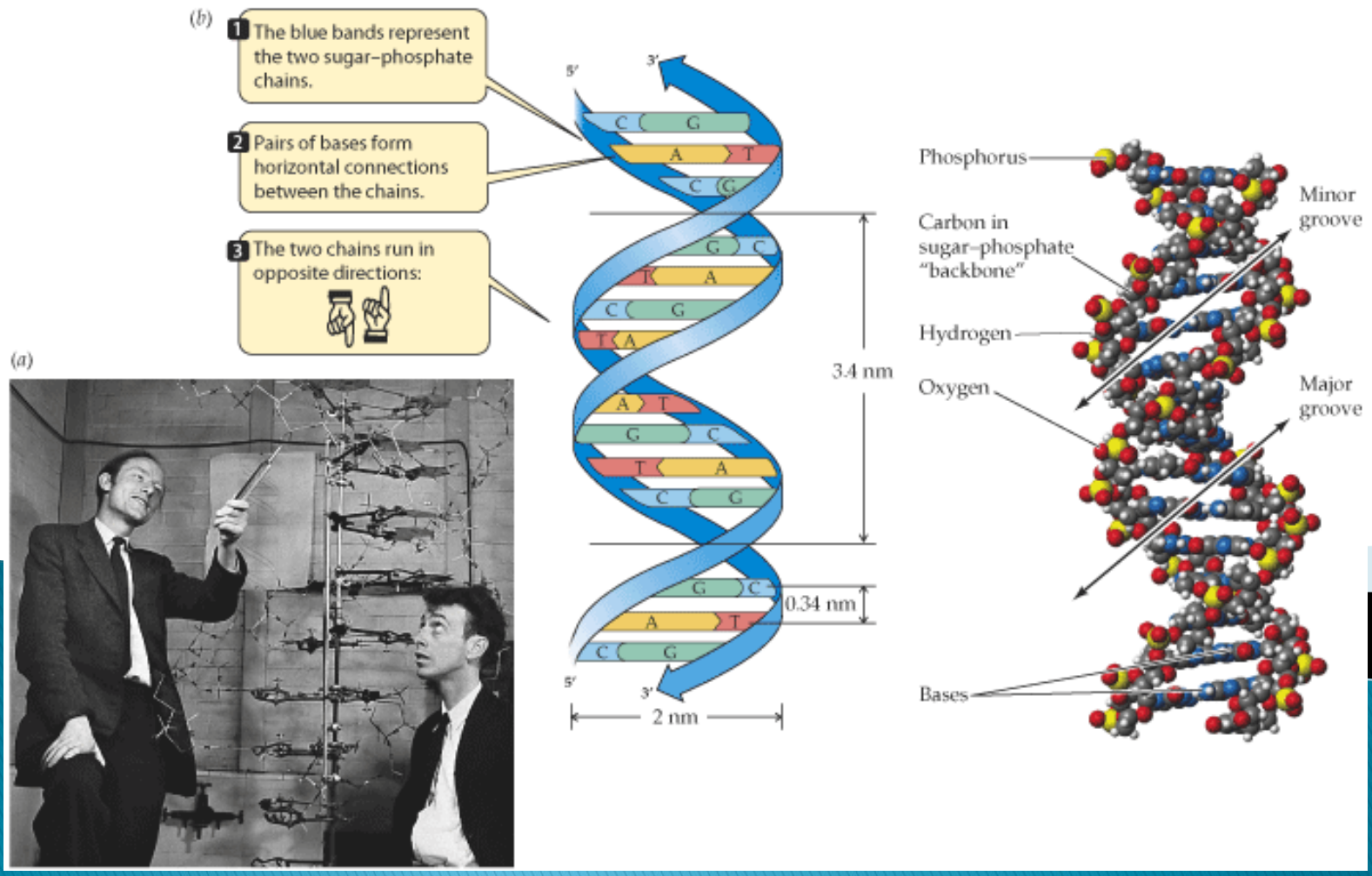
Aim: How was DNA discovered?



1868 – Friedrich Miescher (Sweden)

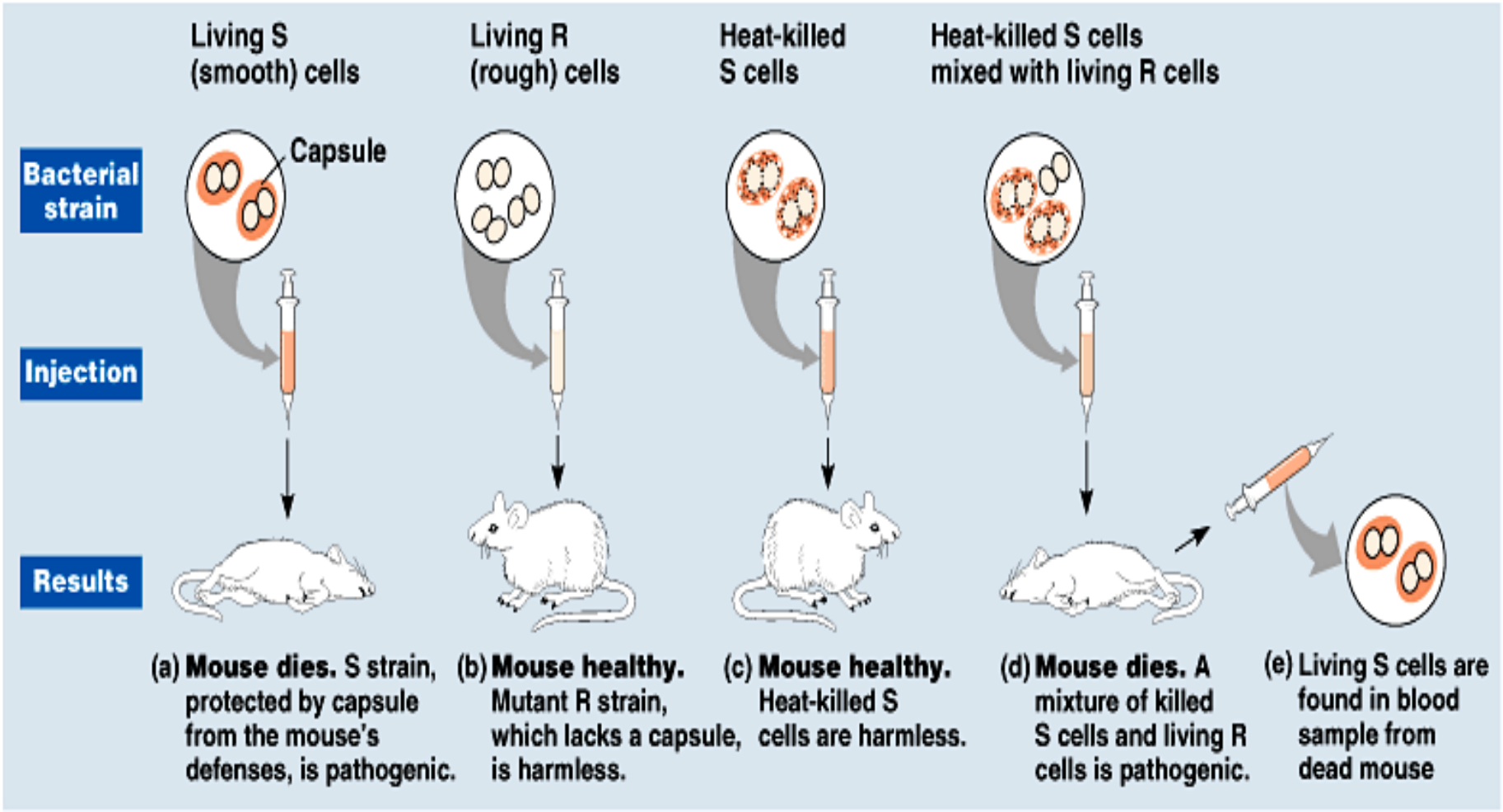
- 1) showed that the nucleus is not only made up of protein:
  - A) attempted to use pepsin (a protein hydrolase) but it would not digest the entire nucleus
  - B) found the nucleus contains phosphorus
- 2) Miescher claimed the nucleus was made up of protein and a non-protein substance he called ‘nuclein’. (It would later be found that nuclein was really DNA)

1914 – Robert Feulgen (German Chemist)

- 1) learned how to stain DNA
- 2) found it exclusively in chromosomes
- 3) found that sex cells contain half the DNA as somatic cells
- 4) perhaps DNA is the hereditary material but he would not commit to this idea

1928 – Frederick Griffin (English bacteriologist)

- – studied two strains of pneumococci:
- R (rough) – harmless
- S (smooth) – deadly
  - Mice + live R bacteria → mice lived
  - Mice + live S bacteria → mice died
  - Mice + dead S bacteria → mice lived
  - Mice + live R + dead S bacteria → mice died
- Somehow live R bacteria had transformed into live S bacteria and killed the mice. What was it???

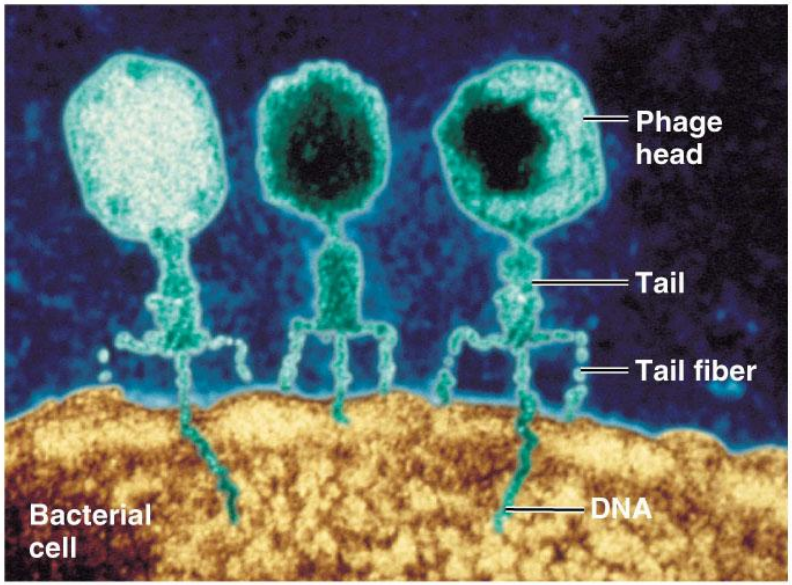

Living S (smooth) cells
Living R (rough) cells
Heat-killed S cells
Heat-killed S cells mixed with living R cells
Bacterial strain
Capsule
Injection
Results
(a) Mouse dies. S strain, protected by capsule from the mouse's defenses, is pathogenic.
(b) Mouse healthy. Mutant R strain, which lacks a capsule, is harmless.
(c) Mouse healthy. Heat-killed S cells are harmless.
(d) Mouse dies. A mixture of killed S cells and living R cells is pathogenic.
(e) Living S cells are found in blood sample from dead mouse

1944 – Avery, MacLead, McCarty

- They were able to identify **DNA** as Griffiths transforming principle through the following experiment.
- took extract (from heated smooth bacteria) and treated it with DNAase (digests DNA) – then mixed with rough bacteria and injected into rats -> the rats lived
- in other side of experiment, treated extract with protease (digests proteins) –then mixed with rough bacteria and injected into rats -> rats died
- This showed that DNA, not protein, has ability to transform cells (for posterity's sake, they were actually mice, not rats)
- Proof was needed and the structure needed to be determined.

1952 – Martha Chase and Alfred D. Hershey

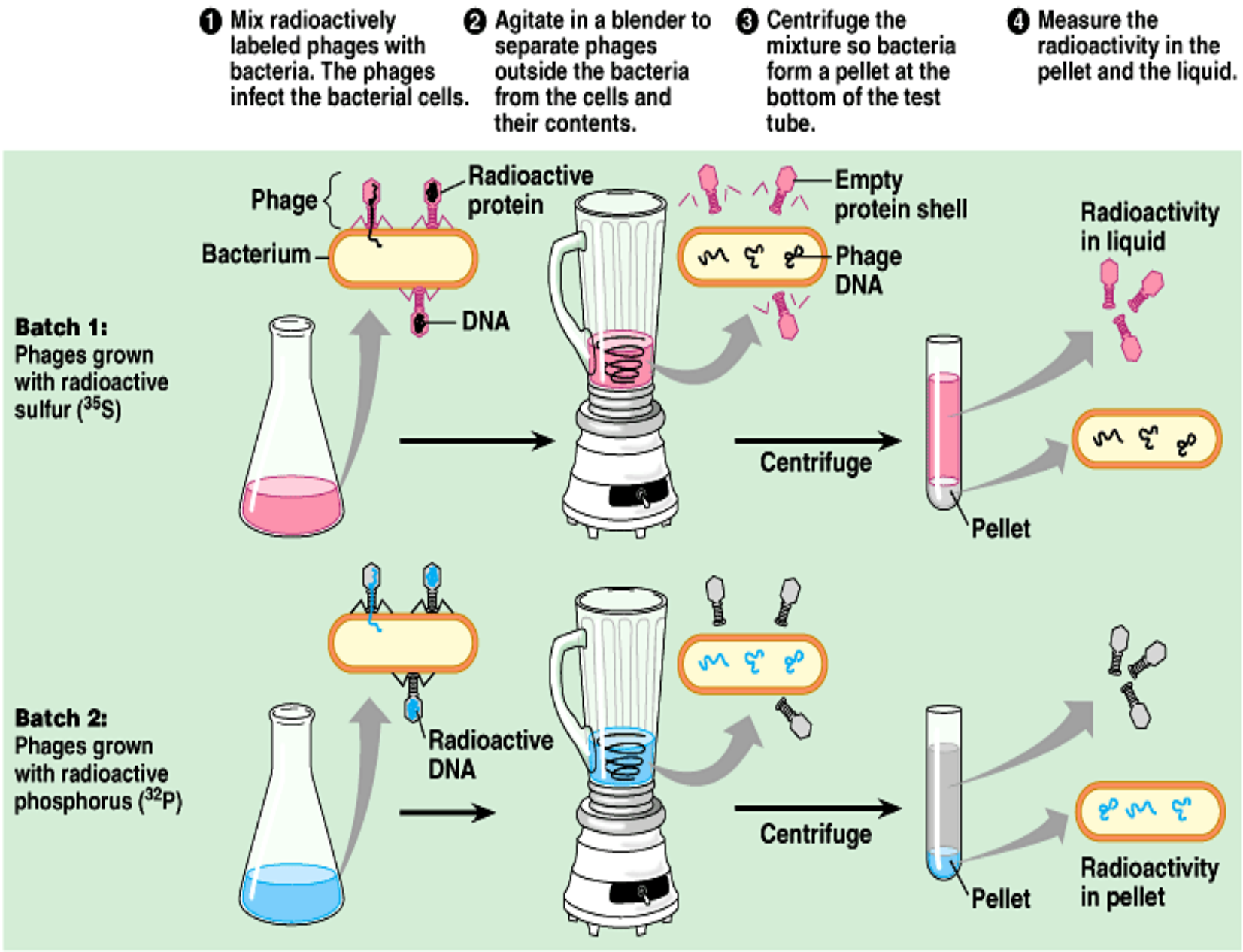
- Carnegie Lab of Genetics
- Studied viruses that attack bacteria.
- These viruses are called bacteriophages.



(a) T2 and related phages use their tail pieces to attach to the host cell and inject their genetic material (TEM).

1952 – Martha Chase and Alfred D. Hershey

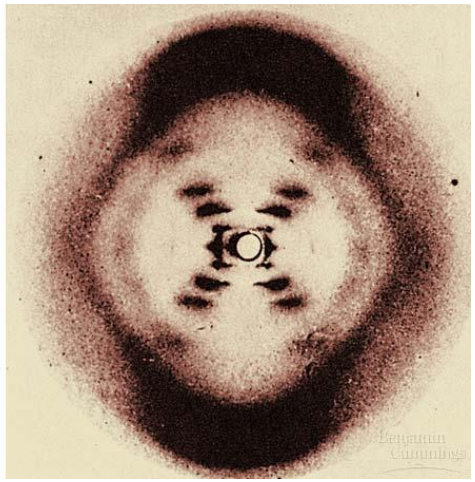
- 1) bacteriophage viruses inject their genetic material into bacteria.
- 2) They tagged one group of bacteria with radioactive P^{32} (DNA) and another group with radioactive S^{35} (protein)
- 3) phage viruses were allowed to infect these radioactive bacteria
- 4) phage viruses incorporated P^{32} into their DNA and S^{35} into their protein coats.
- 5) radioactive viruses were allowed to infect non-radioactive bacteria
- 6) it was found that S^{35} remained outside the bacteria but the P^{32} entered. The new viruses also had radioactive DNA within them
- Conclusion – DNA, not protein, was the hereditary material.

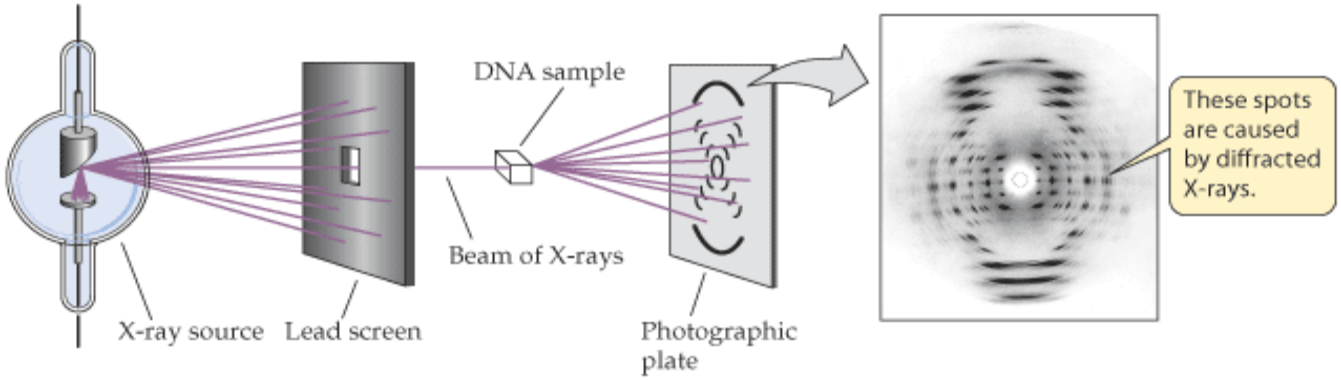

1 Mix radioactively labeled phages with bacteria. The phages infect the bacterial cells.
2 Agitate in a blender to separate phages outside the bacteria from the cells and their contents.
3 Centrifuge the mixture so bacteria form a pellet at the bottom of the test tube.
4 Measure the radioactivity in the pellet and the liquid.
Phage
Radioactive protein
Bacterium
DNA
Empty protein shell
Phage DNA
Radioactivity in liquid
Batch 1:
Phages grown with radioactive sulfur (^{35}S)
Centrifuge
Pellet
Radioactive DNA
Batch 2:
Phages grown with radioactive phosphorus (^{32}P)
Centrifuge
Pellet
Radioactivity in pellet

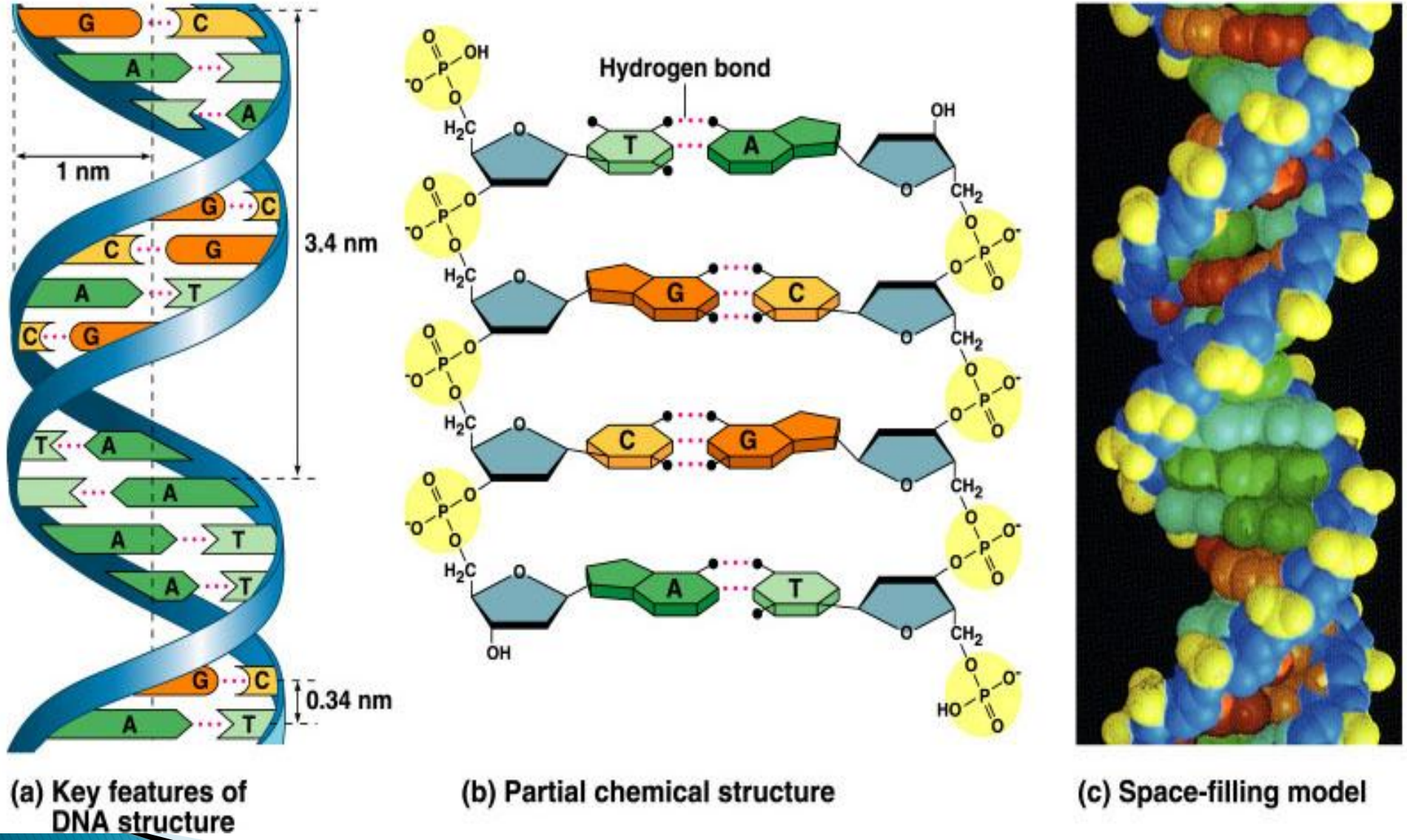
1953 – Watson, Crick, Wilkins, and Franklin

- Watson and Crick assembled all the data into a credible theory.
- Wilkins and Franklin performed critical X-ray defraction studies providing all the necessary data.
- Watson, Crick and Wilkins received a Nobel Prize for their work. Unfortunately, Franklin had died and could not receive the award !!

- DNA is made up of building blocks called nucleotides.
- Each nucleotide contains:
- A) deoxyribose sugar
- B) phosphate group
- C) nitrogen base (purines A,G and pyrimidines C,T)
- Chargaff determined that in a DNA molecule, the number of adenines equals the number of thymines.






1 nm
3.4 nm
0.34 nm
Hydrogen bond
(a) Key features of DNA structure
(b) Partial chemical structure
(c) Space-filling model

- DNA replication is semiconservative
  - Each of the two new daughter molecules will have one old strand, derived from the parent molecule, and one newly made strand

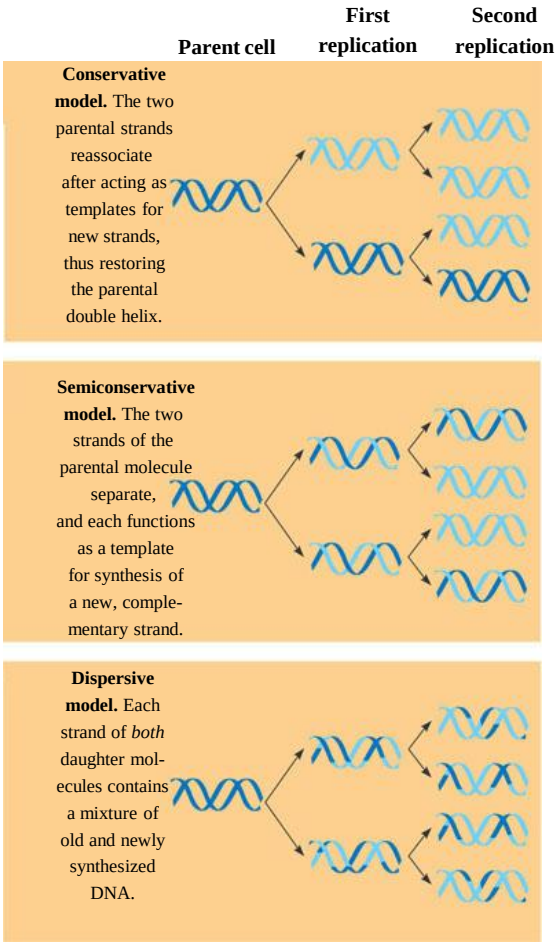
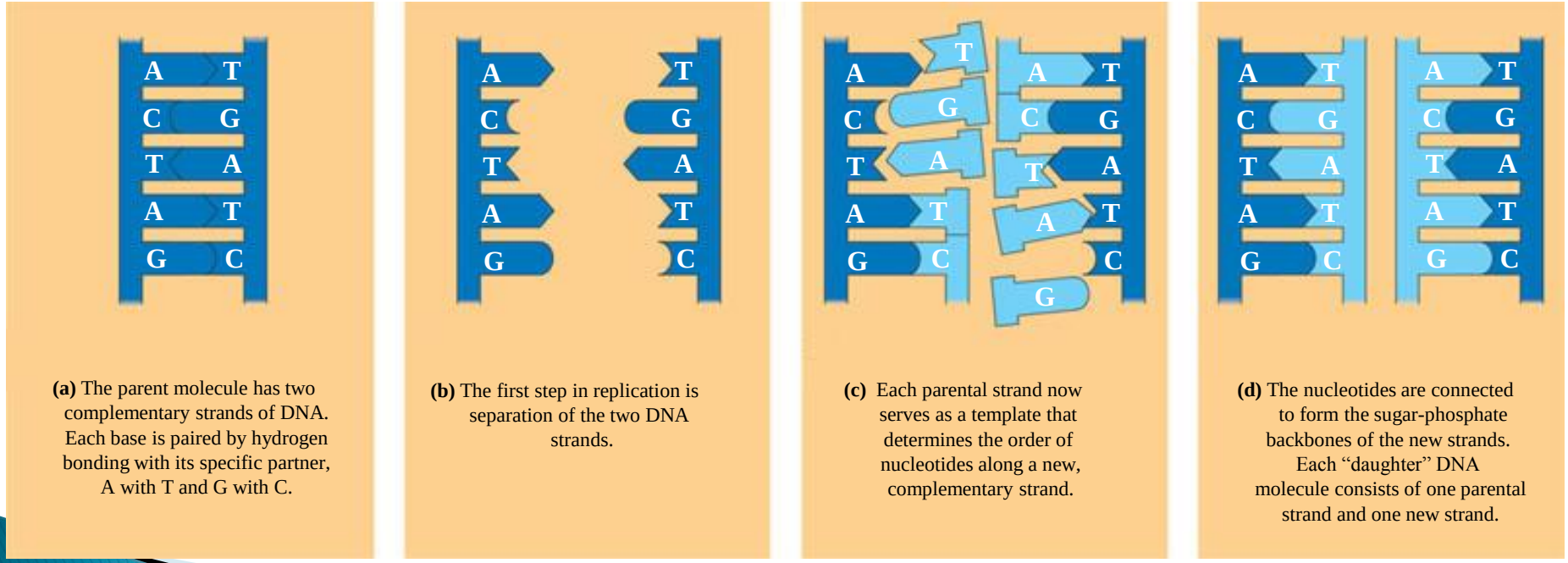


Figure 16.10 a–c

- In DNA replication
  - The parent molecule unwinds, and two new daughter strands are built based on base-pairing rules



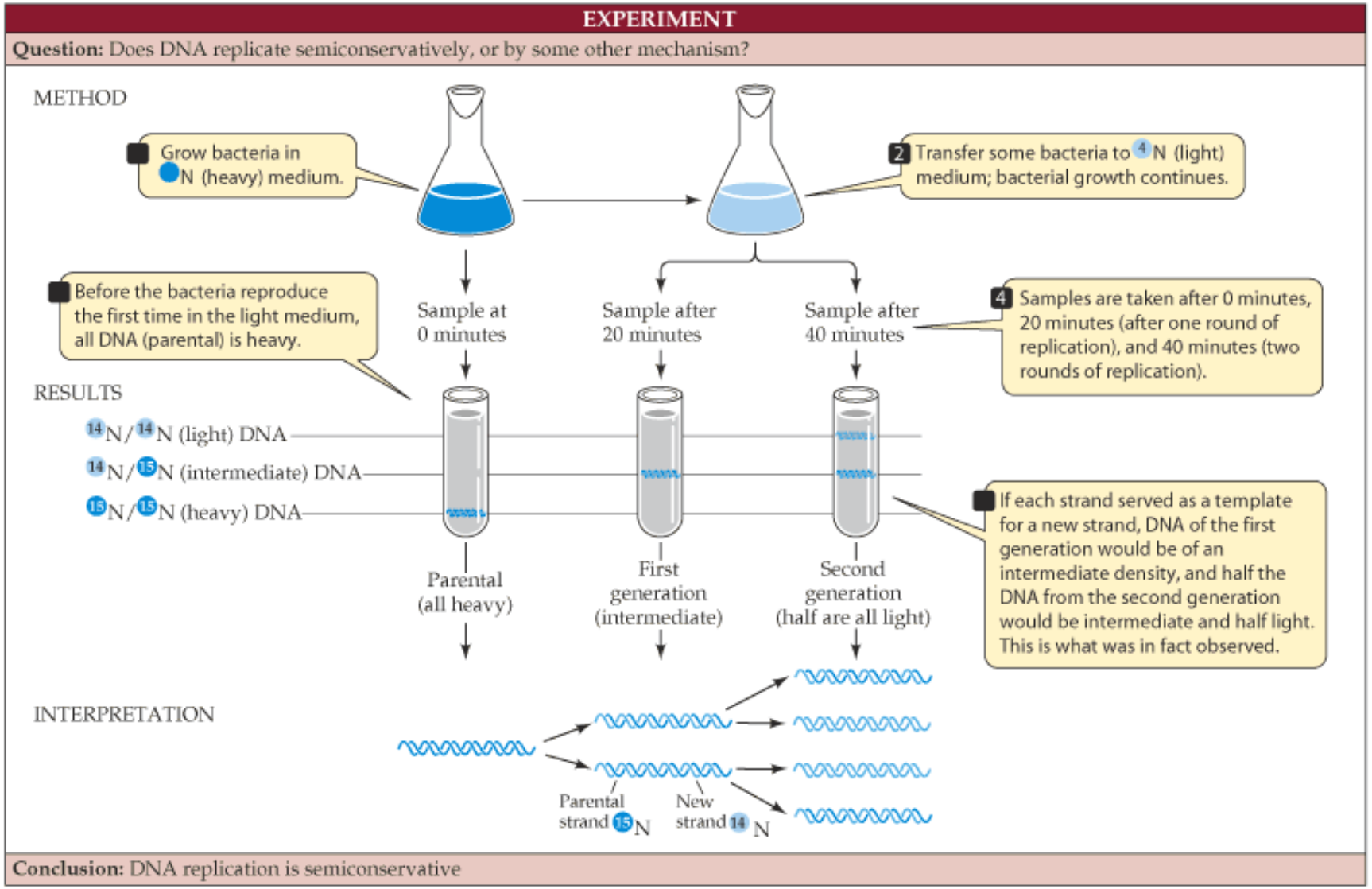
(a) The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

(b) The first step in replication is separation of the two DNA strands.

(c) Each parental strand now serves as a template that determines the order of nucleotides along a new, complementary strand.

(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

Figure 16.9 a–d


EXPERIMENT
Question: Does DNA replicate semiconservatively, or by some other mechanism?
METHOD
1 Grow bacteria in 15N (heavy) medium.
2 Transfer some bacteria to 14N (light) medium; bacterial growth continues.
3 Before the bacteria reproduce the first time in the light medium, all DNA (parental) is heavy.
Sample at 0 minutes
Sample after 20 minutes
Sample after 40 minutes
4 Samples are taken after 0 minutes, 20 minutes (after one round of replication), and 40 minutes (two rounds of replication).
RESULTS
14N/14N (light) DNA
14N/15N (intermediate) DNA
15N/15N (heavy) DNA
Parental (all heavy)
First generation (intermediate)
Second generation (half are all light)
If each strand served as a template for a new strand, DNA of the first generation would be of an intermediate density, and half the DNA from the second generation would be intermediate and half light. This is what was in fact observed.
INTERPRETATION
Parental strand 15N
New strand 14N
Conclusion: DNA replication is semiconservative

Experiments performed by Meselson and Stahl

- Supported the semiconservative model of DNA replication

EXPERIMENT

Matthew Meselson and Franklin Stahl cultured *E. coli* bacteria for several generations on a medium containing nucleotide precursors labeled with a heavy isotope of nitrogen, ^{15}N. The bacteria incorporated the heavy nitrogen into their DNA. The scientists then transferred the bacteria to a medium with only ^{14}N, the lighter, more common isotope of nitrogen. Any new DNA that the bacteria synthesized would be lighter than the parental DNA made in the ^{15}N medium. Meselson and Stahl could distinguish DNA of different densities by centrifuging DNA extracted from the bacteria.

RESULTS

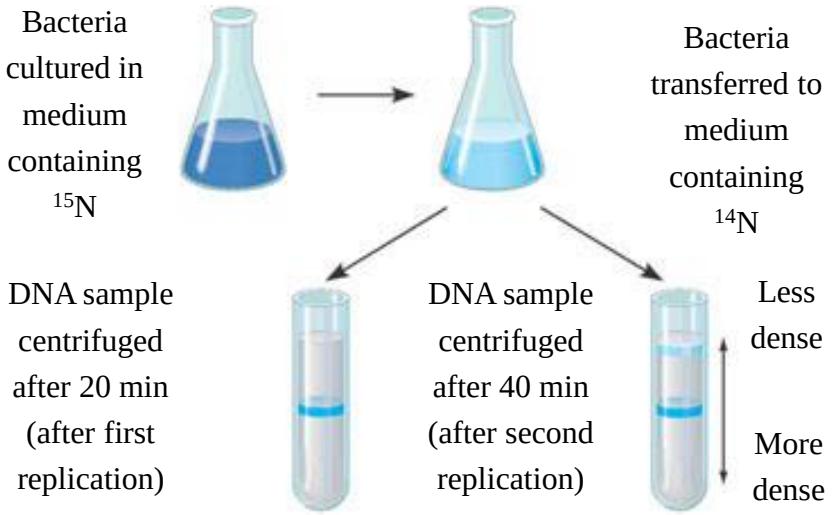


Figure 16.11 The bands in these two centrifuge tubes represent the results of centrifuging two DNA samples from the flask in step 2, one sample taken after 20 minutes and one after 40 minutes.