DNA: The Genetic Material

Chapter 10

DNA as the Genetic Material

- DNA was first extracted from nuclei in 1870
- named ‘nuclein’ after their source.
- Chemical analysis
  - determined that DNA was a weak acid rich in phosphorous.
- Its name provides a lot of information about DNA:
  - deoxyribose nucleic acid:
  - it contains a sugar moiety (deoxyribose),
  - it is weakly acidic,
  - and is found in the nucleus.
- Because of its:
  - nuclear localization
  - subsequent identification as a component of chromosomes
  - it was implicated as a carrier of genetic information.

Are genes composed of DNA or protein?

- Chromosomes are also known to contain protein
  - so early on it was a challenge to demonstrate that DNA was indeed the molecule that contained the genetic information.
- DNA
  - Only four different subunits make up DNA
  - Chromosomes contain less DNA than protein by weight
- Protein
  - 20 different subunits – greater potential variety of combinations
  - Chromosomes contain more protein than DNA by weight
- Classical experimental data confirmed DNA as the genetic material.

Bacterial transformation implicates DNA as the substance of genes

- 1928 – Frederick Griffith – experiments with smooth (S), virulent strain *Streptococcus pneumoniae*, and rough (R), nonvirulent strain
  - Bacterial transformation demonstrates transfer of genetic material

- 1944 – Oswald Avery, Colin MacLeod, and MacIyn McCarty –
  - determined that DNA is the transformation material

Griffith experiment: transformation of bacteria

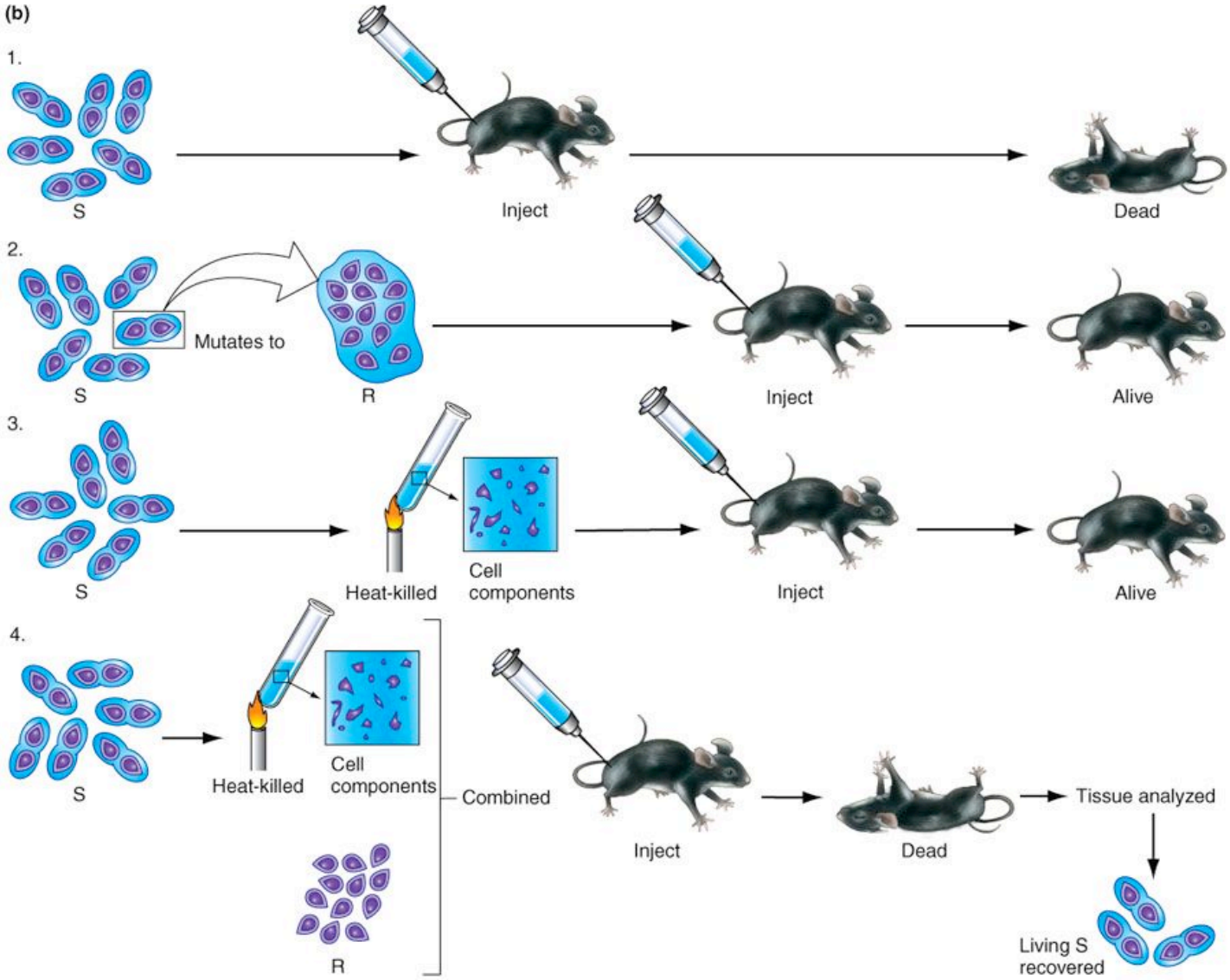
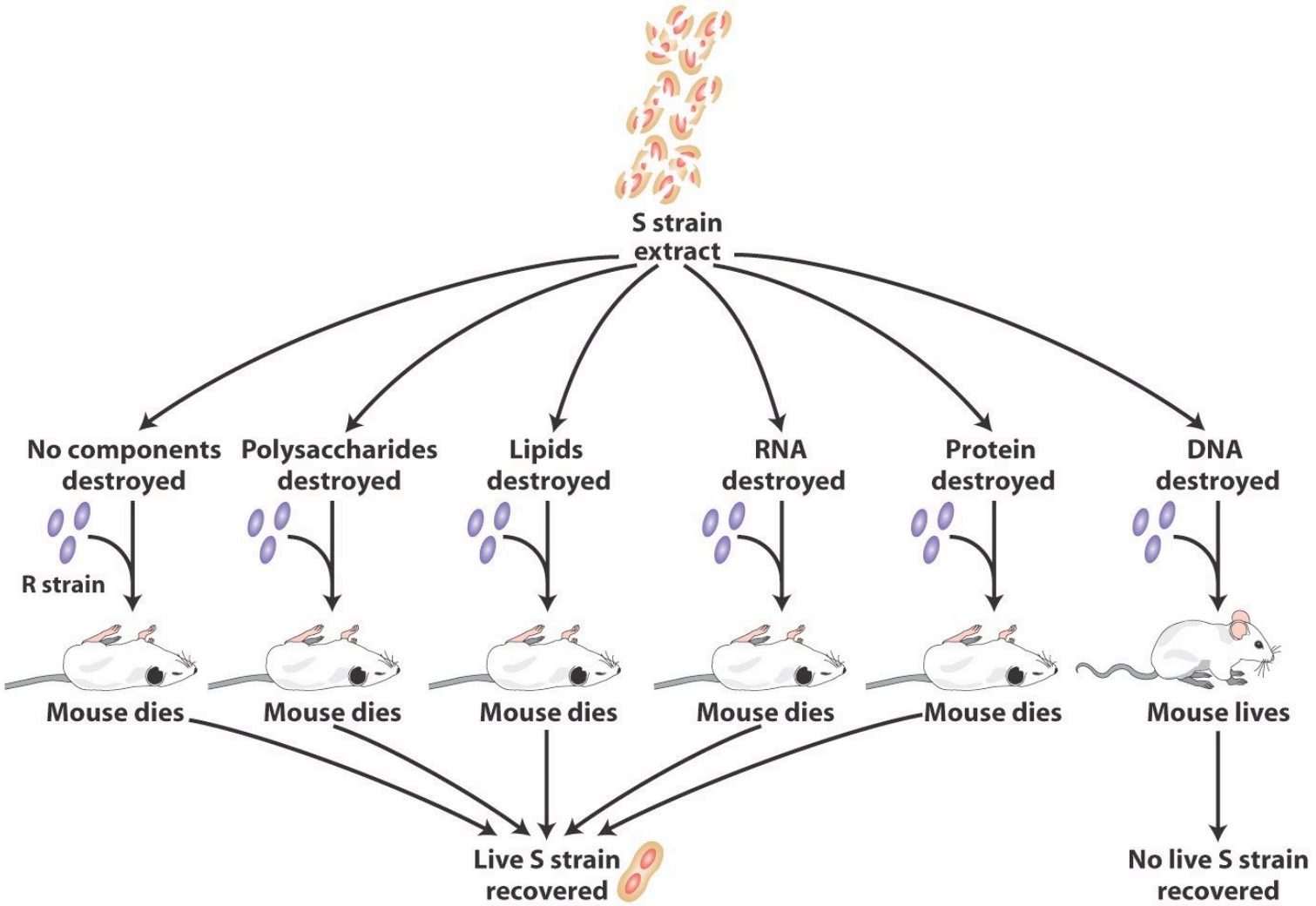


Fig. 6.3 b

Griffith's Experiment

- Griffith observed that live S bacteria could kill mice injected with them.
- When he heat killed the S variants and mixed them with live R variants, and then injected the mixture in the mice, they died.
- Griffith was able to isolate the bacteria from the dead mice, and found them to be of the S variety.
- Thus the bacteria had been *Transformed* from the rough to the smooth version.
- *The ability of a substance to change the genetic characteristics of an organism is known as transformation.*
- Scientists set out to isolate this 'transforming principle' since they were convinced it was the carrier of the genetic information.

Avery, MacLeod, McCarty Experiment: Identity of the Transforming Principle



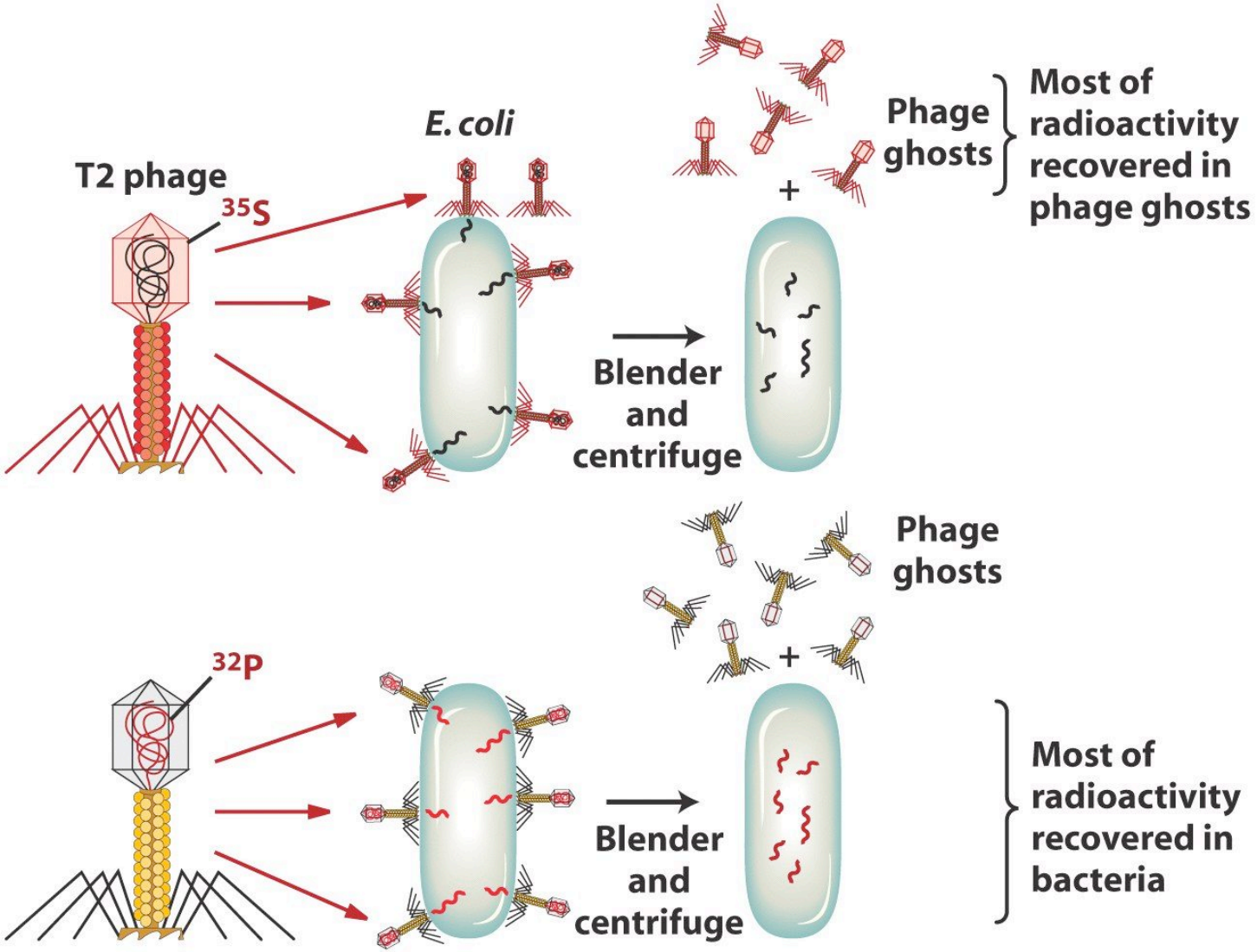
Hershey and Chase experiment

- 1952 – Alfred Hershey and Martha Chase provide convincing evidence that DNA is genetic material

- Waring blender experiment using T2 bacteriophage and bacteria

- Radioactive labels ^{32}P for DNA and ^{35}S for protein

Hersey-Chase Experiment



Hershey and Chase experiment

- Performed in 1952, using bacteriophage, a type of virus that have a very simple structure:
  - an outer core and an inner component.
- The phage are made up of equal parts of protein and DNA.
- It was known that the phage infect by anchoring the outer shell to the cell surface and then deposit the inner components to the cell, infecting it.
- Scientists were interested in finding out whether it was the protein component or the DNA component that got deposited inside the infected cell.
- By incorporating radiolabel either in the protein or the DNA of the infecting phage,
  - they determined that the DNA was indeed introduced into the infected bacteria, causing proliferation of new phage.

The Watson-Crick Model: DNA is a double helix

- 1951 – James Watson learns about x-ray diffraction pattern projected by DNA
- Knowledge of the chemical structure of nucleotides
  - deoxyribose sugar, phosphate, and nitrogenous base
- Erwin Chargaff's experiments demonstrate that
  - ratio of A and T are 1:1
  - ratio of G and C are 1:1
- 1953 – James Watson and Francis crick propose their double helix model of DNA structure

X-ray diffraction patterns produced by DNA fibers – Rosalind Franklin and Maurice Wilkins

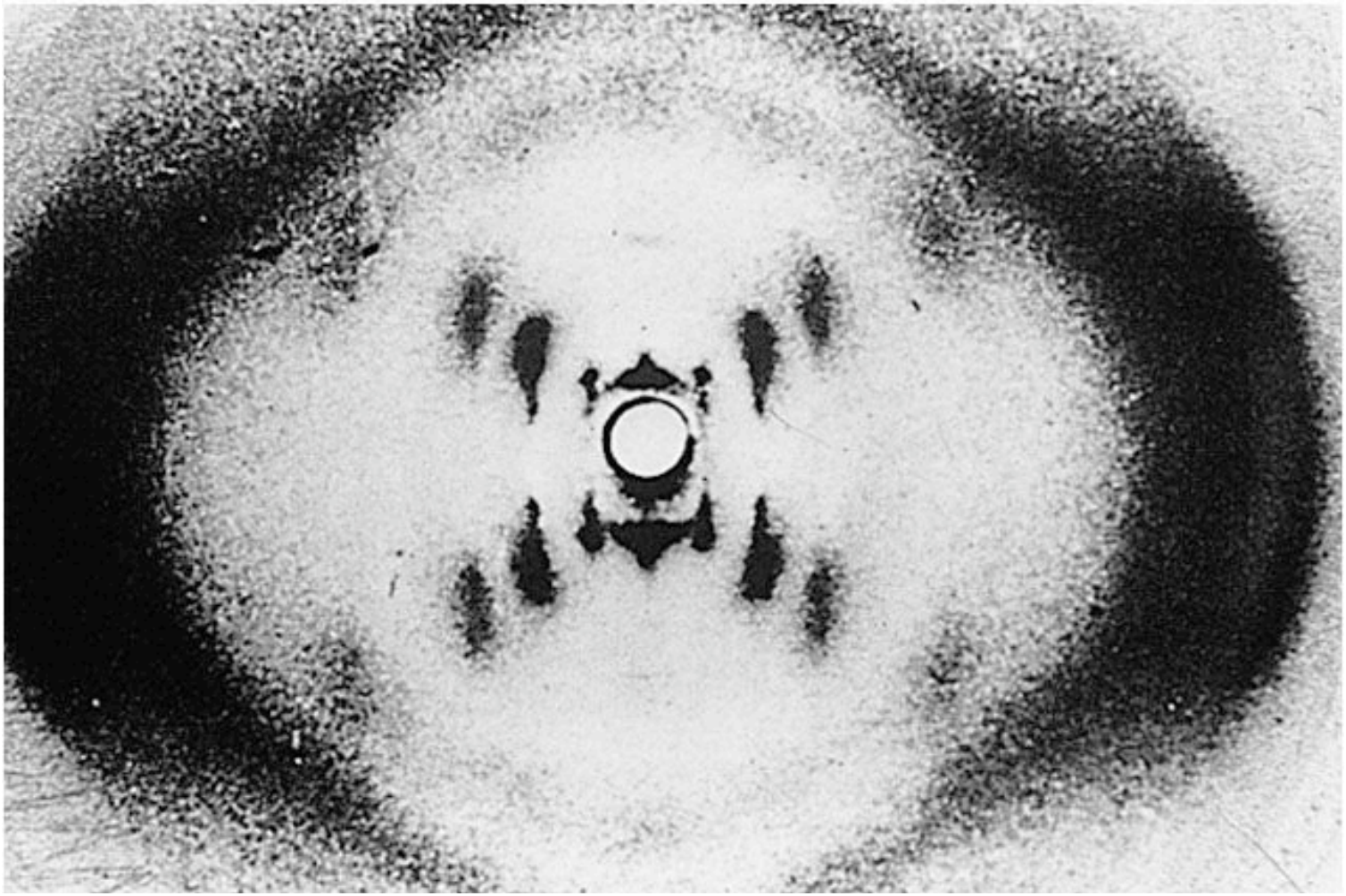
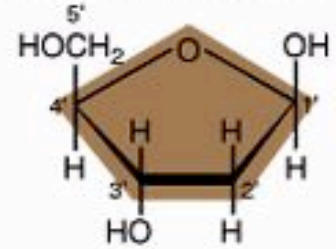


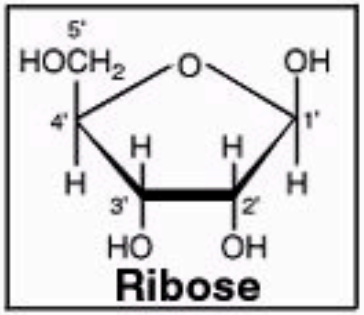
Fig. 6.6

DNA's chemical constituents, part 1

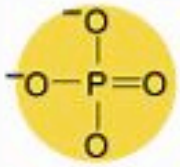
(a) The separate entities

1. Deoxyribose sugar



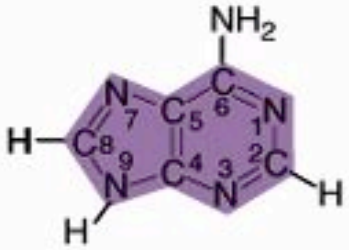


2. A phosphate group

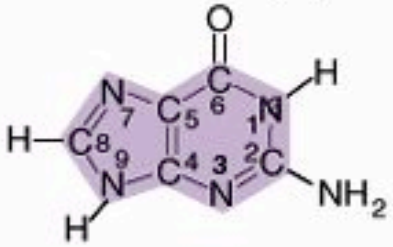


3. Four nitrogenous bases

Purines

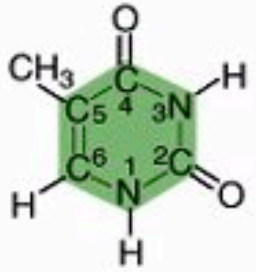


Adenine (A)

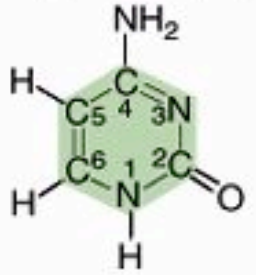


Guanine (G)

Pyrimidines



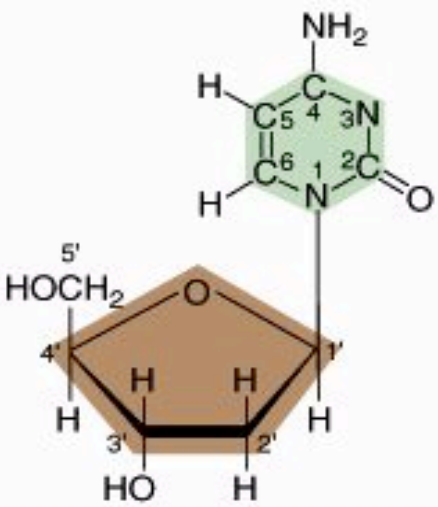
Thymine (T)



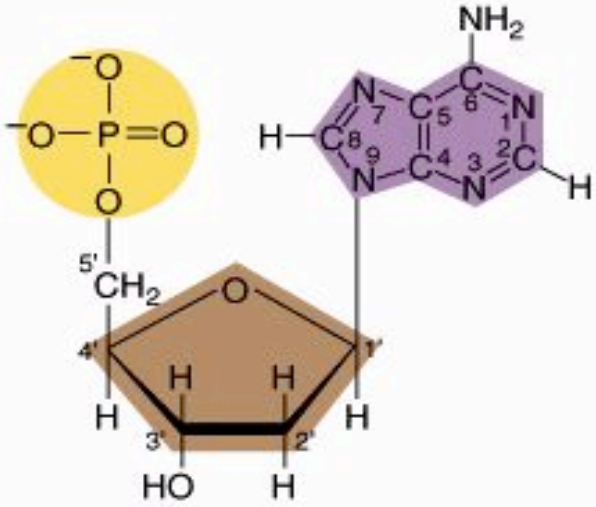
Cytosine (C)

DNA's chemical constituents, part 2

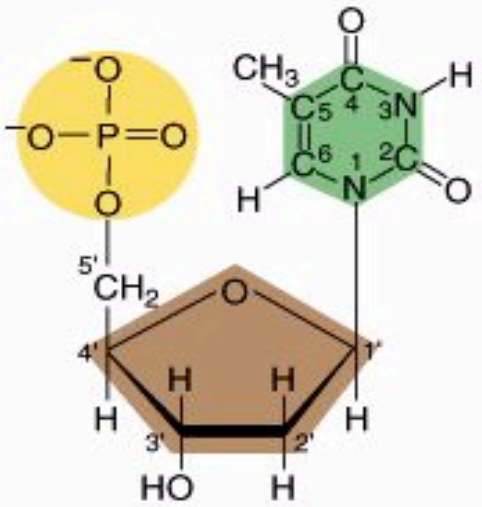
(b) Assembly into a nucleotide



Nucleoside



Purine nucleotide



Pyrimidine nucleotide

DNA's chemical constituents, part 2

(c) Nucleotides linked in a directional chain

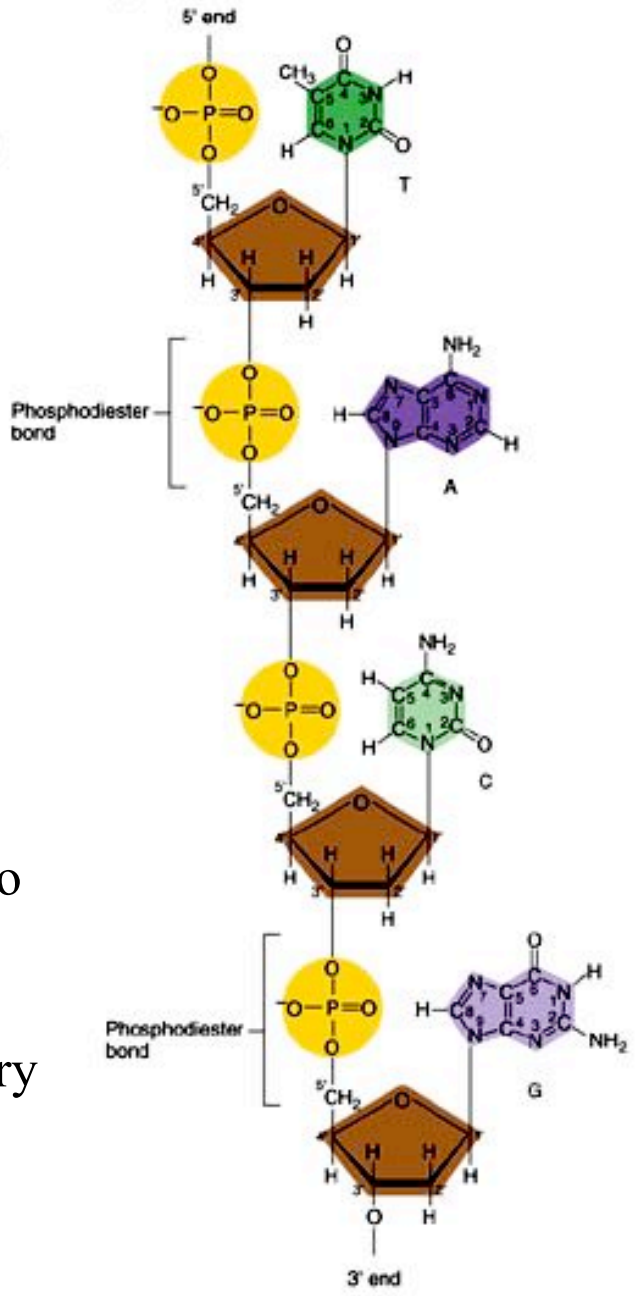
DNA is polar: 5’ to 3’

Beta N-glycosidic bond connects the sugar to the nitrogen base.

A phosphodiester bond connects one nucleotide to the next.

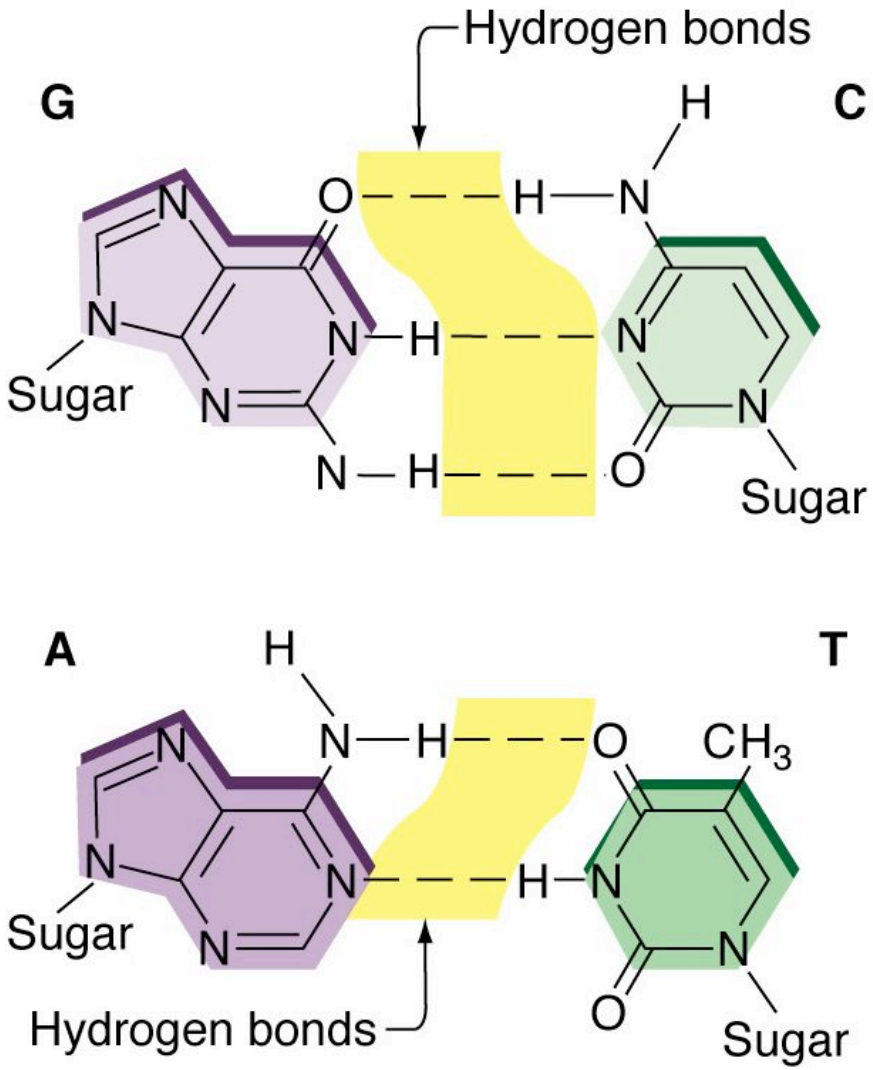
The sugar phosphate backbone is identical in every DNA molecule.

In any DNA purine = pyrimidine



Watson-Crick Model

- Double helical structure.
- The two strands are antiparallel
- It is a right handed helix, this structure is called the B DNA.
- Complementary base pairing:
  - three hydrogen bonds between C and G;
  - two hydrogen bonds between A and T.
- The arrangement of the nitrogen bases determines the genetic message.
- At each position, there are 4 possibilities,
  - therefore for a 100 base pair long molecule of DNA,
  - there are 4^{100} variations possible.



- Complementary base pairing:

– three hydrogen bonds between C and G;

–two hydrogen bonds between A and T.

Chargaff's Rules

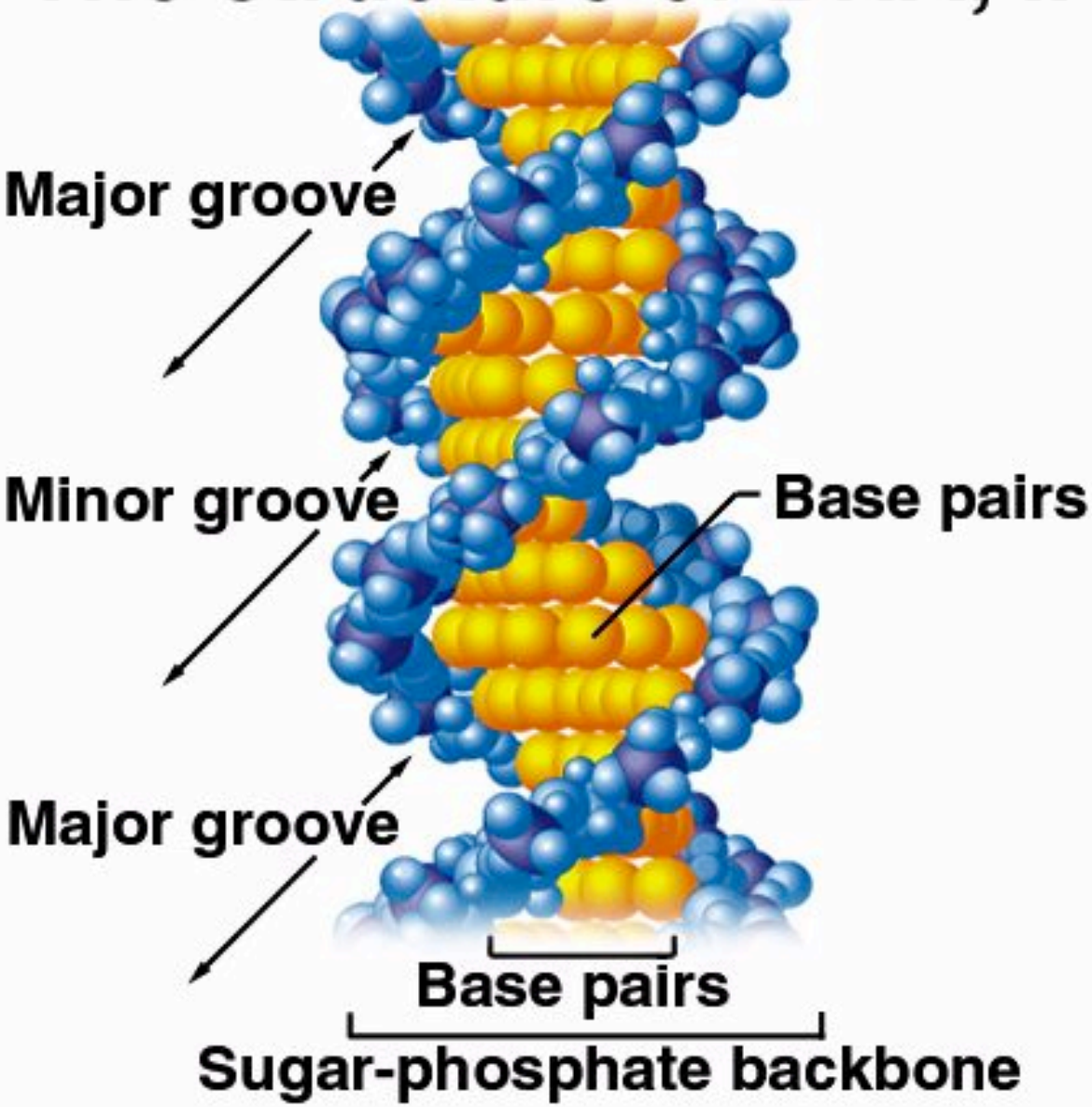
- Ratios of nucleotides
- A=T and G=C
- A+T does not have to equal G+C

TABLE 6.1 Chargaff's Data on Nucleotide Base Composition in the DNA of Various Organisms

	Percentage of Base in DNA				Ratios	
Organism	*A*	*T*	*G*	*C*	*A:T*	*G:C*
Staphylococcus afermentams	12.8	12.9	36.9	37.5	0.99	0.99
Escherichia coli	26.0	23.9	24.9	25.2	1.09	0.99
Yeast	31.3	32.9	18.7	17.1	0.95	1.09
*Caenorhabditis elegans**	31.2	29.1	19.3	20.5	1.07	0.96
*Arabadopsis thaliana**	29.1	29.7	20.5	20.7	0.98	0.99
Drosophila melanogaster	27.3	27.6	22.5	22.5	0.99	1.00
Honeybee	34.4	33.0	16.2	16.4	1.04	0.99
Mus musculus (mouse)	29.2	29.4	21.7	19.7	0.99	1.10
Human (liver)	30.7	31.2	19.3	18.8	0.98	1.03

*Data for *C. elegans* and *A. thaliana* is based on that for close relative organisms.
Note that even though the level of any one nucleotide is different in different organisms, the amount of A always approximately equals the amount of T, and the level of G is always similar to that of C. Moreover, as you can calculate for yourself, the total amount of purines (A plus G) nearly always equals the total amount of pyrimidines (C plus T).

The structure of DNA, II



Question

DNA was extracted from cells of Staphylococcus and found to have 37% cytosine. What percent of guanine does this species have?

1) 37%
2) 13%
3) 74%
4) 26%

Question

What percent of thymine does this species have?

1) 37%
2) 13%
3) 74%
4) 26%

Chromatin Structure

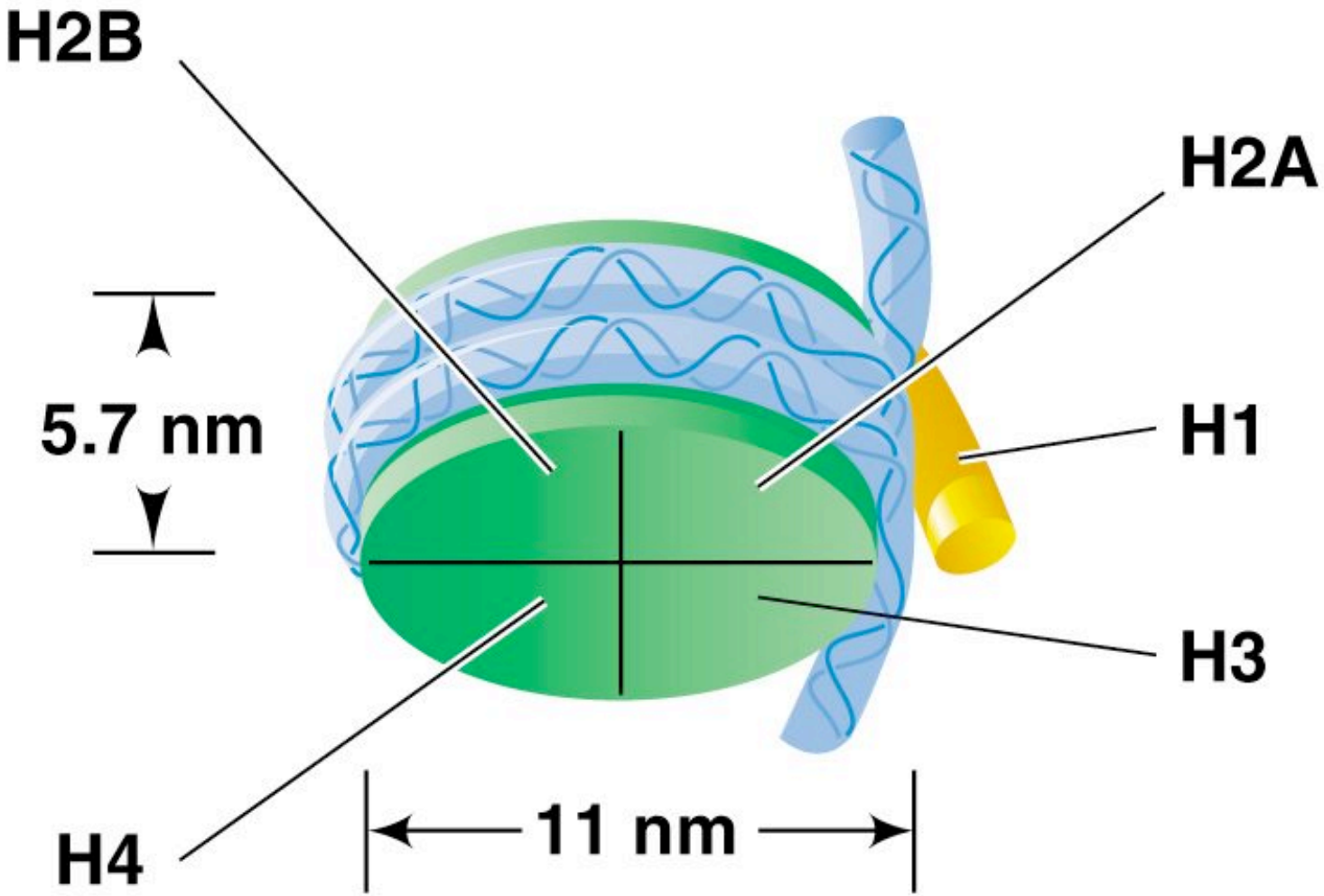
- Histones organize DNA, condense it and prepare it for further condensation by nonhistone proteins.
  - This compaction is necessary to fit large amounts of DNA (2m/6.5ft in humans) into the nucleus of a cell.

- *Nonhistone* is a general name for other proteins associated with DNA.
  - This is a big group, with some structural proteins, and some that bind only transiently.
  - Nonhistone proteins vary widely, even in different cells from the same organism.
  - Most have a net (-) charge, and bind by attaching to histones.

Chromatin Structure

- Both histones and nonhistones are involved in physical structure of the chromosome.
- Histones are abundant, small proteins with a net (+) charge.
  - The five main types are H1, H2A, H2B, H3, and H4.
  - By weight, chromosomes have equal amounts of DNA and histones.
- Histones are highly conserved between species
  - H1 less than the others.

Fig. 10.21 A possible nucleosome structure



Chromatin Structure

- Chromatin formation involves histones and DNA condensation so it will fit into the cell, making a 10-nm fiber
- Chromatin formation has two components:
  - Two molecules each of histones H2A, H2B, H3, and H4 associate to form a nucleosome core
  - and DNA wraps around it 1-3 or 4 times for a 7-fold condensation factor.
    - Nucleosome cores are about 11 nm in diameter.
  - H1 further condenses the DNA by connecting nucleosomes to create chromatin with a diameter of 30nm, for an additional 6-fold condensation.
    - The solenoid model proposes that the nucleosomes form a spiral with 6 nucleosomes per turn

Fig. 10.21
Nucleosomes connected together by linker DNA and H1 histone to produce the "beads-on-a-string" extended

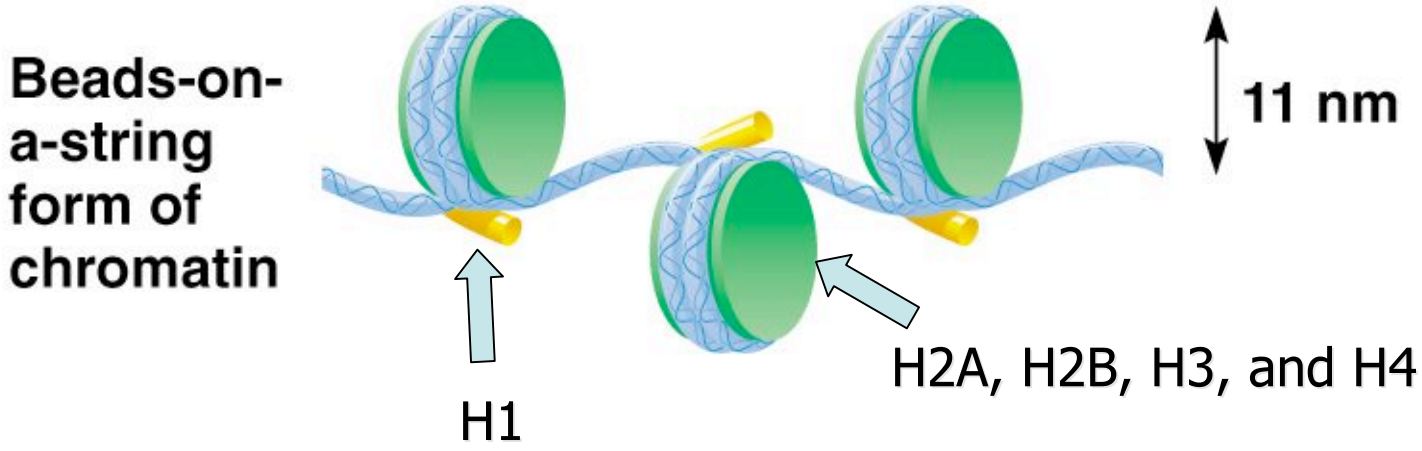
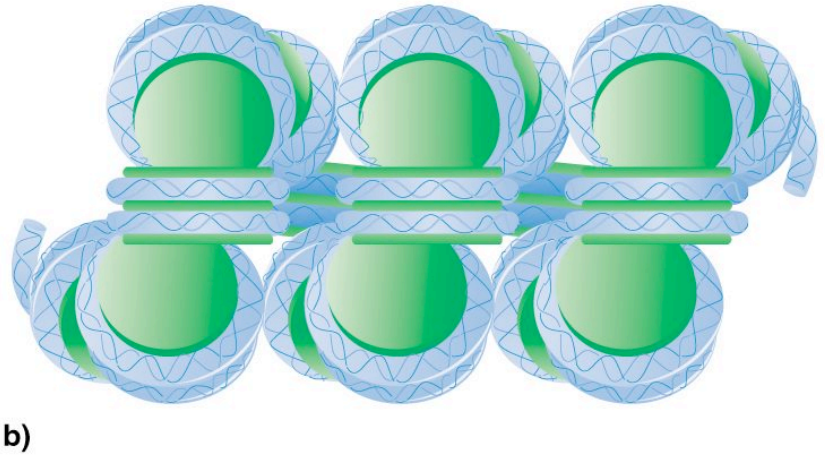


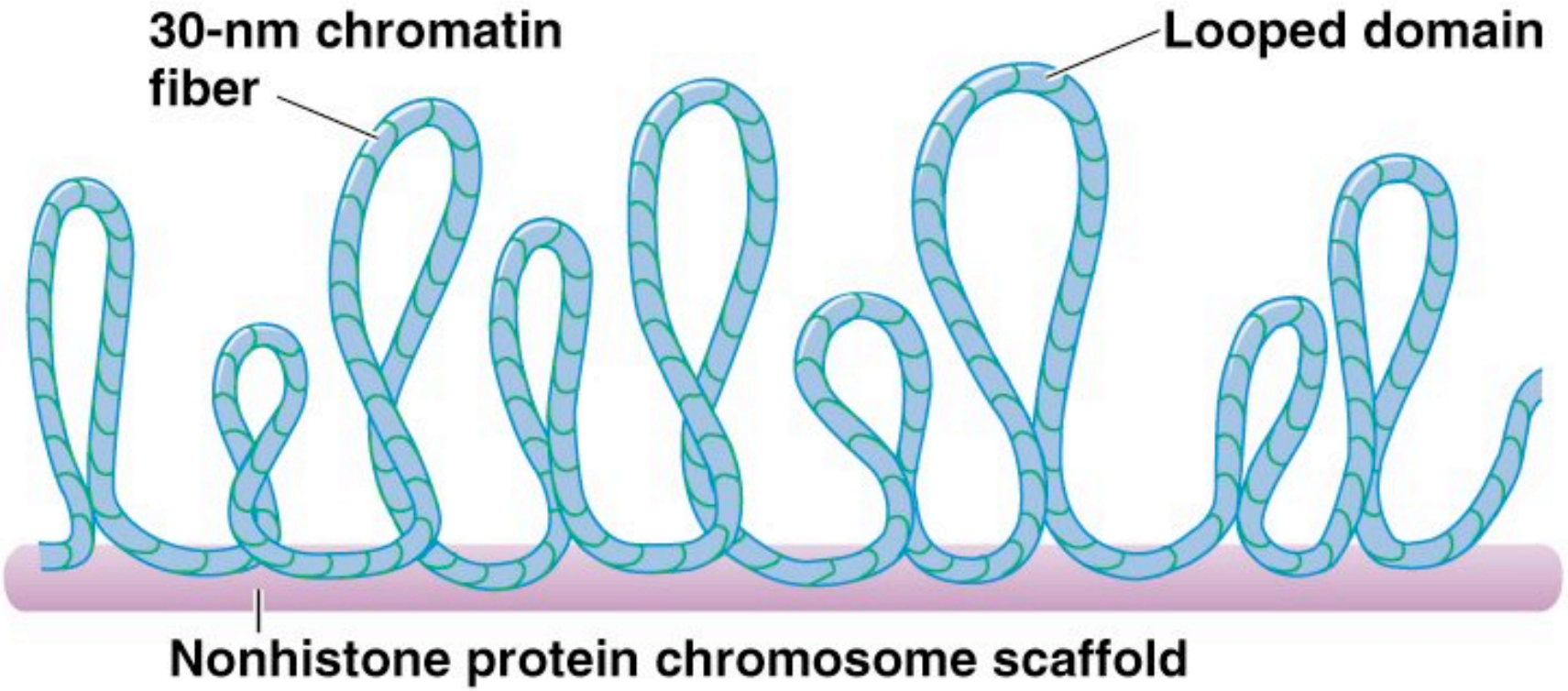
Fig. 10.21
Packaging of nucleosomes into the 30-nm chromatin fiber



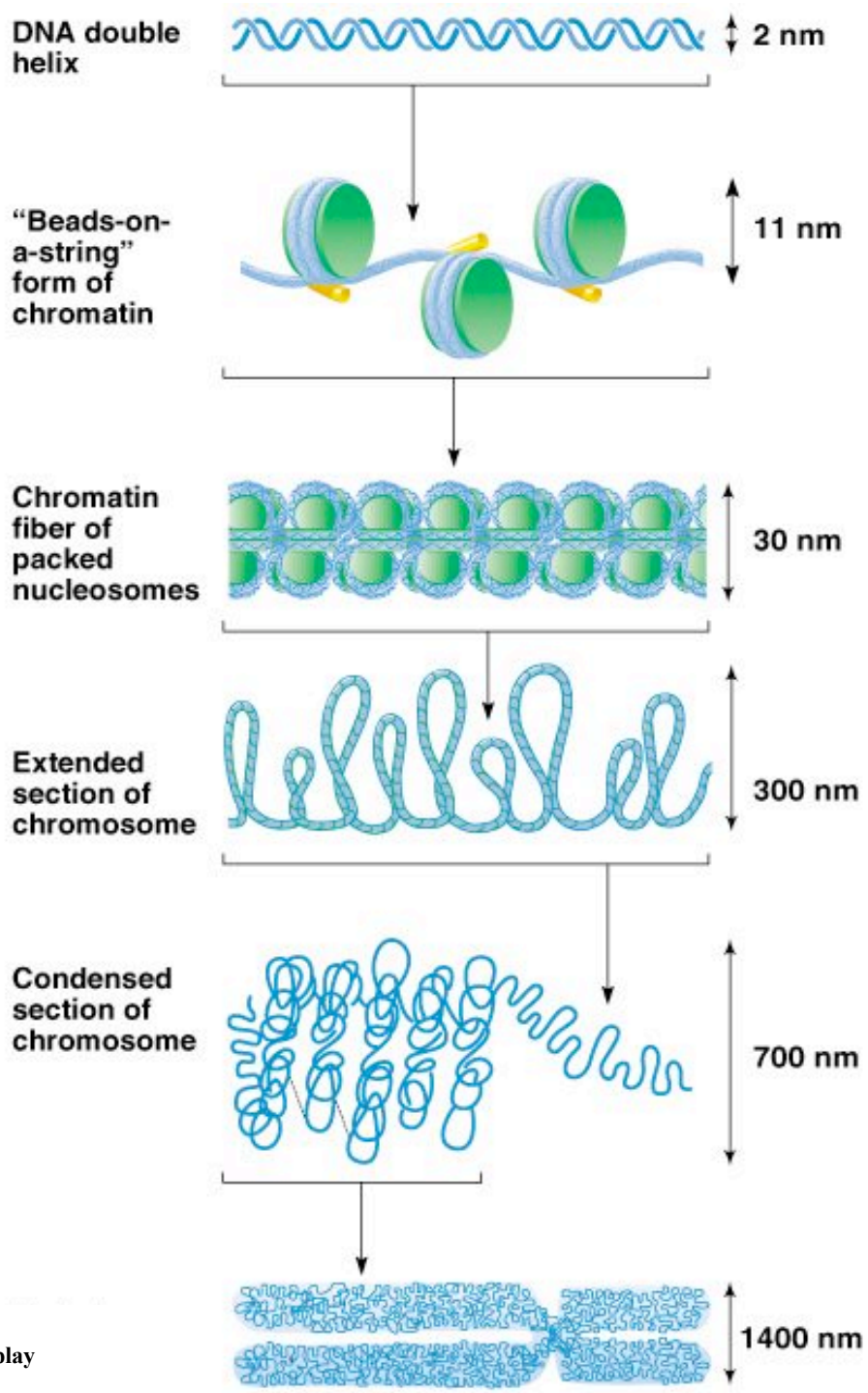
Chromatin Structure

- Beyond the 30-nm filament stage, electron microscopy shows 30–90 loops of DNA attached to a protein scaffold
- SARs (scaffold-associated regions) bind nonhistone proteins to form loops that radiate out in spiral fashion
- Fully condensed chromosome is 10,000-fold shorter and 400-fold thicker than DNA alone.

Fig. 10.24
Model for the organization of 30-nm chromatin fiber into looped domains that are anchored to a nonhistone protein chromosome scaffold



The many different orders of chromatin packing that give rise to the highly condensed metaphase chromosome



Euchromatin & Heterochromatin

- Staining of chromatin reveals two forms:
  - Euchromatin: condenses and decondenses with the cell cycle.
    - It is actively transcribed, and lacks repetitive sequences.
    - Euchromatin accounts for most of the genome in active cells.
  - Heterochromatin remains condensed throughout the cell cycle.
    - It replicates later than euchromatin
    - is transcriptionally inactive.

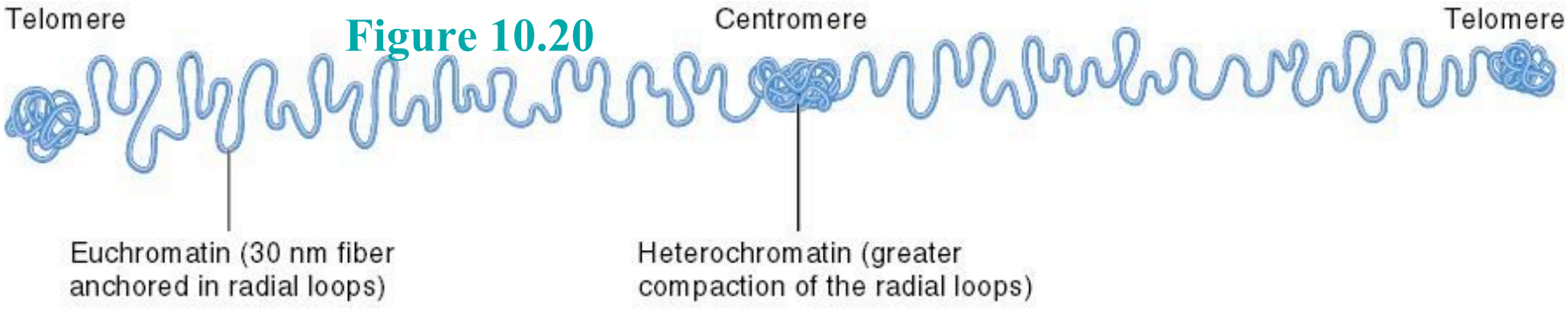
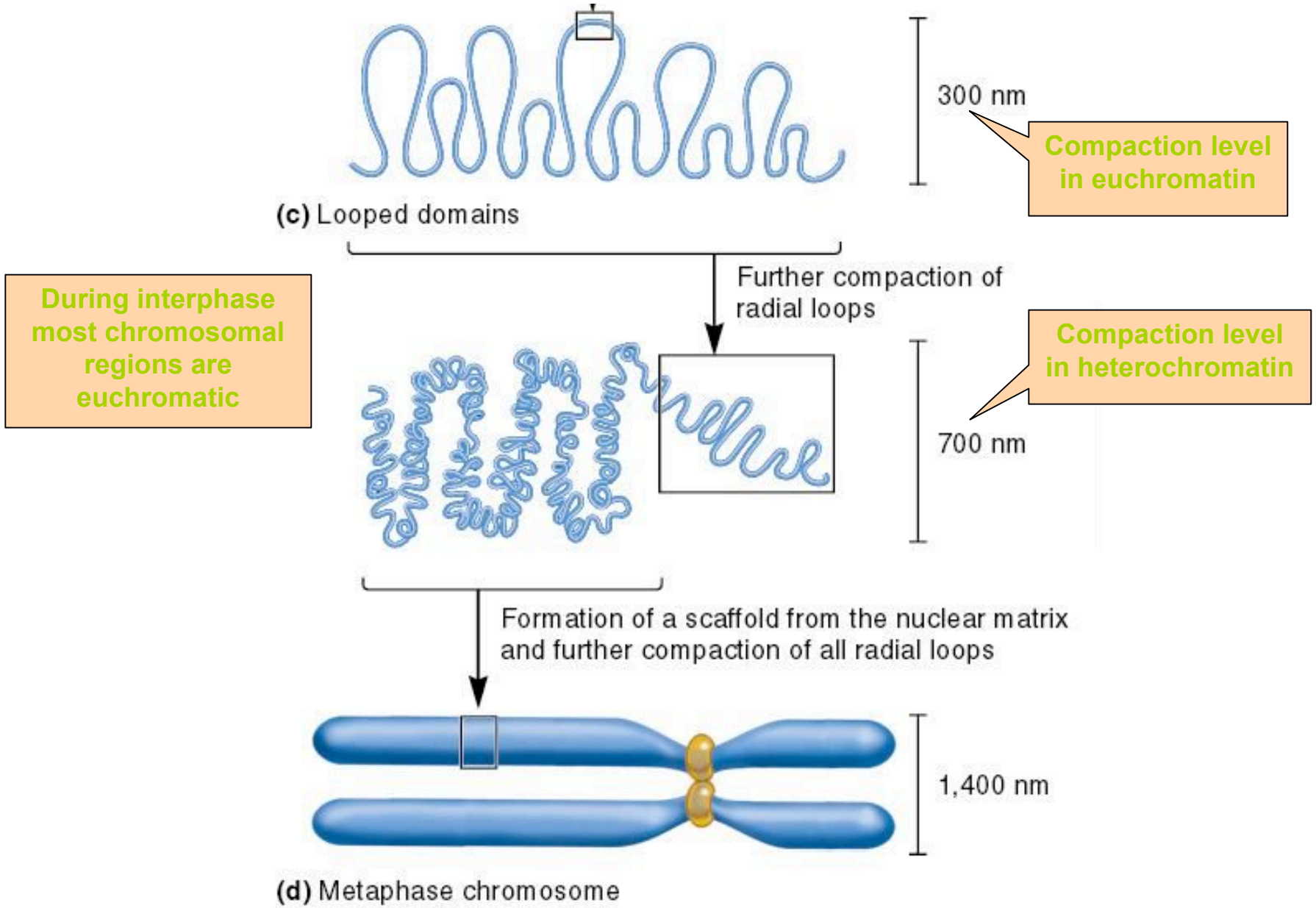


Figure 10.20

- There are two types of heterochromatin
  - Constitutive heterochromatin
    - Regions that are always heterochromatic
    - Permanently inactive with regard to transcription
    - occurs at the same sites in both homologous chromosomes consists mostly of repetitive DNA
      - (e.g., centromeres).
  - Facultative heterochromatin
    - Regions that can interconvert between euchromatin and heterochromatin
    - varies between cell types or developmental stages, or even between homologous chromosomes.
      - Example: Barr body



Centromeric and Telomeric DNA

- eukaryotic chromosomal regions with special functions.
  - Centromeres are the site of the kinetochore, where spindle fibers attach during mitosis and meiosis.
    - They are required for accurate segregation of chromatids.
  - Telomeres are located at the ends of chromosomes, and needed for chromosomal replication and stability.
    - Generally composed of heterochromatin, they interact with both the nuclear envelope and each other.
    - All telomeres in a species have the same sequence.
- Both are Heterochromatin

Unique-Sequence and Repetitive-Sequence DNA

- Prokaryotes have mostly unique-sequence DNA
- Eukaryotes have a mix of unique and repetitive sequences.
  - Unique-sequence DNA includes most of the genes that encode proteins
  - as well as other chromosomal regions.
- Human DNA contains about 65% unique sequences

Dispersed Repetitive Sequences

- There are two types of interspersion patterns found in all eukaryotic organisms:
  - LINEs (long interspersed repeated sequences) with sequences of 5 kb or more. The common example in mammals is LINE-1, with sequences up to 7kb in length, that can act as transposons.
  - SINEs (short interspersed repeated sequences) with sequences of 100–500bp. An example is the *Alu* repeats found in some primates, including humans, where these repeats of 200–300bp make up 9% of the genome.

Homework Problems

Chapter 10

1, 3, 6, 9, 11, 12, 14, 15, 28, 30, 33, 34, 35

- DON'T forget to take the online QUIZ!!
- DON'T forget to submit the online iActivity
  - "Viral Code"