Overview: Life's Operating Instructions

- In 1953, James Watson and Francis Crick introduced an elegant double-helical model for the structure of deoxyribonucleic acid, or DNA
- DNA, the substance of inheritance, is the most celebrated molecule of our time
- Hereditary information is encoded in DNA and reproduced in all cells of the body
- This DNA program directs the development of biochemical, anatomical, physiological, and (to some extent) behavioral traits

Concept 16.1: DNA is the genetic material

- Early in the 20th century, the identification of the molecules of inheritance loomed as a major challenge to biologists

The Search for the Genetic Material: *Scientific Inquiry*

- When T. H. Morgan's group showed that genes are located on chromosomes, the two components of chromosomes—DNA and protein—became candidates for the genetic material
- The key factor in determining the genetic material was choosing appropriate experimental organisms
- The role of DNA in heredity was first discovered by studying bacteria and the viruses that infect them

Evidence That DNA Can Transform Bacteria

- The discovery of the genetic role of DNA began with research by Frederick Griffith in 1928
- Griffith worked with two strains of a bacterium, one pathogenic and one harmless
- When he mixed heat-killed remains of the pathogenic strain with living cells of the harmless strain, some living cells became pathogenic
- He called this phenomenon **transformation**, now defined as a change in genotype and phenotype due to assimilation of foreign DNA
- In 1944, Oswald Avery, Maclyn McCarty, and Colin MacLeod announced that the transforming substance was DNA
- Their conclusion was based on experimental evidence that only DNA worked in transforming harmless bacteria into pathogenic bacteria
- Many biologists remained skeptical, mainly because little was known about DNA

Evidence That Viral DNA Can Program Cells

- More evidence for DNA as the genetic material came from studies of viruses that infect bacteria
- Such viruses, called **bacteriophages** (or **phages**), are widely used in molecular genetics research
- In 1952, Alfred Hershey and Martha Chase performed experiments showing that DNA is the genetic material of a phage known as T2
- To determine this, they designed an experiment showing that only one of the two components of T2 (DNA or protein) enters an *E. coli* cell during infection
- They concluded that the injected DNA of the phage provides the genetic information

Additional Evidence That DNA Is the Genetic Material

- Two findings became known as Chargaff's rules

  - The base composition of DNA varies between species
  - In any species the number of A and T bases are equal and the number of G and C bases are equal
- The basis for these rules was not understood until the discovery of the double helix

Building a Structural Model of DNA: *Scientific Inquiry*

- After DNA was accepted as the genetic material, the challenge was to determine how its structure accounts for its role in heredity
- Maurice Wilkins and Rosalind Franklin were using a technique called X-ray crystallography to study molecular structure
- Franklin produced a picture of the DNA molecule using this technique
- Franklin's X-ray crystallographic images of DNA enabled Watson to deduce that DNA was helical
- The X-ray images also enabled Watson to deduce the width of the helix and the spacing of the nitrogenous bases
- The pattern in the photo suggested that the DNA molecule was made up of two strands, forming a **double helix**
- Watson and Crick built models of a double helix to conform to the X-rays and chemistry of DNA
- Franklin had concluded that there were two outer sugar-phosphate backbones, with the nitrogenous bases paired in the molecule's interior
- Watson built a model in which the backbones were **antiparallel** (their subunits run in opposite directions)
- At first, Watson and Crick thought the bases paired like with like (A with A, and so on), but such pairings did not result in a uniform width
- Instead, pairing a purine with a pyrimidine resulted in a uniform width consistent with the X-ray data
- Watson and Crick reasoned that the pairing was more specific, dictated by the base structures
- They determined that adenine (A) paired only with thymine (T), and guanine (G) paired only with cytosine (C)
- The Watson-Crick model explains Chargaff's rules: in any organism the amount of A = T, and the amount of G = C

Concept 16.2: Many proteins work together in DNA replication and repair

- The relationship between structure and function is manifest in the double helix
- Watson and Crick noted that the specific base pairing suggested a possible copying mechanism for genetic material

The Basic Principle: Base Pairing to a Template Strand

- Since the two strands of DNA are complementary, each strand acts as a template for building a new strand in replication
- In DNA replication, the parent molecule unwinds, and two new daughter strands are built based on base-pairing rules
- Watson and Crick's **semiconservative model** of replication predicts that when a double helix replicates, each daughter molecule will have one old strand (derived or

"conserved" from the parent molecule) and one newly made strand
- Competing models were the conservative model (the two parent strands rejoin) and the dispersive model (each strand is a mix of old and new)
- Experiments by Matthew Meselson and Franklin Stahl supported the semiconservative model
- They labeled the nucleotides of the old strands with a heavy isotope of nitrogen, while any new nucleotides were labeled with a lighter isotope
- The first replication produced a band of hybrid DNA, eliminating the conservative model
- A second replication produced both light and hybrid DNA, eliminating the dispersive model and supporting the semiconservative model

DNA Replication: ***A Closer Look***
- The copying of DNA is remarkable in its speed and accuracy
- More than a dozen enzymes and other proteins participate in DNA replication

Getting Started
- Replication begins at particular sites called **origins of replication**, where the two DNA strands are separated, opening up a replication "bubble"
- A eukaryotic chromosome may have hundreds or even thousands of origins of replication
- Replication proceeds in both directions from each origin, until the entire molecule is copied
- At the end of each replication bubble is a **replication fork**, a Y-shaped region where new DNA strands are elongating
- **Helicases** are enzymes that untwist the double helix at the replication forks
- **Single-strand binding proteins** bind to and stabilize single-stranded DNA
- **Topoisomerase** corrects "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands
- DNA polymerases cannot initiate synthesis of a polynucleotide; they can only add nucleotides to the 3′ end
- The initial nucleotide strand is a short RNA **primer**
- An enzyme called **primase** can start an RNA chain from scratch and adds RNA nucleotides one at a time using the parental DNA as a template
- The primer is short (5–10 nucleotides long), and the 3′ end serves as the starting point for the new DNA strand

Synthesizing a New DNA Strand
- Enzymes called **DNA polymerases** catalyze the elongation of new DNA at a replication fork
- Most DNA polymerases require a primer and a DNA template strand
- The rate of elongation is about 500 nucleotides per second in bacteria and 50 per second in human cells
- Each nucleotide that is added to a growing DNA strand is a nucleoside triphosphate
- dATP supplies adenine to DNA and is similar to the ATP of energy metabolism
- The difference is in their sugars: dATP has deoxyribose while ATP has ribose
- As each monomer of dATP joins the DNA strand, it loses two phosphate groups as a molecule of pyrophosphate

Antiparallel Elongation

- The antiparallel structure of the double helix affects replication
- DNA polymerases add nucleotides only to the free 3′ end of a growing strand; therefore, a new DNA strand can elongate only in the 5′ to 3′ direction
- Along one template strand of DNA, the DNA polymerase synthesizes a **leading strand** continuously, moving toward the replication fork

The DNA Replication Complex

Proofreading and Repairing DNA

- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- In **mismatch repair** of DNA, repair enzymes correct errors in base pairing
- DNA can be damaged by exposure to harmful chemical or physical agents such as cigarette smoke and X-rays; it can also undergo spontaneous changes
- In **nucleotide excision repair**, a **nuclease** cuts out and replaces damaged stretches of DNA

Evolutionary Significance of Altered DNA Nucleotides

- Error rate after proofreading repair is low but not zero
- Sequence changes may become permanent and can be passed on to the next generation
- These changes (mutations) are the source of the genetic variation upon which natural selection operates

Replicating the Ends of DNA Molecules

- Limitations of DNA polymerase create problems for the linear DNA of eukaryotic chromosomes
- The usual replication machinery provides no way to complete the 5′ ends, so repeated rounds of replication produce shorter DNA molecules with uneven ends
- This is not a problem for prokaryotes, most of which have circular chromosomes
- Eukaryotic chromosomal DNA molecules have special nucleotide sequences at their ends called **telomeres**
- Telomeres do not prevent the shortening of DNA molecules, but they do postpone the erosion of genes near the ends of DNA molecules
- It has been proposed that the shortening of telomeres is connected to aging
- If chromosomes of germ cells became shorter in every cell cycle, essential genes would eventually be missing from the gametes they produce
- An enzyme called **telomerase** catalyzes the lengthening of telomeres in germ cells
- The shortening of telomeres might protect cells from cancerous growth by limiting the number of cell divisions
- There is evidence of telomerase activity in cancer cells, which may allow cancer cells to persist

Concept 16.3 A chromosome consists of a DNA molecule packed together with proteins

- The bacterial chromosome is a double-stranded, circular DNA molecule associated with a small amount of protein

- Eukaryotic chromosomes have linear DNA molecules associated with a large amount of protein
- In a bacterium, the DNA is “supercoiled” and found in a region of the cell called the **nucleoid**
- **Chromatin**, a complex of DNA and protein, is found in the nucleus of eukaryotic cells
- Chromosomes fit into the nucleus through an elaborate, multilevel system of packing
- Chromatin undergoes changes in packing during the cell cycle
- At interphase, some chromatin is organized into a 10-nm fiber, but much is compacted into a 30-nm fiber, through folding and looping
- Though interphase chromosomes are not highly condensed, they still occupy specific restricted regions in the nucleus
- Most chromatin is loosely packed in the nucleus during interphase and condenses prior to mitosis
- Loosely packed chromatin is called **euchromatin**
- During interphase a few regions of chromatin (centromeres and telomeres) are highly condensed into **heterochromatin**
- Dense packing of the heterochromatin makes it difficult for the cell to express genetic information coded in these regions
- Histones can undergo chemical modifications that result in changes in chromatin organization