CHAPTER 3
Molecular Genetics DNA Replication
- Watson and Crick DNA model implies a mechanism for replication:
  - a. Unwind the DNA molecule.
  - b. Separate the two strands.
  - c. Make a complementary copy for each strand.
Three possible models were proposed for DNA replication: Conservative model, the dispersive model and the semiconservative model.

- **Conservative model** proposed both strands of one copy would be entirely old DNA, while the other copy would have both strands of new DNA.
- Dispersive model was that dsDNA might fragment, replicate dsDNA, and then reassemble, creating a mosaic of old and new dsDNA regions in each new chromosome.

- Semiconservative model is that DNA strands separate, and a complementary strand is synthesized for each, so that sibling strands have one old and one new strand.
Review

a) The semiconservative model

Parental

First generation

Second generation

b) The conservative model

Parental

First generation

Second generation

c) The dispersive model

Parental

First generation

Second generation
Meselson-Stahl Experiment

Meselson and Stahl (1958) grew *E. coli* in a heavy (not radioactive) isotope of nitrogen, 15N. Because it is heavier, the DNA made by the bacteria containing 15N is more dense than DNA with normal 14N, and so can be separated by CsCl density gradient centrifugation.
Once the *E. coli* were labeled with heavy 15N, the researchers shifted the cells to medium containing normal 14N, and took samples at time points. DNA was extracted from each sample and analyzed in CsCl density gradients.
After one replication cycle in normal 14N medium, all DNA had density intermediate between heavy and normal.
After two replication cycles, there were two bands in the density gradient, one at the intermediate position, and one at the position for DNA containing entirely 14N.
One more time...
Results compared with the three proposed models:

a. Does not fit conservative model, because after one generation there is a single intermediate band, rather than one with entirely 15N DNA (an entire new band) and another with entirely 14N (the old band) DNA.

b. The dispersive model predicted that a single band of DNA of intermediate density would be present in each generation, gradually becoming less dense as increasing amounts of 14N were incorporated with each round of replication. Instead, Meselson and Stahl observed two bands of DNA, with the intermediate form decreasing over time.

c. The semiconservative model fits the data very well.
After Watson and Crick presented the double helix model of DNA, a major question still remained: how does DNA replication actually occur at the molecular level? Three models for DNA replication were proposed: conservative, semi-conservative and dispersive. In the conservative model the two parental DNA strands remain together during replication, so that one progeny molecule contains both parental DNA strands, and the other progeny molecule contains only newly synthesized DNA.
Basic concepts to understand replication.

1. DNA Polymerases, the DNA Replicating Enzymes.

2. How do the DNA polymerases work?

3. A large team of other enzymes and other proteins are needed to carry out DNA replication.
1. DNA Polymerases, the DNA Replicating Enzymes

- In 1955 Arthur Kornberg isolated an enzyme responsible for the synthesis of DNA. This enzyme was originally called the Kornberg enzyme, now known as DNA polymerase I.

- Kornberg also showed that in addition to the enzyme there were several components necessary for DNA synthesis. His reaction mixture included:
  - A DNA fragment to work as a template.
  - All four dNTPs (dATP, dGTP, dTTP, and dCTP).
  - Mg2+ ions.
  - DNAse polymerase I.
2. Roles of DNA Polymerases, how do they work.

All DNA polymerases link dNTPs into DNA chains (Figure 3.4). Main features of the reaction:

An incoming nucleotide is attached by its 5’-phosphate group to the 3’-OH of the growing DNA chain. Energy comes from the release of two phosphates from the dNTP. The DNA chain acts as a primer for the reaction.

The nucleotide is selected by its ability to hydrogen bond with the complementary base in the template strand. The process is fast and accurate. DNA polymerases synthesize only from 5’ to 3’
A better look

New Growing Strand

Template Strand

DNA polymerase

b) Shorthand notation
3. A large team of other enzymes and other proteins are needed to carry out DNA replication.

- **DNA Polymerase I and III** (polymerase and exonuclease activity).
- **Initiator Proteins** binds to Ori C (IHF and FIS).
- **DNA Gyrase** (type two topoisomerase) relaxes supercoiled DNA.
- **DNA Helicase** untwists the DNA separating the double strand.
- Single stranded binding proteins bind to the DNA strands keeping it untwisted and stabilizing it.
- **DNA Primase** binds to helicase forming a complex: Primosome. The DNA primase forms an RNA fragment or template, known as “the primer” (11+-1 nucleotides). It starts with two purine bases, most commonly AG. DNA polymerase III begins synthesizing the DNA.
- **DNA polymerase III** can only DNA only 5 to 3.
- **TBP ter binding protein**
<table>
<thead>
<tr>
<th>Gene Product or Function</th>
<th>Gene</th>
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<tbody>
<tr>
<td>DNA polymerase I</td>
<td>polA</td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td>dnaE, dnaQ, dnaX, dnaN, dnaD holA→E</td>
</tr>
<tr>
<td>Initiator protein; binds to oriC</td>
<td>dnaA</td>
</tr>
<tr>
<td>IHF protein (DNA binding protein); binds to oriC</td>
<td>himA</td>
</tr>
<tr>
<td>FIS protein (DNA binding protein); binds to oriC</td>
<td>fis</td>
</tr>
<tr>
<td>Helicase and activator of primase</td>
<td>dnaB</td>
</tr>
<tr>
<td>Complexes with dnaB protein and delivers it to DNA</td>
<td>dnaC</td>
</tr>
<tr>
<td>Primase; makes RNA primer for extension by DNA polymerase III</td>
<td>dnaG</td>
</tr>
<tr>
<td>Single-stranded binding (SSB) proteins; bind to unwound single-stranded arms of replication forks</td>
<td>ssb</td>
</tr>
<tr>
<td>DNA ligase; seals single-stranded gaps</td>
<td>lig</td>
</tr>
<tr>
<td>Gyrase (type II topoisomerase); replication swivel to avoid tangling of DNA as replication fork advances</td>
<td>gyrA, gyrB</td>
</tr>
<tr>
<td>Origin of chromosomal replication</td>
<td>oriC</td>
</tr>
<tr>
<td>Terminus of chromosomal replication</td>
<td>ter</td>
</tr>
<tr>
<td>TBP (ter binding protein); stalls replication forks</td>
<td>tus</td>
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Initiation of Replication

1. The replication of a DNA molecule begins at special sites, **origins of replication**. In bacteria, this is a single specific sequence of nucleotides that is recognized by the replication enzymes. *E. coli* has one origin, **oriC**, which has:
   
   a. A minimal sequence of about 245 bp required for initiation.
   
   b. Three copies of a 13-bp AT-rich sequence.
   
   c. Four copies of a 9-bp sequence.
2. Initiator proteins **DnaA** attaches to the 9 bp area.

3. DNA helicase binds to initiator proteins **DnaA** on the DNA, and denatures the AT-rich region using ATP as an energy source.

4. Helicase denatures the DNA.

5. Single-strand DNA-binding proteins (SSBs) bind the ssDNA formed by helicase, preventing reannealing.
6. The Helicase binds to the primase enzyme to form a primosome, which synthesizes a short (5–10 nucleotides) RNA primer. The primer is used as a starting point for the DNA polymerase to begin the synthesis of DNA.
RNA PRIMER
(RNA nucleotides)

Helicase + Primase = Primosome

DNA NUCLEOTIDE

DNA POLYMERASE
Semidiscontinuous DNA Replication

- When DNA denatures at the ori, replication forks are formed. DNA replication is usually bidirectional, but we will look at the events at just one replication fork (Figure 3.6).

  - Single-strand DNA-binding proteins (SSBs) bind the ssDNA formed by helicase, preventing reannealing.
b. Primase synthesizes a RNA primer on each template strand.

c. DNA polymerase III adds nucleotides to the 3’ end of the primer, synthesizing a new strand complementary to the template, and displacing the SSBs. DNA is made in opposite directions on the two template strands.

d. The Helicase denaturing the DNA causes tighter winding in other parts of the circular chromosome. Gyrase relieves this tension.
lagging strand synthesis
5' 3'
3' 5'
leading strand synthesis
parental strand
new strand
RNA primer
Elongation

- The new strand made in the 5’ 3’ direction as movement of the replication fork is the **leading strand**, while new strand made in the opposite direction (in fragments) is the **lagging strand**. Leading strand needs only one primer, while lagging needs a series of primers.

- The leading strand is synthesized **continuously**, while lagging strand is synthesized **discontinuously**, in the form of Okazaki fragments. DNA replication is therefore **semidiscontinuous**.
Each okasaki fragment requires an RNA primer to begin, and is extended by DNA polymerase III.
These fragments are gradually joined together. First, the DNA polymerase I uses the 3’-OH (remember the 3’ ending does not have a phosphate) of the adjacent DNA fragment as a primer, and simultaneously removes the RNA primer while “refilling” the primer region in the form of DNA. This is called exonuclease activity. The fragments are then bonded by DNA ligase (Figure 3.7).
Replication of circular DNA.

Some circular chromosomes (e.g., *E. coli*) are circular throughout replication, creating a theta-like (θ) shape. As the strands separate on one side of the circle, positive supercoils form elsewhere in the molecule.

2. Topoisomerases relieve the supercoils, allowing the DNA strands to continue separating as the replication forks advance.
DNA replication in Eukaryotic cells.

- DNA replication is very similar in both prokaryotes and eukaryotes, except that eukaryotes have more than one chromosome.
- Eukaryotic chromosomes contain multiple origins, at which DNA denatures and replication then proceeds bidirectionally until an adjacent replication fork is encountered. The DNA replicated from a single origin is called a replicon, or replication unit (Figure 3.12).
(b) A micrograph of a replicating, eukaryotic chromosome
In eukaryotes, replicon size is smaller than it is in prokaryotes, replication is slower, and each chromosome contains many replicons. Number and size of replicons vary with cell type.

Not all origins within a genome initiate DNA synthesis simultaneously. Cell-specific patterns of origin activation are observed, so that chromosomal regions are replicated in a predictable order in each cell cycle (Figure 3.13).
Eukaryotic origins of replication are generally not well characterized; those of the yeast *Saccharomyces cerevisiae* are among the best understood.

In yeast we find chromosomal DNA fragments (about 100bp) that are known as ARSs (autonomously replicating sequences).

ARSs are yeast replicators.

DNA replication in other eukaryote cells is not very well understood.

DNA and histones must be doubled in each cell cycle. G1 prepares the cell for DNA replication, chromosome duplication occurs during S phase, G2 prepares for cell division, and segregation of progeny chromosomes occurs during M phase, allowing the cell to divide.

6. Cell cycle control is complex, and only outlined here. Yeasts, in which chromosomal replication is well studied, serve as a eukaryotic model organism.
In eukaryotic cells DNA and histones must be doubled in each cell cycle. G1 prepares the cell for DNA replication, chromosome duplication occurs during S phase, G2 prepares for cell division, and segregation of progeny chromosomes occurs during M phase, allowing the cell to divide.

The same steps described for DNA replication in prokaryotic cells seem to be the same for eukaryotic cells: denaturation, semiconservative and semidiscontinuous replication, however less is known about the enzymes and proteins involved in eukaryotic DNA replication.
Three DNA polymerases are used to replicate nuclear DNA: Pol α (alpha), Pol δ (delta) and Pol ε (epsilon) are involved in the DNA replication. Other DNA pols replicate mitochondrial or chloroplast DNA, or are used in DNA repair.

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<tr>
<th>Eukaryotic DNA Polymerases</th>
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<tr>
<td><strong>Polymerase Types</strong></td>
</tr>
<tr>
<td>α, δ, ε</td>
</tr>
<tr>
<td>γ</td>
</tr>
<tr>
<td>η, κ, λ, ζ</td>
</tr>
<tr>
<td>(lesion-replicating polymerases)</td>
</tr>
<tr>
<td>α, β, δ, ε, σ, λ, μ, φ, θ</td>
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Limitations in the DNA polymerase create problems for the linear DNA of eukaryotic chromosomes. The usual replication machinery provides no way to complete the 5’ ends of daughter DNA strands. When the ends of chromosomes are replicated and the primers are removed from the 5’ ends, there is no adjacent DNA strand to serve as a primer, and so a single-stranded region is left at the 5’ end of the new strand. Repeated rounds of replication produce shorter and shorter DNA molecules.
Parental DNA

- RNA priming and DNA synthesis

  - Leading strand
  - RNA primer

- Removal of primers and filling of gaps with DNA where a 3’ end is available

  - Gap remains unfilled

- Further rounds of replication

  - Shorter and shorter daughter molecules
If the gap is not addressed, chromosomes would become shorter with each round of replication (Figure 3.14). Blackburn and Greider have shown that Eukaryotic cells have evolved a mechanism to restore shortened telomeres and chromosome lengths are maintained by the action of the enzymes telomerases.

Telomerase is an RNA-Dependent DNA Polymerase That Carries Its Own Template.
The ends of eukaryotic chromosomal DNA molecules, the telomeres, have special nucleotide sequences.

In human telomeres, this sequence is typically TTAGGG, repeated between 100 and 1,000 times.

Telomeres protect genes from being eroded through multiple rounds of DNA replication.
- **Telomerase** uses a short molecule of RNA as a template to extend the 3’ end of the telomere.

- There is now room for primase and DNA polymerase to extend the 5’ end.

- It does not repair the 3’-end “overhang,” but it does lengthen the telomere.
An excellent animation showing chromatin formation can be found following the link or pasting the link on your browser.

Chromosome coiling animation.
(http://www.biostudio.com/demo_freeman_dna_coiling.htm)