# CH 15:

**Transformation**, now defined as a change in genotype and phenotype due to assimilation of foreign DNA

Two findings became known as **Chargaff's rules**:

1. Base composition of DNA varies between species
2. In any species, the number of A and T bases are equal, and the number of G and C bases are equal (A=T, G=C)

The **semiconservative model** predicts that when a double helix replicates, each daughter molecule will have one old strand (derived or "conserved" from the parent molecule) and one newly made strand

- Beat out **conservative model** (the two parent strands rejoin)
- and the **dispersive model** (each strand is a nearly equal mix of old and new)

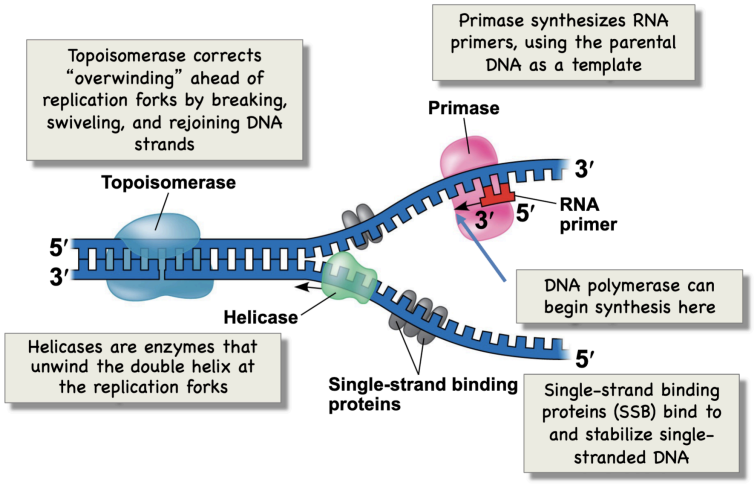
Replication begins at particular sites called **origins of replication**, where the two DNA strands are separated, opening up a replication "bubble"

- 2 replication forks
- Grow until fused -(eukaryotes)

At each end of a replication bubble is a **replication fork**, a Y-shaped region where new DNA strands are elongating

- **DNA polymerases** are enzymes that add new nucleotides to the 3' end of a pre-existing chain (called a primer) according to a template
- **Primase** enzyme synthesizes an **RNA primer**- this is necessary because RNA polymerases can initiate from scratch (de novo), but DNA polymerases can't
- **Helicase** enzyme disrupts hydrogen bonds between complementary bases and unwinds the double helix at the replication forks
- **Single-strand binding protein** (SSB) binds and stabilizes single- stranded DNA
- **Topoisomerase** enzyme corrects (relaxes) "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands

***The DNA Replication Toolkit***

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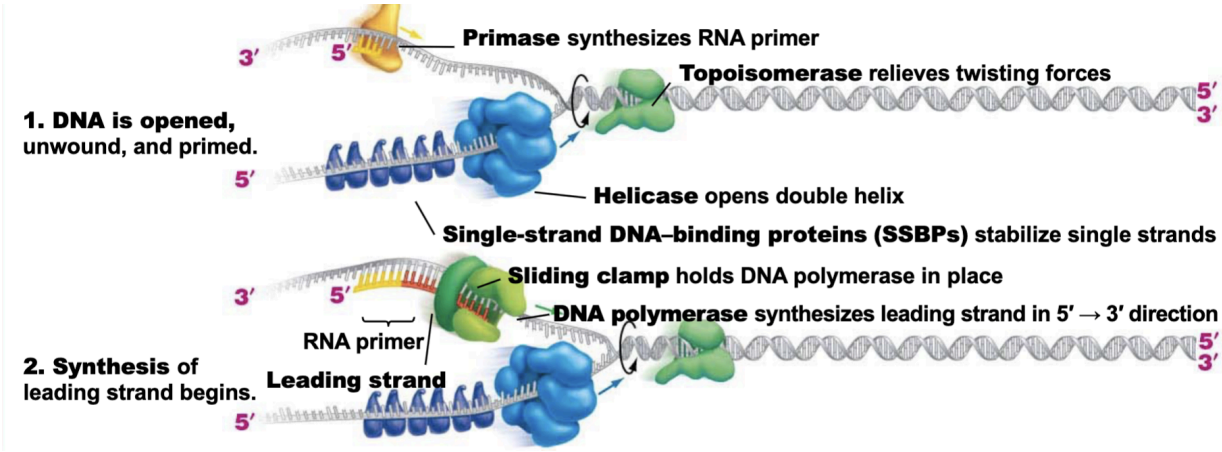
**DNA polymerases** add nucleotides to a DNA chain according to information provided by a **template** strand - ONLY ADD

- DNA synthesis requires a **primer** (a pre-existing DNA or RNA strand that is base paired to a template strand) (5-10 nucleotides long)
- primase start an RNA chain from scratch (de novo) and adds ribonucleotides using the parental DNA as template

new DNA strand can elongate only in the 5 prime to 3 prime direction

- Along one template strand of DNA, the DNA polymerase synthesizes a **leading strand** continuously, moving in the same direction as the replication fork
- The **lagging strand** is synthesized discontinuously (in pieces) in the direction opposite fork movement

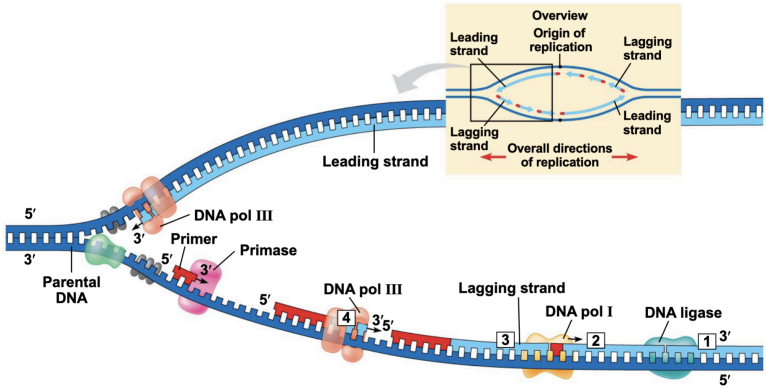



**Sliding clamp** binds the polymerase and fits around the DNA like a doughnut – this helps to hold the polymerase to the primer terminus

To elongate the other new strand, called the **lagging strand**, DNA polymerase must work in the direction away from the replication fork

- The lagging strand is synthesized as a series of segments called Okazaki fragments, which are joined together by DNA ligase

***Summary of Bacterial DNA Replication***




Only two (Pol I and Pol III) are involved in copying the genome

- **Pol III is specialized for bulk DNA replication at the fork** - extremely rapid rate of synthesis
  - complex enzyme (10 different polypeptides) including **two polymerase cores**- this allows leading and lagging strand synthesis to occur at the same time
- Also has 3' to 5' **exonuclease activity**- this allows it to **proofread**

- **Pol I is specialized for Okazaki fragment processing** and DNA repair - **REMOVING**
    - Slow rate of synthesis
    - single polypeptide with 3 different enzyme activities:
        - DNA polymerase activity
        - 5' to 3' exonuclease activity- this allows it to remove an RNA primer and replace it with DNA (nick translation)
        - 3' to 5' exonuclease activity- **proofread** (more later)
    - An **exonuclease** can degrade DNA (break phosphodiester bonds) from a free end
        - usually directional

The proteins that participate in DNA replication form a large complex called **The Replisome**

- dimeric Pol III polymerase that simultaneously synthesizes leading and lagging strands

**3' End Replication Issue:**

- Once the RNA primer is removed from the nascent 5' end, it can't be replaced because there is no primer
- Each cell division shortens the DNA molecule

Eukaryotic chromosomes have "buffers" (telomeres) at their ends

- **Telomeres** consist of a short nucleotide sequence (TTAGGG in humans) and proteins that bind these repeat sequences (shelterin proteins)
- NO genes
- Telomeres do not prevent the shortening of DNA molecules
- Postpone the erosion of genes near the ends of DNA molecules
- Cell enters senescence w/ no telomeres

• A unique enzyme called Telomerase catalyzes the lengthening of telomeres

• Telomerase is a **reverse transcriptase** that contains its own RNA template (reverse transcriptase makes DNA using an RNA template)

- (TERT) can make DNA complementary to an RNA template
- Telomerase RNA component (TERC)
    - CAAUCCCAAUC- provides the template for TERT
- No telomeres for normal somatic cells - **tumor surpressor**
- Progressive telomere shortening in somatic cells is a marker for cell age
- Most cancer cells also express telomerase, which allows them to divide indefinitely

**Telomeres act like cap, telomerase makes the strand longer to prevent it from shaving off**
**No telomeres means cell will shorten**

three distinct excision repair pathways specialized for different types of damage

- Mismatch Repair (MMR)
- Base Excision Repair (BER)
- **Nucleotide Excision Repair (NER)**
    - NER repairs the greatest variety of DNA damage In eukaryotes, 8 protein complexes cooperate to carry out NER
    - **Xeroderma pigmentosum (XP**) is a rare autosomal recessive disorder caused by an inherited defect in any of the eight NER protein complexes - skin cancer