CHAPTER 14: DNA: THE GENETIC MATERIAL

CHAPTER SYNOPSIS

Scientific advances are a result of proper experimental design mixed with insight and a little luck. The events leading to the discovery of DNA as the material of heredity are especially good examples of how individual experiments build upon one another to answer a larger scientific question. Among the first experiments were those that indicated that the hereditary material was stored within the nucleus of every cell. Although this now seems intuitive, there are many structures within a cell that segregate during meiosis other than the chromosomes. The role of the nucleus was further clarified by observing embryonic development after physical manipulation of the nucleus. Several different kinds of experiments were performed to prove that the hereditary material was nucleic acid rather than protein. Among these were the Griffith and Avery experiments in which nonvirulent bacteria were made virulent by a nonprotein-transforming principle. The Hershey Chase experiments indicated that it was the DNA within viruses and not their protein exteriors that was the infecting material that killed bacteria.

Chemical analysis of nucleic acids illustrated their structure but did not hint as to how these units were assembled into a working blueprint. Chargaff determined that DNA was not a simple repeating polymer and that the proportions of the adenine and thymine nitrogenous bases were always equal as were the proportions of guanine and cytosine. X-ray diffraction of impure samples of DNA by Rosalind Franklin gave Watson and Crick sufficient information to construct their three-dimensional model of the DNA molecule. A key point of the model was the complementarity of the DNA strands, a result of the bonding of their bases, adenine to thymine and guanine to cytosine. The Meselson Stahl experiments began to explain DNA replication by determining that it was a semiconservative process; each strand served as a template for the production of a new one and each old and new strand then intertwined to become a new helix. Double-stranded DNA replication is complicated since new nucleotides must be added to both the 5′ to 3′ strand and the 3′ to 5′ strand at the same time, but DNA polymerase can only add onto the 3′ end. The 5′ to 3′ or leading strand is replicated simply by adding nucleotides as the old strands unzip. The 3′ to 5′ lagging strand is replicated in batches via discontinuous synthesis. Segments called Okazaki fragments are made in the usual way. These are then stitched, in order, to the lagging strand by DNA ligase. Since one strand is processed continuously and the other discontinuously, replication as a whole is semidiscontinuous.

The relationship between DNA and proteins was determined by Beadle and Tatum using nutrient deficient strains of mold. They found that each mutated gene was responsible for the production of a single enzyme in a biochemical pathway and postulated the one gene-one enzyme hypothesis. Later experiments showed that the proteins coded for by DNA were composed of amino acid units strung together; somehow the sequence of DNA was related to the protein sequence of amino acids.

CHAPTER OBJECTIVES

- Describe the experiments that first supported the hypothesis that a cell's hereditary material is located in the nucleus.
- Understand the theory and conclusions associated with the Griffith and Avery experiments using *Pneumococcus* and mice.
- Explain the evidence that supports the identity of DNA as hereditary material.
- Identify the three subunits of DNA and describe how they are put together to construct an intact molecule.

- Understand the importance of Chargaff's rules and the complementary nature of nucleotide bases.
- Describe Watson and Crick's three-dimensional model of DNA based upon Franklin's X-ray crystallography.
- Know what is meant by semiconservative replication of DNA and how it was determined.
- Explain the process of semidiscontinuous replication in both strands of the DNA double helix.
- Understand the one gene-one polypeptide hypothesis and the experimental evidence behind it.

KEY TERMS

antiparallel strand
bacteriophage
base pair
Chargaff's rules
complementarity
DNA ligase
DNA polymerase III
double helix
gene
gyrase
helicase
initiator protein
lagging strand
leading strand
model organism
nucleic acid
nucleotide
Okazaki fragment
one-gene/one-enzyme hypothesis
one-gene/one-polypeptide
phosphodiester bond
primase
purine
pyrimidine
replication origin
replication unit
replicon
RNA primer
semiconservative replication
semidiscontinuous replication
single-stranded binding protein
transformation

CHAPTER OUTLINE

14.0 Introduction

I. CONNECTION BETWEEN HEREDITARY TRAITS AND CHROMOSOMES

A. Chain of Experiments Led to Understanding Molecular Mechanisms of Heredity fig 14.1

B. Journey Often Erratic, Path Not Always Direct

14.1 What is the genetic material?

I. THE HAMMERLING EXPERIMENT: CELLS STORE HEREDITARY INFORMATION IN THE NUCLEUS

A. Hammerling's Experiment with *Acetabularia*
   1. Large, unicellular alga used as model organism
   2. Preliminary experiment
      a. Large green alga cell with distinct foot, stalk, and cap
      b. Cap lacking nucleus amputated: Cap regenerates
      c. Foot with nucleus amputated: No foot regenerated
      d. Hypothesized hereditary information stored in foot

B. Surgery on Single Cells
   1. To test hypothesis used two species that looked different fig 14.2
      a. *A. crenulata*: Disk-shaped cap, *A. mediterranea*: Flower-shaped cap
      b. *A. crenulata* stalk onto *A. mediterranea* foot
      c. Regenerated cap looked similar to *A. crenulata*
      d. Amputated regenerated cap, next cap looked like *A. mediterranea*
      e. Further supported that hereditary information in foot

2. Initial flower-shaped cap somewhat intermediate in shape
   a. First cap used information that was already present
   b. Subsequent caps used information provided by new foot
   c. Now know that instructions for first cap based on remaining mRNA

II. TRANSPLANTATION EXPERIMENTS: EACH CELL CONTAINS A FULL SET OF GENETIC INSTRUCTIONS

A. Briggs and King: Frog Nucleus Transplant Experiments
   1. Removed nucleus from frog egg: No development
   2. Added nucleus from another egg: Development occurred
   3. Concluded nucleus directed development fig 14.3

B. Successfully Transplanting Nuclei
   1. Inclusive whether nucleus could direct development of entire adult
   2. Eggs with transplanted nuclei often developed abnormally
   3. Gurdon transplanted nucleus of another species from tadpoles into eggs
      a. Eggs usually developed normally
      b. Nucleus at later stage retained information to direct development

C. Totipotency in Plants
   1. Stewart fragmented mature carrot tissue
   2. Individual cells developed roots, became adult plants when placed on solid medium
   3. Concluded each cell has full set of genetic material, can generate entire adult

III. THE GRIFFITH EXPERIMENT: HEREDITY INFORMATION CAN PASS BETWEEN ORGANISMS

A. Genes Hold Hereditary Information

B. Discovery of Transformation
   1. Griffith injected mice with various strains of one bacteria fig 14.4
      a. Virulent, coated bacteria (S form, smooth colonies) lethal to mice
      b. Nonvirulent, coatless strain (R form, rough colonies) not lethal
      c. Coat necessary for infection
   2. Questioned toxic effect of coat itself
      a. Injected mice with dead coated bacteria
      b. Mice remained healthy
   3. Dead S form and live R form bacteria mixed and injected
      a. Mice died, had live S form bacteria in blood
      b. Factor passed from one strain to other transforming it to virulent strain

IV. THE AVERY AND HERSHEY-CHASE EXPERIMENTS: THE ACTIVE PRINCIPLE IS DNA

A. The Avery Experiments
   1. Utilized same bacterium as Griffith
   2. Removed 99.98% of protein from dead S/live R mixture
   3. Transformation activity unaltered
   4. Properties of transforming principle resembled those of DNA
      a. Purified principle analyzed to resemble elements of DNA
      b. Principle had same density as DNA with ultracentrifugation
      c. Extracting lipid and protein did not alter activity
      d. Protein- or RNA-digesting enzymes did not affect activity
      e. DNA-digesting enzymes destroyed activity
   5. Concluded principle was indeed DNA

B. The Hershey-Chase Experiment: Some Viruses Direct Their Heredity with DNA
   1. Examined bacteriophage viruses that attack bacteria
      a. Bacteriophages possess either DNA or RNA, surrounded by protein coat
      b. Lytic virus injects viral genetic material into bacteria
      c. Causes production and release of more viruses when cell lyses
   2. Experiments to determine if the genetic material was DNA or protein fig 14.5
      a. Used DNA bacteriophage called T2
         1) Labeled bacteriophage DNA with ^{32}P and protein coat with ^{35}S
         2) Viruses infect bacteria, attached viruses shaken off
         3) Agitation removed ^{35}S from bacterial preparation
         4) Found ^{32}P injected into bacterial cells
      b. Concluded genetic material in bacteriophages was DNA

14.2 What is the structure of DNA?

I. THE CHEMICAL NATURE OF NUCLEIC ACIDS

A. Miescher Isolated Material from Cell Nuclei
   1. White substance isolated from human cells, fish sperm
   2. Had unique proportions of nitrogen and phosphorus
   3. Named substance "nuclein"

B. Levene's Analysis: DNA Is a Polymer
   1. Nuclein found to be acidic, renamed nucleic acid
   2. Levene determined primary structure fig 14.6
      a. Phosphate group PO_4
      b. Five carbon sugars
      c. Nitrogen containing base: Purine or pyrimidine
         1) Purines = adenine (A), guanine (G)
         2) Pyrimidines = thymine (T), cytosine (C), RNA contains uracil (U) not T
   3. DNA and RNA composed of repeating units
      a. Called nucleotides
      b. Nitrogen base distinguishes nucleotide identity
   4. Numbering scheme for sugar structure fig 14.7
      a. A prime (′) indicates that the carbon is located on the sugar molecule
      b. Phosphate attaches to 5′ carbon
      c. Base attaches to 1′ carbon
      d. Free hydroxyl, (–OH) attaches to 3′ carbon
   5. Phosphate at 5′ C, hydroxyl at 3′ C enables chains to form
   6. Sugars linked by phosphodiester bond fig 14.8
   7. Nucleotide chain possesses definite direction
      a. One end of chain with free 5′ phosphate group
      b. Other end of chain with free 3′ hydroxyl group
      c. Sequences conventionally written in 5′ to 3′ direction
   8. Levene's early analysis found all four nucleotides present in equal amounts
      a. Assumed DNA a polymer of four repeating units
      b. DNA had structural role and protein had hereditary role
      c. Found to be wrong

C. Chargaff's Analysis: DNA Is Not a Simple Repeating Polymer
   1. Found base amounts differed, depended on source tbl 14.1
   2. Composition of nucleotides varied in complex ways
      a. Suggested that DNA not a simple repeating polymer
      b. Found proportions of certain nucleotides equal to others

3. Chargaff's rules
   a. Proportion of adenine (A) equal to thymine (T)
   b. Proportion of guanine (G) equal to cytosine (C)
   c. Proportion of purine (A + G) equal to pyrimidine (C + T)

II. THE THREE-DIMENSIONAL STRUCTURE OF DNA

A. Franklin: X-ray Diffraction Patterns of DNA fig 14.9a
   1. In X-ray crystallography, molecule bombarded with X-rays
      a. Resulting pattern of diffractions caused by DNA fibers fig 14.9b
      b. Not precise since DNA sample was in fibers not true crystals
   2. Initial analysis of DNA fig 14.9c
      a. Spring-like spiral with helical diameter of 2 nanometers
      b. Complete turn made every 3.4 nanometers

B. Watson and Crick: A Model of the Double Helix fig 14.10
   1. Constructed models to determine shape
   2. Model of double helix fit all known data
      a. Bases pointed inward toward one another
      b. Large purine always paired with small pyrimidine
      c. Hydrogen bonds between bases stabilize antiparallel strands
         1) One strand ran 5′ to 3′
         2) Other strand ran 3′ to 5′
      d. Model explained Chargaff's results
         1) Adenine, thymine form two bonds
         2) Guanine, cytosine form three bonds

14.3 How does DNA replicate?

I. THE MESELSON-STAHL EXPERIMENT: DNA REPLICATION IS SEMICONSERVATIVE

A. Model Dependent on Complementarity of Strands
   1. Sequence of one chain determines sequence of its partner
   2. Each chain is complementary mirror image of other
   3. Unzipping molecule allows each strand to form daughter strands with same sequence
   4. Replication called semiconservative
      a. Sequence of strand conserved
      b. Duplex itself not conserved
      c. One strand of original goes into each of daughter strands

B. Using Heavy Isotopes to Density-Label DNA Strands
   1. Labeled generations of bacteria with heavy nitrogen ^{15}N
   2. DNA of new bacteria denser than other bacteria grown on ^{14}N medium
   3. Transferred ^{15}N bacteria onto ^{14}N medium, collected DNA at intervals

C. Separating DNA Strands by Density
   1. Experimental procedure
      a. Separated DNA strands in cesium chloride
      b. Ultracentrifuge used to spin solution
      c. Cesium ions form density gradient
      d. DNA strands migrate to position that matches density of cesium ions
   2. Experimental results
      a. ^{15}N strands are denser than ^{14}N strands
      b. ^{15}N strands migrate further down tube

D. The Key Result: Replication Alters DNA Density fig 14.11
   1. Initial bacteria all dense
   2. After one round of replication density intermediate between ^{15}N and ^{14}N
   3. After another round, grouped into intermediate and light classes
      a. Intermediate group same as after first round
      b. Light group was equal to all ^{14}N-DNA

E. Interpreting the Results
   1. After first round of replication DNA had one heavy, one light strand
   2. When hybrid replicated to hybrids formed
      a. One had one light and one heavy strand
      b. Other had two light strands fig 14.12
   3. Confirmed Watson-Crick model of semiconservative replication

II. THE REPLICATION PROCESS

A. DNA Replication Must Be Fast and Accurate
   1. Replication begins at one or more origins of replication fig 14.13
   2. DNA replicating enzymes include DNA polymerase III fig 14.14
   3. Catalyzes reaction to add nucleotides to complementary strands tbl 14.2

B. DNA Polymerase III
   1. DNA polymerase I is small, enzyme that plays supporting role
   2. DNA polymerase III is key enzyme, larger, more complex fig 14.15
      a. Contains 10 different polypeptide chains
      b. Is a dimer with two similar multisubunit complexes
   3. Variety of proteins have unique duties
      a. Large α subunit catalyses 5′ to 3′ addition of nucleotides
      b. Smaller ε subunit proofreads 3′ to 5′ strand for mistakes
      c. Ring-shaped β_2 dimer subunit clamps polymerase III complex around DNA helix
      d. Moves at rate of 1,000 nucleotides per second

C. The Need for a Primer
   1. DNA polymerase III cannot link first nucleotide in a newly synthesized strand
      a. RNA polymerase, primase constructs RNA primer
      b. Ten nucleotides complementary to DNA parent template
   2. DNA polymerase III recognizes primer, adds new nucleotides
   3. RNA nucleotides in primer replaced by DNA nucleotides

D. The Two Strands of DNA Are Assembled in Different Ways
   1. DNA polymerase III can only add on to 3′ end
   2. Replication occurs only in 5′ ¾→ 3′ direction
   3. New strands oriented in opposite directions, replicated different ways
   4. Replication of leading strand, 5′ to 3′ strand fig 14.16
      a. Elongates towards replication fork
      b. New strand grows from 3′ end
   5. Lagging strand, 3′ to 5′ strand replication
      a. Elongates away from replication fork
      b. Synthesized discontinuously in small batches
      c. Segments called Okazaki fragments
      d. 5′ ¾→ 3′ synthesis catalyzed by DNA polymerase III
      e. DNA ligase attaches fragment to lagging strand
   6. Overall replication process is termed semidiscontinuous

E. The Replication Process
1. Occurs in five steps
2. Opening up the DNA double helix
a. Stage One: Initiating replication
1) Binding of initiator proteins to replication origin
2) Starts process that opens helix
b. Stage two: Unwinding the duplex
1) Untwisted by helicase enzyme
2) Bind to one strand, push aside other strand
c. Stage three: Stabilizing the single strands
1) Single-strand binding proteins protect strands from cleavage
2) Prevent rewinding
d. Stage four: Relieving the torque generated by unwinding
1) If replication proceeds at 1,000 nucleotides/second helix rotates 100 times/second
2) Resulting twisting, torque, relieved by gyrases (topisomerases)
3) Cleave strand of helix, swivels around intact strand, reseals strand
3. Building a primer
a. DNA polymerases require 3' primers to initiate replication
b. Short series of RNA added by RNA polymerase called primase
c. Multisubunit complex called a primasome
d. Starting chains on exposed templates induces errors
e. RNA marks initial stretch as temporary, later removes
4. Assembling the complementary strand fig 14.17
a. DNA polymerase III binds to replication fork
b. Leading strand complexes with one half of the dimer
c. Lagging strand loops around, complexes with other half of dimer
d. Formation of complementary sequences on both strands at same time
5. Removing the primer
a. DNA polymerase I removes RNA primer
b. Fills in gap and gaps between Okazaki fragments
6. Joining the Okazaki fragments
a. Gaps between Okazaki fragments filled in
b. DNA ligase joins fragments to lagging strand

III. EUKARYOTIC DNA REPLICATION

A. DNA Packaged into Nucleosomes within Chromosomes fig 14.18
1. Individual zone of replication called replication unit or replicon
2. Each has own origin of replication
3. Multiple units may be replicating DNA at once fig 14.19
4. Multiple origins increases speed of replication

B. Replication Regulation Ensures Only One Copy Made

14.4 What is a gene?

I. THE ONE-GENE/ONE-POLYPEPTIDE HYPOTHESIS

A. Garrod: Genetic Disorders Can Involve Specific Enzymes
1. Examined several diseases
a. Behaved like products of simple recessive alleles
b. Concluded they were Mendelian traits
c. Originated as change in heredity in ancestor to family

2. Example: Alkaptonuria
   a. Urine contains homogensic acid that oxidizes and blackens on exposure to air
   b. Acid in normal urine broken down by enzymes
   c. Postulated that affected patients lack enzymes

B. Beadle and Tatum: Genes Specify Enzymes
1. Surmised that information in DNA coded for enzymes
2. Set out to create and examine mutations in chromosomes fig 14.20
3. A defined system
   a. Chose proper organism, a bread mold grown readily on a defined medium
   b. Defined medium contains known ingredients
   c. Used X-rays to induce mutations in mold spores
   d. Expected spores be damaged in areas associated with normal growth fig 14.20
      1) Lose ability to synthesize one or more compounds
      2) Affect ability to grow on normal medium
   e. Allowed progeny to grow on complete medium
      1) Contains all possible nutrients
      2) Strains unable to produce own nutrients still grew
4. Isolating growth-deficient mutant
   a. Grow progeny on minimal medium to test for deficiencies
   b. Cells unable to make metabolite would not grow
   c. Identified numerous growth-deficient mutants
5. Identifying the deficiencies
   a. Individually replace chemicals to determine deficiency
   b. Determine enzymes involved in deficiencies
      1) Each site coded for different enzyme in pathway
      2) Arginine mutants clustered in three areas fig 14.21

C. One-Gene/One-Polypeptide
1. Isolated a mutant strain for each enzyme in arginine pathway
   a. Mutation always located at one of a few chromosomal sites
   b. Each enzyme mutation occurred at different site
2. Concluded genes produce effects by encoding enzyme structure
   a. Original hypothesis: One-gene/one-enzyme
   b. Current relationship: One-gene/one-polypeptide
3. Enzymes responsible for catalyzing synthesis of all parts of an organism
   a. Mediate assembly of all biomolecules
   b. DNA, therefore, specifies structure of organism itself

II. HOW DNA ENCODES PROTEIN STRUCTURE

A. Sanger: Proteins Consist of Defined Sequences of Amino Acids
1. Identified amino acid sequence of insulin
2. First demonstration of protein structure
3. Information for enzymes is ordered list of amino acids

B. Ingram: Single Amino Acid Changes in a Protein Can Have Profound Effects
1. Analyzed normal and sickle-cell hemoglobin fig 14.22
2. Single amino acid substitution between hemoglobins
3. Change from glutamic acid to valine

C. Modern Understanding of Heredity
   1. Hereditary traits defined by changes in protein structure
      a. Result from alteration in protein's amino acid sequence
      b. Dictated by order of nucleotides
   2. Example: Sickle-cell anemia
      a. Mutation replaces single thymina with an adenine
      b. Results in replacement of glutamic acid with valine
   3. Gene: A sequence of nucleotides that usually encodes a particular protein
   4. Some genes encode for special forms of RNA, involved in protein synthesis

INSTRUCTIONAL STRATEGY

PRESENTATION ASSISTANCE:

This chapter illustrates the additive effects of scientific discovery and the need for scientists to openly communicate with one another (*i.e.* publish) very well. Mendel, Darwin, and Einstein are rare exceptions in the scientific world. Even Watson and Crick used someone else's data to derive their model of DNA.

There are a lot of names presented in this chapter. Most of them are important within the historical construct of biology. Plus it is much easier to describe an experiment as "The Griffith Experiment" than to talk about the experiment that used virulent bacteria and so forth. After all, if psychology students learn about Skinner, and English students about Emily Dickinson, why can't biology students be familiar with a few of the biggies in their field?

Students frequently get confused with directionality in the DNA helix even though it seems simple that one strand runs 5′ to 3′ and the other 3′ to 5′. They also expect one strand to always be the sense strand. Sense strand recognition is explained in the next chapter.

Many students confuse nucleotide base names with amino acid names (*i.e.* thymine and thymidine).

Some hints just in case students can't seem to keep the mechanisms of base pairing straight:

(1) A and T are both angular letters and with the addition of U, they have an upright orientation. C and G are curved letters and both open towards the right.
(2) A and G are in the same class and both have horizontal lines in their middles.
(3) Structurally, the class of base with the shorter name (purine) is larger (having a double ring) while the longer name (pyrimidine) is the smaller molecule.

VISUAL RESOURCES:

One could construct all sorts of interesting visual aids associated with DNA replication using zippers and/or Velcro®. The latter would be especially useful to show semiconservative replication using different color strips as it sticks together quickly and pulls apart almost faster (we all know how zippers get stuck at the most inopportune moments). Velcro® sewn into a circle would also illustrate bacterial DNA replication readily. One circle should be simply basted so it can be "nicked" easily.

Sigma sells an interesting, humorous, albeit slightly juvenile book called *BIOKIT: A Journey Into Life* that may give you some ideas regarding presentation of this material to very inexperienced students.

Variously colored pop-it beads are handy for showing amino acid sequence.