DNA Replication
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.

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

Cell Cycle
Mitosis

G2 → G1 → G1 + S + G2 = interphase

Variable lengths. Total time 15 minutes → 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.

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)
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’.

![Exonuclease Diagram]

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

![Ligase Diagram]
Putting it All Together 1

primase makes RNA primer

polymerase extends
Putting it All Together 2

exonuclease removes RNA primer

polymerase fills gap

ligase seals nick
Replicating Linear Chromosomes
When Replication Forks Meet

- Replication begins and is bidirectional.
- Replication origin
- Replication bubbles fuse where they meet.
- Synthesis starts at second origin and is also bidirectional.
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
Lagging and Leading Strands

Gyrase cleaves and swivels duplex to release mechanical stress of unwinding.

Helicase unwinds DNA at the replication fork.

SSB proteins bind and stabilize single-stranded DNA at replication fork.

RNA primer initiates new-strand synthesis.

DNA polymerase complex

Primosome

DNA ligase joins Okazaki fragments on lagging strand.
Replicating Circular DNA Molecules

θ Bidirectional Replication
Replicating Circular DNA Molecules
Rolling Circle Replication

1. A nuclease makes a cut yielding a 3′–OH group and a 5′–P group.
2. Nucleotides are added to the 3′–OH group, displacing the 5′–P-terminated strand.
3. Elongation of the 3′ end continues.
4. The 5′–P-terminated strand also is copied.
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.

![Diagram of DNA replication]

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.