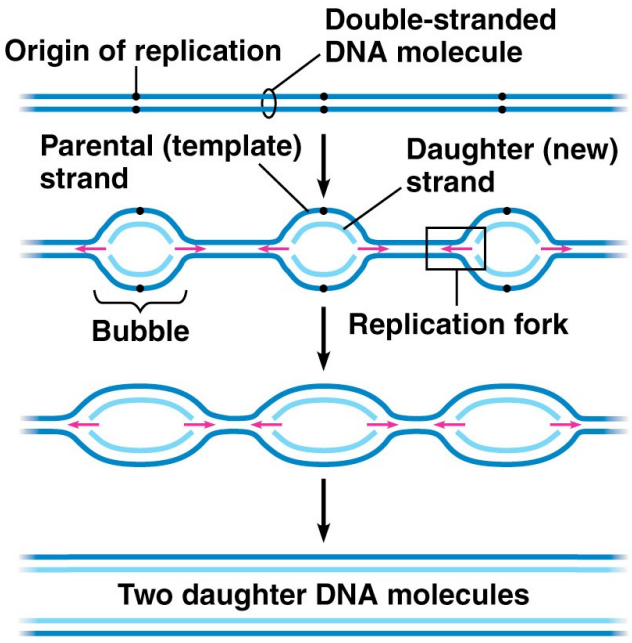
MCB 150

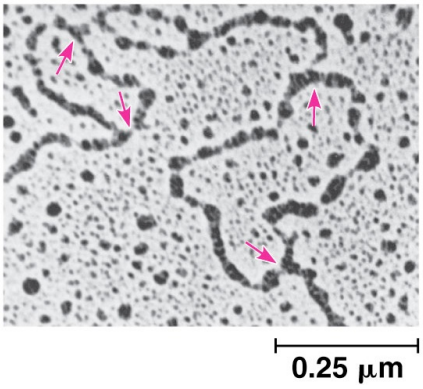
The Molecular and Cellular Basis of Life

DNA replication

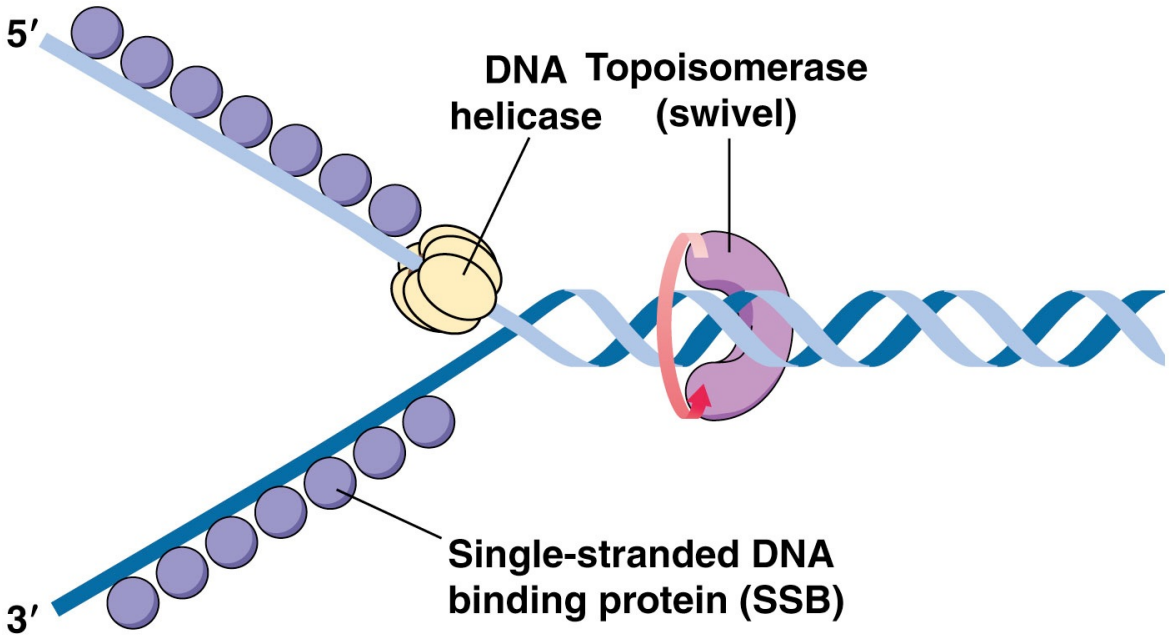
Today's Learning Catalytics Session ID is:
61635784

Eukaryotes must initiate replication in multiple locations to finish prior to cell division:



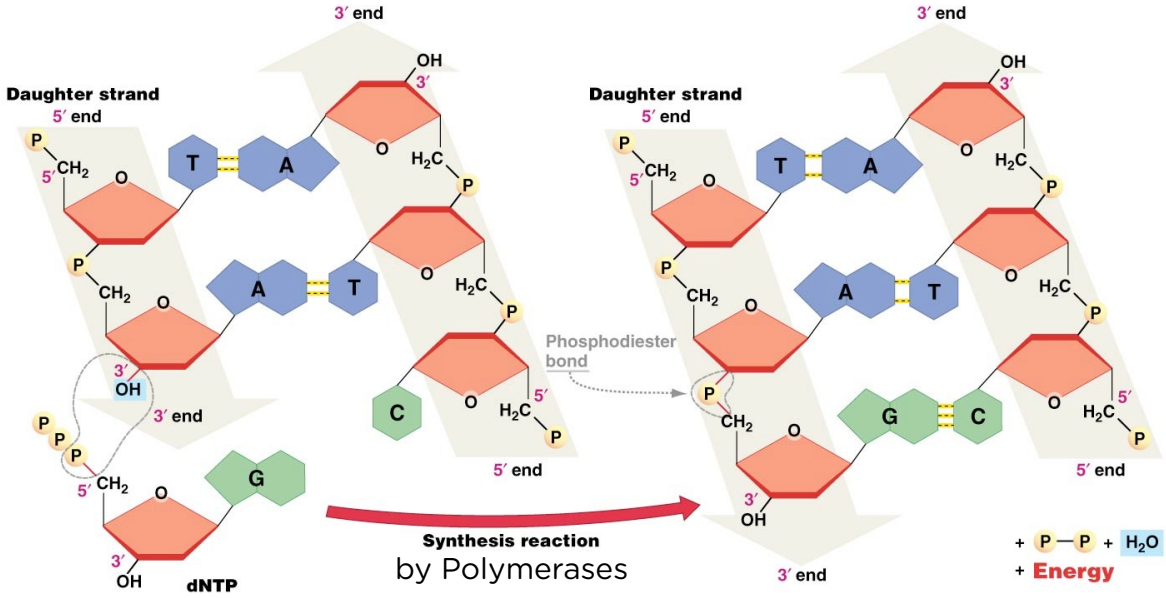


Strands are separated by Helicase enzymes, and are kept single-stranded by Single-Stranded DNA Binding Protein:



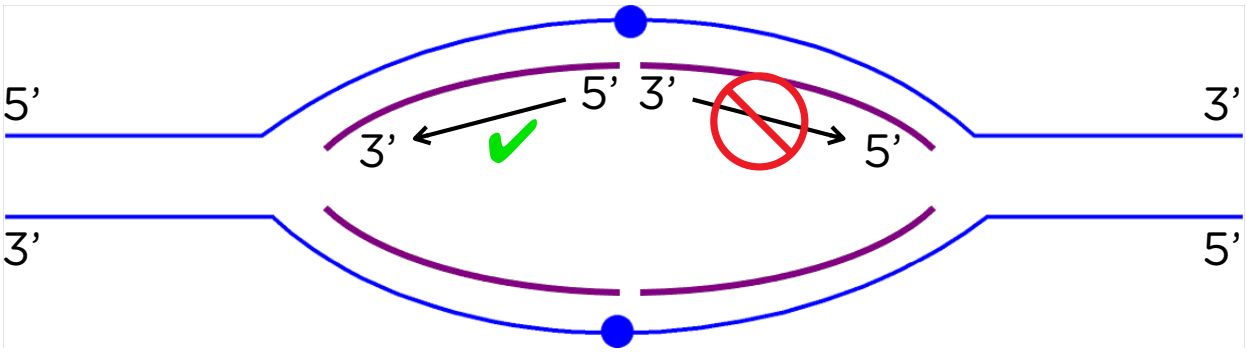
DNA strand synthesis:

1. Incoming dNTP is hybridized to parental template
2. Phosphodiester bond formed with 3’ end of chain



DNA Replication is Bidirectional:

- new DNA needs to be synthesized on both strands on both sides of the *ori*



- BUT… synthesis ONLY occurs in the 5’ to 3’ direction, and the new strands have to be ANTIPARALLEL to the template!

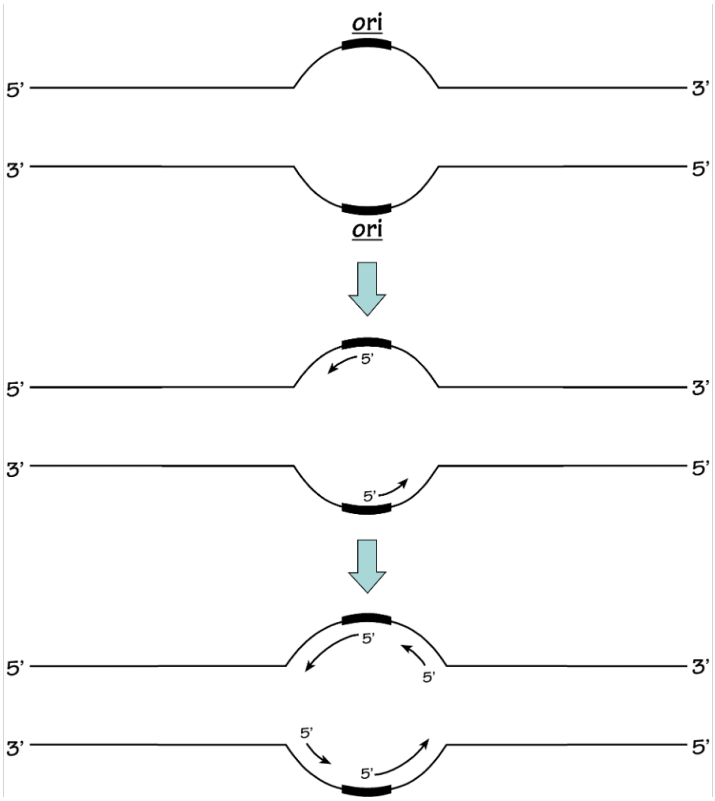
To solve this problem, DNA synthesis on one side of the *ori* begins at the *ori* and proceeds normally

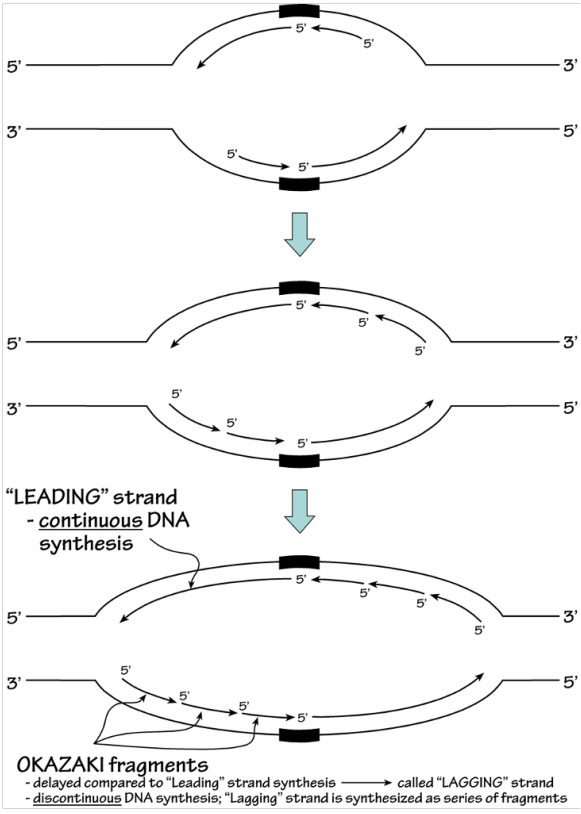
- called the LEADING strand

But DNA synthesis on the other side of the *ori* starts a short distance away from the *ori* and works back toward the *ori*

- called the LAGGING strand
- small fragments of DNA are called Okazaki fragments

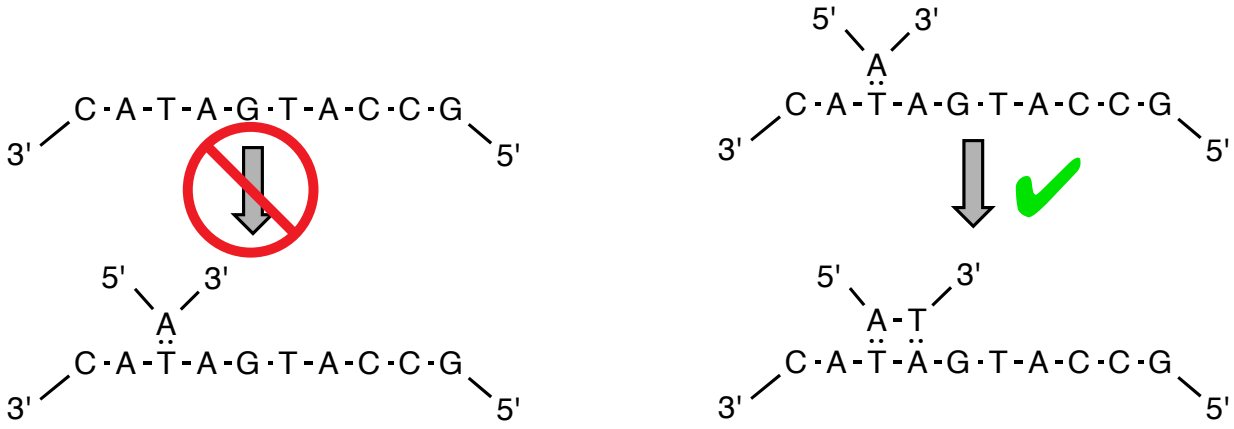
This way, all synthesis occurs 5’→3’





Problem #2: DNA polymerases cannot start a new DNA strand from scratch!

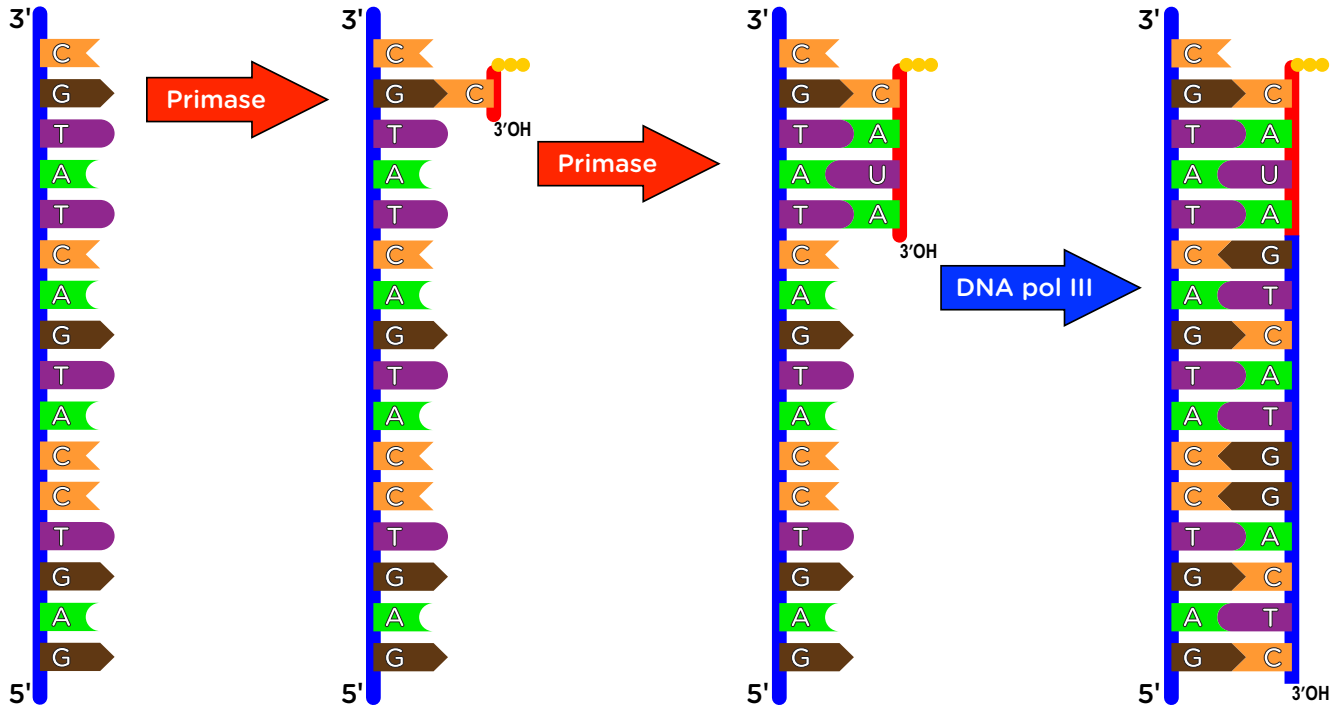
- They absolutely require a free 3′-OH group to which to add the incoming dNTPs



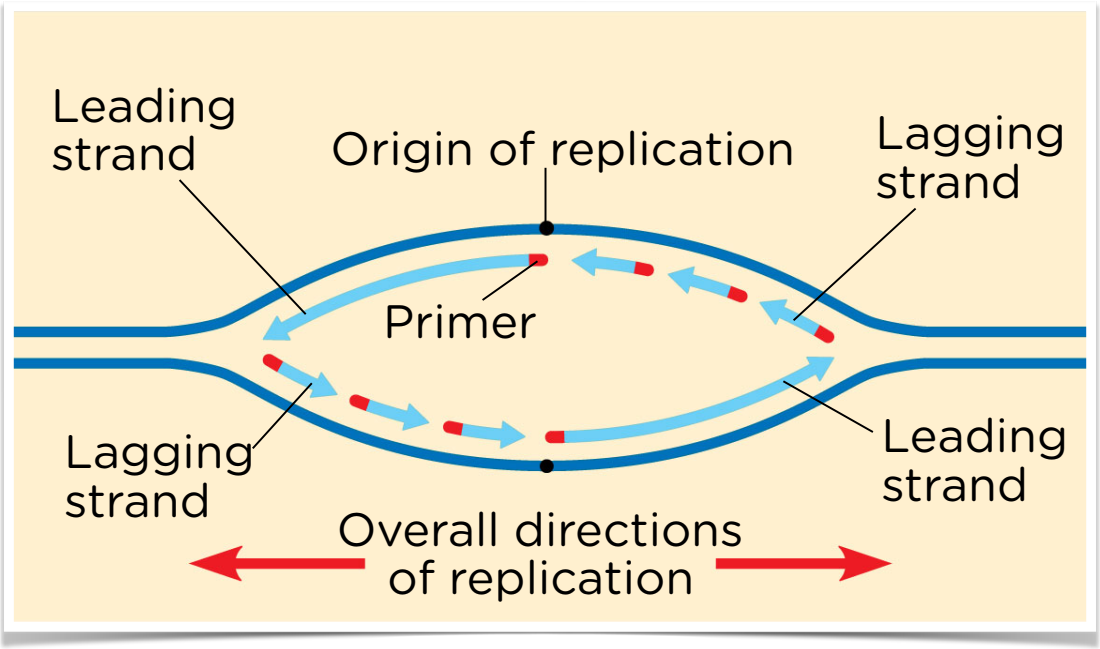
Solution to Problem #2: RNA synthesizing enzymes can use a single-stranded DNA template to make an RNA strand from scratch

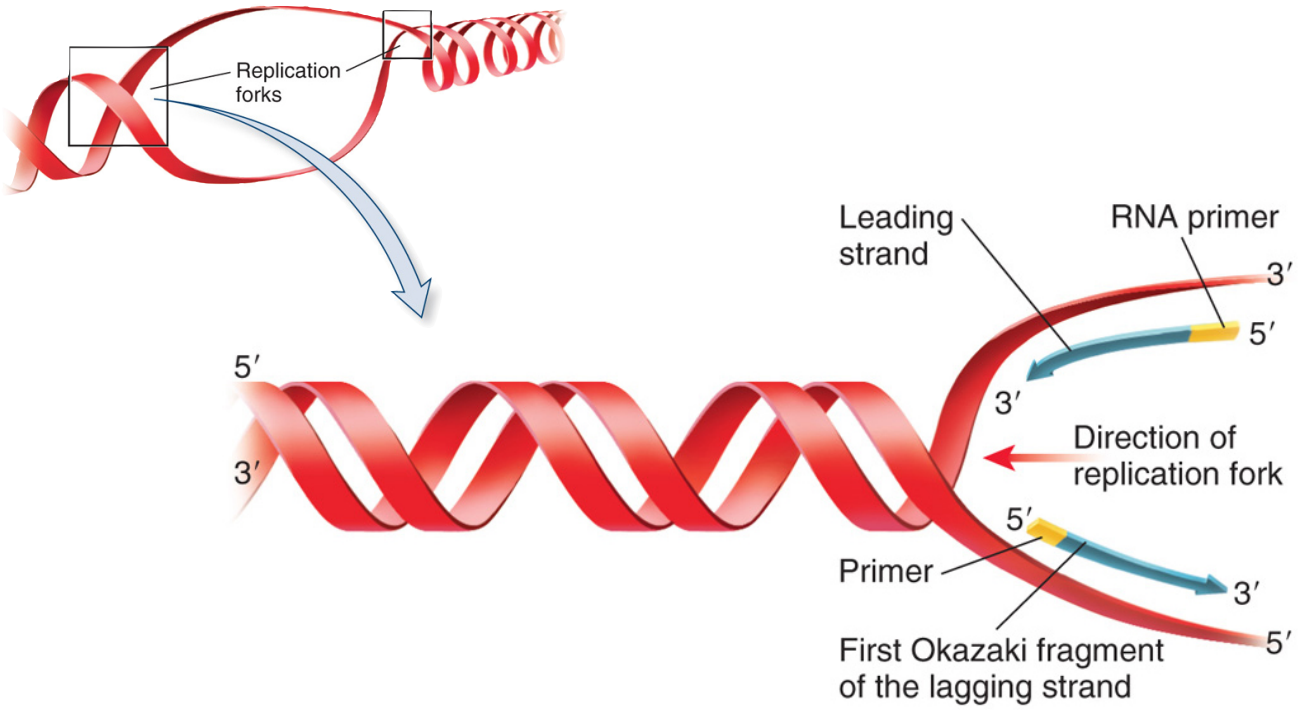
- The special DNA-dependent, RNA-synthesizing enzyme used in DNA Replication is called **Primase**
- Primase creates a short (5–10 nucleotide) strand of RNA opposite an ss-DNA template called a **primer**
- This gives the major DNA-dependent, DNA-synthesizing enzyme (**DNA polymerase III** in *E. coli*) what it needs—a free 3′-OH group

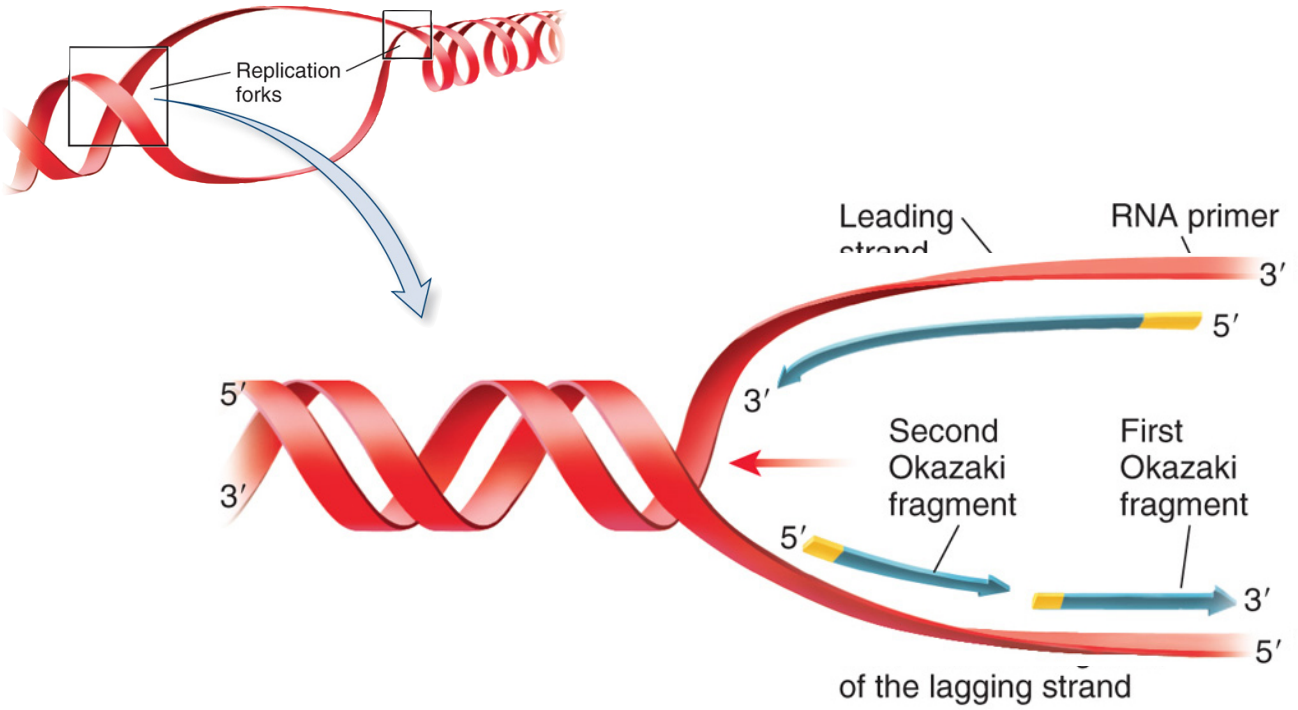
"Priming the Pump" of DNA Synthesis:

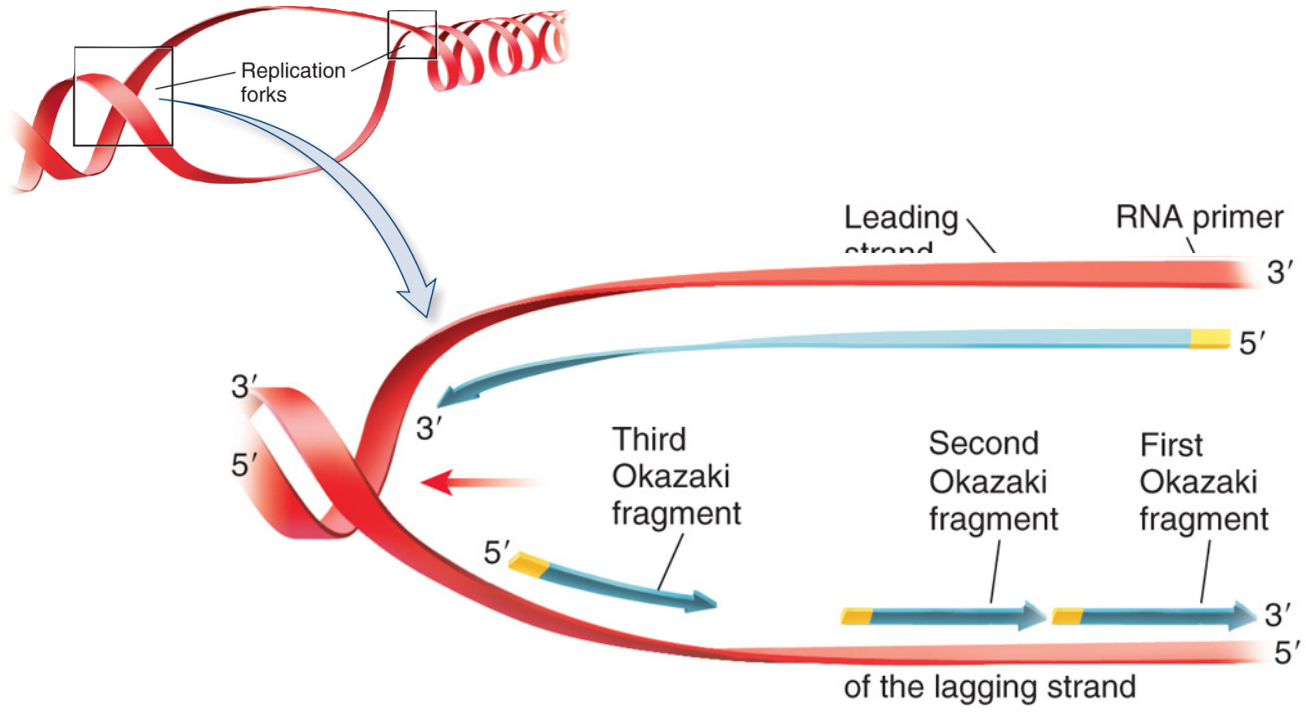


Every fragment is primed:









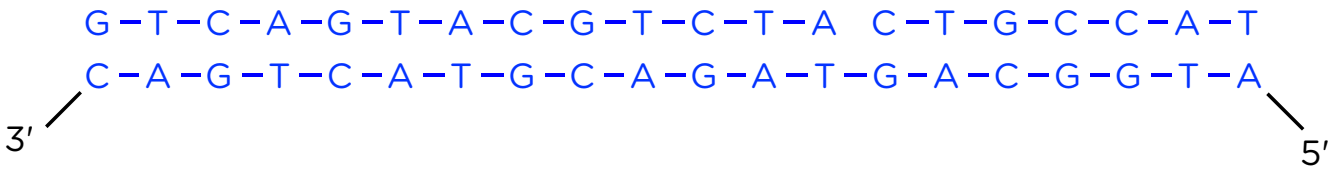
RNA primers must now be removed, or the genome would be littered with RNA bases:

G–T–C–A–G–T–A–C G–U–C–U–A–C–T–G–C–C–A–T
C–A–G–T–C–A–T–G–C–A–G–A–T–G–A–C–G–G–T–A
3′ 5′

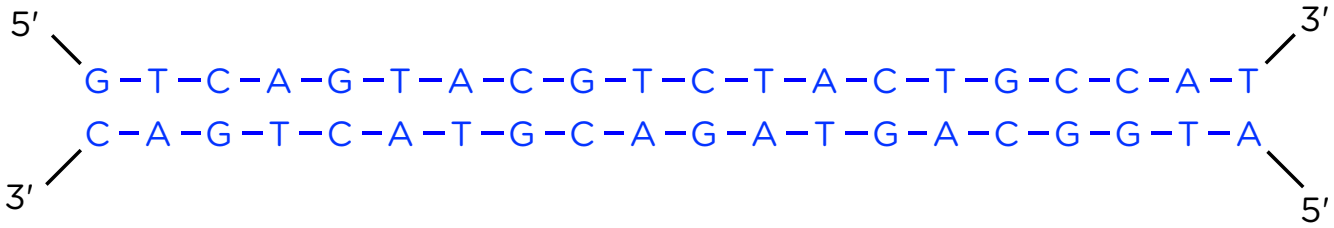
RNA nucleotides removed and replaced with DNA nucleotides by DNA polymerase I:

G–T–C–A–G–T–A–C–G–T–C–T–A C–T–G–C–C–A–T
C–A–G–T–C–A–T–G–C–A–G–A–T–G–A–C–G–G–T–A
3′ 5′

One last problem: Backbone of new chain has "nicks" in it where no covalent linkage exists between nucleotides



These "nicks" are sealed by DNA Ligase (or just Ligase):



Some important terminology:

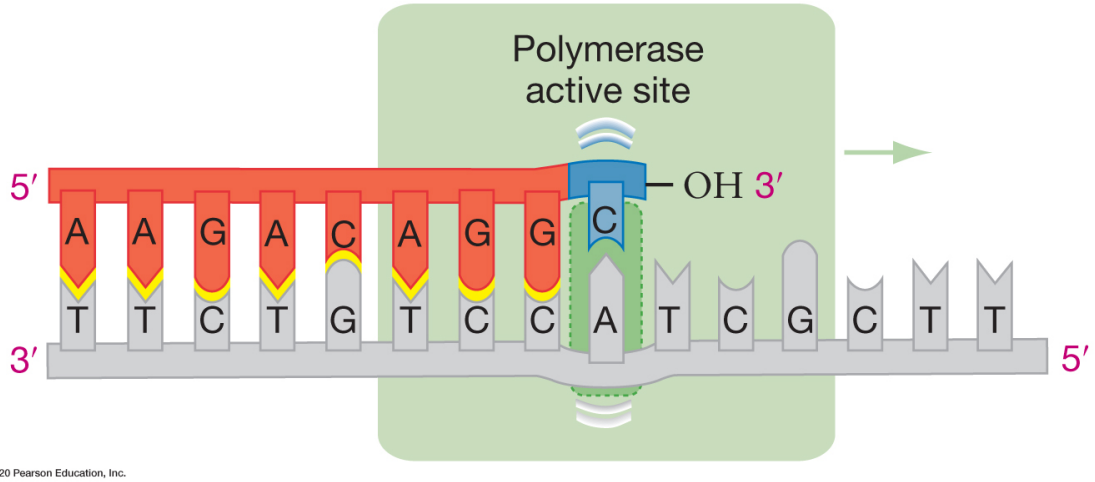
- "X"-dependent "Y"-synthesizing enzyme
  - "X" = what it uses as a template
  - "Y" = what it is making
- An enzyme that degrades (hydrolyzes) a phosphodiester linkage = a nuclease
- A nuclease that hydrolyzes nucleic acid from the end of a chain = an exonuclease
- A nuclease that hydrolyzes nucleic acid internally (i.e., not at one end or the other) = an endonuclease

Some important terminology:

- If an exonuclease starts at the 5′ end, working toward the 3′ end, it is called a 5′–3′ exonuclease
- If an exonuclease starts at the 3′ end, working toward the 5′ end, it is called a 3′–5′ exonuclease
- DNA polymerase I's ability to remove primers is due to its 5′–3′ exonuclease activity, which is a separate enzymatic activity from its DNA synthesizing ability

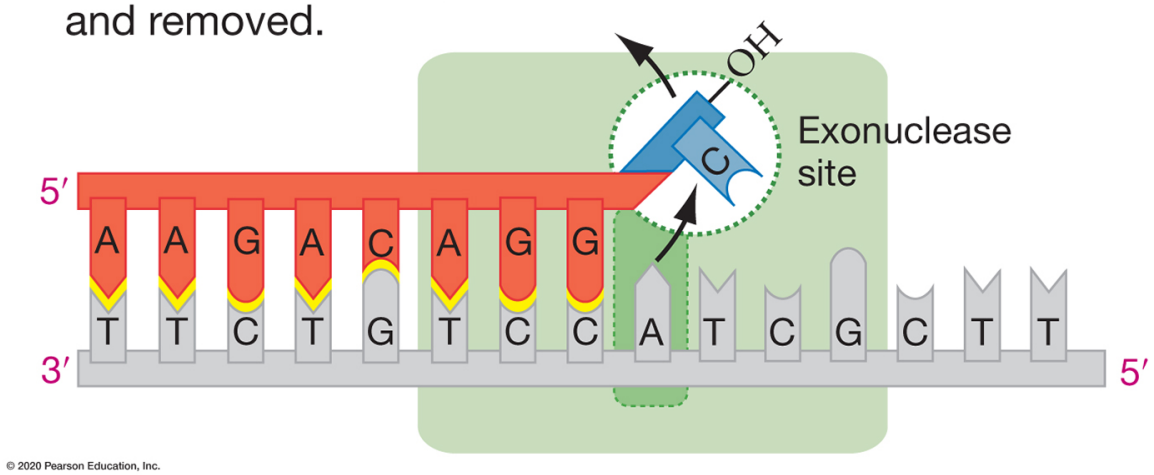
Proofreading: an example of 3′–5′ exonuclease activity

(a) DNA polymerase adds a mismatched deoxyribonucleotide.



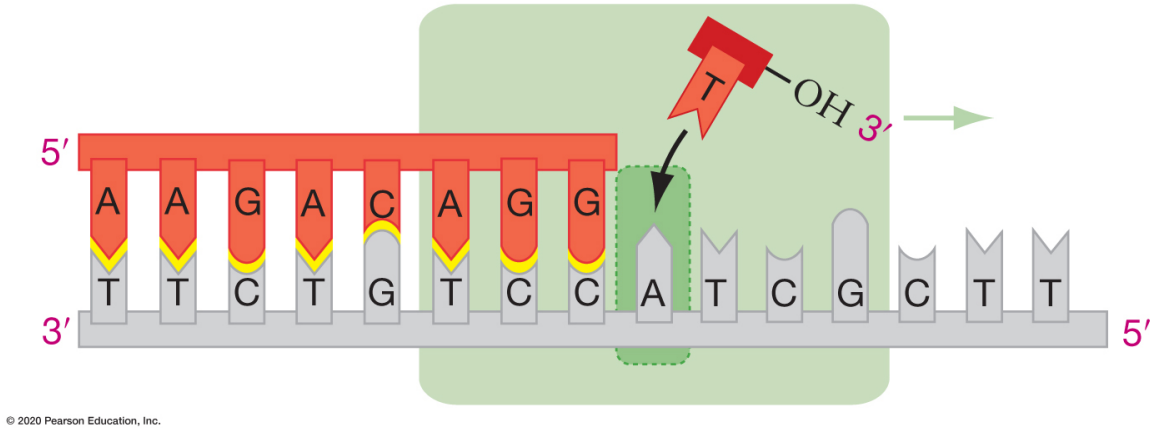
Proofreading: an example of 3′–5′ exonuclease activity

(b) The mismatch is displaced into an exonuclease site and removed.

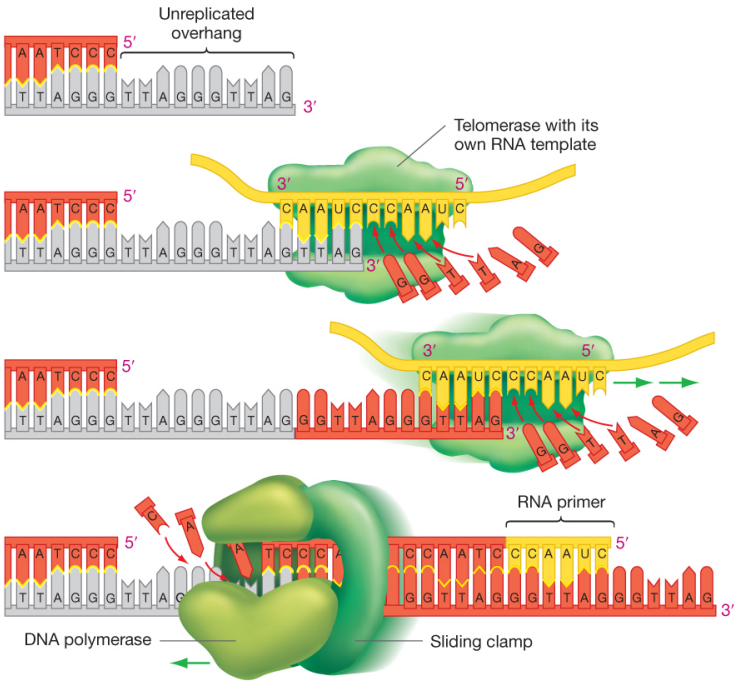


Proofreading: an example of 3′–5′ exonuclease activity

(c) Polymerase adds the correct deoxyribonucleotide.



The “end replication problem” workaround:



1. A strand of parental DNA remains unreplicated after the RNA primer is removed from the end of the lagging strand (see Figure 15.12, steps 3 and 4).

2. Telomerase extends unreplicated end. Telomerase binds to 3′ end of the overhanging strand of parental DNA and, using its own internal RNA template, extends the strand.

3. Telomerase shifts and repeats activity. Telomerase extends the DNA strand by shifting down the newly synthesized DNA and adding additional repeats, multiple times.

4. Extended single-stranded DNA acts as template. Standard DNA synthesis on this template creates double-stranded DNA to prevent chromosome shortening.