

Chapter 17 Gene Regulation in Eukaryotes

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Epigenetics and the environment. Female honeybees that are fed royal jelly throughout the entire larval stage and into adulthood develop into queen bees. The larger queen bee is shown with a blue disk labeled 68. By comparison, those larvae that do not receive this diet become smaller worker bees. These differences in development are caused by epigenetic modifications.

If female bees are fed royal jelly, they develop into queen bees. This change in development is due to epigenetic modifications.



- Like prokaryotes, eukaryotic organisms derive many benefits from **gene regulation**
 - Respond to changing environmental conditions
 - Express only those proteins needed at particular times in life and cell cycle
- In addition, gene expression control is used in multicellular organisms to define tissues and cells with specific functions within the organism
- Gene expression control in eukaryotes is similar to that in prokaryotes
- Regulation can occur at different points in the pathway of gene expression, such as
 - Transcription
 - Rate of translation, including microRNA control
 - Protein modification
 - Stability of mRNA and protein

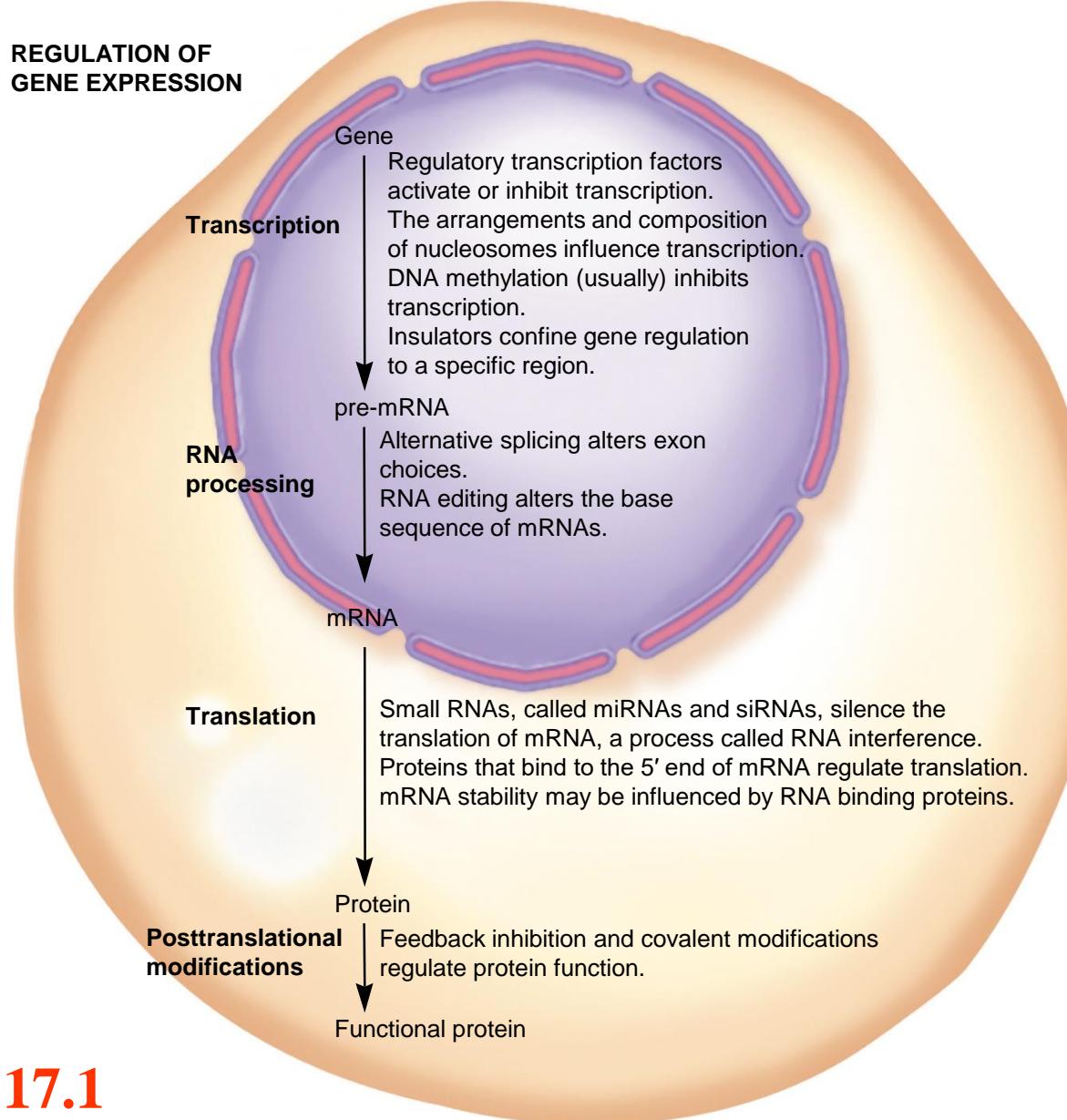
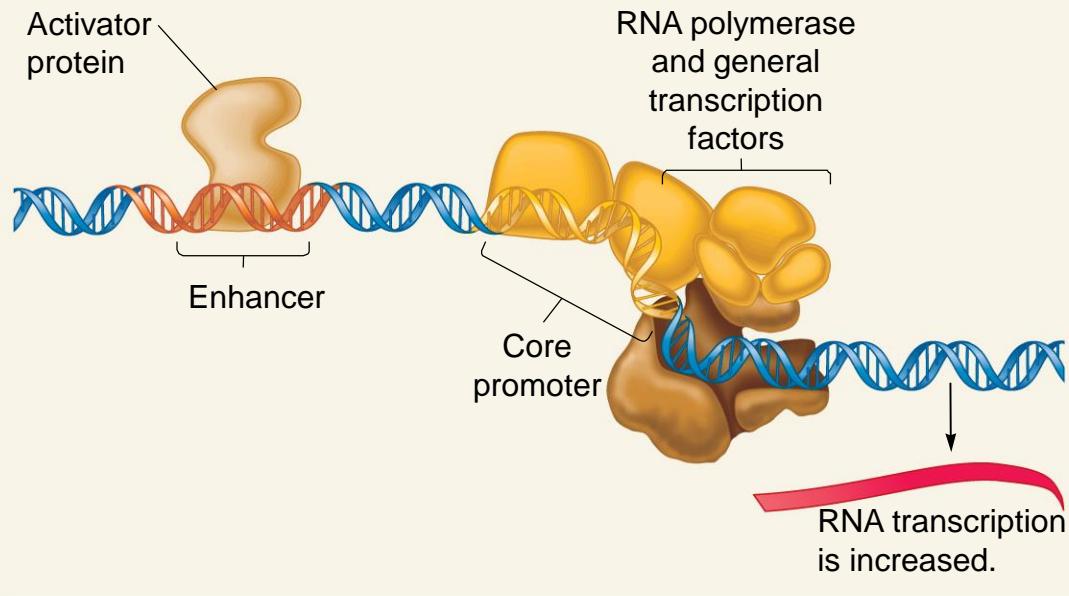


Figure 17.1

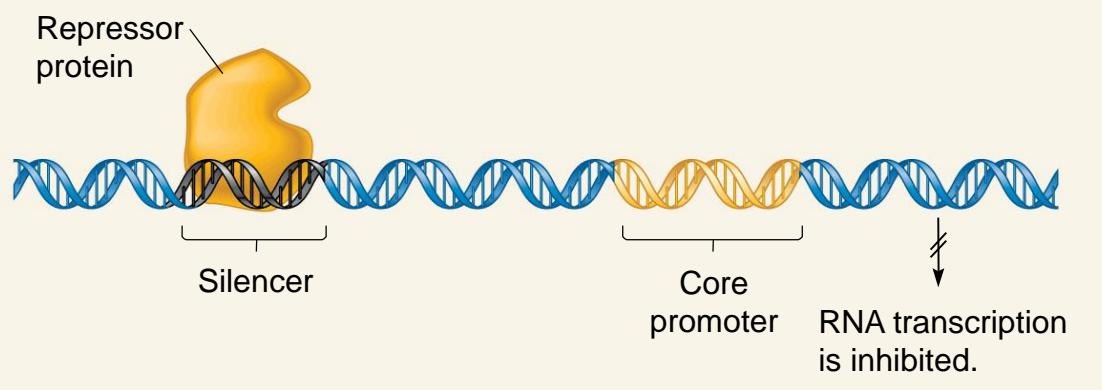
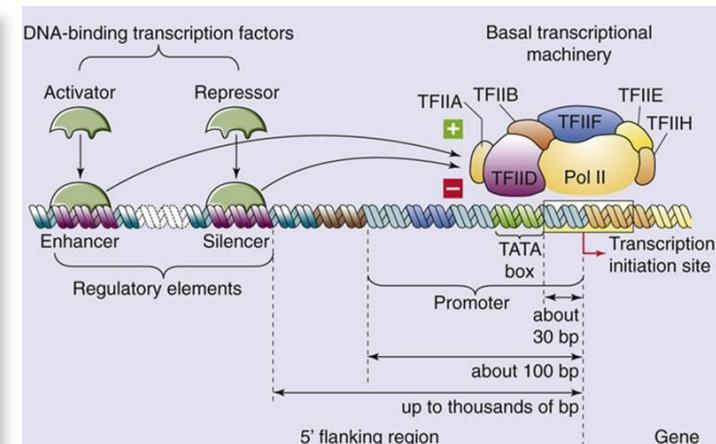
17.1 Regulatory Transcription Factors

- **Transcription factors** are proteins that influence the ability of RNA polymerase to transcribe a given gene
- There are two main types:
 1. **General transcription factors**
 - Required for the binding of the RNA pol to the core promoter and its progression to the elongation stage
 2. **Regulatory transcription factors**
 - Serve to regulate the rate of transcription
 - They influence the ability of RNA pol to begin transcription of a particular gene

- **Regulatory transcription factors** recognize elements located near the core promoter
 - These sequences are known as **control elements** or **regulatory elements**
- The binding of these proteins to these elements, affects the transcription of an associated gene
 - A regulatory protein that increases the rate of transcription is termed an **activator**
 - The sequence it binds is called an **enhancer**
 - A regulatory protein that decreases the rate of transcription is termed a **repressor**
 - The sequence it binds is called a **silencer**



(a) Gene activation



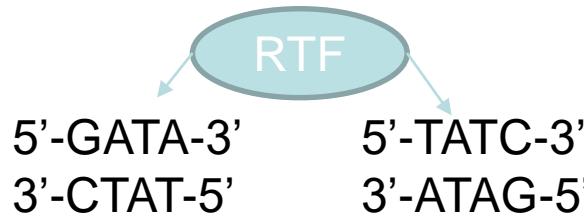
(b) Gene repression

Figure 17.2

- Note: A protein that is a repressor for one gene may be an activator for another
- Genes may have sequences for many different regulatory transcription factors
 - These sequences may overlap so that the binding of a sequence by one transcription factor may preclude the binding of others
 - The result, in terms of transcription, is therefore dependent on what sites are occupied
- **Combinatorial control**
 - Control of transcription controlled by many factors
 - Regulatory transcription factors
 - **Activators**
 - **Repressors**
 - **Small effector molecules** may modulate regulatory transcription factor activity
 - **Nucleosome** arrangements around promoters
 - Affected by regulatory transcription factors
 - **DNA methylation**

Enhancers and Silencers

- The binding of a transcription factor to an enhancer increases the rate of transcription
 - This **up-regulation** can be 10x to 1,000x
- The binding of a transcription factor to a silencer decreases the rate of transcription
 - This is called **down-regulation**
- Many response elements are **orientation independent** or **bidirectional**
 - They can function in the forward or reverse orientation



- Most response elements are located within 200 bp upstream of the promoter
 - However, some are found at various other sites
 - Up to 100,000 bp away!
 - Downstream from the promoter
 - Even within introns

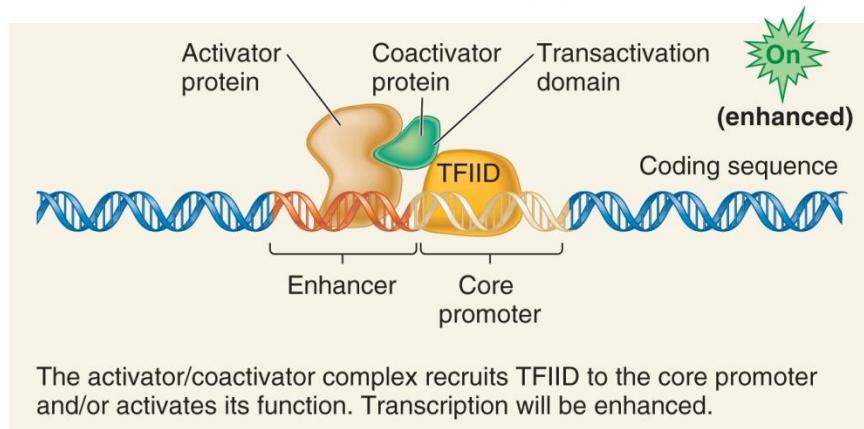
TFIID and Mediator

- The net effect of a transcription factor is to influence the function of RNA polymerase
 - However, most regulatory transcription factors do not bind to RNA polymerase directly
 - So how do they affect its function?
- Two common protein complexes that communicate the effects of regulatory transcription factors are
 - **TFIID** – a general transcription factor that recruits RNA pol to the promoter
 - **Mediator** - a protein complex that mediates between RNA pol II and the regulatory transcription factors

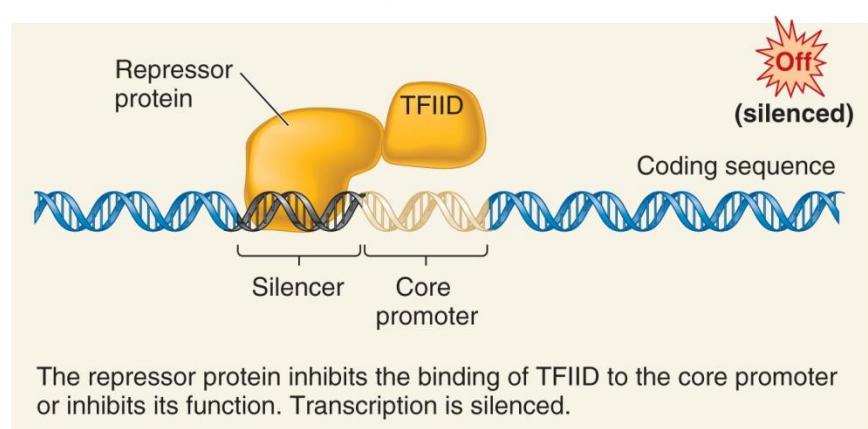
TFIID

- TFIID is a general transcription factor
- It binds the TATA box and recruits RNA pol II to the core promoter
- Activator proteins enhance the function of TFIID
 - Recruit TFIID to the TATA box, or
 - Help it to recruit RNA pol II, or
 - Interact with **coactivators** that act on TFIID

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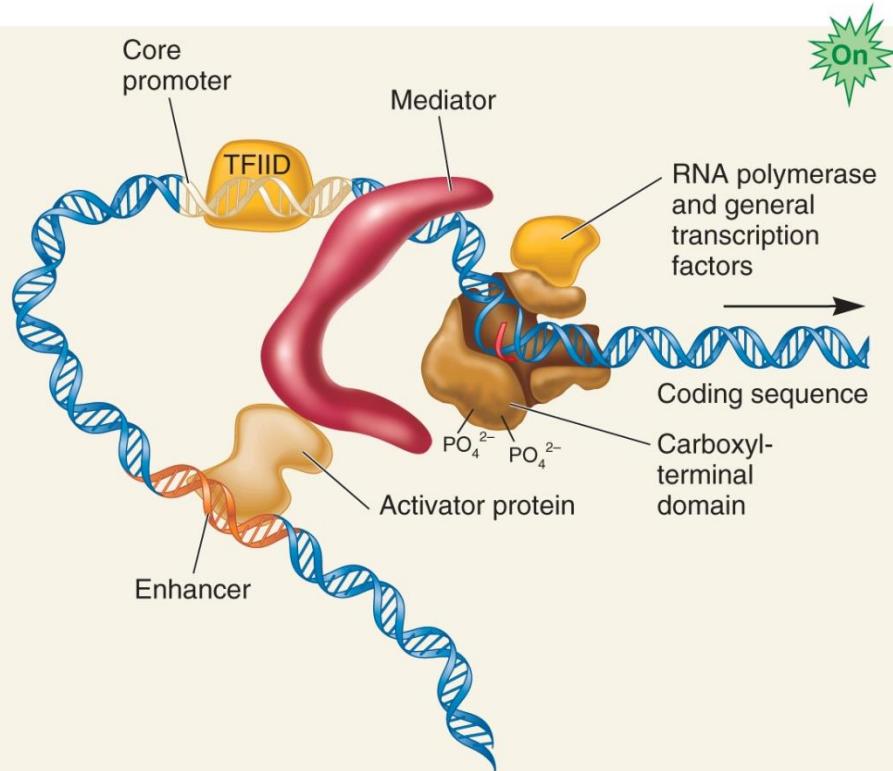
(a) Transcriptional activation via TFIID



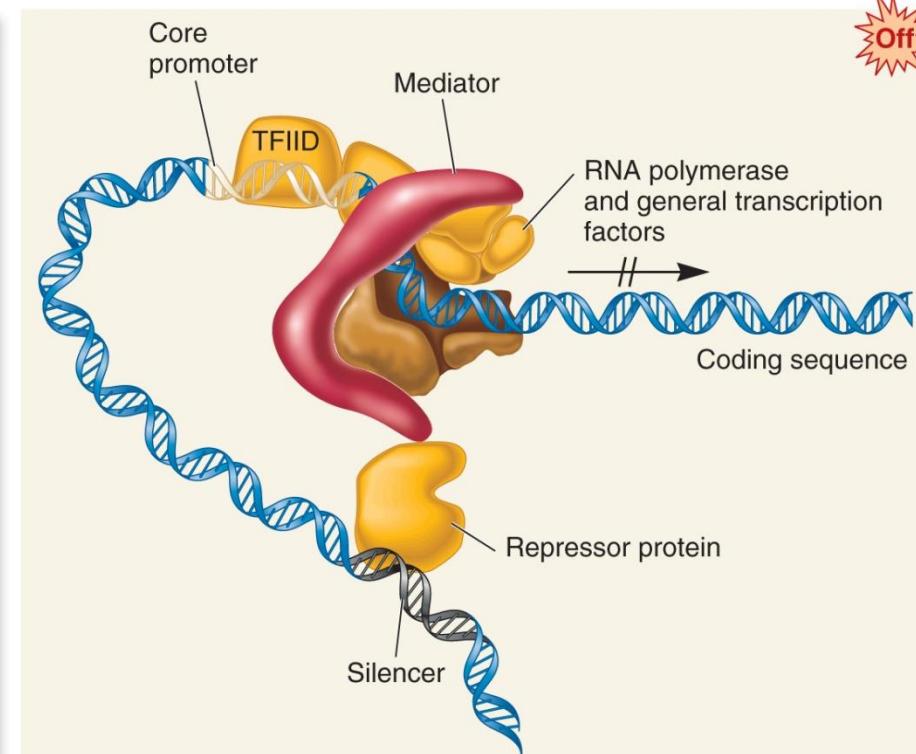
(b) Transcriptional repression via TFIID

Mediator

- Mediator acts to mediate the interactions between RNA pol II and regulatory transcription factors
 - Controls whether RNA pol II can progress to the elongation stage
- **Transcriptional activators** stimulate mediator
 - Switching from initiation to elongation
- **Transcriptional repressors** inhibit mediator
 - Prevent switch to elongation stage, preventing transcription



The activator protein interacts with mediator. This results in the phosphorylation of the carboxyl-terminal domain of RNA polymerase. Some general transcription factors are released, and RNA polymerase proceeds to the elongation phase of transcription.



The repressor protein interacts with mediator in a way that prevents the phosphorylation of RNA polymerase. Therefore, it cannot proceed to the elongation phase of transcription.

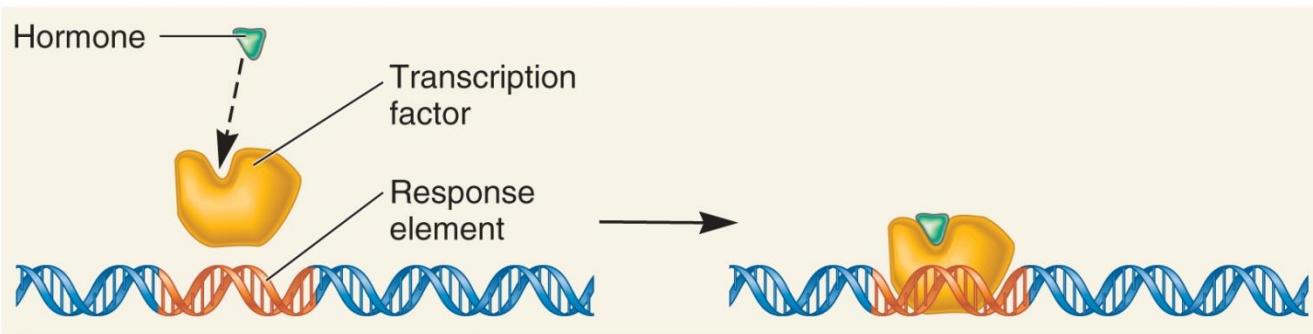
(a) Transcriptional activation via mediator

(b) Transcriptional repression via mediator

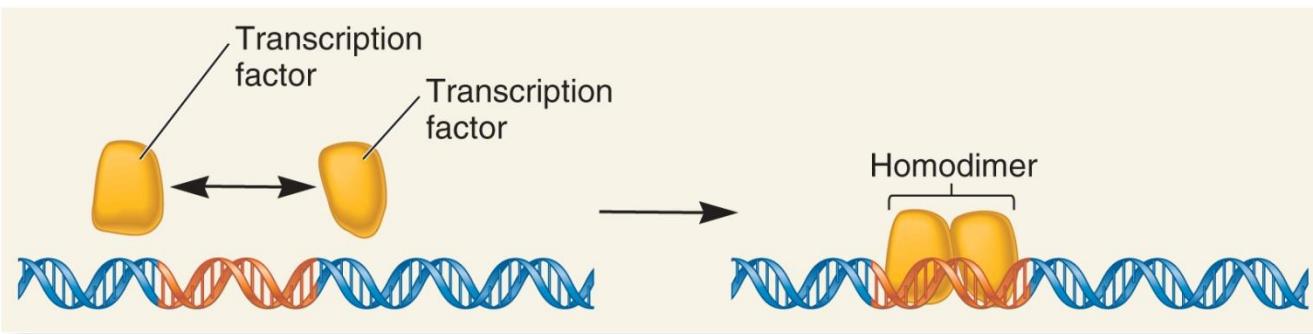
Figure 17.4

Modulation of Regulatory Transcription Factors

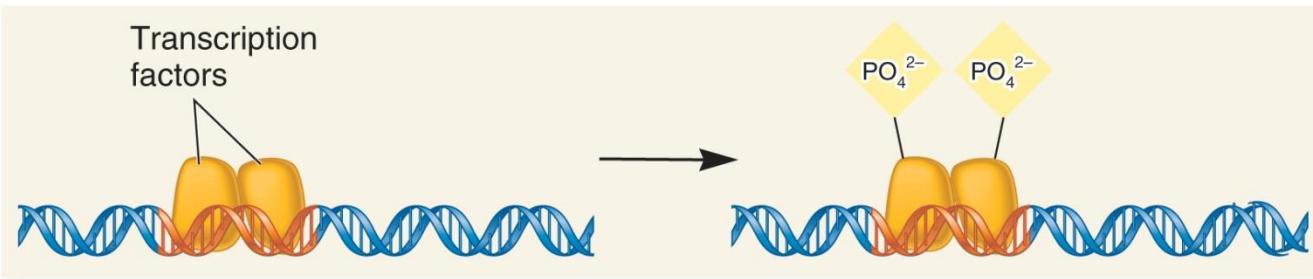
- There are three common ways that the function of regulatory transcription factors can be affected
 1. Binding of a small effector molecule
 2. Protein-protein interactions
 3. Covalent modifications
- Refer to Figure 17.5



(a) Binding of a small effector molecule such as a hormone



(b) Protein–protein interaction



(c) Covalent modification such as phosphorylation

Figure 17.5



Steroid Hormones and Regulatory Transcription Factors

- Steroid hormones are produced by endocrine glands
 - Secreted into the bloodstream
 - Lipids, so they pass through cell plasma membrane
- The ultimate action of a steroid hormone is to affect gene transcription
 - The receptors for steroid hormones are regulatory transcription factors inside the cell
 - The hormone binds to its receptor
 - This complex then acts as a transcription factor

- Cells respond to steroid hormones in different ways
- Example: **Glucocorticoids**
 - Influence nutrient metabolism in most cells
 - Steps of action
 1. Hormone diffuses through plasma membrane
 2. Binds to glucocorticoid receptors
 3. HSP90 is released, exposing a nuclear localization signal (NLS)
 4. Two glucocorticoid receptors dimerize and enter the nucleus
 5. The homodimer binds a glucocorticoid response element (GRE)
 6. This activates transcription

**Heat shock proteins leave
when hormone binds to
receptor**

**Formation of
homodimer**

**Nuclear localization
signal is exposed**

**Glucocorticoid
Response
Elements**

**Transcription
activated or
repressed**

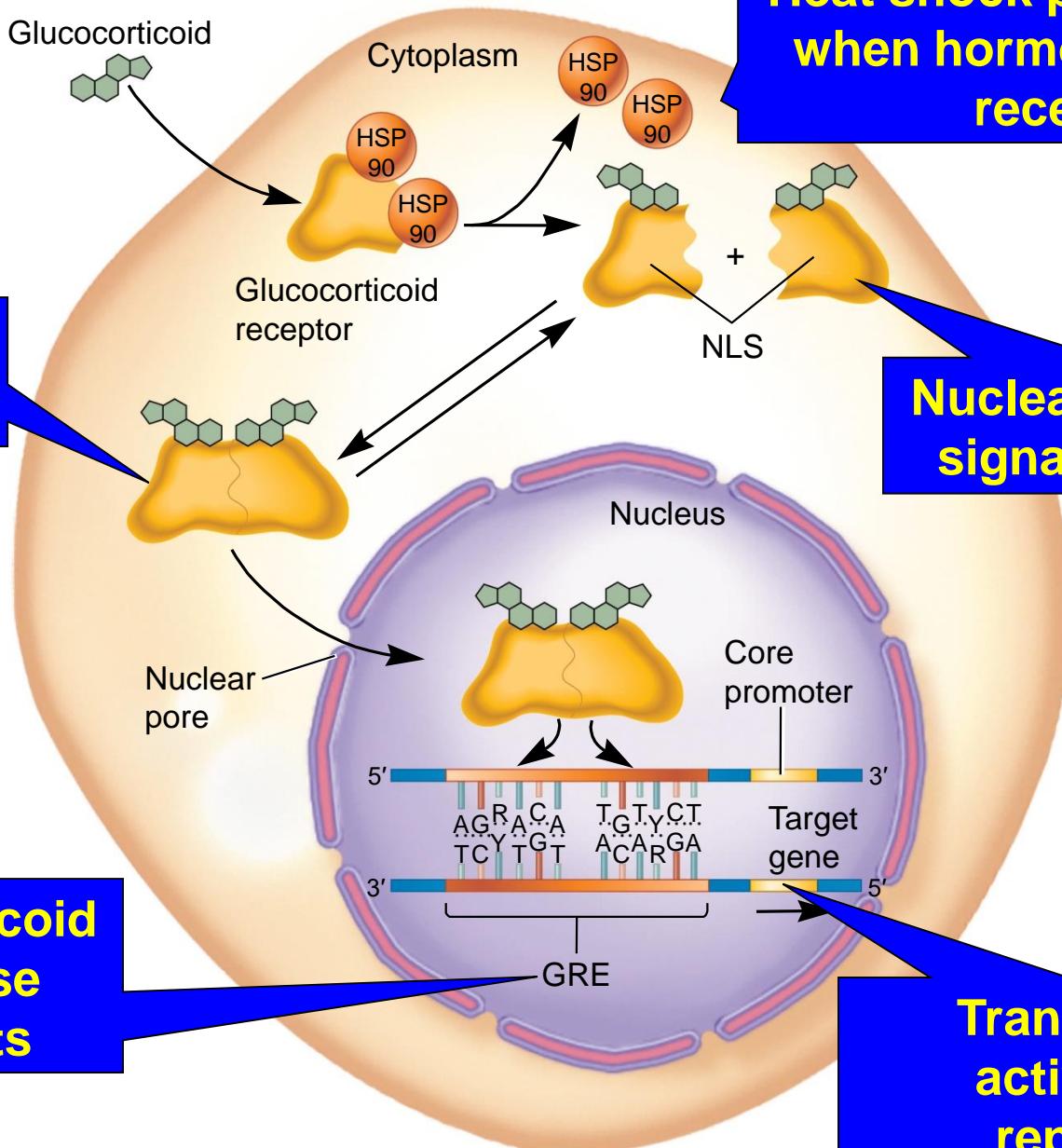
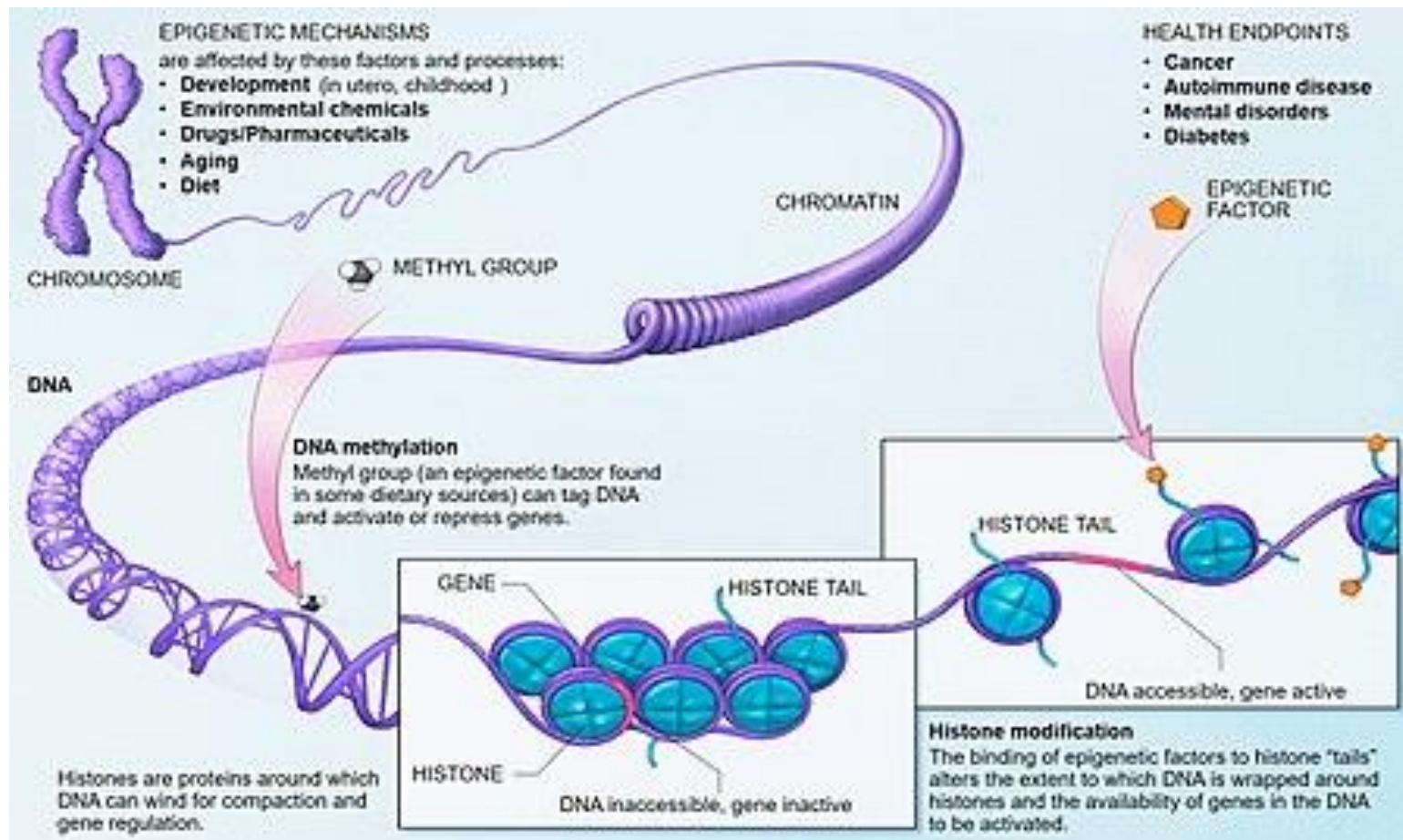


Figure 17.6

17.2 Chromatin Remodeling, Histone Variation, and Histone Modification



Chromatin Remodeling

- **ATP-dependent modification of chromatin**
 - Complex of proteins that use ATP to
 - Reposition nucleosomes
 - Remove nucleosomes
 - Change composition of nucleosomes
 - Histone proteins are members of families
 - Each member of the family may have a different role in chromatin compaction
 - Creates
 - **Open conformation** – more transcription
 - **Closed conformation** – less transcription
- Chromatin-remodeling complexes change the position and composition of nucleosomes
- Many different families of remodelers
 - SWI/SNF family, ISWI family, INO80 family, Mi-2 family
- All remodelers have **DNA translocases**
 - Move along DNA

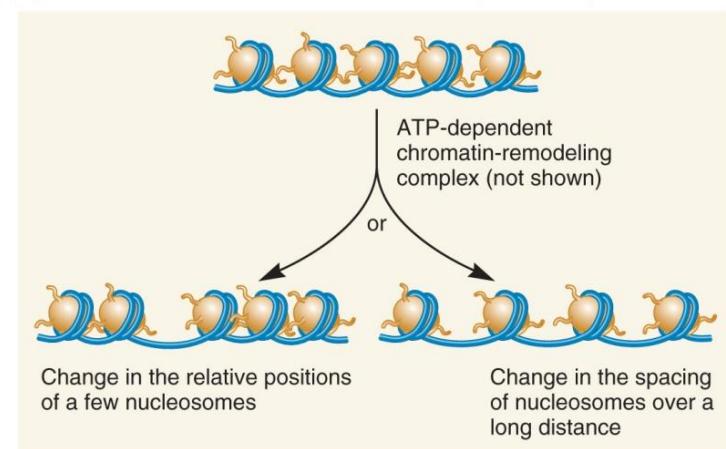
Constitutive heterochromatin:

- constitute ~ 10-20 % of nuclear DNA
- highly compacted, transcriptionally/Recombinatorially inert

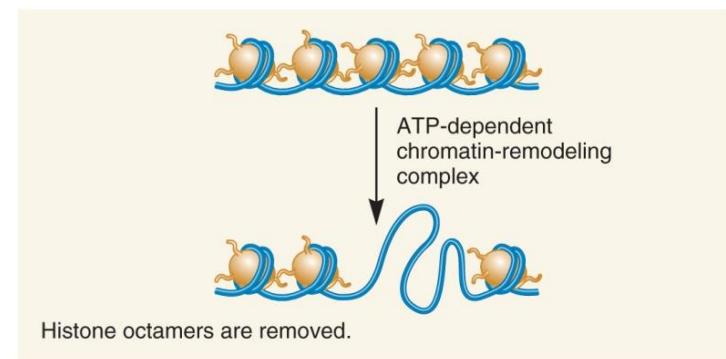
**Euchromatin + facultative heterochromatin:**

- constitute ~ 80% of nuclear DNA
- less condensed, rich in genes, however,
- only small fraction of euchromatin is transcriptionally active
- the rest is transcriptionally inactive/silenced (but can be activated in certain tissues or developmental stages)
- these inactive regions are also known as "facultative heterochromatin"

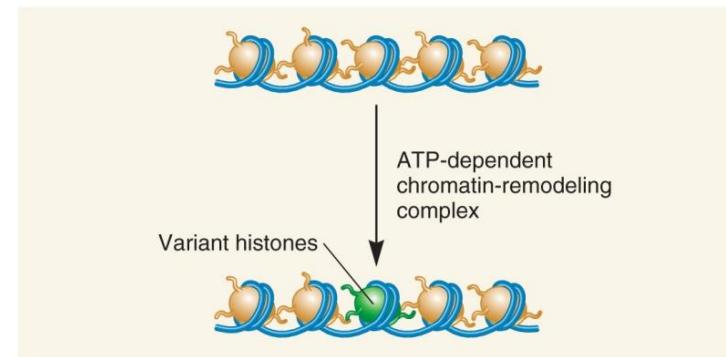
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(a) Change in nucleosome position



(b) Histone eviction



(c) Replacement with histone variants



Histone Variants

- Five standard histone genes: H1, H2A, H2B, H3, and H4
- But duplications and mutations have created many histone variants
 - The human genome has 70 histone genes!
 - Some variants play specialized roles in chromatin structure and function
 - Example: Histone cenH3 is found at the centromere
 - Example: macroH2A is found along the inactive X chromosome

TABLE 17.1**Standard Human Histones and Examples of Histone Variants**

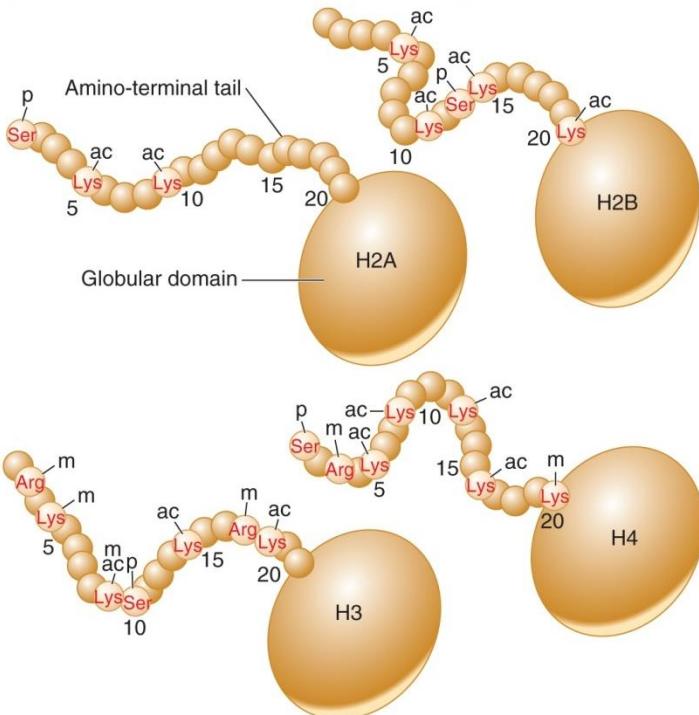
Histone	Type	Number of Genes in Humans	Function
H1	Standard	11	Standard linker histone*
H1 ⁰	Variant	1	Linker histone associated with chromatin compaction and gene repression
H2A	Standard	15	Standard core histone
MacroH2A	Variant	1	Core histone that is abundant on the inactivated X chromosome in female mammals. Plays a role in chromatin compaction
H2A.Z	Variant	1	Core histone that is usually found in nucleosomes that flank the transcriptional start site of promoters. Plays a role in gene transcription
H2A.Bbd	Variant	1	Core histone that promotes the open conformation of chromatin. Plays a role in gene activation
H2A.X	Variant	1	Plays a role in DNA repair
H2B	Standard	17	Standard core histone
spH2B	Variant	1	Core histone found in the telomeres of sperm cells
H3	Standard	10	Standard core histone
cenH3	Variant	1	Core histone found at centromeres. Involved with the binding of kinetochore proteins
H3.3	Variant	2	Core histone that promotes the open conformation of chromatin. Plays a role in gene activation
H4	Standard	14	Standard core histone

*H1 in mammals is found in five subtypes.

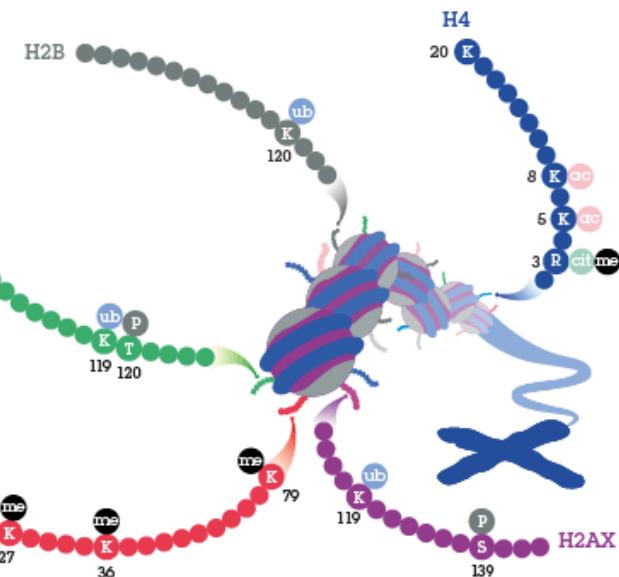
Table 17.1

The Histone Code

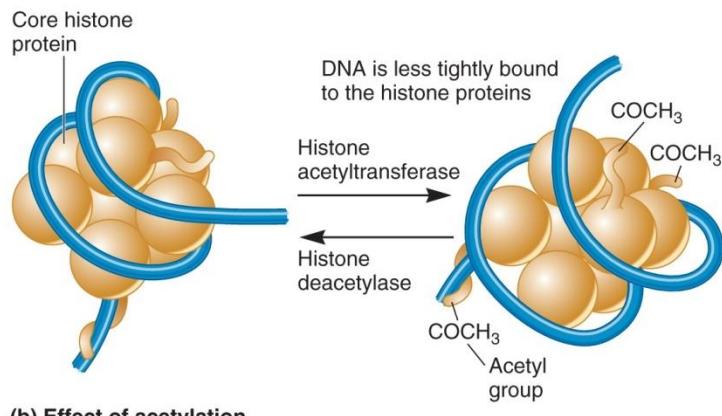
- The histone code also controls transcription
- Histones can be modified multiple ways:
 - Acetylation
 - Methylation
 - Phosphorylation
- There are 50+ histone-modifying enzymes!
- Histone modifications alter chromatin conformation and protein interactions



(a) Examples of possible histone modifications



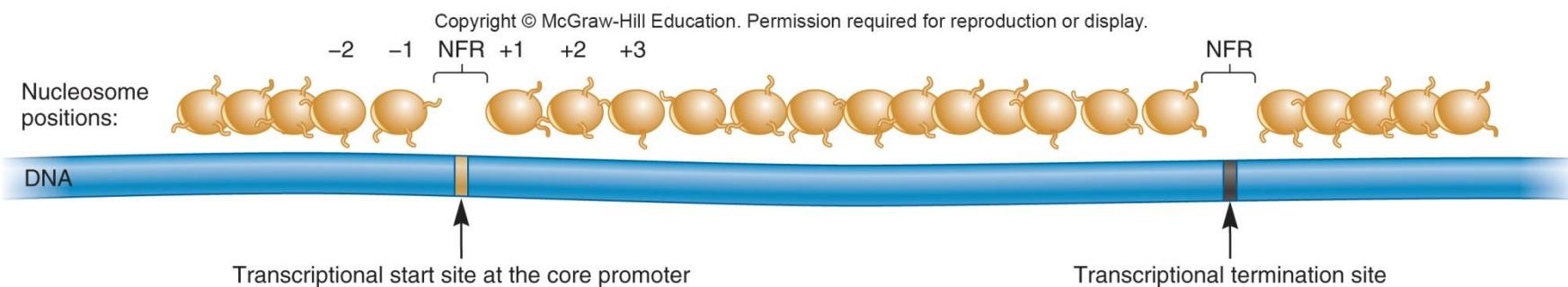
- ac Acetylation
- me Methylation
- p Phosphorylation
- cit Citrullination
- ub Ubiquitination



(b) Effect of acetylation

Position of Nucleosomes Around Genes

- Many eukaryotic genes have a common pattern of nucleosome organization
 - Active genes
 - **Nucleosome free region (NFR)** around the core promoter
 - Around 150 bp long
 - NFR flanked by two well-positioned nucleosomes
 - The **-1 and +1 nucleosomes**
 - Mark the transcription start site



A nucleosome-free region (NFR) is found at the beginning and end of many genes. Nucleosomes tend to be precisely positioned near the beginning and end of a gene, but are less regularly distributed elsewhere.



Transcriptional Activation

- Transcriptional activation involves changes in nucleosome locations, nucleosome composition, and histone modifications
- Some transcriptional activators recruit chromatin-remodeling enzymes and histone-modifying enzymes to the promoter
- Refer to Figure 17.10 for a general scheme for how transcriptional activators facilitate transcription

Many genes are flanked by nucleosome-free regions (NFR) and well-positioned nucleosomes.

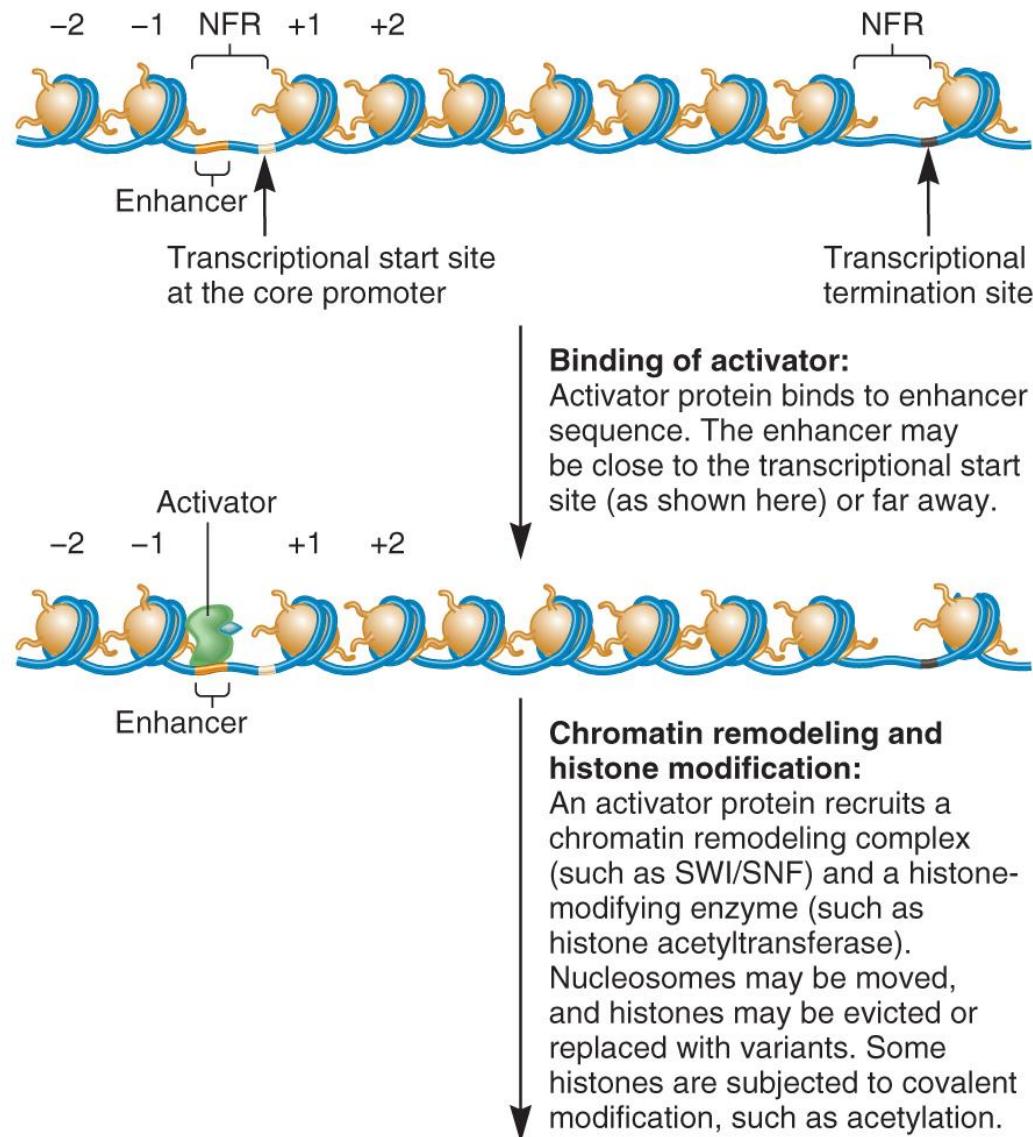


Figure 17.10

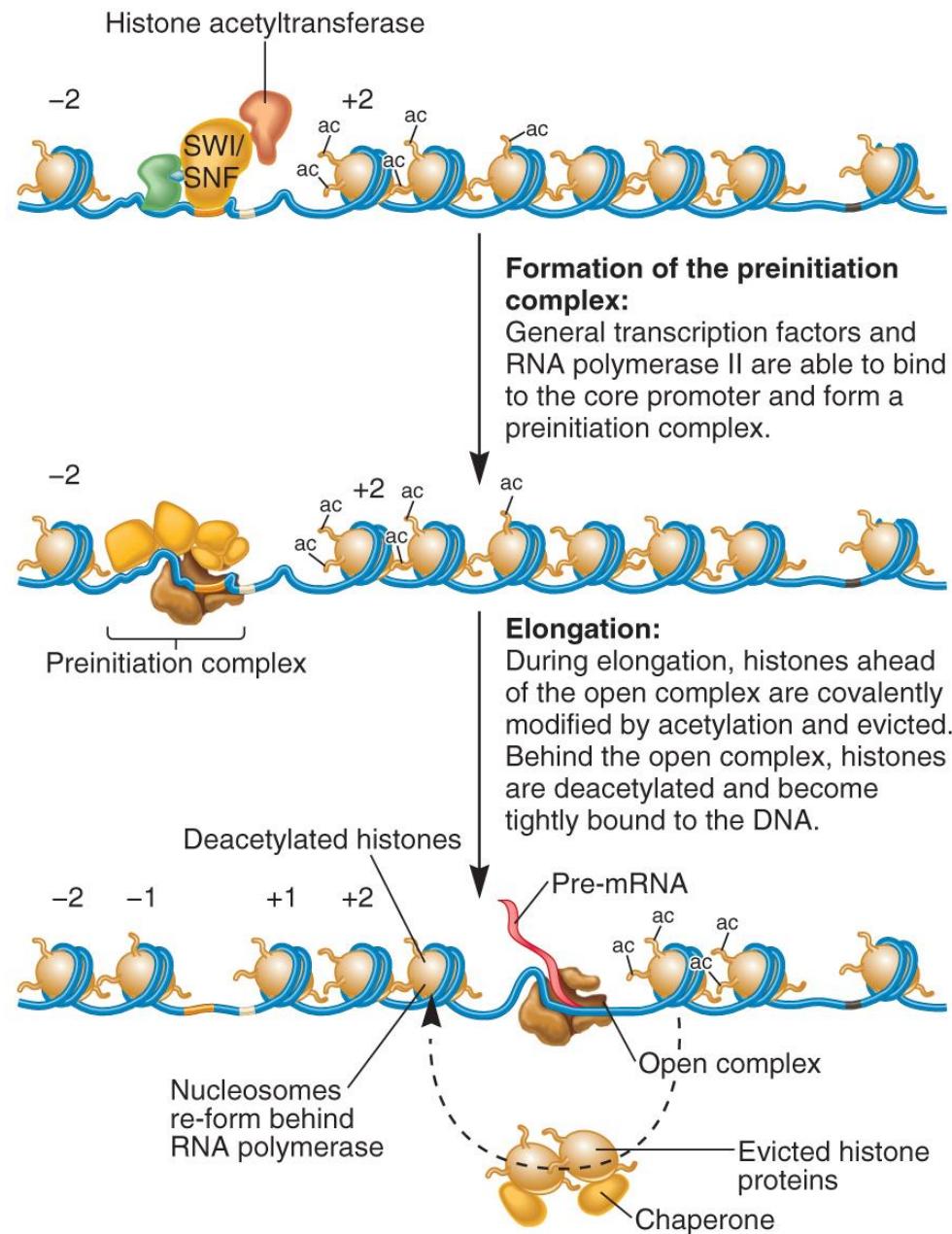
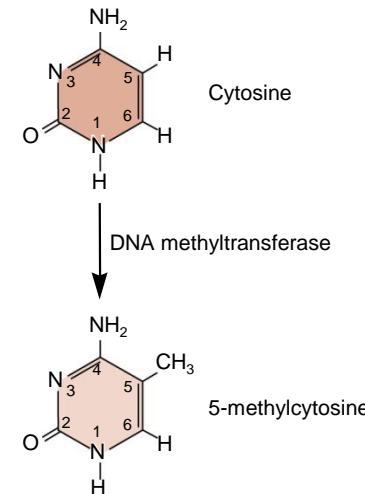


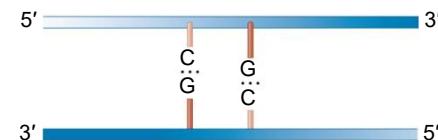
Figure 17.10

17.3 DNA Methylation

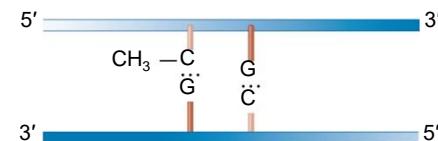
- Carried out by the enzyme **DNA methyltransferase**
- Methyl group added to a cytosine
- It is common in some eukaryotic species, but not all
 - Yeast and *Drosophila* have little DNA methylation
 - Vertebrates and plants have abundant DNA methylation
 - In mammals, ~ 2 to 7% of the DNA is methylated
- Refer to Figure 17.11



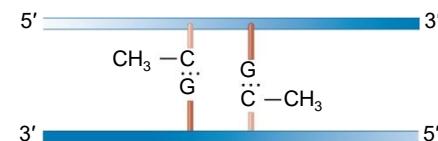
(a) The methylation of cytosine



(b) Unmethylated



(c) Hemimethylated

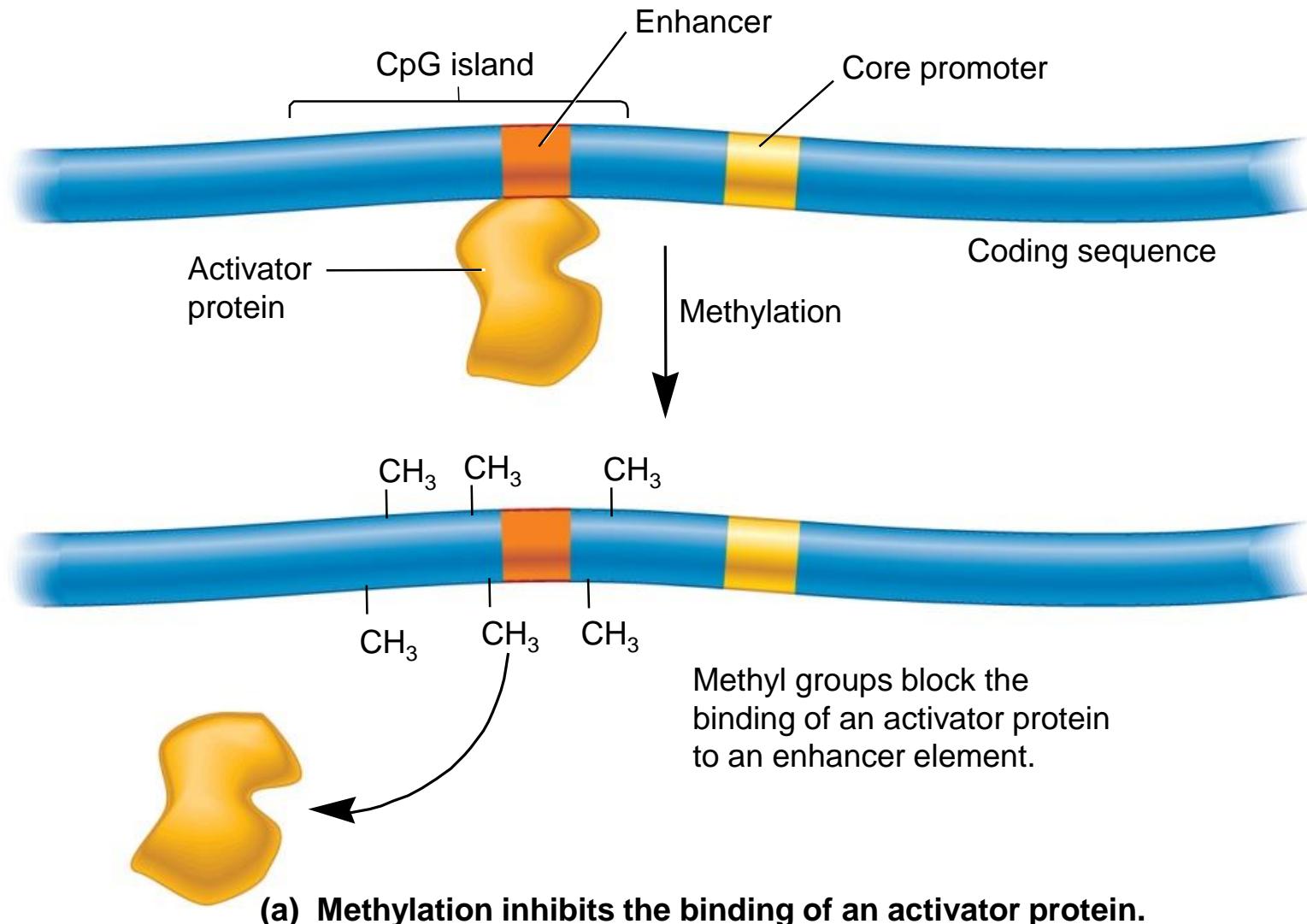


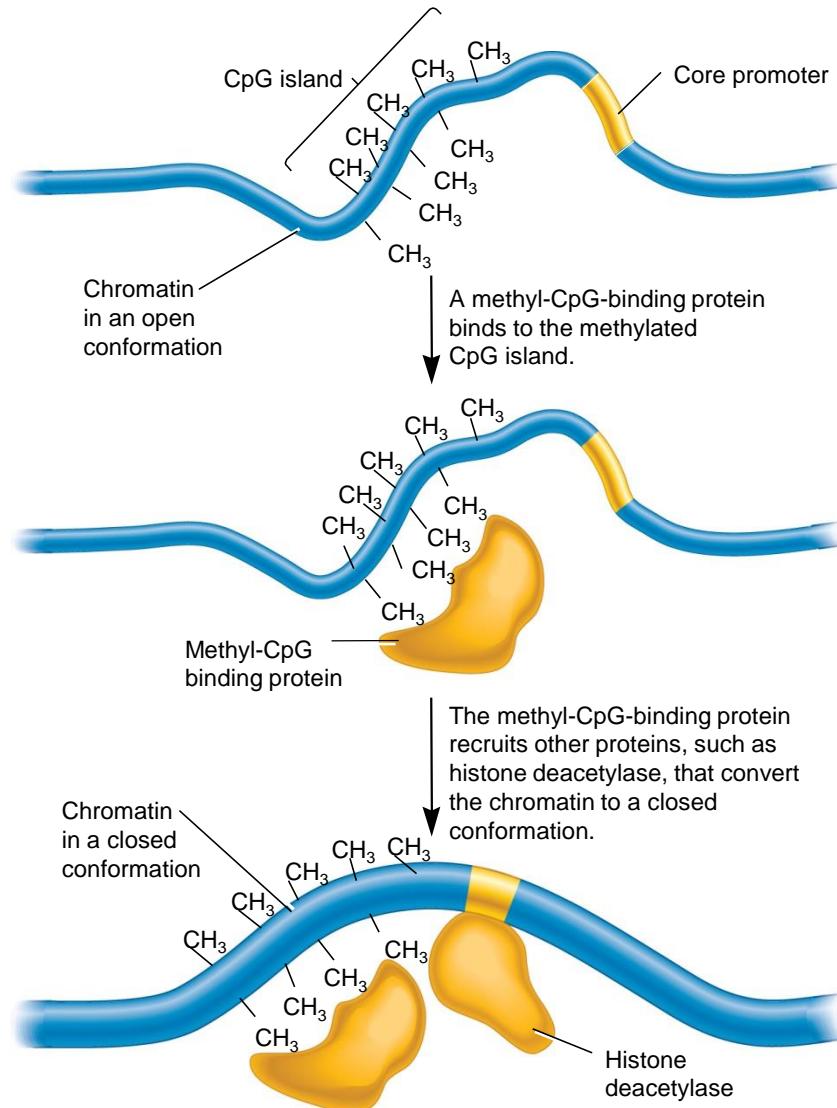
(d) Fully methylated

- In vertebrates and plants, many genes contain **CpG islands** near their promoters
- These CpG islands are 1,000 to 2,000 nucleotides long and contain high number of CpG sites
 - The CpG islands are unmethylated in **housekeeping genes** (genes that tend to be expressed in most cells)
 - The CpG islands tend to be methylated in **tissue-specific genes** when they are not expressed

- Methylation results in

- Proteins binding to the methylated cytosine and **inhibiting transcription**
- A chromatin structure that silences gene expression



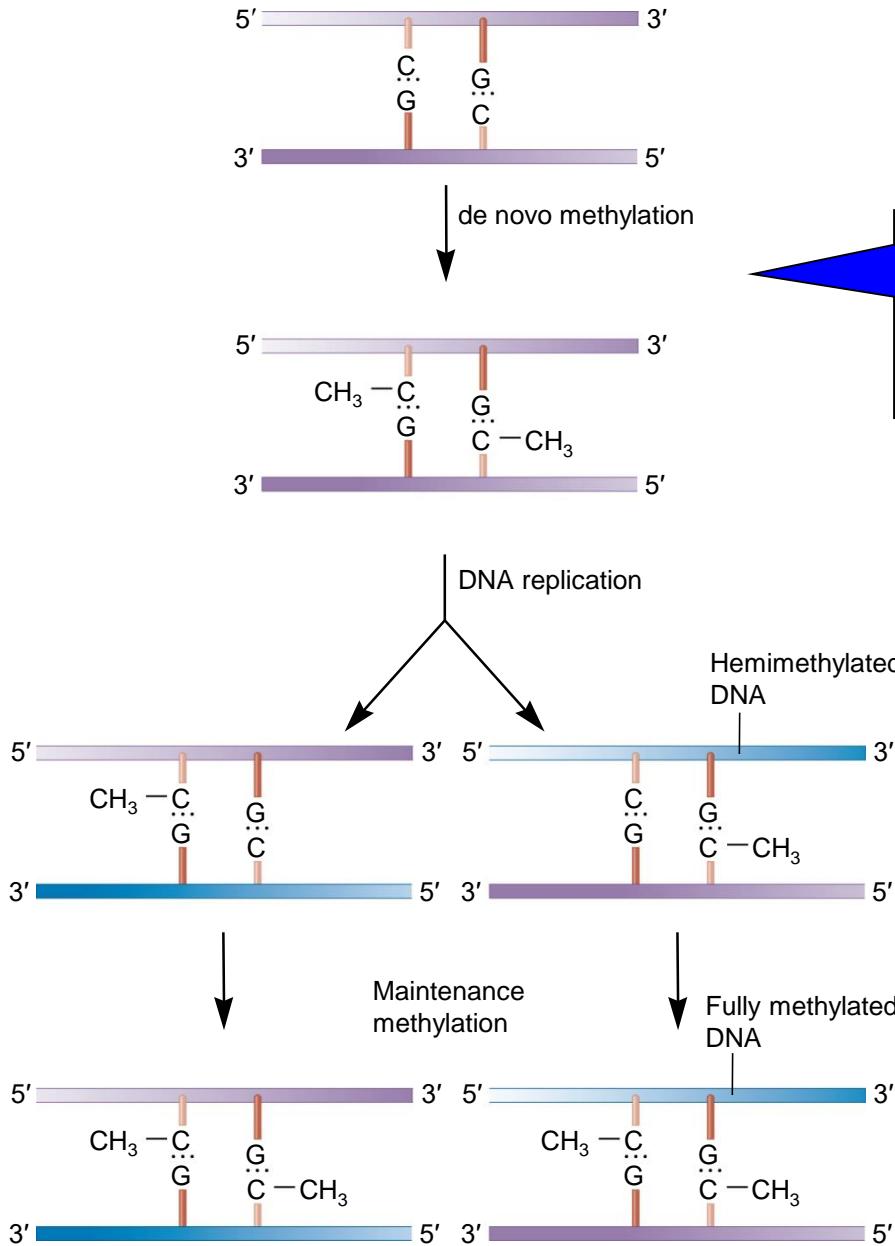


(b) Methyl-CpG-binding protein recruits other proteins that change the chromatin to a closed conformation.

Figure 17.12b

DNA Methylation is Heritable

- Methylated DNA sequences are inherited during cell division
 - Specific genes are methylated in gametes from mother or father (**de novo methylation**)
 - The methylation pattern is maintained in the resulting offspring (**maintenance methylation**)
- Refer to Figure 17.13

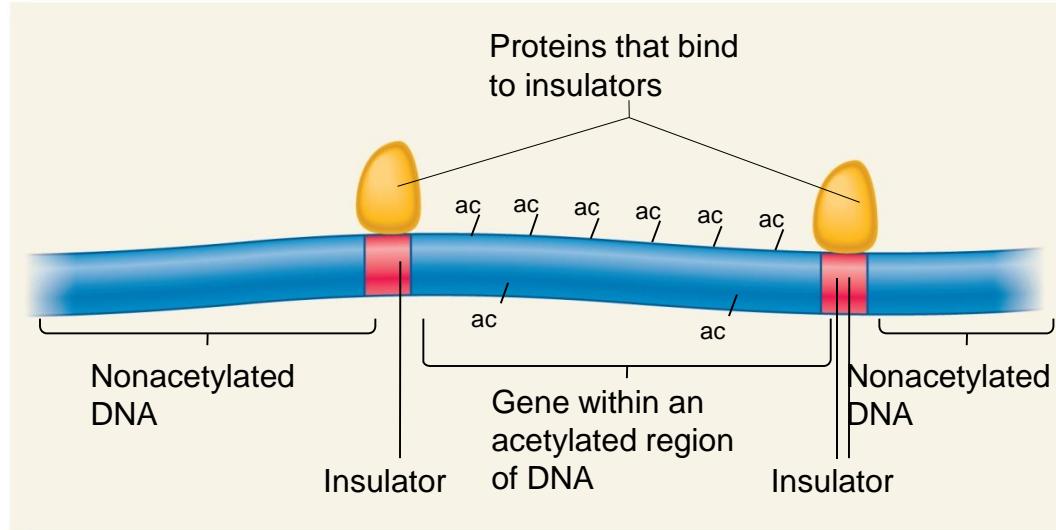


DNA methylase converts hemi-methylated to fully-methylated DNA. A frequent event.

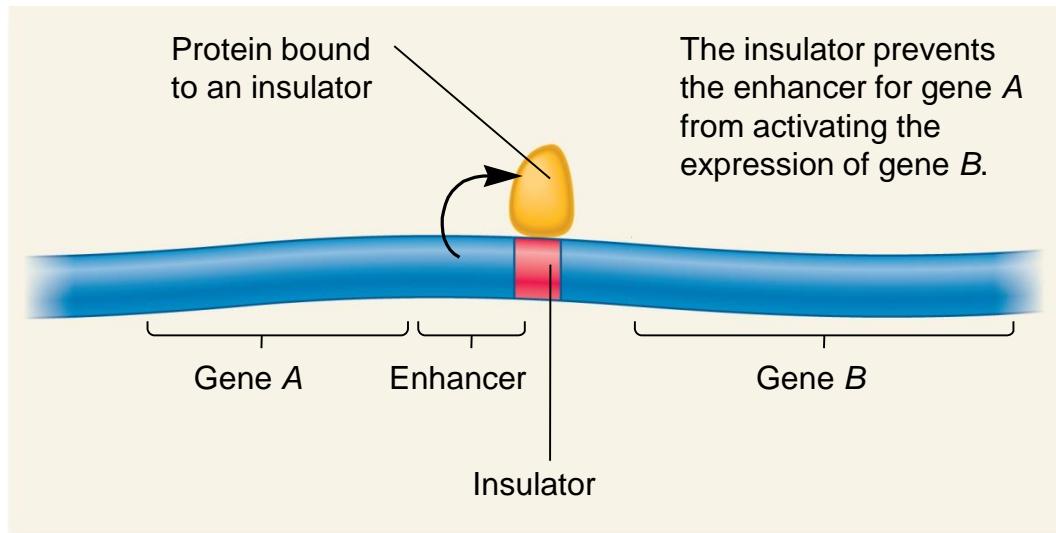
Figure 17.13

17.4 Insulators

- An **insulator** is a segment of DNA that functions as a boundary between two genes
- Insulator sequences that bind proteins, which
 - Act as barrier to chromatin or histone remodeling
 - Block effects of enhancers by formation of loops



(a) Insulators as a barrier to changes in chromatin structure



(b) Insulator that blocks the effects of a neighboring enhancer

17.5 Epigenetic Regulation

- Conrad Waddington coined “epigenetics” in 1941 – since then it’s been used to describe a wide variety of phenomena
 - Change in gene expression not based on the DNA sequence itself
 - Long-term maintenance of the change in expression
 - Passed from cell to cell
 - May be passed from generation to generation
- **Epigenetics** – The study of mechanisms that lead to changes in gene expression, can be passed from cell to cell, are reversible, and do not change the DNA sequence

Different Types of Molecular Changes underlie Epigenetic Gene Regulation

- Different molecular changes can underlie epigenetic phenomena
 - DNA methylation
 - Chromatin remodeling
 - Covalent histone modification
 - Localization of histone variants
 - Feedback loops

TABLE 17.2**Molecular Mechanisms that Underlie Epigenetic Gene Regulation**

Type of Modification	Description
DNA methylation	Methyl groups may be attached to cytosine bases in DNA. When this occurs near promoters, transcription is often inhibited.
Chromatin remodeling	Nucleosomes may be moved to new locations or evicted. When such changes occur in the vicinity of promoters, the level of transcription may be altered. Also, larger scale changes in chromatin structure may occur such as those that happen during X-chromosome inactivation in female mammals.
Covalent histone modification	Specific amino acid side chains found in the amino terminal tails of histones can be covalently modified. For example, they can be acetylated or phosphorylated. Such modifications may enhance or inhibit transcription.
Localization of histone variants	Histone variants may become localized to specific locations, such as near the promoters of genes, and affect transcription.
Feedback loop	The activation of a gene that encodes a transcription factor may result in a feedback loop in which that transcription factor continues to stimulate its own expression.

Table 17.2

Epigenetic Regulation – Either Developmental or Environmental

- Many epigenetic modifications are a normal part of development
 - Example: **Genomic imprinting**
 - The maternal *Igf2* allele is silenced, but the paternal allele is active
 - Example: **X-chromosome inactivation**
 - In each cell of the early embryo, one X chromosome becomes a Barr body
- However, **environmental agents** can also affect epigenetic regulation
 - Temperature, diet, or toxins

TABLE 17.3
Factors that Promote Epigenetic Changes

Factor	Examples
Programmed changes during development	
Genomic imprinting	Certain genes, such as <i>Igf2</i> described in Chapter 6, undergo different patterns of DNA methylation during oogenesis and spermatogenesis. Such patterns affect whether the maternal or paternal allele is expressed in offspring.
X-chromosome inactivation	As described in Chapter 6, X-chromosome inactivation occurs during embryogenesis in female mammals.
Cell differentiation	The differentiation of cells into particular cell types involves epigenetic changes such as DNA methylation and covalent histone modification.
Environmental agents	
Temperature	In flowering plants, cold winter temperatures cause specific types of covalent histone modifications that are thought to affect the expression of specific genes the following spring. This process may be necessary for germination and flowering in the spring.
Diet	The different diets of queen and worker bees alter DNA methylation patterns, which affect the expression of many genes. Such effects may underlie the different body types of queen and worker bees.
Toxins	Cigarette smoke contains a variety of toxins that affect DNA methylation and covalent histone modifications in lung cells. These epigenetic changes may play a role in the development of lung cancer. In addition, metals, such as chromium and cadmium, and certain chemicals found in pesticides and herbicides, cause epigenetic changes that can affect gene expression.

Table 17.3

<https://www.ibiology.org/speakers/c-david-allis/>

Genomic Imprinting Occurs During Gamete Formation

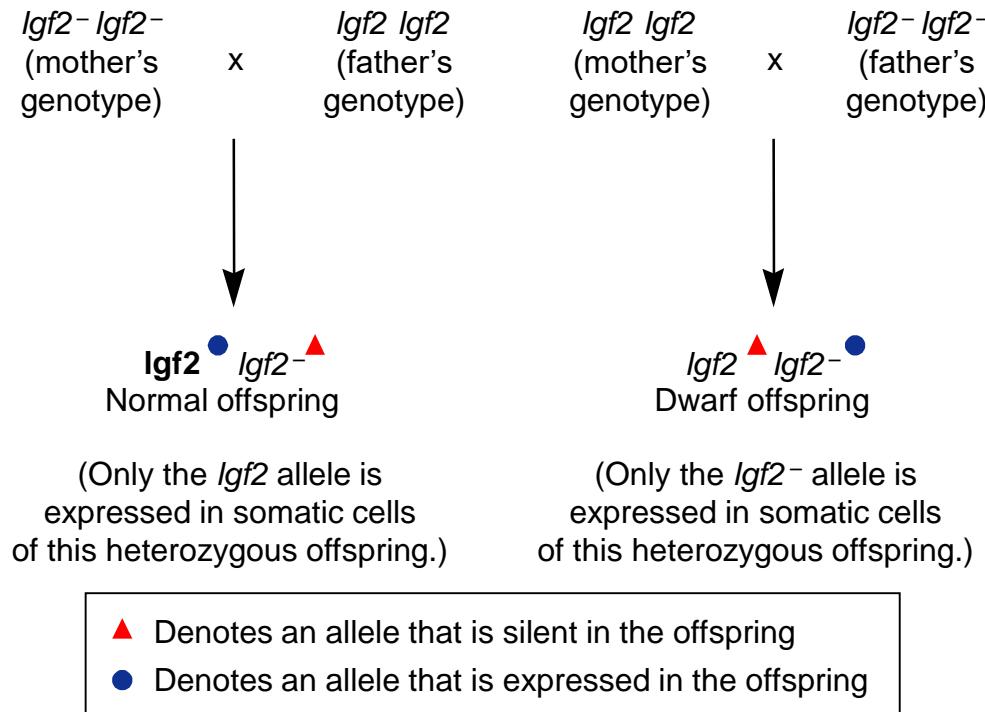
- Epigenetic changes play key roles in the development of plants and animals
- Example: **Genomic imprinting of the *Igf2* gene**
 - The allele from the father is expressed, the mother's alleles is not
 - Based on patterns of DNA methylation which occurs in two regions near the gene
 - The **imprinting control region (ICR)**
 - The **differentially methylated region (DMR)**

Genomic Imprinting

- **Genomic imprinting** - expression of a gene depends on whether it is inherited from the male or the female parent
 - Several mammalian genes are imprinted
 - Biological significance not always clear
- Phenotypes controlled by imprinted genes have a non-Mendelian pattern of inheritance
 - Offspring express either the maternally-inherited or the paternally-inherited allele but not both
 - This is termed **monoallelic expression**

- Example: ***Igf-2* in mice**
 - *Igf-2* encodes a growth hormone called insulin-like growth factor 2
 - Functional *Igf-2* gene is necessary for normal size
 - Imprinting causes **expression of the paternal allele** but not the maternal allele
 - The paternal allele is transcribed into RNA
 - The maternal allele is not transcribed
 - *Igf-2* ⁻ is a mutant allele that makes a defective protein
 - This causes a mouse to be dwarf
 - But **only if inherited from the father**

From chapter 6



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Figure 6.7

- Imprinting can be divided into **three stages**
 1. **Establishment** of the imprint during gametogenesis
 2. **Maintenance** of the imprint during embryogenesis and in the adult somatic cells
 3. **Erasure and reestablishment** of the imprint in the germ cells

Establishment of the imprint

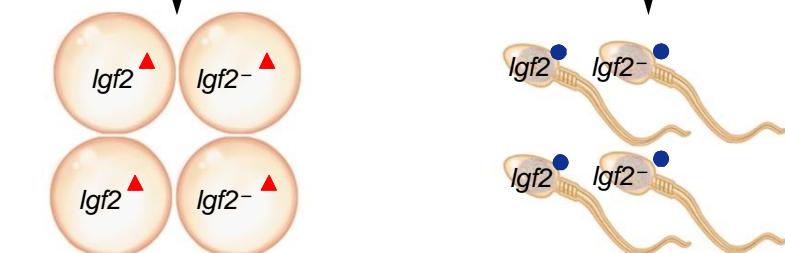
In this example, imprinting occurs during gametogenesis in the *Igf2* gene, which exists in the *Igf2* allele from the male and the *Igf2⁻* allele from the female. This imprinting occurs so that only the paternal allele is expressed.

**Maintenance of the imprint**

After fertilization, the imprint pattern is maintained throughout development. In this example, the maternal *Igf2⁻* allele will not be expressed in the somatic cells. Note that the offspring on the left is a female and the one on the right is a male; both are normal in size.

**Erasure and reestablishment**

During gametogenesis, the imprint is erased. The female mouse produces eggs in which the gene is silenced. The male produces sperm in which the gene can be transcribed into mRNA.



Eggs carry
silenced alleles

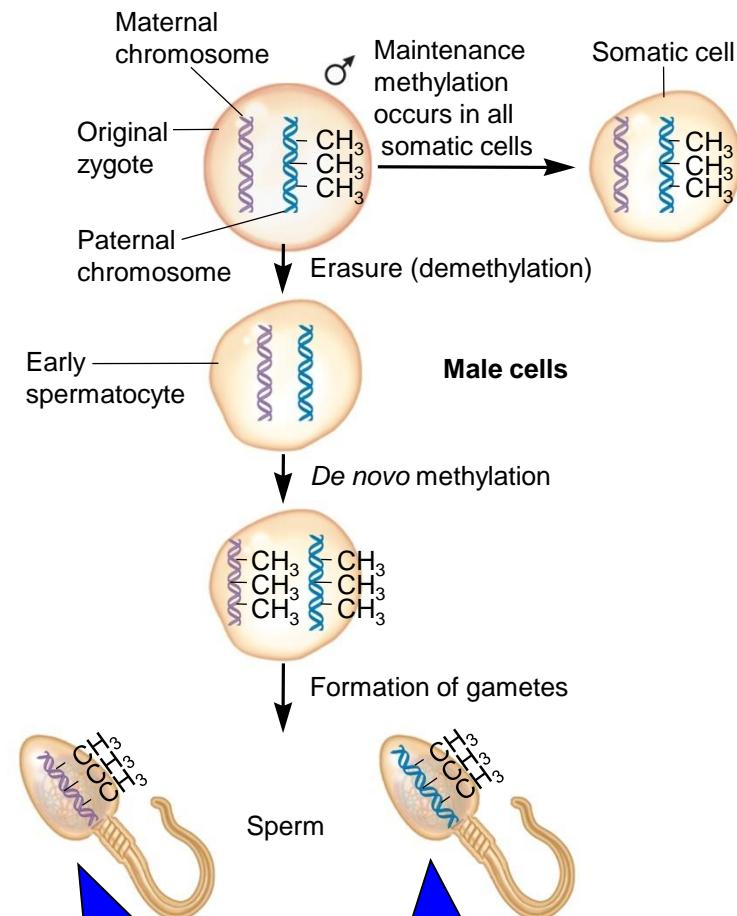
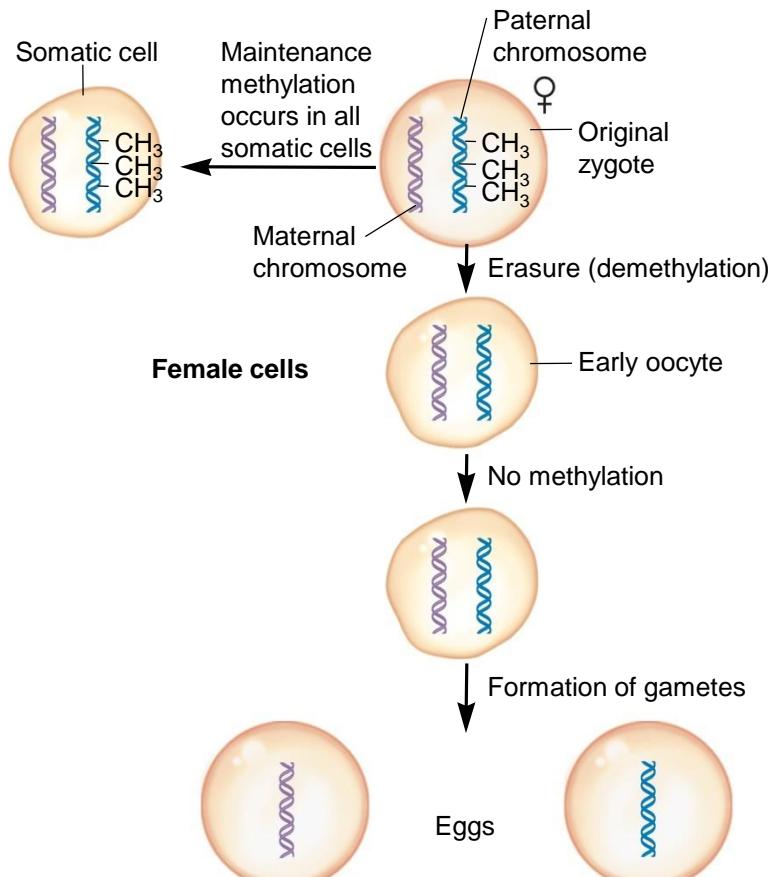
- ▲ Silenced allele
- Transcribed allele

Sperm carry
expressed alleles

Figure 6.8

From chapter 6

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Female gametes have
unmethylated ICR

Male gametes have
methylated ICR



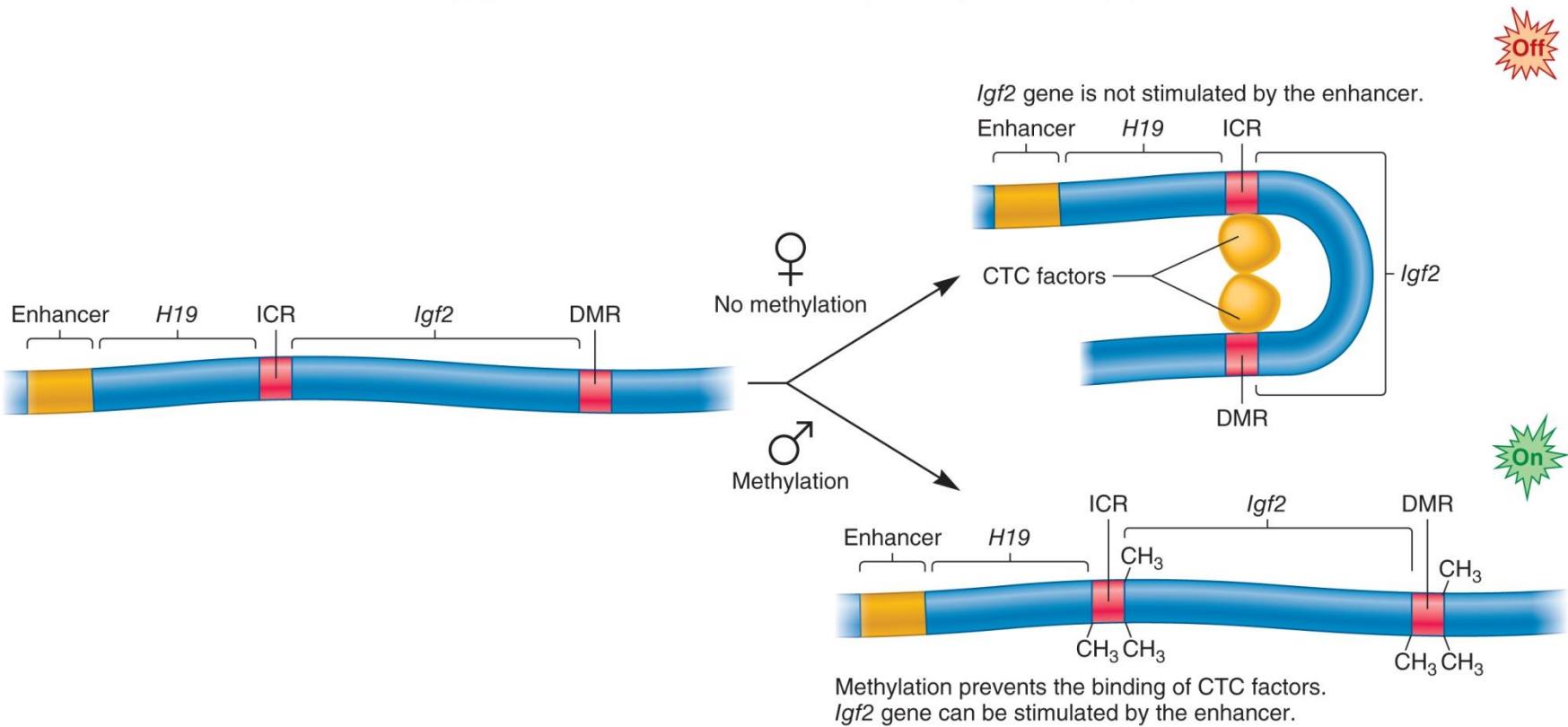


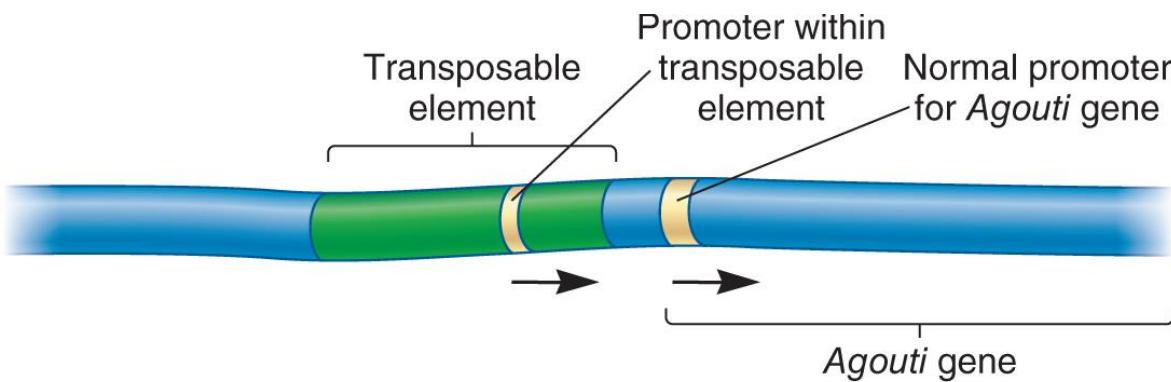
Figure 17.15

Environmental Agents and Epigenetic Changes

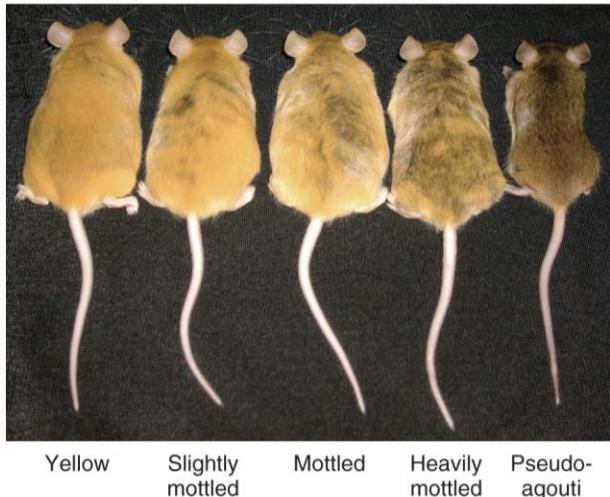
- Environmental agents can also cause long-lasting epigenetic changes
 - Example: **The *Agouti* gene in mice**
 - Controls deposition of yellow pigment on fur
 - Melanocytes in a hair follicle start out making black pigment, then yellow (using *Agouti* gene), then black again
 - Wildtype expression makes brown-looking fur
 - No *Agouti* expression – black fur
 - High *Agouti* expression – yellow fur

| Black
| Yellow
| black

- Unusual allele of *Agouti* – ***A^{v/y}* allele**
 - These mice have wide phenotypic variation
 - May be yellow, mottled, or pseudo-agouti
 - Allele has an inserted transposable element upstream of the promoter
 - TEs are very sensitive to epigenetic modifications
- If pregnant *A^{v/y}* mice are exposed to different diets, the color of their pups will change
 - S-adenosyl methionine is used for DNA methylation
 - Folic acid, vitamin B₁₂, betaine, and choline affect levels of SAM
 - Mothers fed these supplements had pups with darker fur

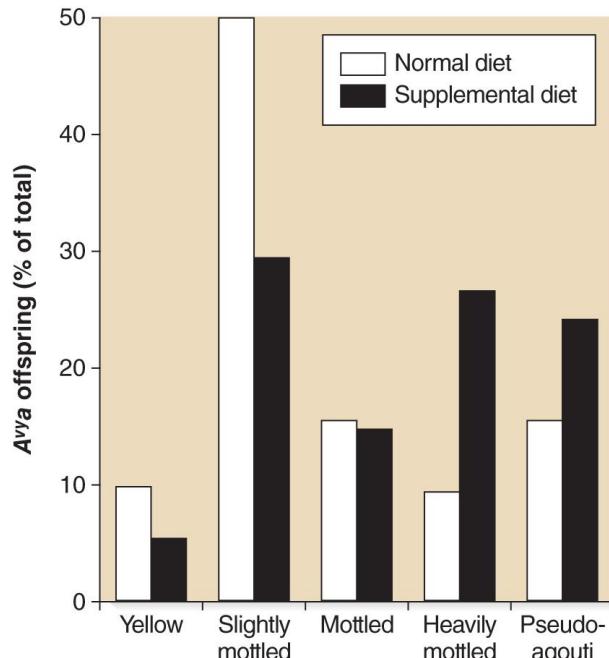


(a) The insertion of a transposable element to create the *A^{v/y}* allele

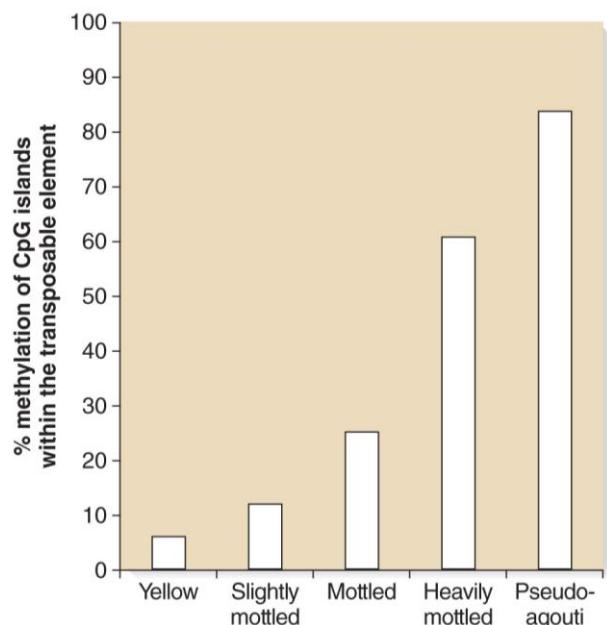


(b) Range in coat-color phenotypes in *A^va* mice due to epigenetic changes

b: From: D.C. Dolinoy et al., "Maternal genistein alters coat color and protects *Avy* mouse offspring from obesity by modifying the fetal epigenome," *Environ Health Perspect.* 2006 Apr; 114(4): 567-572. Reproduced with permission



(c) Effect of diet on coat color



(d) Level of DNA methylation of CpG islands within the TE among mice with different coat colors

17.6 Regulation of RNA Processing, RNA Stability, and Translation

Regulation After Transcription

- Although regulation of gene expression primarily occurs at the transcriptional level, control also occurs at other levels before and after translation
 - Alternative splicing
 - RNA stability
 - RNA interference
 - Translational regulation

TABLE 17.4

Gene Regulation via RNA Processing and Translation

Effect	Description
Alternative splicing	Certain pre-mRNAs can be spliced in more than one way, leading to polypeptides that have different amino acid sequences. Alternative splicing is often cell-specific so that a protein can be fine-tuned to function in a particular cell type. It is an important form of gene regulation in multicellular eukaryotic species.
RNA stability	The amount of RNA is greatly influenced by the half-life of RNA transcripts. A long polyA tail on mRNAs promotes their stability due to the binding of polyA-binding protein. Some RNAs with a relatively short half-life contain sequences that target them for rapid degradation. Some RNAs are stabilized by specific RNA-binding proteins that usually bind near the 3' end.
RNA interference	Double-stranded RNA can mediate the degradation of specific mRNAs in the cell or prevent them from being translated. This is a mechanism of gene regulation. Also, it probably provides eukaryotic cells with protection from invasion by certain types of viruses and may prevent the movement of transposable elements.
Translational regulation of mRNAs	Some mRNAs are regulated via binding proteins that inhibit the ability of the ribosomes to initiate translation. These proteins usually bind at the 5' end of the mRNA, thereby preventing the ribosome from binding.

Alternative Splicing

- Often, the pre-mRNA can be spliced in more than one way
 - In most cases, large sections of the coding region are the same, resulting in two alternative versions of a protein that have similar functions
 - Some exons always used – **constitutive exons**
 - Some are not – **alternative exons**
 - Nevertheless, there will be enough differences in amino acid sequences to provide each protein with its own unique characteristics

- Example: Alternative splicing for the α -tropomyosin gene
- α -tropomyosin protein found in both smooth and striated muscle
 - Functions in the regulation of cell contraction
 - Smooth and striated muscle regulate their contractions in subtly different ways
 - Produce different forms of α -tropomyosin by alternative splicing

Found in the mature mRNA from all cell types



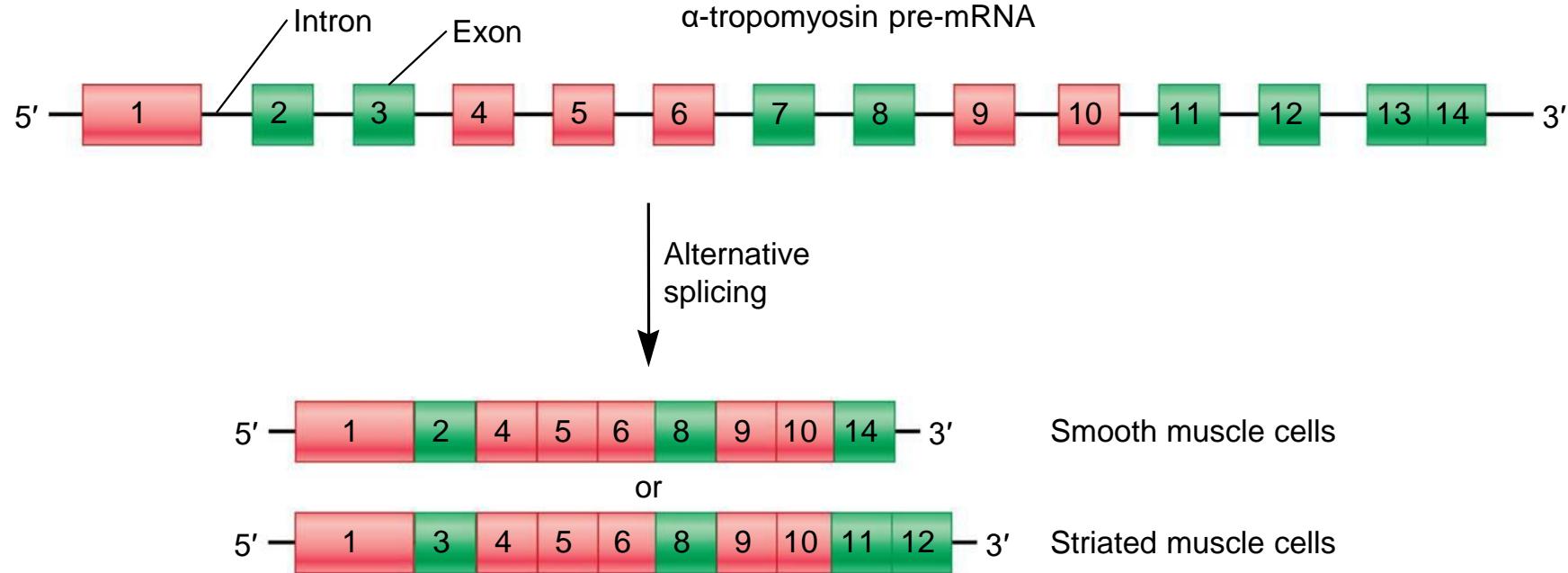
Constitutive exons

Not found in all mature mRNAs



Alternative exons

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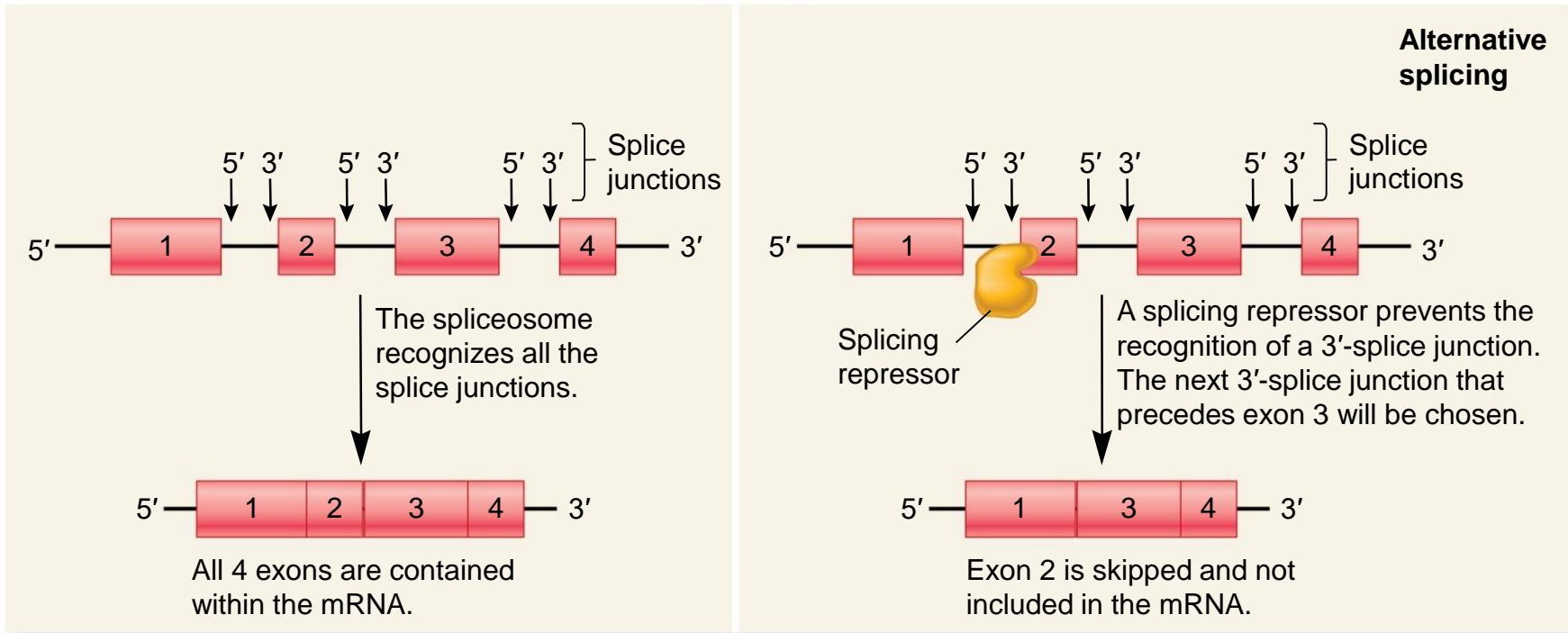


Alternatively spliced versions vary in function to meet the needs of the different cell types

- The degree of splicing and alternative splicing varies greatly among different species
 - Baker's yeast contains about 6,300 genes
 - ~ 300 (i.e., 5%) encode mRNAs that are spliced
 - Only a few of these 300 have been shown to be alternatively spliced
 - Humans contain ~ 25,000 genes
 - Most of these encode mRNAs that are spliced
 - It is estimated that about 70% are alternatively spliced
 - Note: Certain mRNAs can be alternatively spliced to produce dozens of different mRNAs

- Alternative splicing is not a random event
 - The specific pattern of splicing is regulated in a given cell
- It involves proteins known as **splicing factors**
 - These play a key role in the choice of splice sites
- One example of splicing factors is the **SR proteins**
 - At their C-terminal end, they have a domain that is rich in serine (S) and arginine (R)
 - It is involved in protein-protein recognition
 - At their N-terminal end, they have an RNA-binding domain

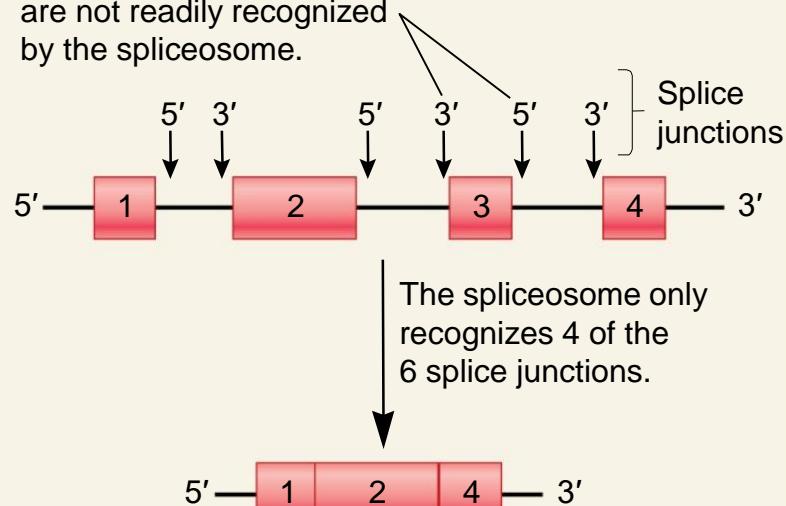
- Splicing factors modulate the ability of spliceosomes to recognize or choose the splice sites
- This can occur in two ways
 - 1. Some splicing factors *inhibit* the ability of a spliceosome to recognize a splice site – **exon skipping**
 - 2. Some splicing factors *enhance* the ability of a spliceosome to recognize a splice site
- Refer to Figure 17.18



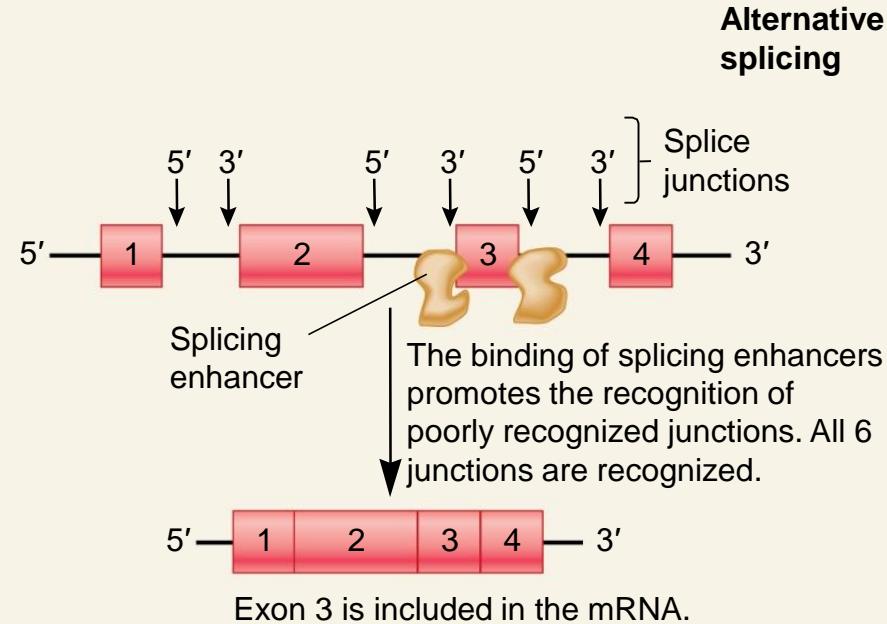
(a) Splicing repressors

Figure 17.18a

These 2 splice junctions
are not readily recognized
by the spliceosome.



The spliceosome only
recognizes 4 of the
6 splice junctions.



The binding of splicing enhancers
promotes the recognition of
poorly recognized junctions. All 6
junctions are recognized.

(b) Splicing enhancers

Figure 17.18b

Stability of mRNA

- The stability of eukaryotic mRNA varies considerably
 - Several minutes to several days
- The stability of mRNA can be regulated so that its half-life is shortened or lengthened
 - This will greatly influence the mRNA concentration
 - And consequently gene expression
- Factors that can affect mRNA stability include
 1. Length of the polyA tail
 2. Destabilizing elements

- Length of the polyA tail
 - Most newly made mRNA have a polyA tail that is about 200 nucleotides long
 - Tail recognized by **polyA-binding protein** which binds to the polyA tail and enhances stability
 - As an mRNA ages, its polyA tail is shortened by the action of cellular nucleases
 - If the tail is too short, polyA-binding protein can no longer bind
 - mRNA will rapidly degraded

- Destabilizing elements
 - Found especially in mRNAs that have short half-lives
 - These elements can be found anywhere on the mRNA
 - However, they are most common at the **3' untranslated region (3' UTR)**
 - The 3' end between the stop codon and the polyA tail
 - Example: **AU-rich element (ARE)** is found in many short-lived RNAs
 - Refer to Figure 17.19

AU-rich element (ARE)
Recognized and bound by cellular proteins. These proteins influence mRNA degradation

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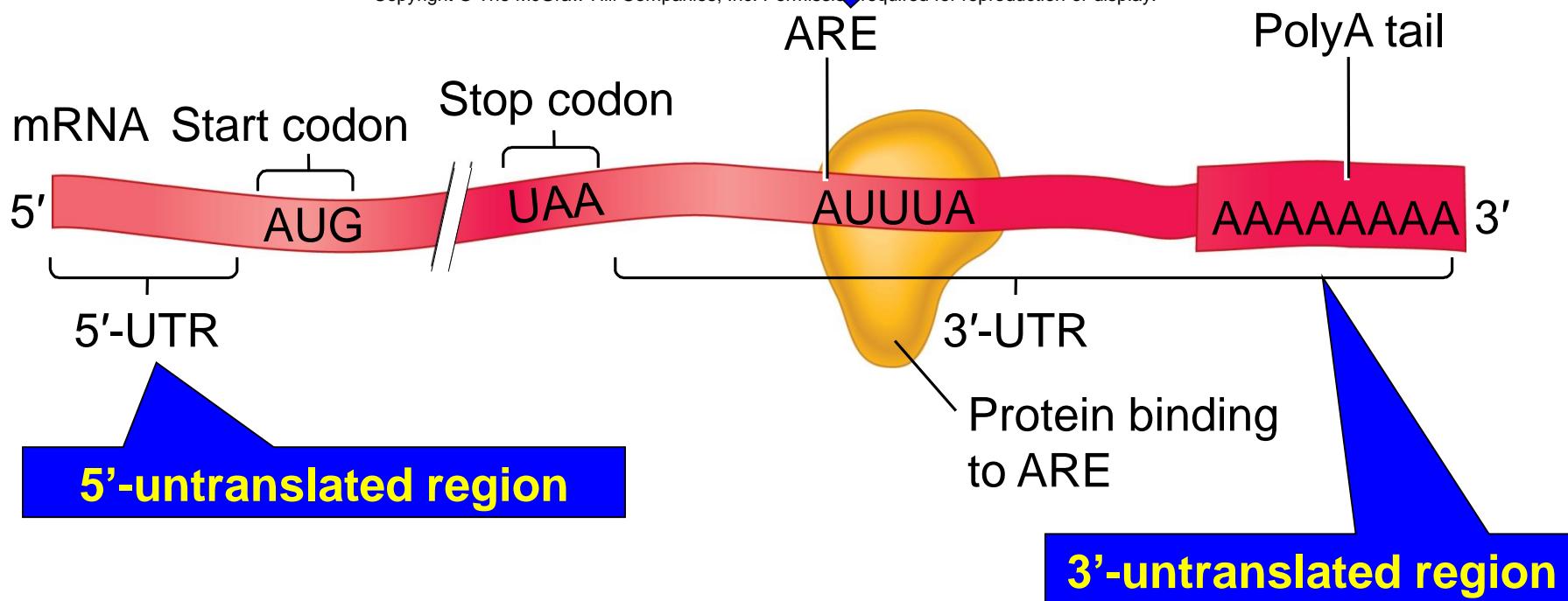


Figure 17.19

RNA Interference (RNAi)

- RNA that promotes RNAi can come from two sources:
 1. MicroRNAs
 2. Short interfering RNAs
- **MicroRNAs (miRNAs)**
 - RNAs that are transcribed from genes and form a hairpin structure
 - They play key roles in regulating gene expression, especially during development
 - Over 1000 genes in humans encode miRNAs

- **Short Interfering RNAs (siRNAs)**
 - Originate from two separate RNA molecules that come together to form dsRNA
 - Can be exogenous
 - From a virus
 - From a researcher wanting to study gene function
 - Play a key role in some viral infections
 - Also useful experimental tools
- Refer to Figure 17.20 for the mechanism of RNA interference, from either miRNA or siRNA

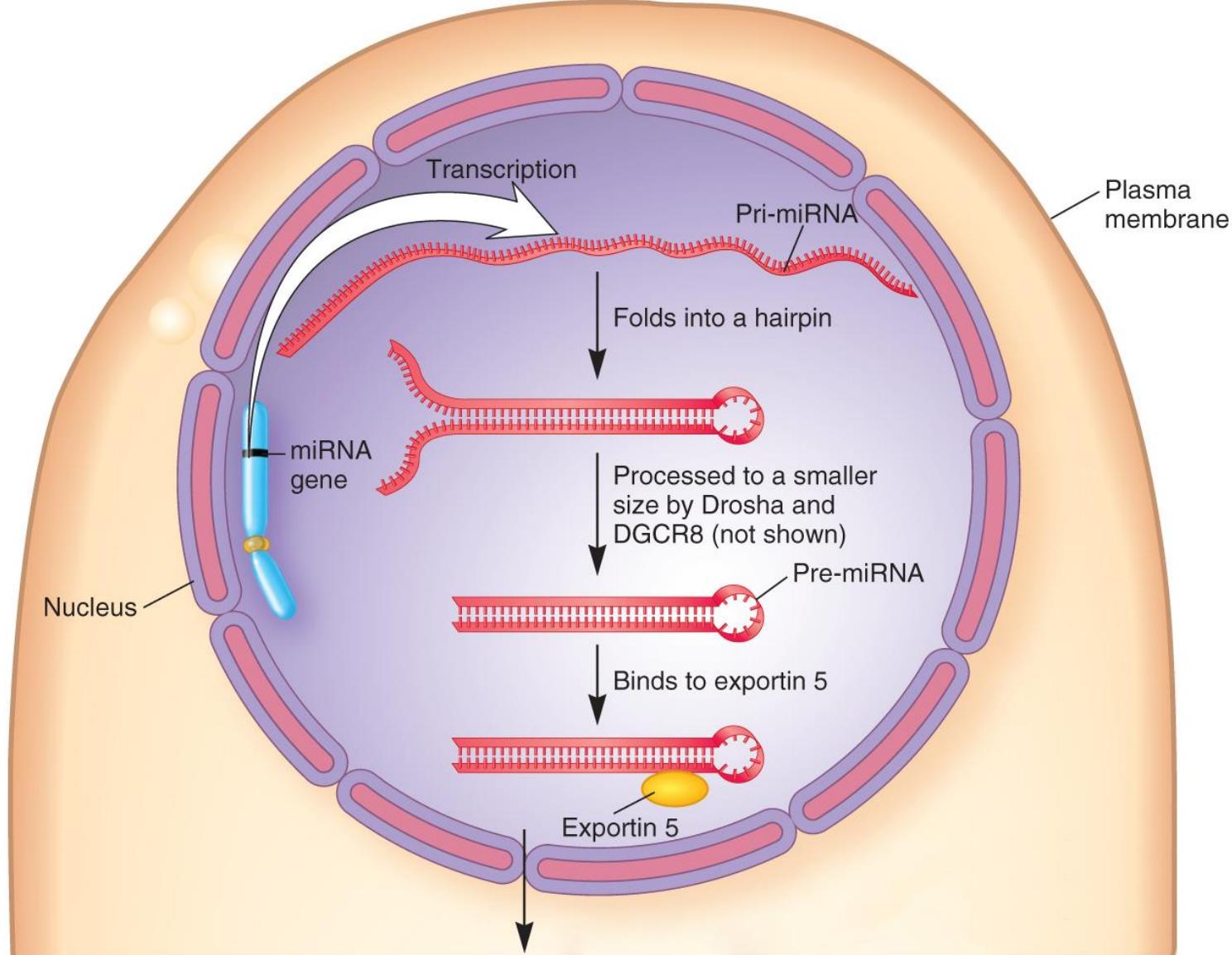


Figure 17.20

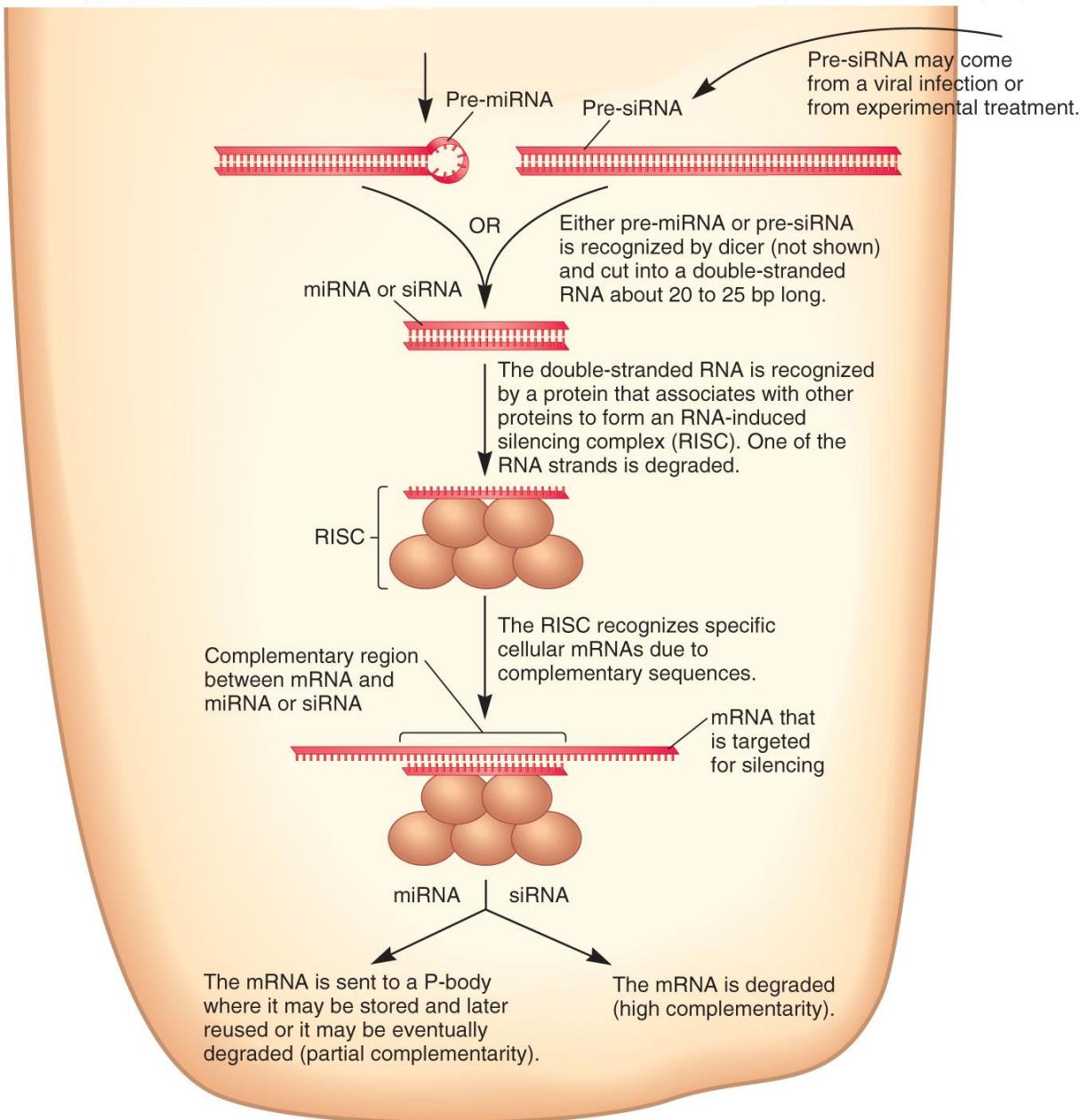


Figure 17.20

Benefits of RNA Interference

- Represents a newly identified form of gene regulation
- May offer a defense mechanism against certain viruses
 - Many RNA viruses have either dsRNA genome or exist as a dsRNA during their life cycle
- May play a role in silencing certain transposable elements
 - Random insertion may place an element near a cellular promoter which will produce a silencing RNA

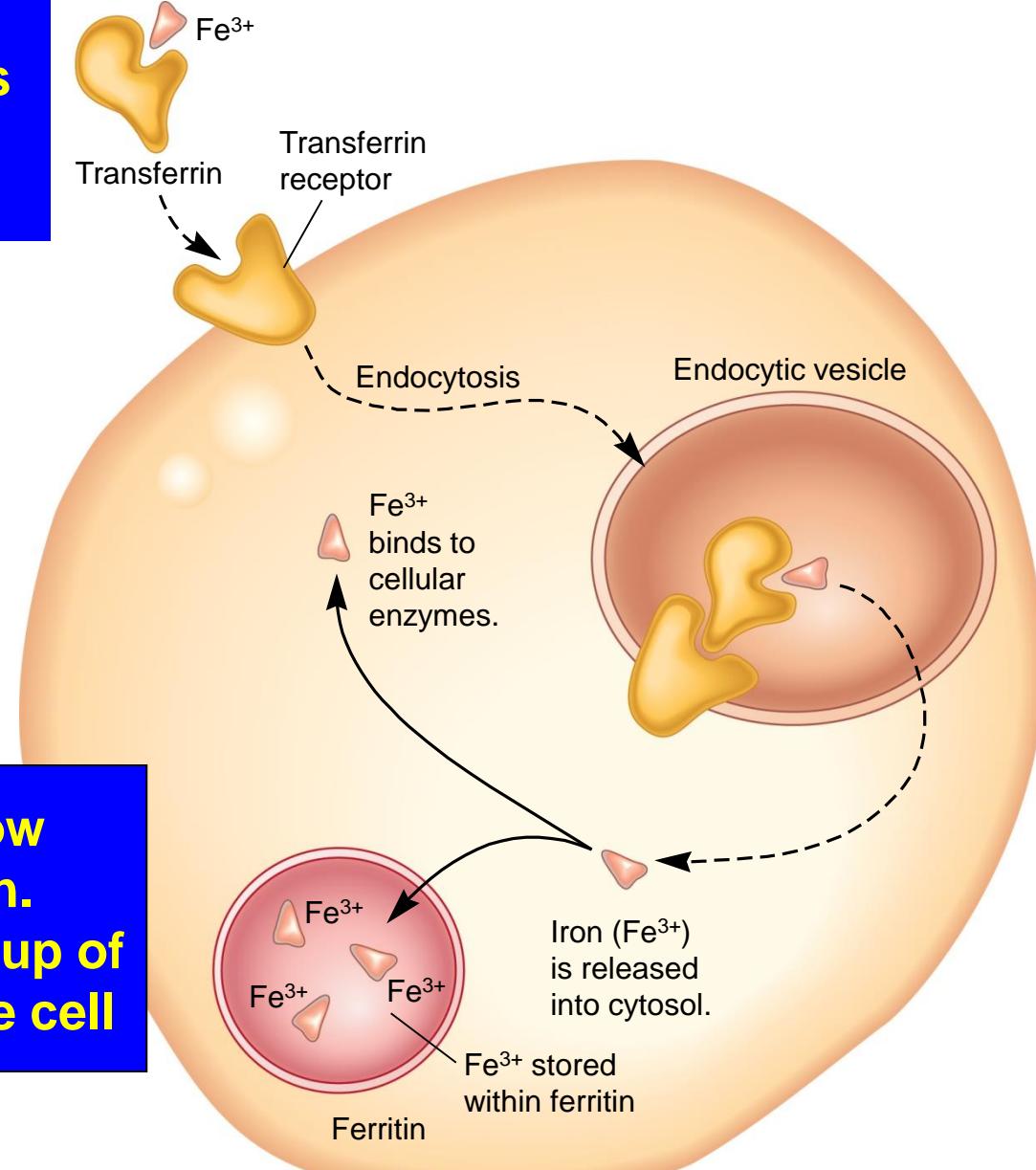
RNA-Binding Proteins Regulate Translation

- Example: **Regulation of iron assimilation**
 - Iron is an essential element for the survival of living organisms
 - It is required for many different enzymes
 - But it can be toxic
- Regulation of iron assimilation provides an example how the translation of specific mRNAs can be modulated
- Refer to Figure 17.21

- Control of transferrin receptor and ferritin levels is important to prevent toxic levels of iron to be present in the cytoplasm
- **Iron regulatory protein (IRP)** binds to transferrin receptor mRNA and to ferritin mRNA and controls their translation
 - IRP binds to a regulatory element within the mRNA known as the **iron response element (IRE)**
 - IRE is found in the 5'-UTR in ferritin mRNA
 - IRE is found in the 3'-UTR in transferrin receptor mRNA

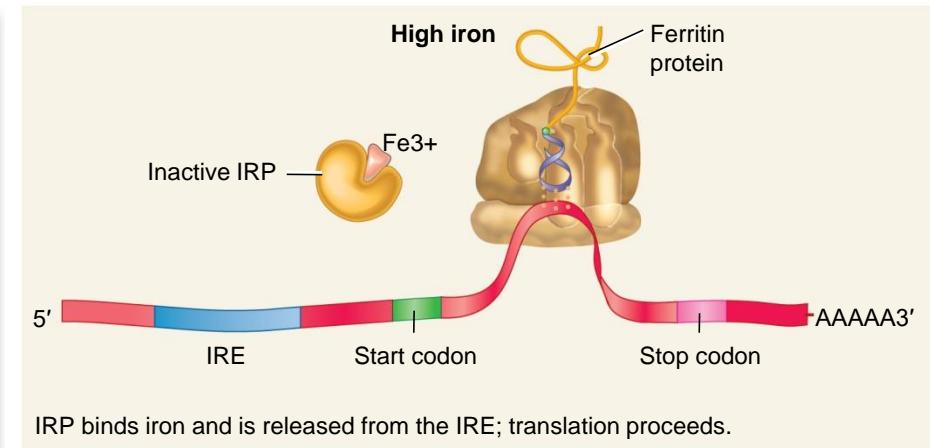
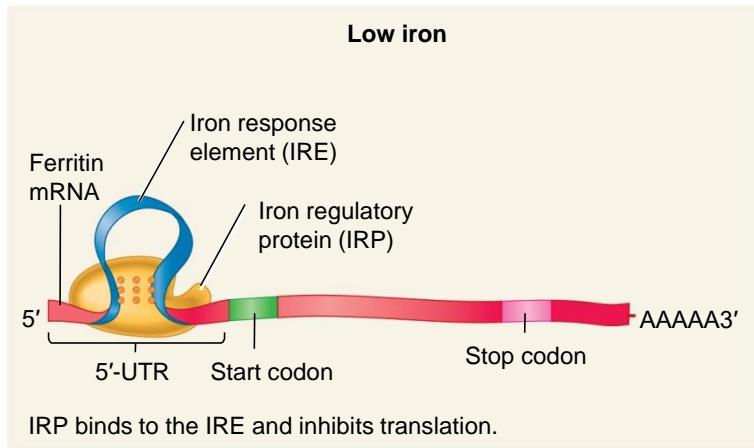
Transferrin is a protein that carries iron through the bloodstream

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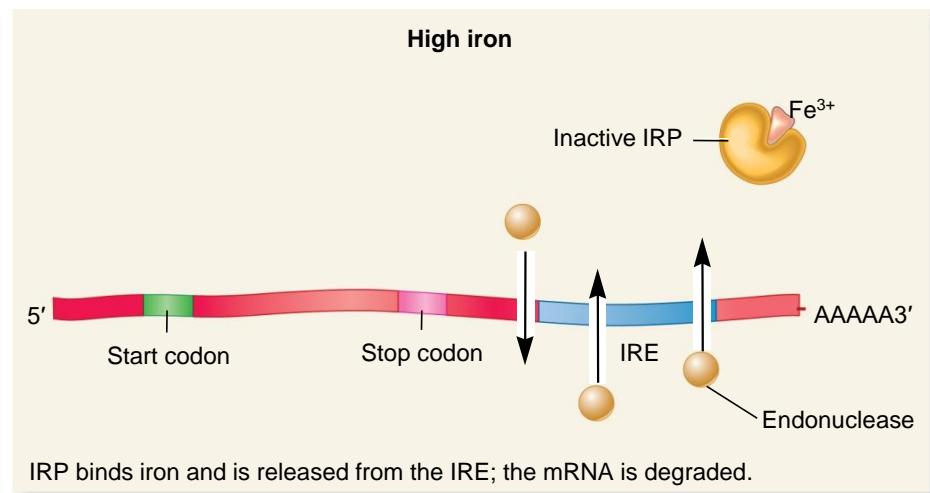
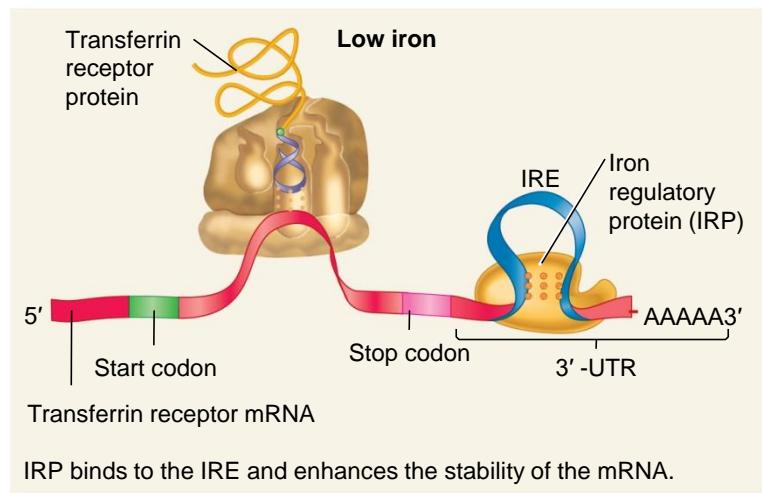


Ferritin is a hollow spherical protein.
Prevents toxic buildup of too much iron in the cell

Figure 17.21



(a) Regulation of ferritin mRNA



(b) Regulation of transferrin receptor mRNA