9 DNA and Its Role in Heredity
Concept 9.1 DNA Structure Reflects Its Role as the Genetic Material

Scientists had criteria for DNA to be accepted as the genetic material, including that it:

- Be present in the cell nucleus and in chromosomes
- Doubles in the cell cycle
- Is twice as abundant in diploid cells
- Has same the pattern of transmission as its genetic information
Stained chromosomes

5 μm
Chromosomes contain DNA, but also contain proteins, so scientists had to determine whether proteins carried genetic information.

Viruses, such as bacteriophages, contain DNA and a little protein.

When a virus infects a bacterium, it injects only its DNA into it, and changes the genetic program of the bacterium.

This provides further evidence for DNA, and not protein, as the genetic material.
Figure 9.2  Viral DNA and Not Protein Enters Host Cells (Part 1)

Bacteriophage T2

0.1 μm
Figure 9.2 Viral DNA and Not Protein Enters Host Cells (Part 2)
Transformation experiments showed that DNA from one strain of bacteria could genetically transform another strain.

DNA can be made to pass through any cell membrane, including egg cells.

It can genetically transform an organism, resulting in a transgenic version of that organism.
Figure 9.3 Transformation of Eukaryotic Cells (Part 1)

INVESTIGATION

HYPOTHESIS

DNA can transform eukaryotic cells.

METHOD

PRINCIPLES OF LIFE, Figure 9.3 (Part 1)
Transformation was achieved by adding the DNA in a solution of calcium chloride (CaCl₂) at pH 7. In other experiments, the type or amount of DNA, pH, or CaCl₂ concentration was varied. The transformation efficiency was calculated as the percentage of cells that produced colonies on a medium containing thymidine. Explain these data.

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<th>Transformation conditions</th>
<th>Efficiency (%)</th>
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<td>Mammalian DNA with TK gene</td>
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<tr>
<td>10 µg</td>
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<tr>
<td>Bacterial virus DNA with TK gene</td>
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<tr>
<td>20 µg</td>
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After identifying DNA as the genetic material, scientists hoped to answer two questions about the structure:

1. How is DNA replicated between cell divisions?
2. How does it direct the synthesis of specific proteins?
Concept 9.1 DNA Structure Reflects Its Role as the Genetic Material

DNA structure was discovered through the work of many scientists.

One crucial piece of evidence came from X-ray crystallography.

A purified substance can be made to form crystals; the pattern of diffraction of X rays passed through the crystallized substance shows position of atoms.
Rosalind Franklin:

Prepared crystallographs from uniformly oriented DNA fibers—her images suggested a spiral model.
Figure 9.4 X-Ray Crystallography Helped Reveal the Structure of DNA (Part 1)
Figure 9.4  X-Ray Crystallography Helped Reveal the Structure of DNA (Part 2)
Chemical composition also provided clues:

DNA is a polymer of nucleotides: deoxyribose, a phosphate group, and a nitrogen-containing base.

The bases form the differences:

- Purines: adenine (A), guanine (G)
- Pyrimidines: cytosine (C), thymine (T)
In 1950 Erwin Chargaff found that in the DNA from many different species:

Amount of A = amount of T

Amount of C = amount of G

Or, the abundance of purines = the abundance of pyrimidines—Chargaff’s rule.
Purines = Pyrimidines
Model building is the assembly of 3-D models of possible molecular structures.

Francis Crick and James Watson used model building and combined all the knowledge of DNA to determine its structure.

Franklin’s X-ray crystallography convinced them the molecule was **helical**.

Modeling also showed that DNA strands are **anti-parallel**.
Watson and Crick suggested that:

- Nucleotide bases are on the interior of the two strands, with a sugar-phosphate backbone on the outside.

- Per Chargaff’s rule, a purine on one strand is paired with a pyrimidine on the other.

These **base pairs** (A-T and G-C) have the same width down the helix.
Concept 9.1 DNA Structure Reflects Its Role as the Genetic Material

Four key features of DNA structure:

• It is a *double-stranded helix* of uniform diameter.

• It is *right-handed*.

• It is *antiparallel*.

• Outer edges of nitrogenous bases are exposed in the *major* and *minor* grooves.
Figure 9.5 DNA Is a Double Helix (Part 2)
Concept 9.1 DNA Structure Reflects Its Role as the Genetic Material

Grooves exist because the backbones of the DNA strands are not evenly spaced relative to one another.

The exposed outer edges of the base pairs are accessible for hydrogen bonding.

_Surfaces of A-T and G-C base pairs are chemically distinct._

_Binding of proteins to specific base pair sequences is key to DNA–protein interactions_, and necessary for replication and gene expression.
Figure 9.6 Base Pairs in DNA Can Interact with Other Molecules
DNA has four important functions—double-helical structure is essential:

- **Storage of genetic information**—millions of nucleotides; base sequence encodes huge amounts of information
- **Precise replication during cell division** by complementary base pairing
Concept 9.1 DNA Structure Reflects Its Role as the Genetic Material

- **Susceptibility to mutations**—a change in information—possibly a simple alteration to a sequence

- **Expression of the coded information as the phenotype**—nucleotide sequence is transcribed into RNA and determines sequence of amino acids in proteins
Semiconservative replication means that each parental strand serves as a template for a new strand.

Conservative replication would show that the intact parental DNA (both strands) serves as a template.

Evidence from radioactively-labeled strands supports semiconservative replication.
Two steps in DNA replication:

The double helix is unwound, making two template strands available for new base pairing.

New nucleotides form base pairs with template strands and linked together by phosphodiester bonds. Template DNA is read in the 3’-to-5’ direction.
During DNA synthesis, *new nucleotides are added to the 3′ end of the new strand*, which has a free hydroxyl group (—OH).

**Deoxyribonucleoside triphosphates** (dNTPs), or *deoxyribonucleotides*, are the building blocks—two of their phosphate groups are released and the third bonds to the 3′ end of the DNA chain.
Figure 9.7 Each New DNA Strand Grows by the Addition of Nucleotides to Its 3′ End
DNA replication begins with the binding of a large protein complex—the *pre-replication complex*—to a specific site on the DNA molecule.

The complex contains *DNA polymerase*, which catalyzes addition of nucleotides.

The complex binds to a region on the chromosome called the *origin of replication* (*ori*).
When the pre-replication complex binds to ori, the DNA unwinds and replication proceeds in two directions.

The replication fork is the site where DNA unwinds to expose bases.

Eukaryotic chromosomes are linear and have multiple origins of replication, which speed up replication.
Figure 9.8 The Origin of DNA Replication (Part 1)
Figure 9.8 The Origin of DNA Replication (Part 2)

(B) Eukaryotic

ori

Initiation of replication

ori

ori

PRINCIPLES OF LIFE, Figure 9.8 (Part 2)
DNA replication begins with a short **primer**—a starter strand.

The primer is complementary to the DNA template.

**Primase**—an enzyme—synthesizes DNA one nucleotide at a time.

DNA polymerase adds nucleotides to the 3’ end.
Figure 9.9 DNA Forms with a Primer
DNA polymerases are larger than their substrates, the dNTPs, and the template DNA.

The enzyme is shaped like an open right hand—the “palm” brings the active site and the substrates into contact.

The “fingers” recognize the nucleotide bases.
Figure 9.10 DNA Polymerase Binds to the Template Strand (Part 1)
Figure 9.10 DNA Polymerase Binds to the Template Strand (Part 2)
A single replication fork opens up in one direction.

- The two DNA strands are antiparallel—the 3′ end of one strand is paired with the 5′ end of the other.
- DNA replicates in a 5′-to-3′ direction.
Figure 9.11  The Two New Strands Form in Different Ways
One new strand, the **leading strand**, is oriented to grow at its 3’ end as the fork opens.

The **lagging strand** is oriented so that its exposed 3’ end gets farther from the fork.

Synthesis of the lagging strand occurs in small, *discontinuous stretches*—**Okazaki fragments**.
Figure 9.11 The Two New Strands Form in Different Ways
Each Okazaki fragment requires its own primer, synthesized by the primase.

DNA polymerase adds nucleotides to the 3’ end, until reaching the primer of the previous fragment.

A different DNA polymerase then replaces the primer with DNA.

The final phosphodiester linkage between fragments is catalyzed by DNA ligase.
Figure 9.12 The Lagging Strand Story (Part 2)

DNA polymerase

Okazaki fragment

Gap
DNA polymerase works very fast:

It is **processive**—it catalyzes many sequential polymerization reactions each time it binds to DNA.
Okazaki fragments are added to RNA primers to replicate the lagging strand.

When the last primer is removed no DNA synthesis occurs because there is no 3’ end to extend—a single-stranded bit of DNA is left at each end.

These are cut after replication and the chromosome is slightly shortened after each cell division.
Figure 9.13  Telomeres and Telomerase (Part 1)
Telomeres are repetitive sequences at the ends of eukaryotic chromosomes.

These repeats prevent the chromosome ends from being joined together by the DNA repair system.

Telomerase contains an RNA sequence—it acts as a template for telomeric DNA sequences.

Telomeric DNA is lost over time in most cells, but not in continuously dividing cells like bone marrow and gametes.
DNA polymerases can make mistakes in replication, but most errors are repaired.

Cells have two major repair mechanisms:

• **Proofreading**—as DNA polymerase adds nucleotides, it has a proofreading function and if bases are paired incorrectly, the nucleotide is removed.

• **Mismatch repair**—after replication other proteins scan for mismatched bases missed in proofreading, and replace them with correct ones.
Figure 9.14 DNA Repair Mechanisms (Part 1)
Figure 9.14 DNA Repair Mechanisms (Part 2)
Concept 9.2 DNA Replicates Semiconservatively

Copies of DNA sequences can be made by the **polymerase chain reaction (PCR)** technique, which uses:

- A double-stranded DNA sample
- Two short primers complementary to the ends of the sequence to be amplified
- The four dNTPs
- A DNA polymerase that works at high temperatures
- Salts and a buffer to maintain pH
Figure 9.15 The Polymerase Chain Reaction
Concept 9.3 Mutations Are Heritable Changes in DNA

*Mutations are changes in the nucleotide sequence of DNA that are passed on from one cell, or organism, to another.*

Mutations occur by a variety of processes.

Errors that are not corrected by repair systems are passed on to daughter cells.
Mutations are of two types:

**Somatic mutations** occur in somatic (body) cells—passed on by mitosis but not to sexually produced offspring.

**Germ line mutations** occur in germ line cells that give rise to gametes. A gamete passes a mutation on at fertilization.
Most genomes include genes and regions of DNA that are not expressed:

- Genes are transcribed into RNAs, for translation into amino acid sequences or into RNAs with catalytic functions.

The *coding regions* of a gene contain sequences within the transcribed region that are translated.

- Genomes also contain regions of DNA that are not expressed.
Concept 9.3 Mutations Are Heritable Changes in DNA

Mutations are discussed in terms of their effects on protein-coding gene function:
Figure 9.16  Mutation and Phenotype

DNA → mRNA → Protein

**Normal allele:**
Codes for a functional protein

**Silent mutation:**
Codes for a functional protein

**Loss-of-function mutation:**
Codes for a nonfunctional protein

**Gain-of-function mutation:**
Codes for a protein with new function

*PRINCIPLES OF LIFE, Figure 9.16*
At the molecular level there are two categories of mutations:

A **point mutation** results from the gain, loss, or substitution of a single nucleotide.

**Chromosomal mutations** are more extensive—they may change the position or cause a DNA segment to be duplicated or lost.
Other mutations result in altered amino acid sequences and have drastic phenotypic effects:

- Sickle-cell disease—allele differs from normal by one base pair
- A gain-of-function mutation as in the TP53 gene, which gains cancer-causing function
Chromosomal mutations:

- **Deletions**—result in the removal of part of the genetic material and can have severe or fatal consequences.

- **Duplications**—homologous chromosomes break in different places and recombine with wrong partners; one may have two copies of segment and the other may have none.
Figure 9.17 Chromosomal Mutations

(A) A sequence of bases (ABCDEFG) is shown with a deletion of CD, resulting in the sequence ABEDFG.

(B) A sequence of bases (ABCDEFG) is shown with a duplication of a segment, resulting in the sequence ABCDDEFG.

The diagrams illustrate the process of chromosomal mutations with specific deletions and duplications.
Chromosomal mutations:

**Inversions**—result from breaking and rejoining, but segment is “flipped”

**Translocations**—segment of DNA breaks off and is inserted into another chromosome; this can lead to duplications and deletions
Mutations are caused in two ways:

**Spontaneous mutations** occur with no outside influence, and are permanent.

**Induced mutations** are due to an outside agent, a mutagen.
Spontaneous mutations—several mechanisms that alter DNA:

- *Errors in replication by DNA polymerase*—most errors are repaired but some become permanent
- *Nucleotide bases can have different structures*—may form *tautomers*; a rare tautomer can pair with the wrong base
Spontaneous mutations:

- *Chemical reactions may change bases* (e.g., loss of amino group—*deamination*)
- *Imperfect meiosis*—nondisjunction of homologous chromosomes may occur
- *Gene sequences can be disrupted*—random chromosome breakage and rejoining
(A) A spontaneous mutation

Cytosine (common tautomer) → Cytosine (rare tautomer)
*Induced* mutation—caused by mutagens:

- *Chemicals can alter nucleotide bases* (e.g., nitrous acid can cause deamination)
  - Some chemicals add other groups to bases (e.g., benzopyrene adds a group to guanine and prevents base pairing). DNA polymerase will then add any base there.
Radiation damages DNA:

- Ionizing radiation, such as X rays, creates free radicals—highly reactive—can change bases, break sugar phosphate bonds.

- UV radiation (from sun or tanning beds) is absorbed by thymine, causing it to form covalent bonds with adjacent nucleotides—disrupts DNA replication.
(B) An induced mutation

Deamination by $\text{HNO}_2$

Deaminated form of cytosine (= uracil)
Figure 9.18 Spontaneous and Induced Mutations (Part 3)

(C) The consequences of either mutation

Original sequence

Template strand

Newly replicated strands

Mutated sequence

Replication is normal

PRINCIPLES OF LIFE, Figure 9.18 (Part 3)
DNA sequencing revealed that mutations occur most often at certain base pairs.

If 5-methylcytosine loses an amino acid, it becomes thymine, a natural base for DNA.

During mismatch repair, it is repaired correctly only half of the time.
Figure 9.19 5-Methylcytosine in DNA Is a “Hotspot” for Mutations

PRINCIPLES OF LIFE, Figure 9.19
Mutations can be artificial or natural:

- Human-made chemicals (e.g., nitrites) or naturally occurring substances (e.g., molds)
- Radiation from nuclear reactions, bombs, or from the sun
Mutations can have benefits:

- Provide the raw material for evolution in the form of genetic diversity
- Diversity may benefit the organism immediately—if mutation is in somatic cells
- May cause an advantageous change in offspring
Possible costs of mutations:

- Some germ line and somatic cell mutations are harmful or lethal.

- Mutations in oncogenes stimulate cell division in cancer, and mutations in tumor suppressor cells fail to inhibit growth.

- Public health policy includes bans on ozone-depleting chemicals and on cigarette smoking, which cause mutations that lead to cancer.