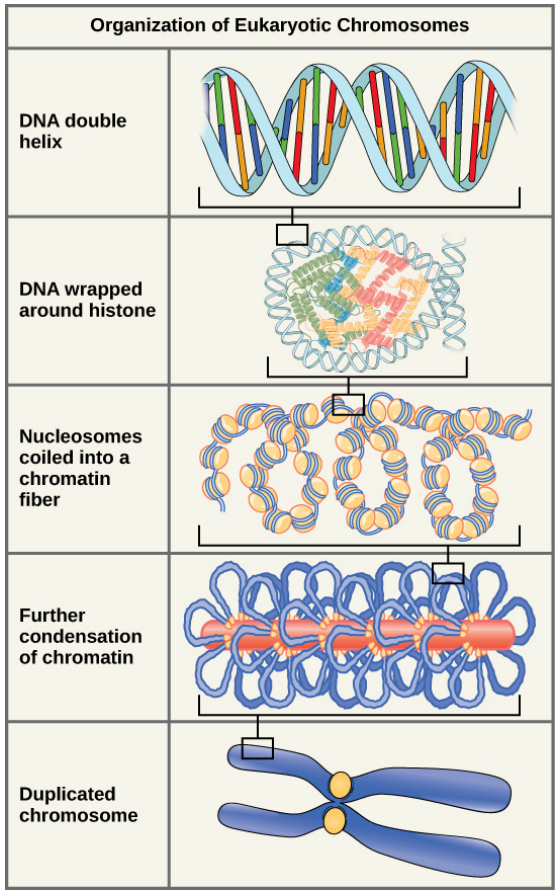

Figure 14.11 These figures illustrate the compaction of the eukaryotic chromosome.

14.3 Basics of DNA Replication

By the end of this section, you will be able to do the following:

- Explain how the structure of DNA reveals the replication process
- Describe the Meselson and Stahl experiments

The elucidation of the structure of the double helix provided a hint as to how DNA divides and makes copies of itself. In their 1953 paper, Watson and Crick penned an incredible understatement: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." With specific base pairs, the sequence of one DNA strand can be predicted from its complement. The double-helix model suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied. What was not clear was how the replication took place. There were three models suggested (Figure 14.12): *conservative, semi-conservative, and dispersive.*

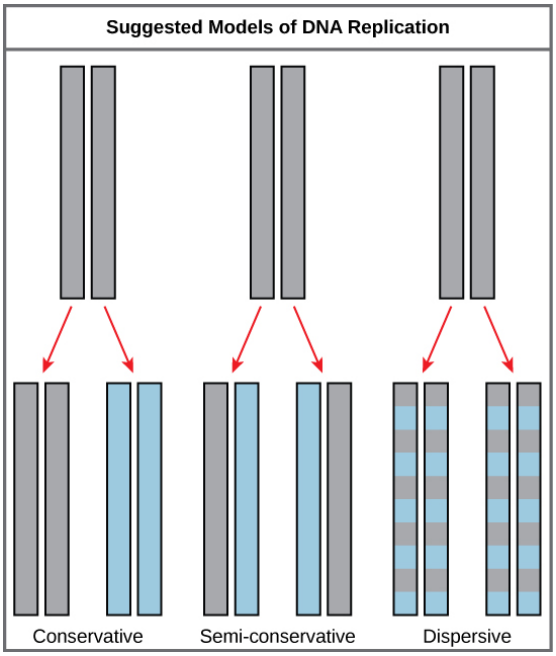

Figure 14.12 The three suggested models of DNA replication. Gray indicates the original DNA strands, and blue indicates newly synthesized DNA.

In conservative replication, the parental DNA remains together, and the newly formed daughter strands are together. The semi-conservative method suggests that each of the two parental DNA strands acts as a template for new DNA to be synthesized; after replication, each double-stranded DNA includes one parental or "old" strand and one "new" strand. In the dispersive model, both copies of DNA have double-stranded segments of parental DNA and newly synthesized DNA interspersed.

Meselson and Stahl were interested in understanding how DNA replicates. They grew *E. coli* for several generations in a medium containing a "heavy" isotope of nitrogen (^{15}N), which gets incorporated into nitrogenous bases, and eventually into the DNA (Figure 14.13).

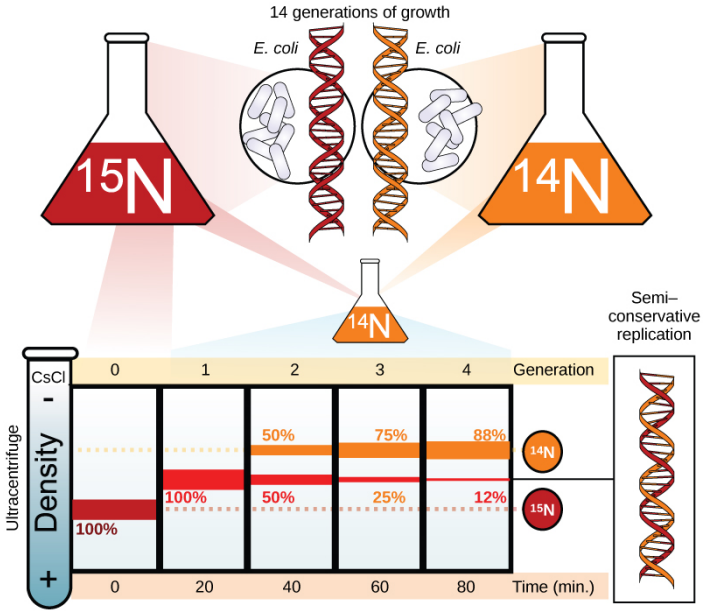

Figure 14.13 Meselson and Stahl experimented with *E. coli* grown first in heavy nitrogen (^{15}N) then in ^{14}N. DNA grown in ^{15}N (red band) is heavier than DNA grown in ^{14}N (orange band), and sediments to a lower level in cesium chloride solution in an ultracentrifuge. When DNA grown in ^{15}N is switched to media containing ^{14}N, after one round of cell division the DNA sediments halfway between the ^{15}N and ^{14}N levels, indicating that it now contains fifty percent ^{14}N. In subsequent cell divisions, an increasing amount of DNA contains ^{14}N only. These

data support the semi-conservative replication model. (credit: modification of work by Mariana Ruiz Villareal)

The *E. coli* culture was then placed into medium containing ^{14}N and allowed to grow for several generations. After each of the first few generations, the cells were harvested and the DNA was isolated, then centrifuged at high speeds in an ultracentrifuge. During the centrifugation, the DNA was loaded into a *gradient* (typically a solution of salt such as cesium chloride or sucrose) and spun at high speeds of 50,000 to 60,000 rpm. Under these circumstances, the DNA will form a band according to its *buoyant density*: the density within the gradient at which it floats. DNA grown in ^{15}N will form a band at a higher density position (i.e., farther down the centrifuge tube) than that grown in ^{14}N. Meselson and Stahl noted that after one generation of growth in ^{14}N after they had been shifted from ^{15}N, the single band observed was intermediate in position in between DNA of cells grown exclusively in ^{15}N and ^{14}N. This suggested either a semi-conservative or dispersive mode of replication. The DNA harvested from cells grown for two generations in ^{14}N formed two bands: one DNA band was at the intermediate position between ^{15}N and ^{14}N, and the other corresponded to the band of ^{14}N DNA. These results could only be explained if DNA replicates in a semi-conservative manner. And for this reason, therefore, the other two models were ruled out.

During DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied. The new strands will be complementary to the parental or "old" strands. When two daughter DNA copies are formed, they have the same sequence and are divided equally into the two daughter cells.

LINK TO LEARNING

View this video (http://openstax.org/l/DNA_replicatio2) on DNA replication.

14.4 DNA Replication in Prokaryotes

By the end of this section, you will be able to do the following:

- Explain the process of DNA replication in prokaryotes
- Discuss the role of different enzymes and proteins in supporting this process

DNA replication has been well studied in prokaryotes primarily because of the small size of the genome and because of the large variety of mutants that are available. *E. coli* has 4.6 million base pairs in a single circular chromosome and all of it gets replicated in approximately 42 minutes, starting from a single site along the chromosome and proceeding around the circle in both directions. This means that approximately 1000 nucleotides are added per second. Thus, the process is quite rapid and occurs without many mistakes.

DNA replication employs a large number of structural proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme **DNA polymerase**, also known as DNA pol, which adds nucleotides one-by-one to the growing DNA chain that is complementary to the template strand. The addition of nucleotides requires energy; this energy is obtained from the nucleoside triphosphates ATP, GTP, TTP and CTP. Like ATP, the other **NTPs** (nucleoside triphosphates) are high-energy molecules that can serve both as the source of DNA nucleotides and the source of energy to drive the polymerization. When the bond between the phosphates is "broken," the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain. In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I is an important accessory enzyme in DNA replication, and along with DNA pol II, is primarily required for repair.

How does the replication machinery know where to begin? It turns out that there are specific nucleotide sequences called *origins of replication* where replication begins. In *E. coli*, which has a single origin of replication on its one chromosome (as do most prokaryotes), this origin of replication is approximately 245 base pairs long and is rich in AT sequences. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called *helicase* unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called *replication forks* are formed. Two replication forks are formed at the origin of replication and these get extended bi-directionally as replication proceeds. **Single-strand binding proteins** coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

DNA polymerase has two important restrictions: it is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be only extended in this direction). It also requires a free 3'-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3'-OH group is not available. Then how does it add the first nucleotide? The problem is solved with the