Peter Pristaš

Molecular biology

DNA structure, organization and replication

Life attributes

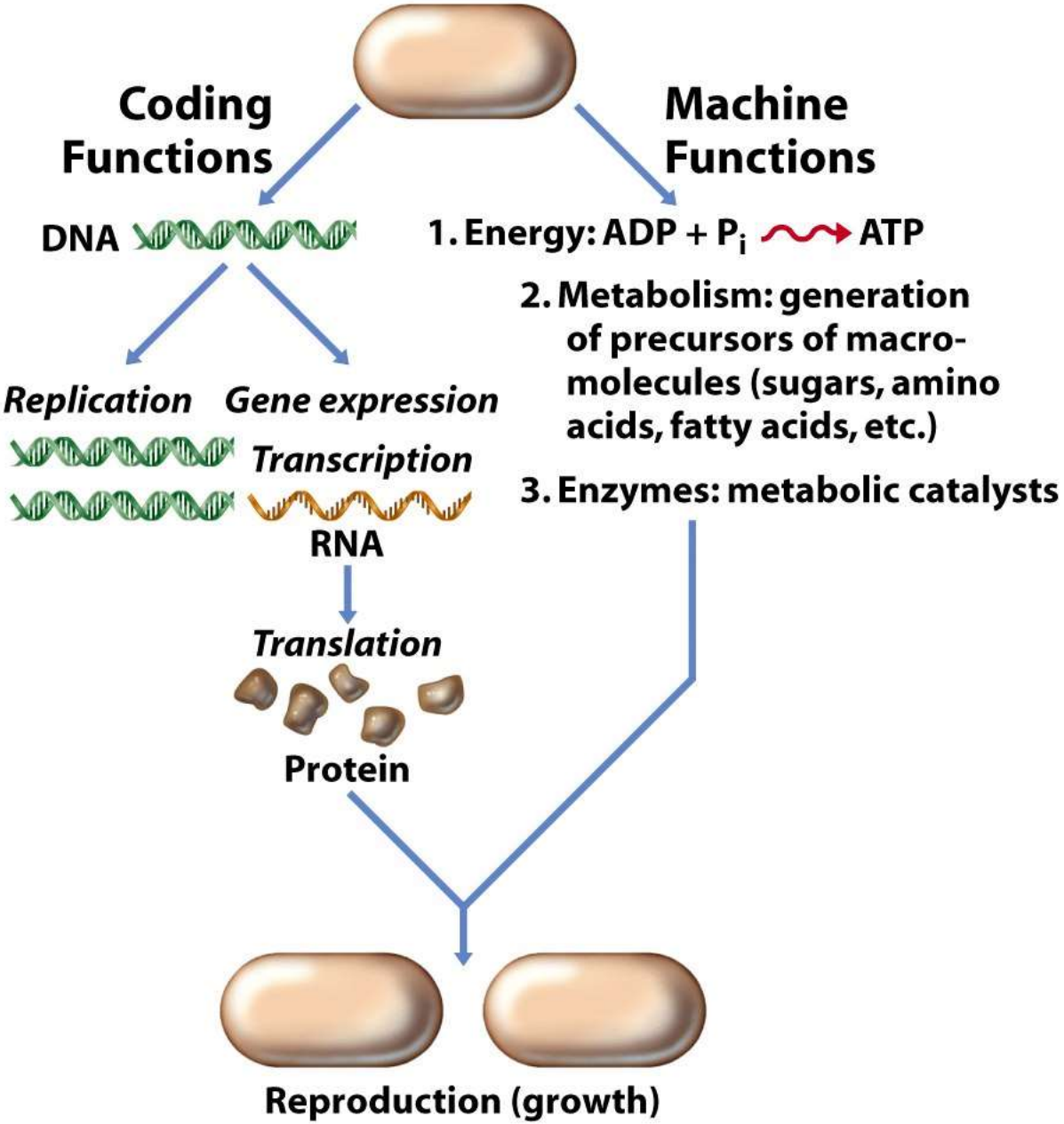

Figure 1-4 Brock Biology of Microorganisms 11/e

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DNA is the Genetic Material

- DNA and RNA were first described by Friedrich Miescher in 1869. He isolated a phosphorus-containing material from the nuclei of cells found in pus from discarded surgical bandages, and called in “nuclein”. He later found the same material in salmon sperm. Later it was recognized that DNA and RNA are slightly different in structure.
- For long time it seemed impossible for complex phenotypic traits to be coded in a simple linear fashion. DNA was known to be a linear polymer of just 4nucleotides, and it was thought to be just a scaffold for the actual genes. Proteins were considered the most likely genetic material, or perhaps some undiscovered substance.
- Definitive proof of DNA’s central role came in the 1950’s, but important experiments were done earlier.

DNA as genetic material

- Griffith discovers transformation - 1928
- Attempting to develop a vaccine
- Isolated two strains of Streptococcus pneumoniae
- Rough strain was harmless
- Smooth strain was pathogenic

DNA as genetic material

1. Mice injected with live cells of harmless strain R.

Mice live. No live R cells in their blood.

2. Mice injected with live cells of killer strain S.

Mice die. Live S cells in their blood.

3. Mice injected with heat-killed S cells.

Mice live. No live S cells in their blood.

4. Mice injected with live R cells *plus* heat-killed S cells.

Mice die. Live S cells in their blood.

- What happened in the fourth experiment?
  - The harmless R cells had been transformed by material from the dead S cells
  - Descendents off the transformed cells were also pathogenic

DNA as genetic material

- Oswald & Avery
  - What is the transforming material?
    - Cell extracts treated with protein-digesting enzymes could still transform bacteria
    - Cell extracts treated with DNA-digesting enzymes lost their transforming ability

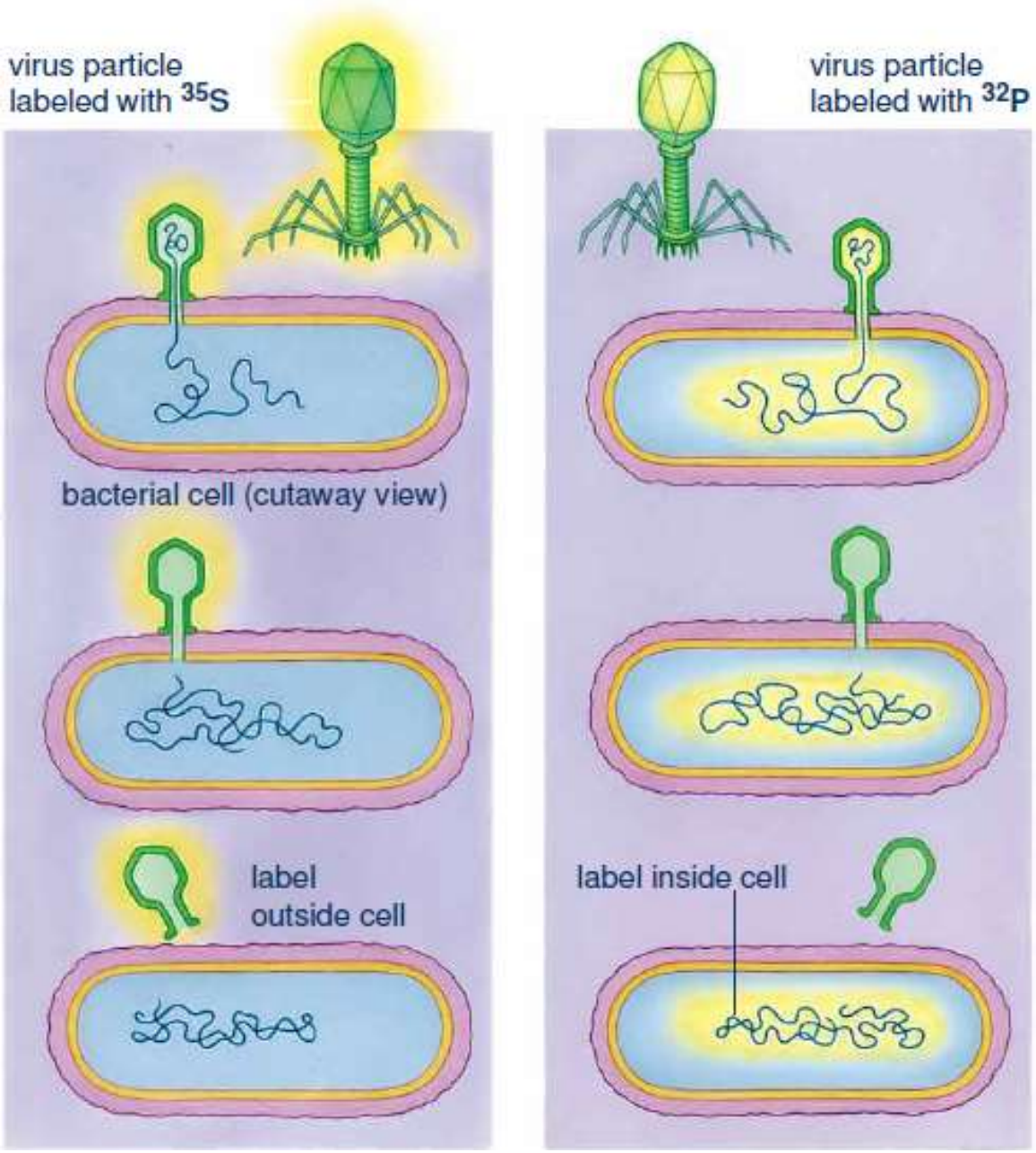
- **Concluded that DNA, not protein, transforms bacteria**

Hershey and Chase results

32P-labelled DNA entered the bacterial cells when the phage infected them, and that the new generation of phage contained a significant amount of that labeled DNA.

In contrast, the 35S-labelled protein stayed outside the cells during an infection, and none of it ended up in the new phage.

This implies that DNA is necessary for phage replication.



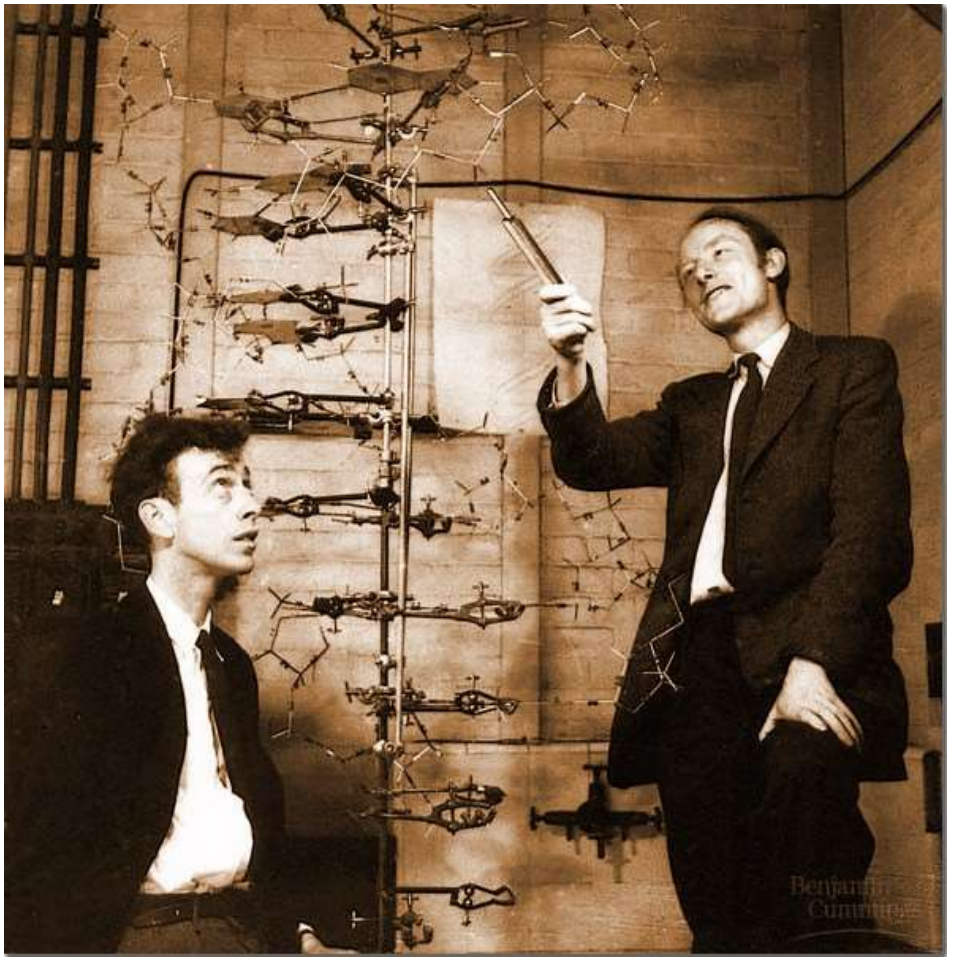
Structure of DNA

- Once the importance of DNA was recognized, it was necessary to deduce how the DNA molecule is structured. A race between various lab groups ensued, and in 1953 James Watson and Francis Crick published a model of DNA structure. Their work was based on X-ray crystallography data provided by Maurice Wilkins and Rosalind Franklin.
- DNA consists of two anti-parallel chains twisted into a helix. The nitrogenous bases are paired in the center of the molecule, and the phosphate-sugar backbones are on the outside.
- Although DNA is the genetic material of all living cells, some viruses use RNA as their genetic material.

DNA as genetic material

Discovering the structure of DNA

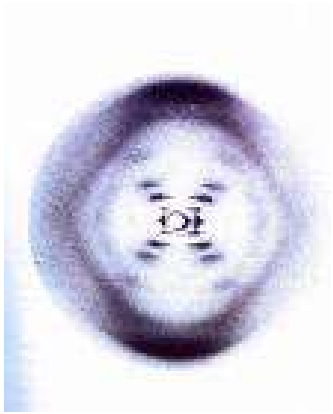
- Structure was discovered in 1953 by James Watson and Francis Crick



DNA as genetic material

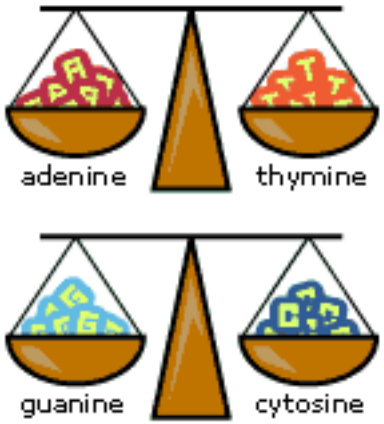
Discovering the structure of DNA

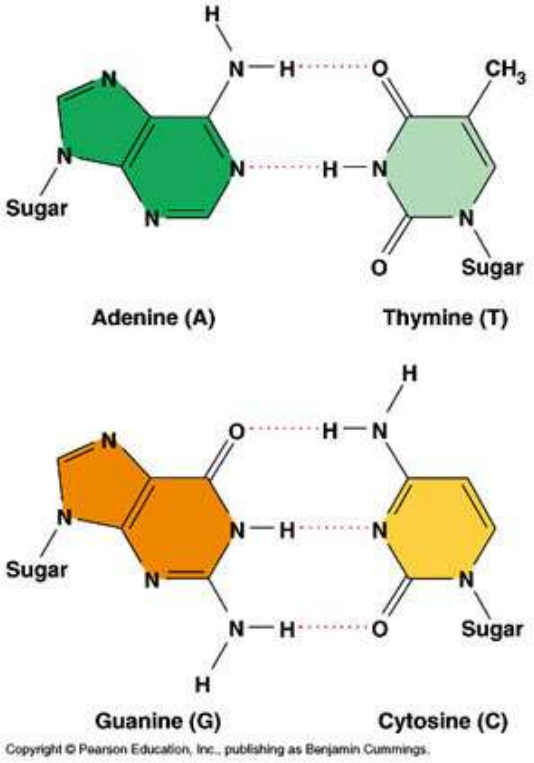
Rosalind Franklin's DNA image



"Chargaff's rule"

A = T & C = G

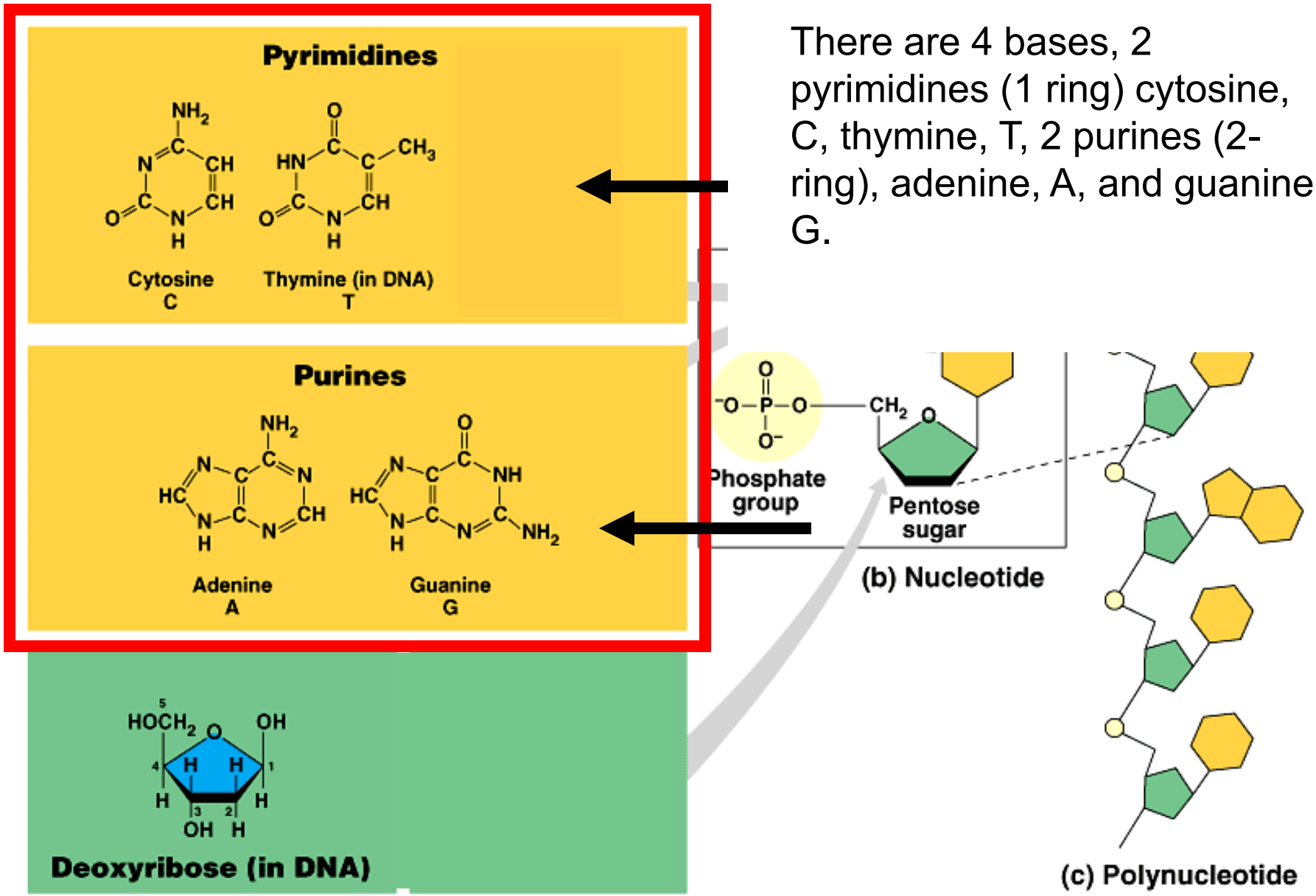




DNA Structure

- DNA is macromolecule composed of subunits called nucleotides.
- Each nucleotide of DNA has 3 parts: a nitrogenous base, a sugar, and a phosphate group.
- The phosphate group, PO_4, links two sugar molecules in the backbone. Each phosphate carries a negative charge. This causes DNA to have an overall negative charge.
- The sugar in the DNA is deoxyribose - it has 5 carbons, numbered 1' through 5'.
  - the nitrogenous base is attached to the 1' carbon
  - the 2' carbon has a free -OH group in the case of RNA, but a -H group in the case of DNA. The lack of the oxygen atom makes DNA far less reactive than RNA.
  - the 3' carbon has an -OH group on it that links to the phosphate group on the next base. The "end" of the DNA molecule is a free 3' OH group.
  - the 5' carbon is attached to the phosphate group.

There are 4 bases, 2 pyrimidines (1 ring) cytosine, C, thymine, T, 2 purines (2-ring), adenine, A, and guanine, G.



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DNA Structure

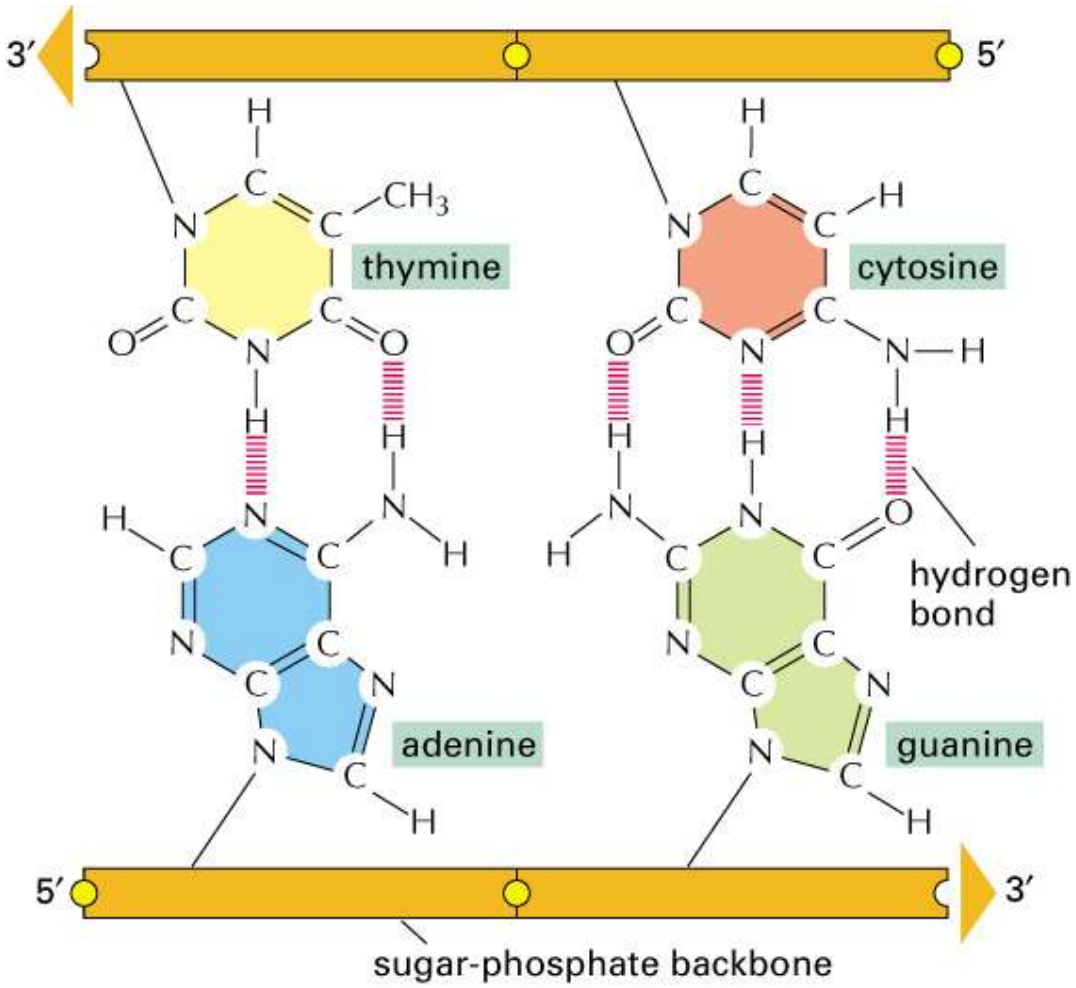
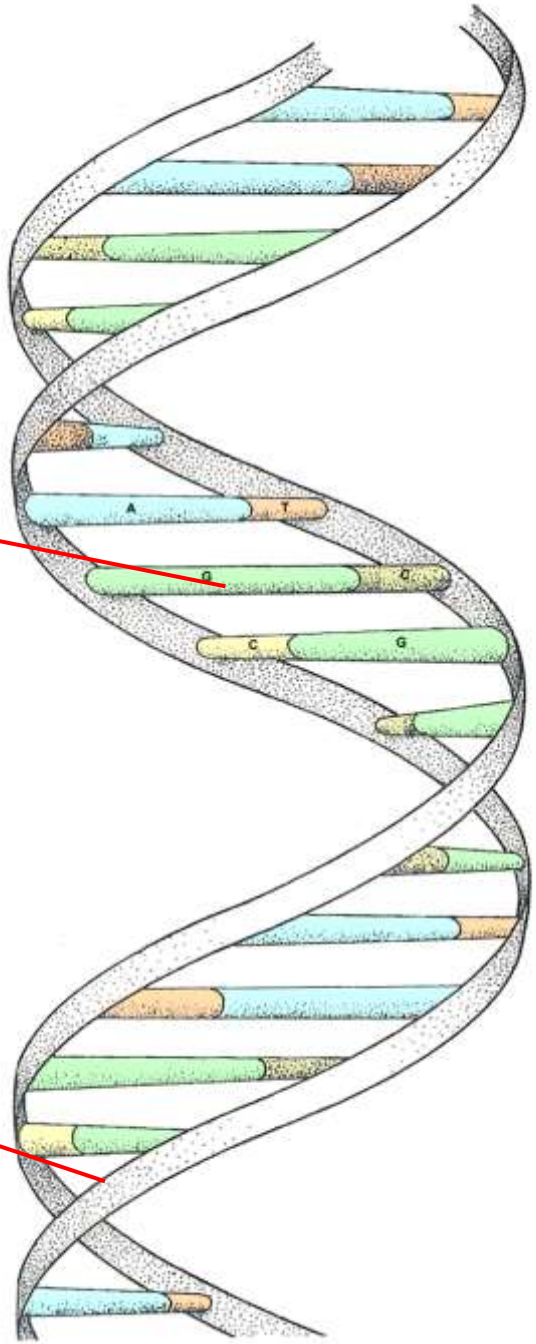


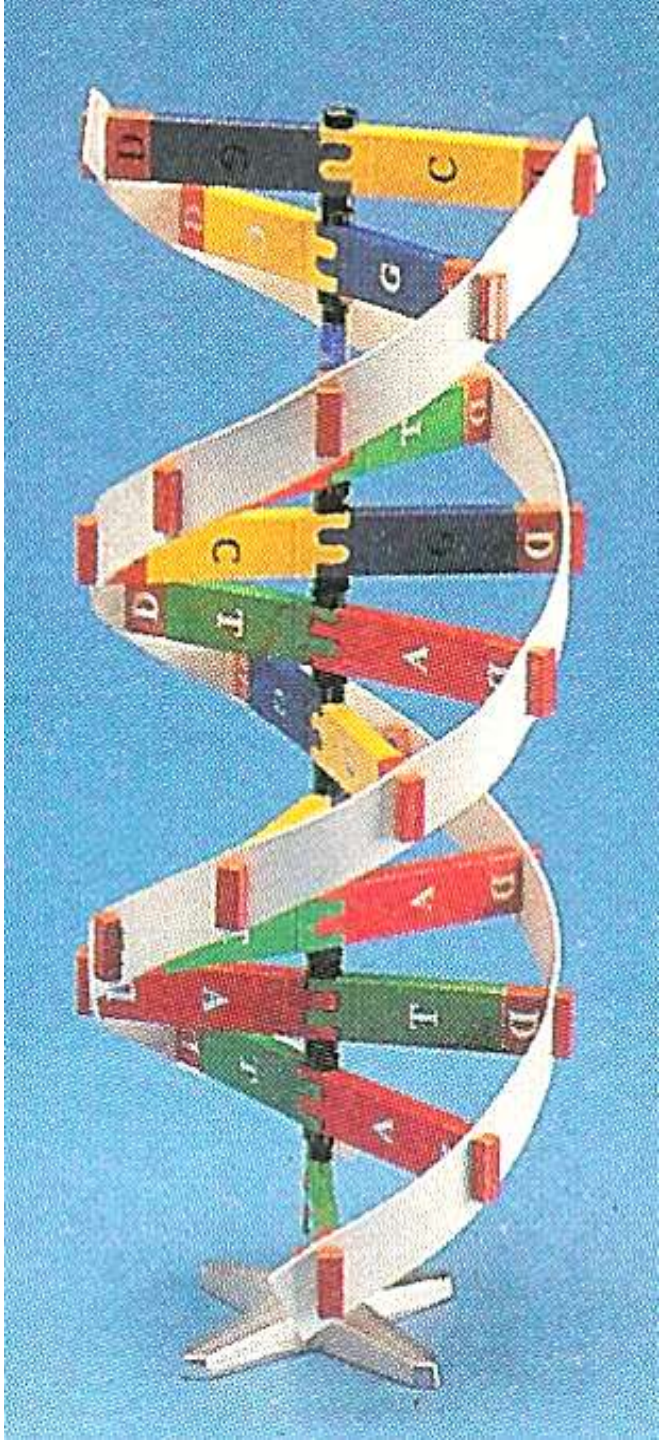
Figure 4–4. Molecular Biology of the Cell, 4th Edition.

The Double Helix

bases

sugar-phosphate chain





DNA Structure

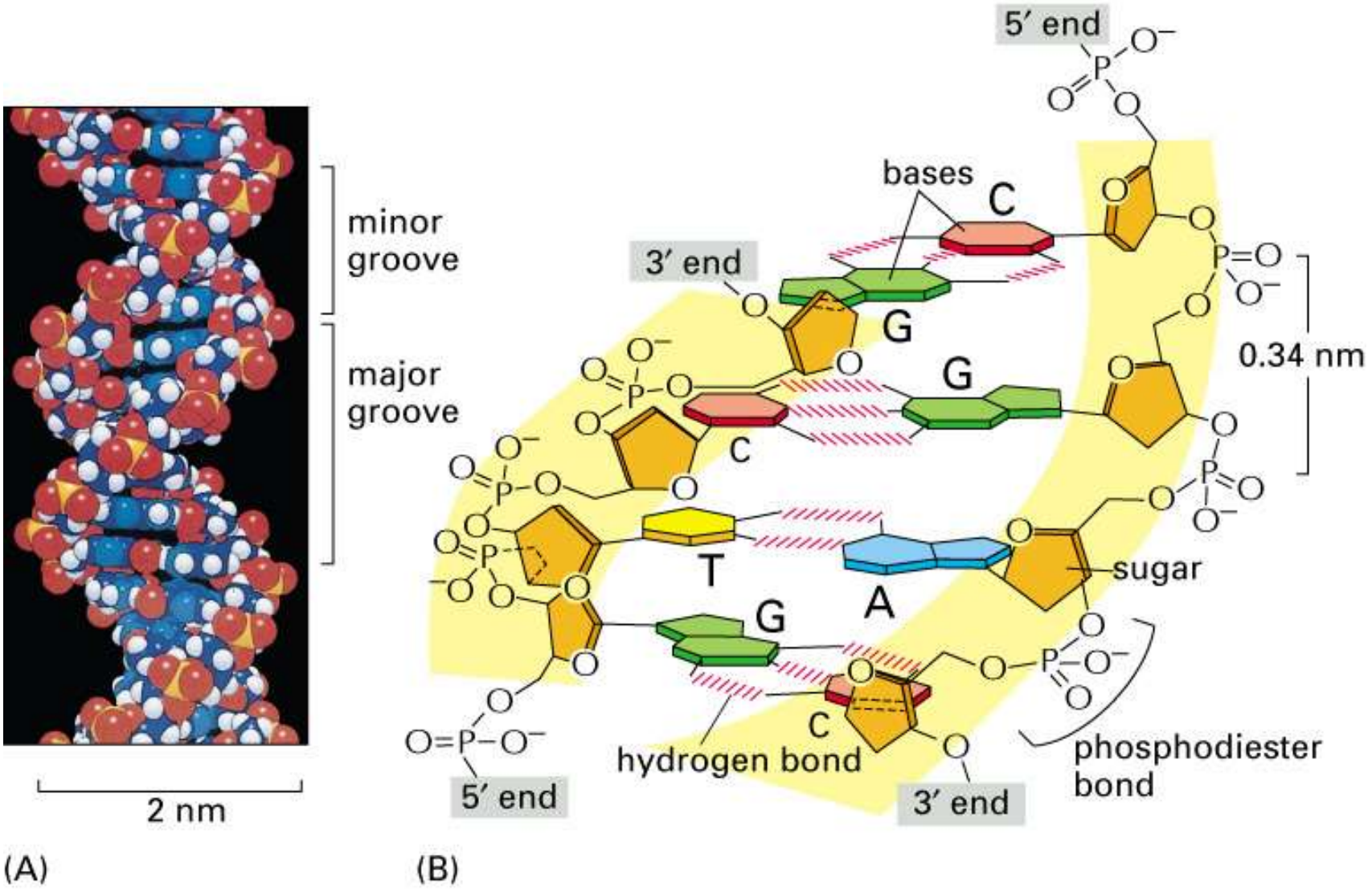


Figure 4–5. Molecular Biology of the Cell, 4th Edition.

DNA structure overview

- complementary strands (antiparallel)
- sugar phosphate backbone held together with hydrogen bonding between bases
- size is expressed in nucleotide bases pairs. Escherichia coli has 4600 kbp. (Escherichia coli chromosome is > 1mm, about 500X longer than the cell itself)
- each bp takes up to 0.34 nm, and each helix turn is 10 bp (or 34 Angstroms)

DNA organization

- Procaryotes and Eukaryotes
  - procaryote – microscopic, unicellular organisms, lack nuclei and membrane-bound organelles
  - eucaryote – unicellular (microscopic) and multicellular, nucleus and membrane-bound organelles
- Viruses
  - acellular, parasitic particles composed of a nucleic acid and protein

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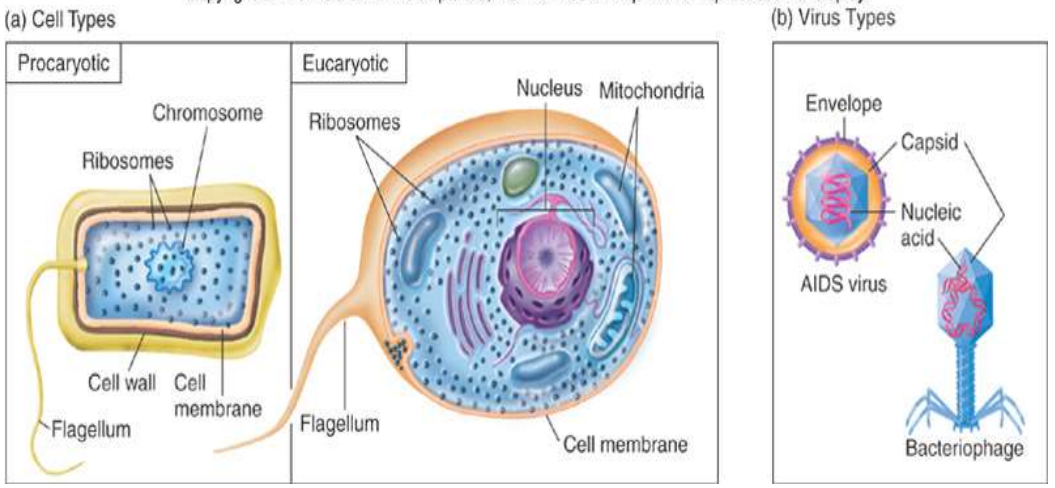
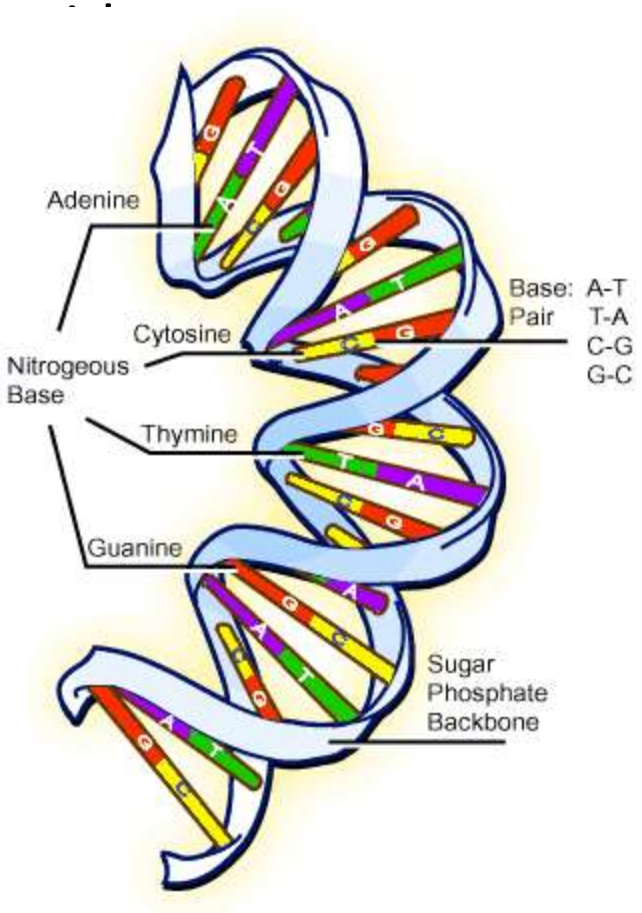


DNA Organization

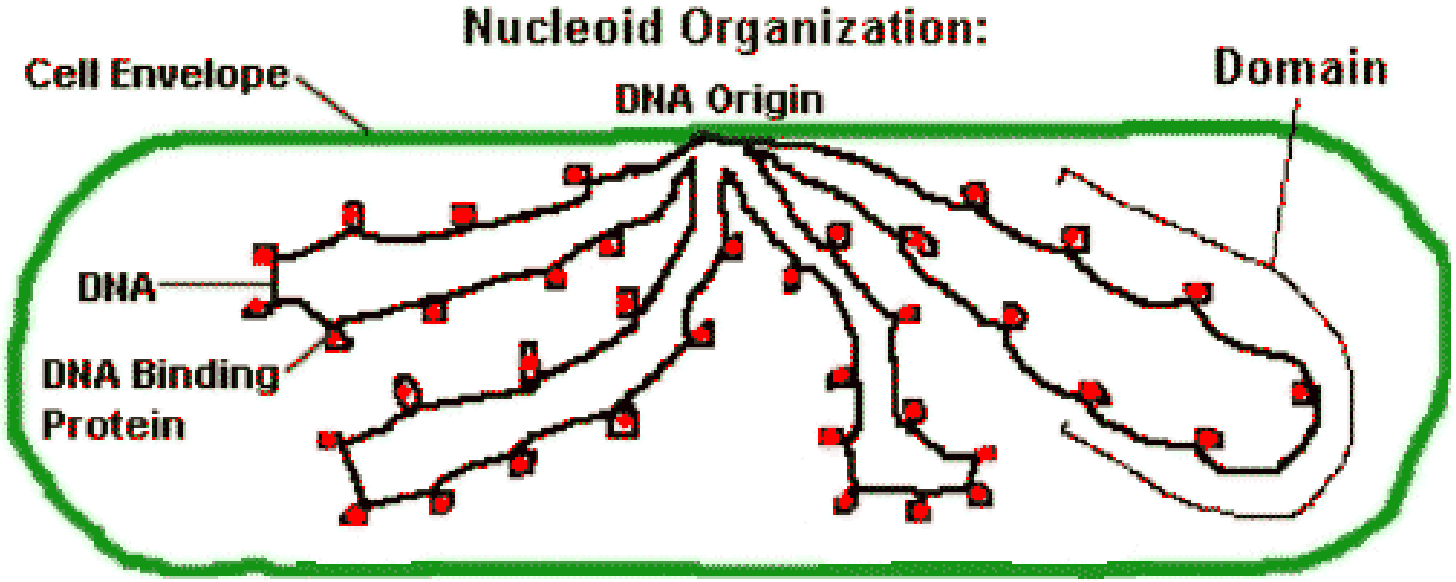
- In prokaryotes: naked circular DN
  negative supercoiling
  - Negative supercoiling is introduced
    gyrase (topoisomerase II)
  - Topoisomerase I relaxes supercoilin
    of single-strand nicks
- In eukaryotes: linear DNA packag
  around histones in units called
  nucleosomes
  - The coiling around histones causes
    supercoiling

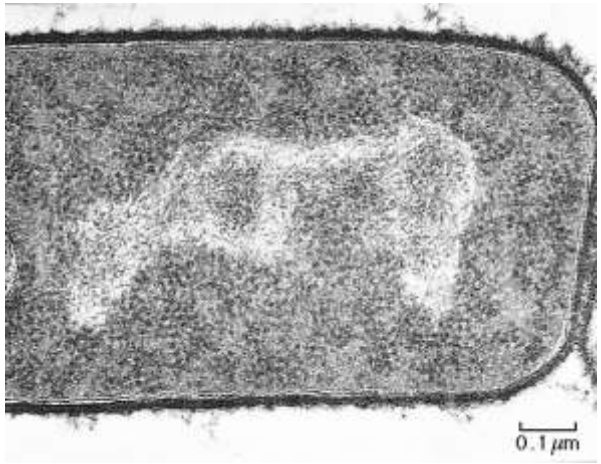


DNA - Bacterial Chromosome

- Commonly circular ds DNA
  - *E. coli* about 1.2 mm in circumference
- No nuclear envelope
- Attached to plasma membrane
- Found in nucleoid region
  - Associated with DNA-binding proteins
    - Small positively charged, similar to histones HU and H
- May have small circular DNA plasmids outside chromosome

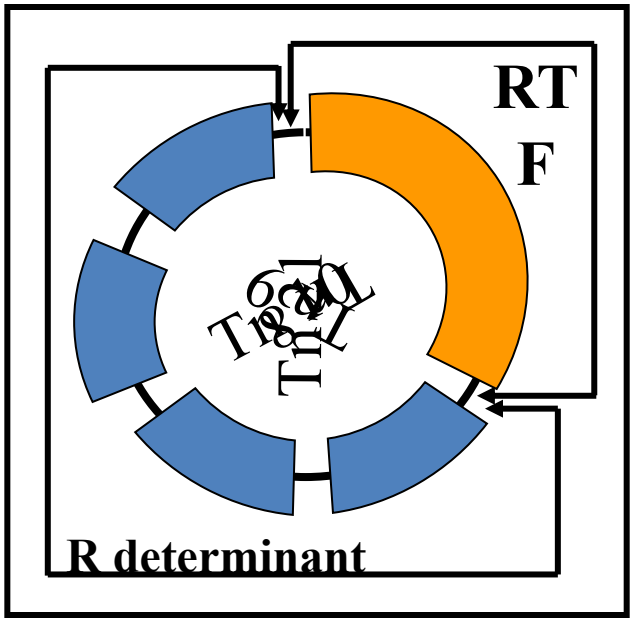
DNA - Bacterial Chromosome





Plasmids

- Definition: Extrachromosomal genetic elements that are capable of autonomous replication (replicon)
- Phenotypic effects
  - Fertility
  - Bacteriocinogenic plasmid
  - Resistance plasmid (R factors)



DNA Organization In Eukaryotes

- DNA protein complex called chromatin
  - Human chromosomes about 19,000 to 73,000 microns in length, total about 2 meters/cell
  - Nucleus about 5-10 microns in diameter
  - Condensation about 10,000X

Chromatin Structure

- Chromatin proteins subdivided into histones and nonhistones
- Histones
  - Very high contents of lysine + arginine (20-30%)
  - Amino acid sequences very conserved between species
    - Histone IV differs by one amino acid between pea and cow

TABLE 12.2 CATEGORIES AND PROPERTIES OF HISTONE PROTEINS

Histone Type	Lysine-Arginine Content	Molecular Weight (Da)
H1	Lysine-rich	23,000
H2A	Slightly lysine-rich	14,000
H2B	Slightly lysine-rich	13,800
H3	Arginine-rich	15,300
H4	Arginine-rich	11,300

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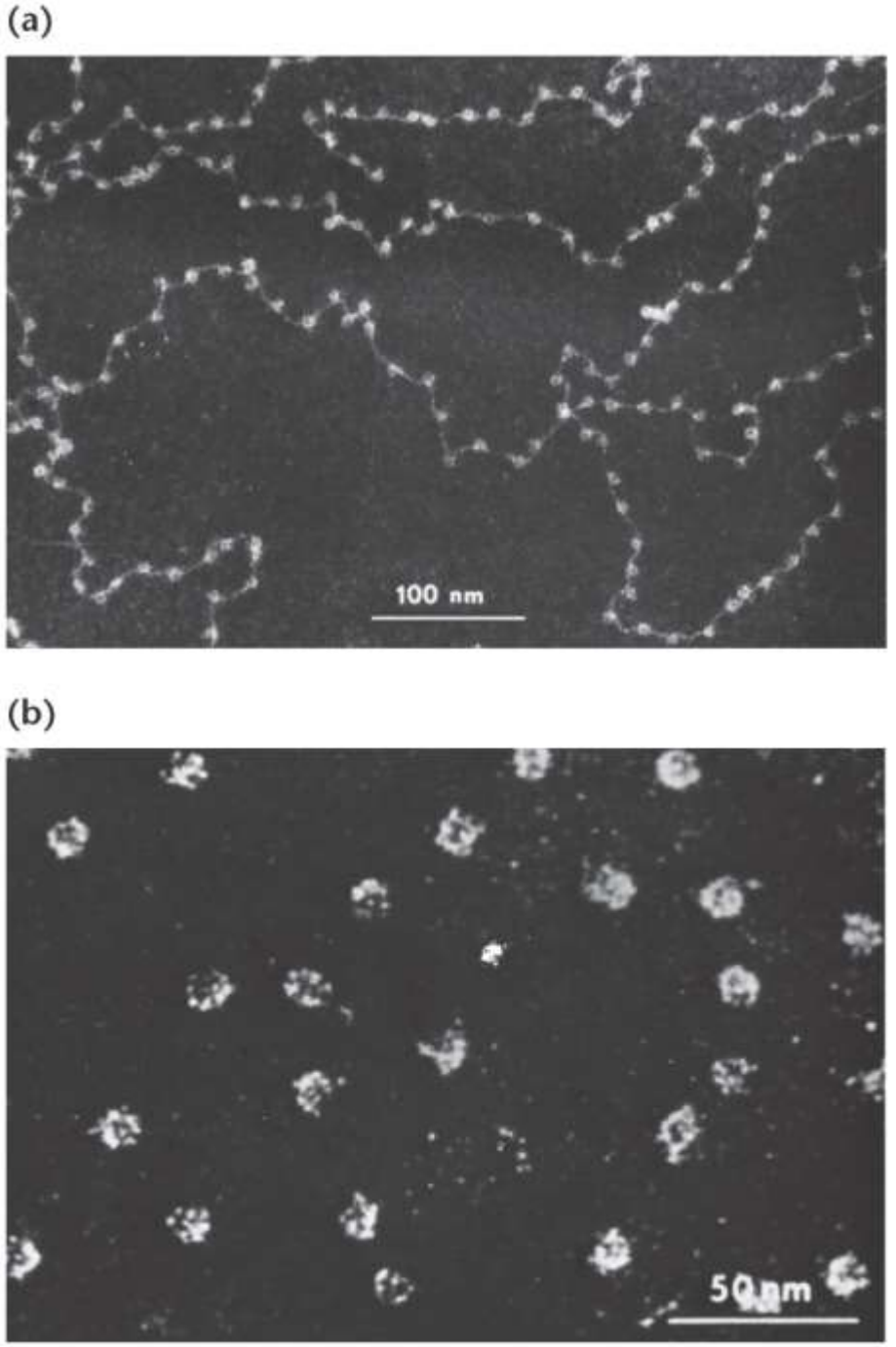
Chromatin Structure

- Olins and Olins used electron microscopy to observe “beads on a string” in the mid 1970s
- Nuclease studies revealed most sensitive sites on DNA in chromatin to be spaced at multiples of about 200 bp
- Studies showed that histones H2A, H2B, H3 and H4 could interact to form tetramers/octamers

EM Studies

- Beads on a string
- Originally called Nu bodies, now nucleosomes



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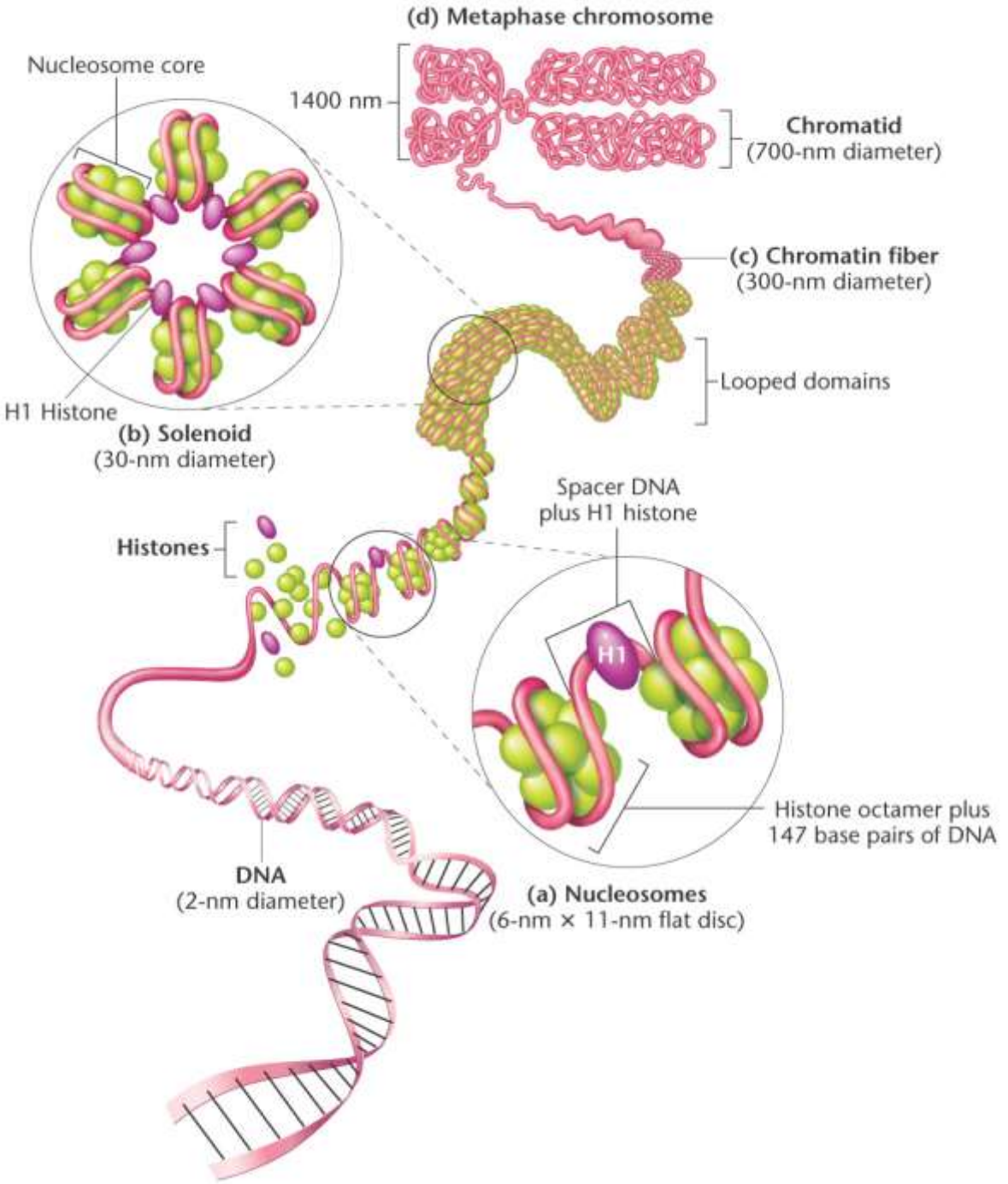
Nucleosomes

- Basic building block of eukaryotic chromatin structure
  - Octamer of 2 each of H2A, H2B, H3 and H4
  - About 147 bp of DNA wrapped around histone core particle
  - Linker DNA between core particles gives total of about 200 bp per nucleosome
  - Histone H1 is on the outside at the point of DNA entry/exit to the core particle
- Humans have about 25 million nucleosomes/cell

Levels of DNA Condensation

- DNA
- 11 nm fiber
- 30 nm fiber
- 300 nm fiber
- 700 nm fiber



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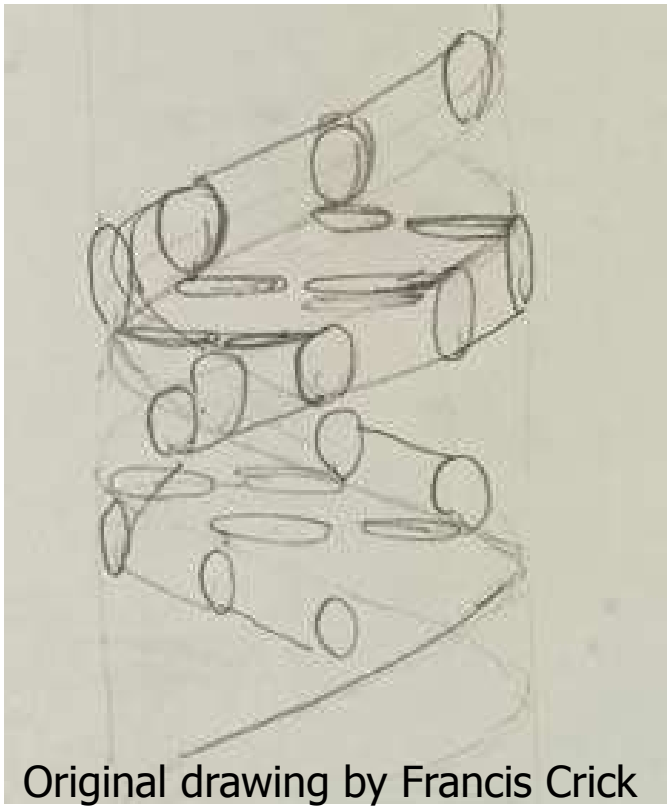
Chromatin Remodeling

- Chromatin structure is dynamic
- Induced change in chromatin structure
  - Replication, gene expression
- Histone modification
  - Acetylation by histone acetyltransferase (HAT)
  - Methylation by methyl transferases
  - Phosphorylation by kinases
- DNA modifications
  - Methylation of cytosine (5-methyl C) in CpG islands

Heterochromatin

- 1928, staining differences in nuclei lead to terms euchromatin and heterochromatin
- Heterochromatin
  - Dark staining
  - Genetically inactive

`It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material'

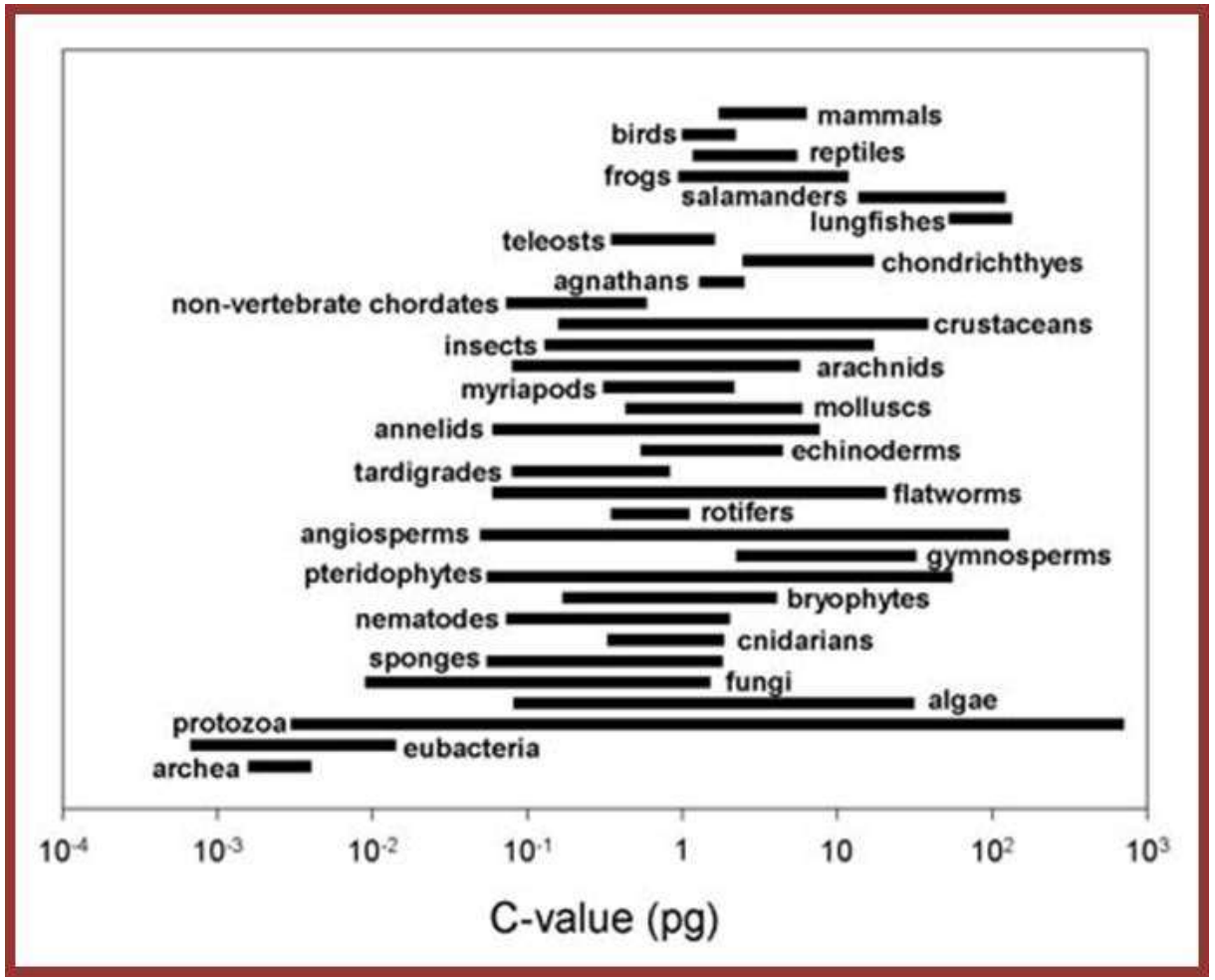


Original drawing by Francis Crick

Watson & Crick

Nature (1953)

DNA Organization In Eukaryotes

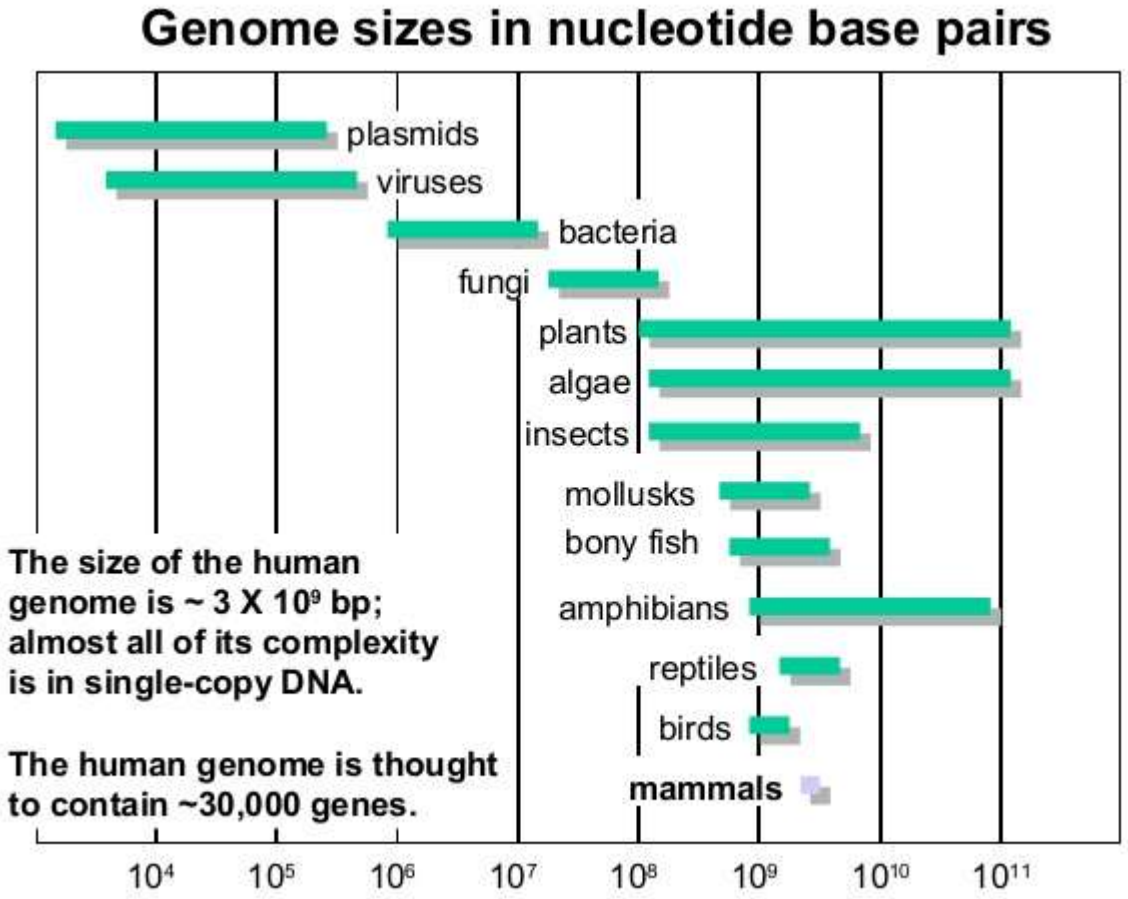


DNA Organization In Eukaryotes

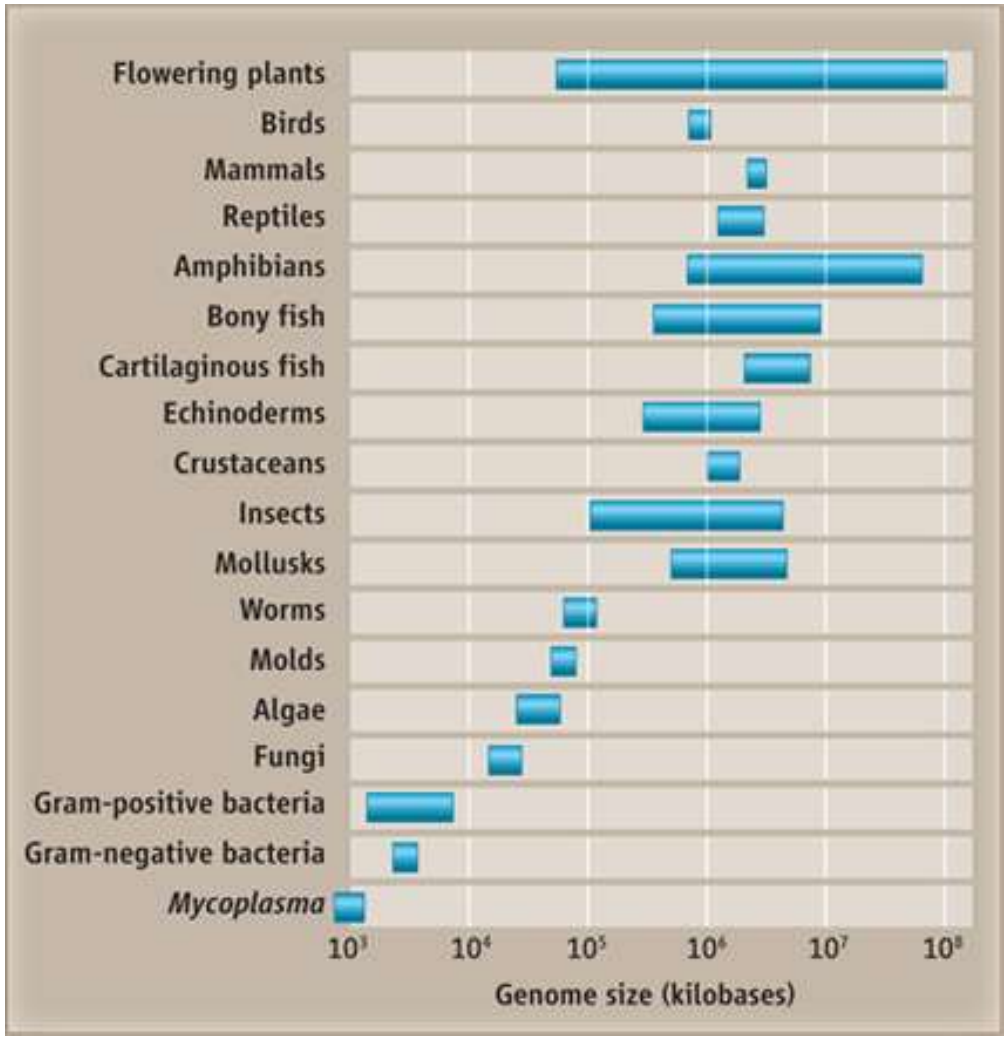
- Nuclear DNA
- Mitochondrial DNA
- Chloroplast DNA

DNA Organization In Eukaryotes

- Nuclear DNA - genome size



C-value paradox



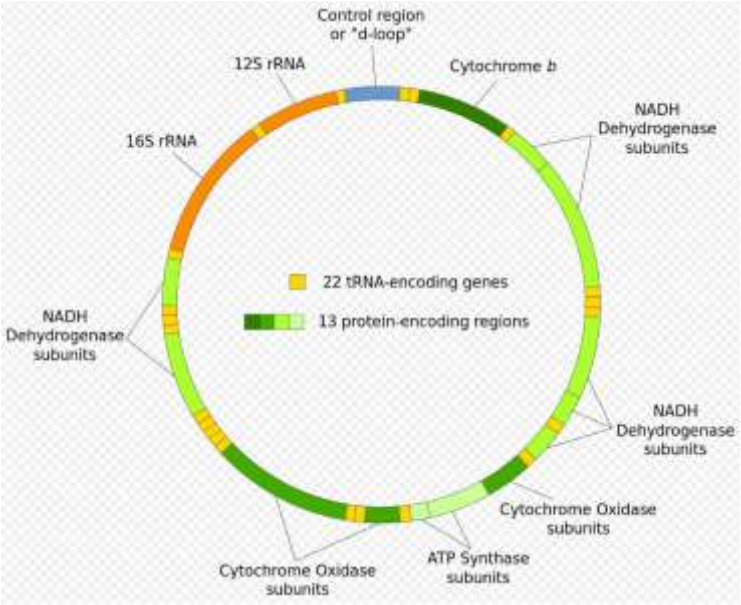
DNA Organization In Eukaryotes

- Mitochondrial DNA

Genome Type	Kingdom	Introns	Size	Shape	Description
1	Animal	No	11–28kbp	Circular	Single molecule
2	Fungi, Plant, Protista	Yes	19–1000kbp	Circular	Single molecule
3	Fungi, Plant, Protista	No	20–1000kbp	Circular	Large molecule and small plasmid like structures
4	Protista	No	1–200kbp	Circular	Heterogeneous group of molecules
5	Fungi, Plant, Protista	No	1–200kbp	Linear	Homogeneous group of molecules
6	Protista	No	1–200kbp	Linear	Heterogeneous group of molecules

DNA Organization In Eukaryotes

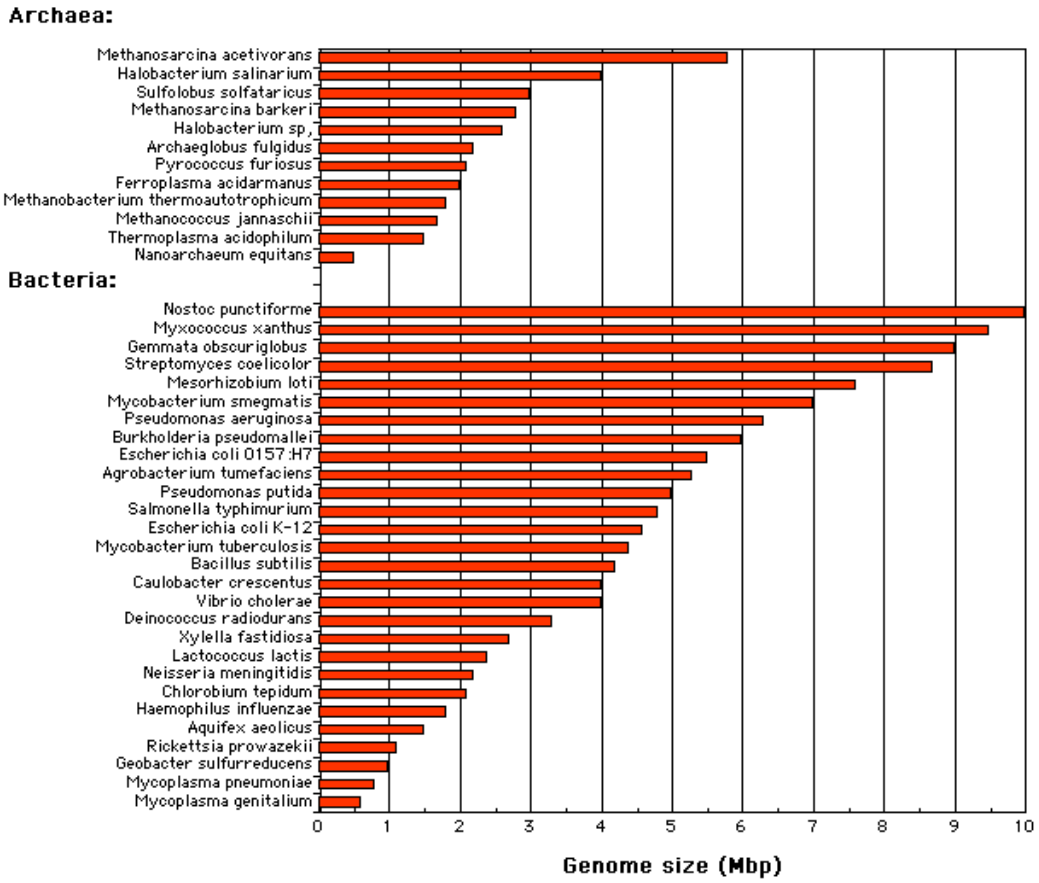
- Mitochondrial DNA



- Circular
- Small in size ~16 kb in man
- 5-10 copies of mtDNA / mitochondrion
- ~1,000 mitochondria / cell
- ~1% of cellular DNA
- Encode:
  - 13 proteins
  - large and small rRNA
  - tRNAs
- NO INTRONS- polycistronic mRNAs
- Mitochondrial genetic code has different genetic code as compared to that in nucleus
  - UGA = tryptophan not STOP
  - AGA = STOP not arginine
  - AUA = methionine not isoleucine

DNA Organization In Prokaryotes

- genome size



DNA Organization In Eukaryotes

- Chloroplast DNA

Chloroplast genome (cpDNA)

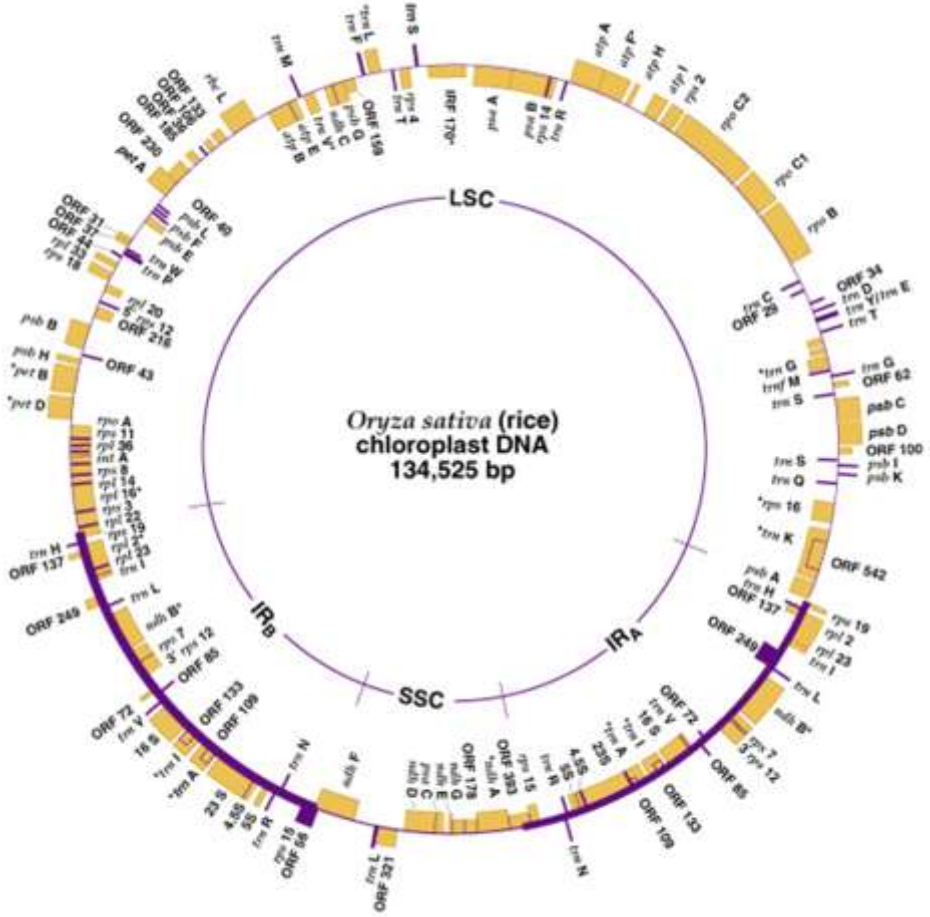
- Uses universal genetic code
- Genes for transcription and translation
- Larger than mtDNA
- Multiple copies in multiple nucleoids
- Genes for
  - rRNA (4.5S, 5S, 16S, 23S)
  - Transcription/translation proteins
  - Photosynthesis and electron transport
- Some genes contain introns
- Inverted repeats (IR_A, IR_B)

DNA Organization In Eukaryotes

- Chloroplast DNA

Table 5.1 Size of the chloroplast DNA in different plant species

Plant species	Chloroplast DNA size (kb)
Nicotiana tobaccum	130
Cucumis sativus	155
Pisum sativum	124
Triticum aestivum	135
Zea mays	139
Chlamydomonas reinhardii	195



DNA Replication

Before a cell divides, the DNA strands unwind and separate

Each strand makes a new partner by adding the appropriate nucleotides

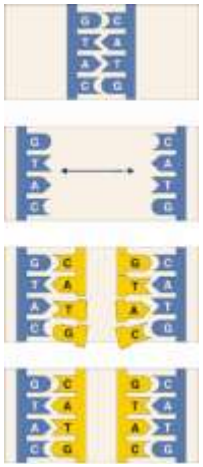
The result is that there are now two double-stranded DNA molecules in the nucleus

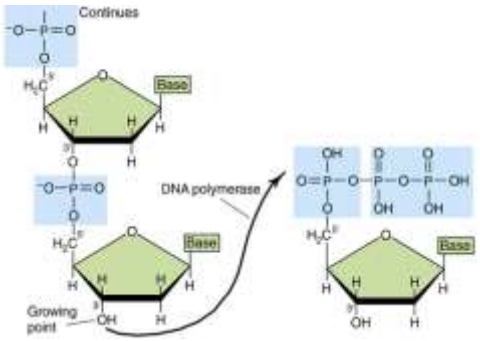
So that when the cell divides, each nucleus contains identical DNA

This process is called **replication**

DNA Structure Helps Explain How It Duplicates

- DNA is two nucleotide strands held together by hydrogen bonds
- Hydrogen bonds between two strands are easily broken
- Each single strand then serves as template for new strand
- addition of a nucleotide





DNA replication

- The biological process of replication is a multi enzyme process.
- DNA polymerase is main enzyme
- Because of the anti parallel nature of DNA replication is asymetrical

DNA polymerases: enzymes that make DNA

1. Both bacteria and eukaryotes contain multiple DNA polymerases.

2. The ones that actually replicate the DNA are called “DNA replicases.”

3. All have the same type of synthetic activity:
   a) each can extend a DNA chain by adding nucleotides one at a time to a 3’ OH end

   b) the choice of dNTPs dictated by base pairing with the template strand

Five DNA polymerases in *E. coli*

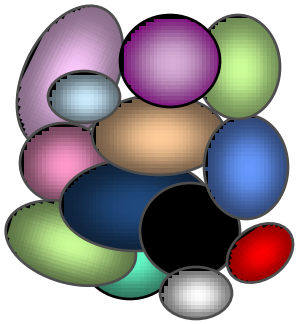
1. pol I: (encoded by *polA* gene)
    a) Major repair enzyme for damaged DNA
    b) Plays secondary role in semiconservative replication.
    c) Most abundant (400/cell)
    d) Molecular mass of 103 kD

pol II: (encoded by *polB* gene)

a) Minor DNA repair enzyme

b) Molecular mass of 90 kDa

Five DNA polymerases in *E. coli*



3. pol III: (encoded by *polC/dnaE* gene):

   a) REPLICASE; *de novo* synthesis of new strands of DNA

   b) Contains many subunits
   c) There are 10-20/cell
   c) Molecular mass of 900 kDa

   c) Has no 5’ to 3’ exonuclease activity

Five DNA polymerases in *E. coli*

4. pol IV: (encoded by *dinB* gene)
    a) SOS repair enzyme of damaged DNA

5. pol V: (encoded by *umuD'*$_2$*C* gene)
    a) SOS repair enzyme of damaged DNA

Eukaryotic DNA polymerases

Five identified in mammals.

Enzyme	α	δ	ε	β	γ
Location	Nuclear	Nuclear	Nuclear	Nuclear	Mitochondrial
function	priming of both strands	elongation of both strands	repair & replication	repair	replication
3'-5' exonuc.	No	Yes	Yes	No	Yes
relative activity	80%			10-15%	2-15%

PRIMASE REPLICASE

6. DNA synthesis has an extraordinary high fidelity: between $\underline{10}^{-8}$ and $\underline{10}^{-10}$

   1 error per genome (4200 kb) per 1000 bacterial replications. Substitutions; frame shift

7. Proofreading function: all bacterial DNA polymerases have a 3'-5' exonuclease activity. Operates in the reverse direction from synthesis.

8. In proofreading the excised base is replaced by a different active site of the enzyme than the one used for the original synthesis.

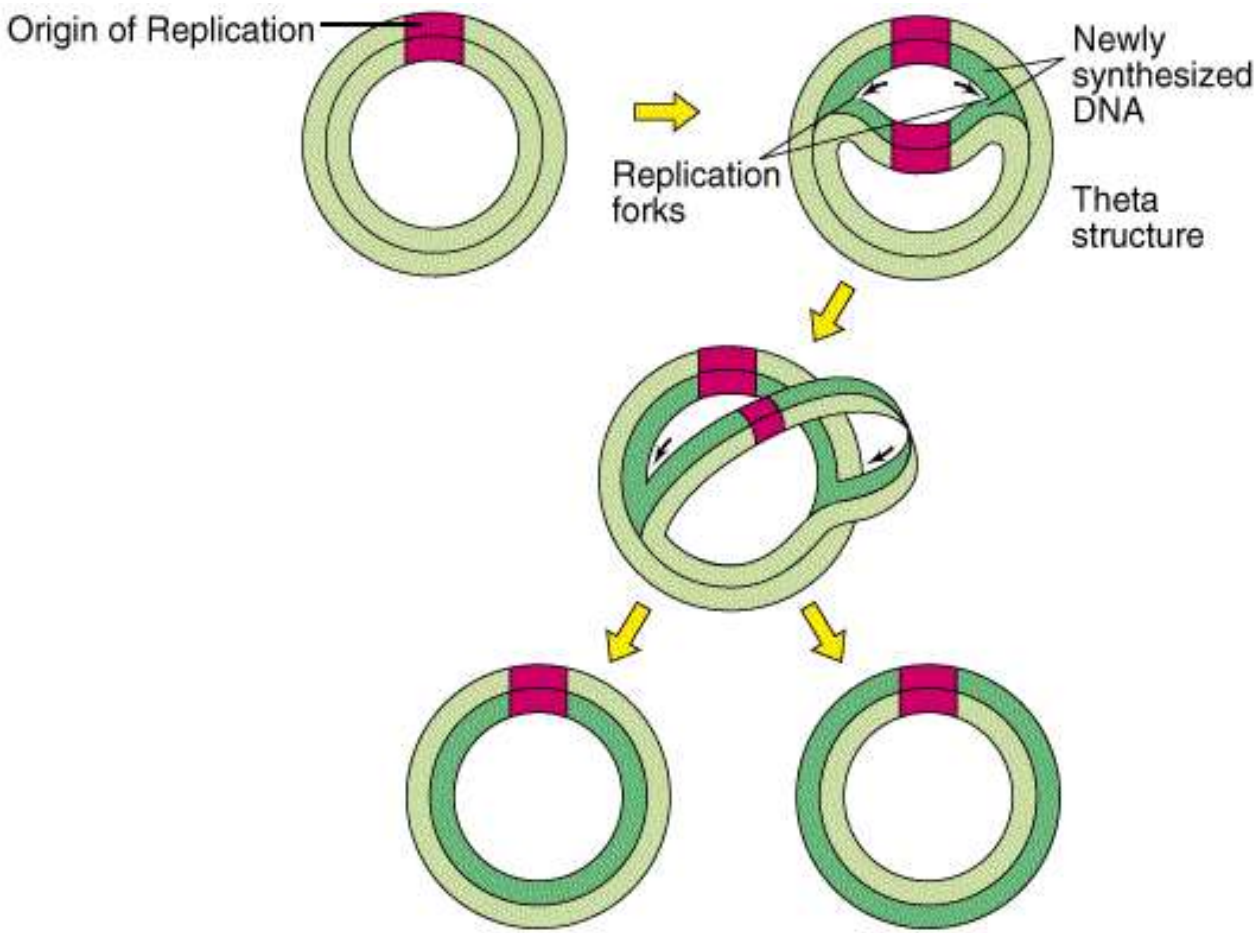
DNA Replication

Primosome: a protein complex that initiates synthesis of a DNA strand.

Replisome: complex of proteins engaged in elongation of the newly synthesized DNA strand.

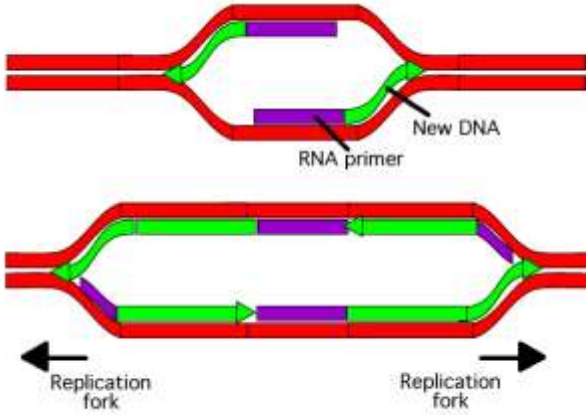
Assembles at the replication fork.

DNA Replication



Semi-conservative Replication

- Watson and Crick recognized that the double stranded DNA molecule could replicate by unwinding, then synthesizing a new strand for each of the old stands.
- This mode of replication is called “semi-conservative”. It means that after one DNA molecule has replicated to become 2 DNA molecules, each new molecule consists of one old strand (from the original molecule) and one new strand.
- The information from each old strand can be used to create the new strands, since A always pairs with T, and G always pairs with C.
- DNA replication starts at specific locations “origins of replication”, and proceeds in both directions.



Semi-conservative Replication

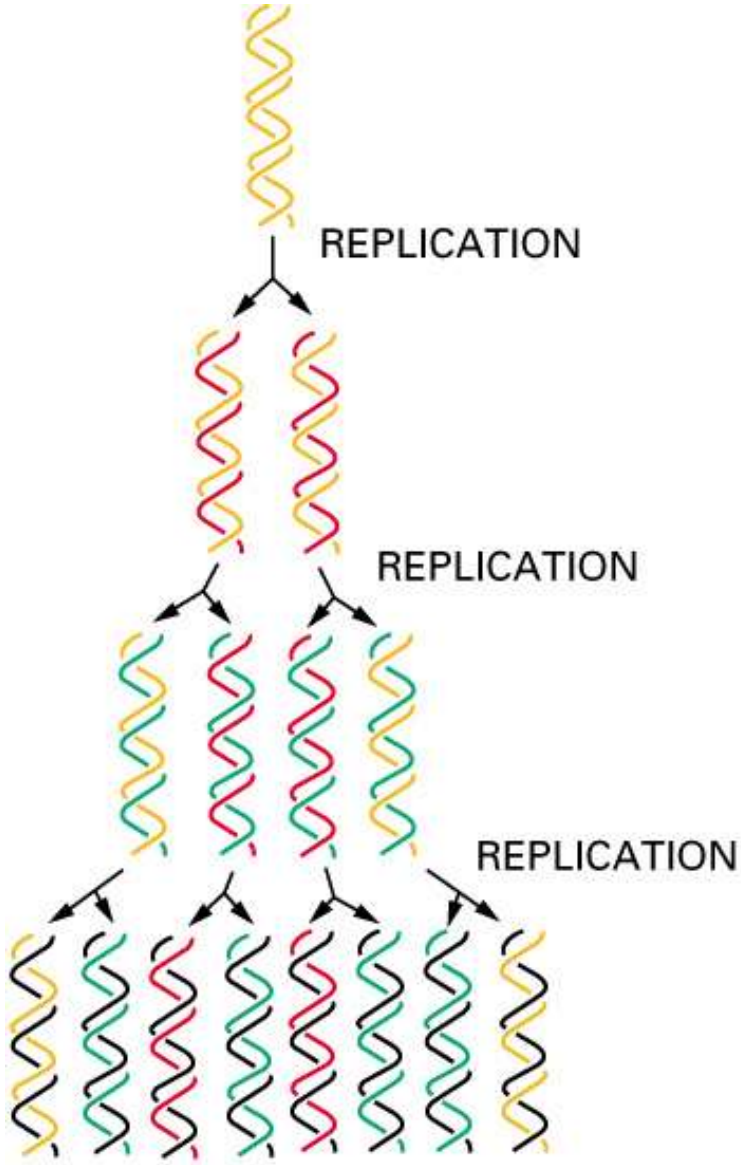
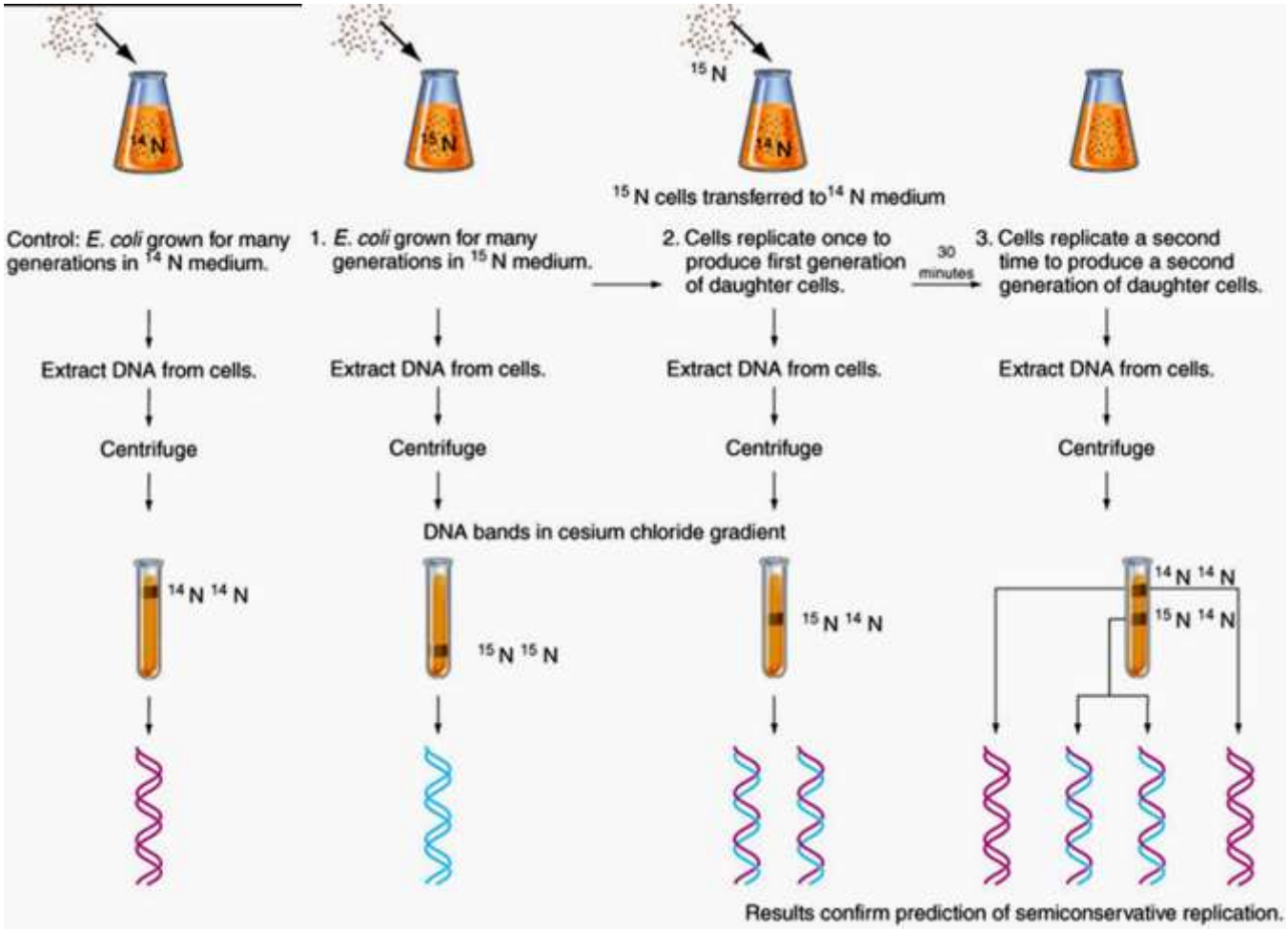


Figure 5–5. Molecular Biology of the Cell, 4th Edition.

Semi-conservative Replication

Meselson-Stahl experiments



Bidirectional replication

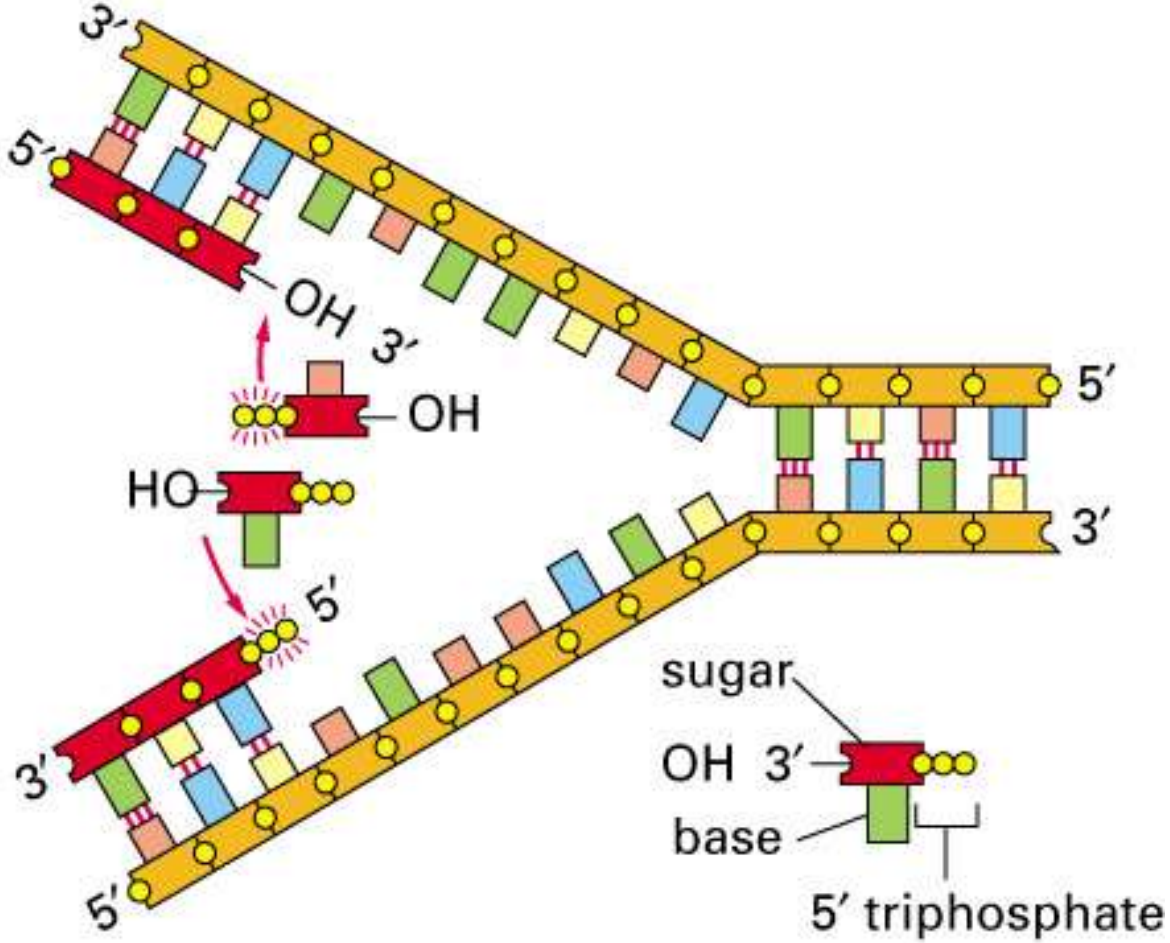


Figure 5–7. Molecular Biology of the Cell, 4th Edition.

Semi-discontinuous Replication

- All known DNA pols work in a 5'>>3' direction
- Solution?
  - Okazaki fragments

Why does DNA replication only occur in the 5’ to 3’ direction?

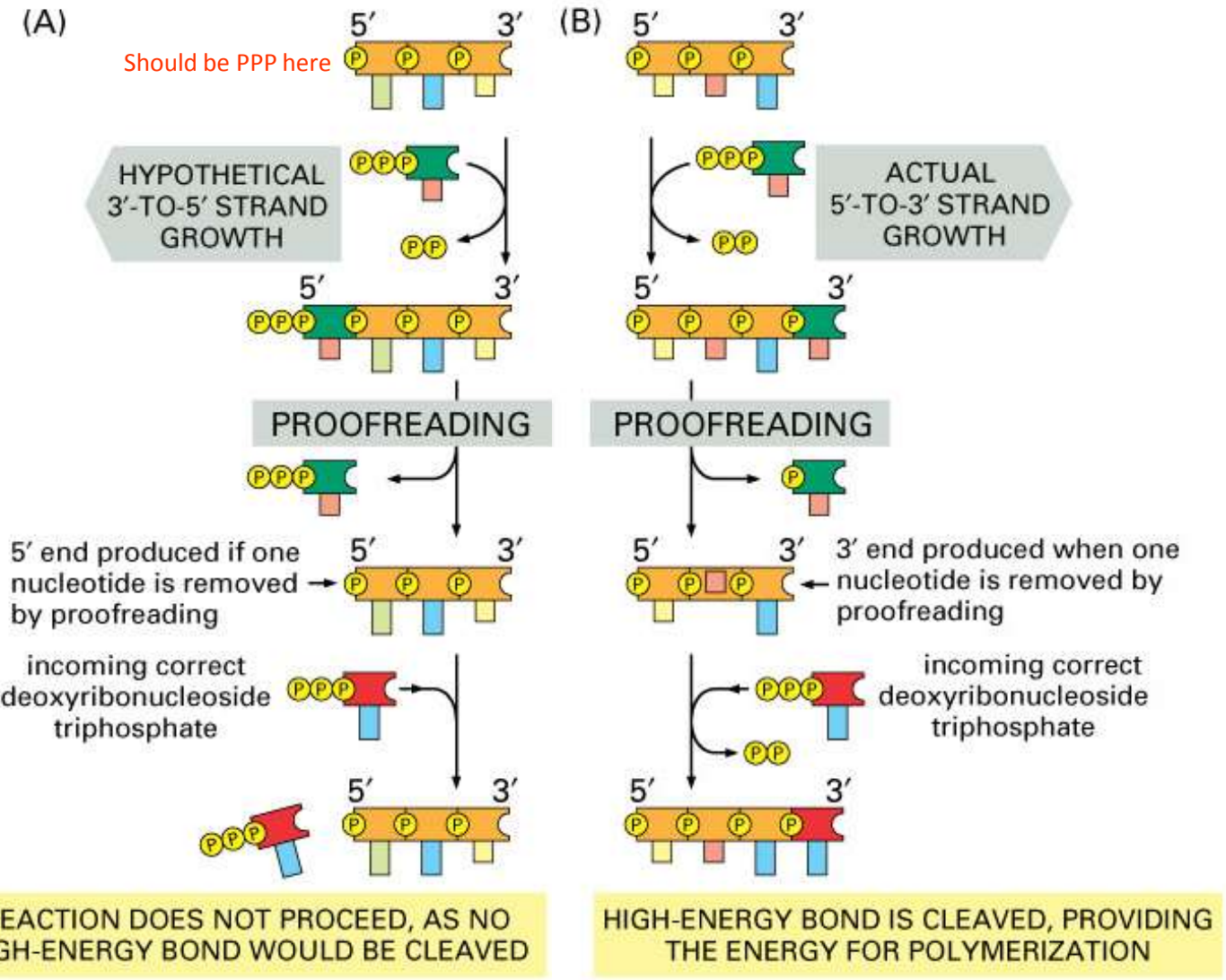


Figure 6-15 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Okazaki Experiment

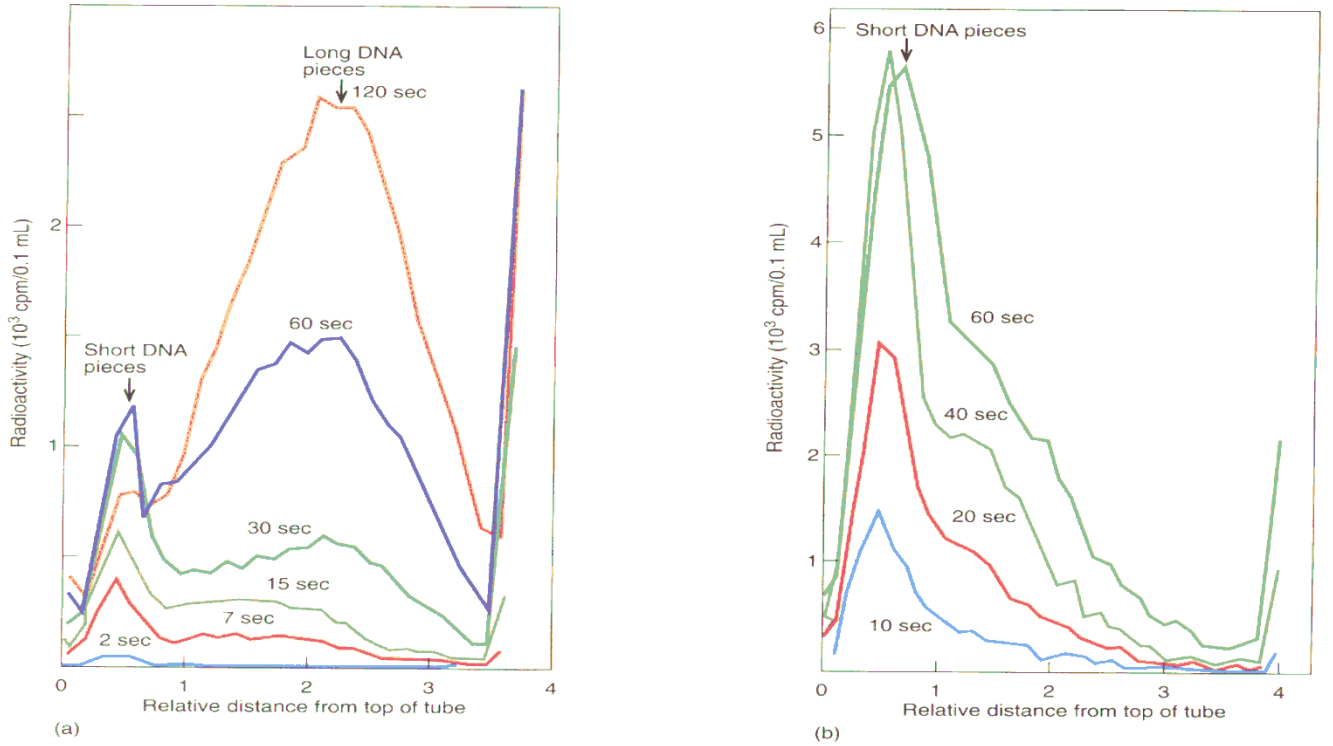


Figure 20.7 Experimental demonstration of at least semidiscontinuous DNA replication. (**a**) Okazaki and his colleagues labeled replicating phage T4 DNA with very short pulses of radioactive DNA precursor and separated the product DNAs according to size by ultracentrifugation. At the shortest times, the label went primarily into short DNA pieces (found near the top of the tube), as the discontinuous model predicted. (**b**) When these workers used a mutant phage with a defective DNA ligase gene, short DNA pieces accumulated even after relatively long labeling times (1 min in the results shown here).

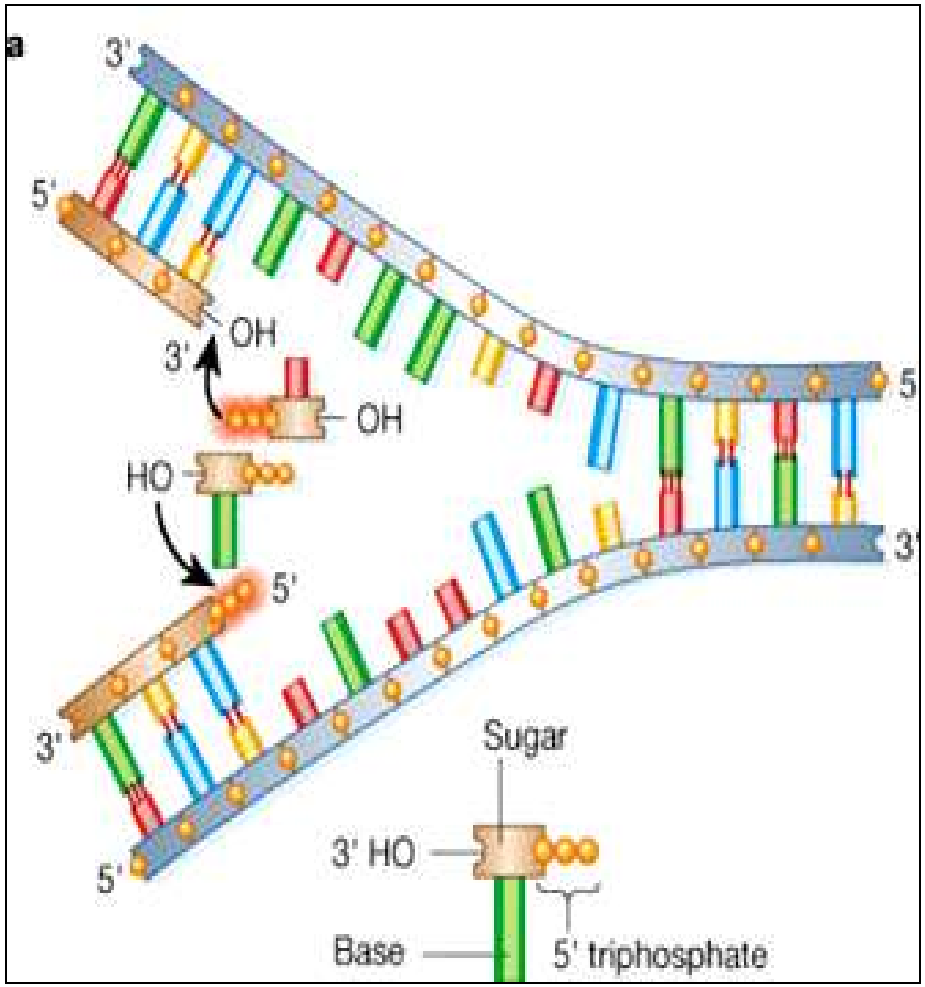
100-**200 nucleotides** in eukaryotes and about 1000-**2000 nucleotides**

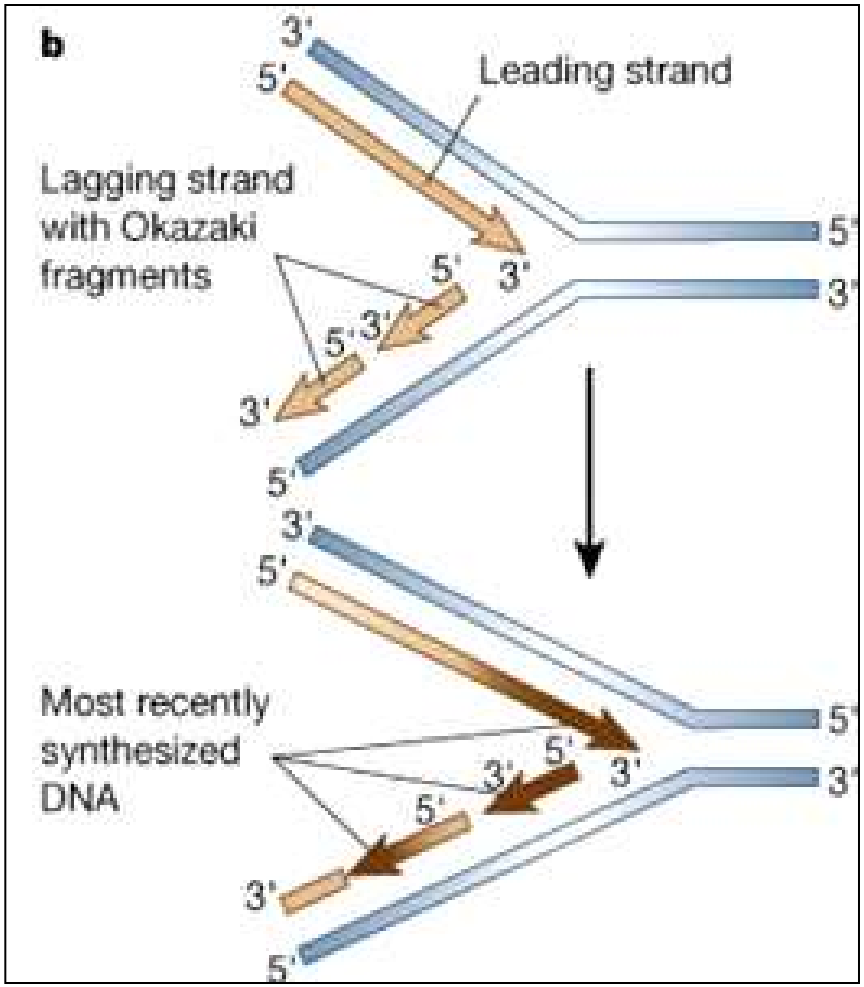
DNA Replication fork is assymetrical

- Leading and lagging strands are different
- The leading strand is continuous
- The lagging strand has Okazaki fragments or is synthesized discontinuously

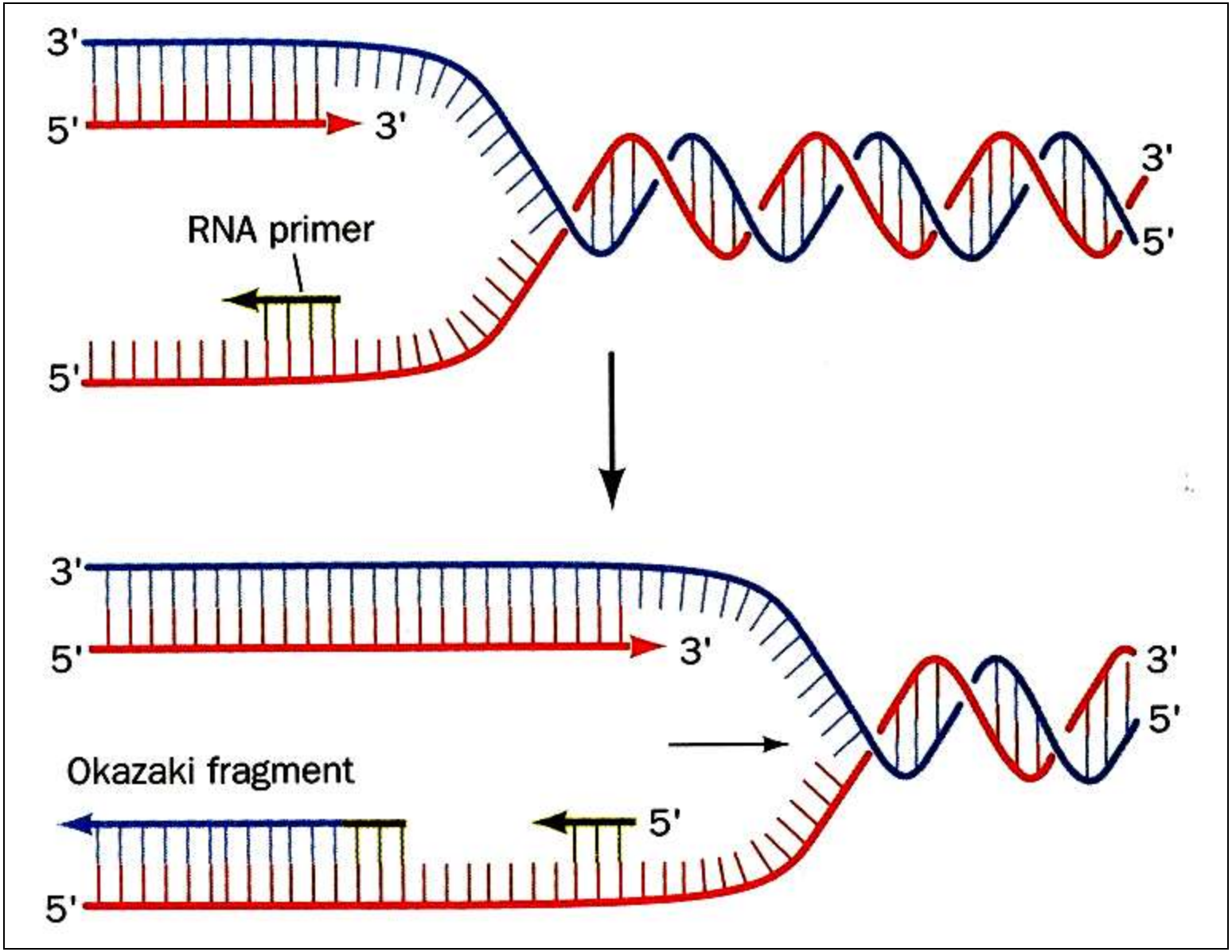
Semi-discontinuous replication

New strand synthesis always in the 5′-3′ direction





RNA primers required



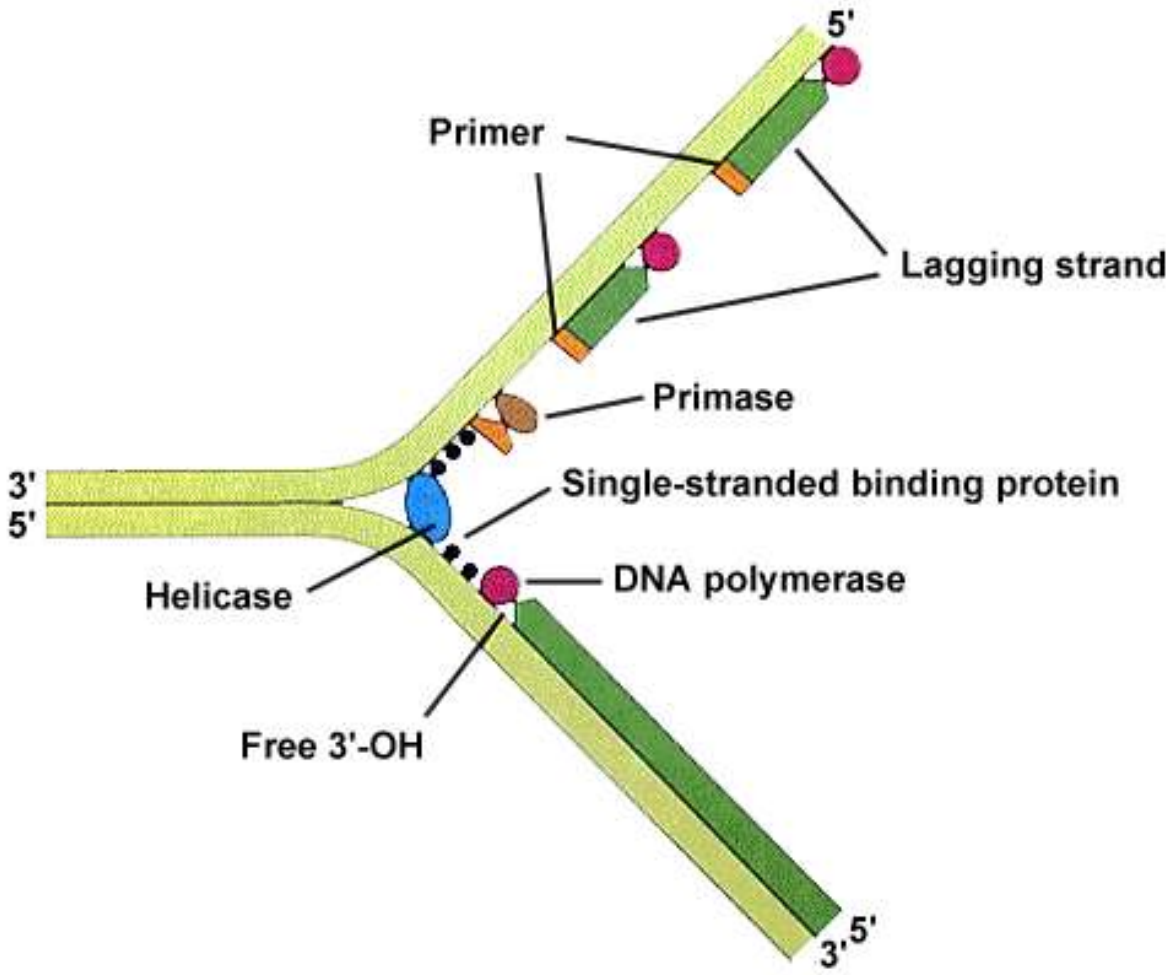
Features of DNA Replication

- DNA replication is semiconservative
  - Each strand of template DNA is being copied.
- DNA replication is semidiscontinuous
  - The leading strand copies continuously
  - The lagging strand copies in segments (Okazaki fragments) which must be joined
- DNA replication is bidirectional
  - Bidirectional replication involves two replication forks, which move in opposite directions

Replication fork

- Many different proteins are needed to open up the DNA helix for replication
- Two strands must be separated first
- Helicases do this
- Many different helicases have been identified
- Single stranded binding proteins are important in maintaining the single stranded nature at the replication fork

Bidirectional replication



DNA polymerase needs a primer

- Primase synthesizes a short RNA primer.
- DNA polymerase needs a 3’ OH to add its first base

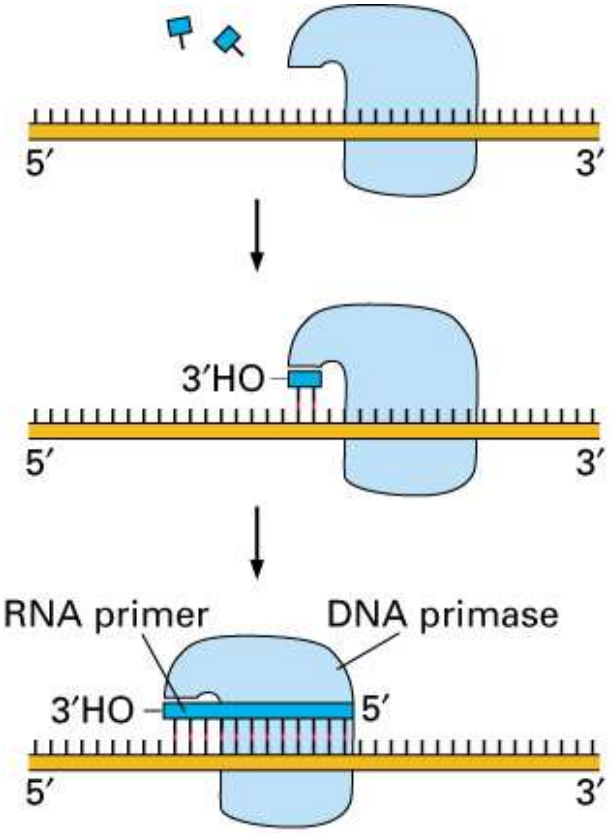


Figure 5–12. Molecular Biology of the Cell, 4th Edition.

Replication fork

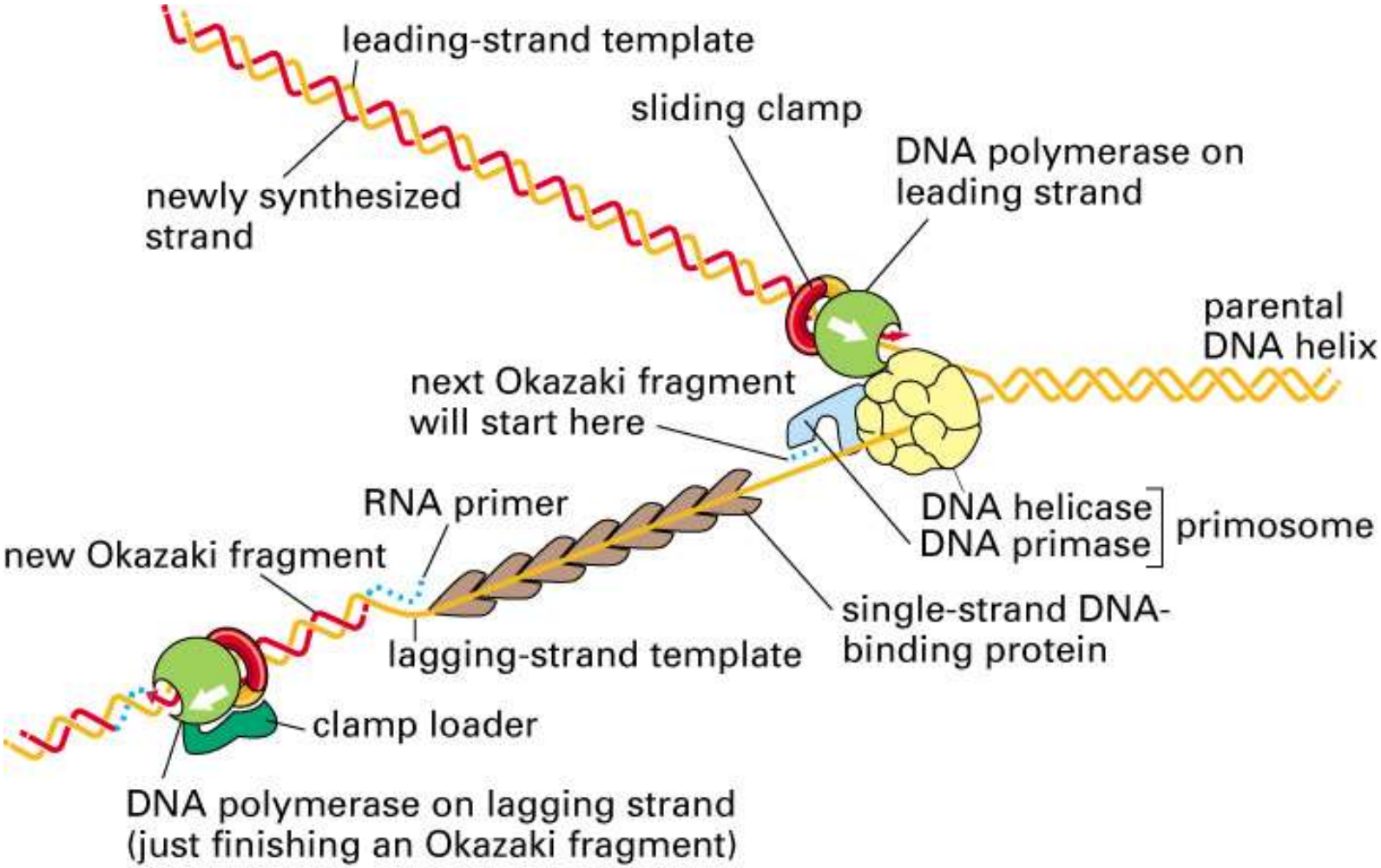


Figure 5–21. Molecular Biology of the Cell, 4th Edition.

Replication fork

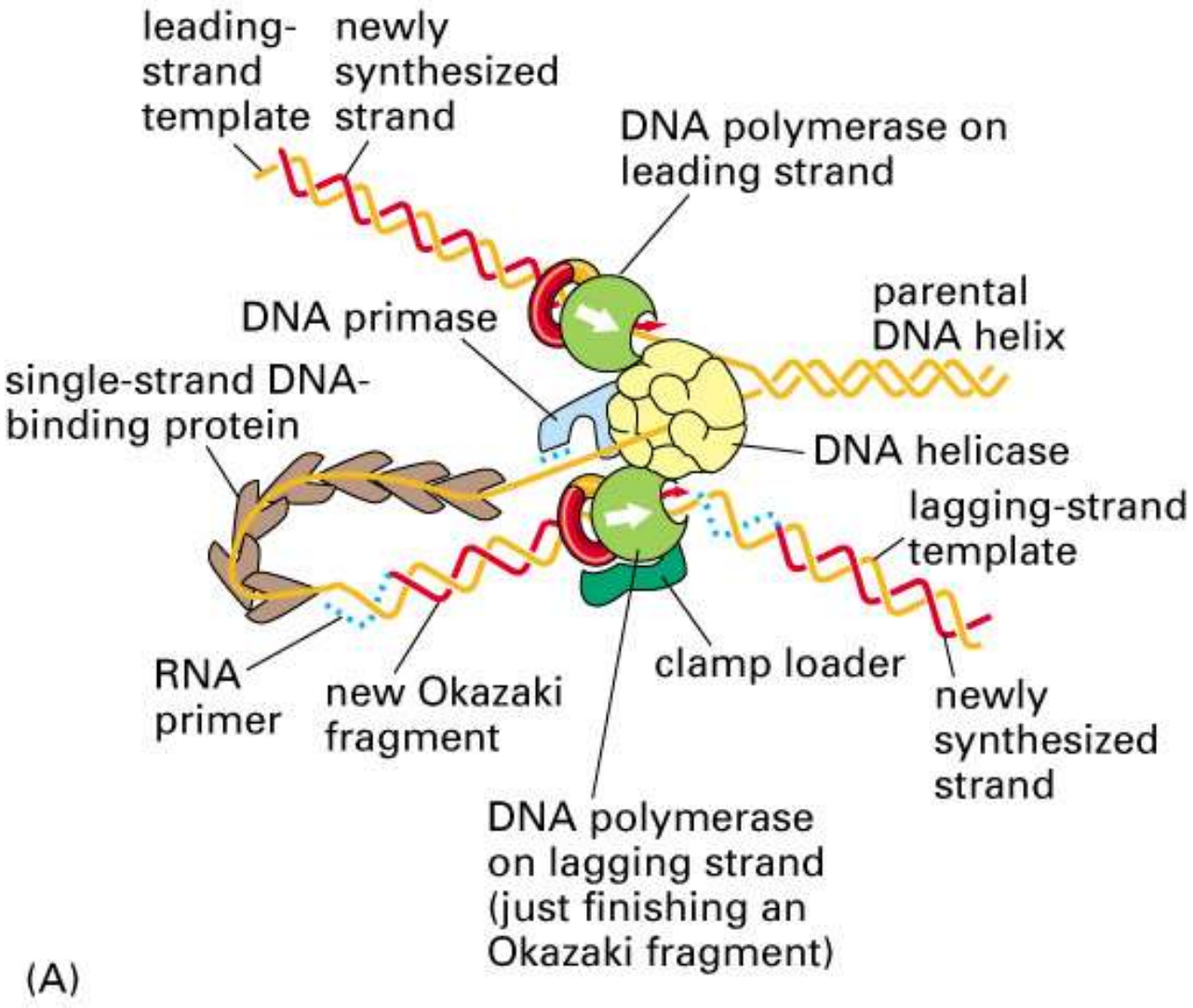


Figure 5–22 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Replication fork

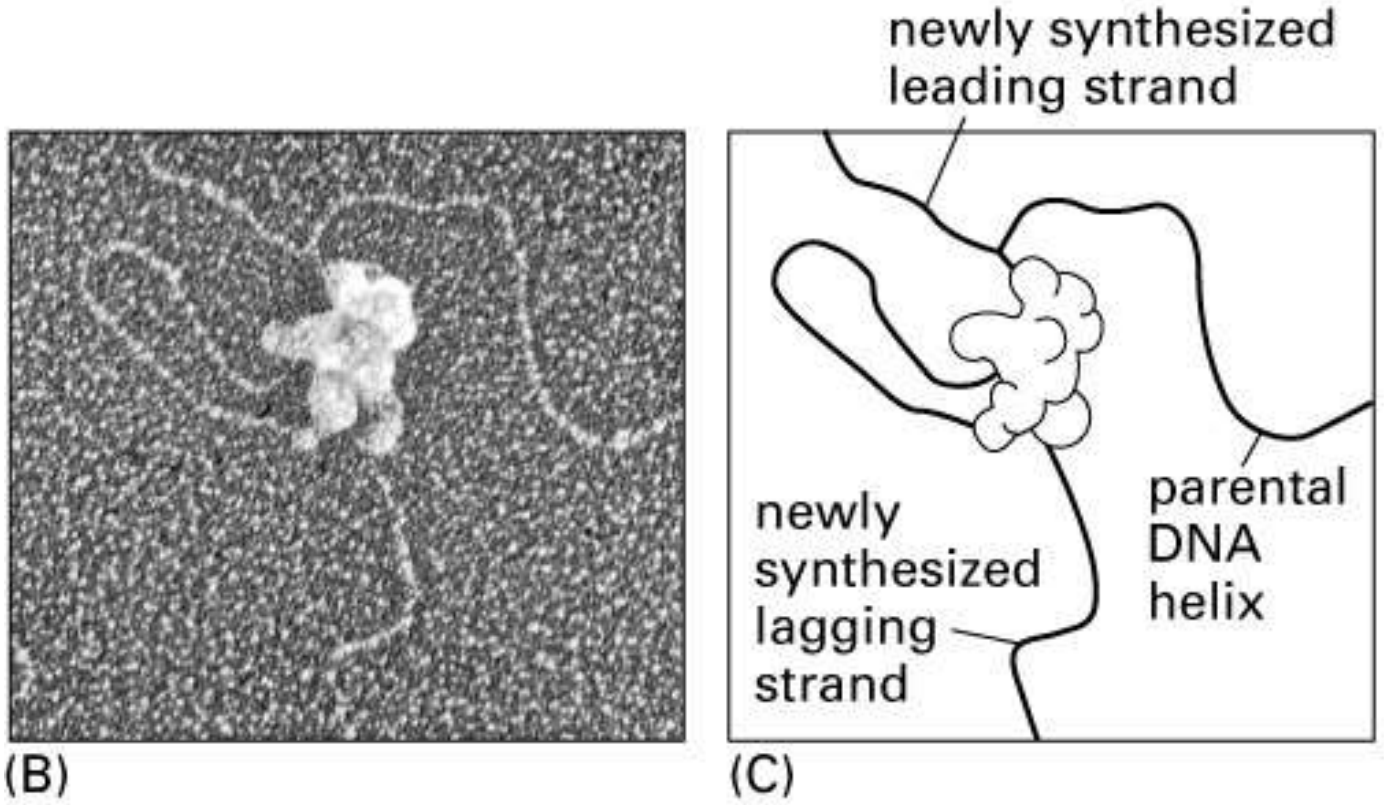


Figure 5–22 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

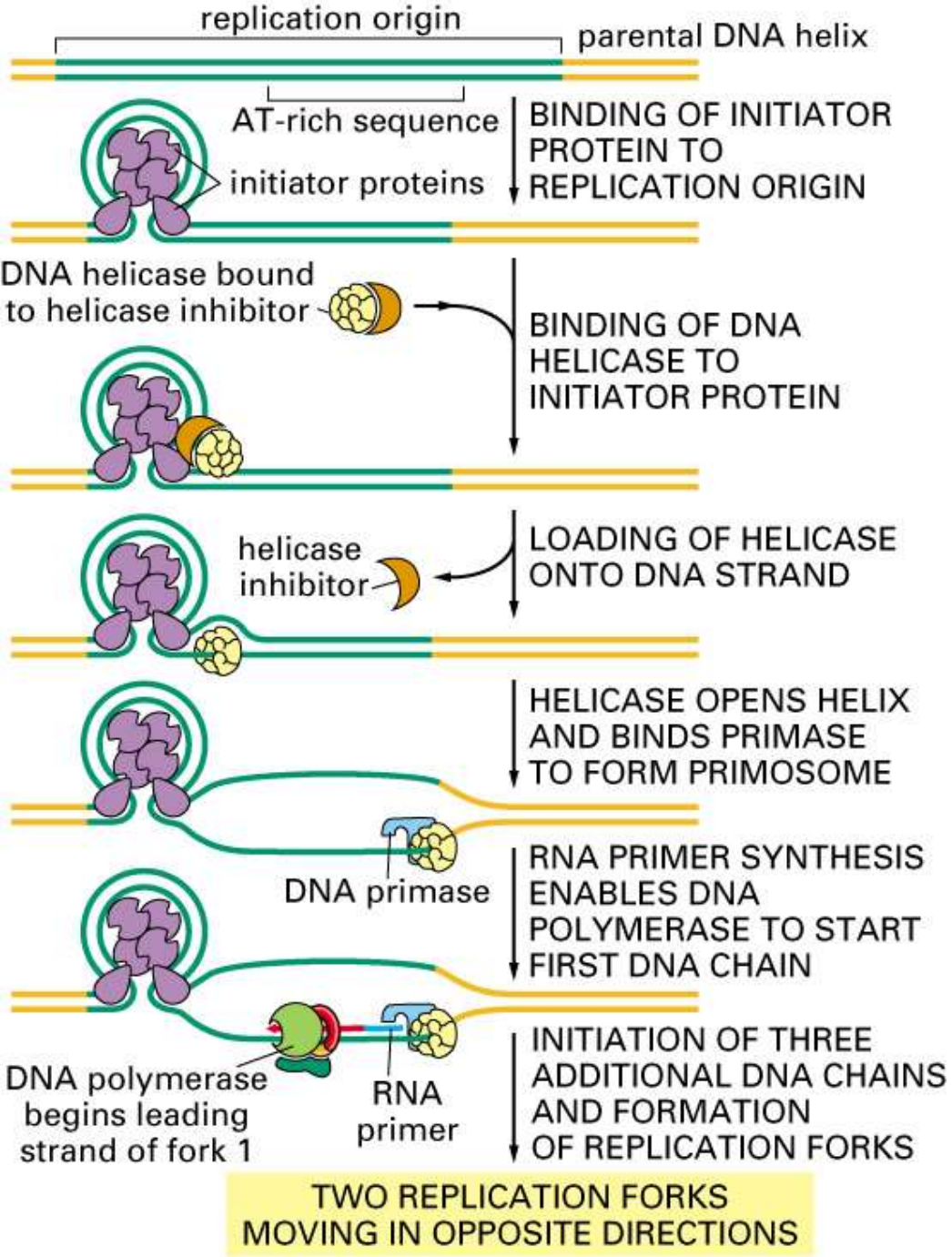


Figure 5–31. Molecular Biology of the Cell, 4th Edition.

Initiation occurs at replication origins

Topoisomerases

- This enzyme prevents DNA tangling during replication.
- 3 dimensional structure …Alpha helical
- 1 complete turn every 10 base pairs
- During replication there is a need to follow the DNA structure.
- This produces Tortional stress
- The cell uses topoisomerases to relieve this.

Topoisomerases

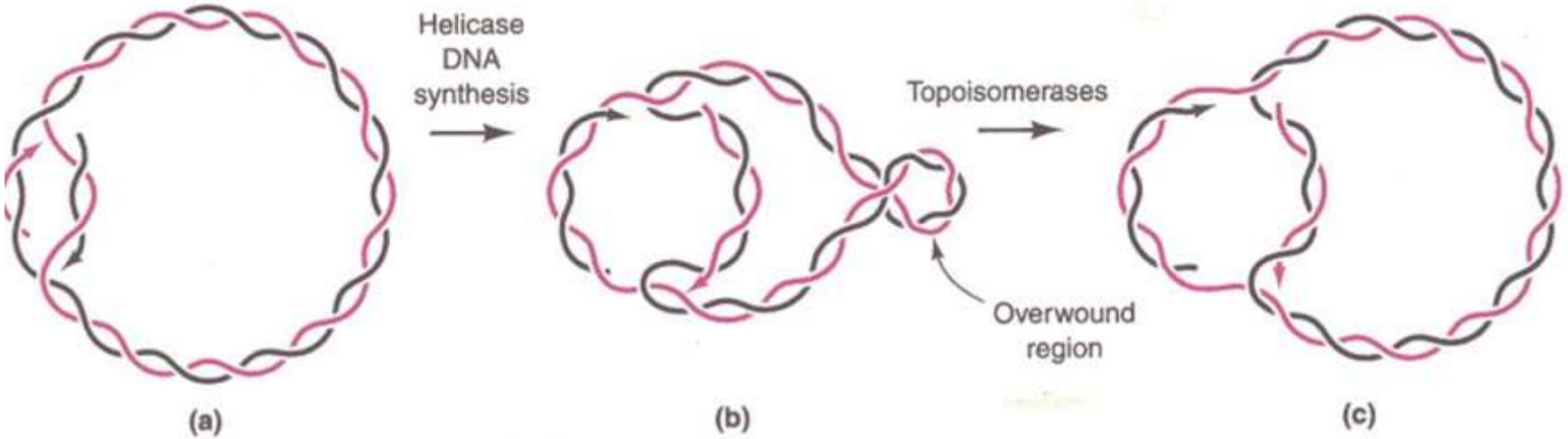


Figure 7-4 Relaxation of DNA overwinding by topoisomerases. DNA replication is shown after it has begun on a circular DNA molecule. In this example a replication fork is working at both sides of the growing "bubble" (a). As the replication bubble grows (b), an overwound region appears ahead of the replication bubble. Through the action of topoisomerases the overwound region is relaxed (c). Illustration provided by C. Ullsperger, A. Vologodskii, and N. Cozzarelli.

Topoisomer

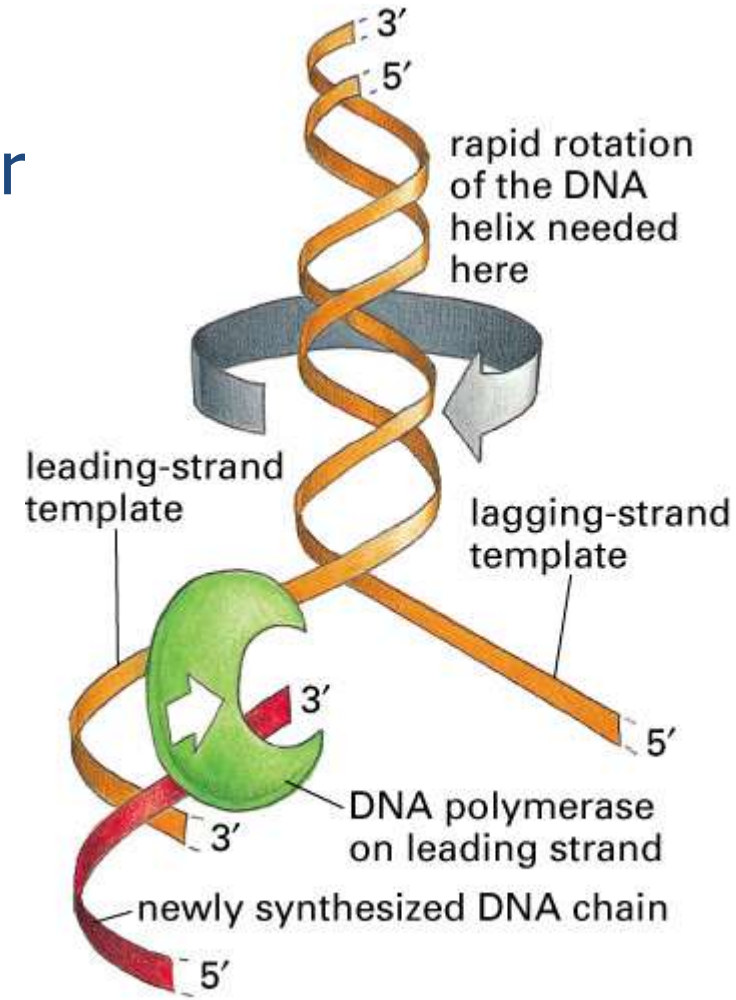
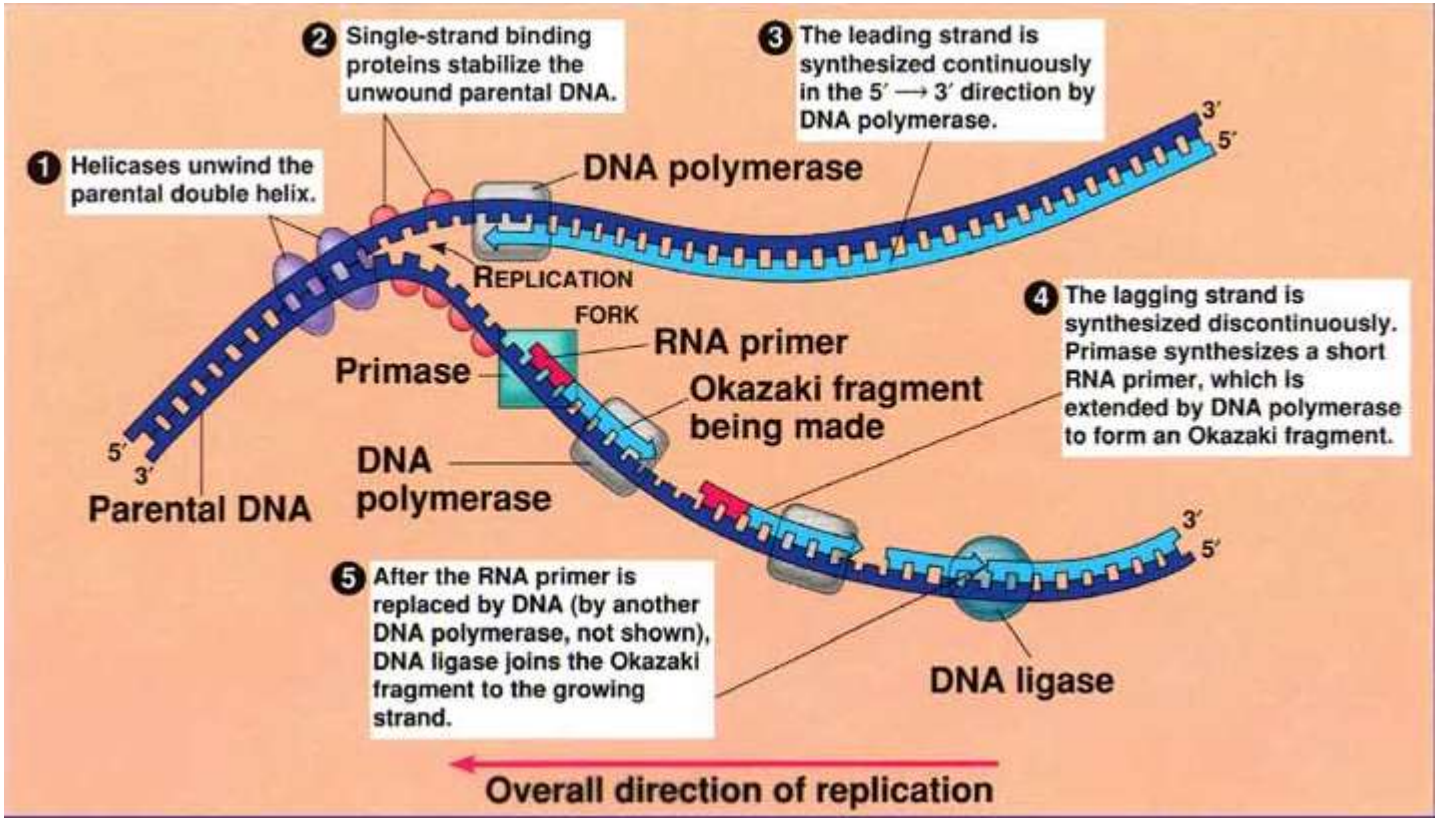
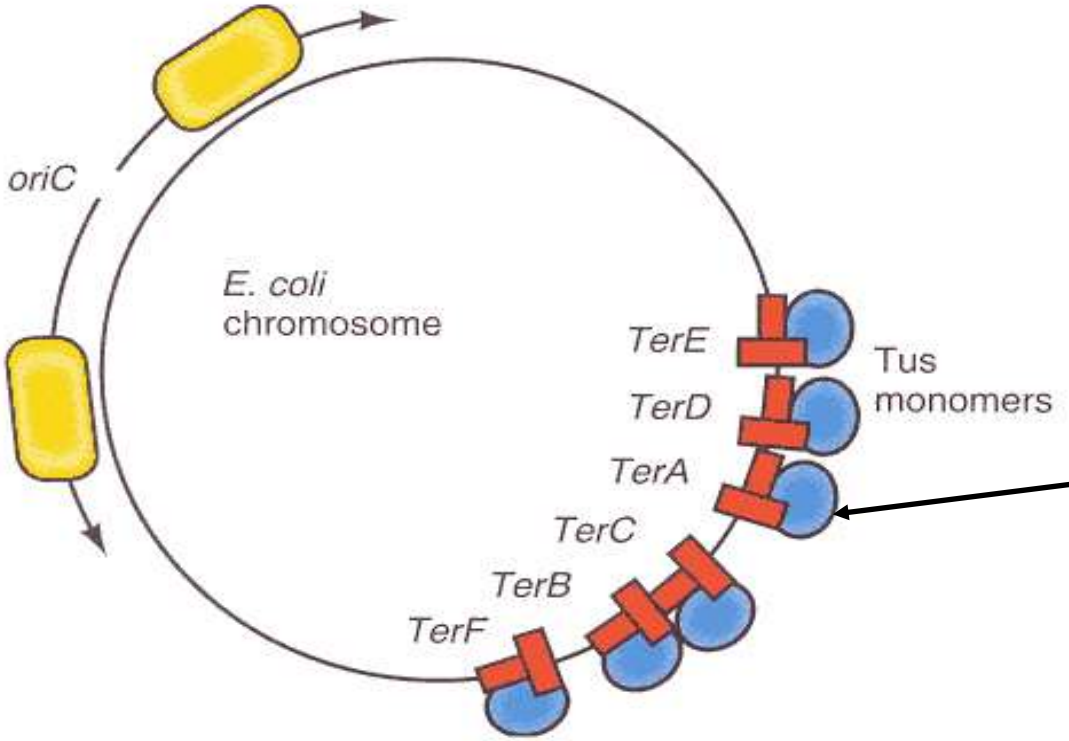


Figure 5–24. Molecular Biology of the Cell, 4th Edition.

Overview of replication



Termination of Replication



Tus Protein-arrests replication fork motion

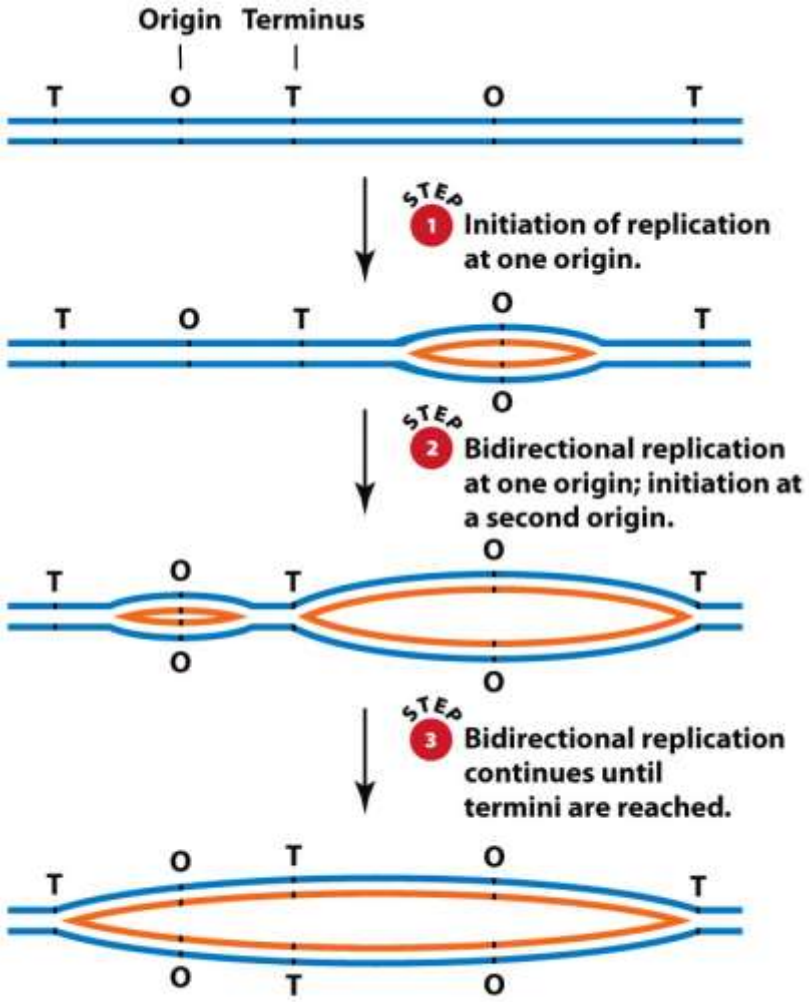
- Occurs @ specific site opposite *ori c*
- ~350 kb
- Flanked by 6 nearly identical *non-palindromic**, 23 bp terminator (*ter*) sites

DNA replication in eucaryotes

Multiple ori

Linear chromosomes

telomeres



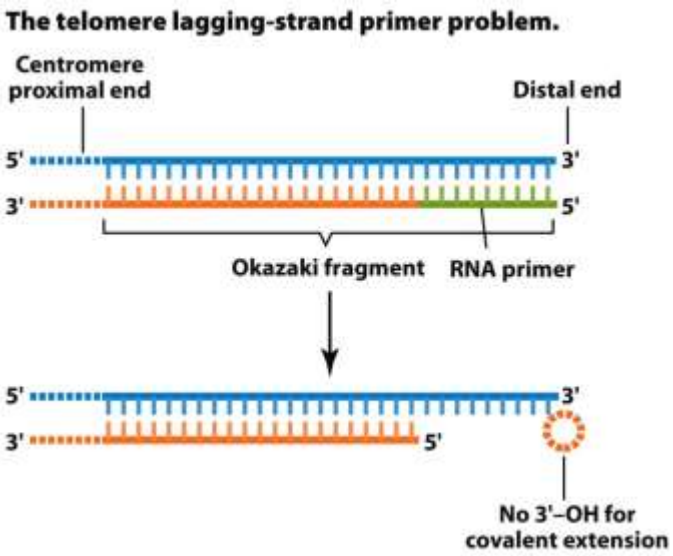
Diagrammatic interpretation of the replication of the DNA molecules visualized in *(a)* and *(b)*.

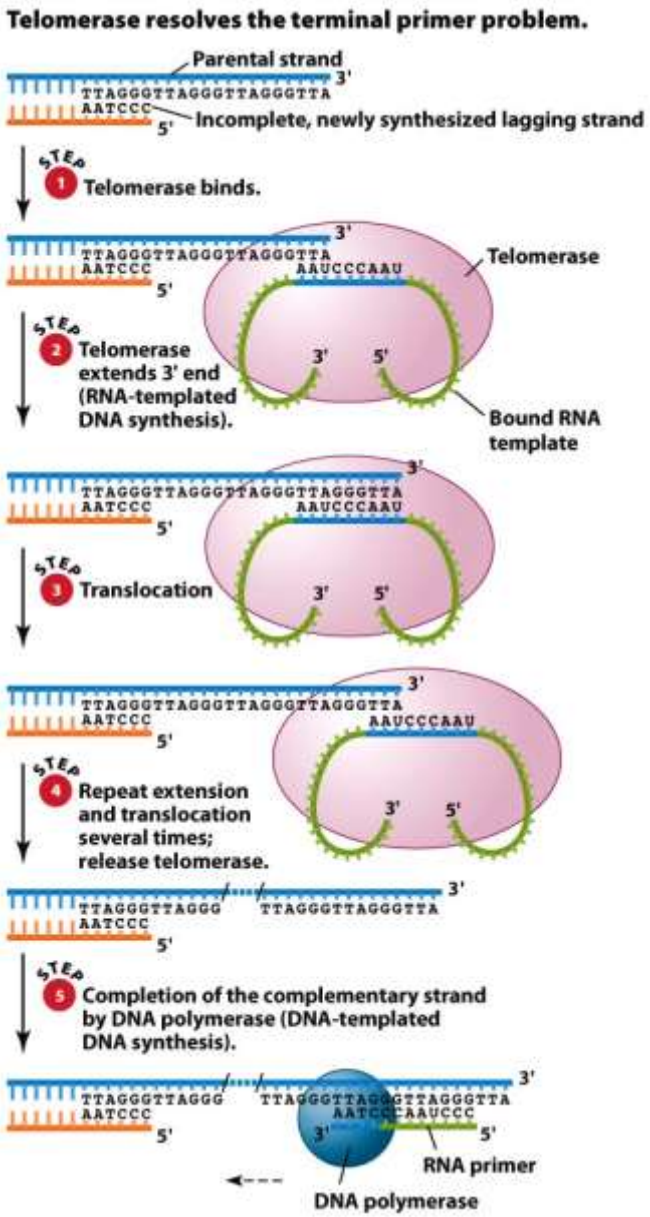
DNA replication in eucaryotes

Multiple ori

Linear chromosomes

telomeres





Telomeric DNA

- Telomeres found on ends of eukaryotic chromosomes
  - Render natural chromosome ends “inert” with regard to interactions by other chromosome ends such as those resulting from chromosome breakage
- Sequence composed of short repeat segments
  - 50 repeats of GGGGTT in *Tetrahymena*
  - GGGATT in humans

FIDELITY OF REPLICATION

- Expect $1/10^{3\text{-}4}$, get $1/10^{8\text{-}10}$.
- Factors
  - 3’→5’ exonuclease activity in DNA pols
  - Use of “tagged” primers to initiate synthesis
  - Battery of repair enzymes
  - Cells maintain balanced levels of dNTPs

Replication occurs at origins

FC174 phage

***OriC* origin of bacterial chromosomal replication**

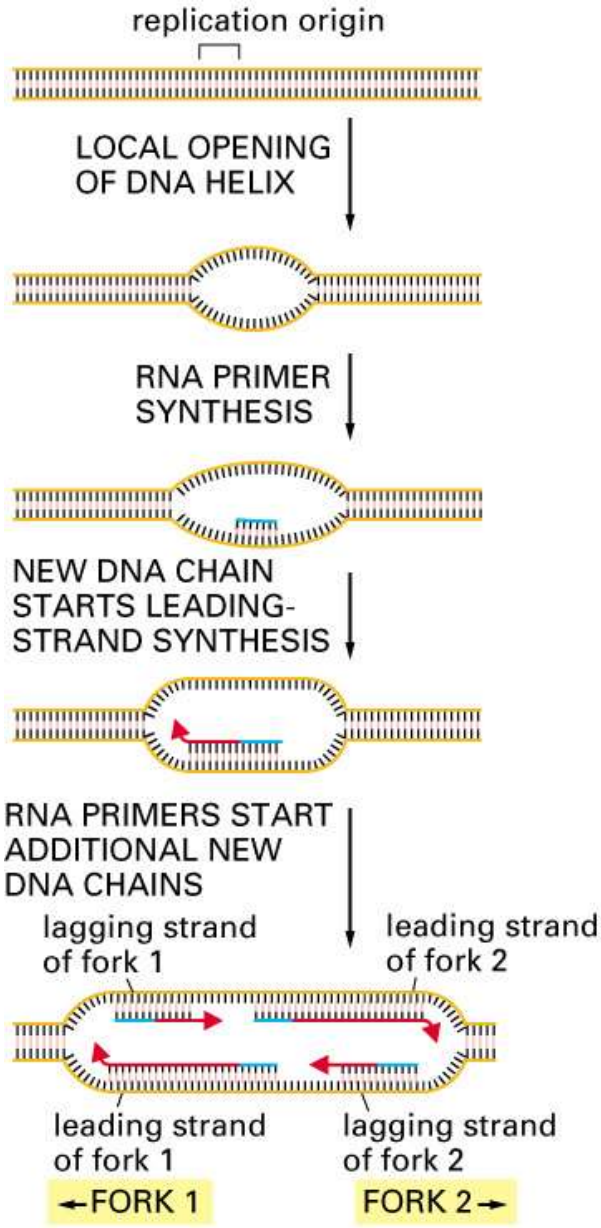


Figure 5–29. Molecular Biology of the Cell, 4th Edition.

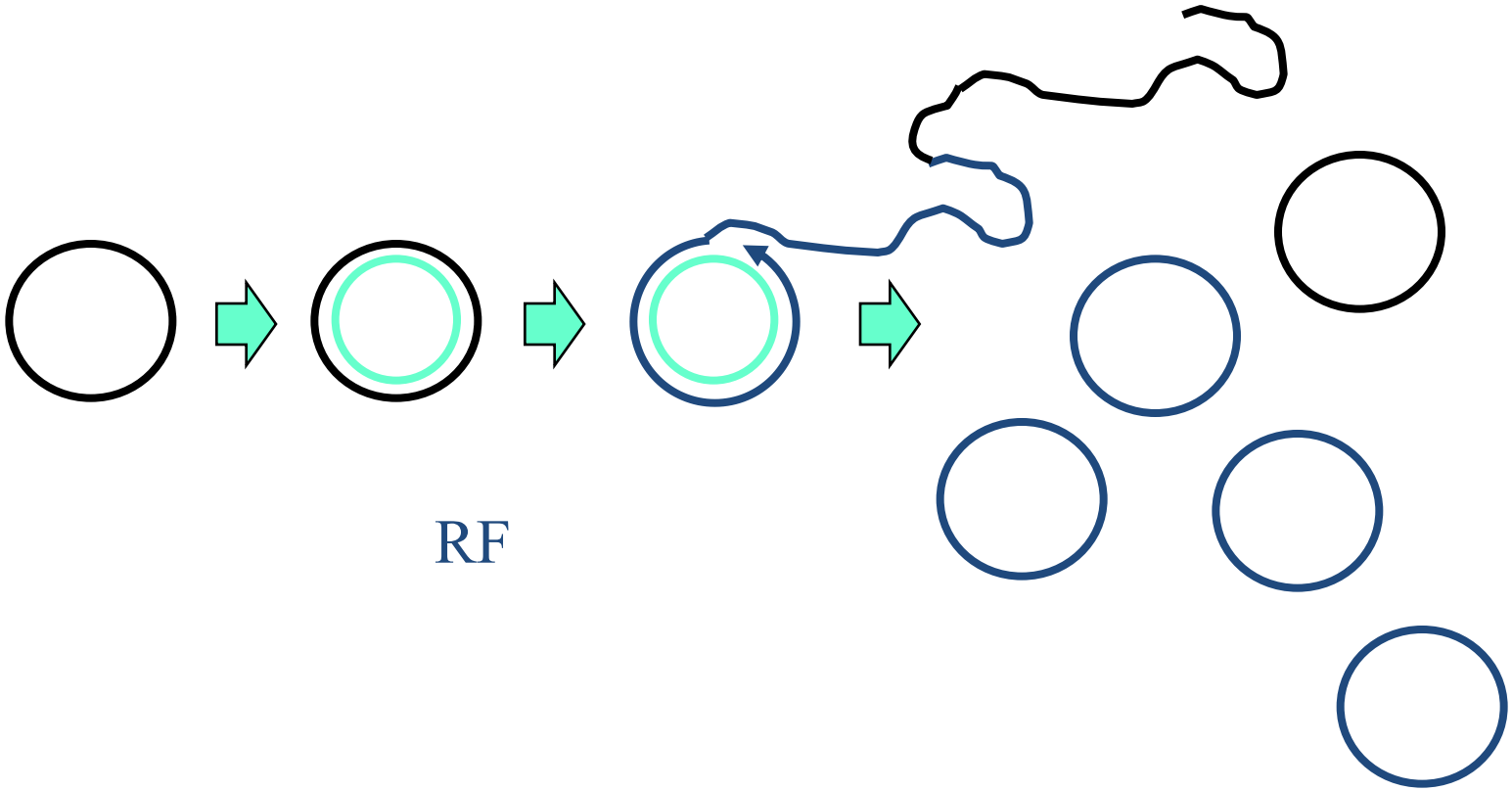
Rolling circle replication

FC174 phage: each strand synthesized separately
(unidirectional replication fork)

OriC origin of bacterial chromosomal replication

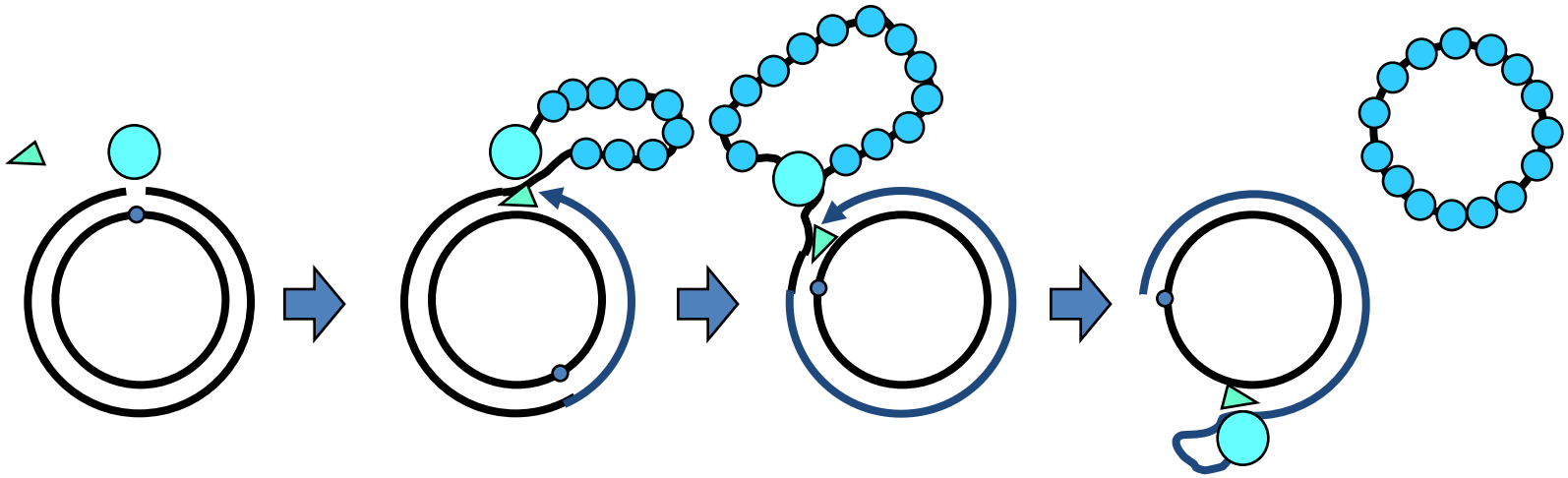
bidirectional

ΦX174 phage as a simple model for replication



Rolling circle replication is a model for leading strand synthesis.

ΦX174 as a simple model for replication



gene A protein

SSB

cooperative

Rep helicase .

pol III