**Study Plan: Episode 5—Genetic Transfer**

1. **Read the UNIT OVERVIEW presented in this Study Guide.**
2. **View the video "Genetic Transfer"**
3. **Read UNIT OBJECTIVES and KEY CONCEPTS sections of this study guide.**
4. **View the video a second time, this time taking notes. Pay particular attention to topics identified by the UNIT OBJECTIVES or KEY CONCEPTS as significant.**
5. **Read Chapter 10 in Microbes and Society (pages 193 – 215).**
6. **View YouTube video clips posted in Moodle regarding the types of genetic transfer.**
7. **Return to the Unit Objectives and Key Concepts listed in this Study Guide. Do you feel you have achieved each objective? Review sections of the text or video pertinent to material you don't feel you have mastered.**
8. **Post any questions you have about Genetic Engineering on the Student Forum.**
9. **Check your answers to the review questions. Refer to the appropriate sections in your texts.**

**Unit Overview**

Genetic information is passed from parent to offspring by ***vertical gene transfer*** (the video tends to use the term “transmission” , which means the same thing). In plants and animals, the recombination of genetic information – the definition of sex - is linked to reproduction. Think of your family tree – with several generations laid out on the tree you can easily “see” vertical gene transfer. In bacteria, vertical gene transfer does occur, but bacteria reproduce clonally, passing their entire genome to daughter cells. Simply put, bacteria replicate their DNA and then split into two cells that are genetically identical to the original cell and now to each other.

Recombination is a separate process, occurring when small pieces of bacterial DNA are transferred horizontally to another individual in the same generation. Horizontal gene transfer is one-way and involves only a fraction of the genome, but it allows bacteria to share important genetic traits. ***Transduction, conjugation,*** and ***transformation*** are the principal vehicles of horizontal gene transfer in bacteria.

The microbes’ talent for sharing genetic traits with other organisms in the same generation has many important applications. The study of microbial gene transfer is used to study virulence and antibiotic resistance in pathogens, yielding valuable insights into better methods for disease control and treatment. *Corynebacterium diphtheriae* (causative agent of diphtheria) has to produce a toxin to kill cells in the course of an infection. The gene for that toxin is transmitted from one cell to another by a virus that infects this genus of bacteria (bacterial viruses are called bacteriophages). This process is called **transduction**. Phage conversion is the term to describe a bacteria that has gained information from a bacteriophage. Transduction is not to be confused with **transducktion**, which is when a student from the University of Oregon takes classes at Lane Community College.

Horizontal gene transfer techniques, learned from and often mediated by microbes, are used by scientists to introduce genes of interest into other prokaryotic cells or into eukaryotic cells. Bacteria transformed with the human gene for insulin produce high quality insulin and are much easier to care for than the pigs formerly used for this purpose! This powerful new tool has the potential to revolutionize medicine and transform plant and animal husbandry.

The long-term ecological and social effects of this technology must be carefully considered and regulatory policies governing its use considered. Genetic additions or deletions that could be engineered pose very difficult ethical choices. For example, should we pursue research designed to lengthen the human lifespan, when so many humans living today die young because of malnutrition, disease, or social conflict?

**Unit Objectives**

* Distinguish between vertical and horizontal gene transfer
* Explain the two broad mechanisms that lead to genetic variability in bacteria and how that differs from eukaryotic cells
* Describe the three ways genetic information is transferred between bacteria and implications for drug resistance
  + Transformation, conjugation, and transduction.
* Explain the importance of horizontal gene transmission in drug (antibiotic) resistance.

**Key Concepts**

* Bacteriocin
* Binary fission
* Biotechnology
* Competence factor
* Conjugation
* DNA ligase
* DNA polymerase
* Endonucleases
* F-factor
* Lysogenic cycle
* Mutagen
* Mutation
* Plasmid
* Transduction
* Transformation

**Key Concepts**

**Vertical Gene Transmission**

* Bacterial, as well as other organisms, transmit their genes "vertically" i.e., to their descendents.
* Vertical transmission in bacteria occurs during asexual reproduction. In asexual reproduction the chromosome is replicated and identical copies are inherited by the daughter cells produced through binary fission. This produces clones of bacteria. In bacteria, this process is called binary fission.
* Asexual reproduction in eukaryotic cells involves mitosis. Mitosis is the process eukaryotic cells use to create daughter cells with the same number of chromosomes as the parent cell so that all cells of a multicellular organism have the same genome. Bacteria (prokaryotic organisms) do not undergo mitosis.
* In organisms that reproduce via sexual reproduction there is a mixing of the parent's genetic contributions which leads to genetic variability.
* Some eukaryotic microbes, as well as all multicellular organisms, undergo sexual reproduction.
* Baker's yeast, *Saccharomyces cerevisiae,* is a fungus that undergoes both asexual (mitosis) and sexual (meiosis) reproduction
* For sexually reproduction to occur a diploid cell (one with 2 copies of each chromosome) first undergoes a process called meiosis which reduces the number of chromosomes to 1 or each type. Meiosis produces haploid gametes (i.e. sperm and egg cells).
* At the time of fertilization the two gametes fuse to form a zygote or a new diploid cell with its own unique mix of genes.
* Bacteria do not sexually reproduce. *They obtain genetic variability through mutations and horizontal gene transfer.*
* Recall that mutations lead to changes to the sequence of bases in the DNA and these changes are inherited.

**Genetic Exchange among Bacteria: Horizontal Gene Transmission:**

* Genetic exchange among bacteria is not an essential part of their life cycle but it allows for cells to acquire new genetic abilities or traits such as drug resistance or toxin production.
* In horizontal gene transfer bacteria only share a portion of the donor cell's genome.
* There are three processes for horizontal gene transfer: Transduction, conjugation, and transformation.
* **Transduction**: A bacteriophage (“phage” for short), a virus that targets bacteria, mediates **transduction**. (Bacteriophage literally means “bacteria eater”). The bacteriophage injects its DNA into a poor, unsuspecting, helpless bacterium. The bacterium essentially becomes a virus factory producing hundreds of new viruses. Sometimes, small segments of bacterial DNA are accidentally included in the newly produced phage particles. Genetic exchange can occur when this phage infects a new host and transmits the DNA from another bacterium. Bacterial toxins, such as the diphtheria toxin, can be was acquired this way. (The same is true about the toxin in *E. coli* O157:H7 – the *E. coli* obtained the toxin gene from another bacterium – *Shigella* sp. This is a side note added by the instructor.)
* **Conjugation** is genetic transfer from a donor cell to a recipient cell via a joining tube called the sex pilus (plural is “pili”). This method allows the sharing of plasmids. Plasmids are small extra-chromosomal circular pieces of DNA containing commonly around 20 genes. The plasmids may contain toxin or antibiotic resistant genes. Plasmids replicate independently of the bacterial chromosome (another instructor side-note). Once contact has been made between two cells a single strand of the plasmid is transferred through the pilus from the donor cell to the recipient cell. The complementary strand of DNA is made very quickly by the host cell DNA polymerases.
* The best-studied conjugative plasmid is the F plasmid in *Escherichia coli* (F = fertility factor). Conjugation begins when an F+ cell forms a sex pilus that attaches to an F- cell. The F plasmid is nicked and copies itself, producing a single strand of plasmid DNA that enters the recipient cell. The recipient becomes F+ and is now capable of transferring DNA to another recipient.
* The number of F+ cells rapidly increases, which can quickly spread drug resistant genes in a population
* In **transformation**, a recipient cell that is capable of "picking up free-floating DNA pieces" (the cell is referred to as being competent) from the environment takes up DNA that originated from another bacterium that died and the DNA is incorporated into the recipient’s genome. Some bacteria undergo natural transformation. Most do not.
* This was discovered when Frederick Griffith performed a series of experiments involving two strains of *Streptococcus pneumoniae*.
  + One strain (S) had a smooth appearance due to a capsule on the outside of the cell. When the S strain was injected into live mice the mice would die (making them unsuitable for barbecuing).
  + The other strain (R) had a rough appearance because it lacked the capsule. When the R strain was injected into live mice the mice did not die (they remained fresh and tasty).
  + If the S strain was killed with heat and injected into live mice the mice did not die.
  + Interestingly, when the heat-killed S strain and live R strain were mixed together and injected into the mice the mice would die.
  + The term transformation was used to describe this phenomenon. The R strain was bringing on bits of DNA from the heat killed strain and incorporating that DNA into its own genome. This resulted in the conversion of the R strain into an S strain that killed the mice.
  + In order for transformation to take place the bacteria have to be “competent”, meaning that they have the ability to take up exogenous DNA (from outside the cell).
* Note: a piece of transferred DNA must be incorporated into the chromosome or a plasmid to become a permanent part of the recipient cell's genome. (Incorporation occurs by a process called recombination.)
* In order for bacteria to take up the DNA from the environment around them they must be competent. Very few bacteria are competent, but those that are include some important pathogens.
  + *Streptococcus* indiscriminately takes up DNA from any source. *Streptococcus* is responsible for strep throat, Scarlet fever, one type of pneumonia, and necrotizing fasciitis.
  + *Neisseria* sp. and *Haemophilus* sp. are only capable of taking up DNA from other bacteria of their same species. *Neisseria* sp. are responsible for gonorrhea and one type of meningitis.
* In artificial transformation, bacterial cells are treated in the laboratory to make them able to take up DNA from their environment. Recombinant DNA technology (genetic engineering) utilizes artificial transformation (electric current can be used as in this video or chemical treatment of the bacterial cells can be used – instructors addition).
* One thing that the videos fail to mention is that the frequency of conjugation, transformation, and transduction are fairly low. So while we do get the spread of antibiotic resistance through these mechanisms it is not as often as you might have been led to believe. If it were more often we would have a lot more bacteria that are antibiotic resistant.
* In **transposition**, DNA pieces can hop from location on the DNA to another location in the host cell DNA. This is a problem if antibiotic genes jump from the host chromosome to a conjugative plasmid. Thus, transposition works in conjunction with conjugation – DNA is not transferred directly from one bacterium to another by transposition.

**Implications/Population Dynamics in Bacteria**

* A genetic change conferring antibiotic resistance to one or a few bacterial cells can rapidly sweep through an entire population of bacteria by means of horizontal gene transfer combined with the rapid growth rate of microorganisms and strong natural selection. However, in a situation in which there are no antibiotics these resistant bacteria have no selective advantage and some may actually be weaker under normal circumstances.
* Antibiotic-resistant genes can be horizontally transferred to one's normal flora or resident bacteria making them hard to treat if they ever end up in the wrong place and cause disease.
* Urinary tract infections (introduced via catheters) and pneumonias (introduced from respirators) acquired in the hospital are often from ones' normal flora.
* Drug-resistant strains of bacteria are more common in the hospital setting.
* Since such a high proportion of the hospital population receives antibiotics at one point, drug resistant microbes are selected for at a higher rate.
* Antibiotic resistant bacteria are harder to treat.
* Hospital acquired infections are called nosocomial infections.

**Recombinant DNA technology makes use of horizontal gene transfer and bacterial enzymes and plasmids**

* Recombinant DNA technology is a collection of procedures for manipulating DNA *in vitro* (literally “in glass”; it means in a laboratory experiment) and putting the DNA into a cell.
* *E coli* (normal flora strain) is the most common host for recombinant DNA because it is easy to maintain and cultivate, it grows rapidly, and it and been thoroughly studied; its genetic regulation is well understood.
* Figure 10.12 provides a good summary of how Genetic Engineering is currently being used in our society. Figure 10.10 and 10.11 Demonstrates how DNA can be put together into a plasmid that can be used to modify another organism.
* Gene cloning is a basic tool of DNA technology. DNA cloning involves the following:
  + Obtaining the DNA or gene to be cloned.
  + Putting the gene into a "cloning vector" (commonly a plasmid). The plasmid is cut with bacterial enzymes called restriction enzymes; these enzymes cut DNA in specific locations, based on the DNA sequence.
* Using artificial horizontal gene transfer to get the cloning vector with desired gene into an appropriate host cell (usually a bacterium or a yeast cell).
* Testing to assure the desired gene has been inserted into the host cell (host cell now is considered to be a “recombinant”).
* The recombinant cells are now allowed to divide and can be used to:
* Make many copies of the gene of interest
* To produce large amount of the cloned gene's protein product
* A genetically modified organism (i.e., plant with pest-resistance gene)
* Study the function of the gene in development or disease
* Gene cloning was the way a researcher originally got large amounts of a gene to study, but now, one can use a technique called the Polymerase Chain Reaction (PCR).
* PCR is a procedure that makes many copies of a DNA sequence overnight (actually, in just a couple of hours). This automated technique allows one to copy DNA without using a living cell.
* The researcher introduces the four different nucleotides in DNA, a DNA polymerase (enzyme to copy the DNA) from a heat-tolerant bacterium, the gene to copy, and short segments of DNA called primers.

**Applications of Recombinant DNA Technology:**

* Recombinant DNA technology produces huge amounts of DNA for use in sequencing. DNA sequencing answers questions about gene structure, gene function, and the relatedness of genes and organisms. The Human Genome Project sequenced the entire human genome.
* PCR (Polymerase Chain Reaction) has widespread application from forensics to medical research.
* Recombinant DNA technology produces medically-useful proteins such as insulin for treating diabetes, growth hormone for treating pituitary dwarfism, growth factors that stimulate the production of white blood cells (instructor’s addition) and the hepatitis B vaccine.
* Recombinant DNA technology allows us to engineer (genetically alter) microorganisms, plants, and animals. For example, plants can be engineered to resist disease and insect damage. The video presented the example of the engineered cassava root that was developed to battle problems in Zimbabwe with infected cassava plants. The cassava mosaic virus is transmitted by white flies from one plant to another. The virus infects the plant producing a mottled appearance of the leaves (thus the appearance of a mosaic) and it weakens the roots which is valuable food source for the native people. Much of this work was done by Victor Masona.
  + *Agrobacterium* was used to transfer certain genes to the cassava plants in the lab. In essence viral DNA was used to vaccinate the plant. However, at the time of the video these transgenic plants were field tested but the Zimbabwe government would not let them be used studies had been performed.
  + *Agrobacterium* is typically used to transfer DNA into plant cells and has been used to genetically engineer other drops.

**Review Questions**

**Multiple Choice**

**1. Which process(es) leads to genetic variability in bacteria?**

* 1. Vertical gene transmission
  2. Mutations
  3. Meiosis and sexual reproduction
  4. Horizontal gene transmission
  5. B and D

**2. Which process(es) are not found in eukaryotic cells?**

* 1. Vertical gene transmission
  2. Mutations
  3. Meiosis and sexual reproduction
  4. Horizontal gene transmission
  5. B and D

**3. What form of horizontal gene transmission uses a bacteriophage to shuttle genes?**

* 1. Transformation
  2. Conjugation
  3. Transposition
  4. Transduction
  5. Transcontinental

**4. What is the type of horizontal gene transmission that can occur if there is free floating DNA in the vicinity of bacterial cells?**

* 1. Transformation
  2. Conjugation
  3. Transposition
  4. Transduction
  5. Transcontinental

**5. What is the type of horizontal gene transmission that can occur when a plasmid is transferred across a sex pilus?**

* 1. Transformation
  2. Conjugation
  3. Transposition
  4. Transduction
  5. Transcontinental

1. **What bacterium was Frederick Griffith using when he reported transformation in bacteria?**
   1. *Staphylococcus*
   2. *Corynebacterium*
   3. *Neisseria*
   4. *Streptococcus*
   5. *Haemophilus*

**True/False**

1. \_\_\_\_\_Genetically modified plants have been genetically engineered to express a gene from another organism.
2. \_\_\_\_\_ The rapid spread of antibiotic resistance though a population of bacteria is due to mutations.
3. \_\_\_\_\_ A nosocomial infection caused by a drug-resistant bacterium is harder to treat.
4. \_\_\_\_\_ A plasmid is commonly used as a cloning vector.

**Fill In**

1. \_\_\_\_\_\_\_\_\_\_\_ is a process whereby DNA from one bacterium is introduced into a second bacterium by a virus.
2. A \_\_\_\_\_\_\_\_\_\_\_ is an extra-chromosomal piece of DNA in a bacterial cell.

**Discussion Questions**

1. While you learned about the different means of transmitting DNA from one bacterium to another explain why we do not see more antibiotic resistance developing in all strains of pathogens.

**Answers**

**Multiple Choice**

1. E 2. D 3. D 4. A 5. B 6. D

True/False

1. T 2. F. 3. T 4. T

**Fill In**

1. Transduction 2. plasmid

**Discussion**

1. Start with the actually frequency at which the different means of horizontal transfer actually takes places and whether DNA is transferred between every genus of bacteria or are there limitations to these mechanisms.