The Genetic Material

Lecture 1
Brooker Chapters 3, 9 & 10

BIO 184
Dr. Tom Peavy

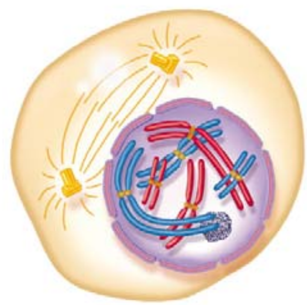
HOW DO WE KNOW
DNA
IS THE
GENETIC MATERIAL????

What are the requirements of "Genetic Material"?

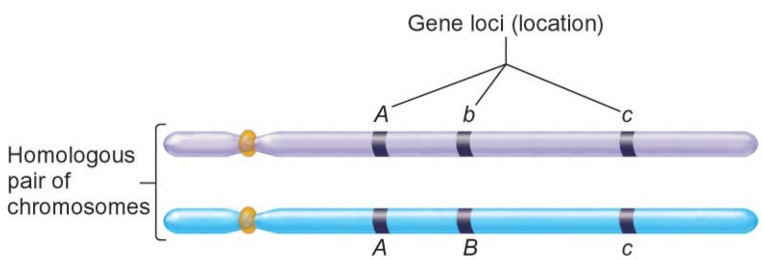
Evidence that Genes Reside within Chromosomes

- 1667- Anton van Leeuwenhoek (microscopy)
  - Hypothesis: spermatozoa ("sperm animals") enter the egg to achieve fertilization
  - Homunculus (spermists vs ovists)

- Late 1800's – microscopy studies
  - egg and sperm nuclei unite and contribute equally (e.g. frogs, sea urchins)
  - dyes used to stain the nucleus and observed long, threadlike bodies = Chromosomes ("colored bodies)
  - Mitosis described (nucleus is equally partitioned into daughter cells)
  - Sex Determination (♂ and ♀ chromosomes)



- Homologous Chromosomes: The pair of chromosomes in a diploid individual that have the same overall genetic content.
  - One member of each homologous pair of chromosomes is inherited from each parent.

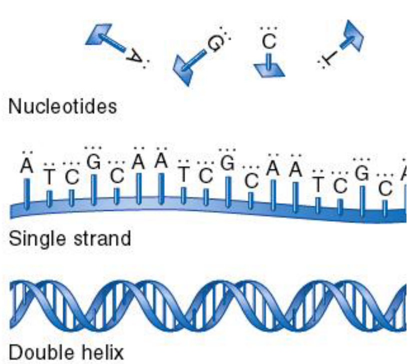


Chromosome theory of Inheritance
(Sutton and Boveri 1902)

- Chromosomes are in pairs and genes, or their alleles, are located on chromosomes
- Homologous chromosomes separate during meiosis so that alleles are segregated
- Meiotic products have one of each homologous chromosome but not both
- Fertilization restores the pairs of chromosomes

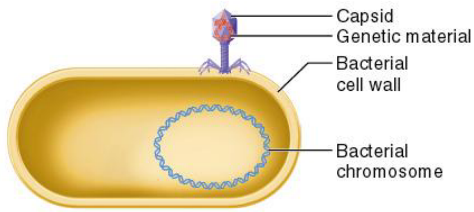
Chromosomes

- Approximately 40% DNA and 60% protein



Evidence for DNA as Genetic Material

- Used simple experimental organisms to study question
  – Bacteria with single circular chromosome without a nucleus (prokaryotes)
  – Bacteriophage (“bacteria eaters”)

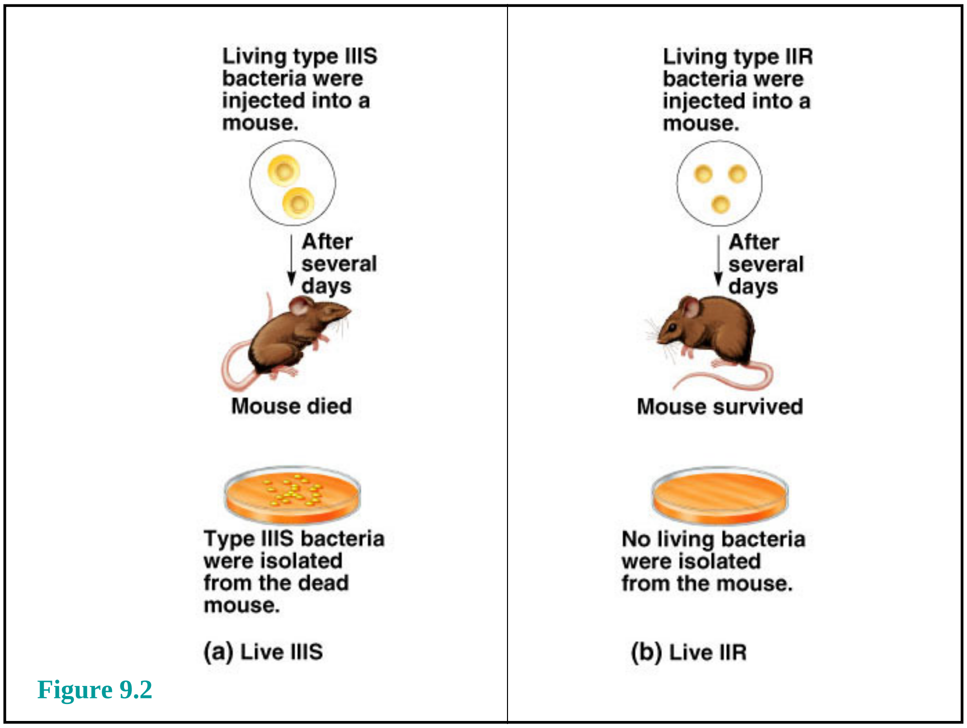


Frederick Griffith Experiments

- In 1928, Griffith studied the bacterium *Streptococcus pneumoniae*

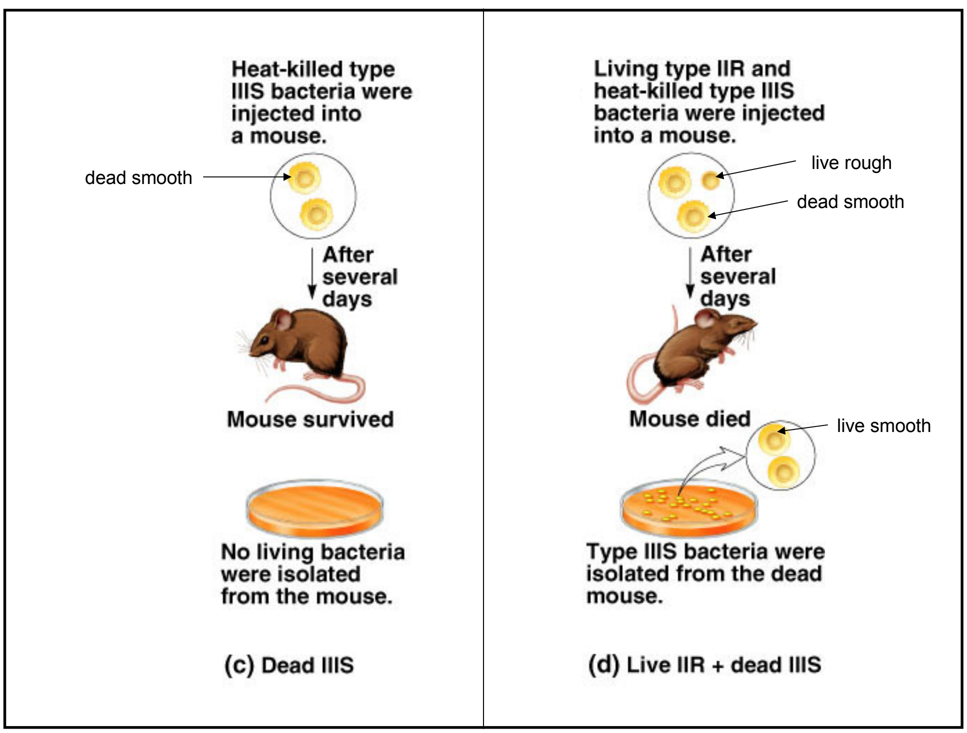
- *S. pneumoniae* comes in two strains
  – S → Smooth (strain IIIS)
    - Secretes a polysaccharide capsule (evades immune system)
    - Produce smooth colonies on solid media

  – R → Rough (strain IIR)
    - Unable to secrete a capsule
    - Produce colonies with a rough appearance



Living type IIIS bacteria were injected into a mouse.
After several days
Mouse died
Type IIIS bacteria were isolated from the dead mouse.
(a) Live IIIS
Living type IIR bacteria were injected into a mouse.
After several days
Mouse survived
No living bacteria were isolated from the mouse.
(b) Live IIR

Figure 9.2



Heat-killed type IIIS bacteria were injected into a mouse.
dead smooth
After several days
Mouse survived
No living bacteria were isolated from the mouse.
(c) Dead IIIS
Living type IIR and heat-killed type IIIS bacteria were injected into a mouse.
live rough
dead smooth
After several days
Mouse died
live smooth
Type IIIS bacteria were isolated from the dead mouse.
(d) Live IIR + dead IIIS

The Experiments of Avery, MacLeod & McCarty

- realized that Griffith's observations could be used to identify the genetic material or "transforming principle"

- In essence, the formation of the capsule is guided by the bacteria's genetic material
  - Transformed bacteria *acquired* information to make the capsule
  - *Variation* exists in ability to make capsule
  - The information required to create a capsule is *replicated* and *transmitted* from mother to daughter cells

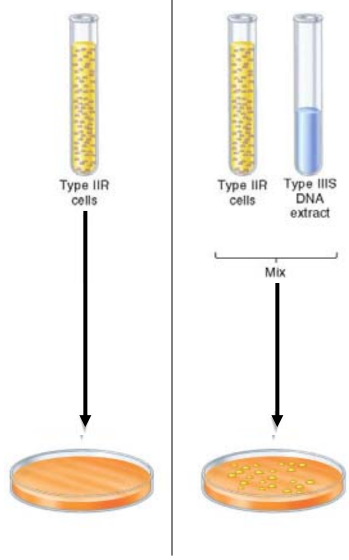
The Experiments of Avery, MacLeod & McCarty

- At the time of their experimentation in the 1940s, it was known that DNA, RNA, proteins and carbohydrates are the major constituents of living cells

- Prepared cell extracts from smooth cells (IIIS) and added to rough cells (IIR) for transformation in culture medium

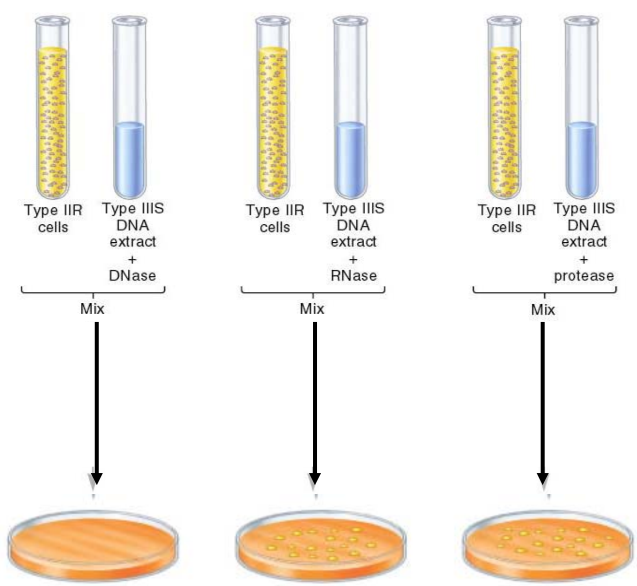
- Only the DNA <u>enriched</u> extract was able to convert rough cells into smooth cells

Figure 9.3



Method

- Allow sufficient time for the DNA extract to be taken up by the rough cells
- Add antibody that aggregates rough bacteria (not transformed)
- Gentle centrifugation (removes aggregated rough bacteria)
- Plate remaining cells in supernatant and incubate overnight (i.e. smooth cells will grow, if any)



Still....

More Verification was Needed

Hershey and Chase Experiment (1952)

- Studied the bacteriophage T2
  - It is relatively simple since its composed of only two macromolecules
    - DNA and protein

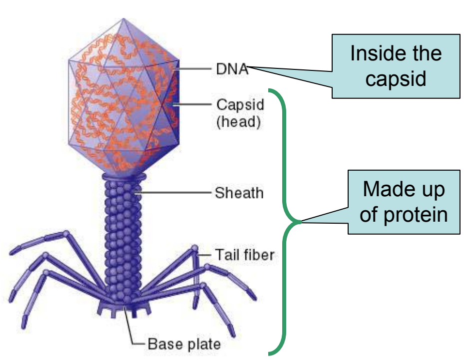


Figure 9.4

Figure 9.5

Life cycle of the T2 bacteriophage

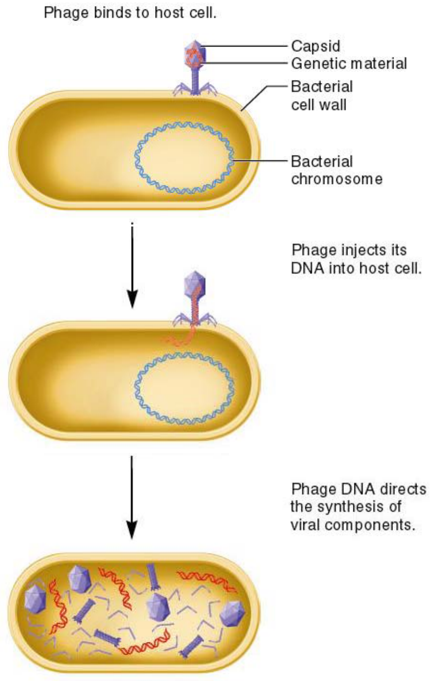
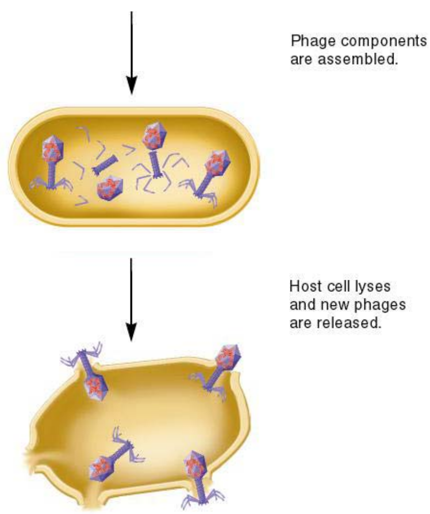


Figure 9.5

Life cycle of the T2 bacteriophage



Hypothesis

– Only the genetic material of the phage is injected into the bacterium
  - Isotope labeling will reveal if it is DNA or protein

Method

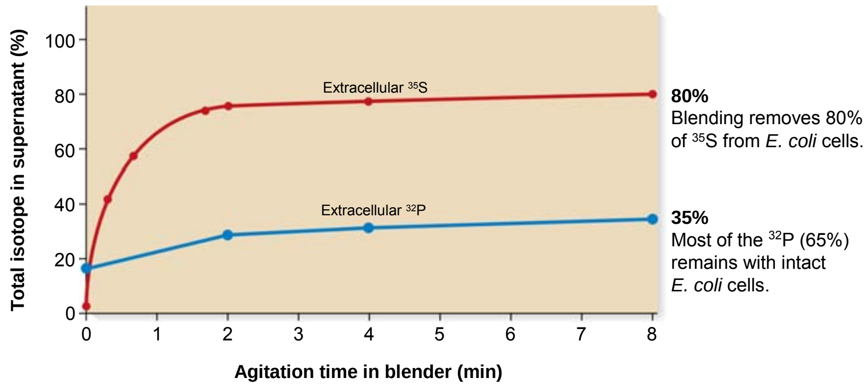
– Used radioisotopes to distinguish DNA from proteins
  - ^{32}P labels DNA specifically
  - ^{35}S labels protein specifically

– The two different Radioactively-labeled phages were used to infect non-radioactive *Escherichia coli* cells separately

– After allowing sufficient time for infection to proceed, the residual phage particles were sheared off the cells
  - Phage ghosts and *E. coli* cells were separated

– Radioactivity was monitored using a scintillation counter

RESULTS



Data from A. D. Hershey and Martha Chase (1952) Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage. *Journal of General Physiology* 36, 39–56.

TABLE 9.1

Examples of DNA- and RNA-Containing Viruses

Virus	Host	Nucleic Acid
Tomato bushy stunt virus	Tomato	RNA
Tobacco mosaic virus	Tobacco	RNA
Influenza virus	Humans	RNA
HIV	Humans	RNA
f2	*E. coli*	RNA
Qβ	*E. coli*	RNA
Cauliflower mosaic virus	Cauliflower	DNA
Herpesvirus	Humans	DNA
SV40	Primates	DNA
Epstein-Barr virus	Humans	DNA
T2	*E. coli*	DNA
M13	*E. coli*	DNA

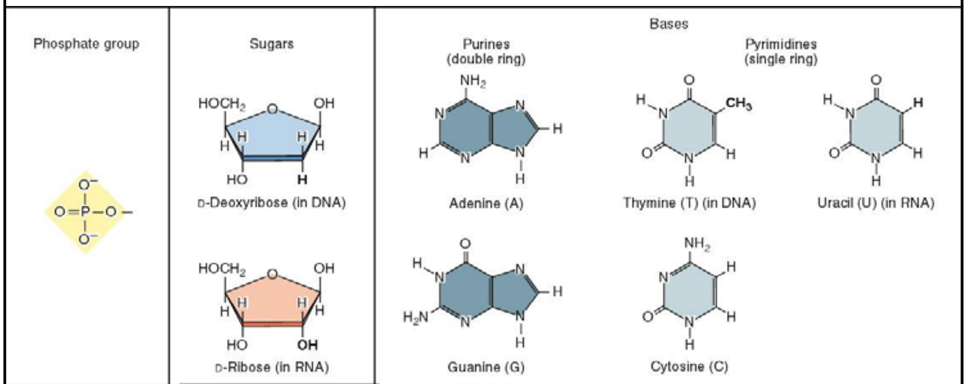
RNA can also serve as the genetic material in many viruses

THE STRUCTURE OF
DNA AND RNA

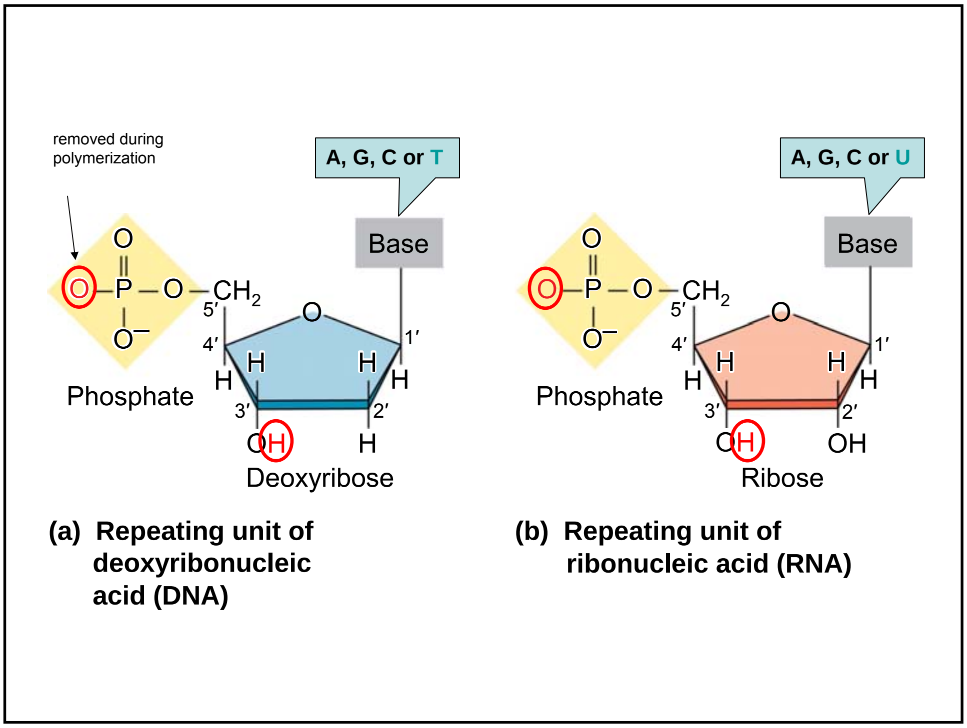
Nucleotides

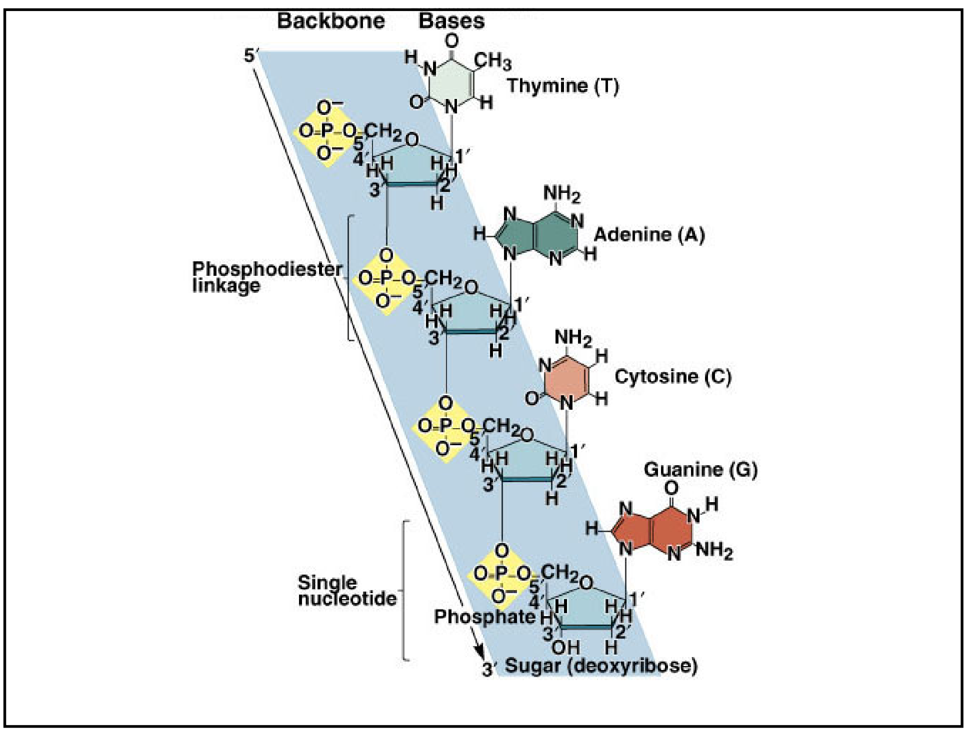
- The nucleotide is the repeating structural unit of DNA and RNA
- It has three components
  - A phosphate group
  - A pentose sugar
  - A nitrogenous base

Repeating unit comprised of: phoshate group + pentose sugar + nitrogenous base



- Nucleotides are covalently linked together by phosphodiester bonds
  - A phosphate connects the 5' carbon of one nucleotide to the 3' carbon of another
- Therefore the strand has directionality
  - 5' to 3'
- The phosphates and sugar molecules form the backbone of the nucleic acid strand
  - The bases project from the backbone


removed during polymerization
A, G, C or T
Base
Phosphate
Deoxyribose
A, G, C or U
Base
Phosphate
Ribose
(a) Repeating unit of deoxyribonucleic acid (DNA)
(b) Repeating unit of ribonucleic acid (RNA)

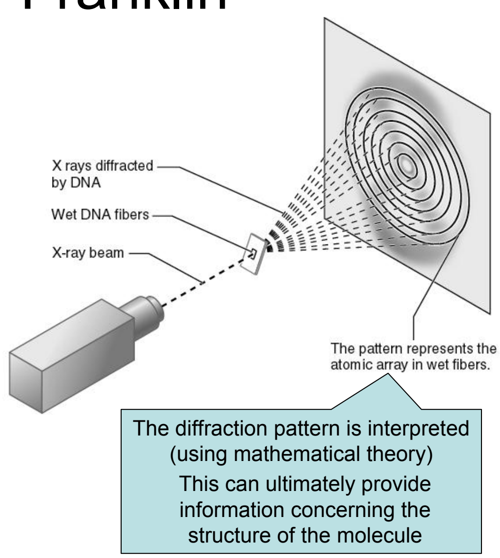

Backbone
Bases
Thymine (T)
Adenine (A)
Phosphodiester linkage
Cytosine (C)
Guanine (G)
Single nucleotide
Phosphate
Sugar (deoxyribose)

Discovery of the Structure of DNA

- In 1953, James Watson and Francis Crick discovered the double helical structure of DNA
- The scientific framework for their breakthrough was provided primarily by:
  – Rosalind Franklin (X-ray diffraction)
  – Erwin Chargaff (chemical composition)

Rosalind Franklin

- She used X-ray diffraction to study wet fibers of DNA
- The diffraction pattern she obtained suggested several structural features of DNA
  – Helical
  – More than one strand
  – 10 base pairs per complete turn



Erwin Chargaff's Experiment

- Chargaff pioneered many of the biochemical techniques for the isolation, purification and measurement of nucleic acids from living cells
- It was already known then that DNA contained the four bases: A, G, C and T
- Chargaff analyzed the the base composition of DNA in different species to see if there was a pattern

Base Content in the DNA from a Variety of Organisms*

	Percentage of Bases (based on molarity)			
Organism	*Adenine*	*Thymine*	*Guanine*	*Cytosine*
Escherichia coli	26.0	23.9	24.9	25.2
Streptococcus pneumoniae	29.8	31.6	20.5	18.0
Yeast	31.7	32.6	18.3	17.4
Turtle red blood cells	28.7	27.9	22.0	21.3
Salmon sperm	29.7	29.1	20.8	20.4
Chicken red blood cells	28.0	28.4	22.0	21.6
Human liver cells	30.3	30.3	19.5	19.9

*When the base compositions from different tissues within the same species were measured, similar results were obtained. These data were compiled from several sources. See E. Chargaff and J. Davidson, Eds. (1995) *The Nucleic Acids*. Academic Press, New York.

Chargaff's rule

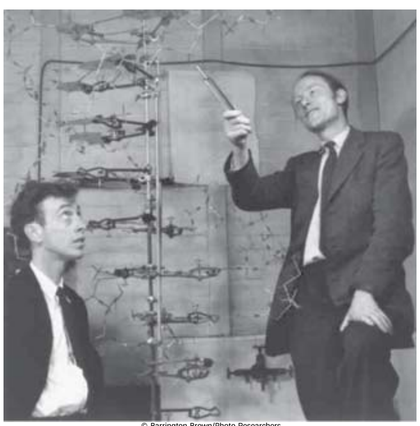
Percent of adenine = percent of thymine (A=T)

Percent of cytosine = percent of guanine (C=G)

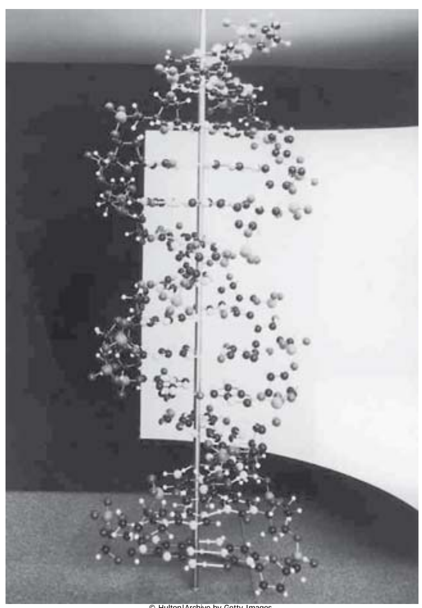
A+G = T+C   (or purines = pyrimidines)

Modeling the structure of DNA based on data observations

(Watson, Crick and Maurice Wilkins were awarded the Nobel Prize in 1962 for their discoveries)



(a) Watson and Crick



(b) Original model of the DNA double helix

The DNA Double Helix

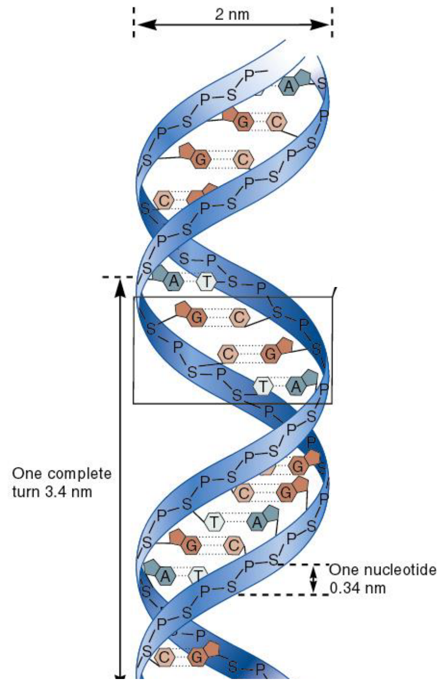
- General structural features
  - Two strands are twisted together around a common axis
  - There are 10 bases per complete twist
  - The two strands are antiparallel
    - One runs in the 5' to 3' direction and the other 3' to 5'
  - The helix is primarily right-handed in the B form
    - As it spirals away from you, the helix turns in a clockwise direction

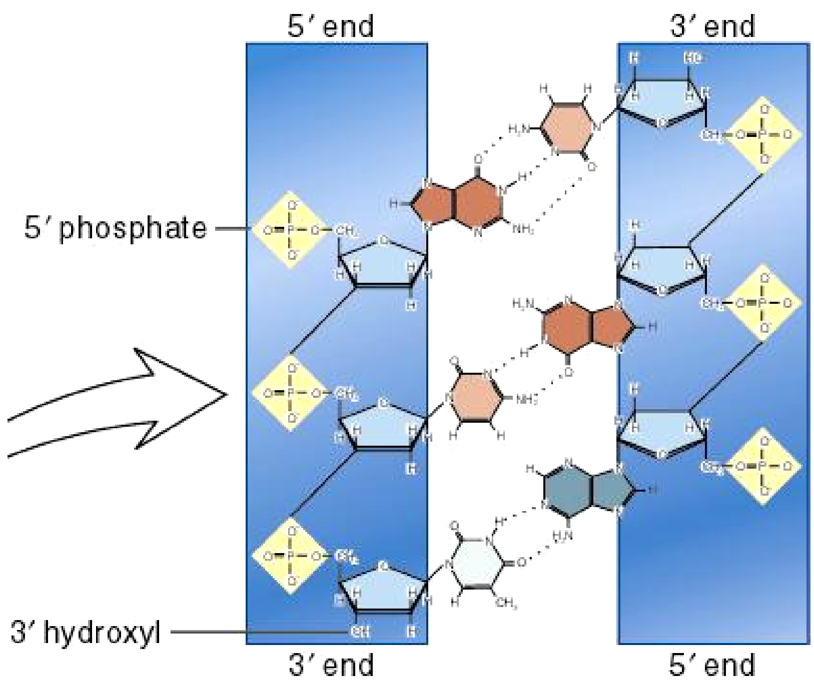
The DNA Double Helix

- General structural features
  - The double-bonded structure is stabilized by
    - 1. Hydrogen bonding between complementary bases
      - A bonded to T by two hydrogen bonds
      - C bonded to G by three hydrogen bonds
    - 2. Base stacking
      - Within the DNA, the bases are oriented so that the flattened regions are facing each other

Key Features

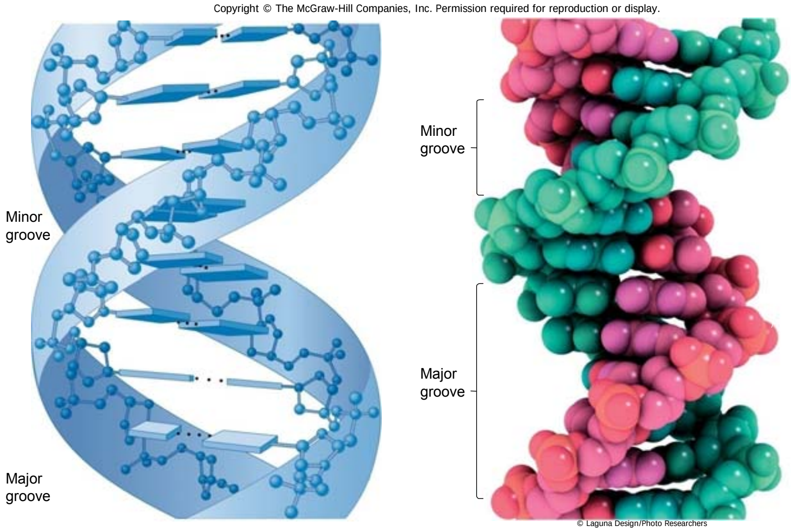
- Two strands of DNA form a right-handed double helix.
- The bases in opposite strands hydrogen bond according to the A/T and G/C rule.
- The 2 strands are antiparallel with regard to their 5′ to 3′ directionality.
- There are ~10.0 nucleotides in each strand per complete 360° turn of the helix.





The DNA Double Helix

- General structural features
  - There are two asymmetrical grooves on the outside of the helix
    - 1. Major groove
    - 2. Minor groove
    - Certain proteins can bind within these grooves
      - They can thus interact with a particular sequence of bases



(a) Ball-and-stick model of DNA (b) Space-filling model of DNA

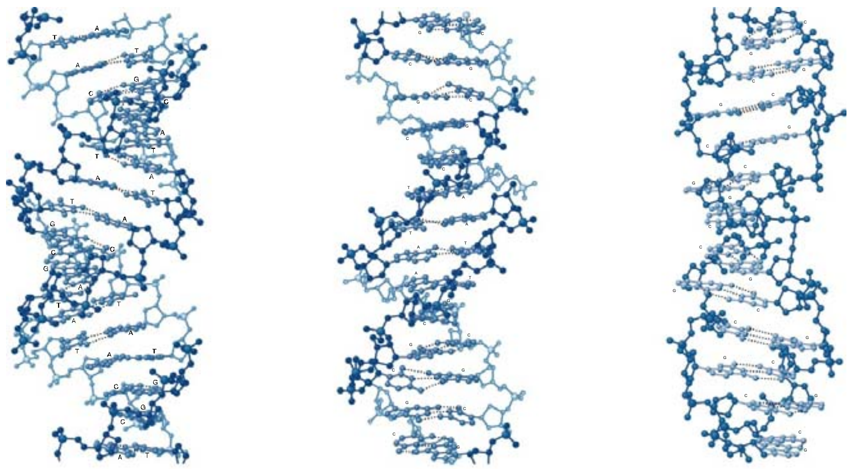
Figure 9.17

DNA Can Form Alternative Types of Double Helices

- The DNA double helix can form different types of secondary structure
  - The predominant form found in living cells is B-DNA
  - However, under certain *in vitro* conditions, A-DNA and Z-DNA double helices can form

- A-DNA
  - Right-handed helix
  - 11 bp per turn
  - Occurs under conditions of low humidity
  - Little evidence to suggest that it is biologically important
- Z-DNA
  - Left-handed helix
  - 12 bp per turn
  - Its formation is favored by
    - Alternating purine/pyrimidine sequences, at high salt concentrations (e.g. GCGCGCGCGC)
  - Evidence from yeast suggests that it may play a role in transcription and recombination

9-50



A- DNA B- DNA Z- DNA

(note the tilt of the bases for A- and Z-type DNA)

DNA Can Form a Triple Helix

• synthetic DNA oligomers (short pieces) were found to complex to double stranded DNA forming a triplex

•found to occur in nature during some instances of recombination and also during telomerase activity (extension of DNA ends)

RNA Structure

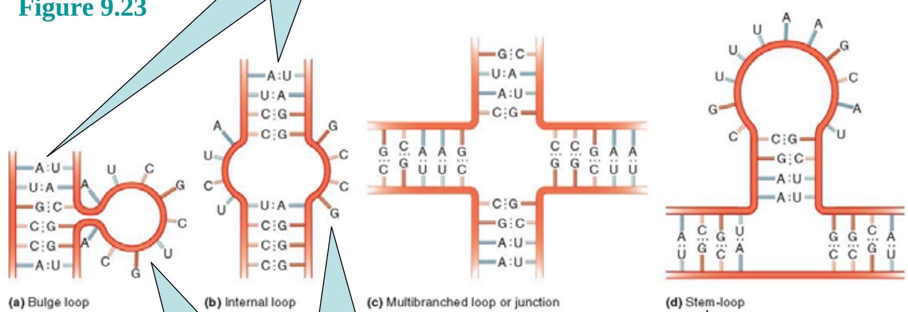
- The primary structure of an RNA strand is much like that of a DNA strand
- RNA strands are typically several hundred to several thousand nucleotides in length
- In RNA synthesis, only one of the two strands of DNA is used as a template

- Although usually single-stranded, RNA molecules can form short double-stranded regions
  - This secondary structure is due to complementary base-pairing
    - A to U and C to G
  - This allows short regions to form a double helix
- RNA double helices typically
  - Are right-handed (11-12 base pairs per turn)
- Different types of RNA secondary structures are possible

Complementary regions

Held together by hydrogen bonds

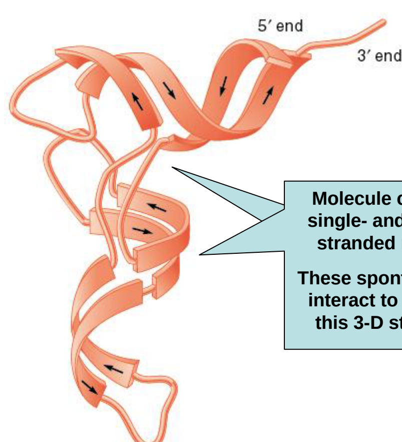
Figure 9.23



Noncomplementary regions

Have bases projecting away from double stranded regions

Also called hair-pin



Molecule contains single- and double-stranded regions

These spontaneously interact to produce this 3-D structure

Figure 9.24

(a) Ribbon model

- the tertiary structure of $tRNA^{phe}$

(transfer RNA carrying the amino acid phenylalanine)

GENOME/ CHROMOSOME ORGANIZATION

Prokaryotic vs. Eukaryotic

What are the essential differences?

How would this impact chromosome organization?

- The main function of the genetic material is to store the 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. Replication of chromosomes
  - 3. Proper segregation of chromosomes
  - 4. Compaction of chromosomes
    - So they can fit within living cells

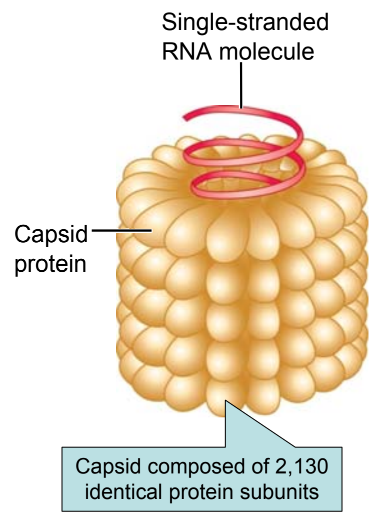
Viral Genomes

- The genome can be
  - DNA <u>or</u> 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

- During an infection process, mature viral particles need to be assembled

- Viruses with a simple structure may self-assemble
  - Genetic material and capsid proteins spontaneously bind to each other
  - Example: Tobacco mosaic virus



- Complex viruses, such as T2 bacteriophages, undergo a process called directed assembly
  - Virus assembly requires proteins that are not part of the mature virus itself

- The noncapsid proteins usually have two main functions
  - 1. Carry out the assembly process
    - Scaffolding proteins that are not part of the mature virus
  - 2. Act as proteases that cleave viral capsid proteins
    - This yields smaller capsid proteins that assemble correctly

BACTERIAL CHROMOSOMES

- The bacterial chromosome is found in a region of the cell called the nucleoid (not enclosed in membrane)

- 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

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

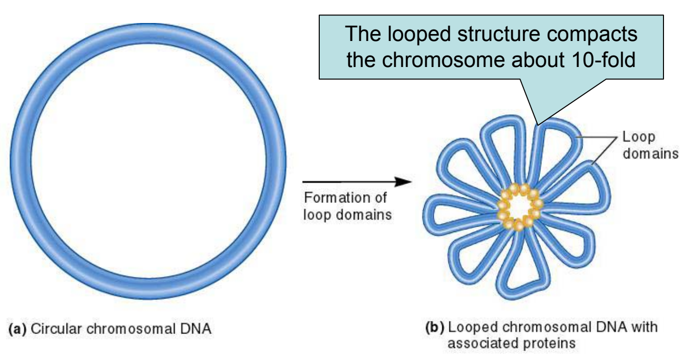


Figure 10.5

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

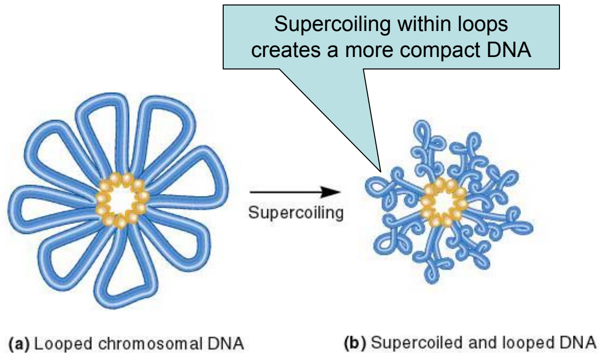
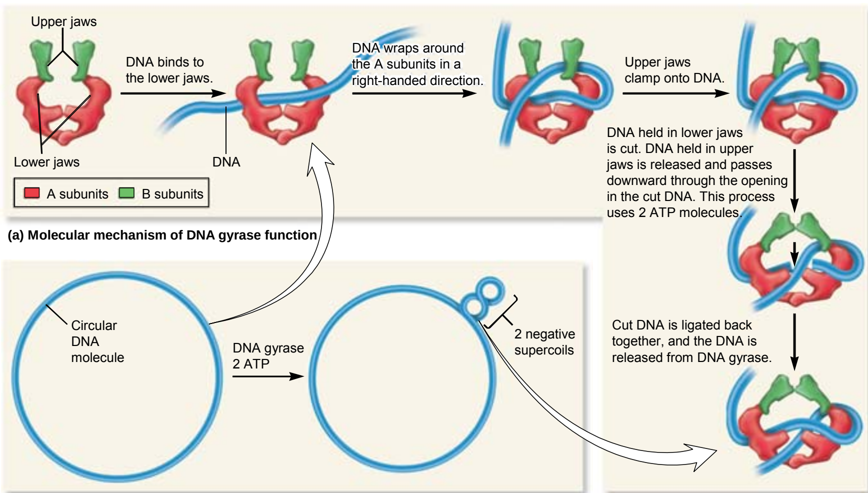
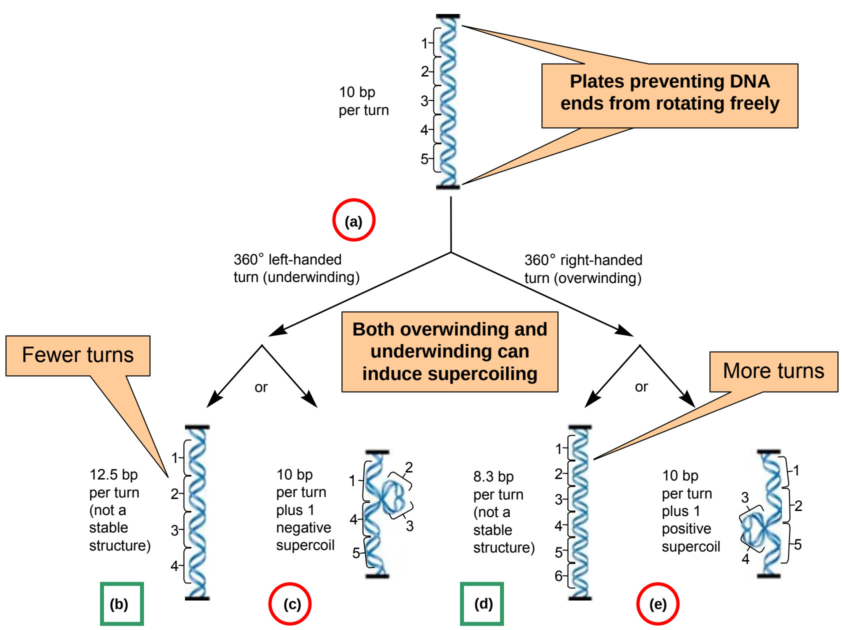


Figure 10.6

- 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

How Gyrase Works





These two DNA conformations do not occur in living cells

These three DNA conformations are **topoisomers** of each other

- The chromosomal DNA in bacteria is negatively supercoiled
  - In *E. coli*, there is one negative supercoil per 40 turns of the double helix

- Negative supercoiling has two major effects
  - 1. Helps in the compaction of the chromosome
  - 2. Creates tension that may be released by DNA strand separation

- Two main classes of drugs inhibit gyrase and other bacterial topoisomerases
  - 1. Quinolones
  - 2. Coumarins

  (note: these do not inhibit eukaryotic topoisomerases)

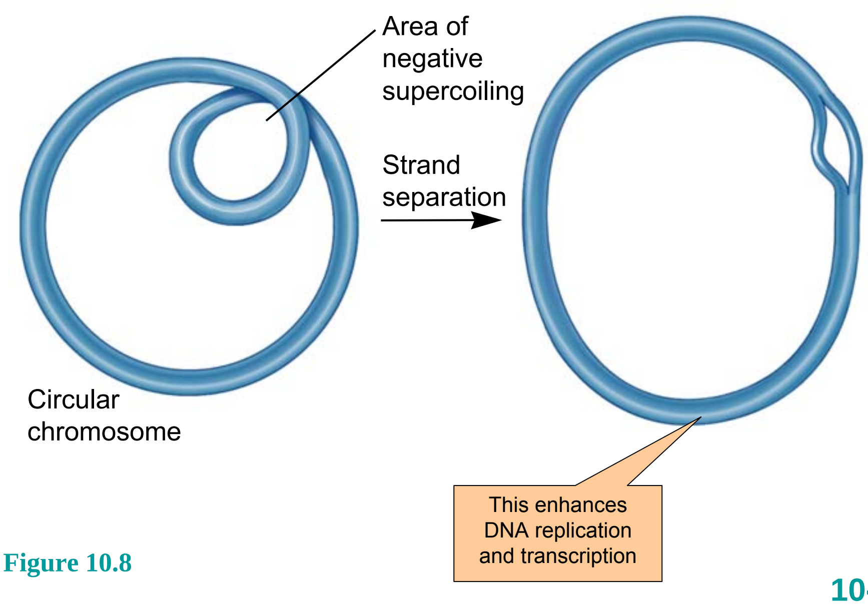


Figure 10.8

10-17

EUKARYOTIC CHROMOSOMES

- Eukaryotic genomes vary substantially in size
  - 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

Variation in Eukaryotic Genome Size

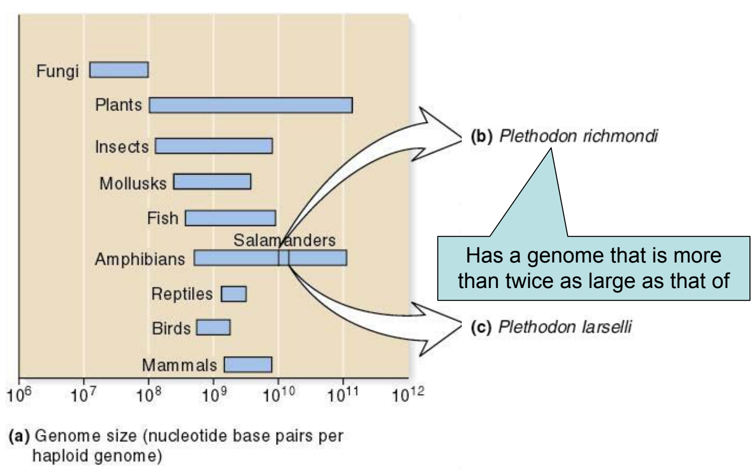


Figure 10.10

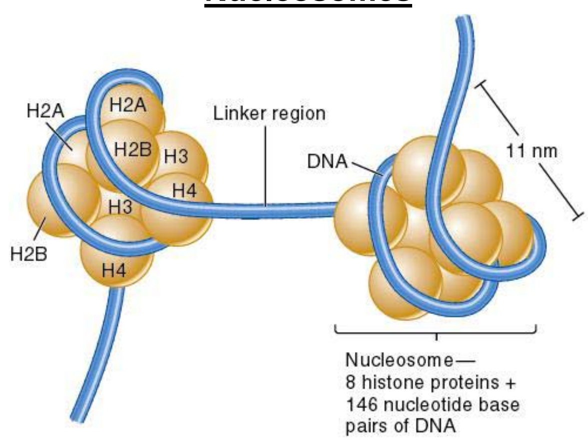
Eukaryotic Chromatin Compaction

-Problem-

- If stretched end to end, a single set of human chromosomes will be over 1 meter long- but cell's nucleus is only 2 to 4 μm in diameter!!!

- How does the cell achieve such a degree of chromatin compaction?

First Level= Chromatin organized as repeating units
Nucleosomes

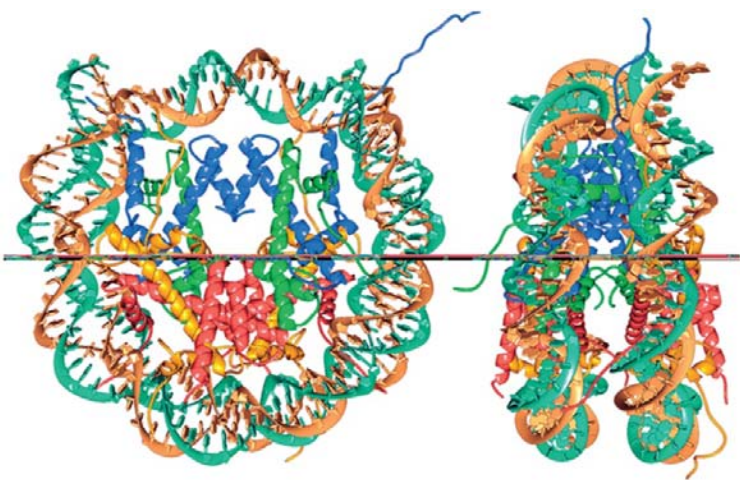


- Double-stranded DNA wrapped around an octamer of histone proteins

- Connected nucleosomes resembles "beads on a string"
  - seven-fold reduction of DNA length

- 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

Nucleosome core particle



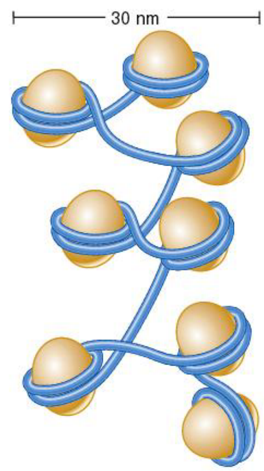
(b) Molecular model for nucleosome structure

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

Histone H1 plays a role in this compaction (non-histone proteins also play a role)

The 30 nm fiber shortens the total length of DNA another **seven-fold**

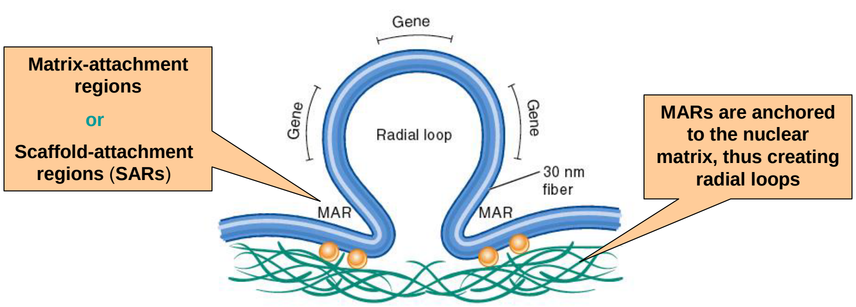
These two events compact the DNA
7x7 =49 (~50 fold compaction)



Three-dimensional zigzag model

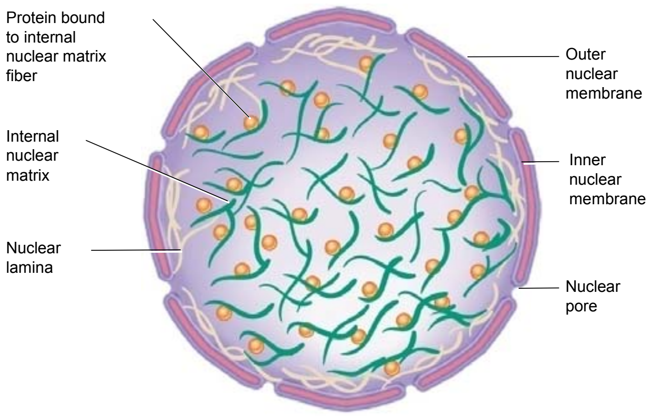
Further Compaction of the Chromosome

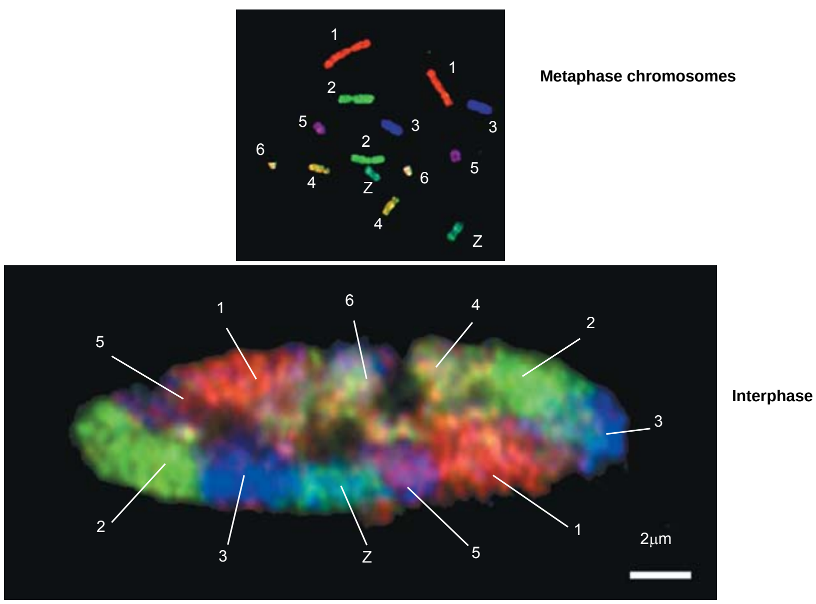
- A third level of compaction involves interaction between the 30 nm fiber and the nuclear matrix



The nuclear matrix is composed of two parts:

- Nuclear lamina (Fibers that line the inner nuclear membrane)
- Internal matrix proteins (Connected to nuclear lamina and fills interior of nucleus)



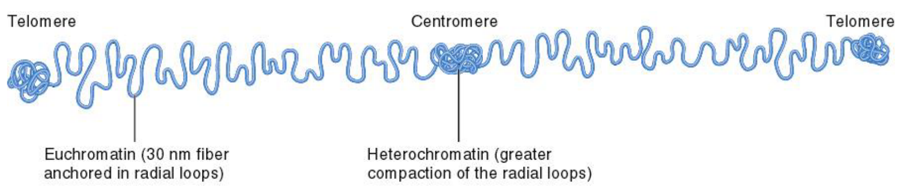


Each of seven types of chicken chromosomes is labeled a different color. Each occupies a specific territory during interphase.

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

Figure 10.20



- 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

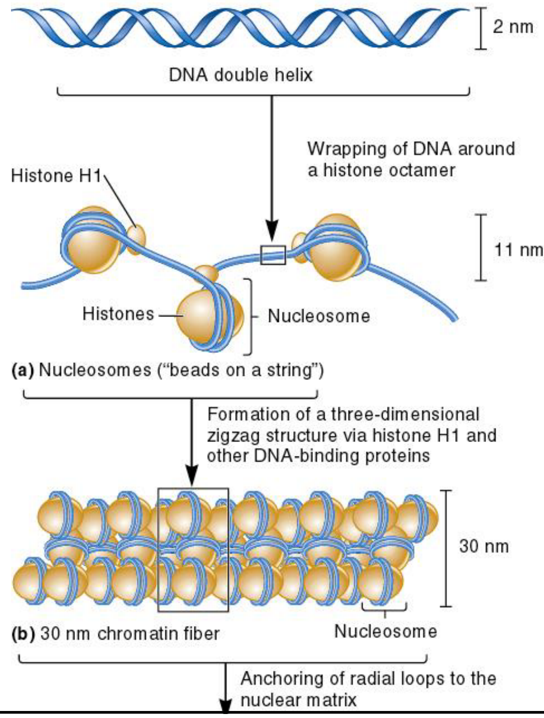
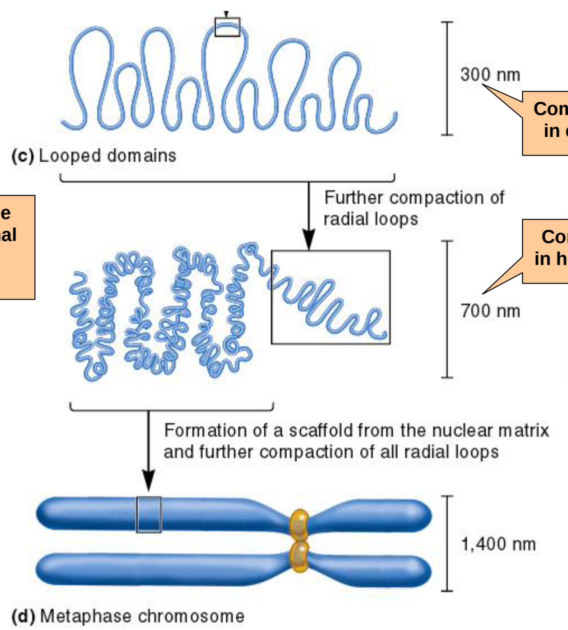


Figure 10.21



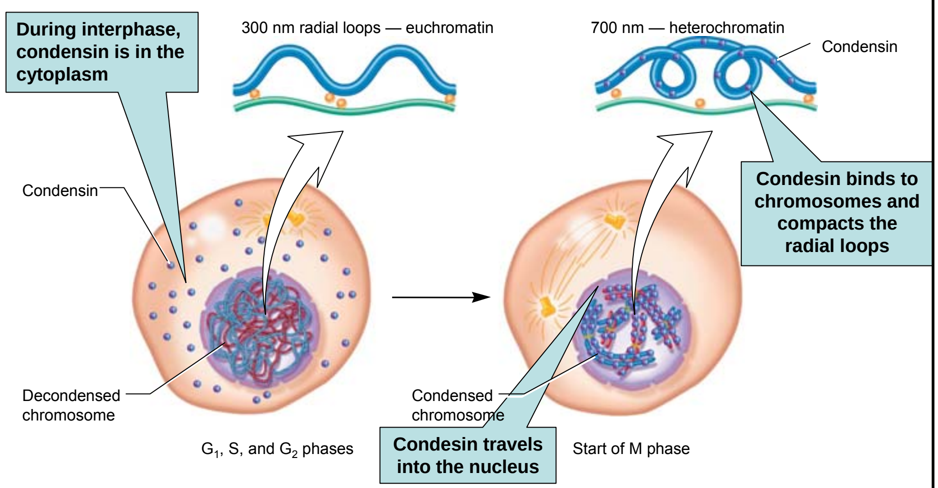
Compaction level in euchromatin

Compaction level in heterochromatin

During interphase most chromosomal regions are euchromatic

Figure 10.21

Condensin plays a critical role in condensation



Cohesin plays a critical role in sister chromatid alignment