1.3.1: DNA replication

Learning Objectives

- Summarize the process of DNA replication, including bidirectional synthesis and explain the Meselson-Stahl experiment that demonstrated semiconservative replication.
- Recall that sister chromatids are identical copies of each other produced during S phase.
- Explain how telomerase replicates ends of chromosomes and the cellular significance of chromosome shortening, addressing the specific function of telomerase RNA.

DNA Replication

When the cell enters S (synthesis) phase in the cell cycle (G1-S-G2-M) all the chromosomal DNA is replicated. Enzymes called DNA polymerases synthesize new strands by adding nucleotides to the 3'-OH group present on the previous nucleotide. For this reason, they are said to work in a 5' to 3' direction. DNA polymerases use a single strand of DNA as a template upon which it will synthesize the complementary sequence. This produces two new double-stranded molecules from one double helix. But how are the new and old strands distributed? The answer to this question was elucidated by classic experiments by Meselson and Stahl.

Experiment that demonstrated semiconservative DNA replication

For an overview of the experiment, watch:
Now, listen to the following story about these classic experiments by one of the scientists involved:
The Mechanisms of Replication

Like many molecular events we will study, replication can be divided into three stages: initiation, elongation, and termination.

Initiation

DNA replication in both prokaryotes and eukaryotes begins at an **Origin of Replication** (Ori). Origins are specific sequences on specific positions on the chromosome. In *E. coli*, the OriC origin is ~245 bp in size. Chromosome replication begins with the binding of the DnaA initiator protein to an AT-rich 9-mer in OriC and melts (disrupts the hydrogen bonding between) the two strands.

In prokaryotes, with a small, simple, circular chromosome, only one origin of replication is needed to replicate the whole genome. For example, *E. coli* has a ~4.5 Mb genome (chromosome) that can be duplicated in ~40 minutes assuming a single origin, bi-directional replication, and a speed of ~1000 bases/second/fork for the polymerase. At least five prokaryotic DNA polymerases have been discovered to date. The primary DNA polymerase for replication in *E. coli* is DNA Polymerase III (Pol III). Pol I is also involved in the basic mechanism of DNA replication, primarily to fill in gaps created during lagging strand synthesis (defined 3 pages ahead) or through error-correcting mechanisms. DNA polymerase II and the recently discovered Pol IV and Pol V do not participate in chromosomal replication, but rather are used to synthesize DNA when certain types of repair is needed at other times in the cellular life cycle.

Thinking about sequences
Why would an initiator binding site in the OriC be an AT-rich region?

However, in larger, more complicated eukaryotes, with multiple linear chromosomes, more than one origin of replication is required per chromosome to duplicate the whole chromosome set in the 8-hours of S-phase of the cell cycle. For example, the human diploid genome has 46 chromosomes ($6 \times 10^9$ basepairs). The shortest chromosomes are ~50 Mbp long and so could not possibly be replicated from one origin. Additionally, the rate of replication fork movement is slower, only ~100 base/second. Thus, eukaryotes contain multiple origins of replication distributed over the length of each chromosome to enable the duplication of each chromosome within the observed time of S-phase (Fig 2.9).

![Figure 2.9: Part of a eukaryote chromosome showing multiple Origins (1, 2, 3) of Replication, each defining a replicon (1, 2, 3). Replication may start at different times in S-phase. Here #1 and #2 begin first then #3. As the replication forks proceed bi-directionally, they create what are referred to as “replication bubbles” that meet and form larger bubbles. The end result is two semi-conservatively replicated duplex DNA strands. (Original-Locke-CC:AN)](https://bio.libretexts.org/Courses/University_of_Arkansas_Little_Rock/BIOL3300_Genetics/01%3A_Chemistry_to_Chromoso...)

**Elongation**

As DNA polymerase proceeds along the template, the nucleotide that base pairs with each base on the template is covalently bonded to the 3' end of the growing strand. Note that the energy is provided by the nucleotide triphosphate itself; two phosphates are released and one phosphate remains as a part of the phosphodiester bond.

**Termination**

In prokaryotes, elongation proceeds bidirectionally until the replication forks meet. RNA primers are removed by a specialized DNA polymerase and then DNA is synthesized in their place. The resulting DNA fragments are then "sealed" together with DNA ligase.

In eukaryotes, replication also proceeds bidirectionally until adjacent forks meet, or the fork encounters the end of the chromosome.

**Limitations of the 5'-to-3' activity of DNA polymerase**

Recall that enzymes are specific to their substrates. In the case of DNA polymerase, the structure only allows it to add
nucleotides to the 3' end of existing DNA, which presents some questions:

1. If the enzyme can only add nucleotides to existing DNA, how will it get started?

2. Because DNA is double stranded, each strand needs to be used as a template, but these strands are antiparallel. How can one complex make new DNA in opposite directions?

3. How will the 3’ end be replicated when there is no longer a place for a primer on the complementary strand?

Obstacle #1: How to begin

Once oriC has been opened and the helicases have attached to the two sides of the replication fork, the replication machine, aka the replisome can begin to form. However, before the DNA polymerases take positions, they need to be primed. DNA polymerases are unable to join two individual free nucleotides together to begin forming a nucleic acid; they can only add onto a pre-existing strand of at least two nucleotides. Therefore, a specialized RNA polymerase (RNAP’s do not have this limitation) known as primase is a part of the replisome, and reads creates a short RNA strand termed the primer for the DNA polymerase to add onto. Although only a few nucleotides are needed, the prokaryotic primers may be as long as 60 nt depending on the species. The helicase will continue to travel in front of the fork to unwind new DNA and allow primase to add new primers as needed.

Obstacle #2: Make two strands in opposite directions at the same time

Because DNA is being unwound in the direction of fork movement, both strands need to be synthesized in the unwound region at the same time. The two subunits that are adding nucleotides are actually tethered together, so they cannot travel in opposite directions.
Helicase opens up the double stranded DNA and leads the rest of the replication machine along. So, in the single-stranded region trailing the helicase, if we look left to right, one template strand is 3’ to 5’ (in blue), while the other is 5’ to 3’ (in red). Since we know that nucleic acids are polymerized by adding the 5’ phosphate of a new nucleotide to the 3’ hydroxyl of the previous nucleotide (5’ to 3’, in green), this means that one of the strands, called the leading strand, is being synthesized in the same direction that the replication machine moves. No problem there.

The other strand is problematic: looked at linearly, the newly synthesized strand would be going 3’ to 5’ from left to right, but DNA polymerases cannot add nucleotides that way. How do cells resolve this problem? A number of possibilities have been proposed, but the current model is depicted here. The replication machine consists of the helicase, primases, and two DNA polymerase III holoenzymes moving in the same physical direction (following the helicase). In fact, the pol III complexes are physically linked through τ subunits.

In order for the template strand that is 5’ to 3’ from left to right to be replicated, the strand must be fed into the...
polymerase backwards. This can be accomplished either by turning the polymerase around or by looping the DNA around. As the Figure shows, the current model is that the primase is also moving along left to right, so it has just a short time to quickly synthesize a short primer before having to move forward with the replisome and starting up again, leaving intermittent primers in its wake. Because of this, Pol III is forced to synthesize only short fragments of the chromosome at a time, called Okazaki fragments after their discoverer. Pol III begins synthesizing by adding nucleotides onto the 3’ end of a primer and continues until it hits the 5’ end of the next primer. It does not (and can not) connect the strand it is synthesizing with the 5’ primer end.

DNA replication is called a semi-discontinuous process because while the leading strand is being synthesized continuously, the lagging strand is synthesized in fragments. This leads to two major problems: first, there are little bits of RNA left behind in the newly made strands (just at the 5’ end for the leading strand, in many places for the lagging); and second, Pol III can only add free nucleotides to a fragment of single stranded DNA; it cannot connect another fragment. Therefore, the new “strand” is not whole, but riddled with missing phosphodiester bonds.

The first problem is resolved by DNA polymerase I. Unlike Pol III, Pol I is a monomeric protein and acts alone, without additional proteins. There are also 10-20 times as many Pol I molecules as there are Pol III molecules, since they are needed for so many Okazaki fragments. DNA Polymerase I has three activities: (1) like Pol III, it can synthesize a DNA strand based on a DNA template, (2) also like Pol III, it is a 3’-5’ proofreading exonuclease, but unlike Pol III, (3) it is also a 5’-3’ exonuclease. The 5’-3’ exonuclease activity is crucial in removing the RNA primer. The 5’-3’ exonuclease binds to double-stranded DNA that has a single-stranded break in the phosphodiester backbone such as what happens after Okazaki fragments have been synthesized from one primer to the next, but cannot be connected. This 5’-3’ exonuclease then removes the RNA primer. The polymerase activity then adds new DNA nucleotides to the upstream Okazaki fragment, filling in the gap created by the removal of the RNA primer. The proofreading exonuclease acts just like it does for Pol III, immediately removing a newly incorporated incorrect nucleotide. After proofreading, the overall error rate of nucleotide incorporation is approximately 1 in $10^7$.

Even though the RNA has been replaced with DNA, this still leaves a fragmented strand. The last major player in the DNA replication story finally appears: DNA ligase. This enzyme has one simple but crucial task: it catalyzes the attack of the 3’-OH from one fragment on the 5’ phosphate of the next fragment, generating a phosphodiester bond.

See the whole complex in action in this animation:
Obstacle #3: How to copy the end?

The ends of linear chromosomes present a problem – at each end one strand cannot be completely replicated because there is no primer to extend. Although the loss of such a small sequence would not be a problem, the continued rounds of replication would result in the continued loss of sequence from the chromosome end to a point were it would begin to loose essential gene sequences. Thus, this DNA must be replicated. Most eukaryotes solve the problem of synthesizing this unreplicated DNA with a specialized DNA polymerase called telomerase, in combination with a regular polymerase. Telomerases are RNA-directed DNA polymerases. They are a riboprotein, as they are composed of both protein and RNA. These enzymes contain a small piece of RNA that serves as a portable and reusable template from which the complementary DNA is synthesized. The RNA in human telomerases uses the sequence 3-AAUCCC-5’ as the template, and thus our telomere DNA has the complementary sequence 5’-TTAGGG-3’ repeated over and over 1000’s of times. After the telomerase has made the first strand a primase synthesizes an RNA primer and a regular DNA polymerase can then make a complementary strand so that the telomere DNA will ultimately be double stranded to the original length. Note: the number of repeats, and thus the size of the telomere, is not set. It fluctuates after each round of the cell cycle. Because there are many repeats at the end, this fluctuation maintains a length buffer – sometimes it’s longer, sometimes it’s shorter – but the average length will be maintained over the generations of cell replication.
Figure \(\PageIndex{5}\): Telomere replication showing the completion of the leading strand and incomplete replication of the lagging strand. The gap is replicated by the extension of the 3' end by telomerase and then filled in by extension of an RNA primer. (Original-Locke-CC:AN)

In the absence of telomerase, as is the case in human somatic cells, repeated cell division leads to the "Hayflick limit", where the telomeres shorten to a critical limit and then the cells enter a senescence phase of non-growth. The activation of telomerase expression permits a cell and its descendants to become immortal and bypass the Hayflick limit. This happens in cancer cells, which can form tumors as well as in cells in culture, such as HeLa cells, which can be propagated essentially indefinitely. HeLa cells have been kept in culture since 1951.

Contributors and Attributions

Todd Nickle and Isabelle Barrette-Ng Online Open Genetics

E.V. Wong, Cells - Molecules and Mechanisms (https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/Book%3A_Cells_-_Molecules_and_Mechanisms_(Wong)/7%3A_DNA/7.3%3A_Prokaryotic_Replication)