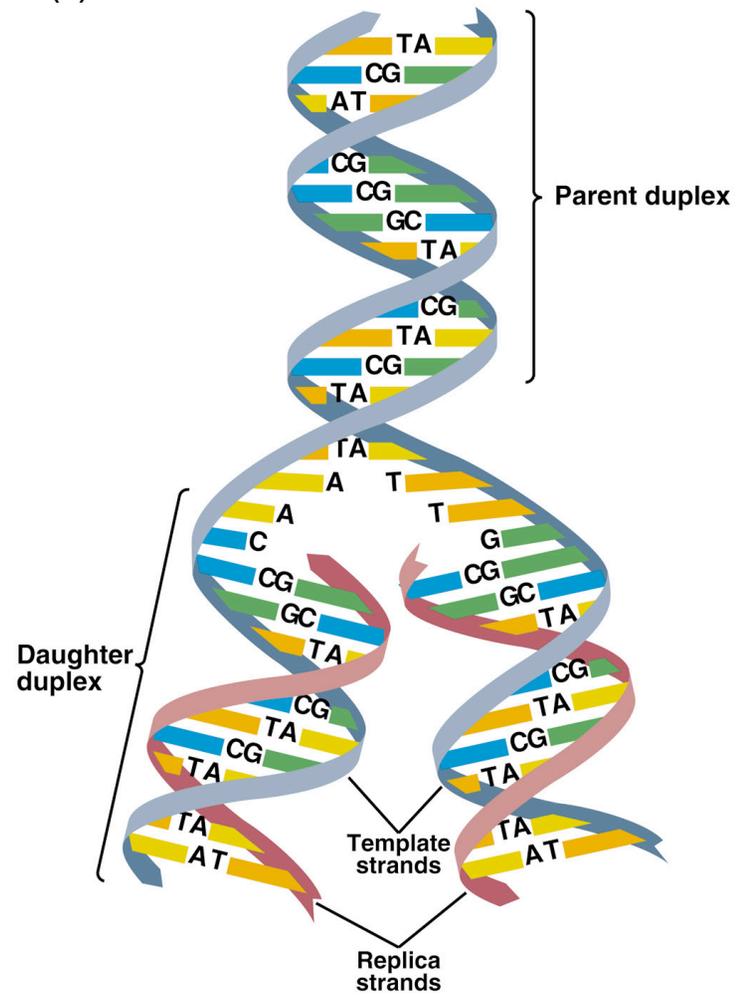


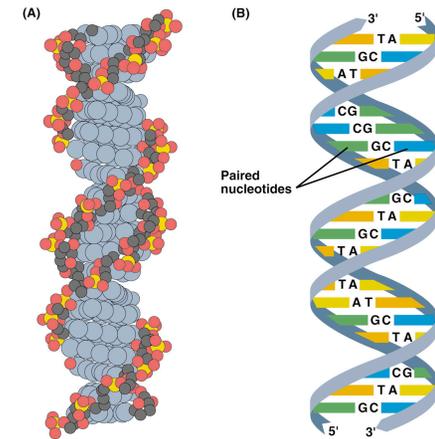
# DNA Replication

(A)



# Reminder

- Genes are DNA.
- DNA is a double-stranded molecule.
- The genetic information in a gene is encoded in the sequence of bases on one strand of DNA.



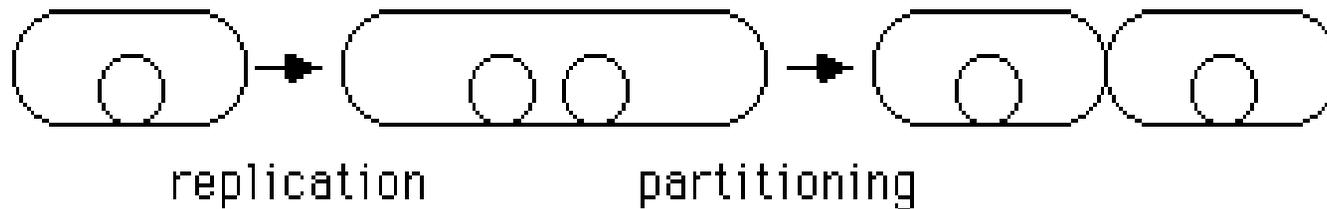
```
1      10      20      30      40      50      60      70      80      90      100
AcatttgcttctgacacaactgtgttcactagcaactcaaacagacaccATGGTGACCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGC
101
AAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGgtttggtatcaaggttacaagacagggtttaaggagaccaatagaaactgggcatgtggag
201
acagagaagactccttgggtttctgataggcactgactctctctgcctattgggtctatccccacccttagGCTGCTGGTGGTCTACCCTTGGACCCAGA
```

When a cell divides, both daughter cells must receive a complete set of genes, so the DNA molecules (chromosomes) must replicate before division.

# Asexual Reproduction

## Review from Introductory Biology

### Prokaryotes

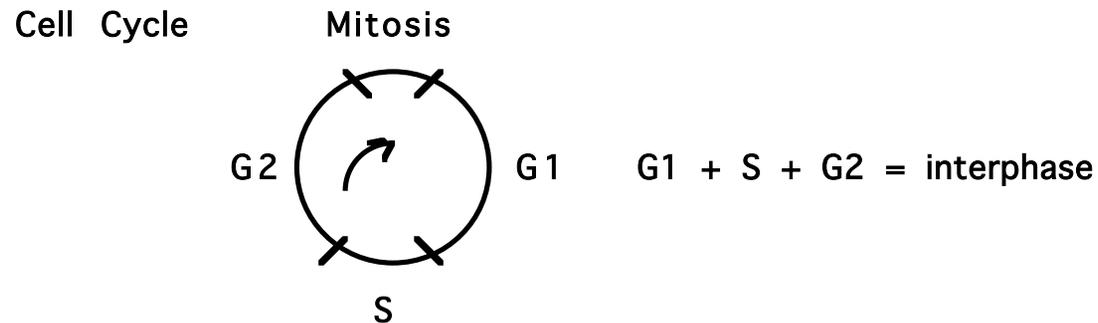


1. The entire genome is on one circular chromosome = DNA molecule.
2. The chromosome replicates once to produce two chromosomes that are identical (except for rare mutations).
3. The two identical daughter chromosomes move toward opposite end of the cell.
4. When the cell divides the daughter chromosomes are partitioned one to each daughter cell.

# Asexual Reproduction (cont.)

## Eukaryotes

### Asexual reproduction by mitosis



Variable lengths. Total time 15 m inutes --> days  
Animal cells in culture ca. 1 day

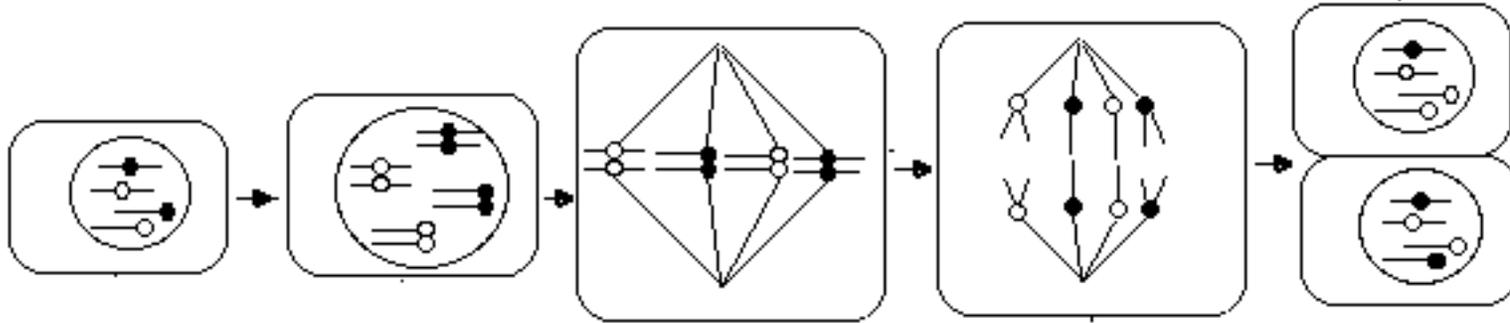
DNA replicates during S

Gene expression occurs during G1 and G2 (and S?)

Nuclear division (mitosis) occurs during Mitosis

Cell division (cytokinesis) occurs at the end of Mitosis

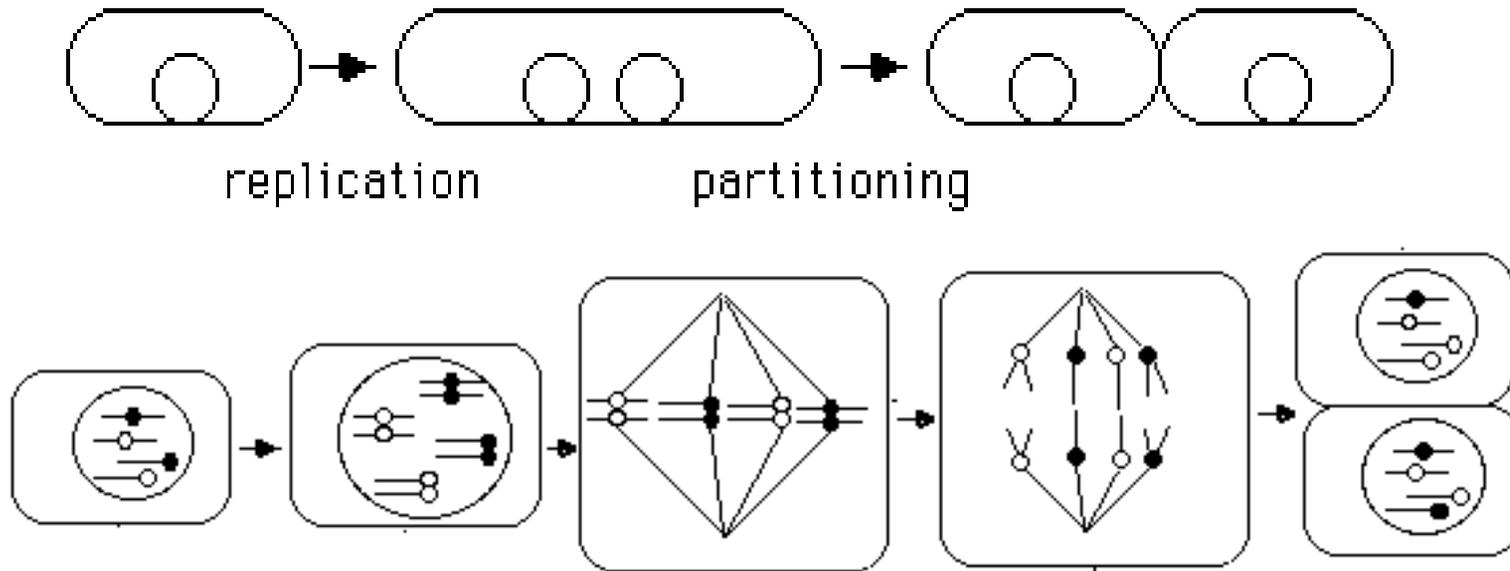
## Mitosis (continued)



The genome is divided among a number of chromosomes.

1. Each chromosome replicates once in the S phase to produce two sister chromatids (identical DNA molecules).
2. During mitosis the the kinetochore regions of each pair of sister chromatids are attached by chromosome fibers to opposite poles of the cell.
3. Chromosome fibers contract pulling sister chromatids to opposite ends of the cell.
4. During cytokinesis the sister chromatids are partitioned one to each daughter cell.

# Asexual Reproduction

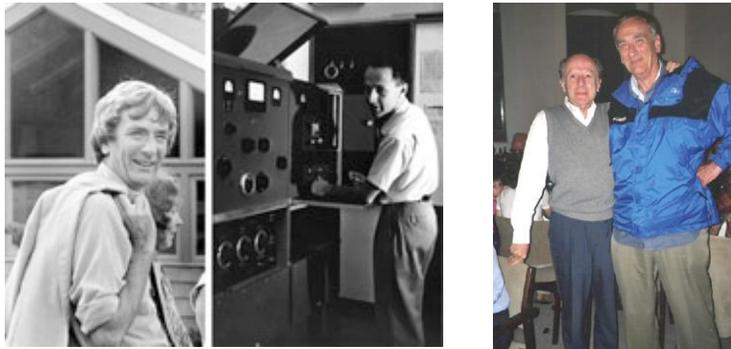


Note that the end result of asexual reproduction in prokaryotes and eukaryotes is the same genetically:

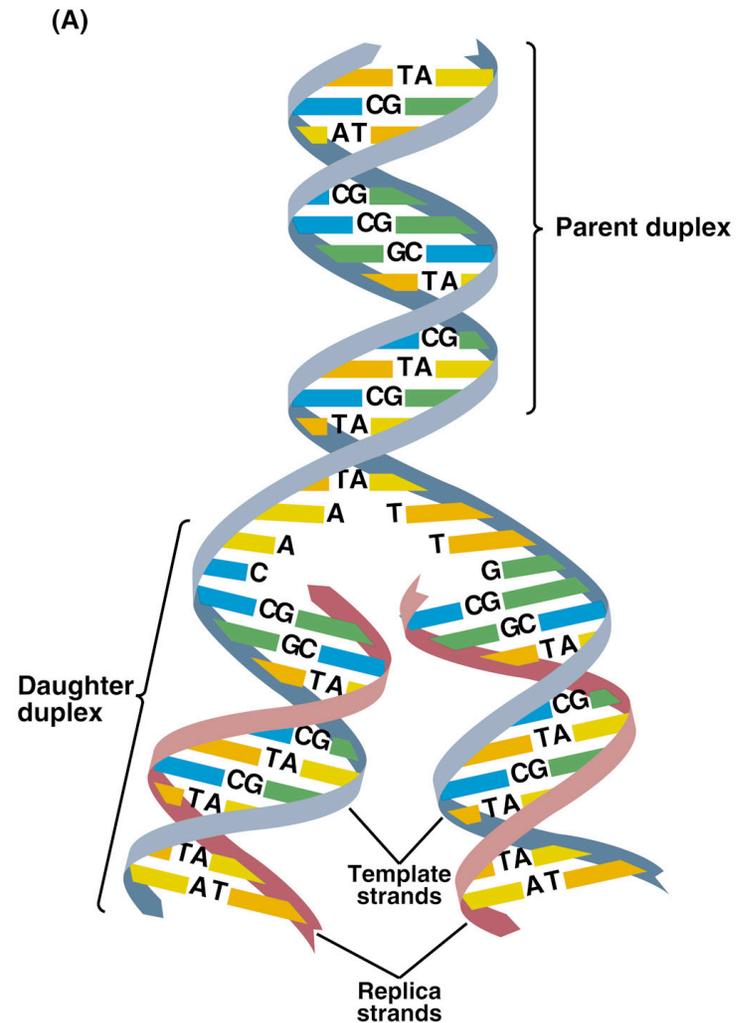
- Each daughter cell gets a complete copy of the parental cell genome.
- The daughter cells are genetically identical, except for new mutations that occur during the cell cycle (mainly during DNA replication).
- The daughter cells constitute a clone.

# DNA Replication is Semiconservative

1. The strands separate.
2. A new strand is made using each old strand as a template according to the rules of base pairing.

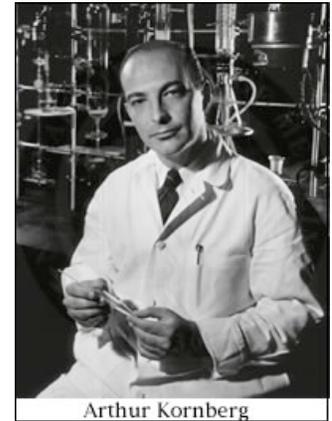


Model proposed by Watson and Crick,  
verified by Matt Meselson and Frank  
Stahl



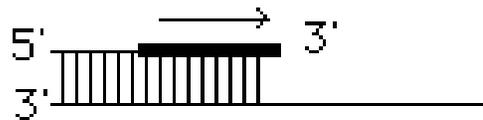
# DNA Replication: Enzyme Activities

**Many enzymes are required for DNA replication. We will only consider enzyme activities, not specific enzymes. Enzymes with these activities are also used for DNA manipulation in the lab.**



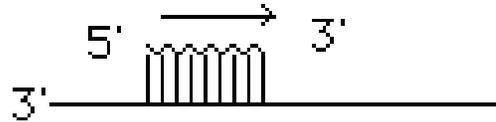
Arthur Kornberg

- 1. Helicase** unwinds double-helical DNA.
- 2. Single-strand binding protein** binds single strand to keep DNA unwound.
- 3. DNA polymerase** adds new nucleotides (nucleoside triphosphates) to 3' end of existing DNA strand (or RNA primer). Elongates chains 5' to 3' only.



# DNA Replication: Enzyme Activities

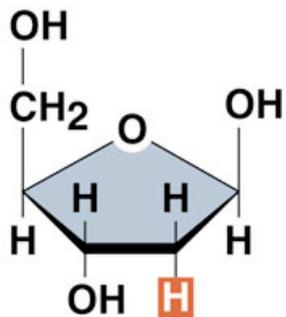
**4. Primase** makes a single strand of ca. 20 bp off RNA using a DNA strand as a template.



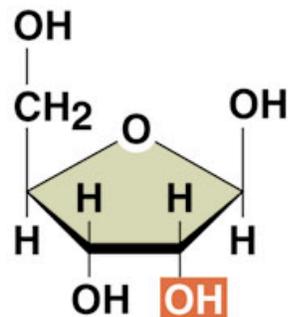
Reminder:

RNA is like DNA except

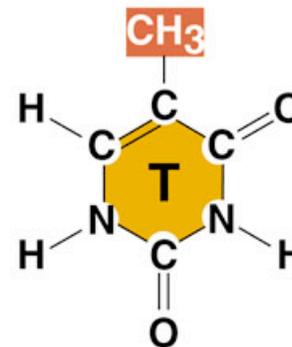
- single-stranded
- ribose instead of deoxyribose
- uracil instead of thymine (U pairs with A just as T does)



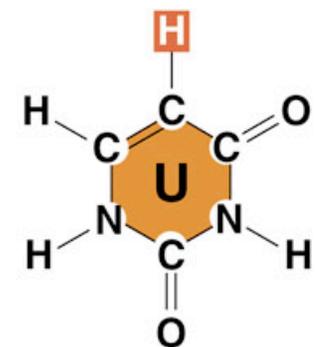
Deoxyribose



Ribose



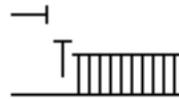
Thymine



Uracil

# DNA Replication: Enzyme Activities

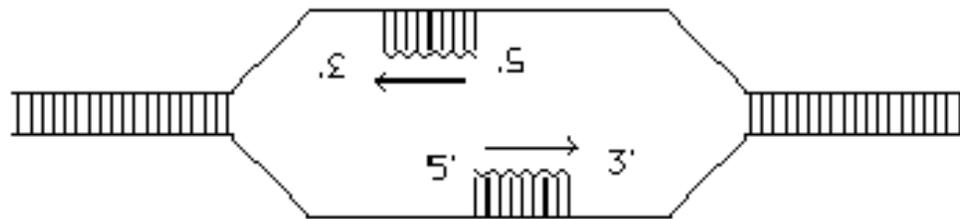
**5. Exonuclease** removes nucleotides from the end of a DNA strand; different enzymes work 5' to 3' or 3' to 5'.



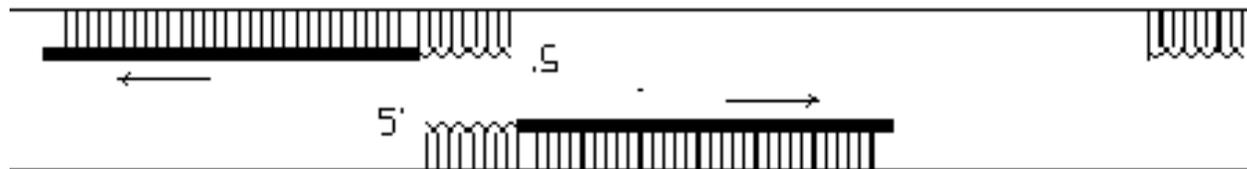
**6. Ligase** joins ends of single DNA strands by making new phosphate bonds.



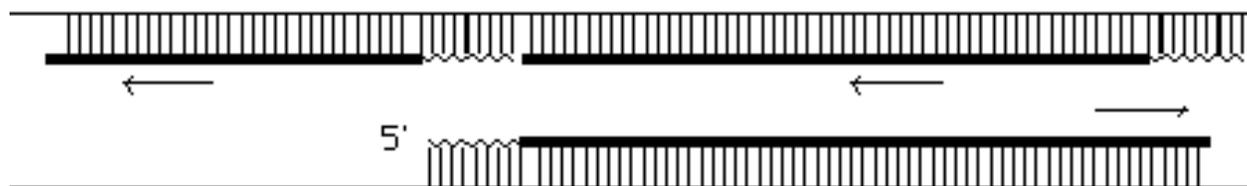
# Putting it All Together 1



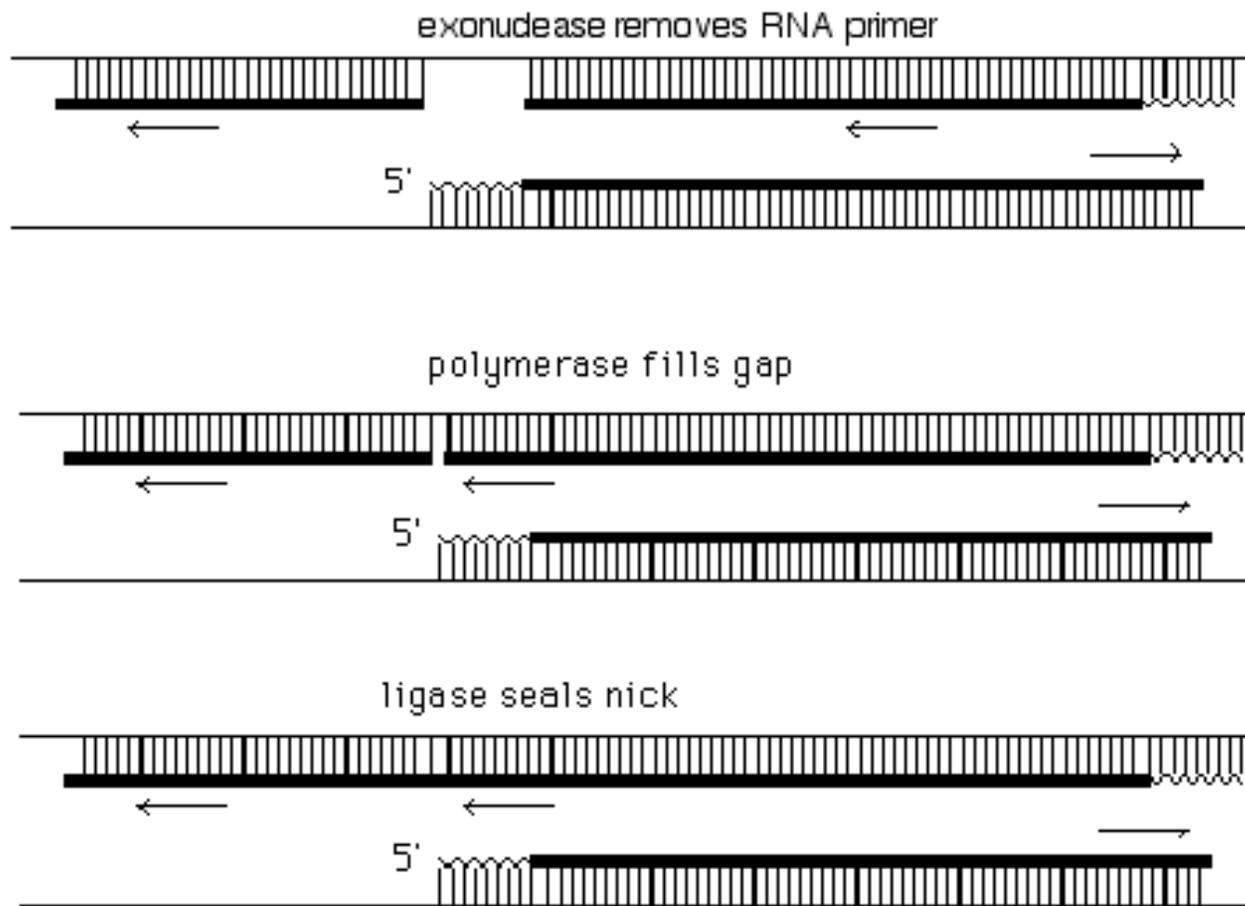
primase makes RNA primer



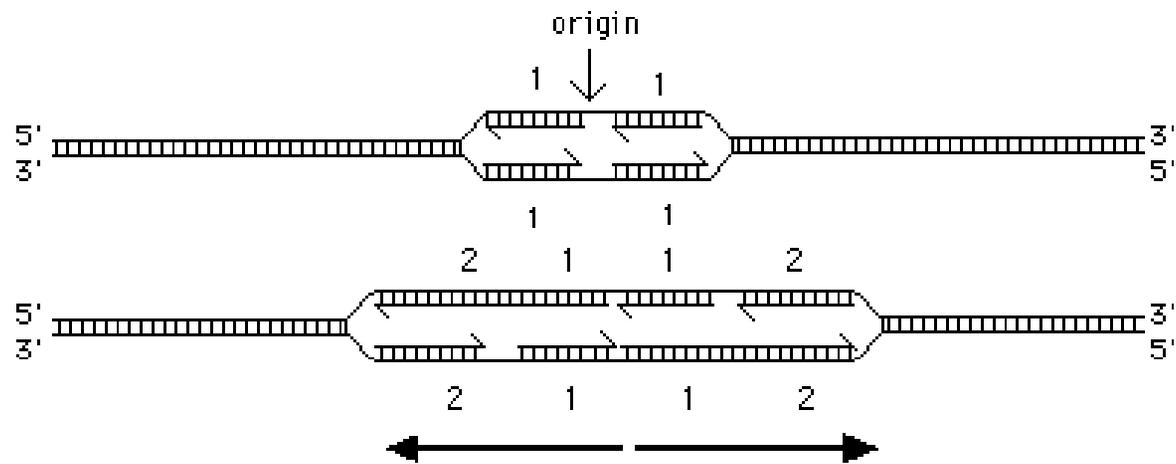
polymerase extends



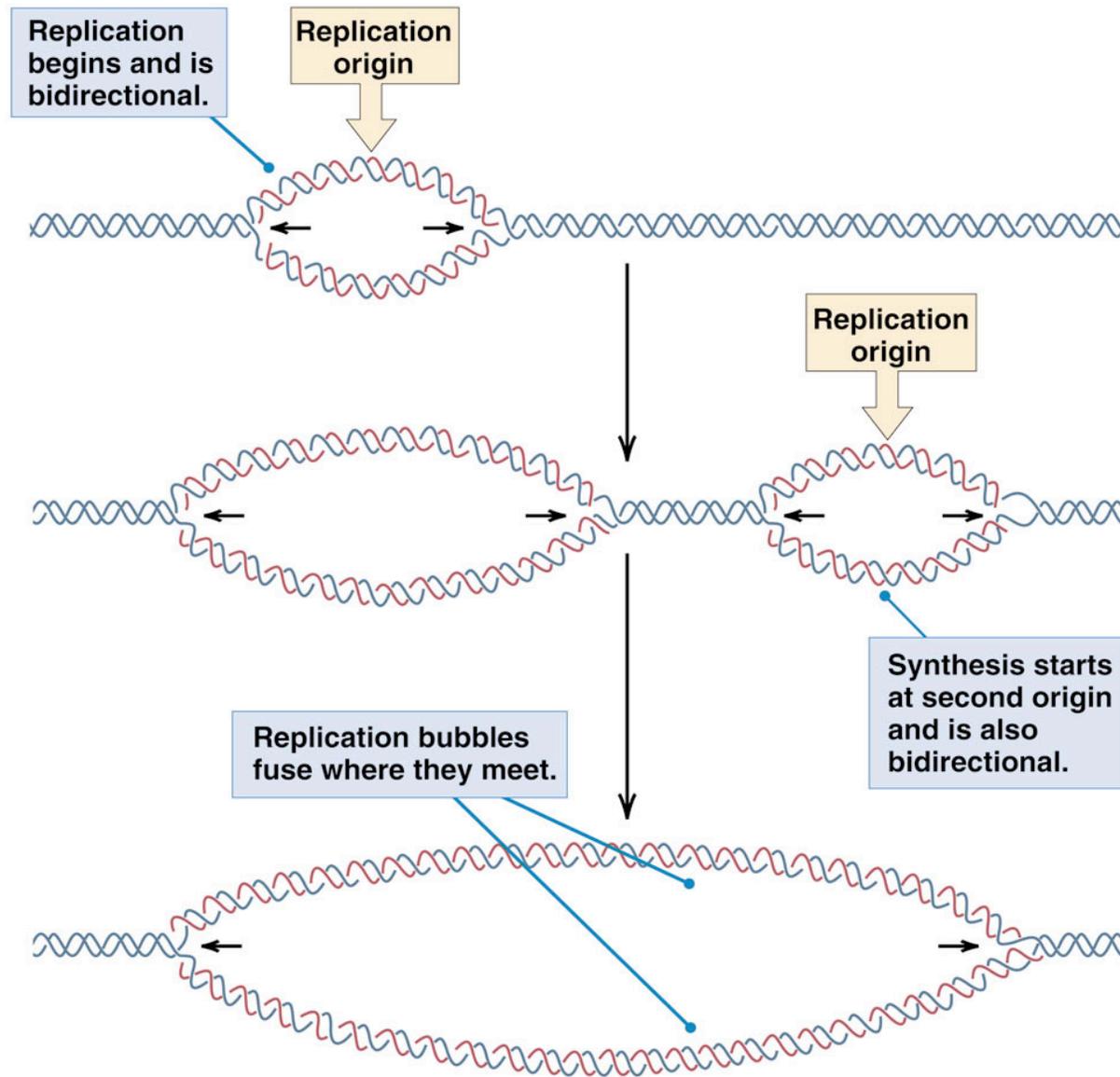
# Putting it All Together 2



# Replicating Linear Chromosomes



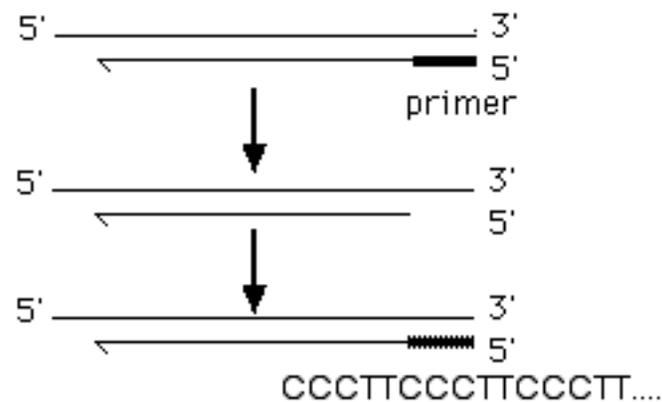
# When Replication Forks Meet



# Enzyme Activities to Finish the Job

**7. Gyrase (a topoisomerase)** relaxes supercoils produced when the molecule is twisted during replication. Also facilitates unwinding at beginning of replication.

**8. Telomerase** uses a short RNA template to add short DNA repeats to the short ends of linear chromosomes when the last primer is removed using RNA template.



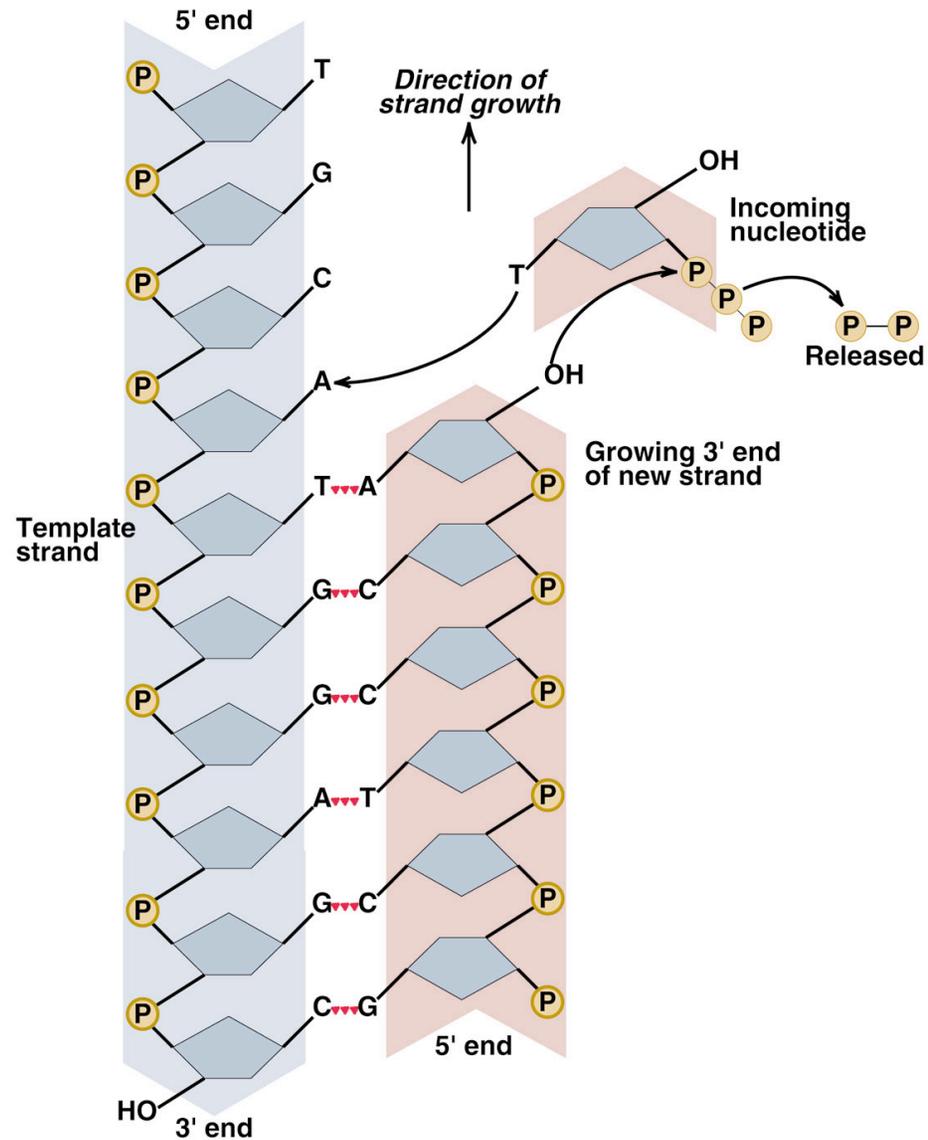
# Enzyme Activities for Biotechnology

**These enzyme activities, plus a few others, are also used to manipulate DNA, for example:**

- PCR
- Making recombinant DNA
- Detecting mutations at the molecular level

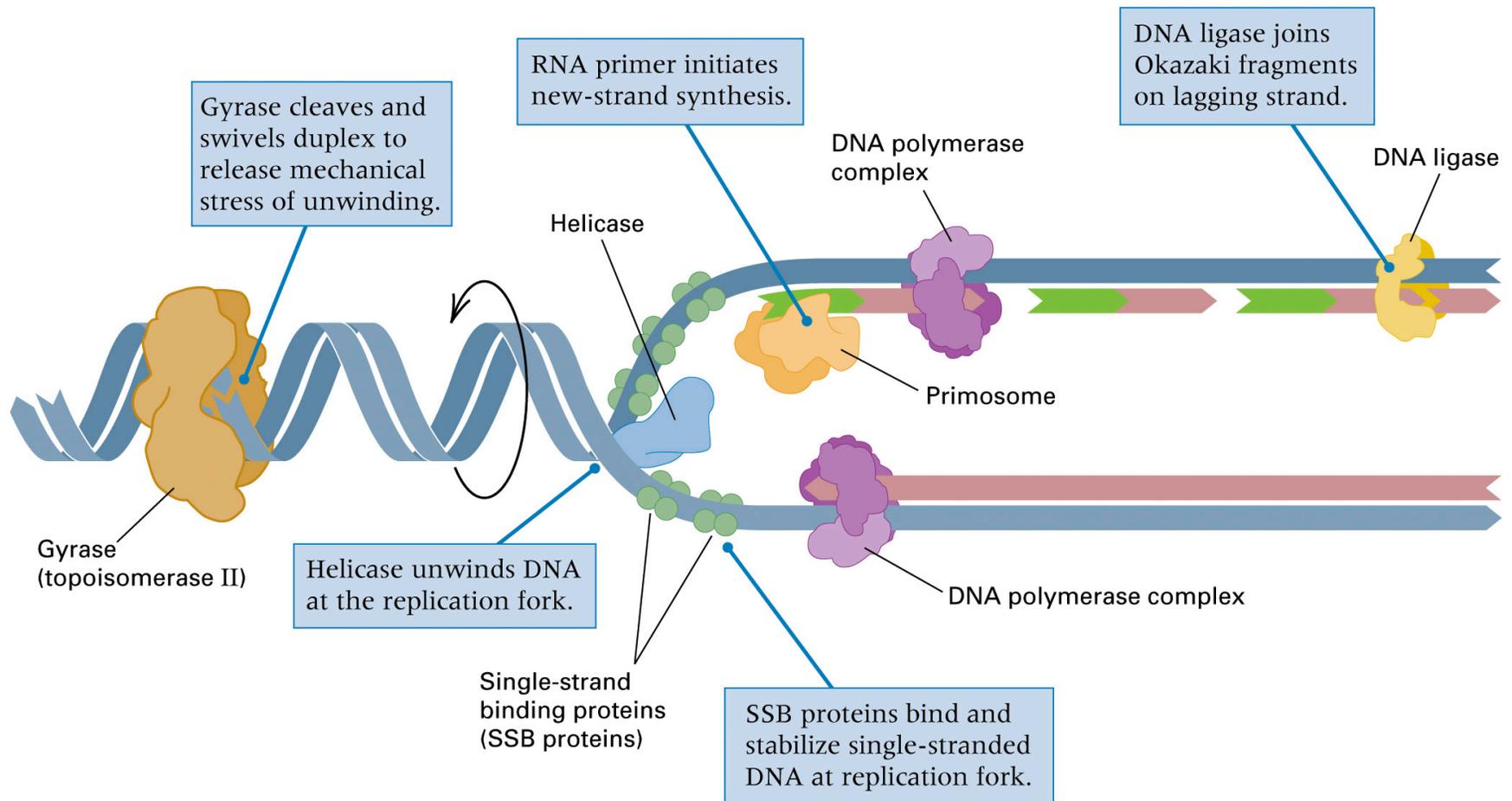
# DNA Replication Details

## Polymerase



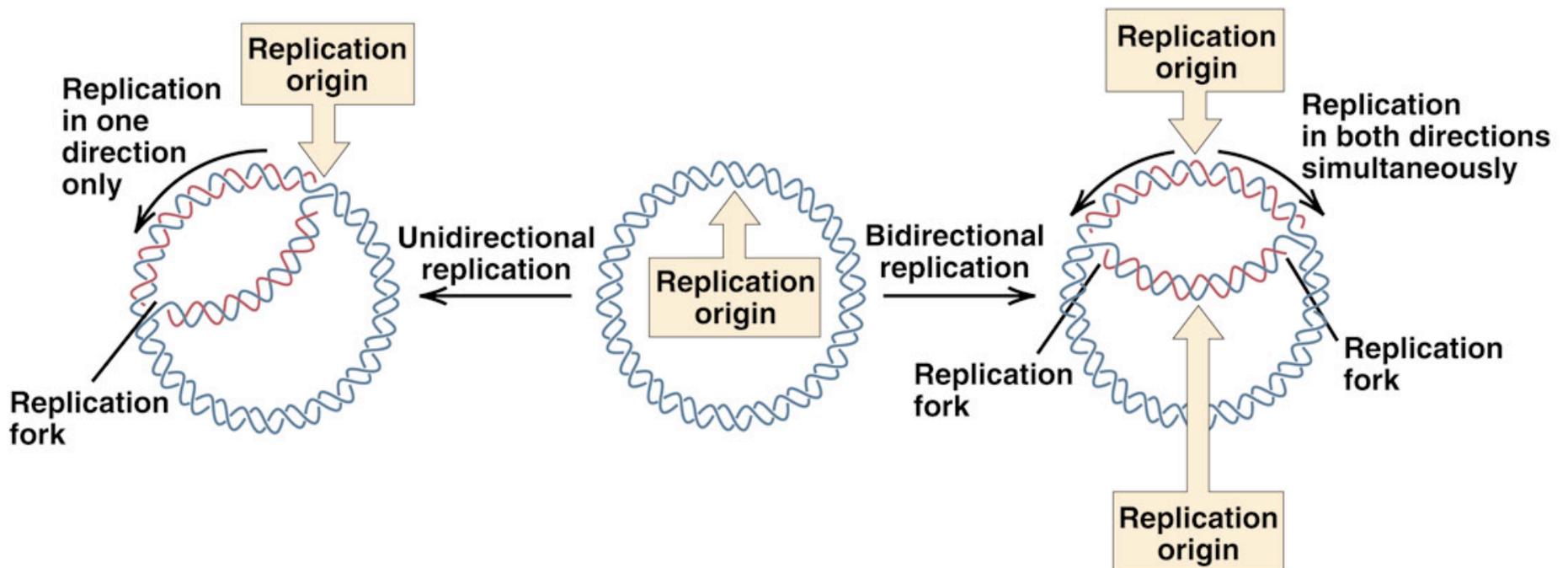
# DNA Replication Details

## Lagging and Leading Strands



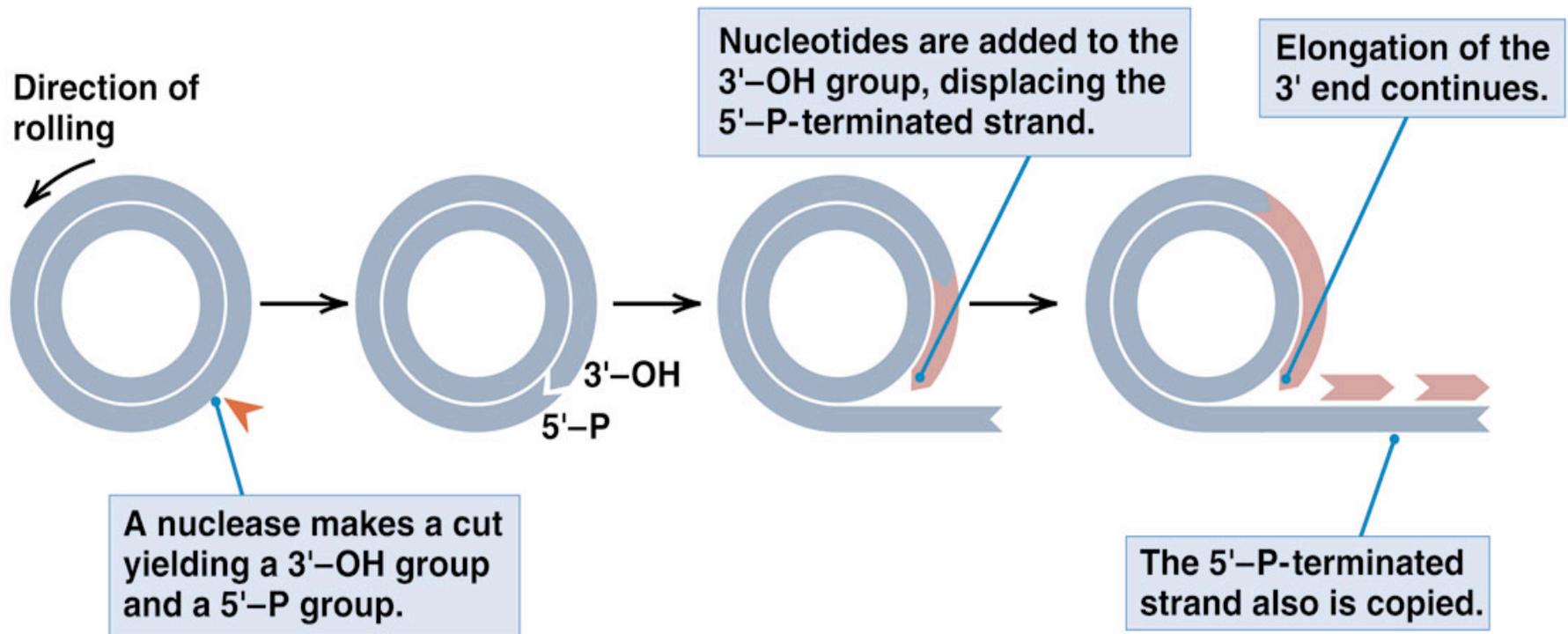
# Replicating Circular DNA Molecules

## θ Bidirectional Replication



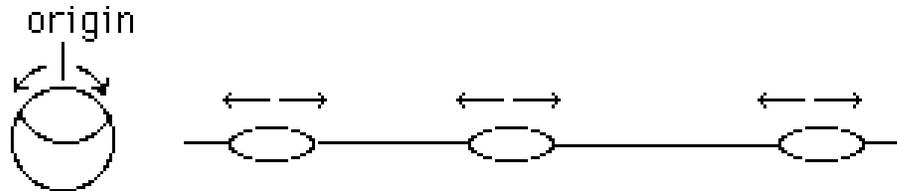
# Replicating Circular DNA Molecules

## Rolling Circle Replication



# DNA Replication is Very Fast

*E. Coli* completes replication of its 3.3 Mbp circular genome.  
Has two growing points so rate is about 690 bp/second.



Human chromosome replication rate is ca. 25 bp/second.  
To complete replication of its 2.9 Gbp genome in a reasonable time it  
has one origin every 90 kbp.