4.1 DNA: THE HEREDITARY MATERIAL

Section 4.1 Questions

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Understanding Concepts

1.

Scientist	Experiment	Contribution
Joachim Hammerling	Experimented using green alga *Acetabularia*; observed that regeneration of new appendages was driven by the nucleus-containing "foot" of the alga.	The nucleus was known to contain DNA, therefore, the experiment put forth the possibility that DNA is the hereditary material.
Frederick Griffith	Experimented using mice and two different strains of pneumococcus bacteria (virulent and nonvirulent); observed that when heat-treated virulent pneumococcus was mixed with nonvirulent pneumococcus and then injected into healthy mice, death resulted.	Griffith discovered the process of transformation. A chemical component found in the dead pathogenic cells was able to cause the death of healthy mice when mixed with nonvirulent cells. Griffith was not aware what the chemical component was, but his experiment set the stage for further experimentation that investigated whether DNA or proteins were responsible for transformation.
Oswald Avery, Maclyn McCarty, and Colin MacLeod	Building on the experimental work of Griffith, they ruptured heat-killed encapsulated cells to release their contents. RNA, DNA, protein, and purified polysaccharide coats were isolated and tested for transforming ability.	Nonvirulent cells mixed with purified DNA from dead virulent cells produced colonies of virulent cells. This result was not observed when the experiment was performed with RNA or protein, hence, DNA was the "transforming principle" of pneumococcus bacteria.
Alfred Hershey and Martha Chase	Used radioactively labelled viruses to infect bacterial cells. Observed that the infected bacterial cells contained radioactive phosphorus, not radioactive sulfur.	Viruses (bacteriophages) infect bacterial cells by injecting their hereditary material into the cell. DNA is rich in phosphorus, while proteins are rich in sulfur. The infected bacterial cells contained radioactive phosphorus, indicating that DNA from the virus had been injected. DNA was the hereditary material.

2. Hammerling's experiment cannot be used as conclusive scientific evidence that DNA is the hereditary material, because the nucleus contains both protein and DNA. Hammerling hypothesized that the nucleus was responsible for regeneration of the alga, but he could not conclusively show which chemical component of the nucleus was responsible.

Applying Inquiry Skills

3. (a) If infected cells in Hershey and Chase's experiments contained radioactive sulfur in their cell walls and no radioactive phosphorous, it would indicate that the hereditary information was protein. Proteins are high in sulfur, while DNA is rich in phosphorous.
   (b) If infected cells in Hershey and Chase's experiments contained neither radioactive phosphorous nor sulfur, this would indicate that neither protein nor DNA was the hereditary material.
   (c) If Hammerling observed that after grafting an *A. crenulata* stalk to an *A. mediterranea* foot the cap that grew was that of the *A. crenulata* species, he might have concluded that the hereditary information was found in the stalk of the alga. Since the nucleus is found in the foot of the alga, it may have led Hammerling to further hypothesize that the nucleus did not contain the hereditary information.

(d) If Hammerling observed that after grafting an *A. crenulata* stalk to an *A. mediterranea* foot no cap was regenerated, he might have concluded that the hereditary information was found in the cap of the alga. He may have further hypothesized that the nucleus did not contain the hereditary information since it is found in the foot of the alga.

Making Connections

4. *Acetabularia* was a model organism for Hammerling's experiment for the following reasons: it is a large unicellular organism (5 cm), making it easy to work with; it has distinctive features such as the stalk, foot, and cap region, hence, Hammerling could easily observe change as a result of experimental manipulation; it replicates quickly, therefore, the waiting period is short to obtain results; and it is a unicellular alga, therefore, ethical issues surrounding its use are not present.

   Some characteristics that make an organism attractive for experimental research include rate of replication, cost, availability, simplicity, and lack of ethical issues surrounding its use. Humans do not make ideal research subjects for many reasons. First, the human body is very complex and it is difficult to isolate one variable. Second, not many people would be willing to subject themselves to research unless they had a personal stake in the outcome. Safety would be a concern—experimental procedures and protocols need to have been tried on more primitive organisms first. Finally, the cost of experimentation on humans is also a concern.
5. It is important to study the historic experiments that revealed genetic principles as well as the principles themselves for many reasons. By studying the experiments, we gain an understanding of the nature of scientific research and experimentation, we appreciate the evolutionary nature of scientific knowledge that we currently have, and we give the scientific principles more value when we understand how they have come to be. For example, we now have a clear understanding of the structure of DNA as it was elucidated by Watson and Crick; yet we can appreciate the knowledge that was gathered by Franklin, Wilkins, and Chargraff that was used by Watson and Crick in their model of DNA. Chargraff and Franklin's experimental data lend credence to Watson and Crick's hypothesis. Another example that illustrates the nature of scientific research and experimentation is Avery, McCarty, and MacLeod's experimentation. These scientists built on knowledge gained by Griffith in his experiments. Scientific knowledge builds on itself. Finally, the evolutionary nature of science is recognized when we study the experiments performed by Hammerling up to Watson and Crick's model of DNA. Science is tentative in nature.
6. Experiments need to be repeated to
   - Establish validity of experimental results.
   - Eliminate anomalies in the data.

   Experiments need not be repeated since
   - Resources such as money, personnel, and time are valuable and limited.
   - The pursuit of new knowledge is sacrificed.

   Avery, McCarty, and MacLeod did repeat some of Griffith's work but then took the experiment one step further. By repeating the work, they were able to observe what Griffith had done firsthand. This led them to further hypothesize and design new experiments that illustrated that DNA was responsible for the transforming principle. Hence, they took what Griffith had done and built on that knowledge base with further experimentation.

4.2 DNA STRUCTURE

Explore an Issue Debate: Competition Drives Science

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Statement: Competition is the key driving force of science, followed by collaboration.
Student answers will vary. Some points and counterpoints that could be used in a position paper to support or not support the statement are as follows.

Point	Counterpoint
Competition due to patriotism has driven and drives science. Fritz Haber proclaimed during WWI that he was first a patriot to Germany and a scientist to the world second. Using this form of reasoning, he felt no guilt for his role in the development of deadly gases used to attack Germany's enemies.	Phillip Morrison, a lead scientist in the Manhattan Project, has stated that the scientists who built the atomic bomb were more interested in making the project work and not necessarily thinking of its use. Many scientists worked on the project and if it were not for the international assortment of world-class scientists working together on the project, it would have not been completed in two years. In addition, he does not feel that the scientists should bear responsibility for Hiroshima. It was the government who chose to use the scientific tool in the manner that it did. The scientists were interested in the science of the fission reaction. They were not acting patriotically but rather as a scientific body driven by curiosity.
Privately funded endeavours progress at a faster pace because of the underlying principle of competition in the free market. Craig Venter founded Celera Genomics in attempt to be first to decipher the human genome. The publicly funded project headed by Eric Lander was not progressing at a fast enough rate.	Celera Genomics did use some of the sequences that had already been deciphered by Eric Lander's team. Instead of repeating the work, they chose to incorporate the sequences published on the Internet into their project. Hence, collaboration allowed Craig Venter to have a competitive edge.
Scientists who work in academia strive to attain tenure. A key criterion that universities use to make the decision about whether tenure should be granted is the number of articles published by the scientist. Scientific fraud has been uncovered in many cases, illustrating that some scientists will falsify data or place their names on papers that they were not necessarily a part of.	Cases of scientific fraud are rare. It is difficult to quantify the percentage of data that is published that may not be scientifically sound. In addition, scientific journals possess a sophisticated review process by which they review the articles that they are considering publishing.

Try This Activity: The Directionality of DNA Strands: A Whole-Class Simulation

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Students will have a hard time seeing the hydrogen and phosphodiester bonds if they are crunched together. Use as much space as possible and have students spread out. Outstretched hands will better represent the bonding.

Section 4.2 Questions

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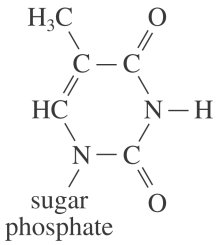
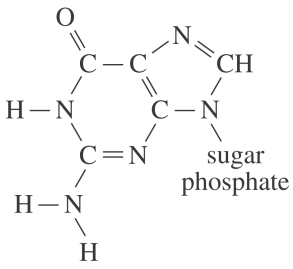
Understanding Concepts

1. nucleotide: a molecule that consists of a five-carbon sugar (deoxyribose or ribose) with a nitrogenous base attached to their 1' carbon and a phosphate group attached to their 5' carbon
   complementary base pairing: pairing of the nitrogenous base of one strand of DNA with the nitrogenous base of another strand; adenine (A) pairs with thymine (T); guanine (G) pairs with cytosine (C)
   phosphodiester bond: a bond formed between the 3' carbon of one sugar and the 5' carbon of the next sugar by way of an intervening phosphate group
   glycosyl bond: a bond between a sugar and another organic molecule by way of an intervening nitrogen or oxygen atom

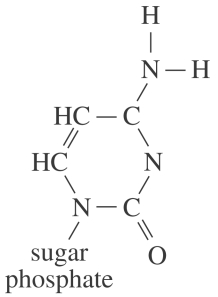
2. Despite the fact that a purine is opposite to a pyrimidine, this base pairing cannot occur because the pairing does not allow for hydrogen bonding.



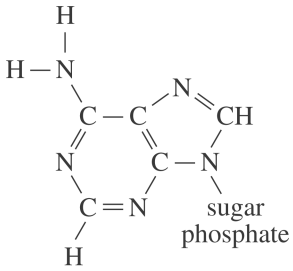
thymine



guanine



cytosine



adenine

3. 3'–TACGGAAT–5'
4. DNA is a nucleic acid polymer that consists of monomer units called nucleotides. Each nucleotide comprises a deoxyribose sugar, a nitrogenous base, and a phosphate group. The phosphate group is negatively charged. The four nitrogenous bases are adenine, thymine, guanine, and cytosine. A DNA molecule is double stranded and takes the shape of a helix, which is approximately 2 nm wide. A complete helical turn occurs every 3.4 nm. The two strands run antiparallel to each other. The deoxyribose sugar and the phosphate group form the backbone of each strand. The nitrogenous bases pair via hydrogen bonding. Adenine only pairs with thymine on the opposite strand and vice versa, while guanine only pairs with cytosine on the opposite strand and vice versa.
5. A purine is a nitrogenous base that is double ringed, while a pyrimidine is a nitrogenous base that contains a single ring. Adenine and guanine are purines. Thymine and cytosine are pyrimidines.

Applying Inquiry Skills

6. thymine = 20%, adenine = 20%, guanine = 30%, cytosine = 30%
Since adenine is complementary to thymine, the percentage of thymine must equal the percentage of adenine, which is 20%. Therefore, adenine and thymine comprise 40% (20% adenine + 20% thymine) of the molecule. The remaining 60% [100% (total molecule) – 40% (adenine + thymine)] of the molecule comprises guanine and cytosine. Since guanine and cytosine are complementary, there must be an equal amount of each at 30% (60/2 = 30%).
7. sample A = double stranded (percentage of A = T and percentage of C = G)
sample B = single stranded (percentage of G is not equal to C)
sample C = single stranded (percentage of G is not equal to C, and percentage of A is not equal to T)
8. $$\frac{3\times10^{9}\ \text{nucleotides}}{10\ \text{nucleotides per turn}}$$
$= 3 \times 10^{8}$ turns

3×10^{8} turns $\times$ 3.4 nm/turn
$= 1.02 \times 10^{9}$ nm

$$(1.02 \times 10^{9}\ \text{nm}) \times \frac{10^{-9}\ \text{m}}{1\ \text{m}} = 1.02\ \text{m}$$

A human cells DNA would be 1.02 m long. It would contain 3×10^{8} turns.

9. $75 \text{ mm} \times \frac{10^6 \text{ nm}}{1 \text{ mm}}$
$= 7.5 \times 10^7 \text{ nm}$

$(7.5 \times 10^7 \text{ nm}) \times \frac{1 \text{ turn}}{3.4 \text{ nm}}$
$= 2.2 \times 10^7 \text{ turns}$
$(2.2 \times 10^7 \text{ turns}) \times \frac{10 \text{ nucleotides}}{1 \text{ turn}}$
$= 2.2 \times 10^8 \text{ nucleotides}$
This molecule would contain 2.2×10^8 nucleotides.

Making Connections

10. Erwin Chargaff's experiments demonstrated that the proportion of thymine was equal to the proportion of adenine and that the proportion of guanine was equal to the proportion of cytosine. In addition, Chargaff found that the proportion of adenine plus guanine was equal to that of thymine plus cytosine. If Watson and Crick's hypothesis was correct, then it violated Chargaff's data. The hypothesis did not explain why the proportion of T is equal to A or why the proportion of G is equal to C. The hypothesis implied that the occurrence of each nucleotide is independent of another nucleotide; hence, the proportions of each nucleotide would not be related or dependent on the proportions of another nucleotide. Watson and Crick's hypothesis also violated Rosalind Franklin's findings. Rosalind Franklin discovered that the width of a DNA molecule is constant. If cytosine bonded to cytosine, thymine to thymine, and so on, then the width of the DNA molecule would vary because at some points you would have two purines bonded to each other and other points, two pyrimidines. Purines are larger than pyrimidines, therefore, the width of the DNA molecule would change.

4.3 DNA REPLICATION AND REPAIR

Section 4.3 Questions

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Understanding Concepts

1.

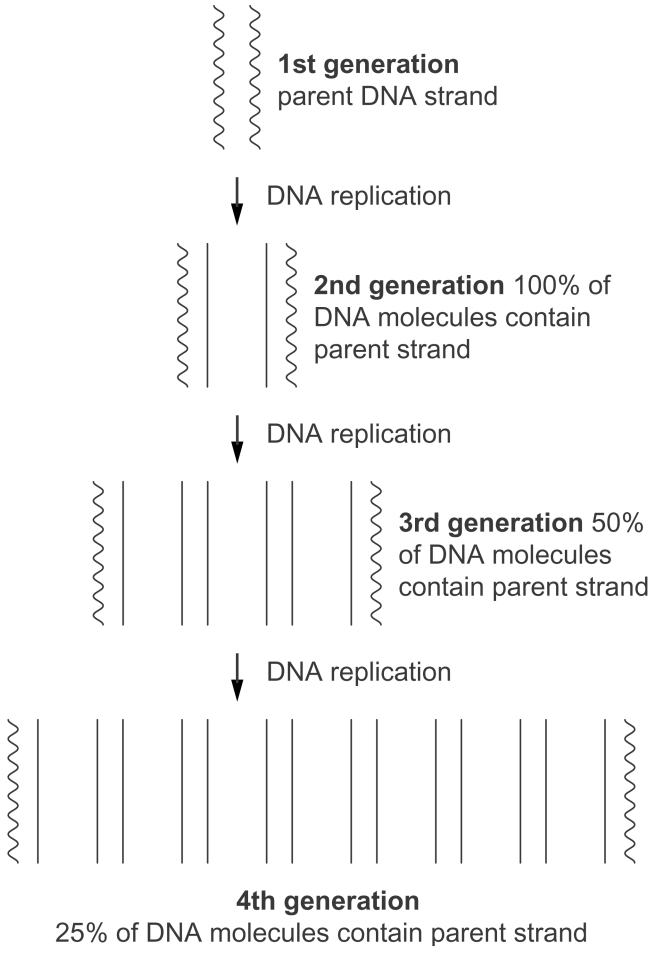
Enzyme	Function
DNA gyrase	The bacterial enzyme that relieves the tension produced by the unwinding of DNA during replication
DNA helicase	The enzyme that unwinds double-helical DNA by disrupting hydrogen bonds
DNA polymerase I	An enzyme that removes RNA primers and replaces them with the appropriate deoxyribonucleotides during DNA replication
DNA polymerase III	The enzyme responsible for synthesizing complementary strands of DNA during DNA replication
DNA ligase	The enzyme that joins DNA fragments together by catalyzing the formation of a bond between the 3′ hydroxyl group and a 5′ phosphate group on the sugar-phosphate backbones
primase	The enzyme that builds RNA primers

2.

Leading Strand	Lagging Strand
• Complementary strand is built toward replication fork • Built continuously • Primase needs to add only one RNA primer that DNA polymerase will use to build toward the replication fork	• Complementary strand is built away from replication fork • Built discontinuously in small sections known as Okazaki fragments • Primase continuously adds primers as replication fork travels along DNA molecule • Gaps between Okazaki fragments are joined by DNA ligase

The two mechanisms must exist since DNA strands run antiparallel to each other. One complementary strand to be built will be running in the 5' to 3' direction, toward the replication fork, while the other complementary strand to be built will be running in the 3' to 5' direction. Since DNA polymerase III can only add nucleotides to a 3' end of a DNA strand, the two complementary strands are built in opposing directions, resulting in different mechanisms.

3. Initially, Meselson and Stahl grew bacteria for 17 generations in ^{15}N. The DNA isolated from the bacteria at this point appeared as a heavy band in the density gradient. Both strands contained ^{15}N. The bacteria were then switched to a medium that contained ^{14}N, a lighter isotope of nitrogen. After one generation of replication, half the strands consisted of ^{15}N and the other half consisted of ^{14}N. These DNA molecules appeared as an intermediate band in the density gradient. The DNA was harvested from the bacteria after a second generation of replication. Since DNA replication is semiconservative, half the DNA molecules strictly comprised ^{14}N, composing the light band, while the other half was of intermediate nature. In total 25% of the DNA strands comprised of ^{15}N and 75% comprised ^{14}N.
4. A replication fork is the region where the enzymes replicating a DNA molecule are bound to the untwisted, single-stranded DNA, whereas a replication bubble is formed when two replication forks are in close proximity to each other, resulting in a bubble of single-stranded DNA between them.
5. It is necessary for eukaryotic DNA to have multiple replication origin sites due to its size. It would take too long for DNA replication to occur if replication started at one end of a DNA molecule and proceeded to the other end. Multiple replication origin sites allow for efficiency in replication.
6. Both parental strands of a DNA molecule can be used as a template because of the complementary nature of DNA. Since A is bonded to T, and G bonded to C only, it does not matter which strand is used since their newly built complements will be identical to the original complementary strand of the template strand used.
7. After four generations of replication, 25% of the DNA molecules would contain one of the original parent strands.

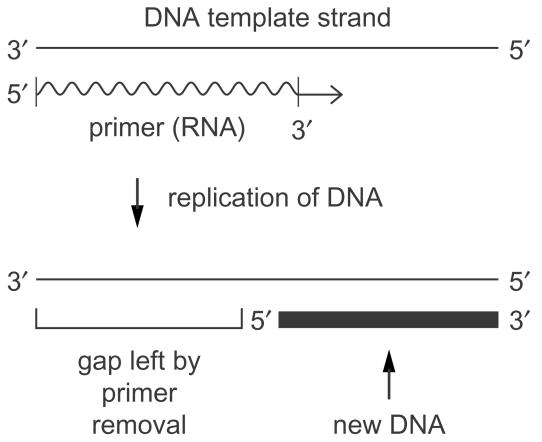


Applying Inquiry Skills

8. If DNA replication were conservative, then Meselson and Stahl would have observed only two bands in their density gradient. One band would have been heavy (^{15}N), representing the parent strands. The new daughter strands would have been light (^{14}N). Since the parent strands would have been conserved as parents, no DNA molecules of intermediary nature would have appeared.

Making Connections

9. Student answers will vary. Some key information related to telomeres and their role in aging follows.
Telomeres are the noncoding DNA regions found at the ends of eukaryotic chromosomes. They are approximately 8 to 14 kilobasepairs long and in human chromosomes comprise the repeating nucleotide sequence 5'–TTAGGG–3'. Telomeres play an important role during DNA replication by protecting the coding region of DNA from being degraded because of the mechanism by which DNA replicates. During DNA replication the leading strand is replicated continually. To replicate the lagging strand, DNA polymerization starts from several RNA primers that are elongated to create DNA fragments termed Okazaki fragments. The RNA primers are eventually degraded and replaced by DNA nucleotides. Removal of the terminal RNA primer on the lagging strand leaves a gap that ordinarily is filled in by extension of an Okazaki fragment. Since DNA polymerase III can only build in the 5' to 3' direction, the terminal RNA primer is removed, but DNA nucleotides cannot be inserted since DNA polymerase cannot build in the 3' to 5' direction. Therefore, after each replication of DNA, the length of a chromosome shortens by one primer length.



DNA polymerase cannot fill the gap left by the primer removal because of the lack of a 3′ end.

Normal human cells undergo a finite number of cell divisions. It has been proposed that telomere shortening acts as a molecular clock that triggers cell death. Once the telomeric length reaches a certain critical point, a signal is sent that stops the cell from further dividing, leading to cell death. Cell death is linked to aging. Studies have shown that people with progeria (accelerated aging syndrome) have a lower average telomeric length in comparison with normal individuals of the same age (Lindsay et al., 1991). In addition, repair of DNA damage in telomeric regions decreases with age (Kruk et al., 1995).

Telomerase is the enzyme that is responsible for building up telomeric DNA. Telomerase activity has been detected in some cancer cells and other immortal cell lines. Further study of telomeres and telomerase is required to clearly illustrate the role they play in aging and cancer.

10. Watson and Crick recognized that DNA replication must be highly accurate to preserve the DNA sequence responsible for the coding of all proteins. If cells divided and numerous errors were made in the replication of the DNA, then new cells would contain erroneous DNA that could possibly code for nonfunctioning proteins. Nonfunctioning proteins would be disadvantageous for the cell and the organism. They believed that complementary base pairing pointed to semiconservative replication. Since adenine complements thymine and guanine complements cytosine, an opposite strand can be built using one strand as a template. The opposing strand will be identical to the original opposing parent strand that is now being used as a template. The likelihood of an error occurring is strongly diminished since a template strand that is a parent strand is being used. The enzyme responsible for building the opposing strand has no choice but to add the appropriate complementary base pair. Errors are kept to a minimum, increasing the viability of the new cell containing the newly replicated DNA.