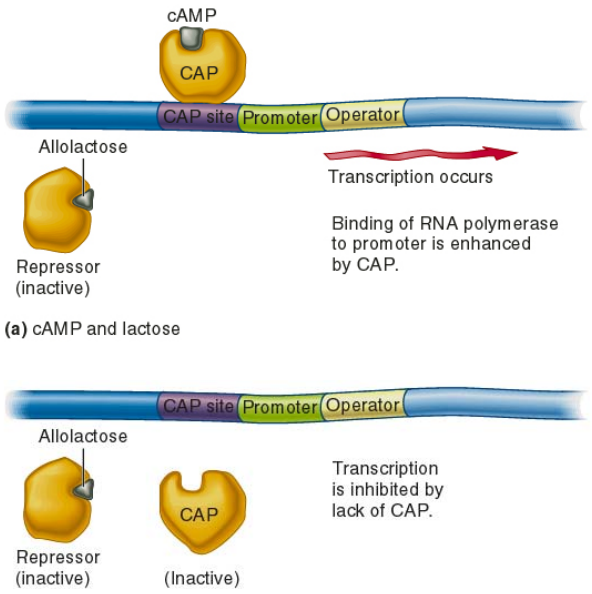


Figure 14.8

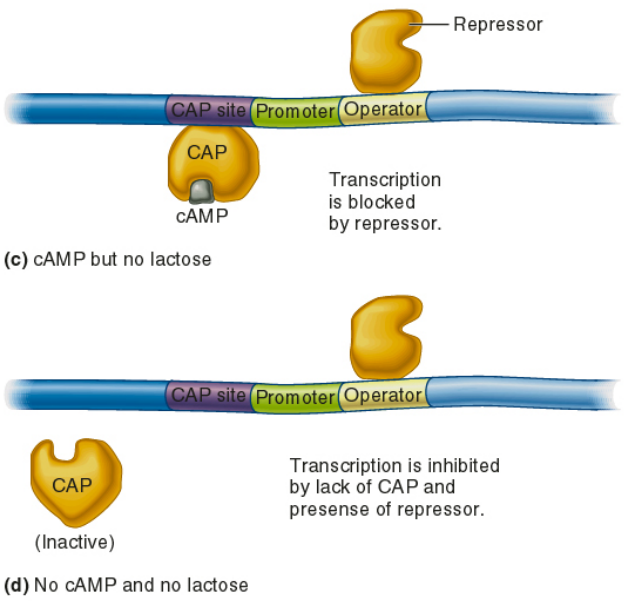
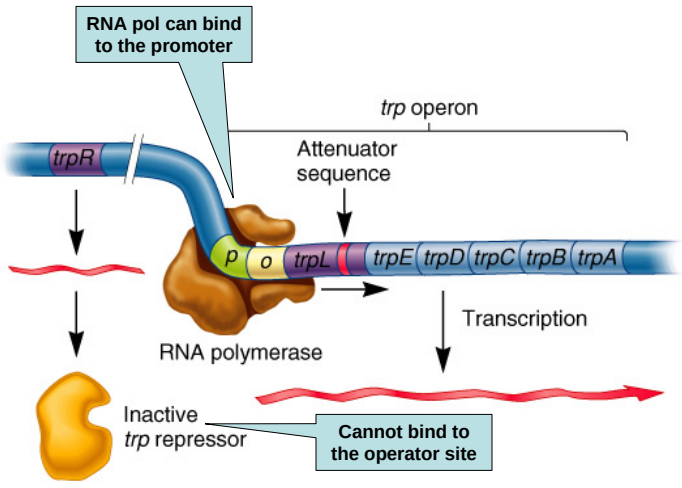


Figure 14.8

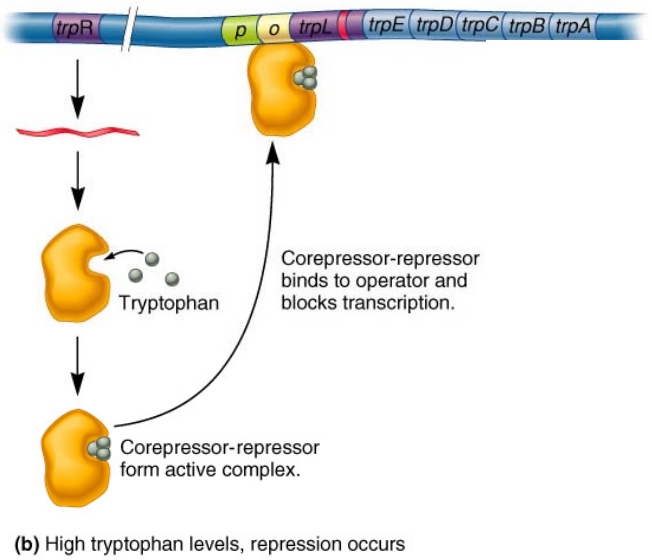
The *trp* Operon

- The *trp* operon = involved in the biosynthesis of the amino acid tryptophan
  - The genes *trpE*, *trpD, trpC, trpB* and *trpA* encode enzymes involved in tryptophan biosynthesis
  - The genes *trpR* and *trpL* are involved in regulation
    - *trpR* → Encodes the *trp* repressor protein
      - Functions in repression
    - *trpL* → Encodes a short peptide called the *L*eader peptide
      - Functions in attenuation



(a) Low tryptophan levels, transcription of the entire *trp* operon occurs

Figure 14.13 **Organization of the *trp* operon and regulation via the *trp* repressor protein**



(b) High tryptophan levels, repression occurs

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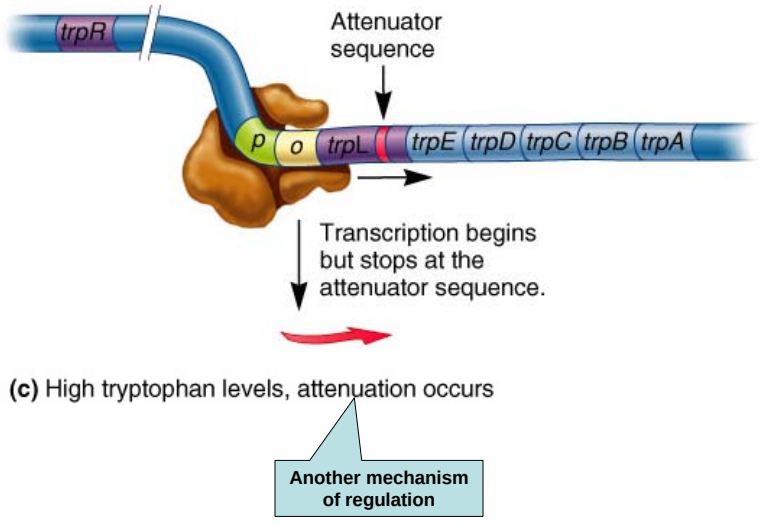


(c) High tryptophan levels, attenuation occurs

Another mechanism of regulation

Figure 14.13 **Organization of the *trp* operon and regulation via the *trp* repressor protein**

- Attenuation occurs in bacteria because of the coupling of transcription and translation
- During attenuation, transcription actually begins but it is terminated before the entire mRNA is made
  - A segment of DNA, termed the attenuator, is important in facilitating this termination
  - In the case of the *trp* operon, transcription terminates shortly past the *trpL* region (Figure 14.13*c*)
  - Thus attenuation inhibits the further production of tryptophan
- The segment of *trp* operon immediately downstream from the operator site plays a critical role in attenuation
  - The first gene in the *trp* operon is *trpL*
    - It encodes a short peptide termed the *L*eader peptide

- Region 2 is complementary to regions 1 and 3
- Region 3 is complementary to regions 2 and 4
  - Therefore several stem-loops structures are possible

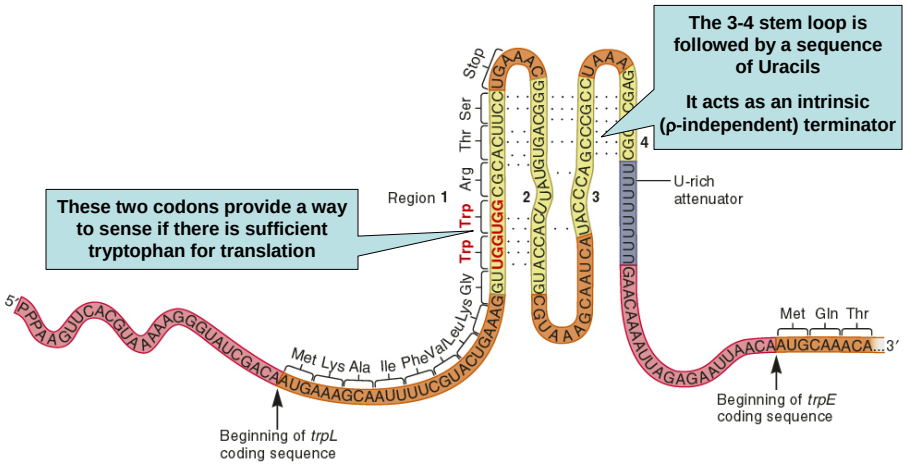


Figure 14.14 **Sequence of the *trpL* mRNA produced during attenuation**

- Therefore, the formation of the 3-4 stem-loop causes RNA pol to terminate transcription at the end of the *trpL* gene
- Conditions that favor the formation of the 3-4 stem-loop rely on the translation of the *trpL* mRNA
- There are three possible scenarios
  - 1. No translation
  - 2. Low levels of tryptophan
  - 3. High levels of tryptophan

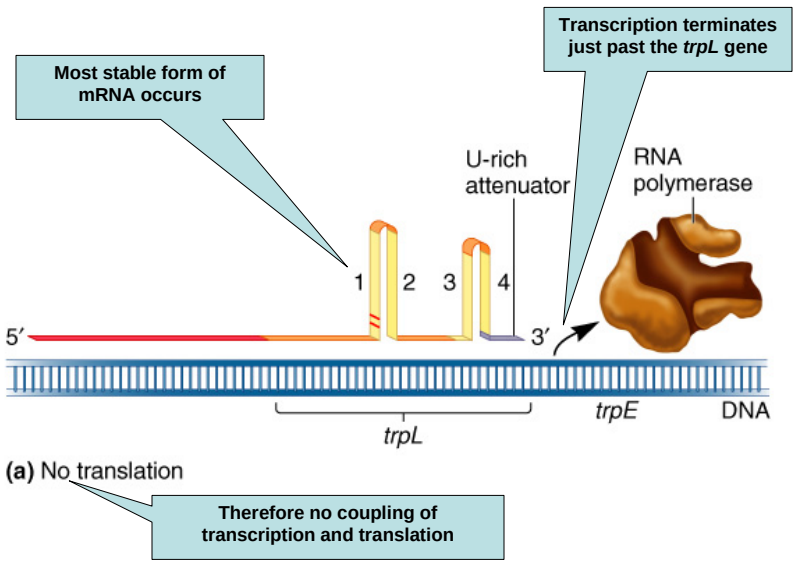
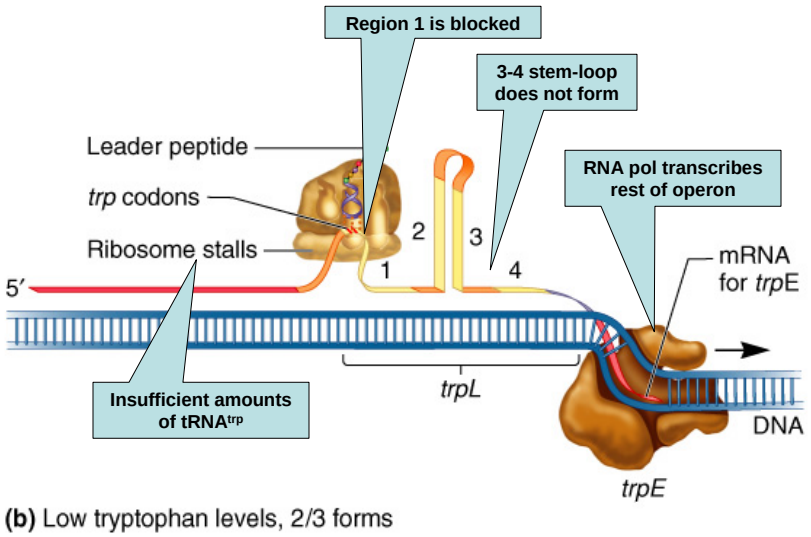


Figure 14.15 **Possible stem-loop structures formed from *trpL* mRNA under different conditions of translation**



(b) Low tryptophan levels, 2/3 forms

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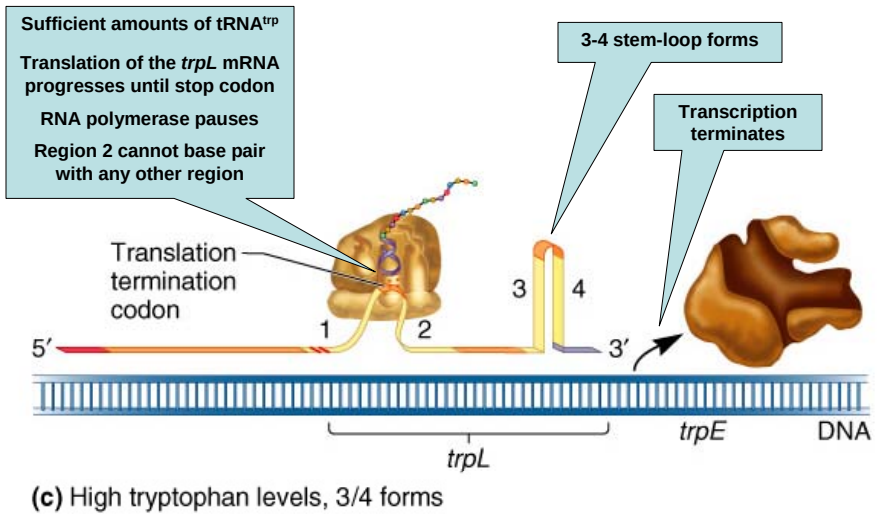

(c) High tryptophan levels, 3/4 forms

Figure 14.15 **Possible stem-loop structures formed from *trpL* mRNA under different conditions of translation**

Inducible *vs* Repressible Regulation

- The study of many operons revealed a general trend concerning inducible versus repressible regulation
  - Operons involved in catabolism (ie. breakdown of a substance) are typically inducible
    - The substance to be broken down (or a related compound) acts as the inducer
  - Operons involved in anabolism (ie. biosynthesis of a substance) are typically repressible
    - The inhibitor or corepressor is the small molecule that is the product of the operon

Transcriptional regulation:

- Repressors → Bind to DNA and inhibit transcription (confers Negative Control)
- Activators → Bind to DNA and increase transcription (confers Positive control)

- Effector molecules bind to regulatory proteins and not to DNA directly (either increase or inhibit transcription)

  - Inducers increase transcription by either:
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  - Inhibitors of transcription (2 types)
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Translational Regulation

- For some bacterial genes, the translation of mRNA is regulated by the binding of proteins
- A translational regulatory protein recognizes sequences within the mRNA
- In most cases, these proteins act to inhibit translation
  - These are known as translational repressors

Translational Regulation

- Translational repressors inhibit translation in one of two ways
  - 1. Binding next to the Shine-Dalgarno sequence and/or the start codon
    - This will sterically hinder the ribosome from initiating translation
  - 2. Binding outside the Shine-Dalgarno/start codon region
    - They stabilize an mRNA secondary structure that prevents initiation
- Translational repression is also found in eukaryotes

- A second way to regulate translation is via the synthesis of antisense RNA
  - An RNA strand that is complementary to mRNA

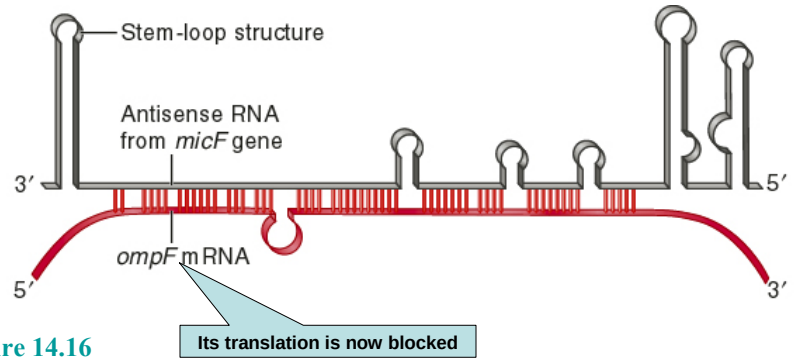

Figure 14.16

Posttranslational Regulation

- A common mechanism to regulate the activity of metabolic enzymes is feedback inhibition
- The final product in a pathway often can inhibit the an enzyme that acts early in the pathway

- Enzyme 1 is an allosteric enzyme, with two different binding sites
  - Catalytic site → binds substrate
  - Regulatory site → binds final product of the pathway

- If the concentration of product 3 becomes high
- It will bind to enzyme 1
- Thereby inhibiting its ability to convert substrate 1 into product 1

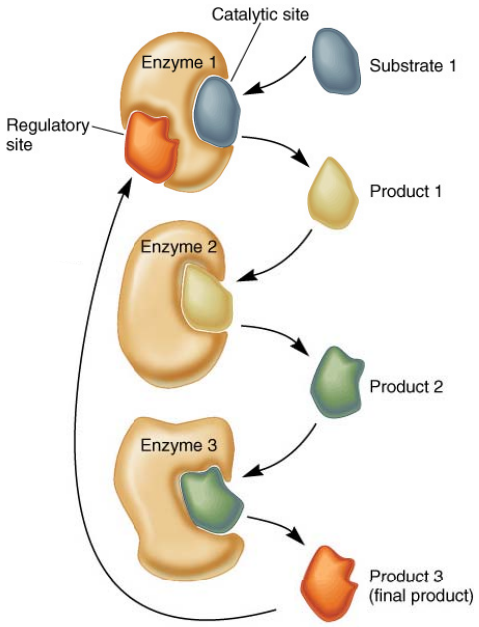

Figure 14.17

Posttranslational Regulation

- A second strategy to control the function of proteins is by the covalent modification of their structure
- Some modifications are irreversible
  - Proteolytic processing
  - Attachment of prosthetic groups, sugars, or lipids
- Other modifications are reversible and transiently affect protein function
  - Phosphorylation ($–PO_4$)
  - Acetylation ($–COCH_3$)
  - Methylation ($–CH_3$)