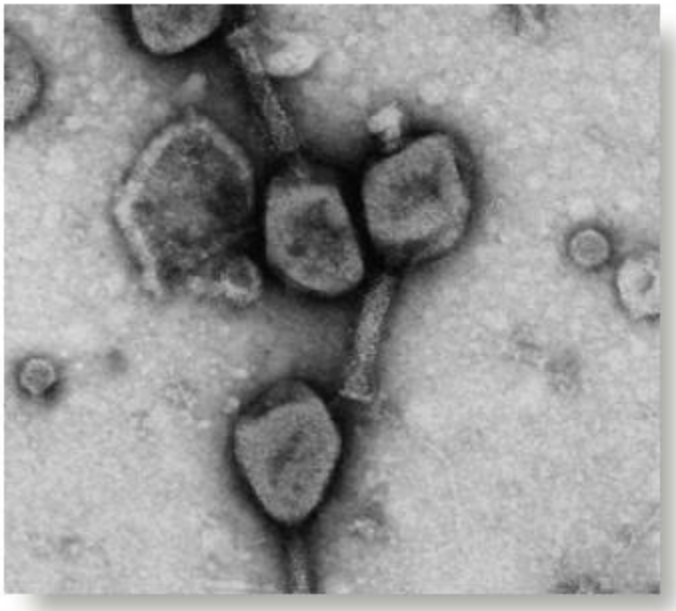


Figure 5-21 Bacteriophage T4. Enlargement of the *E. coli* phage T4 showing details of structure: note head, tail, and tail fibers. [Photograph from Jack D. Griffith.]

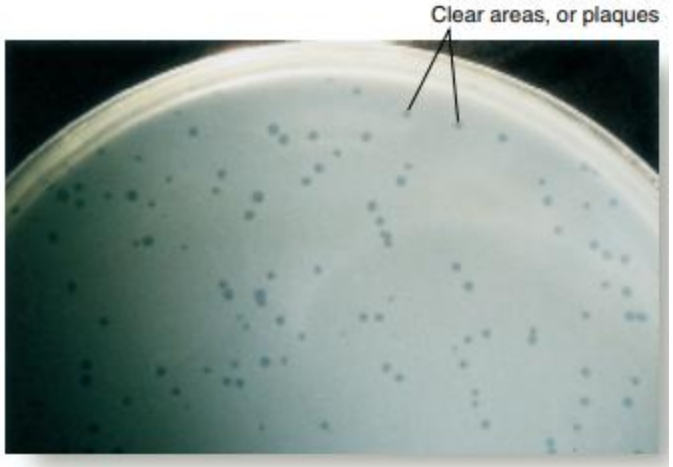


Figure 5-24 Phage plaques. Through repeated infection and production of progeny phage, a single phage produces a clear area, or plaque, on the opaque lawn of bacterial cells.

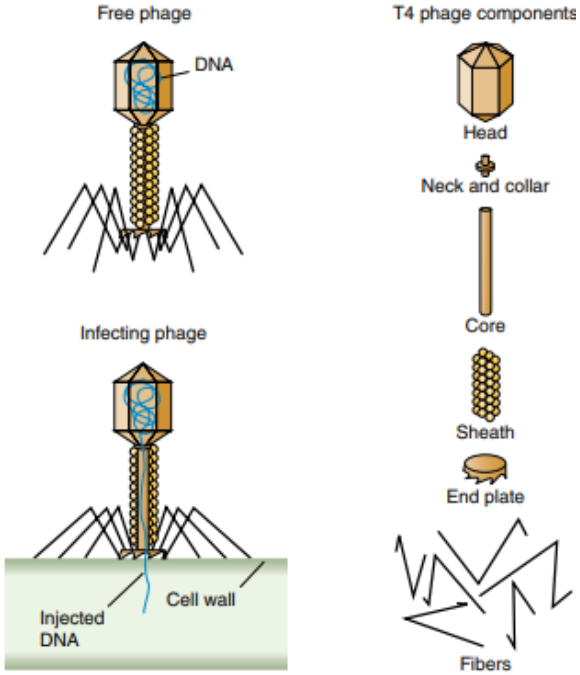


Figure 5-20 An infecting phage injects DNA through its core structure into the cell. The left half of the figure shows bacteriophage T4 as a free phage, then in the process of infecting an *E. coli* cell. The major structural components of

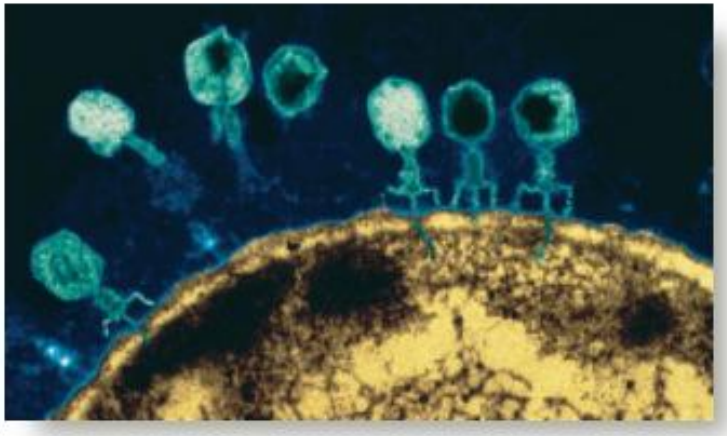


Figure 5-22 Micrograph of a bacteriophage attaching to a bacterium and injecting its DNA. [Dr. L. Caro/Science Photo Library, Photo Researchers.]

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.

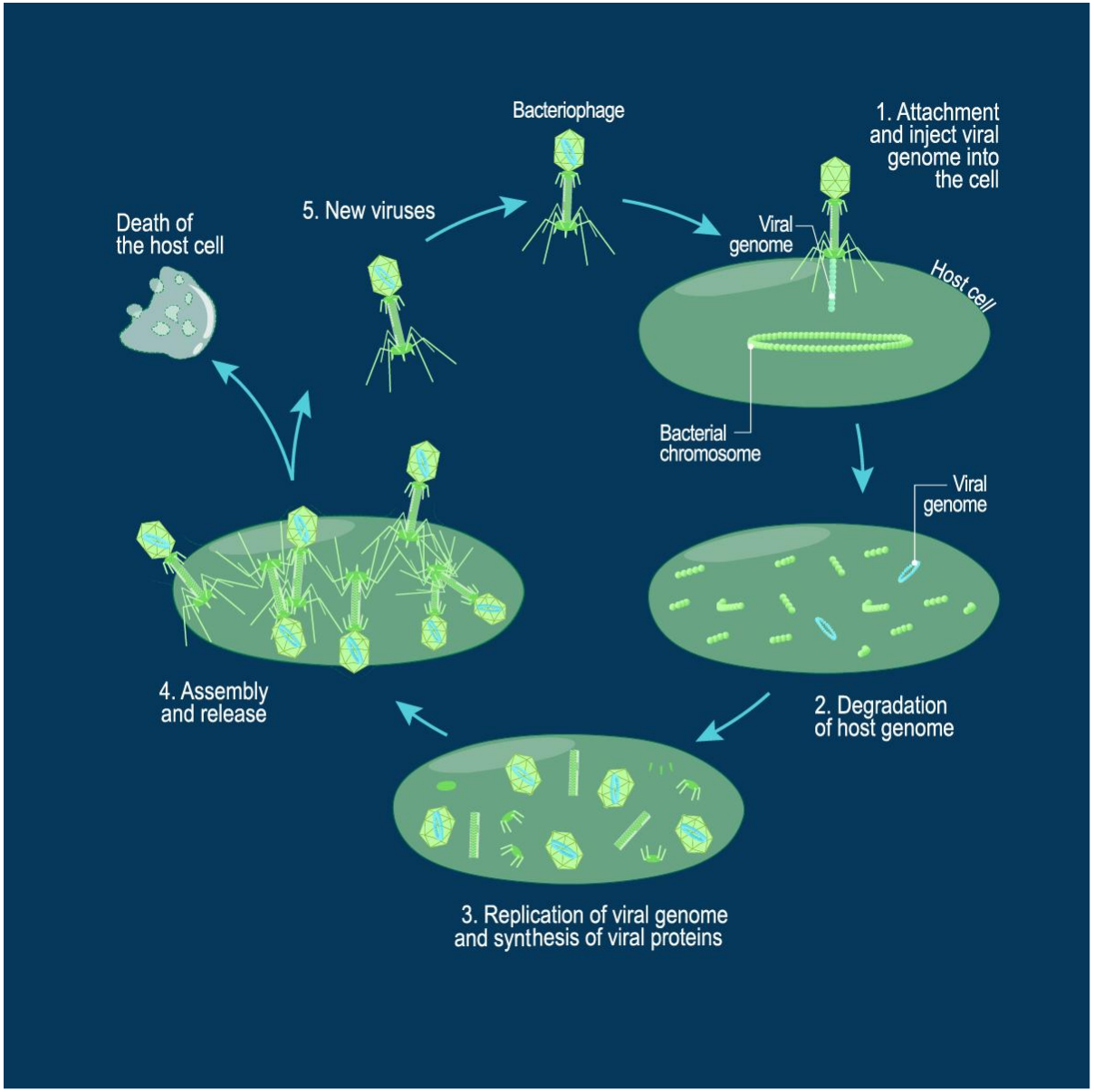
Their DNA either integrates into the host chromosome to replicate with it or replicates like a plasmid, separately in the cytoplasm.

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.*



In the lytic cycle (Figure 2), sometimes referred to as virulent infection, the infecting phage ultimately kill the host cell to produce many of their own progeny. Immediately following injection into the host cell, the phage genome synthesizes early proteins that break down the host DNA, allowing the phage to take control of the cellular machinery.

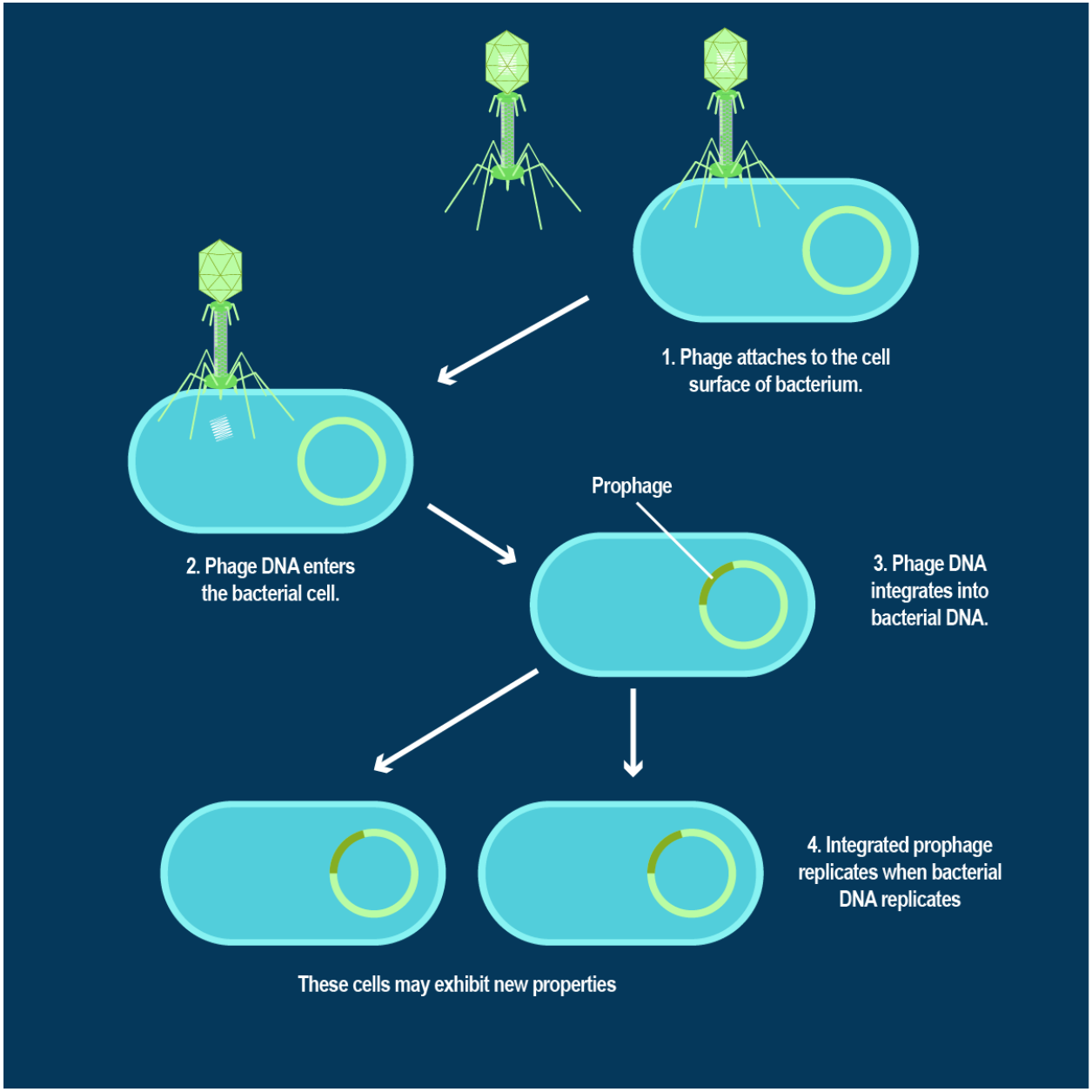
Some bacterial viruses, called *temperate phages, can establish* a nonlytic association with their host cells that does not kill the cell.

When bacteriophage infects *E. coli,* the viral DNA may be integrated into the host-cell chromosome rather than being replicated.

The integrated viral DNA, called a *prophage, is replicated as part of the cell's DNA* from one host-cell generation to the next.

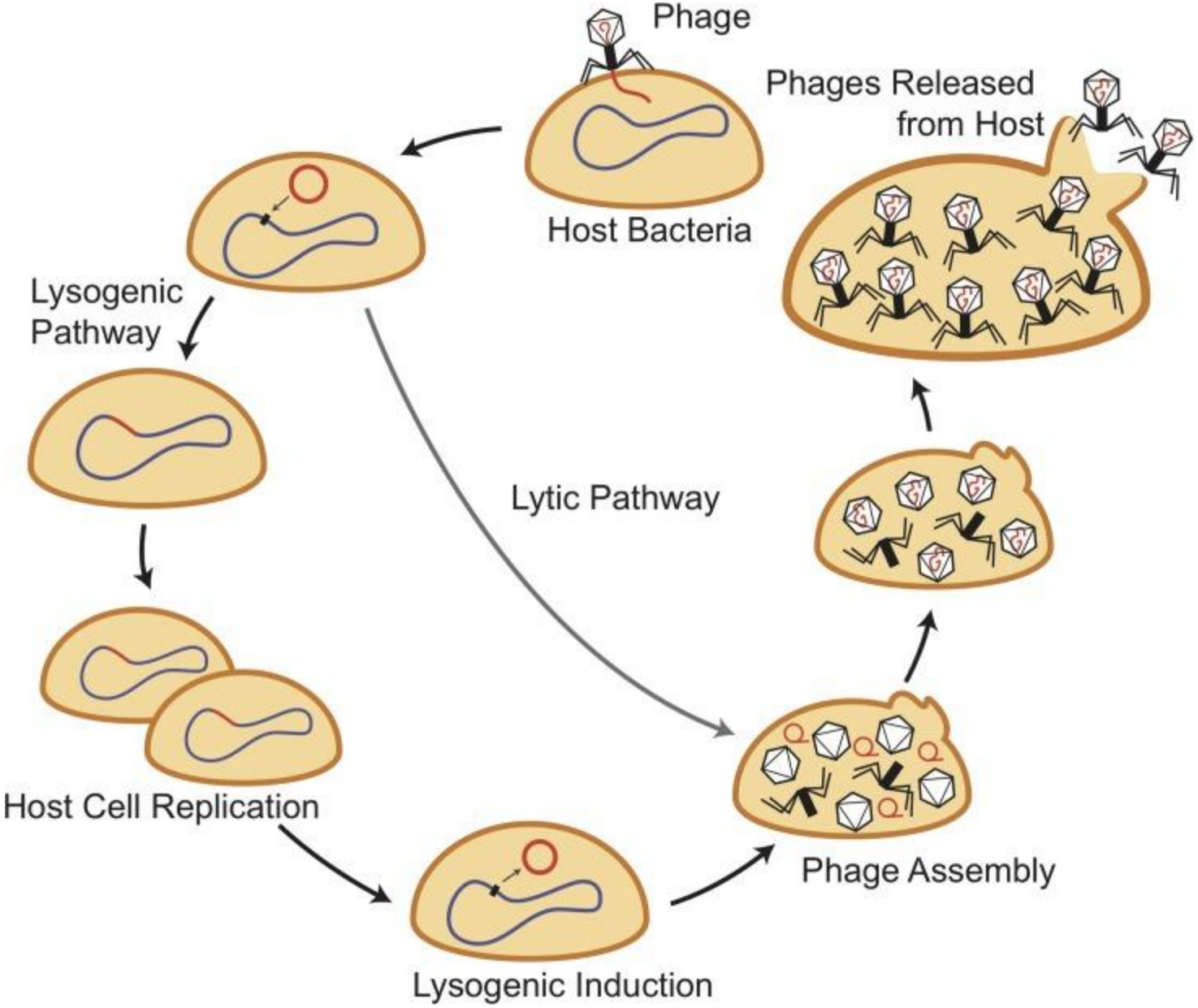
This phenomenon is referred to as **lysogeny.**

Under certain conditions, the prophage DNA is activated, leading to its excision from the host-cell chromosome, entrance into the lytic cycle, and subsequent production and release of progeny virions.



The lysogenic cycle (Figure 3), sometimes referred to as temperate or non-virulent infection, does not kill the host cell, instead using it as a refuge where it exists in a dormant state. Following the injection of the phage DNA into the host cell, it integrates itself into the host genome, with the help of phage-encoded integrases, where it is then termed a prophage. The prophage genome is then replicated passively along with the host genome as the host cell divides for as long as it remains there and does not form the proteins required to produce progeny. As the phage genome is generally comparatively small, the bacterial hosts are normally relatively unharmed by this process

https://youtu.be/hFwA0aBX5bE


Phage
Host Bacteria
Phages Released
from Host
Lysogenic
Pathway
Lytic Pathway
Host Cell Replication
Phage Assembly
Lysogenic Induction

Generalized transduction

By what mechanisms can a phage carry out generalized transduction?

In 1965, K. Ikeda and J. Tomizawa threw light on this question in some experiments on the *E. coli* phage P1.
They found that when a donor cell is lysed by P1, the bacterial chromosome is broken up into small pieces.

Occasionally, the newly forming phage particles mistakenly incorporate a piece of the bacterial DNA into a phage head in place of phage DNA.

This event is the origin of the transducing phage.
A phage carrying bacterial DNA can infect another cell.
That bacterial DNA can then be incorporated into the recipient cell's chromosome by recombination.
Because genes on any of the cut-up parts of the host genome can be transduced, this type of transduction is by necessity of the **generalized** type.

Generalized transduction can be used to obtain bacterial linkage information when genes are close enough that the phage can pick them up and transduce them

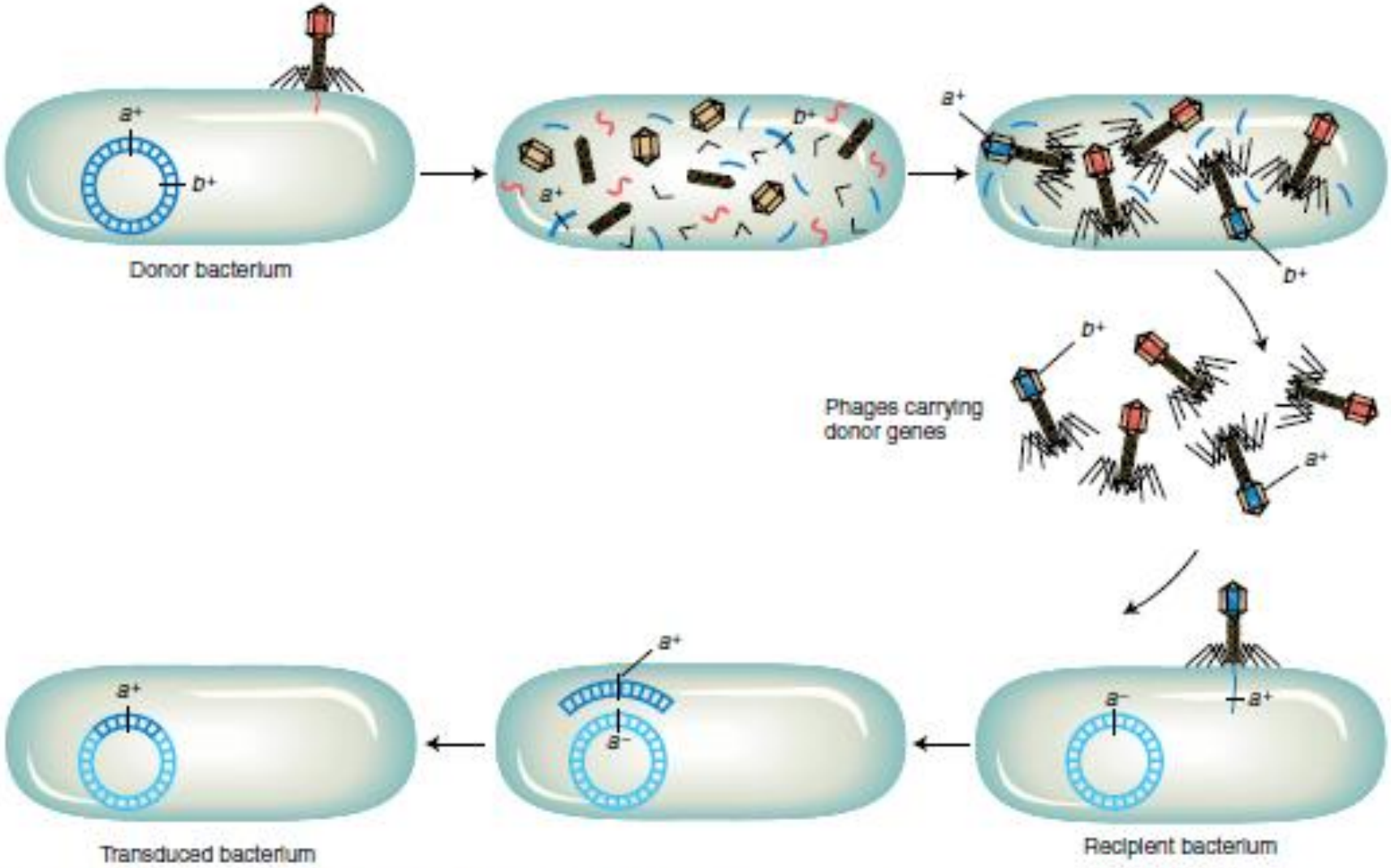


Figure 5-27 **The mechanism of generalized transduction.** In reality, only a very small minority of phage progeny (1 in 10,000) carry donor genes.

Phages P1 and P22 both belong to a phage group that shows generalized transduction.

- Suppose that we wanted to find the linkage between *met and arg in E. coli.*
- *We could grow phage P1 on a donor met+ arg+* strain, and then allow P1 phages from lysis of this strain to infect a *met-arg- strain.*
- *First, one donor allele is selected,* say, *met.*
- *Then, the percentage of met+ colonies* that is also *arg+ is measured.*
- *Strains transduced to both met and arg are called* ***cotransductants.*** The greater the cotransduction frequency, the *closer two genetic markers*
  must be.

Experiment	Selected marker	Unselected markers
1	*leu*$^+$	50% are *azi*^{r}; 2% are *thr*$^+$
2	*thr*$^+$	3% are *leu*$^+$; 0% are *azi*^{r}
3	*leu*$^+$ and *thr*$^+$	0% are *azi*^{r}

Using an extension of this approach, we can estimate the *size* of the piece of host chromosome that a phage can pick up, as in the following type of experiment, which uses P1 phage:

donor *leu*$^+$ *thr*$^+$ *azi*^{r} ⟶ recipient *leu*$^-$ *thr*$^-$ *azi*^{s}

In this experiment, P1 phage grown on the *leu*$^+$ *thr*$^+$ *azi*^{r} donor strain infect the *leu*$^-$ *thr*$^-$ *azi*^{s} recipient strain. The strategy is to select one or more donor markers in the recipient and then test these transductants for the presence of the unselected markers. Results are outlined in Table 5-3. Experiment 1 in Table 5-3 tells us that *leu* is relatively close to *azi* and distant from *thr*, leaving us with two possibilities:

or

Experiment 2 tells us that *leu* is closer to *thr* than *azi* is, so the map must be

By selecting for *thr*$^+$ and *leu*$^+$ together in the transducing phages in experiment 3, we see that the transduced piece of genetic material never includes the *azi* locus because the phage head cannot carry a fragment of DNA that big. P1 can only cotransduce genes less than approximately 1.5 minutes apart on the *E. coli* chromosome map.

Mechanism of specialized transduction

- λ **INSERTION: The interrupted-mating experiments** described above showed that the prophage is part of the lysogenic bacterium's chromosome.

- How is the prophage inserted into the bacterial genome?

- In 1962, Allan Campbell proposed that it inserts by a single crossover between a circular chromosome and the circular *E. coli chromosome.*

- *The* crossover point would be between a specific site in λ, the λ **attachment site, and an attachment** site in the bacterial chromosome located between the genes *gal and*
- *bio, because* λ *integrates at that position in the E. coli* chromosome.

- The crossing-over is mediated by a phage encoded recombination system.

Integration of the prophage into the *E. coli chromosome* should increase the genetic distance between flanking bacterial markers for *gal and bio. In fact, studies show that lysogeny does increase time-of-entry or recombination distances* between the bacterial genes.

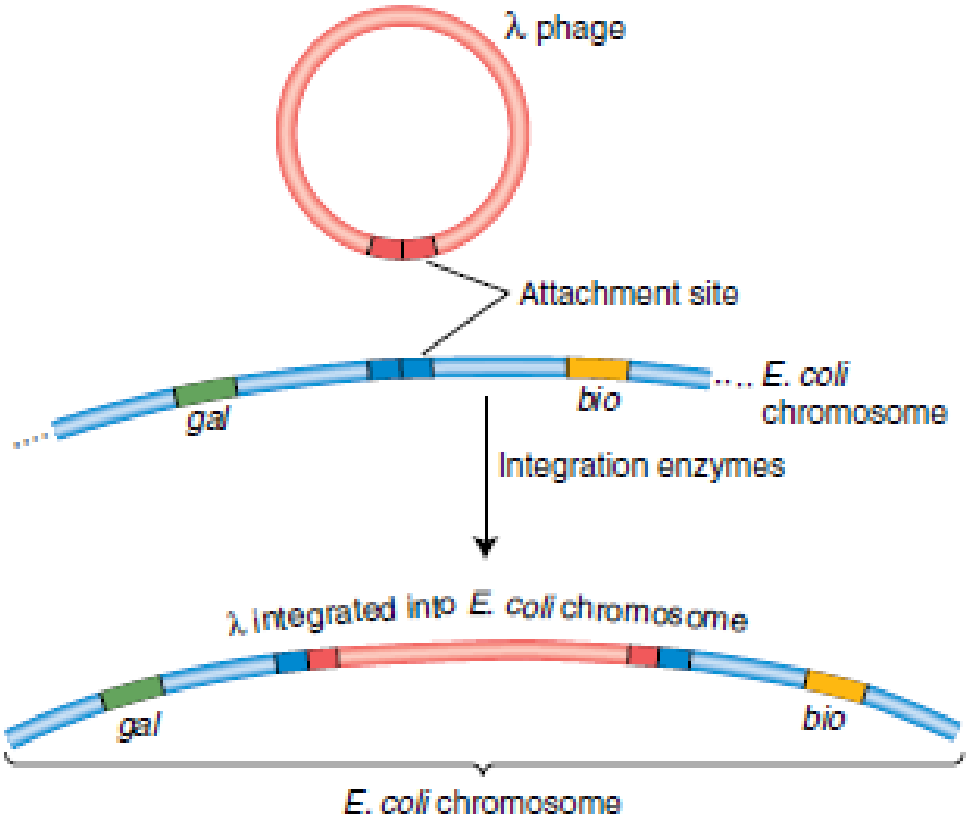
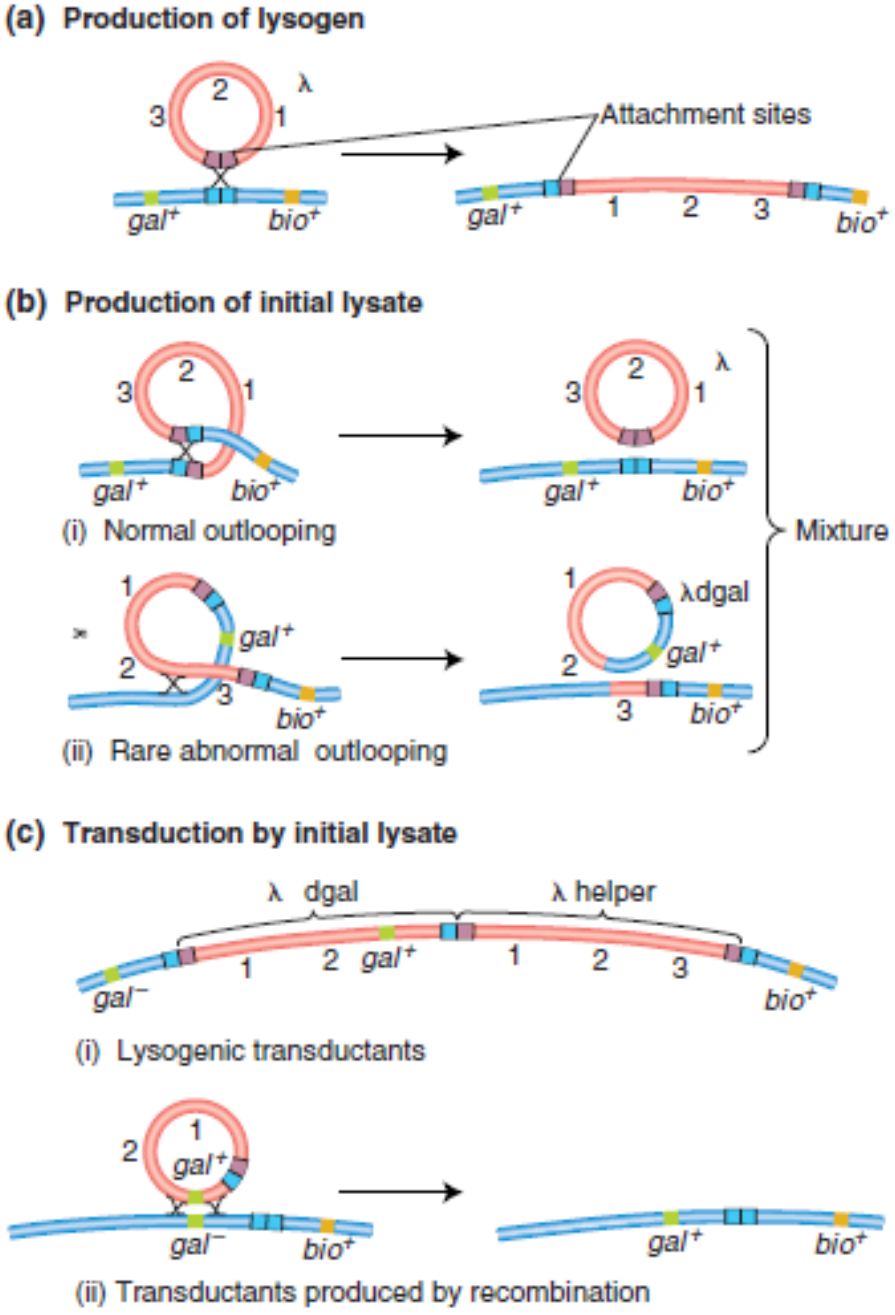


Figure 5-30 Model for the integration of phage λ into the *E. coli* chromosome. Reciprocal recombination takes place between a specific attachment site on the circular λ DNA and a specific region on the bacterial chromosome between the *gal* and *bio* genes.

- As a prophage, λ always inserts between the *gal region and* the *bio region of the host chromosome* and in transduction experiments, as expected, can transduce only the *gal and bio genes.*

- How does carry away neighboring genes?
- The explanation lies again in an imperfect reversal of the Campbell insertion mechanism, as for generalized transduction.
- The recombination event between specific regions of and the bacterial chromosome is catalyzed by a specialized enzyme system.
- The attachment site and the enzyme that uses this site as a substrate dictate that integrates only at that point in the chromosome.
- Furthermore, during lysis the prophage normally excises at precisely the correct point to produce a normal circular chromosome.

- Very rarely, excision is abnormal owing to faulty outlooping and can result in phage particles that now carry a nearby gene and leave behind some phage genes


(a) Production of lysogen
λ
2
3
1
Attachment sites
gal+
bio+
gal+
1
2
3
bio+
(b) Production of initial lysate
2
3
1
λ
gal+
bio+
(i) Normal outlooping
Mixture
1
gal+
2
3
bio+
λdgal
(ii) Rare abnormal outlooping
(c) Transduction by initial lysate
λ dgal
λ helper
gal−
1
2
gal+
1
2
3
bio+
(i) Lysogenic transductants
2
1
gal+
gal−
bio+
gal+
bio+
(ii) Transductants produced by recombination

How are other phages, which act as specialized transducers, able to carry only certain host genes to recipient cells?

The short answer is that specialized transducers insert into the bacterial chromosome at one position only.

When they exit, a faulty outlooping occurs.

Hence they can pick up and transduce only genes that are close by.

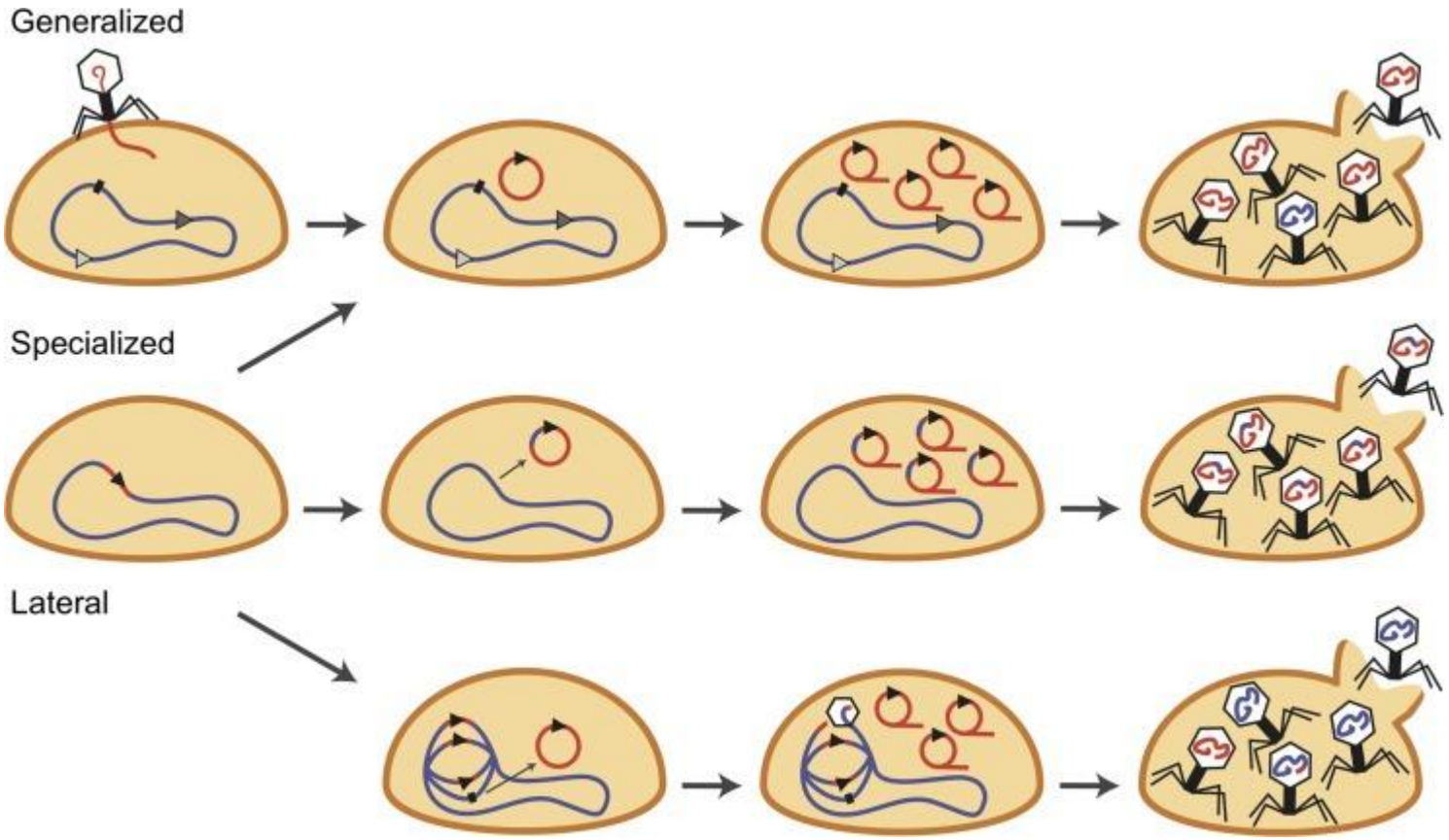
The resulting phage genome is defective because of the genes left behind, but it has also gained a bacterial gene *gal or bio.*

These phages are referred to as dgal (-defective *gal) or dbio.*

The abnormal DNA carrying nearby genes can be packaged into phage heads and can infect other bacteria.

In the presence of a second, normal phage particle in a double infection, the dgal can integrate into the chromosome at the attachment site.

In this manner, the *gal* genes in this case are transduced into the second host.


Generalized
Specialized
Lateral

overview

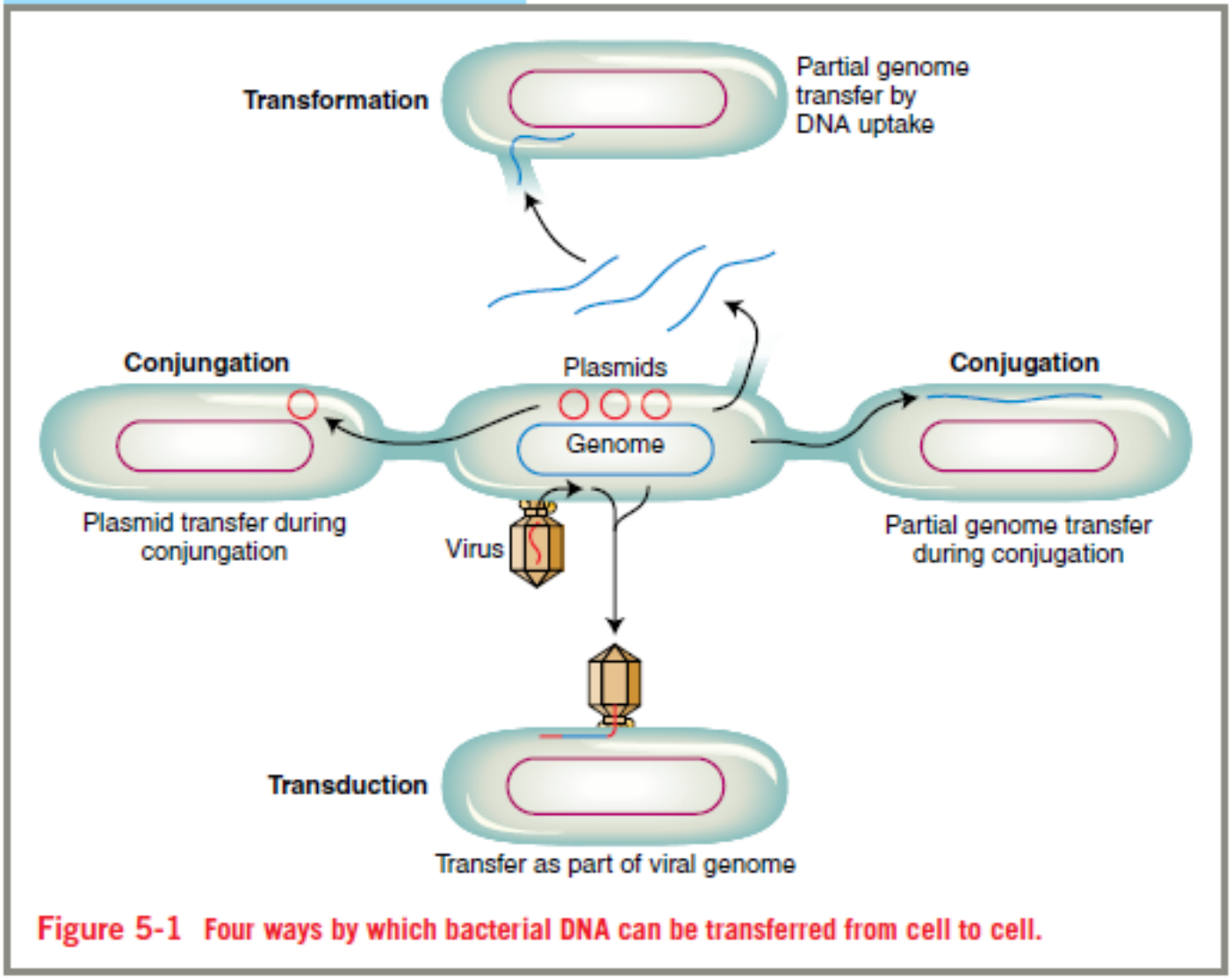


Figure 5-1 Four ways by which bacterial DNA can be transferred from cell to cell.