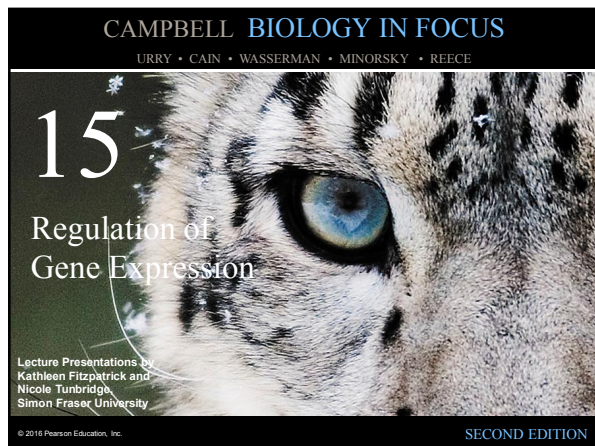




Overview: Beauty in the Eye of the Beholder

- Prokaryotes and eukaryotes alter gene expression in response to their changing environment
- Multicellular eukaryotes also develop and maintain multiple cell types

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Figure 15.1

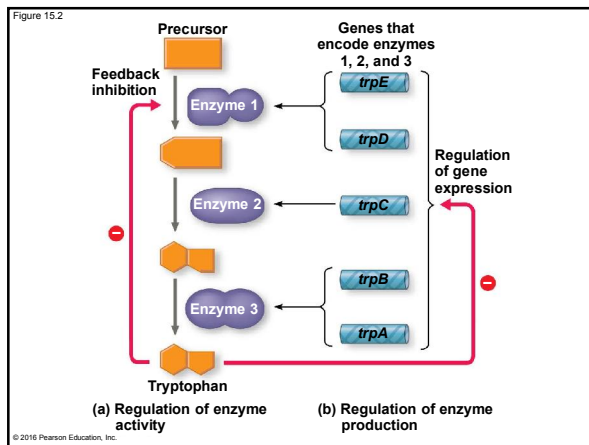


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Concept 15.1: Bacteria often respond to environmental change by regulating transcription

- Natural selection has favored bacteria that produce only the gene products needed by the cell
- A cell can regulate the production of enzymes by feedback inhibition or by gene regulation
- Gene expression in bacteria is controlled by a mechanism described as the operon model

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Operons: The Basic Concept

- A group of functionally related genes can be coordinately controlled by a single “on-off switch”
- The regulatory “switch” is a segment of DNA called an **operator**, usually positioned within the promoter
- An **operon** is the entire stretch of DNA that includes the operator, the promoter, and the genes that they control

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- The operon can be switched off by a protein **repressor**
- The repressor prevents gene transcription by binding to the operator and blocking RNA polymerase
- The repressor is the product of a separate **regulatory gene**

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- The repressor can be in an active or inactive form, depending on the presence of other molecules
- A **corepressor** is a molecule that cooperates with a repressor protein to switch an operon off
- The *trp* (tryptophan) operon is an example of such an operon

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- By default, the *trp* operon is on and the genes for tryptophan synthesis are transcribed
- When tryptophan is present, it binds to the *trp* repressor protein, which then turns the operon off
- The repressor is active only in the presence of its corepressor tryptophan; thus the *trp* operon is turned off (repressed) if tryptophan levels are high

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Figure 15.3

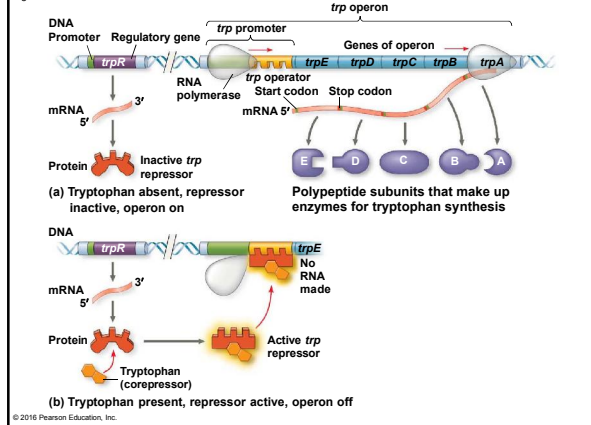


Figure 15.3-1

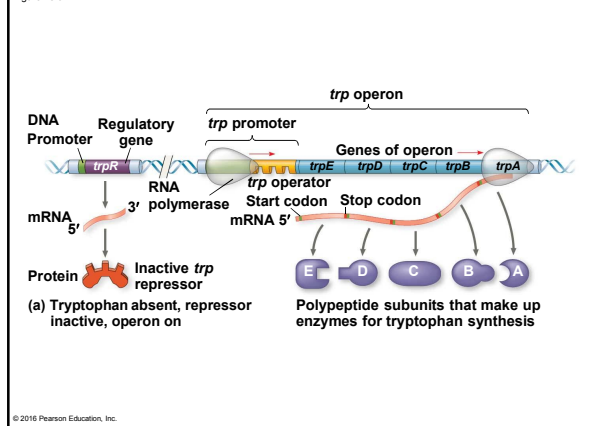
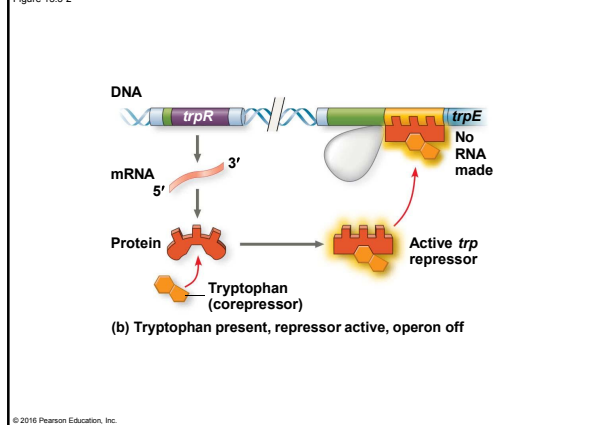


Figure 15.3-2



Repressible and Inducible Operons: Two Types of Negative Gene Regulation

- A repressible operon is one that is usually on; binding of a repressor to the operator shuts off transcription
- The *trp* operon is a repressible operon
- An inducible operon is one that is usually off; a molecule called an inducer inactivates the repressor and turns on transcription

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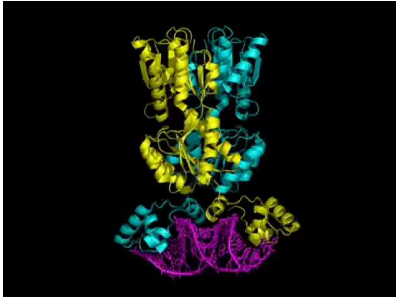
- The *lac* operon is an inducible operon and contains genes that code for enzymes that function in the use of lactose
- By itself, the *lac* repressor is active and switches the *lac* operon off
- A molecule called an **inducer** inactivates the repressor to turn the *lac* operon on

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- For the *lac* operon, the inducer is allolactose, formed from lactose that enters the cell
- Enzymes of the lactose pathway are called inducible enzymes
- Analogously, the enzymes for tryptophan synthesis are said to be repressible enzymes

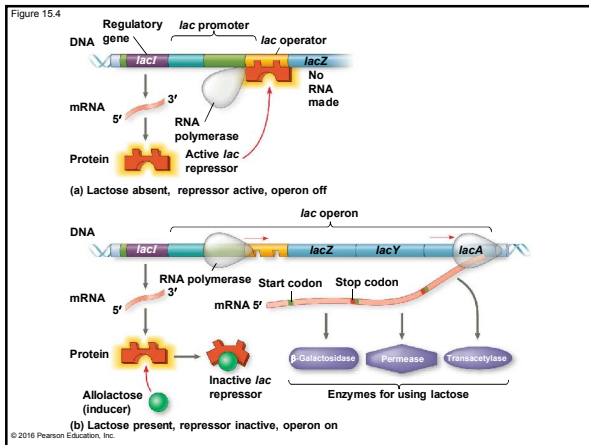
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Video: *lac* Repressor Model



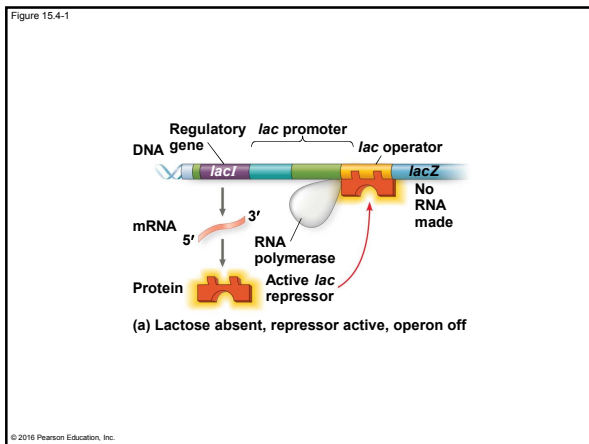
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Figure 15.4



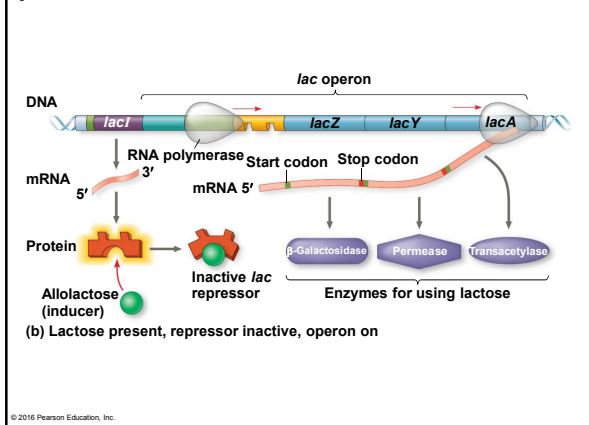
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Figure 15.4-1



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Figure 15.4-2



- Inducible enzymes usually function in catabolic pathways; their synthesis is induced by a chemical signal
- Repressible enzymes usually function in anabolic pathways; their synthesis is repressed by high levels of the end product
- Regulation of the *trp* and *lac* operons involves negative control of genes because operons are switched off by the active form of the repressor

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Positive Gene Regulation

- E. coli* will preferentially use glucose when it is present in the environment
- When glucose is scarce, CRP (cAMP receptor protein) acts as an **activator** of transcription
- CRP is activated by binding with **cyclic AMP (cAMP)**
- Activated CRP attaches to the promoter of the *lac* operon and increases the affinity of RNA polymerase, thus accelerating transcription

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- When glucose levels increase, CRP detaches from the *lac* operon, and transcription proceeds at a very low rate, even if lactose is present
- CRP helps regulate other operons that encode enzymes used in catabolic pathways

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Figure 15.5

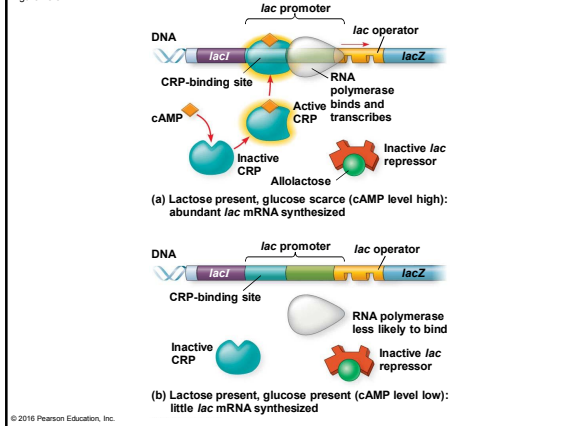


Figure 15.5-1

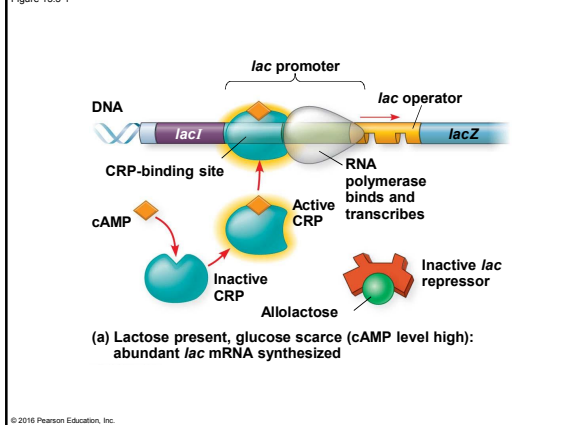
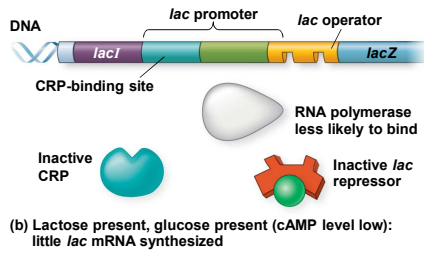


Figure 15.5-2



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Concept 15.2: Eukaryotic gene expression is regulated at many stages

- All organisms must regulate which genes are expressed at any given time
- In multicellular organisms, regulation of gene expression is essential for cell specialization

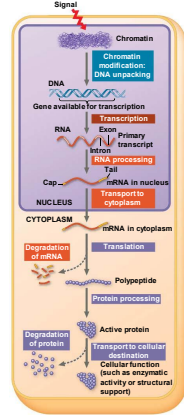
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Differential Gene Expression

- Almost all the cells in an organism contain an identical genome
- Differences between cell types result from **differential gene expression**, the expression of different genes by cells with the same genome
- Abnormalities in gene expression can lead to imbalances and diseases, including cancer
- Gene expression is regulated at many stages

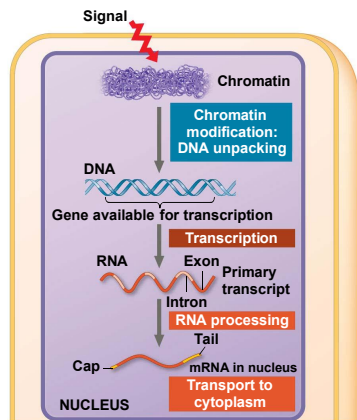
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Figure 15.6



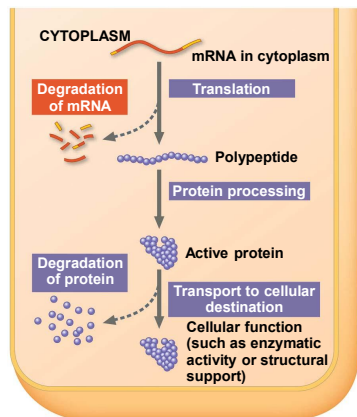
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Figure 15.6-1



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Figure 15.6-2



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- In all organisms, gene expression is commonly controlled at transcription
- The greater complexity of eukaryotic cell structure and function provides opportunities for regulating gene expression at many additional stages

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Regulation of Chromatin Structure

- The structural organization of chromatin packs DNA into a compact form and helps regulate gene expression in several ways
- The location of a gene promoter relative to nucleosomes and scaffold attachment sites can influence gene transcription

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- Genes within highly condensed heterochromatin are usually not expressed
- Chemical modifications to histone proteins and DNA can influence chromatin structure and gene expression

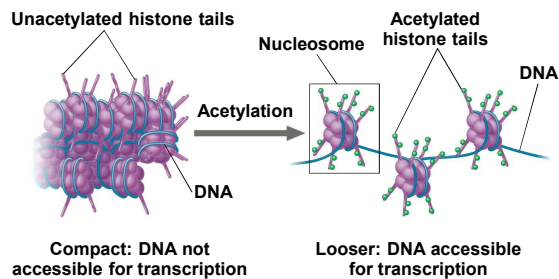
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Histone Modifications and DNA Methylation

- In **histone acetylation**, acetyl groups are attached to positively charged lysines in histone tails
- This generally loosens chromatin structure, promoting the initiation of transcription
- The addition of methyl groups (methylation) can condense chromatin and lead to reduced transcription

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Figure 15.7



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- **DNA methylation** is the addition of methyl groups to certain bases in DNA, usually cytosine
- Individual genes are usually more heavily methylated in cells where they are not expressed
- Once methylated, genes usually remain so through successive cell divisions
- After replication, enzymes methylate the correct daughter strand so that the methylation pattern is inherited

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Epigenetic Inheritance

- Though chromatin modifications do not alter DNA sequence, they may be passed to future generations of cells
- The inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called **epigenetic inheritance**
- Epigenetic modifications can be reversed, unlike mutations in DNA sequence

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Regulation of Transcription Initiation

- Chromatin-modifying enzymes provide initial control of gene expression by making a region of DNA either more or less able to bind the transcription machinery

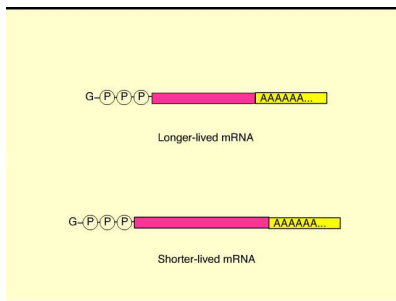
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Organization of a Typical Eukaryotic Gene

- Associated with most eukaryotic genes are multiple **control elements**, segments of noncoding DNA that serve as binding sites for transcription factors that help regulate transcription
- Control elements and the transcription factors they bind are critical for precise regulation of gene expression in different cell types

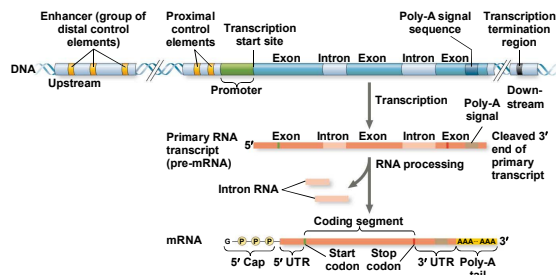
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Animation: mRNA Degradation



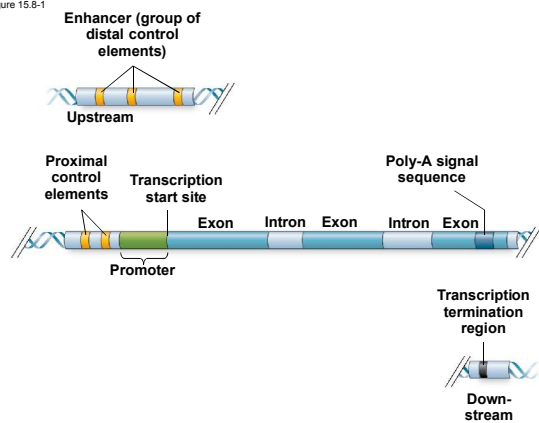
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Figure 15.8



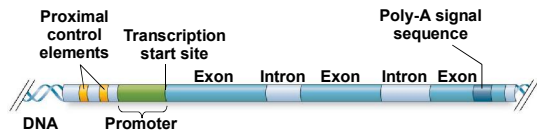
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Figure 15.8-1



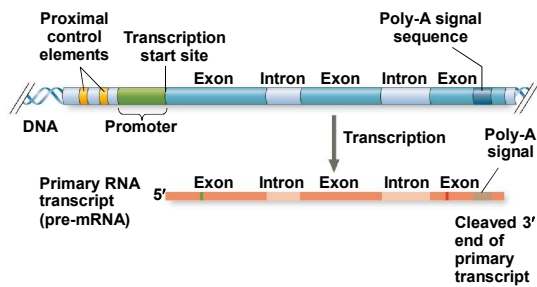
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Figure 15.8-2-#1



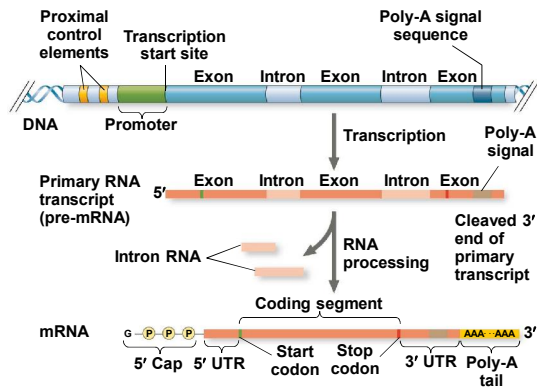
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Figure 15.8-2-#2



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Figure 15.8-2-#3



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The Roles of General and Specific Transcription Factors

- General transcription factors act at the promoter of all genes
- Some genes require specific transcription factors that bind to control elements close to or farther away from the promoter

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General Transcription Factors at the Promoter

- To initiate transcription, eukaryotic RNA polymerase requires the assistance of transcription factors
- A few transcription factors bind to a DNA sequence, but many bind to proteins
- The interaction of general transcription factors and RNA polymerase II with a promoter usually leads to only a low rate of initiation

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Enhancers and Specific Transcription Factors

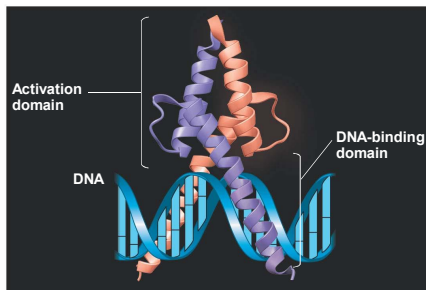
- Proximal control elements are located close to the promoter
- Distal control elements, groupings of which are called **enhancers**, may be far away from a gene or even located in an intron

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- An activator is a protein that binds to an enhancer and stimulates transcription of a gene
- Activators have two domains, one that binds DNA and a second that activates transcription
- Bound activators facilitate a sequence of protein-protein interactions that result in transcription of a given gene

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Figure 15.9

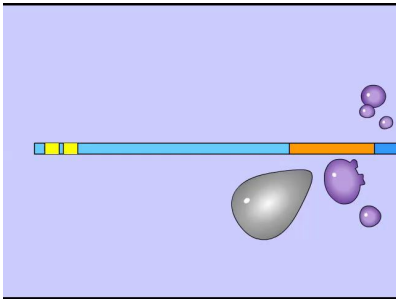


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- Bound activators are brought into contact with a group of mediator proteins through DNA bending
- The mediator proteins in turn interact with proteins at the promoter
- These protein-protein interactions help to assemble and position the initiation complex on the promoter

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Animation: Transcription Initiation



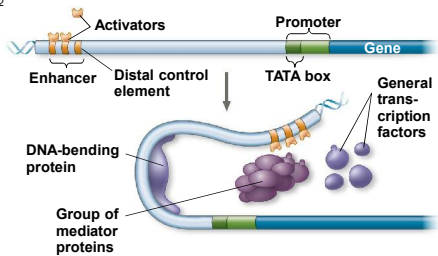
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Figure 15.10-s1

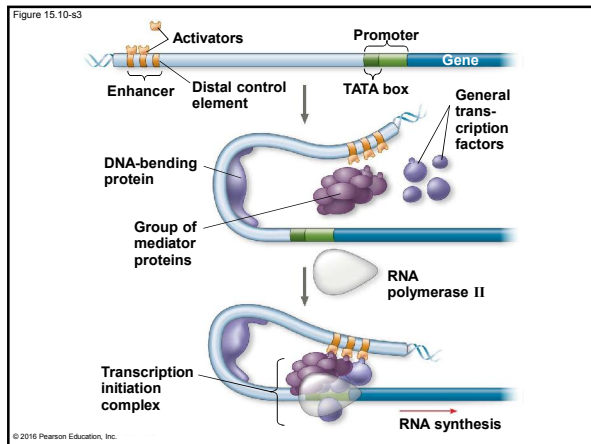


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Figure 15.10-s2

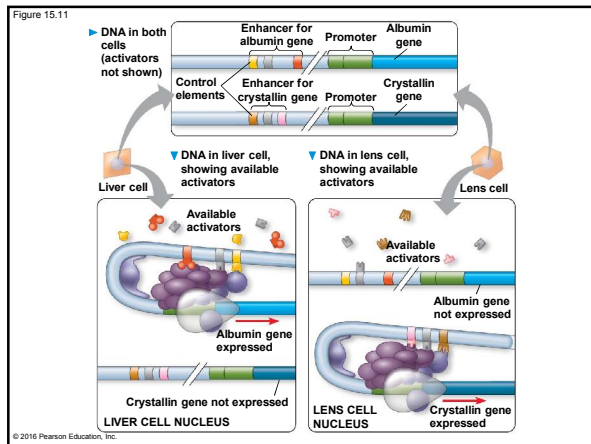


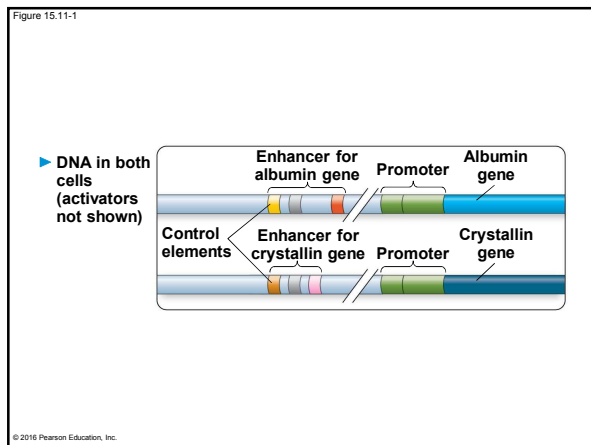
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- Some transcription factors function as repressors, inhibiting expression of a particular gene by a variety of methods
 - Some activators and repressors act indirectly by influencing chromatin structure to promote or silence transcription
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- Combinatorial Control of Gene Activation**
- A particular combination of control elements can activate transcription only when the appropriate activator proteins are present
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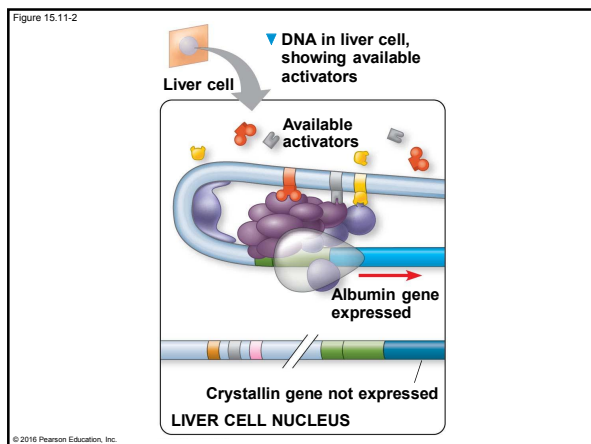
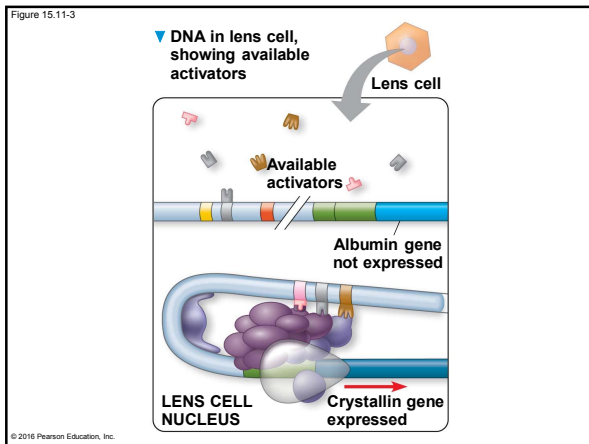


Figure 15.11-3



Coordinately Controlled Genes in Eukaryotes

- Eukaryotic genes that are co-expressed are, for the most part, not organized into operons
- These genes can be scattered over different chromosomes, but each has the same combination of control elements
- Copies of the activators recognize specific control elements and promote simultaneous transcription of the genes

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Mechanisms of Post-Transcriptional Regulation

- Transcription alone does not account for gene expression
- Regulatory mechanisms can operate at various stages after transcription
- Such mechanisms allow a cell to fine-tune gene expression rapidly in response to environmental changes

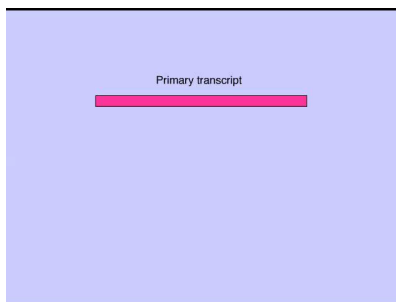
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RNA Processing

- In **alternative RNA splicing**, different mRNA molecules are produced from the same primary transcript, depending on which RNA segments are treated as exons and which as introns
- Alternative splicing can significantly expand the repertoire of a eukaryotic genome

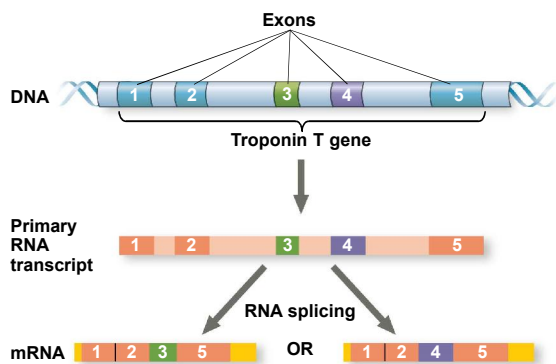
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Animation: RNA Processing



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Figure 15.12



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Initiation of Translation and mRNA Degradation

- The initiation of translation of selected mRNAs can be blocked by regulatory proteins that bind to sequences or structures of the mRNA
- Alternatively, translation of all mRNAs in a cell may be regulated simultaneously
- For example, translation initiation factors are simultaneously activated in an egg following fertilization

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- The life span of mRNA molecules in the cytoplasm is important in determining the pattern of protein synthesis in a cell
- Eukaryotic mRNA generally survives longer than prokaryotic mRNA
- Nucleotide sequences that influence the life span of mRNA in eukaryotes reside in the untranslated region (UTR) at the 3' end of the molecule

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Protein Processing and Degradation

- After translation, various types of protein processing, including cleavage and chemical modification, are subject to control
- The length of time each protein functions in a cell is regulated by means of selective degradation
- To mark a particular protein for destruction, the cell commonly attaches molecules of ubiquitin to the protein, which triggers its destruction

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Concept 15.3: Noncoding RNAs play multiple roles in controlling gene expression

- Only a small fraction of DNA encodes proteins, and a very small fraction of the non-protein-coding DNA consists of genes for RNAs such as rRNA and tRNA
- A significant amount of the genome may be transcribed into noncoding RNAs (ncRNAs)
- A large and diverse population of RNA molecules in the cell play crucial roles in regulating gene expression

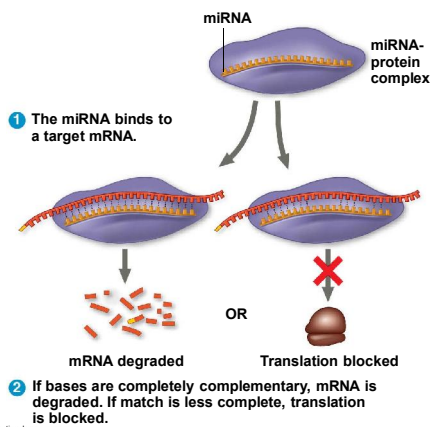
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Effects on mRNAs by MicroRNAs and Small Interfering RNAs

- **MicroRNAs (miRNAs)** are small, single-stranded RNA molecules that can bind to complementary mRNA sequences
- These can degrade the mRNA or block its translation

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Figure 15.13



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- Another class of small RNAs are called **small interfering RNAs (siRNAs)**
- siRNAs and miRNAs are similar but form from different RNA precursors
- The phenomenon of inhibition of gene expression by siRNAs is called **RNA interference (RNAi)**

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Chromatin Remodeling and Effects on Transcription by ncRNAs

- In some yeasts, siRNAs are required for the formation of heterochromatin at the centromeres of chromosomes
- The siRNA system interacts with other noncoding RNAs and with chromatin-modifying enzymes
- This leads to condensation of centromere chromatin into heterochromatin

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- A class of small ncRNAs called piwi-associated RNAs (piRNAs) also induces formation of heterochromatin
- They block expression of transposons, parasitic DNA elements in the genome
- The role of ncRNAs adds to the complexity of the processes involved in regulation of gene expression

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Concept 15.4: Researchers Can Monitor Expression of Specific Genes

- Cells of a given multicellular organism differ from each other because they express different genes from an identical genome
- The most straightforward way to discover which genes are expressed by cells of interest is to identify the mRNAs being made

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Studying the Expression of Single Genes

- We can detect mRNA in a cell using **nucleic acid hybridization**, the base pairing of a strand of nucleic acid to its complementary sequence
- The complementary molecule in this case is a short, single-stranded DNA or RNA called a **nucleic acid probe**
- Each probe is labeled with a fluorescent tag to allow visualization

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- The technique allows us to see the mRNA in place (*in situ*) in the intact organism and is thus called ***in situ* hybridization**

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Figure 15.14

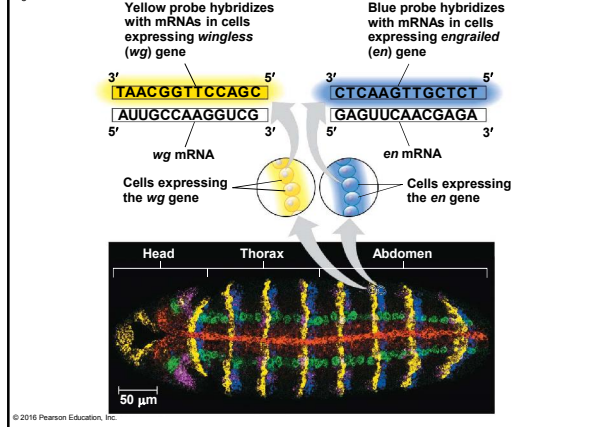


Figure 15.14-1

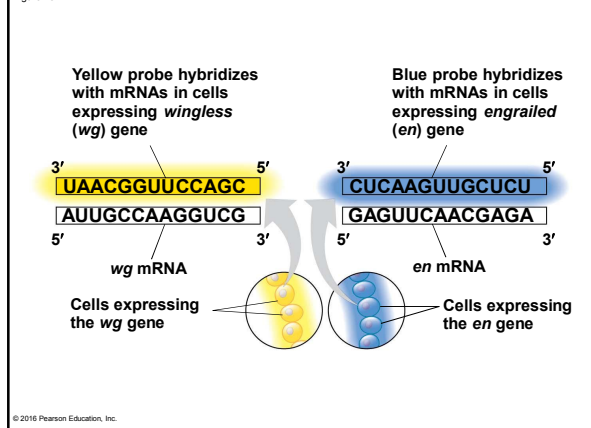
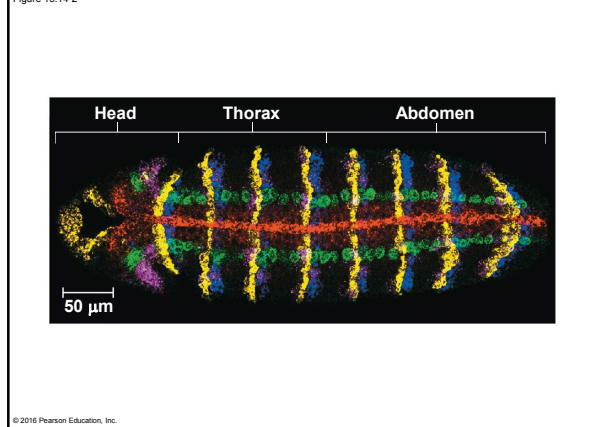


Figure 15.14-2

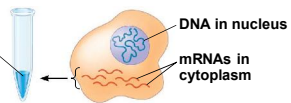


- Another widely used method for comparing the amounts of specific mRNAs in several different samples is **reverse transcriptase–polymerase chain reaction (RT-PCR)**
- RT-PCR turns sample sets of mRNAs into double-stranded DNAs with the corresponding sequences

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Figure 15.15-s1

- 1 Test tube containing reverse transcriptase and mRNA

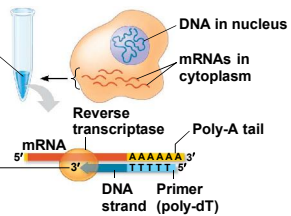


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Figure 15.15-s2

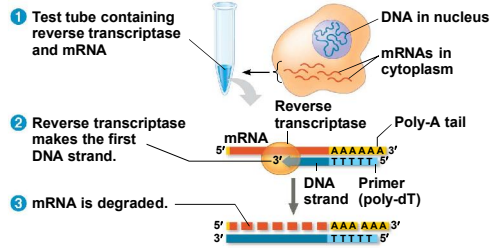
- 1 Test tube containing reverse transcriptase and mRNA

- 2 Reverse transcriptase makes the first DNA strand.



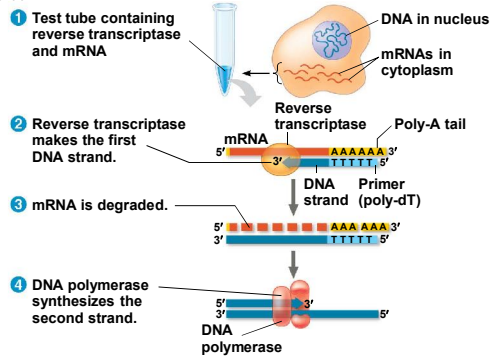
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Figure 15.15-a3



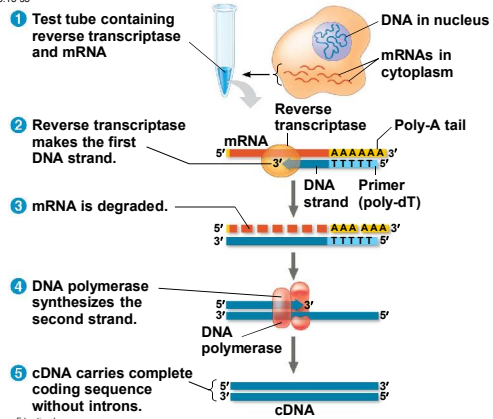
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Figure 15.15-a4



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Figure 15.15-a5



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- RT-PCR relies on the activity of reverse transcriptase, which can synthesize a DNA copy of an mRNA, called a **complementary DNA (cDNA)**
- Once the cDNA is produced, PCR is used to make many copies of the sequence of interest, using primers specific to that sequence

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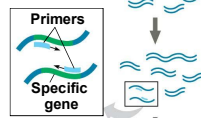
Figure 15.16

Technique

1 cDNA synthesis

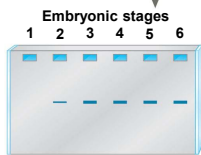
mRNAs

2 PCR amplification



Results

3 Gel electrophoresis



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Studying the Expression of Groups of Genes

- A major goal of biologists is to learn how genes act together to produce and maintain a functioning organism
- Large groups of genes are studied by a systems approach
- The aim is to identify networks of gene expression across an entire genome

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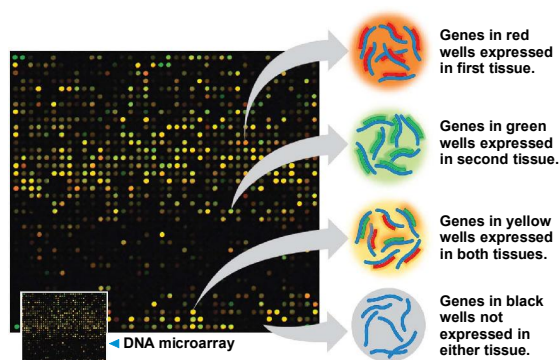
- Genome-wide expression studies can be carried out using **DNA microarray assays**
- A microarray—also called a DNA chip—contains tiny amounts of many single-stranded DNA fragments affixed to the slide in a grid
- mRNAs from cells of interest are isolated and made into cDNAs labeled with fluorescent molecules

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- Usually, cDNAs from two different samples are labeled with different fluorescent tags and tested on the same microarray
- The experiment can identify subsets of genes that are being expressed differently in one sample compared to another

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Figure 15.17



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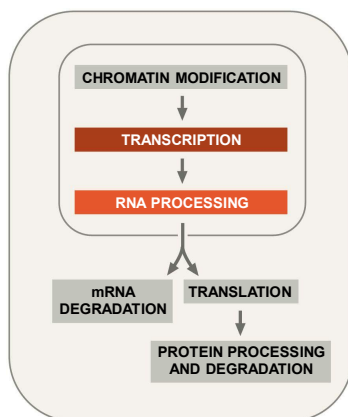
- An alternative to microarray analysis is simply to sequence cDNA samples from different tissues or stages to discover which genes are expressed
- This is called RNA sequencing, or RNA-seq
- This method is becoming more widespread as the cost of sequencing decreases

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- Studies of genes that are expressed together in some tissues but not others may contribute to a better understanding of diseases and suggest new diagnostic tests or therapies

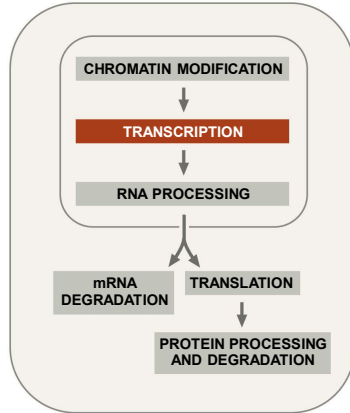
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Figure 15.UN01



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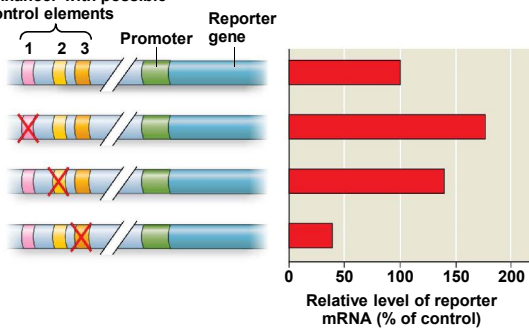
Figure 15.UN02



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Figure 15.UN03-1

Enhancer with possible control elements



Data from J. N. Walters et al., Regulation of human microsomal prostaglandin E synthase-1 by IL-1 β requires a distal enhancer element with a unique role for C/EBP β , *Biochemical Journal* 443:561–571 (2012).

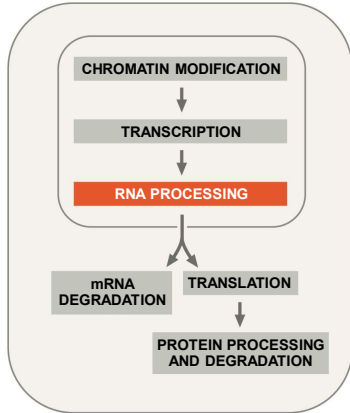
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Figure 15.UN03-2



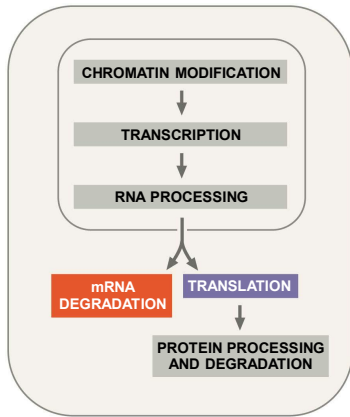
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Figure 15.UN04



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Figure 15.UN05



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Figure 15.UN06

5' ...CUCAUCACCGGC... 3'
3' GAGTAGTGGCCG 5'

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Figure 15.UN07

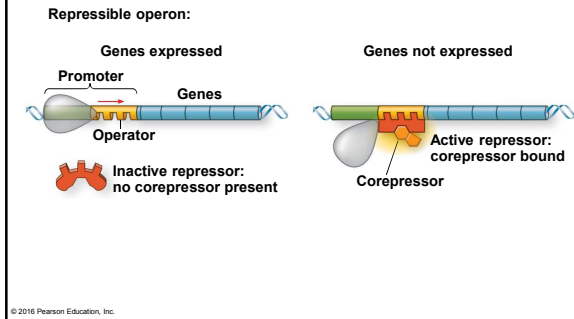


Figure 15.UN08

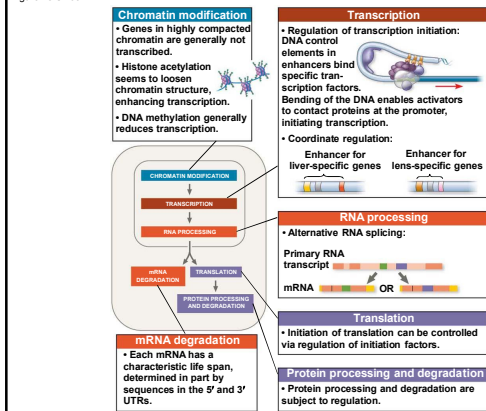


Figure 15.UN08-1

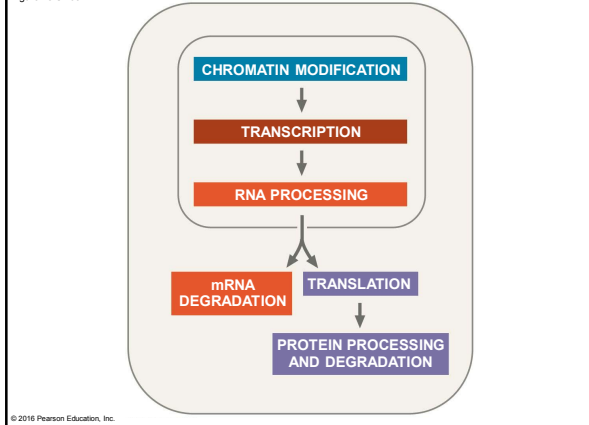


Figure 15.UN08-2

Chromatin modification

- Genes in highly compacted chromatin are generally not transcribed.
- Histone acetylation seems to loosen chromatin structure, enhancing transcription.
- DNA methylation generally reduces transcription.

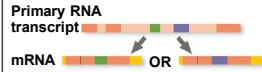


mRNA degradation

- Each mRNA has a characteristic life span, determined in part by sequences in the 5' and 3' UTRs.

RNA processing

- Alternative RNA splicing:



Translation

- Initiation of translation can be controlled via regulation of initiation factors.

Protein processing and degradation

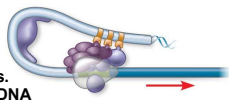
- Protein processing and degradation are subject to regulation.

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Figure 15.UN08-3

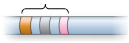
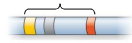
Transcription

- Regulation of transcription initiation: DNA control elements in enhancers bind specific transcription factors. Bending of the DNA enables activators to contact proteins at the promoter, initiating transcription.
- Coordinate regulation:



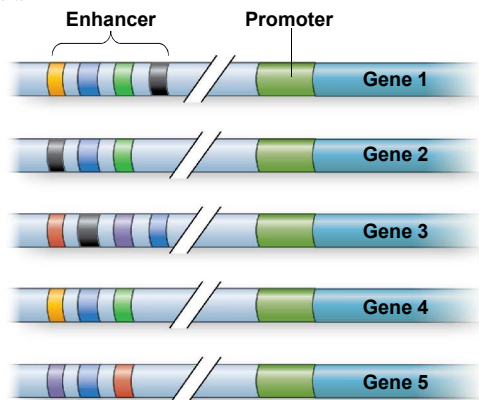
Enhancer for liver-specific genes

Enhancer for lens-specific genes



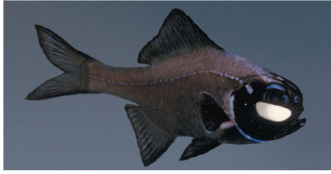
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Figure 15.UN09



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Figure 15.UN10



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