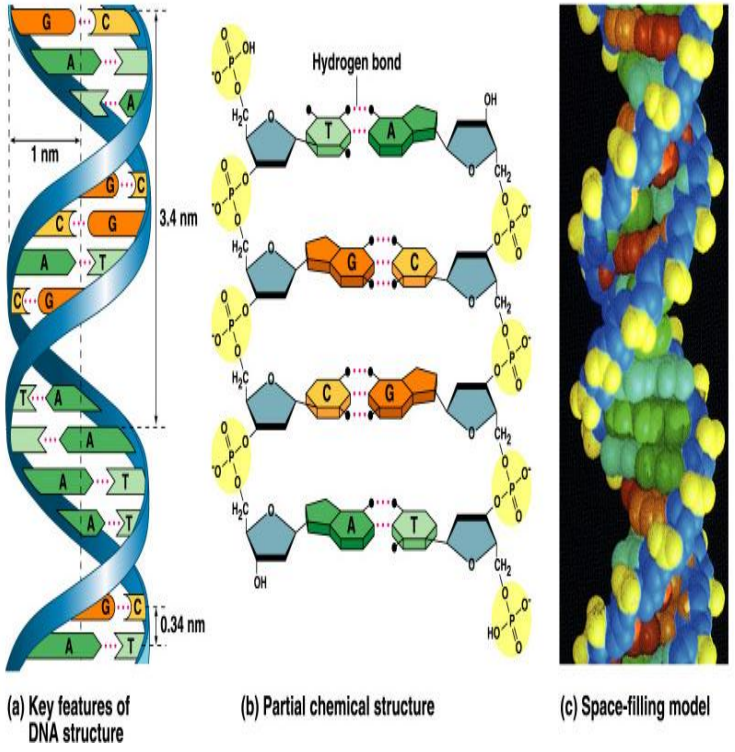
Aim: How is DNA replicated?

DNA Structure

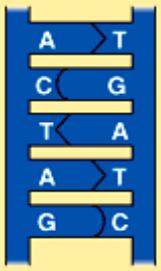
- DNA molecules are arranged as a double helix with an anti-parallel arrangement.
- Genes are found on DNA and differ in the:
  - Length of the chain
  - Sequence (arrangement) of the nitrogen bases.



According to Watson and Crick, in order to replicate DNA:

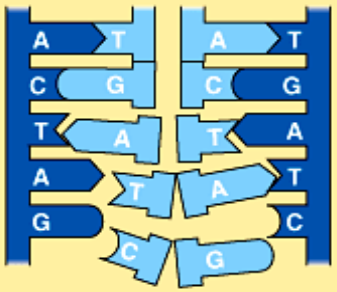
1) Hydrogen bonds between nitrogen bases must be broken

2) Complimentary bases must be added to the exposed bases. (semi-conservative replication)

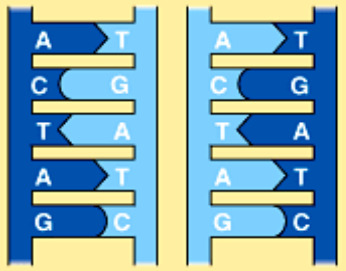


(a) The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

(b) The first step in replication is separation of the two DNA strands.



(c) Each parental strand now serves as a template that determines the order of nucleotides along a new complementary strand.

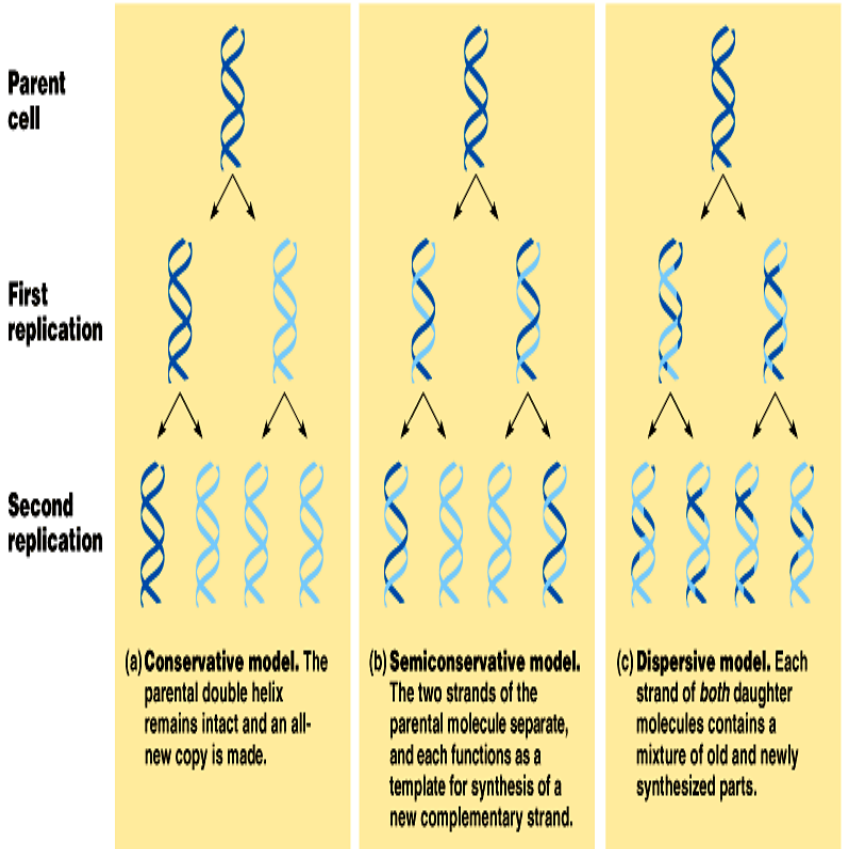


(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

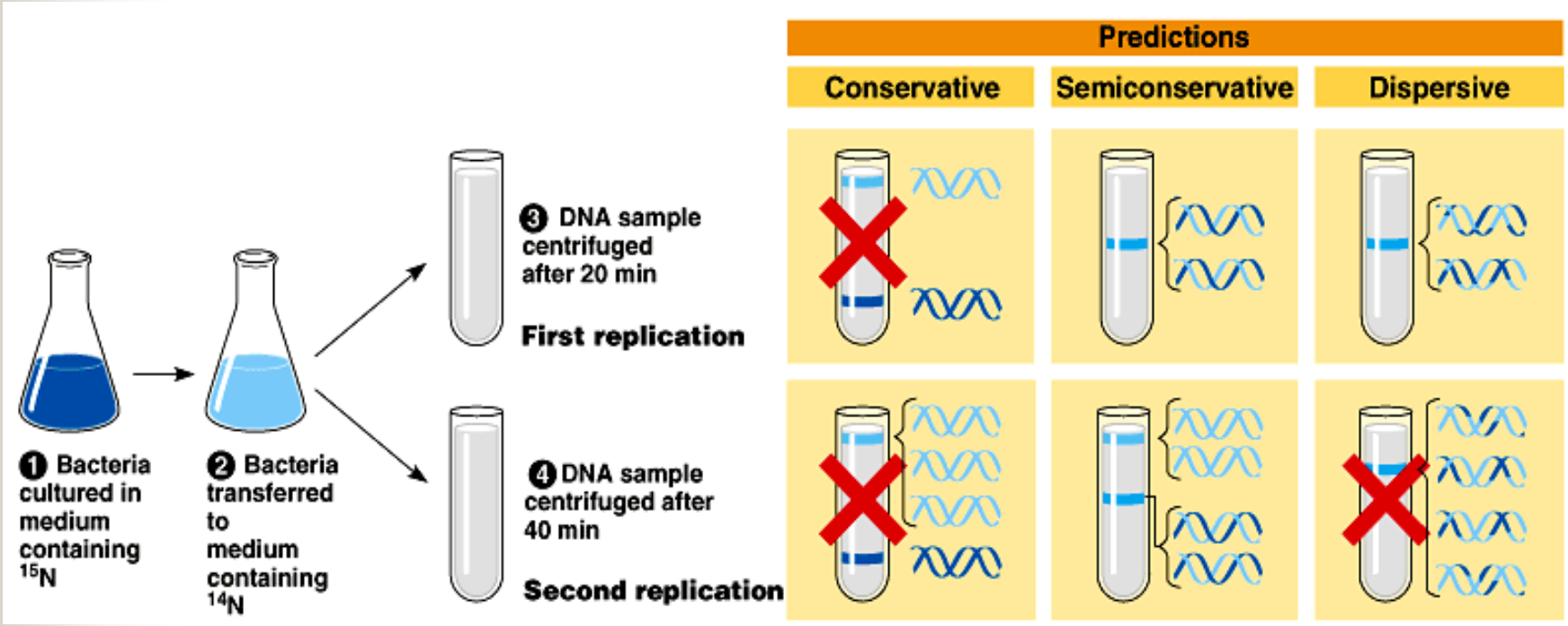
Experimental evidence for semi-conservative DNA replication

- 1) 1957 – Arthur Kornberg (Washington University in St. Louis)
  - **A) extracted DNA polymerase enzyme from bacteria. Polymerase enzymes create polymers (large molecules made up of hundreds of building blocks)**
  - **B) found that DNA polymerase synthesizes DNA in vitro.**

- 2) 1958 – Matthew Meselson and Franklin Stahl (California Institute of Technology)
  - A) used DNA containing radioactive heavy N^{15} as a template and normal N^{14} for replication.
  - B) 2nd generation DNA contained both N^{15} and N^{14} (50 % each)



- Conclusion: DNA replication involves a semi-conservative process.
- Each new DNA molecule contains one parental strand and one new strand.
- DNA must unzip and replicate.



What is the general mechanism of DNA replication?

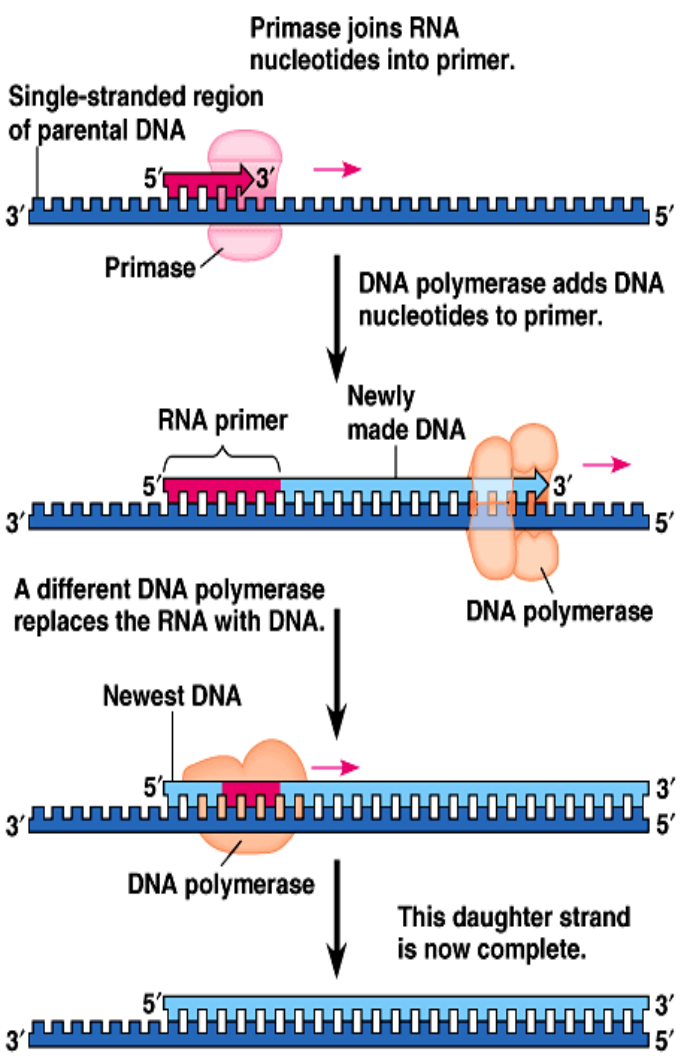
- Key events in DNA replication for all organisms:
- 1) Replication of strands occurs in only one direction, along the 3' → 5' template strand of the original DNA molecule. The new strand builds in a 5' → 3' direction.
- 2) All organisms have mechanisms that locate and correct errors in replication.
- 3) All organisms have ways of preventing the strands from tangling.
- 4) Eukaryotes must first unwind DNA (50 base-pairs per second)

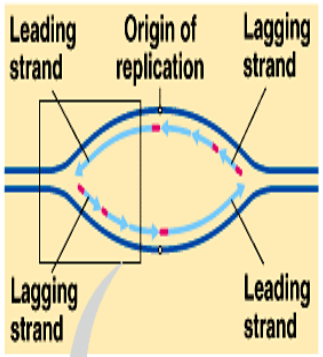
What is the general mechanism of DNA replication?

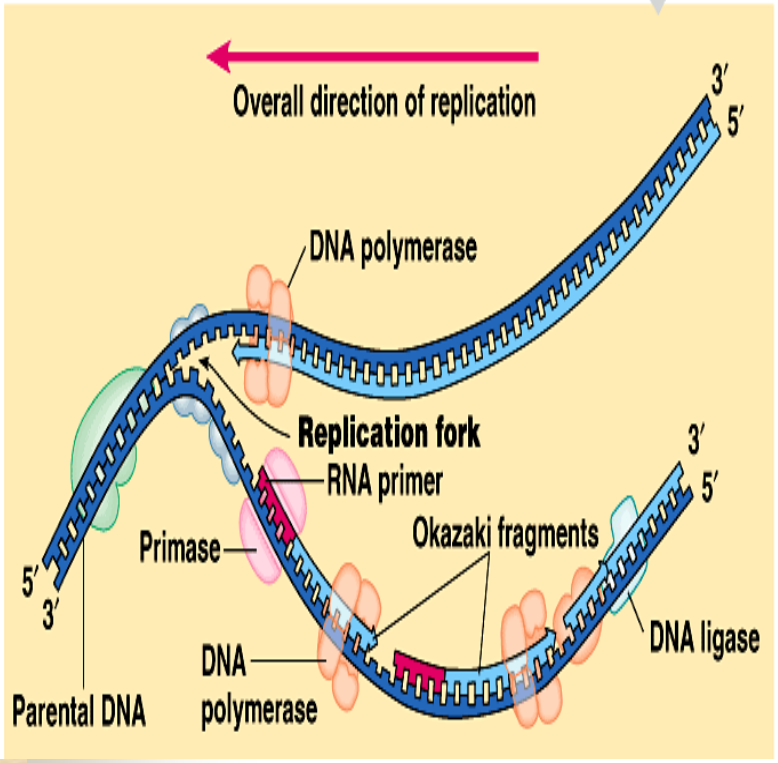
- 5) Eukaryotes have extremely long chromosomes and duplicated their DNA along many spots (replication bubbles) simultaneously.
- Of note:
  - **A) DNA polymerase III adds 500 base-pairs per second in prokaryotes with only 1 error per billion pairs added. In humans, 50 base-pairs per second are added.**
  - **B) DNA replication is basically the same in prokaryotes (bacteria) and eukaryotes.**

DNA replication in E. coli bacteria

- 1) DNA B enzme binds to the initiation site on DNA
- 2) DNA gyrases (helicases) relax supercoiling.
- 3) DNA rep enzyme “unzips” the double strand by breaking Hydrogen bonds. ATP is required.
- 4) SSB (single-strand binding) proteins keep the two strands from reattaching.

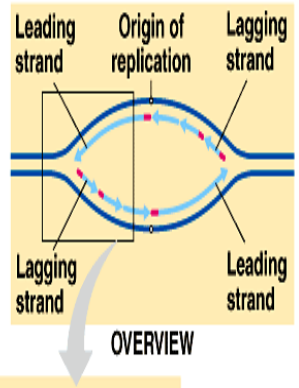


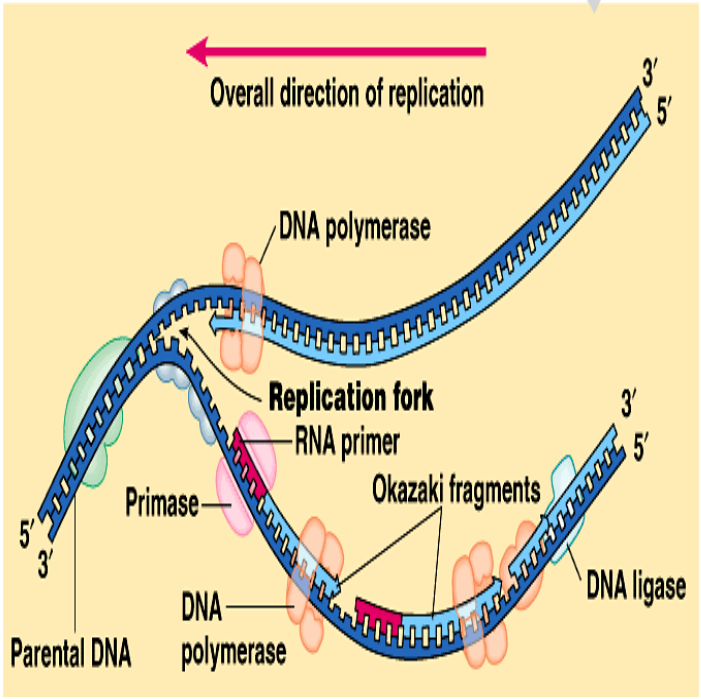




- 5) On the leading strand, DNA polymerase III follows the rep enzyme along the 3' → 5' template, adding complimentary bases as it goes.
- DNA polymerase III has 4 active sites, one for each nitrogen base.
- Energy is released as nitrogen bases are added.

- 6) On the lagging strand, there is no initiation site. So…
  - A) RNA primase enzyme adds RNA nucleotides complimentary to the template. These ‘primers’ are 10 bases long
  - B) From the RNA primers, DNA poly III produces new DNA fragments 1000 – 2000 bases long. These are called OKAZAKI FRAGMENTS





- 7) DNA polymerase I removes RNA primers and replaces them with DNA nucleotides.
- 8) DNA ligase welds together the Okazaki fragments.