In Name of the GOD

DNA structure and replication

By Dr. S. ABBASI

Course outlines

- 1- Proof that DNA Carries the Genetic Information and DNA structure
- 2- DNA replication in Prokaryotic – Initiation
- 3- DNA replication – Elongation and Termination
- 4- DNA replication in Eukaryotic
- 5- DNA Mutation and Repair
- 6- PCR Technique and Applications
- 7- Mid-Term Exam
- 8- Transcription in Prokaryotic - Initiation
- 9- Transcription – Elongation and Termination
- 10-Transcription in Eukaryotic – Initiation
- 11-Transcription – Elongation and Termination
- 12-Post-Transcriptional Modification
- 13-DNA Translation in Prokaryotic
- 14-DNA Translation in Eukaryotic
- 15-Cell Organelles
- 16-Gene cloning Technology

- هدف كلي
- شناخت كافي از ساختمان و عملكرد مولكولي سلول

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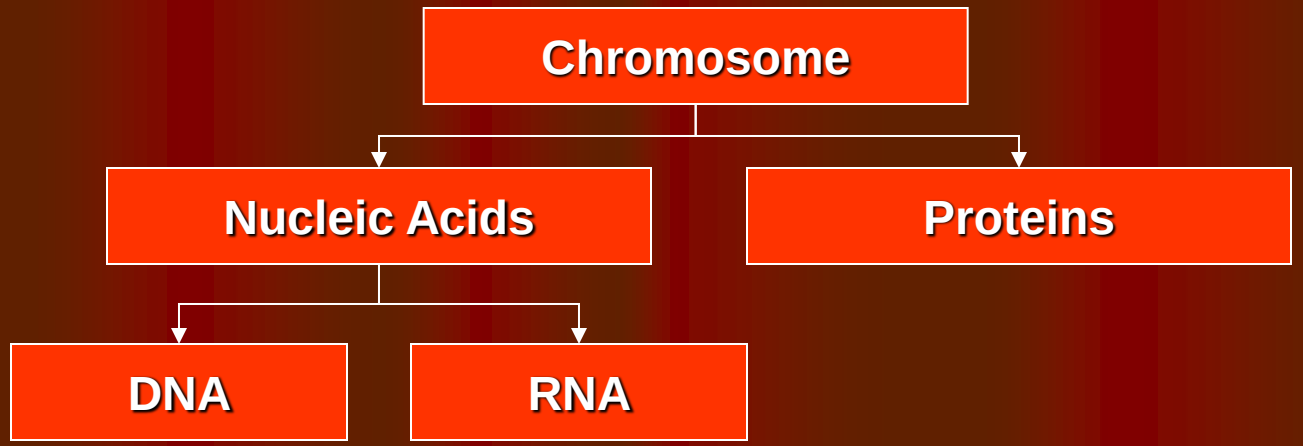
questions

- What is Phenotype and what is Genotype?

- What is prokaryotic cells? And Eukaryotic cells?

Function of the genetic material

- **1-The genotypic function, replication –** store genetic information and transmit the information from parents to offspring accurately
- **2-The phenotypic function, gene expression –** genetic material dictate the growth and differentiation of the organism
- **3-The evolutionary function, mutation –** undergo change so that organisms can adapt modifications in the environment



Discovery of Transformation in Bacteria

The Griffith's experiment with pneumococci

- **Experiment**

(1) Injecting Type IIIS pneumococci into mice

(2) Injecting heat-killed Type IIIS pneumococci into mice

(3) Injecting Type IIR pneumococci into mice

(4) Injecting heat-killed Type IIIS pneumococci plus live Type IIR pneumococci into mice

Findings

(1) In 2 & 3 None of the mice dead

(2) **Live Type IIIS** were recovered from carcasses

Discovery

Type IIIS phenotype of the transformed was passed on to progeny cells

And It was due to permanent inherited change in the genotype of the cells

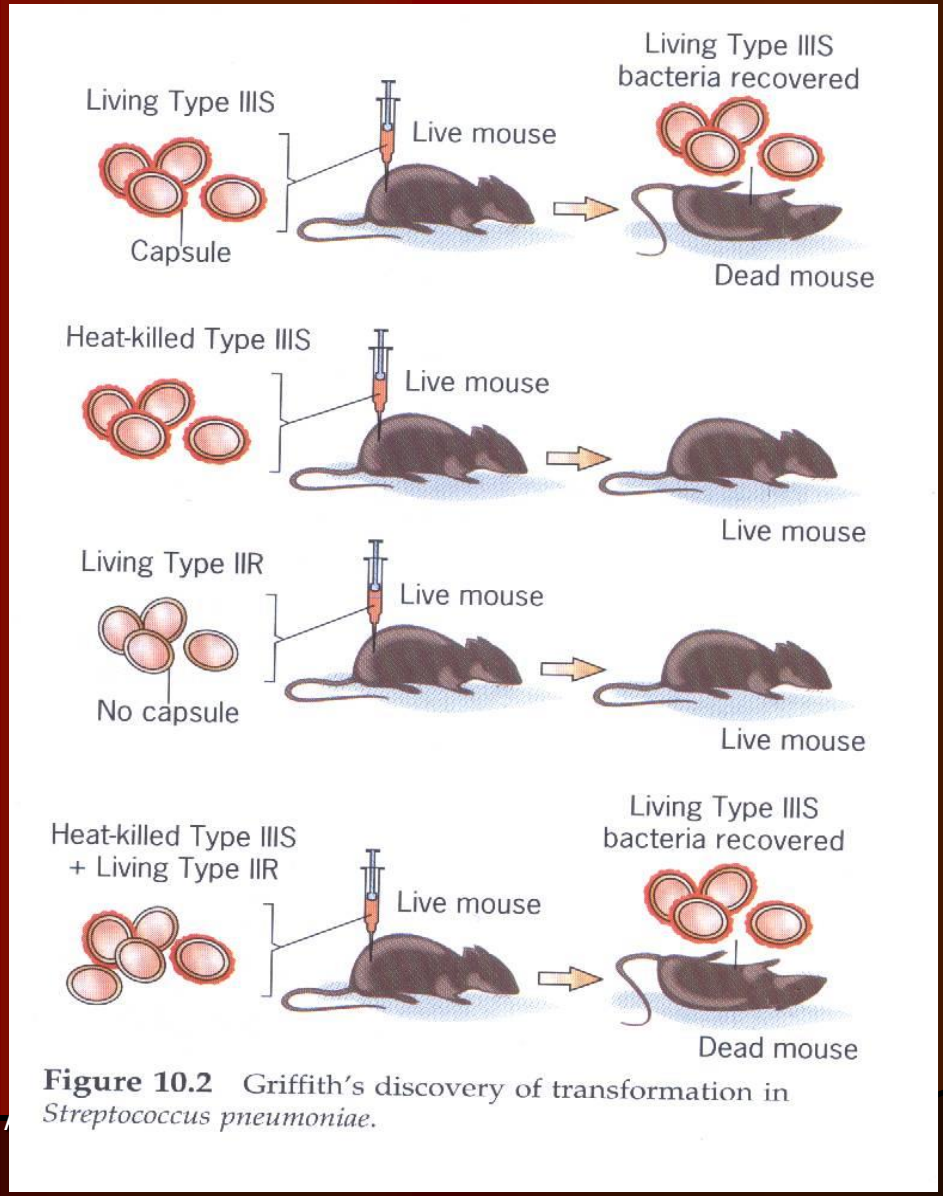


Figure 10.2 Griffith's discovery of transformation in *Streptococcus pneumoniae*.

Proof that DNA Mediates Transformation

The Avery, McLeod and McCarty's experiment

- **Experiment**

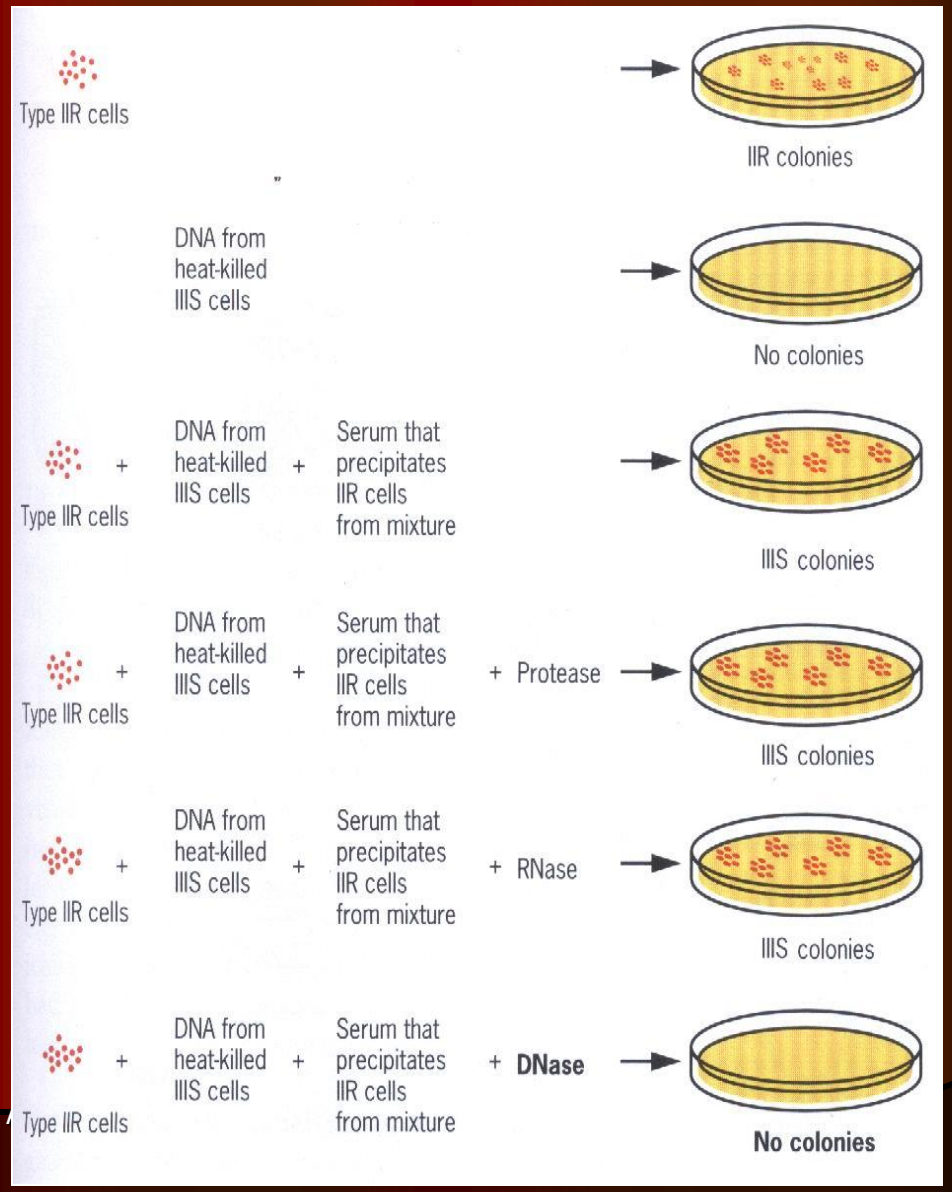
  Highly purified DNA from Type IIIS cells was treated with DNase, RNase and proteases. DNA was then tested for its ability to transform Type IIR to IIIS

- **Findings**

  Only DNase treatment has any effect on the transforming activity of the DNA preparation

- **Discovery**

  Genetic material is DNA rather than protein or RNA



Proof that DNA Carries the Genetic Information in Bacteriophage T2

The Hershey and Chase's experiment

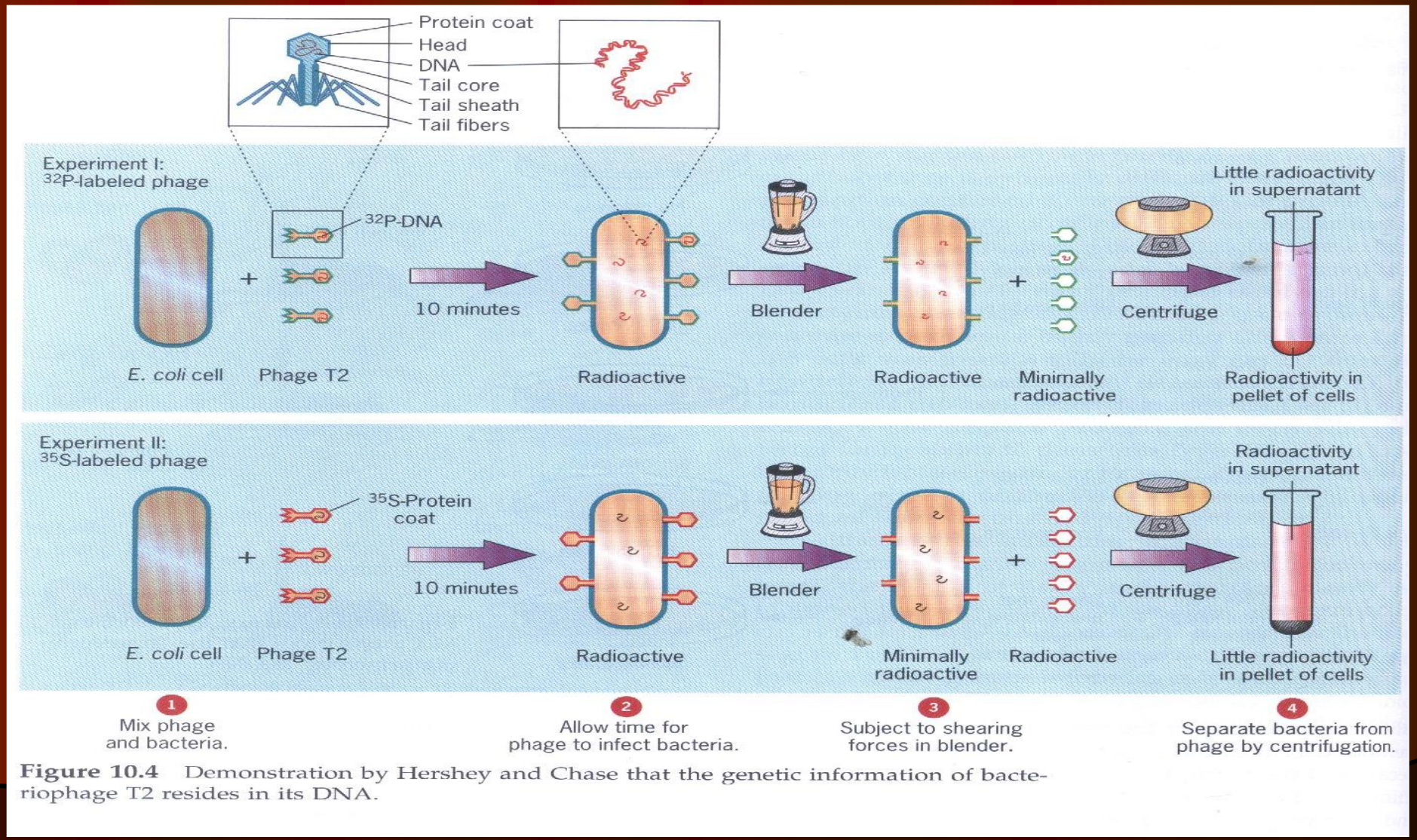


Figure 10.4 Demonstration by Hershey and Chase that the genetic information of bacteriophage T2 resides in its DNA.

Proof that DNA Carries the Genetic Information in Bacteriophage T2

The Hershey and Chase's experiment

- **Experiment**

(1) 32P labeled T2 phage were mixed with *E. coli* cells → shearing → centrifugation

(2) ^{35}S labeled T2 phage were mixed with *E. coli* cells → shearing → centrifugation

Findings

DNA of the virus enters the host cell, whereas the protein coat remains outside the cell

- **Discovery**

Genetic material of a particular bacterial virus was present in DNA

Nucleic Acids

- Two types of nucleic acids – DNA and RNA
- Composed of repeating subunits - nucleotides
- Each nucleotide is composed of
  - A phosphate group
  - A five-carbon sugar (pentose)
  - A cyclic nitrogen-containing compound (base)
- Two type of pentose – deoxyribonucleic acid and ribonucleic acid
- Five type of bases – adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U)
- Double-ring bases – A and G → purines
- Single-ring bases – C, T and U → pyrimidines

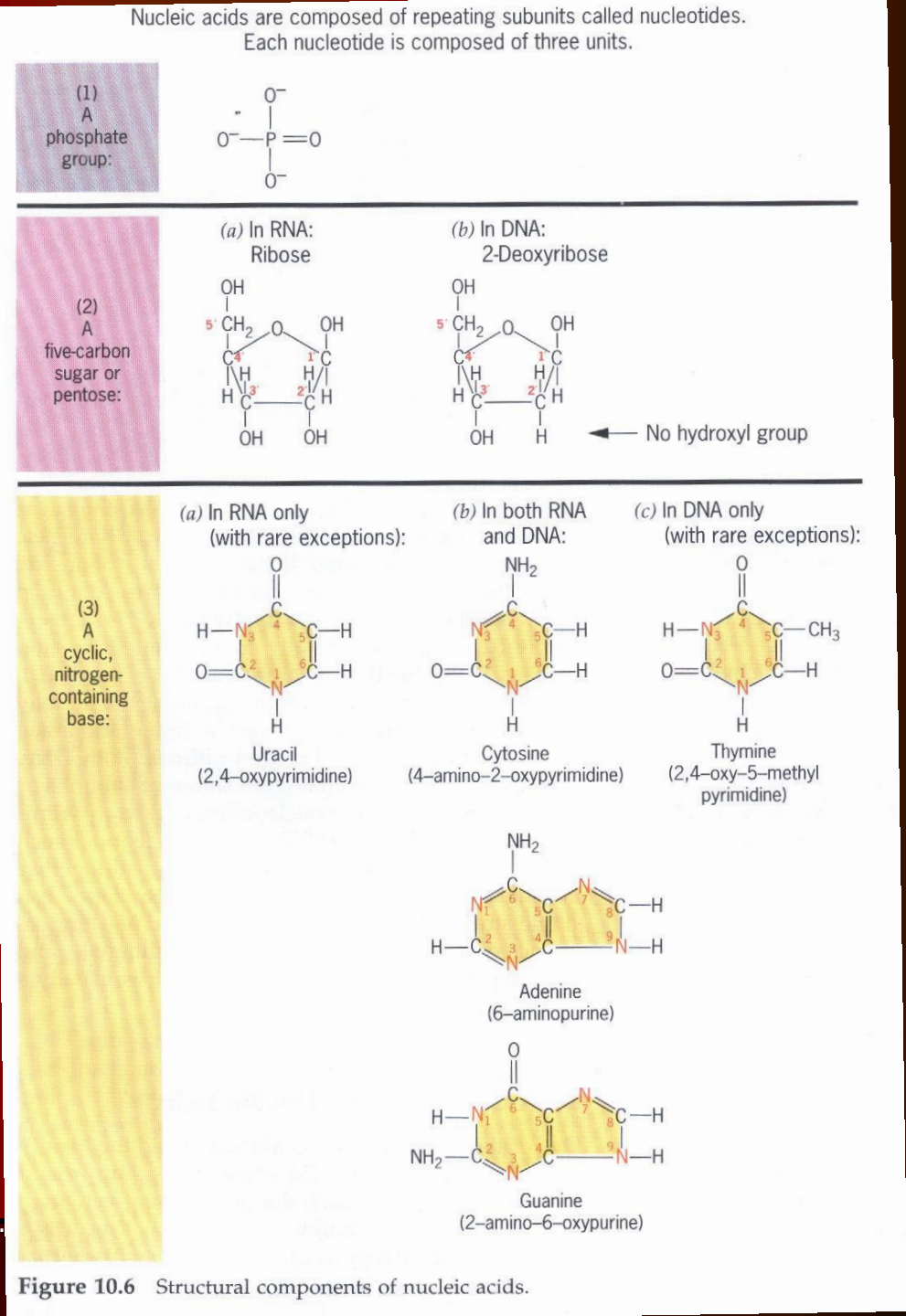
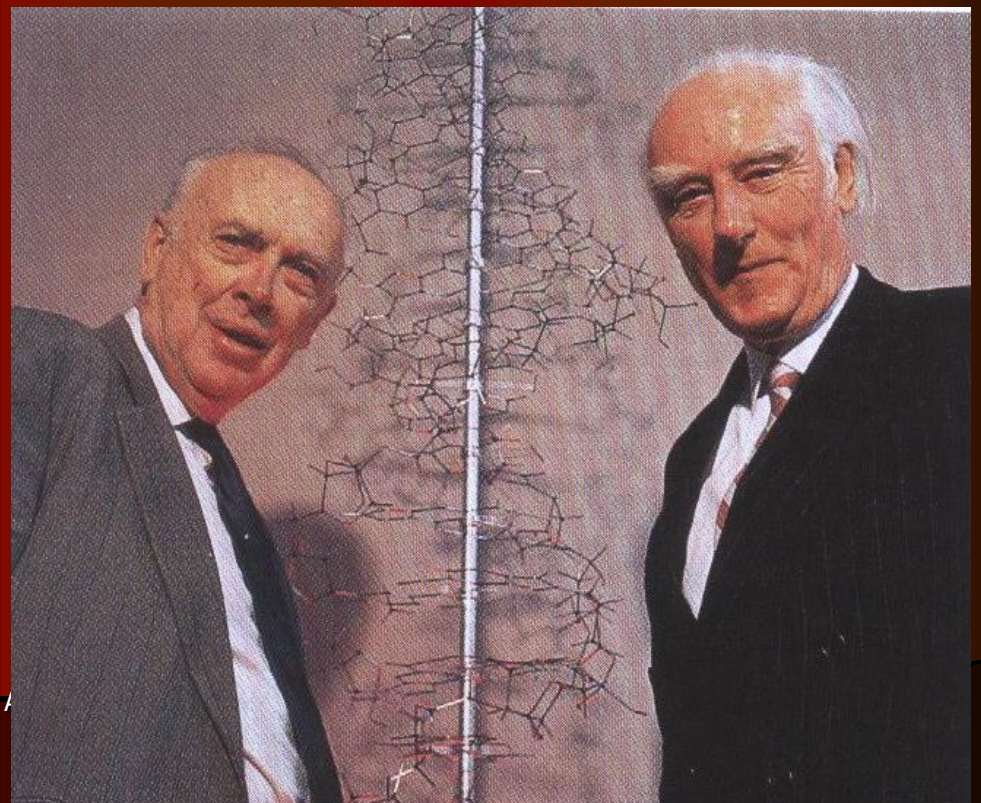


Figure 10.6 Structural components of nucleic acids.

DNA structure

- Discovered by Watson and Crick (1953)
- 1-Right handed double helix
- 2-Two polynucleotide chains are coiled about one another in a spiral
- 3-Polynucleotide consists of nucleotides linked together by phosphodiester bonds
- 4-Two polynucleotide strands are held together in helical form by hydrogen bonding (HB)
- 5-Specific base-pairing. A with T (2 HB) and G with C (3 HB).
- 6- with Major and Miner Groove



DNA structure

Structures of the four common deoxyribonucleotides present in DNA

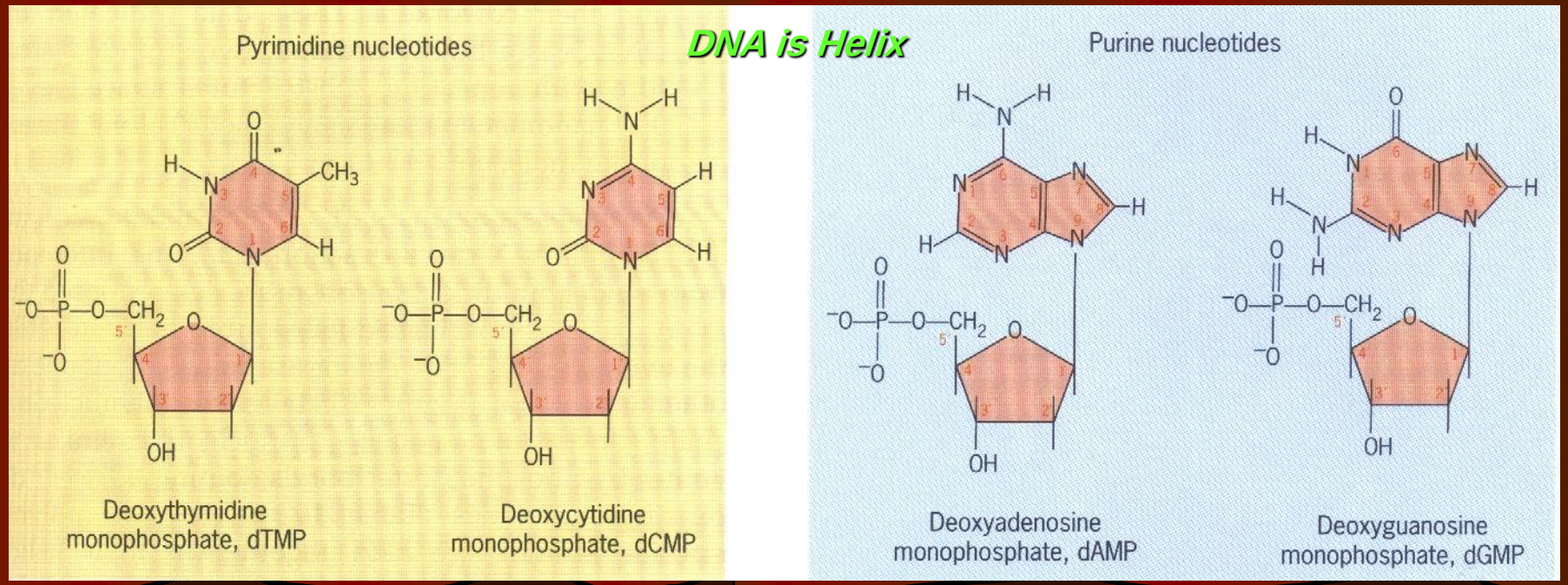
1- CHARGAFF's Base Ratio 1945-1956 by chromatography

A=T, C=G

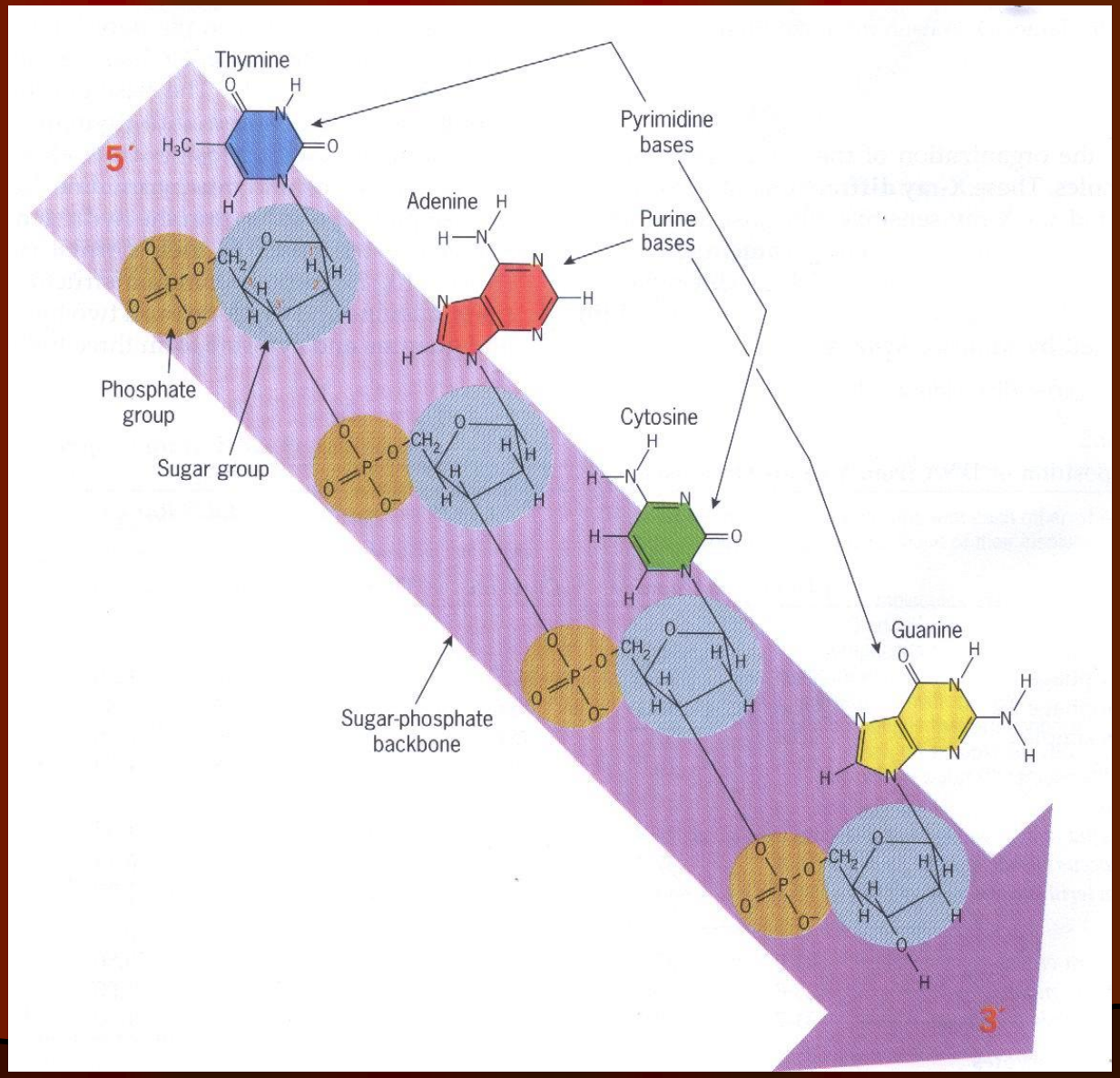
(A+G)=(T+C)

2-Rosalind Franklin 1953 by x-ray diffraction pattern

DNA is Helix

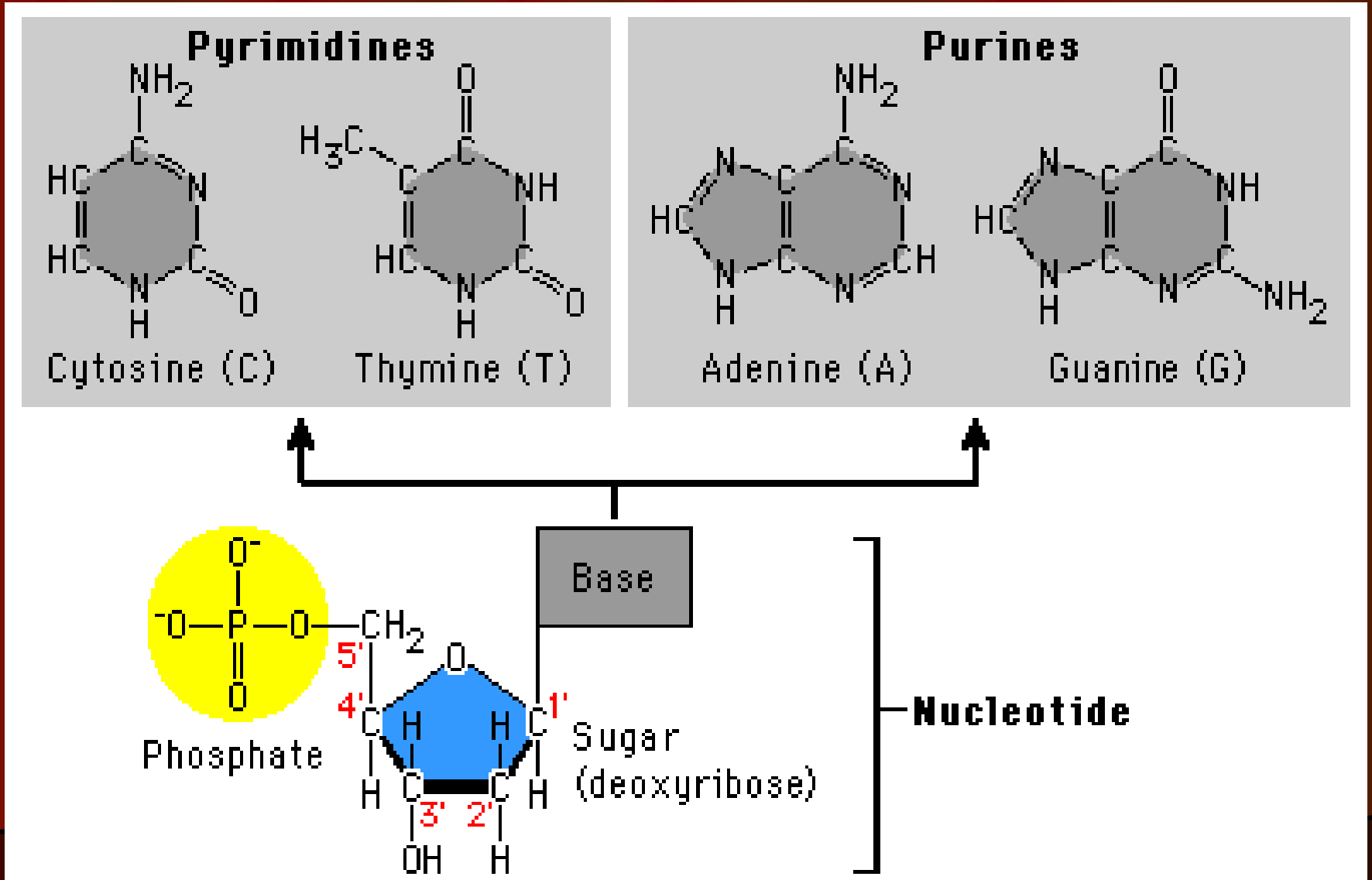


DNA structure



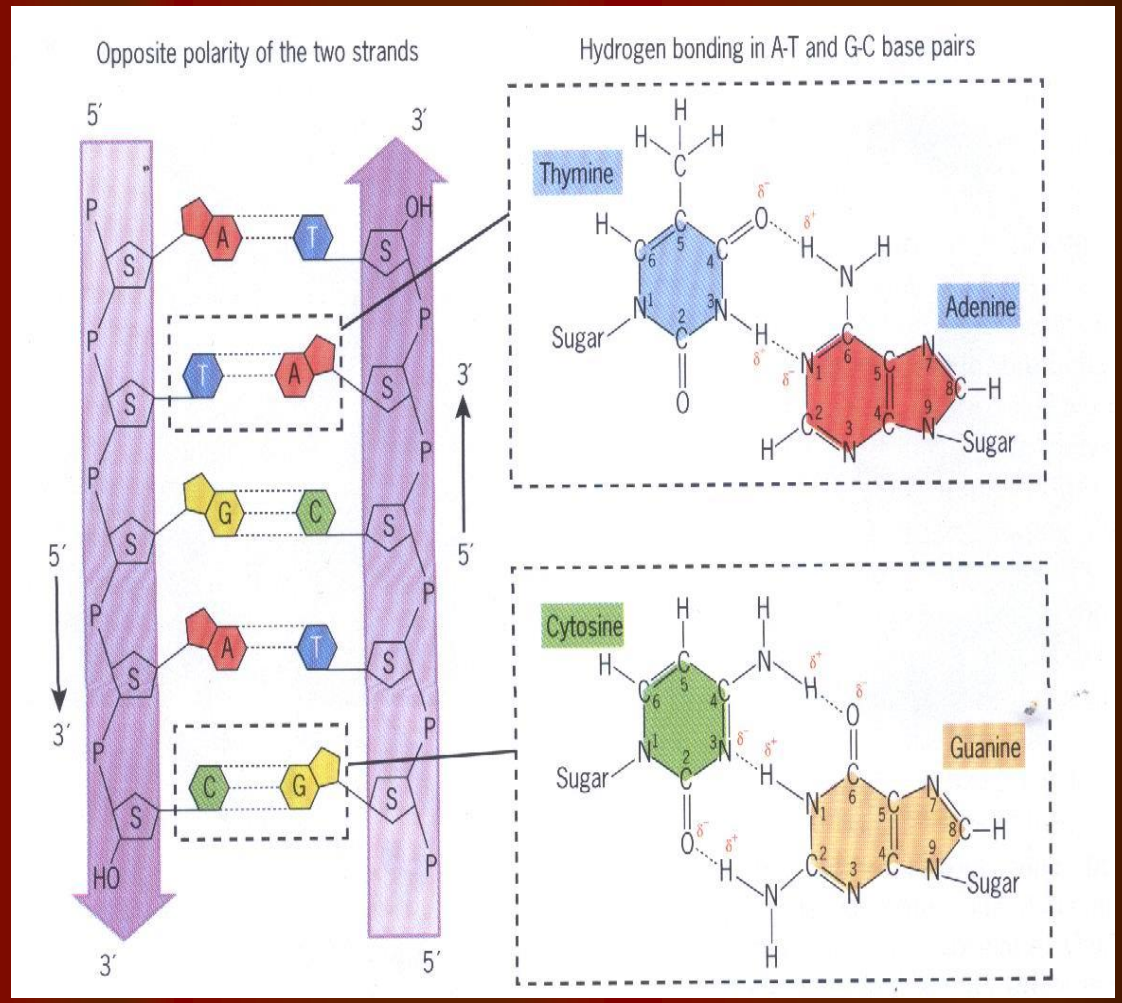
Formation of a polynucleotide chain by joining nucleotides with phosphodiester linkages

Nucleotide structure



DNA structure

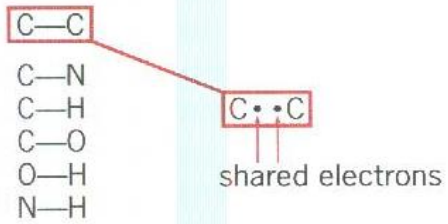
- 7-The **complementarity** of two strands of double helix makes DNA uniquely suited to store and transmit genetic information from generation to generation
- 8-Base pairs in DNA are stacked about 0.34nm apart
- 9-10 base per turn (360º) of double helix
- 10-The sugar-phosphate backbones of the two complementary strands are **antiparallel.** One strand go from **5' → 3'** and the other from **3' → 5'**
- 11-DNA stability count on the large number of hydrogen bonds between base pairs



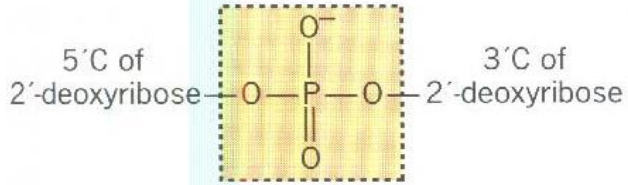
DNA structure

Chemical bonds important in DNA structure

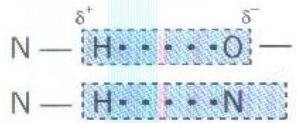
(a) *Covalent bonds*
Strong chemical bonds formed by sharing of electrons between atoms.
(1) In bases and sugars



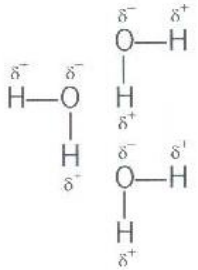
(2) In phosphodiester linkages



(b) *Hydrogen bonds*
A weak bond between an electronegative atom and a hydrogen atom (electropositive) that is covalently linked to a second electronegative atom.

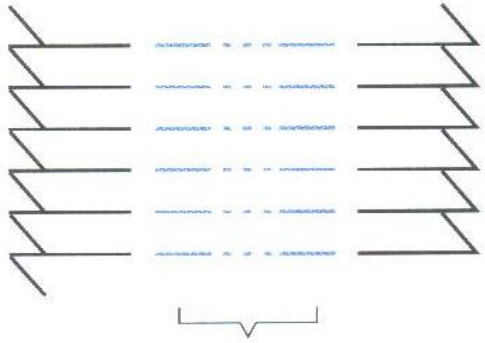


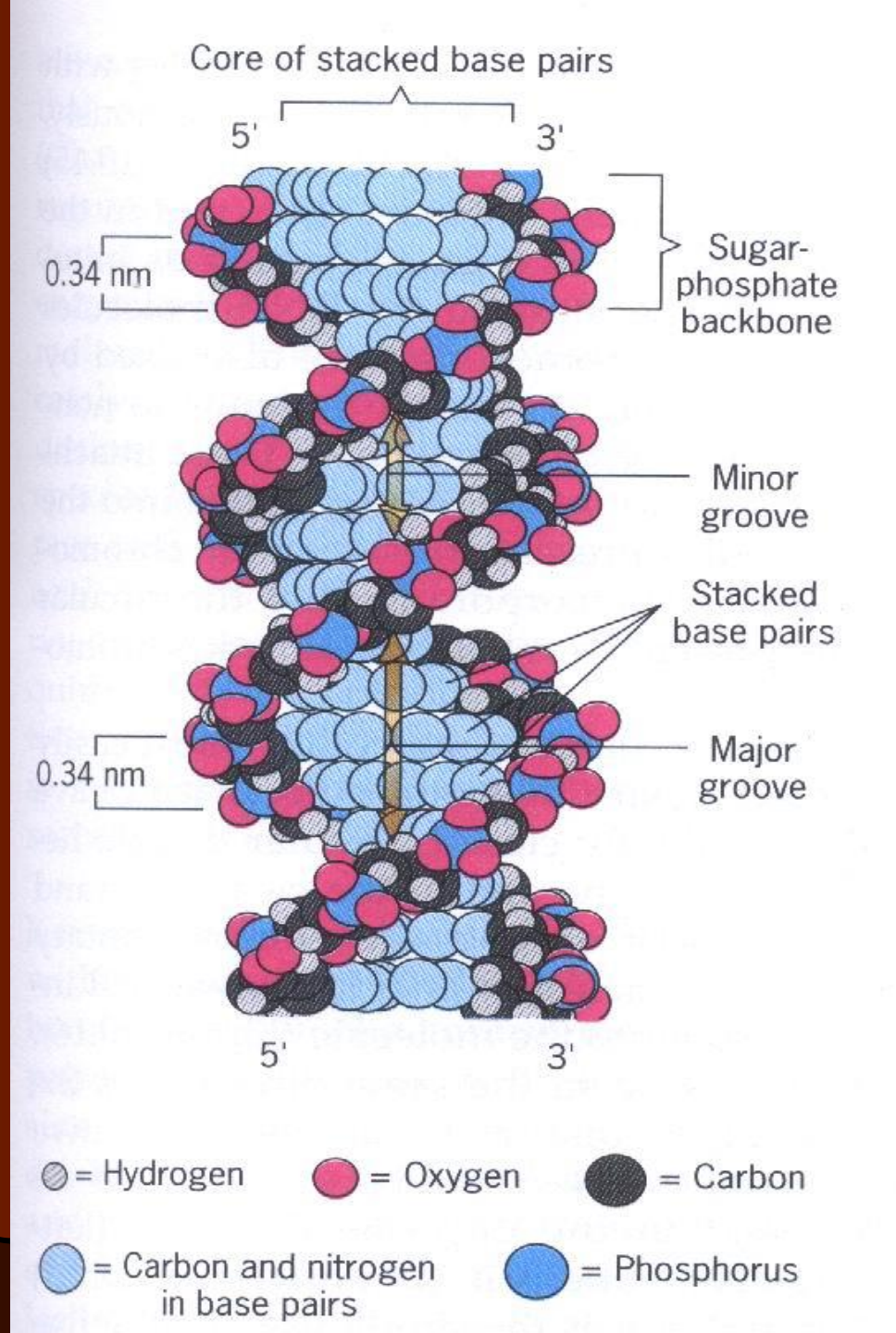
(c) *Hydrophobic "bonds"*
The association of nonpolar groups with each other when present in aqueous solutions because of their insolubility in water.

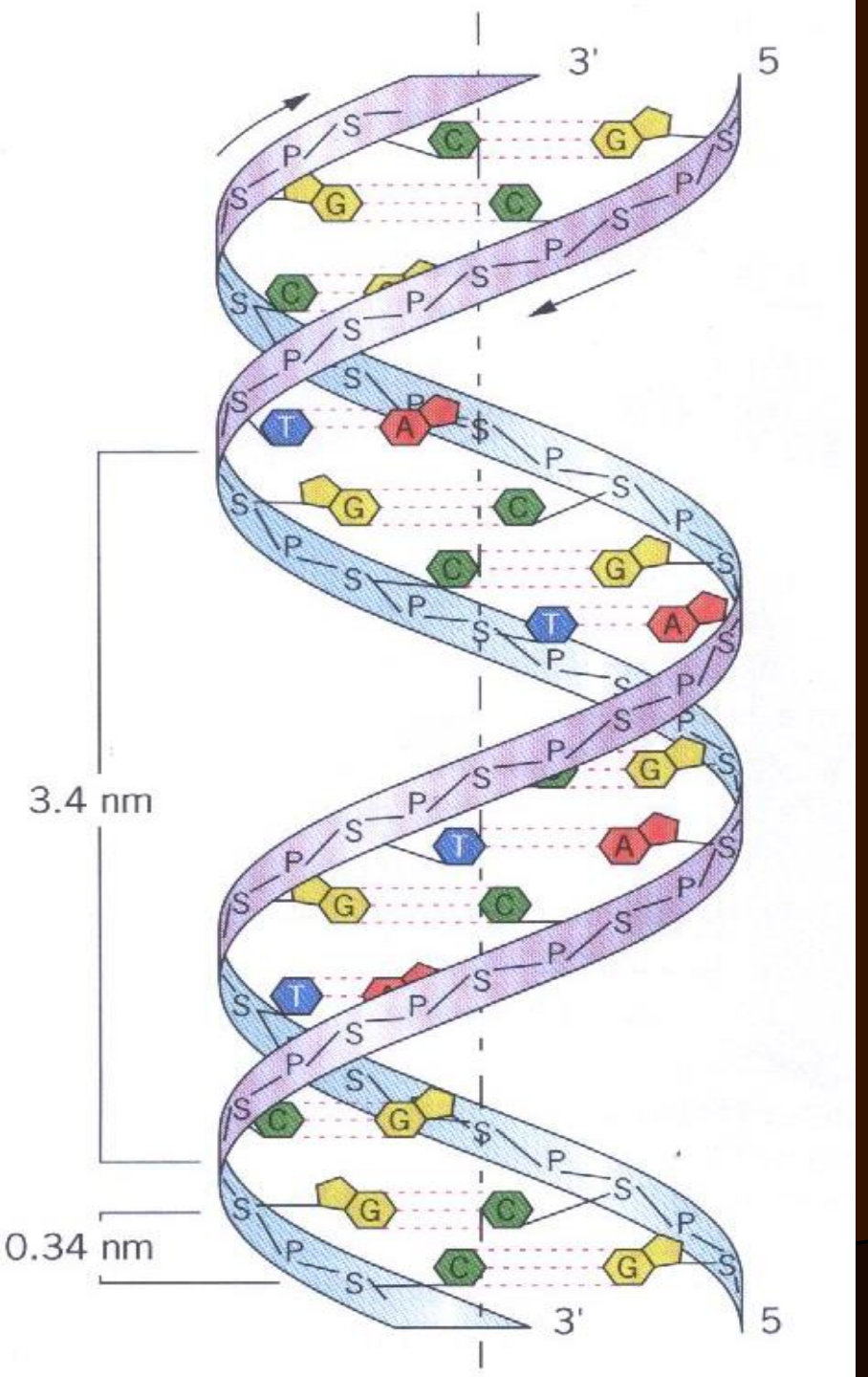


Water molecules are very polar (δ^- O and δ^+ Hs)
Compounds that are similarly polar are very soluble in water ("hydrophilic").
Compounds that are nonpolar (no charged groups) are very insoluble in water ("hydrophobic").

The stacked base pairs provide a hydrophobic core.

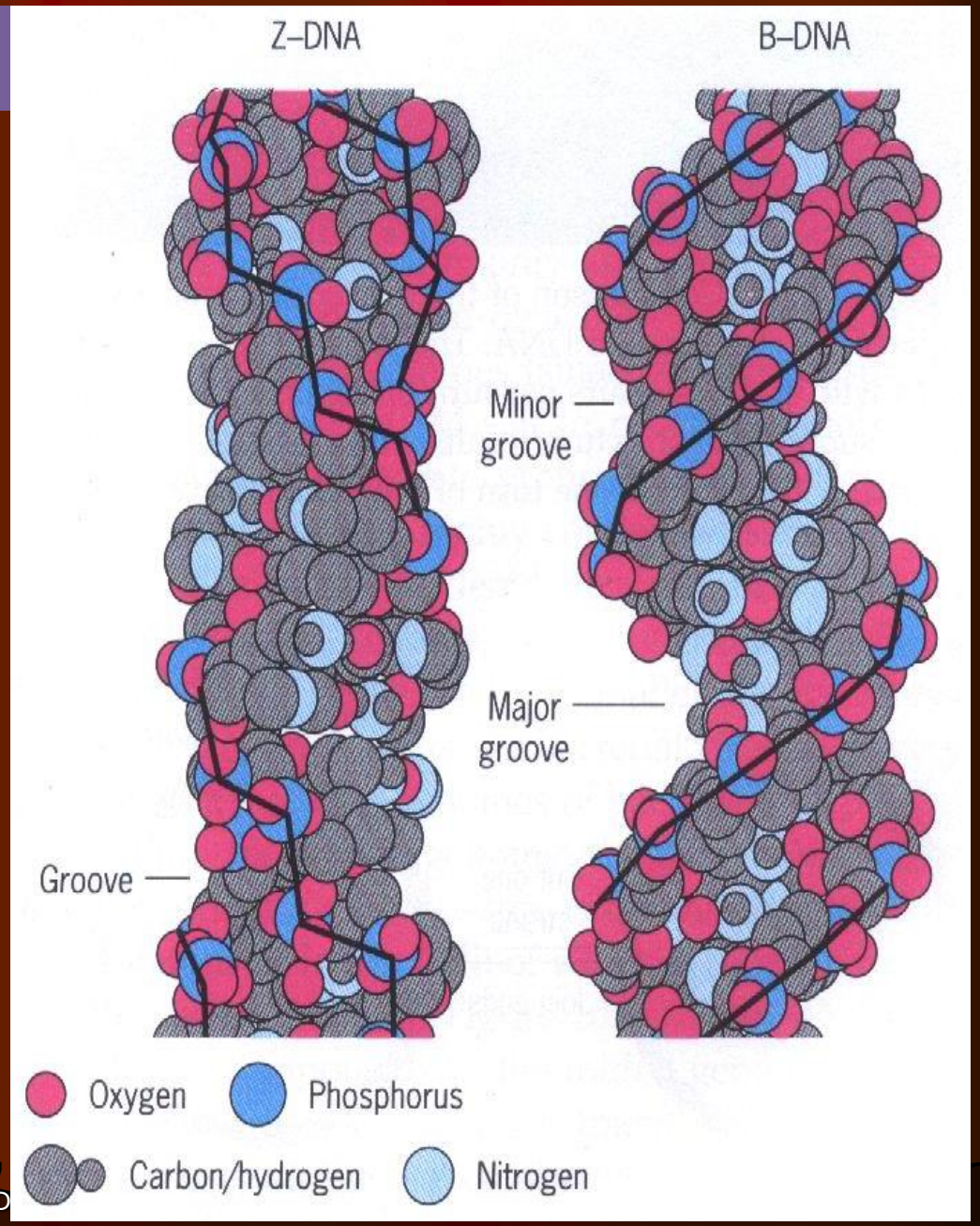



Core of stacked base pairs
5'
3'
0.34 nm
Sugar-phosphate backbone
Minor groove
Stacked base pairs
Major groove
0.34 nm
5'
3'
= Hydrogen
= Oxygen
= Carbon
= Carbon and nitrogen in base pairs
= Phosphorus


3'
5
3.4 nm
0.34 nm
3'
5

DNA structure: Alternate form

- Three forms of DNA structure
  - A-DNA
  - B-DNA
  - Z-DNA
- A-DNA – in high concentration of salts or in a partially dehydrated state
- B-DNA – under physiological conditions (in the aqueous protoplasm of living cells with low concentration of salts)
- Z-DNA – discovered by x-ray diffraction analysis of crystals form by DNA oligomers (existence in living cells is not proven)



DNA structure: Alternate form

Alternate forms of DNA

Helix Form	*Helix Direction*	*Base Pairs per Turn*	*Helix Diameter*
A	Right-handed	11	2.3 nm
B	Right-handed	10	1.9 nm
Z	Left-handed	12	1.8 nm

DNA structure: Negative Supercoils *In vivo*

- **Supercoils** are introduced into a DNA molecule when one or both strands are cleaved and when the complementary strand at one end are rotated or twisted around each other with the other end held fixed in space
- Supercoils are introduced into and removed from DNA molecules by enzymes that play essential roles in **DNA replication**

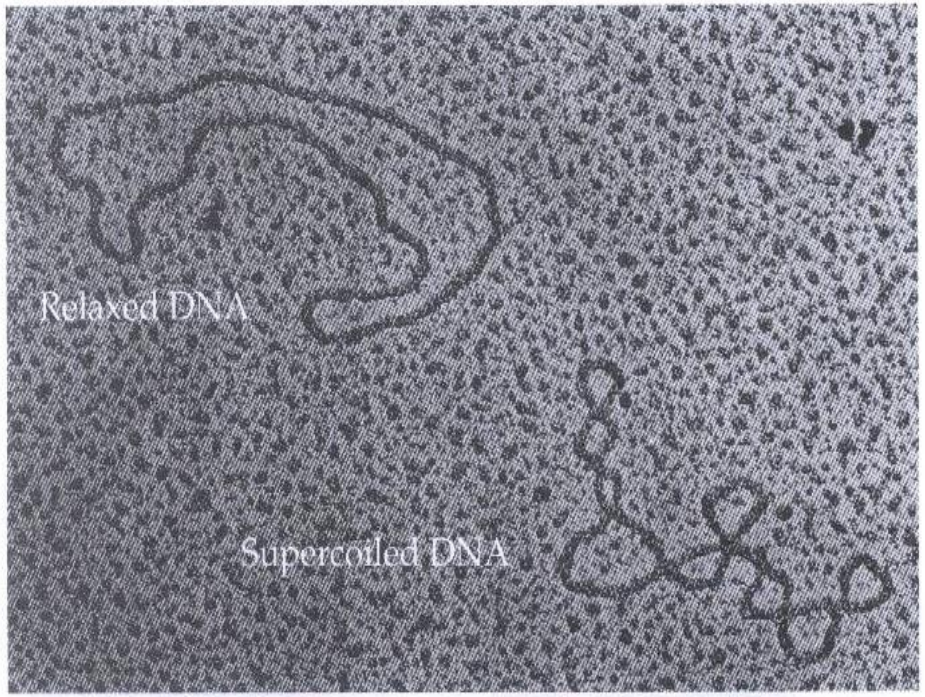


Figure 10.15 Comparison of the relaxed and negatively supercoiled structures of DNA. The relaxed structure is B-DNA with 10.4 base pairs per turn of the helix. The negatively supercoiled structure results when B-DNA is underwound, with less than one turn of the helix for every 10.4 base pairs.

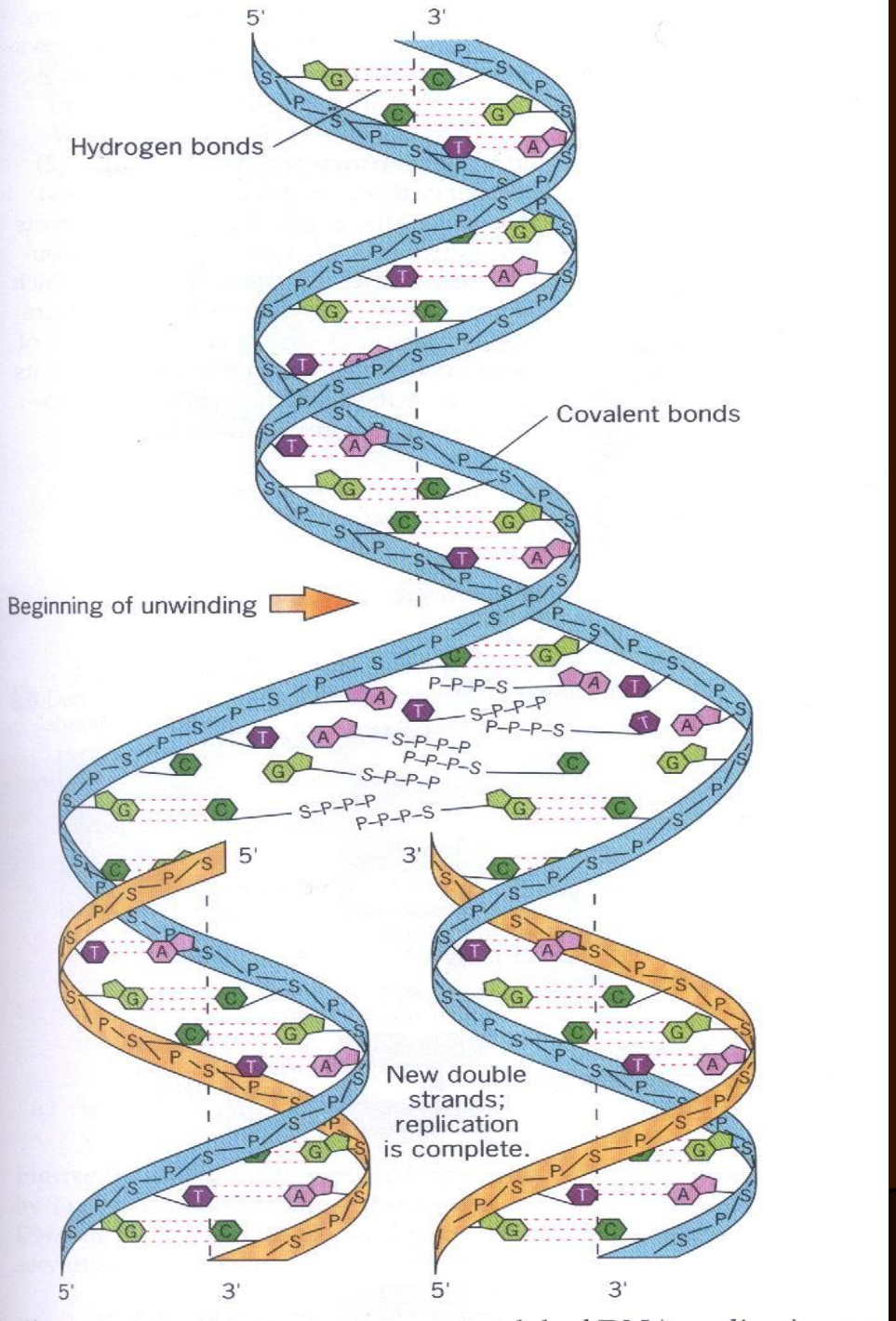
Basic features of DNA replication

- In human – DNA synthesis at 3,000 nucleotides per minute
- In bacteria – DNA synthesis at 30,000 nucleotides per minute
- Fidelity of DNA replication – 1 mistake per billion nucleotides incorporated
- DNA synthesis involves three steps
  - Chain initiation
  - Chain extension or elongation
  - Chain termination

Three characteristics for replication:
1-semi-concervative
2-semi-continouce
3-bidirectional

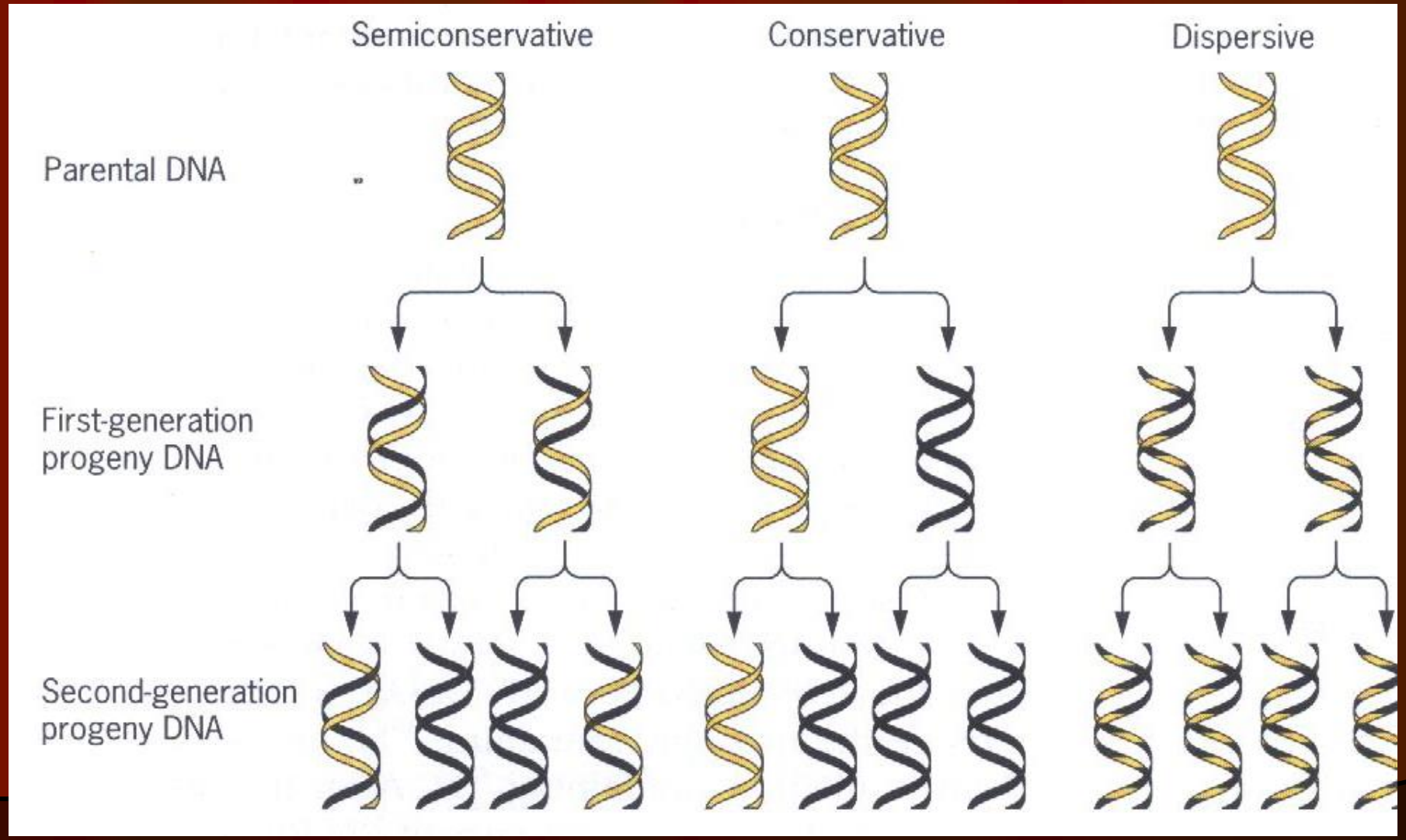
Semiconservative Replication

- In semi conservative replication, each of the parental strands is conserved and serves as a template for the synthesis of the new complementary strand
- The base sequence in each progeny strand is determined by the hydrogen-bonding potentials of the bases in the parental strand



Semiconservative Replication

Meselson and Stahl's hypothesis



Semiconservative Replication

Meselson and Stahl's experiment

- **Experiment**

  Grew E. coli cells for many generations in a medium in which the heavy isotope of ^{15}N, had been substituted for the normal, light isotope, ^{14}N.

  Proposed three mechanisms of replication – **semi-conservative, conservative or dispersive**

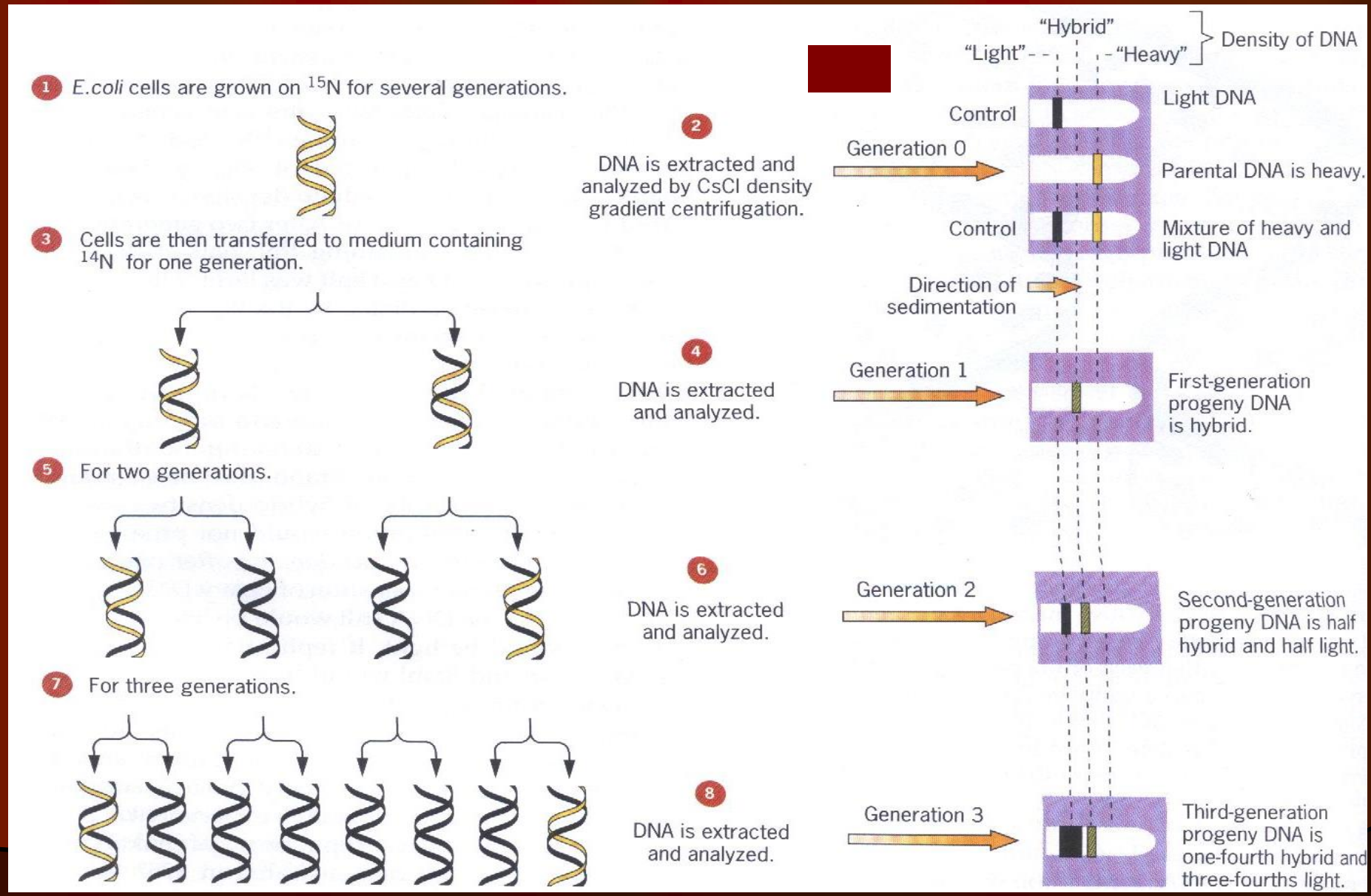
- **Findings**

  Refer to following diagram

- **Discovery**

  Semi-conservative DNA replication

Semiconservative Replication



GENOME

Prokaryotic (E.Coli)

a-DNA (+ Proteins such as Hu 9000d,HLP_1,H,H_1)

- 4639 Kb=2400 genes (1897 coding for known proteins + 379 unknown proteins+21 rRNA + 84 tRNA
- 1/3 cell vol. called NUCLEOID
- Super-coiled

b- PLASMIDs(1-25Kb)

1-Fertility P.
2-Resistance p.
3-Col P. (produces colicine and kills the others ei. E.Col_1)
4-Degradative P.(producing unusual products ; salicylic, toluene,ei.Tol P)
5-Virolence P. (Ti P.)

Eukaryotics (Saccharomyces cervisiae)

12520Kb=16Chromosomes
Protein Coding Genes 5800

rRNA	120-130
tRNA	262
Other RNA	37

Conserved sequences in Yeast Centromeric DNA (125bp)
BOXI 5'-PuTCACPuTG-3'
AT-rich region (90%AT)
BOXII 5'-TGNTTTCCGAAA-3'
In human 170-171 nu =ALPHOID sequence with CENP-B box
PyTTCGTTGGAAPuCGGGA
Kinetochore(5x171nu+5xproteins called CENP-B Proteins)
TELOMERES
5'AGGGTT-3' $+TRF_1$

- Differences:
- 1- larger
- 2-in nucleus
- 3-small and large tandom repeats
- 4-DNA divided into different sizes
- 5-# and size of chromosome is stable in one species
- 6-DNA is linear + Histon and non Histon Proteins
- 7- 2n chromosomes

- 1973 Chromatin + endonuclease =200X length
- 1975 R. Kornberg : chromomere or beads structure

In human 46 chromosome double stranded

Nucleosome

1- chromatosome

A- core

I. Histons 2x (H4=102aa, H3=135aa, H2A=121-148aa, H2B=129-156aa)

II. Non-histons :a-nucleoplasm in attached to H2A, H2B.

b- N1 protein attached to H3, H4)

III. 146 bp with 1.8 rounds

B-accessory

I. 10 bp from each sides

2. H1 Histon(H5 , H0 in birds and are not in yeast and protistas)

2-linker

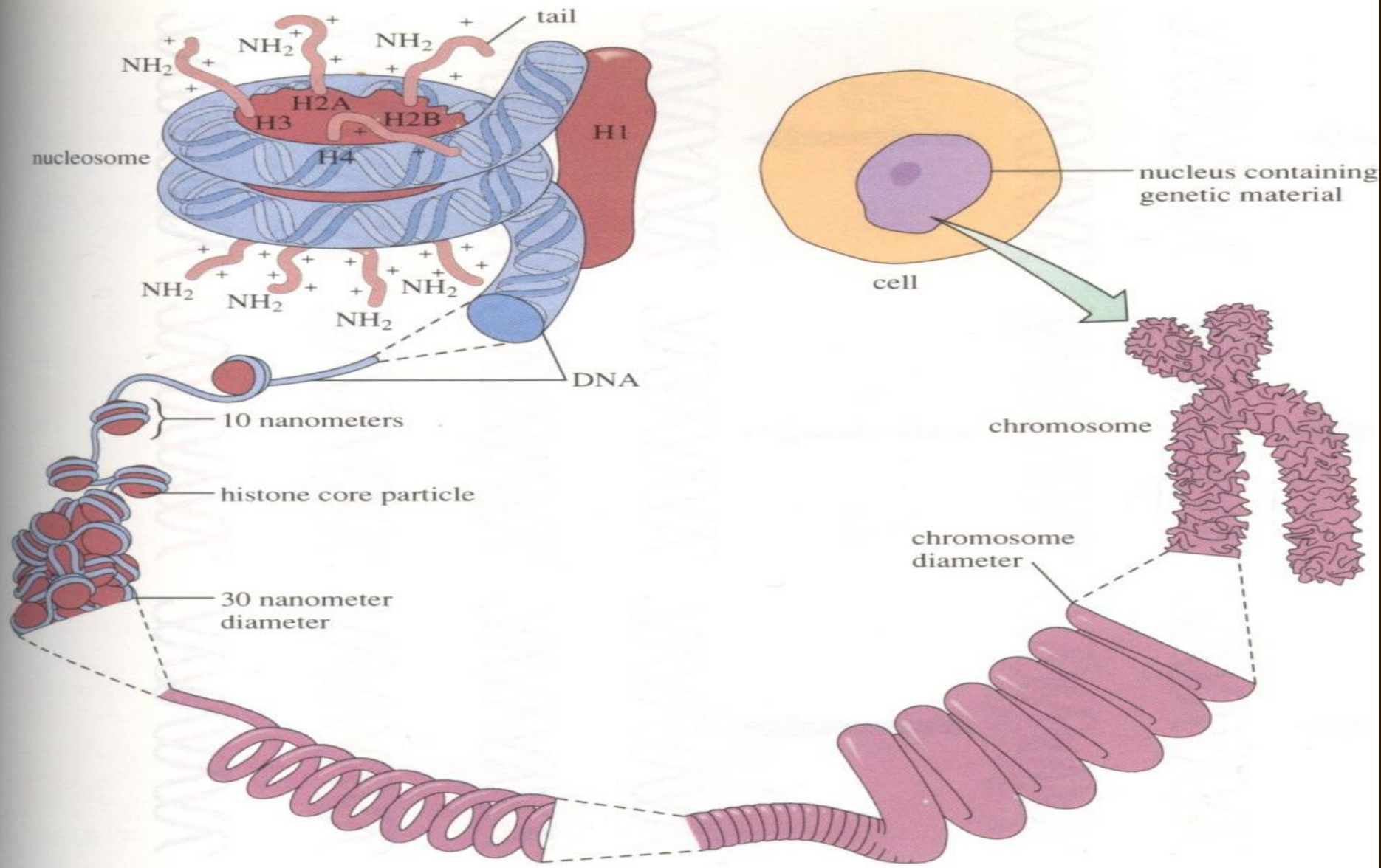


Figure 7.11

One hundred forty-six nucleotides of DNA entwine in two loops around an octomer of histone proteins, forming a nucleosome. Nucleosomes are in turn wound into a cable three times thicker than an individual nucleosome. The DNA and associated histone proteins form chromatin, which comprise chromosomes. When DNA is transcribed, it becomes unwound from its protein support.

Origin of replication (Prokaryotes)

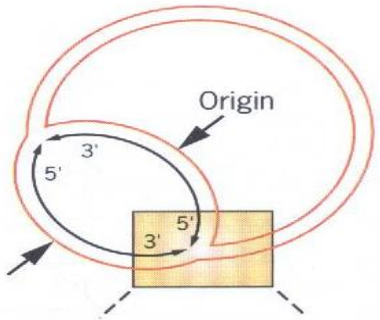
Called Ori C

- **1- Methylation in GA*TC at N^6 A by DAM Methylase (enzyme)10 mins after replication finished**
- **2- Consensus sequences**

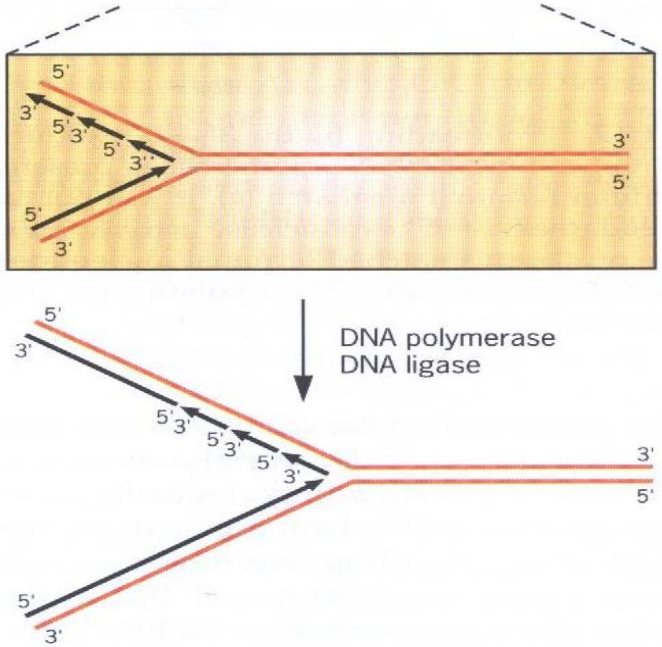
13 mer GA*TCTNTTNTTTT (left)

9 mer TTATNCANA (right)

Total of 245bp and AT-rich



(a) Relatively low-resolution techniques such as autoradiography
electron microscopy show that at the macromolecular level b
nascent DNA chains are extended in the same overall directio
each replication fork.



(b) High-resolution biochemical techniques such as pulse-labeling
density gradient analysis show that replication of the lagging
strand is discontinuous—short fragments are synthesized in
5' → 3' direction and subsequently joined by DNA ligase.

Figure 11.18 Continuous synthesis of the leading str
and discontinuous synthesis of the lagging strand at a
DNA replication fork.

NUCLEASE

- **Breaks Phosphodiester bonds**

1-Hydrolysis Ester bonds (3’ carbon Sugar and Phosphate) ei; Phosphodiesterase

2-Hydrolysis Ester bonds (5’ carbon Sugar and Phosphate) ei; Nuclease

OR

1-Ribonuclease

2-Deoxyribonuclease

Each divided to 2 groups :a-Exonuclease

b-Endonuclease (Restriction Endonuclease)

Semiconservative DNA Replication

- Important terms
  - Replisome – complete replication apparatus present at the replication fork that carries out the semiconservative replication
  - Origin – site of initiation of replication
  - Leading strand – continuously synthesize strand
  - Lagging strand (Okazaki fragments) – discontinuosly synthesize strand
  - DNA helicase
  - Single-stranded DNA-binding (SSB) proteins
  - DNA primase
  - RNA primer
  - DNA polymerases – have an absolute requirement for a free 3'-hydroxyl – 5' → 3' synthesis
  - DNA ligase