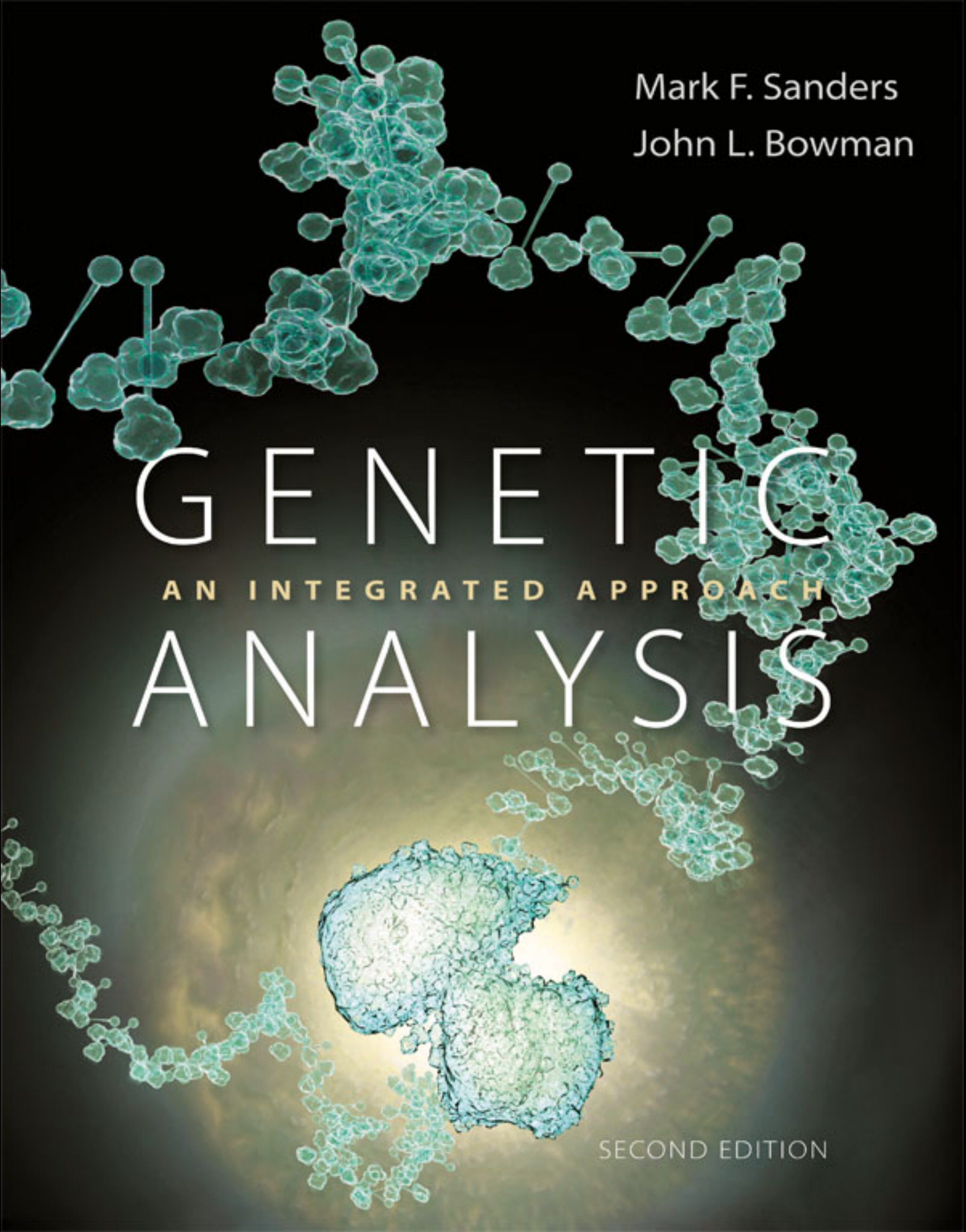


7

DNA Structure and Replication

Lectures by Dr. Tara Stoulig
Southeastern Louisiana University

7.1 DNA Is the Hereditary Molecule of Life

- Our contemporary understanding of hereditary transmission is rooted in the knowledge that **DNA IS THE HEREDITARY MOLECULE FOR ALL THE ORGANISMS**
- "hereditary molecule" means that carries and transmit the genetic information of a species
- Long before DNA role was established researchers had identified five essential characteristics of hereditary material:

Features of Hereditary Material

1. **Localized to the** nucleus and a **component of chromosomes**
2. Able to **accurately replicate itself** so that daughter cells contain the same information as parent cells
3. Present in **stable form in cells**
4. **Sufficiently complex** to contain information needed for structure, function, development, and reproduction of an organism
4. **Mutable**; undergoing a low rate of mutations that introduces genetic variation and serves as a foundation for evolutionary change

Chromosomes Contain DNA: historical background

- DNA was first noticed in **1869 by Friedrich Meischer**: isolated it (together with proteins) from nuclei of white blood cells. He called it **"nuclein"**
- In the 1870s, **microscopic studies** identified fusion of male and female nuclei during reproduction and **chromosomes were observed** soon after.
- In 1895, **Edmund Wilson** first **suggested that DNA might be the hereditary material**. He observed that sperm and eggs contribute the same number of chromosomes during reproduction. He made a connection (speculation) between the substance observed by Meischer and the chromatin of chromosomes

Rediscovery of Mendel

- In 1900, **Mendel's hereditary p**rinciples (1856) were rediscovered
- In 1903, **Walter Sutton and Theodor Boveri** independently described the parallels between chromosome partitioning into gametes and the inheritance of genes
- By 1920, DNA was identified as the principal component of nuclein and basic chemistry of DNA was determined.
- In 1923, DNA was localized to chromosomes and made a candidate for the hereditary material

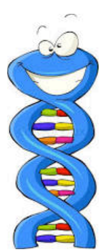
- HOWEVER……..

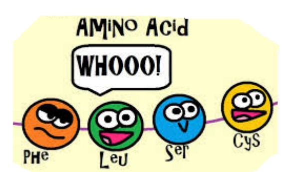
Is DNA the unique Candidate for the Hereditary Material????

NO, because:

- Proteins are also found in chromosomes
- RNA is in the nucleus and near chromosomes
- Lipids and Carbohydrates were considered to be candidates

At that time the best candidate was protein with 20 different aa!!!





The Transformation Factor by Griffith

- Frederick Griffith identified two strains of *Pneumococcus* bacteria: S, which caused fatal pneumonia in mice (pathogen), and R, which did not cause disease (not pathogen)

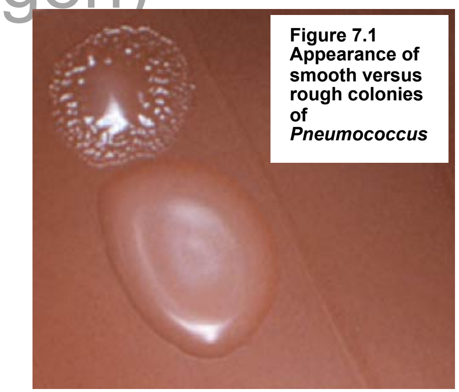
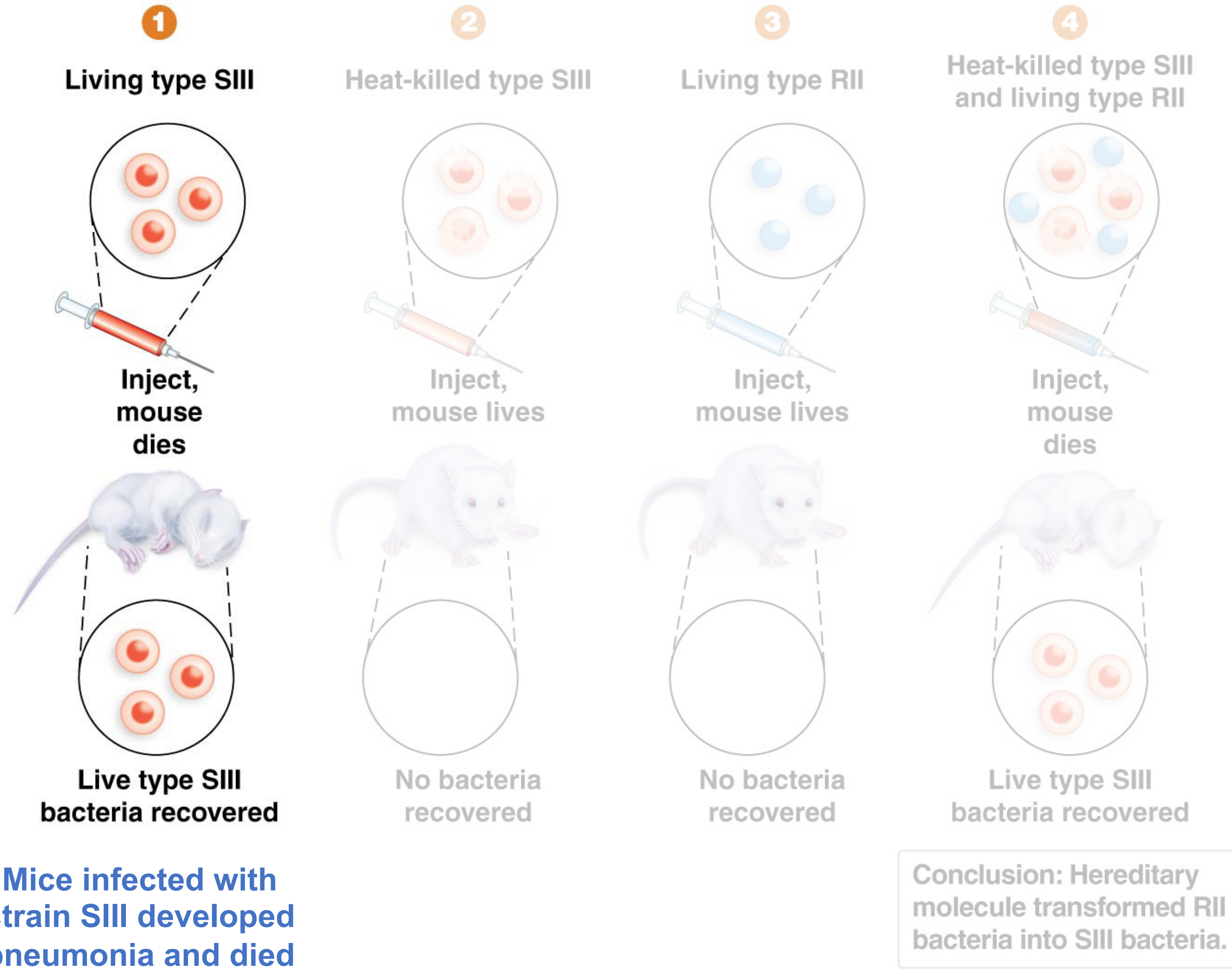


Figure 7.1 Appearance of smooth versus rough colonies of *Pneumococcus*

- A single gene mutation can convert an S (smooth) strain to an R (rough) strain *of the same antigenic type*. R is vulnerable to the immunitary mice system
- Example: ~~RII into SIII~~ SII into RII

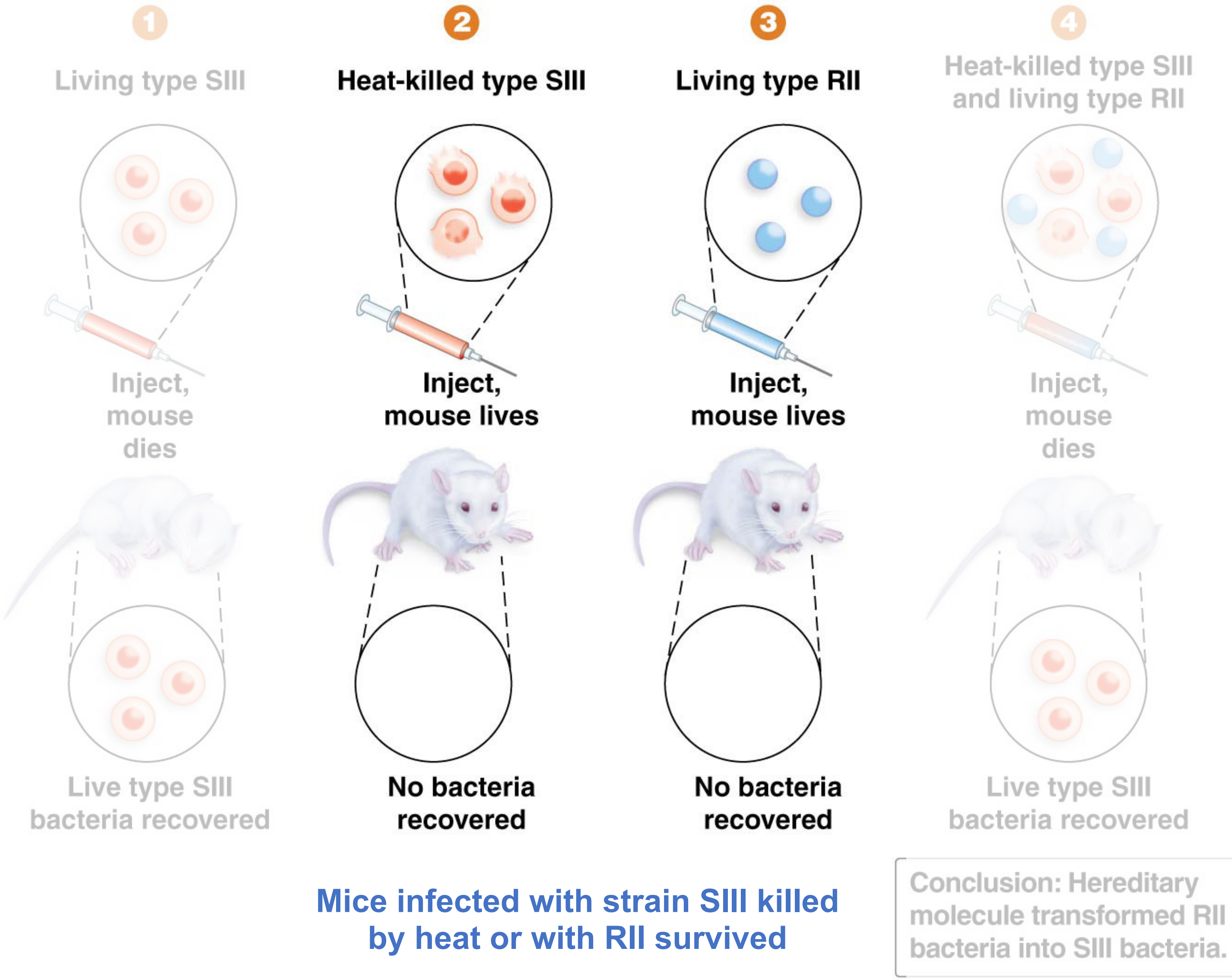
Griffith's Experimental Results



Mice infected with strain SIII developed pneumonia and died

Figure 7.2 Frederick Griffith's experiment identifying a "transformation factor" responsible for heredity.

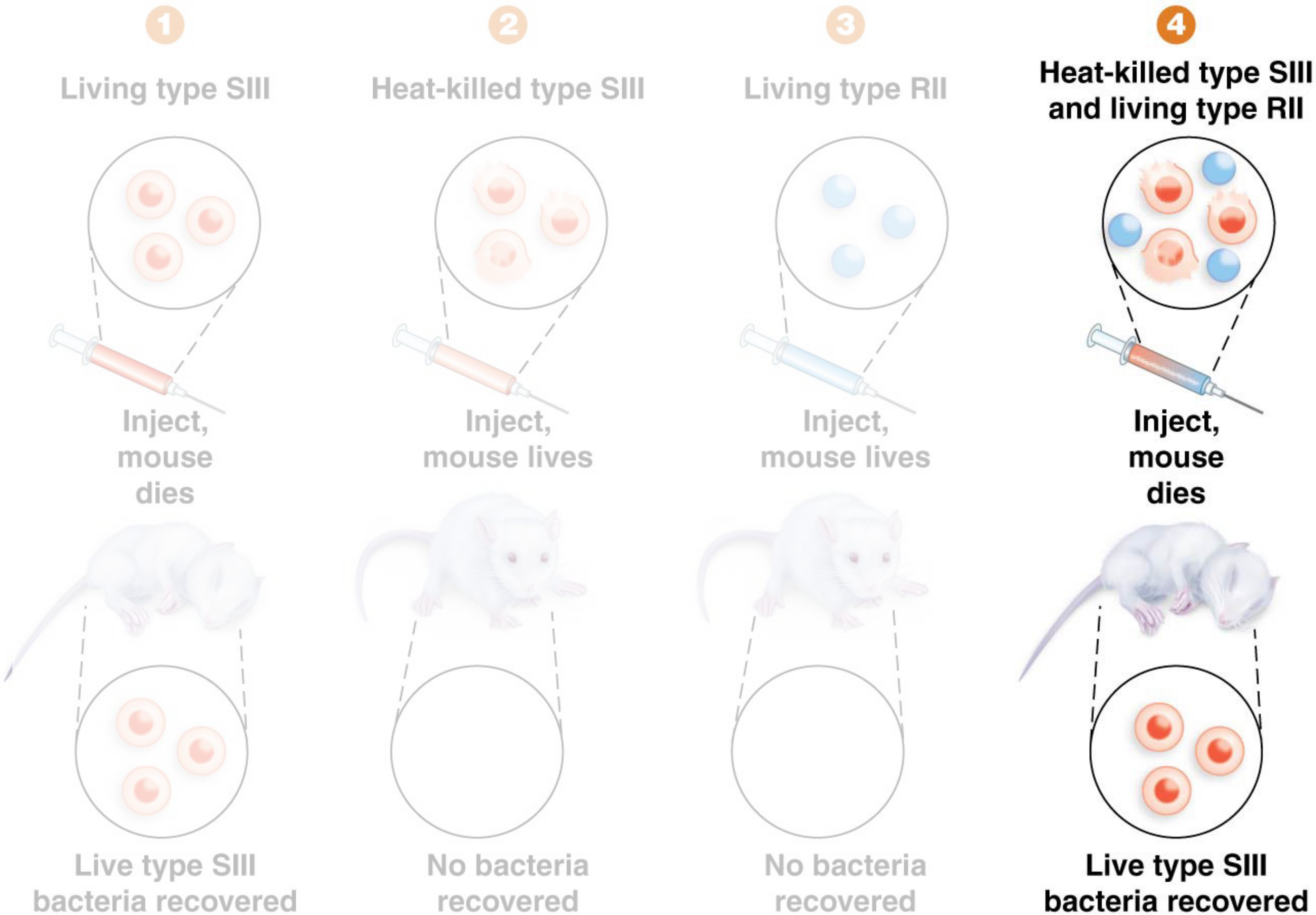
Griffith's Experimental Results



Mice infected with strain SIII killed by heat or with RII survived

Figure 7.2 Frederick Griffith's experiment identifying a "transformation factor" responsible for heredity.

Griffith's Experimental Results



Conclusion?

Conclusion: Hereditary molecule transformed RII bacteria into SIII bacteria.

Figure 7.2 Frederick Griffith's experiment identifying a "transformation factor" responsible for heredity.

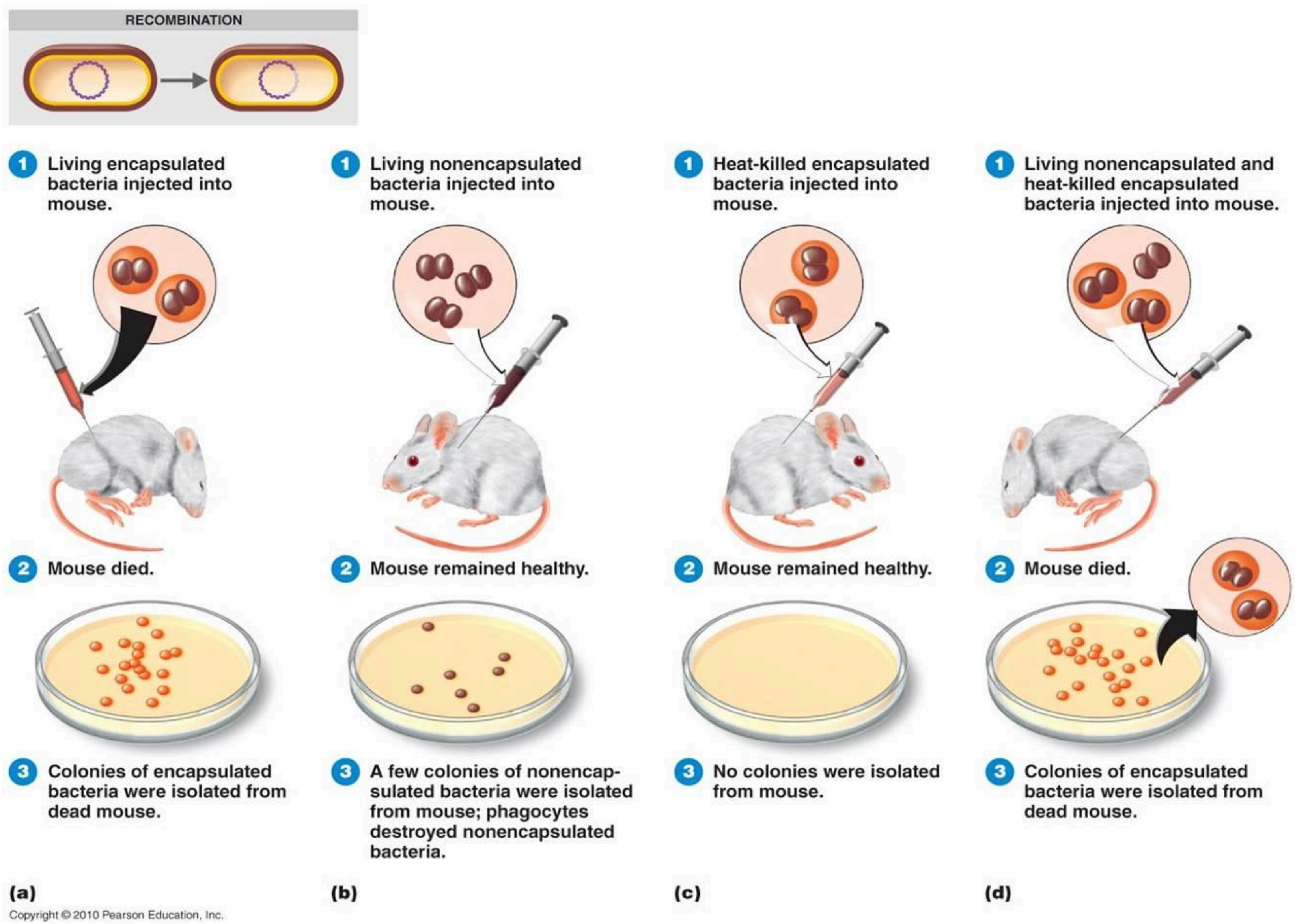
Griffith's Hypothesis

- Griffith proposed the **transformation factor** as the molecule that transformed the RII (non pathogen) into SIII (pathogen). He said the transforming factor carried hereditary information, but **he could not identify the molecule**

.........NOWADAYS:

- Griffith had described the process of ***transformation***, used by bacteria **to transfer DNA**, in his experiment (to make *R non pathogen bacteria* with a very fragile coating capsule able to produce a smooth and resistant capsule thanks to the acquisition of a wt allele from *S pathogen bacteria*)

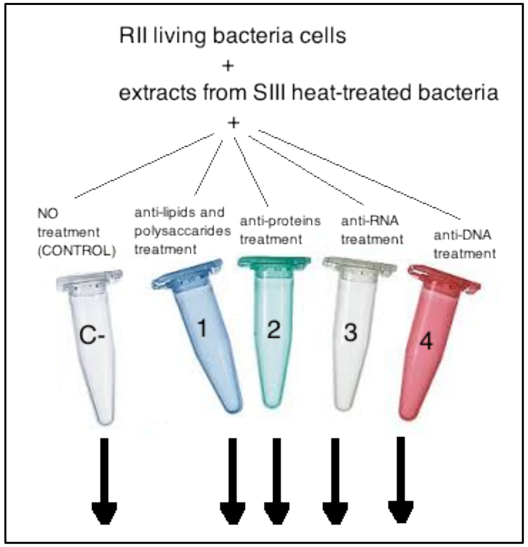
A MORE DETAILED VERSION OF THE GRIFFITH'S EXPERIMENT with the current knowledge

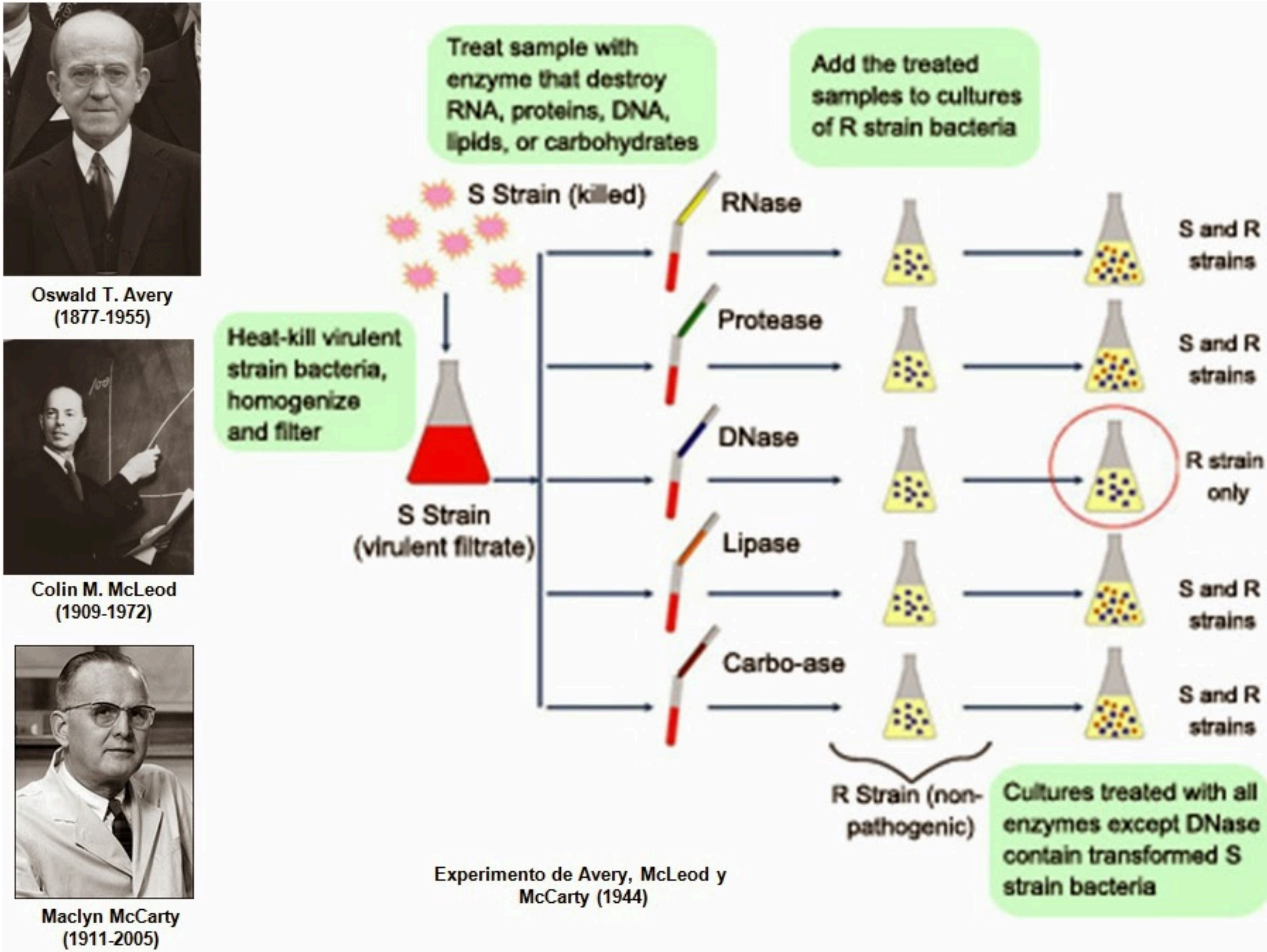


DNA is the Transformation Factor: a more elegant experiment inspired by Griffith's data

- Avery, MacLeod, and McCarty wanted to prove what is the transformation factor and developed:
- In vitro transformation procedure (living RII bacteria + purified cellular extracts from the heat-killed SIII bacteria) **BECAUSE THEY WERE SURE THAT IN THE MIX WILL BE THE TRANSFORMATION FACTOR**
- THEY TREAT the extract of heat-killed SIII bacteria with reagents to destroy:

1. RNA
2. proteins
3. DNA
4. lipids
5. polysaccharides





All aliquots have S transformed bacteria except the one with the DNA destroyed

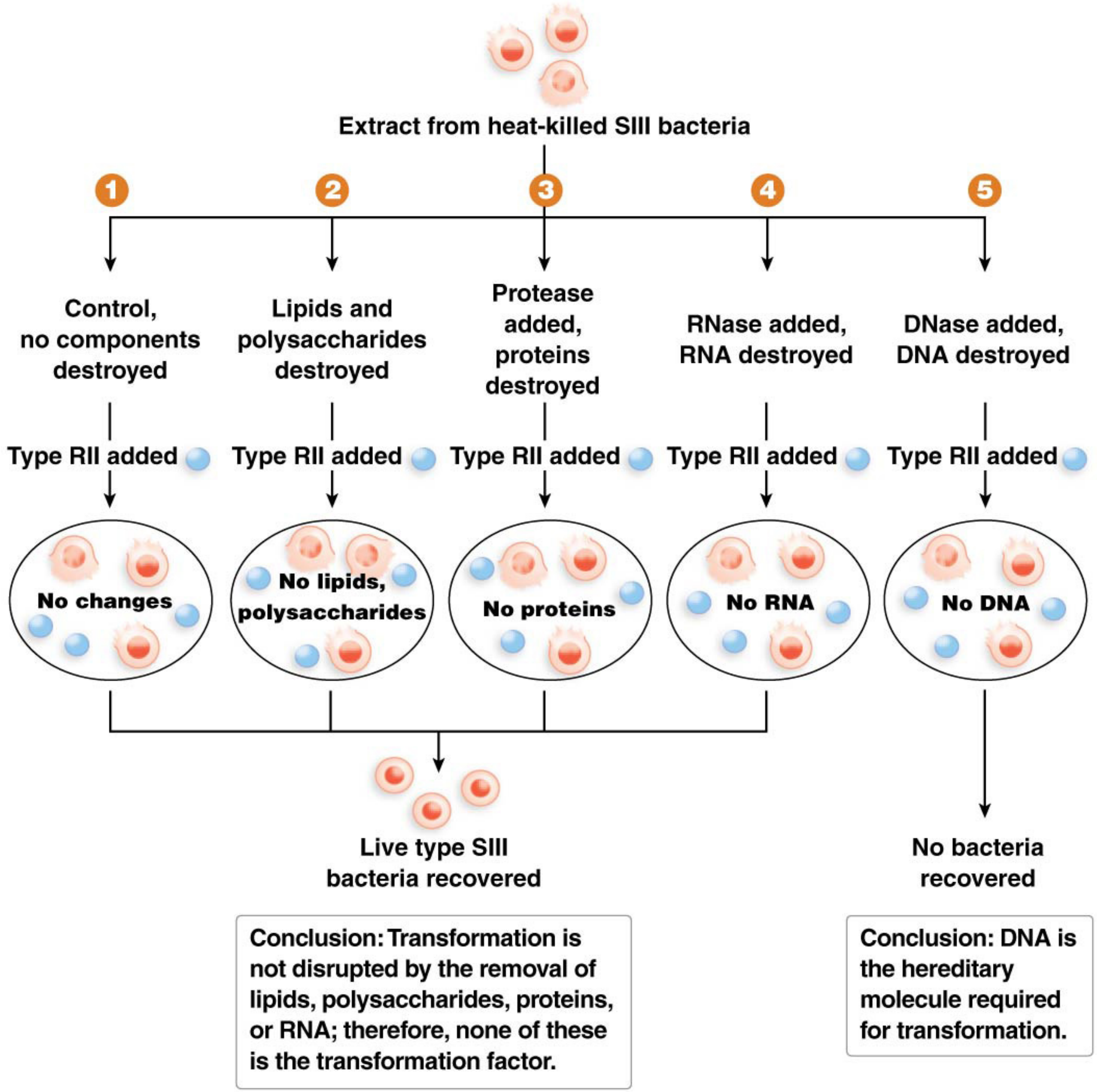
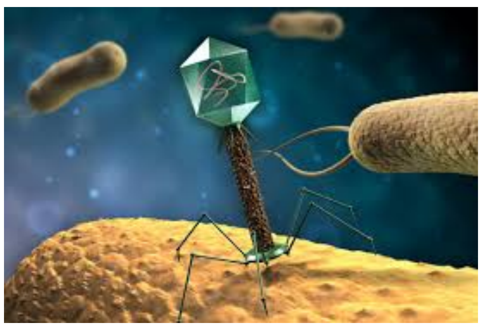


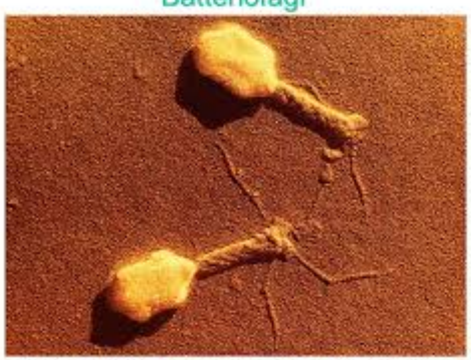
Figure 7.3 Avery, MacLeod, and McCarty's use of in vitro transformation to identify DNA as the most likely hereditary molecule.

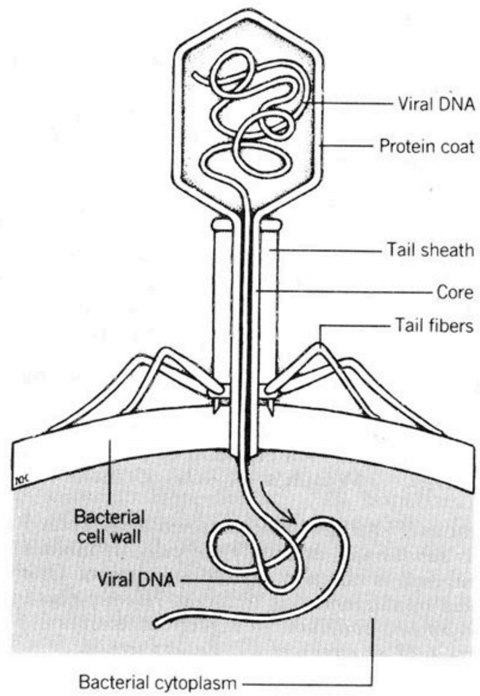
Is DNA the Hereditary Molecule??

- 1952, Hershey and Chase
- **Bacteriophages** (**phages**) are viruses that infect bacteria. They used T2-phage



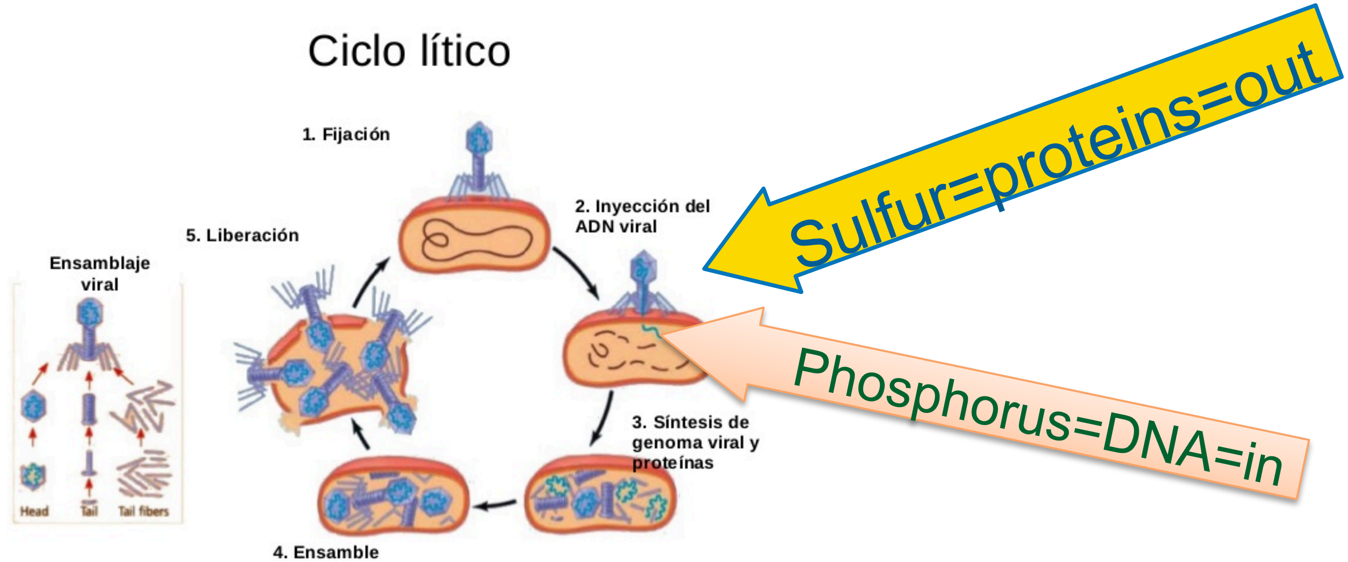
Batteriofagi





Phage Infection of Bacteria: some info

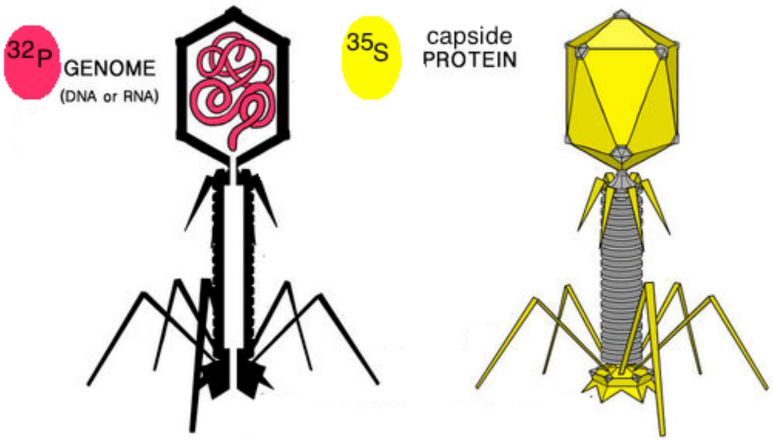
- Phages must infect bacterial hosts to reproduce
- Infection: the phage injects **DNA into** the bacterial cell and leaves its **protein shell outside,** on the bacteria surface



- The **phage DNA replicates** in the bacterium and produces proteins that are assembled **into progeny** phage – these are released by lysis of the host cell

Hershey and Chase Experiments

- **Proteins** contain large amounts of **sulfur** but almost no phosphorus
- **DNA** contains large amounts of **phosphorus** but no sulfur

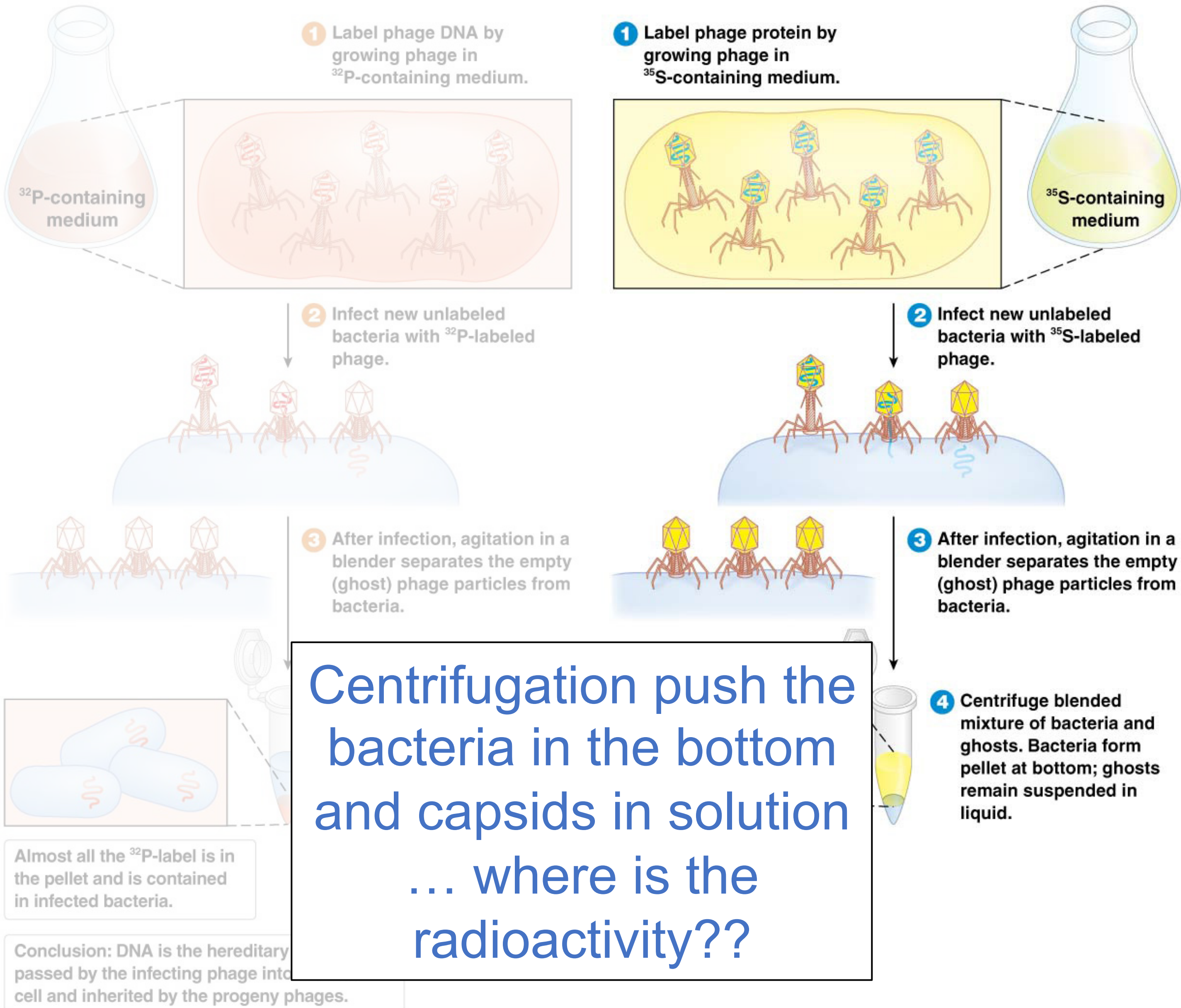


- Hershey and Chase **separately labeled** phages:

i) viral DNA (with radioactive phosphorus ^{32}P) and then traced each radioactive label in the course of infection

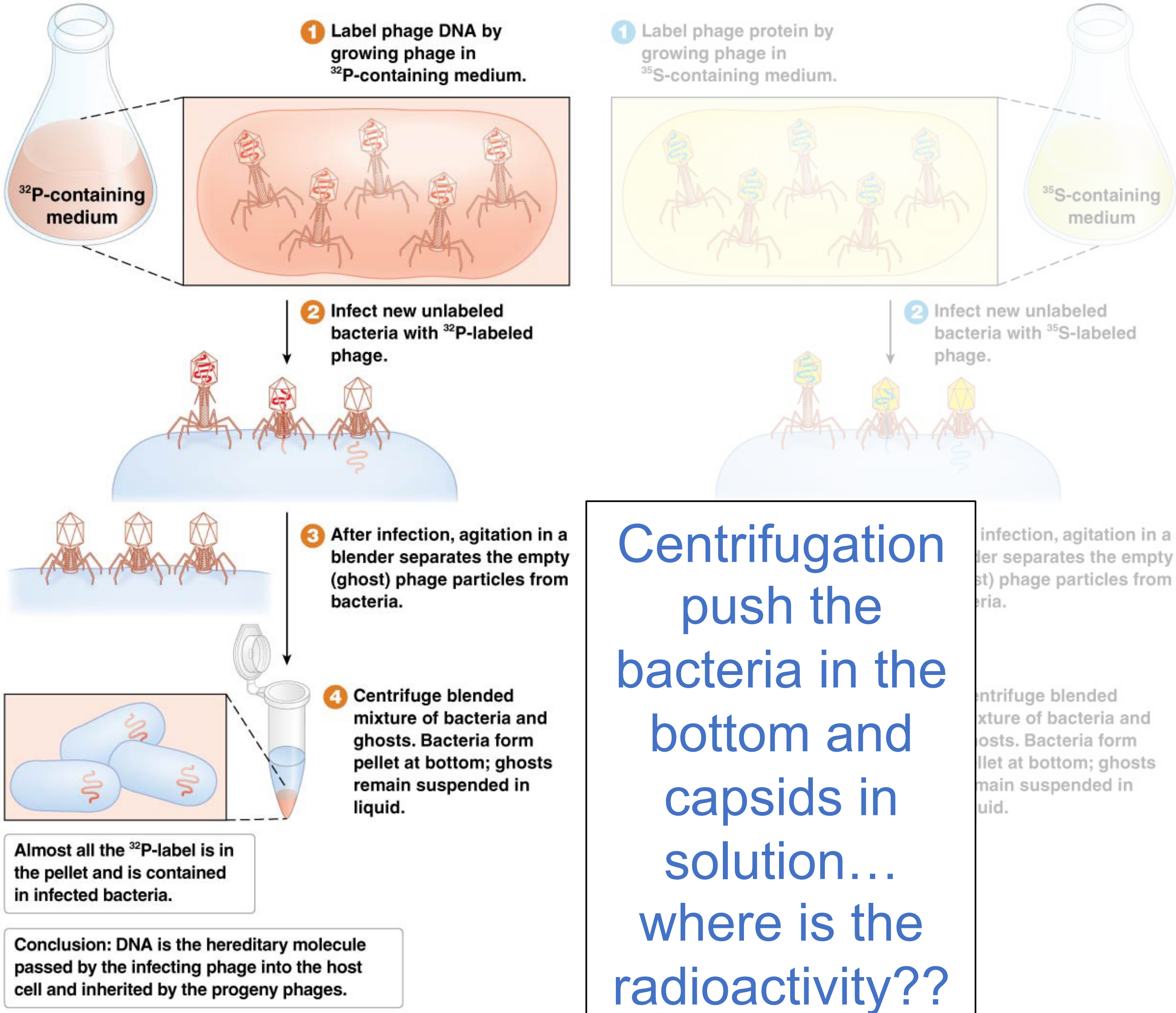
ii) capside proteins (with radioactive sulfur ^{35}S)

Figure 7.4 Hershey-Chase experiment showing DNA to be the molecule in bacteriophages that causes lysis of infected bacterial cells.



Centrifugation push the bacteria in the bottom and capsids in solution … where is the radioactivity??

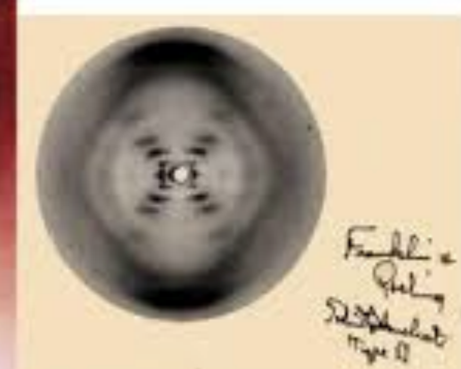
Figure 7.4 Hershey-Chase experiment showing DNA to be the molecule in bacteriophages that causes lysis of infected bacterial cells.



Centrifugation push the bacteria in the bottom and capsids in solution… where is the radioactivity??

Hershey-chase showed that viral DNA is responsible for the infection of bacteria cells and DNA is the molecule inherited by the viral progeny

DNA Structure



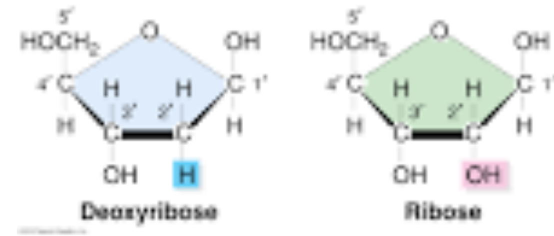
7.2 The DNA: basic informations

- **Watson and Crick's elaborated a model** of the secondary structure of DNA
- It is composed of four kinds of **nucleotides**, joined by **covalent** phosphodiester **bond**
- **Two** complementary and antiparallel Strands

  that come together to form a **double helix**

DNA Nucleotides

- A DNA **nucleotide** is composed of:



➢ a **sugar**,

➢ **one** of four nitrogenous **bases**,

➢ and **up to three phosphate groups** (3P if free nucleotides, 1P if in chain)

- **Deoxyribose is the sugar of DNA nucleotides**; it has five carbons, identified as 1′, 2′, 3′, 4′, and 5′
- **Sugar structure in nucleotide**: A nucleotide base is attached to the 1′ carbon, an OH (hydroxyl) group is attached to the 3′ carbon, and one to three phosphates are attached to the 5′ carbon

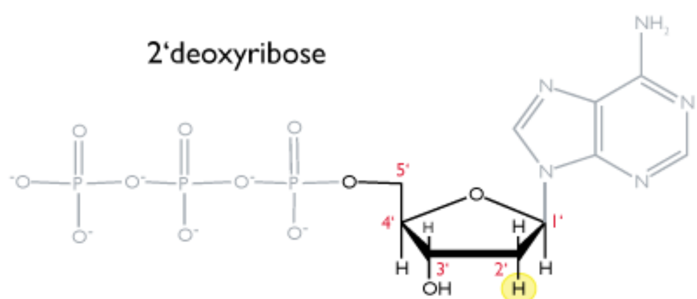
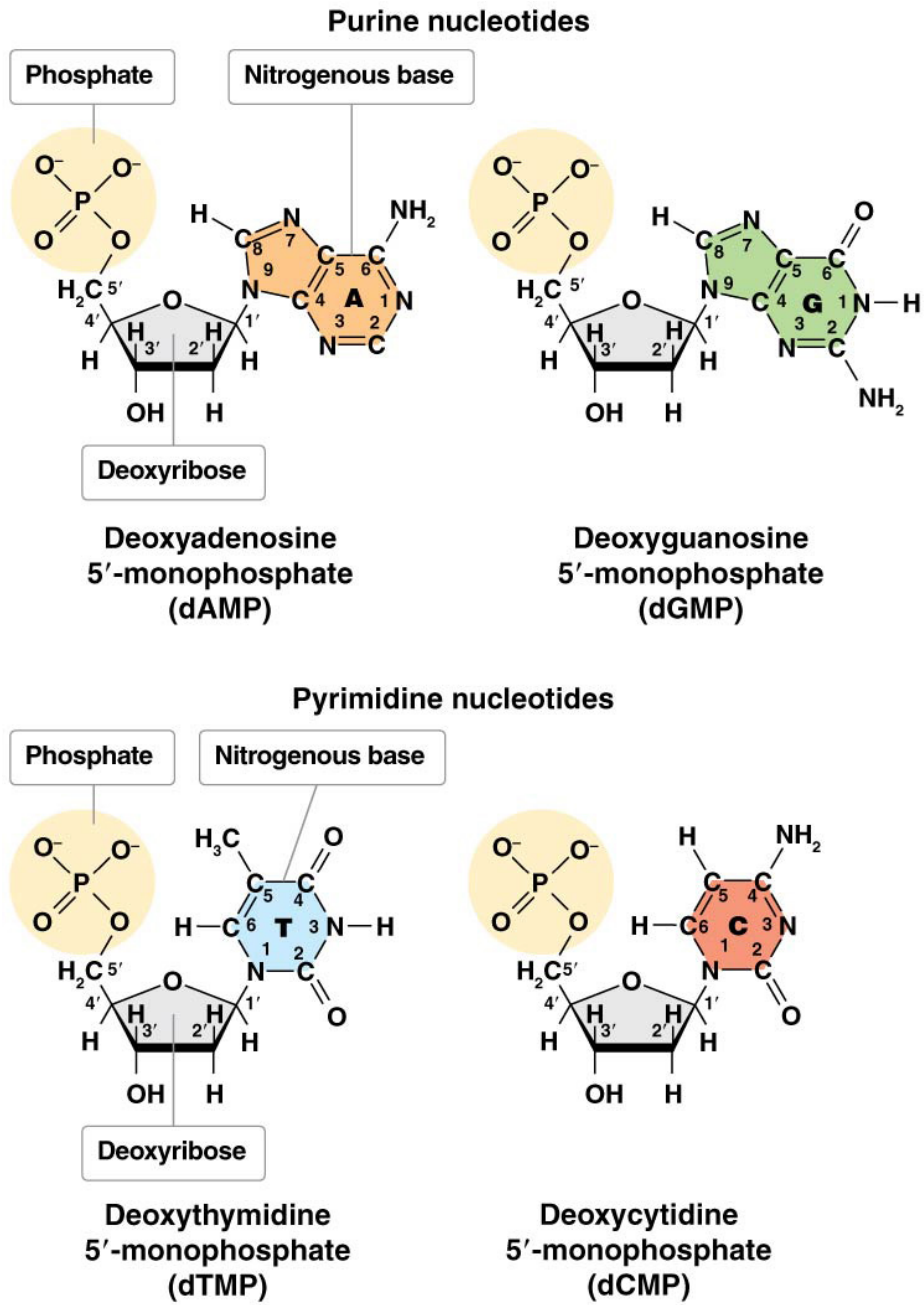
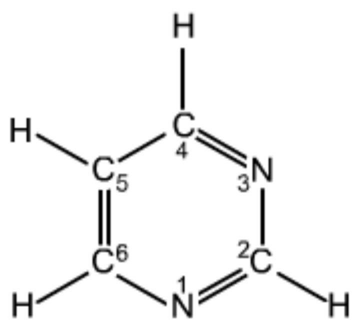


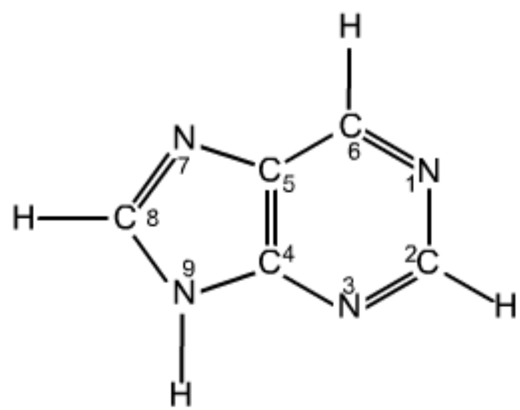
Figure 7.5 Components and structures of DNA nucleotide monophosphates.



Nitrogenous Bases



Pyrimidine ring



Purine ring

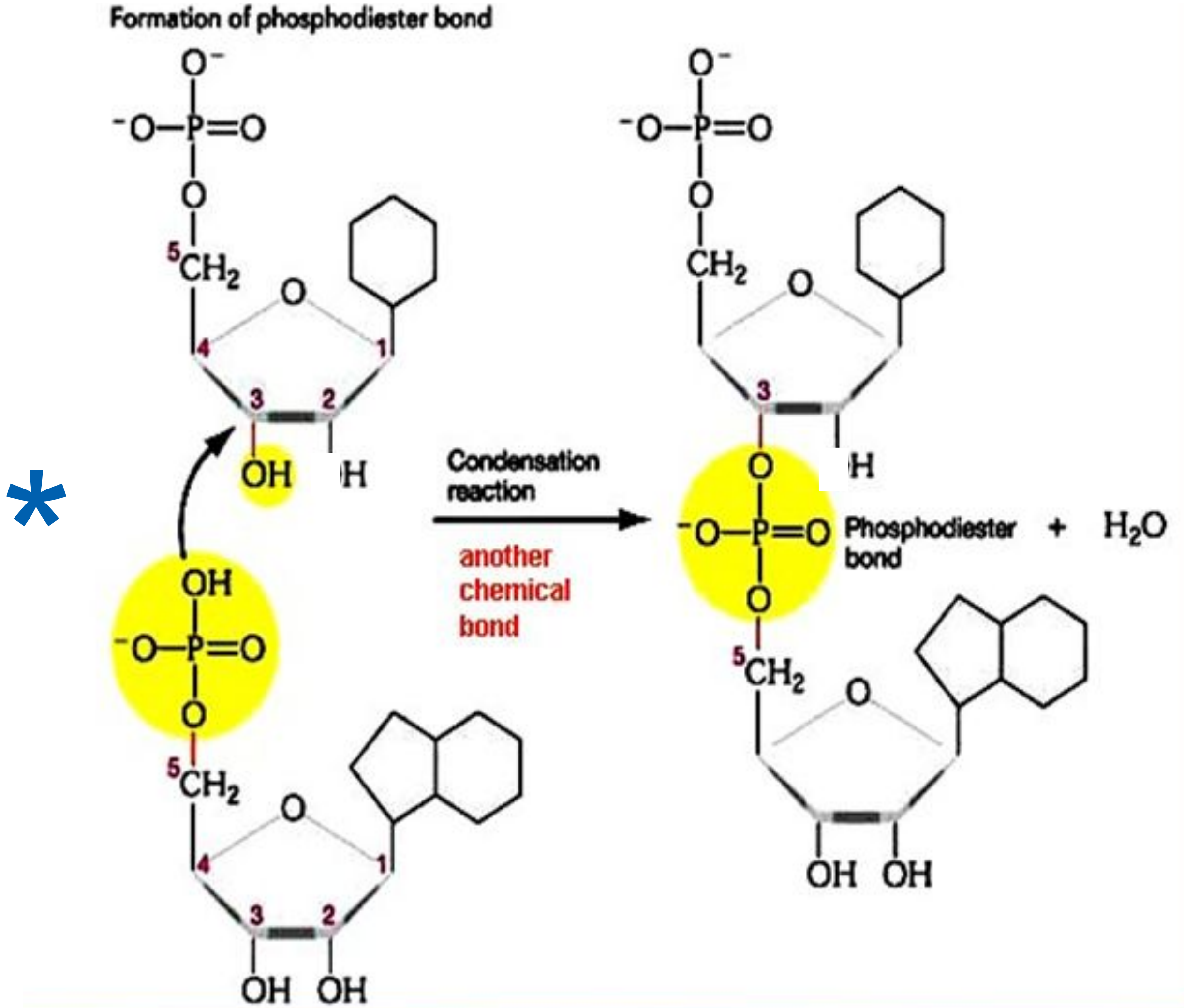
Nitrogenous Bases

A nitrogenous base is one of three essential components in a nucleotide. There are two different types of nitrogenous bases, called pyrimidines and purines. Purines have two heterocyclic rings; pyrimidines have only one.

The numbers identify the nitrogen and carbon atoms in the chemical structures.

STOP | NEXT 1 2 3 4 5 6 7 8 9 10 11 12

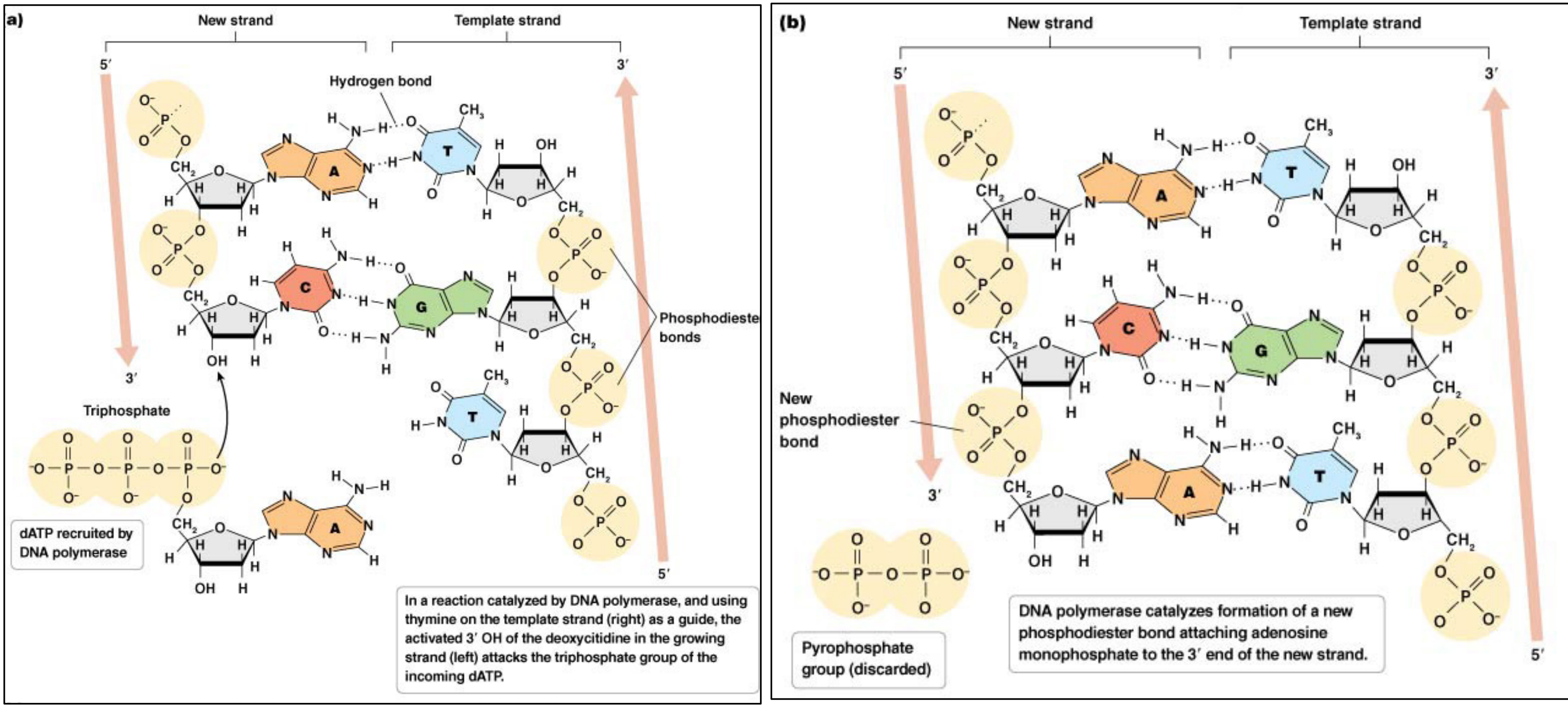
Phosphodiester bond in DNA: made by DNA polymerase *



Phosphodiester bond:

- phosphate group of a nucleotide and the OH group on the sugar of the other nucleotide
- Nitrogenous bases are not participating in the bond

Figure 7.6 DNA strand elongation: three fosphates-free nucleotide incorporated into a chain of DNA in elongation



Complementary DNA Nucleotide Pairing

- The two strands of a double helix form a stable structure that follows two rules:
  1. The bases of one strand are ***complementary*** to the bases in the corresponding strand (A pairs with T and G pairs with C)
  - one purine with one pyrimidine
  - **hydrogen (H) bonds between the bases** on the antiparallel strands
  - Two H bonds form between A and T; three H bonds form between G and C
  2. The two strands are ***antiparallel*** with respect to their 5′ and 3′ ends

The Twisting Double Helix: notice the details

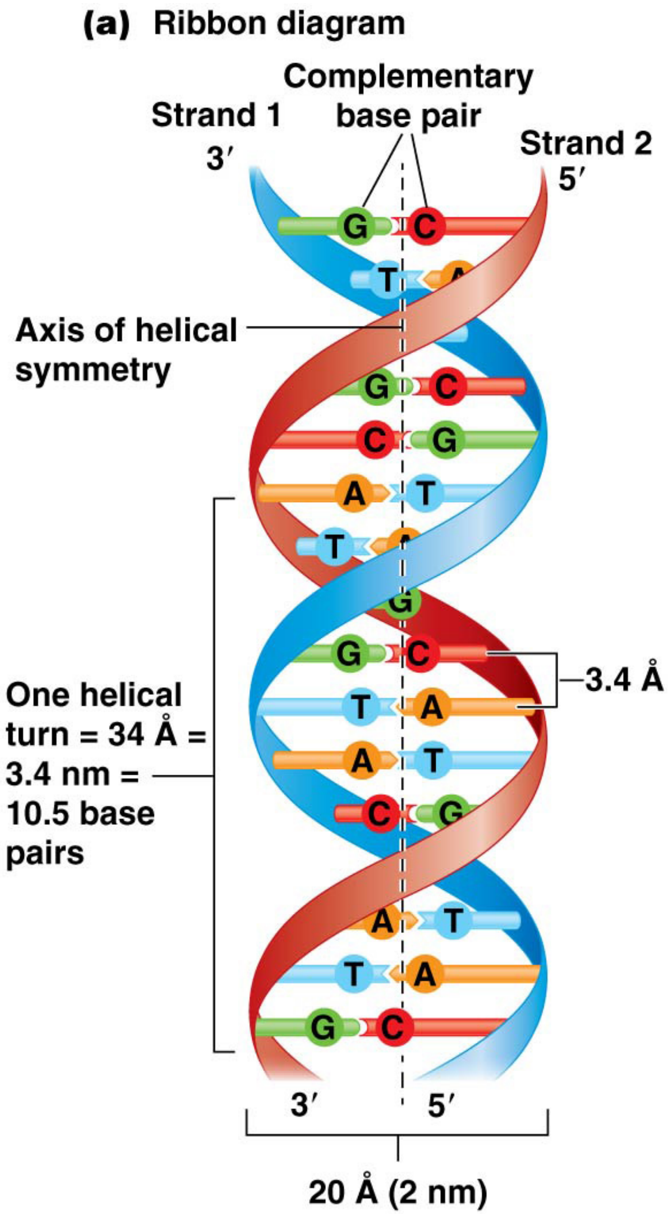
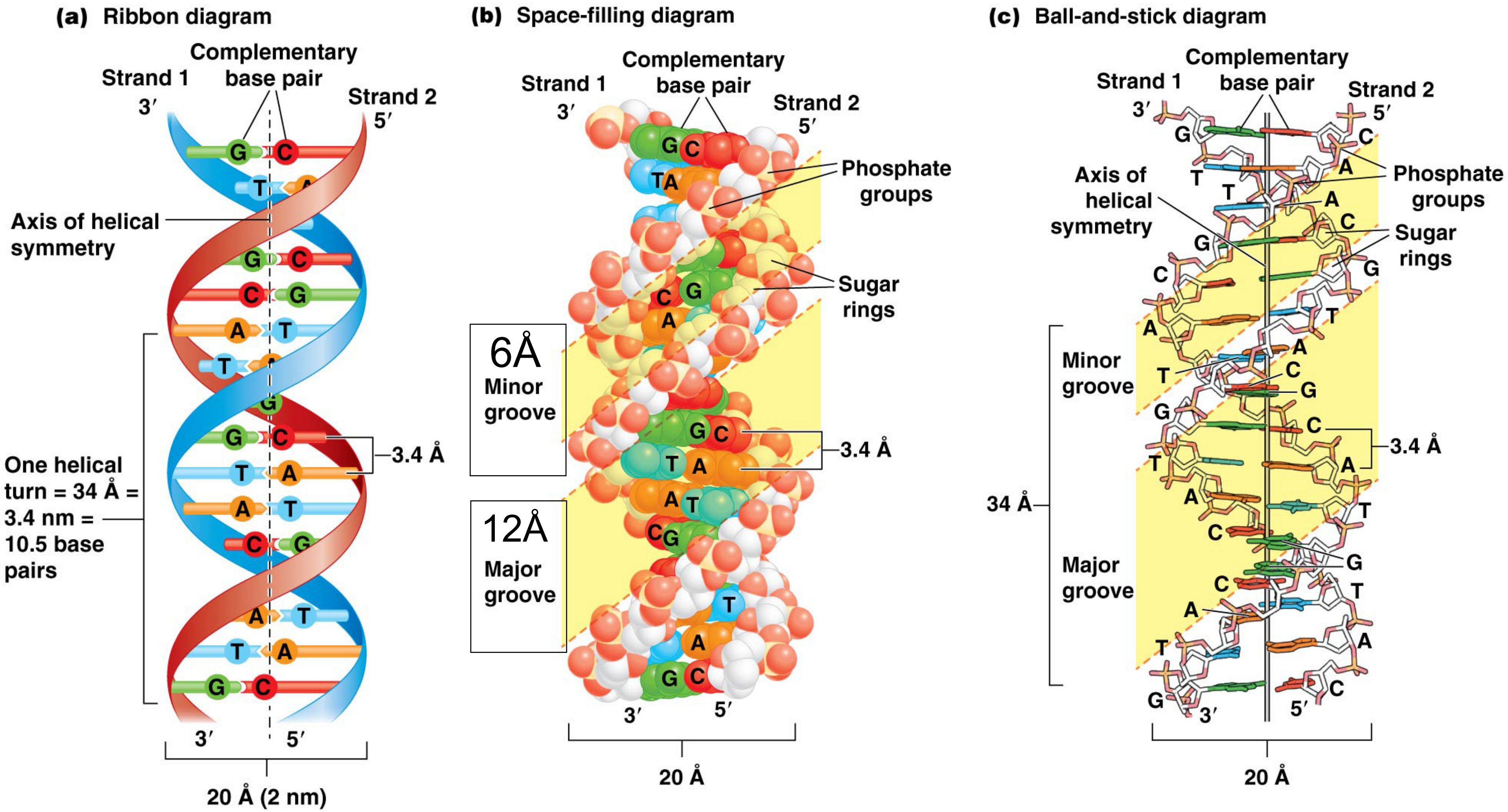


Figure 7.7a The DNA double helix.

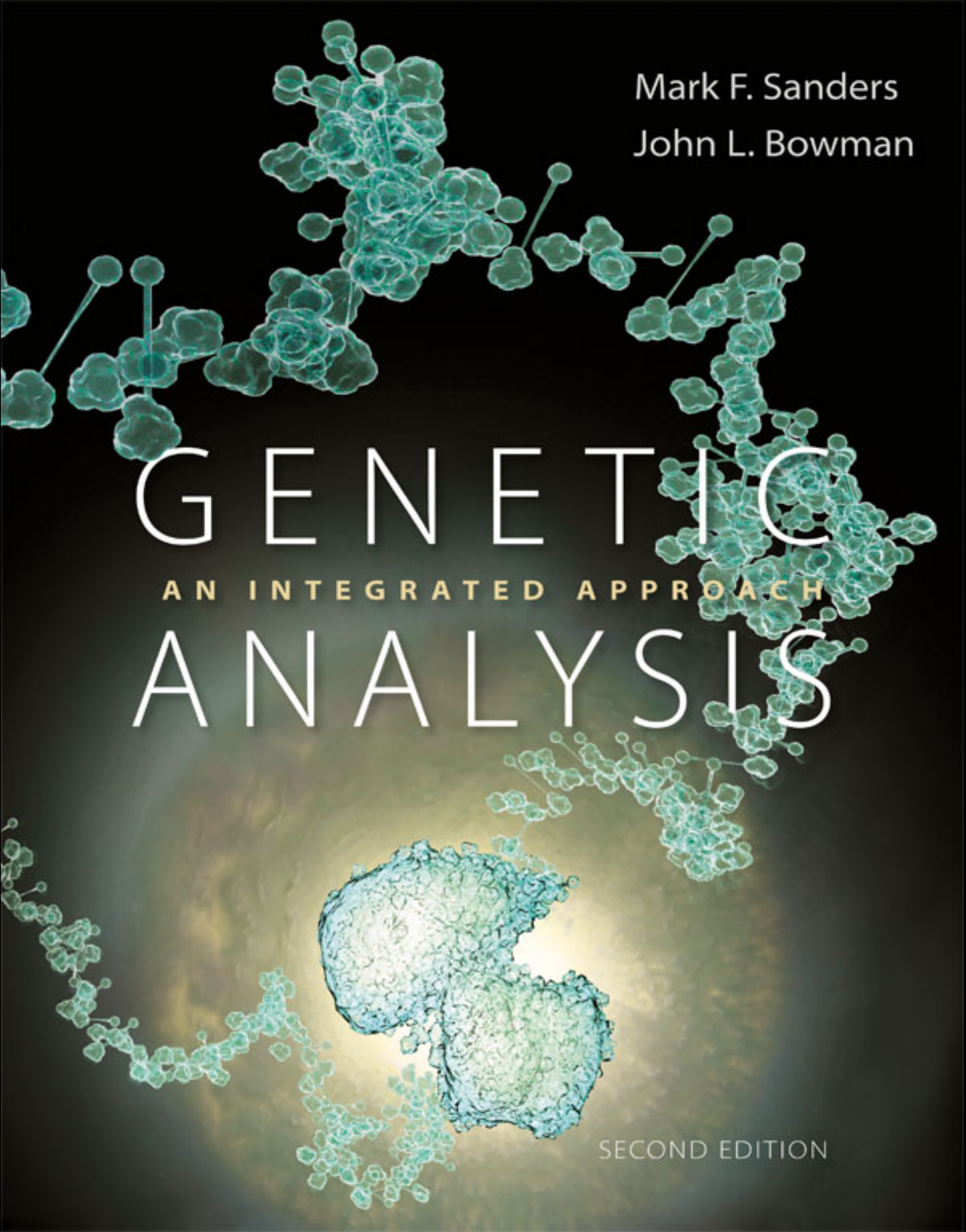
- The DNA double helix has an **axis of helical symmetry**, an imaginary line that passes lengthwise through the core of the helix
- The **diameter** of the molecule is 20Å, where 1Å is 10^{-10} m
- Nucleotide base pairs are spaced along the DNA duplex at intervals of 3.4Å
- The twist in the double helix is because of the **base stacking (to maintain the base planes parallel)**

Major and minor groove



Major and Minor Grooves

- Base-pair stacking creates gaps between the sugar-phosphate backbones that partially expose the nucleotides
- The **major groove**, approximately 12Å wide, alternates with the **minor groove**, approximately 6Å wide
- These grooves are **regions where DNA binding proteins** can make **direct contact** with nucleotides



11

DNA Structure and Replication

Lectures by Dr. Tara Stoulig
Southeastern Louisiana University

11.1 Viruses Are Infectious Particles Containing Nucleic Acid Genomes

- **Virus** is a non-cellular infectious particle
- Viruses are particles made of a **protein structure** with **genetic material inside**
- Viruses have a small **nucleic acid genome** with a limited number of genes
- The nucleic acid can be single-stranded (ss) or double-stranded (ds) DNA or RNA
- A virus must infect a **host cell** in order to express its genetic material and replicate using host cell proteins and ribosomes

Viral Genomes

- The content, structural configuration, and size of viral genomes **vary from one virus to another**

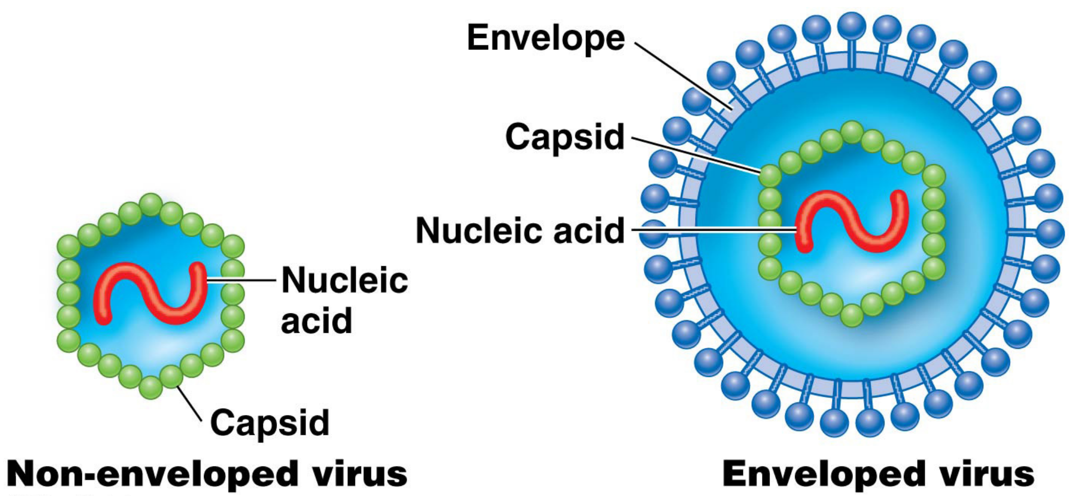
- Nucleic acids of viruses are **not associated with proteins**

- Viral genomes range in size from a few thousand bases (or base pairs if double stranded) to more than 200,000 base pairs

- Content ranges **from 5 to nearly 300 genes**

Viral Protein Packaging

- **Caspid**—protein coat that contains viral genetic material
- A **non-enveloped virus** contains genetic material in only a protein shell
- An **enveloped virus** has an envelope of host cell cytoplasmic membrane surrounding the caspid



Viruses diversity

ALGUNAS INFECCIONES VIRALES IMPORTANTES EN LA MEDICINA HUMANA

Tipo de ácido nucleico	Nombre de la familia y esquema de la morfología	Nombre del virus y características de la enfermedad
ADN	Adenovirus 	*Varios tipos de adenovirus.* Infección de los ojos, del tracto respiratorio y a veces del tracto intestinal.
	Herpesvirus 	*Virus del herpes simple.* Lesiones vesiculares en la piel, mucosas y ojos. Causa común de meningoencefalitis.
	Poxvirus 	*Virus de la varicela.* Se caracteriza por una erupción vesicular. Puede dar complicaciones como neumonía. En el adulto puede causar una infección vesicular localizada llamada herpes zoster.
ARN	Flavivirus 	*Virus del dengue.* Fiebre alta, dolores de cabeza, huesos, músculos y articulaciones. Puede haber una erupción en la piel. *Virus de la fiebre amarilla.* Fiebre, dolor de cabeza náuseas, vómitos, hemorragia e ictericia.
	Togavirus 	*Virus de la rubéola.* Síntomas respiratorios y erupción en la piel.
	Picornavirus 	*Virus de la polio* (3 tipos). Puede dar una enfermedad leve; fiebre baja y malestar general. Puede producir meningitis o se puede presentar la poliomielitis paralítica. *Rhinovirus* (más de 100 tipos). Causa el resfriado común.
	Reovirus 	*Rotavirus.* Gastroenteritis, principalmente en niños pequeños.
	Ortomixovirus 	*Virus de la influenza.* Causa la gripe o influenza.
	Paramixovirus 	*Virus del sarampión.* Se caracteriza por un cuadro respiratorio agudo con fotofobia, catarro, tos y una erupción en la piel. *Virus de las paperas.* Afecta las glándulas parótidas, puede afectar los testículos y los ovarios.
	Rhabdovirus 	*Virus de la rabia.* Tiene afinidad por el sistema nervioso y produce daños en el cerebro. Es casi siempre mortal.
	Retrovirus 	*Virus de la inmunodeficiencia humana (VIH).* Ataca el sistema inmune. Causa el síndrome de inmunodeficiencia adquirida (SIDA).

11.2 Bacterial genome

- Bacteria generally have a single chromosome of double-stranded DNA
- Most bacterial species contain circular chromosomes
- **Structural genes** contain DNA sequences that encode bacterial proteins essential for normal bacterial functions and metabolism
- **Intergenic regions** separate one gene from the next

Table 11.2 Chromosome Diversity among Bacteria

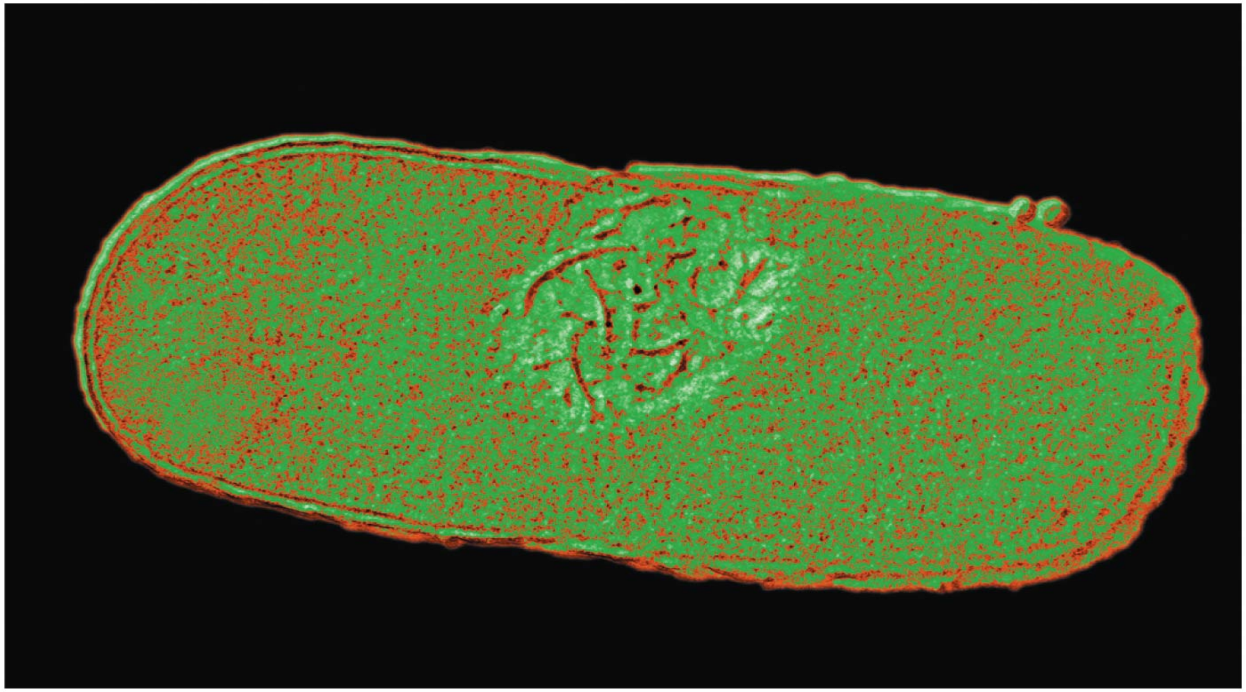
Table 11.2 Chromosome Diversity among Bacteria

Species	Genome Size (in Mb)	Number of Chromosomes	Chromosome Form(s)
Mycoplasma genitalium	0.58	1	Circular
Borrelia burgdorferi	1.4	2	One circular, one linear
Haemophilus influenzae	1.83	1	Circular
Vibrio cholerae	4.0	2	Both circular
Escherichia coli	4.2	1	Circular
Agrobacterium tumefaciens	5.7	4	Three circular, one linear
Sinorhizobium meliloti	6.7	3	All circular

- 4.2 Mb of genome in E.coli measures 1200μm and the cell measures are 0.5μm width x 2μm length… how can the genome fit into the cell?
- Condensation/compaction

Bacterial Chromosome Compaction

- **Nucleoid:** small region in which bacterial chromosome/s is/are densely packed
- The chromosome is organized into a series of **tight loops** (efficient packaging of relatively long DNA molecules into very small spaces)



Bacterial Chromosome Compaction

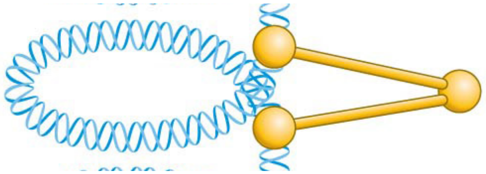
- Bacterial chromosomes are compacted in two ways:

  1. Proteins help organize the DNA into loops that pack the chromosome into the nucleoid

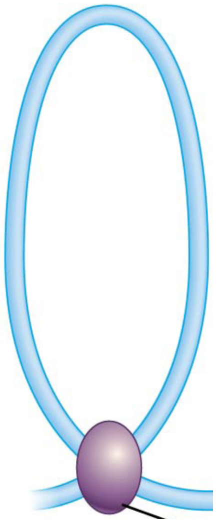
  2. The circular DNA undergoes *supercoiling*

1. Proteins Organizing the DNA

- **Structural maintenance of chromosome (SMC) proteins** holds DNA in coils or V shapes

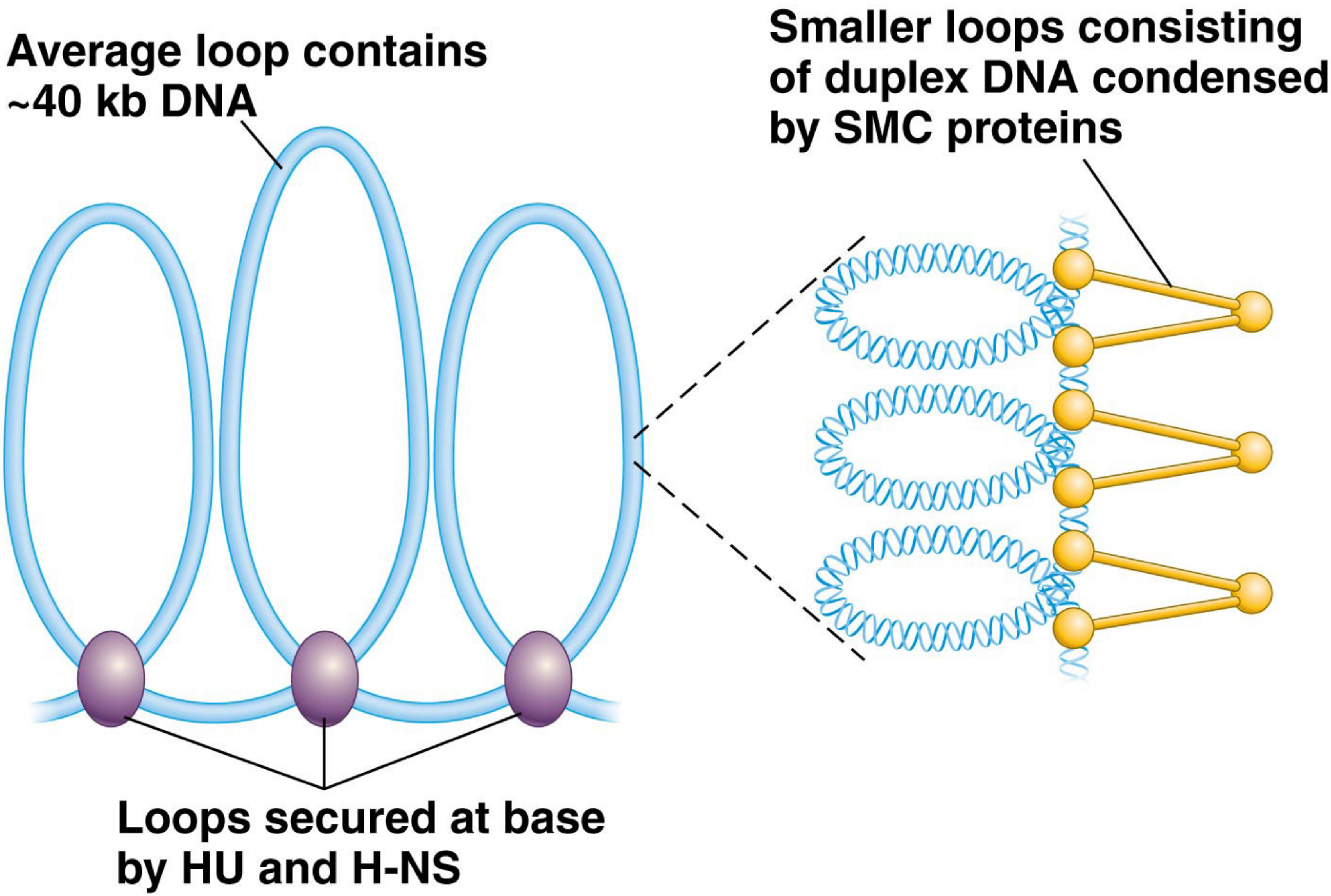


- **Small nucleoid-associated proteins** participate in the DNA bending that contributes to folding and condensation of chromosomes

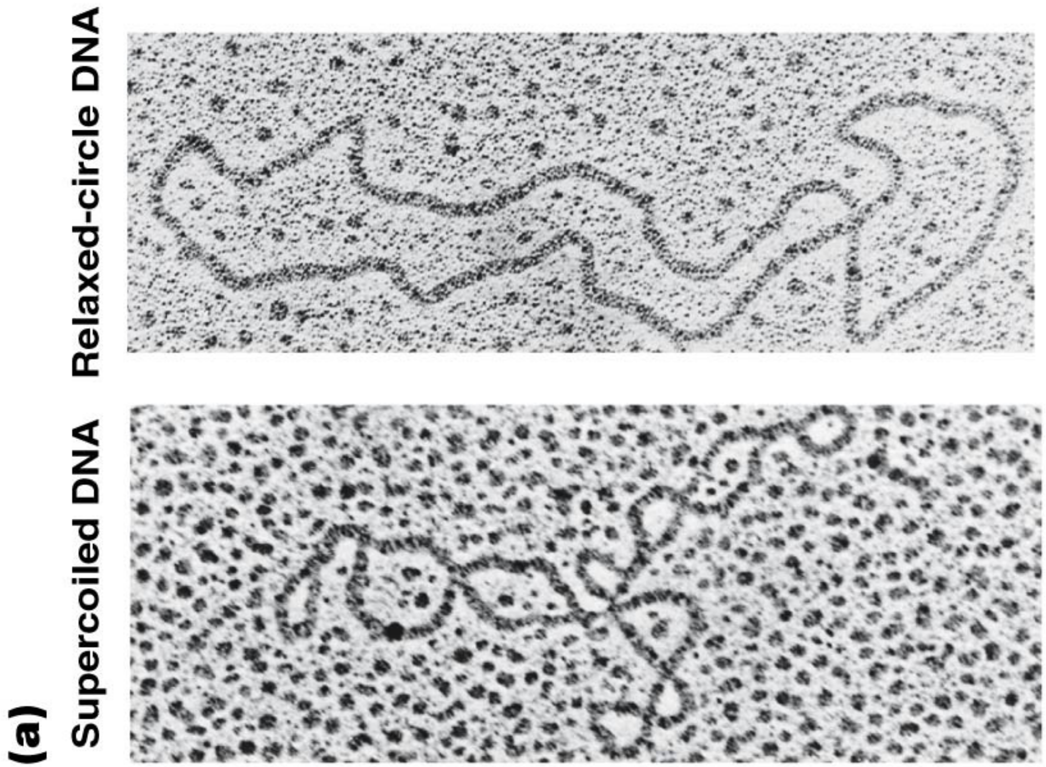


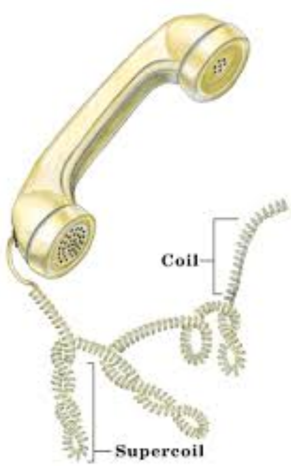
- Other proteins also interact in the nucleoid to compact DNA but are still under investigation

Figure 11.4 Bacterial chromosome condensation by proteins.



2. Supercoiling: twisting the DNA around on itself





By the extensive twisting DNA looks like:

- Convoluted
- Overlapping itself
- Not flat on a plane

Topoisomerases

- **Positive supercoiling** twists the DNA so that it is *over-rotated* (12.5 bp/helical twist)
- **Negative supercoiling** twists the DNA so that it is *under-rotated* (8.3 bp/helical twist)
- **Topoisomerases** (enzymes) partially **unwind** supercoiled DNA **to relieve torsional stresses** that could result from supercoiling

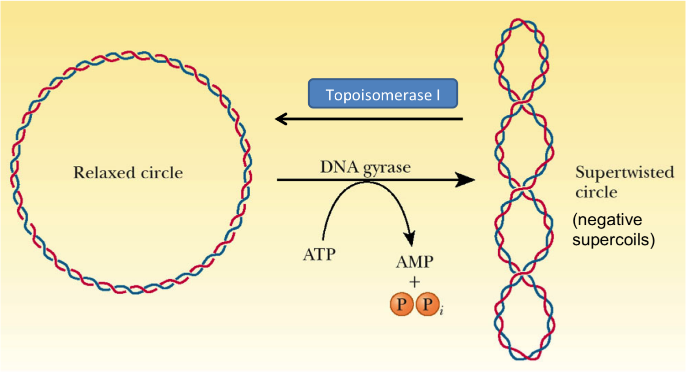


Fig. 10-9, p.248

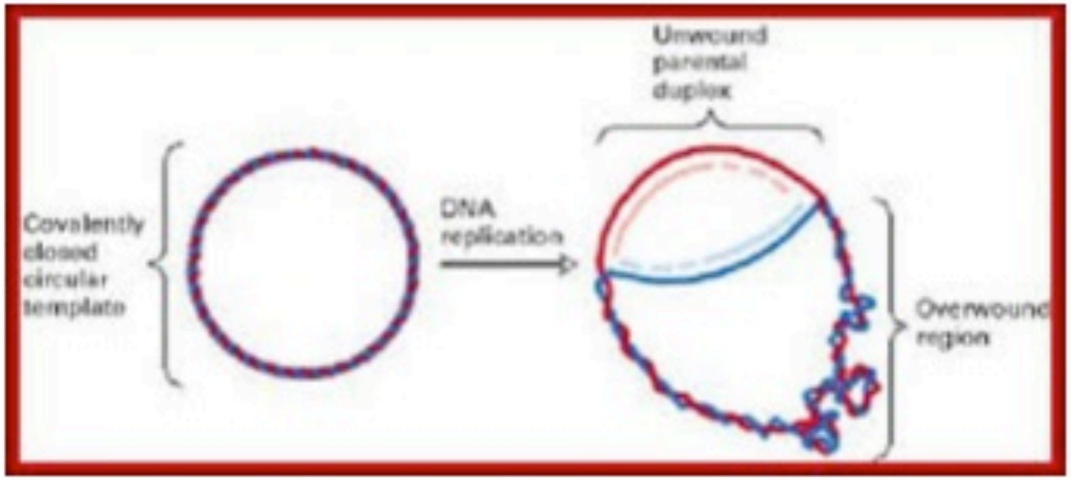
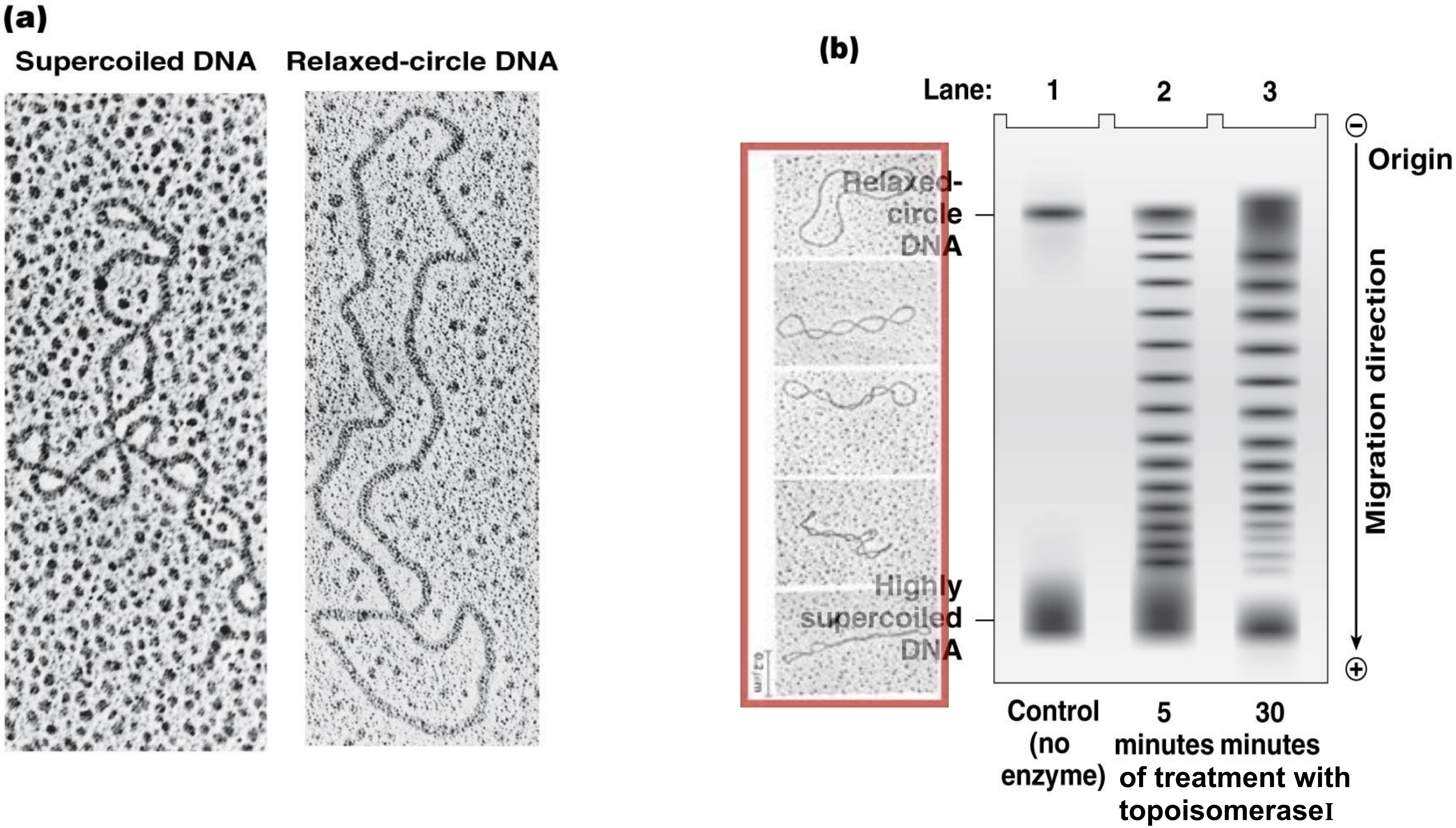
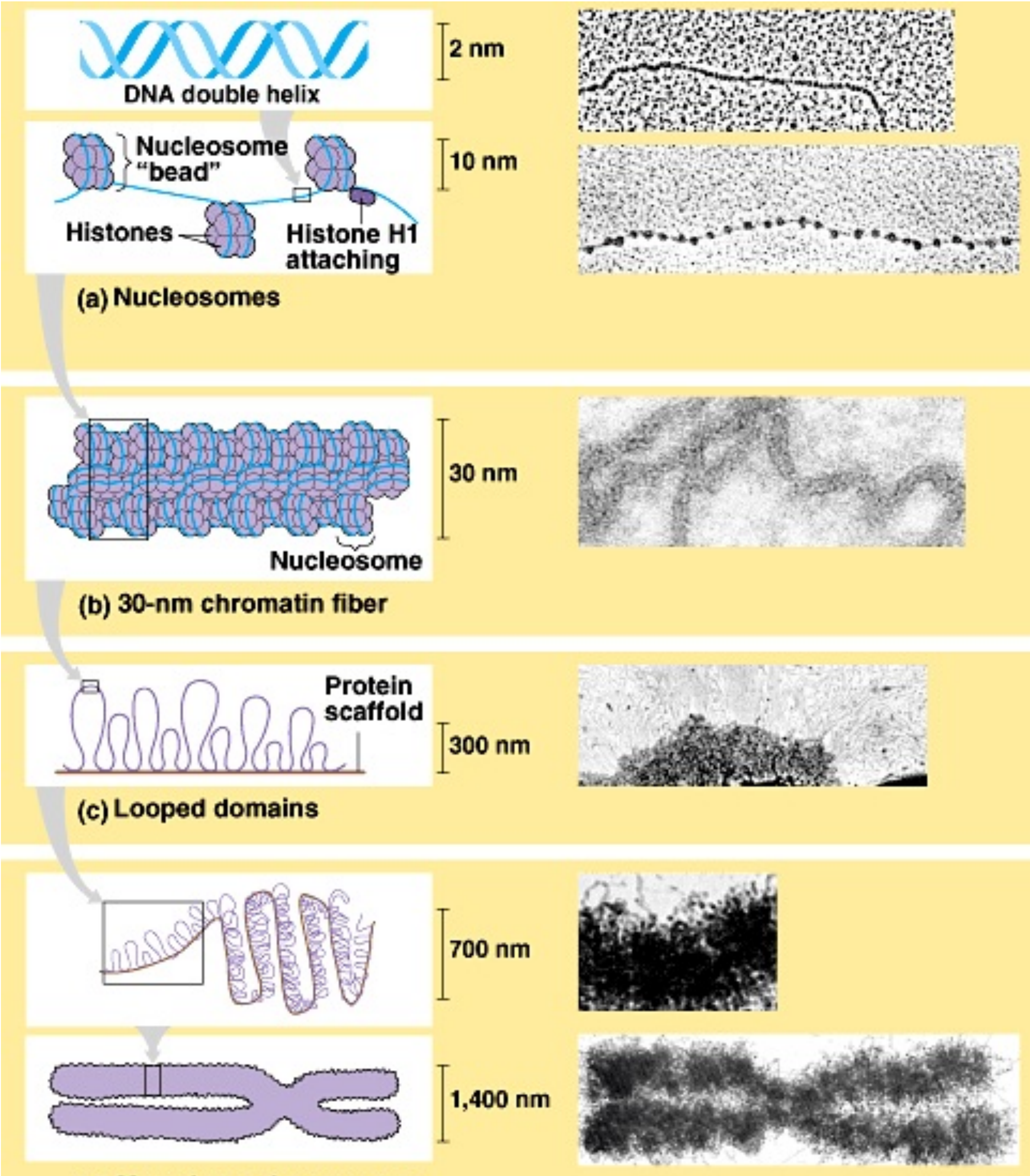

Covalently closed circular template
DNA replication
Unwound parental duplex
Overwound region

Figure 11.5 Circular DNA of bacteria



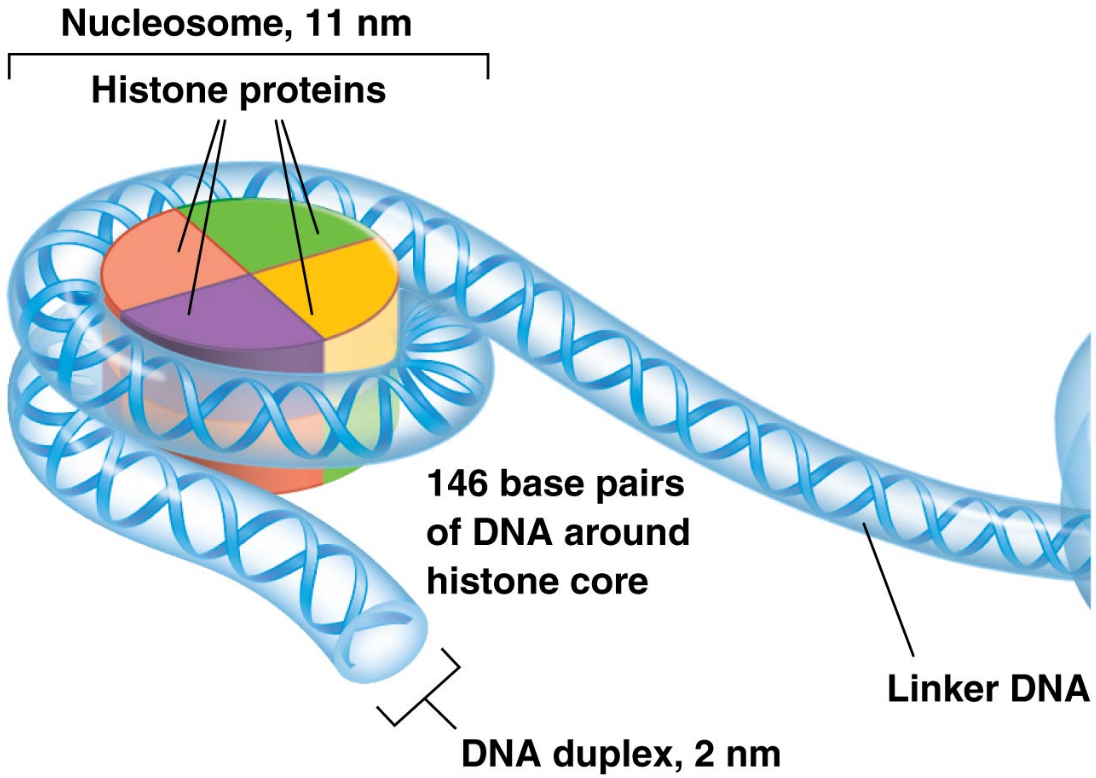
11.3 Eukaryotic Chromosomes Are Organized into Chromatin

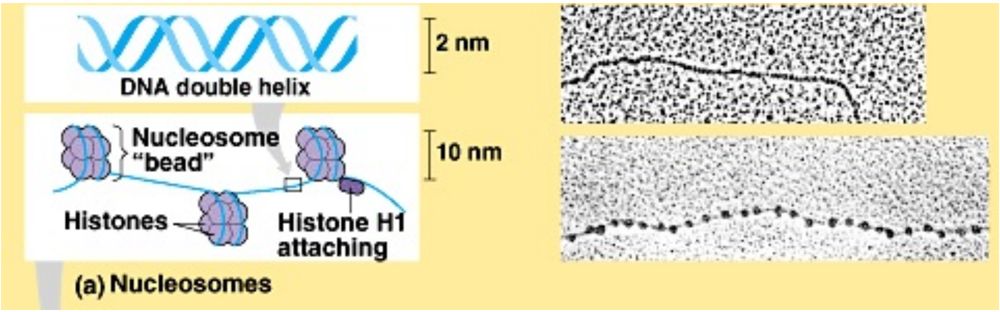
- Eukaryotes manage massive amount of DNA and multiple chromosomes and need organization
- **Chromatin:** eukaryotic chromosomes organization (DNA and associated proteins)



Histones

- five types of histone proteins in chromatin: **H1**, **H2A**, **H2B**, **H3**, and **H4**; they are highly conserved among eukaryotes
- **Nucleosome core particles**: basic units of histone protein organization with two molecules each of histones H2A, H2B, H3, and H4 (octamer)
- A span of DNA ~146 bp long (**core DNA**) wraps around each octamer to form a **nucleosome**
- The wrapping of DNA around the octamer is the first level of **DNA condensation**, and compacts the DNA **about sevenfold**





(a) Nucleosomes

Figure 11.6b Condensing the Nuclear Material

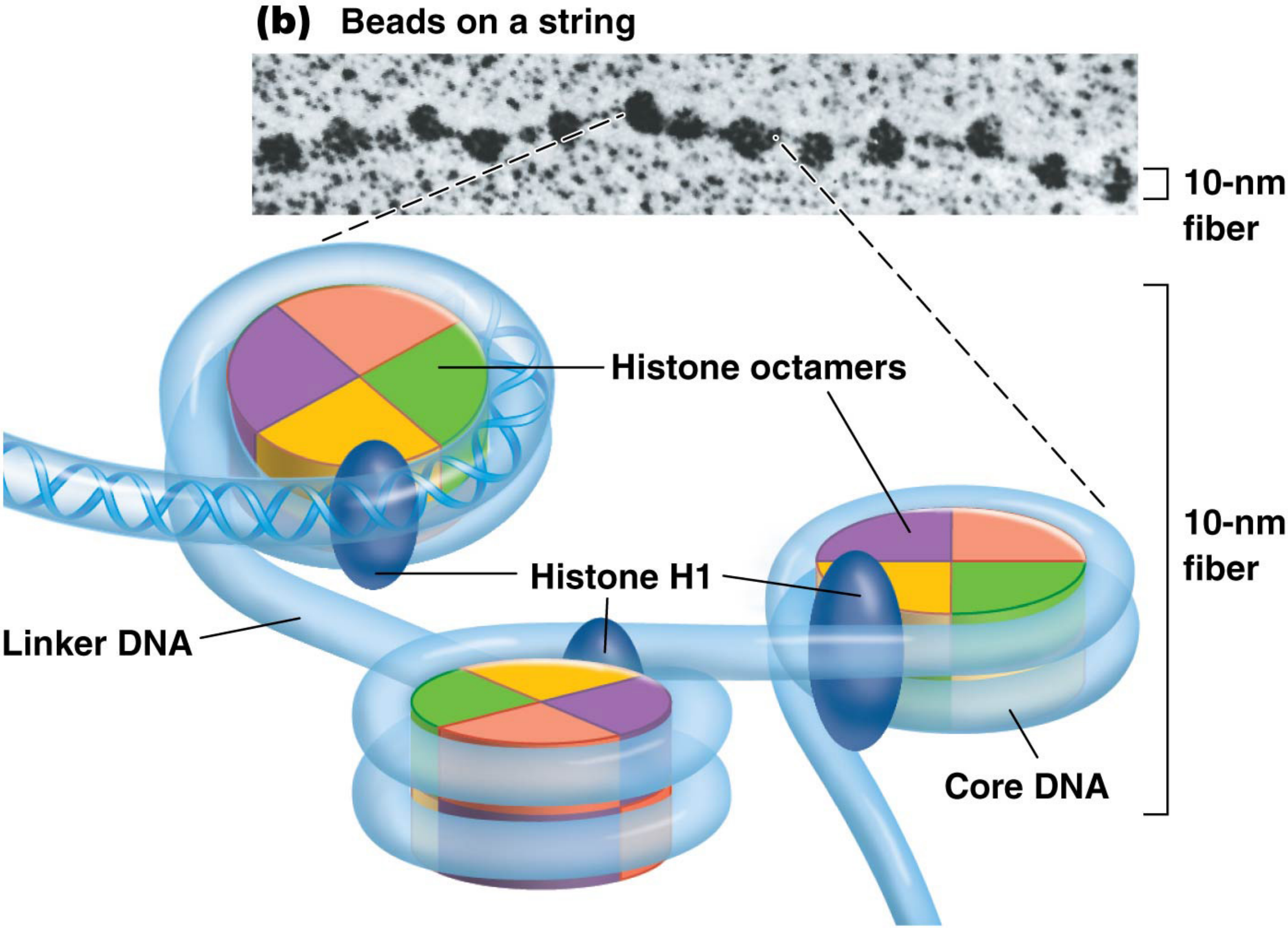
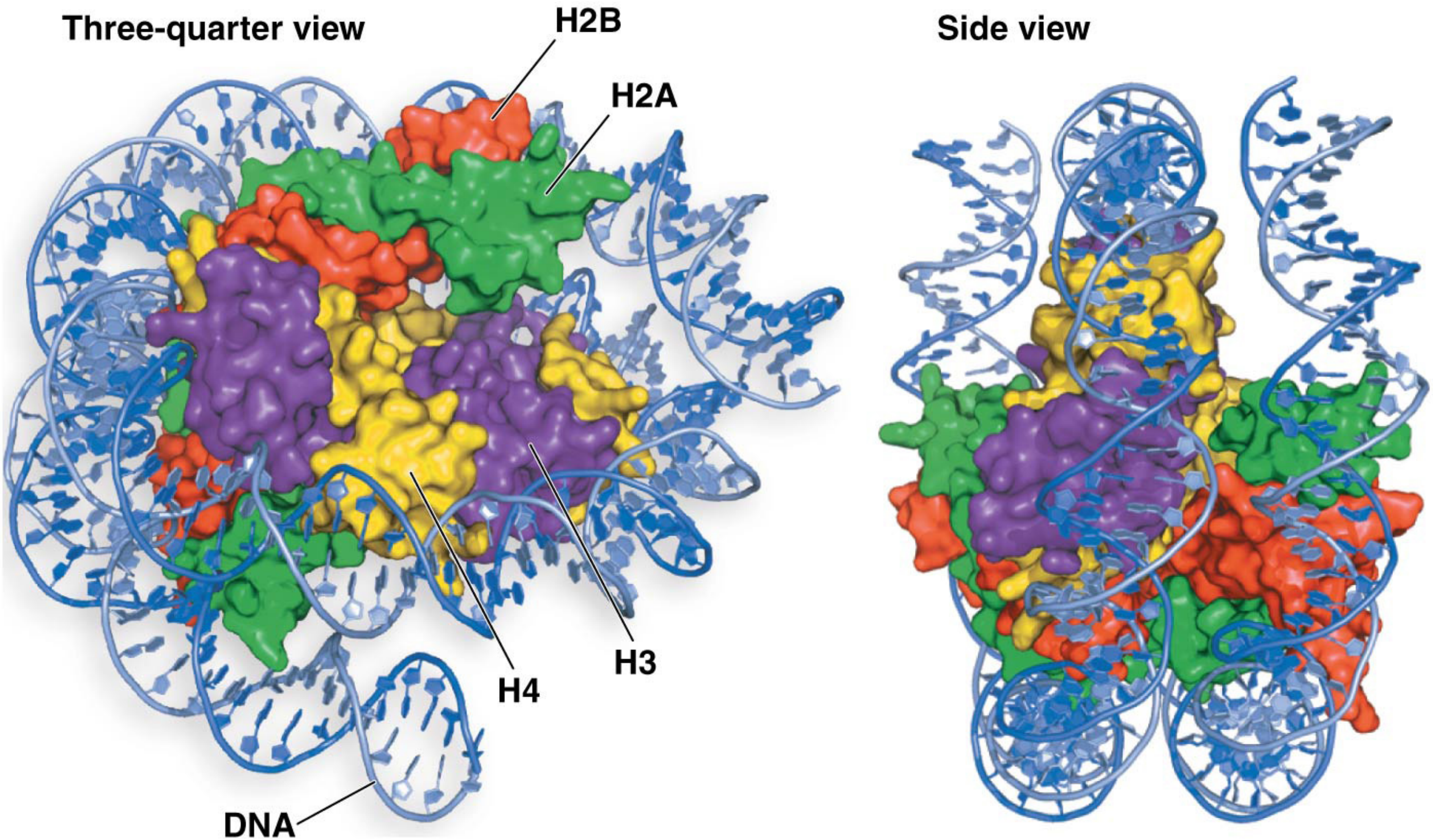
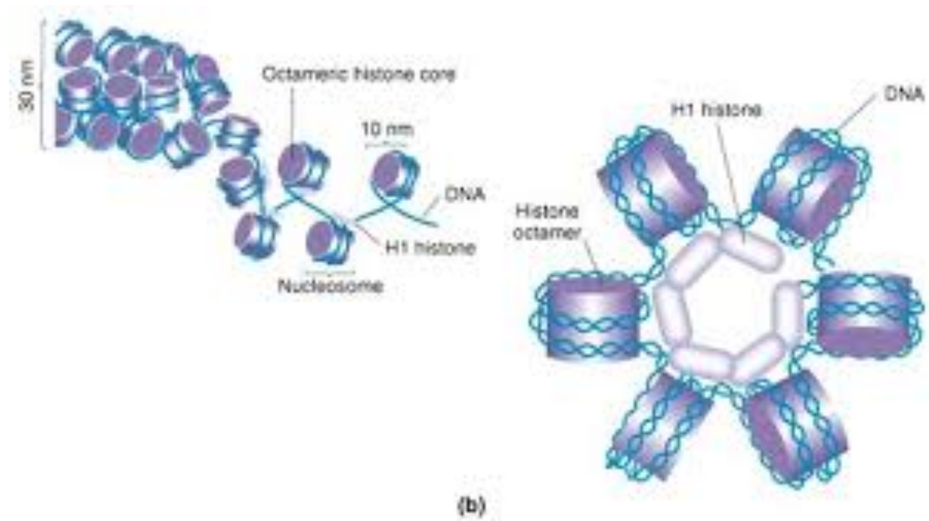


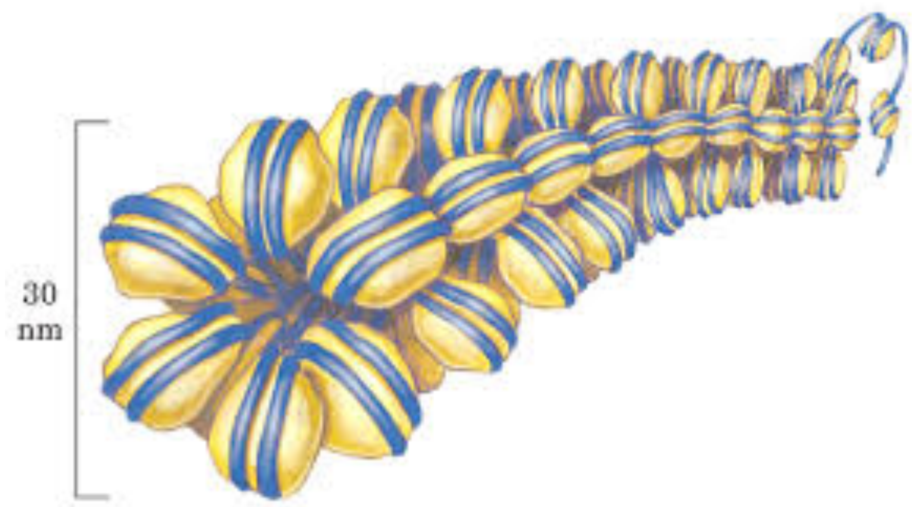
Figure 11.7 Rendering of X-Ray crystal structure of a nucleosome



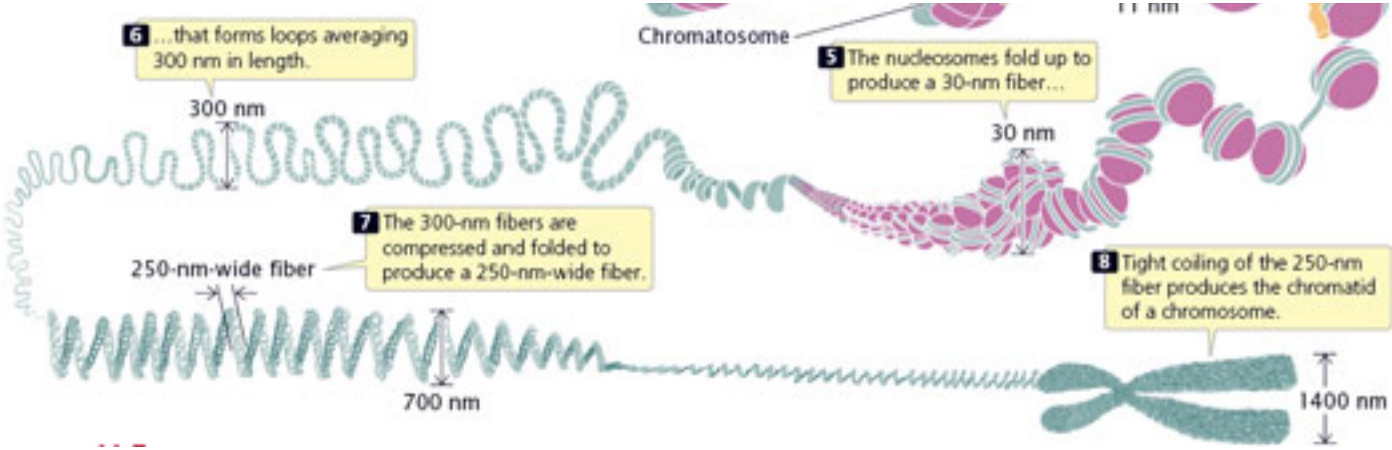
Solenoid Structure

- Instead, a **30-nm fiber** forms when the 10-nm fiber coils into a **solenoid structure**, with six to eight nucleosomes per turn and histone H1 stabilizing the solenoid in the center



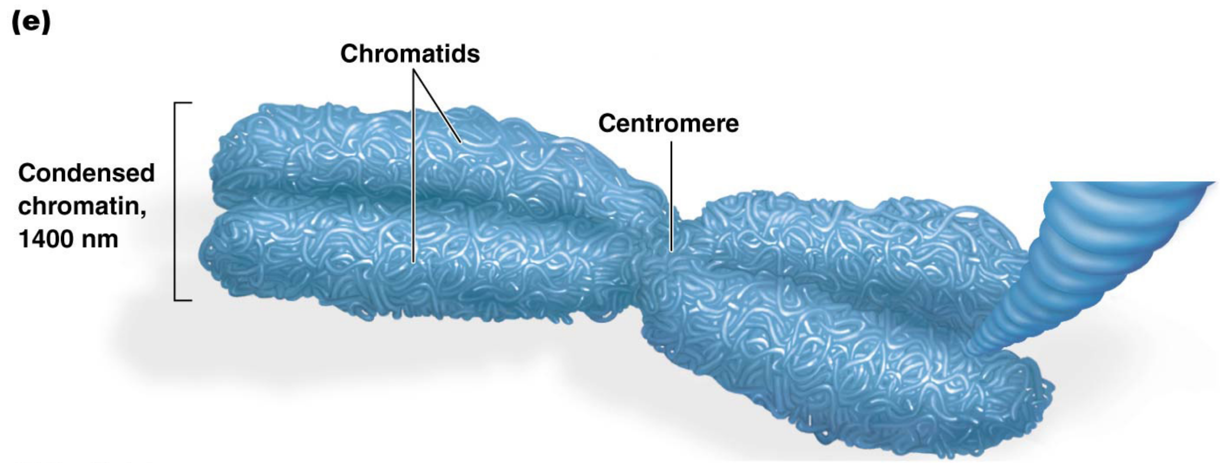


Higher-Order Chromatin Organization and Chromosome Structure

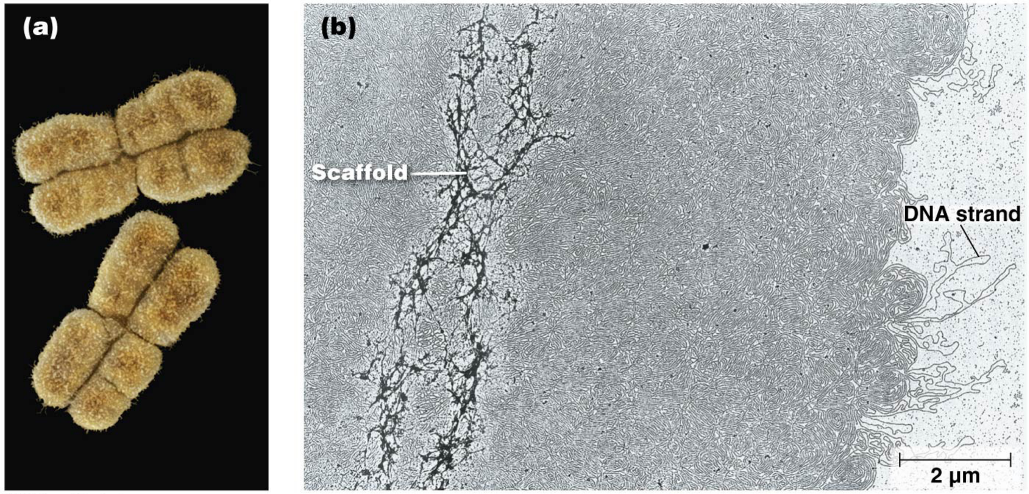


- Interphase chromosome structure results from the formation of **looped domains** of chromatin
- The loops are **constituted by** 30-nm fiber of DNA looped on non-histonic proteins
- The loops of chromatin form the **300-nm fiber**

Figure 11.6e Condensing the Nuclear Material



- The chromatin loops, with **continued condensation**, form the sister chromatids
- **Chromosome scaffold**: filamentous non-histone protein framework that gives chromosomes their shape (to anchor DNA loops)



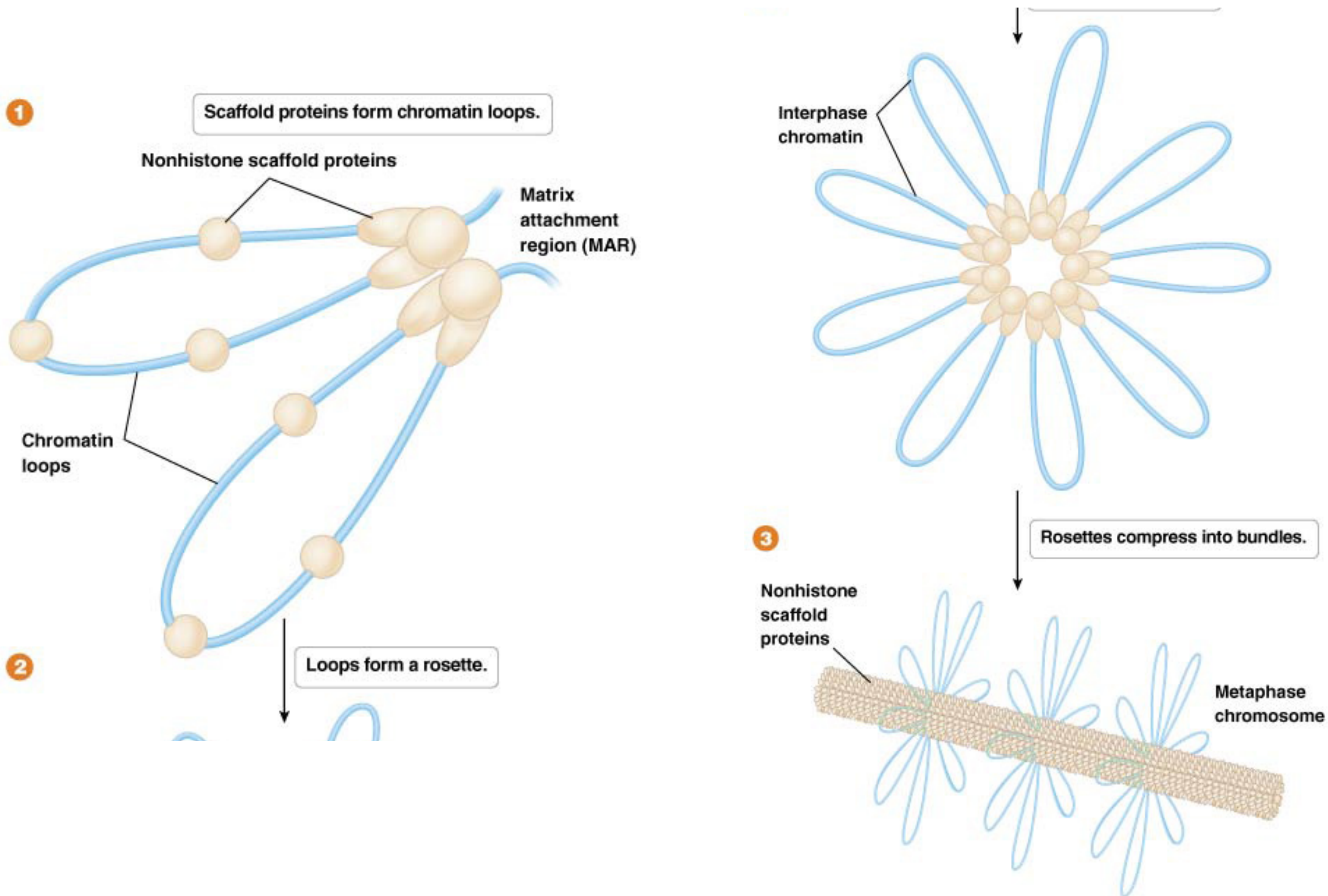
Higher Order Chromatin Condensation

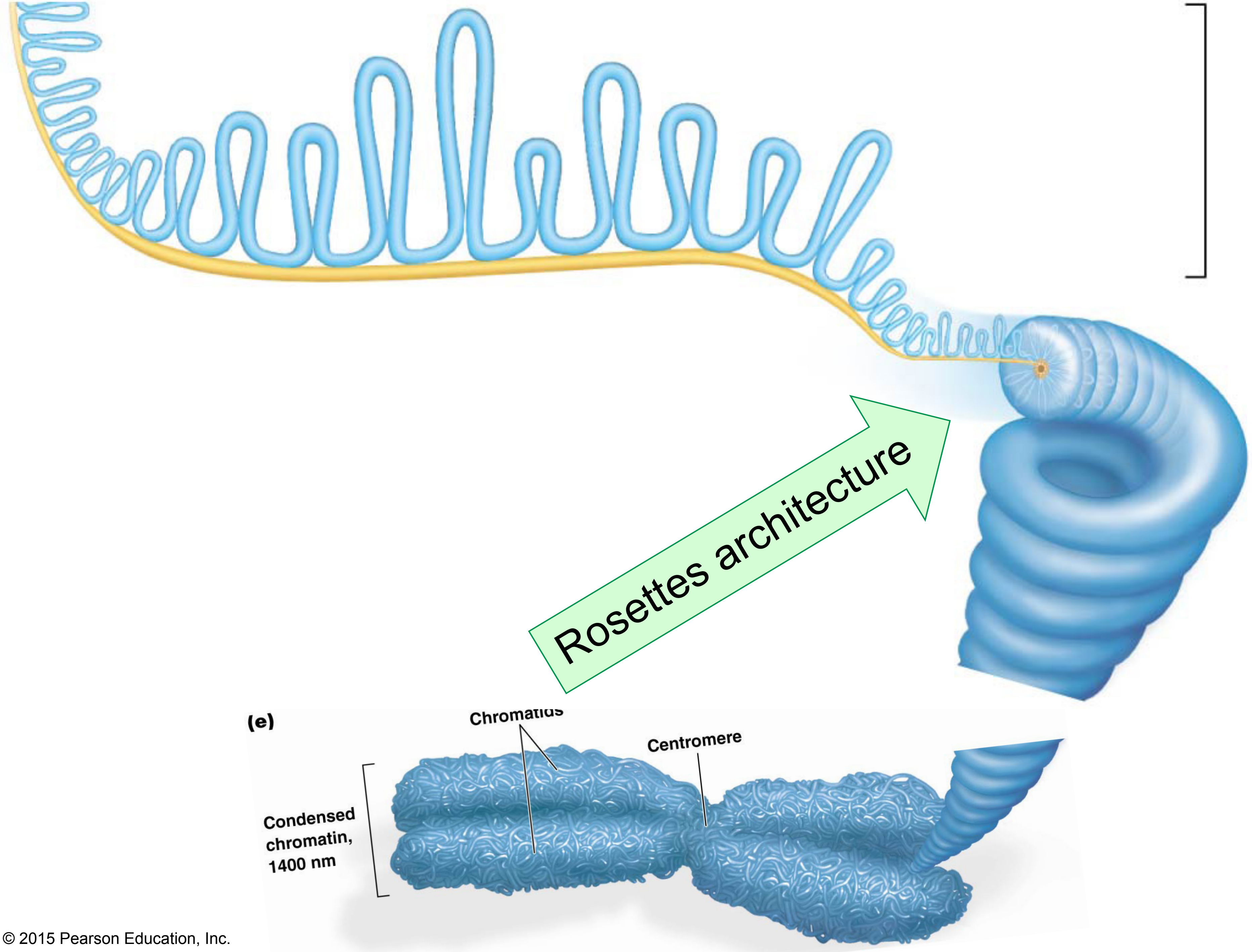
- Chromatin loops of 20 to 100 kb are anchored to the chromosome scaffold by nonhistone proteins at sites called **MARs** (**matrix attachment regions**)

- The **radial loop–scaffold model** suggests that the loops gather into “rosettes” and are further compressed by nonhistone proteins

- Metaphase chromatin is compacted 250-fold compared to the 300-nm fiber

Figure 11.9 The radial loop–scaffold model of chromatin condensation.




Rosettes architecture
(e)
Chromatids
Centromere
Condensed chromatin, 1400 nm