Chapter 9

Topics

- Genetics
- Flow of Genetics
- Regulation
- Mutation
- Recombination

Genetics

- Genome
  - Chromosome
  - Gene
  - Protein
- Genotype
- Phenotype

Terms and concepts

- gene
  - Fundamental unit of heredity
  - DNA segment which codes for protein or RNA
- clone
  - population of cells that are genetically identical
- genome
  - all genes present in a cell or virus
    - haploid – one set of genes (eg., bacteria)
    - diploid – two sets of genes (eg., humans)
- genotype
  - specific set of genes an organism possesses
- phenotype
  - set of observable characteristics

The sum total of genetic material ~~of a cell~~ is referred to as the genome.

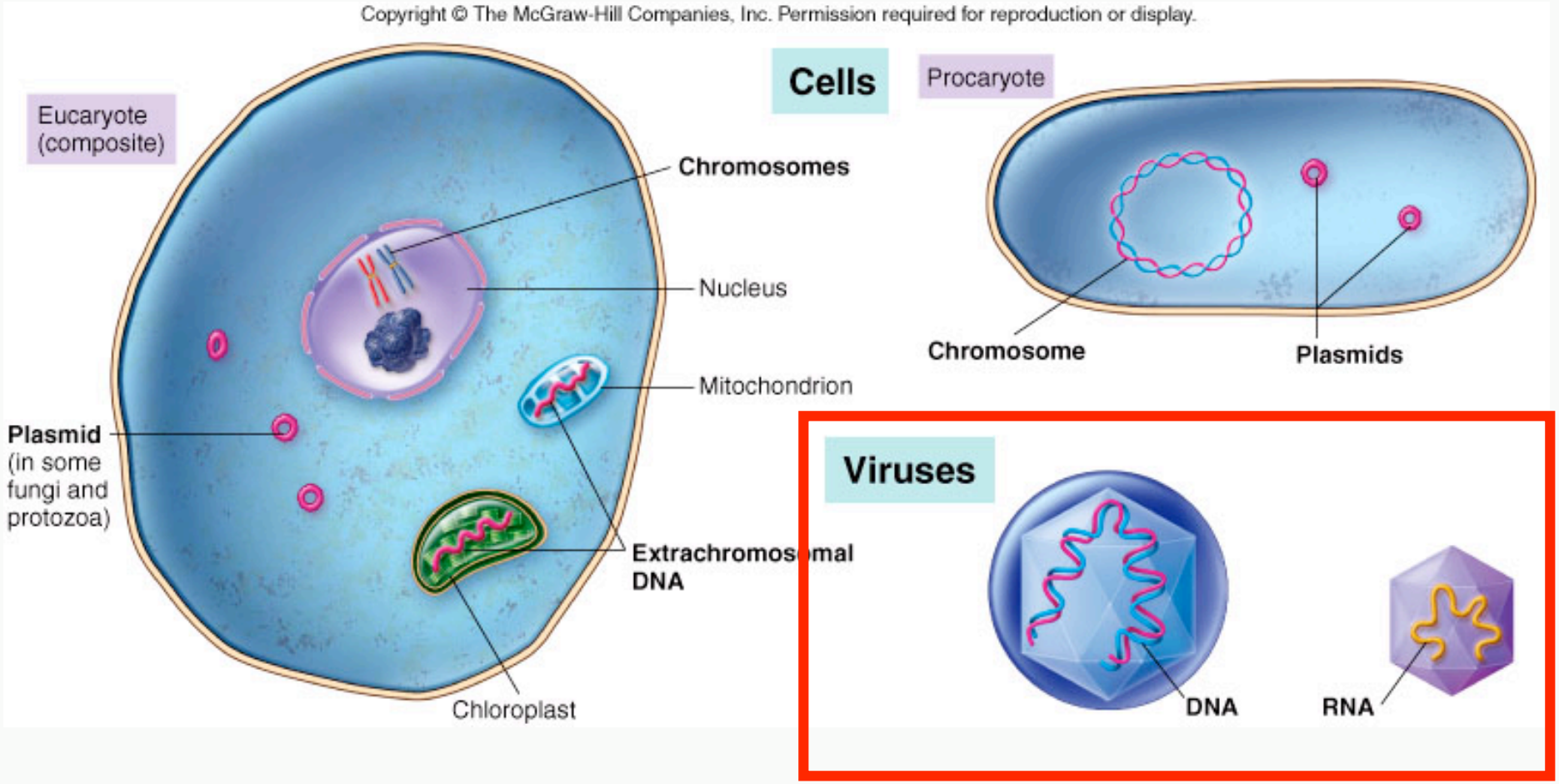
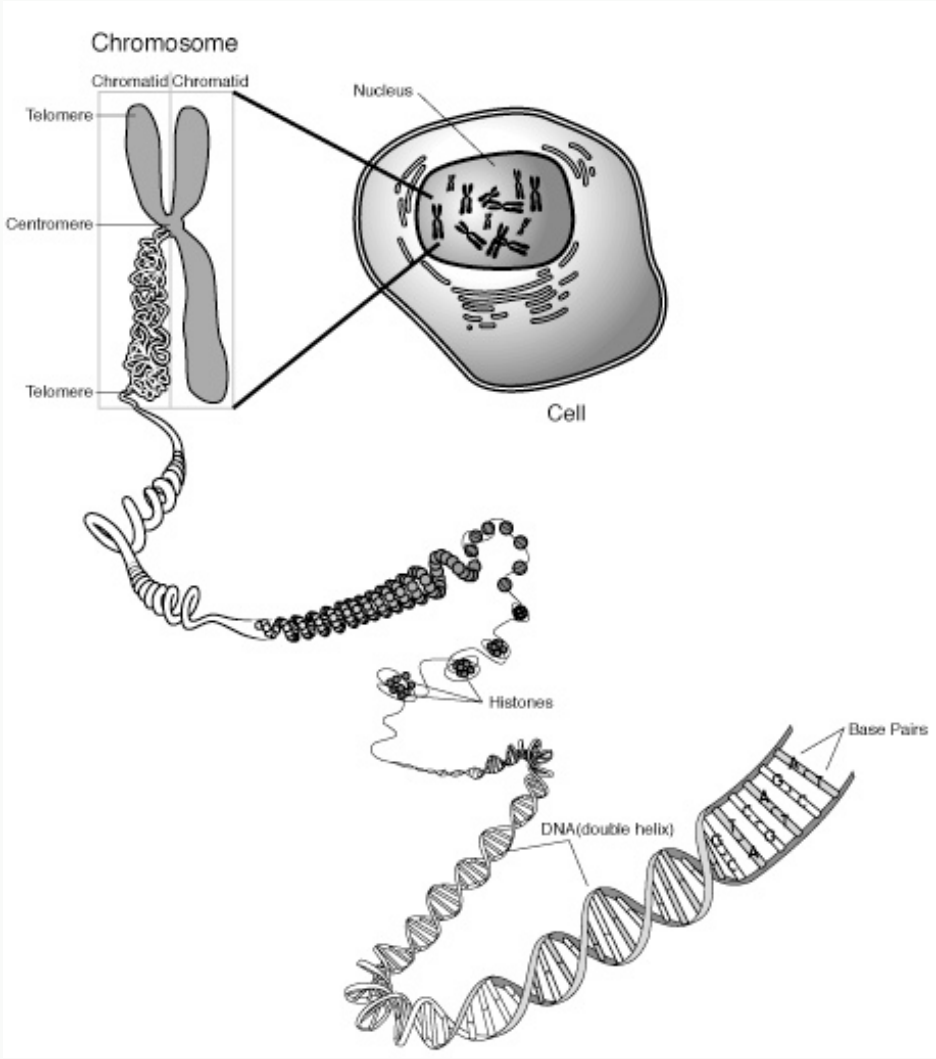


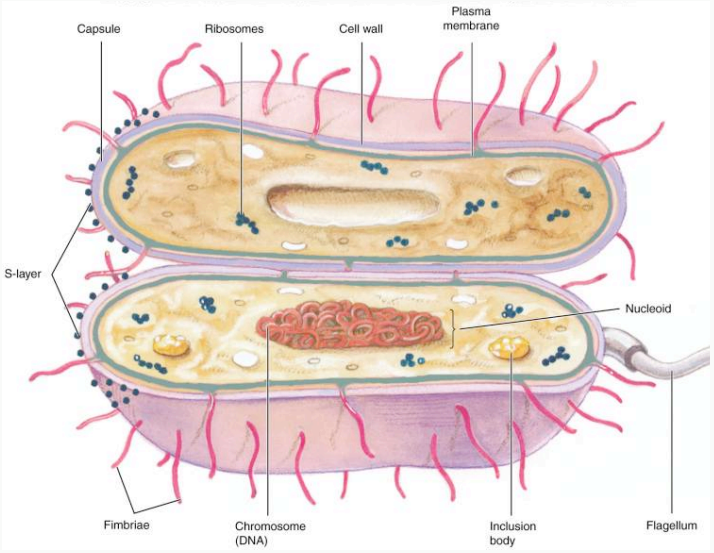
Fig. 9.2 The general location and forms of the genome

DNA and Chromosomes



The Organization of DNA in Cells

- Chromosomes – neatly packaged DNA molecule.



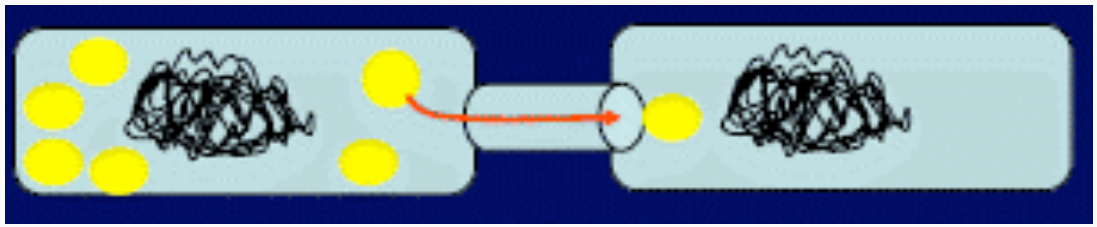
- organization differs in prokaryotic and eukaryotic cell types

Chromosome

- Prokaryotic
  - Histonelike proteins condense DNA
- Eukaryotic
  - Histone proteins condense DNA
- Subdivided into basic “informational packets” called genes

Plasmids

- usually small, closed circular DNA molecules
- exist and replicate independently of chromosome
- not required for growth and reproduction
- may carry genes that confer selective advantage (e.g., drug resistance)



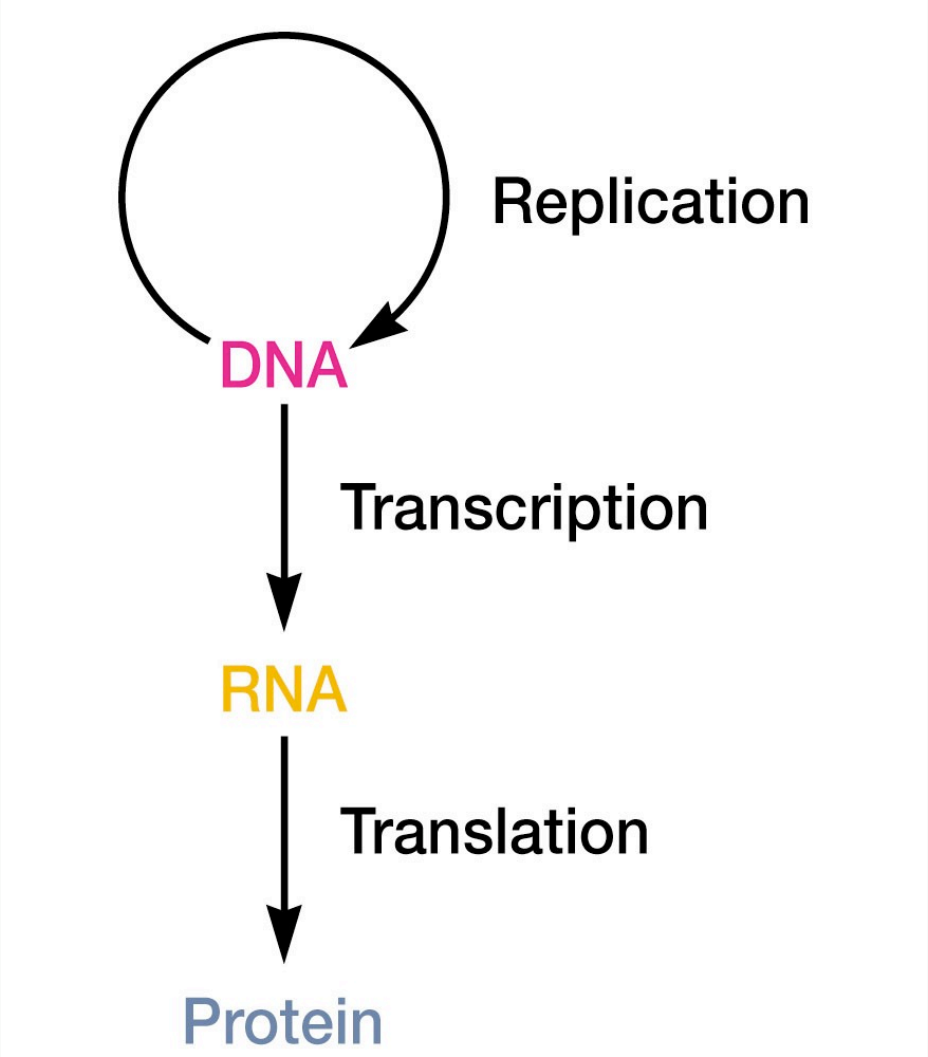
Genes

- Three categories
  - Structural
  - Regulatory
  - Encode for functional/regulatory RNAs
- Genotype
  - Specific set of genes an organism possesses
- Phenotype
  - Set of observable characteristics

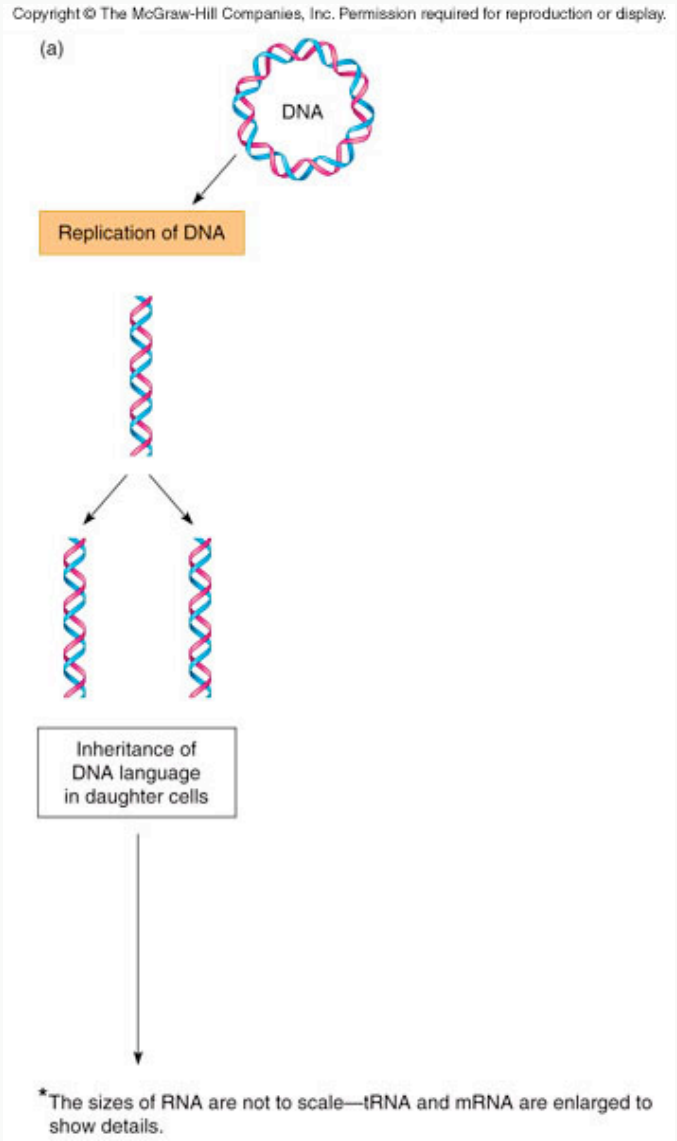
Flow of Genetics

- NA replication (DNA => DNA; RNA => RNA)
- Gene Expression (DNA =>RNA=>Protein)
  - Replication
  - Transcription
  - Translation
  - Post-translational modification

The Central Dogma



Representation of the flow of genetic information.



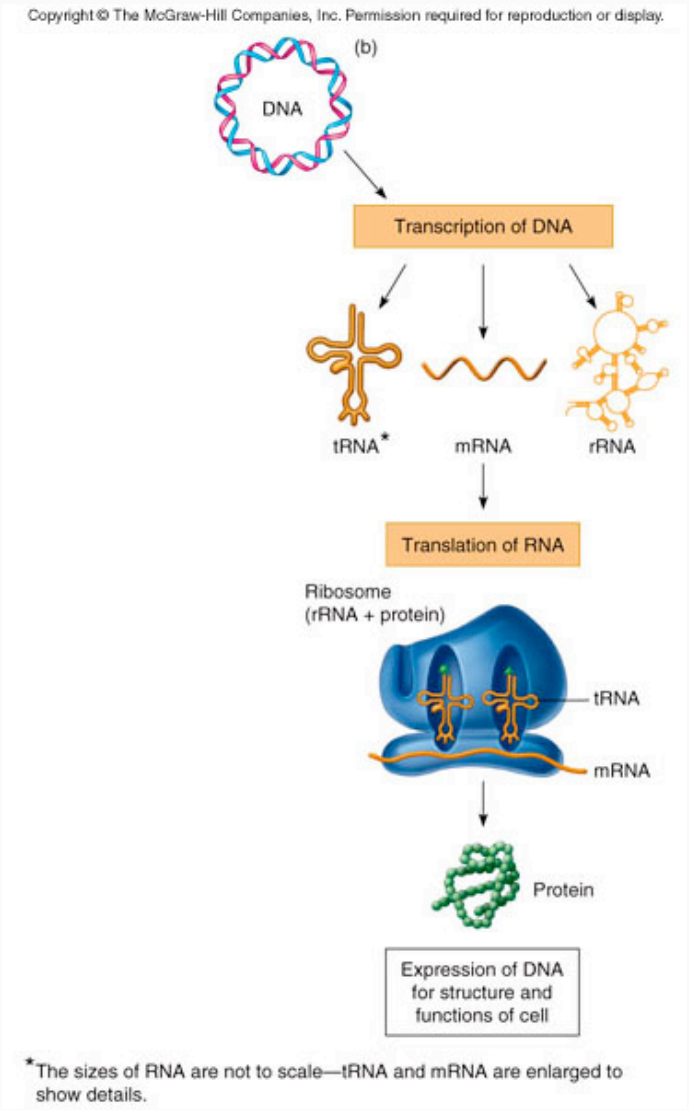


Fig. 9.9 Summary of the flow of genetic information in cell.

DNA

- **Structure -** √

Fig. 9.3 An *Escherichia coli* cell disrupted to release its DNA molecule.

- Replication - today

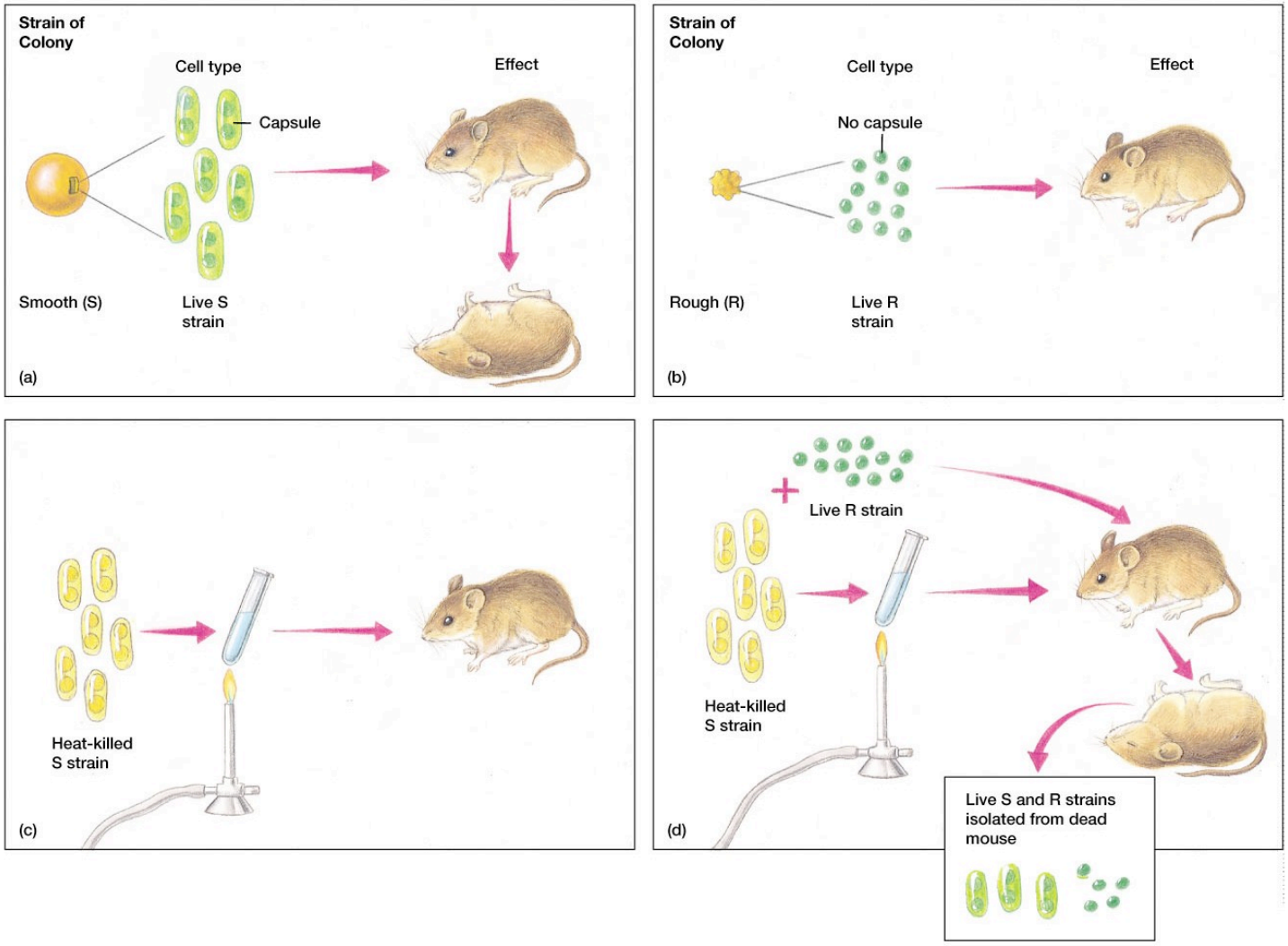
Structure

- Nucleotide
  - Phosphate
  - Deoxyribose sugar
  - Nitrogenous base
- Double stranded helix
  - Antiparallel arrangement

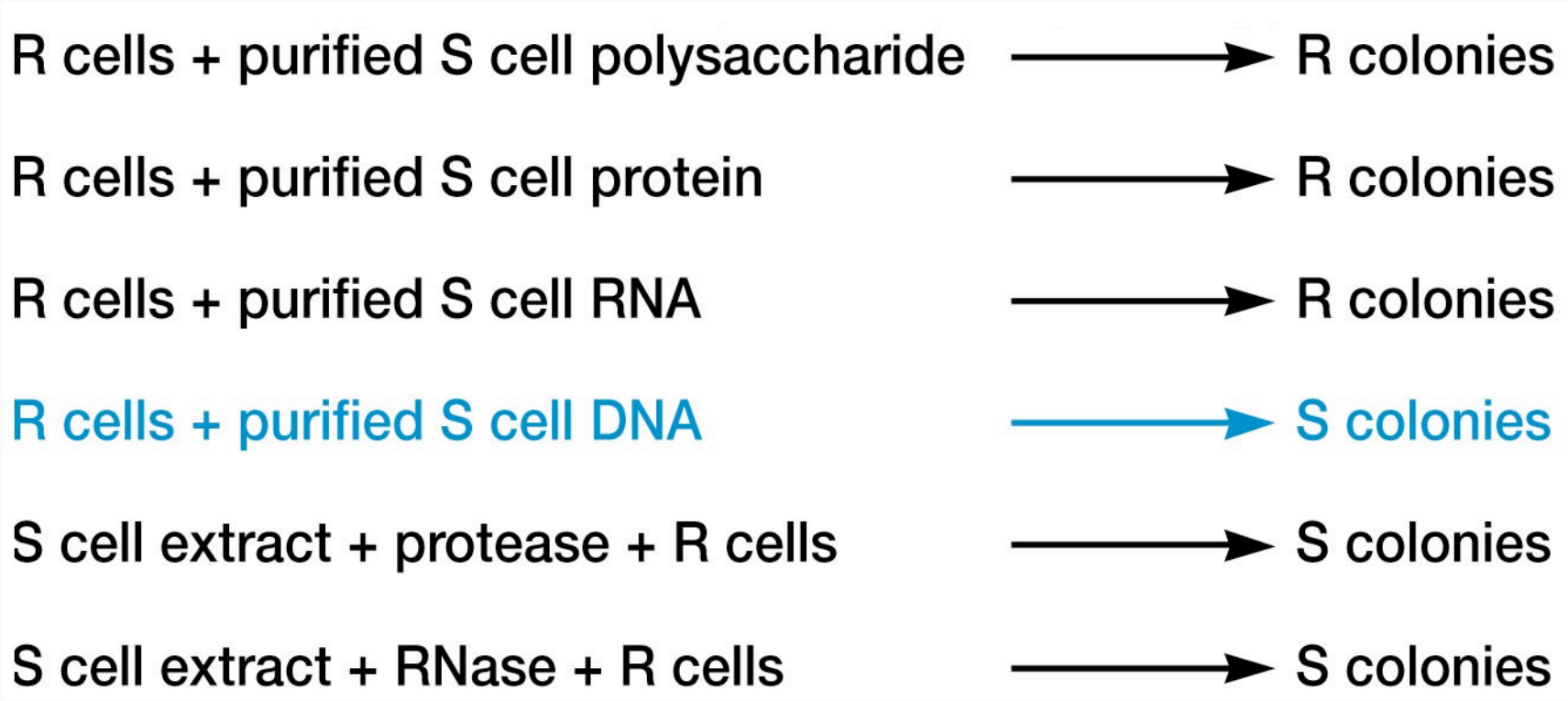
DNA as Genetic Material

- established by several critical experiments
  - Fred Griffith (1928)
  - Oswald T. Avery, C. M. MacLeod, and M. J. McCarty (1944)
  - Alfred D. Hershey and Martha Chase (1952)

Griffith's Experiment

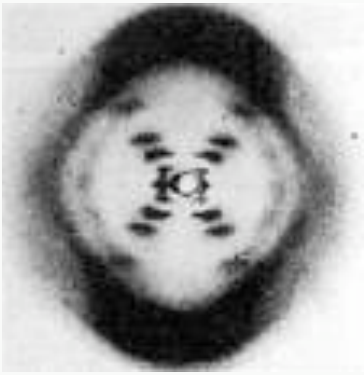


Griffith's Experiment: Transforming principle



Structure of DNA

Rosalind Franklin



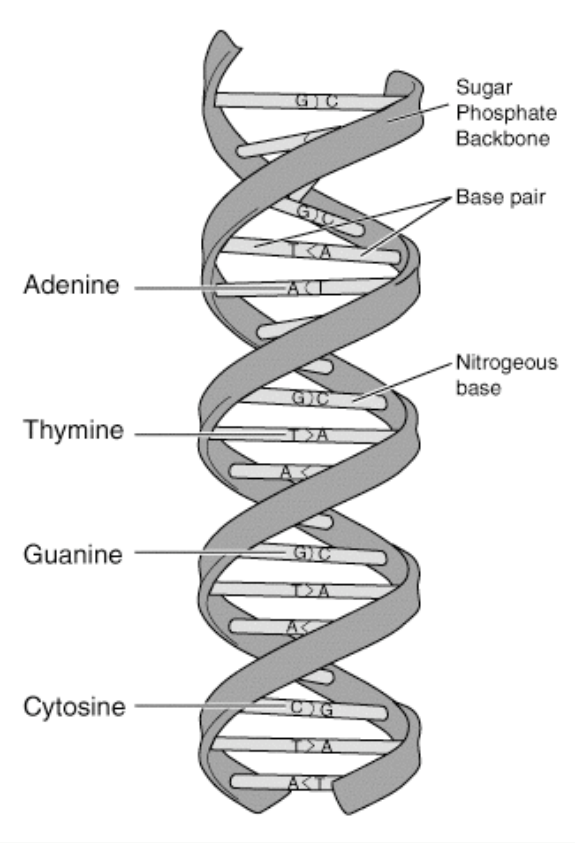
X-ray analysis
of DNA structure

"for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material"

Francis Harry Compton Crick

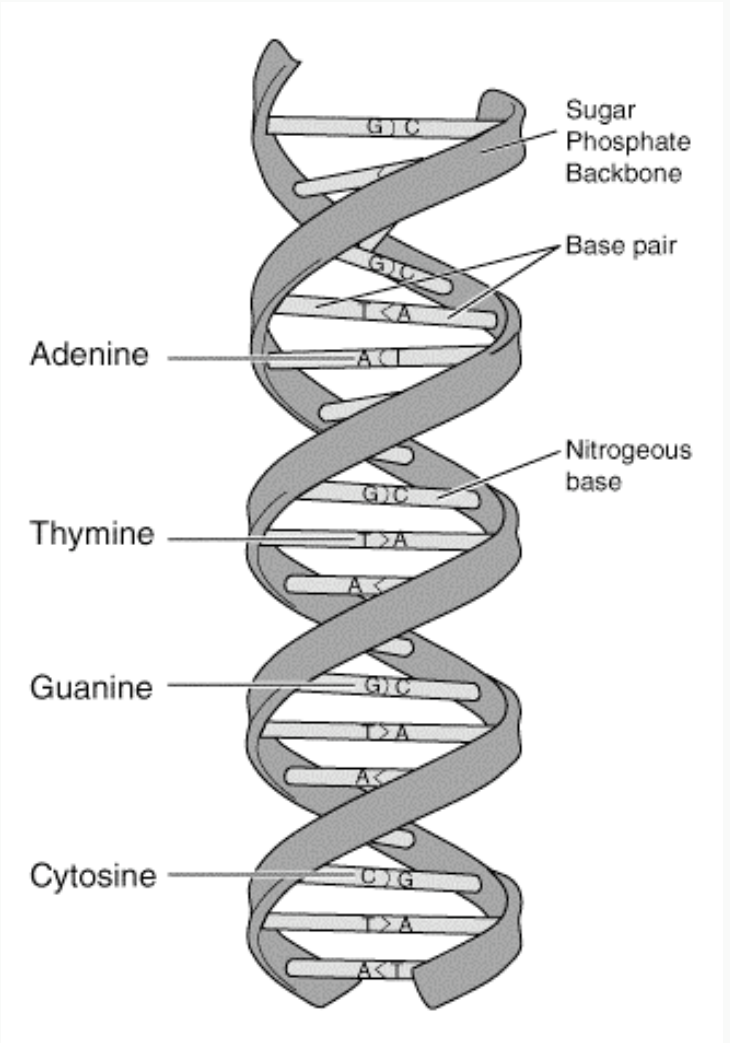
James Dewey Watson

Maurice Hugh Frederick Wilkins

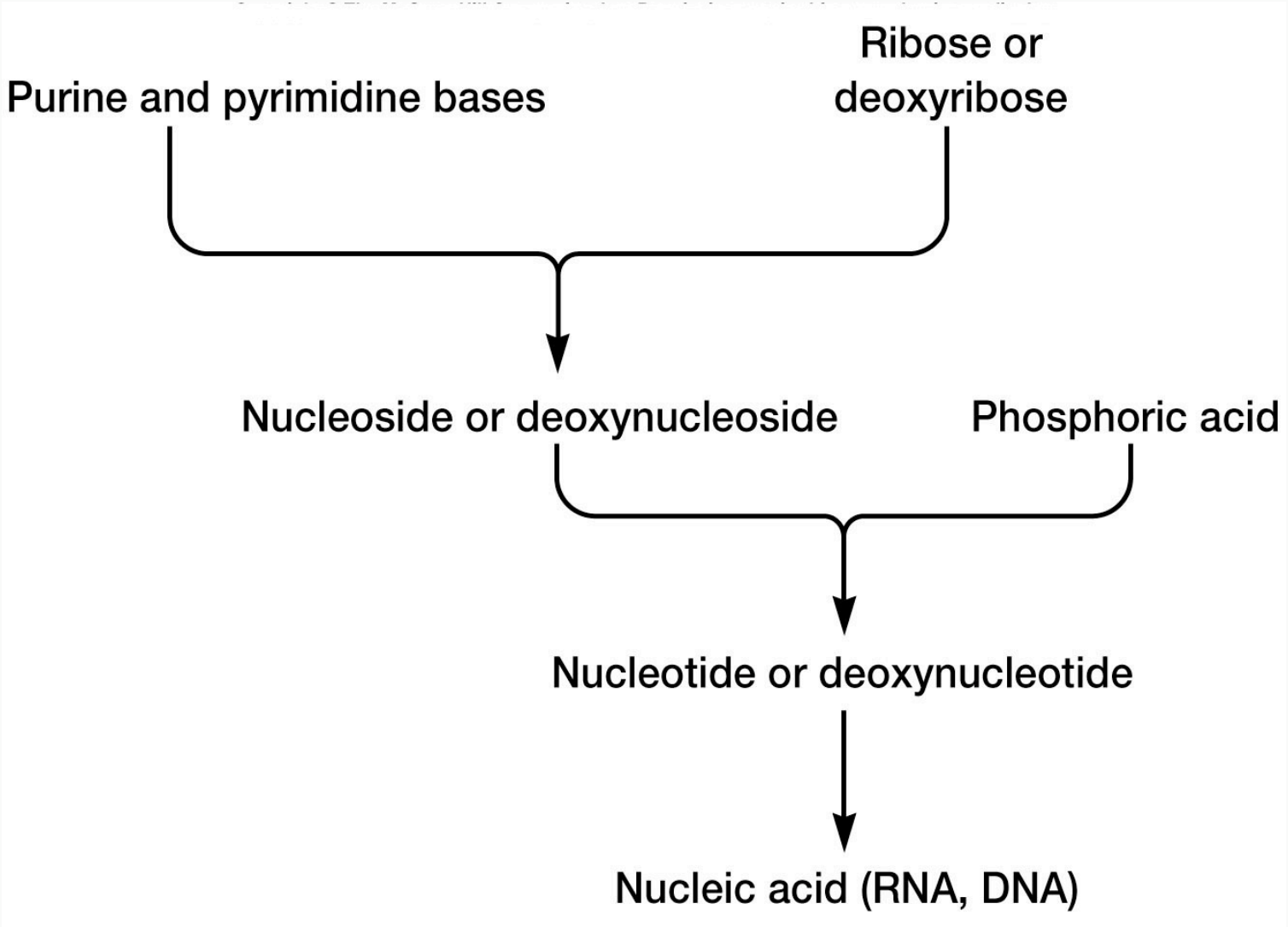


DNA Structure

- nitrogenous bases
  - A, T, G, C
- pentose sugar
  - deoxyribose
- chain of nucleotides linked by phosphodiester bonds
- usually a double helix, composed of two complementary strands
  - base pairing rules
    - A with T
    - G with C



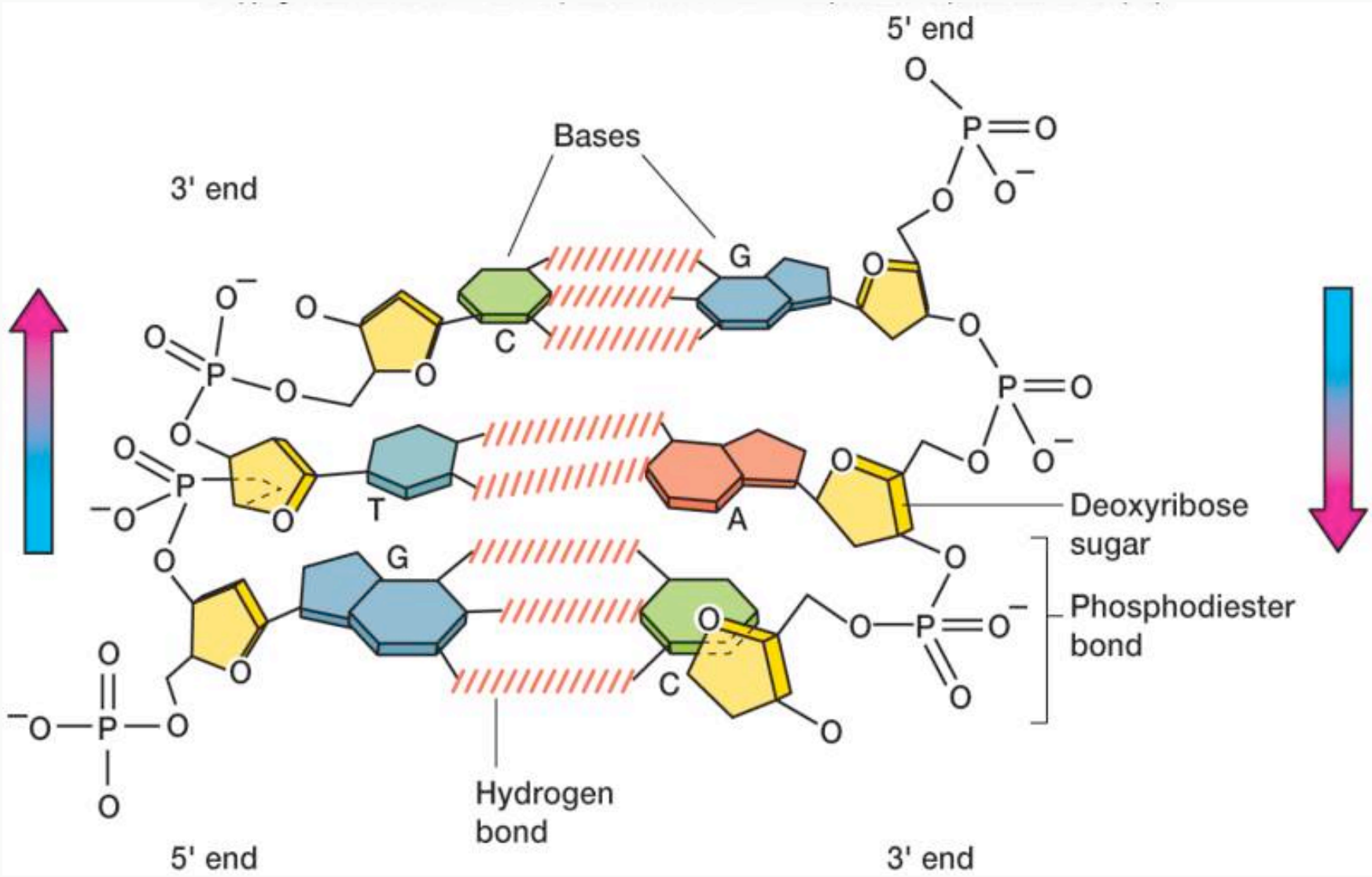
Nucleic Acid Structure



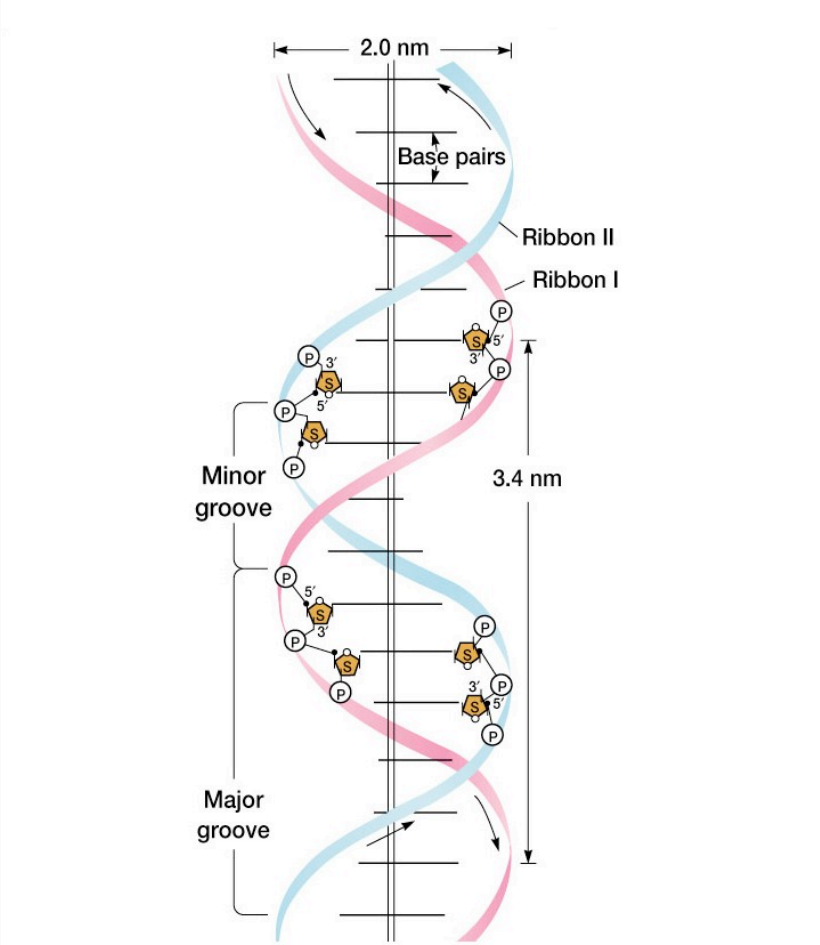
Nitrogenous bases

- Purines
  - Adenine
  - Guanine
- Pyrimidines
  - Thymine
  - Cytosine

DNA Structure



DNA Structure



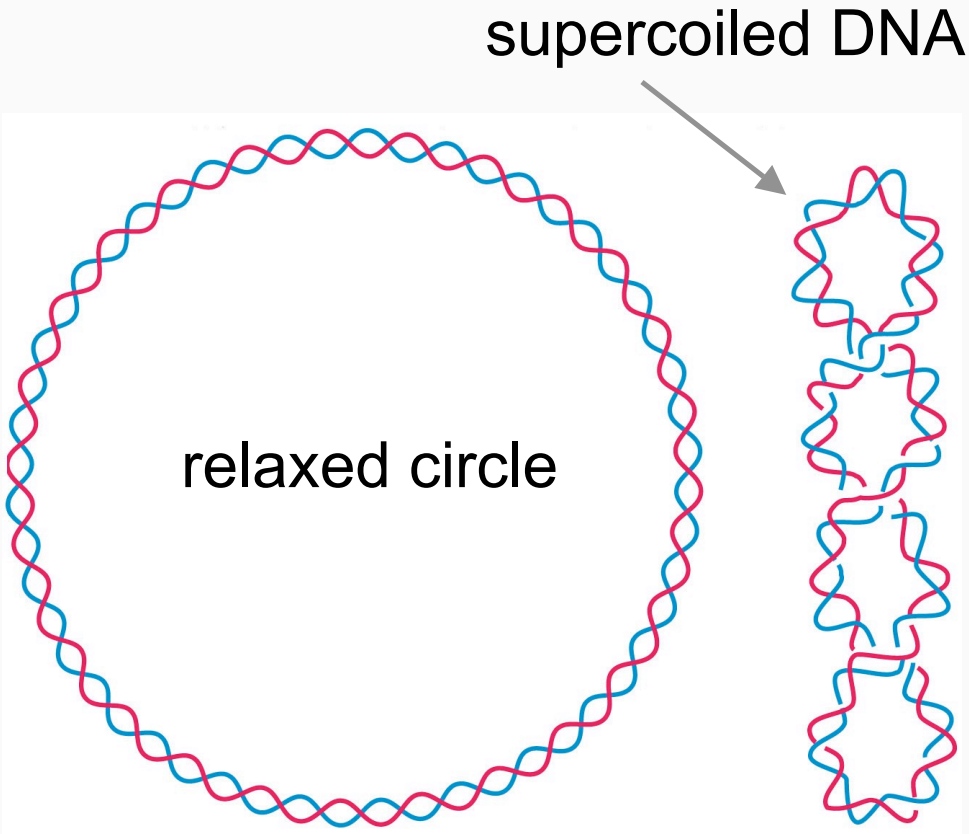
two polynucleotide chains are anti-parallel

RNA Structure

- nitrogenous bases
  - A, G, C, U (instead of T)
- pentose sugar
  - ribose
- usually consists of single strand of nucleotides linked by phosphodiester bonds
  - can coil back on itself
    - forms hairpin-shaped structures with complementary base pairing and helical organization
    - base pairing rules
      - A with U
      - G with C

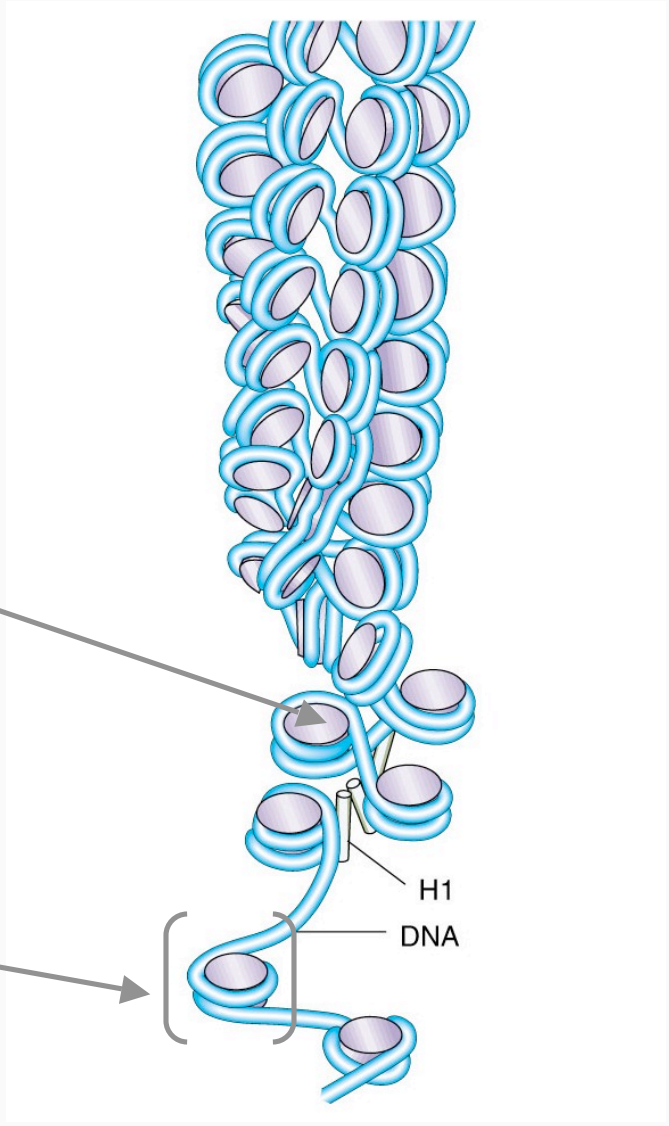
Prokaryotic chromosome

- usually exists as closed circular, supercoiled molecule associated with basic proteins

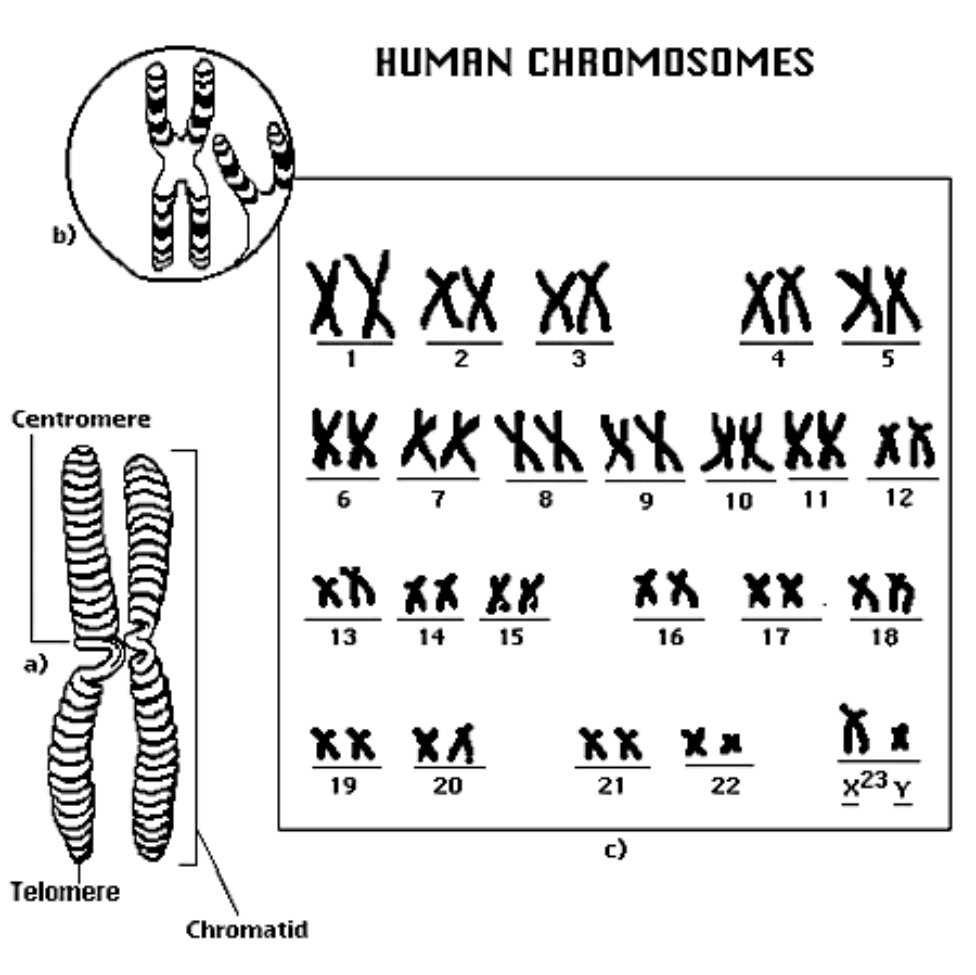


Eukaryotic DNA

- linear molecules
- associated with histones
- coiled into repeating units called nucleosomes



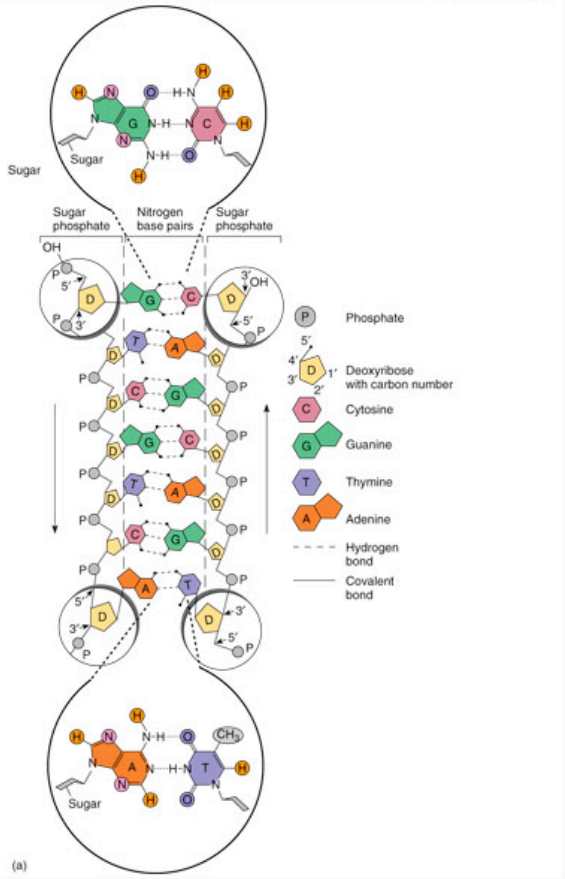
Eukaryotic Chromosomes



A chromosome is formed from a single DNA molecule that contains many genes.

A chromosomal DNA molecule contains three specific nucleotide sequences which are required for replication: a DNA *replication origin*;
a *centromere* to attach the DNA to the mitotic spindle.;
a *telomere* located at each end of the linear chromosome.

Purines and pyrimidines pair (A-T or G-C) and the sugars (backbone) are linked by a phosphate.



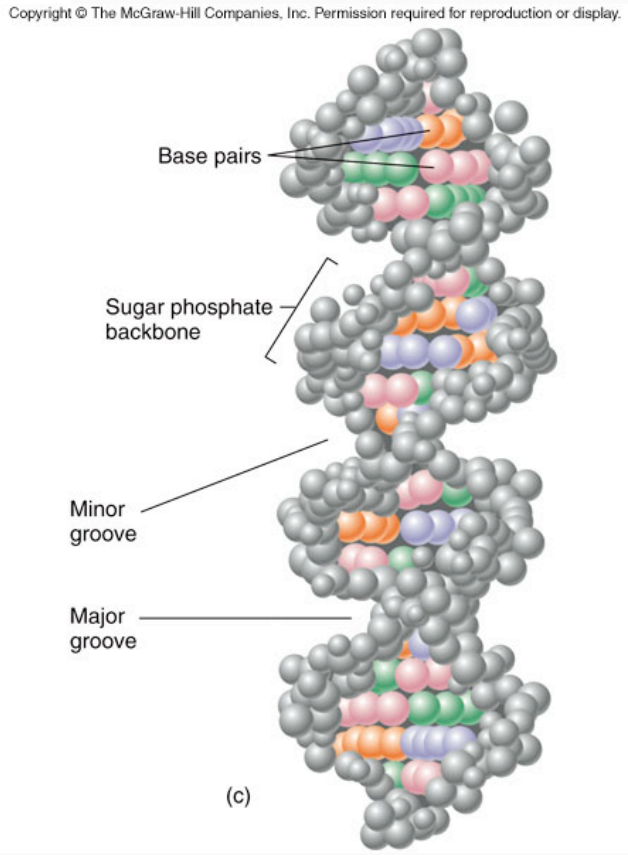


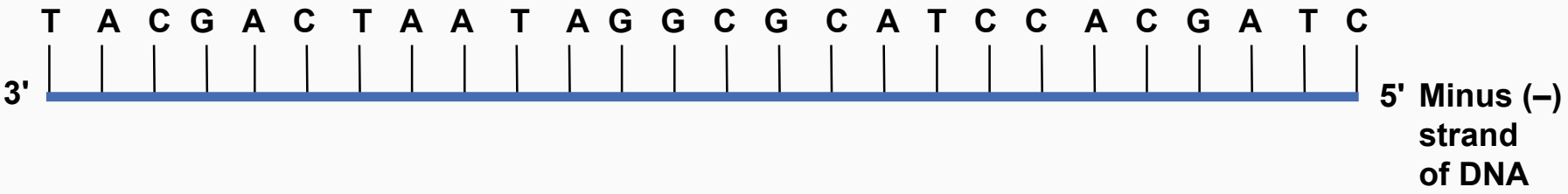
Fig. 9.4 Three views of DNA structure

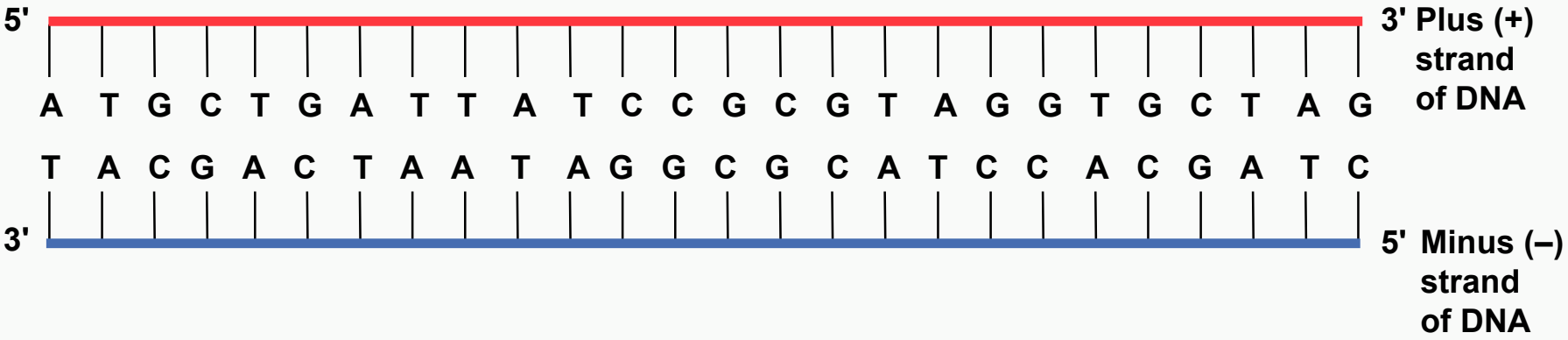
Replication

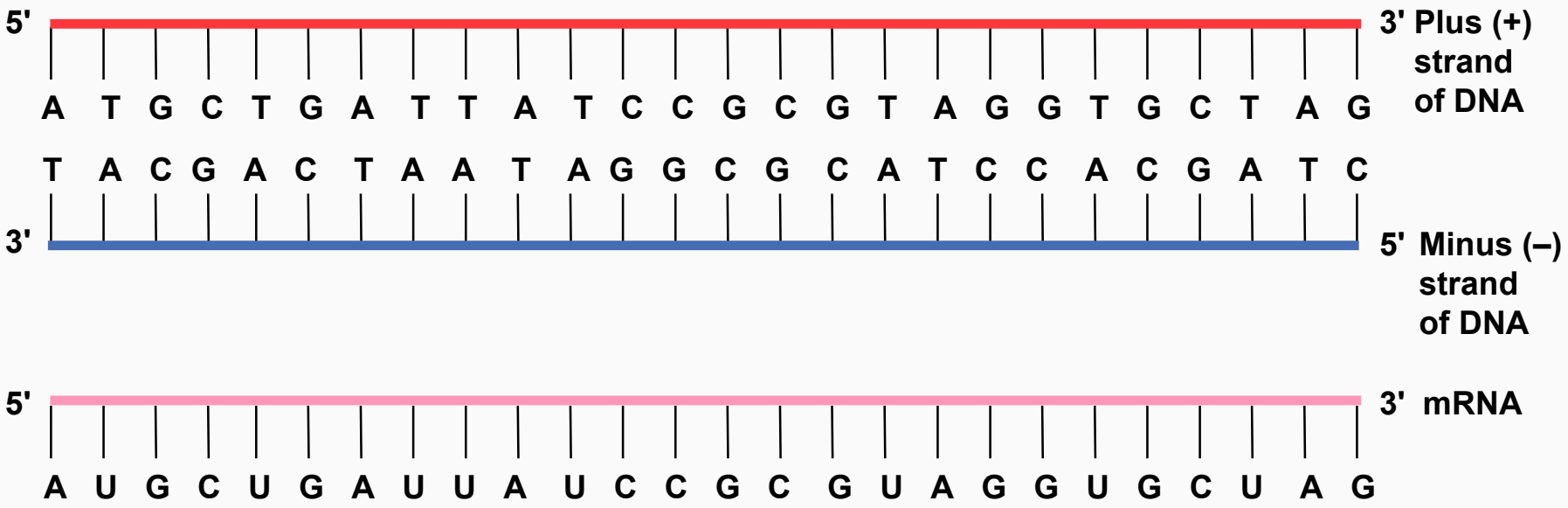
- Nature: Semiconservative
- Involved Enzymes (in Primosome & Replisome)
- Leading strand
- Lagging strand
  - Okazaki fragments

Semiconservative

- New strands are synthesized in 5’ to 3’ direction


T A C G A C T A A T A G G C G C A T C C A C G A T C
3'
5'
Minus (–) strand of DNA


5'
3' Plus (+) strand of DNA
A T G C T G A T T A T C C G C G T A G G T G C T A G
T A C G A C T A A T A G G C G C A T C C A C G A T C
3'
5' Minus (–) strand of DNA


5'
3' Plus (+) strand of DNA
A T G C T G A T T A T C C G C G T A G G T G C T A G
T A C G A C T A A T A G G C G C A T C C A C G A T C
3'
5' Minus (–) strand of DNA
5'
3' mRNA
A U G C U G A U U A U C C G C G U A G G U G C U A G

Semiconservative replication of DNA synthesizes a new strand of DNA from a template strand.

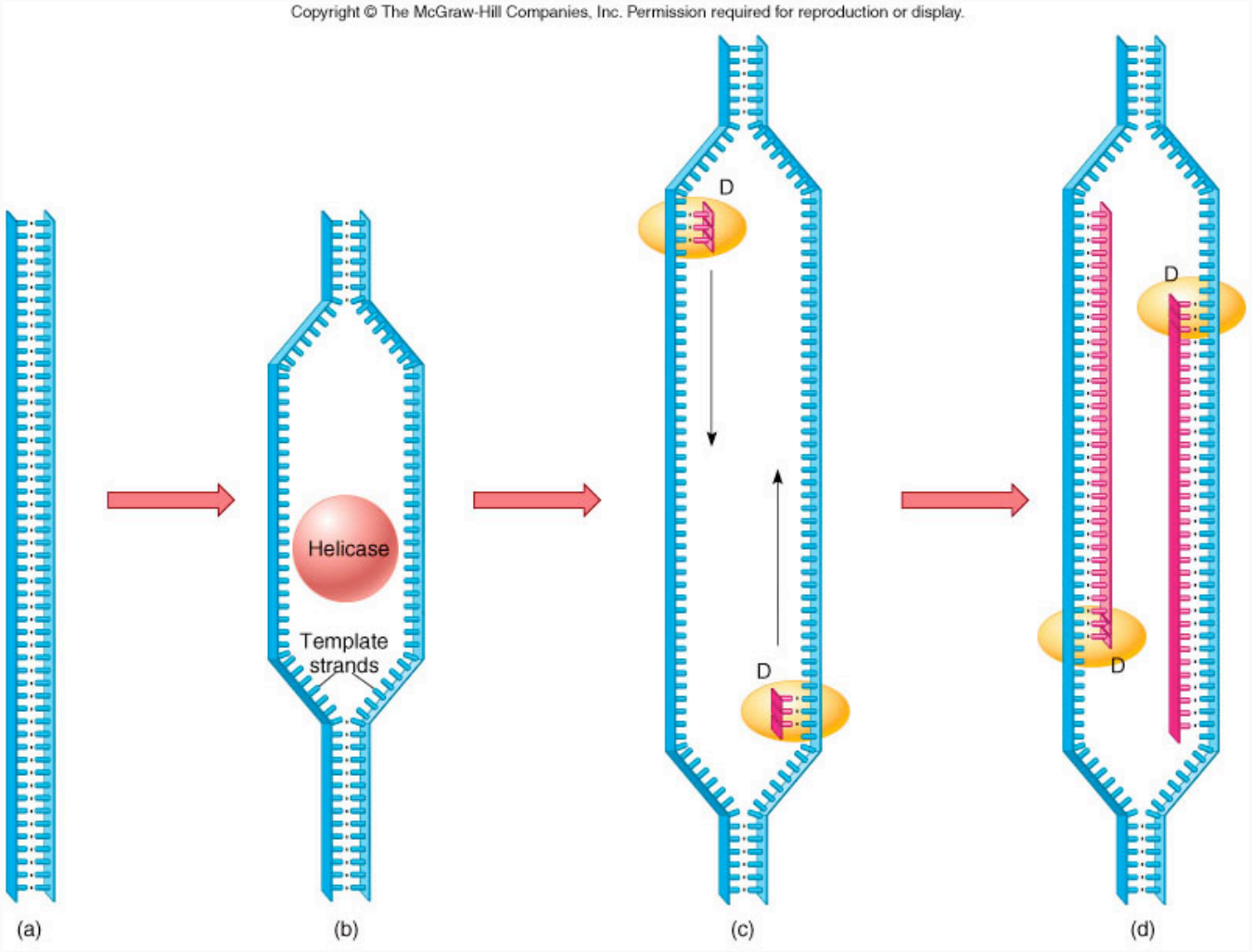


Fig. 9.5 Simplified steps to show the semiconservative replication of DNA

Enzymes

- Helicase
- DNA polymerase III
- Primase
- DNA polymerase I
- Ligase
- Gyrase

The function of important enzymes involved in DNA replication.

TABLE 9.1 Some Enzymes Involved in DNA Replication and Their Functions

Enzyme	Function
Helicase	Unzipping the DNA helix
Primase	Synthesizing an RNA primer
DNA polymerase III	Adding bases to the new DNA chain; proofreading the chain for mistakes
DNA polymerase I	Removing primer, closing gaps, repairing mismatches
Ligase	Final binding of nicks in DNA during synthesis and repair
Gyrase	Supercoiling

Table 9.1 Some enzymes involved in DNA replication

Leading strand

- DnaA,DnaB proteins at Origin or Replication (circular genetic element) or
- RNA primer (linear genetic element) initiate the 5' to 3' synthesis of DNA in a continuous manner

Lagging strand

- Because the direction of synthesis (5' -> 3') is opposite to fork movement, the lagging strand is synthesized in form of multiple DNA (Okazaki) fragments
  - Primer synthesis performed by RNA polymerase (Primase),
  - DNA synthesis performed by DNA polymerase III
  - RNA primer removal and fill-in with DNA by DNA polymerase I
- Okazaki fragments are ligated together by DNA ligase to form one continuous strand

The steps associated with the DNA replication process.

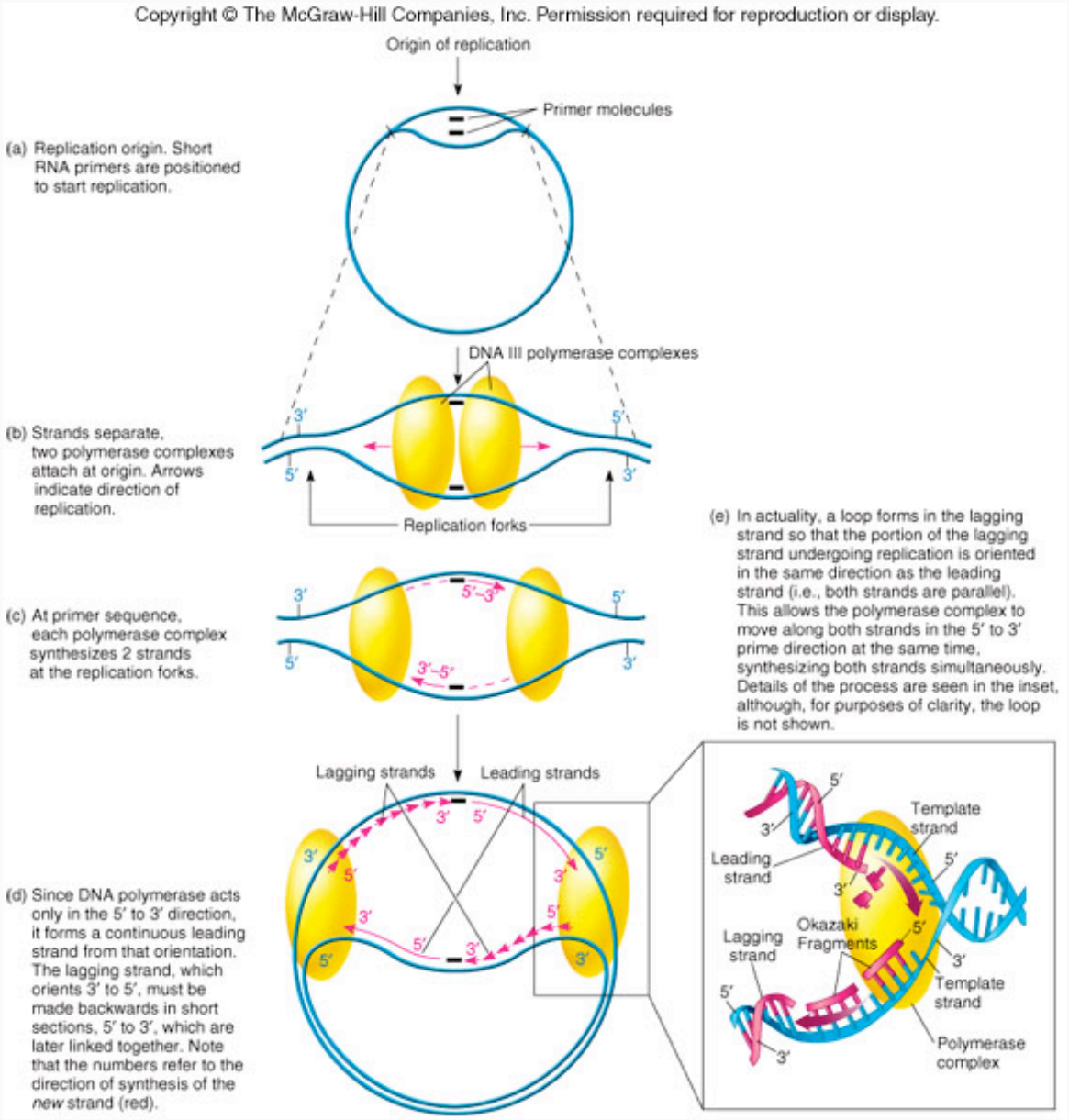
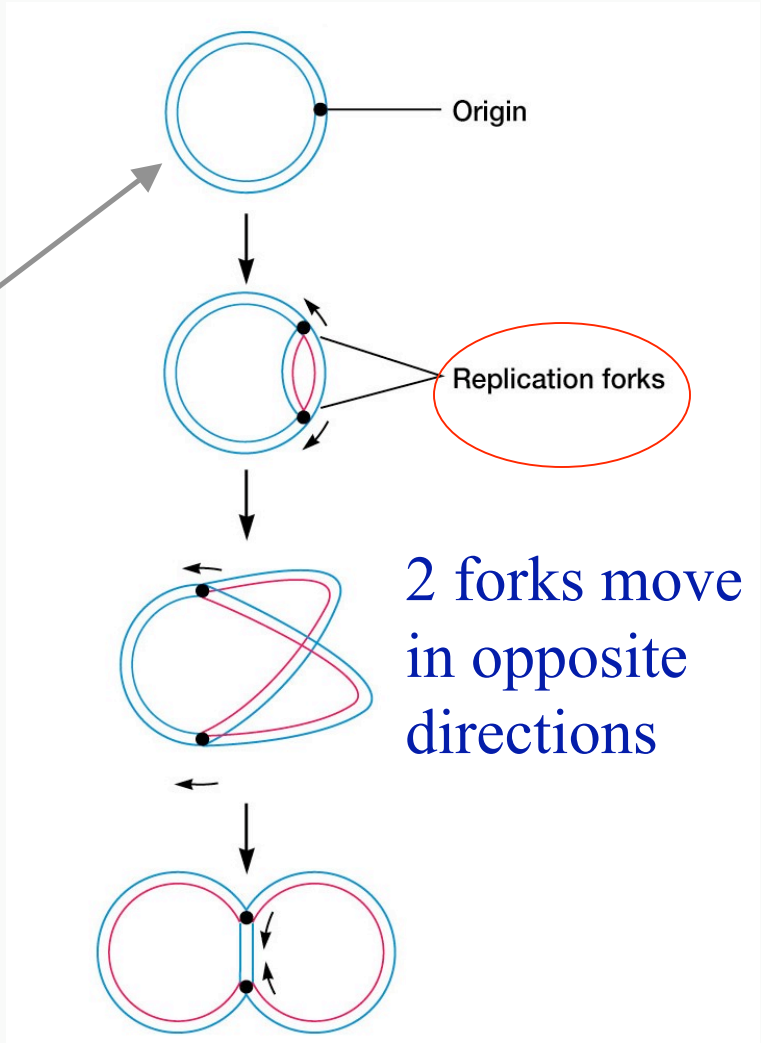


Fig. 9.6 The bacterial replicon: a model for DNA Synthesis

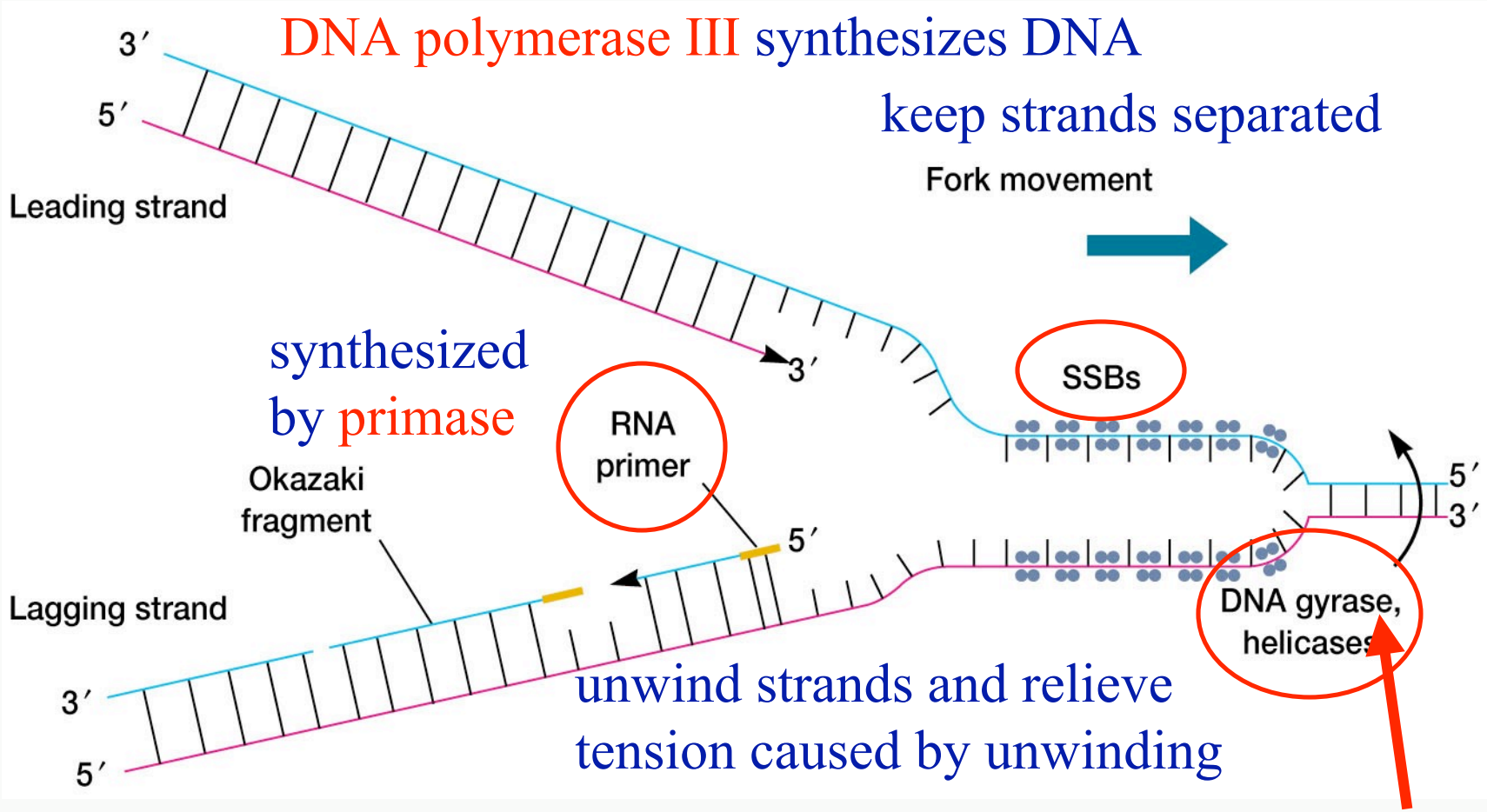
Patterns of DNA synthesis…

- in procaryotes
  - bidirectional from a single origin of replication
  - replicon
    - portion of the genome that contains an origin and is replicated as a unit



Mechanism of DNA Replication

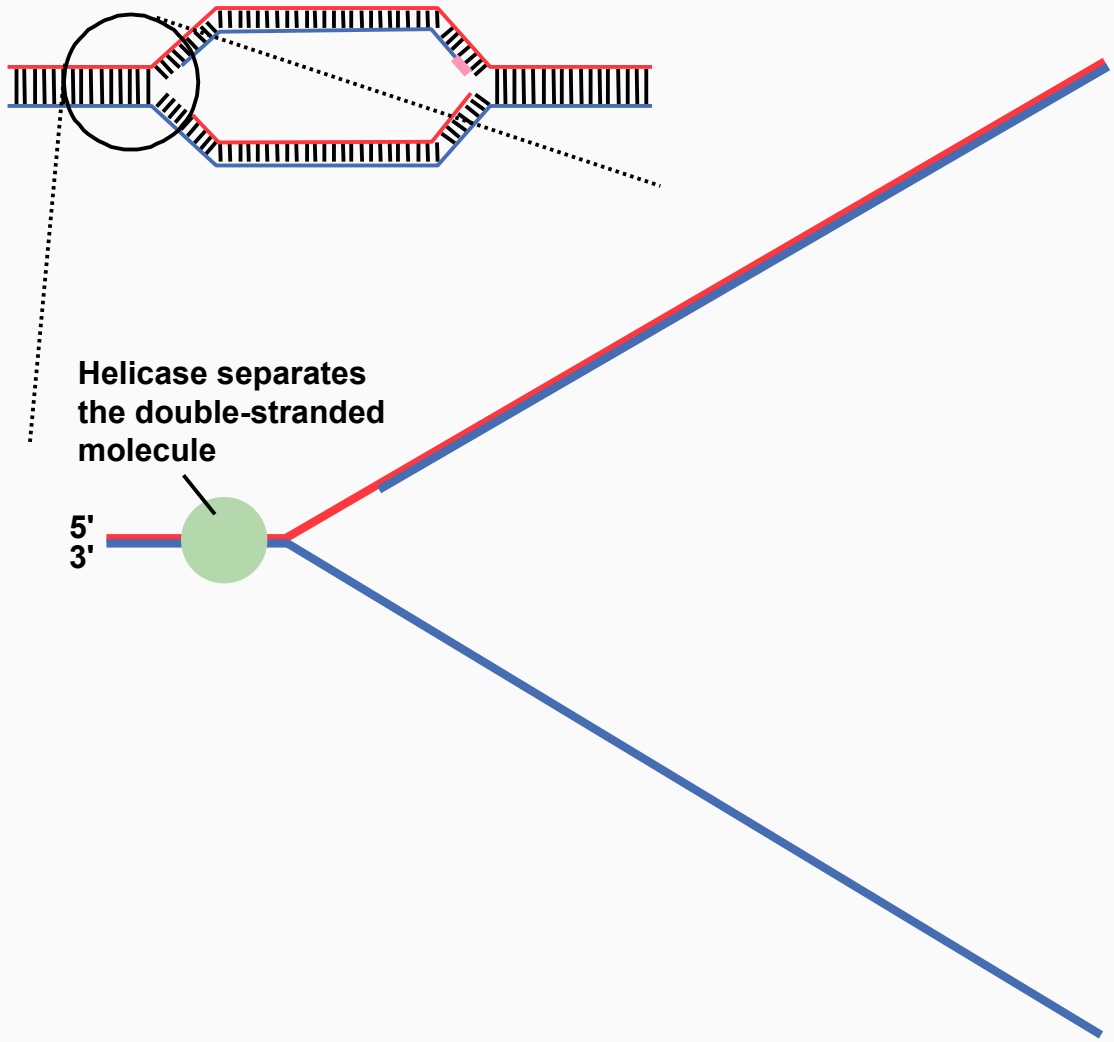
in bacteria

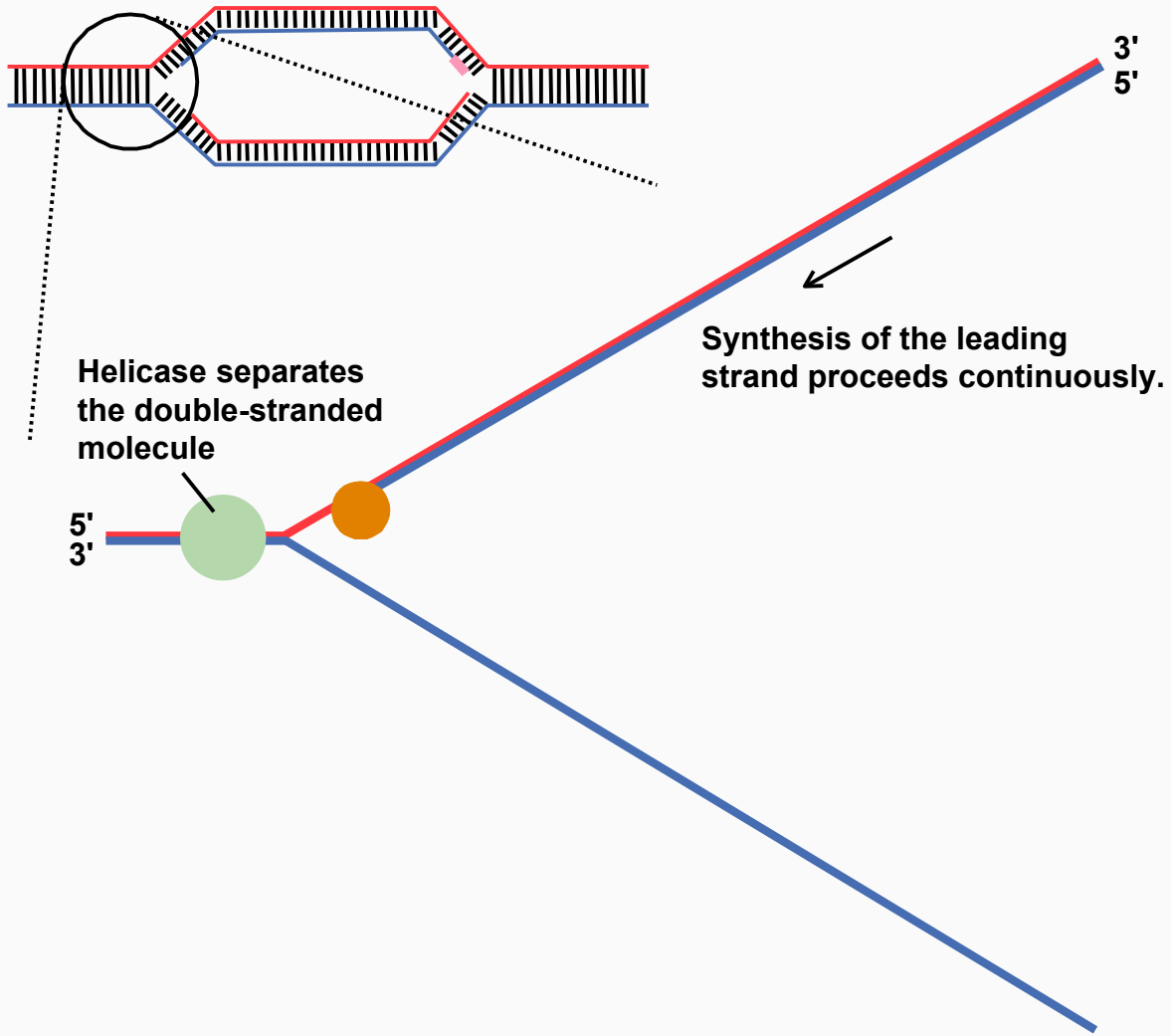


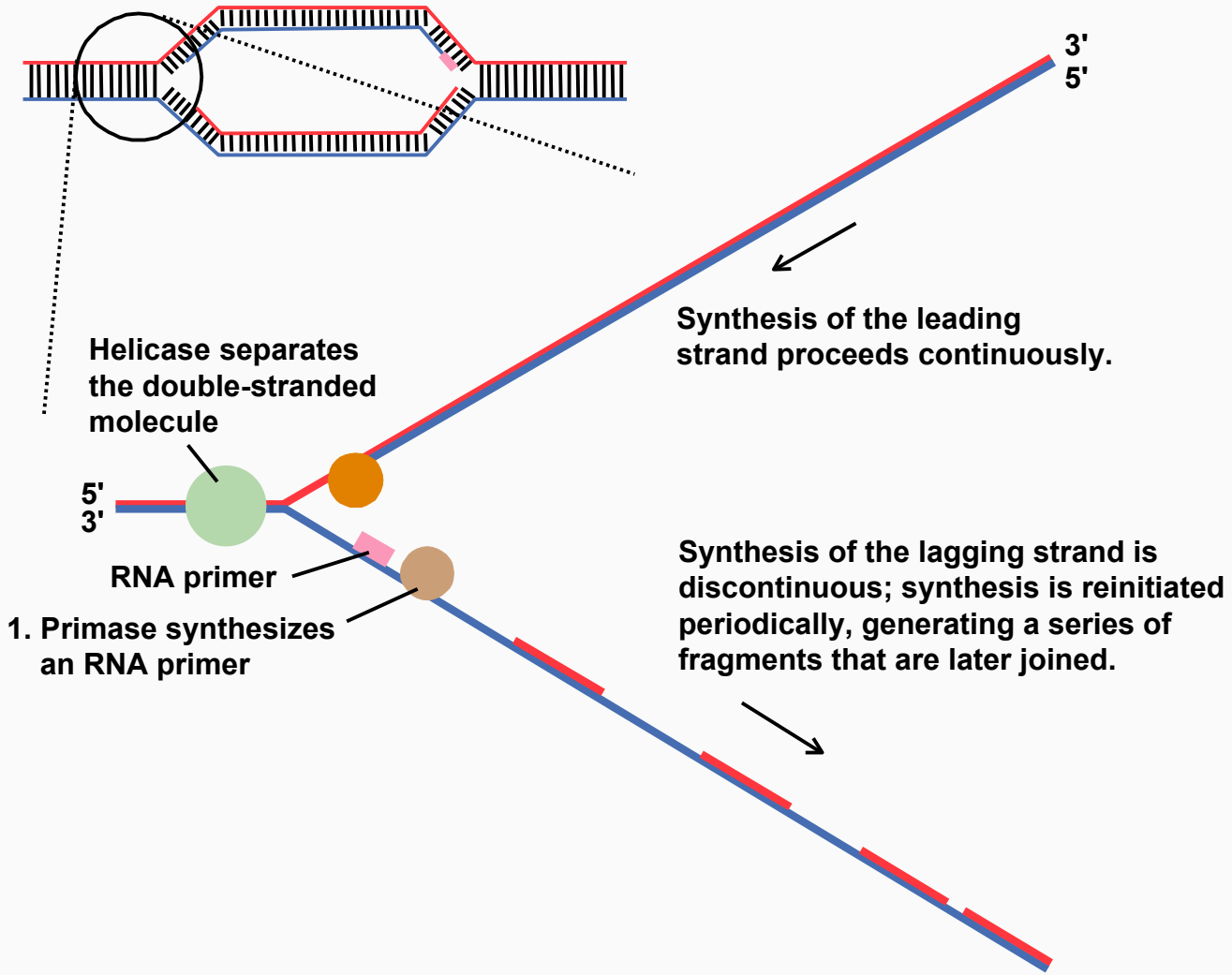
Quinolone Target

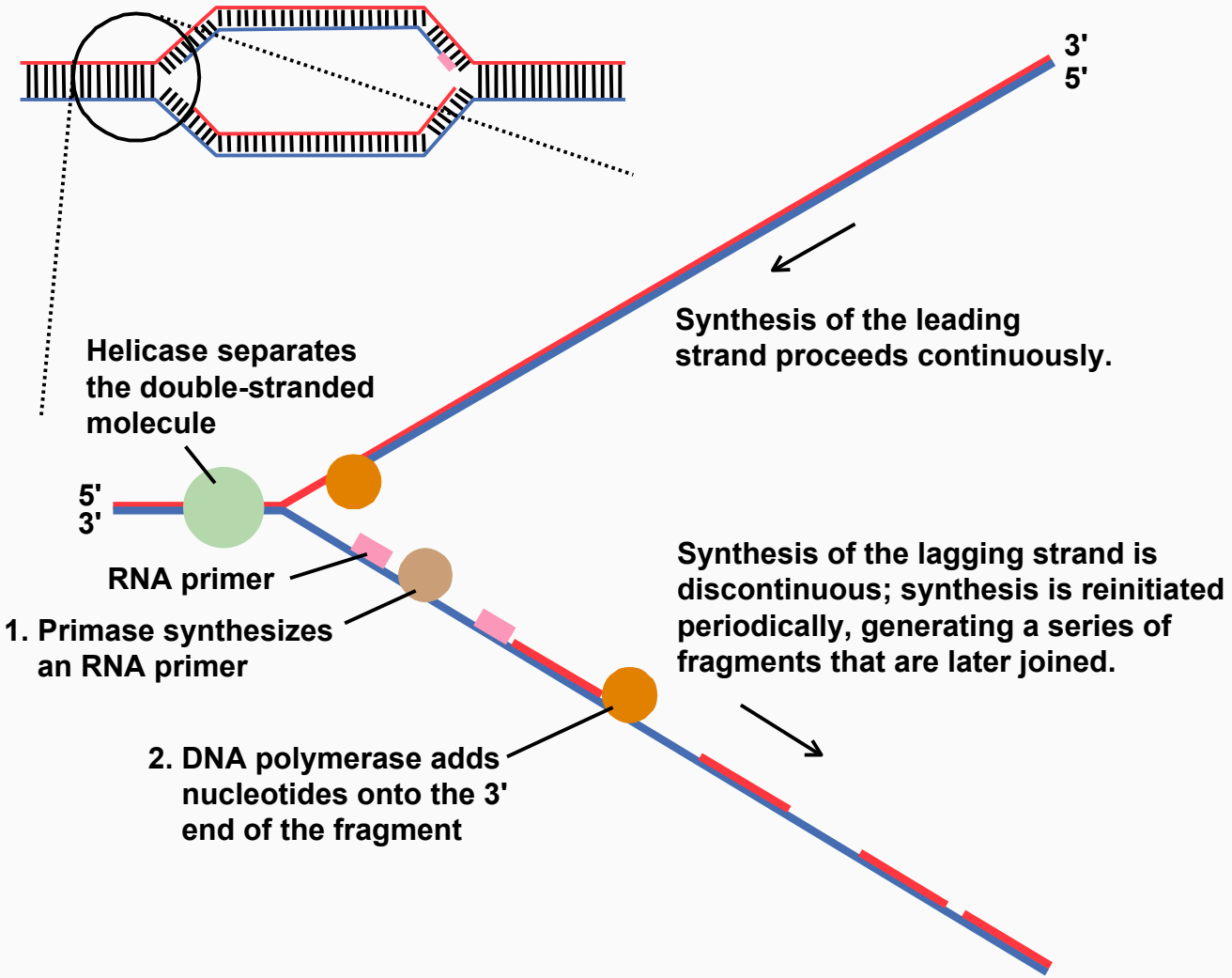
Some amazing facts

- ≥ 30 proteins required to replicate *E. coli* chromosome
- occurs with great fidelity
  - error frequency = 10^{-9} or 10^{-10} per base pair replicated
  - due to proofreading activity of DNA polymerases III and I
- occurs very rapidly
  - 750 to 1,000 base pairs/second in procaryotes
  - 50-100 base pairs/second in eucaryotes


Helicase separates
the double-stranded
molecule
5'
3'


3'
5'
Synthesis of the leading
strand proceeds continuously.
Helicase separates
the double-stranded
molecule
5'
3'


3'
5'
Synthesis of the leading strand proceeds continuously.
Helicase separates the double-stranded molecule
5'
3'
RNA primer
1. Primase synthesizes an RNA primer
Synthesis of the lagging strand is discontinuous; synthesis is reinitiated periodically, generating a series of fragments that are later joined.


3'
5'
Synthesis of the leading strand proceeds continuously.
Helicase separates the double-stranded molecule
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3'
RNA primer
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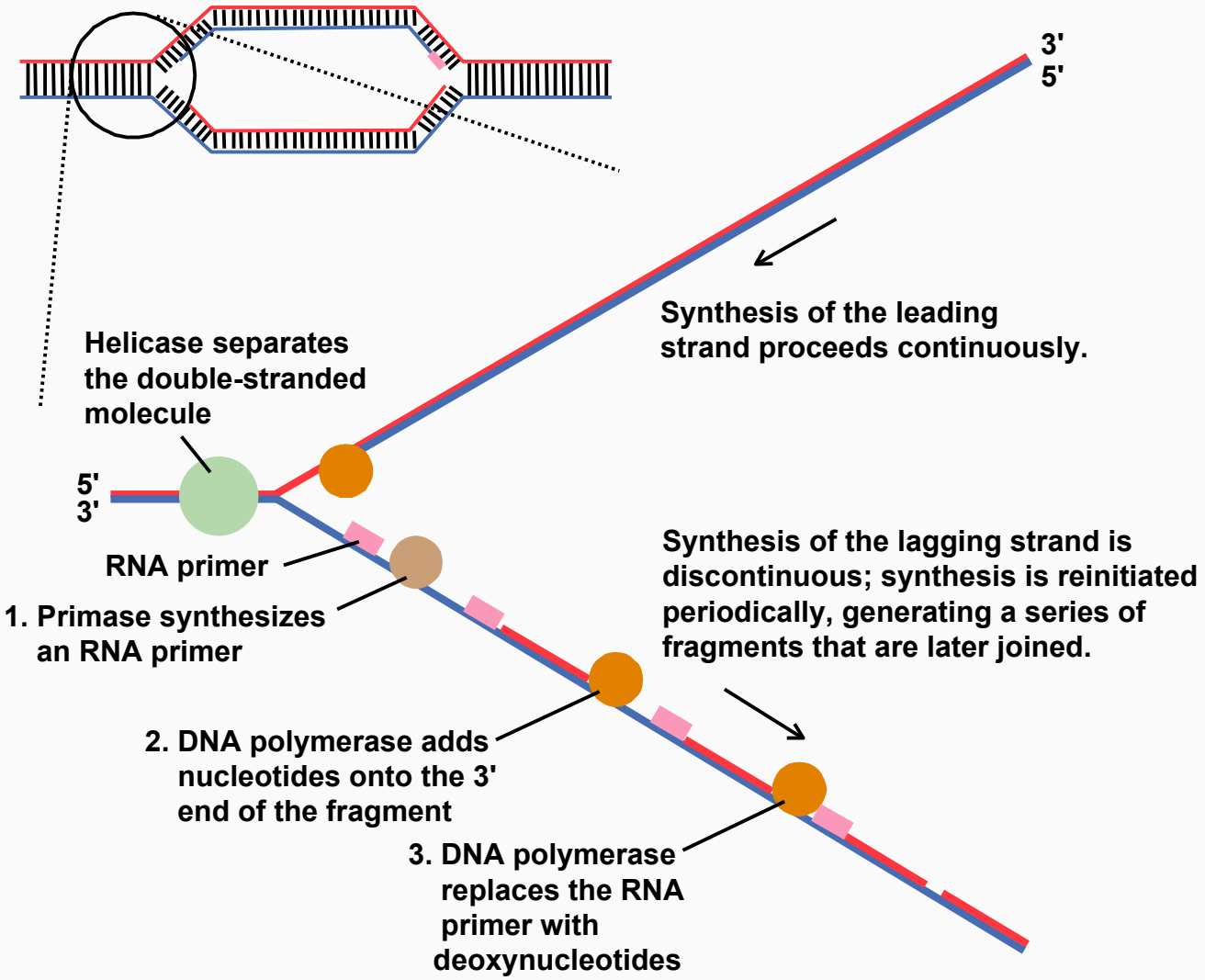
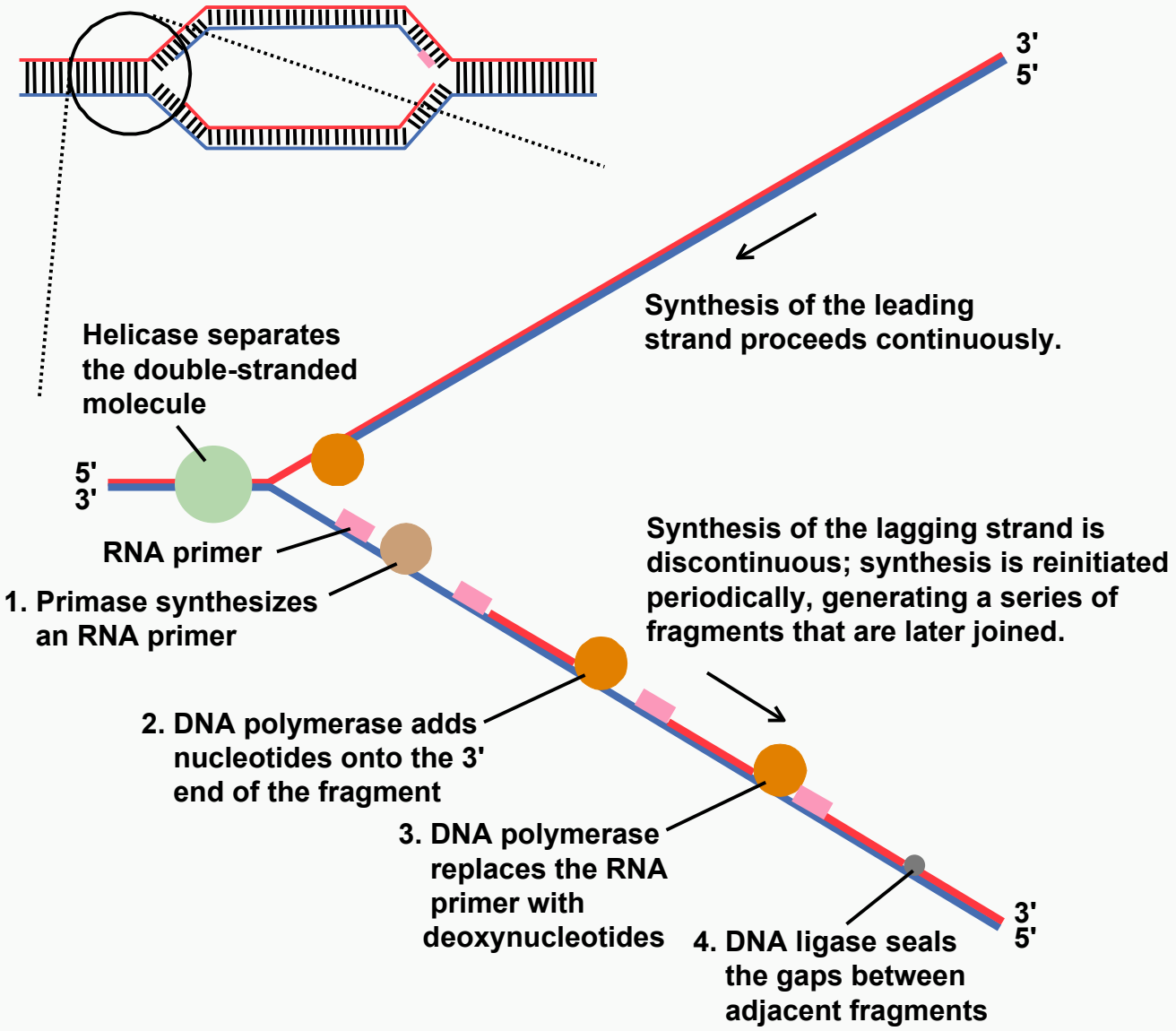
2. DNA polymerase adds nucleotides onto the 3' end of the fragment


3'
5'
Synthesis of the leading strand proceeds continuously.
Helicase separates the double-stranded molecule
5'
3'
RNA primer
1. Primase synthesizes an RNA primer
Synthesis of the lagging strand is discontinuous; synthesis is reinitiated periodically, generating a series of fragments that are later joined.
2. DNA polymerase adds nucleotides onto the 3' end of the fragment
3. DNA polymerase replaces the RNA primer with deoxynucleotides


3'
5'
Synthesis of the leading strand proceeds continuously.
Helicase separates the double-stranded molecule
5'
3'
RNA primer
1. Primase synthesizes an RNA primer
Synthesis of the lagging strand is discontinuous; synthesis is reinitiated periodically, generating a series of fragments that are later joined.
2. DNA polymerase adds nucleotides onto the 3' end of the fragment
3. DNA polymerase replaces the RNA primer with deoxynucleotides
4. DNA ligase seals the gaps between adjacent fragments
3'
5'

Replication processes from other biological systems (some Gram-positive bacterial genomes, plasmids, viruses) involve a rolling cycle.

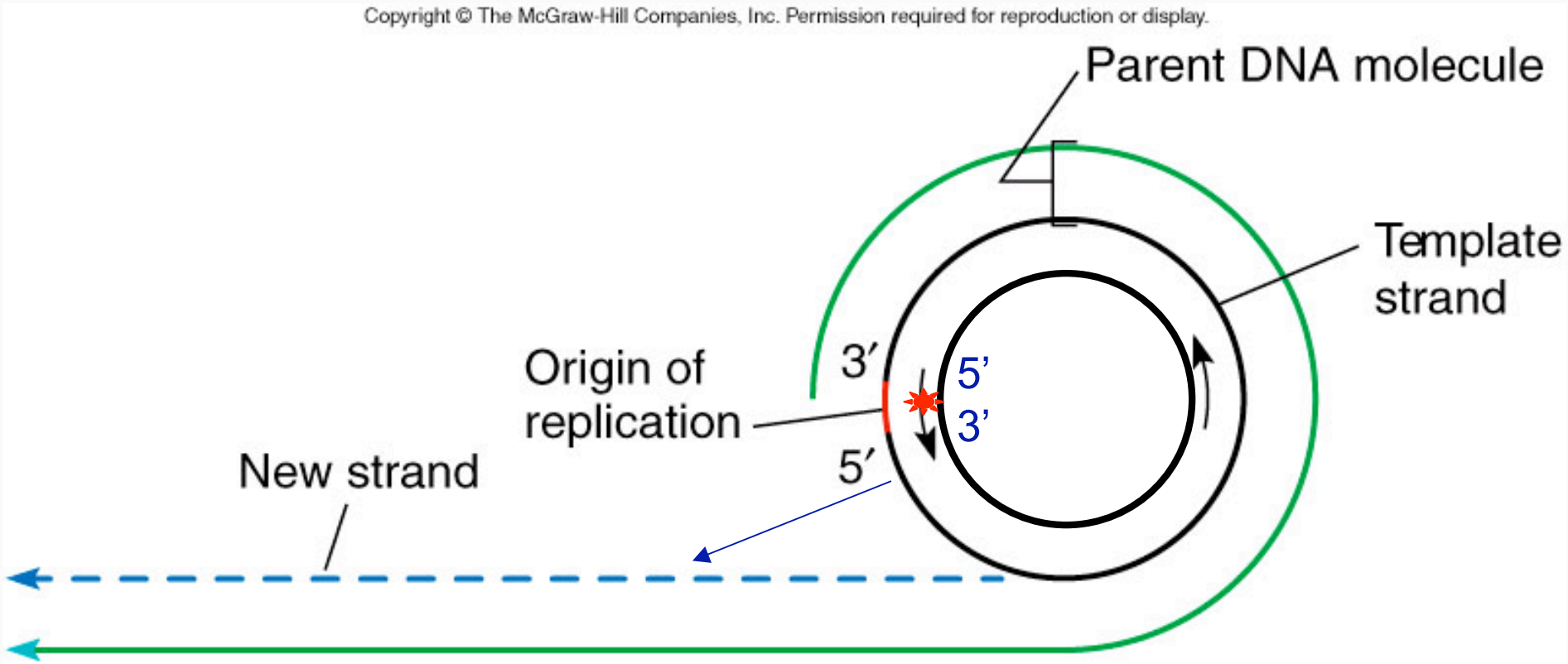
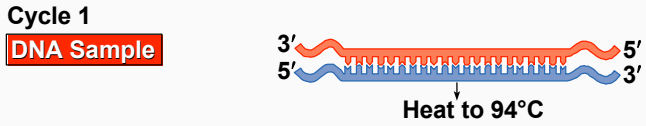


Fig. 9.8 Simplified model of rolling circle DNA Replication

RNA

- Transcription
  - Message RNA (mRNA)
  - Transfer RNA (tRNA)
  - Ribosomal RNA (rRNA)
- Codons (nucleotide triplett)

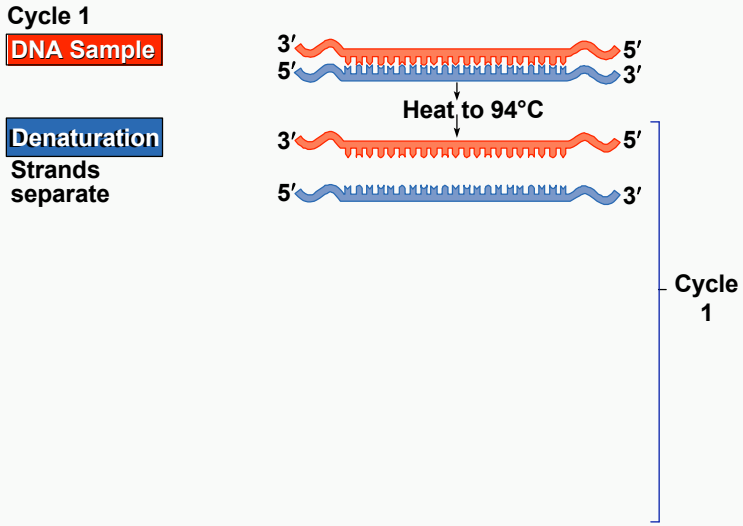
(a) In cycle 1, the DNA to be amplified is denatured, primed, and replicated by a polymerase that can function at high temperature. The two resulting strands then serve as templates for a second cycle of denaturation, priming, and synthesis.*



*For simplicity's sake, we have omitted the elongation of the complete original parent strand during the first cycles. Ultimately, templates that correspond only to the smaller fragments dominate and become the primary population of replicated DNA.

Polymerase Chain Reaction

(a) In cycle 1, the DNA to be amplified is denatured, primed, and replicated by a polymerase that can function at high temperature. The two resulting strands then serve as templates for a second cycle of denaturation, priming, and synthesis.*



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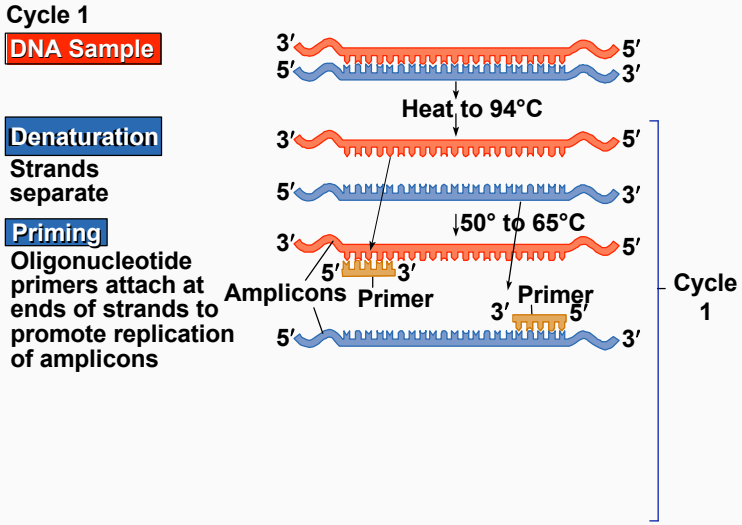

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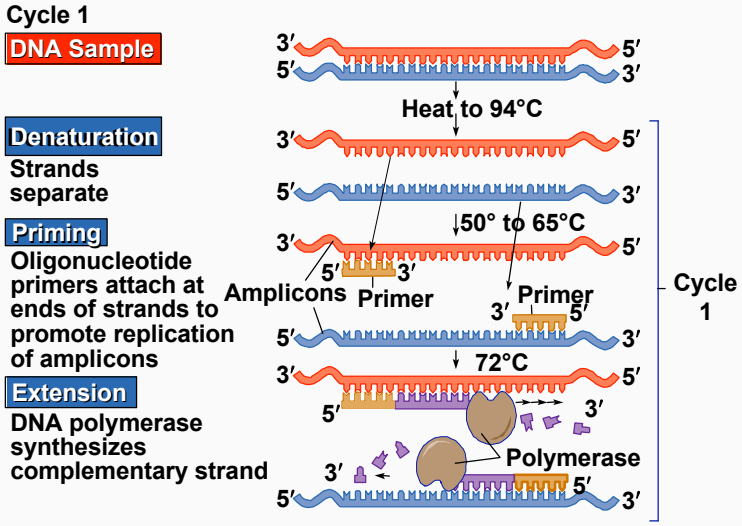

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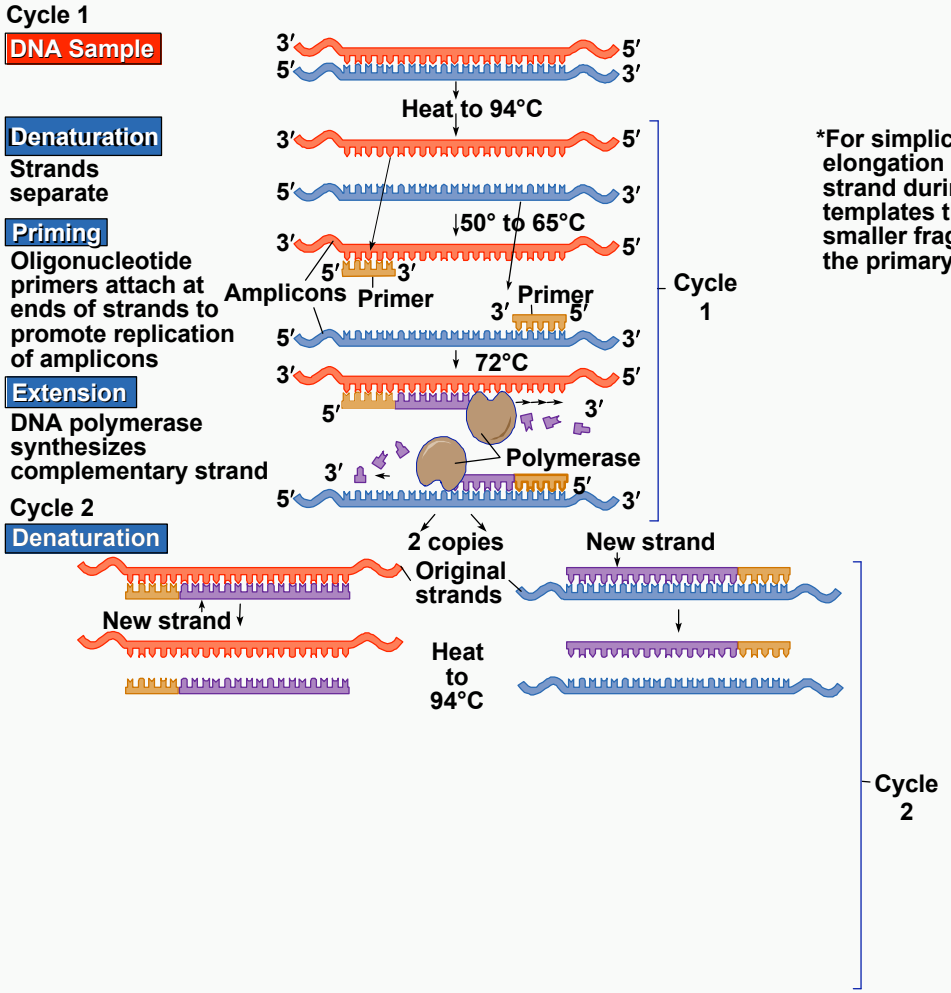

*For simplicity's sake, we have omitted the elongation of the complete original parent strand during the first cycles. Ultimately, templates that correspond only to the smaller fragments dominate and become the primary population of replicated DNA.

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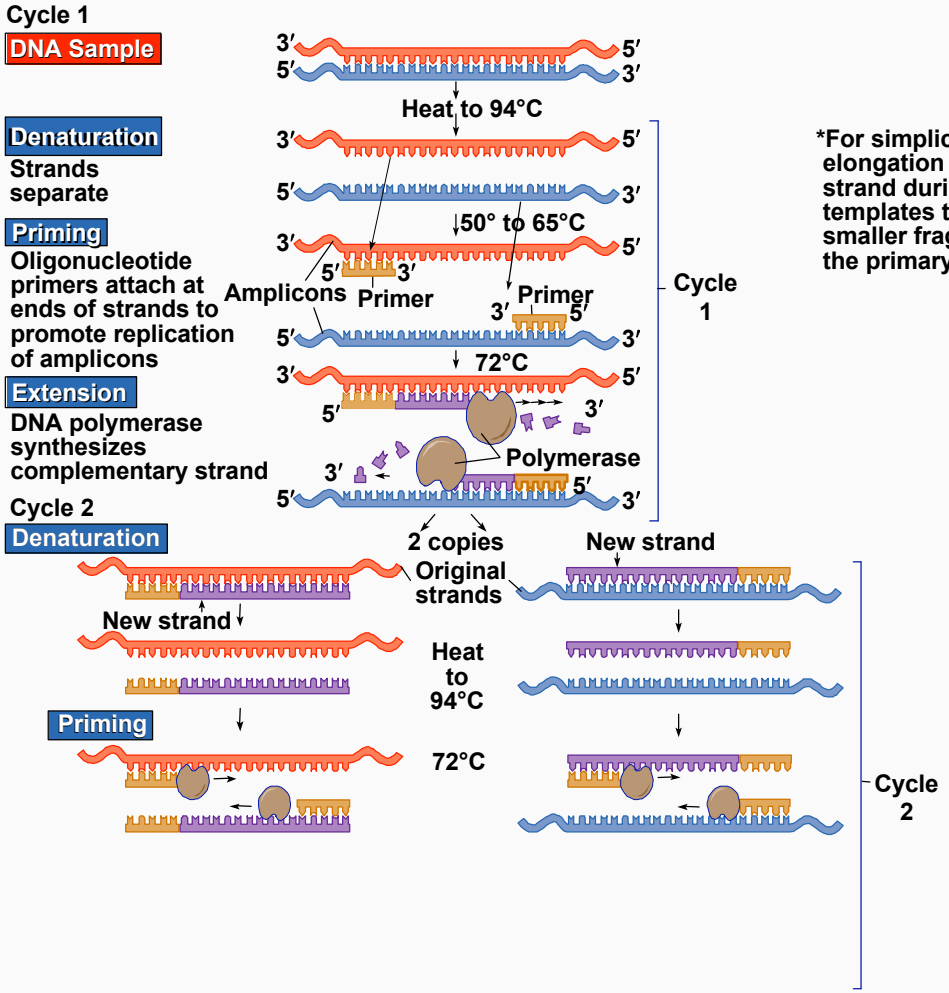

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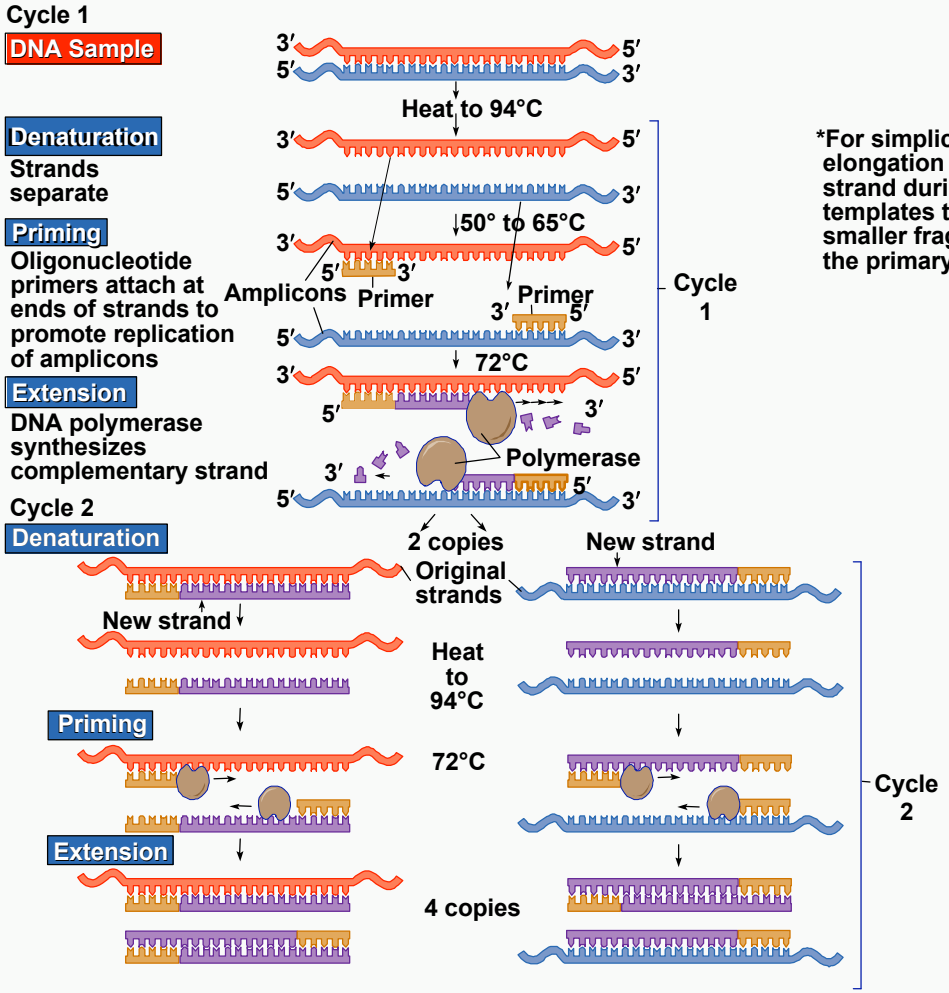

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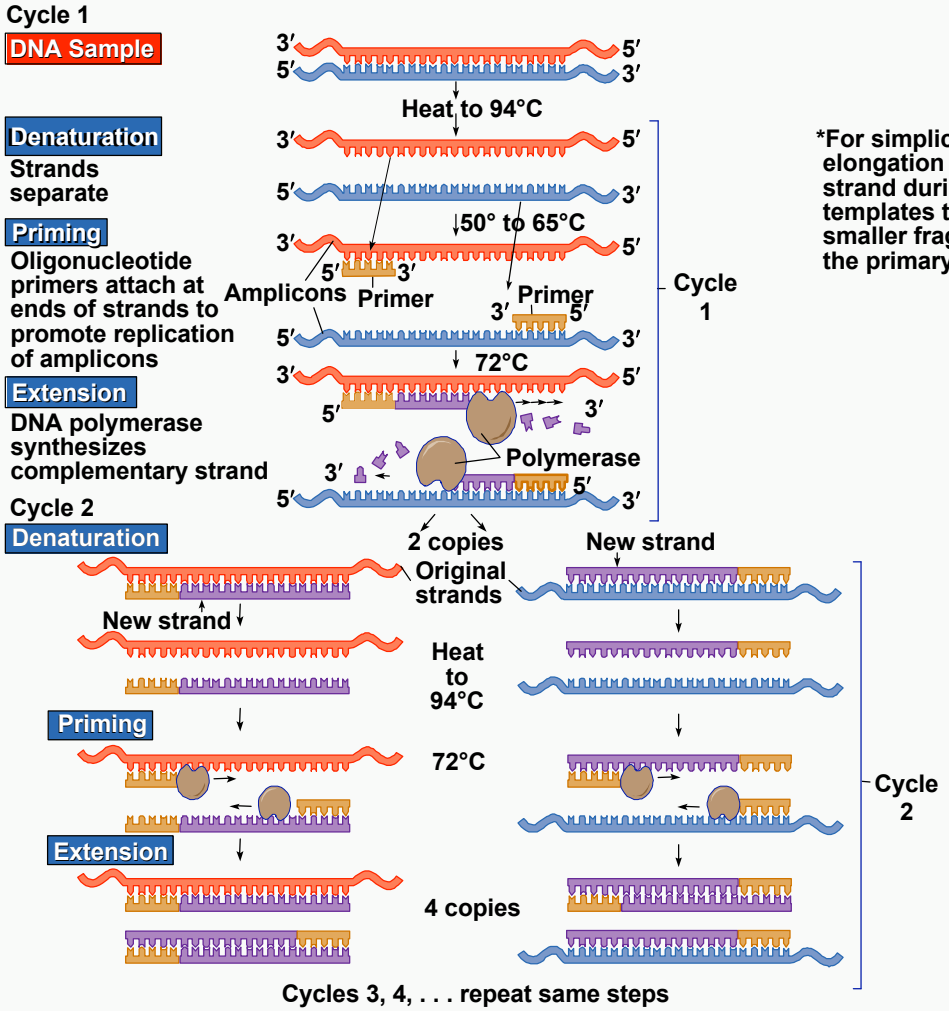

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(b) A view of the process after 6 cycles, with 64 copies of amplified DNA. Continuing this process for 20 to 40 cycles can produce millions of identical DNA molecules.