

Unit 4 - Gene Expression and Genetic Technology

- 4A. Control of Gene Expression
- 4B. Biotechnology
- 4C. Genomics
- 4D. Genes and Development

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Module 4A – Control of Gene Expression

- Every cell contains thousands of genes which code for proteins.
- However, every gene is not actively producing proteins at all times.
- In this module, we will examine some of the factors that help regulate when a gene is active, and how strongly it is expressed.

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Objective # 1

Explain the importance of gene regulation in both prokaryotes and eukaryotes.

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Objective 1

Prokaryotes:

- are unicellular or colonial
- evolved to quickly exploit transient resources
- main role of gene regulation is to allow cells to adjust to changing conditions
- in the same cell, different genes are active at different times

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Objective 1

Eukaryotes:

- mostly complex multicellular organisms
- evolved the ability to maintain a stable internal environment (homeostasis)
- main role of gene regulation is to allow specialization and division of labor among cells
- at the same time, different genes are active in different cells

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Objective # 2

List and describe the different levels of gene regulation, and identify the level where genes are most commonly regulated.

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Objective 2

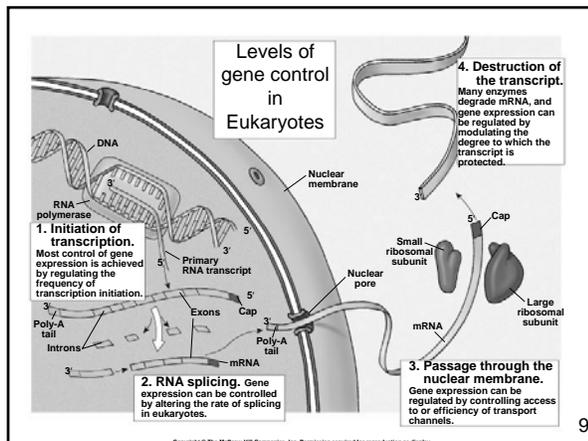
- To be expressed, a gene must be transcribed into m-RNA, the m-RNA must be translated into a protein, and the protein must become active.
- Gene regulation can theoretically occur at any step in this process.

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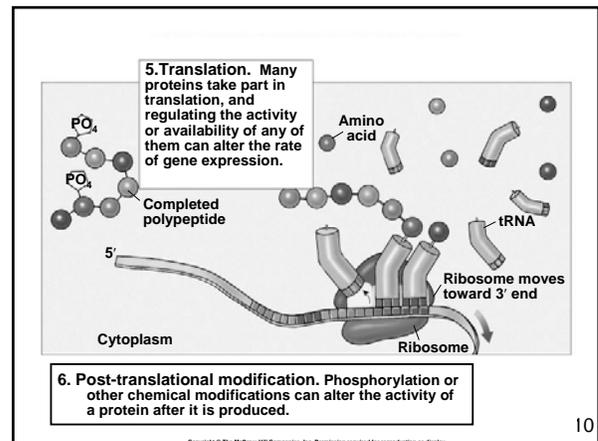
Objective 2

- We can classify levels of gene regulation into 2 main categories:
 - Transcriptional controls - factors that regulate transcription
 - Posttranscriptional controls – factors that regulate any step in gene expression after transcription is complete

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Objective 2

- It is most efficient to regulate genes at the first step of gene expression – namely transcription.
- Both prokaryotes and eukaryotes rely primarily on transcriptional controls to regulate gene expression.

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Objective # 3

Explain what regulatory proteins do and describe how they identify specific sequences on the DNA double helix.

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Objective 3

- In order to regulate transcription, special proteins called regulatory proteins must bind to specific regions of the DNA called regulatory sequences.
- How does each regulatory protein recognize the appropriate regulatory sequence on the DNA?

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Objective 3

- Biologists used to think the DNA double helix had to unwind and separate before proteins could “read” the sequence of base pairs.
- However, careful study has revealed 2 helical grooves winding around the DNA molecule. The deeper one is called the major groove and the shallower one is the minor groove.

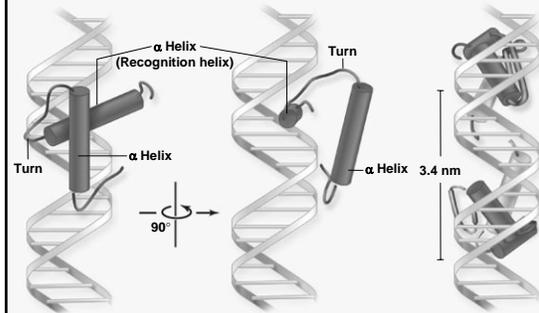
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Objective 3

- Regulatory proteins can read DNA sequences by inserting bent regions of the protein chain, called “DNA-binding motifs”, into the major groove.
- Several common DNA-binding motifs are shared by many different regulatory proteins. One example is the helix-turn-helix motif:

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The Helix-Turn-Helix Motif



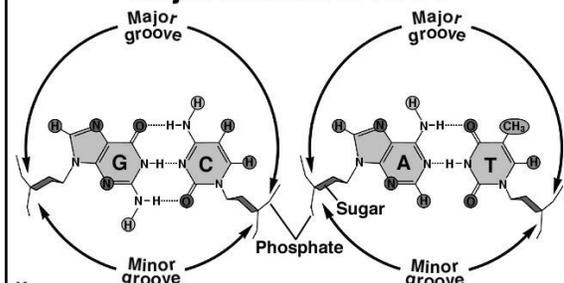
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Objective 3

- Once the DNA-binding motif is inserted into the major groove, it examines the chemical groups that project into the groove.
- Since each of the 4 possible base pairs has a different set of chemical groups that extend into the groove, the regulatory protein can determine the base pair sequence by examining these groups:

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Major Groove of DNA



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Objective # 4

Distinguish between negative control systems and positive control systems.

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Objective 4

Gene regulation relies on both negative and positive control systems:

- In negative control systems, the regulatory protein is a repressor which binds to DNA and blocks transcription.
- In positive control systems, the regulatory protein is an activator which binds to DNA and promotes transcription.

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Objective 4

We will look at 3 regulatory systems.

- 1) In regulation by repression:
 - The repressor alone cannot bind to DNA and block transcription.
 - However, when a co-repressor binds to the repressor, the repressor is activated. Once activated, the repressor can bind to DNA and block transcription.

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Objective 4

- 2) In regulation by induction:
 - The repressor alone can bind to DNA and block transcription.
 - When an inducer binds to the repressor, the repressor is inactivated and can no longer bind to DNA and block transcription.

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Objective 4

- 3) In regulation by activation:
 - The activator alone cannot bind to DNA and stimulate transcription.
 - When an inducer joins with the activator, the activator can bind to DNA and stimulate transcription.

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Regulatory Proteins

Regulation by Repression

When a microorganism such as E. coli is grown on glucose, it must synthesize all of its amino acids. If an amino acid such as histidine is added to the medium, the organism can turn off the synthesis of the enzymes needed to make histidine.

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Objective # 5

Describe the process of gene regulation in prokaryotic cells, and provide examples of both negative and positive transcriptional control in a prokaryotic cell such as *E. coli*.

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Objective 5

Prokaryotes can quickly turn production of specific proteins on or off in response to environmental conditions. This rapid response is facilitated by 3 important mechanisms:

- simultaneous transcription and translation
- short-lived m-RNAs
- operons

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Objective 5

Operons:

- In prokaryotes, functionally related genes are often located next to each other and are transcribed as a unit.
- For example, in *E. coli* 5 different enzymes are needed to synthesize the amino acid tryptophan. The genes that code for these enzymes are located together.

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Objective 5

- A single promoter serves all 5 genes. Recall that the promoter is the region where RNA polymerase binds to DNA and begins transcription
- The genes are transcribed as a unit, producing one long mRNA molecule (polycistronic mRNA) which contains the code to make all 5 enzymes.

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Objective 5

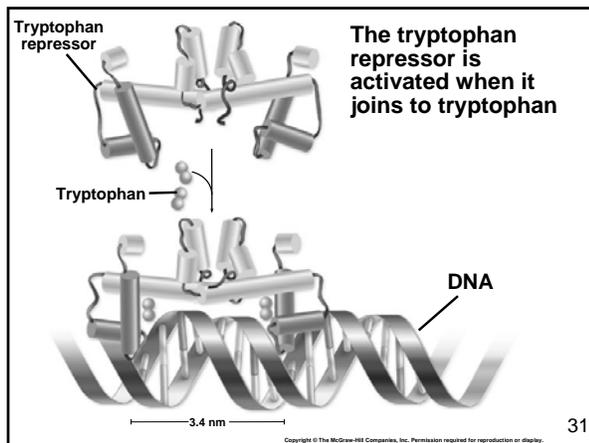
- There is also a single regulatory switch, called the operator.
- The operator is positioned within the promoter, or between the promoter and the protein coding genes. It controls access of RNA polymerase to the genes.
- All together, the operator, promoter, and the genes they control is called an operon.

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Objective 5

- Transcription of the 5 coding genes in the tryptophan operon is blocked when a transcriptional repressor binds to the operator.
- The repressor binds to the operator only when it is activated by the presence of tryptophan:

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Objective 5

Because transcription of the coding genes in the tryptophan operon can be blocked by a repressor, it is an example of negative transcriptional control:

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Objective 5

- Other regulatory proteins act as transcriptional activators by enhancing the ability of RNA polymerase to join with the promoter.
- For example, CAP (catabolite activator protein) stimulates transcription of genes that allow *E. coli* to use other food sources when glucose is not present:

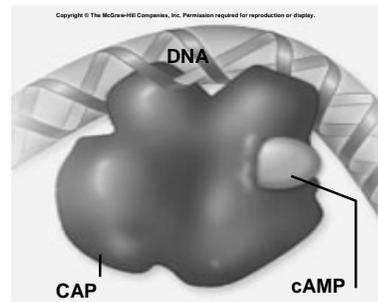
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Objective 5

- Low levels of glucose lead to high levels of cAMP.
- The cAMP binds to CAP, altering its shape so it can bind to DNA near any of several promoters. This bends the DNA, making it easier for RNA polymerase to join with the promoter and initiate transcription:

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cAMP binds to CAP, altering its shape so it can bind to DNA:



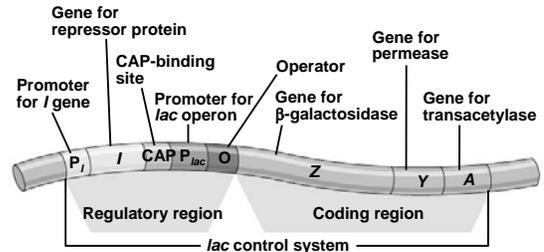
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Objective 5

- The lactose operon is present in *E. coli*. It controls the production of 3 enzymes needed to digest lactose (a disaccharide made of glucose and galactose).
- The lac operon consists of a coding region and a regulatory region:

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lac Operon of *E. coli*



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Objective 5

- Since glucose is the preferred food for *E. coli*, it makes sense to produce the 3 enzymes needed to digest lactose only when lactose is present AND glucose is absent.
- Therefore, the lac operon is affected by the levels of both lactose and glucose in the cell.

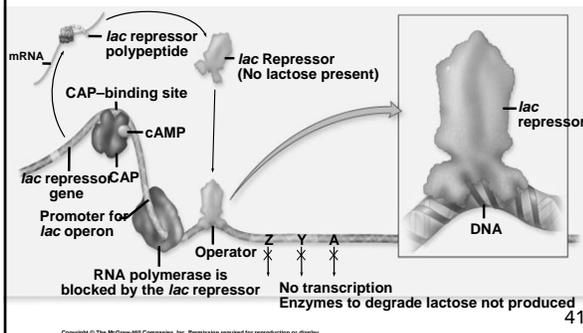
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Objective 5

- How lactose affects the lac operon:
 - When lactose is absent, the lac repressor binds to the operator and blocks transcription of the genes needed to digest lactose:

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When lactose is absent, the lac repressor binds to the operator and blocks transcription:



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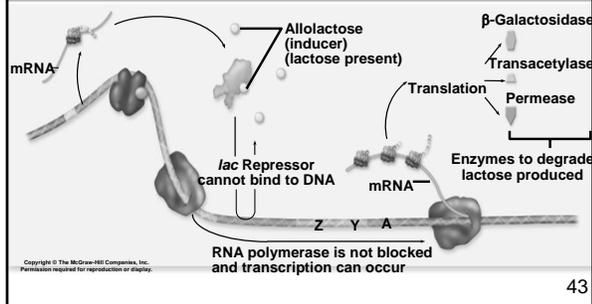
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Objective 5

- When lactose is present, a metabolite of lactose called allolactose binds to the repressor and inactivates it. Therefore, the repressor can no longer bind to the operator and block transcription:

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When lactose is present, a metabolite of lactose called allolactose binds to the repressor and inactivates it:



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Lac Operon

The expression of genes can be transcriptionally regulated. Such regulation is a critical feature of both eukaryotic and prokaryotic organisms.

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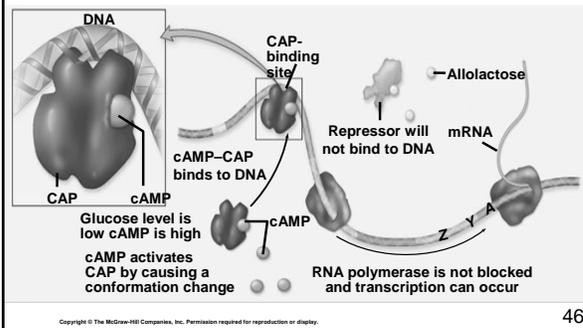
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Objective 5

- How glucose affects the lac operon:
 - When the glucose level is low, cAMP is high. The cAMP binds to CAP, altering its shape so it can bind to DNA near any of several promoters. This bends the DNA, making it easier for RNA polymerase to join with the promoter and initiate transcription

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When glucose is low, CAP binds to DNA and stimulates transcription:



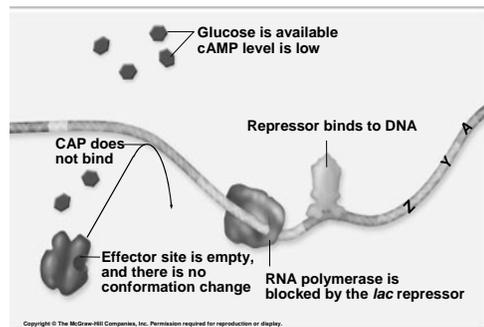
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Objective 5

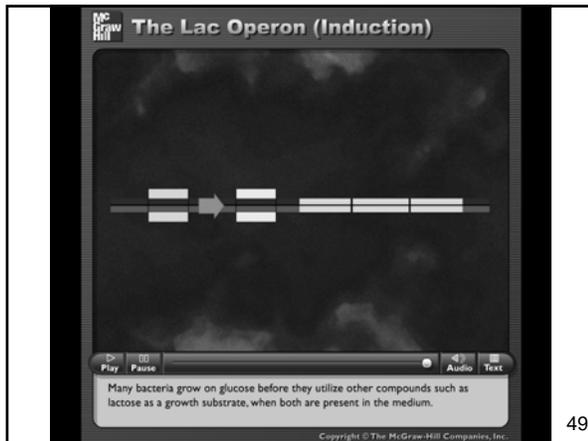
- When the glucose level is high, cAMP is low. Therefore, CAP is not activated and transcription is not stimulated.
- In addition, high glucose inhibits transport of lactose into the cell. Low lactose allows the lac repressor to bind to the operator and block transcription. This mechanism, called inducer exclusion, is the primary way glucose inhibits transcription of the lac operon.

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When glucose is high, CAP does not bind to DNA but the lac repressor does:



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Objective # 6

Describe the process of gene regulation in eukaryotic cells and compare it with gene regulation in prokaryotic cells.

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Objective 6

- In eukaryotes, gene regulation is more complex and more likely to involve posttranscriptional controls.
- However, like in prokaryotes, transcriptional controls are still the primary method of gene regulation in eukaryotes, and they will be the main focus of our discussion.

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Objective 6

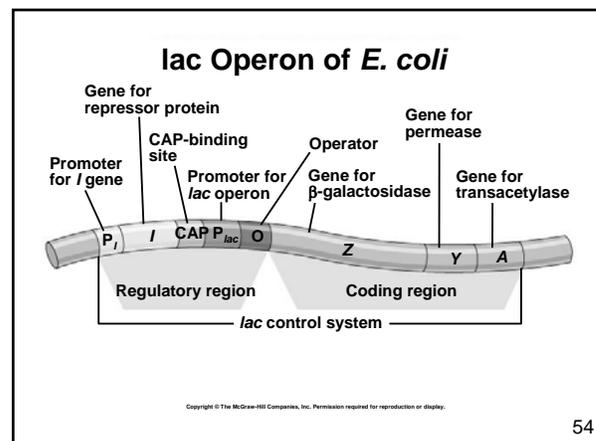
- In prokaryotes, functionally related genes are grouped together to form operons.
- Eukaryotes lack operons.

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Objective 6

- In addition, in prokaryotes regulatory molecules control transcription by binding to DNA sequences within or very close to the promoter.
- This limits the number of regulatory molecules that can affect transcription:

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Objective 6

- In eukaryotes, on the other hand, a much larger number of regulatory molecules are required in order for RNA polymerase to bind to the promoter and initiate transcription.
- Because many more regulatory molecules are involved, more complex regulation is possible.

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Objective 6

- The regulatory molecules involved in binding RNA polymerase to the promoter and initiating transcription in eukaryotes are called transcription factors.
- Transcription factors can be either general or specific.

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Objective 6

- General transcription factors are also called basal factors. They bind to the promoter to form an initiation complex.
- The initiation complex captures and stabilizes RNA polymerase on the promoter. This initiates transcription at the basal level. The basal level can be increased or decreased through the action of specific transcription factors.

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Objective 6

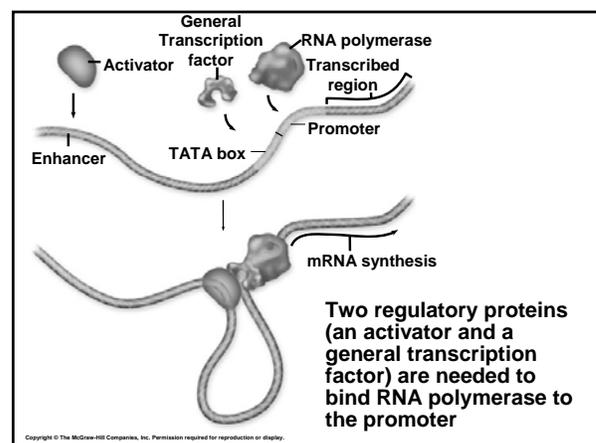
- Specific transcription factors include activators, coactivators, and repressors.
- Activators bind to regulatory sequences on the DNA called enhancers that may be located far away from the promoter.
- Because enhancers can be scattered anywhere in the genome, many different activators can affect the transcription of a single gene.

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Objective 6

- Activators act to increase the rate of transcription by interacting with the general transcription factors (also called basal factors) either directly or with the help of coactivators.

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Objective 6

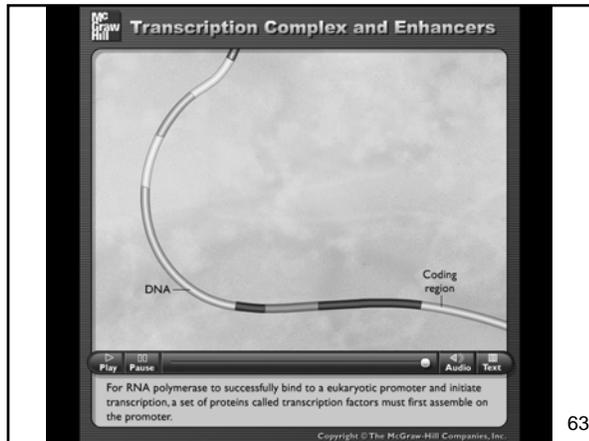
- In more complex systems, several activators may interact with many different coactivators and basal factors in order to bind RNA polymerase to the promoter and initiate transcription.
- Anything that affects the availability of a particular activator, coactivator or basal factor will affect the rate of transcription.

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Objective 6

- A third group of specific transcription factors are called repressors. These proteins bind to regulatory sequences called silencers located adjacent to or overlapping enhancers.
- Binding of a repressor to a silencer blocks binding of the activator to the enhancer and therefore inhibits transcription:

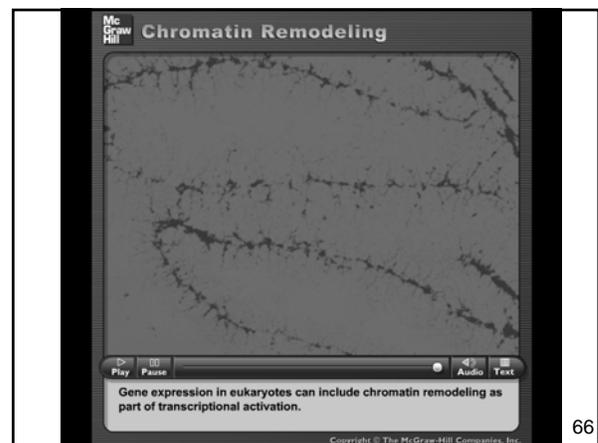
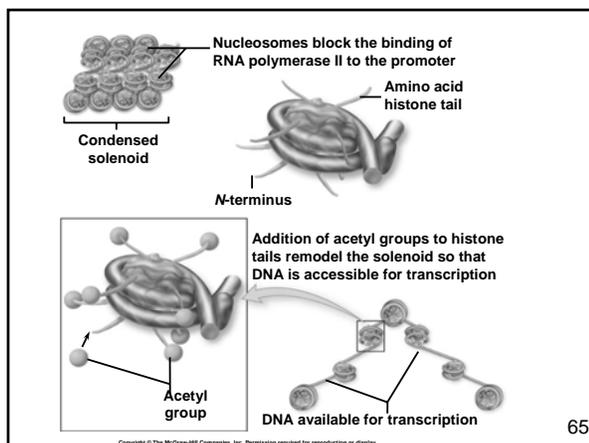
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Objective 6

- Alterations to chromatin structure may also help regulate gene transcription in eukaryotes.
- For example, addition of acetyl groups (called acetylation) to histone proteins can alter chromatin structure, making genes accessible for transcription:

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Objective 6

- Although less common than transcriptional control of gene expression, various types of posttranscriptional control may also occur in eukaryotes:

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RNA interference is a mechanism of silencing gene expression after the gene has been transcribed. This interference is due to the presence of antisense RNA, which is complementary to a specific

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McGraw-Hill Control of Gene Expression in Eukaryotes

Initiation of transcription

DNA

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Most of the control of gene expression in eukaryotes is achieved by regulating the frequency of transcription initiation.

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