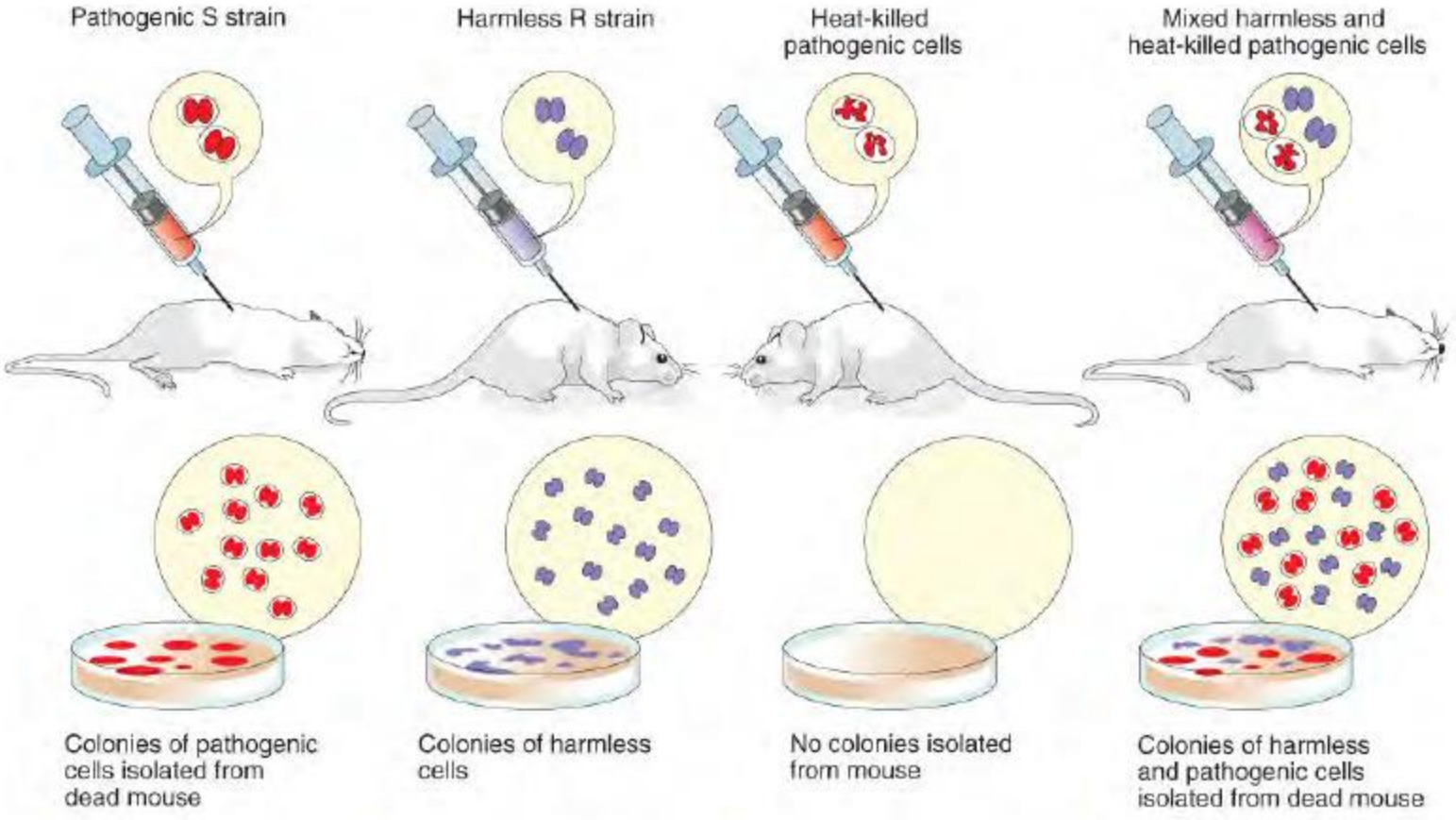
Griffith's Transformation Experiment



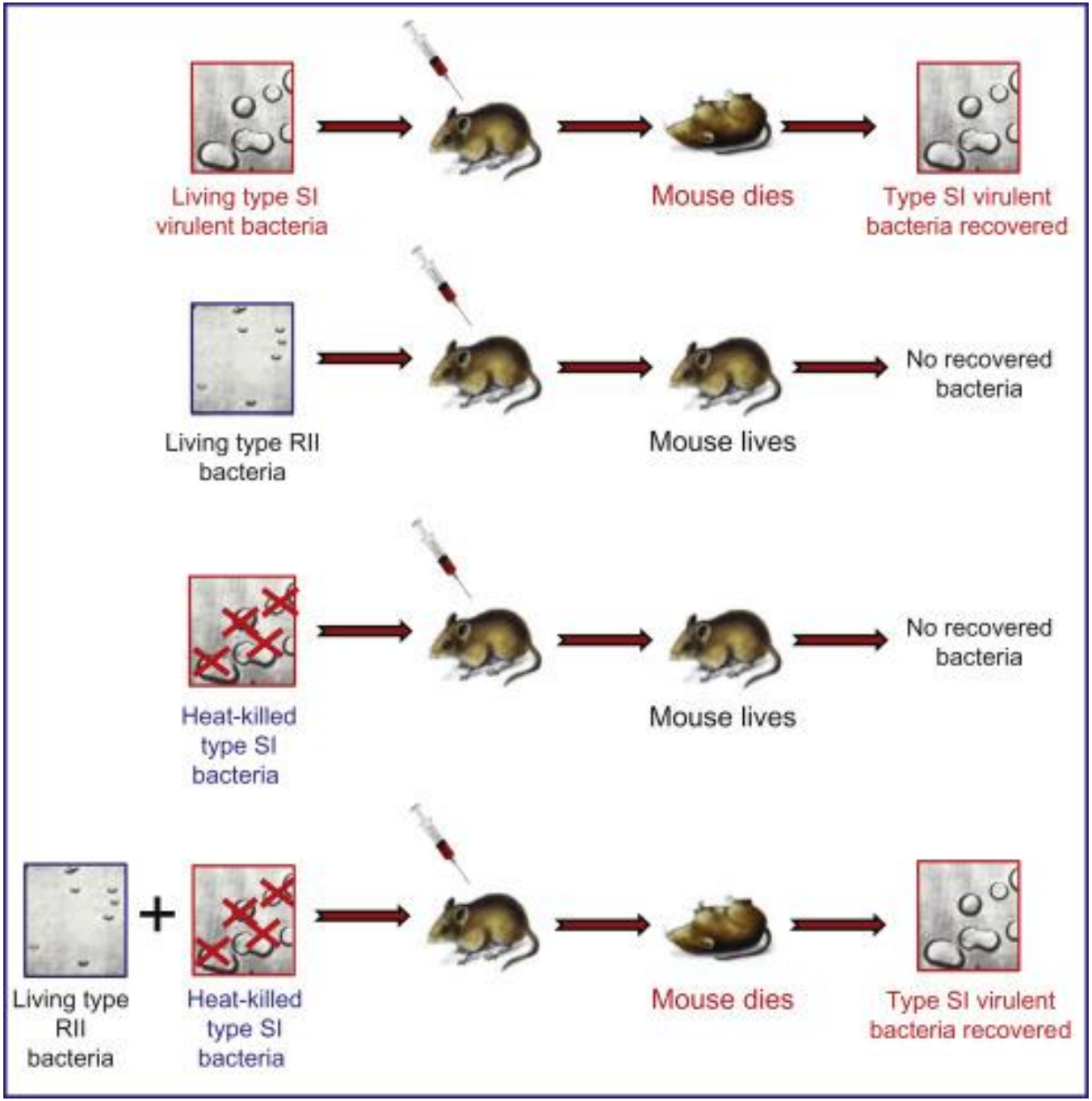
(A) When Griffith injected S strain (encapsulated, pathogenic) cells into the mouse, it developed pneumonia and died.

(B) An injection of R strain (unencapsulated,harmless) cells did no harm to the mouse.

(C) Furthermore, an injection of heat-killed S strain cells did no harm because the cells were dead.

(D) But when Griffith injected a mixture of live R strain and heat-killed S strain cells into the mouse, it died. When Griffith cultivated the organism from the blood, he found live S strain cells.

1928!


Living type SI virulent bacteria
Mouse dies
Type SI virulent bacteria recovered
Living type RII bacteria
Mouse lives
No recovered bacteria
Heat-killed type SI bacteria
Mouse lives
No recovered bacteria
Living type RII bacteria
+
Heat-killed type SI bacteria
Mouse dies
Type SI virulent bacteria recovered

Miescher and many others suspected that nuclein (nucleic acid) was associated in some way with cell inheritance,

But the first direct evidence that DNA is the bearer of genetic information came in 1944 through a discovery made by Oswald T. Avery, Colin MacLeod, and Maclyn McCarty.

These investigators found that DNA extracted from a virulent (disease-causing) strain of the bacterium *Streptococcus pneumoniae, also known as* pneumococcus, genetically transformed a nonvirulent strain of this organism into a virulent form.

Avery and his colleagues concluded that the DNA extracted from the virulent strain carried the inheritable genetic message for virulence. Not everyone accepted these conclusions, because protein impurities present in the DNA could have been the carrier of the genetic information.

This possibility was soon eliminated by the finding that treatment of the DNA with proteolytic enzymes did not destroy the transforming activity, but treatment with deoxyribonucleases (DNA-hydrolyzing enzymes) did.

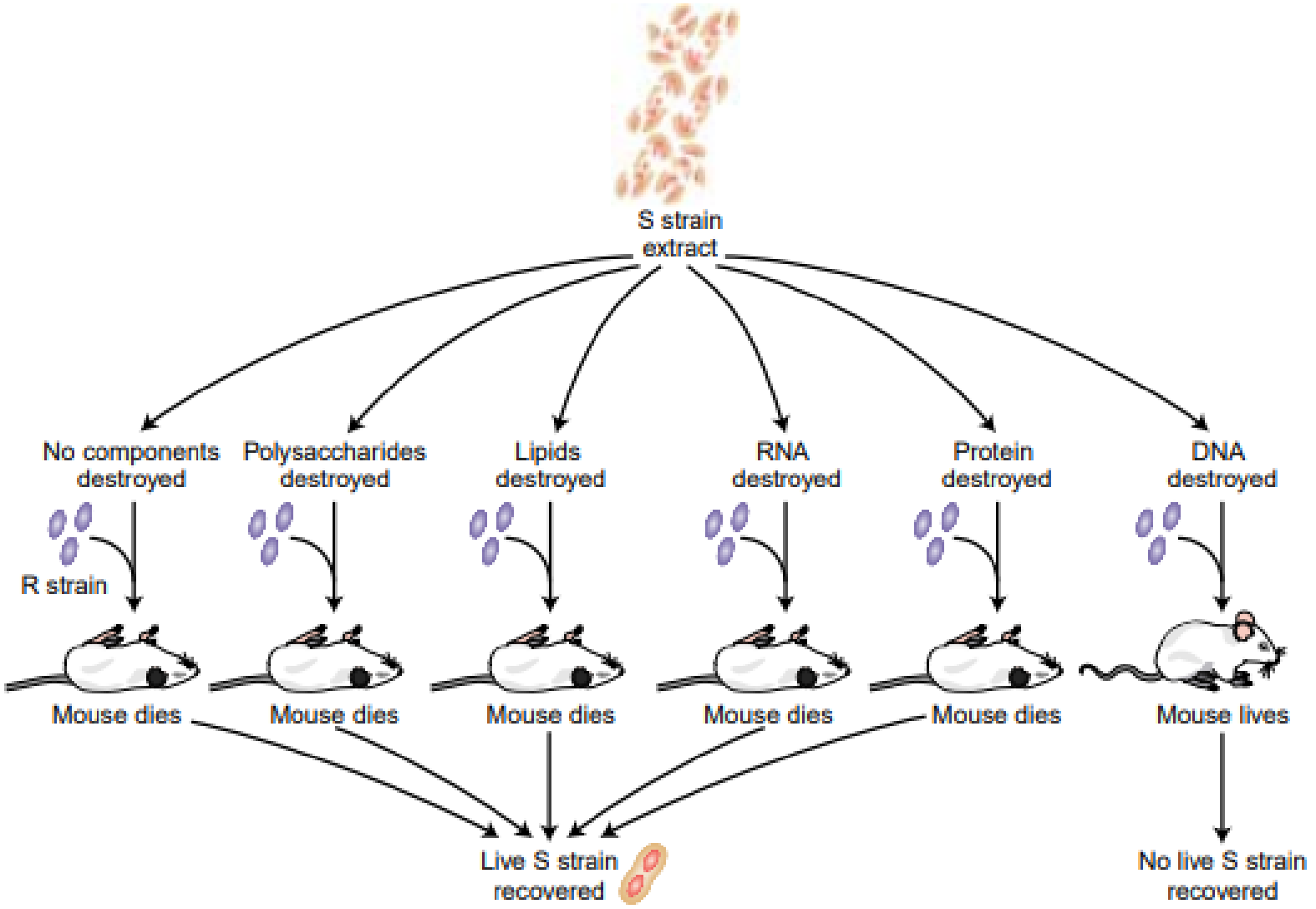


Figure 7-3 **DNA is the agent transforming the R strain into virulence.** If the DNA in an extract of heat-killed S strain cells is destroyed, then mice injected with a mixture of the heat-killed cells and the live nonvirulent strain R are no longer killed.

The Avery-MacLeod-McCarty experiment. (a) When injected into mice, the encapsulated strain of pneumococcus is lethal,

(b) whereas the nonencapsulated strain, (c) like the heat-killed encapsulated strain, is harmless. **(d)**

Earlier research by the bacteriologist Frederick Griffith had shown that adding heat-killed virulent bacteria (harmless to mice) to a live nonvirulent strain permanently transformed the latter into lethal, virulent, encapsulated bacteria.

(e) Avery and his colleagues extracted the DNA from heat-killed virulent pneumococci, removing the protein as completely as possible, and added this DNA to nonvirulent bacteria.

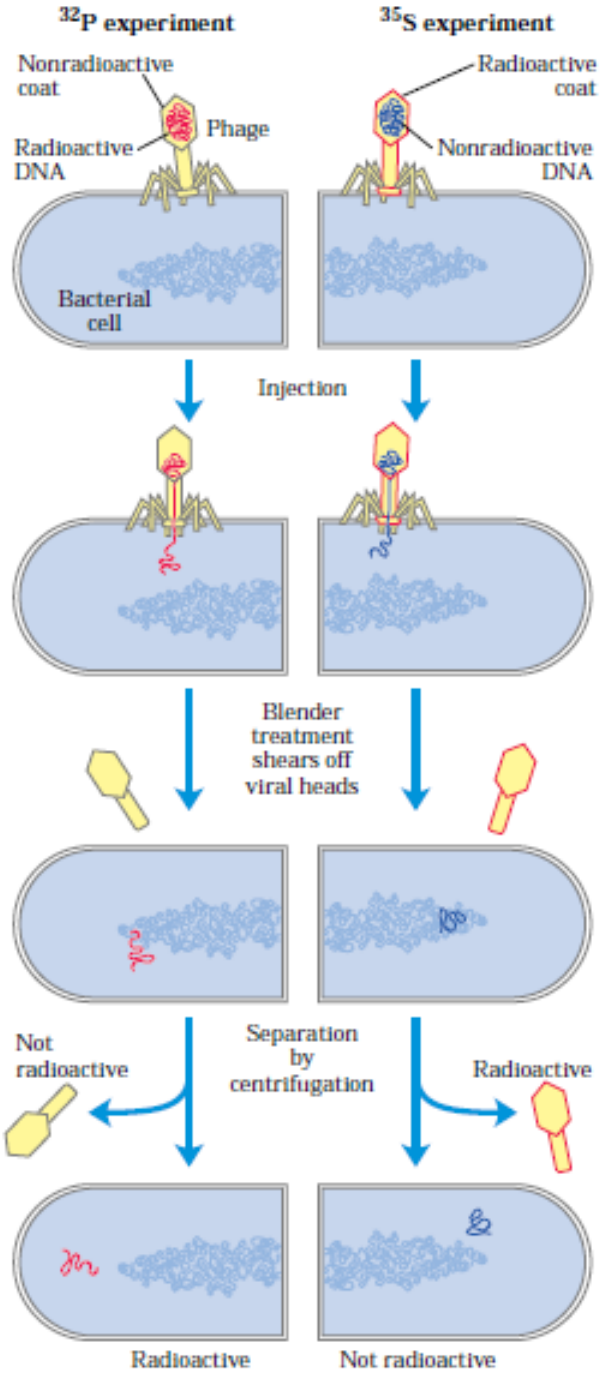
The DNA gained entrance into the nonvirulent bacteria, which were permanently transformed into a virulent strain.

- **The Hershey-Chase experiment.**

- Two batches of isotopically labeled bacteriophage T2 particles were prepared. One was labeled with ^{32}P in the phosphate groups of the DNA, the other with ^{35}S in the sulfur-containing amino acids of the protein coats (capsids).
- (**Note that DNA contains no sulfur and viral protein contains no phosphorus**.)

- The two batches of labeled phage were then allowed to infect separate suspensions of unlabeled bacteria.
- Each suspension of phage-infected cells was agitated in a blender to shear the viral capsids from the bacteria.
- The bacteria and empty viral coats (called "ghosts") were then separated by centrifugation.
- The cells infected with the ^{32}P-labeled phage were found to contain ^{32}P, indicating that the labeled viral DNA had entered the cells; the viral ghosts contained no radioactivity.
- The cells infected with 35S-labeled phage were found to have no radioactivity after blender treatment, but the viral ghosts contained ^{35}S.

- Progeny virus particles (not shown) were produced in both batches of bacteria some time after the viral coats were removed, indicating that the genetic message for their replication had been introduced by viral DNA, not by viral protein.


32P experiment
35S experiment
Nonradioactive coat
Phage
Radioactive DNA
Bacterial cell
Radioactive coat
Nonradioactive DNA
Injection
Blender treatment shears off viral heads
Not radioactive
Separation by centrifugation
Radioactive
Radioactive
Not radioactive

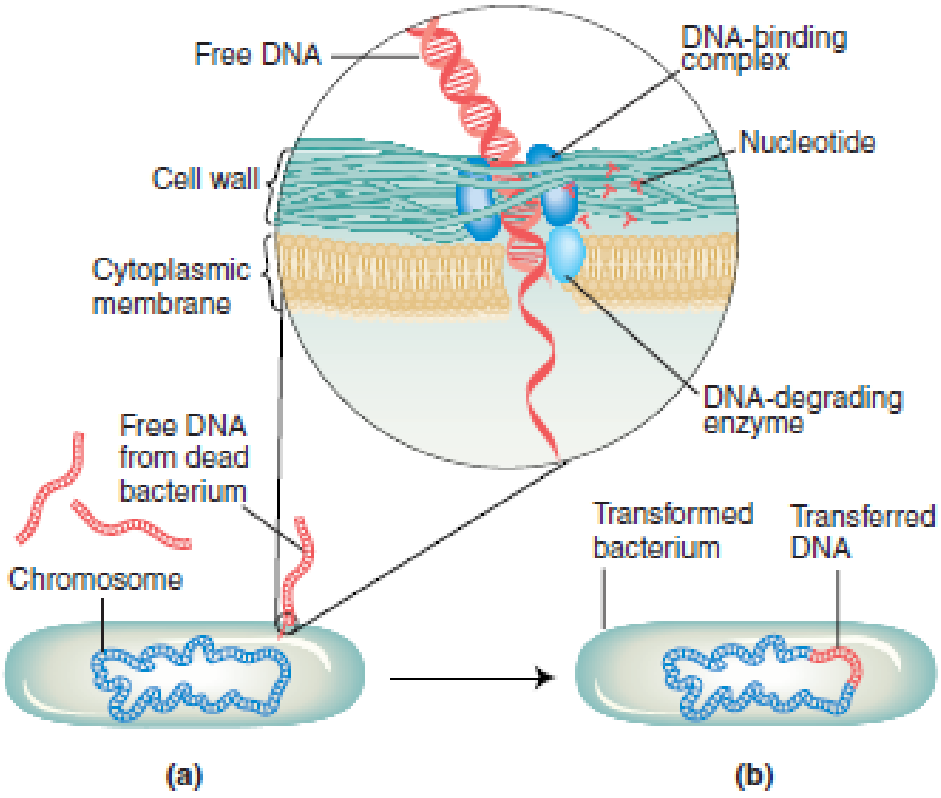


Figure 5-19 Transformation. Bacterium undergoing transformation (a) picks up free DNA released from a dead bacterial cell. As DNA-binding complexes on the bacterial surface take up the DNA (inset), enzymes break down one strand into nucleotides; a derivative of the other strand may integrate into the bacterium's chromosome (b). [After

Chromosome mapping using transformation

Transformation can be used to provide information on bacterial gene linkage. When DNA (the bacterial chromosome) is extracted for transformation experiments, some breakage into smaller pieces is inevitable.

If two donor genes are located close together on the chromosome, there is a good chance that sometimes they will be carried on the same piece of transforming DNA.

Hence both will be taken up, causing a **double transformation.**

Conversely, if genes are widely separated on the chromosome, they will be carried on separate transforming segments.

Any double transformants will most likely arise from separate independent transformations

- **Integration of foreign DNA during natural transformation of *Acinetobacter* sp. by homology-facilitated illegitimate recombination**

- The active uptake of extracellular DNA and its genomic integration is **termed natural transformation** and constitutes a major horizontal gene-transfer mechanism in prokaryotes.

- Chromosomal DNA transferred within a species can be integrated effectively by homologous recombination, whereas foreign DNA with low or no sequence homology would rely on illegitimate recombination events, which are rare.

Species such as *S. pneumoniae* are naturally competent; competence is mediated by cell cycle, quorum sensing via secretion of a competence stimulating peptide, and specific competence proteins.

Not all bacteria are naturally competent, however, but can be forced to take up DNA using nonphysiological techniques.

E. coli was the first bacterium to be forced to become competent.

Forced competency was discovered stepwise; initially, helper phage-mediated transformation was used.

Later it was found that **calcium ions** improved competence.

It was then established that extensive treatment with a calcium chloride solution eliminated the need for phage entirely.

$CaCl_2$-based chemical competency allowed for successful transformation using recipient and donor DNA from *E. coli*,

https://www.youtube.com/watch?v=7Ul9RVYG5CM

Transformation of *Escherichia coli* by Electroporation

The term “transformation” refers cellular ingestion of foreign DNA. In nature, transformation can occur in certain types of bacteria.

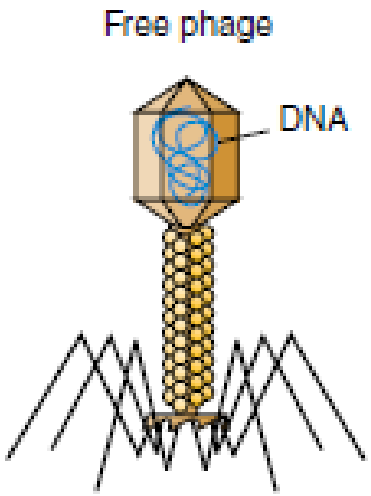
In molecular biology, however, transformation is artificially induced through the creation of pores in the bacterial cell walls.

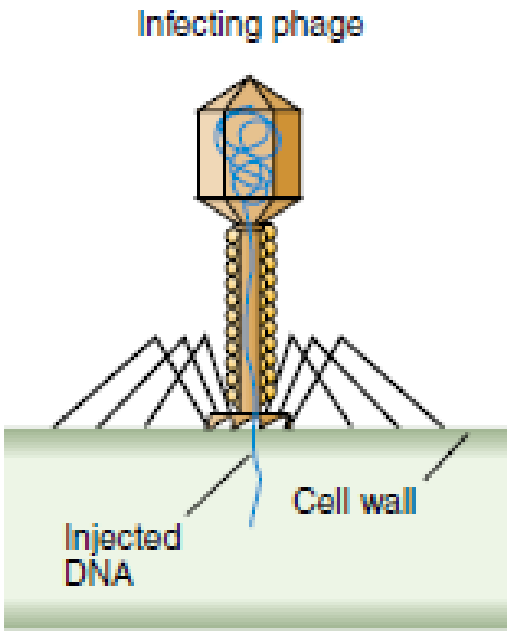
Bacterial cells that are able to take up DNA from the environment are called **competent cells.**

Electrocompetent cells can be produced in the laboratory and transformation of these cells can be achieve via the application of an electrical field that creates pores in the cell wall through which DNA can pass.

Preparing electrocompetent bacteria is considerably easier than preparing cells for transformation by chemical methods.

Bacteria are simply grown to mid-log phase, chilled, centrifuged, washed extensively with ice-cold buffer or H_2O to reduce the ionic strength of the cell suspension, and then suspended in an ice-cold buffer containing 10% glycerol.

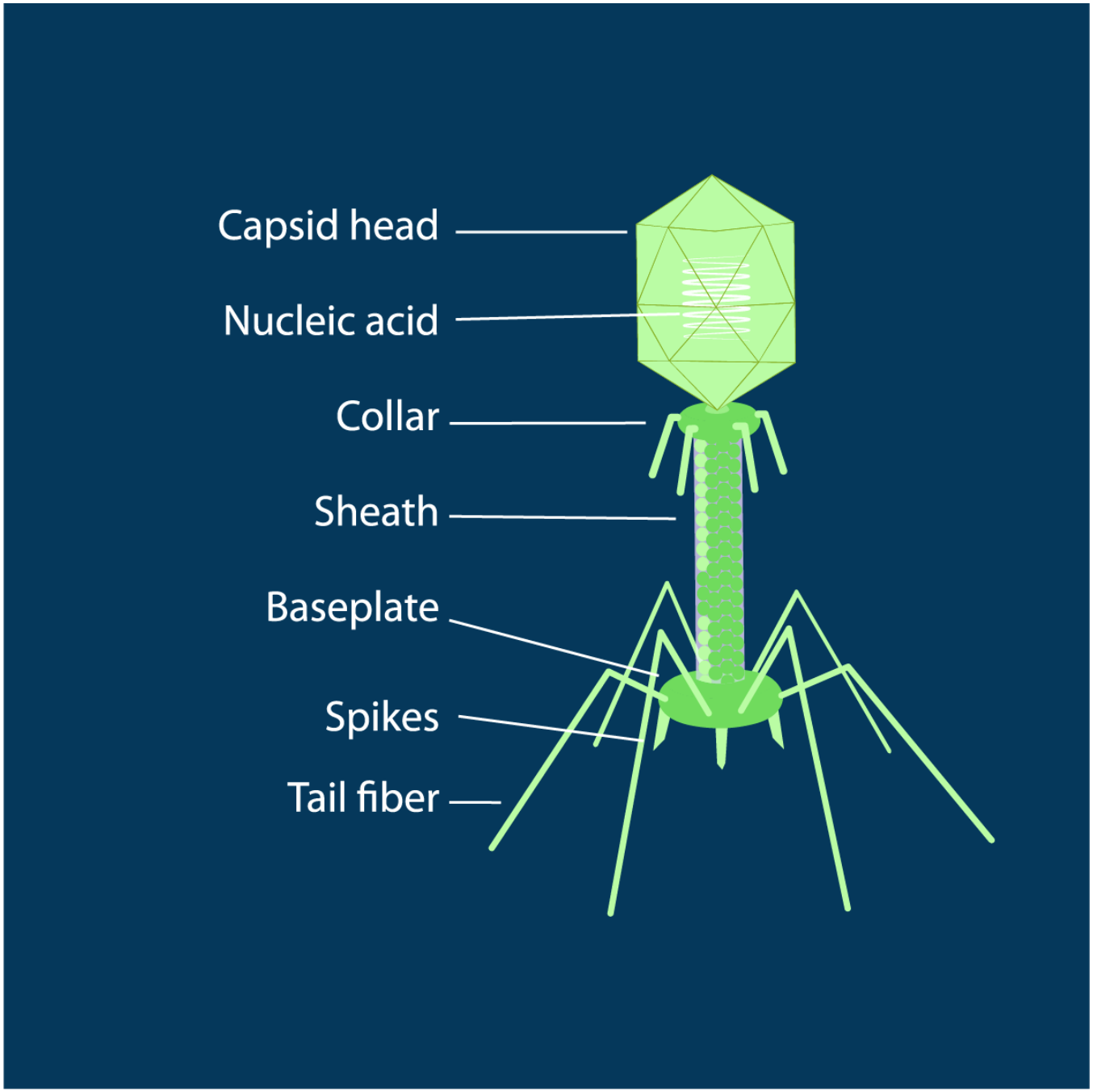




Most bacteria are susceptible to attack by **bacteriophages.**
A phage consists of a nucleic acid "chromosome" (DNA or RNA) surrounded by a coat of protein molecules.

Phage types are identified not by species names but by symbols, for example, phage T4, phage , and so forth.

Figure 5-22 Micrograph of a bacteriophage attaching to a bacterium and injecting its DNA. [Dr. L. Caro/Science Photo Library,


Capsid head
Nucleic acid
Collar
Sheath
Baseplate
Spikes
Tail fiber

Infection of bacteria by phages

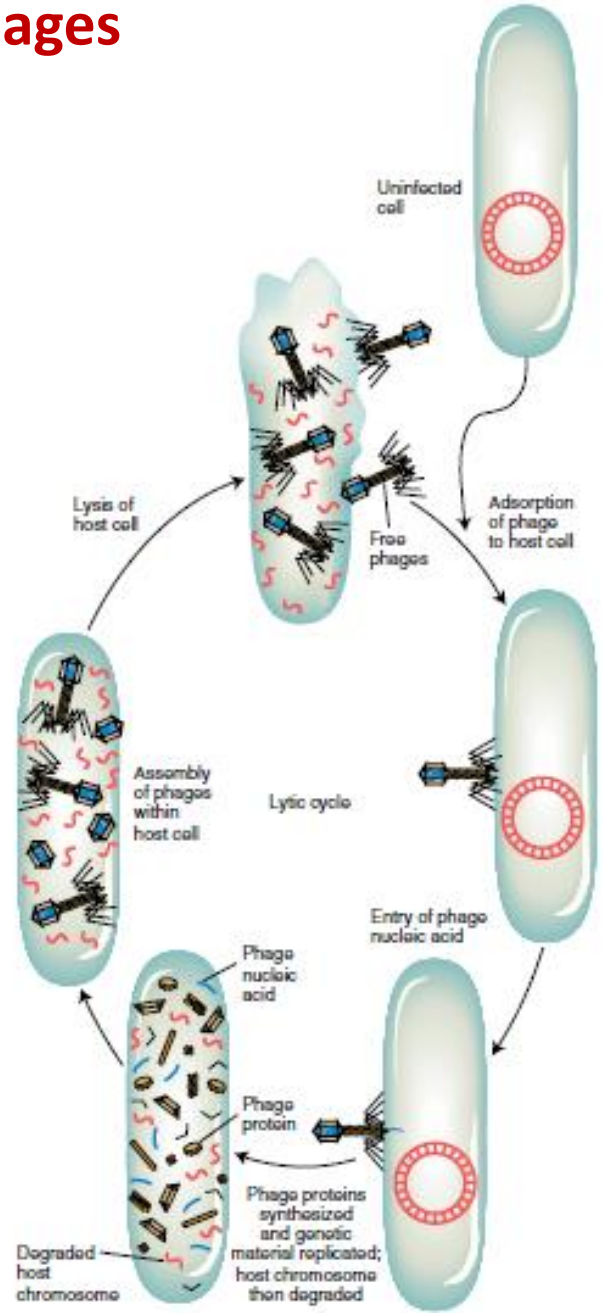
During infection, a phage attaches to a bacterium and injects its genetic material into the bacterial cytoplasm.

The phage genetic information then takes over the machinery of the bacterial cell by turning off the synthesis of bacterial components and redirecting the bacterial synthetic machinery to make more phage components.

Newly made phage heads are individually stuffed with replicates of the phage chromosome.

Ultimately, many phage descendants are made, and these are released when the bacterial cell wall breaks open.

This breaking-open process is called **lysis.**



Transduction

Some phages are able to pick up bacterial genes and carry them from one bacterial cell to another: a process known as **transduction.**

Lederberg and Zinder had discovered a new type of gene transfer, mediated by a virus.

They were the first to call this process *transduction.*

As a rarity in the lytic cycle, virus particles sometimes pick up bacterial genes and transfer them when they infect another host.

Transduction has subsequently been demonstrated in many bacteria.

In 1951, Joshua Lederberg and Norton Zinder were testing for recombination in the bacterium *Salmonella typhimurium.*

The researchers used two different strains: one was *phe- trp- tyr-, and the other was Met- his-. T*hey are all auxotrophic.
When either strain was plated on a minimal medium, no wild-type cells were observed.

However, after the two strains were mixed, wild-type prototrophs appeared at a frequency of about 1 in 10^5.

Thus far, the situation seems similar to that for recombination in *E. coli.*

However, in this case, the researchers also recovered recombinants from a U-tube experiment, in which conjugation was prevented by a filter separating the two arms.

By varying the size of the pores in the filter, they found that the agent responsible for gene transfer was the same size as a known phage of *Salmonella, called* **phage P22.**

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To understand the process of transduction we need to distinguish two types of phage cycle.

Virulent phages are those that immediately lyse and kill the host.

Temperate phages can remain within the host cell for a period without killing it.

Their DNA either integrates into the host chromosome to replicate with it or replicates like a plasmid, separately in the cytoplasm.

A phage integrated into the bacterial genome is called a **prophage.**

A bacterium harboring a quiescent phage is called **lysogenic.** Occasionally a lysogenic bacterium lyses spontaneously.

Only temperate phages can transduce.

There are two kinds of transduction: generalized and specialized.
Generalized transducing phages can carry *any part of* the bacterial chromosome, whereas **specialized transducing** phages carry only certain *specific parts.*