# DNA replication

When a cell divides, either as part of reproduction (in the case of prokaryotes) or as part of growth (in the case of multicellular eukaryotes) the genetic information needed to make and run the cell must also be divided into the two daughter cells. Since each cell typically contains all the information needed to make a complete organism, the information, ie. the DNA, must be completely replicated prior to cell division. Since the information is the instruction manual for creating and running the organism, it must be copied accurately. The basic principles involved in DNA replication are the same for prokaryotes and eukaryotes, however the enzymes have different names and work at different speeds. (Be careful of the use of Greek letters alpha - epsilon in both prokaryotes and eukaryotes to refer to different protein complexes.) The initiation and termination of genome replication are somewhat different in each. You should understand the basics in both and keep in mind that there are variations that we will not cover, notably mitochondrial genomes and viruses, which deviate widely from the standard model. During DNA replication, the machinery is almost completely unconcerned with the information content of the DNA that is being replicated. It is the difference between photocopying an article and reading it. Note that the DNA sequence is not the only thing that must be replicated. Epigenetic information, patterns of DNA methylation, and in eukaryotes, a nucleosome pattern, must also be replicated.

Reading:

**MBoC(6th) Ch5**: *DNA REPLICATION MECHANISMS* and *THE INITIATION AND COMPLETION OF DNA REPLICATION IN CHROMOSOMES*.

There is more material covered in the chapters than you need to know. Use the text and illustrations below to help focus.

Need to know and understand

- The replication fork and the enzymes active in running it
- DNA-dependent DNA polymerases, several involved in both eukaryotes and prokaryotes
- direction of polymerization - DNA grows at 3' ends only
- Basic requirements - template, primer, DNA polymerase, dNTPs
- Processivity - average number of nucleotides a polymerase will add before falling off the DNA.
- origin of replication (ori)
- replicon - the length of DNA synthesized from one ori during one cycle of replication
- Okazaki fragments
- replication fork
- bidirectional synthesis: two replication forks from single ori moving in opposite directions
- leading strand, lagging strand
- The basics are the same in both prokaryotes and eukaryotes, but initiation and termination are different. The enzymes involved have much the same function, but different names.

*E. coli* DNA replication

- 100,000 nt/min, 30 minutes to replicate the genome
- $10^{-6}$ - $10^{-8}$ error rate
- DNA dependent DNA polymerases (Pol I, II, III) and activities of each (odd combinations: Pol II - repair only, Pol I - 5' ->3' exonuclease).
- Initiation
  - ori - origin of replication (only one).
  - DnaA protein binding sites

DnaB helicase and DnaC - prepare open ss bubble at *ori*
SSB single stranded binding proteins stabilize the single stranded bubble
RNA primer added by DnaG Primase

Elongation

bidirectional
topoisomerases - makes sure the DNA remains relaxed and untangled. DNA gyrase is a Topoisomerase II
DNA helicase - open the DNA in front of the replication fork
SSBs stabilize the ssDNA
replisome - PolIII + helicase + primosome
Primosome - complex of Primase (DnaG) + helicase (DnaB) + other proteins, adds RNA primers at specific intervals
Pol III DNA polymerase holoenzyme adds complementary nucleotides to 3' end of growing DNA strand. It functions as a dimer at the replication fork - one for the leading strand, the other for the lagging strand. They are teathered together]. By functioning as an obligate dimer, the synthesis of the leading and lagging strand are synchronized.
Pol I DNA polymerase removes RNA primers (RnaseH also does this) and adds complementary nucleotides to 3' end of growing DNA strand.
*E.coli* Ligase ligates the nicks in the DNA

Eukaryotic DNA replication

Much slower, 500 -5000 nt/min
Much more accurate
Principles and processes are the same as in prokaryotes but enzymes have different names
6 DNA polymerases (α, β, γ, δ, ε). α, δ, ε required for nuclear DNA replication. γ is mitochondrial. β most active in non-mitotic cells (repair?).

Initiation

origin of replication - ARS (autonomously replicating sequence). Many on each chromosome, between 50-300 kb apart.
Many more polymerase molecules/cell (As a result, the genome can sometimes be replicated faster than *E.coli*, eg. the early *Drosophila* embryo genome is replicated in 3 minutes).
ARS + proteins binding in G1 phase of cell-cycle forms the ORC (origin recognition complex)
ORC + protein kinases form the pre-replication complex, which can bind DNA polymerase.
The kinase activity prevents the formation of new ORCs following replication but before next G1 phase.

Elongation

Primase lays down RNA primer.
Pol α synthesizes the first bits of DNA added to the RNA primer. ie. the very first part of the leading strand and the first parts of all the Okazaki fragments. Polymerase activity then switches to pol δ (the equivalent of pol III in the prokaryotes).
Accessory protein RFC (in mammals) is the clamp loading protein (equivalent to γ in the prokaryotes).
Accessory protein PCNA (in mammals) is the sliding clamp protein (equivalent to β in the prokaryotes). PCNA is used as a marker in cancer diagnosis. Antibodies against PCNA are found in the blood of patients with Systemic Lupus.

The problem of linear DNA ends

Impossible to synthesize ends of linear DNA by normal replication. The lagging strand is incomplete. So telomeres get shorter.

Telomeres are recovered by Telomerase, which is not active in most adult somatic cells.
Telomerase contains an internal RNA primer that binds to 3' end of chromosomal DNA and has an RNA-dependent DNA polymerase activity (reverse transcriptase) that adds to the free 3' end. After this, the lagging strand is extended by primase and polymerase followed by removal of primer.

DNA replication in Medicine. Genotoxins in Chemotherapy. Learn a bit about how they act on the genome - understanding more will come as you study DNA repair pathways and the cell cycle. Antibiotics that inhibit DNA synthesis in bacteria. Hmm, for now pretty much restricted to the quinolones, e.g. Ciprofloxacin. These are Big-Pharma derivatives of the original nalidixic acid.

Focus illustrations: Source: Molecular Biology of the Cell: Sixth Edition (2015) Alberts et al., Garland Science, NY

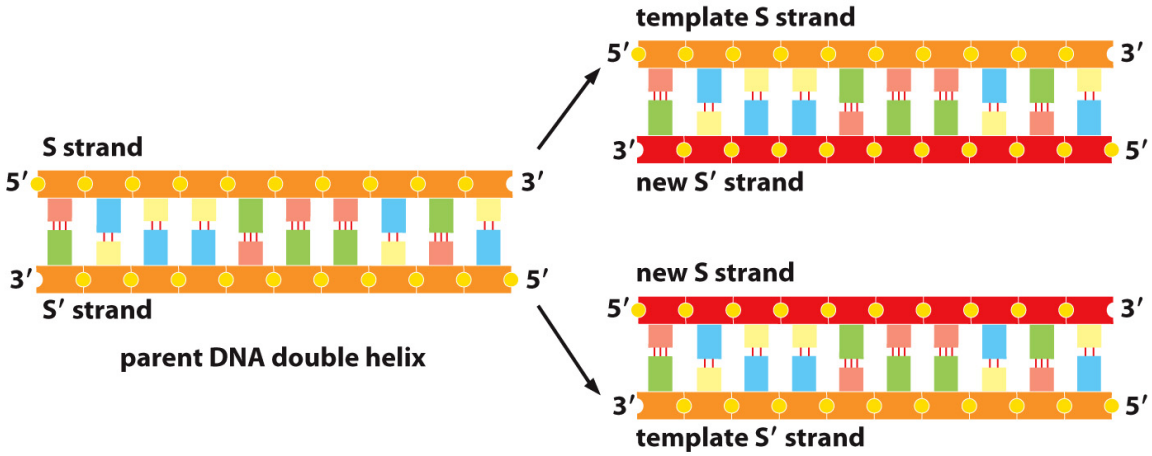


Figure 5-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–2 The DNA double helix acts as a template for its own duplication. Because the nucleotide A will pair successfully only with T, and G only with C, each strand of DNA can serve as a template to specify the sequence of nucleotides in its complementary strand by DNA base-pairing. In this way, a doublehelical DNA molecule can be copied precisely.

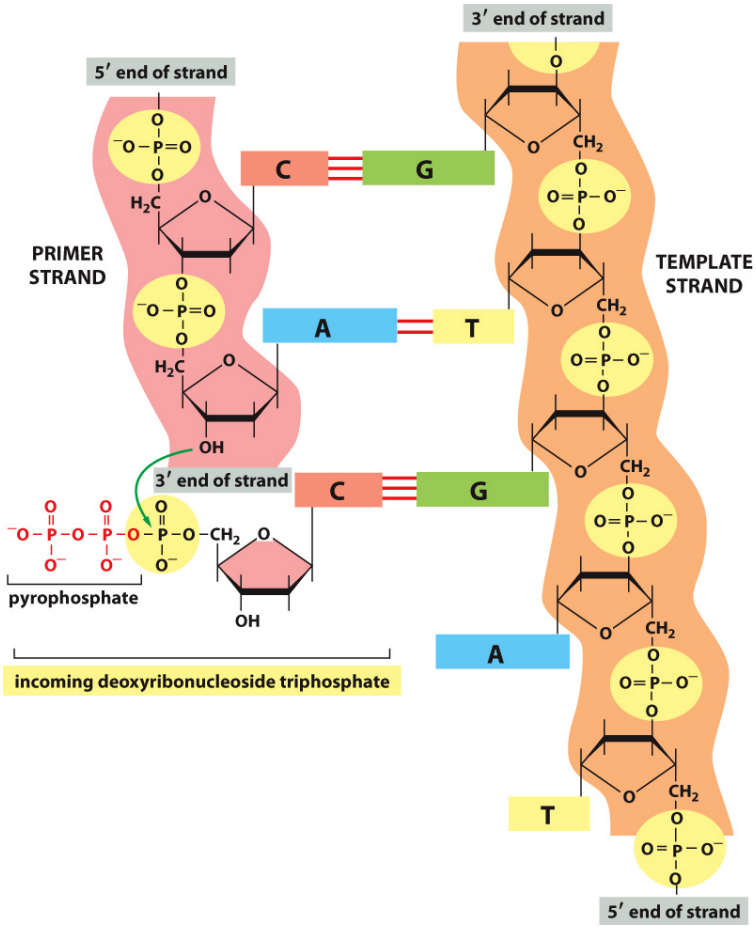


Figure 5-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–3 The chemistry of DNA synthesis. The addition of a deoxyribonucleotide to the 3′ end of a polynucleotide chain (the primer strand) is the fundamental reaction by which DNA is synthesized. As shown, base-pairing between an incoming deoxyribonucleoside triphosphate and an existing strand of DNA (the template strand) guides the formation of the new strand of DNA and causes it to have a complementary nucleotide sequence. The way in which complementary nucleotides base-pair is shown in Figure 4–4.

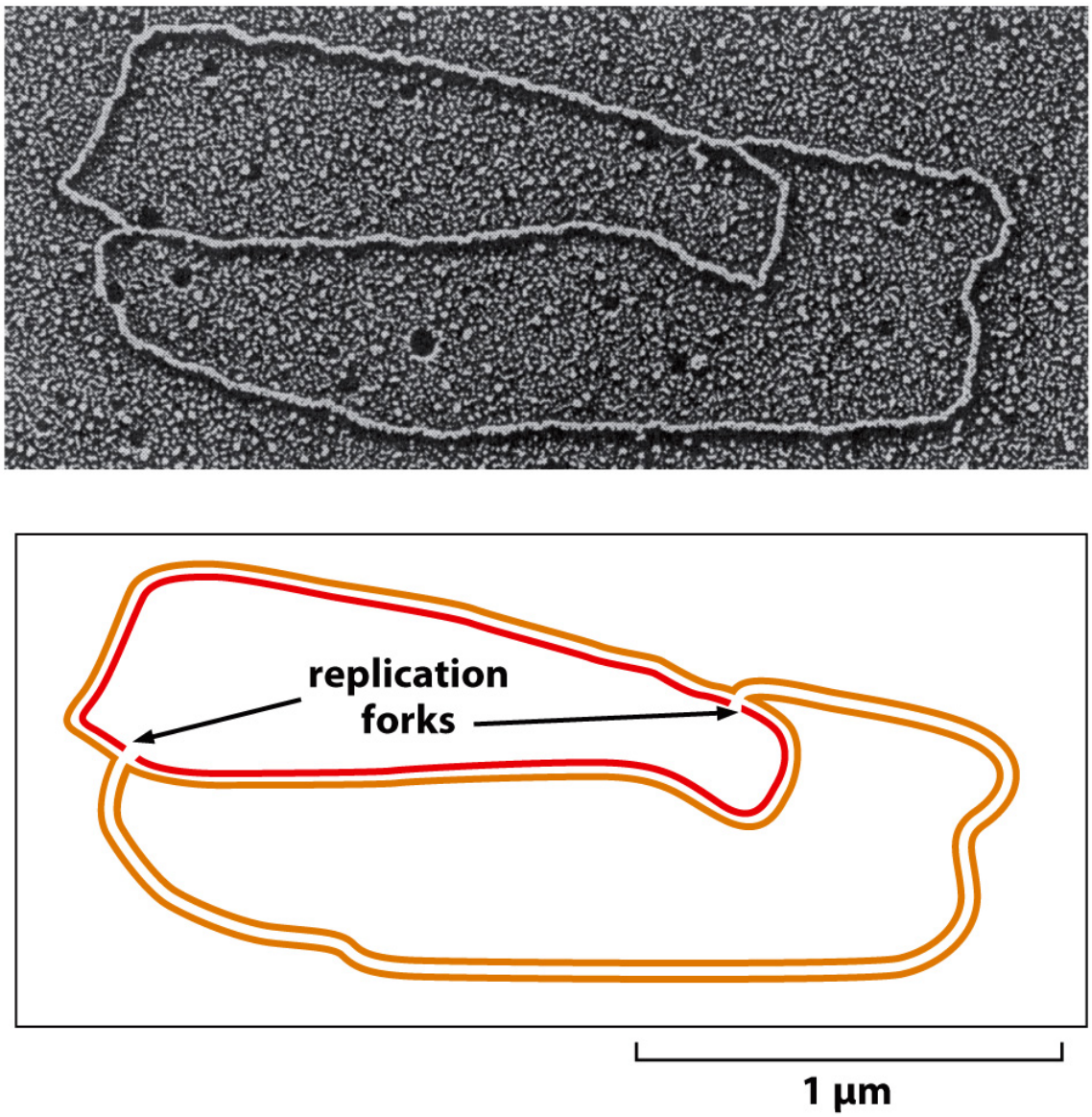


Figure 5-6 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–6 Two replication forks moving in opposite directions on a circular chromosome. An active zone of DNA replication moves progressively along a replicating DNA molecule, creating a Y-shaped DNA structure known as a replication fork: the two arms of each Y are the two daughter DNA molecules, and the stem of the Y is the parental DNA helix. In this diagram, parental strands are orange; newly synthesized strands are red.

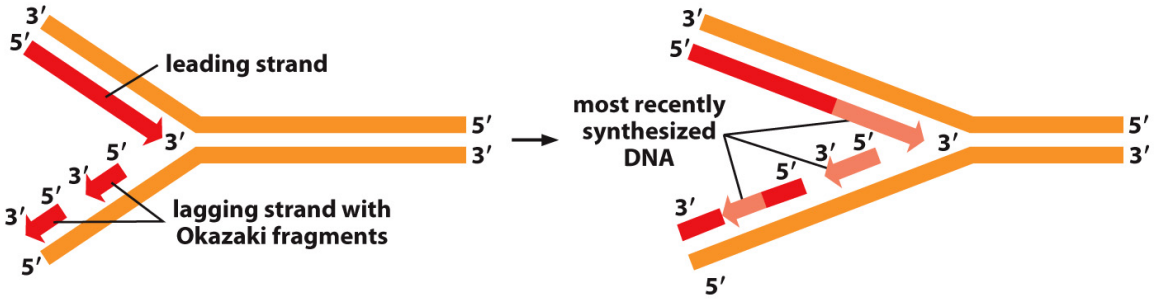


Figure 5-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–7 The structure of a DNA replication fork. Left, replication fork with newly synthesized DNA in red and arrows indicating the 5′-to-3′ direction of DNA synthesis. Because both daughter DNA strands are polymerized in the 5′-to-3′ direction, the DNA synthesized on the lagging strand must be made initially as a series of short DNA molecules, called Okazaki fragments, named after the scientist who discovered them. Right, the same fork a short time later. On the lagging strand, the Okazaki fragments are synthesized sequentially, with those nearest the fork being the most recently made.

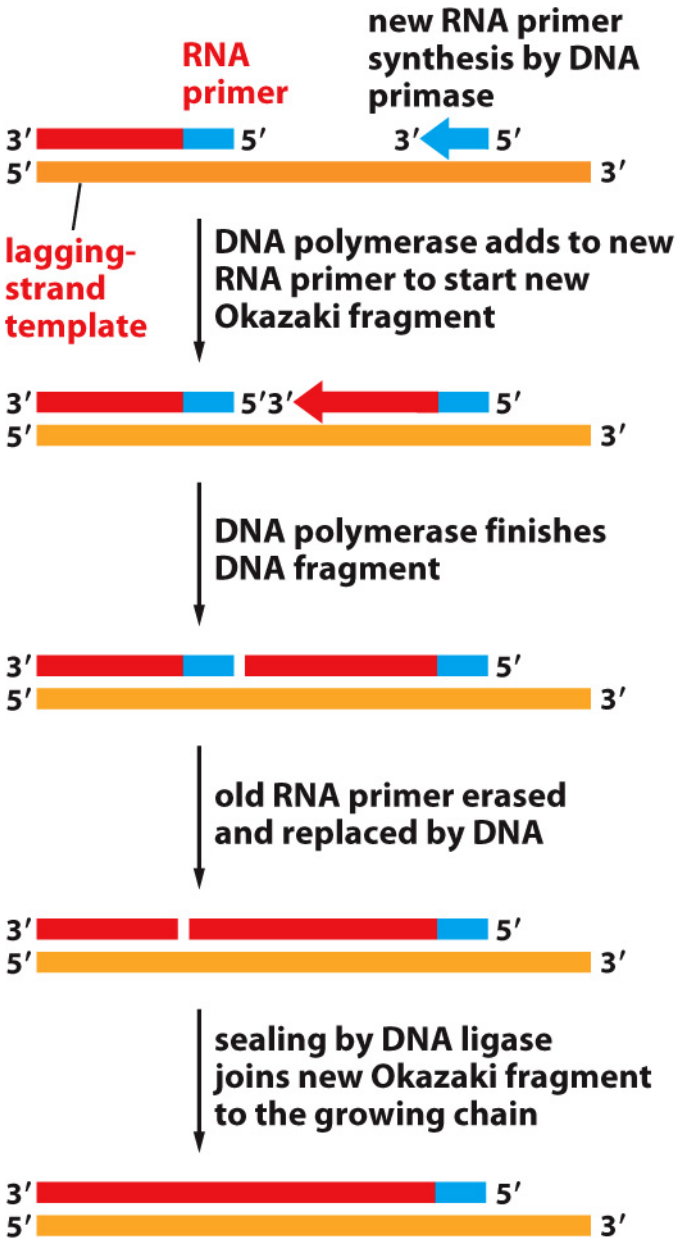


Figure 5-11 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–11 The synthesis of one of many DNA fragments on the lagging strand. In eukaryotes, RNA primers are made at intervals spaced by about 200 nucleotides on the lagging strand, and each RNA primer is approximately 10 nucleotides long. This primer is erased by a special DNA repair enzyme (an RNAse H) that recognizes an RNA strand in an RNA/DNA helix and fragments it; this leaves gaps that are filled in by DNA polymerase and DNA ligase.

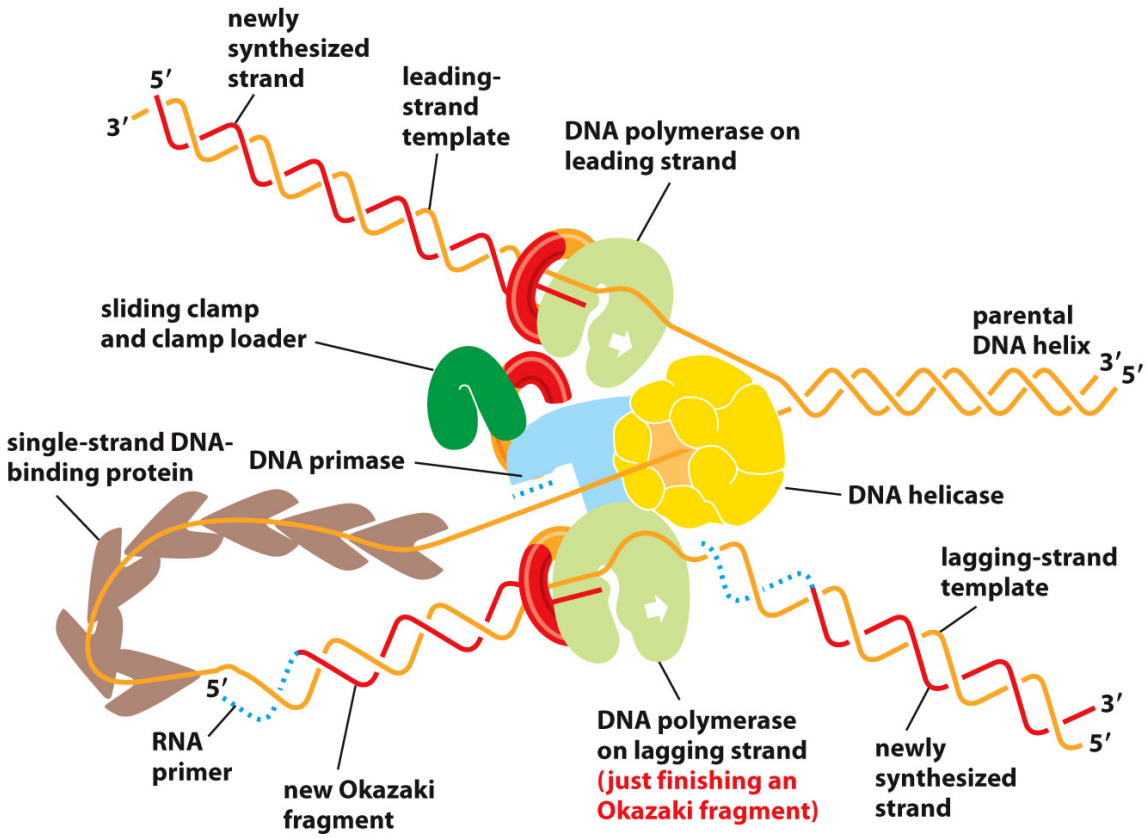


Figure 5-18a Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–18 A bacterial replication fork. (A) This schematic diagram shows a current view of the arrangement of replication proteins at a replication fork when DNA is being synthesized. The lagging-strand DNA is folded to bring the lagging-strand DNA polymerase molecule into a complex with the leading-strand DNA polymerase molecule. This folding also brings the 3′ end of each completed Okazaki fragment close to the start site for the next Okazaki fragment. Because the lagging-strand DNA polymerase molecule remains bound to the rest of the replication proteins, it can be reused to synthesize successive Okazaki fragments. In this diagram, it is about to let go of its completed DNA fragment and move to the RNA primer that is just being synthesized. Additional proteins (not shown) help to hold the different protein components of the fork together, enabling them to function as a well-coordinated protein machine (Movie 5.4 and Movie 5.5).

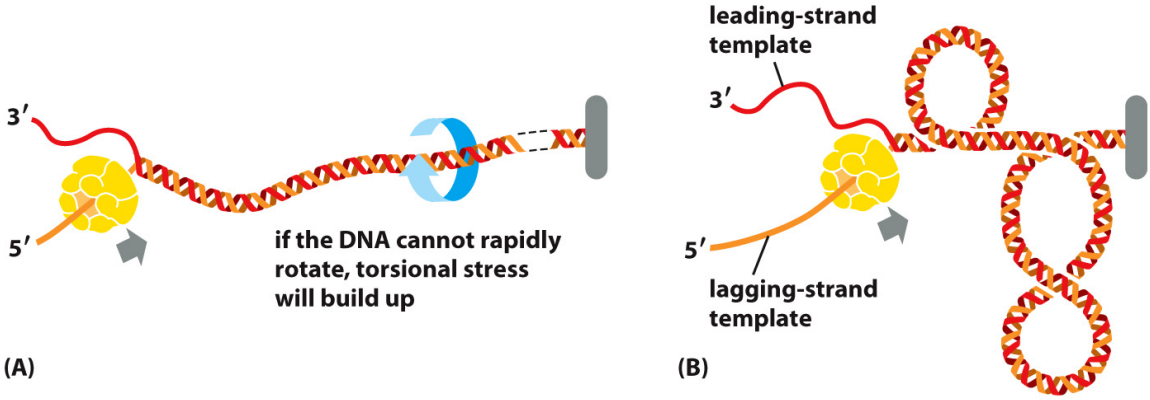


Figure 5-20 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–20 The "winding problem" that arises during DNA replication. (A) For a bacterial replication fork moving at 500 nucleotides per second, the parental DNA helix ahead of the fork must rotate at 50 revolutions per second. (B) If the ends of the DNA double helix remain fixed (or difficult to rotate), tension builds up in front of the replication fork as it becomes overwound. Some of this tension can be taken up by supercoiling, whereby the DNA double helix twists around itself (see Figure 6–19). However, if the tension continues to build up, the replication fork will eventually stop because further unwinding requires more energy than the helicase can provide. Note that in (A), the dotted line represents about 20 turns of DNA.

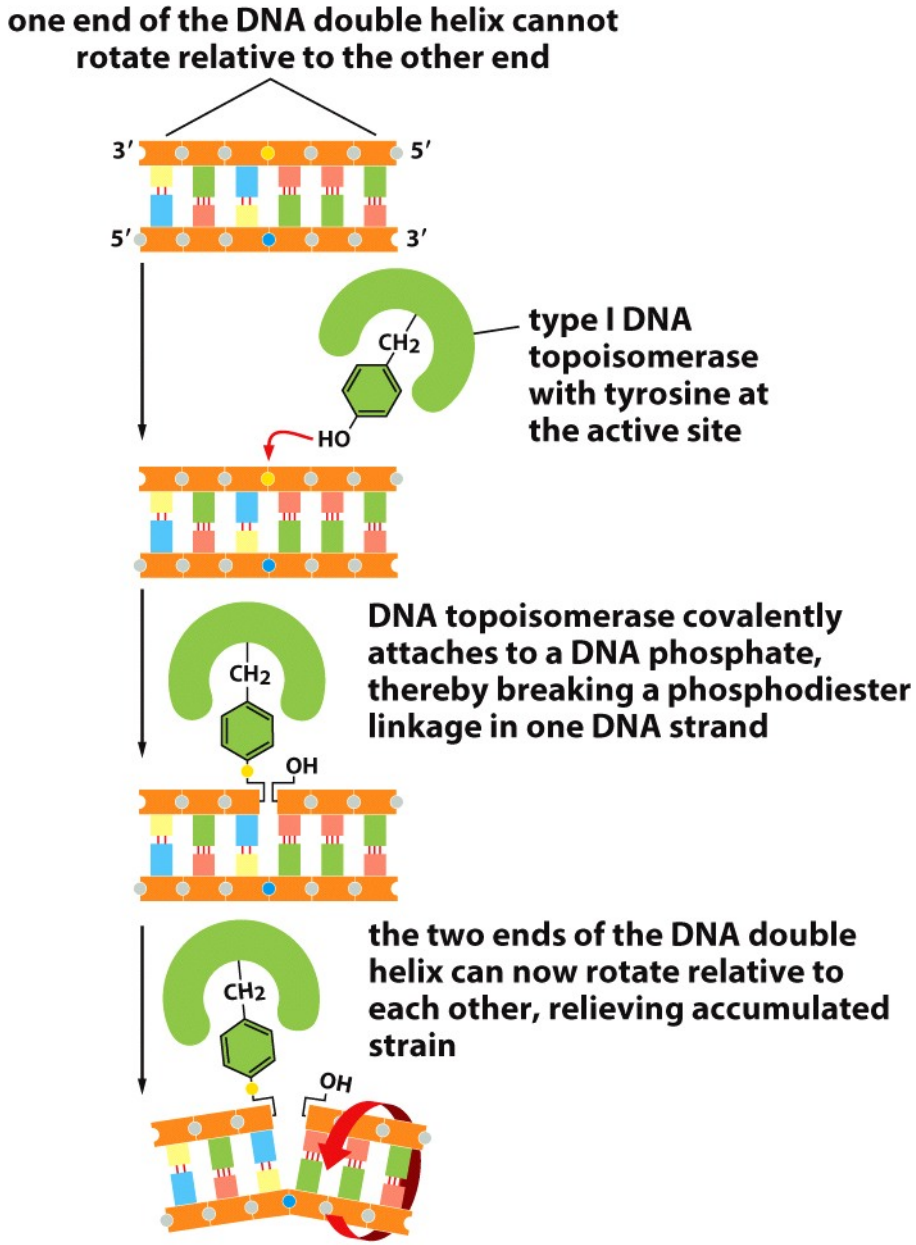


Figure 5-21 (part 1 of 2) Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–21 The reversible DNA nicking reaction catalyzed by a eukaryotic DNA topoisomerase I enzyme. As indicated, these enzymes transiently form a single covalent bond with DNA; this allows free rotation of the DNA around the covalent backbone bonds linked to the blue phosphate.

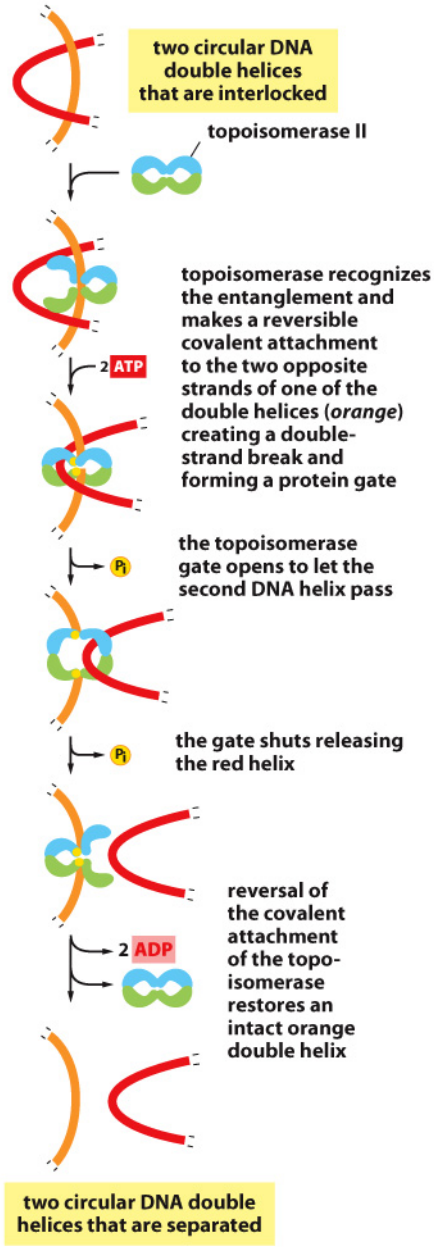


Figure 5-22 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–22 The DNA-helix-passing reaction catalyzed by DNA topoisomerase II. Unlike type I topoisomerases, type II enzymes hydrolyze ATP (red), which is needed to release and reset the enzyme after each cycle. Type II topoisomerases are largely confined to proliferating cells in eukaryotes; partly for that reason, they have been effective targets for anticancer drugs. Some of these drugs inhibit topoisomerase II at the third step in the figure and thereby produce high levels of double-strand breaks that kill rapidly dividing cells. The small yellow circles represent the phosphates in the DNA backbone that become covalently bonded to the topoisomerase (see Figure 5–21).

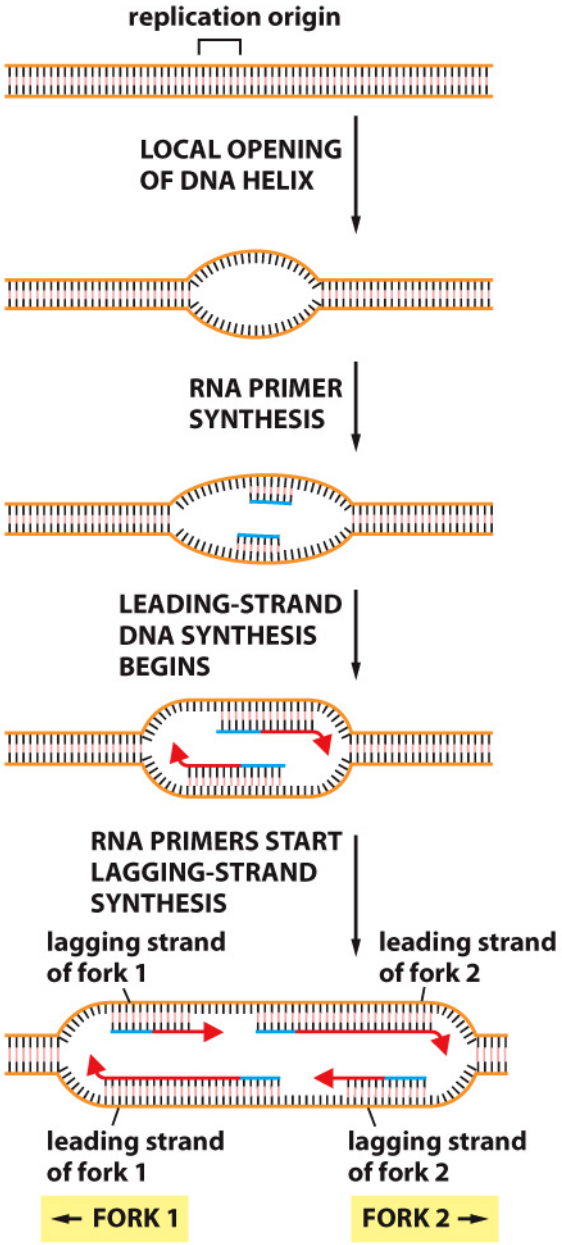


Figure 5-23 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–23 A replication bubble formed by replication-fork initiation. This diagram outlines the major steps in the initiation of replication forks at replication origins. The structure formed at the last step, in which both strands of the parental DNA helix have been separated from each other and serve as templates for DNA synthesis, is called a replication bubble.

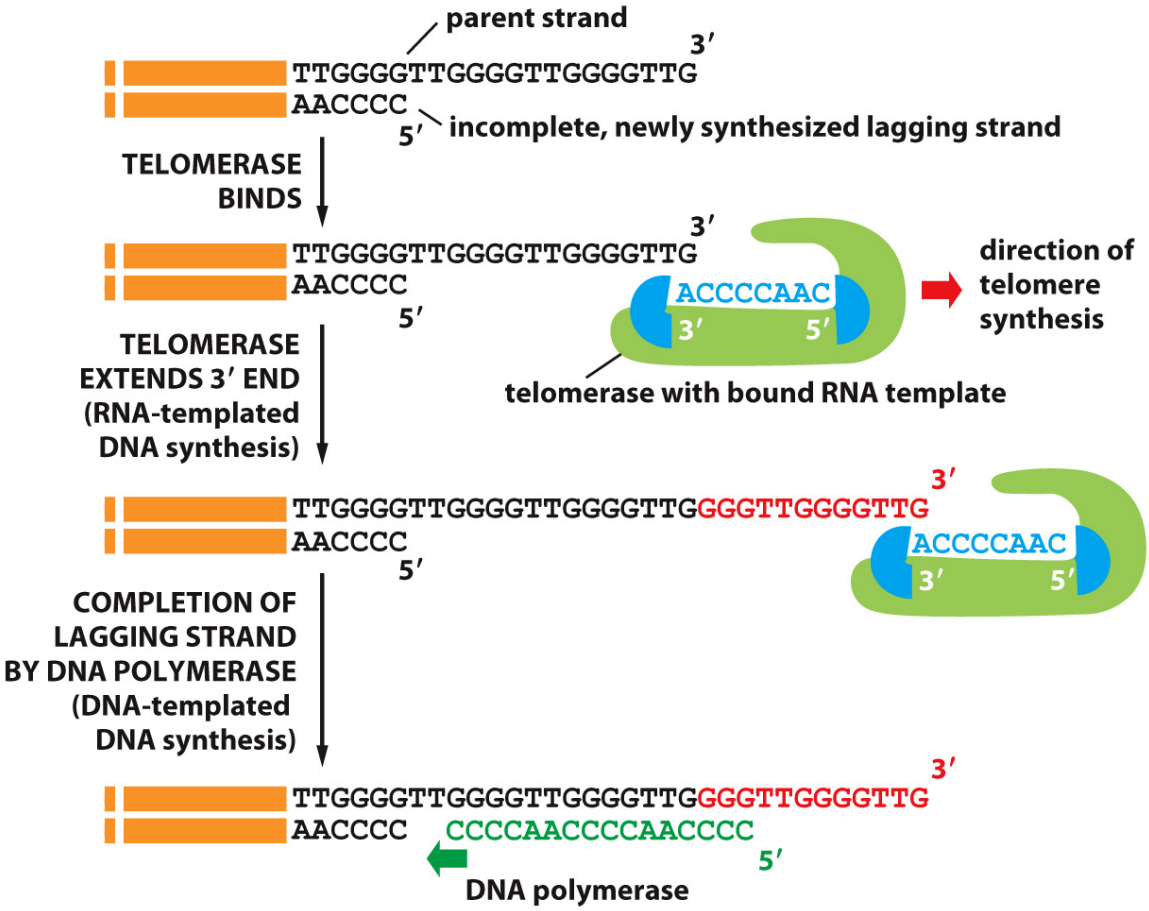


Figure 5-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–34 Telomere replication. Shown here are the reactions that synthesize the repeating sequences that form the ends of the chromosomes (telomeres) of diverse eukaryotic organisms. The 3′ end of the parental DNA strand is extended by RNA-templated DNA synthesis; this allows the incomplete daughter DNA strand that is paired with it to be extended in its 5′ direction. This incomplete, lagging strand is presumed to be completed by DNA polymerase α, which carries a DNA primase as one of its subunits (Movie 5.6). The telomere sequence illustrated is that of the ciliate Tetrahymena, in which these reactions were first discovered.