# GENE EXPRESSION AND BIOTECHNOLOGY

## Bacteria
## (Chapter 27)

**YOU MUST KNOW…**

- The key ways in which prokaryotes differ from eukaryotes with respect to genome, membrane-bound organelles, size, and reproduction.
- How horizontal acquisition of genetic information occurs in prokaryotes via transformation, conjugation, and transduction.
- How these mechanisms plus mutation contribute to genetic diversity in prokaryotes. (EK 3.C.2)

### *Diverse structural and metabolic adaptations have evolved in prokaryotes (27.1)*

- Life is divided into three domains: **Archaea, Bacteria, and Eukarya**. Both domain Bacteria and domain Archaea are made up of prokaryotes.
- Prokaryotes are perhaps 1/10 the size of a typical eukaryotic cell.
- As you read the following sections, study Figure 6.5. Prokaryotes have *no true nuclei* or internal compartmentalization. The DNA is concentrated in a *nucleoid region* and has little associated protein. The small genome consists of a single circular chromosome. Prokaryotes reproduce through an asexual process called **binary fission** and have short generation times.
- In addition to their one major chromosome, prokaryotic cells may also possess smaller, circular, self-replicating pieces of DNA called **plasmids**. In biotechnology plasmids are often used to carry the gene of interest into a prokaryotic cell.
- Outside their cell membranes, most prokaryotes possess a cell wall that contains **peptidoglycans**. (It is worth noting that cell walls are found in three kingdoms – plants have cell walls of cellulose, fungi have cell walls of chitin, and bacteria have cell walls of peptidoglycans.)
- Prokaryotes use appendages called **pili** that adhere to each other or to surrounding surfaces. About half of the prokaryotes are **motile**, because they possess whiplike **flagella**.
    - Because the flagellum of a bacterium is structurally different from the eukaryotic flagellum, this is another example of analogous structures.

### *Rapid reproduction, mutation, and genetic recombination promote genetic diversity in prokaryotes (27.2)*

- It is important for you to know that there are many ways for genetic information to be transferred between bacterial cells. In eukaryotes, the sexual processes of meiosis and fertilization combine DNA from two individuals. Meiosis and fertilization do not occur in

prokaryotes. However, genetic recombination – the combining of DNA from two different sources – can occur in prokaryotes through the three mechanisms explained below. When the individuals are different species, this movement is called **horizontal gene transfer**, also referred to as **horizontal acquisition of genetic information**. Note that all three processes result in increased genetic variation without sexual reproduction.

- Three mechanisms by which bacteria can transfer genetic material between each other are:
    - **Transformation**, in which a prokaryote takes up DNA from its environment. The foreign DNA is integrated into the chromosome by the exchange of homologous DNA segments. Investigation 8 utilizes transformation to introduce a gene into *E. coli*.
    - **Transduction** is a process in which a bacteriophage (virus) transfers genes between one prokaryote and another. A random piece of DNA is accidentally packaged into the head of the bacteriophage, which is then introduced to the next host during infection. Recombination may occur when the newly introduced DNA replaces its homologous section in the host cell's chromosome.
    - **Conjugation** occurs when genes are directly transferred from one prokaryote to another when they are temporarily joined by a "mating bridge". Once again recombination may occur when the newly introduced DNA replaces it homologous section in the host cell's chromosome.
- Although **mutations** are rare, they are the major source of genetic variation in prokaryotes due to the short generation time and large population sizes of bacteria.

# Regulation of Gene Expression
# (Chapter 18)

**YOU MUST KNOW…**

- Genes can be activated by *inducer* molecules, or they can be inhibited by the presence of a *repressor* as they interact with regulatory proteins or sequences. (EK 3.B.1)
- A *regulatory gene* is a sequence of DNA that codes for a regulatory protein such as a repressor protein.
- How the components of an operon function to regulate gene expression in both repressible and inducible operons.
- How positive and negative control function in gene expression. (EK 3.B.1)
- The impact of DNA methylation and histone acetylation on gene expression.
- How timing and coordination of specific events are regulated in normal development, including pattern formation and induction. (EK 2.E.1)
- The role of microRNAs in control of cellular functions.
- The role of gene regulation in embryonic development and cancer.

## *Bacteria often respond to environmental change by regulating transcription (18.1)*

- In bacteria, genes are often clustered into units called *operons*, which allow the expression of several related genes to be controlled as a unit. Figure 18.3 shows a repressible operon with an inactive repressor. Locate each part of the operon and the regulatory gene as you read the accompanying text.
- An **operon** consists of three parts:
    - An **operator** that controls the access of RNA polymerase to the genes. The operator is found within the promoter site or between the promoter and the protein coding genes of the operon.
    - The **promoter**, which is where RNA polymerase attaches.
    - The **genes of the operon**. This is the entire stretch of DNA required for all the enzymes produced by the operon.
- Located some distance from the operon is a regulatory gene. **Regulatory genes** produce repressor proteins that may bind to the operator site. When a regulatory protein occupies the operator site, RNA polymerase is blocked from the genes of the operon. In this situation the operon is off.
- A **repressible operon** is normally *on* but can be inhibited. This type of operon is normally anabolic, building an essential organic molecule. The repressor protein produced by the regulatory gene is inactive. If the organic molecule being produced by the operon is provided to the cell, the molecule can act as a **corepressor** and bind to the repressor protein, activating it.

The activated repressor protein binds to the operator site, shutting down the operon. This type of repressible operon is shown in Figure 5.9. Figure 18.3b in your book shows the corepressor turning off the operon.

- An **inducible operon** is normally *off* but can be activated. This type of operon is normally catabolic, breaking down food molecules for energy. The repressor protein produced by the regulatory gene is active. To turn an inducible operon on, a specific small molecule, called an **inducer**, binds to and inactivates the repressor protein. With the repressor out of the operator site, RNA polymerase can access the genes of the operon. The *lac* operon is an example of an inducible operon and can be seen in Figure 18.4 in your book.

### *Eukaryotic gene expression is regulated at many stages (18.2)*

- The expression of eukaryotic genes can be turned off and on at any point along the pathway from gene to functional protein. Further, the differences between cell types are not caused by different genes being present but by **differential gene expression**, the expression of different genes by cells with the same genome.
- Recall that the fundamental packaging unit of DNA, the nucleosome, consists of DNA bound to small proteins termed histones. The more tightly bound DNA is to its histones, the less accessible it is for transcription. This relationship is governed by two chemical interactions:
    - **DNA methylation** is the addition of methyl groups to DNA. It causes the DNA to be more tightly packaged, thus reducing gene expression.
    - In **histone acetylation**, acetyl groups are added to amino acids of histone proteins, thus making the chromatin less tightly packed and encouraging transcription.
- Notice that methylation occurs primarily on DNA and reduces gene expression, whereas acetylation occurs on histones and increases gene expression.
- **Epigenetic inheritance** is the inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence. The DNA sequence is not changed, just its expression.
- *Transcription initiation* is another important control point in gene expression. At this stage, DNA control elements that bind transcription factors are involved in regulation.
- The **transcription initiation complex** greatly enhances gene expression. Study Figure 18.10 and note its essential elements. DNA sequences far from this gene, termed **enhancer regions**, are bound to the promoter region by proteins termed **activators**.
- The control of gene expression may also occur after transcription and just after translation, when proteins are processed.
- Coordinately controlled genes, such as the genes coding for the enzymes of a metabolic pathway, are expressed together. This is possible even though the genes in a given pathway may be scattered on different chromosomes. All of the genes that code for the enzymes of the pathway share the same control elements. In general, eukaryotes do not have operons.

**CONNECT WITH THE CURRICULUM FRAMEWORK**

**Big Idea 3**

The control of gene expression is an extremely important topic, so spend enough time to understand different ways genes are controlled in both prokaryotes (with operons) and eukaryotes. Any factor that changes gene expression will affect cells, the organism, and populations. (Los 3.18-3.23)

### *Noncoding RNAs play multiple roles in controlling gene expression (18.3)*

- Recent research has indicated that large, diverse populations of RNA molecules in the cell play crucial roles in regulating gene expression. Small molecules of single-stranded RNA can complex with proteins and influence gene expression.
    - Two types of RNA, *micro RNAs* (*miRNAs*) and *small interfering RNAs* (*siRNAs*), can bind to mRNA and degrade the mRNA or bind to mRNA and block its translation. The blocking of gene expression in this manner is called RNA interference (RNAi).

### *A program of differential gene expression leads to the different cell types in a multicellular organism (18.4)*

- The zygote undergoes transformation through three interrelated processes:
    - **Cell division** is the series of mitotic divisions that increases the number of cells.
    - **Cell differentiation** is the process by which cells become specialized in structure and function.
    - **Morphogenesis** gives an organism its shape.
- What controls differentiation and morphogenesis?
    - **Cytoplasmic determinants** are maternal substances in the egg that influence the course of early development. These are distributed unevenly in the early cells of the embryo and result in different effects.
    - **Cell-cell signals** result from molecules, such as growth factors, produced by one cell influencing neighboring cells, a process called **induction**, which causes cells to differentiate.
- **Determination** is the series of events that lead to observable differentiation of a cell. Differentiation is caused by cell-cell signals and is irreversible.
- **Pattern formation** sets up the body plan and is a result of cytoplasmic determinants and inductive signals. This is what determines head and tail, left and right, back and front. Uneven distribution of substances called **morphogens** plays a role in establishing these axes.
- **Homeotic genes** are master control genes that control pattern formation.

# Viruses
# (Chapter 19)

**YOU MUST KNOW…**

- The components of a virus.
- The differences between lytic and lysogenic cycles.
- How viruses can introduce genetic variation into host organisms. (LO 3.29)
- Mechanisms that introduce genetic variation into viral populations. (LO 3.30)

## *A virus consists of a nucleic acid surrounded by a protein coat (19.1)*

- Smaller than ribosomes, the tiniest viruses are about 20 nm across.
- The two essential components of a virus are a protein shell or **capsid** that surrounds **the genetic material** (either double- or single-stranded DNA or double- or single-stranded RNA).
- Many viruses found in animals have membranous **viral envelopes** that surround the capsid and aid the viruses in infecting their hosts. (See Figure 19.8)
- **Bacteriophages**, or **phages**, are viruses that infect bacterial cells.

## *Viruses replicate only in host cells (19.2)*

- Viruses have a limited **host range**. This means they can infect only a very limited variety of hosts. *Example*: Human cold virus infects only cells of the upper respiratory tract.
- Viral reproduction occurs only in host cells. Two variations have been studied in bacteriophages. Read the following, then study Figure 19.6.
    - The **lytic cycle** ends in the death of the host cell by rupturing it (lysis). In this cycle, a bacteriophage injects its DNA into a host cell and takes over the host cell's machinery to synthesize new copies of the viral DNA as well as protein coats. These self-assemble, and the bacterial cell is lysed, releasing multiple copies of the virus.
    - In the **lysogenic cycle** the bacteriophage's DNA becomes incorporated into the host cell's DNA and is replicated along with the host cell's genome. The viral DNA is known as a **prophage**. Under certain conditions, the prophage will enter the lytic cycle, described on the previous page.
- **Retroviruses** are RNA viruses that use the enzyme **reverse transcriptase** to transcribe DNA from an RNA template. The new DNA then permanently integrates into a chromosome in the nucleus of an animal cell. The host transcribes the viral DNA into RNA that may be used to synthesize viral proteins or may be released from the host cell to infect more cells. *Example*: HIV is a retrovirus.
- Viruses have the ability to introduce genetic change into organisms as well as to undergo rapid genetic change themselves. Moving from one host to another, viruses may pick up pieces of the

first host's DNA and carry it to the next cell to be infected. This is very common in bacteria infected by viruses, where the process is called **transduction**.

- RNA viruses lack replication error-checking mechanisms and thus have higher rates of mutation. Mutations may accumulate rapidly and give rise to diverse clones of the virus within one organism, as occurs in humans with AIDS, or result in new genetic strains that may cause disease. This rapid mutation of viruses explains why there is no vaccine against the common cold.

# DNA Tools and Biotechnology
# (Chapter 20)

**YOU MUST KNOW…**

- The terminology of biotechnology.
- How plasmids are used in bacterial transformation to clone genes.
- The key ideas that make PCR possible and applications of this technology.
- How gel electrophoresis can be used to separate DNA fragments or protein molecules.
- Information that can be determined from DNA gels results, such as fragment sizes and RFLP analysis.

## *DNA sequencing and cloning are valuable tools in genetic engineering and biological inquiry (20.1)*

- The key to unlocking the concepts of biotechnology is to understand the terms. Know the following commonly used terms:
    - **Genetic engineering** is the process of manipulating genes and genomes.
    - **Biotechnology** is the process of manipulating organisms or their components for the purpose of making useful products.
    - **Recombinant DNA** is DNA that has been artificially made, using DNA from different sources – and often different species. An example is the introduction of a human gene into an E. coli bacterium.
    - **Gene cloning** is the process by which scientists can produce multiple copies of specific segments of DNA that they can then work with in the lab. Many bacteria have DNA outside the main circular chromosome in plasmids. A **plasmid** is a small, circular extra-chromosome loop of DNA. Plasmids are often used in biotechnology.
    - **Restriction enzymes** are used to cut strands of DNA at specific locations (called **restriction sites**). They are mostly derived from bacteria where they serve the important function of protection against invading viruses.
    - When a DNA molecule is cut by restriction enzymes, the result will always be asset of **restriction fragments**, which may have at least one single-stranded end, called a **sticky end**. Sticky ends can form hydrogen bonds with complementary single-stranded pieces of DNA. These unions can be sealed with the enzyme **DNA ligase**. Follow this important step in Figure 20.6 in your book.
- Follow the steps that may occur to clone a gene in Figure 20.5.
    - *Identify and isolate the gene of interest and a **cloning vector***. The vector will carry the DNA sequence to be cloned and is often a bacterial plasmid.

- *Cut both the gene of interest and the vector with the same restriction enzyme*. This gives the plasmid and the human gene matching sticky ends.
- *Join the two pieces of DNA*. Form recombinant plasmids by mixing the plasmids with the DNA fragments. The human DNA fragments can be sealed into the plasmid using DNA ligase.
- *Get the vector carrying the gene of interest into a host cell*. The plasmids are taken up by the bacterium by *transformation*. The process of transformation is a key part of Investigation 8.
- *Select for cells that have been transformed*. The bacterial cells carrying the clones must be identified or selected. This can be done by linking the gene of interest to an antibiotic resistance gene or a *reporter gene* such as green fluorescent protein. In AP Investigation 8, we use an ampicillin-resistant plasmid. Any bacterial cells that do not pick up the plasmid by transformation will be killed when grown on agar with the antibiotic ampicillin.

- For certain applications the next step is finding the gene of interest among the many colonies present after transformation. A process known as **nucleic acid hybridization** can be used to find the gene. If we know at least part of the nucleotide sequence of the gene of interest, we can synthesize a **probe** complementary to it. For example, if the known sequence is G-G-C-T-A-A, then we would synthesize the complementary probe C-C-G-A-T-T. If we make the probe radioactive or fluorescent, the probe will be easy to track, taking us to the proper gene of interest.
- **PCR** (polymerase chain reaction) is a method used to amplify a particular piece of DNA without the use of cells. PCR is used to amplify DNA when the source is impure or scanty (as it would be at a crime scene). Figure 20.8 shows the basic steps of the PCR procedure.
- **Gel electrophoresis** is a lab technique used to separate macromolecules, primarily DNA and proteins. The principles of this separation of DNA include (refer to Figure 20.7):
  - An *electric current* is applied to the field. DNA is negatively charged and migrates to the positive electrode.
  - A gel made of a polymer is used as a matrix to separate molecules by size. The gel allows smaller molecules to move more easily than larger fragments of DNA.
  - The DNA must be stained or tagged for visualization.
- **Restriction fragment length polymorphisms (RFLPs)** result from small differences in DNA sequences and can be detected by electrophoresis. The difference in banding patterns after electrophoresis allows for diagnosis of disease or is used to answer paternity and identity questions.
- The process just described leads to a genomic library. A **genomic library** is a set of thousands of recombinant plasmid clones, each of which has a piece of the original genome being studied. A **cDNA library** is made up of complementary DNA made from mRNA transcribed by reverse transcriptase. This technique rids the gene of introns but may not contain every gene in the organism.

### *Biologists use DNA technology to study gene expression and function (20.2)*

- Genome-wide studies of gene expression are made possible by the use of **DNA microarray assays**. DNA microarray chips work as follows:
    - Small amounts of single-stranded DNA (ssDNA) fragments representing different genes are fixed to a glass slide in a tight grid, termed a DNA *chip*.
    - The mRNA molecules from the cells being tested are isolated and used to make cDNA using reverse transcriptase, then tagged with a fluorescent dye.
    - The cDNA bonds to the ssDNA on the chip, indicating which genes are "on" in the cell (actively producing mRNA). This enables researchers, for example, to see differences in gene expression between breast cancer tumors and noncancerous breast tissue.

### *Cloned organisms and stem cells are useful for basic research and other applications (20.3)*

- In animal cloning the nucleus of an egg is removed and replaced with the diploid nucleus of a body cell, a process termed *nuclear transplantation*. The ability of a body cell to successfully form a clone decreases with embryonic development and cell differentiation.
- The major goal of most animal cloning is reproduction, but not for humans. In humans, the major goal is the production of **stem cells**. A stem cell can both reproduce itself indefinitely and, under the proper conditions, produce other specialized cells. Stem cells have enormous potential for medical applications.
- **Embryonic stem cells** are *pluripotent*, which means "capable of differentiating into many different cell types". The ultimate aim is to use them for the repair of damaged or diseased organs, such as insulin-producing pancreatic cells for people with diabetes or certain kinds of brain cells for people with Parkinson's disease.