DNA

The Genetic Material

Functions of the Genetic Material

The genetic material must **replicate**, control the **growth and development** of the organism, and allow the organism to **adapt to changes in the environment**.

- Genotypic Function: Replication
- Phenotypic Function: Gene Expression
- Evolutionary Function: Mutation (Gene modifications)

1928 - Frederick Griffith's Transformation Experiments

- Two strains of *Streptococcus pneumoniae*
  - **S → Smooth**
    - Secrete a polysaccharide capsule
    - Produce smooth colonies on solid media
    - **virulent**
  - **R → Rough**
    - Unable to secrete a capsule
    - Produce colonies with a rough appearance
    - **avirulent**
- Two coat types
  - II
  - III
- Four possible phenotypes
  - IIS or IIIS or IIR or IIIR

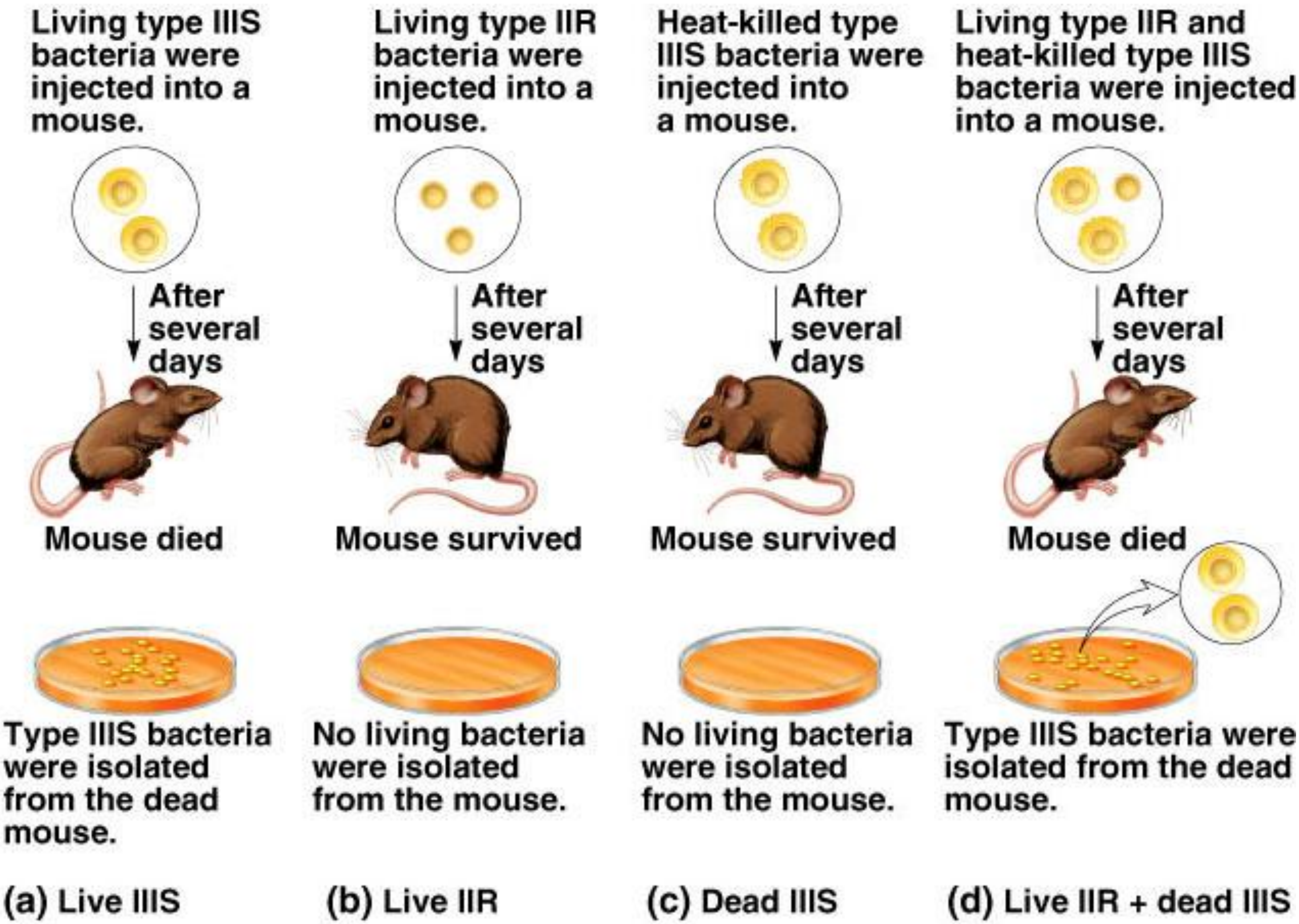
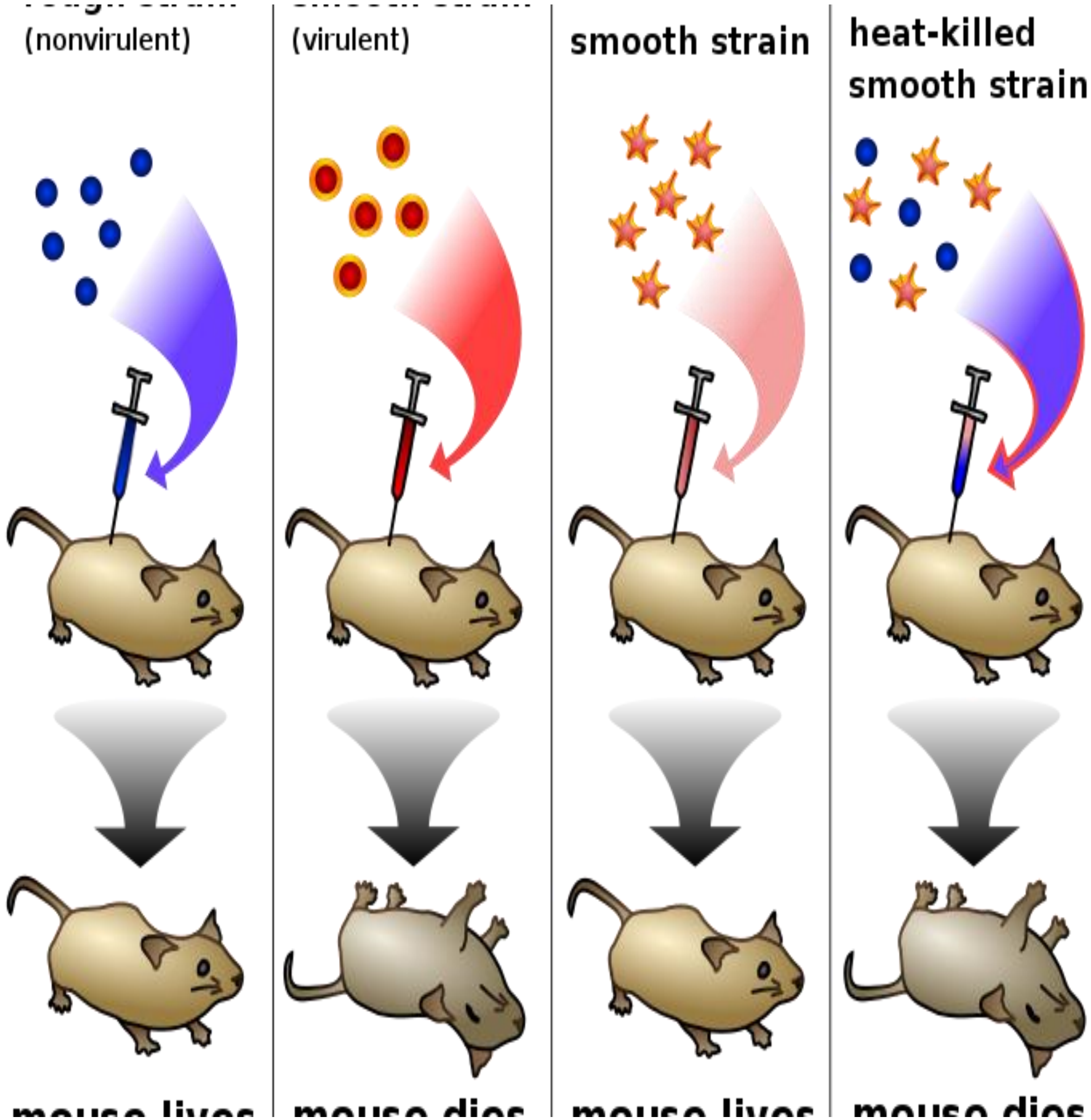

Living type IIIS bacteria were injected into a mouse.
Living type IIR bacteria were injected into a mouse.
Heat-killed type IIIS bacteria were injected into a mouse.
Living type IIR and heat-killed type IIIS bacteria were injected into a mouse.
After several days
After several days
After several days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

Figure 9.2

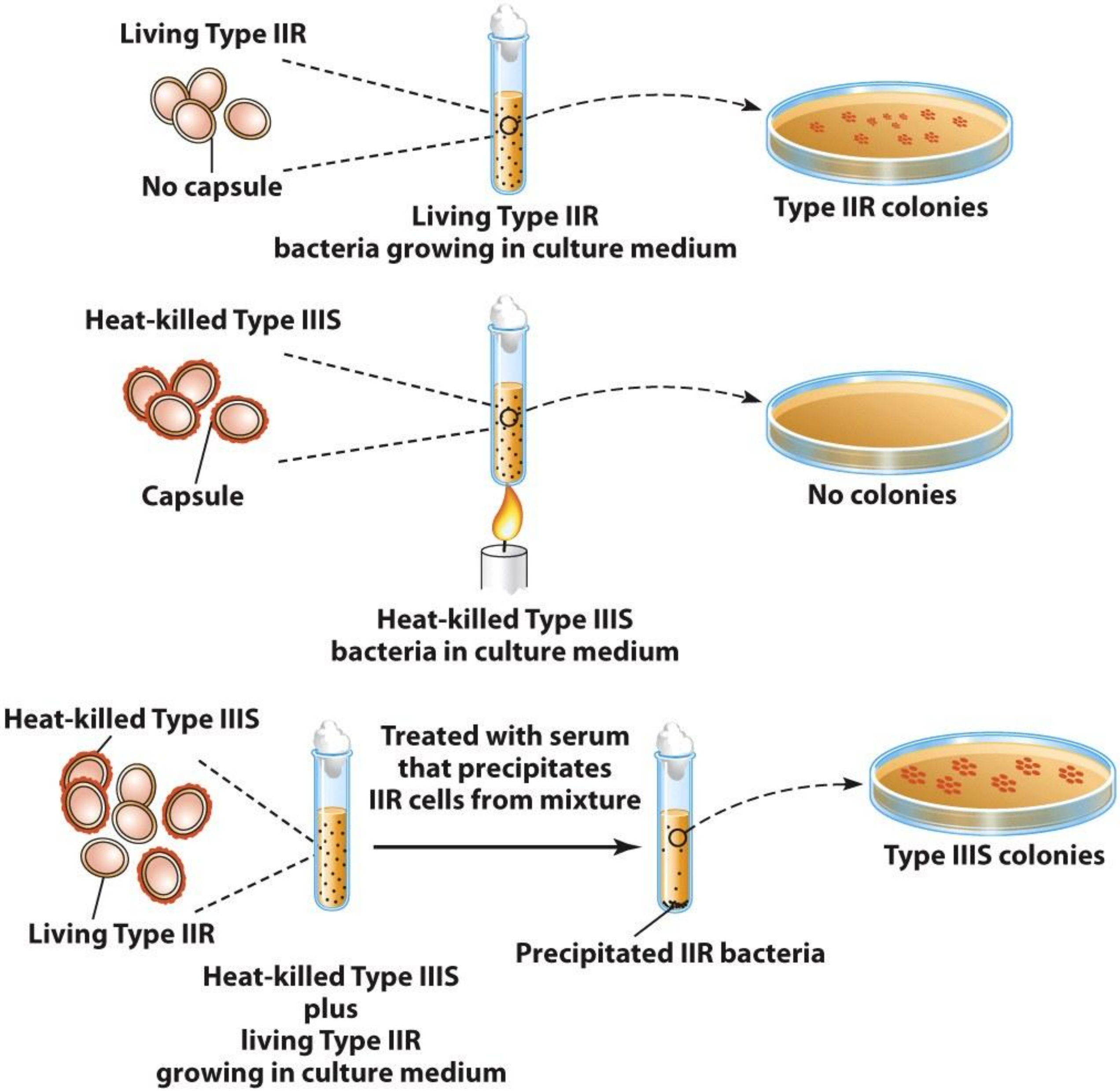

(nonvirulent)
(virulent)
smooth strain
heat-killed
smooth strain

SUMMARY

Griffith's Conclusions

- Something from the dead type IIIS transformed type IIR into type IIIS
- Called this process transformation
- The unknown substance was termed the transforming principle

Sia and Dawson's experiment (in vitro) in 1931



1944 - Avery, MacLeod & McCarty Identify the Genetic Material

- Griffith's transforming principle was the genetic material
- Transformation assay to identify actual biomolecule
- Major constituents - DNA, RNA, proteins, carbohydrates, & lipids
- Made cell extracts from type IIIS cells containing each of these macromolecules

Avery's Experiments

- Mixed each extract with type IIR cells to test for transformation
- Only extract containing purified DNA transformed type IIR to type IIIS
- Verify that DNA, not RNA or protein, is the genetic material

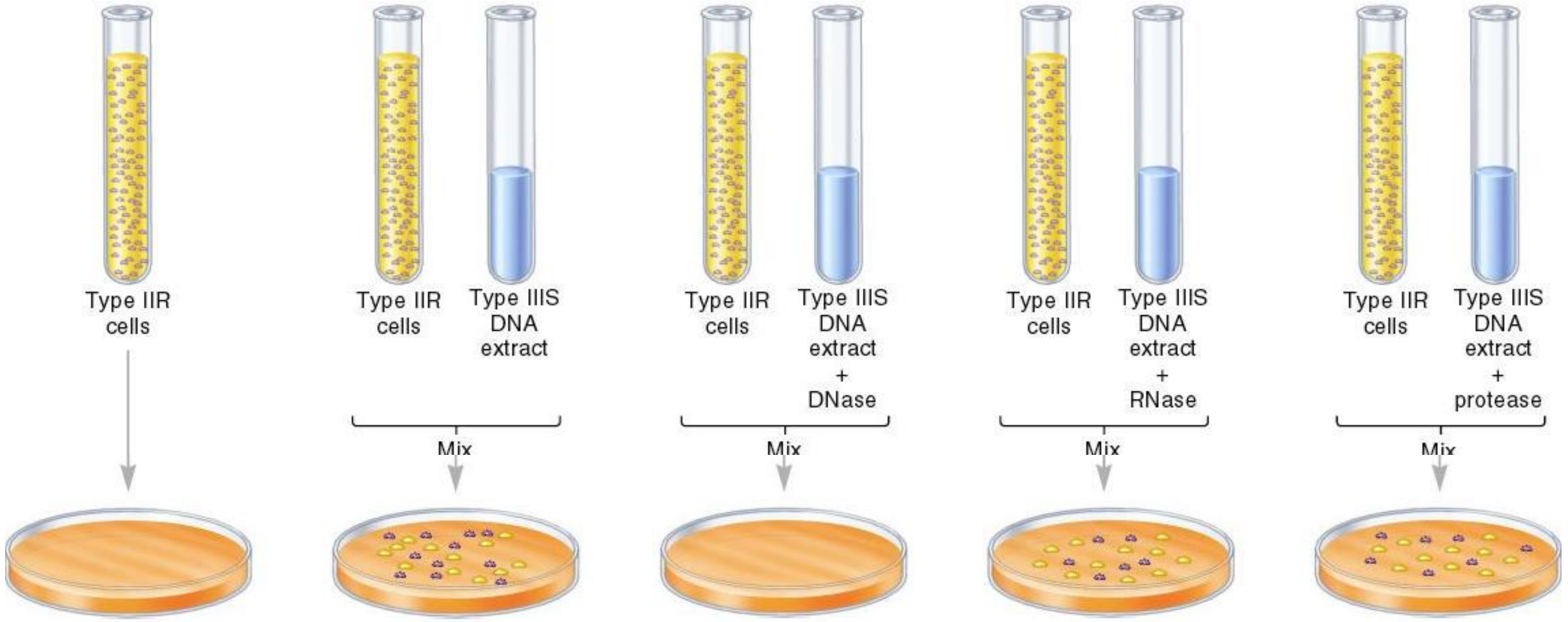


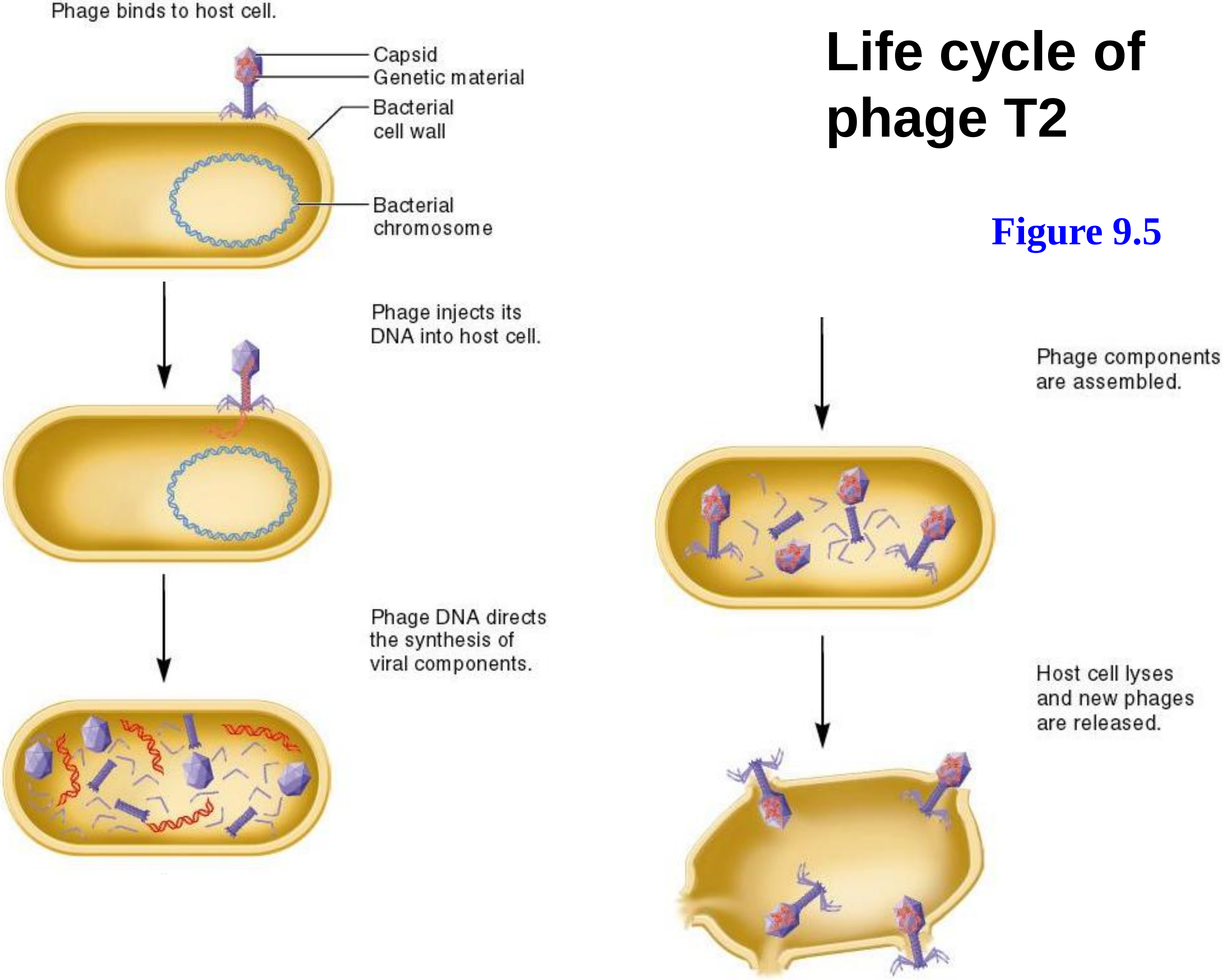
Figure 9.3

The Hershey and Chase experiment:

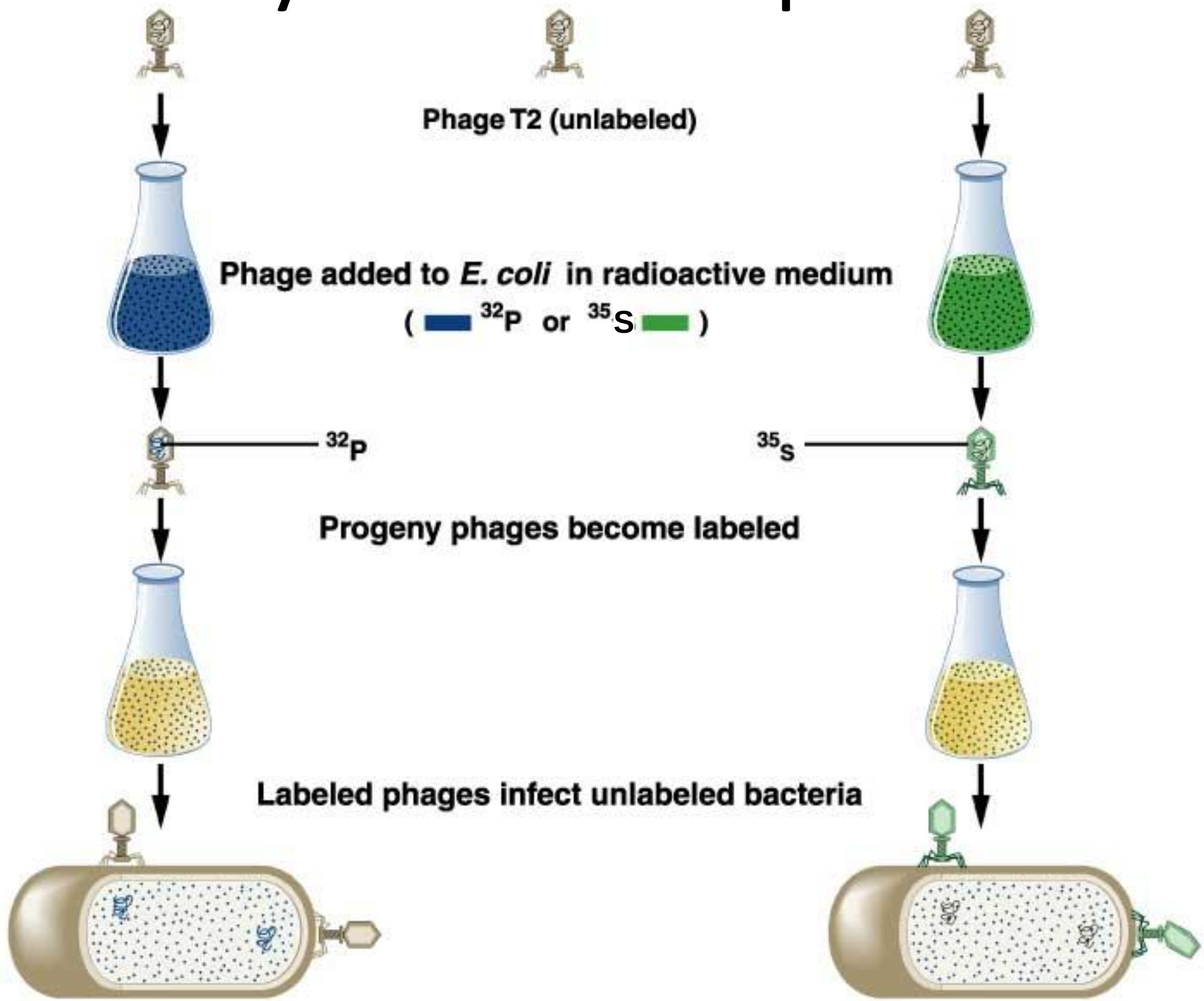
- 1952- Hershey & Chase Confirm DNA is Genetic Material Using Phage T2
- Used radioisotopes to distinguish DNA from proteins
  - ^{32}P labels DNA specifically
  - ^{35}S labels protein specifically
- Infect non-radioactive *E. coli* with *r*adioactively-labeled phages
- Remove phage coats from cells
- Is ^{32}P or ^{35}S inside bacteria?

Life cycle of phage T2

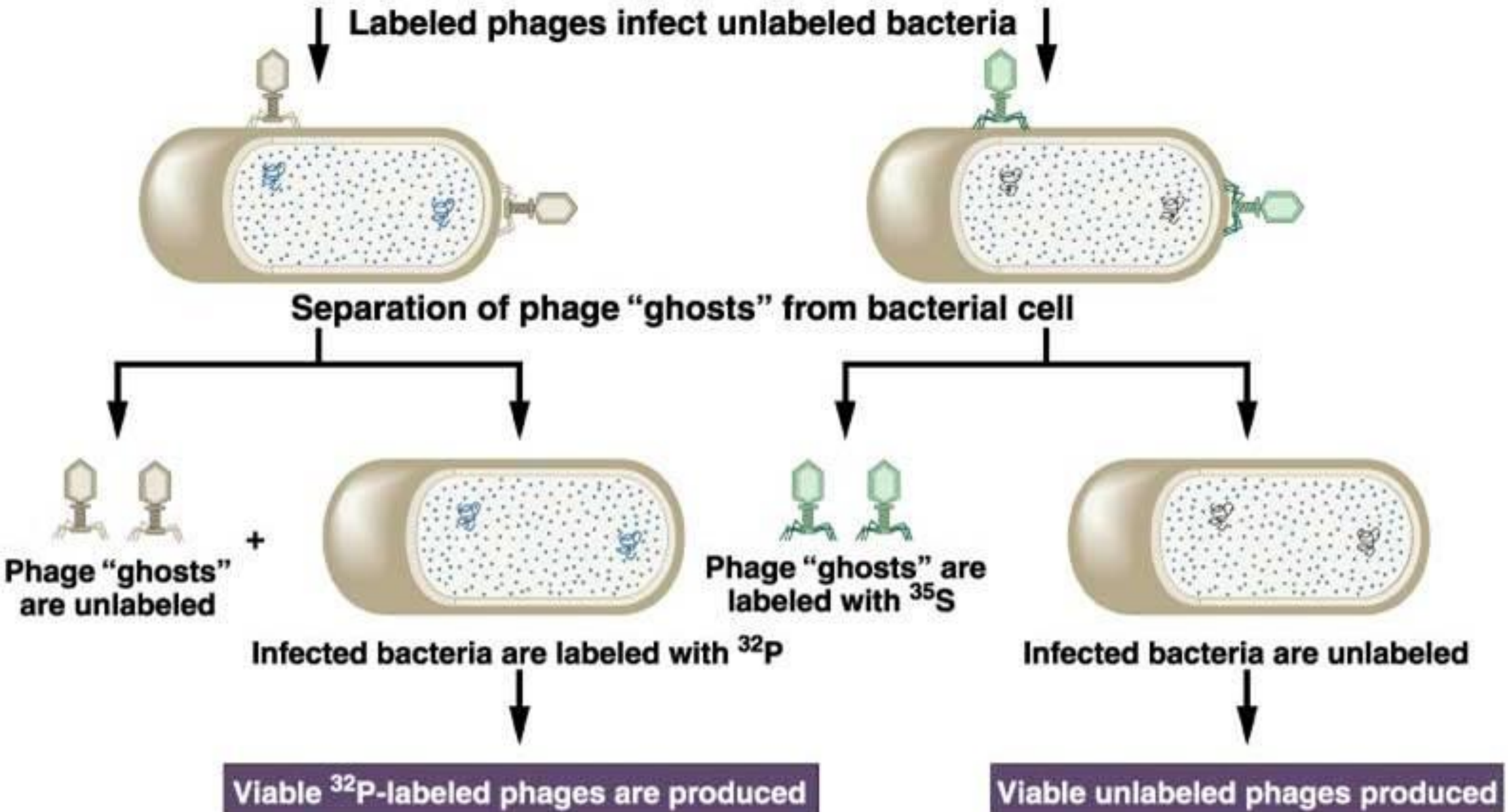
Figure 9.5



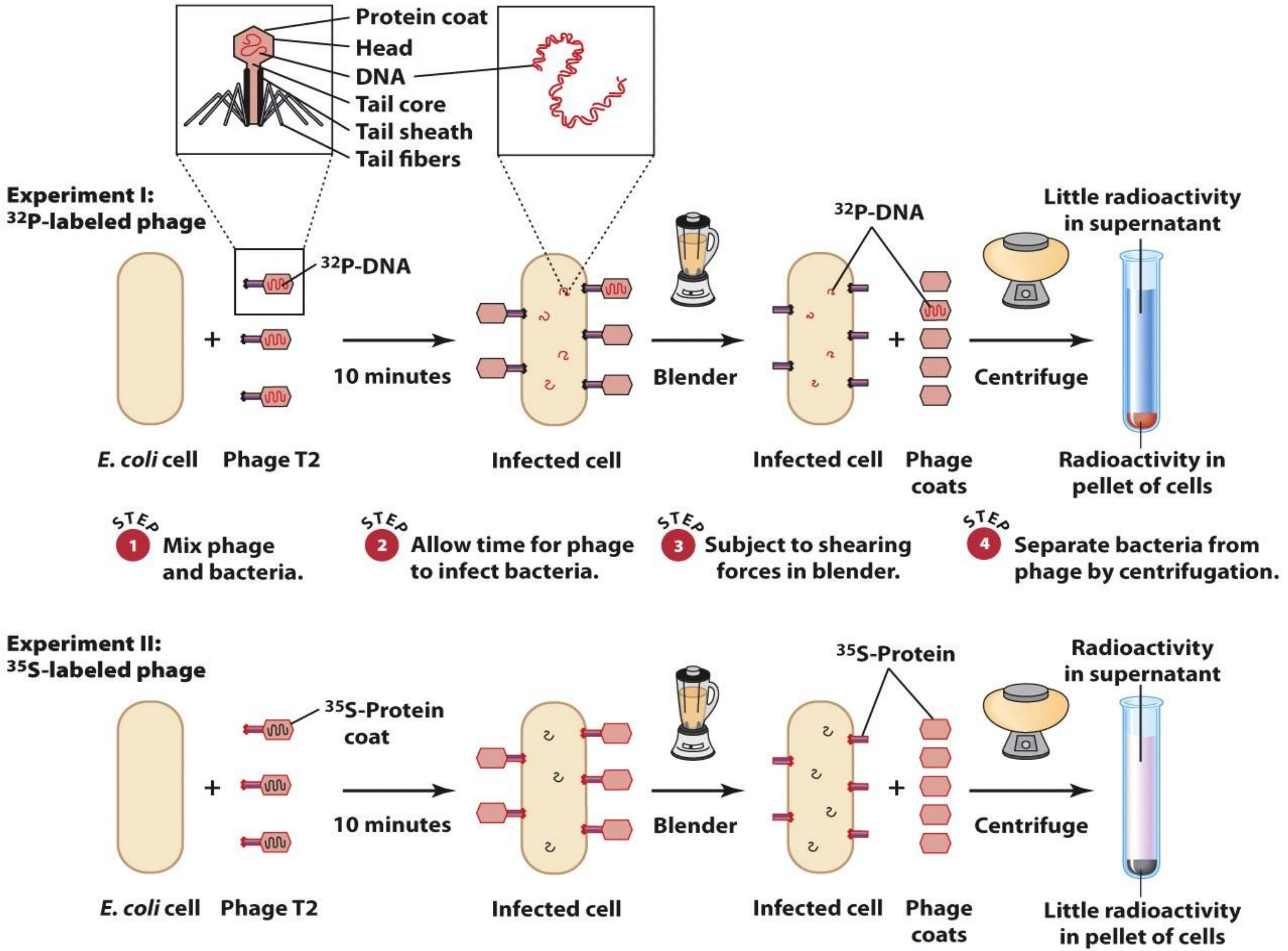
Hershey & Chase Experiment



Hershey & Chase Experiment



Summary of Hershey & Chase Experiment

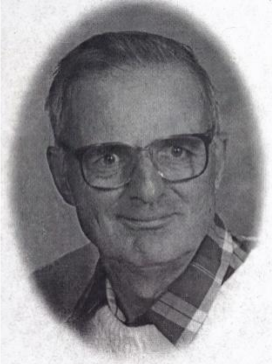


The Discovery of RNA as Viral Genetic Material

All known cellular organisms have DNA as their genetic material.

Some viruses, however, use RNA instead.

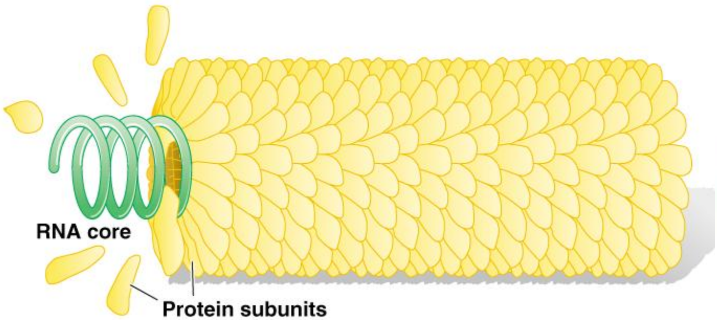
Gierer and Schramm Experiment (1956)



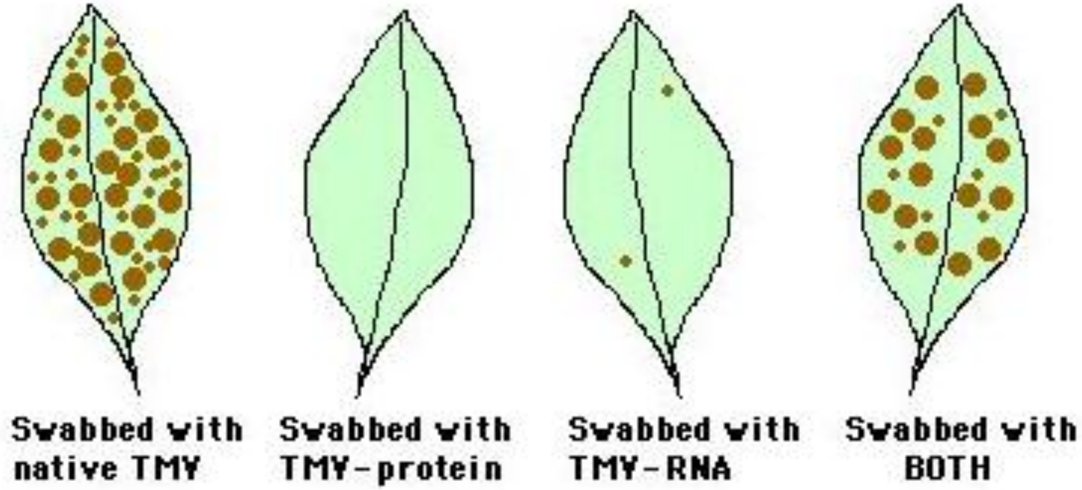
Tobacco mosaic virus (TMV) is composed of RNA and protein; it contains no DNA.

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 .



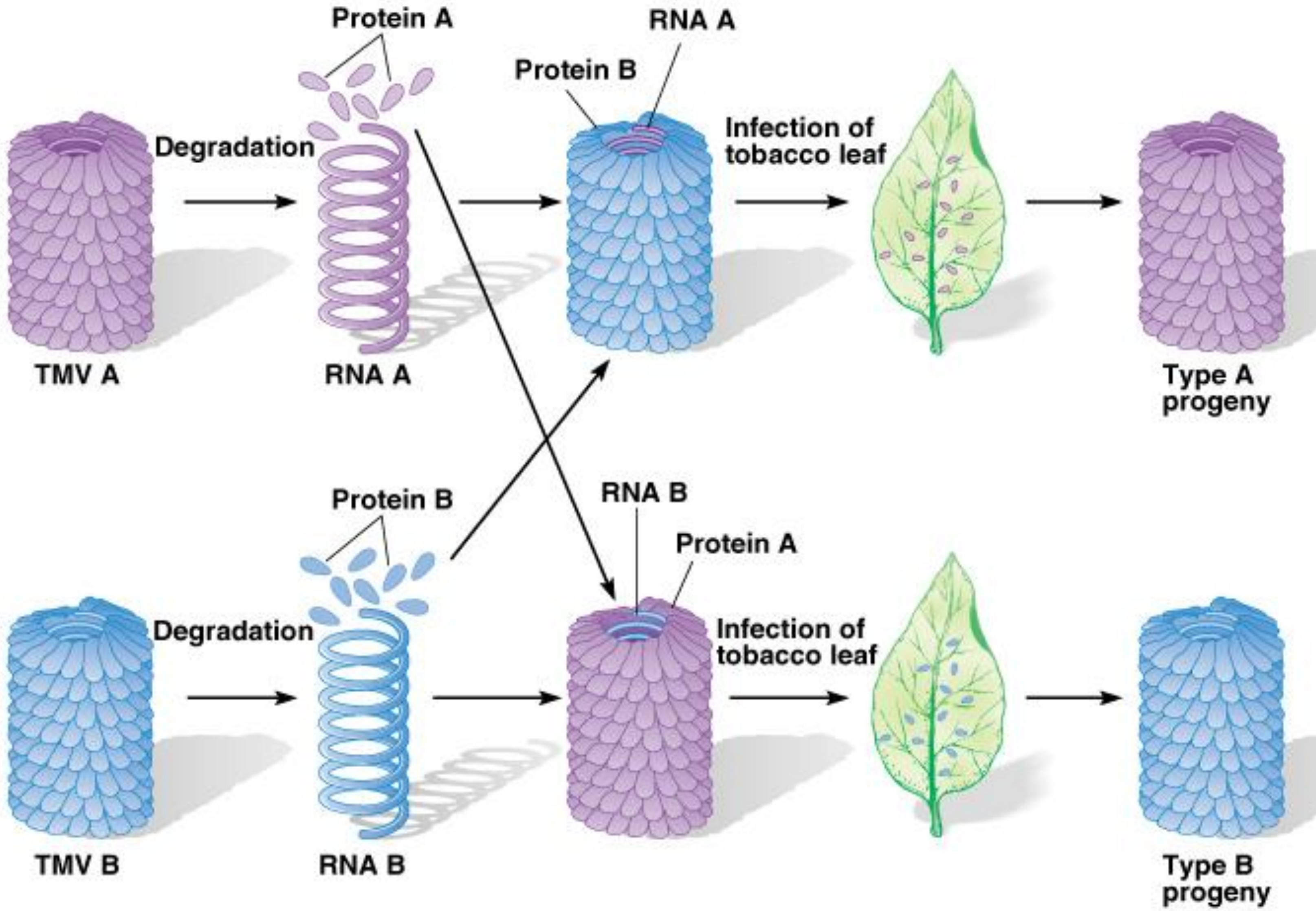
Typical tobacco mosaic virus (TMV) particle



Fraenkel-Conrat & co-workers Experiment (1957)

- In 1957 **Fraenkel-Conrat & co-workers** 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.

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



Final conclusion

- *The genetic information of* ***most living*** *organisms is stored in deoxyribonucleic acid (DNA).*
- *In some viruses, the genetic information is present in ribonucleic acid (RNA).*

The Composition of DNA and RNA

DNA and RNA are polymers composed of monomers called nucleotides.

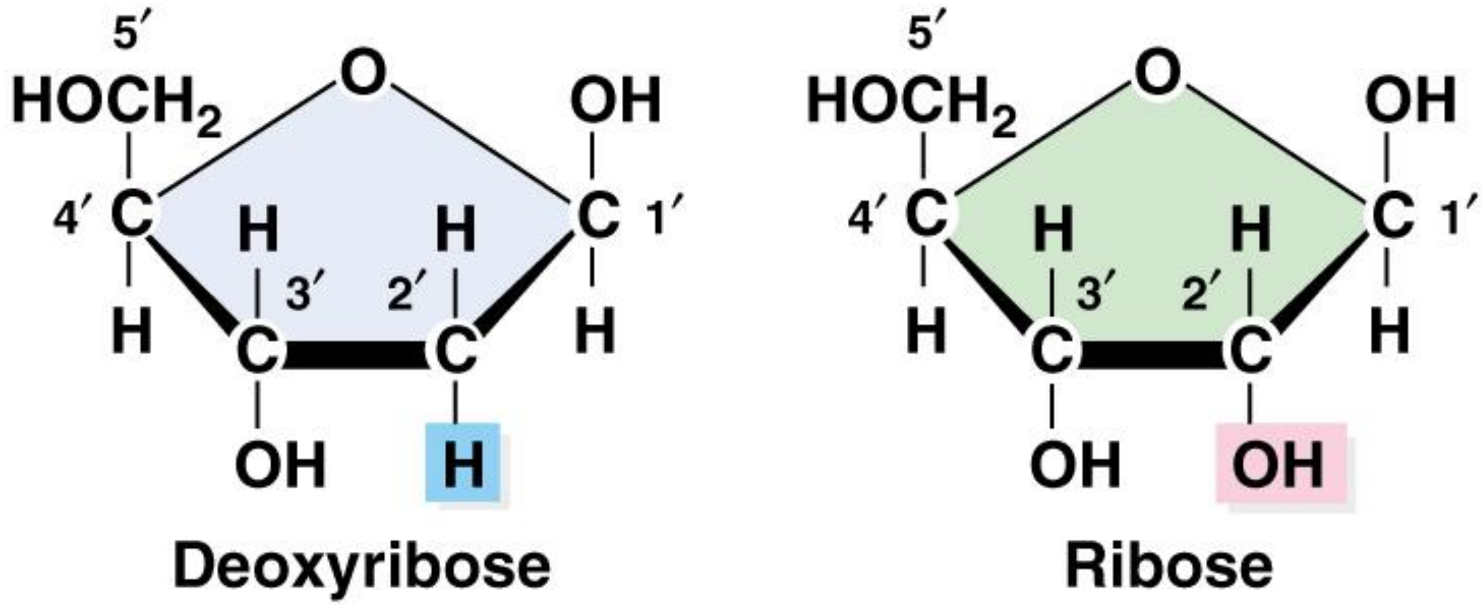
Each nucleotide has three parts:

a. A pentose (5-carbon) sugar.

b. A nitrogenous base.

c. A phosphate group.

The pentose sugar in RNA is ribose, and in DNA it's deoxyribose. The only difference is at the 2' position, where RNA has a hydroxyl (OH) group, while DNA has only a hydrogen.

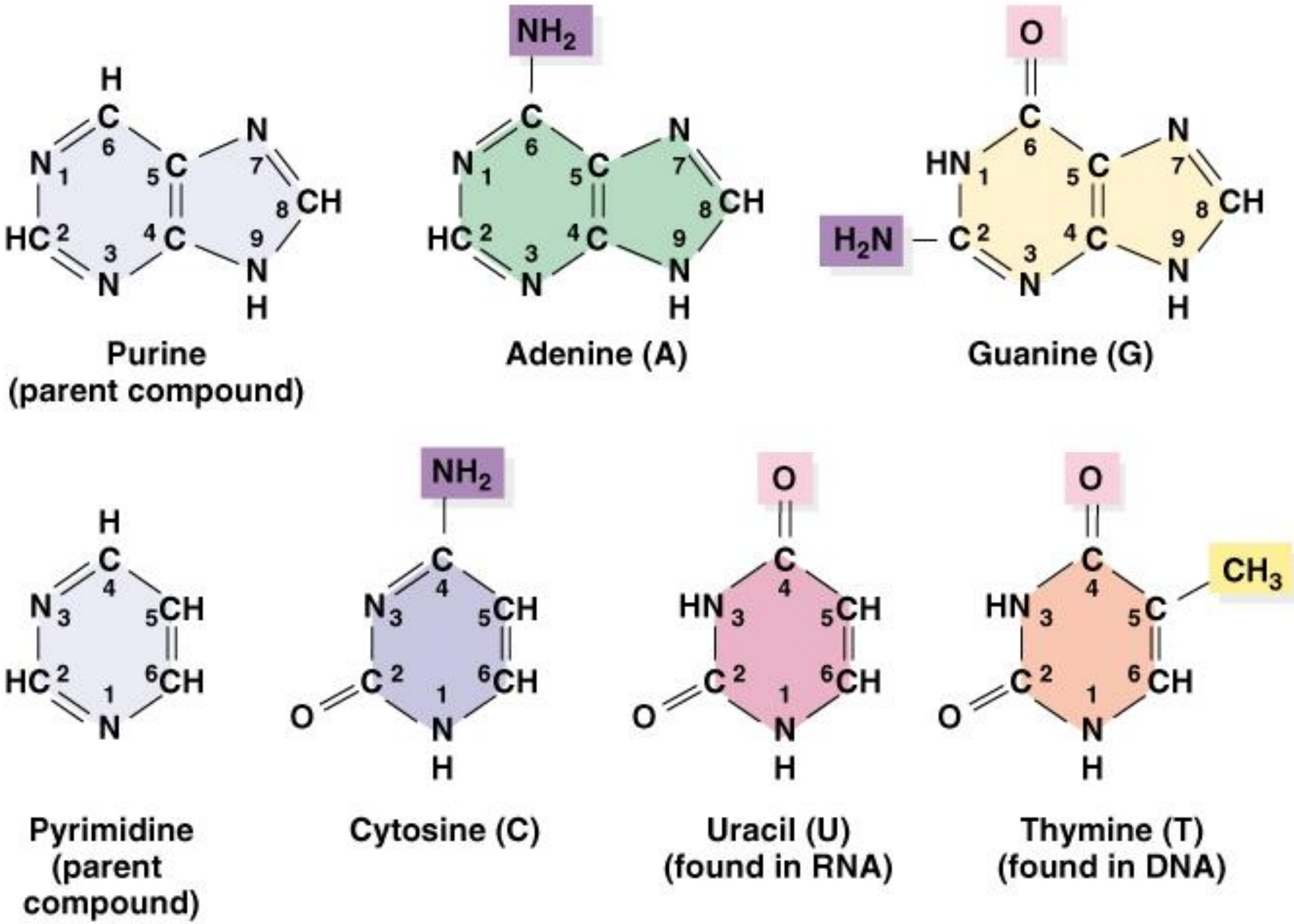


Structures of deoxyribose and ribose in DNA and RNA

There are two classes of nitrogenous bases

a. **Purines** (double-ring, nine-membered structures) include adenine (A) and guanine (G).

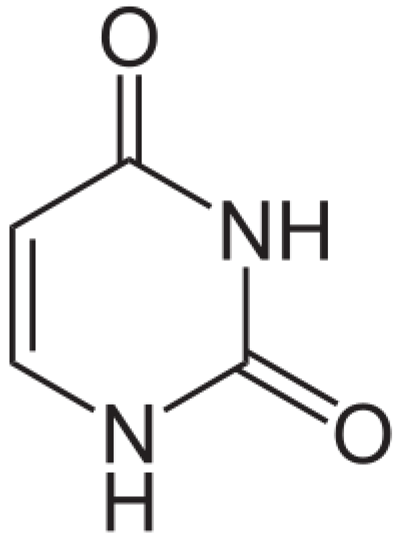
b. **Pyrimidines** (one-ring, six-membered structures) include cytosine (C), thymine (T) in DNA and uracil (U) in RNA.

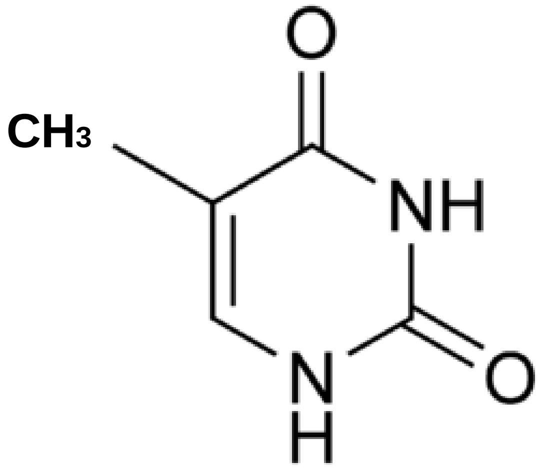


Structures of the nitrogenous bases in DNA and RNA

Why Thymine not Uracil in DNA?

First Reason

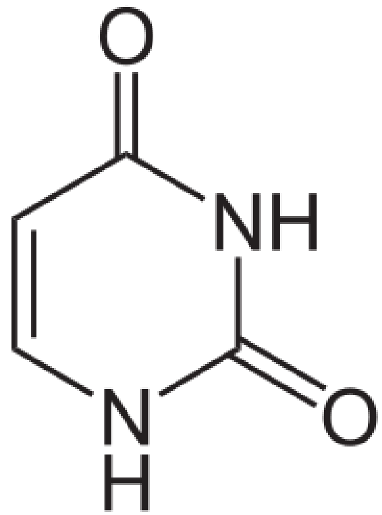


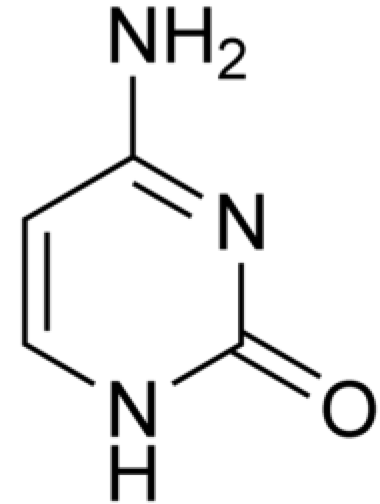


- Despite uracil's tendency to pair with adenine, it can also pair with any other base, including itself.

- By adding a methyl group (which is hydrophobic) and turning it into thymine, its position is reorganized in the double-helix, not allowing those wrong pairings to happen.

Second Reason

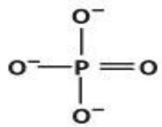




- Cytosine can deaminate to produce uracil. The only difference between them is the change from an O in uracil to an NH2 in cytosine.
- The problem is that, if uracil were a component of DNA, the repair systems would not be able to distinguish original uracil from uracil originated by deamination of cytosine.
- So using thymine instead makes it way easier and more stable, as any uracil inside DNA must come from a cytosine and so it can be replaced by a new cytosine.

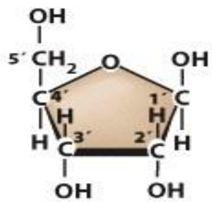
Nucleic acids are composed of repeating subunits called nucleotides. Each nucleotide is composed of three units.

(1) A phosphate group:

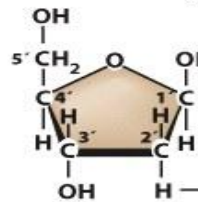


(2) A five-carbon sugar or pentose:

(a) **In RNA: Ribose**



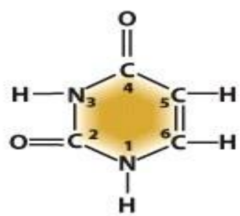
(b) **In DNA: 2-Deoxyribose**



No hydroxyl group

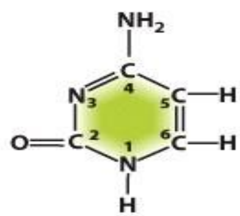
(3) A cyclic, nitrogen-containing base:

(a) **In RNA only (with rare exceptions):**



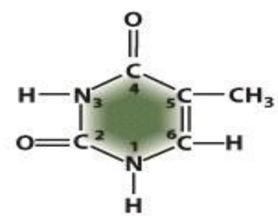
Uracil

(b) **In both RNA and DNA:**



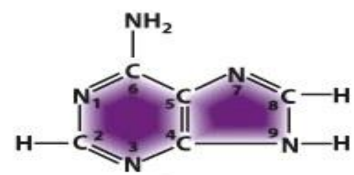
Cytosine

(c) **In DNA only (with rare exceptions)**

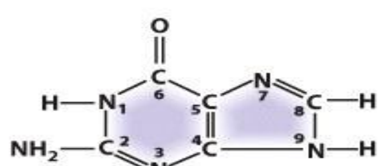


Thymine

Pyrimidines



Adenine

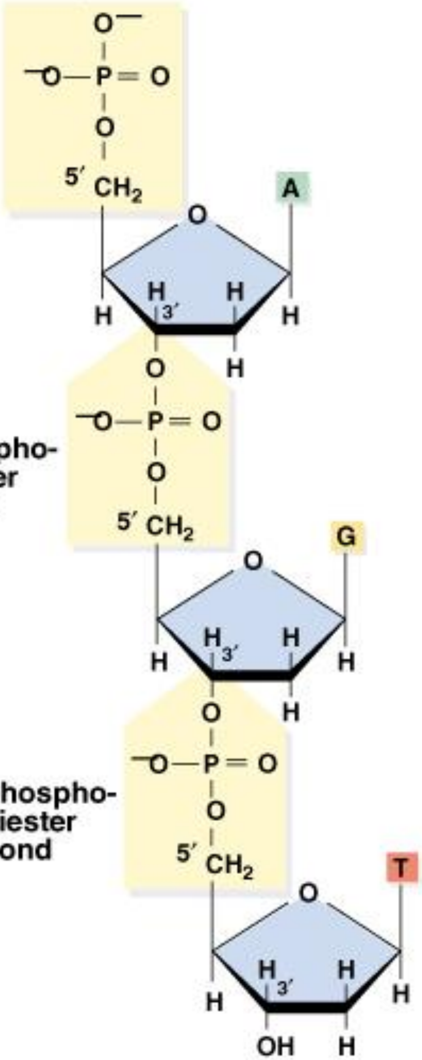


Guanine

Purines

b) DNA polynucleotide chain

5¢ end



Phospho-diester bond

Phospho-diester bond

3¢ end

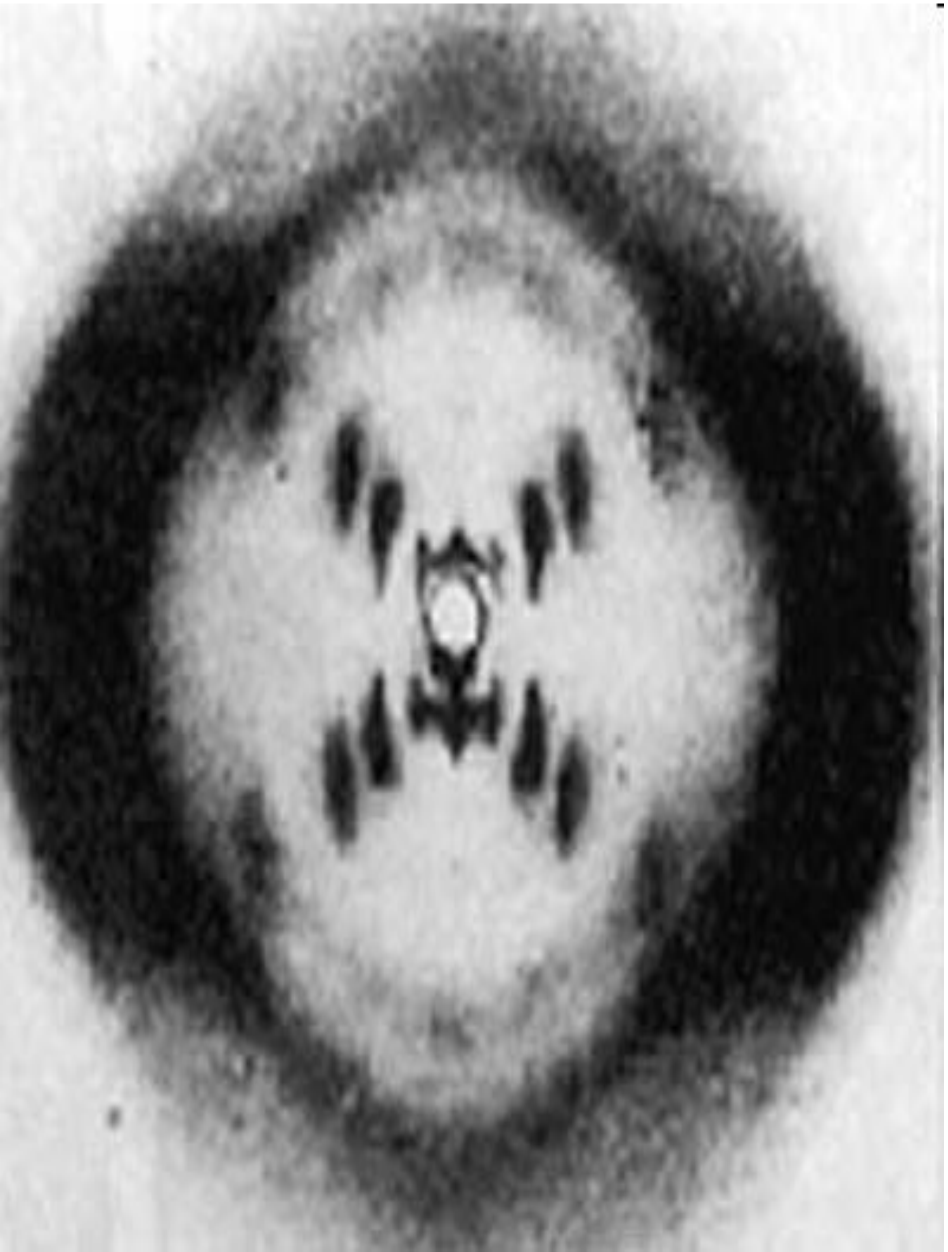
Watson & Crick's DNA Model

Two additional sources of data assisted Watson and Crick with their model:

a. **Erwin Chargaff's ratios** obtained for DNA derived from a variety of sources showed that the amount of purine always equals the amount of pyrimidine, and further, that the amount of G equals C, and the amount of A equals T.

b. **Rosalind Franklin's X ray diffraction** images of DNA showed a helical structure with regularities at 0.34 nm and 3.4 nm along the axis of the molecule (Figure 8.9).

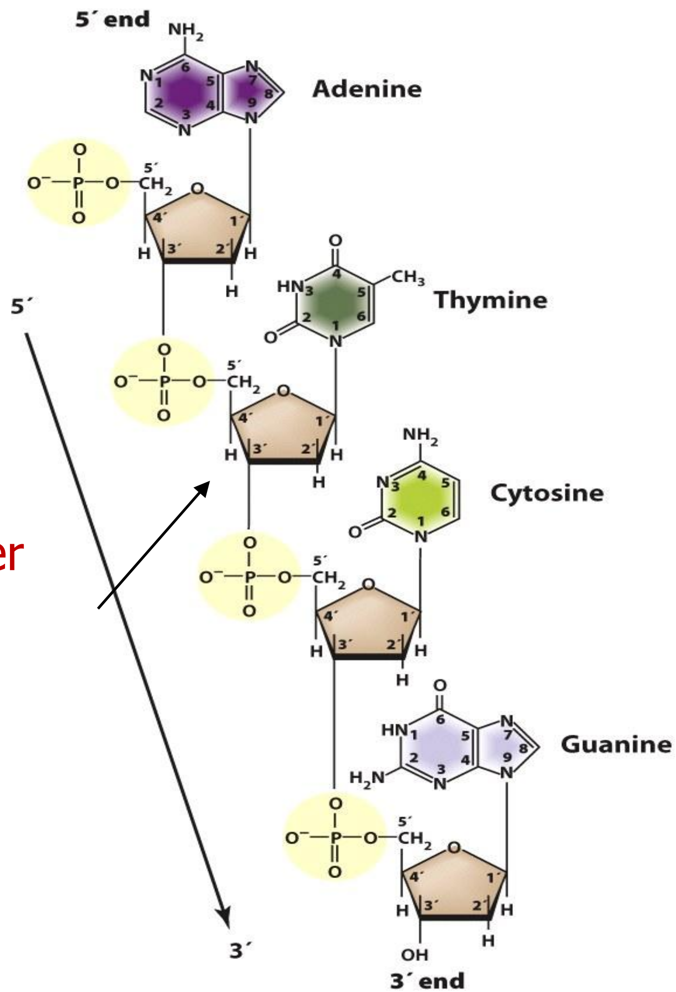
Wilkins & Franklin's Photographs



- X-ray diffraction to study the structures of molecules
- 1952 Wilkins & Franklin developed high-quality X-ray diffraction photographs of strands of DNA which suggested that the DNA resembled a tightly coiled helix and was composed of two or three chains of nucleotides

The Double Helix

Phosphodiester
[C-O-P-O-C]



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Polynucleotide chain

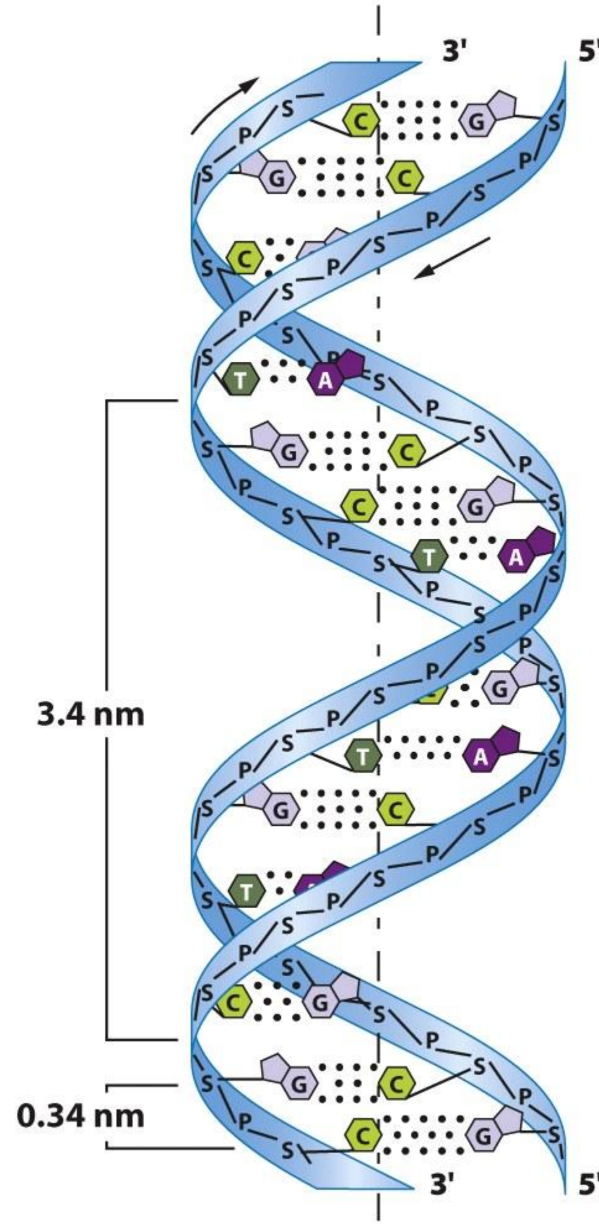


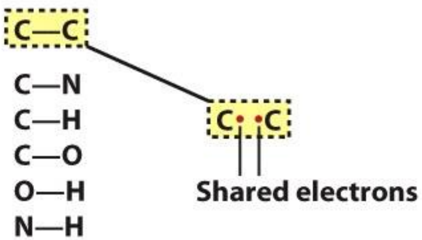
TABLE 9.2

Chemical Bonds Important in DNA Structure

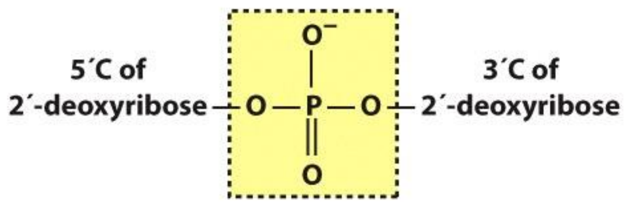
(*a*) *Covalent bonds*

Strong chemical bonds formed by sharing of electrons between atoms.

(1) In bases and sugars

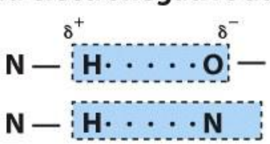


(2) In phosphodiester linkages



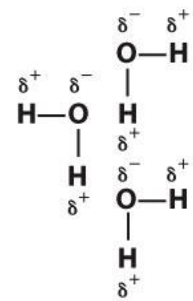
(*b*) *Hydrogen bonds*

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



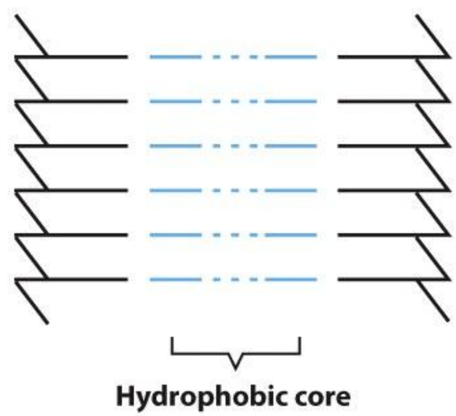
(*c*) *Hydrophobic "bonds"*

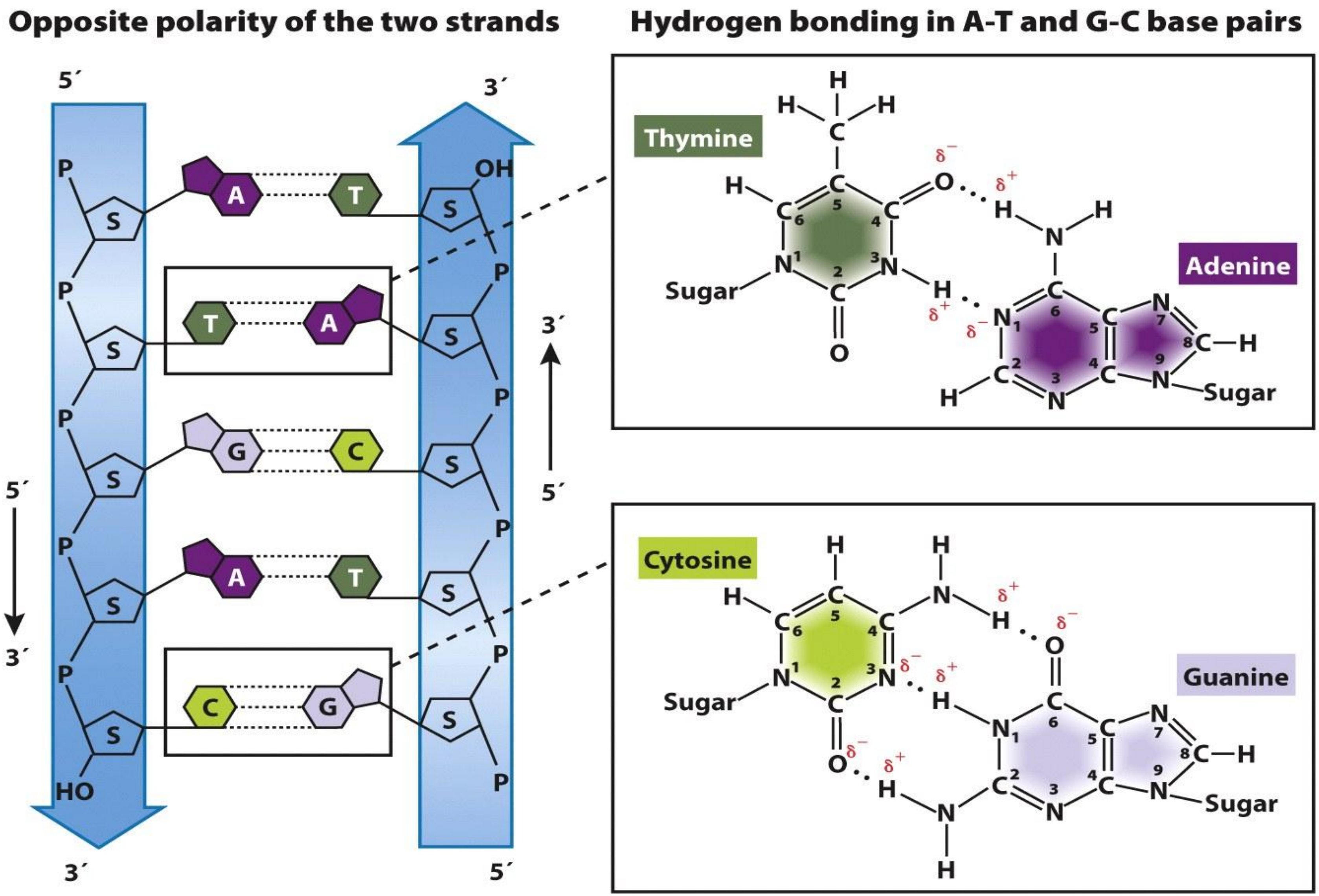
The association of nonpolar groups with each other when present in aqueous solutions because of their insolubility in water.



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

The stacked base pairs provide a hydrophobic core.





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Complementary and Antiparallel

DNA Structure

- Complementary Base Pairs (A with T, G with C)
- Anti-parallel Strands
- Right-handed double helix (B-DNA)

Different DNA Structures

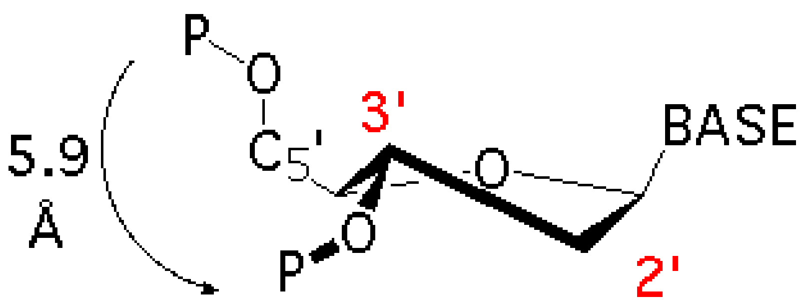
X ray diffraction studies show that DNA can exist in different forms

a. A-DNA is the dehydrated form, and so it is not usually found in cells. It is a right-handed helix with 10.9 bp/turn. A-DNA has a deep and narrow major groove, and a wide and shallow minor groove.

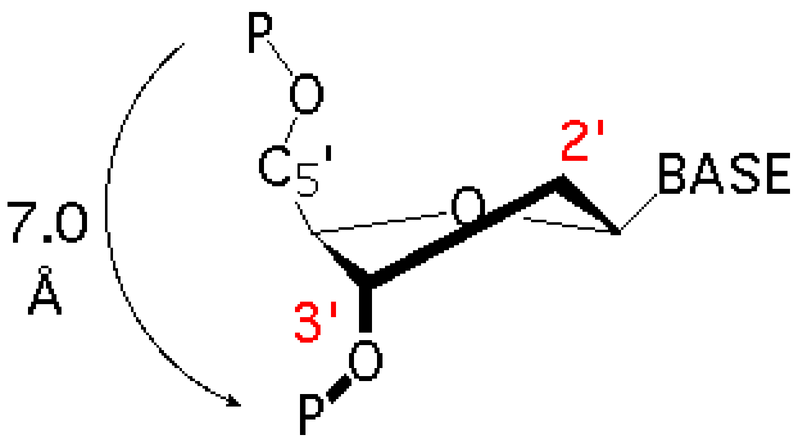
b. B-DNA is the hydrated form of DNA, the kind normally found in cells. It is also a right-handed helix, with only 10.0 bp/turn. B-DNA has a wide major groove and a narrow minor groove, and its major and minor grooves are of about the same depth.

c. Z-DNA is a left-handed helix with a zigzag sugar-phosphate backbone that gives it its name. It has 12.0 bp/turn in helix. Z-DNA has a deep minor groove, and a very shallow major groove. Its existence in living cells has not been proven.

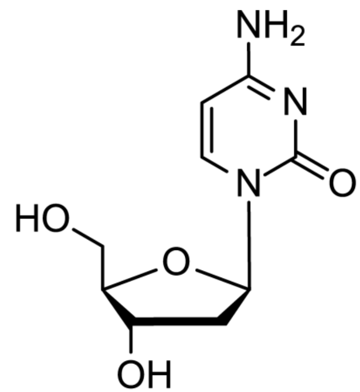
	A form	*B form*	*Z form*
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines



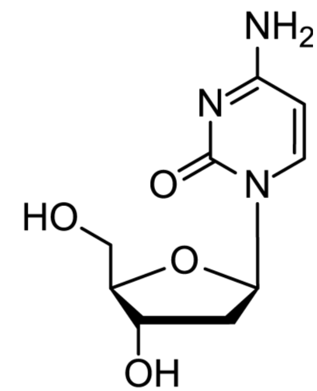
C3'-endo (A-Conformation)



C2'-endo (B-Conformation)

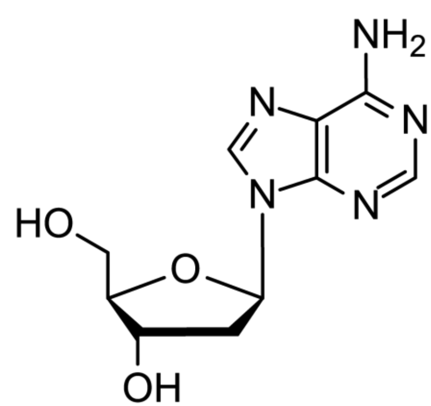


anti

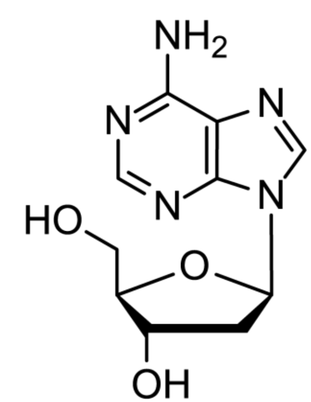


syn

deoxycytidine (a pyrimidine nucleoside)



anti



syn

deoxyadenosine (a purine nucleoside)

TABLE 9.3

Alternate Forms of DNA

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

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DNA replication

DNA-RNA duplexes

Elevated content of C:G

THE GENETIC CODE

Most genetic code tables designate the codons for amino acids as mRNA sequences. Important features of the genetic code include:

- **Each codon consists of three bases (triplet).** There are 64 codons. They are all written in the 5' to 3' direction.
- **61 codons code for amino acids.** The other three (UAA, UGA, UAG) are stop codons (or nonsense codons) that terminate translation.
- **There is one start codon** (initiation codon), AUG, coding for methionine. Protein synthesis begins with methionine (Met) in eukaryotes, and formyl methionine (fmet) in prokaryotes.
- **The code is unambiguous**. Each codon specifies no more than one amino acid.

- **The code is degenerate**. More than one codon can specify a single amino acid.
- All amino acids, except Met and tryptophan (Trp), have more than one codon.
- For those amino acids having more than one codon, the first two bases in the codon are usually the same. **The base in the third position often varies.**
- **The code is almost universal** (the same in all organisms). Some minor exceptions to this occur in mitochondria and some organisms.
- The code is commaless (contiguous). There are no spacers or "commas" between codons on an mRNA.
- Neighboring codons on a message are non-overlapping.