16 GENE REGULATION

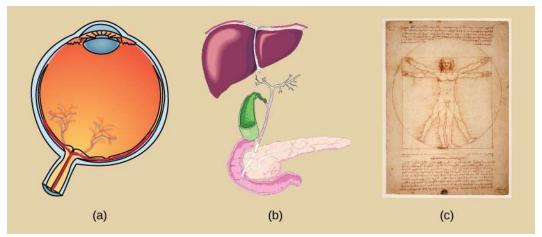


Figure 16.1 The genetic content of each somatic cell in an organism is the same, but not all genes are expressed in every cell. The control of which genes are expressed dictates whether a cell is (a) an eye cell or (b) a liver cell. It is the differential gene expression patterns that arise in different cells that give rise to (c) a complete organism.

Chapter Outline

16.1: Regulation of Gene Expression

16.2: Prokaryotic Gene Regulation

16.3: Eukaryotic Epigenetic Gene Regulation

16.4: Eukaryotic Transcriptional Gene Regulation

16.5: Eukaryotic Post-transcriptional Gene Regulation

16.6: Eukaryotic Translational and Post-translational Gene Regulation

16.7: Cancer and Gene Regulation

Introduction

Most people know that regular exercise is important to maintain good health. It promotes cardiovascular health and helps to prevent obesity. Scientists have now discovered that long-term endurance training also changes how genes are expressed in muscle tissue. In a recent study, 23 healthy people each exercised one leg for 45 minutes four days a week while resting the other leg. After three months, muscles from participants' legs were biopsied, and scientists analyzed the activity level of over 20,000 genes in the tissue samples.

They found that for each participant the exercised leg had reduced inflammation and improved metabolism compared with the non-exercised leg. These differences were accompanied by changes in genes associated with metabolism and inflammation. However, the actual nucleotide sequences of the genes weren't changed. Instead, some genes were methylated, which simply means methyl groups were attached to certain nucleotides along the sequence. This, essentially, turned the genes "off" or otherwise changed how they were expressed. DNA methylation is an example of epigenetics, which is a process that alters genes without affecting the nucleotide sequence of the genes. The full research article can be found here (http://openstaxcollege.org/l/32endurance).

16.1 | Regulation of Gene Expression

In this section, you will explore the following question:

• How does prokaryotic gene regulation differ from eukaryotic gene regulation?

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Structure and function in biology result from the presence of genetic information and the correct expression of this information. In the chapter on DNA structure and function, we explored how genes are translated into proteins, which in turn determine the nature of the cell. But how does a cell know when to "turn on" its DNA? With few exceptions, each cell in your body contains identical genetic information. If each cell has the same exact DNA make up, how is it that a liver cell differs from a nerve or muscle cell?

As we will discover, although each cell shares the same genome and DNA sequence, each cell does not express exactly the same genes. Many factors determine when and how genes are expressed in a given cell. Even the type of chromosome a gene is located on, like whether it is a sex chromosome or not, can determine its expression pattern, as can mutations or changes in DNA sequence and other external factors. In prokaryotes, gene expression is regulated primarily at the level of transcription, when DNA is copied into RNA. However, eukaryotes have evolved regulatory mechanisms in gene expression at multiple levels. In all cases, regulation of gene expression determines the type and amount of protein produced in the cell. Errors in regulatory processes can result in many human diseases and conditions, including cancer.

Gene expression regulation occurs at different points in prokaryotes and eukaryotes. Prokaryotic organisms express their entire genome in every cell, but not necessarily all at the same time. In general, a gene is expressed only when its specific protein product is needed. Remember that each cell in an organism carries the same DNA as every other cell. Yet cells of eukaryotic organisms each express a unique subset of DNA depending on cell type. To express a protein, DNA is first transcribed into RNA, which is then translated into proteins. In prokaryotic cells, transcription and translation occur almost simultaneously. In eukaryotic cells, transcription occurs in the nucleus, separate from the translation that occurs in the cytoplasm along ribosomes attached to endoplasmic reticulum. As stated above, gene expression in prokaryotes is regulated at the level of transcription, whereas in eukaryotes, gene expression is regulated at multiple levels, including the epigenetic (DNA), transcriptional, pre- and post-transcriptional, and translational levels.

The science of epigenetics studies heritable changes in the genome that do not affect the underlying DNA gene sequences.

The content presented in this section supports the learning objectives outlined in Big Idea 3 of the $AP^{\$}$ Biology Curriculum Framework. The $AP^{\$}$ learning objectives merge essential knowledge content with one or more of the seven science practices. These objectives provide a transparent foundation for the $AP^{\$}$ Biology course, along with inquiry-based laboratory experiences, instructional activities, and $AP^{\$}$ exam questions.

Big Idea 3	Living systems store, retrieve, transmit and respond to information essential to life processes.
Enduring Understanding 3.B	Expression of genetic information involves cellular and molecular mechanisms.
Essential Knowledge	3.B.1 Gene regulation results in differential gene expression, leading to cell specialization
Science Practice	7.1 The student can connect phenomena and models across spatial and temporal scales.
Learning Objective	3.18 The student is able to describe the connection between the regulation of gene expression and observed differences between different kinds of organisms.

For a cell to function properly, necessary proteins must be synthesized at the proper time. All cells control or regulate the synthesis of proteins from information encoded in their DNA. The process of turning on a gene to produce RNA and protein is called **gene expression**. Whether in a simple unicellular organism or a complex multi-cellular organism, each cell controls

when and how its genes are expressed. For this to occur, there must be a mechanism to control when a gene is expressed to make RNA and protein, how much of the protein is made, and when it is time to stop making that protein because it is no longer needed.

Although genetic differences between species and between individuals within a species are often responsible for phenotypic differences, another mechanism that can create phenotypic differences is differences in gene expression. For example, although every cell in an organism contains the same genes, the bone cells in the organism appears different from the fat cells due to differences in which genes are expressed by which cell. Similarly, although mice and humans share approximately 97.5% of their genes, they are very different organisms because different genes are turned on at different times during development and in different cells. Even organisms that share 100% identity in their genomes (a.k.a clones) can eventually appear different if they express their genes differently in response to different environmental conditions, for example. Even among humans, identical twins can possess different birthmarks, wrinkles, or other features that arise during development sometimes due to differential gene expression.

The regulation of gene expression conserves energy and space. It would require a significant amount of energy for an organism to express every gene at all times, so it is more energy efficient to turn on the genes only when they are required. In addition, only expressing a subset of genes in each cell saves space because DNA must be unwound from its tightly coiled structure to transcribe and translate the DNA. Cells would have to be enormous if every protein were expressed in every cell all the time.

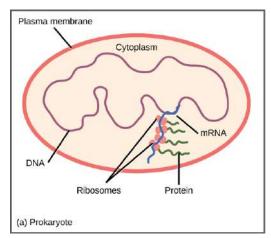
The control of gene expression is extremely complex. Malfunctions in this process are detrimental to the cell and can lead to the development of many diseases.

Prokaryotic versus Eukaryotic Gene Expression

To understand how gene expression is regulated, we must first understand how a gene codes for a functional protein in a cell. The process occurs in both prokaryotic and eukaryotic cells, just in slightly different manners.

Prokaryotic organisms are single-celled organisms that lack a cell nucleus, and their DNA therefore floats freely in the cell cytoplasm. To synthesize a protein, the processes of transcription and translation occur almost simultaneously. When the resulting protein is no longer needed, transcription stops. As a result, the primary method to control what type of protein and how much of each protein is expressed in a prokaryotic cell is the regulation of DNA transcription. All of the subsequent steps occur automatically. When more protein is required, more transcription occurs. Therefore, in prokaryotic cells, the control of gene expression is mostly at the transcriptional level.

Eukaryotic cells, in contrast, have intracellular organelles that add to their complexity. In eukaryotic cells, the DNA is contained inside the cell's nucleus and there it is transcribed into RNA. The newly synthesized RNA is then transported out of the nucleus into the cytoplasm, where ribosomes translate the RNA into protein. The processes of transcription and translation are physically separated by the nuclear membrane; transcription occurs only within the nucleus, and translation occurs only outside the nucleus in the cytoplasm. The regulation of gene expression can occur at all stages of the process (Figure 16.2). Regulation may occur when the DNA is uncoiled and loosened from nucleosomes to bind transcription factors (epigenetic level), when the RNA is transcribed (transcriptional level), when the RNA is processed and exported to the cytoplasm after it is transcribed (post-transcriptional level), when the RNA is translated into protein (translational level), or after the protein has been made (post-translational level).



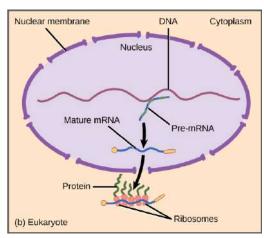


Figure 16.2 Prokaryotic transcription and translation occur simultaneously in the cytoplasm, and regulation occurs at the transcriptional level. Eukaryotic gene expression is regulated during transcription and RNA processing, which take place in the nucleus, and during protein translation, which takes place in the cytoplasm. Further regulation may occur through post-translational modifications of proteins.

The differences in the regulation of gene expression between prokaryotes and eukaryotes are summarized in **Table 16.1**. The regulation of gene expression is discussed in detail in subsequent modules.

Differences in the Regulation of Gene Expression of Prokaryotic and Eukaryotic Organisms

Prokaryotic organisms	Eukaryotic organisms
Lack nucleus	Contain nucleus
DNA is found in the cytoplasm	DNA is confined to the nuclear compartment
RNA transcription and protein formation occur almost simultaneously	RNA transcription occurs prior to protein formation, and it takes place in the nucleus. Translation of RNA to protein occurs in the cytoplasm.
Gene expression is regulated primarily at the transcriptional level	Gene expression is regulated at many levels (epigenetic, transcriptional, nuclear shuttling, post-transcriptional, translational, and post-translational)

Table 16.1

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Prokaryotic cells can only regulate gene expression by controlling the amount of transcription. As eukaryotic cells evolved, the complexity of the control of gene expression increased. For example, with the evolution of eukaryotic cells came compartmentalization of important cellular components and cellular processes. A nuclear region that contains the DNA was formed. Transcription and translation were physically separated into two different cellular compartments. It therefore became possible to control gene expression by regulating transcription in the nucleus, and also by controlling the RNA levels and protein translation present outside the nucleus.

Some cellular processes arose from the need of the organism to defend itself. Cellular processes such as gene silencing developed to protect the cell from viral or parasitic infections. If the cell could quickly shut off gene expression for a short period of time, it would be able to survive an infection when other organisms could not. Therefore, the organism evolved a new process that helped it survive, and it was able to pass this new development to offspring.

Cytochrome c oxidase is a highly conserved protein found in bacteria and in the mitochondria of eukaryotes. Based on your knowledge of evolutionary relationships, which of the following statements would you expect to be true of the cytochrome c oxidase protein sequence?

- a. The bacterial protein will be more similar to the human protein than the yeast protein.
- b. The yeast protein will be more similar to the human protein than the bacterial protein.
- c. The bacterial protein will be more similar to the yeast protein than the human protein.
- d. The bacterial and yeast proteins will share a similar sequence, but the human protein will be unrelated.



Think About It

How does controlling gene expression alter the overall protein level in the cell?

16.2 | Prokaryotic Gene Regulation

In this section, you will explore the following question:

 What are operons and what are the roles of activators, inducers, and repressors in regulating operons and gene expression?

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The regulation of gene expression in prokaryotic cells occurs at the transcriptional level. Simply stated, if a cell does not transcribe the DNA's message into mRNA, translation (protein synthesis), does not occur. Bacterial genes are often organized into common pathways or processes called operons for more coordinated regulation of expression. For example, in *E. coli*, genes responsible for lactose metabolism are located together on the bacterial chromosome. (The operon model includes several components, so when studying how the operon works, it is helpful to refer to a diagram of the model. See **Figure 16.3** and **Figure 16.4**.) The operon includes a regulatory gene that codes for a repressor protein that binds to the operator, which prevents RNA polymerase from transcribing the gene(s) of interest. An example of this is seen in the structural genes for lactose metabolism. However, if the repressor is inactivated, RNA polymerase binds to the promoter, and transcription of the structural genes occurs.

There are three ways to control the transcription of an operon: inducible control, repressible control, and activator control.

The *lac* operon is an example of inducible control because the presence of lactose "turns on" transcription of the genes for its own metabolism. The *trp* operon is an example of repressible control because it uses proteins bound to the operator sequence to physically prevent the binding of RNA polymerase. If tryptophan is not needed by the cell, the genes necessary to produce it are turned off. Activator control (typified by the action of Catabolite Activator Protein) increases the binding ability of RNA polymerase to the promoter. Certain genes are continually expressed via this regulatory mechanism.

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 of the AP[®] Biology Curriculum Framework. The learning objectives listed in the Curriculum Framework provide a transparent foundation for the AP[®] Biology course, an inquiry-based laboratory experience, instructional activities, and AP[®] exam questions. A learning objective merges required content with one or more of the seven science practices.

Big Idea 3	Living systems store, retrieve, transmit and respond to information essential to life processes.
Enduring Understanding 3.B	Expression of genetic information involves cellular and molecular mechanisms.
Essential Knowledge	3.B.1 Gene regulation results in differential gene expression, leading to cell specialization
Science Practice	1.4 The student can use representations and models to analyze situations or solve problems qualitatively and quantitatively
Learning Objective	3.21 The student can use representations to describe how gene regulation influences cell products and function.
Essential Knowledge	3.B.2 A variety of intercellular and intracellular signal transmissions mediate gene expression.
Science Practice	1.4 The student can use representations and models to analyze situations or solve problems qualitatively and quantitatively.
Learning Objective	3.23 The student can use representations to describe mechanisms of the regulation of gene expression.

The DNA of prokaryotes is organized into a circular chromosome supercoiled in the nucleoid region of the cell cytoplasm. Proteins that are needed for a specific function, or that are involved in the same biochemical pathway, are encoded together in blocks called **operons**. For example, all of the genes needed to use lactose as an energy source are coded next to each other in the lactose (or *lac*) operon.

In prokaryotic cells, there are three types of regulatory molecules that can affect the expression of operons: repressors, activators, and inducers. **Repressors** are proteins that suppress transcription of a gene in response to an external stimulus, whereas **activators** are proteins that increase the transcription of a gene in response to an external stimulus. Finally, inducers are small molecules that either activate or repress transcription depending on the needs of the cell and the availability of substrate.

The trp Operon: A Repressor Operon

Bacteria such as *E. coli* need amino acids to survive. **Tryptophan** is one such amino acid that *E. coli* can ingest from the environment. *E. coli* can also synthesize tryptophan using enzymes that are encoded by five genes. These five genes are next to each other in what is called the **tryptophan** (*trp*) **operon** (**Figure 16.3**). If tryptophan is present in the environment, then *E. coli* does not need to synthesize it and the switch controlling the activation of the genes in the *trp* operon is switched off. However, when tryptophan availability is low, the switch controlling the operon is turned on, transcription is initiated, the genes are expressed, and tryptophan is synthesized.

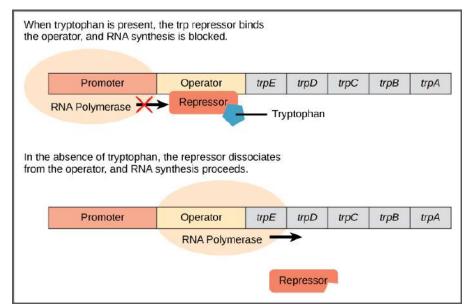


Figure 16.3 The five genes that are needed to synthesize tryptophan in *E. coli* are located next to each other in the *trp* operon. When tryptophan is plentiful, two tryptophan molecules bind the repressor protein at the operator sequence. This physically blocks the RNA polymerase from transcribing the tryptophan genes. When tryptophan is absent, the repressor protein does not bind to the operator and the genes are transcribed.

A DNA sequence that codes for proteins is referred to as the coding region. The five coding regions for the tryptophan biosynthesis enzymes are arranged sequentially on the chromosome in the operon. Just before the coding region is the **transcriptional start site**. This is the region of DNA to which RNA polymerase binds to initiate transcription. The promoter sequence is upstream of the transcriptional start site; each operon has a sequence within or near the promoter to which proteins (activators or repressors) can bind and regulate transcription.

A DNA sequence called the operator sequence is encoded between the promoter region and the first *trp* coding gene. This **operator** contains the DNA code to which the repressor protein can bind. When tryptophan is present in the cell, two tryptophan molecules bind to the *trp* repressor, which changes shape to bind to the *trp* operator. Binding of the tryptophan–repressor complex at the operator physically prevents the RNA polymerase from binding, and transcribing the downstream genes.

When tryptophan is not present in the cell, the repressor by itself does not bind to the operator; therefore, the operon is active and tryptophan is synthesized. Because the repressor protein actively binds to the operator to keep the genes turned off, the *trp* operon is negatively regulated and the proteins that bind to the operator to silence *trp* expression are **negative regulators**.





Watch this video (http://openstaxcollege.org/l/trp_operon) to learn more about the *trp* operon.

What would happen if bacteria did not have *trp* R?

- a. The cell would not be able to break down tryptophan.
- b. The cell will gradually produce more tryptophan over time.
- c. The cell would not be able to make tryptophan.
- d. The cell would make tryptophan when it was not needed.

Catabolite Activator Protein (CAP): An Activator Regulator

Just as the *trp* operon is negatively regulated by tryptophan molecules, there are proteins that bind to the operator sequences that act as a **positive regulator** to turn genes on and activate them. For example, when glucose is scarce, *E. coli* bacteria can turn to other sugar sources for fuel. To do this, new genes to process these alternate genes must be transcribed. When glucose levels drop, cyclic AMP (cAMP) begins to accumulate in the cell. The cAMP molecule is a signaling molecule that is involved in glucose and energy metabolism in *E. coli*. When glucose levels decline in the cell, accumulating cAMP binds to the positive regulator **catabolite activator protein (CAP)**, a protein that binds to the promoters of operons that control the processing of alternative sugars. When cAMP binds to CAP, the complex binds to the promoter region of the genes that are needed to use the alternate sugar sources (**Figure 16.4**). In these operons, a CAP binding site is located upstream of the RNA polymerase binding site in the promoter. This increases the binding ability of RNA polymerase to the promoter region and the transcription of the genes.

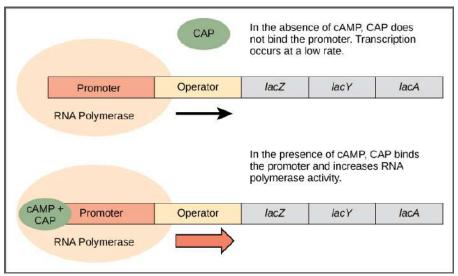


Figure 16.4 When glucose levels fall, *E. coli* may use other sugars for fuel but must transcribe new genes to do so. As glucose supplies become limited, cAMP levels increase. This cAMP binds to the CAP protein, a positive regulator that binds to an operator region upstream of the genes required to use other sugar sources.

The lac Operon: An Inducer Operon

The third type of gene regulation in prokaryotic cells occurs through **inducible operons**, which have proteins that bind to activate or repress transcription depending on the local environment and the needs of the cell. The *lac* operon is a typical inducible operon. As mentioned previously, *E. coli* is able to use other sugars as energy sources when glucose concentrations are low. To do so, the cAMP–CAP protein complex serves as a positive regulator to induce transcription. One such sugar source is lactose. The *lac* operon encodes the genes necessary to acquire and process the lactose from the local environment. CAP binds to the operator sequence upstream of the promoter that initiates transcription of the *lac* operon. However, for the *lac* operon to be activated, two conditions must be met. First, the level of glucose must be very low or non-existent. Second, lactose must be present. Only when glucose is absent and lactose is present will the *lac* operon be transcribed (**Figure 16.5**). This makes sense for the cell, because it would be energetically wasteful to create the proteins to process lactose if glucose was plentiful or lactose was not available.



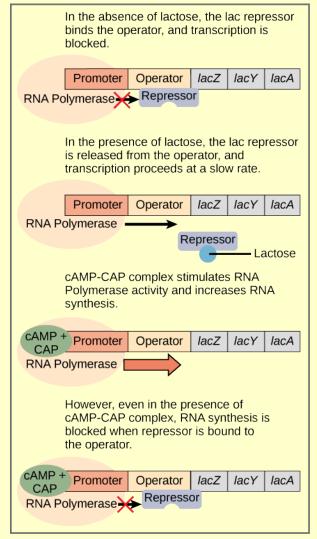


Figure 16.5 Transcription of the *lac* operon is carefully regulated so that its expression only occurs when glucose is limited and lactose is present to serve as an alternative fuel source.

In *E. coli*, the *trp* operon is on by default, while the *lac* operon is off. Why do you think that this is the case?

- a. The trp operon is inducible and is positively regulated. Therefore it is ON by default whereas the lac operon is repressible and is OFF by default.
- b. The trp operon synthesizes tryptophan which is essential for the cell and therefore remains ON, whereas the lac operon synthesizes enzymes for the breakdown of a sugar that is not always available and remains OFF by default.
- c. The trp operon is constitutive and remains ON by default, whereas the lac operon is repressible and therefore is OFF by default.
- d. The lac operon undergoes transcriptional attenuation and therefore is OFF by default, whereas the trp operon is not regulated by any such mechanism and is ON by default.

If glucose is absent, then CAP can bind to the operator sequence to activate transcription. If lactose is absent, then the repressor binds to the operator to prevent transcription. If either of these requirements is met, then transcription remains off.

Only when both conditions are satisfied is the *lac* operon transcribed (Table 16.2).

Signals that Induce or Repress Transcription of the *lac* Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	-	-	+	No
+	-	+	-	Some
-	+	-	+	No
-	+	+	-	Yes

Table 16.2





Watch an animated tutorial (http://openstaxcollege.org/l/lac_operon) about the workings of *lac* operon here.

The *E. coli* bacteria can have several mutations that affect the lac operon system. One mutation inhibits the ability of RNA polymerase to bind to the lac operon. How would this affect the cell?

- a. The cell would make more lactose.
- b. There would be no lactose outside of the cell.
- c. The cell would not be able to process tryptophan.
- d. The cell would not be able to process lactose.

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Activity

Modeling the Operon. Use construction paper or more elaborate materials, such as Styrofoam noodles, electrical tape, and Velcro tabs, to create a model of the *lac* and *trp* operons that include a regulator, promoter, operator, and structural genes. Then use the model to show how the presence of substrate, e.g., allolactose or tryptophan, can change the activity of the operons. As an extension of the activity, use the model to make predictions about the effects of mutations in any of the regions on gene expression.

Think About It

In *E. coli*, the *trp* operon is on by default, while the *lac* operon is off by default. Why do you think this is the case?

16.3 | Eukaryotic Epigenetic Gene Regulation

In this section, you will explore the following question:

• What is the science of epigenetics and how is this process regulated?

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One reason that eukaryotic gene expression is more complex than prokaryotic gene expression is because the processes of transcription and translation are physically separated within the eukaryotic cell. Eukaryotic cells also package their genomes in a more sophisticated way compared with prokaryotic cells. Consequently, eukaryotic cells can regulate gene expression at multiple levels, beginning with control of access to DNA. Because genomic DNA is folded around histone proteins to create nucleosome complexes, nucleosomes physically regulate the access of proteins, such as transcription factors and enzymes, to the underlying DNA. Methylation of DNA and histones causes nucleosomes to pack tightly together, preventing transcription factors from binding to the DNA. Methylated nucleosomes contain DNA that is not expressed. On the other hand, histone acetylation results in loose packing of nucleosomes, allowing transcription factors to bind to DNA. Acetylated nucleosomes contain DNA that may be expressed.

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Epigenetic Control: Regulating Access to Genes within the Chromosome

As stated earlier, one reason why eukaryotic gene expression is more complex than prokaryotic gene expression is because the processes of transcription and translation are physically separated. Unlike prokaryotic cells, eukaryotic cells can regulate gene expression at many different levels. Eukaryotic gene expression begins with control of access to the DNA. This form of regulation, called epigenetic regulation, occurs even before transcription is initiated.

The human genome encodes over 20,000 genes; each of the 23 pairs of human chromosomes encodes thousands of genes. The DNA in the nucleus is precisely wound, folded, and compacted into chromosomes so that it will fit into the nucleus. It is also organized so that specific segments can be accessed as needed by a specific cell type.

The first level of organization, or packing, is the winding of DNA strands around histone proteins. Histones package and order DNA into structural units called nucleosome complexes, which can control the access of proteins to the DNA regions (**Figure 16.6a**). Under the electron microscope, this winding of DNA around histone proteins to form nucleosomes looks like small beads on a string (**Figure 16.6b**). These beads (histone proteins) can move along the string (DNA) and change the structure of the molecule.

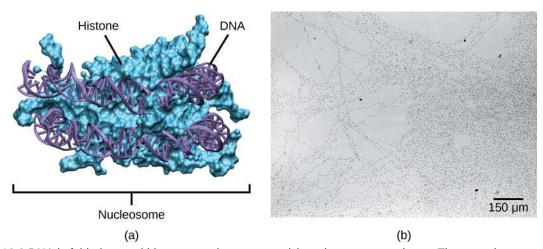
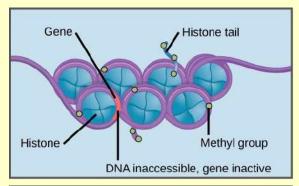


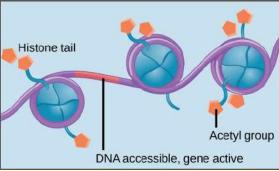
Figure 16.6 DNA is folded around histone proteins to create (a) nucleosome complexes. These nucleosomes control the access of proteins to the underlying DNA. When viewed through an electron microscope (b), the nucleosomes look like beads on a string. (credit "micrograph": modification of work by Chris Woodcock)

If DNA encoding a specific gene is to be transcribed into RNA, the nucleosomes surrounding that region of DNA can slide down the DNA to open that specific chromosomal region and allow for the transcriptional machinery (RNA polymerase) to initiate transcription (**Figure 16.7**). Nucleosomes can move to open the chromosome structure to expose a segment of DNA, but do so in a very controlled manner.

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Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.



Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

Figure 16.7 Nucleosomes can slide along DNA. When nucleosomes are spaced closely together (top), transcription factors cannot bind and gene expression is turned off. When the nucleosomes are spaced far apart (bottom), the DNA is exposed. Transcription factors can bind, allowing gene expression to occur. Modifications to the histones and DNA affect nucleosome spacing.

In females, one of the two X chromosomes is inactivated during embryonic development because of epigenetic changes to the chromatin. What impact do you think these changes would have on nucleosome packing?

- a. Methylation of DNA and hypo-acetylation of histones causes the nucleosomes to pack loosely together, inactivating one of the X chromosomes.
- b. Methylation of histones and hyper-acetylation of DNA causes the nucleosomes to pack tightly together, inactivating one of the X chromosome due to transcriptional repression.
- c. Methylation of DNA and hypo-acetylation of histones causes the nucleosomes to pack tightly together, inactivating one of the X chromosomes.
- d. Acetylation of DNA and hyper-methylation of histones causes the nucleosomes to pack tightly together, inactivating one of the X chromosomes.

How the histone proteins move is dependent on signals found on both the histone proteins and on the DNA. These signals are tags added to histone proteins and DNA that tell the histones if a chromosomal region should be open or closed (**Figure 16.8** depicts modifications to histone proteins and DNA). These tags are not permanent, but may be added or removed as needed. They are chemical modifications (phosphate, methyl, or acetyl groups) that are attached to specific amino acids in the protein or to the nucleotides of the DNA. The tags do not alter the DNA base sequence, but they do alter how tightly wound the DNA is around the histone proteins. DNA is a negatively charged molecule; therefore, changes in the charge of the histone will change how tightly wound the DNA molecule will be. When unmodified, the histone proteins have a large positive charge; by adding chemical modifications like acetyl groups, the charge becomes less positive.

The DNA molecule itself can also be modified. This occurs within very specific regions called CpG islands. These are stretches with a high frequency of cytosine and guanine dinucleotide DNA pairs (CG) found in the promoter regions of genes. When this configuration exists, the cytosine member of the pair can be methylated (a methyl group is added). This modification changes how the DNA interacts with proteins, including the histone proteins that control access to the region.

Highly methylated (hypermethylated) DNA regions with deacetylated histones are tightly coiled and transcriptionally inactive.

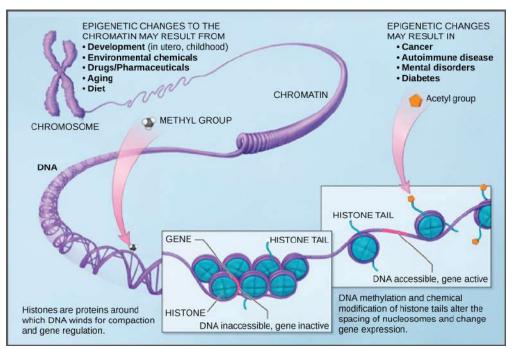


Figure 16.8 Histone proteins and DNA nucleotides can be modified chemically. Modifications affect nucleosome spacing and gene expression. (credit: modification of work by NIH)

This type of gene regulation is called epigenetic regulation. Epigenetic means "around genetics." The changes that occur to the histone proteins and DNA do not alter the nucleotide sequence and are not permanent. Instead, these changes are temporary (although they often persist through multiple rounds of cell division) and alter the chromosomal structure (open or closed) as needed. A gene can be turned on or off depending upon the location and modifications to the histone proteins and DNA. If a gene is to be transcribed, the histone proteins and DNA are modified surrounding the chromosomal region encoding that gene. This opens the chromosomal region to allow access for RNA polymerase and other proteins, called **transcription factors**, to bind to the promoter region, located just upstream of the gene, and initiate transcription. If a gene is to remain turned off, or silenced, the histone proteins and DNA have different modifications that signal a closed chromosomal configuration. In this closed configuration, the RNA polymerase and transcription factors do not have access to the DNA and transcription cannot occur (Figure 16.7).

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Think About It

In females, one of the two X chromosomes is inactivated during embryonic development because of epigenetic changes to the chromatin. What impact do you think these changes will have on nucleosome packaging and, consequently, gene expression?





View this video (http://openstaxcollege.org/l/epigenetic_reg) that describes how epigenetic regulation controls gene expression.

Explain how the study of epigenetics can lead to improved treatment of cancer.

- a. Epigenetics would allow new body parts to be synthesized that could replace those damaged by cancer.
- b. Epigenetics could change the genetic code of all cells in the body to prevent them from becoming cancerous.
- c. New therapies could be made that changes the genetic code of harmful cancer genes.
- d. New therapies could be made that do not require altering the cancer cell's DNA.

16.4 | Eukaryotic Transcriptional Gene Regulation

In this section, you will explore the following question:

• What is the role of transcription factors, enhancers, and repressors in gene regulation?

Connection for AP® Courses

To start transcription, general transcription factors must first bind to a specific area on the DNA called the TATA box and then recruit RNA polymerase to that location. In addition, other areas on the DNA called enhancer regions help augment transcription. Transcription factors can bind to enhancer regions to increase or prevent transcription.

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 of the AP^{\otimes} Biology Curriculum Framework. The Learning Objectives listed in the Curriculum Framework provide a transparent foundation for the AP^{\otimes} Biology course, an inquiry-based laboratory experience, instructional activities, and AP^{\otimes} exam questions. A Learning Objective merges required content with one or more of the seven Science Practices

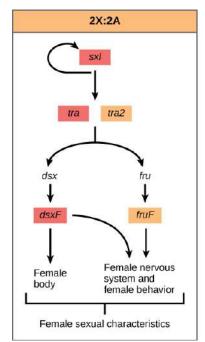
Big Idea 3	Living systems store, retrieve, transmit and respond to information essential to life processes.
Enduring Understanding 3.B	Expression of genetic information involves cellular and molecular mechanisms.
Essential Knowledge	3.B.1 Gene regulation results in differential gene expression, leading to cell specialization
Science Practice	7.1 The student can connect phenomena and models across spatial and temporal scales.
Learning Objective	3.18 The student is able to describe the connection between the regulation of gene expression and observed differences between different kinds of organisms
Essential Knowledge	3.B.1 Gene regulation results in differential gene expression, leading to cell specialization

Science Practice	7.1 The student can connect phenomena and models across spatial and temporal scales
Learning Objective	3.19 The student is able to describe the connection between the regulation of gene expression and observed differences between individuals in a population
Essential Knowledge	3.B.1 Gene regulation results in differential gene expression, leading to cell specialization.
Science Practice	6.2 The student can construct explanations of phenomena based on evidence produced through scientific practices
Learning Objective	3.20 The student is able to explain how the regulation of gene expression is essential for the processes and structures that support efficient cell function.
Essential Knowledge	3.B.1 1 Gene regulation results in differential gene expression, leading to cell specialization.
Science Practice	1.4 The student can use representations and models to analyze situations or solve problems qualitatively and quantitatively.
Learning Objective	3.21 The student can use representations to describe how gene regulation influences cell products and function.

The Science Practice Challenge Questions contain additional test questions for this section that will help you prepare for the AP exam. These questions address the following standards: [APLO 3.18]

Like prokaryotic cells, the transcription of genes in eukaryotes requires the actions of an RNA polymerase to bind to a sequence upstream of a gene to initiate transcription. However, unlike prokaryotic cells, the eukaryotic RNA polymerase requires other proteins, or transcription factors, to facilitate transcription initiation. Transcription factors are proteins that bind to the promoter sequence and other regulatory sequences to control the transcription of the target gene. RNA polymerase by itself cannot initiate transcription in eukaryotic cells. Transcription factors must bind to the promoter region first and recruit RNA polymerase to the site for transcription to be established.

The activity of transcription factors can regulate differential gene expression in cells, resulting in the development of different cell products and functions. For example, scientists have found that primary sexual characteristics is regulated by several genes **Figure 16.9**. In the fruit fly *Drosophila*, the *slx* gene determines sex. This gene is expressed when the organism has two copies of the X chromosome. The gene product for *slx* binds to the mRNA of the *tra* gene and regulates its splicing. In the presence of *slx*, *tra* is spliced into its female form and influences the expression of *dsx* and *fru* to result in female sexual characteristics. In the absence of *slx*, *tra* is spliced into its male form and male sexual characteristics result.



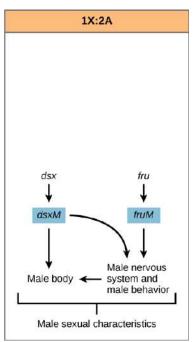


Figure 16.9 In *Drosophila melanogaster*, the sex is determined by a series of splicing events in sex determination genes on a cell-by-cell basis without any involvement of sex hormones (which circulate throughout the entire body). The primary sex-determination gene is *Sex lethal (Sxl)*, which is transcribed only when the *X/A* ratio (the *X* chromosome-to-autosome ratio) equals or exceeds 1. As females have two *X* chromosomes and males have one, *Sxl* is transcribed only in females (see the figure, left part). Sxl is a splicing repressor and binds to its target, the primary RNA of the *transformer (tra)* gene, which undergoes differential splicing depending on the presence (female) or absence (male) of Sxl, yielding a protein-coding *tra* mRNA only in females. The Tra protein binds to the primary RNAs of *doublesex (dsx)* and *fruitless (fru)*. In females, Tra promotes splicing to occur near its binding site, whereas in males it uses an alternative, default splice site. The *dsx* primary RNA thus produces female-specific mRNA and male-specific mRNA, both of which encode functional Dsx proteins, DsxF and DsxM, respectively. The presence (female) and absence (male) of Tra similarly results in female-type *fru* mRNA and male-type *fru* mRNA, but here, only the male-type *fru* mRNA encodes a functional protein.





View the process of transcription—the making of RNA from a DNA template—at **this site** (http://openstaxcollege.org/l/transcript_RNA).

Describe the major events that occur during eukaryotic transcription.

- a. DNA unwinds, transcription factors bind, the termination complex forms, and DNA polymerase adds nucleotides to the mRNA.
- b. DNA unwinds, transcription factors bind, and RNA polymerase adds nucleotides to the mRNA.
- c. The transcription complex forms, transcription factors add nucleotides to the forming mRNA, and the mRNA disconnects from the DNA.
- d. Elongation occurs, followed by the formation of the transcription initiation complex and the disconnection of the mRNA strand from DNA.

The Promoter and the Transcription Machinery

Genes are organized to make the control of gene expression easier. The promoter region is immediately upstream of the coding sequence. This region can be short (only a few nucleotides in length) or quite long (hundreds of nucleotides long). The longer the promoter, the more available space for proteins to bind. This also adds more control to the transcription process. The length of the promoter is gene-specific and can differ dramatically between genes. Consequently, the level of control of gene expression can also differ quite dramatically between genes. The purpose of the promoter is to bind transcription factors that control the initiation of transcription.

Within the promoter region, just upstream of the transcriptional start site, resides the TATA box. This box is simply a repeat of thymine and adenine dinucleotides (literally, TATA repeats). RNA polymerase binds to the transcription initiation complex, allowing transcription to occur. To initiate transcription, a transcription factor (TFIID) is the first to bind to the TATA box. Binding of TFIID recruits other transcription factors, including TFIIB, TFIIE, TFIIF, and TFIIH to the TATA box. Once this complex is assembled, RNA polymerase can bind to its upstream sequence. When bound along with the transcription factors, RNA polymerase is phosphorylated. This releases part of the protein from the DNA to activate the transcription initiation complex and places RNA polymerase in the correct orientation to begin transcription; DNA-bending protein brings the enhancer, which can be quite a distance from the gene, in contact with transcription factors and mediator proteins (Figure 16.10).

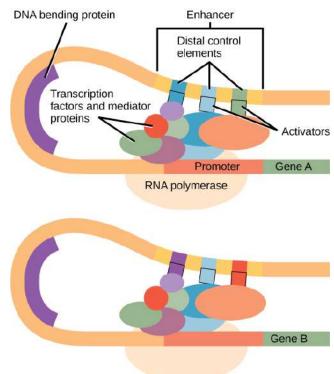


Figure 16.10 An enhancer is a DNA sequence that promotes transcription. Each enhancer is made up of short DNA sequences called distal control elements. Activators bound to the distal control elements interact with mediator proteins and transcription factors. Two different genes may have the same promoter but different distal control elements, enabling differential gene expression.

In addition to the general transcription factors, other transcription factors can bind to the promoter to regulate gene transcription. These transcription factors bind to the promoters of a specific set of genes. They are not general transcription factors that bind to every promoter complex, but are recruited to a specific sequence on the promoter of a specific gene. There are hundreds of transcription factors in a cell that each bind specifically to a particular DNA sequence motif. When transcription factors bind to the promoter just upstream of the encoded gene, it is referred to as a *cis-acting element*, because it is on the same chromosome just next to the gene. The region that a particular transcription factor binds to is called the **transcription factor binding site**. Transcription factors respond to environmental stimuli that cause the proteins to find their binding sites and initiate transcription of the gene that is needed.

Enhancers and Transcription

In some eukaryotic genes, there are regions that help increase or enhance transcription. These regions, called **enhancers**,

are not necessarily close to the genes they enhance. They can be located upstream of a gene, within the coding region of the gene, downstream of a gene, or may be thousands of nucleotides away.

Enhancer regions are binding sequences, or sites, for transcription factors. When a DNA-bending protein binds, the shape of the DNA changes (Figure 16.10). This shape change allows for the interaction of the activators bound to the enhancers with the transcription factors bound to the promoter region and the RNA polymerase. Whereas DNA is generally depicted as a straight line in two dimensions, it is actually a three-dimensional object. Therefore, a nucleotide sequence thousands of nucleotides away can fold over and interact with a specific promoter.

Turning Genes Off: Transcriptional Repressors

Like prokaryotic cells, eukaryotic cells also have mechanisms to prevent transcription. Transcriptional repressors can bind to promoter or enhancer regions and block transcription. Like the transcriptional activators, repressors respond to external stimuli to prevent the binding of activating transcription factors.



Think About It

How can cells in a multicellular eukaryotic organism be of different types given that they all share the same genome?

16.5 | Eukaryotic Post-transcriptional Gene Regulation

In this section, you will explore the following question:

· How is gene expression controlled through post-transcriptional modifications of RNA molecules?

Connection for AP® Courses

Post-transcriptional regulation can occur at any stage after transcription. One important post-transcriptional mechanism is RNA splicing. After RNA is transcribed, it is often modified to create a mature RNA that is ready to be translated. As we studied in previous chapters, processing messenger RNA involves the removal of introns that do not code for protein. Spliceosomes remove the introns and ligate the exons together, often in different sequences than their original order on the newly transcribed (immature) messenger RNA. A GTP cap is added to the 5'-end and a poly-A tail is added to the 3'-end. This mature messenger RNA then leaves the nucleus and enters the cytoplasm. Once in the cytoplasm, the length of time the messenger RNA resides there before being degraded—a characteristic lifespan or "shelf-life" of the molecule called RNA stability—can be altered to control the amount of protein that is synthesized. RNA stability is controlled by several factors, including microRNAs (miRNA or RNAi, RNA interference); miRNAs always decrease stability and promote decay of messenger RNA.

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 of the AP[®] Biology Curriculum Framework. The learning objectives listed in the Curriculum Framework provide a transparent foundation for the AP[®] Biology course, an inquiry-based laboratory experience, instructional activities, and AP[®] exam questions. A learning objective merges required content with one or more of the seven science practices.

Big Idea 3	Living systems store, retrieve, transmit and respond to information essential to life processes.
Enduring Understanding 3.A	Heritable information provides for continuity of life.
Essential Knowledge	3.A.1 DNA, and in some cases RNA, is the primary source of heritable information.

Science Practice	6.5 The student can evaluate alternative scientific explanations.
Learning Objective	3.1 The student is able to construct scientific explanations that use the structures and mechanisms of DNA and RNA to support the claim that DNA and, in some cases, RNA are the primary source of heritable information.
Essential Knowledge	3.A.1 DNA, and in some cases RNA, is the primary source of heritable information.
Science Practice	6.4 The student can make claims and predictions about natural phenomena based on scientific theories and models.
Learning Objective	3.6 The student can predict how a change in a specific DNA or RNA sequence can result in changes in gene expression.

RNA is transcribed, but must be processed into a mature form before translation can begin. This processing after an RNA molecule has been transcribed, but before it is translated into a protein, is called post-transcriptional modification. As with the epigenetic and transcriptional stages of processing, this post-transcriptional step can also be regulated to control gene expression in the cell. If the RNA is not processed, shuttled, or translated, then no protein will be synthesized.

RNA Splicing, the First Stage of Post-transcriptional Control

In eukaryotic cells, the RNA transcript often contains regions, called introns, that are removed prior to translation. The regions of RNA that code for protein are called exons (**Figure 16.11**). After an RNA molecule has been transcribed, but prior to its departure from the nucleus to be translated, the RNA is processed and the introns are removed by splicing.

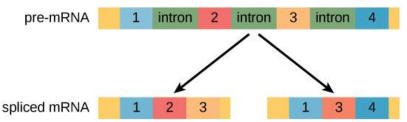


Figure 16.11 Pre-mRNA can be alternatively spliced to create different proteins.

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Alternative RNA Splicing

In the 1970s, genes were first observed that exhibited alternative RNA splicing. Alternative RNA splicing is a mechanism that allows different protein products to be produced from one gene when different combinations of introns, and sometimes exons, are removed from the transcript (Figure 16.12). This alternative splicing can be haphazard, but more often it is controlled and acts as a mechanism of gene regulation, with the frequency of different splicing alternatives controlled by the cell as a way to control the production of different protein products in different cells or at different stages of development. Alternative splicing is now understood to be a common mechanism of gene regulation in eukaryotes; according to one estimate, 70 percent of genes in humans are expressed as multiple proteins through alternative splicing.

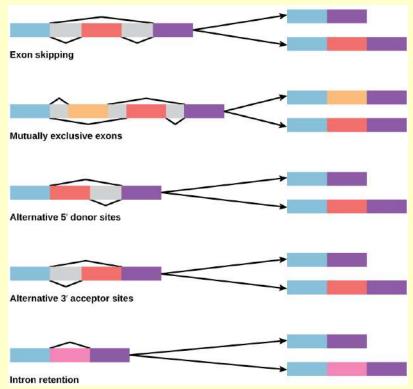


Figure 16.12 There are five basic modes of alternative splicing.

Does alternative gene splicing increase or decrease the flexibility of gene expression? Explain your answer.

- a. Flexibility increases because mRNA can be altered after transcription is completed.
- b. Flexibility increases because genes can be divided and recombined into new genes.
- c. Flexibility decreases because the mRNA molecule becomes smaller.
- d. Flexibility decreases because DNA is degraded during alternative splicing.





Visualize how mRNA splicing happens by watching the process in action in this video (http://openstaxcollege.org/l/mRNA_splicing).

Several human diseases are caused by an error in mRNA splicing. Explain why this occurs.

- a. Once an mRNA is spliced, the original mRNA cannot be created again.
- b. Spliced RNA cannot produce proper proteins.
- c. Splicing does not occur at all.
- d. Splicing occurs in the wrong location on mRNA.



Think About It

What is an evolutionary advantage of alternative gene splicing of introns during post-transcriptional modification of mRNA?

Control of RNA Stability

Before the mRNA leaves the nucleus, it is given two protective "caps" that prevent the end of the strand from degrading during its journey. The **5' cap**, which is placed on the 5' end of the mRNA, is usually composed of a methylated guanosine triphosphate molecule (GTP). The **poly-A tail**, which is attached to the 3' end, is usually composed of a series of adenine nucleotides. Once the RNA is transported to the cytoplasm, the length of time that the RNA resides there can be controlled. Each RNA molecule has a defined lifespan and decays at a specific rate. This rate of decay can influence how much protein is in the cell. If the decay rate is increased, the RNA will not exist in the cytoplasm as long, shortening the time for translation to occur. Conversely, if the rate of decay is decreased, the RNA molecule will reside in the cytoplasm longer and more protein can be translated. This rate of decay is referred to as the RNA stability. If the RNA is stable, it will be detected for longer periods of time in the cytoplasm.

Binding of proteins to the RNA can influence its stability. Proteins, called **RNA-binding proteins**, or RBPs, can bind to the regions of the RNA just upstream or downstream of the protein-coding region. These regions in the RNA that are not translated into protein are called the **untranslated regions**, or UTRs. They are not introns (those have been removed in the nucleus). Rather, these are regions that regulate mRNA localization, stability, and protein translation. The region just before the protein-coding region is called the **5' UTR**, whereas the region after the coding region is called the **3' UTR** (**Figure 16.13**). The binding of RBPs to these regions can increase or decrease the stability of an RNA molecule, depending on the specific RBP that binds.

Figure 16.13 The protein-coding region of mRNA is flanked by 5' and 3' untranslated regions (UTRs). The presence of RNA-binding proteins at the 5' or 3' UTR influences the stability of the RNA molecule.

RNA Stability and microRNAs

In addition to RBPs that bind to and control (increase or decrease) RNA stability, other elements called microRNAs can bind to the RNA molecule. These **microRNAs**, or miRNAs, are short RNA molecules that are only 21–24 nucleotides in length. The miRNAs are made in the nucleus as longer pre-miRNAs. These pre-miRNAs are chopped into mature miRNAs by a protein called **dicer**. Like transcription factors and RBPs, mature miRNAs recognize a specific sequence and bind to the RNA; however, miRNAs also associate with a ribonucleoprotein complex called the **RNA-induced silencing complex (RISC)**. RISC binds along with the miRNA to degrade the target mRNA. Together, miRNAs and the RISC complex rapidly destroy the RNA molecule.

16.6 | Eukaryotic Translational and Post-translational Gene Regulation

In this section, you will explore the following question:

• What are different ways in which translational and post-translational control of gene expression take place?

Connection for AP® Courses

Changing the status of the RNA or the protein itself can affect the amount of protein produced, the function of the protein, or how long the protein resides in the cell. Modifications such as phosphorylation and environmental stimuli can affect the stability and function of the protein.

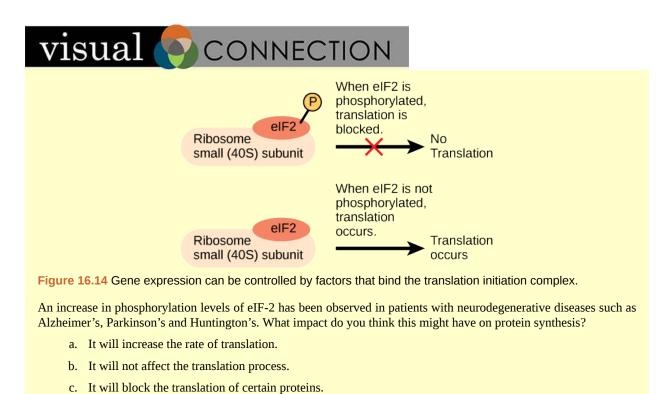
Information presented and the examples highlighted in the section support concepts outlined in Big Idea 4 of the $AP^{\$}$ Biology Curriculum Framework. The Learning Objectives listed in the Curriculum Framework provide a transparent foundation for the $AP^{\$}$ Biology course, an inquiry-based laboratory experience, instructional activities, and $AP^{\$}$ exam questions. A Learning Objective merges required content with one or more of the seven Science Practices.

Big Idea 4	Biological systems interact, and these systems and their interactions possess complex properties.
Enduring Understanding 4.A	Interactions within biological systems lead to complex properties.
Essential Knowledge	4.A.3 Interactions between external stimuli and regulated gene expression result in specialization of cells, tissues and organs.
Science Practice	1.3 The student can refine representations and models of natural or man-made phenomena and systems in the domain.
Learning Objective	4.7 The student is able to refine representations to illustrate how interactions between external stimuli and gene expression result in specialization of cells, tissues, and organs.

After the RNA has been transported to the cytoplasm, it is translated into protein. Control of this process is largely dependent on the RNA molecule. As previously discussed, the stability of the RNA will have a large impact on its translation into a protein. As the stability changes, the amount of time that it is available for translation also changes.

The Initiation Complex and Translation Rate

Like transcription, translation is controlled by proteins that bind and initiate the process. In translation, the complex that assembles to start the process is referred to as the **initiation complex**. The first protein to bind to the RNA to initiate translation is the **eukaryotic initiation factor-2 (eIF-2)**. The eIF-2 protein is active when it binds to the high-energy molecule **guanosine triphosphate (GTP)**. GTP provides the energy to start the reaction by giving up a phosphate and becoming **guanosine diphosphate (GDP)**. The eIF-2 protein bound to GTP binds to the small **40S ribosomal subunit**. When bound, the methionine initiator tRNA associates with the eIF-2/40S ribosome complex, bringing along with it the mRNA to be translated. At this point, when the initiator complex is assembled, the GTP is converted into GDP and energy is released. The phosphate and the eIF-2 protein are released from the complex and the large **60S ribosomal subunit** binds to translate the RNA. The binding of eIF-2 to the RNA is controlled by phosphorylation. If eIF-2 is phosphorylated, it undergoes a conformational change and cannot bind to GTP. Therefore, the initiation complex cannot form properly and translation is impeded (**Figure 16.14**). When eIF-2 remains unphosphorylated, it binds the RNA and actively translates the protein.



Chemical Modifications, Protein Activity, and Longevity

d. It will produce multiple fragments of polypeptides.

Proteins can be chemically modified with the addition of groups including methyl, phosphate, acetyl, and ubiquitin groups. The addition or removal of these groups from proteins regulates their activity or the length of time they exist in the cell. Sometimes these modifications can regulate where a protein is found in the cell—for example, in the nucleus, the cytoplasm, or attached to the plasma membrane.

Chemical modifications occur in response to external stimuli such as stress, the lack of nutrients, heat, or ultraviolet light exposure. These changes can alter epigenetic accessibility, transcription, mRNA stability, or translation—all resulting in changes in expression of various genes. This is an efficient way for the cell to rapidly change the levels of specific proteins in response to the environment. Because proteins are involved in every stage of gene regulation, the phosphorylation of a protein (depending on the protein that is modified) can alter accessibility to the chromosome, can alter translation (by altering transcription factor binding or function), can change nuclear shuttling (by influencing modifications to the nuclear pore complex), can alter RNA stability (by binding or not binding to the RNA to regulate its stability), can modify translation (increase or decrease), or can change post-translational modifications (add or remove phosphates or other chemical modifications).

The addition of an ubiquitin group to a protein marks that protein for degradation. Ubiquitin acts like a flag indicating that the protein lifespan is complete. These proteins are moved to the **proteasome**, an organelle that functions to remove proteins, to be degraded (**Figure 16.15**). One way to control gene expression, therefore, is to alter the longevity of the protein.

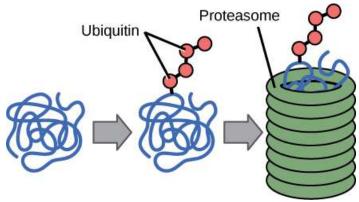


Figure 16.15 Proteins with ubiquitin tags are marked for degradation within the proteasome.



Think About It

How can environmental stimuli such as ultraviolet light exposure or nutrient deficiency modify gene expression?

16.7 | Cancer and Gene Regulation

In this section, you will explore the following questions:

- · How can changes in gene expression cause cancer?
- · How can changes to gene expression at different levels disrupt the cell cycle?

Connection for AP® Courses

Cancer is a disease of altered gene expression that can occur at every level of control, including at the levels of DNA methylation, histone acetylation, and activation of transcription factors. By understanding how each stage of gene regulation works in normal cells, we can understand what goes wrong in diseased states. For example, changes in the activity of the tumor suppressor gene *p*53 can result in cancer. Phosphorylation and other protein modifications have also been implicated in cancer.

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 of the $AP^{\$}$ Biology Curriculum Framework. The learning objectives listed in the Curriculum Framework provide a transparent foundation for the $AP^{\$}$ Biology course, an inquiry-based laboratory experience, instructional activities, and $AP^{\$}$ exam questions. A Learning Objective merges required content with one or more of the seven Science Practices.

Big Idea 3	Living systems store, retrieve, transmit and respond to information essential to life processes.
Enduring Understanding 3.B	Expression of genetic information involves cellular and molecular mechanisms.

Essential Knowledge	3.B.2 A variety of intercellular and intracellular signal transmissions mediate gene expression.
Science Practice	6.2 The student can construct explanations of phenomena based on evidence produced through scientific practices.
Learning Objective	3.22 The student is able to explain how signal pathways mediate gene expression, including how this process can affect protein production.
Essential Knowledge	3.B.2 A variety of intercellular and intracellular signal transmissions mediate gene expression.
Science Practice	1.4 The student can use representations and models to analyze situations or solve problems qualitatively and quantitatively.
Learning Objective	3.23 The student can use representations to describe mechanisms of the regulation of gene expression.

Cancer is not a single disease but includes many different diseases. In cancer cells, mutations modify cell-cycle control and cells don't stop growing as they normally would. Mutations can also alter the growth rate or the progression of the cell through the cell cycle. One example of a gene modification that alters the growth rate is increased phosphorylation of cyclin B, a protein that controls the progression of a cell through the cell cycle and serves as a cell-cycle checkpoint protein.

For cells to move through each phase of the cell cycle, the cell must pass through checkpoints. This ensures that the cell has properly completed the step and has not encountered any mutation that will alter its function. Many proteins, including cyclin B, control these checkpoints. The phosphorylation of cyclin B, a post-translational event, alters its function. As a result, cells can progress through the cell cycle unimpeded, even if mutations exist in the cell and its growth should be terminated. This post-translational change of cyclin B prevents it from controlling the cell cycle and contributes to the development of cancer.

Cancer: Disease of Altered Gene Expression

Cancer can be described as a disease of altered gene expression. There are many proteins that are turned on or off (gene activation or gene silencing) that dramatically alter the overall activity of the cell. A gene that is not normally expressed in that cell can be switched on and expressed at high levels. This can be the result of gene mutation or changes in gene regulation (epigenetic, transcription, post-transcription, translation, or post-translation).

Changes in epigenetic regulation, transcription, RNA stability, protein translation, and post-translational control can be detected in cancer. While these changes don't occur simultaneously in one cancer, changes at each of these levels can be detected when observing cancer at different sites in different individuals. Therefore, changes in **histone acetylation** (epigenetic modification that leads to gene silencing), activation of transcription factors by phosphorylation, increased RNA stability, increased translational control, and protein modification can all be detected at some point in various cancer cells. Scientists are working to understand the common changes that give rise to certain types of cancer or how a modification might be exploited to destroy a tumor cell.

Tumor Suppressor Genes, Oncogenes, and Cancer

In normal cells, some genes function to prevent excess, inappropriate cell growth. These are tumor suppressor genes, which are active in normal cells to prevent uncontrolled cell growth. There are many tumor suppressor genes in cells. The most studied tumor suppressor gene is p53, which is mutated in over 50 percent of all cancer types. The p53 protein itself functions as a transcription factor. It can bind to sites in the promoters of genes to initiate transcription. Therefore, the mutation of p53 in cancer will dramatically alter the transcriptional activity of its target genes.





Watch this animation (http://openstaxcollege.org/l/p53_cancer) to learn more about the use of p53 in fighting cancer.

Treatment of cancer is often called a "fight against biology." Explain why the use of p53 supports this statement.

- a. because normal cells are often negatively affected by cancer treatments, including p53
- b. because cancer cells are always affected by current cancer treatments, including p53
- c. because normal cells are often negatively affected by cancer treatments, with the exception of p53
- d. because cancer cells often aren't affected by cancer treatments, with the exception of p53

Proto-oncogenes are positive cell-cycle regulators. When mutated, proto-oncogenes can become oncogenes and cause cancer. Overexpression of the oncogene can lead to uncontrolled cell growth. This is because oncogenes can alter transcriptional activity, stability, or protein translation of another gene that directly or indirectly controls cell growth. An example of an oncogene involved in cancer is a protein called myc. **Myc** is a transcription factor that is aberrantly activated in Burkett's Lymphoma, a cancer of the lymph system. Overexpression of myc transforms normal B cells into cancerous cells that continue to grow uncontrollably. High B-cell numbers can result in tumors that can interfere with normal bodily function. Patients with Burkett's lymphoma can develop tumors on their jaw or in their mouth that interfere with the ability to eat.

Cancer and Epigenetic Alterations

Silencing genes through epigenetic mechanisms is also very common in cancer cells. There are characteristic modifications to histone proteins and DNA that are associated with silenced genes. In cancer cells, the DNA in the promoter region of silenced genes is methylated on cytosine DNA residues in CpG islands. Histone proteins that surround that region lack the acetylation modification that is present when the genes are expressed in normal cells. This combination of DNA methylation and histone deacetylation (epigenetic modifications that lead to gene silencing) is commonly found in cancer. When these modifications occur, the gene present in that chromosomal region is silenced. Increasingly, scientists understand how epigenetic changes are altered in cancer. Because these changes are temporary and can be reversed—for example, by preventing the action of the histone deacetylase protein that removes acetyl groups, or by DNA methyl transferase enzymes that add methyl groups to cytosines in DNA—it is possible to design new drugs and new therapies to take advantage of the reversible nature of these processes. Indeed, many researchers are testing how a silenced gene can be switched back on in a cancer cell to help re-establish normal growth patterns.

Genes involved in the development of many other illnesses, ranging from allergies to inflammation to autism, are thought to be regulated by epigenetic mechanisms. As our knowledge of how genes are controlled deepens, new ways to treat diseases like cancer will emerge.

Cancer and Transcriptional Control

Alterations in cells that give rise to cancer can affect the transcriptional control of gene expression. Mutations that activate transcription factors, such as increased phosphorylation, can increase the binding of a transcription factor to its binding site in a promoter. This could lead to increased transcriptional activation of that gene that results in modified cell growth. Alternatively, a mutation in the DNA of a promoter or enhancer region can increase the binding ability of a transcription factor. This could also lead to the increased transcription and aberrant gene expression that is seen in cancer cells.

Researchers have been investigating how to control the transcriptional activation of gene expression in cancer. Identifying how a transcription factor binds, or a pathway that activates where a gene can be turned off, has led to new drugs and new ways to treat cancer. In breast cancer, for example, many proteins are overexpressed. This can lead to increased phosphorylation of key transcription factors that increase transcription. One such example is the overexpression of the epidermal growth factor receptor (EGFR) in a subset of breast cancers. The EGFR pathway activates many protein kinases that, in turn, activate many transcription factors that control genes involved in cell growth. New drugs that prevent the

activation of EGFR have been developed and are used to treat these cancers.

Cancer and Post-transcriptional Control

Changes in the post-transcriptional control of a gene can also result in cancer. Recently, several groups of researchers have shown that specific cancers have altered expression of miRNAs. Because miRNAs bind to the 3' UTR of RNA molecules to degrade them, overexpression of these miRNAs could be detrimental to normal cellular activity. Too many miRNAs could dramatically decrease the RNA population leading to a decrease in protein expression. Several studies have demonstrated a change in the miRNA population in specific cancer types. It appears that the subset of miRNAs expressed in breast cancer cells is quite different from the subset expressed in lung cancer cells or even from normal breast cells. This suggests that alterations in miRNA activity can contribute to the growth of breast cancer cells. These types of studies also suggest that if some miRNAs are specifically expressed only in cancer cells, they could be potential drug targets. It would, therefore, be conceivable that new drugs that turn off miRNA expression in cancer could be an effective method to treat cancer.

Cancer and Translational/Post-translational Control

There are many examples of how translational or post-translational modifications of proteins arise in cancer. Modifications are found in cancer cells from the increased translation of a protein to changes in protein phosphorylation to alternative splice variants of a protein. An example of how the expression of an alternative form of a protein can have dramatically different outcomes is seen in colon cancer cells. The c-Flip protein, a protein involved in mediating the cell death pathway, comes in two forms: long (c-FLIPL) and short (c-FLIPS). Both forms appear to be involved in initiating controlled cell death mechanisms in normal cells. However, in colon cancer cells, expression of the long form results in increased cell growth instead of cell death. Clearly, the expression of the wrong protein dramatically alters cell function and contributes to the development of cancer.

New Drugs to Combat Cancer: Targeted Therapies

Scientists are using what is known about the regulation of gene expression in disease states, including cancer, to develop new ways to treat and prevent disease development. Many scientists are designing drugs on the basis of the gene expression patterns within individual tumors. This idea, that therapy and medicines can be tailored to an individual, has given rise to the field of personalized medicine. With an increased understanding of gene regulation and gene function, medicines can be designed to specifically target diseased cells without harming healthy cells. Some new medicines, called targeted therapies, have exploited the overexpression of a specific protein or the mutation of a gene to develop a new medication to treat disease. One such example is the use of anti-EGF receptor medications to treat the subset of breast cancer tumors that have very high levels of the EGF protein. Undoubtedly, more targeted therapies will be developed as scientists learn more about how gene expression changes can cause cancer.



Clinical Trial Coordinator

A clinical trial coordinator is the person managing the proceedings of the clinical trial. This job includes coordinating patient schedules and appointments, maintaining detailed notes, building the database to track patients (especially for long-term follow-up studies), ensuring proper documentation has been acquired and accepted, and working with the nurses and doctors to facilitate the trial and publication of the results. A clinical trial coordinator may have a science background, like a nursing degree, or other certification. People who have worked in science labs or in clinical offices are also qualified to become a clinical trial coordinator. These jobs are generally in hospitals; however, some clinics and doctor's offices also conduct clinical trials and may hire a coordinator.



Think About It

New drugs are being developed that decrease DNA methylation and prevent the removal of acetyl groups from histone proteins. Explain how these drugs could affect gene expression to help kill tumor cells.

How can understanding the gene expression in a cancer cell tell you something about that specific form of cancer?

KEY TERMS

3' UTR 3' untranslated region; region just downstream of the protein-coding region in an RNA molecule that is not translated

5' cap a methylated guanosine triphosphate (GTP) molecule that is attached to the 5' end of a messenger RNA to protect the end from degradation

5' UTR 5' untranslated region; region just upstream of the protein-coding region in an RNA molecule that is not translated

activator protein that binds to prokaryotic operators to increase transcription

catabolite activator protein (CAP) protein that complexes with cAMP to bind to the promoter sequences of operons that control sugar processing when glucose is not available

cis-acting element transcription factor binding sites within the promoter that regulate the transcription of a gene adjacent to it

dicer enzyme that chops the pre-miRNA into the mature form of the miRNA

DNA methylation epigenetic modification that leads to gene silencing; commonly found in cancer cells

enhancer segment of DNA that is upstream, downstream, perhaps thousands of nucleotides away, or on another chromosome that influence the transcription of a specific gene

epigenetic heritable changes that do not involve changes in the DNA sequence

eukaryotic initiation factor-2 (eIF-2) protein that binds first to an mRNA to initiate translation

gene expression processes that control the turning on or turning off of a gene

guanine diphosphate (GDP) molecule that is left after the energy is used to start translation

guanine triphosphate (GTP) energy-providing molecule that binds to eIF-2 and is needed for translation

histone acetylation epigenetic modification that leads to gene silencing; commonly found in cancer cells.

inducible operon operon that can be activated or repressed depending on cellular needs and the surrounding environment

initiation complex protein complex containing eIF2-2 that starts translation

lac operon operon in prokaryotic cells that encodes genes required for processing and intake of lactose

large 60S ribosomal subunit second, larger ribosomal subunit that binds to the RNA to translate it into protein

microRNA (miRNA) small RNA molecules (approximately 21 nucleotides in length) that bind to RNA molecules to degrade them

myc oncogene that causes cancer in many cancer cells

negative regulator protein that prevents transcription

operator region of DNA outside of the promoter region that binds activators or repressors that control gene expression in prokaryotic cells

operon collection of genes involved in a pathway that are transcribed together as a single mRNA in prokaryotic cells

poly-A tail a series of adenine nucleotides that are attached to the 3' end of an mRNA to protect the end from degradation

positive regulator protein that increases transcription

post-transcriptional control of gene expression after the RNA molecule has been created but before it is translated into

protein

post-translational control of gene expression after a protein has been created

proteasome organelle that degrades proteins

repressor protein that binds to the operator of prokaryotic genes to prevent transcription

RISC protein complex that binds along with the miRNA to the RNA to degrade it

RNA stability how long an RNA molecule will remain intact in the cytoplasm

RNA-binding protein (RBP) protein that binds to the 3' or 5' UTR to increase or decrease the RNA stability

small 40S ribosomal subunit ribosomal subunit that binds to the RNA to translate it into protein

trans-acting element transcription factor binding site found outside the promoter or on another chromosome that influences the transcription of a particular gene

transcription factor protein that binds to the DNA at the promoter or enhancer region and that influences transcription of a gene

transcription factor binding site sequence of DNA to which a transcription factor binds

transcriptional start site site at which transcription begins

trp operon series of genes necessary to synthesize tryptophan in prokaryotic cells

tryptophan amino acid that can be synthesized by prokaryotic cells when necessary

untranslated region segment of the RNA molecule that are not translated into protein. These regions lie before (upstream or 5') and after (downstream or 3') the protein-coding region

CHAPTER SUMMARY

16.1 Regulation of Gene Expression

While all somatic cells within an organism contain the same DNA, not all cells within that organism express the same proteins. Prokaryotic organisms express the entire DNA they encode in every cell, but not necessarily all at the same time. Proteins are expressed only when they are needed. Eukaryotic organisms express a subset of the DNA that is encoded in any given cell. In each cell type, the type and amount of protein is regulated by controlling gene expression. To express a protein, the DNA is first transcribed into RNA, which is then translated into proteins. In prokaryotic cells, these processes occur almost simultaneously. In eukaryotic cells, transcription occurs in the nucleus and is separate from the translation that occurs in the cytoplasm. Gene expression in prokaryotes is mostly regulated at the transcriptional level, whereas in eukaryotic cells, gene expression is regulated at the epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels.

16.2 Prokaryotic Gene Regulation

The regulation of gene expression in prokaryotic cells occurs at the transcriptional level. There are three ways to control the transcription of an operon: repressive control, activator control, and inducible control. Repressive control, typified by the *trp* operon, uses proteins bound to the operator sequence to physically prevent the binding of RNA polymerase and the activation of transcription. Therefore, if tryptophan is not needed, the repressor is bound to the operator and transcription remains off. Activator control, typified by the action of CAP, increases the binding ability of RNA polymerase to the promoter when CAP is bound. In this case, low levels of glucose result in the binding of cAMP to CAP. CAP then binds the promoter, which allows RNA polymerase to bind to the promoter better. In the last example—the *lac* operon—two conditions must be met to initiate transcription. Glucose must not be present, and lactose must be available for the *lac* operon to be transcribed. If glucose is absent, CAP binds to the operator. If lactose is present, the repressor protein does not bind to its operator. Only when both conditions are met will RNA polymerase bind to the promoter to induce transcription.

16.3 Eukaryotic Epigenetic Gene Regulation

In eukaryotic cells, the first stage of gene expression control occurs at the epigenetic level. Epigenetic mechanisms control access to the chromosomal region to allow genes to be turned on or off. These mechanisms control how DNA is packed into the nucleus by regulating how tightly the DNA is wound around histone proteins. The addition or removal of chemical modifications (or flags) to histone proteins or DNA signals to the cell to open or close a chromosomal region. Therefore, eukaryotic cells can control whether a gene is expressed by controlling accessibility to transcription factors and the binding of RNA polymerase to initiate transcription.

16.4 Eukaryotic Transcriptional Gene Regulation

To start transcription, general transcription factors, such as TFIID, TFIIH, and others, must first bind to the TATA box and recruit RNA polymerase to that location. The binding of additional regulatory transcription factors to *cis*-acting elements will either increase or prevent transcription. In addition to promoter sequences, enhancer regions help augment transcription. Enhancers can be upstream, downstream, within a gene itself, or on other chromosomes. Transcription factors bind to enhancer regions to increase or prevent transcription.

16.5 Eukaryotic Post-transcriptional Gene Regulation

Post-transcriptional control can occur at any stage after transcription, including RNA splicing, nuclear shuttling, and RNA stability. Once RNA is transcribed, it must be processed to create a mature RNA that is ready to be translated. This involves the removal of introns that do not code for protein. Spliceosomes bind to the signals that mark the exon/intron border to remove the introns and ligate the exons together. Once this occurs, the RNA is mature and can be translated. RNA is created and spliced in the nucleus, but needs to be transported to the cytoplasm to be translated. RNA is transported to the cytoplasm through the nuclear pore complex. Once the RNA is in the cytoplasm, the length of time it resides there before being degraded, called RNA stability, can also be altered to control the overall amount of protein that is synthesized. The RNA stability can be increased, leading to longer residency time in the cytoplasm, or decreased, leading to shortened time and less protein synthesis. RNA stability is controlled by RNA-binding proteins (RPBs) and microRNAs (miRNAs). These RPBs and miRNAs bind to the 5' UTR or the 3' UTR of the RNA to increase or decrease RNA stability. Depending on the RBP, the stability can be increased or decreased significantly; however, miRNAs always decrease stability and promote decay.

16.6 Eukaryotic Translational and Post-translational Gene Regulation

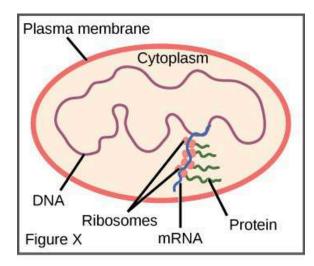
Changing the status of the RNA or the protein itself can affect the amount of protein, the function of the protein, or how long it is found in the cell. To translate the protein, a protein initiator complex must assemble on the RNA. Modifications (such as phosphorylation) of proteins in this complex can prevent proper translation from occurring. Once a protein has been synthesized, it can be modified (phosphorylated, acetylated, methylated, or ubiquitinated). These post-translational modifications can greatly impact the stability, degradation, or function of the protein.

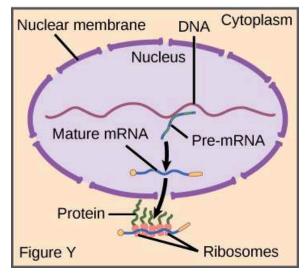
16.7 Cancer and Gene Regulation

Cancer can be described as a disease of altered gene expression. Changes at every level of eukaryotic gene expression can be detected in some form of cancer at some point in time. In order to understand how changes to gene expression can cause cancer, it is critical to understand how each stage of gene regulation works in normal cells. By understanding the mechanisms of control in normal, non-diseased cells, it will be easier for scientists to understand what goes wrong in disease states including complex ones like cancer.

REVIEW QUESTIONS

- **1.** Control of gene expression in eukaryotic cells occurs at which level(s)?
 - a. only the transcriptional level
 - b. epigenetic and transcriptional levels
 - c. epigenetic and transcriptional and translational levels
 - d. epigenetic and transcriptional, translational, and post-translational levels





What do figures X and Y in the graphic illustrate?

- a. Transcription and translation in a eukaryotic cell (figure X) and a prokaryotic cell (figure Y).
- b. Transcription and translation in a prokaryotic cell (figure X) and a eukaryotic cell (figure Y).
- c. Transcription in a eukaryotic cell (figure X) and translation in a prokaryotic cell (figure Y).
- d. Transcription in a prokaryotic cell (figure X) and translation in a eukaryotic cell (figure Y)
- **3.** If glucose is absent but lactose is present, the *lac* operon will be:
 - a. activated
 - b. repressed
 - c. partially activated
 - d. mutated
- **4.** What would happen if the operator sequence of the *lac* operon contained a mutation that prevented the repressor protein from binding the operator?

- a. In the presence of lactose, the lac operon will not be transcribed.
- In the absence of lactose, the lac operon will be transcribed.
- The cAMP-CAP complex will not increase RNA synthesis.
- d. The RNA polymerase will not bind the promoter.
- **5.** What would happen if the operator sequence of the *trp* operon contained a mutation that prevented the repressor protein from binding to the operator?
 - a. In the absence of tryptophan, the genes trpA-E will not be transcribed.
 - In the absence of tryptophan, only genes trpE and trpD will be transcribed.
 - In the presence of tryptophan, the genes trpA-E will be transcribed.
 - d. In the presence of tryptophan, the trpE gene will not be transcribed.
- **6.** What are epigenetic modifications?
 - a. the addition of reversible changes to histone proteins and DNA
 - b. the removal of nucleosomes from the DNA
 - c. the addition of more nucleosomes to the DNA
 - d. mutation of the DNA sequence
- **7.** Which of the following statements about epigenetic regulation is false?
 - a. Histone protein charge becomes more positive when acetyl groups are added.
 - b. DNA molecules are modified within CpG islands.
 - c. Methylation of DNA and histones causes nucleosomes to pack tightly together.
 - d. Histone acetylation results in the loose packing of nucleosomes.
- 8. Which of the following is true of epigenetic changes?
 - a. They only allow gene expression.
 - b. They allow movement of histones.
 - c. They change the DNA sequence.
 - d. They are always heritable.
- **9.** The binding of what is required for transcription start?
 - a. a protein
 - b. DNA polymerase
 - c. RNA polymerase
 - d. a transcription factor
- **10.** What would be the outcome of a mutation that prevented DNA binding proteins from being produced?

- a. decreased transcription because transcription factors would not bind to transcription binding sites
- b. decreased transcription because enhancers would not be able to bind to transcription factors
- c. increased transcription because repressors would not be able to bind to promoter regions
- d. increased transcription because RNA polymerase would be able to increase binding to promoter regions
- **11.** What will result from the binding of a transcription factor to an enhancer region?
 - a. decreased transcription of an adjacent gene
 - b. increased transcription of a distant gene
 - c. alteration of the translation of an adjacent gene
 - d. initiation of the recruitment of RNA polymerase
- **12.** Which of the following are involved in post-transcriptional control?
 - a. control of RNA splicing
 - b. ubiquitination
 - c. proteolytic cleavage
 - d. phosphorylation
- **13.** Gene A is thought to be associated with color blindness. The protein corresponding to gene A is isolated. Analysis of the protein recovered shows there are actually two different proteins that differ in molecular weight that correspond to gene A. What is one reason why there may be two proteins corresponding to the gene?
 - a. One protein had a 5' cap and a poly-A tail in its mRNA, and the other protein did not.
 - b. One protein had a 5' UTR and a 3' UTR in its RNA, and the other protein did not.
 - c. The gene was alternatively spliced.
 - d. The gene produced mRNA molecules with differing stability.
- **14.** Binding of an RNA binding protein will change the stability of the RNA molecule in what way?
 - a. increase
 - b. decrease
 - c. neither increase nor decrease
 - d. either increase or decrease
- **15.** A mutation in the 5'UTR that prevents any proteins from binding to the region will:

- a. increase or decrease the stability of the RNA molecule
- b. prevent translation of the RNA molecule
- c. prevent splicing of the RNA molecule
- d. increase or decrease the length of the poly-A tail
- **16.** Post-translational modifications of proteins can affect which of the following?
 - a. mRNA splicing
 - b. 5'capping
 - c. 3'polyadenylation
 - d. chemical modifications
- **17.** A mutation is found in eIF-2 that impairs the initiation of translation. The mutation could affect all but one of the following functions of eIF-2. Which one would not be affected?
 - a. The mutation prevents eIF-2 from binding to RNA
 - b. The mutation prevents eIF-2 from being phosphorylated.
 - The mutation prevents eIF-2 from binding to GTP.
 - d. The mutation prevents eIF-2 from binding to the 40S ribosomal subunit.
- **18.** The addition of a ubiquitin group to a protein does what?
 - a. increases the stability of the protein
 - b. decreases translation of the protein
 - c. increases translation of the protein
 - d. marks the protein for degradation
- **19.** What are cancer-causing genes called?
 - a. transformation genes
 - b. tumor suppressor genes
 - c. oncogenes
 - d. protooncogenes
- **20.** Targeted therapies are used in patients with a certain gene expression pattern. A targeted therapy that prevents the activation of the estrogen receptor in breast cancer would be beneficial to what type of patient?
 - a. patients who express the EGFR receptor in normal cells
 - b. patients with a mutation that inactivates the estrogen receptor
 - c. patients with over-expression of ER alpha in their tumor cells
 - d. patients with over-expression of VEGF, which helps in tumor angiogenesis
- **21.** In a new cancer treatment, a cold virus is genetically

modified so that it binds to, enters, and is replicated in cells, causing them to burst. The modified cold virus cannot replicate when wildtype p53 protein is present in the cell. How does this treatment treat cancer without harming healthy cells?

- a. The modified virus only infects and enters cancer cells.
- The modified virus replicates in normal and cancer cells.
- c. The modified virus only infects and enters normal cells.
- d. The modified virus replicates only in cancer cells.
- **22.** A drug designed to switch silenced genes back on in cancer cells would result in what?

CRITICAL THINKING QUESTIONS

- **24.** Which best distinguishes prokaryotic and eukaryotic cells?
 - a. Prokaryotes possess a nucleus whereas eukaryotes do not, but eukaryotes show greater compartmentalization that allows for greater regulation of gene expression.
 - Eukaryotic cells contain a nucleus whereas prokaryotes do not, and eukaryotes show greater compartmentalization that allows for greater regulation of gene expression.
 - c. Prokaryotic cells are less complex and perform highly-regulated gene expression whereas eukaryotes perform less-regulated gene expression.
 - d. Eukaryotic cells are more complex and perform less-regulated gene expression whereas prokaryotic cells perform highly-regulated gene expression.
- **25.** Which statement is correct regarding the distinction between prokaryotic and eukaryotic gene expression?

- a. prevent methylation of DNA and deacetylation of histones
- b. prevent methylation of DNA and acetylation of histones
- prevent deacetylation of DNA and methylation of histones
- d. prevent acetylation of DNA and demethylation of histones
- **23.** What are positive cell-cycle regulators that can cause cancer when mutated called?
 - a. transformation genes
 - b. tumor suppressor genes
 - c. oncogenes
 - d. mutated genes
 - a. Prokaryotes regulate gene expression at the level of transcription whereas eukaryotes regulate at multiple levels including epigenetic, transcriptional and translational.
 - Prokaryotes regulate gene expression at the level of translation whereas eukaryotes regulate at the level of transcription to manipulate protein levels.
 - Prokaryotes regulate gene expression with the help of repressors and activators whereas eukaryotes regulate expression by degrading mRNA transcripts, thereby controlling protein levels.
 - d. Prokaryotes control protein levels using epigenetic modifications whereas eukaryotes control protein levels by regulating the rate of transcription and translation.
- **26.** All the cells of one organisms share the genome. However, during development, some cells develop into skin cells while others develop into muscle cells. How can the same genetic instructions result in two different cell types in the same organism? Thoroughly explain your answer.
- **27.** Which of the following statements describes prokaryotic transcription of the lac operon?
 - a. When lactose and glucose are present in the medium, transcription of the lac operon is induced.
 - b. When lactose is present but glucose is absent, the lac operon is repressed.
 - c. Lactose acts as an inducer of the lac operon when glucose is absent.
 - d. Lactose acts as an inducer of the lac operon when glucose is present.

- **28.** The lac operon consists of regulatory regions such as the promoter as well as the structural genes lacZ, lacY, and lacA, which code for proteins involved in lactose metabolism. What would be the outcome of a mutation in one of the structural genes of the *lac* operon?
 - a. Mutation in structural genes will stop transcription.
 - b. Mutated lacY will produce an abnormal β galactosidase protein.
 - c. Mutated lacA will produce a protein that will transfer an acetyl group to $\,\beta\,$ galactosidase.
 - d. Transcription will continue but lactose will not be metabolized properly.
- **29.** In some diseases, alteration to epigenetic modifications turns off genes that are normally expressed. Hypothetically, how could you reverse this process to turn these genes back on?
- **30.** Flowering Locus C (FLC) is a gene that is responsible for flowering in certain plants. FLC is expressed in new seedlings, which prevents flowering. Upon exposure to cold temperatures, FLC expression decreases and the plant flowers. FLC is regulated through epigenetic modifications. What type of epigenetic modifications are present in new seedlings and after cold exposure?
 - a. In new seedlings, histone acetylations are present; upon cold exposure, methylation occurs.
 - In new seedlings, histone deacetylations are present; upon cold exposure, methylation occurs.
 - c. In new seedlings, histone methylations are present; upon cold exposure, acetylation occurs.
 - d. In new seedlings, histone methylations are present; upon cold exposure, deacetylation occurs.
- **31.** A mutation within the promoter region can alter gene transcription. Describe how this can happen.
 - a. Mutated promoters decrease the rate of transcription by altering the binding site for the transcription factor.
 - Mutated promoters increase the rate of transcription by altering the binding site for the transcription factor.
 - Mutated promoters alter the binding site for transcription factors to increase or decrease the rate of transcription.
 - d. Mutated promoters alter the binding site for transcription factors and thereby cease transcription of the adjacent gene.
- **32.** What could happen if a cell had too much of an activating transcription factor present?

- The transcription rate would increase, altering cell function.
- The transcription rate would decrease, inhibiting cell functions.
- The transcription rate decreases due to clogging of the transcription factors.
- d. The transcription rate increases due to clogging of the transcription factors.
- **33.** The *wnt* transcription pathway is responsible for key changes during animal development. Based on the transcription pathway shown below. In this diagram, arrows indicate the transformation of one substance into another. Square lines, or the lines with no arrowheads, indicate inhibition of the product below the line. Based on this, how would increased *wnt* gene expression affect the expression of Bar-1?

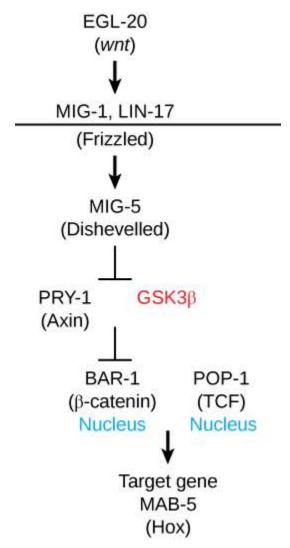


Figure 16.16

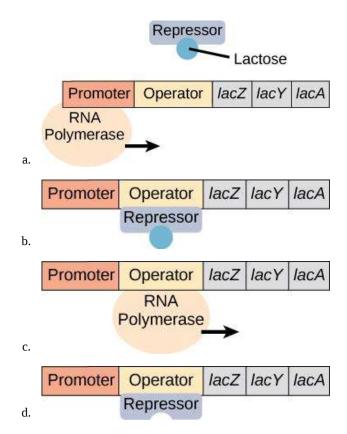
34. Describe how RBPs can prevent miRNAs from degrading an RNA molecule.

- a. RBPs can bind first to the RNA, thus preventing the binding of miRNA, which degrades RNA.
- b. RBPs bind the miRNA, thereby protecting the mRNA from degradation.
- c. RBPs methylate miRNA to inhibit its function and thus stop mRNA degradation.
- RBPs direct miRNA degradation with the help of a DICER protein complex.
- **35.** How can external stimuli alter post-transcriptional control of gene expression?
 - a. UV rays can alter methylation and acetylation of proteins.
 - b. RNA binding proteins are modified through phosphorylation.
 - c. External stimuli can cause deacetylation and demethylation of the transcript.
 - d. UV rays can cause dimerization of the RNA binding proteins.
- **36.** Protein modifications can alter gene expression in many ways. Describe how phosphorylation of proteins can alter gene expression.
 - a. Phosphorylation of proteins can alter translation, RNA shuttling, RNA stability or post transcriptional modification.
 - Phosphorylation of proteins can alter DNA replication, cell division, pathogen recognition and RNA stability.
 - c. Phosphorylated proteins affect only translation and can cause cancer by altering the p53 function.
 - d. Phosphorylated proteins affect only RNA shuttling, RNA stability, and post-translational modifications.
- **37.** Changes in epigenetic modifications alter the accessibility and transcription of DNA. Describe how environmental stimuli, such as ultraviolet light exposure, could modify gene expression.
 - a. UV rays could cause methylation and deacetylation of the genes that could alter the accessibility and transcription of DNA.
 - The UV rays could cause phosphorylation and acetylation of the DNA and histones which could alter the transcriptional capabilities of the DNA.
 - UV rays could cause methylation and phosphorylation of the DNA bases which could become dimerized rendering no accessibility of DNA.
 - d. The UV rays can cause methylation and acetylation of histones making the DNA more tightly packed and leading to inaccessibility.

- **38.** New drugs are being developed that decrease DNA methylation and prevent the removal of acetyl groups from histone proteins. Explain how these drugs could affect gene expression to help kill tumor cells.
 - These drugs maintain the demethylated and the acetylated forms of the DNA to keep transcription of necessary genes "on".
 - The demethylated and the acetylated forms of the DNA are reversed when the silenced gene is expressed.
 - c. The drug methylates and acetylates the silenced genes to turn them back "on".
 - d. Drugs maintain DNA methylation and acetylation to silence unimportant genes in cancer cells.
- **39.** How can understanding the gene expression pattern in a cancer cell tell you something about that specific form of cancer?
 - Understanding gene expression patterns in cancer cells will identify the faulty genes, which is helpful in providing the relevant drug treatment.
 - b. Understanding gene expression will help diagnose tumor cells for antigen therapy.
 - c. Gene profiling would identify the target genes of the cancer-causing pathogens.
 - d. Breast cancer patients who do not express EGFR can respond to anti-EGFR therapy.
- **40.** Explain what personalized medicine is and how it can be used to treat cancer.
 - a. Personalized medicines would vary based on the type of mutations and the gene's expression pattern.
 - b. The medicines are given based on the type of tumor found in the body of an individual.
 - c. The personalized medicines are provided based only on the symptoms of the patient.
 - d. The medicines tend to vary depending on the severity and the stage of the cancer.

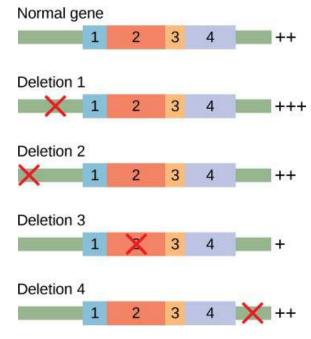
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- **41.** Which of the following is found in both prokaryotes and eukaryotes?
 - a. 3' poly-A tails
 - b. 5' caps
 - c. promoters
 - d. introns
- **42.** The enzyme ployadenylate polymerase catalyzes the addition of adenosine monophosphate to the 3' ends of mRNAs to form a poly-A tail. If the enzyme were blocked so that it could not function, the result would be:
 - a. increased mRNA stability in eukaryotes, and decreased mRNA stability in prokaryotes
 - decreased mRNA stability in eukaryotes, and no effect in prokaryotes
 - no effect in eukaryotes, and increased mRNA stability in prokaryotes
 - d. no effect in eukaryotes, and decreased mRNA stability in prokaryotes
- **43.** Describe two ways in which gene regulation differs and two ways in which it is similar in prokaryotes and eukaryotes.
 - a. Prokaryotes show co-transcriptional translation whereas eukaryotes perform transcription prior to translation; in both cell types, regulation occurs through the binding of transcription factors, activators, and repressors.
 - Prokaryotes perform transcription prior to translation whereas eukaryotes show cotranscriptional translation (the processes occur in the same organelle).
 - c. Prokaryotes show co-transcriptional translation that is regulated prior to translation whereas eukaryotes perform transcription prior to translation that is regulated only at the level of transcription. In both domains, transcription factors, activators, and repressors provide regulation.
 - d. Prokaryotes show co-transcriptional translation that occurs in the nucleus whereas eukaryotes show transcription prior to translation. In both cell types, regulation occurs using transcription factors, activators, and repressors.
- **44.** Lactose digestion in *E. coli* begins with its hydrolysis by the enzyme $\,\beta$ -galactosidase. The gene encoding $\,\beta$
- -galactosidase, lacZ, is part of a coordinately regulated operon containing other genes required for lactose utilization. Which of the following figures correctly depicts the interactions at the *lac* operon when lactose is not being utilized?



- **45.** What would be the result of a mutation in the repressor protein that prevented it from binding lactose?
 - a. The repressor will bind to lactose when it is removed from the operator.
 - b. The repressor will bind the operator in the presence of lactose.
 - c. The repressor will not bind the operator in the presence of lactose.
 - d. The repressor will not bind the operator in the absence of lactose.
- **46.** What type of modification might be observed in the GR gene in all newborn rats?
 - a. The DNA will have many methyl molecules.
 - b. The DNA will have many acetyl molecules.
 - c. The DNA will have few methyl groups.
 - d. The histones will have many acetyl groups.
- **47.** What type of modification will be observed in the GR gene in the highly nurtured rats?
 - a. The DNA will have many methyl molecules.
 - b. The DNA will have many acetyl molecules.
 - c. The DNA will have few methyl groups.
 - d. The histones will have few acetyl groups.

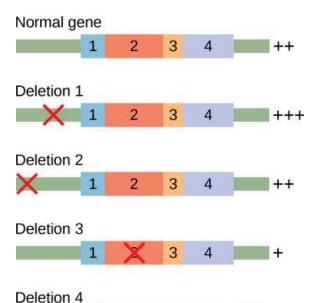
48.



The level of transcription of a gene is tested by creating deletions in the gene as shown in the illustration. These modified genes are tested for their level of transcription: (++) normal transcription levels; (+) low transcription levels; (+++) high transcription levels. Which deletion is in an enhancer involved in regulating the gene?

- a. deletion 1
- b. deletion 2
- c. deletion 3
- d. deletion 4

49.



Which deletion is in a repressor involved in regulating the

3

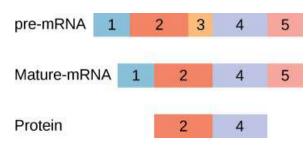
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1

gene?

- a. deletion 1
- b. deletion 2
- c. deletion 3
- d. deletion 4

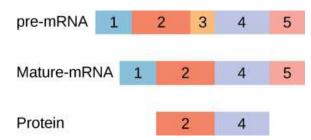
50.



The diagram provided shows different regions (1-5) of a pre-mRNA molecule, a mature-mRNA molecule, and the protein corresponding to the mRNA. A mutation in which region is most likely to be damaging to the cell?

- a. 1
- b. 2
- c. 3
- d. 5

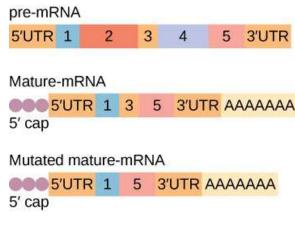
51.



What do regions 1 and 5 correspond to?

- a. exons
- b. introns
- c. promoters
- d. untranslated regions

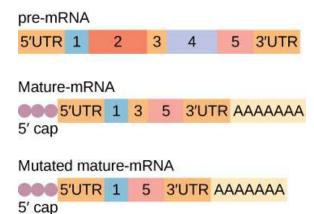
52.



What are regions 1 through 5 in the diagram?

- a. 1, 3, and 5 are exons; 2 and 4 are introns.
- b. 2 and 4 are exons; 1,3, and 5 are introns.
- c. 1 and 5 are exons; 2, 3, and 4 are introns.
- d. 2, 3, and 4 are exons; 1 and 5 are introns.

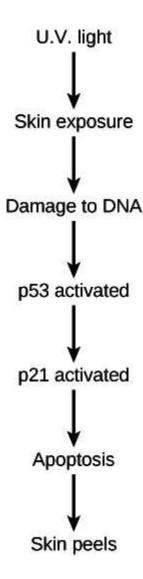
53.



A mutation results in the formation of the mutated maturemRNA as indicated in the diagram. Describe what type of mutation occurred and what the likely outcome of the mutation is.

- a. Mutation in the GU-AG sites of introns produced a non-functional protein.
- b. A transversion mutation in the introns led to alternative splicing, producing a functional protein.
- c. A transversion mutation in the GU-AG site mutated this mRNA, producing a non-functional protein.
- d. Transition mutations in the introns could produce a functional protein.

54.



The diagram illustrates the role of p53 in response to UV exposure. What would be the result of a mutation in the p53 gene that inactivates it?

- a. Skin will peel in response to UV exposure.
- Apoptosis will occur in response to UV exposure.
- c. No DNA damage will occur in response to UV exposure.
- d. No peeling of skin will occur in response to UV exposure.

55. Which of the following will not occur in response to UV exposure if a p53 mutation inactivates the p53 protein?

- 1. Damage to DNA
- 2. p53 activation
- 3. p21 activation
- 4. Apoptosis

- a. 1, 2, and 3
- b. 3 and 4
- c. 3
- d. 2, 3, and 4

56.



What happens when tryptophan is present?

- a. The repressor binds to the operator, and RNA synthesis is blocked.
- b. RNA polymerase binds to the operator, and RNA synthesis is blocked.
- c. Tryptophan binds to the repressor, and RNA synthesis proceeds.
- d. Tryptophan binds to RNA polymerase, and RNA synthesis proceeds.

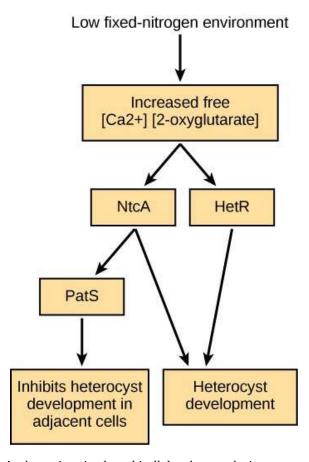
57.



What happens in the absence of tryptophan?

- a. RNA polymerase binds to the repressor
- b. the repressor binds to the promoter
- c. the repressor dissociates from the operator
- d. RNA polymerase dissociates from the promoter

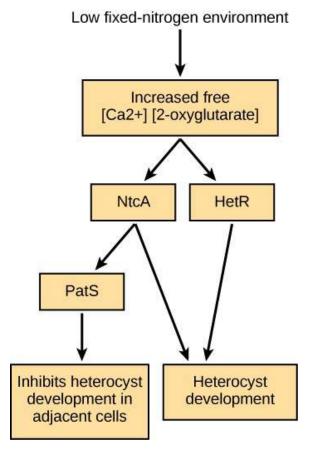
58.



Anabaena is a simple multicellular photosynthetic cyanobacterium. In the absence of fixed nitrogen, certain newly developing cells along a filament express genes that code for nitrogen-fixing enzymes and become nonphotosynthetic heterocysts. The specialization is advantageous because some nitrogen-fixing enzymes function best in the absence of oxygen. Heterocysts do not carry out photosynthesis but instead provides adjacent cells with fixed nitrogen and receives fixed carbon and reduced energy carriers in return. As shown in the diagram above, when there is low fixed nitrogen in the environment, an increase in the concentration of free calcium ions and 2-oxyglutarate stimulates the expression of genes that produce two transcription factors (NtcA and HetR) that promote the expression of genes responsible for heterocyst development. HetR also causes production of a signal, PatS, that prevents adjacent cells from developing as heterocysts. Based on your understanding of the ways in which signal transmission mediates cell function, which of the following predictions is most consistent with the information given above?

- In an environment with low fixed nitrogen, treating the *Anabaena* cells with a calciumbinding compound should prevent heterocyst differentiation.
- b. A strain that overexpresses the patS gene should develop many more heterocysts in a low nitrogen environment.
- In an environment with abundant fixed nitrogen, free calcium levels should be high in all cells, preventing heterocysts from developing.
- d. In environments with abundant fixed nitrogen, loss of the hetR gene should induce heterocyst development.

59.



Which of the following statements about *Anabaena* is false?

- a. Decreasing the concentration of free calcium ions will prevent heterocyst development.
- b. In the presence of fixed nitrogen, NtcA will not be expressed.
- Low fixed nitrogen levels result in increased PatS levels.
- d. A mutation in NtcA that makes it nonfunctional will also allow adjacent cells to develop as heterocysts.

SCIENCE PRACTICE CHALLENGE QUESTIONS

60. The operon model describes expression in prokaryotes. **Describe** this model and the essential difference in the

way in which expression is regulated in eukaryotes.