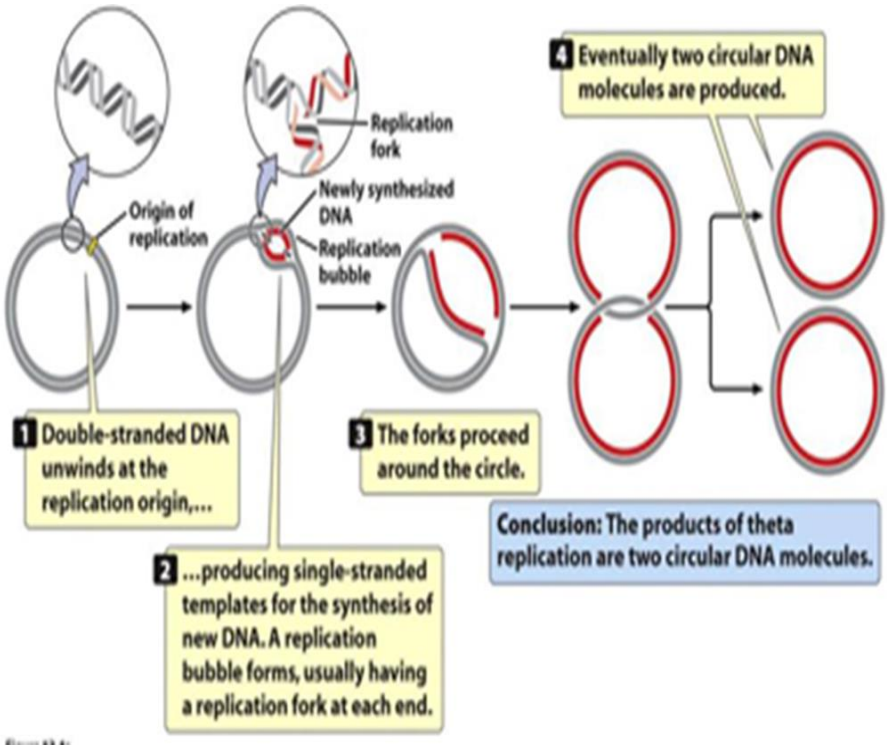
Mode of replication

Individuals unit of replication are called replicons .each of which contains a replication origin. Bacterial chromosomes have a single replication origin whereas eukaryotic chromosomes contain many or multiple origin.

Theta replication : a common type of replication that take place in circular DNA , such as that found in E.coli and other bacteria, is called theta replication. Double-stranded DNA begins to unwind at the replication origin, producing single-stranded nucleotide strands that then serve as templates on which new DNA can be synthesised. The unwinding of the double helix generates a loop , termed a replication bubble. The point of unwinding , where the two single nucleotides strands separate from the double stranded DNA helix is called a replication fork. If there are two replication forks, one at each end of the replication bubble, the forks proceed outward in both directions on a process called bidirectional replication, simultaneously unwinding and replicating the DNA until they eventually meet.



Liner eukaryotic replication

The larger linear chromosomes in eukaryotic cells , however , contain far too much DNA to be replicated speedily from a single origin. Eukaryotic replication proceed at a rate ranging from 500 to 5000 nucleotides per minute at each replication fork(considerably slower than bacterial replication). Typical eukaryotic replicons are from 20,000 to 300,000 base pairs in length. At each replication origin, the DNA unwinds and produces a replication bubble. Replication takes place on both strands at each end of the bubble. With the two replication forks spreading outward.

Eventually , replication forks of adjacent replicons run in to each other, and the replicons fuse to form long stretches of newly synthesized DNA

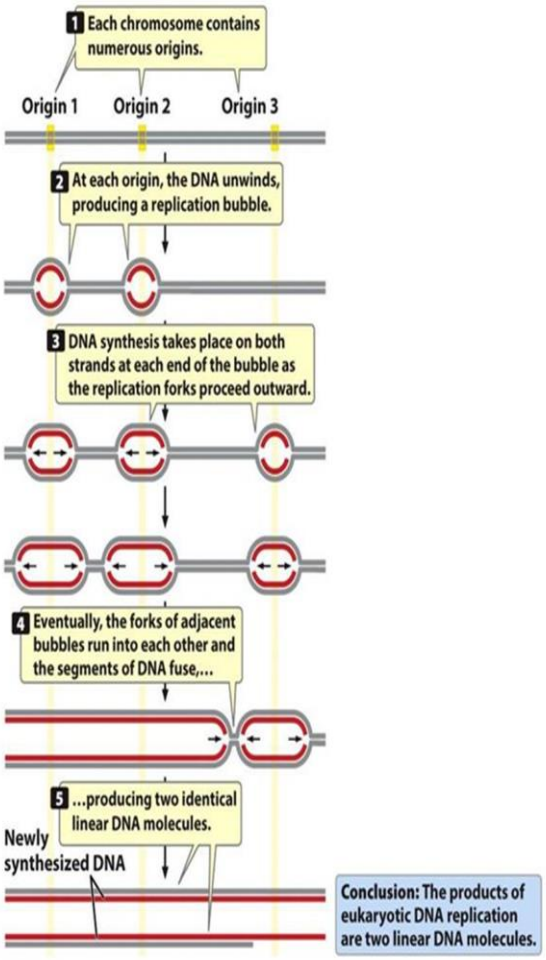
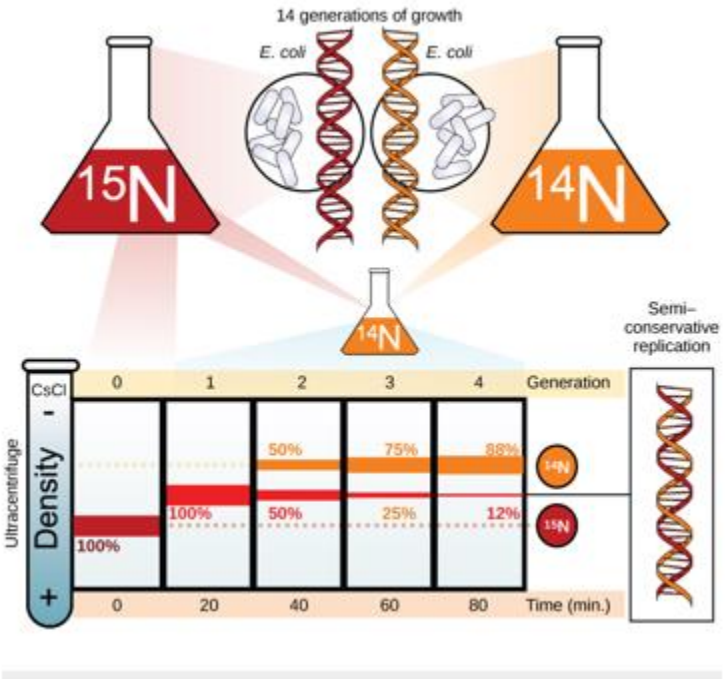


Figure 12.6
Genetics: A Conceptual Approach, Fifth Edition

Meselson and Stahl experament

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 (15N), which gets incorporated into nitrogenous bases, and eventually into the DNA



Meselson and Stahl experimented with E. coli grown first in heavy nitrogen (15N) then in 14N. DNA grown in 15N (red band) is heavier than DNA grown in 14N (orange band), and sediments to a lower level in cesium chloride solution in an ultracentrifuge

The E. coli culture was then placed into medium containing 14N 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 15N will form a band at a higher density position (i.e., farther down the centrifuge tube) than that grown in 14N. Meselson and Stahl noted that after one generation of growth in 14N after they had been shifted

from 15N, the single band observed was intermediate in position in between DNA of cells grown exclusively in 15N and 14N. This suggested either a semi-conservative or dispersive mode of replication. The DNA harvested from cells grown for two generations in 14N formed two bands: one DNA band was at the intermediate position between 15N and 14N, and the other corresponded to the band of 14N 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.

DNA replication in prokaryotes

DNA replication has been extremely well studied in prokaryotes primarily because of the small size of the genome and the 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 minuits starting from a single origin of replication proceeding around the circle in both directions. This means that approximately 1000 nucleotides are added per second. The process is quite rapid and occurs without many mistakes

DNA replication employs a large number of proteins and enzymes each of which plays a critical role during the processes. 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 are complementary to the template strand.

The addition of nucleotides requires energy, this energy is obtained from the nucleotides that have three phosphates attached to them , similar to ATP which has three phosphate groups attached. 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 ans DNA polIII. It is now known that DNA polIII is the enzyme required for DNA synthesis, DNA polI and DNA polII are primarily required for repair.

There are specific nucleotides 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) , it is approximately 245 base pairs long and is rich in AT sequence. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called helicase unwind the DNA by breaking the hydrogen bonds between the nitrogenous base pairs.ATP hydrolysis is required for this process. As the DNA opens, Y-shaped structures called replication forks are formed. Two replication forks are formed at the origin of replication and these get extended bi-directionally are 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 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 make 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 help of a primer that provides the free3-OH end.

Another enzyme, RNA primase, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA. Because this sequence primes the DNA synthesis, it is appropriately called the primer.DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand.

The replication fork moves at the rate of 1000 nucleotides per second.DNA polymerase can only extend in the 5 to 3 direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel, that is one strand is in the 5 to 3 direction and the other is oriented in the 3 to 5 direction.

One strand which is complementary to the 3 to 5 parental DNA strand is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. This continuously synthesized strand is

known as the leading strand. The other strand, complementary to the 5 to 3 parental DNA, is extended away from the replication fork, in small fragments known as Okazaki fragments, each requiring a primer to start the synthesis. The strand with the Okazaki fragments is known as the lagging strand

The leading strand can be extended by one primer alone , whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall directions of the lagging strand will be 3 to 5 and that of the leading strand 5 to 3.

A protein called the sliding clamp holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place.

Topoisomerase prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up, it does so by causing temporary nicks in the DNA helix and then resealing it.as synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA polI , and the gaps are filled in by deoxyribonucleotides. The nicks that remain between the newly synthesized DNA and the previously synthesized DNA are sealed by the enzyme DNA ligase that catalyses the formation of phosphodiester linkage between the 3-OH end of one nucleotides and the 5 phosphate end of the other fragment,

Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division