PROFESSIONAL DEVELOPMENT

AP® Biology

From Gene to Protein—
A Historical Perspective

Curriculum Module

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Introduction

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A "Big Idea" in biology is that living systems store, retrieve, transmit, and respond to information critical to life processes. Heritable information provides for continuity of life, and the storage and transfer of this information are necessary for life to continue. In most cases, this information is passed from parent to offspring via deoxyribonucleic acid, or DNA. This double-stranded molecule provides a simple and elegant solution for the transmission of heritable information: By using each strand as a template, existing information can be preserved and duplicated with high fidelity. For information in DNA to direct cellular processes, it must be transcribed (DNA→RNA) and translated (RNA→ polypeptide). The protein products determine the metabolism and thus the cellular activities and phenotypes upon which evolution operates.

Although all cells of an organism contain the same complement of DNA, some genes are continually expressed, whereas expression of others is regulated to allow more efficient energy utilization and increased metabolic fitness for the organism. Gene expression is controlled by environmental signals and developmental cascades that involve both regulatory and structural genes.

But how do we know what we know? What scientific evidence supports the claim that DNA is *the* molecule of heredity? What key experiments allowed scientists to conclude that DNA is able to store, retrieve, and transmit information necessary for living systems? Why do changes in genotype result in changes in phenotype? Why do gene regulatory mechanisms in bacteria provide useful tools for modeling control systems in eukaryotes? This Curriculum Module asks students to explore these questions and many more as they trace the pathway from gene to protein from a historical perspective.

This Curriculum Module begins with students drawing their own conclusions from the experiments of Frederick Griffith and those of Alfred Hershey and Martha Chase that identify the source of genetic information. Next, students read an original article describing the proposed model of the structure of DNA by James Watson and Francis Crick, which was published in *Nature*; the students then construct their own model through a formative assessment. Students extract DNA from living cells and make observations about its chemical makeup. Using data gleaned from experiments by Mathew Meselson and Franklin Stahl, students will create visual representations that describe the process of DNA replication. They build on the "one gene–one polypeptide" concept to model transcription and translation and also use biotechnology to induce a new phenotype in bacteria. Finally, students will examine the *lac* and *trp* models of regulation of gene expression in bacteria to model control systems in eukaryotes. The authors chose the information and approach based on the organizing principles of Big Ideas and Enduring Understandings that provide depth of study. Peppered among the instructional strategies are activities designed to help students answer the question, "How do we know what we know about DNA?" with "This is why we know what we know."

Each learning environment is different. Each school has its own mix of students with different abilities and interests. Each classroom is different, even among AP® Biology courses with common and required elements of content and skill. In all cases, students learn best by *doing*. The lessons presented in this module are intended to be used as strategies or guides, and each teacher should tap into his or her own expertise to make the content rich, engaging, challenging, relevant, and unique within the curriculum and cognitive frameworks. The instructional activities are *examples* of how teachers can engage students by accommodating their different learning styles, knowledge bases, and abilities and, at the same time, provide depth of content and skills. All activities are inquiry based and serve to make the course less teacher focused and more student driven. Sample AP Biology Exam questions pertaining to the module are also included.

Prerequisite Knowledge

Biochemistry

Understanding biological processes at the molecular level allows students to study biology at a deeper, more conceptual level. The relationship between structure and function (a key theme in biology) begins at the molecular level. Carbon-based molecules—carbohydrates, lipids, proteins, and nucleic acid—make up the bulk of organic matter essential to living systems. The structure and function of polypeptides, especially enzymes, should be reviewed in some inquiry-based manner (e.g., teacher-provided questions or student-generated visual representations) because the processes of DNA replication, transcription, and translation are enzyme catalyzed. Additionally, enzymes and proteins also play important roles in gene regulation. In this Curriculum Module, the study of the chemical makeup of DNA precedes the study of how it works.

Energy and Metabolism

The nature of the transfer of energy in living systems is a fundamental theme in Advanced Placement® study. For example, the concept of energy coupling of catabolic (exothermic) and anabolic (endothermic) processes—as well as the role of nucleoside triphosphates, such as ATP and GTP—allows students to explore DNA beyond Watson and Crick. The role that these processes play should be relatively familiar to students if teachers follow a sequence put forth by information in their textbook, but the processes should be reviewed through an examination of diagrams such as ATP recycling and coupling of reactions. This foundation will allow students to appreciate the anabolic processes of DNA synthesis (replication), transcription, and translation. It is strongly suggested that students perform AP LAB 2: Enzyme Catalysis in the AP Lab Manual.

Mitosis

In eukaryotic organisms, heritable information is packaged into chromosomes that are passed from one generation of cells to the next. Mitosis provides a mechanism that ensures each daughter cell receives an identical and complete set of chromosomes; thus, mitosis ensures fidelity in the transmission of heritable information. Additionally, mitosis allows for asexual reproduction of organisms in which progeny are genetically identical to the parental cell. Since chromosomes duplicate in mitosis, their chemical constituent, DNA, also must be able to replicate. Students will ultimately link DNA replication to the behavior of chromosomes during mitosis.

Meiosis

Sexual reproduction of diploid organisms involves the recombination of heritable information from both parents through fusion of gametes during fertilization. Meiosis produces haploid gametes and increases genetic variation through random assortment of maternal and paternal chromosomes and random exchanges between homologous chromosomes. Meiosis followed by fertilization provides a spectrum of possible phenotypes for natural selection and evolution. DNA provides the genotype, while translation of its information into polypeptides provides the phenotype. Ultimately, students will link DNA replication to the behavior of chromosomes (duplication) observed in the first stage of meiosis.

Mendelian Genetics

Some traits (phenotypes) are products of action from single genes. Single gene traits provided the experimental system through which Mendel described a model of inheritance from parent to progeny. Mendel's "factors" have been identified as genes—or discrete sequences of DNA with information to produce polypeptides. Students must make connections between Mendel, mitosis and meiosis, DNA, and phenotype. Furthermore, the principles of Mendelian genetics can be applied to many observable phenotypes, including human genetic disorders and the ethical, social, and medical issues surrounding them.

Lesson 1: Protein vs. DNA

Plan the Lesson

Connections to Course Outline

The content of this lesson addresses the following areas of the AP Biology course outline:

- Big Idea: Living systems store, retrieve, transmit, and respond to information critical to living systems.
- Enduring Understanding: DNA, not protein, is the source of heritable information.

Objectives

- Students investigate two key historical experiments to identify *the* source of genetic information: protein or DNA.
- Students are able to analyze data provided by Frederick Griffith and draw conclusions about the discovery of an unknown "transforming factor" in bacteria.
- Students are able to analyze data provided by the Hershey–Chase experiments with bacteriophage T2 and draw conclusions supporting the identification of nucleic acid, not protein, as *the* source of genetic information.
- Students extract DNA from living cells and make observations about its chemical makeup.

Common Student Misconceptions

Students, like scientists of the early twentieth century, have difficulty distinguishing between the "language of DNA" and the "language of proteins." Both contain information, but only the information contained in DNA sequences of nucleotides is passed from parent to offspring. Additionally, students often confuse the double helix of DNA with the alpha helix secondary structure of protein. With respect to the chemical components of DNA and protein, students might want to include both sulfur and phosphorous; sulfur is a component of proteins containing the amino acid cysteine, while phosphorous is a component of nucleic acids.

Protein or DNA? How did scientists discover the source of heritable genetic information? What evidence supported the theory that nucleic acid enables living systems to store, retrieve, and transmit information critical to life processes from one generation to the next? By asking questions, teachers can engage students to seek answers. In the first two instructional activities, students examine the work of Griffith and the Hershey–Chase experiments in the quest to identify the source of genetic information. In the third activity, students extract DNA from living cells and make observations about its chemical makeup.

Teach the Lesson

Instructional activity I: Griffith's experiments

This first activity asks students to examine the work of Frederick Griffith and draw conclusions about how his research helped identify the source of genetic material. Students are asked to address the *inquiry* question below and answer the questions provided in the *experimental analysis*. Students may work in pairs or small groups.

Instructional time is approximately 20 minutes.

Background Information

Once T. H. Morgan and his co-researchers showed that Mendel's traits (genes) are located on chromosomes, the two chemical components of chromosomes—DNA and protein—became the candidates for *the* genetic material. Until the 1940s, the case for protein seemed more likely (Campbell and Reece 2005, 293). Why? Because scientists had previously discovered that polypeptides contain a "language" based on a 20-letter amino acid alphabet from which myriad proteins could be synthesized. Furthermore, little was known about the structure and function of nucleic acids. Even after Rosalind Franklin produced the X-ray diffraction photograph that Watson and Crick used to model the structure of DNA, it could be argued that the helical shape of Franklin's molecule supported protein (Campbell and Reece, 293). Students often have this same misperception, confusing the alpha helix secondary structure of protein with the double helix of DNA.

Teaching Tips

Before proceeding with the work of Griffith and the Hershey–Chase experiments, do the following:

1. Review the four structures of protein by asking students to use a visual representation to describe how interactions between R-groups can determine myriad three-dimensional shapes.
2. Ask the students for reasons why Franklin's X-ray diffraction photograph could have been interpreted as a secondary structure, alpha helix protein. Follow up by asking them to describe the effects of increased temperature or low pH on protein

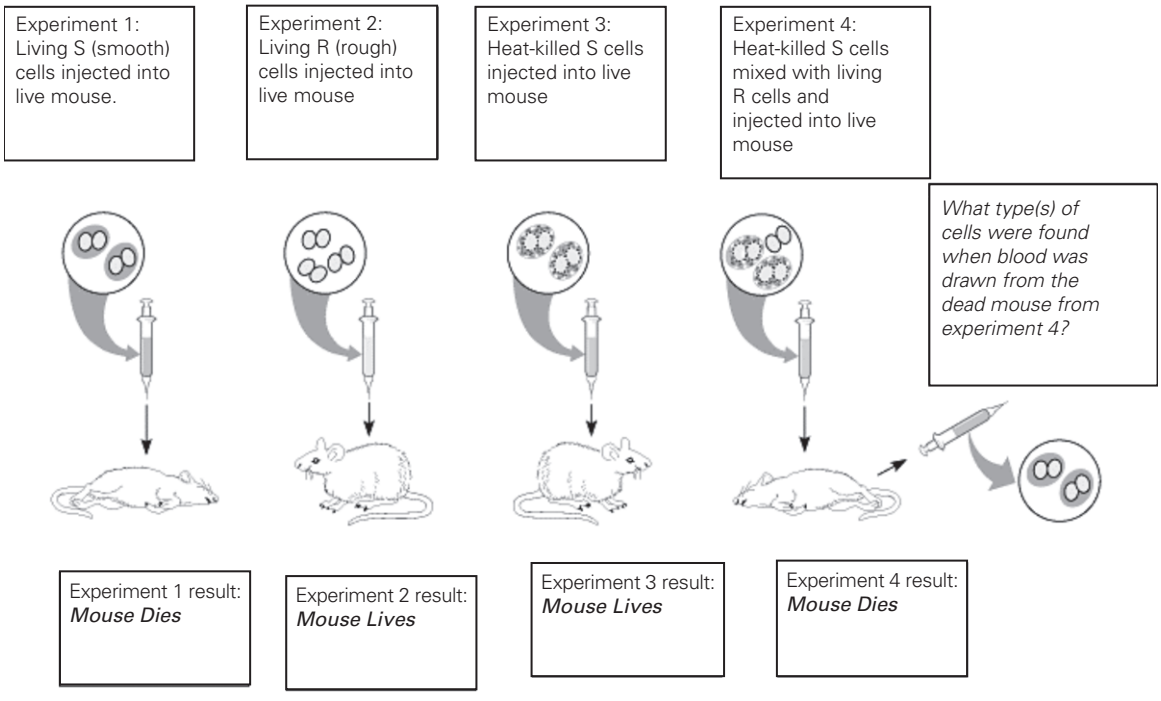
structure and enzymatic activity. (Students should have gleaned this information as prerequisite material in AP LAB 2: Enzyme Catalysis.)

A key factor in correctly identifying DNA as *the* genetic material was the choice of experimental organisms. Why are bacteria and the viruses that infect them simpler to study than Mendel's pea plants, Morgan's fruit flies, or humans? What role can luck play in discovery? In 1928, Frederick Griffith, who was studying *Streptococcus pneumoniae* to find a vaccine for pneumonia, made a startling observation. Griffith had two strains of the bacterium, a pathogenic or disease-causing strain and a second harmless one. Bacteria of the "S," or "smooth," strain are pathogenic because a protein capsule protects them from an animal's defense system; bacteria of the "R," or "rough," strain lack a capsule and are nonpathogenic. When Griffith killed the pathogenic bacteria with heat and then mixed the cell remains with living "R" bacteria, he made a startling discovery (described in Figure 1). Griffith called this phenomenon *transformation* (Campbell and Reece, 294). Little did Griffith know that his work in 1928 would provide a foundation for genetic engineering and recombinant DNA technology in the twenty-first century.

Inquiry for Students

How does information resulting from Griffith's experiments with *Streptococcus pneumoniae* support the idea that a heritable material (the identity of which was unknown in 1928) transformed living, nonpathogenic "R" bacteria into pathogenic "S" bacteria?

Figure 1: Griffith's experiments with two strains of Streptococcus pneumoniae



Experimental Analysis

The student groups should:

1. Form inferences about each of Griffith's four experiments.
2. Draw conclusions regarding each of the two bacterial strains from their experimental inferences.
3. Share their conclusions with each other and with you.

Give the student groups a few minutes to think about comments made by you or their classmates. They should ask themselves if their conclusions to the inquiry support a "transforming factor" observed by Griffith in *S. pneumonia* and then explain why or why not. This information can be shared and discussed as a class.

At first glance, students likely would have predicted that mixing heat-killed pathogenic "S" bacteria with nonvirulent but living "R" bacteria would produce living cells (and not harm the mouse) because neither bacterial strain is harmful by itself. You should point out that Griffith's results were surprising even to him. A common student misconception is that the heat treatment denatured not only the protein capsule but the genetic material as well. Students should describe why Griffith's "transforming factor" was different from proteins because (1) the heat treatment did not denature the unidentified factor; (2) the factor was able to transfer information from one type of bacterial cell to another; and (3) the factor transformed the host cells by giving them new properties.

Summary

This activity asks students to examine the work of Fredrick Griffith and to draw conclusions about why his experimental results helped to identify the source of genetic information. The identification of a "transforming factor" began a quest to determine its molecular composition, beginning with the Hershey–Chase experiments. If the heat treatment *had* destroyed DNA, then all parts would have been damaged beyond repair and function; no mice would have survived the Griffith experiment.

Instructional Activity II: Hershey–Chase Experiments

This second activity asks students to examine the work of Hershey and Chase in an effort to identify the source of genetic information *and* draw conclusions to support the hypothesis that DNA, not protein, is *the* genetic material. Students are asked to address the *inquiry* questions below and to answer the questions provided in the *experimental analysis*. Students may work in pairs or small groups.

Instructional time is approximately 30 minutes.

Background Information

The results of Griffith's work began a quest among scientists to identify his "transforming factor." Additional evidence for DNA, not protein, as *the* genetic material came from experiments using viruses that infect bacteria called phages. Bacteriophages serve as tools for research in molecular genetics because they are much simpler organisms than cells, consisting of little more than DNA enclosed by a protective coat. To reproduce, bacteriophages infect bacterial cells and take over the host's metabolic machinery.

In 1952, Alfred Hershey and Martha Chase performed a series of experiments with a bacteriophage known as T2. Phage T2 infects *Esherichia coli*, a common bacterium that lives in the intestines of animals. Previous studies had shown that T2 was composed of DNA and protein; that it could turn an *E. coli* cell into a T2-producing factory; and that somehow T2 could reprogram its host cell to produce viruses (Campbell and Reece, 295). But a key question remained unanswered: Which component, DNA or protein, was responsible for the observed behavior of T2?

Teaching Tips

At this point you should do the following:

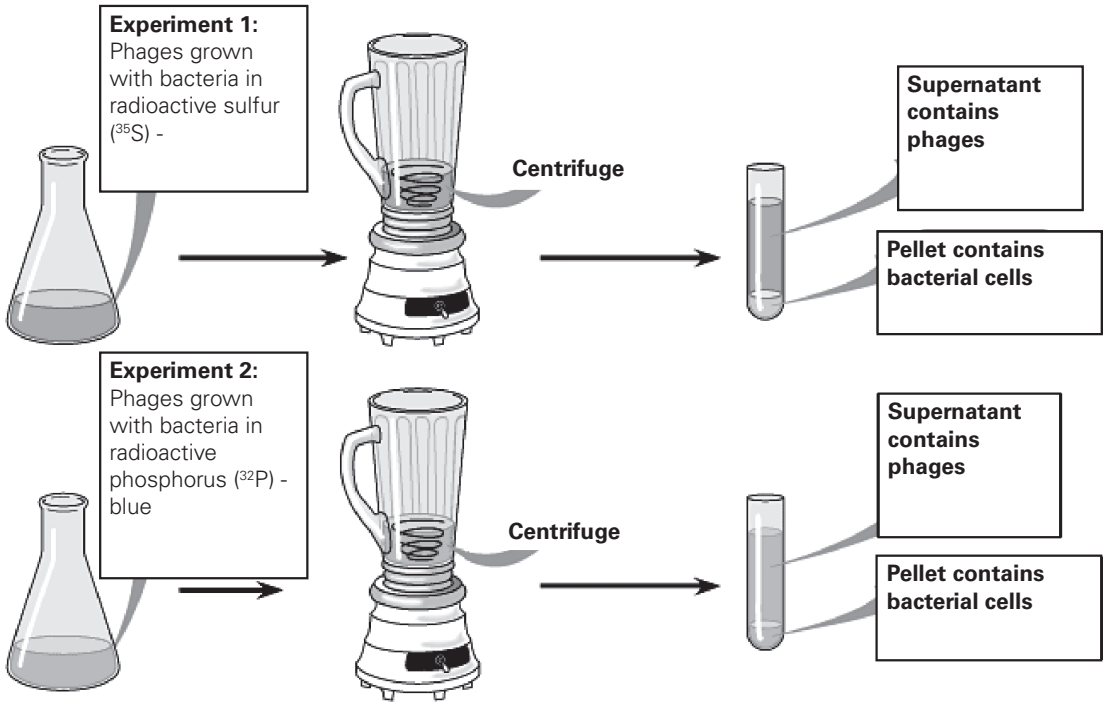
1. Review the elements that comprise nucleic acid and protein.
2. Reiterate that Hershey and Chase used radioactive sulfur and phosphorous to trace the fates of protein and DNA, respectively, of T2 phages that infected bacterial cells. Ask students why Hershey and Chase used radioactive sulfur and phosphorous in their experiment. (Students should answer that proteins can contain sulfur if they have the amino acid cysteine with a sulfhydryl group in its side chain, while phosphorous is a component of the phosphate groups that comprise DNA monomers.)

Inquiry for Students

Is DNA or protein the genetic material of phage T2?

How does evidence resulting from the Hershey–Chase experiments support that DNA, not protein, is *the* heritable material?

Figure 2: Design of Hershey–Chase experiments with bacteriophage grown with *Escherichia coli*



Experimental Analysis

Each student group should:

1. Through narrative or visual representation, compare the design of the Hershey–Chase experiment 1 to experiment 2. Why did they use radioactive sulfur in experiment 1 and radioactive phosphorus in experiment 2?
2. Discuss the results of the Hershey–Chase experiments with T2. Where was radioactive sulfur found at the end of experiment 1? Where was it found at the end of experiment 2?
3. Draw conclusions from the results assuming that the material (DNA or protein) that was found in the bacterial cells is, in fact, *the* genetic material.
4. Based on the Hershey–Chase experiments, is it reasonable to assume that Griffith's "transforming factor" was DNA, not protein? Why or why not? What is the connection between the two experiments?
5. Share their responses with another student group.

Give the student groups a few minutes to think about the comments made by their classmates. Students should ask themselves if their conclusions to the inquiry justify that DNA, not protein, is *the* genetic material. You should ask a member of each group to share the group's responses with the class. A common misconception is that proteins contain *both* sulfur and phosphorous. You can review the structure of proteins and their amino acid monomers; because phosphorous is *not* an element in amino acids, it cannot be found in proteins.

Summary

Students describe, through narrative or visual representation, and discuss with the class how the Hershey–Chase experiments support the conclusion that DNA, not protein, is *the* genetic material. If students have difficulty with this conclusion, you should then incorporate a "think-pair-share" learning strategy with pairs of students in which they revisit the conclusions they drew earlier. You should circulate among the groups, asking questions to facilitate and clarify student understanding. The Hershey–Chase experiments also expose students to viruses as carriers of genetic information. The next step for students—as it was for scientists—is to investigate the physical and chemical nature of DNA.

Instructional Activity III: DNA Extraction

This third activity is modified from Carolina Biological Supply Company's *DNA Necklace Kit* (product number 211138, available for purchase at http://www.carolina.com/product/211138.do), but you can purchase the necessary supplies from other commercial vendors. You should also consider tapping into resources at local colleges or biotechnology companies that often donate supplies to high schools.

This activity asks students to extract DNA from their own cells in order to investigate the chemical properties of DNA along with other cellular components. Students address the *inquiry* questions below, follow the procedural steps, and answer the questions provided in the *experimental analysis*. Students may work in pairs or small groups.

This lab activity can be modified by asking students to design their own experiment. You can tell students what the materials in the lab kit do: The sports drink contains salt that is compatible with the osmotic environment of the cells; lysis solution dissolves cell membrane barriers, particularly phospholipids; and ethanol causes DNA to precipitate out of solution. Students can also investigate the roles of the reagent as a pre-lab assignment.

Instructional time is approximately 40 minutes.

Background Information

With Griffith's "transforming factor" and Hershey and Chase's identification of DNA, not protein, as *the* genetic material, the mysteries contained in Charles Darwin's discussion of variation and Gregor Mendel's study of heritable traits were beginning to be resolved. The obvious next step for scientists interested in the mystery of life was to further investigate the physical and chemical nature of DNA.

Students can learn the abstract concept that DNA is made up of nucleotide subunits, and they can also memorize the structure of those nucleotides, along with how they are arranged in the molecule. However, showing students their own DNA (or another organism's DNA) is an invaluable first step because doing so demonstrates that DNA is real. Additionally, it helps them discover some of the important chemical properties of DNA. How can DNA, the largest of biological molecules, be extracted and isolated from living tissue? If one strand of DNA is so thin (2 nm) that it can't be seen with a microscope, how can it be visualized by the naked eye?

DNA extraction is a routine procedure that is essential for molecular analysis. It involves three basic steps: (1) collecting cells, (2) removing membrane lipids by adding a detergent, and (3) precipitating the DNA with an alcohol, usually ethanol. Since DNA is insoluble in alcohol, multiple molecules will aggregate together. Cellular and DNA-bound histone proteins can be removed prior to precipitation by adding a protease. DNA can also be resolubilized in a slightly alkaline buffer.

Teaching Tips

1. Though DNA is the largest of biological molecules, it is only about 50 trillionths of an inch long, and a single molecule cannot be visualized even with a light microscope. Ask students: How might we be able to see DNA without the naked eye?
2. Review the structure of cell membranes with students. In order to remove DNA from the cell and nucleus, these structures must essentially be dissolved. Ask students which types of solutions could be used to do this and ask them to explain their reasoning. Students can review by the "think-pair-share" learning strategy or as a class discussion.
3. Review with students the other types of molecules that are present in solutions once cell membranes are dissolved (namely proteins). How can DNA be isolated from these other molecules?

Inquiry for Students

How can DNA, a submicroscopic molecule, be visualized with the naked eye?

What must be done to extract and isolate DNA from human cheek cells?

What can we conclude about the chemical nature of DNA through isolation techniques?

Materials

15 mL test tubes, small disposable cups, sports drink, 70 percent ethanol, disposable plastic pipettes, cell lysis (detergent) solution, 1.5 mL microcentrifuge tubes and test tube racks, colored string.

Procedure

The students will:

1. Obtain a 15mL test tube and label it with the student's name.
2. Obtain a small cup of sports drink and swish it around in mouth for *one full minute*. While swishing, they should gently and continuously scrape the sides of their cheeks with molars.
3. Spit the drink (with the collected cheek cells) back into the small cup.
4. Pour the contents of the cup into the labeled test tube (discard the cup).
5. Holding the test tube at an angle, they will use the provided plastic pipette to add 2mL of detergent solution to the collected cheek cells.
6. Cap the test tube, and invert it five to eight times.
7. Allow this to stand for two minutes.
8. Using the provided pipette, add the cold alcohol by letting it run gently down the side of the test tube (holding the test tube at an angle). Add the alcohol until the total volume reaches 12–13mL. They should have two distinct layers. The students should NOT mix the cheek cell solution with the alcohol!
9. Observe as strands of a translucent solid begin to precipitate where the alcohol layer meets the cheek cell solution.
10. Place the 15mL test tube in a test tube rack and let it stand undisturbed for 15 minutes. During this time the solid will continue to precipitate out.
11. Use a plastic pipette to transfer the solid DNA into a smaller test tube. To do so, the students should place the pipette near the DNA and draw the DNA into the pipette (along with some alcohol). They should NOT move the pipette up and down into the bottom layer.

Experimental Analysis

Each student group will answer the following questions:

1. As discussed, DNA is a very thin molecule. However, DNA was able to be visualized in this investigation. How was this possible?
2. DNA in a single human cell totals three meters in length. How is it able to fit inside the nucleus of a cell?
3. What was the chemical purpose of using cell detergent in the investigation? Where is DNA located in eukaryotic cells? What, then, is the first step in isolating it from nuclei?
4. How was ethanol used in the investigation? How was the sports drink used in the investigation?
5. Share their ideas with other lab pairs. You will then ask several pairs to share their findings with the entire class.

You should provide student pairs with a few minutes to discuss their ideas. In a whole class discussion, reflect upon the inquiry questions provided at the beginning of the lab and ask students to make some conclusions about the chemical structure of DNA based upon this investigation. If students are unable to make correct conclusions about the chemical structure of DNA, then you should address and clarify within the student pairs.

A common student misconception is that DNA is too small to be seen with the naked eye; in response, you can ask students to approximate how many cheek cells they collected. How did the sports drink help in the collection of cheek cells? Finally, students should be asked to come up with ideas on their own about why it would be useful to be able to isolate DNA. Many students will make the connection between DNA and criminal investigations.

Summary

This laboratory investigation exposes students to one of biotechnology's most fundamental but critical techniques—the isolation of DNA. The process allows students to glean information about the chemical nature of nucleic acid and the structure of DNA.

Lesson 2: The Watson and Crick Model of DNA

Plan the Lesson

Connections to Course Outline

The content of this lesson addresses the following areas of the AP Biology course outline:

- Big Idea: Livings systems store, retrieve, transmit, and respond to information critical to living systems.
- Enduring Understanding: The information language of DNA is encoded in its structure.

Objectives

- Students read a firsthand account of the discovery of the structure of DNA written by Watson and Crick (published in *Nature*) and highlight the key concepts.
- Students are able to use information presented in the *Nature* article to construct a model of DNA.
- Students are able to use their constructed models to determine how the structure of DNA serves its function as a carrier of genetic information.

Common Student Misconceptions

Common student misconceptions about the structure of DNA include the following:

- that a molecule of DNA consists of chains of nitrogen bases linked together, rather than identifying DNA as a polymer of nucleotides consisting of a nitrogen base, a five-carbon sugar (deoxyribose), and a phosphate group;
- that the nitrogen bases randomly pair with each other, rather than adenine pairing with thymine, and cytosine with guanine;

- that the two strands of a DNA double helix run in a *parallel* direction, not *antiparallel*, where the "leading" strand runs in a 3′→5′ direction, and the opposite or "lagging" strand runs from 5′→3′; in addition, students often incorrectly identify the two types of chemical bonds (hydrogen and covalent) present in DNA and also confuse ribose and deoxyribose sugars.

Teach the Lesson

Instructional Activity I: The Watson and Crick Model of DNA

In this activity, students will read the original *Nature* article, "A Structure for Deoxyribose Nucleic Acid" by Watson and Crick, in order to determine how "they knew what they knew." Students will develop a preliminary model of the structure of DNA based solely on information provided in the article. Students should read the article once, annotating areas of importance. Then, they will reread the article with a classmate and share areas that each thought were noteworthy. Finally, students will share their annotations with the class.

Instructional time: 50 minutes of class time for reading and discussion (or assign the first part of reading for homework).

Background Information

Historical evidence supported that DNA, not protein, is *the* genetic material. However, several questions remained unanswered: How could the structure of DNA account for its role in inheritance? What is the "language" of its information? How is information stored in DNA retrieved and transmitted to a new generation of cells? How are the instructions embedded in DNA translated into polypeptides that determine cellular activities and phenotypes?

By the early 1950s, scientists had determined the arrangement of covalent bonds in a nucleic acid polymer and began working on DNA's three-dimensional structure. Erwin Chargaff analyzed the nitrogenous base composition from several different organisms. Although his results showed molecular diversity among species, they also showed a regularity: In the DNA of each species he studied, the amount of the nitrogenous base adenine (A) approximately equaled the amount of thymine (T), and the amount of cytosine (C) approximately equaled the amount of guanine (G) (Campbell and Reece, 296). What could these observations suggest? What are possible relationships between (A) and (T), and between (C) and (G)? An X-ray diffraction photograph of DNA taken by Rosalind Franklin provided clues to the molecule's pattern and shape.

Enter James Watson and Francis Crick. These "young, bold, brash," and relatively unknown scientists collaborated at Cambridge University where Crick had been studying protein structure with a technique called X-ray crystallography. Watson, an American, was familiar with the type of patterns helical molecules produce upon X-ray diffraction. One glance at Franklin's photograph told him that DNA was helical in shape and that

its dimensions suggested a double-stranded molecule, not a single-stranded alpha helix protein. With this information, Watson and Crick began constructing a model much like puzzle makers assemble pieces to put a jigsaw together (Campbell and Reece, 297–298).

Inquiry for Students

How did James Watson and Francis Crick know what they knew?

How was their discovery based on a great deal of work by many scientists?

What is the structure of DNA, and how might its structure reveal a possible "copying mechanism"?

Materials

Watson and Crick's *Nature* article, "A Structure for Deoxyribose Nucleic Acid" (April 25, 1953), available for free download at http://www.nature.com/nature/dna50/archive.html.

Student Instructions

Read and annotate the article at least once. Next, read through the article with a friend from class, discussing and clarifying points that each of you found to be important as you read. Understand that you will probably need to reread many parts of this article, but don't get frustrated. Finally, answer the following questions on a separate sheet of paper. (Note: To differentiate instruction, students can also present their answers as descriptive illustrations.) For each question, please cite the specific paragraph in the article where you found the answer.

Analysis Questions

The students will analyze and discuss the following:

1. Describe the structure of DNA monomers by using citations from the article. How is the structure arranged, that is, which of the "parts" are on the outside? Which ones are on the inside of the molecule? Cite the article.
2. How did Linus Pauling's model differ from Watson and Crick's model for DNA? Explain in detail.
3. What type of bond holds the bases together? Cite the article. From what we have talked about this year regarding this bond, how might this bond affect the overall stability of the DNA molecule?
4. In the eighth paragraph, a very important observation is made about base pairing. What is the significance of how bases pair up?
5. Reread the last half of the article. Based on what Watson and Crick say, try to hypothesize what their "possible copying mechanism" might be. Use clues from the article in your explanation.

If students incorrectly visualize the structure of DNA, they cannot understand the processes of replication, transcription, and translation. In this event, you must address misconceptions via direct questioning, providing specific examples, etc.

Give student pairs plenty of time to discuss and answer the questions. If necessary, they can answer the questions for homework. Discuss with students how Watson and Crick's work could be viewed as the synthesis of a great deal of experimental data from many scientists. Finally, based solely on information in the *Nature* article, have student groups diagram the structure of DNA on large poster paper and share the diagram with other groups for critique and evaluation.

Summary

The primary objective of this activity is to provide students with the experience of reading a historical piece in the content area. From this, students acquire an appreciation of the process of science and the ingenuity of not just Watson and Crick, but the many other scientists that led them to model DNA. Students should be able to develop an accurate structural picture of DNA based solely on information from the article.

Instructional Activity II: Formative Assessment

Students can also learn how DNA stores, retrieves, and transmits information through an inquiry-based formative assessment, "From Gene to Protein" (Appendix A). In this activity, the students are provided materials to construct a short strand of DNA based on their study of the Watson and Crick model. After student groups think that they have constructed an accurate model, they answer (in writing) a set of questions prepared by the teacher to assess their understanding. You should spend time with each group and ask several additional questions that have been prepared in advance. A student worksheet is included with the formative assessment in Appendix A.

Summary

Students construct a short sequence of DNA based on the Watson and Crick model. The activity can be expanded for further study: Students can use their constructed DNA sequence to distinguish between DNA and RNA; to model the processes of replication, transcription, and translation; and to predict the effects of DNA mutations on polypeptide synthesis.

Lesson 3: Replication of DNA

Plan the Lesson

Connections to Course Outline

The content of this lesson addresses the following areas of the AP Biology course outline:

- Big Idea: Living systems store, retrieve, transmit, and respond to information critical to living systems.
- Enduring Understanding: The process of DNA replication is semiconservative; that is, when a double helix replicates, the daughter strand will consist of one strand conserved from the parent strand and one newly synthesized strand.

Objectives

- Students investigate experiments by Meselson and Stahl supporting a "semiconservative" mechanism of DNA replication.
- Students are able to use their constructed model of DNA to simulate DNA replication.
- Students are able to link the process of DNA replication to the behavior of chromosomes in mitosis and meiosis.

Common Student Misconceptions

Common student misconceptions include the following:

- that the two strands of a DNA molecule run in a *parallel* direction, not *antiparallel*;
- that the strands separate between covalent bonds between nucleotides making up the "uprights" of the helix, rather than between the hydrogen bonds connecting the nucleotide "rungs" between the two strands;
- that the activities on the "leading" and "lagging" strands are the same;
- that the helix completely unzips during replication, rather than replication occurring within replication bubbles.

Teach the Lesson

Instructional Activity I: Three Models of DNA Replication

The following activity comes in three parts. First, students are asked to describe through visual representation the three models of DNA replication. Second, they are asked to design their own experiment to test the three models of DNA replication and share their ideas with the class. Third, the students compare their lab design with the Meselson–Stahl experiments and draw conclusions about the mechanism of DNA replication and whether it supports the model described by Watson and Crick. It is suggested that you have the students complete all three parts of the lesson.

Instructional time is two 45-minute periods.

Background Information

Heritable information is passed from parent to offspring through mitosis and meiosis followed by fertilization. How does the Watson and Crick model of DNA provide a mechanism for the transmission of genetic information from one generation to the next? How can existing information be both preserved and duplicated with high fidelity?

According to the Watson and Crick model, a molecule of DNA consists of two complementary strands of nucleotides, with each storing information to reconstruct the other. Watson and Crick hypothesized that prior to replication, the hydrogen bonds connecting the strands are broken, and the two chains unwind and separate. Watson writes, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (*Nature* 1953). In the 1950s, three possible models for replication were postulated—conservative, semiconservative, and dispersive. Watson and Crick's model suggested semiconservative replication, with each strand serving as a template for the assembly of a new, complementary strand of nucleotides. However, Watson and Crick's hypothesis remained untested for several years.

In the late 1950s, Matthew Meselson and Franklin Stahl tested not only Watson and Crick's model for replication, but two alternative ones as well. Using *E. coli* as their experimental organism, Meselson and Stahl cultured bacteria for several generations in a medium containing nucleotides precursors labeled with a heavy isotope of nitrogen, ^{15}N. As their DNA replicated, the bacteria incorporated ^{15}N into nucleotides. Meselson and Stahl then transferred the bacteria into a growth medium containing only ^{14}N, the "lighter" and more common form of nitrogen. DNA of different densities could be distinguished from each other by centrifuging DNA extracted from the bacteria (Campbell and Reece, 300).

Part 1

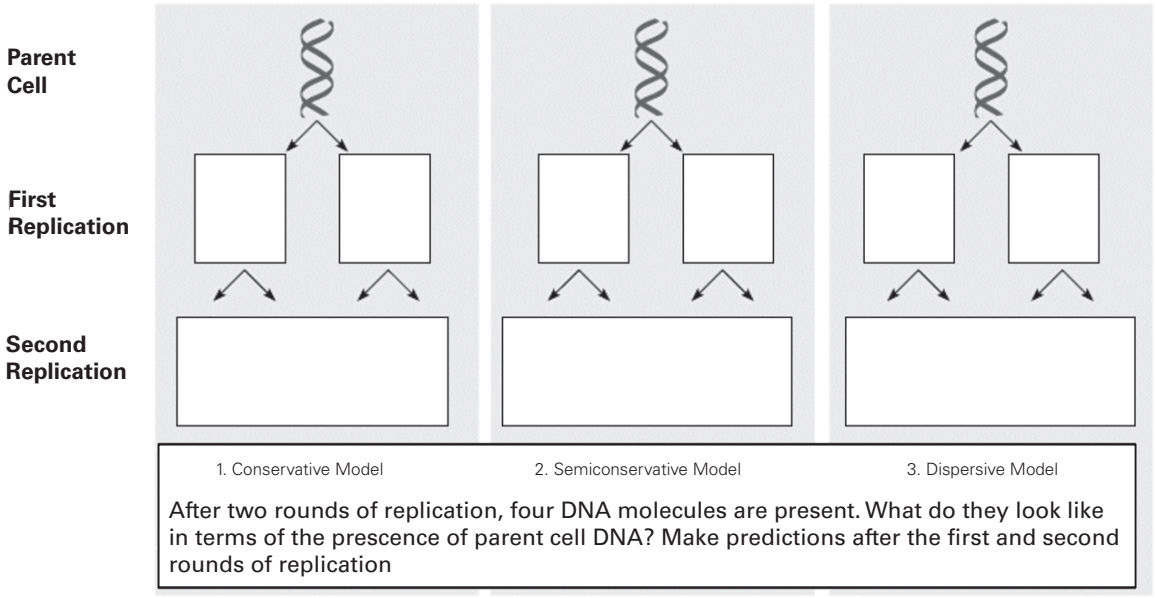
Inquiry for Students

What were the three models of DNA replication under investigation in the 1950s?

Materials

Colored pencils/crayons/markers, DNA models of replication diagram

Figure 3: Student investigation of possible models for DNA replication (DNA Replication, Instructional Activity I, Part A). Based on the terms, conservative, semiconservative, and dispersive, students are to design visual representations of the resulting DNA molecules after rounds of replication.



Student Instructions

In order for DNA to provide for continuity of life, the template parent molecules must remain with new DNA molecules. With a classmate, answer the following questions that relate to the diagram.

1. Define the following terms in the context of how a molecule might be replicated: *conservative, semiconservative,* and *dispersive*.
2. Using the diagram above, design a visual representation for each of the three models of replication. Label or color the DNA from the parent strand and any new DNA.
3. Based on what you know about how biological molecules are synthesized, which model would best support the biological theme of continuity and change? Conservation of energy? Support your answer.

Part 2

Design an experiment to test the three models of DNA replication.

Students can assume that they have access in a laboratory to the following:

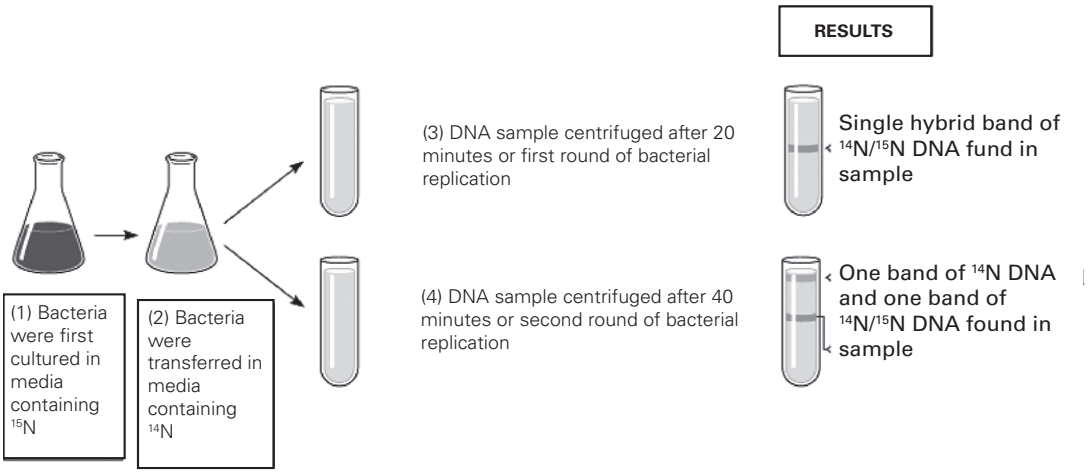
1. an experimental organism of their choice
2. their choice of radioactive isotopes (e.g., ^{14}C, ^{14}N, ^{15}N, ^{32}P)
3. test tubes
4. food/growth media for organisms
5. a centrifuge

Students must justify their choice of organism and istopes. It is suggested that students work with lab partners, but another option is to assign this activity as a homework assignment. They will share their experimental designs with the teacher and their classmates. The design can be a written description similar to the experimental protocol in the AP Lab Manual or presented in the form of a visual representation.

Part 3

To wrap up the discussion in Part 2, you should present the design and results of the Meselson–Stahl experiments investigating the mechanism of DNA replication shown in figure 4. Students are asked what model of replication (conservative, semiconservative, or dispersive) from Part 1 is supported by the Meselson–Stahl results.

Figure 4: Results of Meselsohn–Stahl experiment. Bacteria were first cultured in growth media containing the heavier isotope ^{15}N and then transferred to growth media containing the lighter isotope ^{14}N. A sample was centrifuged after 20 minutes (one round of replication) and a sample was centrifuged after 40 minutes (two rounds of replication). Light (top) and heavy (bottom) DNA will separate via centrifuge. Hybrid (light and heavy DNA) will appear as a single band in the middle.



Experimental Analysis

Students will answer the following:

1. Draw conclusions, in writing, from the results of the Meselson–Stahl experiment illustrated in the image provided.
2. After the first replication, which of the three models is proven to be invalid? Support your answer.
3. After the second round of replication, which of the two remaining models is supported?
4. What evidence from the experiment supports the model of DNA replication described by Watson and Crick?
5. Share your conclusions with your teacher and classmates. Do they agree with your conclusions? Why or why not?
6. If your teacher or classmates disagree with your conclusions, can you refine your ideas? Why or why not?
7. What are ways in which scientists can resolve conflicting ideas with respect to conclusions drawn from experimental results?

A common student misperception is the conservative model of DNA replication; that is, a new strand of DNA is synthesized from scratch. You could address this misconception by discussing that if this were the case, after several replications that information in the original DNA template would be lost—and continuity of heritable information is a key function of DNA.

Challenge Question

Watson and Crick could only hypothesize how their model of DNA could serve the criteria of replication. How did the Meselson–Stahl experiments testing the three hypotheses of replication support Watson and Crick's statement, "It has not escaped our notice that the specific [nitrogenous base] pairing we have postulated immediately suggests a possible copying mechanism for the genetic material"?

You should join each student group for a few minutes as they work on the activity. You should also assess their understanding of the Meselson–Stahl model of DNA replication by asking questions similar to ones in the experimental analysis above. Students can also share their responses to the questions with other groups.

Summary

Students use Meselson and Stahl's model to justify that DNA replication is a semi-conservative process; that is, information in the parent strand (template) conserved in the newly formed molecule. The experimental results also support Watson and Crick's hypothesis about the nature of DNA replication, which will be investigated more thoroughly in the next instructional activity.

Instructional Activity II: Student Model of DNA Replication

The following activity asks students to use the molecule of DNA they constructed as part of the formative assessment in Appendix A to model the process of DNA replication. As they work through the process, students are asked to answer several questions. Students should work in pairs or small groups.

Instructional time is approximately 30 minutes.

Background Information

A complex process, DNA replication involves several enzymes and other molecules. Replication is not as simple as separating or "unzipping" a molecule of DNA between hydrogen bonds forming the double helix and building complementary strands. Although much more is known about how replication occurs in bacteria, the process is fundamentally similar in eukaryotes; thus, replication in bacteria serves as a model for the evolution of the process from bacteria to eukaryotes.

Replication of a DNA molecule begins at special sites called origins of replication. Proteins recognize a particular sequence of nucleotides and separation of the two strands begins at this site, with an enzyme (helicase) opening up a replication "bubble." Since bacteria only have one chromosome, they only have one origin. By comparison, eukaryotic chromosomes have perhaps thousands of replication origins and thousands of bubbles that ultimately fuse into each other. Replication continues in both directions until the entire molecule of DNA is copied.

Remind the students that from their study of the structure of DNA, they confirmed the antiparallel nature of the two strands: The uncoiled DNA consists of a 3′→5′ template strand and a 5′→3′ template strand. Replication enzymes—DNA polymerases—that assemble nucleotides into a new strand can only move in the 3′→5′ direction; thus, the template can only be "read" in the 3′→5′ direction, while the new, complementary strand grows in the antiparallel 5′→3′ direction. The 3′→5′ template strand is referred to as the "leading" strand because replication occurs in a continuous direction, with DNA polymerase following the opening of the replication bubble. However, for the 5′→3′ template, DNA polymerase moves *away* from the uncoiling replication bubble because the enzyme only can add nucleotides to the free 3′ end of a growing DNA strand. If this is true, how can the second strand be copied?

Teaching Tips

Ask the students to come up with an analogy for the phenomenon described above. For example, a student could use a simple scenario: Susie Smith, a student in AP Bio, is trying to ride up an escalator at the local mall. If the escalator is moving upward and Susie rides it upward, she travels in the same direction the escalator is moving; she is riding up the "leading" strand. However, if Susie tries to ride up an escalator that is moving *downward*, she will have trouble traveling up this "lagging" strand.

Follow up by asking how Susie can travel *up* an escalator that is moving *down*. Can she ever make it to the top? (Yes, if she skips steps or travels upward in leaps or bounds.)

In contrast to the leading strand, which elongates continuously, the lagging strand is synthesized as a series of segments—the "leaps and bounds" of the analogy above. As the helix uncoils, DNA polymerase assembles short segments of nucleotides called Okazaki fragments, named for the Japanese scientist who discovered them in the 1960s (Campbell and Reece, 302–303). Another enzyme, ligase, ultimately joins the segments together, forming a single new strand of DNA.

DNA polymerases cannot initiate the synthesis of a polynucleotide but, rather, can only add nucleotides to the 3′ end of a preexisting chain. The first nucleotides of the leading strand and each Okazaki fragment of the lagging strand are initiated by RNA primase, which, unlike DNA polymerase, can start an RNA chain from scratch. Primase joins RNA nucleotides together one at a time, making a short segment of RNA primer complementary to the DNA template strand where replication begins; DNA polymerase then adds a DNA nucleotide to the 3′ end of RNA primer and continues adding nucleotides to the growing strand according to the base-pairing rules. Only one RNA primer is required for DNA polymerase to begin synthesizing the leading strand because there is only one 3′ attachment site. However, each Okazaki fragment is primed separately. Ultimately, appropriate DNA nucleotides replace RNA primers.

Inquiry for Students

Using the molecule of DNA you constructed as part of the embedded classroom assessment, "From Gene to Protein" (Appendix A), can you model the process of DNA replication?

Materials

In addition to the previously constructed molecule of DNA, students will need construction paper, scissors, markers, or other media to construct several additional DNA nucleotides consisting of deoxyribose, phosphate groups, and the nitrogenous bases adenine, thymine, cytosine, and guanine. Students will also need to construct representations of the enzymes used in DNA replication, including helicase, DNA polymerase, and ligase. The teacher may also ask students to construct RNA primers and primase.

Analysis Questions

As students model the process of DNA replication using a constructed molecule, they should answer the following questions in writing. If appropriate, they may use visual representations to answer the questions. They should also discuss their answers within their student groups.

1. Do the strands of DNA in your model run in an *antiparallel* direction? Support your answer.
2. At the first step in replication, did you "unzip" the DNA molecule between the bonds connecting the strands? What type of bonds connect the strands in the double helix? (Bonus: What is the name of the enzyme that does the "unzipping"?)
3. Can you identify the "leading" and "lagging" strands?
4. What is the significance of 3′→5′ and 5′→3′?
5. What is the role of DNA polymerase in replication?
6. How do the activities of replication differ on the leading and lagging strands? Why?
7. In what direction is the template strand "read"? In what direction is the newly synthesized strand assembled?
8. What is a replication "bubble"?
9. Did you correctly mode Okazaki fragments with respect to replication of the lagging strand?
10. Did you correctly pair A–T and C–G when making a strand complementary to the original DNA template strand?
11. What are the roles of RNA primer and RNA primase in DNA replication?
12. What are the roles of telomeres and telomerases in DNA replication in eukaryotes?

You should join each student group for a few minutes as they work on the activity. The teacher assesses their understanding of the process of DNA replication by asking questions similar to ones in the analysis above. You can ask more difficult questions depending on the sophistication of the class. Students can use similar question sets to reflect on whether or not they achieved the objective of the lesson to model accurately the semiconservative process of DNA replication. The most common student misconception is that the strands of DNA run in the same direction (parallel); if this is the case, the concepts of the leading and lagging strands and the difference in replication activities on them will be difficult to understand. At this point, you can work with the students as they review the structure of DNA using the molecule they constructed in Lesson 2 of this module.

Summary

Students are asked to model the process of DNA replication using the DNA molecule they constructed in the formative assessment in Appendix A. As they work through the process, they should be able to describe the semiconservative model of DNA replication, the difference between template and complementary strands, and the activities on the leading and lagging strands. If there are still misconceptions, you should ask a pair of students to diagram DNA replication on the board and have the class walk through the process. Once the students understand replication, you can introduce the concepts of errors in the process.

Instructional Activity III: Errors in DNA Replication

This is an activity that can be done with the class as a group. Ask the students to keep in mind Watson and Crick's model of DNA. Then, ask them to hypothesize what happens if adenine (A) is incorrectly paired with guanine (G), or cytosine (C) with thymine (T). What happens to the dimensions of the rungs and overall shape of the molecule? What effect could improperly paired bases have on the "language" of DNA?

Background Information

DNA has built-in redundancy; that is, *each* strand possesses all the information to synthesize a polypeptide. If one strand is damaged, the *other* strand provides the same information. Although DNA replication occurs with great accuracy, errors do occur. In bacteria, DNA polymerase checks each newly attached nucleotide to make sure that it is correctly paired with its complementary one, that is, (A) with (T), and (C) with (G). If a mistake occurs, DNA polymerases can usually correct it. Unfortunately, if the error is not corrected, it becomes a mutation. Mutations are sequences of nucleotides in a DNA molecule that do not match the original DNA molecule from which it was copied.

Teaching Tips

At this point, you can do the following:

1. Introduce the concept of mutations in which nucleotides are substituted incorrectly, deleted, or inserted. (An effective demonstration uses the DNA molecule students constructed in Lesson 2. The teacher can randomly substitute, delete, or insert nucleotides into the model.)
2. Ask students how "misspellings" of nucleotides changes the "language" of the original DNA sequences. Eventually students will be asked to connect errors in replication to incorrect polypeptide synthesis and effects on the phenotype.
3. Have a student use a model of DNA to demonstrate to the class the effect of the above errors on the structure of the molecule.

Hopefully, students will respond that if any of the above mistakes occur, the "language" of DNA changes. This leads to the next question and the objective of the next activity: What mechanisms does a cell have, if any, for proofreading and repair?

For Further Study

At this point, you can provide more details about the role of DNA polymerase and other repair enzymes in correcting "misspellings." However, a discussion of mutations and repair mechanisms is best saved until the students have a better understanding of transcription and translation.

You can also introduce the concepts of telomeres and telomerase: In eukaryotes with linear chromosomes, a small portion of the cell's DNA cannot be replicated or repaired due to the limitations of DNA polymerases; since DNA polymerase can only add nucleotides to the 3′ end of a preexisting polynucleotide, there is an "end problem." The usual mechanism of replication cannot complete the 5′ ends of daughter DNA strands. Bacteria do not have this problem because their DNA is circular with no ends.

For an instructional activity, you can ask students to follow the end of one strand of a DNA molecule through two rounds of replication. Students should be able to observe that after the first round, the new lagging strand of DNA is shorter than its template. After a second round, both the leading and lagging strands are shorter than the original DNA parental template. With each successive replication, the ends of the newly formed DNA strands become shorter and shorter. The student can repeat the exercise until the concept becomes clear.

You can ask students to describe, using a diagram, the effect of this shortening on DNA replication. How much information in the form of DNA can be "lost" from the ends of the strands during replication without consequences to the cell? Students can hypothesize about how the "end problem" can be fixed. The teacher can then introduce the role of telomeres and telomerases in preserving eukaryotic genes and information.

Summary

This short activity asks students to point out possible sources of errors in the DNA replication process. Students are asked to predict the possible effects of mutation on the "language" of DNA. The lesson also provides an opportunity for advanced students to study the "end problem" of replication on the 5′ ends of daughter DNA strands and the role of telomeres and telomerases in preserving eukaryotic genes and information. The next lesson takes the students through the translation of the genetic information.

Lesson 4: Transcription—DNA→RNA

Plan the Lesson

Connections to Course Outline

The content of this lesson addresses the following areas of the AP Biology course outline:

- Big Idea: Living systems store, retrieve, transmit, and respond to information critical to living systems.
- Enduring Understanding: The direction of the flow of genetic information is from nucleic acid to protein. In order for information stored in DNA to direct cellular processes, it is transcribed onto RNA and, in turn, translated into polypeptides.

Objectives

- Students are able to describe Garrod's observations about "inborn errors of metabolism" and evidence that genes determine traits through enzyme-catalyzed chemical reactions.
- Students are able to justify the revision of Beadle and Tatum's "one gene–one enzyme" model to "one gene–one polypeptide."
- Students can describe through narrative or visual representation the similarities and differences between DNA and RNA.
- Students are able to distinguish between the three types of RNA—mRNA, rRNA, and tRNA—with respect to structure and function.
- Using their constructed models of DNA, students can model the process of transcription of information from DNA→RNA.

Common Student Misconceptions

A common misconception is that both strands of the DNA molecule are transcribed, and that a strand of DNA consists of one gene, not multiple genes. Students also incorrectly identify ribose as the sugar in RNA and forget to substitute uracil for thymine.

Teach the Lesson

Instructional Activity: Transcription—DNA→RNA

The following activity asks students to use a constructed molecule of DNA to model the process of transcription of information from DNA to RNA. Students are asked to address the *inquiry* question below and answer the questions provided in the *analysis*. Students may work in pairs or small groups.

Instructional time is approximately 15 minutes.

Background Information

Genetic information is stored in DNA in the form of specific sequences of nucleotides. But how does this information determine an organism's traits, including the variety of pea shapes Mendel observed in his plants? How does a nucleus control the activities of a cell? How does DNA determine cellular metabolism? How does an organism's genotype determine its phenotype?

In the early 1900s, Archibald Garrod, while investigating causes of inherited metabolic disease in humans, suggested that genes determine traits through enzymes that catalyze specific chemical reactions in the cell. Later research supported Garrod's "inborn errors of metabolism," including studies by Beadle and Tatum who coined the phrase, "one gene–one enzyme" (Campbell and Reece, 309–310). However, not all proteins are enzymes; insulin is an example of a nonenzyme protein, and the inability to synthesize it results in the often severe phenotypic consequences associated with diabetes.

Teaching Tips

At this point, you can pause and ask students why, in light of the above information, was Beadle and Tatum's hypothesis later revised to "one gene–one polypeptide"? What is a simple definition of a "gene"?

A gene does not build a protein directly. Another nucleic acid, RNA, "communicates" the language of DNA into another language—the language of polypeptides. RNA is chemically similar to DNA, but with three significant differences: It is single-stranded, contains ribose instead of deoxyribose as its sugar, and has the nitrogenous base uracil, not thymine. Additionally, RNA exists in three forms: mRNA, rRNA, and tRNA. Getting information from DNA to protein involves all three RNAs and requires two stages: transcription and translation.

This is also a good opportunity to review cell structure by asking students to explore the following scenario: DNA is a double-stranded molecule, and its size prevents it from getting through the nuclear membrane of eukaryotes. Assuming this is true, how can the information stored in DNA direct the synthesis of polypeptides outside the nucleus at the ribosomes attached to endoplasmic reticulum? Students should hypothesize that some kind of intermediate (i.e., RNA) that *can* leave the nucleus is necessary.

In transcription, RNA molecules are created by using DNA as a template. Similar to the action of DNA polymerases, RNA polymerases read the DNA template in the 3′→5′ direction and assemble the RNA nucleotides in the 5′→3′ direction; however, RNA polymerase can start a chain without primer. Specific sequences of nucleotides along the DNA template indicate where transcription of a gene begins and ends. In initiation, RNA polymerase attaches to a "start" sequence of nucleotides, "unzips" the helix, and begins appropriately adding nucleotides one by one, with adenine pairing with uracil, and cytosine pairing with guanine. Termination occurs when RNA polymerase reaches a "stop" sequence of nucleotides. Unlike DNA replication, only one strand of DNA is transcribed.

Although the process of transcription is fundamentally similar in bacteria and eukaryotes, it is important to point out a couple of significant differences. Following transcription in bacteria, the RNA transcript is immediately usable as mRNA. In eukaryotes, it must first undergo processing in various ways, including the excision of noncoding regions (introns), before leaving the nucleus. The nonexcised coding regions (exons) are spliced together and will ultimately be expressed as a polypeptide.

Inquiry for Students

Using a constructed molecule of DNA with at least 12 nucleotides as a template, can you model the process of transcription of information from DNA→RNA?

Materials

Students will have already constructed a molecule of DNA. However, they will need construction paper, scissors, markers, or other media to make several RNA nucleotides consisting of ribose, phosphate groups, and the nitrogenous bases—adenine, uracil, cytosine, and guanine. Students will also need to construct representations of the enzymes helicase and RNA polymerase.

Analysis Questions

1. Describe through narrative or a visual representation *three* differences between DNA and RNA.
2. Describe through narrative or visual representation the structural differences between mRNA, rRNA, and tRNA.
3. How is transcription similar to DNA replication?
4. How is it different?
5. What is the role of RNA polymerase in transcription?
6. Given a sequence of DNA 3′…GATTACAGATTACAGATTACA…5′, what is the sequence of mRNA that can be transcribed?

You should join each student group for a few minutes as they work on the activity. You can assess their understanding of the process of DNA replication by asking questions similar to ones in the experimental analysis above. Students can use similar question sets to reflect on whether or not they achieved the objective of the lesson to model accurately the process of transcription of genetic information from DNA→RNA. Students can critique the work of other groups and provide feedback. Remember, students learn best by teaching.

For further study, students can investigate why introns and RNA splicing exist in the first place. Why do introns exist if their information is never expressed as a gene? Are introns "junk" DNA or "trash"? What's the difference? What is the evolutionary significance of existence of introns?

Summary

Students should be able to model using a visual representation the process of transcription of genetic information from DNA to RNA. In transcription, RNA molecules are created by using DNA as a template. Similar to the action of DNA polymerases, RNA polymerases read the DNA template in the 3′→5′ direction and assemble the RNA nucleotides in the 5′→3′ direction. Unlike DNA replication, only one strand of DNA is transcribed. The next task asks students to translate the information that has been transcribed from DNA to RNA.

Lesson 5: Translation—DNA→RNA→Protein

Plan the Lesson

Connections to Course Outline

The content of this lesson addresses the following areas of the AP Biology course outline:

- Big Idea: Living systems store, retrieve, transmit, and respond to information critical to living systems.
- Enduring Understanding: The direction of the flow of genetic information is from nucleic acid to protein. In order for information stored in DNA to direct cellular processes, it is transcribed onto RNA and, in turn, translated into a sequence of amino acids, or polypeptides.

Objectives

- Students are able to describe through narrative or visual representation the Triplet Code.
- Given a visual representation of a sequence of DNA, students are able to transcribe the sequence to a sequence of RNA.
- Given a visual representation of a sequence of mRNA and a "dictionary" of amino acid codons, students are able to translate the sequence to a primary structure polypeptide.
- Students are able to revise or refine a visual representation of the process of translation of genetic information from DNA→RNA→protein.
- Students are able to create, revise, or refine a visual representation of the process of translation.
- Students are able to conduct an experiment, collect evidence, and draw conclusions supporting that information in DNA, when translated, results in observable phenotypes.

Common Student Misconceptions

Common student misconceptions about the Triplet Code are (1) that one nitrogenous base codes for one amino acid, or (2) that three amino acids code for one nitrogenous base. The Triplet Code states that three nitrogenous bases code for one amino acid. With respect to the process of translation, students often cannot distinguish between the structure and function of the three types of RNA—mRNA, rRNA, and tRNA. They also confuse codons and anti-codons and cannot make the connection DNA→RNA→amino acid sequence (polypeptide). Students have difficulty connecting genotypes with observable phenotypes.

Teach the Lesson

Instructional Activity I: The Triplet Code

This activity begins the study of translation by asking students to perform the first two "warm-up" instructional activities described below with no background information provided by the teacher. (Background information is provided for you, however.) Students should work in pairs or small groups.

Instructional time is short—approximately 10 minutes.

Background Information

For information stored in DNA to direct cellular processes, it must be translated to protein. However, when biologists first made the connection between information in DNA to polypeptide synthesis, they recognized a dilemma: There are only four nucleotide bases (A, T, C, and G), but there are 20 amino acids. What is the connection between nucleotide base sequences of DNA and amino acid sequences? How is DNA's information "decoded"?

Marshall Nirenberg and others cracked the genetic code in the 1960s—nearly 20 years after Watson and Crick determined the structure of DNA (Campbell and Reece, 313). Each amino acid is coded for by a sequence of three nitrogenous bases, hence the Triplet Code. Although taking the four bases in all combinations of triplets (4^3) results in 64 arrangements, some amino acids have more than one code or codon. Additionally, the codon AUG specifies both the amino acid methionine and a "start" codon, while three codons serve as "stops" and do not code for amino acids.

Inquiry

How can four nitrogenous bases (A, T, C, and G) code for the 20 amino acids in polypeptides?

Students work in pairs to answer the following questions in writing. You can present the questions in a worksheet or read them orally, one by one, pausing to allow the students to process and answer each question.

1. If each nitrogenous base codes for one amino acid (4^1), how many amino acids can you code for?
2. What if you take the bases *two* at a time? For example, what if AT specified one amino acid, and TC another? How many amino acids can you code for? In other words, 4^2 = ?
3. If you answered 16, you're close, but that's not enough. There are 20 amino acids, not 16. What if you take the four bases *three* at a time? How many amino acid codons result from 43?
4. If you answered 64, congratulations! You are correct! If each arrangement of three consecutive bases specifies one amino acid, there can be 64 possible code words—more than enough to specify all 20 amino acids and then some. With that information, explain the term *synonymous codons*? What does the word *synonym* mean in an English class?
5. Why is the "language" of DNA said to reside in the Triplet Code?

Common student misconceptions about the Triplet Code are (1) that one nitrogenous base codes for one amino acid, or (2) that three amino acids code for one nitrogenous base. You can address these misconceptions by asking for a student volunteer to go to the board and show, in writing, how he or she deduced that three nitrogenous bases are necessary to code for one amino acid. The student's classmates can critique whether or not the student's reasoning is logical. (You can use this "teaching moment" to describe differences between positive and negative criticism.)

Summary

Students work through a series of questions to determine the connection between nucleotide base sequences of DNA and amino acid sequences. The activity allows students to interpret or define the Triplet Code.

Instructional Activity II: Using the Dictionary

This activity takes the above concept one step further by asking students to transcribe a DNA sequence of nucleotides to a sequence of RNA nucleotides and, in turn, a sequence of amino acids. Students often forget to substitute uracil (U) in RNA to thymine (T) in DNA. Some students might still think that in the Triplet Code, three amino acids code for one nitrogenous base. Additionally, they might have difficulty reading the dictionary of amino acid codons. You should visit each student group to make sure they are correctly interpreting the dictionary.

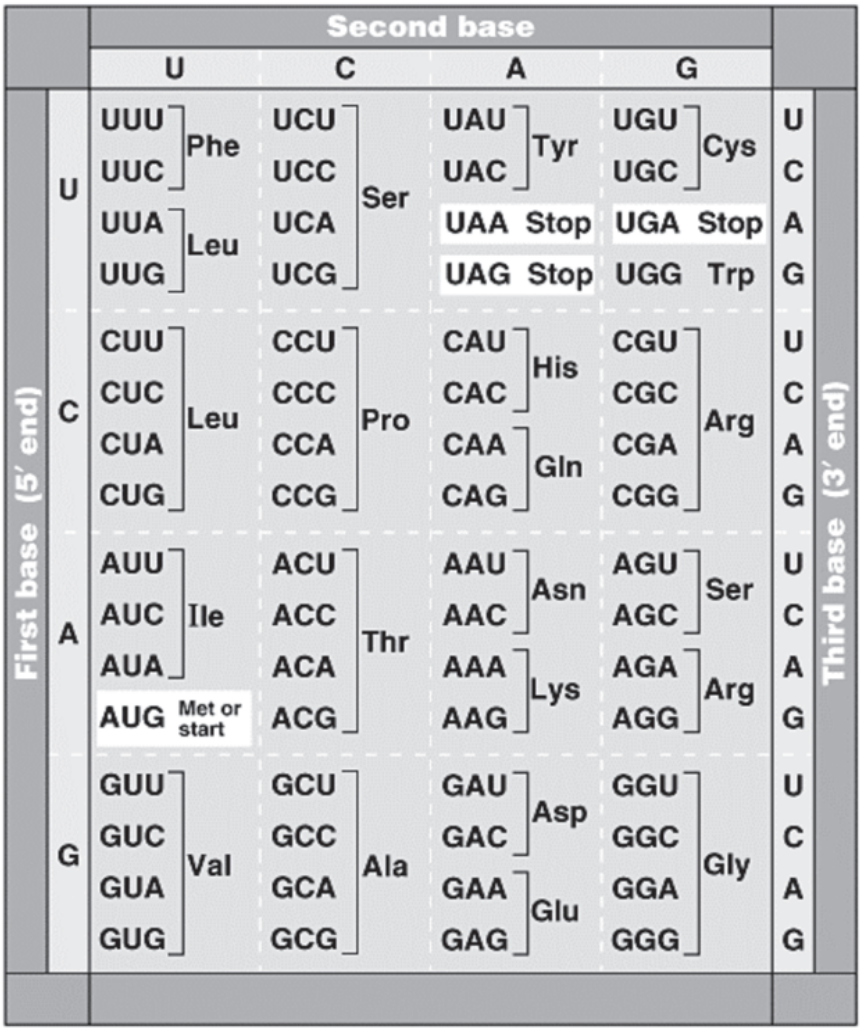
Students work in pairs or small groups to answer the following questions in writing.

Instructional time is short—approximately 10 minutes.

Inquiry for Students

1. Given the DNA sequence 3′…ATTTCAAACGCATACGAT…5′, what is the sequence of mRNA that can be *transcribed* from this sequence?
2. How many amino acids are coded for by this sequence?
3. Using the "dictionary" of the genetic code in the figure below, *translate* your mRNA sequence into a sequence of amino acids.

Figure 5: The Genetic Code. Note that this is this the translation of mRNA codons and is read the 5′ to 3′ direction.



Students can compare their responses to the questions by sharing them with another group. Or, a member of each group can go to the board and illustrate the group's transcription of the given sequence from DNA→RNA *and* the subsequent translation of the mRNA sequence to a sequence of amino acids. You can ask students to resolve any discrepancies in their answers.

Summary

In a quick activity, students are given a short sequence of DNA nucleotides and are asked to write down the sequence of mRNA that can be transcribed from the sequence. Using a dictionary of the genetic code, students are then asked to translate the transcribed mRNA sequence into a sequence of amino acids.

Instructional Activity III: Translation Steps

At this point, many teachers chose to present details of translation through a lecture or a PowerPoint presentation. However, the following inquiry-based activities are examples of more effective strategies for student engagement and learning. The suggested activities raise questions, and questions from students are good indicators of their level of understanding. You can create a rubric of essential features that should be included in each visual representation. Students should work in pairs or small groups. They can find information in their textbook, the Internet, or other sources approved by you.

Background Information

The process of translation is fundamentally the same in bacteria and eukaryotes. A cell interprets a genetic message in DNA and synthesizes a polypeptide. What other "ingredients" are necessary? What are the roles of mRNA, tRNA, rRNA, ribosomes, and amino acids in polypeptide synthesis? Where in a bacterial cell does translation occur? A eukaryotic cell? This is a good time to review cell structure and function.

The basic concept of translation is that as a molecule of mRNA is moved through a ribosome (rRNA and protein), codons are translated one by one into a sequence of amino acids—the primary structure of a protein. To summarize, translation consists of three distinct stages: initiation, elongation, and termination. Initiation begins when a ribosome attaches to a region near the 5′ end of the mRNA. A tRNA with the anticodon UAC carries methionine to the mRNA at the complementary "start" codon AUG. Elongation continues as each mRNA codon is read, with tRNAs carrying specific amino acids to the ribosome. As each new tRNA arrives, the polypeptide chain is elongated by one new amino acid, growing in sequence length according to the codons on the mRNA. When a "stop" codon on mRNA is read, termination occurs with the release of the completed polypeptide. Once the polypeptide is completed, interactions between amino acid R-groups result in secondary and tertiary structures.

Suggested Activities

1. Students can describe key steps in the process of translation by using a visual representation such as a diagram with annotation. The concept can also be presented as a poster project.
2. Students can revise or refine a visual representation of translation.
3. Students can generate a *Jeopardy*-type game to learn and review essential terms associated with the process of translation.
4. Students can design a board game to take players through the steps in translation.
5. Students can generate a PowerPoint presentation or video describing the three stages of translation. For example, group 1 can describe initiation; group 2 can describe elongation; group 3 can describe termination. Students can find a rubic for translation in their textbook.

Both you and the students can assess the effectiveness of the group visual representations or presentations with respect to scientific accuracy, clarity, creativity, and usefulness based on the teacher-generated rubric. Students can make suggestions about how to increase the effectiveness of the presentations.

Summary

Students are asked to model the steps in translations by choosing an appropriate instructional activity designed by you. Each activity can be modified to address different learning styles and time constraints.

Instructional Activity IV: For Further Study

This activity is set out in the pGLO Bacterial Transformation Lab (Appendix B). The experiment consists of three parts. In Part A, the students consider crucial scientific investigative background. In Part B, they follow a procedure to transform bacteria with a gene that encodes for green fluorescent protein. In Part C, they collect data and analyze their results in order to determine how successful they were in causing a phenotypic change in *E. coli* bacteria.

Background Information

For information in DNA to direct cellular processes, it must be translated into polypeptides. The protein products determine the metabolism and thus the cellular activities and phenotypes upon which evolution operates. Because DNA is a universal molecule, its information, when transcribed and translated, can be expressed as a phenotype, and phenotypes are usually observable.

In this experiment, the students will perform a procedure known as genetic transformation—a key technique in biotechnology. What are some possible applications of genetic transformation in agriculture, environmental science, and medicine? The complex, advanced procedure allows the students to transform *E. coli* bacteria with a gene that codes for green fluorescent protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. GFP causes the jellyfish to glow in the dark. Following the transformation procedure, the bacteria express their newly acquired jellyfish gene as an observable phenotype; they produce the fluorescent protein, which causes them to glow a brilliant green color under ultraviolet light. This activity is an opportunity for the students to revisit Griffith's 1928 experiment identifying a "transforming factor" later identified as DNA.

For more thorough background information, along with the complete student experimental procedure and reflection on the activity, refer to Appendix B. It is also strongly suggested that the students perform all three parts of the experiment, including the investigative background; however, if time constraint is an issue, the teacher may perform the actual experimental procedure as a demonstration and ask the students to analyze the results.

Summary

In this extensive laboratory activity, students perform a procedure known as genetic transformation—a key technique in biotechnology—in order to investigate how bacteria can use gene transfer to acquire new, observable phenotypes. If the transformation is successful, the bacteria will incorporate the *pGlo* plasmid and express a new, observable phenotype: The bacteria will glow in the dark. Additionally, some *E. coli* colonies will demonstrate resistance to antibiotic. The experiment demonstrates the universality of DNA and its expression. You should allow time for class discussion of the experimental results by allowing each student group to explain their conclusions. The rest of the class respectfully critiques each group until you are satisfied that each member of the class understands how the information in DNA is expressed as a phenotype. This experiment is a connection to the next lesson, the regulation of gene expression, *and* to natural selection and evolution.

Lesson 6: Gene Regulation—the Operon Model

Plan the Lesson

Connections to the Course Outline

The content of this lesson addresses the following areas of the AP Biology course outline:

- Big Idea: Living systems store, retrieve, transmit, and respond to information critical to living systems.
- Enduring Understanding: Structure and function in biology involve two interacting aspects: the presence of necessary genetic information and the correct expression of this information.

Objectives

- Students are able to explain why regulation of gene expression is necessary.
- Students are able to describe by visual representation the components of the operon.
- Students are able to describe by visual representation the *lac* operon of inducible gene expression.
- Students are able to describe by visual representation the *trp* operon of repressible gene expression.

Common Student Misconceptions

The first challenge for students is to understand that the operator region of the operon controls transcription, and that the promoter region controls translation of the structural genes to polypeptides. Students should be able to explain this difference clearly. A second challenge is to understand that the difference between the *lac* and *trp* operons is small, but significant; students often confuse the inducible response of the *lac* operon with the repressible response of the *trp* operon.

Teach the Lesson

Students can learn how the environment controls gene and protein expression through the inquiry-based embedded classroom assessment "Operon" (Appendix C). Provided materials, students construct models of the *lac* and *trp* operons and use them to develop answers to probing questions provided by the teacher. First, students reveal their understanding of the operon model by expressing the role of each of the components. Next, they are asked what happens if the promoter and operator are reversed and whether or not transcription will occur under the new circumstances. The students are also asked to consider evidence that would imply a mutation of the regulator gene that increases, decreases, or deactivates gene expression. Finally, students are provided with a scenario in which expression does not occur and are asked to apply their model to pose questions that might be tested to explain this observation. You should spend time with each group and ask several additional questions that have been prepared in advance. Details of the embedded classroom assessment, along with the student worksheet, are found in Appendix C.

Background Information

Structure and function in biology involve two interacting aspects: the presence of necessary genetic information and the correct expression of this information. Genetic material controls the production of cell products and, in turn, these products determine the metabolism and nature of the cell. Most cells within an organism contain the same set of genetic instructions, and although some genes are continually expressed, the expression of most genes is regulated. Why? Why do cells place limits on gene to protein pathways? In terms of efficiency, regulation of pathways allows better utilization of energy and resources, thus increasing metabolic fitness. But what signals control gene expression?

Environmental signals and cascades involve both regulatory and structural genes that, when activated or inactivated, control synthesis of polypeptides. Gene regulation has been studied in the bacterium *E. coli*, a common organism that lives in the digestive system of humans. The DNA of *E. coli* contains sequences of DNA, called *operons*, that direct particular biosynthetic pathways and serve as models for gene regulation in both bacteria and eukaryotes. Four components of the operon model are (1) a regulatory gene that produces a repressor protein; (2) the promoter region that serves as a binding site for RNA polymerase; (3) the operator region that can block the action of RNA polymerase; and (4) the structural genes that can be transcribed and translated into polypeptides, including enzymes.

The *lac* operon in *E. coli* controls the breakdown of lactose. In the *lac* model, the repressor protein is usually active, and the structural genes are blocked, preventing transcription and translation of enzymes required for lactose metabolism if lactose is absent. However, when lactose is available, the repressor protein is inactivated, thus allowing the structural genes to be activated to produce enzymes necessary for lactose metabolism. Since a

substance is required to "turn on" the operon, the *lac* operon is an example of an inducible response.

In the *trp* model of an *E. coli* operon, the repressor protein is usually inactive, allowing for the transcription of structural genes necessary for the production of the amino acid tryptophan. When present, tryptophan binds with the repressor protein, inhibiting production of tryptophan synthetase; thus, the bacterium no longer needs to synthesize its own tryptophan. Since the structural genes stop producing enzymes only in the presence of an active repressor, the *trp* operon is an example of a repressible response.

Summary

Students investigate mechanisms of gene expression and regulation by constructing models of the *lac* and *typ* operons and then use their models to develop answers to questions provided by the teacher. Students also use their models to pose questions that might be tested to explain an observation when gene expression does not occur.

Alignment with AP® Exam Questions

After students complete the lessons in this curriculum module, they can use the AP Biology Exam questions to check for understanding of the processes involved in the transfer of information from gene to protein. The questions are intended for use as formative assessments and should not be used as summative assessments. Students can "think-pair-share" by answering questions individually, pairing with another student, and sharing their responses to the practice questions to justify their answers. You should provide time for students to present their answers and rationales for answer choices. You should also give feedback on the accuracy of answers and provide clarification on misunderstandings possibly embedded in the student rationales.

Sample AP Biology Free-Response Questions

2003

Excerpted from Form B, Question 1

A difference between prokaryotes (bacteria) and eukaryotes is seen in the organization of their genetic material.

a. **Discuss** the organization of the genetic material in prokaryotes and eukaryotes.

b. **Contrast** the following activities in prokaryotes and eukaryotes:

- Replication of DNA
- Transcription or translation
- Gene regulation
- Cell division

2005

Excerpted from Question 3

Protein synthesis is vital for cell growth and metabolism.

a. **Describe** transcription and translation.

b. **Identify** similarities between transcription and translation.

c. **Identify** differences between transcription and translation.

d. **Describe** structural changes that can occur to a protein after translation to make it function properly.

2002

Excerpted from Question 1, part (b)

The human genome illustrates both continuity and change. All humans are nearly identical in coding sequences and have many proteins that are identical in structure and function. Nevertheless, each human has a unique DNA fingerprint. **Explain** this apparent contradiction.

References

Campbell, Neil A., and Jane B. Reece. 2005. *Biology* 7th ed. San Francisco, CA: Benjamin Cummings.

College Board. 2001. *AP Biology Laboratory Manual.* New York: The College Board.

Watson, J. D., and F. H. C. Crick. 1953. "Molecular structure of nucleic acids: A structure for deoxyribonucleic acids." *Nature* 171, no. 738 http://www.nature.com/nature/dna50/archive.html.

Appendix A

From Gene to Protein: A Formative Assessment for AP Biology

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Introduction

It is expected that students who successfully complete AP Biology will be able to apply their understanding that living systems store, retrieve, transmit, and respond to information critical to life processes. After completing AP Biology, it is also expected that students will be able to correctly construct a molecule of DNA and use it to model the processes of replication, transcription, and translation. The following activity is a formative assessment that provides students with an opportunity to construct a short strand of DNA based on historical evidence provided by the work of Watson, Crick, Wilkins, Franklin, et al. First, the students should read Watson and Crick's *Nature* article, "A Structure for Deoxyribose Nucleic Acid," available for free download at http://www.nature.com/nature/dna50/archive.html. Additional sources of information can be provided by a textbook, the Internet, films (e.g., *The Double Helix*), or other sources approved by the instructor. The activity can be expanded for further study; for example, the students can use their constructed DNA sequence to distinguish between DNA and RNA; to model the processes of replication, transcription, and translation; and to predict the effects of DNA mutations on polypeptide synthesis.

If students incorrectly visualize the structure of DNA, they cannot understand the processes of replication, transcription, and translation. Common student misconceptions about the structure of DNA are the following:

- A molecule of DNA consists of chains of nitrogen bases linked together, rather than identifying DNA as a polymer of nucleotides consisting of a nitrogen base, a five-carbon sugar (deoxyribose), and a phosphate group.
- The nitrogen bases randomly pair with each other, rather than adenine pairing with thymine, and cytosine with guanine.
- The two strands of a DNA double helix run in a *parallel* direction, not *antiparallel*, where the "leading" strand runs in a 3′ →5′ direction, and the opposite or "lagging" strand runs from 5′ → 3′.

In addition, students often incorrectly identify the two types of chemical bonds (hydrogen and covalent) present in DNA and also confuse ribose and deoxyribose sugars.

Teacher Protocol

Provide small groups of students with construction paper, markers, and scissors. With little guidance, ask them to construct several (at least 24) DNA nucleotides based on the Watson and Crick model. For example, using different colors of construction paper, a student can cut out five-sided polygons to represent deoxyribose, rectangles to represent nitrogenous bases, and circles to represent phosphate groups. Once students have constructed several nucleotides, ask them to assemble a short sequence of DNA. After each group has constructed their model, the instructor will distribute the student worksheets, which are intended to assess whether they have correctly assembled an accurate model of a short sequence of DNA. Once the students have constructed their model of DNA, you can introduce the concept of mutations by adding, inserting, or deleting nucleotides. Ask the students to describe, using a visual representation, the effect of change on the original nucleotide sequence.

After students have had an opportunity to construct their molecule of DNA and answer the questions in the student worksheet, the teacher interacts with the student groups by asking predetermined questions in an *interpretive framework* and assessing the students' understanding of the concepts. An example of an illustrative interpretive framework for this formative assessment follows the student worksheet.

In-Class Formative Assessment

Student Questions (to be answered *after* student groups think have they accurately constructed a model of DNA):

1. The work of Watson, Crick, and others provided evidence that DNA is a polymer of nucleotides. What is a polymer? What is the advantage or significance of polymers as opposed to monomers?
2. Can you identify the monomers that make a polymer of DNA? What's unique about them? How do they differ in structure from the monomers that *comprise* proteins?
3. Two sugars comprise nucleic acids (DNA and RNA). Can you identify *one* structural difference between deoxyribose and ribose? Between deoxyribose and glucose? Can you identify the *fifth* and the *third* carbons of deoxyribose?
4. Beginning with a molecule of deoxyribose, to which carbon did you add a molecule of phosphate? To which carbon did you add a nitrogen base? What type of bond connects the sugar, phosphate and nitrogen base?
5. How many nitrogen bases are there? Can you distinguish between the structures of purines versus pyrimidines? Which two bases are purines? Which two are

pyrimidines? How did you model the different types of bases? (For example, if you cut out rectangles to represent nitrogen bases, did you use two different sizes, one size for adenine and thymine, and another for cytosine and guanine? Why *should* you have done this?)

6. What would happen if different-sized nitrogenous bases bonded together? How could this change the dimensions of the double helix? Would this change the orientation of the two strands?
7. What is the historical scientific evidence that supports that DNA is a double-stranded molecule? Please describe. How can two strands of linked nucleotides form a *double*-stranded molecule, i.e., a "double helix"?
8. What types of bonds connect the nitrogen bases across the middle of the double helix?
9. What are *two* reasons why adenine always pairs with thymine and guanine with cytosine? What scientific evidence supports this pairing of nitrogenous bases?
10. Do your two strands of DNA run in a *parallel* direction or an *antiparallel* direction? Are they oriented in opposite directions? Can you identify the 5′ and 3′ ends of each strand? What does this mean? Does one strand run in a 3′ → 5′ direction and the other in a 5′ → 3′ direction? Why does directionality of the strands even matter?
11. A molecule of DNA must be stable enough to accurately carry genetic information and transmit this information to a new generation of cells, but also be able to change. How does the *structure* of DNA support this phenomenon? In other words, what makes DNA complex and stable enough to carry genetic information but also able to change and transmit information during the processes of cell division? (Remember, changes in DNA provide the "raw material" for evolution.) You may be creative in your answer. For example, can you explain to Captain Zork visiting from the planet Zenon why DNA is *THE* molecule of heredity for earthlings?
12. Challenge Question: What does it mean that DNA has "built-in redundancy"? Why is this an important phenomenon with respect to DNA's ability to carry genetic information?

"From Gene to Protein" Student Worksheet

You (the teacher) are called over to a group of students who claim that they have accurately assembled a strand of DNA. You immediately notice that they have used two types of five-carbon sugars in their model, ribose and deoxyribose.

Q: I notice that you have used both ribose and deoxyribose as the sugars in your molecule. What do they have in common as molecules?

A: Both are five-carbon sugars.

Q: You are correct. However, what is one key difference between them? What does the prefix "de" imply in deoxyribonucleic acid?

A: To take away something. Like detoxify.

A: (hesitantly) That deoxyribose has one less oxygen than ribose?

Q: You're both right. So, if DNA is an acronym for deoxyribonucleic acid, which sugar is a component of a DNA nucleotide?

A: Um, like, deoxyribose.

Q: Great. The rest of your molecule looks good. Please replace your ribose sugars with deoxyribose.

You approach another group, who seems confident that their model accurately meets the requirements of a molecule of DNA. However, you notice that their double helix has "rungs" of different dimensions.

Q: I notice that your molecule has a really strange shape. Some of the "rungs" are longer than others. No fireman would be able to climb up that ladder! Do you remember a key piece of scientific evidence supporting that each rung had the same dimensions?

A: (Students look puzzled.)

Q: Hint: The scientist was a woman who was awarded a Nobel Prize posthumously.

A: Rosalind Franklin! She took an X-ray diffraction photograph of DNA. (Note: This student will ultimately receive a "5" on the AP Biology Exam!)

Q: And?

A: The rungs were all the same size, just like a ladder.

Q: What parts of nucleotides make up the rungs?

A: Nitrogen bases—adenine, thymine, guanine, and cytosine.

Q: Do all nitrogen bases have the same molecular structure?

A: No. Adenine and guanine have two rings, and thymine and cytosine have one ring.

A: And that guy Chargaff discovered that the amount of adenine equals the amount of thymine, and the amount of cytosine is the same as guanine.

Q: So what does scientific evidence tell you about the structure of DNA?

A: That the rungs must be the same size. That A pairs with T and C with G.

Q: Great. Here's a bonus question: Which two nitrogen bases are purines, and which two are pyrimidines?

A: Uh …

A: (gulp)

Q: I'd be sure to look that up before tomorrow's quiz.

While you were assessing the work of the previous group, a student has been frantically waving you over to her side of the room. The constructed double helix of her group looks accurate, the uprights are composed of sugar–phosphate linkages, and the nitrogen bases are correctly paired across the middle. The students have even sketched in two hydrogen bonds between each adenine–thymine pair and three hydrogen bonds between each guanine–cytosine pair. However, each strand has an additional phosphate attached to their 3′ ends, and the two strands run in a parallel direction, not antiparallel.

Q: This looks good except …

A: Huh? (looks crestfallen)

Q: What are the monomers of DNA?

A: Nucleotides.

Q: What are the three parts of a nucleotide?

A: Deoxyribose, phosphate, and nitrogen base.

Q: Great. To which carbon of deoxyribose do you attach a phosphate group?

A: Uh …

Q: Remember the hint I gave you? Think phonetics.

A: Fosfate and … five! They sound the same! The phosphate group is attached to the 5′ carbon of the sugar.

A: And the phosphate of one nucleotide is attached to the sugar of the next nucleotide in the sequence. (Student quickly removes the extra phosphate groups.)

Q: Bingo! I asked you to do a little detective work about how scientists worked out the structure of DNA. Do you remember what Watson and Crick discovered about the directionality of the two strands?

A: One runs up and one runs down?

A: That's lame. The term is "antiparallel." One runs from 3′ to 5′, and the other runs from 5′ to 3′. Like a divided highway.

Q: Did anyone read ahead in the text about DNA replication? The directions 3′ to 5′ and 5′ to 3′ will become significant.

A: (eagerly raising hand) During replication, each template strand is "read" in the 3′ to 5′ direction because of DNA polymerase, and the new strand is assembled 5′ to 3′. When the helix is unzipped by helicase, the activities on the leading strand differ from the activities on the lagging strand. A scientist named Okazaki…

Q: Great. I'm glad you read ahead. The bell is about to ring. Don't disassemble your molecule. We'll use it to study DNA replication tomorrow.

Interpretive Framework

An interpretive framework is a reflection on these questions:

- What are the challenges that students are likely to encounter while trying to answer these questions?
- What might you see or hear that alerts you to these problems?
- How could you respond to help students surmount these barriers?

Each learning environment is different. Each teacher brings his or her own expertise and selection of learning experiences to reach the goal. Each classroom is different. All of these factors require that, for an effective use of a formative assessment, the teacher must personally reflect on the answers to these questions.

Reflection

The first challenge is helping students understand the Watson and Crick model of DNA. Students often think that a molecule of DNA consists of chains of nitrogen bases linked together, rather than nucleotides consisting of a nitrogen base, deoxyribose, and a phosphate group. Students also incorrectly pair the nitrogen bases and sometimes attempt to pair a purine with a purine, or a pyrmidine with a pyrmidine. If this is the case, the teacher can review the evidence (i.e., Franklin's X-ray diffraction photograph and Chargaff's work) Watson and Crick used to determine the dimensions of the DNA molecule and the consequent nitrogen base pairing. The teacher can use the analogy comparing the DNA double helix with a typical ladder students can find in the garage at their houses: The rungs are the same size.

Perhaps the most difficult concept about the structure of DNA for students to understand is the antiparallel directionality of the two strands. This phenomenon was difficult for Watson and Crick to understand as well. Students likely create parallel strands, with each beginning and ending with phosphate groups. This is why it is essential that students construct their

DNA molecules by building individual nucleotides first and then assembling the nucleotides together. By doing so, they are able to visualize the 5′ and 3′ ends of the molecule. Another tactic is to review the structures of sugars; students can number the carbons to identify the 3′ and 5′ carbons. Understanding DNA's directionality is essential if students are to understand the different activities on the leading and lagging strands.

Appendix B

For Further Study—pGLO Bacterial Transformation Lab

Background Information

Genetic transformation has implications in agriculture, environmental science, and medicine. In this experiment, students use a procedure to transform bacteria with a gene that codes for green fluorescent protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. The green fluorescent protein causes jellyfish to glow in the dark. If gene transfer and transformation are successful, the bacteria will express their newly acquired jellyfish gene and produce the fluorescent protein, causing them to glow a brilliant green color under UV light.

Students learn about the process of moving genes from one organism to another with the aid of plasmids. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more traits that may be beneficial to bacterial survival. In nature, bacteria can transfer plasmids back and forth, allowing them to share these beneficial genes. This natural mechanism allows bacteria to adapt to new environments. The recent occurrence of bacterial resistance to antibiotics is due to the transmission of plasmids.

Bio-Rad's pGLO plasmid encodes both the gene for GFP and a gene for resistance to the antibiotic ampicillin. pGLO also incorporates a special gene regulation system in which the gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the nutrient medium. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on plates with an antibiotic. Transformed cells will appear white (wild-type phenotype) on plates without arabinose and fluorescent green when arabinose is included in the nutrient agar medium.

The students will be provided with the tools and a protocol for performing genetic transformation and determining the degree of transformation efficiency in an organism in order to further explore the one gene–one polypeptide hypothesis and the concept of phenotype expression in an organism.

Materials

Refer to Instructor's Guide, *Biotechnology Explorer: pGLO Bacterial Transformation Kit* (see http://www.bio-rad.com/LifeScience/pdf/Bulletin_9563.pdf), for materials and experimental protocol. This kit is available for purchase from Bio-Rad Laboratories at www.bio-rad.com (see the Bio-Rad "life science education" catalog). Alternative bacterial transformation labs can be purchased from other vendors, including Carolina Biological. You should also consider tapping into resources at local colleges or biotechnology companies that often donate supplies to high schools.

Instruction Time: Three parts—one 30-minute discussion of prelab considerations, and two 50-minute laboratory periods on separate days.

Part A: Prelab Considerations

The teacher should discuss the following considerations with students on the day prior to experimentation. There are many considerations that need to be thought through in the process of planning a scientific laboratory investigation. Since scientific laboratory investigations are designed to get information about a question, the first step is to formulate a question for this investigation.

Consideration 1: Can we genetically transform an organism? Which organism?

1. To genetically transform an entire organism, you must insert the new gene into every cell in the organism. Which organism is better suited for total genetic transformation—one composed of many cells, or one composed of a single cell?
2. Scientists often want to know if the genetically transformed organism can pass its new traits on to its offspring and future generations. To get this information, which would be a better candidate for your investigation, an organism in which each new generation develops and reproduces quickly, or one that does this more slowly?
3. Safety is another important consideration in choosing an experimental organism. What traits or characteristics should the organism have (or not have) to be sure it will not harm you or the environment?
4. Based on the above considerations, which would be the best choice for a genetic transformation: a bacterium, earthworm, fish, or mouse? Describe your reasoning.

Consideration 2: How can we tell if cells have been genetically transformed?

Students should recall that the goal of genetic transformation is to change an organism's traits (phenotype). Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pretransformation) phenotype must be made. Ask the students to look at the colonies of *E. coli* on their starter plates and list observable traits or characteristics.

The following pretransformation observations of *E. coli* might provide baseline data to refer to when attempting to determine if any genetic transformation has occurred.

a. Number of colonies

b. Size of:

   1. the largest colony

   2. the smallest colony

   3. the majority of colonies

c. Color of the colonies

d. Distribution of the colonies on the plate

e. Visible appearance when viewed with ultraviolet (UV) light

f. The ability of the cells to live and reproduce in the presence of an antibiotic such as ampicillin

1. Describe how you could use two LB/agar plates, some *E. coli*, and some ampicillin to determine how *E. coli* cells are affected by ampicillin.
2. What would you expect your experimental results to indicate about the effect of ampicillin on the *E. coli* cells?

Consideration 3: The Genes

Genetic transformation involves the insertion of new DNA into the *E. coli* cells. In addition to one large chromosome, bacteria often contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for more than one trait. Scientists can use a process called genetic engineering to insert genes coding for new traits into a plasmid. In this case, the pGLO plasmid carries the GFP gene that codes for the green fluorescent protein and a gene *(bla)* that codes for a protein that gives the bacteria resistance to an antibiotic. The genetically engineered plasmid can then be used to genetically transform bacteria to give them this new trait.

Consideration 4: The Act of Transformation

This transformation procedure involves three main steps. These steps are intended to introduce the plasmid DNA into the *E. coli* cells and provide an environment for the cells to express their newly acquired genes. To move the pGLO plasmid DNA through the cell membrane, you will:

1. Use a transformation solution of CaCl.
2. Carry out a procedure referred to as heat shock.
3. For transformed cells to grow in the presence of ampicillin, you must provide them with nutrients and a short incubation period to begin expressing their newly acquired genes.

Part B: Transformation

Students follow the experimental protocol provided by the teacher.

Review Questions

Have the students answer the following questions before collecting data and analyzing their results.

1. On which of the plates would you expect to find bacteria most like the original nontransformed *E. coli* colonies you initially observed? Explain your predictions.
2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain your predictions.
3. Which plates should be compared to determine if any genetic transformation has occurred? Why?
4. What is meant by a control plate? What purpose does a control serve?

Part C: Data Collection and Analysis

Data Collection

1. Observe the results obtained from the transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates.
2. Carefully observe and draw what you see on each of the four plates. Put your drawings in a data table. Record your data to allow you to compare observations of the "+ pGLO" cells with your observations for the nontransformed *E. coli*.
3. Write down the following observations for each plate.
    a. How much bacterial growth do you see on each plate, relatively speaking?

b. What color are the bacteria?

c. How many bacterial colonies are on each plate (count the spots you see)?

Analysis of Results

The goal of data analysis for this investigation is to determine if genetic transformation has occurred.

1. Which of the traits that you originally observed for *E. coli* did not seem to become altered? List these untransformed traits and how you arrived at this analysis for each trait listed.
2. Original trait—analysis of observations: Of the *E. coli* traits you originally noted, which seem now to be significantly different after performing the transformation procedure? List those traits and describe the changes that you observed.
3. New trait—observed change: If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be inferred about the other genes on the plasmid that you used in your transformation procedure?
4. From the results that you obtained, how could you prove that the changes that occurred were due to the procedure that you performed?

Ask the students about the fluorescent green color that is observed in the *E. coli* colonies, reflecting on the source of the green color. What are the two possible sources of fluorescence within the colonies when exposed to UV light? Ask the students what they observed when they shined the UV light onto a sample of original pGLO plasmid DNA. Which of the two possible sources of the fluorescence can be eliminated? What does this observation indicate about the source of the fluorescence?

Tell the students to look at their four plates again in order to discuss the interactions between genes and the environment. Do they observe some *E. coli* growing on the LB plate that does not contain ampicillin or arabinose? Is it possible to determine whether these bacteria are ampicillin resistant by looking at them on the LB plate? Very often an organism's traits are caused by a combination of its genes and its environment. Ask the students to think about the green color they saw in the genetically transformed bacteria: What two factors must be present in the bacteria's environment in order to see the green color? What do you think each of these two environmental factors is doing to cause the genetically transformed bacteria to turn green? What advantage would there be for an organism to be able to turn on or turn off particular genes in response to certain conditions? Can you think of other examples?

Reflection

If transformation is successful, the bacteria will incorporate the pGlo plasmid and express a new, observable phenotype: The bacteria will glow in the dark. Additionally, some *E. coli* colonies will demonstrate resistance to antibiotics. The experiment demonstrates the universality of DNA and its expression.

Summary

The experiment demonstrates the universality of DNA and its expression.

Appendix C

Operon: A Formative Assessment for AP Biology

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It is expected that students who complete AP Biology successfully can describe the operon model of the mechanisms through which controls are placed by the environment on protein expression. It is expected that they can create, describe, and use models that explain the behavior of biological systems. A goal of this course is to help students practice the critically important skill of posing scientific questions that might extend or refine a model.

Instruction in the *lac* and *trp* operons should be treated separately so that students do not get them confused. However, both are included in each step of this formative assessment because this tool is intended for use after the students have had an opportunity to work toward an understanding of these models of the control of expression.

The level of detail for these processes that is appropriate for AP Biology can be summarized:

> *lac operon:* Repressor is usually active and the structural genes blocked from transcription; corepressor (allolactose) inactivates it, allowing the structural genes to be transcribed into galactosidase.
>
> *trp operon:* Repressor is usually inactive so that the structural genes are transcribed and tryptophan is produced. Tryptophan itself binds with the repressor when it is present, stopping production of tryptophan synthetase.

This assessment assumes that the students have had the opportunity to make a model of the mechanism of gene expression. Simple models can be made from construction paper, or the students can be creative and make more elaborate models. However, each model should contain a regulator, promoter, operator, and structural genes.

In this assessment, the students are asked probing questions in order to reveal their understanding of the operon model by expressing the role of each of these parts of the model. First, they are asked what happens if the promoter and operator are reversed. This should reveal whether or not they understand the role of the operator as the site at which transcription begins. Then they are asked to consider evidence that would imply mutation of the regulator gene that either increases, decreases, or deactivates repression. They are then asked to consider evidence that would imply mutation in the operator. Finally, they

are provided with a scenario in which expression does not occur and asked to apply their model to pose a question that might be tested to explain this observation.

In-class Assignment

Using your understanding of the operon model of the regulation of protein expression and the physical model you have constructed of the gene sequence that controls expression, work with your group to develop answers to these questions. Record your answers in your lab notebook with drawings of your model and annotations that illustrate your reasoning.

Lac operon

1. What happens if the promoter and operator in the *lac* operon are reversed? That is, does the rate of galactosidase increase, decrease, or remain unchanged, and why?
2. What happens if there is a silent mutation of the regulator gene in the *lac* operon?
3. What happens if there is a missense mutation of the regulator gene in the *lac* operon?
4. What happens if there is a nonsense mutation of the regulator gene in the *lac* operon?
5. Could a mutation of the regulator gene be present without producing a change in the rate at which protein is expressed?
6. What happens if there is a mutation in the operator gene in the *lac* operon?
7. If lactose is not broken down when it is present, how could one determine if the problem is one of regulation?

Trp operon

8. What happens if the promoter and operator *trp* operon are reversed? That is, does the rate of tyrptophan synthetase expression increase, decrease, or remain unchanged, and why?
9. What happens if there is silent mutation of the regulator gene in the *trp* operon?
10. What happens if there is missense mutation of the regulator gene in the *trp* operon?
11. What happens if there is nonsense mutation of the regulator gene in the *trp* operon?
12. What happens if there is a mutation in the operator gene in the *trp* operon?

Interpretive Framework

An interpretive framework is a reflection on these questions:

- What are the challenges that the students are likely to encounter while trying to answer these questions?
- What might you see or hear that alerts you to these problems?
- How could you respond to help the students surmount these barriers?

Each learning environment is different. Each teacher brings his or her own expertise and selection of learning experiences to reach the goal. Each classroom is different. For an effective use of a formative assessment, all of these factors require the teacher to personally reflect on the answers to these questions.

Reflection

The first challenge is to understand that the operator region of the operon controls transcription, and that the promoter region controls translation of the structural genes. Students should be able to clearly explain this difference.

The second challenge is to understand that the difference between the two operons is small but significant. Both operons use a regulatory protein, which is encoded in the DNA separately from the rest of the operon. In one case, the regulatory protein (repressor) is active until it is deactivated; in the other case, the regulatory (repressor) protein is inactive until it is activated. In other words, students should be able to demonstrate with their model that the enzymes to break down lactose are normally *not produced* unless lactose is present, and that the enzymes for tryptophan synthesis are normally *produced* unless tryptophan is present. Students should be able to distinguish between the two without confusion. It helps if students can see that one pathway is for the *breakdown* of lactose, and the other is for the *synthesis* of tryptophan.

Question 5 challenges students to think about the role of the structural genes as well as the regulator genes. Students may come up with various hypotheses, but they should be able to demonstrate their hypotheses with their model.

A primary goal of a formative assessment is to provide a platform for teachers to reflect on how their practices relate to student learning progressions. The anticipation of student responses to predetermined questions is a natural component of planning for instruction using embedded classroom assessments. Upon completion of the assessment, both the teacher and the students can reflect upon its effectiveness as a learning tool.

For the Teacher

1. Do you understand the principles behind a formative assessment?
2. Do you think the formative assessment model serves as an effective, inquiry-based teaching tool?
3. Based on the examples, do you think you could/would write your own formative assessment like this and use it to teach a particular concept or concepts?
4. Do you think anticipating, diagnosing, and responding to student thinking through formative assessments are effective teaching/learning tools? Why or why not?
5. If you incorporated a formative assessment like this in your course, did any student misperceptions surprise you? Did you believe that your students would be able to answer your questions and then discover that some could not?
6. Do you think a formative assessment like this will provide greater opportunity (1) for your students to work more collaboratively, and (2) for you to interact and engage with your students on a more personal level?
7. Do you think a formative assessment like this will adequately prepare your students for more formal assessments, i.e., classroom tests and the AP Exam in this discipline?

For the Student

1. Do you feel that you learn more by doing or by listening? Please explain.
2. Do you think a formative assessment provides an opportunity for a better interaction with your teacher while trying to learn a particular concept? Why or why not?
3. Do you enjoy opportunities to work collaboratively with your classmates? Does a formative assessment provide opportunities for more collaborative work?
4. Did the formative assessment capture your attention and interest? Did you feel more engaged in the subject? Did the scenario make the material more relevant to your life? Please explain.
5. Would you be able to write a formative assessment for your peers?

About the Contributors

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Sharon Radford currently teaches at Paideia School in Atlanta, Georgia. She has taught Advanced Placement Biology for over 20 years, and has served as a reader and table leader at the AP Exam reading. She served on the board of directors of the National Association of Biology Teachers as director at large from 2005–2008. In 1992 she was a summer fellow in the Woodrow Wilson program in Bioethics and she was an Access Excellence Fellow in 1995. She participated in the Human Genome Project workshop: Genetics Education for Middle and Secondary Science Teachers; Ethical, Legal, Social and Technological Implications in 1994 and 1995. She was recognized as an outstanding teacher by NABT in 1992 and by the Siemans Corporation in 2005 and 2006.

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