BHARATHIDASAN UNIVERSITY

Tiruchirappalli 620024
Tamilnadu, India

Program: M.Sc., Microbiology

Course Title : Microbial Genetics & Molecular Biology

Course Code: 24MICCC3

Unit I: Molecules of Life

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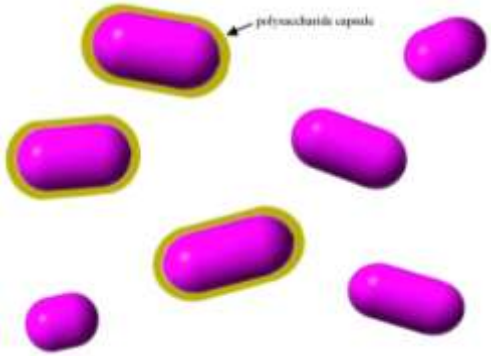
KEY CONCEPT

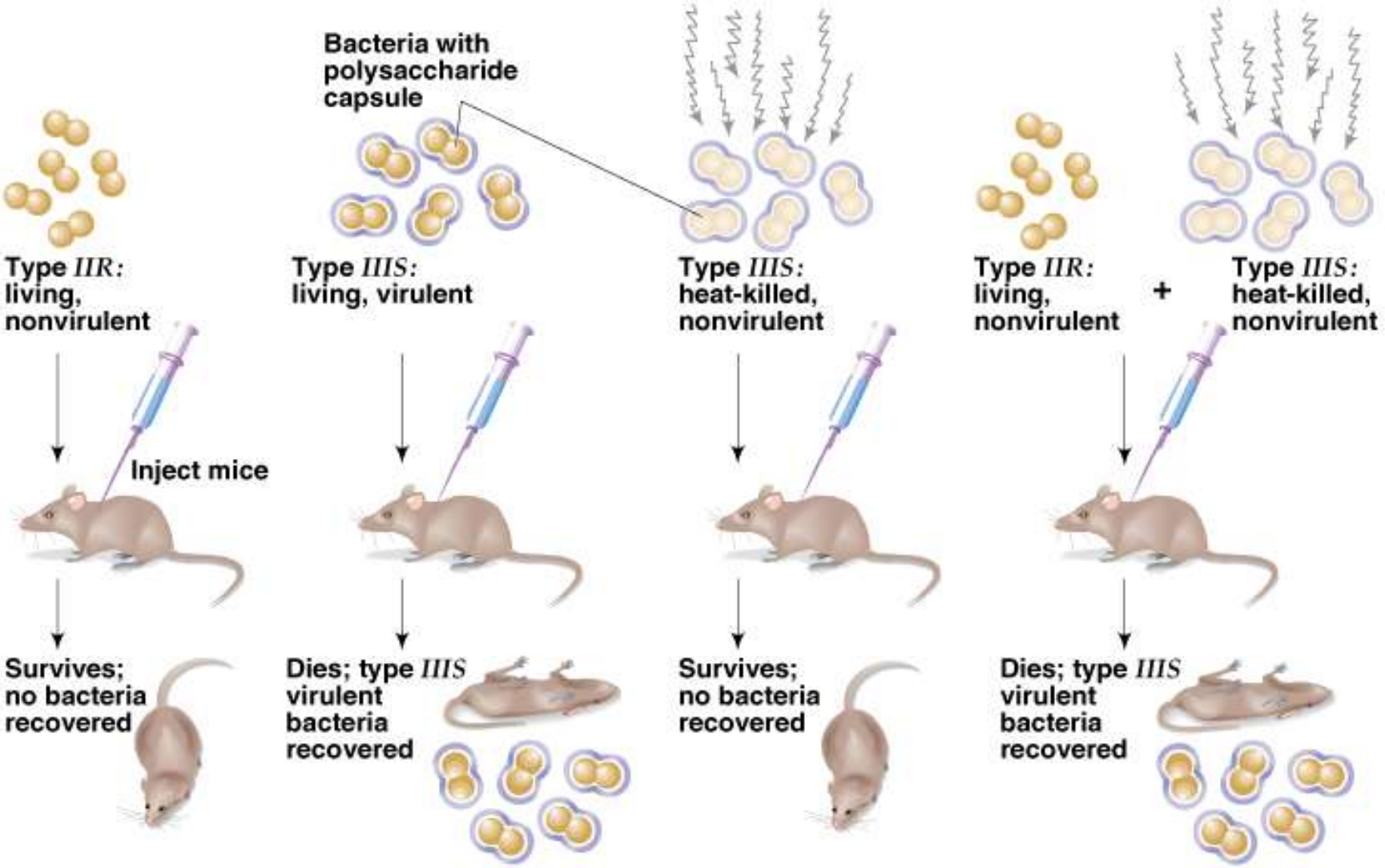
DNA was identified as the genetic material through a series of experiments.

Frederick Griffith (1928)

- Microbiologist studying the bacterium that causes pneumonia to create a vaccine
  - He studied two types
    - » S- smooth- Pneumonia + death
    - » R- rough- Pneumonia + recovery



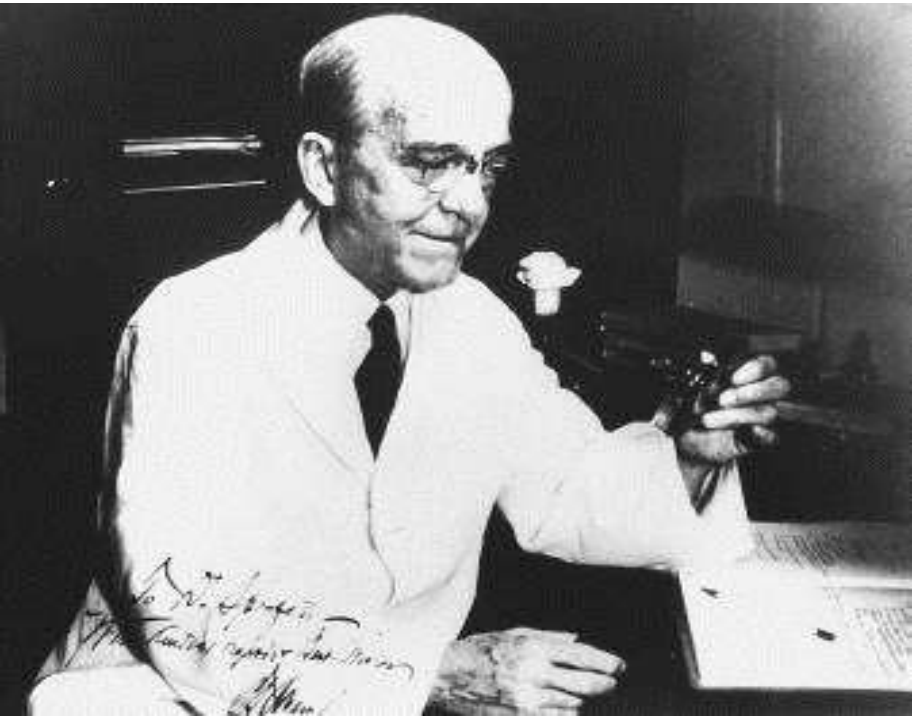
Griffith's transformation experiment



He called this agent the transforming principle, but did not know what it was or how it worked.

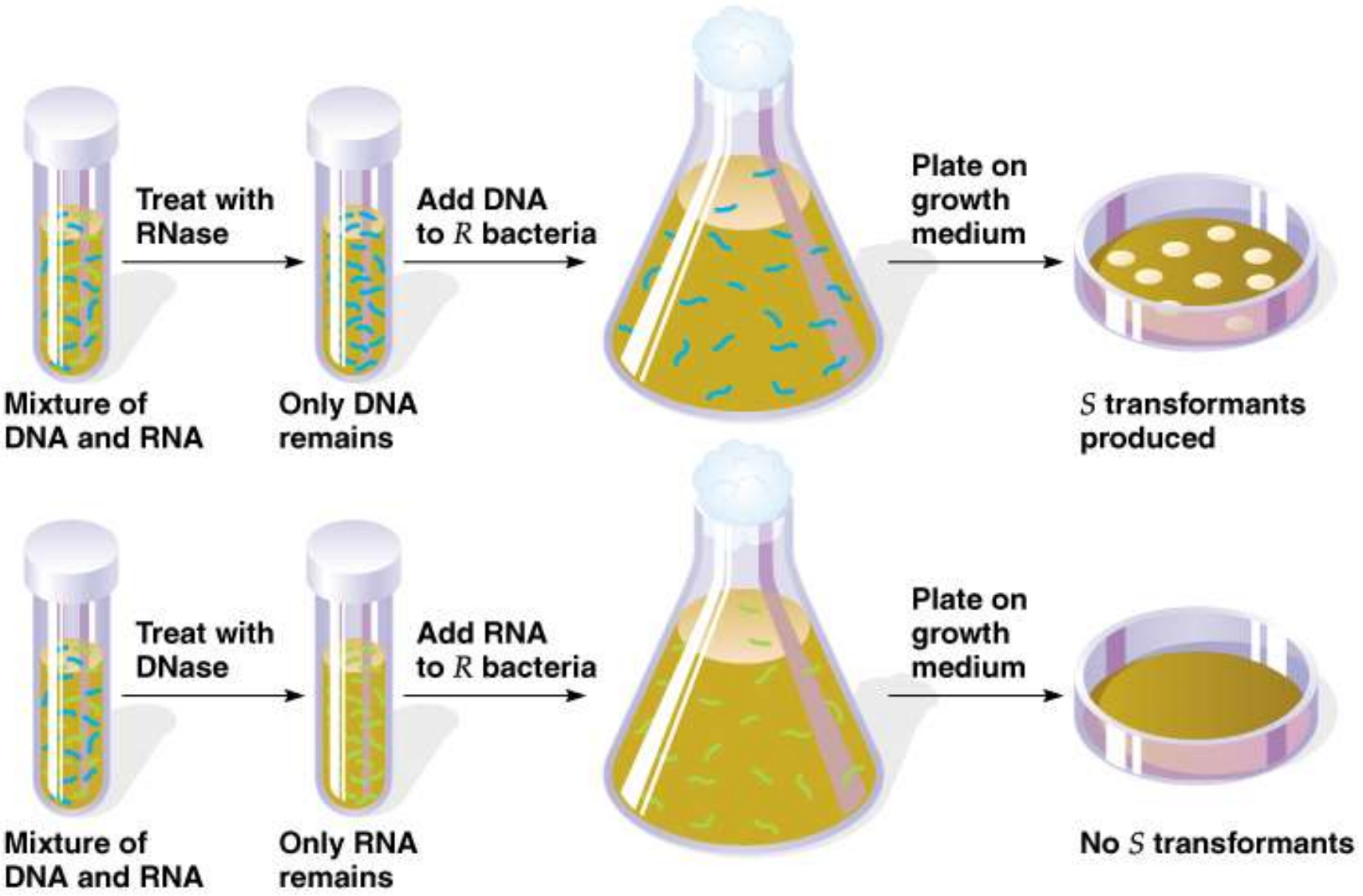
- **<u>Oswald Avery</u>**, a biologist, spent 10 years trying to figure out what Griffith had discovered (1944)
- He combined R bacteria with an extract made from S bacteria and observed R bacteria **turning into** S bacteria.

Avery, MacLeod, and McCarty prepared identical extracts of the heat-killed S-strain and subjected each extract to one of three enzymes:



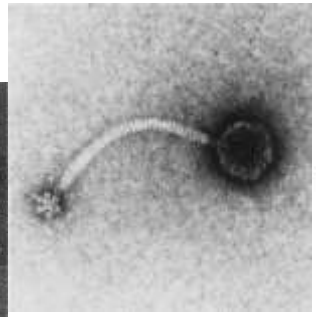
- Broke down the **Heat-killed S bacteria** into its components
  - Carbohydrates
  - Lipids
  - Proteins
  - DNA
  - RNA

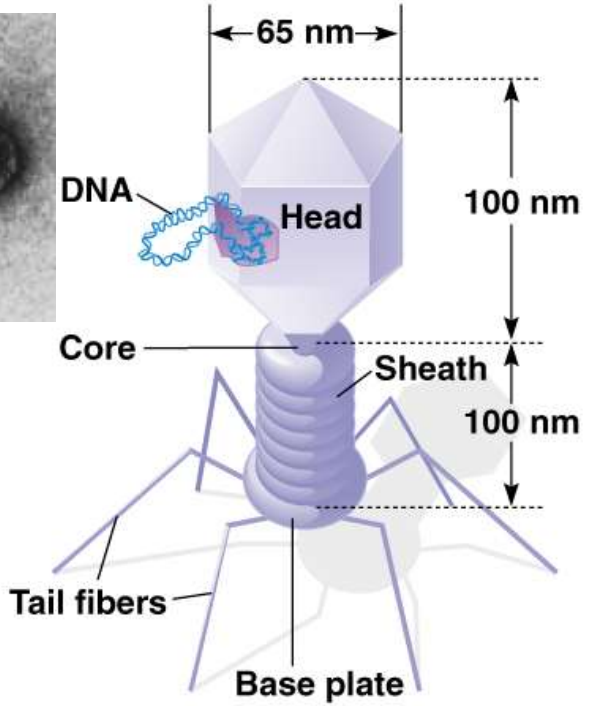
Experiment that showed that DNA, not RNA, was the transforming principle



Identifying DNA as the Material of Heredity: Hershey and Chase

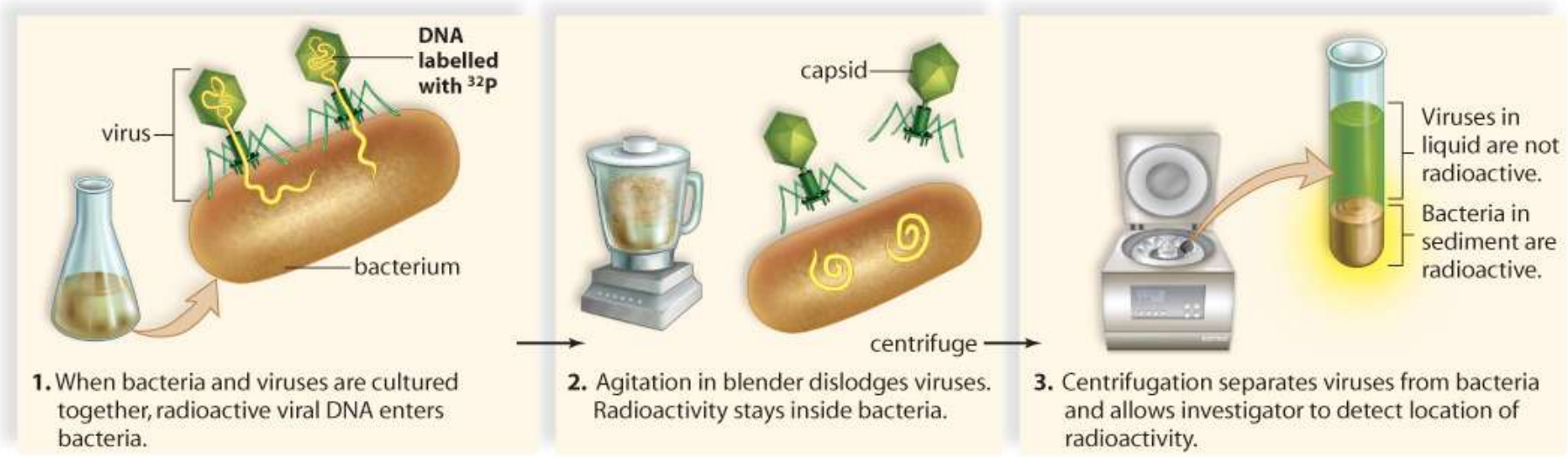
- In 1952, Americans Alfred Hershey and Martha Chase ruled out protein as the hereditary material. Their experiments used T2 bacteriophages, which consist of nucleic material surrounded by a protein coat.
- Hershey and Chase used two different radioactive isotopes to track each molecule (^{35}S for proteins and ^{32}P for DNA).





In their first experiment:

- a virus with DNA radioactively labelled with ^{32}P was allowed to infect bacteria. After agitation and separation, radioactivity was found in the bacteria pellet but not in the liquid medium.

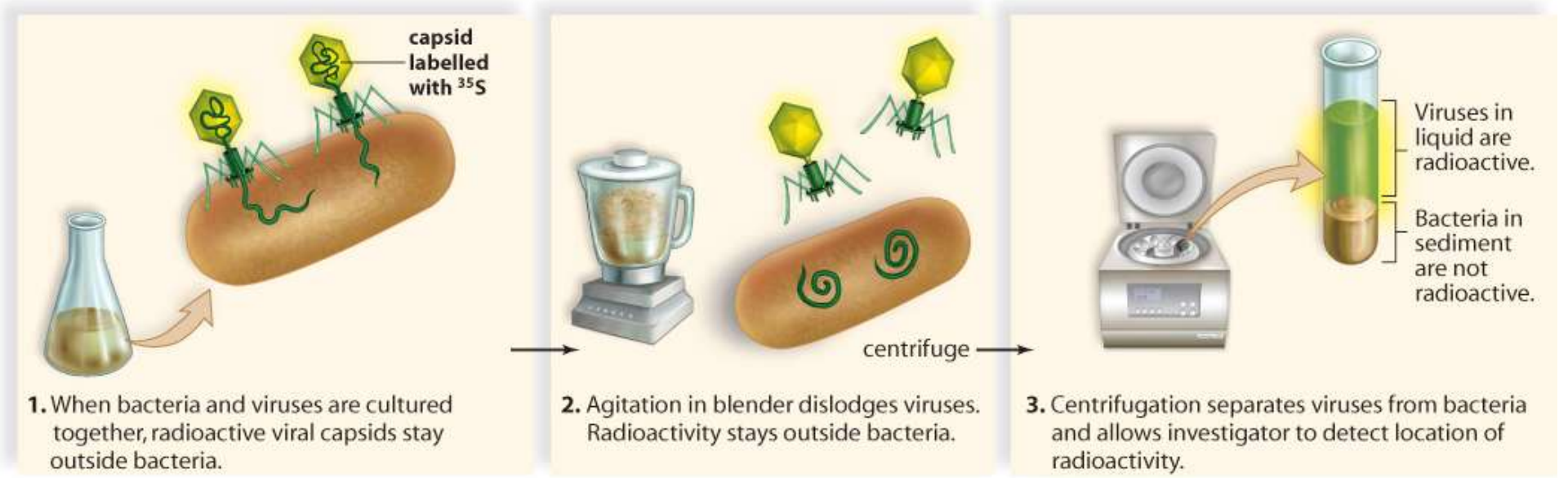


a. Viral DNA is labelled (yellow).

- ✓ Tagged DNA was **found inside the bacteria**; tagged proteins were not.
- ✓ **<u>This confirmed that DNA was the genetic material!</u>**

In their second experiment:

- A virus with its protein coat radioactively labeled with ^{35}S was allowed to infect bacteria. After agitation and separation, radioactivity was found in the liquid medium but not in the bacteria pellet. The results proved that viral DNA held the genetic material needed for viruses to reproduce.



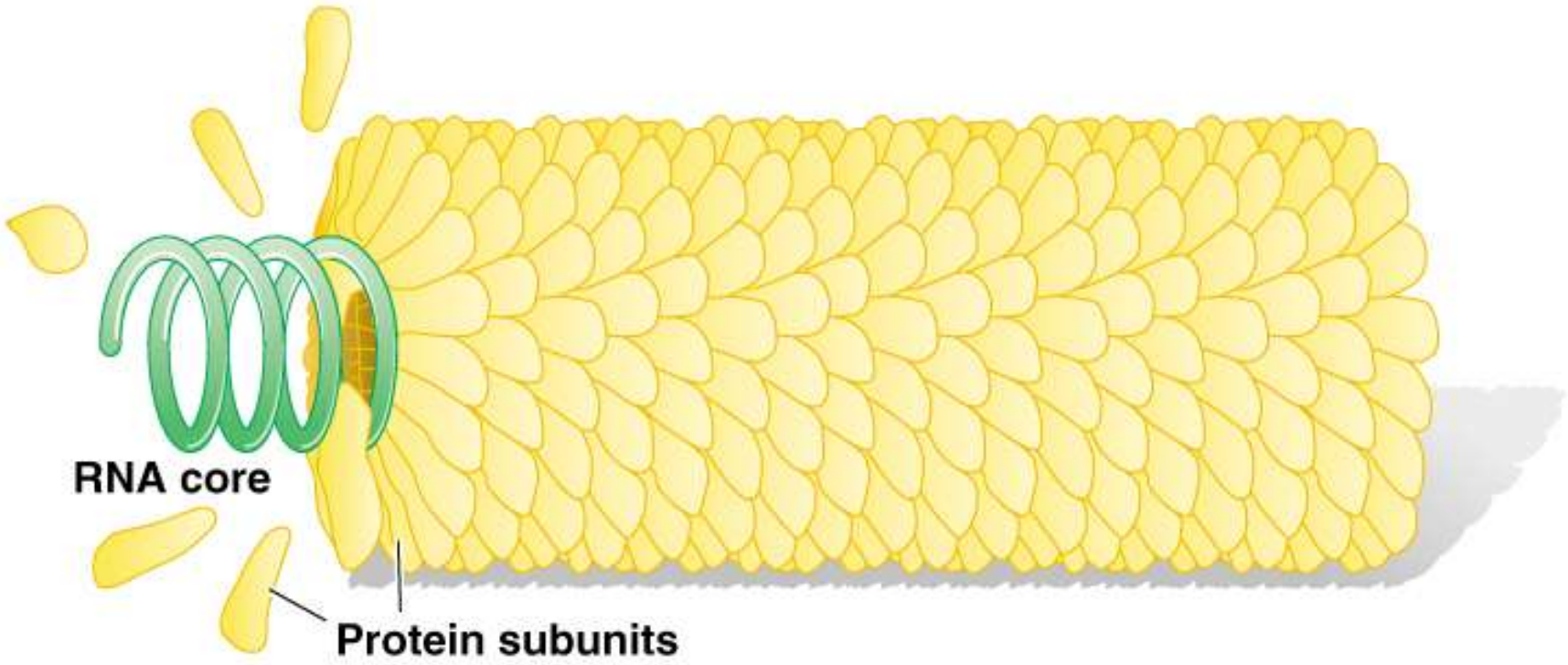
b. Viral capsid is labelled (yellow).

✓ **<u>This confirmed that DNA was the genetic material!</u>**

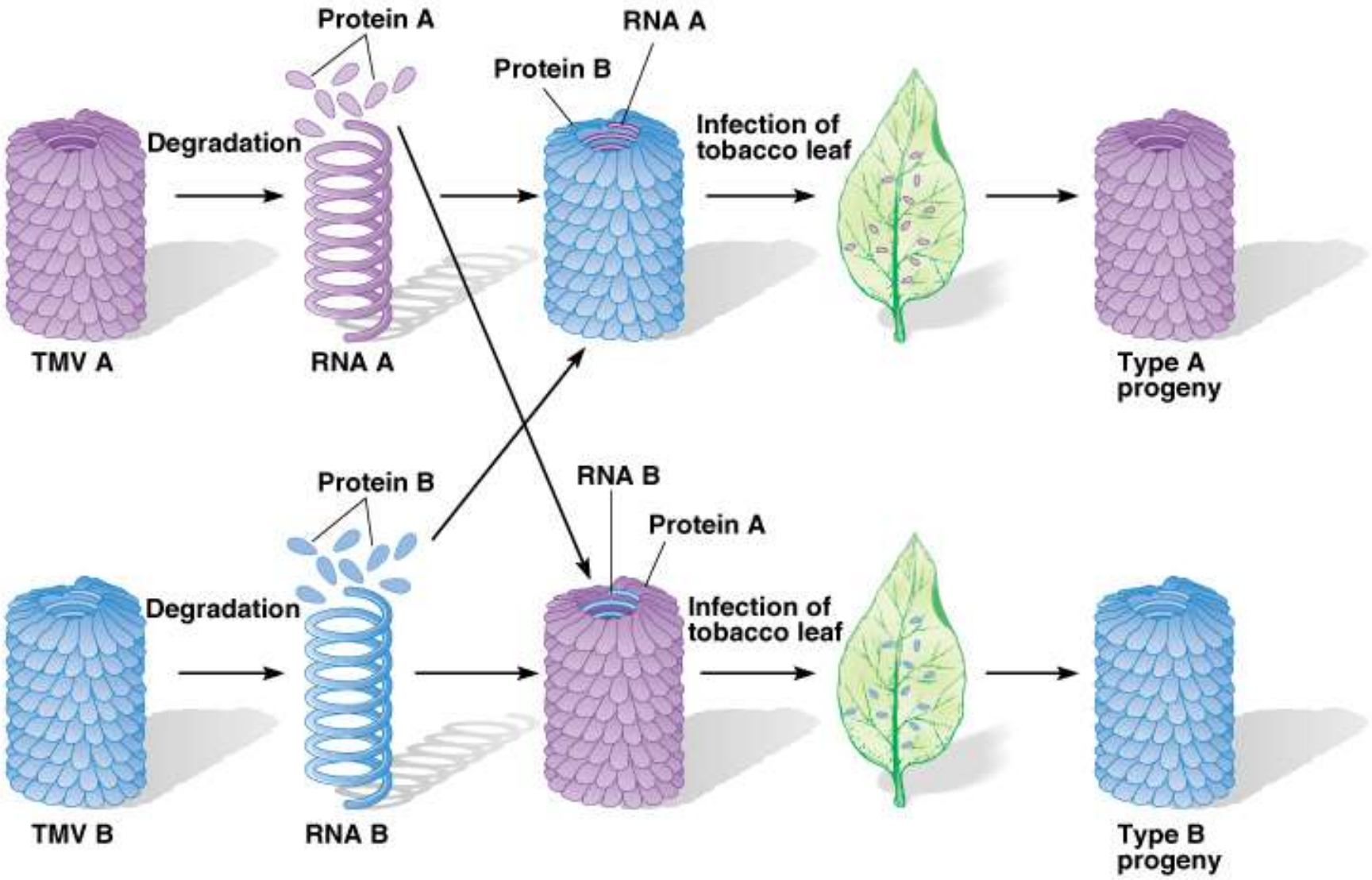
The Discovery of RNA as Viral Genetic Material

1. All known cellular organisms have DNA as their genetic material. Some viruses, however, use RNA instead.
2. Tobacco mosaic virus (TMV) is composed of RNA and protein; it contains no DNA. In 1956 Gierer and Schramm showed that when purified RNA from TMV is applied directly to tobacco leaves, they develop mosaic disease. Pretreating the purified RNA with RNase destroys its ability to cause TMV lesions
2. In 1957 Fraenkel-Conrat and Singer showed that in TMV infections with viruses containing RNA from one strain and protein from another, the progeny viruses were always of the type specified by the RNA, not by the protein.

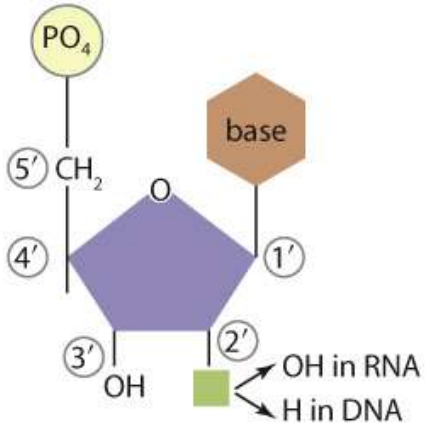
Typical tobacco mosaic virus (TMV) particle



Demonstration that RNA is the genetic material in tobacco mosaic virus (TMV)



Determining the Chemical Composition and Structure of DNA



DNA was discovered in 1869 by Fredrich Miescher. By isolating the nuclei of white blood cells, he extracted an acidic molecule he called *nuclein*.

In the early 1900s, Phoebus Levene isolated two types of nucleic acid: RNA and DNA. In 1919, he proposed that both were made up of individual units called **nucleotides**. Each nucleotide was composed of one of four nitrogen-containing bases, a sugar, and a phosphate group.

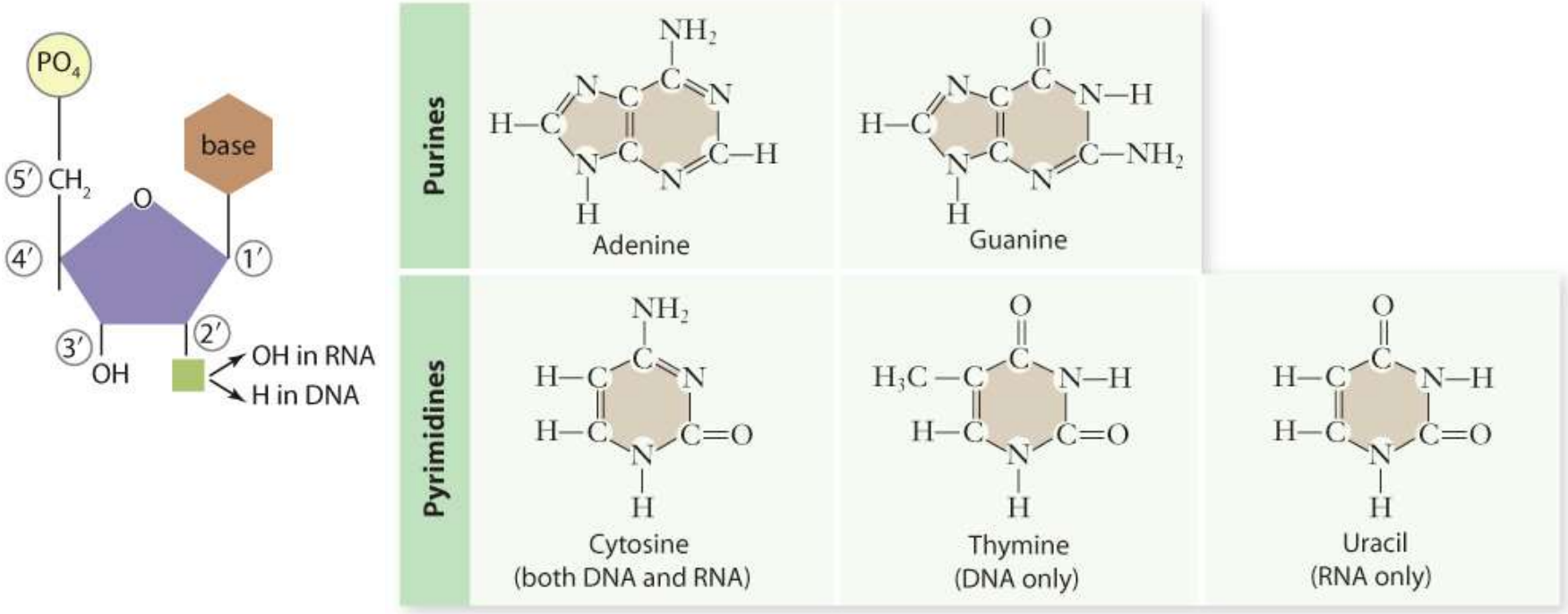
The Chemical Composition of the Nucleotides, DNA, and RNA

In later years, other scientists confirmed and extended Levene's work. DNA and RNA are both made up of a combination of four different nucleotides.

Nucleotides are often identified by referring to their bases:

- DNA has the nucleotides adenine (A), guanine (G), cytosine (C), and thymine (T).
- RNA has the nucleotides adenine (A), guanine (G), cytosine (C), and uracil (U).

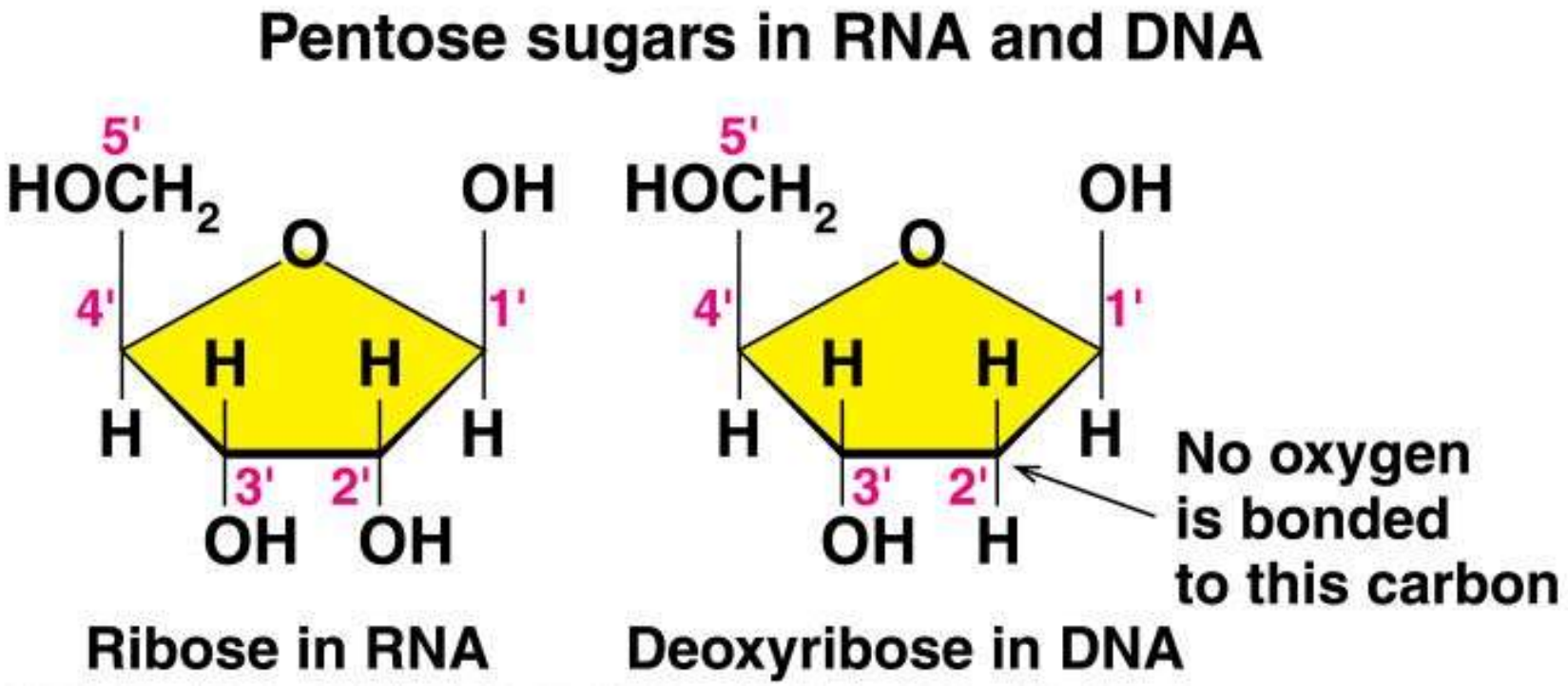
The Chemical Composition of the Nucleotides, DNA, and RNA



The general structure of a DNA nucleotide includes a phosphate group, a deoxyribose sugar group, and a nitrogen-containing base. Nucleotides in RNA have the same basic structure, except a ribose sugar group is used. The sugar groups differ by a hydroxyl group at the 2′ carbon. Both DNA and RNA contain the same purine bases and the cytosine pyrimidine base. However, thymine is only present in DNA, and uracil is only present in RNA.

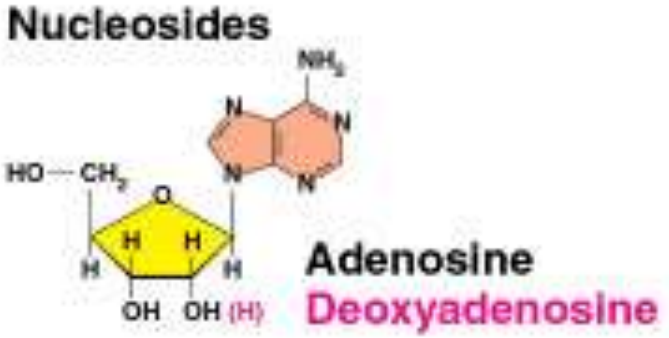
Pentose Sugars

- There are two related **pentose sugars**:
  - RNA contains **ribose**
  - DNA contains **deoxyribose**
- The sugars have their carbon atoms numbered with primes to distinguish them from the nitrogen bases



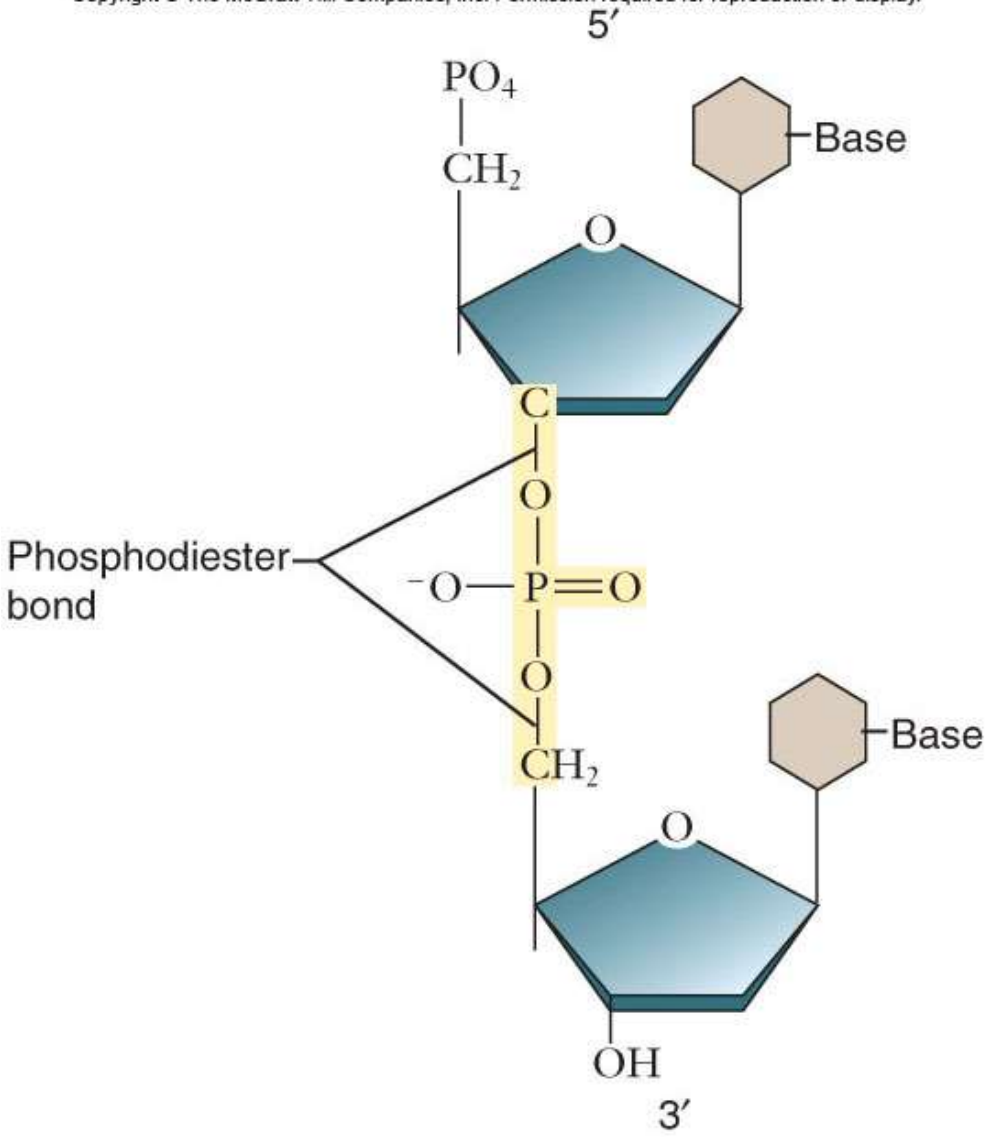
Nucleosides and Nucleotides

- A nucleoside consists of a nitrogen base linked by a glycosidic bond to C1’ of a ribose or deoxyribose
- Nucleosides are named by changing the the nitrogen base ending to *-osine* for purines and *–idine* for pyrimidines
- A nucleotide is a nucleoside that forms a phosphate ester with the C5’ OH group of ribose or deoxyribose
- Nucleotides are named using the name of the nucleoside followed by *5’-monophosphate*

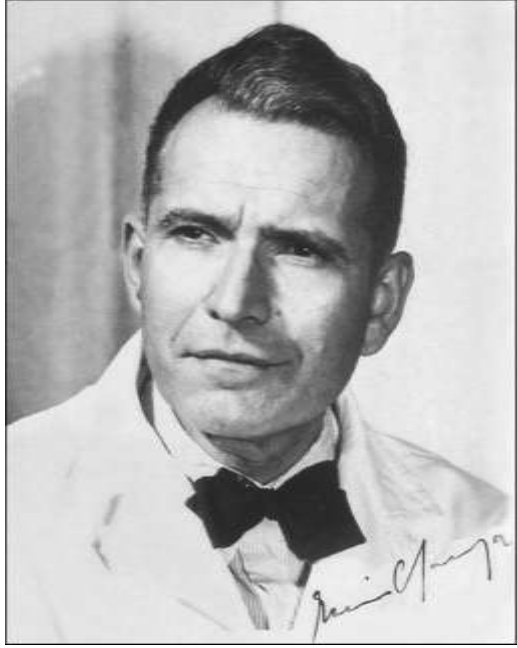


Names of Nucleosides and Nucleotides

Base	Nucleosides	Nucleotides
RNA		
Adenine (A)	Adenosine (A)	Adenosine 5'-monophosphate (AMP)
Guanine (G)	Guanosine (G)	Guanosine 5'-monophosphate (GMP)
Cytosine (C)	Cytidine (C)	Cytidine 5'-monophosphate (CMP)
Uracil (U)	Uridine (U)	Uridine 5'-monophosphate (UMP)
DNA		
Adenine (A)	Deoxyadenosine (A)	Deoxyadenosine 5'-monophosphate (dAMP)
Guanine (G)	Deoxyguanosine (G)	Deoxyguanosine 5'-monophosphate (dGMP)
Cytosine (C)	Deoxycytidine (C)	Deoxycytidine 5'-monophosphate (dCMP)
Thymine (T)	Deoxythymidine (T)	Deoxythymidine 5'-monophosphate (dTMP)



Chargaff's Rule: Closing in on the Structure of DNA



Erwin Chargaff was inspired by Avery, MacLeod, and McCarty's work on DNA and launched a research program to study nucleic acids. By the late 1940s, he had reached two conclusions:

- There is variation in the composition of nucleotides in different species.
- Regardless of the species, DNA maintains certain nucleotide proportions. That is, the amount of A and T nucleotides are equal and the amount of C and G nucleotides are equal. This constant relationship is known as **Chargaff's rule**.

Chargaff's Rule: Closing in on the Structure of DNA

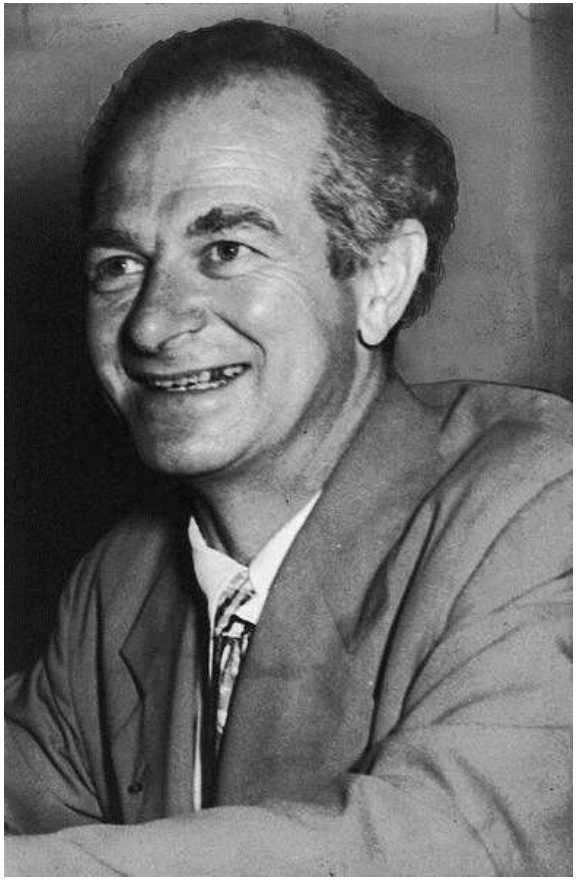
In DNA, the percent composition of adenine is the same as thymine, and the percent composition of cytosine is the same as guanine.

Table 5.1 Percent Composition of Each Base from DNA of Several Species

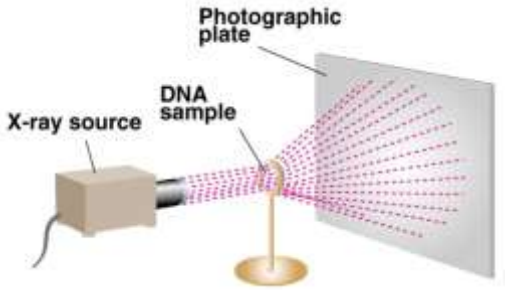
Organism	Adenine	Thymine	Guanine	Cytosine
Mycobacterium tuberculosis	15.1	14.6	34.9	35.4
Escherichia coli	26.0	23.9	24.9	25.2
Yeast	31.3	32.9	18.7	17.1
Drosophila melanogaster	27.3	27.6	22.5	22.5
Mouse	29.2	29.4	21.7	19.7
Human (liver)	30.7	31.2	19.3	18.8

Pauling Discovers a Helical Structure for Proteins

In 1951, Linus Pauling discovered that many proteins have helix-shaped structures. Scientists, including James Watson and Francis Crick, used this information when deducing the structure of DNA.

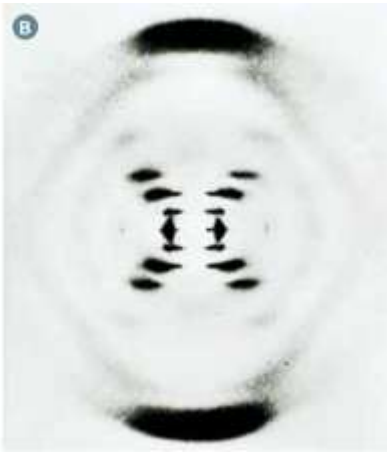


Franklin Determines a Helical Structure for DNA



In the early 1950s, Rosalind Franklin and Maurice Wilkins used X-ray diffraction to analyze DNA samples. Franklin captured high-resolution photographs and, using mathematical theory to interpret them, determined the following:

- DNA has a helical structure.
- The nitrogen bases are on the inside of the DNA helix, and the sugar-phosphate backbone is on the outside.



(A) Rosalind Franklin was a British chemist who was hired to work alongside Maurice Wilkins at the X-ray diffraction facilities at King's College.
(**B**) In the diffraction image of DNA that she produced, the central x-shaped pattern enabled researchers to infer that DNA has a helical structure.

Watson and Crick Build a Three-Dimensional Model for DNA

In the early 1950s, Watson and Crick began working on a description of the structure of DNA using the results and conclusions of their peers.

In 1953, they published a paper that proposed a structure with the following features:

- a twisted ladder, which they called a double-helix. The sugar-phosphate molecules make up the sides or “handrails” of the ladder, and the bases make up the “rungs” of the ladder by protruding inwards.

1962: Nobel Prize in Physiology and Medicine

Watson, J.D. and F.H. Crick, "Molecular Structure of Nucleic Acids: A Structure for Deoxynucleic Acids". *Nature* 171 (1953), p. 738.

James D. Watson

Francis H. Crick

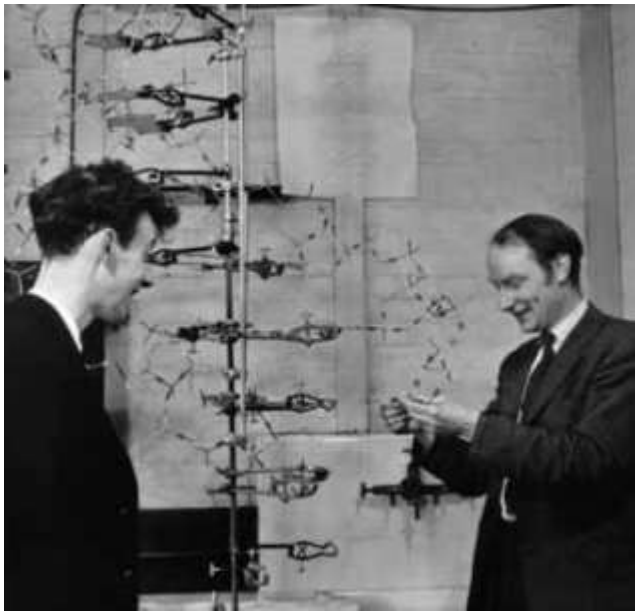
Maurice H. F. Wilkins

What about?
Rosalind Franklin

Watson and Crick Build a Three-Dimensional Model for DNA

- The distance between the sugar-phosphate backbones remains constant over the length of a molecule of DNA. An A nucleotide on one strand always sits across from a T nucleotide (and C across from G) in order to maintain constant distance. These are called *base pairs*.

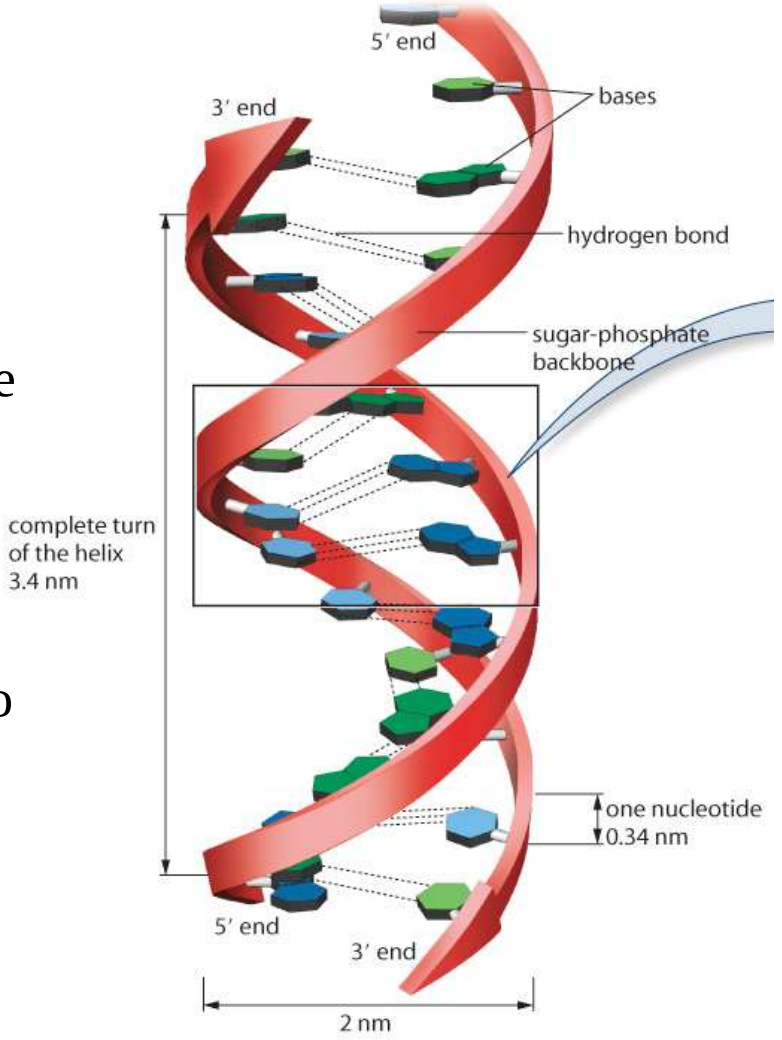
- Different sequences of base pairs can exist, which accounts for the differences between species.



The Modern DNA Model: The DNA Double Helix

Structural features of DNA:

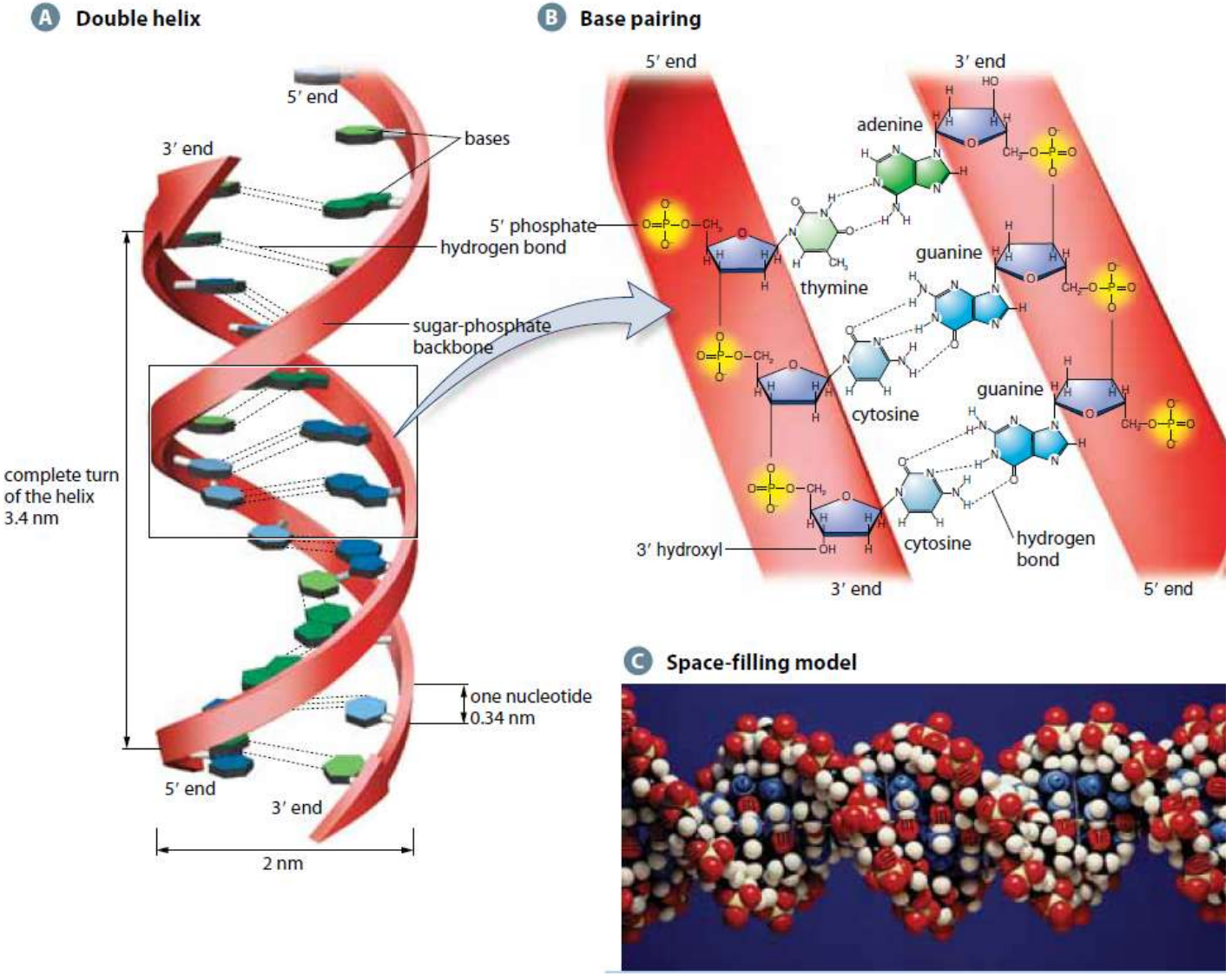
- The double helix is composed of two polynucleotide strands that twist around one another. Each strand has a backbone of alternating phosphate groups and sugars.
- The distance between the sugar-phosphate backbones in each strand is constant.
- The bases of each nucleotide are attached to each sugar and face inward.

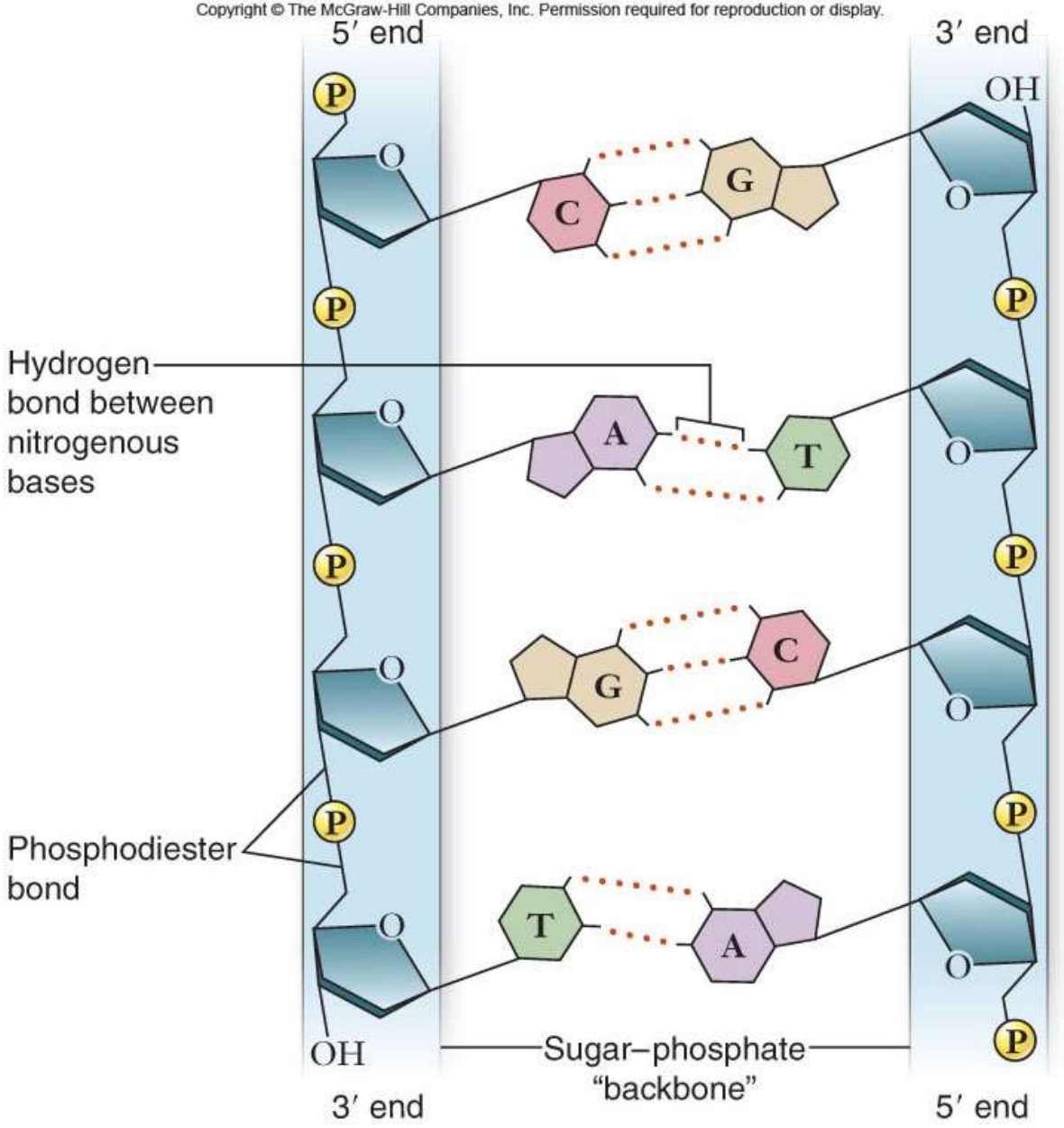


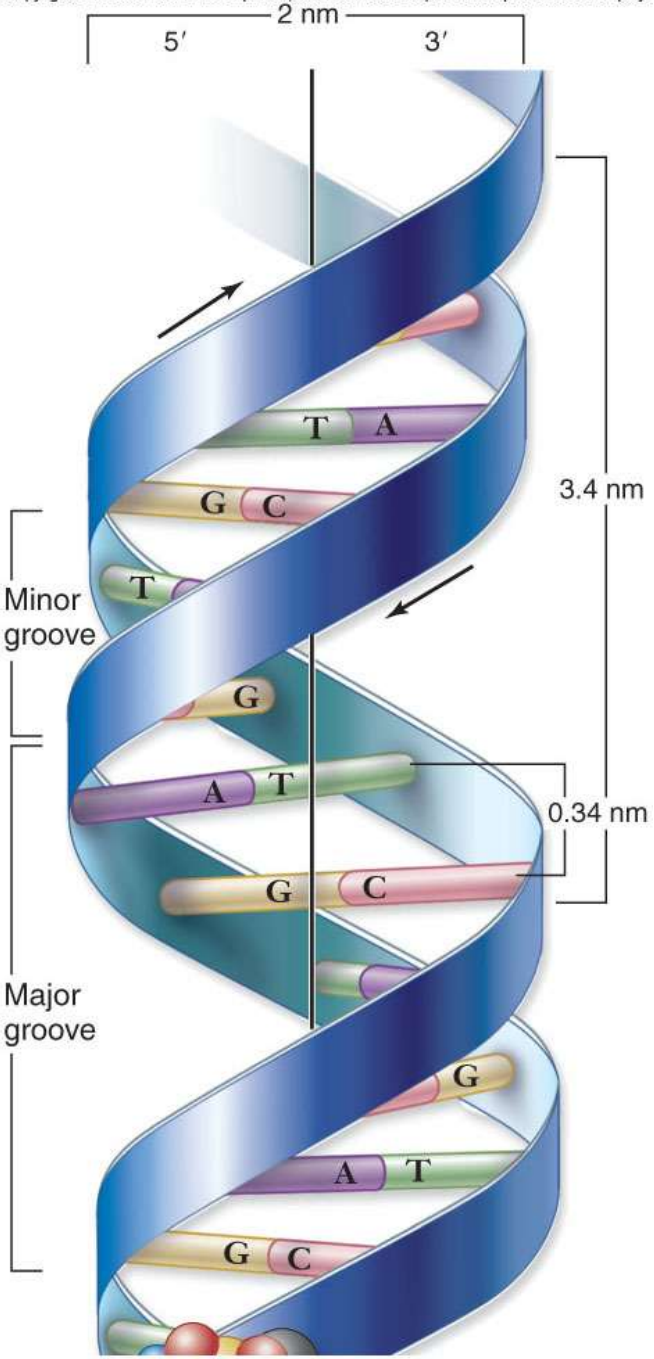
The Modern DNA Model: The DNA Double Helix

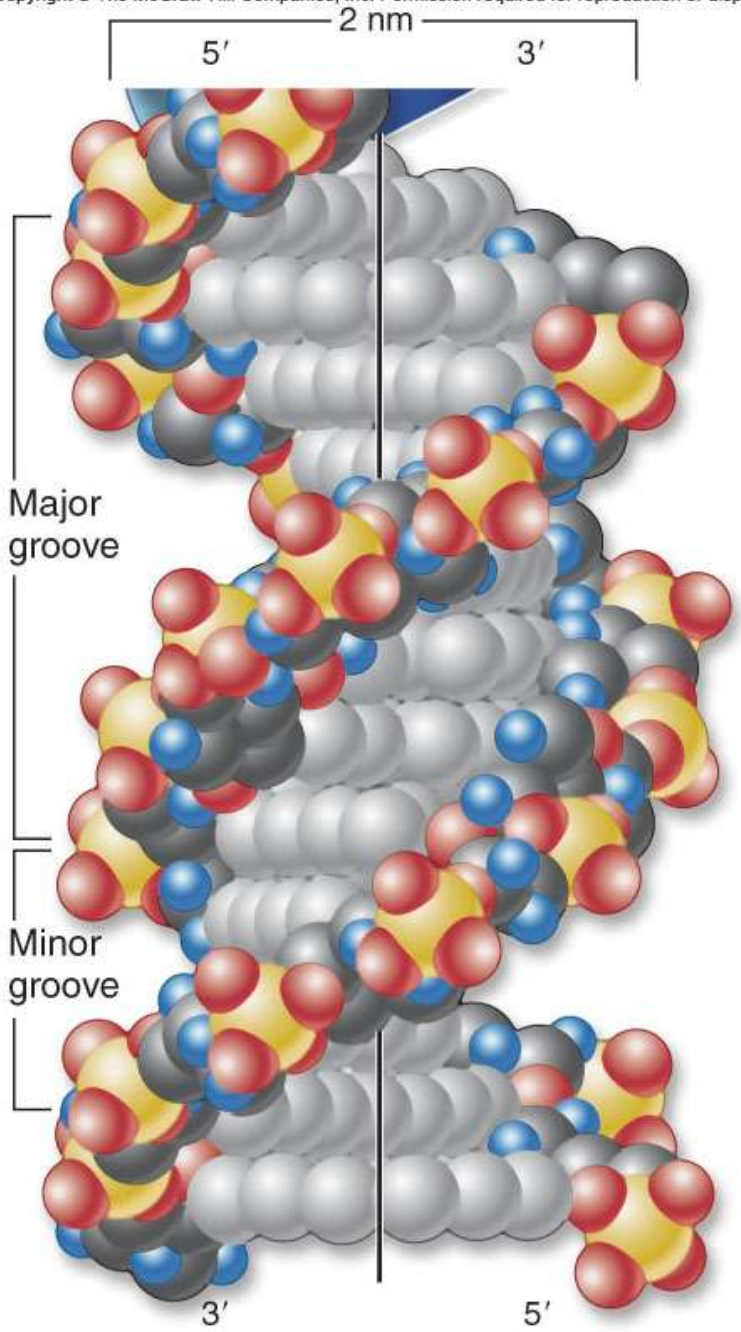
- The two strands are complementary due to **complementary base pairing** of A with T and C with G. Hydrogen bonds link each complementary base pair.
- Each strand has a 5′ end and a 3′ end. The 5′ and 3′ come from the numbering of the carbons in the deoxyribose sugar.
- The two strands are **antiparallel**, where the 5′ end from one strand is across from the 3′ end of the complementary strand.
- The sequence of a DNA strand is written in the 5′ to 3′ direction.

The DNA Double Helix



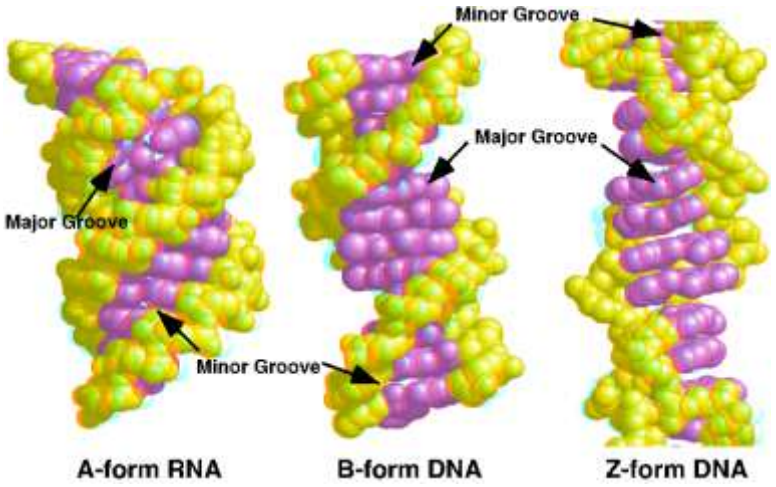






Types of DNA Structures

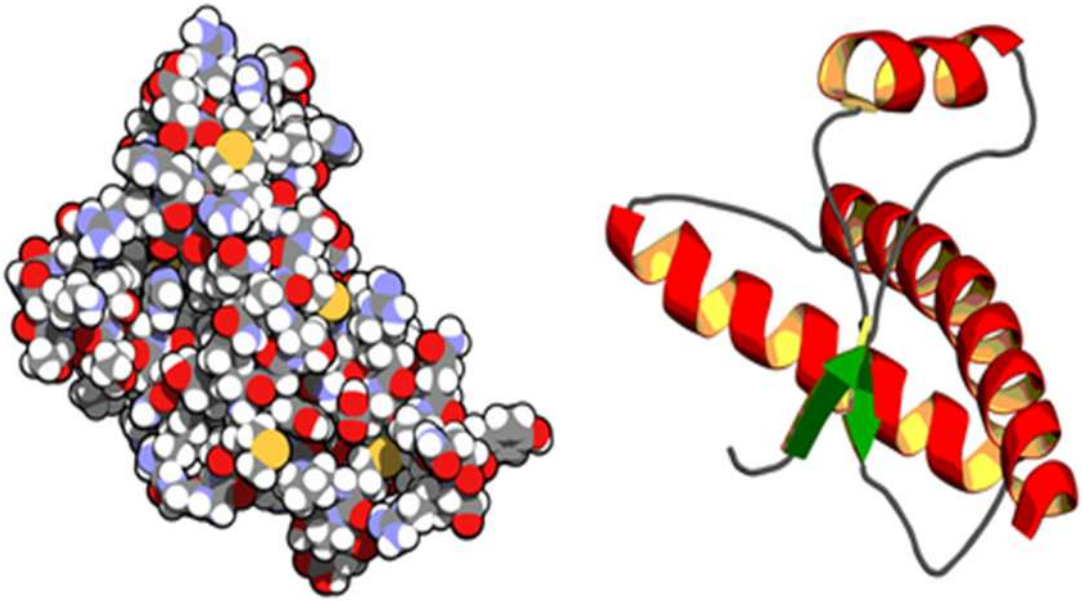
- Three forms of DNA
  - **A form**: right handed helix
  - **B form**: the most likely biological conformation, right handed helix
  - **Z form**: form a left handed helix;



Chemical Properties of DNA

- Factors that affect DNA structure
  - Temperature: denaturation (can be reversible)
  - pH: high pH can denature DNA
  - Salt concentration: lowering salt concentration can denature DNA
  - Chemicals: sodium hydroxide, formamide can also denature DNA

Proteins (Prions)



- **Stanley B. Prusiner** coined the term proin from **Pro**teinaceous **in**fective particle and changed to prion to sound it rhythmic.
- Prion diseases were caused by misfolded proteins.
- Elucidated the gene and mechanism by which wild type protein bring about the clinical disease

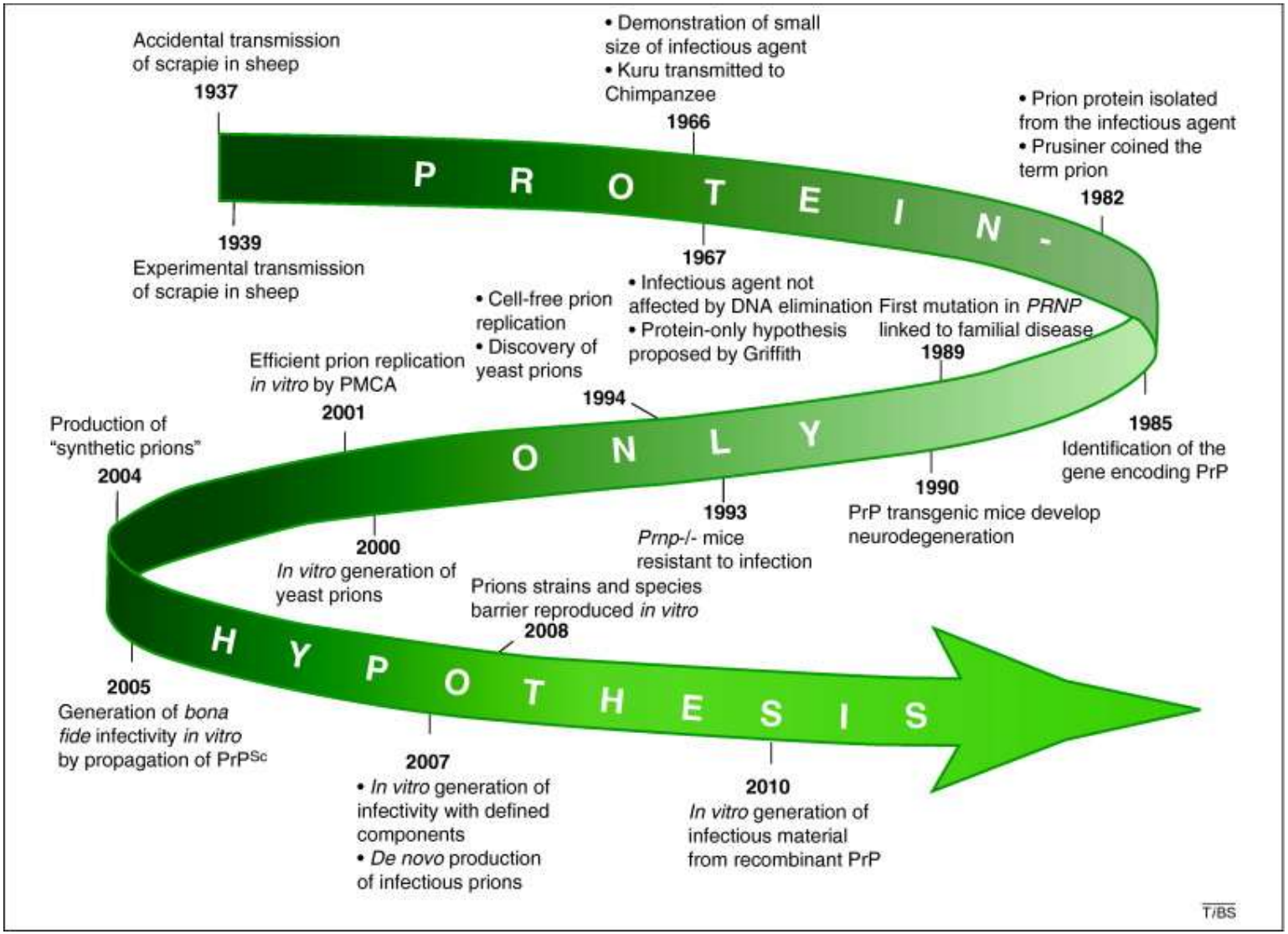
Prion Diseases

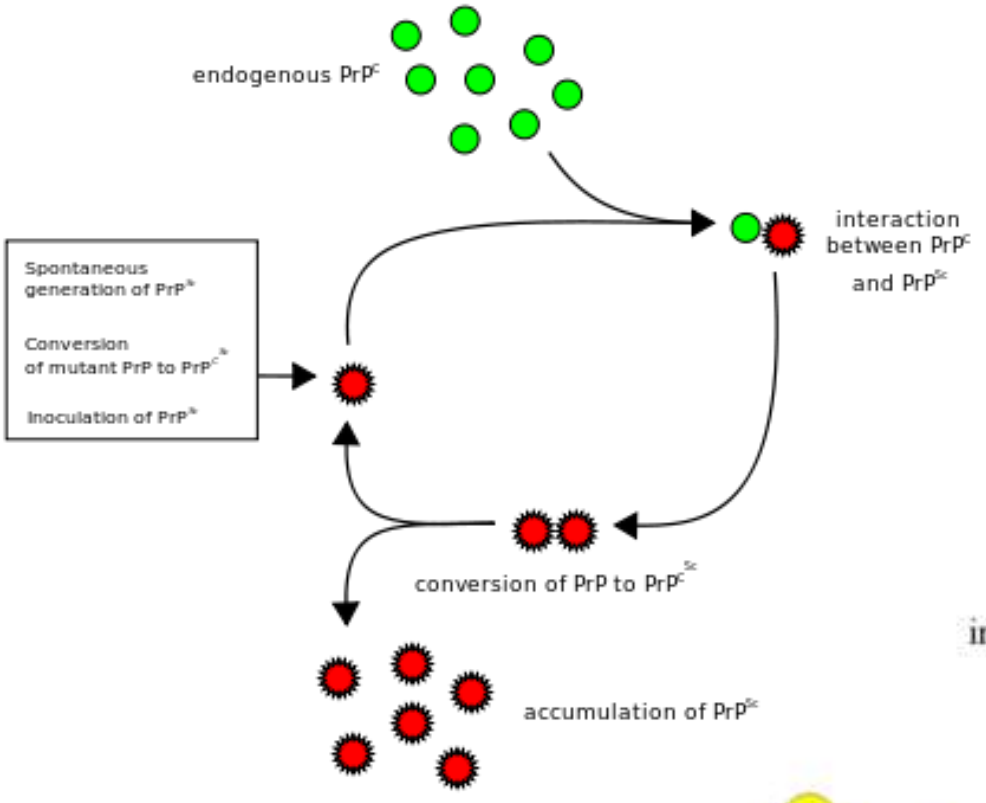
Human

- Kuru
- Fatal Familial Insomnia (FFI)
- Creutzfeldt-Jakob disease (CJD)

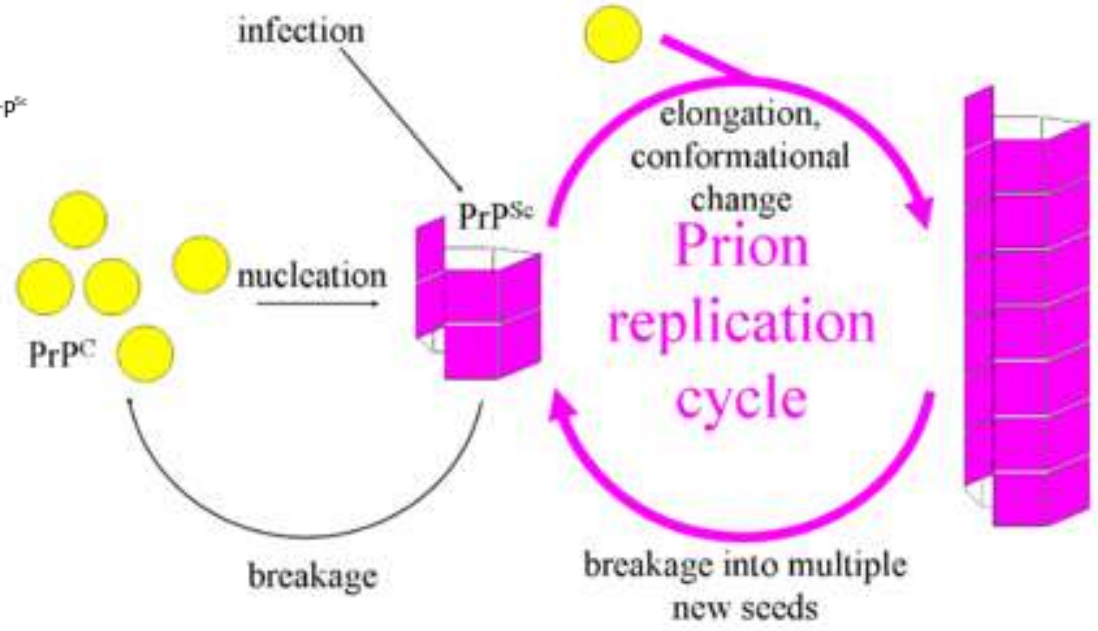
Animal

- Scrapie
- Bovine Spongiform Encephalopathy (BSE)
- Chronic Wasting Disease (CWD)


Accidental transmission of scrapie in sheep
1937
1939
Experimental transmission of scrapie in sheep
• Demonstration of small size of infectious agent
• Kuru transmitted to Chimpanzee
1966
1967
• Infectious agent not affected by DNA elimination
• Protein-only hypothesis proposed by Griffith
• Prion protein isolated from the infectious agent
• Prusiner coined the term prion
1982
First mutation in PRNP linked to familial disease
1989
1985
Identification of the gene encoding PrP
PROTEIN-ONLY
• Cell-free prion replication
• Discovery of yeast prions
1994
Efficient prion replication in vitro by PMCA
2001
Production of "synthetic prions"
2004
2000
In vitro generation of yeast prions
1993
Prnp-/- mice resistant to infection
1990
PrP transgenic mice develop neurodegeneration
Prions strains and species barrier reproduced in vitro
2008
HYPOTHESIS
2005
Generation of bona fide infectivity in vitro by propagation of PrPSc
2007
• In vitro generation of infectivity with defined components
• De novo production of infectious prions
2010
In vitro generation of infectious material from recombinant PrP
TiBS

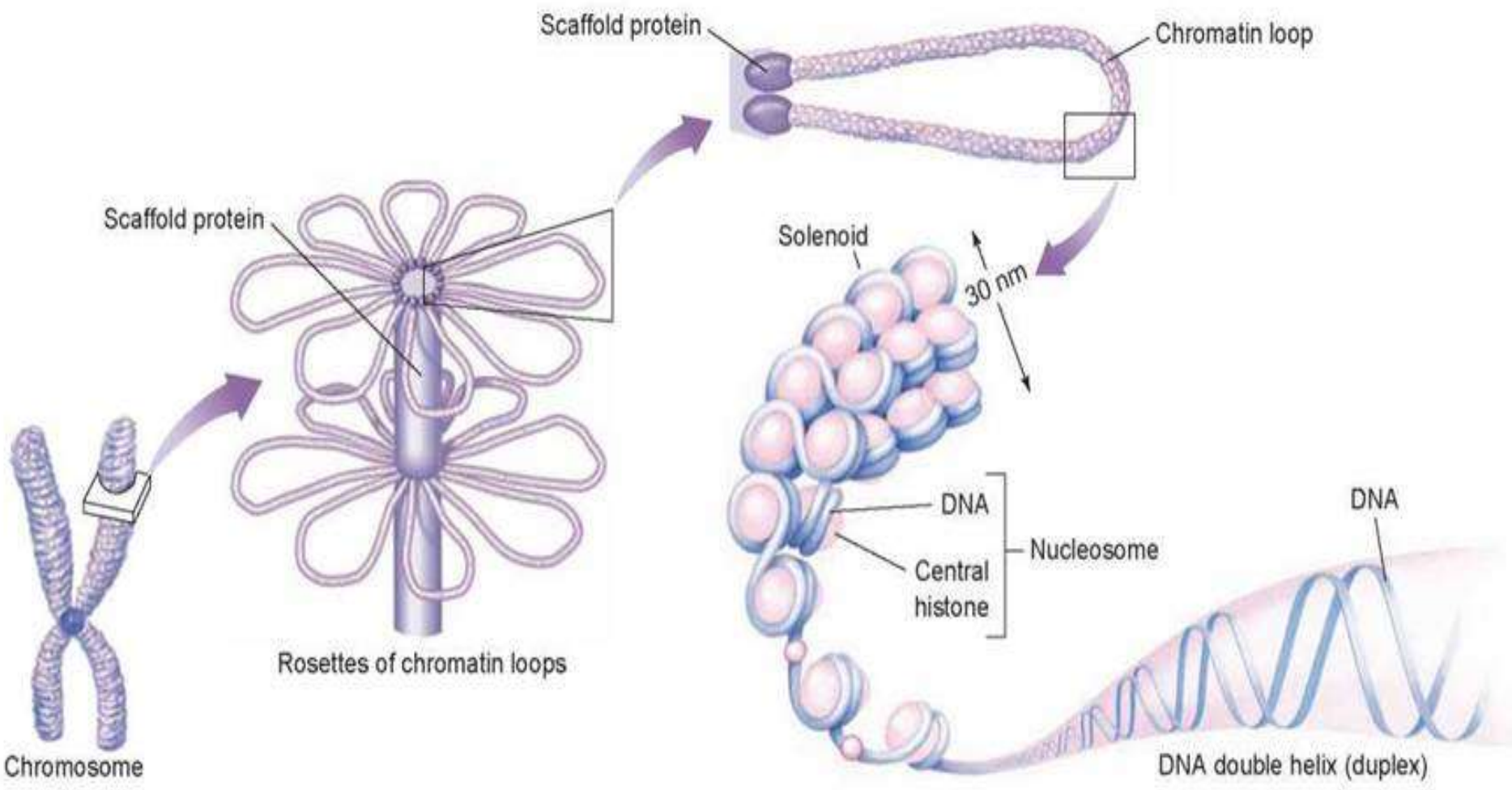


Heterodimer model



Firbril model

CHROMOSOME ORGANIZATION AND MOLECULAR STRUCTURE



INTRODUCTION

- Chromosomes are the structures that contain the genetic material
  - They are complexes of DNA and proteins
- The genome comprises all the genetic material that an organism possesses
  - In bacteria, it is typically a single circular chromosome
  - In eukaryotes, it refers to one complete set of *nuclear* chromosomes
  - Note:
    - Eukaryotes possess a mitochondrial genome
    - Plants also have a chloroplast genome

INTRODUCTION

- The main function of the genetic material is to store information required to produce an organism
  - The DNA molecule does that through its base sequence

- DNA sequences are necessary for
  - 1. Synthesis of RNA and cellular proteins
  - 2. Proper segregation of chromosomes
  - 3. Replication of chromosomes
  - 4. Compaction of chromosomes
    - So they can fit within living cells

VIRAL GENOMES

- Viruses are small infectious particles containing nucleic acid surrounded by a capsid of proteins
- For replication, viruses rely on their host cells
  - ie., the cells they infect
- Most viruses exhibit a limited host range
  - They typically infect only specific types of cells of one host species

- Bacteriophages may also contain a sheath, base plate and tail fibers

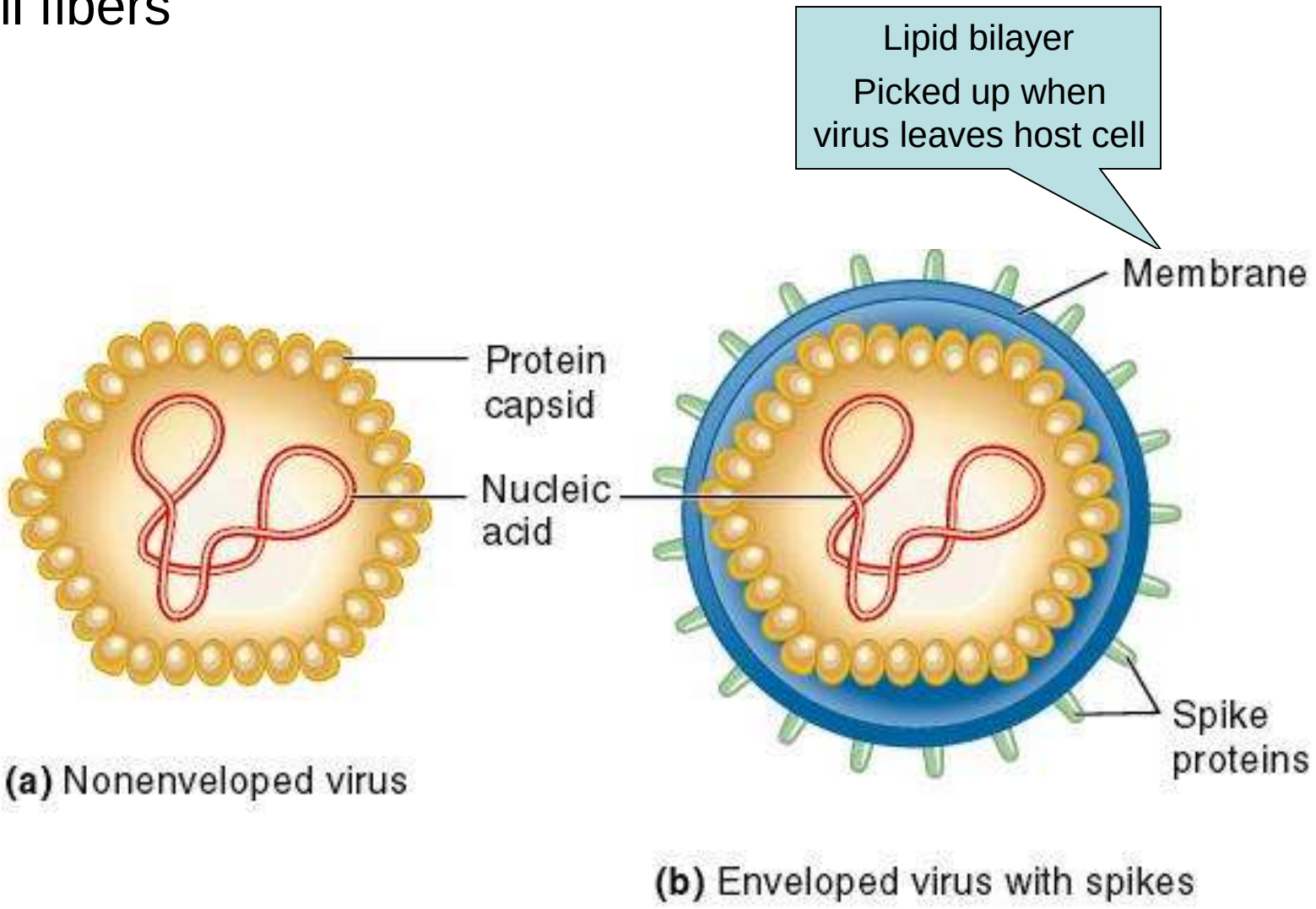


Figure General structure of viruses

Viral Genomes

- A viral genome is the genetic material of the virus
  - Also termed the viral chromosome
- The genome can be
  - DNA or RNA
  - Single-stranded or double-stranded
  - Circular or linear
- Viral genomes vary in size from a few thousand to more than a hundred thousand nucleotides

TABLE 10.1

Characteristics of Selected Viral Genomes

Virus	Host	Type of Nucleic Acid*	Size**	Number of Genes
Parvovirus	Mammals	ssDNA	5.0	5
Phage fd	*E. coli*	ssDNA	6.4	10
Lambda	*E. coli*	dsDNA	48.5	36
T4	*E. coli*	dsDNA	169	>190
Qβ	*E. coli*	ssRNA	4.2	4
TMV	Many plants	ssRNA	6.4	6
Influenza virus	Mammals	ssRNA	13.5	12

*ss refers to single stranded and ds refers to double stranded.

**Number of thousands of nucleotides or nucleotide base pairs.

BACTERIAL CHROMOSOMES

- The bacterial chromosome is found in a region called the nucleoid

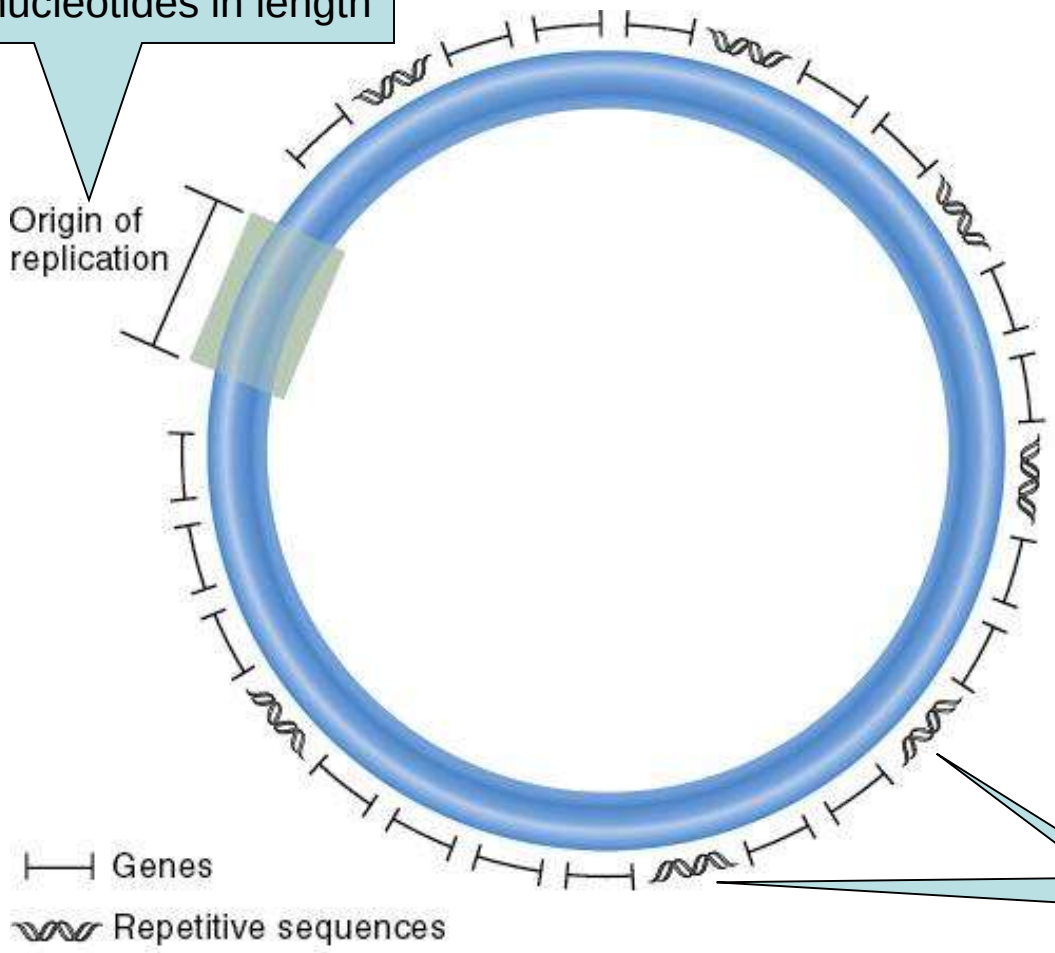
- The nucleoid is not membrane-bounded
  - So the DNA is in direct contact with the cytoplasm

- Bacteria may have one to four identical copies of the same chromosome
  - The number depends on the species and growth conditions

- Bacterial chromosomal DNA is usually a circular molecule that is a few million nucleotides in length
  - *Escherichia coli* → ~ 4.6 million base pairs
  - *Haemophilus influenzae* → ~ 1.8 million base pairs

- A typical bacterial chromosome contains a few thousand different genes
  - Structural gene sequences (encoding proteins) account for the majority of bacterial DNA
  - The nontranscribed DNA between adjacent genes are termed intergenic regions

A few hundred nucleotides in length

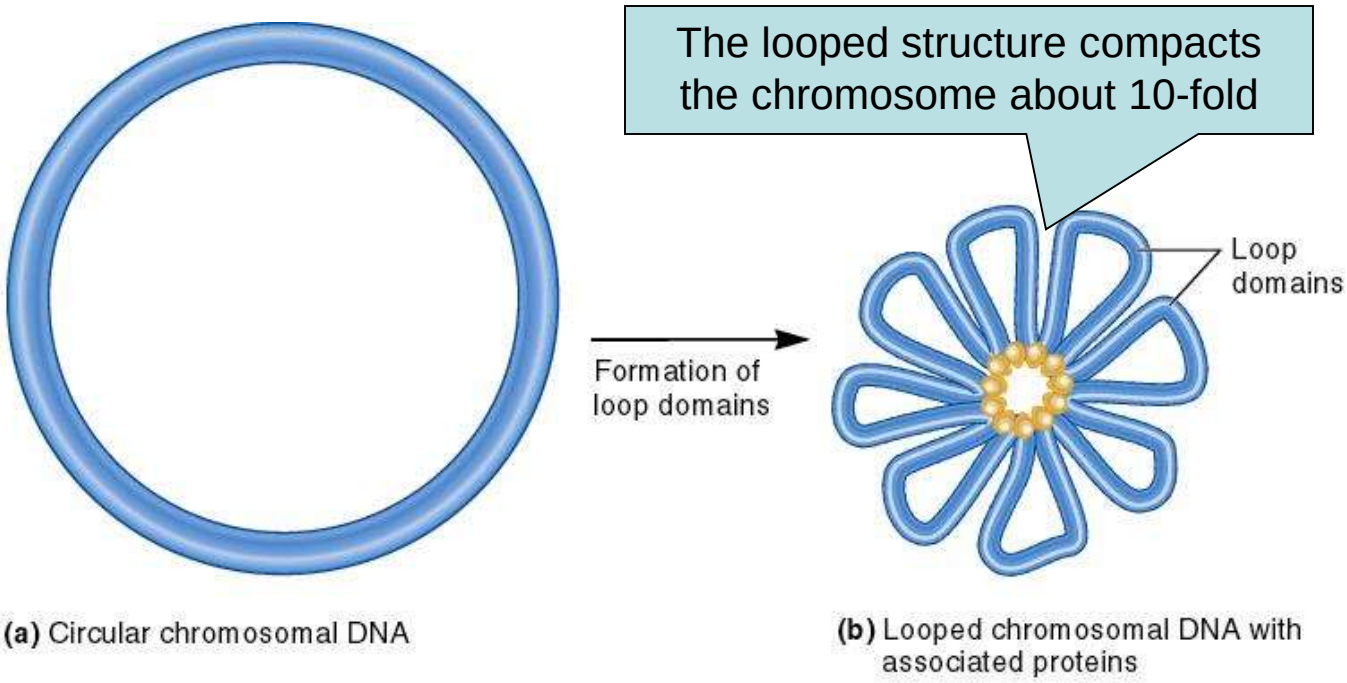


Key features:

- Most, but not all, bacterial species contain circular chromosomal DNA.
- A typical chromosome is a few million base pairs in length.
- Most bacterial species contain a single type of chromosome, but it may be present in multiple copies.
- Several thousand different genes are interspersed throughout the chromosome.
- One origin of replication is required to initiate DNA replication.
- Short repetitive sequences may be interspersed throughout the chromosome.

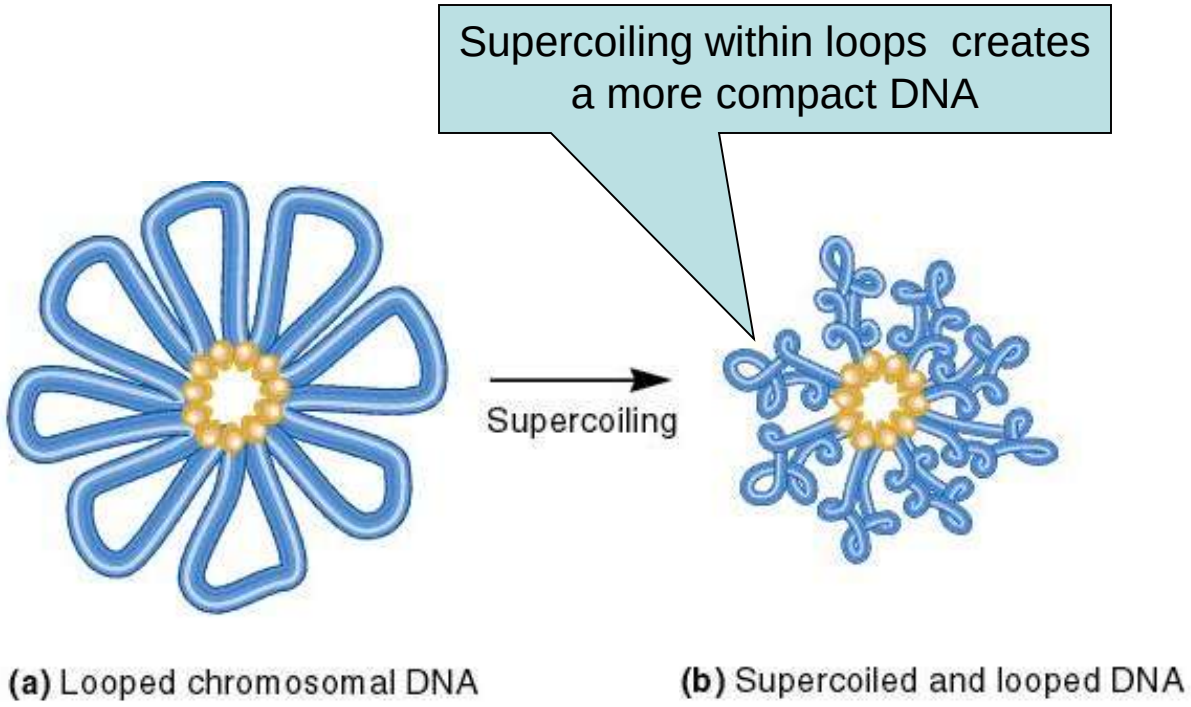
These play roles in DNA folding, DNA replication, and gene expression

- To fit within the bacterial cell, the chromosomal DNA must be compacted about a 1000-fold
  - This involves the formation of loop domains



- The number of loops varies according to the size of the bacterial chromosome and the species
  - *E. coli* has 50-100 with 40,000 to 80,000 bp of DNA in each

- DNA supercoiling is a second important way to compact the bacterial chromosome



- The control of supercoiling in bacteria is accomplished by two main enzymes
  - 1. DNA gyrase (also termed DNA topoisomerase II)
    - Introduces negative supercoils using energy from ATP
    - It can also relax positive supercoils when they occur
  - 2. DNA topoisomerase I
    - Relaxes negative supercoils
- The competing action of these two enzymes governs the overall supercoiling of bacterial DNA

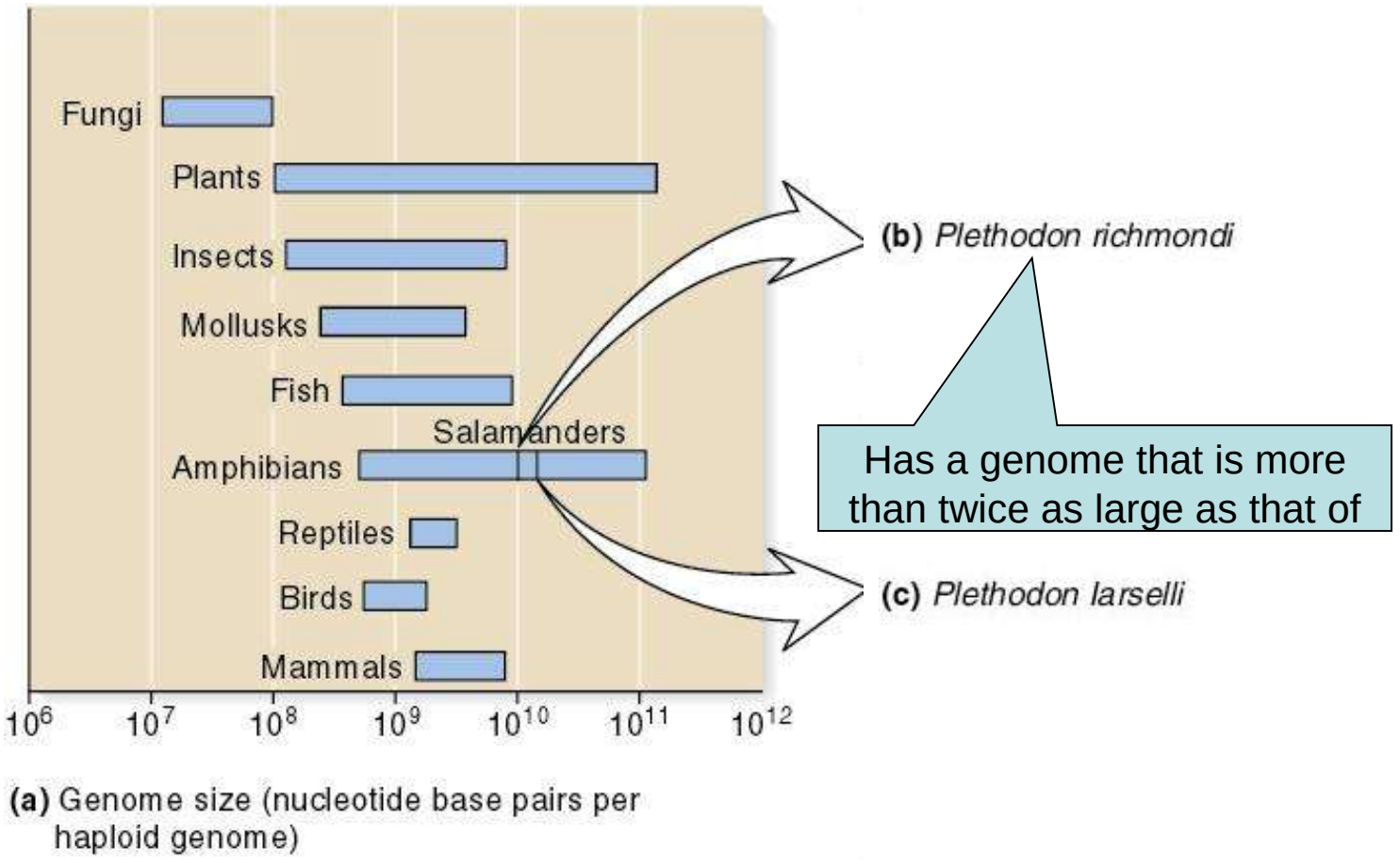
EUKARYOTIC CHROMOSOMES

- Eukaryotic species contain one or more sets of chromosomes
  - Each set is composed of several different linear chromosomes
- The total amount of DNA in eukaryotic species is typically greater than that in bacterial cells
- Chromosomes in eukaryotes are located in the nucleus
  - To fit in there, they must be highly compacted
    - This is accomplished by the binding of many proteins
    - The DNA-protein complex is termed chromatin

- Eukaryotic genomes vary substantially in size

- In many cases, this variation is not related to complexity of the species
  - For example, there is a two fold difference in the size of the genome in two closely related salamander species

  - The difference in the size of the genome is not because of extra genes
    - Rather, the accumulation of repetitive DNA sequences
      - These do not encode proteins

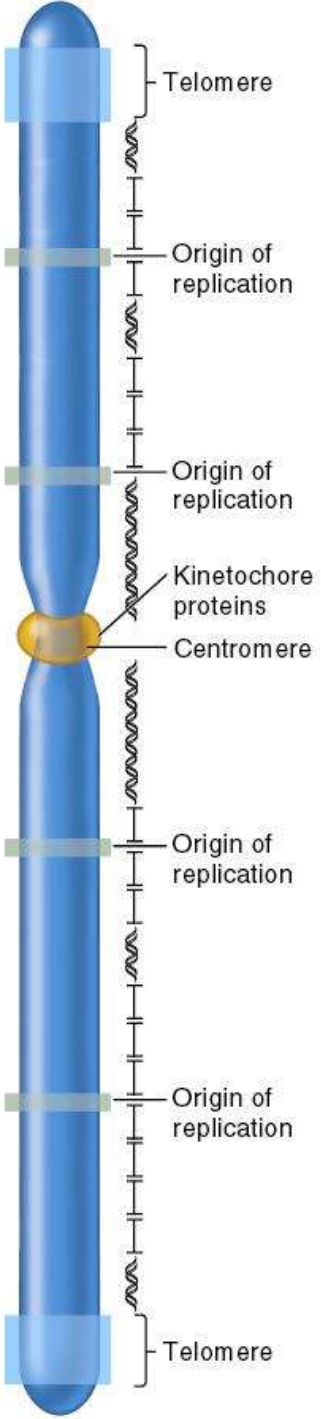


(a) Genome size (nucleotide base pairs per haploid genome)

Organization of Eukaryotic Chromosomes

- A eukaryotic chromosome contains a long, linear DNA molecule

- Three types of DNA sequences are required for chromosomal replication and segregation
  - Origins of replication
  - Centromeres
  - Telomeres



Key features:

- Eukaryotic chromosomes are usually linear.
- A typical chromosome is tens of millions to hundreds of millions of base pairs in length.
- Eukaryotic chromosomes occur in sets. Many species are diploid, which means that somatic cells contain 2 sets of chromosomes.
- Genes are interspersed throughout the chromosome. A typical chromosome contains between a few hundred and several thousand different genes.
- Each chromosome contains many origins of replication that are interspersed about every 100,000 base pairs.
- Each chromosome contains a centromere that forms a recognition site for the kinetochore proteins.
- Telomeres contain specialized sequences located at both ends of the linear chromosome.
- Repetitive sequences are commonly found near centromeric and telomeric regions, but they may also be interspersed throughout the chromosome.

Genes

Repetitive sequences

- Genes are located between the centromeric and telomeric regions along the entire chromosome
  - A single chromosome usually has a few hundred to several thousand genes

- In lower eukaryotes (such as yeast)
  - Genes are relatively small
    - They contain primarily the sequences encoding the polypeptides
    - ie: Very few introns are present

- In higher eukaryotes (such as mammals)
  - Genes are long
    - They tend to have many introns

Repetitive Sequences

- Sequence complexity refers to the number of times a particular base sequence appears in the genome

- There are three main types of repetitive sequences
  - Unique or non-repetitive
  - Moderately repetitive
  - Highly repetitive

Repetitive Sequences

- Unique or non-repetitive sequences
  - Found once or a few times in the genome
  - Includes structural genes as well as intergenic areas
- Moderately repetitive
  - Found a few hundred to a few thousand times
  - Includes
    - Genes for rRNA and histones
    - Origins of replication
    - Transposable elements

Repetitive Sequences

- Highly repetitive
  - Found tens of thousands to millions of times
  - Each copy is relatively short (a few nucleotides to several hundred in length)
  - Some sequences are interspersed throughout the genome
    - Example: *Alu* family in humans
  - Other sequences are clustered together in tandem arrays
    - Example: AATAT and AATATAT sequences in *Drosophila*
    - These are commonly found in the centromeric regions

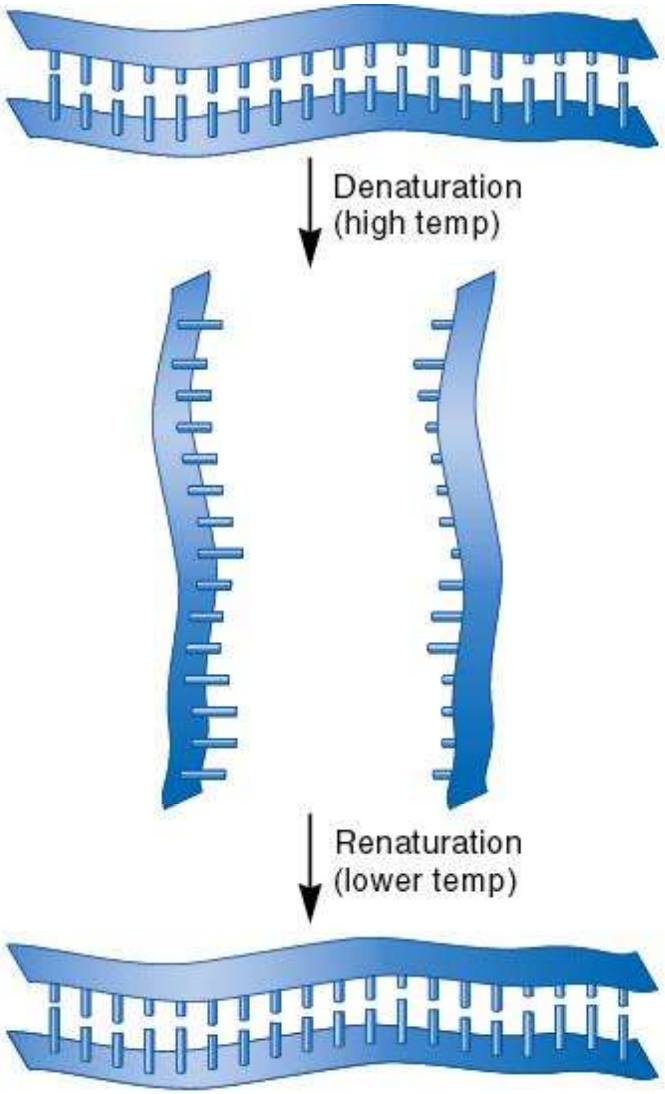


Figure **(a)** Renaturation of DNA strands

Renaturation Experiments

- The rate of renaturation of complementary DNA strands provides a way to distinguish the three different types of repetitive sequences

- The renaturation rate of a particular DNA sequence depends on the concentration of its complementary partner
  - Highly repetitive DNA will be the fastest to renature
    - Because there are many copies of complementary sequences
  - Unique sequences will be the slowest to renature
    - It takes added time for these sequences to find each other

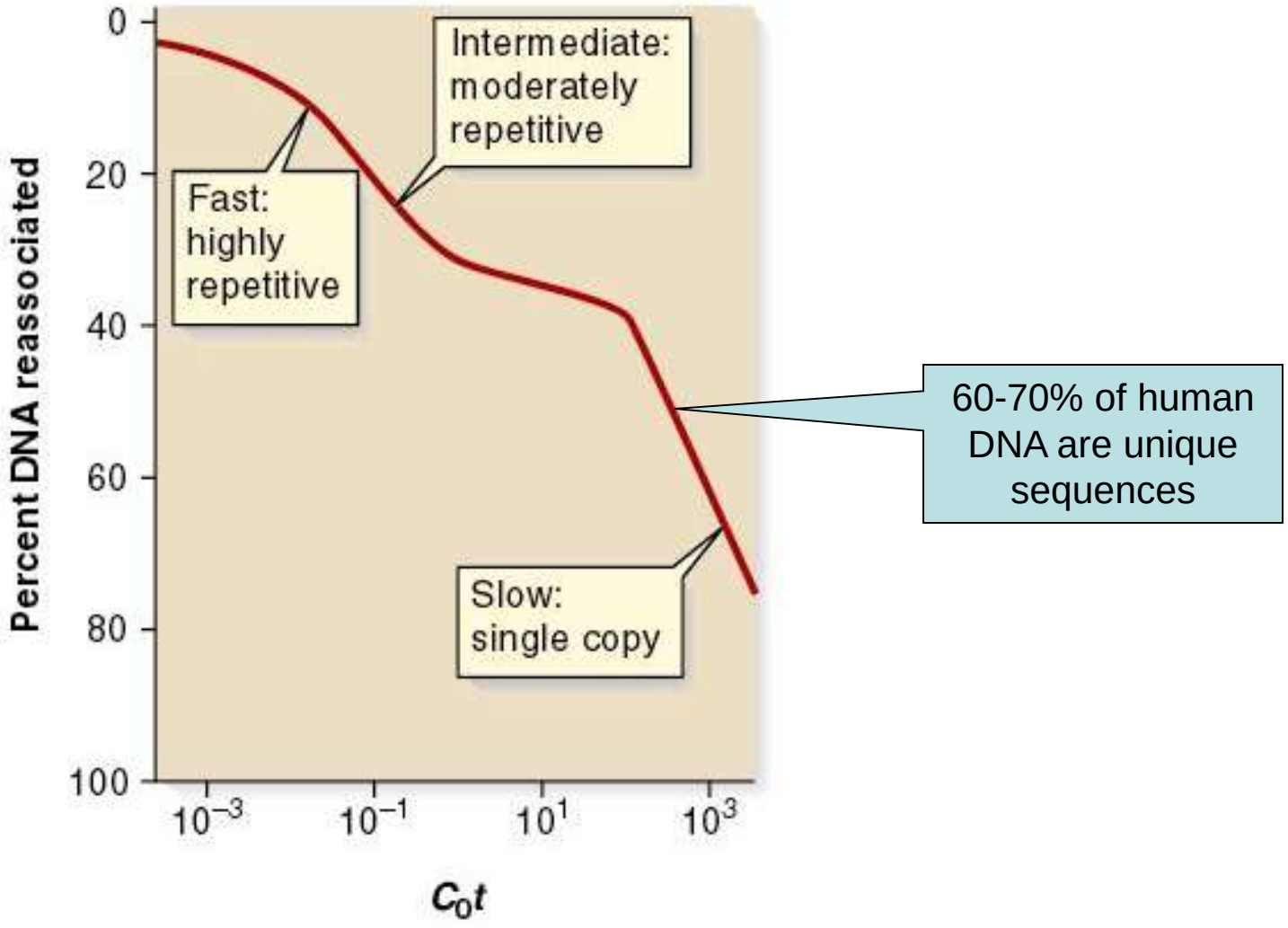
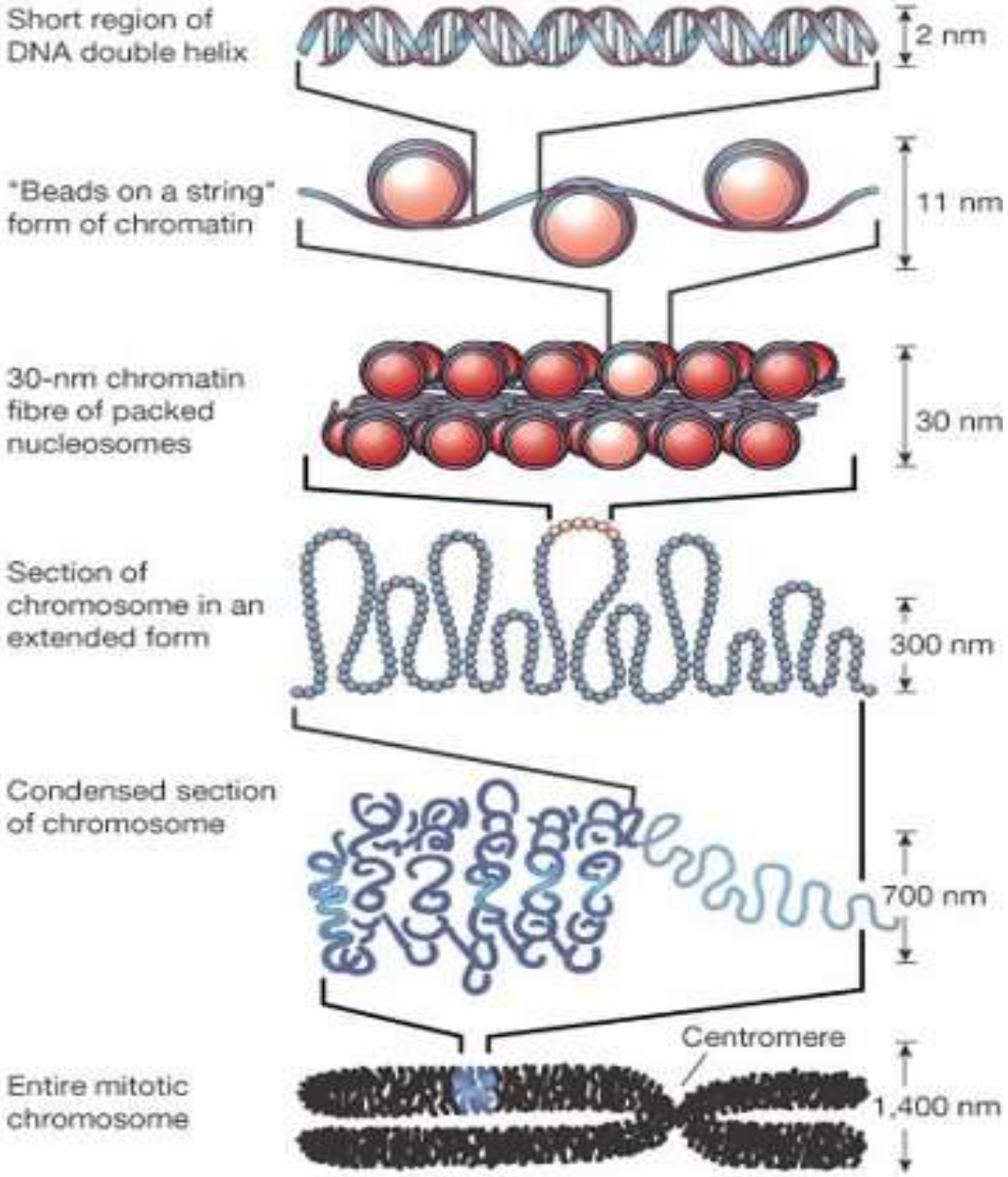


Figure **(b)** Human chromosomal DNA C_0t curve

Eukaryotic Chromatin Compaction

- If stretched end to end, a single set of human chromosomes will be over 1 meter long!
  - Yet the cell's nucleus is only 2 to 4 μm in diameter
    - Therefore, the DNA must be tightly compacted to fit
- The compaction of linear DNA in eukaryotic chromosomes involves interactions between DNA and various proteins
  - Proteins bound to DNA are subject to change during the life of the cell
    - These changes affect the degree of chromatin compaction


Short region of DNA double helix
2 nm
"Beads on a string" form of chromatin
11 nm
30-nm chromatin fibre of packed nucleosomes
30 nm
Section of chromosome in an extended form
300 nm
Condensed section of chromosome
700 nm
Centromere
Entire mitotic chromosome
1,400 nm

Nucleosomes

- The repeating structural unit within eukaryotic chromatin is the nucleosome
- It is composed of double-stranded DNA wrapped around an octamer of histone proteins
  - An octamer is composed two copies each of four different histones
  - 146 bp of DNA make 1.65 negative superhelical turns around the octamer

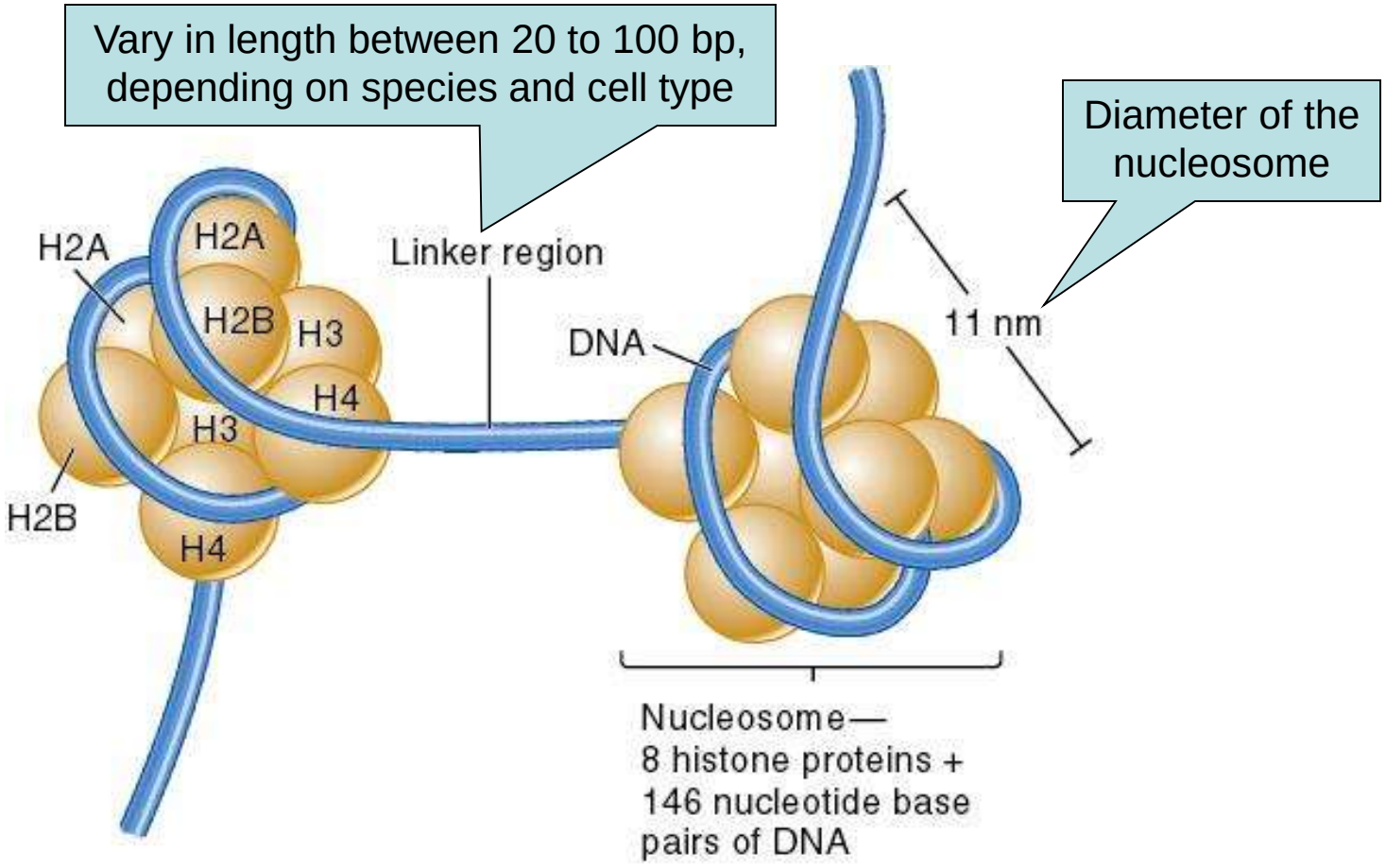


Figure **(a)** Nucleosomes showing core histones

- Overall structure of connected nucleosomes resembles “beads on a string”
  - This structure shortens the DNA length about seven-fold

- Histone proteins are basic
  - They contain many positively-charged amino acids
    - Lysine and arginine
  - These bind with the phosphates along the DNA backbone

- There are five types of histones
  - H2A, H2B, H3 and H4 are the core histones
    - Two of each make up the octamer
  - H1 is the linker histone
    - Binds to linker DNA
    - Also binds to nucleosomes
      - But not as tightly as are the core histones

Play a role in the organization and compaction of the chromosome

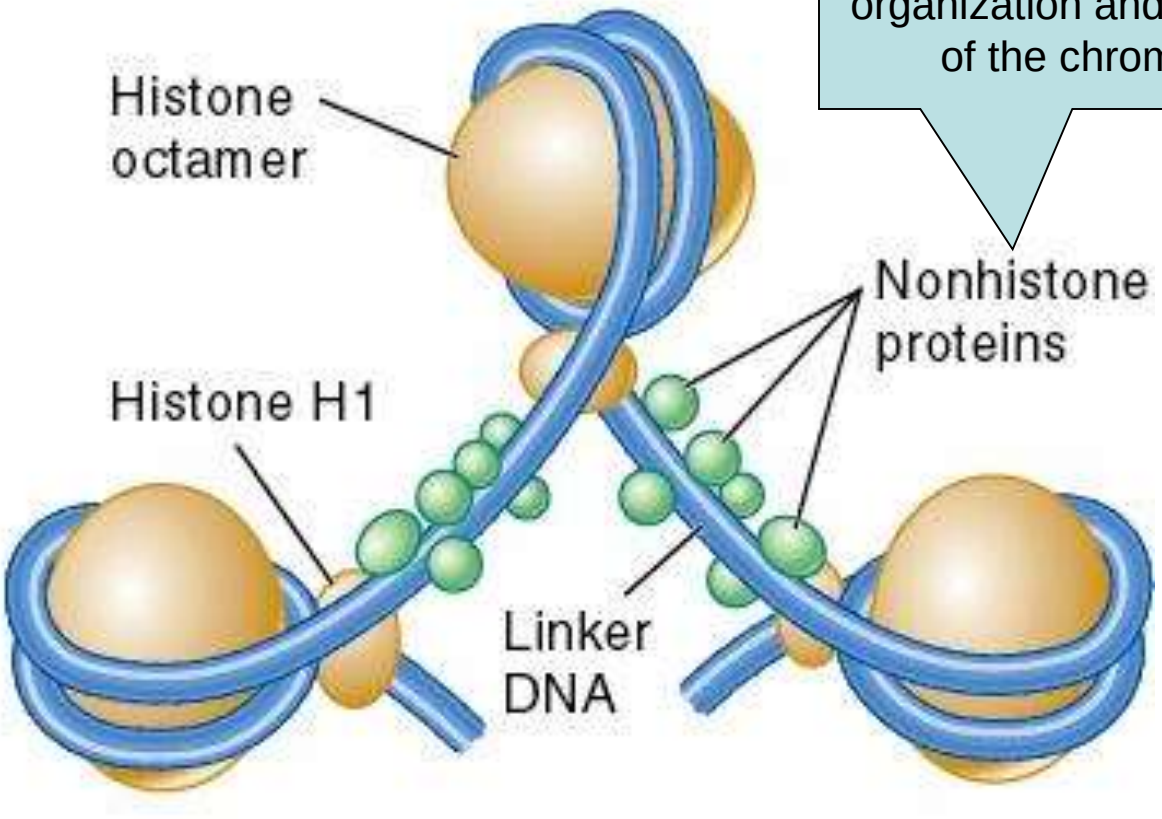


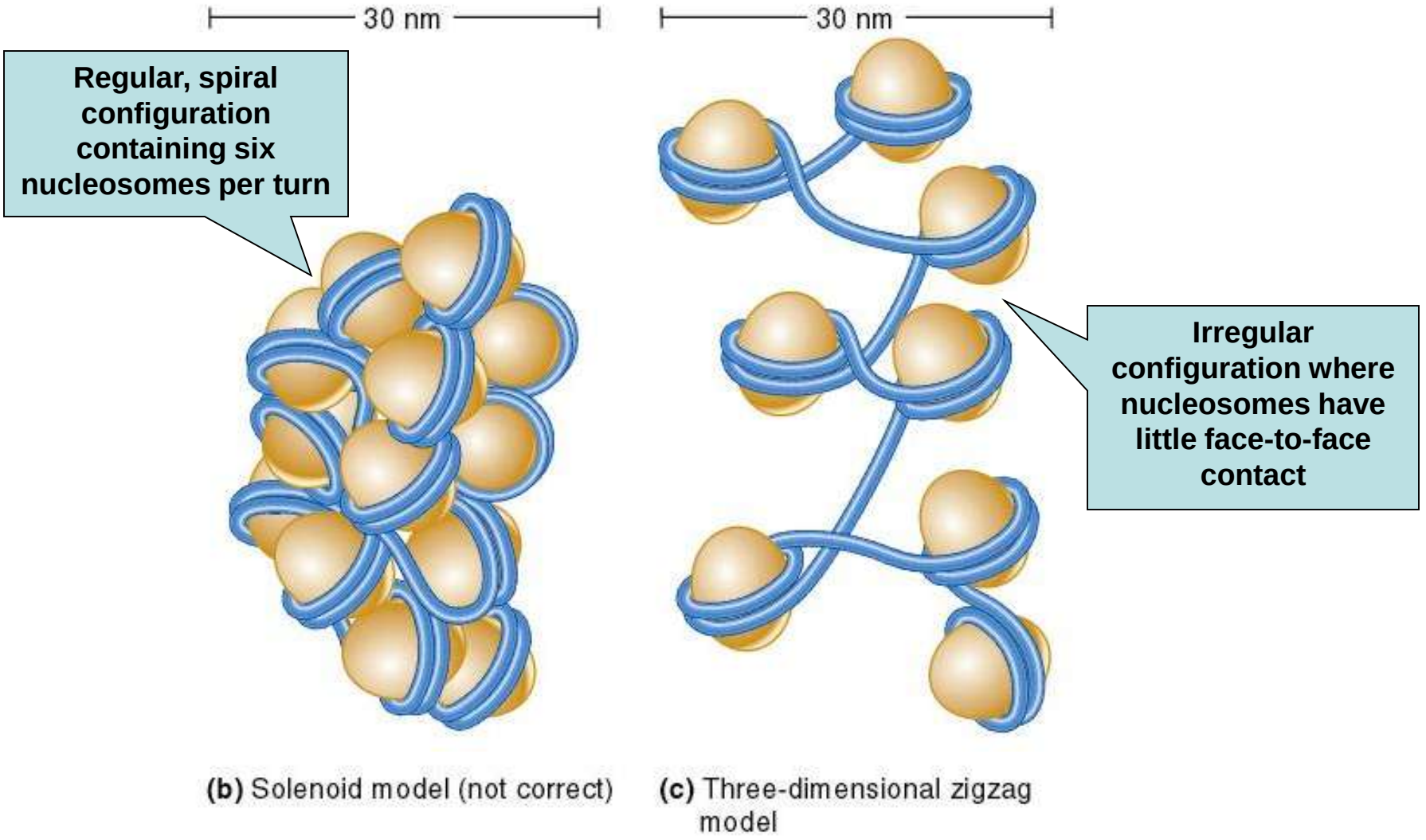
Figure **(b)** Nucleosomes showing linker histones and nonhistone proteins

Nucleosomes Join to Form a 30 nm Fiber

- Nucleosomes associate with each other to form a more compact structure termed the 30 nm fiber

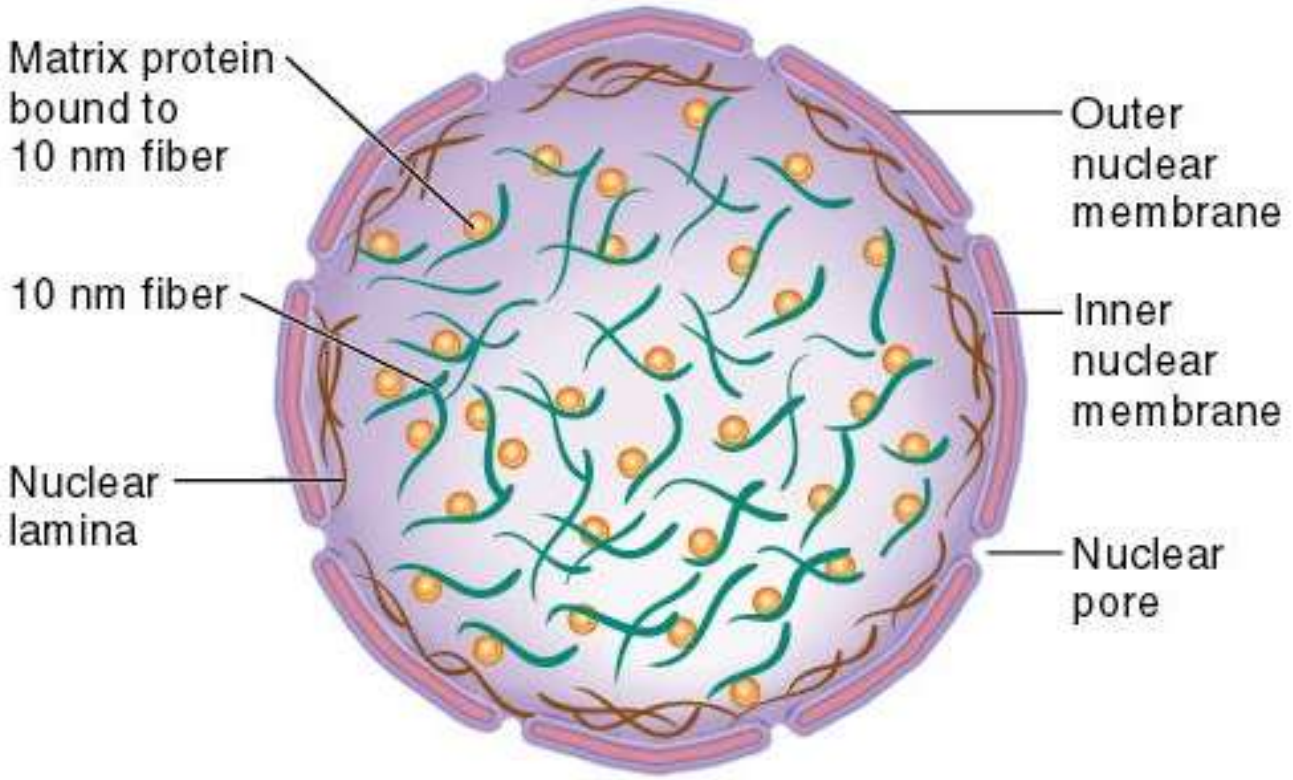
- Histone H1 plays a role in this compaction
  - At moderate salt concentrations, H1 is removed
    - The result is the classic beads-on-a-string morphology
  - At low salt concentrations, H1 remains bound
    - Beads associate together into a more compact morphology

- The 30 nm fiber shortens the total length of DNA another seven-fold
- Its structure has proven difficult to determine
  - The DNA conformation may be substantially altered when extracted from living cells
  - Two models have been proposed
    - Solenoid model
    - Three-dimensional zigzag model


30 nm
30 nm
Regular, spiral configuration containing six nucleosomes per turn
Irregular configuration where nucleosomes have little face-to-face contact
(b) Solenoid model (not correct)
(c) Three-dimensional zigzag model

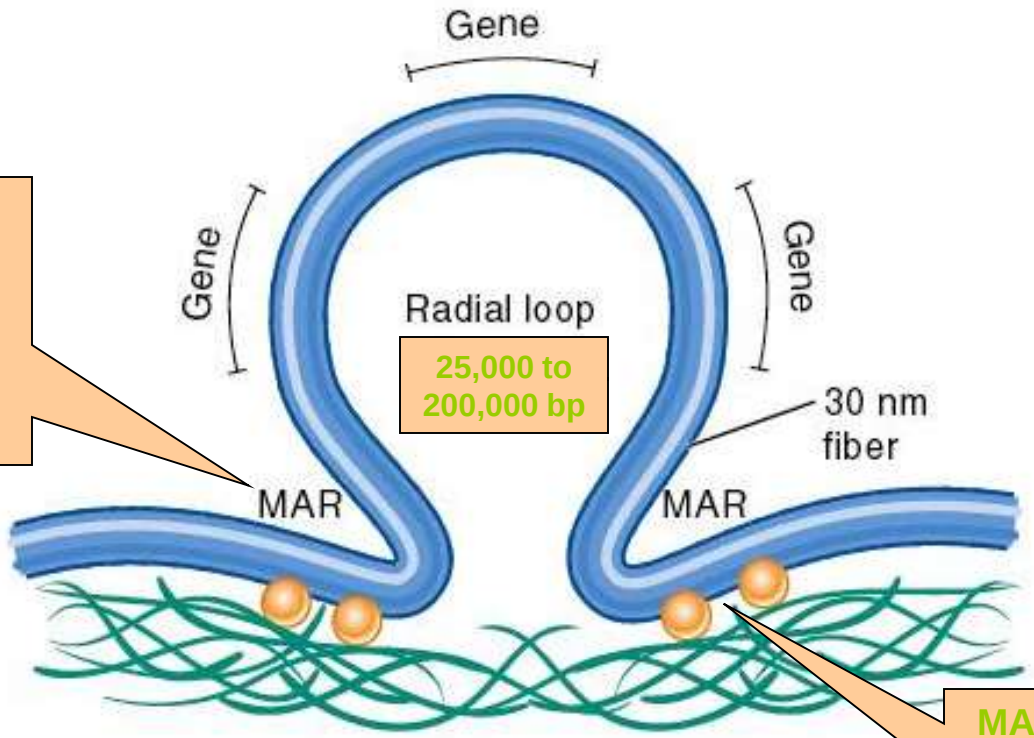
Further Compaction of the Chromosome

- The two events we have discussed so far have shortened the DNA about 50-fold
- A third level of compaction involves interaction between the 30 nm fiber and the nuclear matrix
- The nuclear matrix is composed of two parts
  - Nuclear lamina
  - Internal matrix proteins
    - 10 nm fiber and associated proteins


Matrix protein
bound to
10 nm fiber
10 nm fiber
Nuclear
lamina
Outer
nuclear
membrane
Inner
nuclear
membrane
Nuclear
pore
(a)

Figure

- The third mechanism of DNA compaction involves the formation of radial loop domains



Matrix-attachment regions

or

Scaffold-attachment regions (SARs)

MARs are anchored to the nuclear matrix, thus creating radial loops

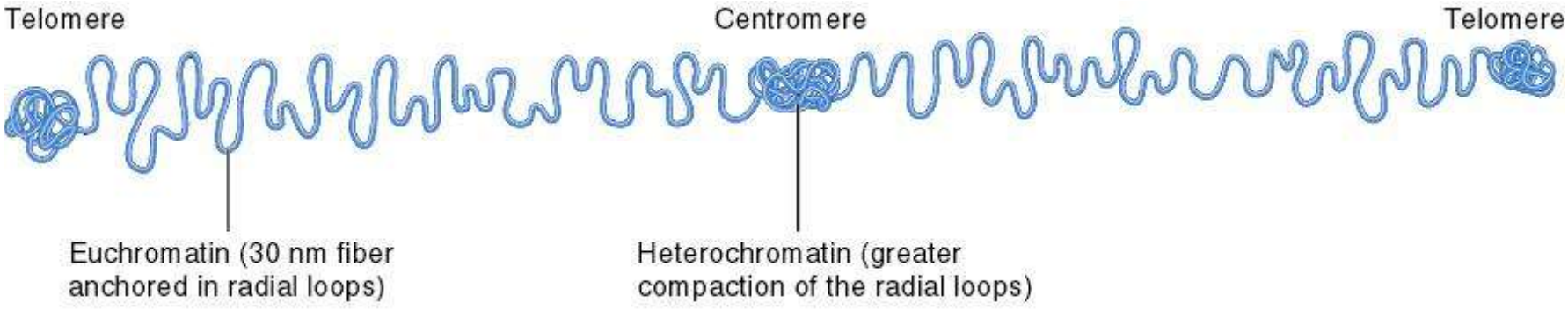
Figure (d)

Further Compaction of the Chromosome

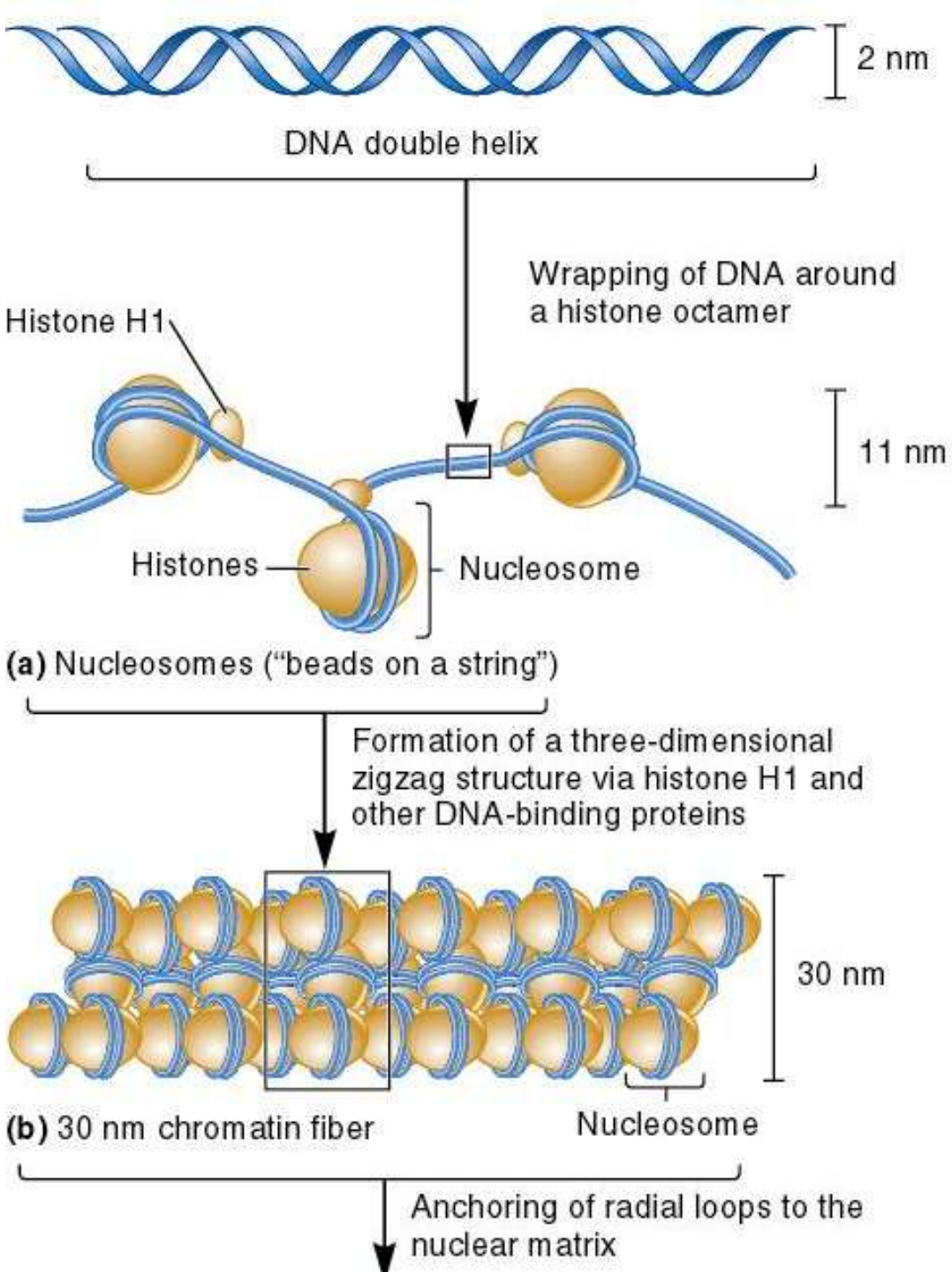
- The attachment of radial loops to the nuclear matrix is important in two ways
  - 1. It plays a role in gene regulation
  - 2. It serves to organize the chromosomes within the nucleus
    - Each chromosome in the nucleus is located in a discrete and nonoverlapping chromosome territory

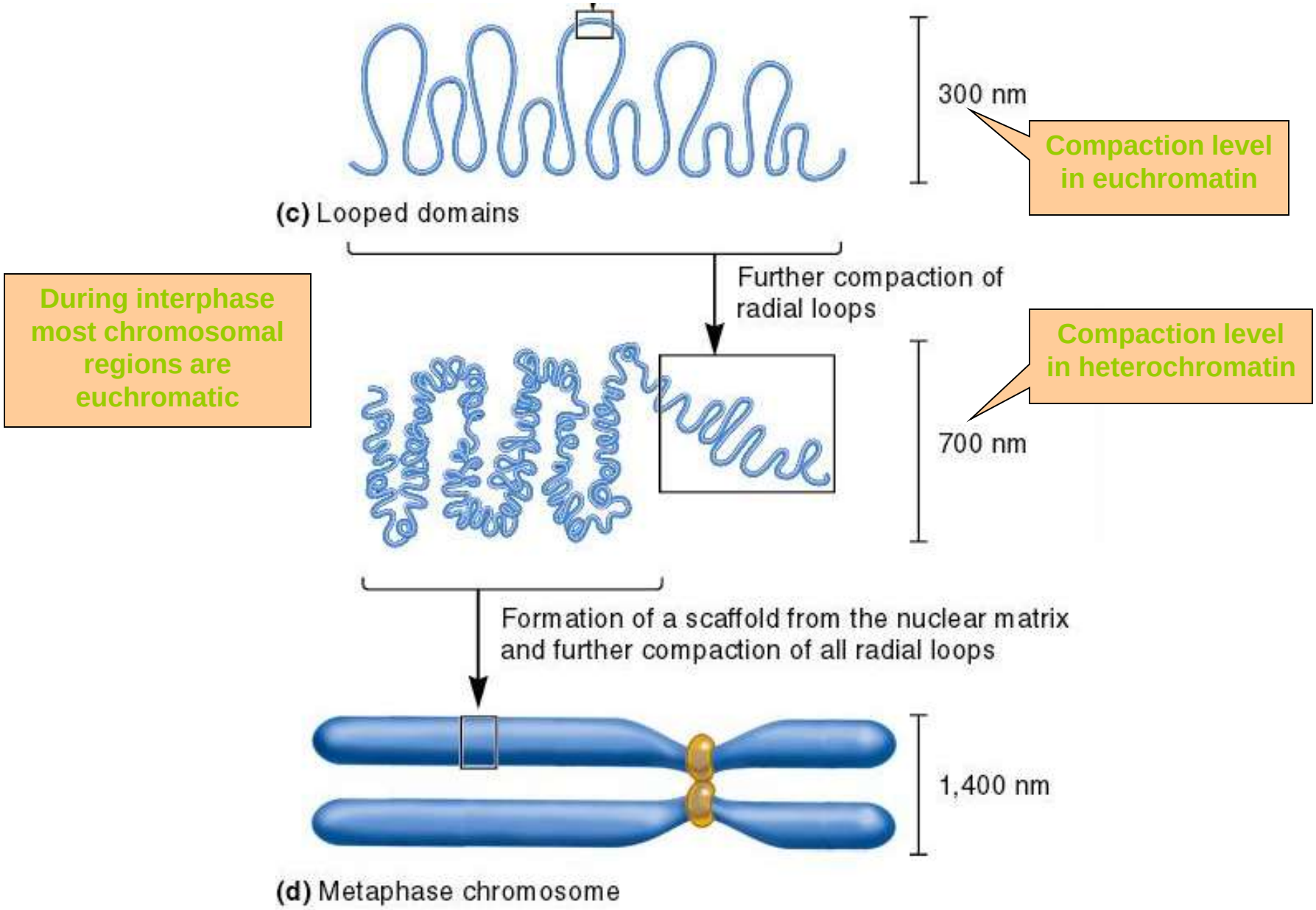
Heterochromatin *vs* Euchromatin

- The compaction level of interphase chromosomes is not completely uniform
  - Euchromatin
    - Less condensed regions of chromosomes
    - Transcriptionally active
    - Regions where 30 nm fiber forms radial loop domains
  - Heterochromatin
    - Tightly compacted regions of chromosomes
    - Transcriptionally inactive (in general)
    - Radial loop domains compacted even further



- There are two types of heterochromatin
  - Constitutive heterochromatin
    - Regions that are always heterochromatic
    - Permanently inactive with regard to transcription
  - Facultative heterochromatin
    - Regions that can interconvert between euchromatin and heterochromatin
    - Example: Barr body


2 nm
DNA double helix
Wrapping of DNA around a histone octamer
Histone H1
11 nm
Histones
Nucleosome
(a) Nucleosomes ("beads on a string")
Formation of a three-dimensional zigzag structure via histone H1 and other DNA-binding proteins
30 nm
(b) 30 nm chromatin fiber
Nucleosome
Anchoring of radial loops to the nuclear matrix


300 nm
Compaction level in euchromatin
(c) Looped domains
Further compaction of radial loops
During interphase most chromosomal regions are euchromatic
Compaction level in heterochromatin
700 nm
Formation of a scaffold from the nuclear matrix and further compaction of all radial loops
1,400 nm
(d) Metaphase chromosome

Metaphase Chromosomes

- As cells enter M phase, the level of compaction changes dramatically
  - By the end of prophase, sister chromatids are entirely heterochromatic
  - Two parallel chromatids have an overall diameter of 1,400 nm
- These highly condensed metaphase chromosomes undergo little gene transcription

Metaphase Chromosomes

- In metaphase chromosomes the radial loops are highly compacted and stay anchored to a scaffold
  - The scaffold is formed from the nuclear matrix
- Histones are needed for the compaction of radial loops

- Two multiprotein complexes help to form and organize metaphase chromosomes
  - Condensin
    - Plays a critical role in chromosome condensation
  - Cohesin
    - Plays a critical role in sister chromatid alignment
- Both contain a category of proteins called SMC proteins
  - Acronym = Structural maintenance of chromosomes
  - SMC proteins use energy from ATP and catalyze changes in chromosome structure

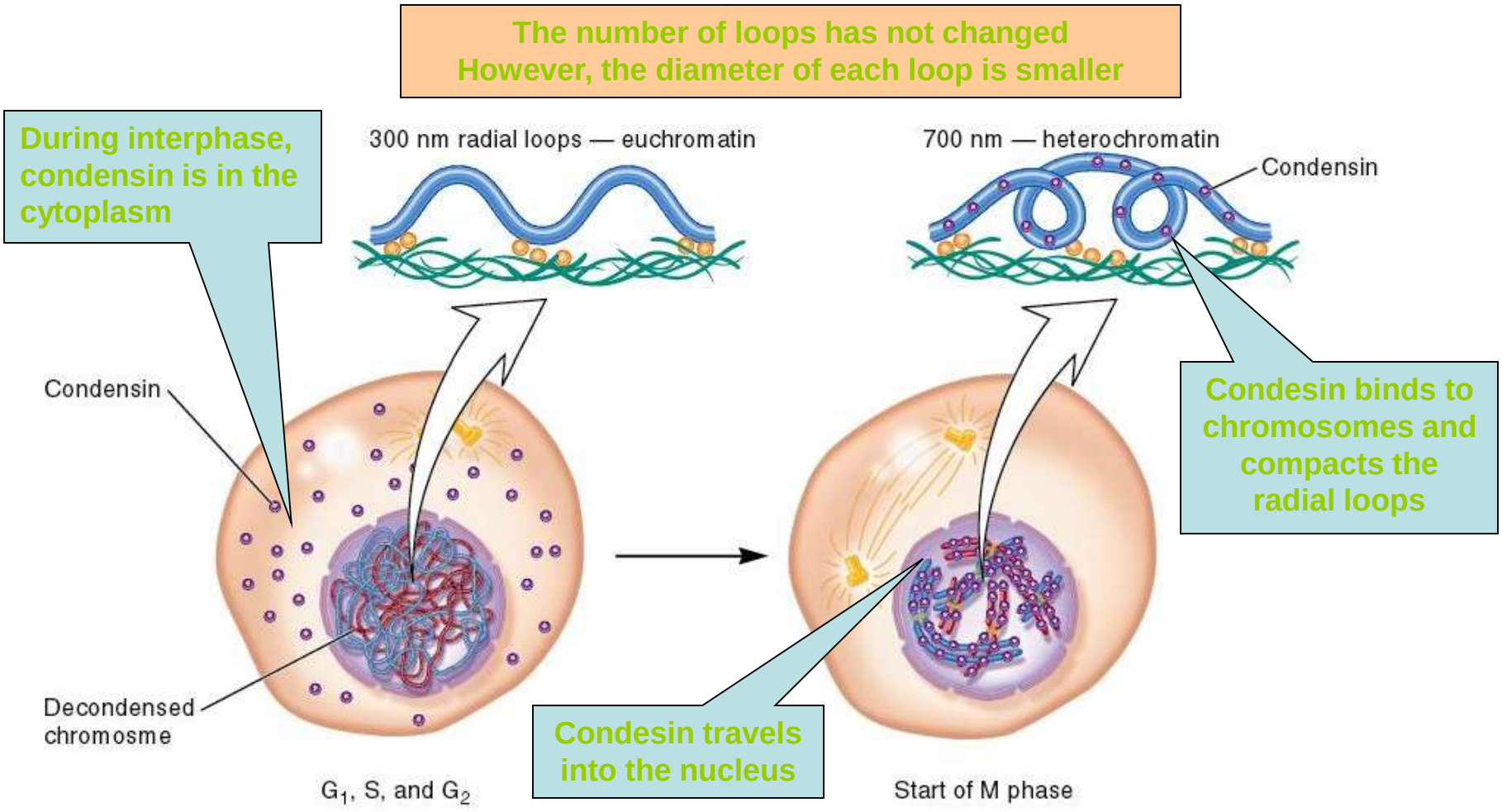


Figure **The condensation of a metaphase chromosome by condensin**

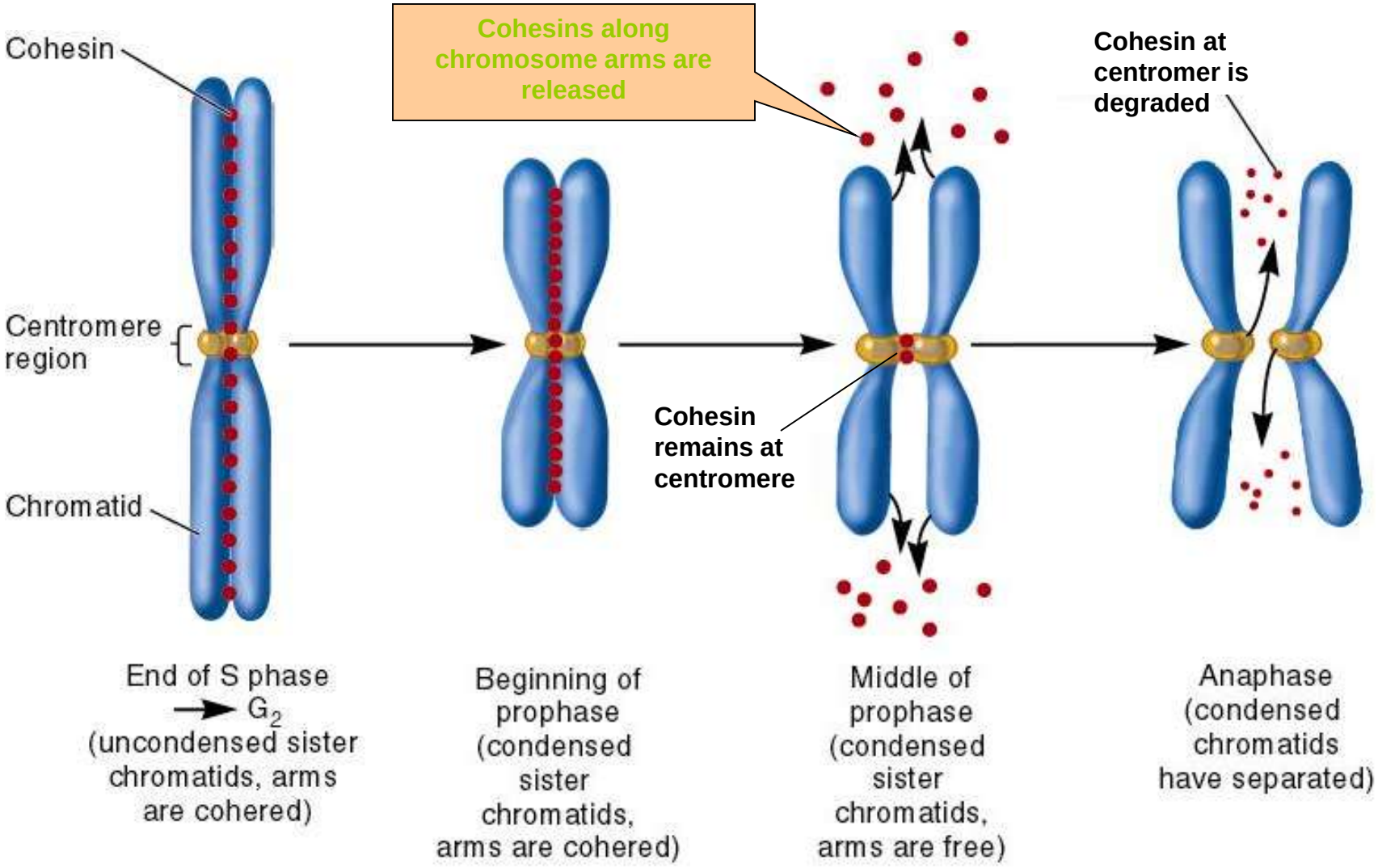


Figure **The alignment of sister chromatids via cohesin**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