

Vehicles: plasmids and bacteriophages

2

A DNA molecule needs to display several features to be able to act as a vehicle for gene cloning. Most important, it must be able to replicate within the host cell, so that numerous copies of the recombinant DNA molecule can be produced and passed to the daughter cells. A cloning vehicle also needs to be relatively small, ideally less than 10 kilobases (kb) in size, as large molecules tend to break down during purification, and are also more difficult to manipulate. Two kinds of DNA molecule that satisfy these criteria can be found in bacterial cells: plasmids and bacteriophage chromosomes. Although plasmids are frequently employed as cloning vehicles, two of the most important types of vector in use today are derived from bacteriophages.

2.1 PLASMIDS

2.1.1 Basic features of plasmids

Plasmids are circular molecules of DNA that lead an independent existence in the bacterial cell (Figure 2.1). Plasmids almost always carry one or more genes, and often these genes are responsible for

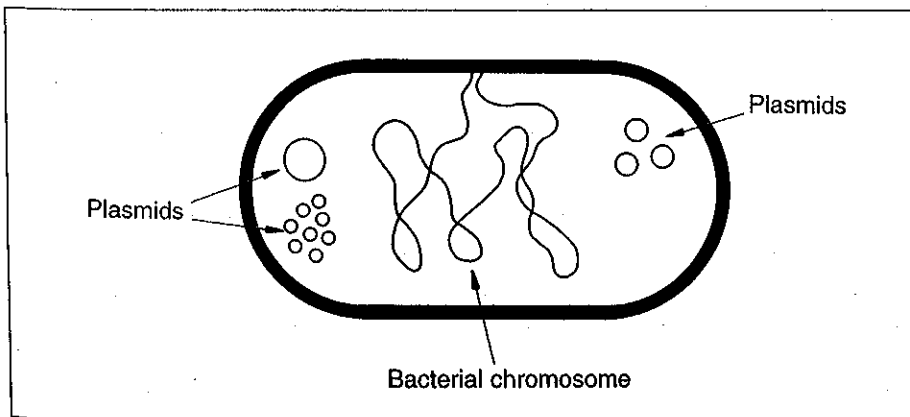
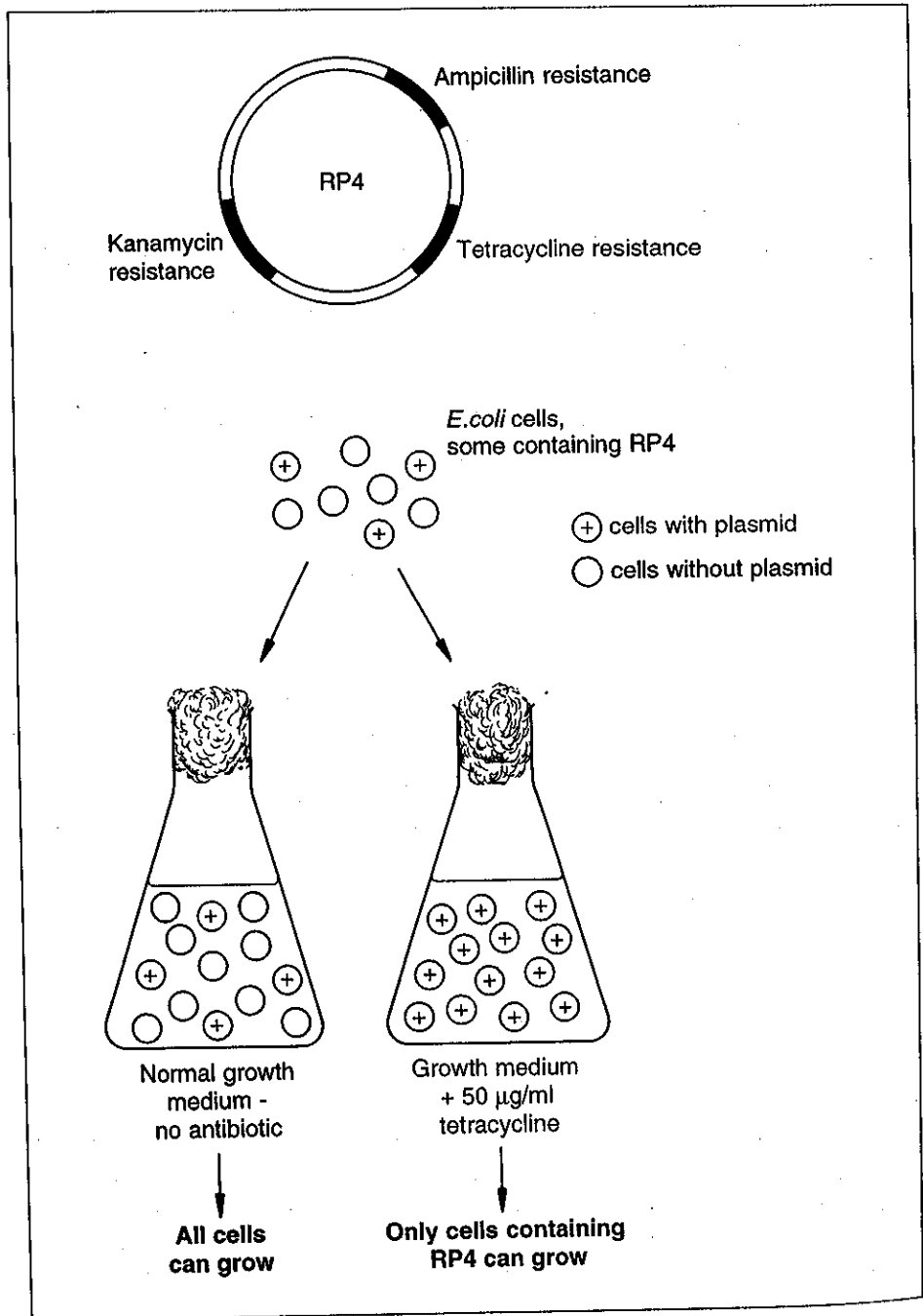


Figure 2.1 Plasmids: independent genetic elements found in bacterial cells.

a useful characteristic displayed by the host bacterium. For example, the ability to survive in normally toxic concentrations of antibiotics such as chloramphenicol or ampicillin is often due to the presence in the bacterium of a plasmid carrying antibiotic-resistance genes. In the laboratory antibiotic resistance is often used as a **selectable marker** to ensure that bacteria in a culture contain a particular plasmid (Figure 2.2).

All plasmids possess at least one DNA sequence that can act as an **origin of replication**, so they are able to multiply within the cell quite independently of the main bacterial chromosome (Figure

Figure 2.2 The use of antibiotic resistance as a selectable marker for a plasmid. RP4 (top) carries genes for resistance to ampicillin, tetracycline and kanamycin. Only those *E. coli* cells that contain RP4 (or a related plasmid) are able to survive and grow in a medium that contains toxic amounts of one or more of these antibiotics.



2.3(a). The smaller plasmids make use of the host cell's own DNA replicative enzymes in order to make copies of themselves, whereas some of the larger ones carry genes that code for special enzymes that are specific for plasmid replication.

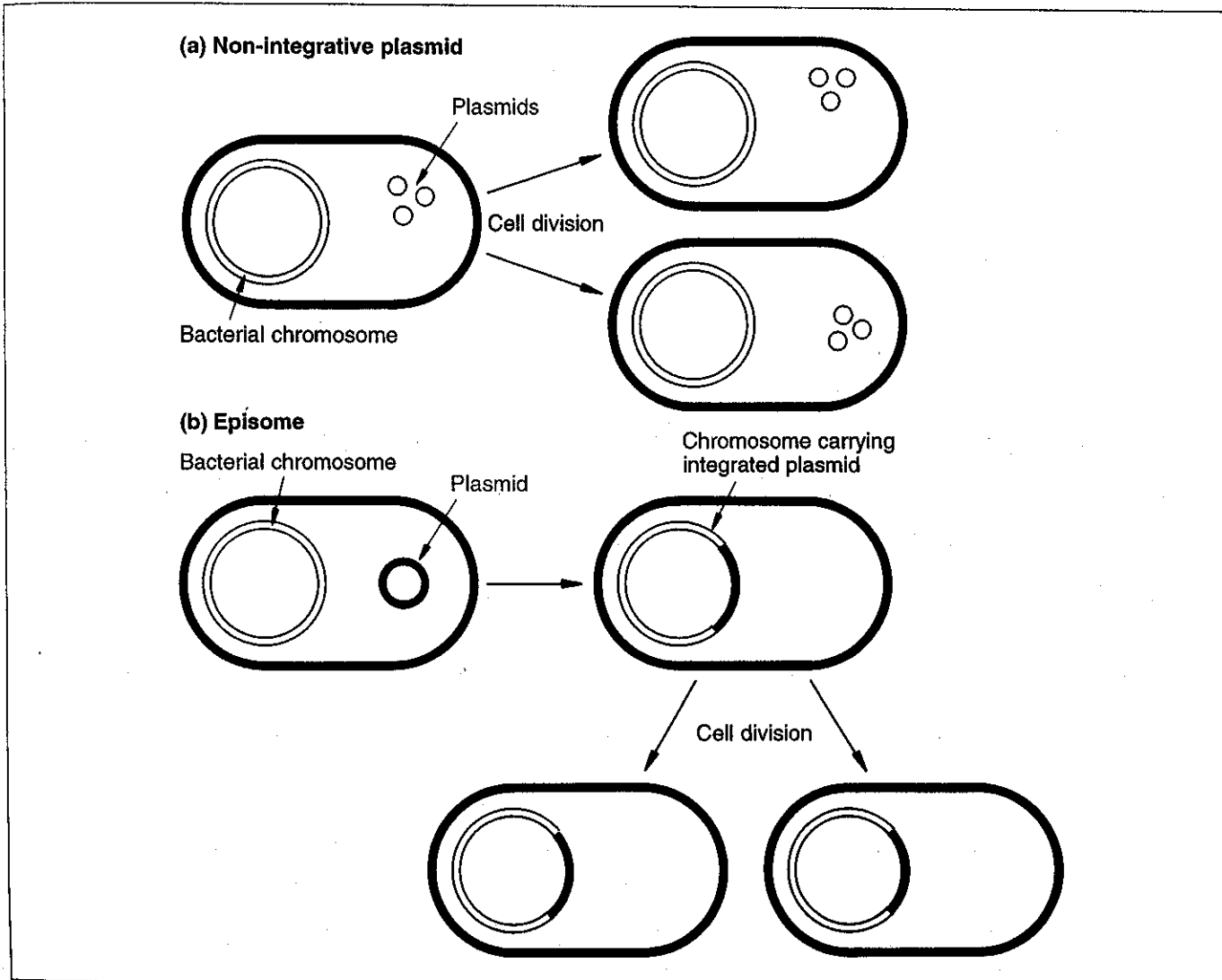


Figure 2.3 Replication strategies for (a) a non-integrative plasmid, and (b) an episome.

A few types of plasmid are also able to replicate by inserting themselves into the bacterial chromosome (Figure 2.3(b)). These integrative plasmids or **episomes** may be stably maintained in this form through numerous cell divisions, but will at some stage exist as independent elements. Integration is also an important feature of some bacteriophage chromosomes and will be described in more detail when these are considered (p. 20).

2.1.2 Size and copy number

These two features of plasmids are particularly important as far as cloning is concerned. We have already mentioned the relevance of

plasmid size and stated that less than 10 kb is desirable for a cloning vehicle. Plasmids range from about 1.0 kb for the smallest to over 250 kb for the largest plasmids (Table 2.1), so only a few will be useful for cloning purposes. However, as described in Chapter 7, larger plasmids may be adapted for cloning under some circumstances.

Table 2.1 Sizes of representative plasmids

Plasmid	Nucleotide length (kb)	Molecular weight (MDa)	Organism
pUC8	2.9	4.8	<i>E. coli</i>
ColE1	6.4	10	<i>E. coli</i>
RP1	9.1	15	Bacteriophages + others
F	9.5	16	<i>E. coli</i>
pOL	117	19	<i>Pseudomonas putida</i>
GT10A15	215	34	<i>Agrobacterium tumefaciens</i>

The **copy number** refers to the number of molecules of an individual plasmid that are normally found in a single bacterial cell. The factors that control copy number are not well understood, but each plasmid has a characteristic value that may be as low as one (especially for the large molecules) or as many as 50 or more. Generally speaking, a useful cloning vehicle needs to be present in the cell in multiple copies so that large quantities of the recombinant DNA molecule can be obtained.

2.1.3 Conjugation and compatibility

Plasmids fall into two groups: conjugative and non-conjugative. Conjugative plasmids are characterized by the ability to promote sexual **conjugation** between bacterial cells (Figure 2.4), a process that can result in a conjugative plasmid spreading from one cell to all the other cells in a bacterial culture. Conjugation and plasmid transfer are controlled by a set of transfer or *tra* genes, which are present on conjugative plasmids but absent from the non-conjugative type. However, a non-conjugative plasmid may, under some circumstances, be cotransferred along with a conjugative plasmid when both are present in the same cell.

Several different kinds of plasmid may be found in a single cell, including more than one different conjugative plasmid at any one time. In fact, cells of *E. coli* have been known to contain up to seven different plasmids at once. To be able to coexist in the same cell, different plasmids must be **compatible**. If two plasmids are incompatible then one or the other will be quite rapidly lost from the cell.

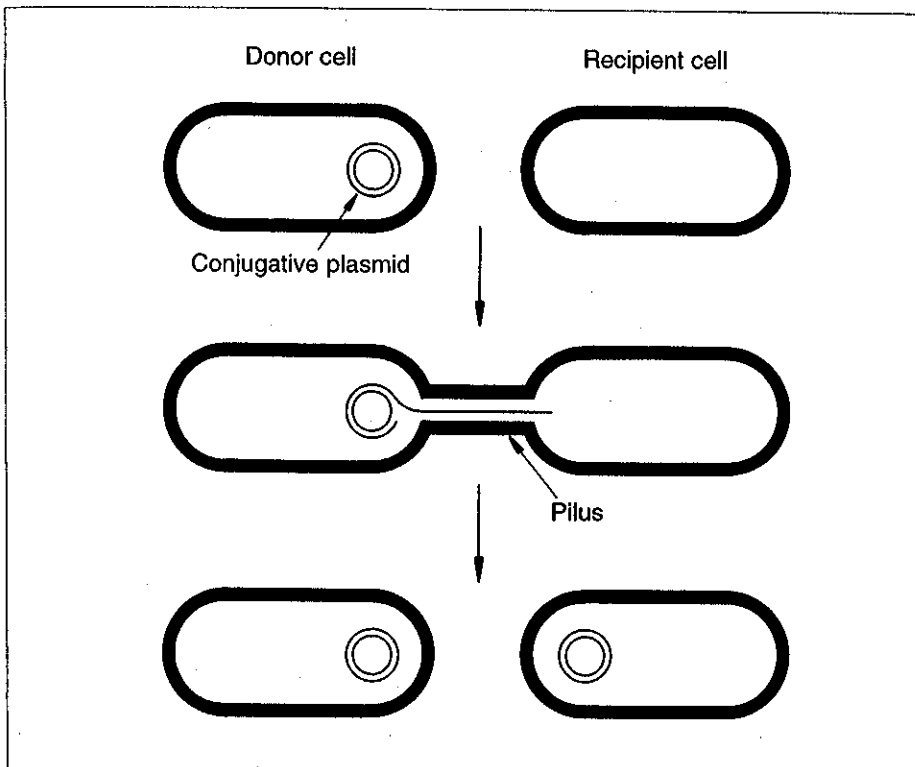


Figure 2.4 Plasmid transfer by conjugation between bacterial cells. The donor and recipient cells attach to each other by a pilus, a hollow appendage present on the surface of the donor cell. A copy of the plasmid is then passed to the recipient cell. Transfer is thought to occur through the pilus, as drawn in the Figure, but this has not been proven and transfer by some other means (e.g. directly across the bacterial cell walls) remains a possibility.

Different types of plasmid can therefore be assigned to different **incompatibility groups** on the basis of whether or not they can coexist, and plasmids from a single incompatibility group are often related to each other in various ways. The basis of incompatibility is not well understood, but events during plasmid replication are thought to underlie the phenomenon.

2.1.4 Plasmid classification

The most useful classification of naturally occurring plasmids is based on the main characteristic coded by the plasmid genes. The five main types of plasmid according to this classification are as follows.

1. **Fertility or 'F' plasmids** carry only *tra* genes and have no characteristic beyond the ability to promote conjugal transfer of plasmids; e.g. F plasmid of *E. coli*.
2. **Resistance or 'R' plasmids** carry genes conferring on the host bacterium resistance to one or more antibacterial agents, such as chloramphenicol, ampicillin and mercury. R plasmids are very important in clinical microbiology as their spread through natural populations can have profound consequences in the treatment of bacterial infections; e.g. RP4, commonly found in *Pseudomonas*, but also occurring in many other bacteria.
3. **Col plasmids** code for colicins – proteins that kill other bacteria; e.g. ColE1 of *E. coli*.

4. **Degradative plasmids** allow the host bacterium to metabolize unusual molecules such as toluene and salicylic acid; e.g. TOL of *Pseudomonas putida*.
5. **Virulence plasmids** confer pathogenicity on the host bacterium; e.g. Ti plasmids of *Agrobacterium tumefaciens*, which induce crown gall disease on dicotyledonous plants.

2.1.5 Plasmids in organisms other than bacteria

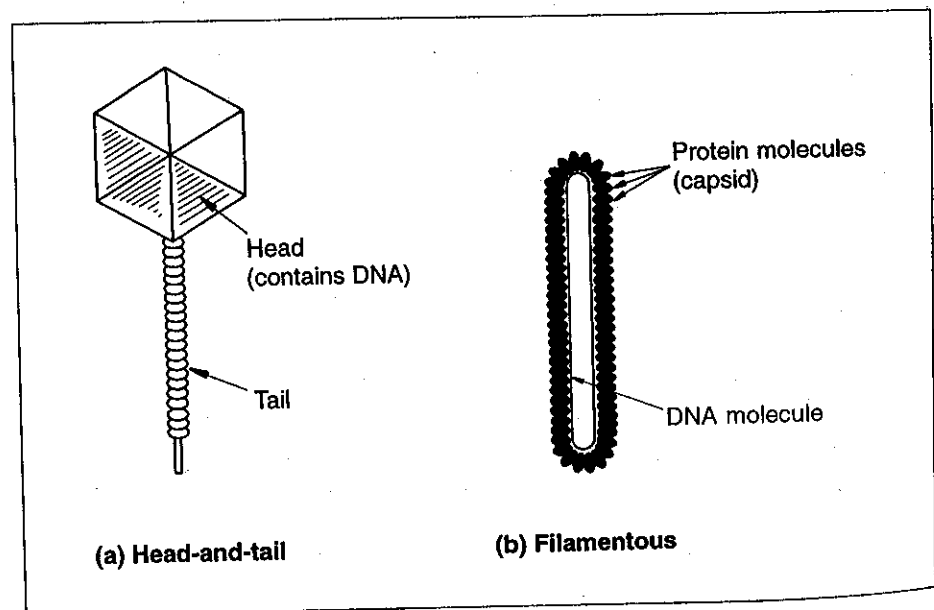
Although plasmids are widespread in bacteria they are by no means so common in other organisms. The best characterized eukaryotic plasmid is the 2 μm circle that occurs in many strains of the yeast *Saccharomyces cerevisiae*. The discovery of the 2 μm plasmid was very fortuitous as it has allowed the construction of vectors for cloning genes with this very important industrial organism as the host (p. 133). However, the search for plasmids in other eukaryotes (e.g. filamentous fungi, plants and animals) has proved disappointing, and it is suspected that many higher organisms simply do not harbour plasmids within their cells.

2.2 BACTERIOPHAGES

2.2.1 Basic features of bacteriophages

Bacteriophages, or phages as they are commonly known, are viruses that specifically infect bacteria. Like all viruses, phages are very simple in structure, consisting merely of a DNA (or occasionally RNA) molecule carrying a number of genes, including several for replication of the phage, surrounded by a protective coat or capsid made up of protein molecules (Figure 2.5).

Figure 2.5 Schematic representations of the two main types of phage structure. (a) Head-and-tail (e.g. λ). (b) Filamentous (e.g. M13).



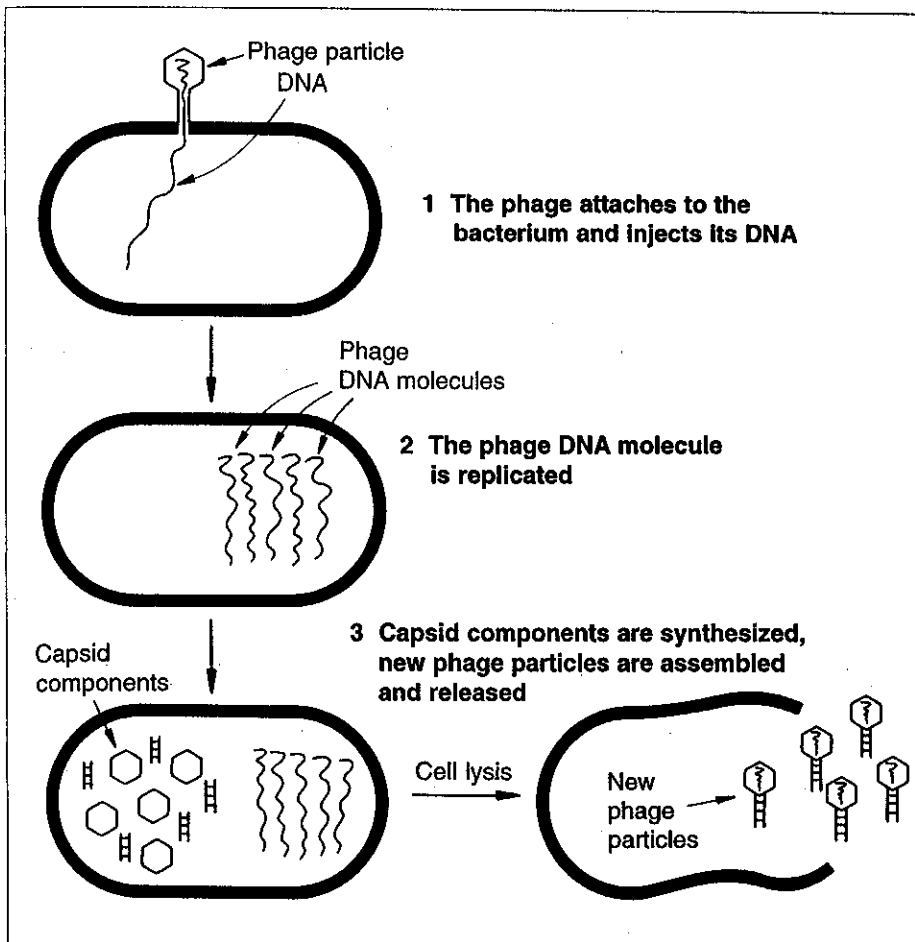


Figure 2.6 The general pattern of infection of a bacterial cell by a bacteriophage.

The general pattern of infection, which is the same for all types of phage, is a three-step process (Figure 2.6).

1. The phage particle attaches to the outside of the bacterium and injects its DNA chromosome into the cell.
2. The phage DNA molecule is replicated, usually by specific phage enzymes coded by genes on the phage chromosome.
3. Other phage genes direct synthesis of the protein components of the capsid, and new phage particles are assembled and released from the bacterium.

With some phage types the entire infection cycle is completed very quickly, possibly in less than 20 minutes. This type of rapid infection is called a **lytic cycle**, as release of the new phage particles is associated with lysis of the bacterial cell. The characteristic feature of a lytic infection cycle is that phage DNA replication is immediately followed by synthesis of capsid proteins, and the phage DNA molecule is never maintained in a stable condition in the host cell.

2.2.2 Lysogenic phages

In contrast to a lytic cycle, **lysogenic** infection is characterized by retention of the phage DNA molecule in the host bacterium, possibly for many thousands of cell divisions. With many lysogenic phages the phage DNA is inserted into the bacterial genome, in a manner similar to episomal insertion (see Figure 2.3(b)). The integrated form of the phage DNA (called the **prophage**) is quiescent, and a bacterium (referred to as a **lysogen**) which carries a prophage is usually physiologically indistinguishable from an uninfected cell. However, the prophage is eventually released from the host genome and the phage reverts to the lytic mode and lyses the cell. The infection cycle of λ , a typical lysogenic phage of this type, is shown in Figure 2.7.

A limited number of lysogenic phages follow a rather different infection cycle. When **M13**, or a related phage, infects *E. coli*, new phage particles are continuously assembled and released from the cell. The M13 DNA is not integrated into the bacterial genome and does not become quiescent. With these phages, cell lysis never occurs, and the infected bacterium can continue to grow and divide, albeit at a slower rate than uninfected cells. Figure 2.8 shows the M13 infection cycle.

Although there are many different varieties of bacteriophage, only λ and M13 have found any real role as cloning vectors. The properties of these two phages will now be considered in more detail.

(a) Gene organization in the λ DNA molecule λ is a typical example of a head-and-tail phage (Figure 2.5(a)). The DNA is contained in the polyhedral head structure and the tail serves to attach the phage to the bacterial surface and to inject the DNA into the cell (Figure 2.7).

The λ DNA molecule is 49 kb in size and has been intensively studied by the techniques of gene mapping and **DNA sequencing**. As a result the positions and identities of most of the genes on the λ DNA molecule are known (Figure 2.9). A feature of the λ genetic map is that genes related in terms of function are clustered together on the genome. For example, all of the genes coding for components of the capsid are grouped together in the left-hand third of the molecule, and genes controlling integration of the prophage into the host genome are clustered in the middle of the molecule. Clustering of related genes is profoundly important for controlling expression of the λ genome, as it allows genes to be switched on and off as a group rather than individually. Clustering is also important in the construction of λ -based cloning vectors (described in Chapter 6).

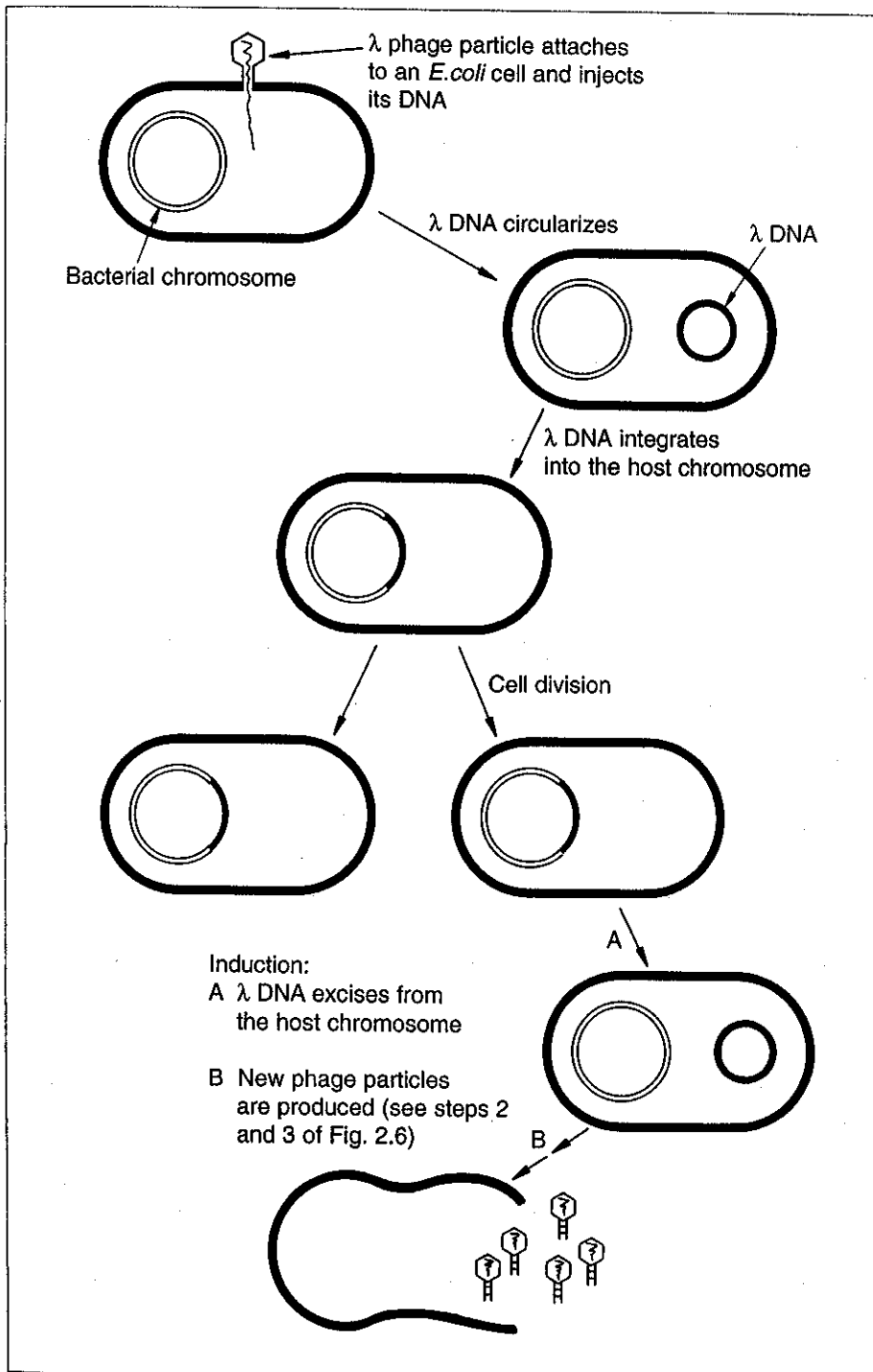
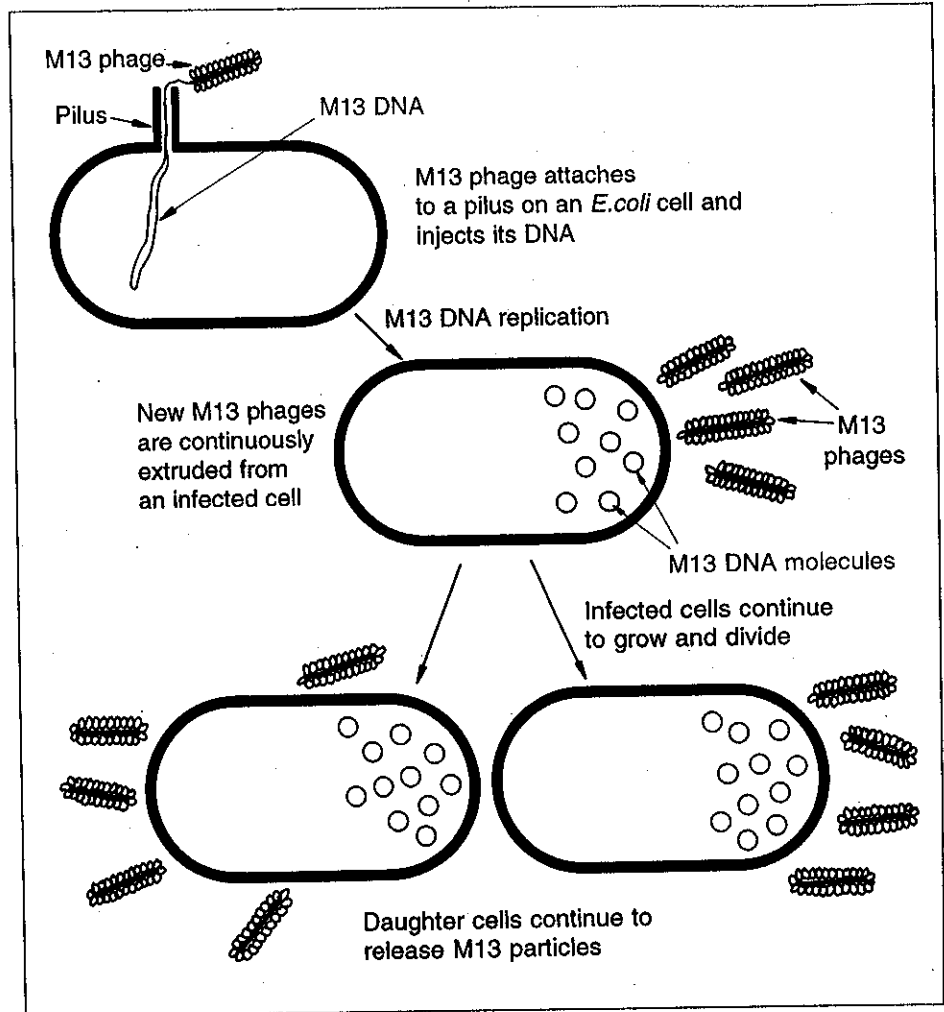


Figure 2.7 The lysogenic infection cycle of bacteriophage λ .

(b) **The linear and circular forms of λ DNA** A second feature of λ that turns out to be of importance in the construction of cloning vectors is the conformation of the DNA molecule. The molecule shown in Figure 2.9 is linear, with two free ends, and represents the DNA present in the phage head structure. This linear molecule consists of two **complementary** strands of DNA, base-paired according to the **Watson-Crick rules** (that is,

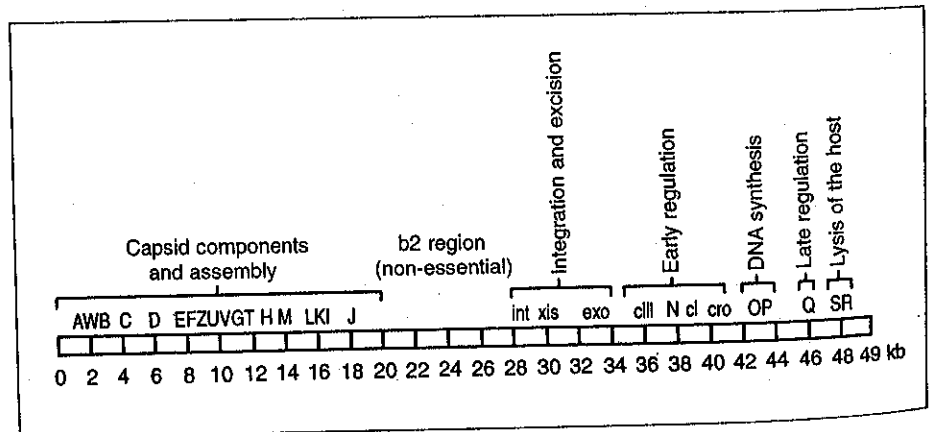
Figure 2.8 The infection cycle of bacteriophage M13.



double-stranded DNA). However, at either end of the molecule is a short 12-nucleotide stretch, in which the DNA is single-stranded (Figure 2.10(a)). The two single strands are complementary, and so can base-pair with one another to form a circular, completely double-stranded molecule (Figure 2.10(b)).

Complementary single strands are often referred to as 'sticky'

Figure 2.9 The λ genetic map, showing the positions of the important genes and the functions of the gene clusters.



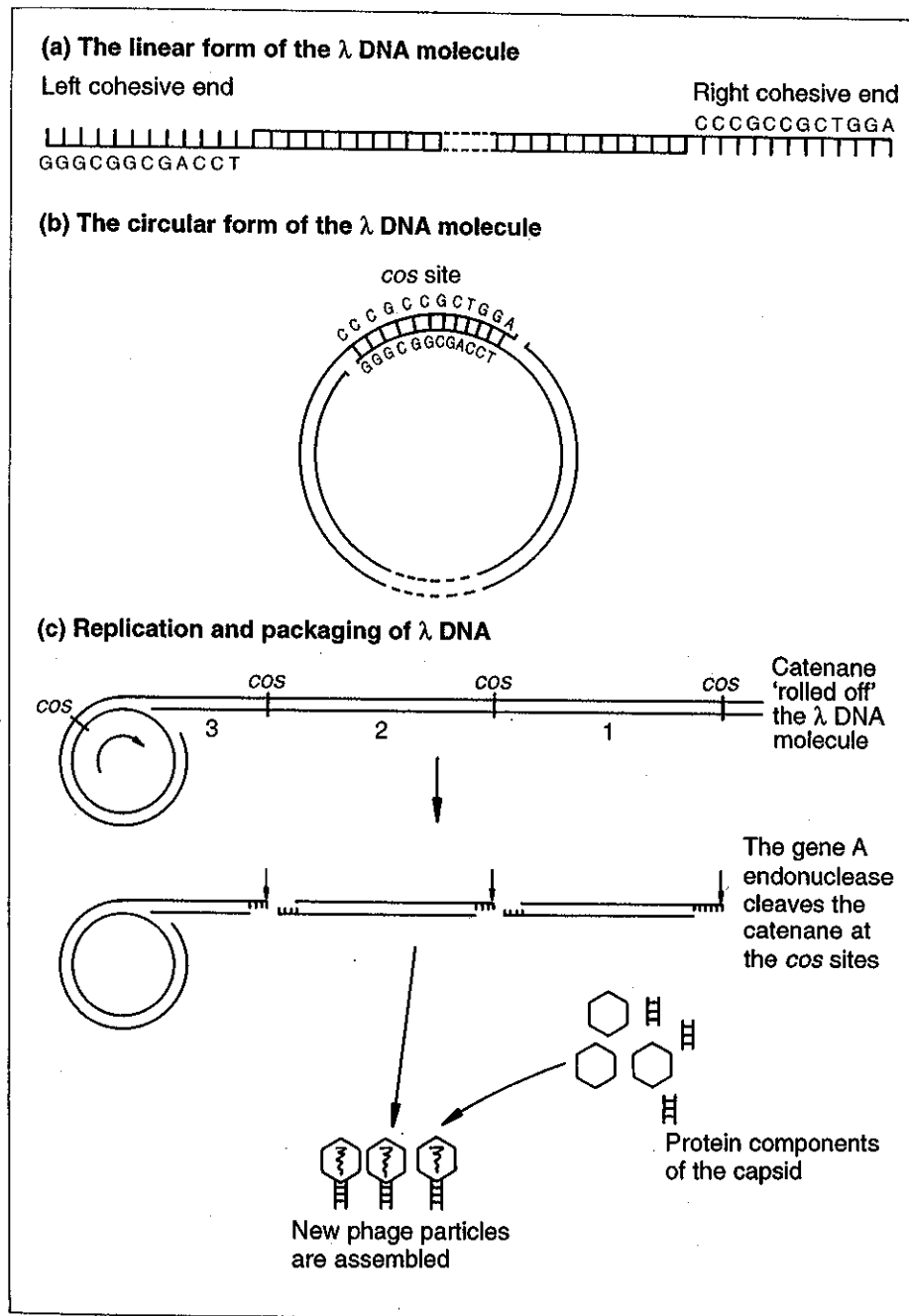


Figure 2.10 The linear and circular forms of λ DNA. (a) The linear form showing the left and right cohesive ends. (b) Base-pairing between the cohesive ends results in the circular form of the molecule. (c) Rolling circle replication produces a catenane of new linear λ DNA molecules, which are individually packaged into phage heads as new λ particles are assembled.

ends or 'cohesive' ends, because base-pairing between them can 'stick' together the two ends of a DNA molecule (or the ends of two different DNA molecules). The λ cohesive ends are called the *cos* sites and they play two distinct roles during the λ infection cycle. First of all, they allow the linear DNA molecule that is injected into the cell to be circularized, which is a necessary prerequisite for insertion into the bacterial genome (Figure 2.7).

The second role of the *cos* sites is rather different, and comes into play after the prophage has excised from the host genome. At this stage a large number of new λ DNA molecules are produced by the

rolling circle mechanism of replication (Figure 2.10(c)), in which a continuous DNA strand is 'rolled off' of the template molecule. The result is a catenane consisting of a series of linear λ genomes joined together at the *cos* sites. The role of the *cos* sites is now to act as recognition sequences for an **endonuclease** which cleaves the catenane at the *cos* sites producing individual λ genomes. This endonuclease (which is the product of gene A on the λ DNA molecule) creates the single-stranded sticky ends, and also acts in conjunction with other proteins to package each λ genome into a phage head structure.

As we shall see in Chapter 6, the cleavage and packaging processes recognize just the *cos* sites and the DNA sequences to either side of them. Changing the structure of the internal regions of the λ genome, for example by inserting new genes, has no effect on these events so long as the overall length of the λ genome is not altered too greatly.

(c) M13 – a filamentous phage M13 is an example of a filamentous phage (Figure 2.5(b)) and is completely different in structure from λ . Furthermore, the M13 DNA molecule is much smaller than the λ genome, being only 6407 nucleotides in length. It is circular, and is unusual in that it consists entirely of single-stranded DNA.

The smaller size of the M13 DNA molecule means that it has room for fewer genes than the λ genome. This is possible because the M13 capsid is constructed from multiple copies of just three proteins (requiring only three genes), whereas synthesis of the λ head-and-tail structure involves over 15 different proteins. In addition, M13 follows a simpler infection cycle than λ and does not need genes for insertion into the host genome.

Injection of an M13 DNA molecule into an *E. coli* cell occurs via the **pilus**, the structure that connects two cells during sexual conjugation (see Figure 2.4). Once inside the cell the single-stranded molecule acts as the template for synthesis of a complementary strand, resulting in normal double-stranded DNA (Figure 2.11(a)). This molecule is not inserted into the bacterial genome, but instead replicates until over 100 copies are present in the cell (Figure 2.11(b)). When the bacterium divides, each daughter receives copies of the phage genome, which continues to replicate, thereby maintaining its overall numbers per cell. As shown in Figure 2.11(c), new phage particles are continuously assembled and released, about 1000 new phages being produced during each generation of an infected cell.

(d) The attraction of M13 as a cloning vehicle Several features of M13 make this phage attractive as the basis for a cloning vehicle. The genome is less than 10 kb in size, well within the range

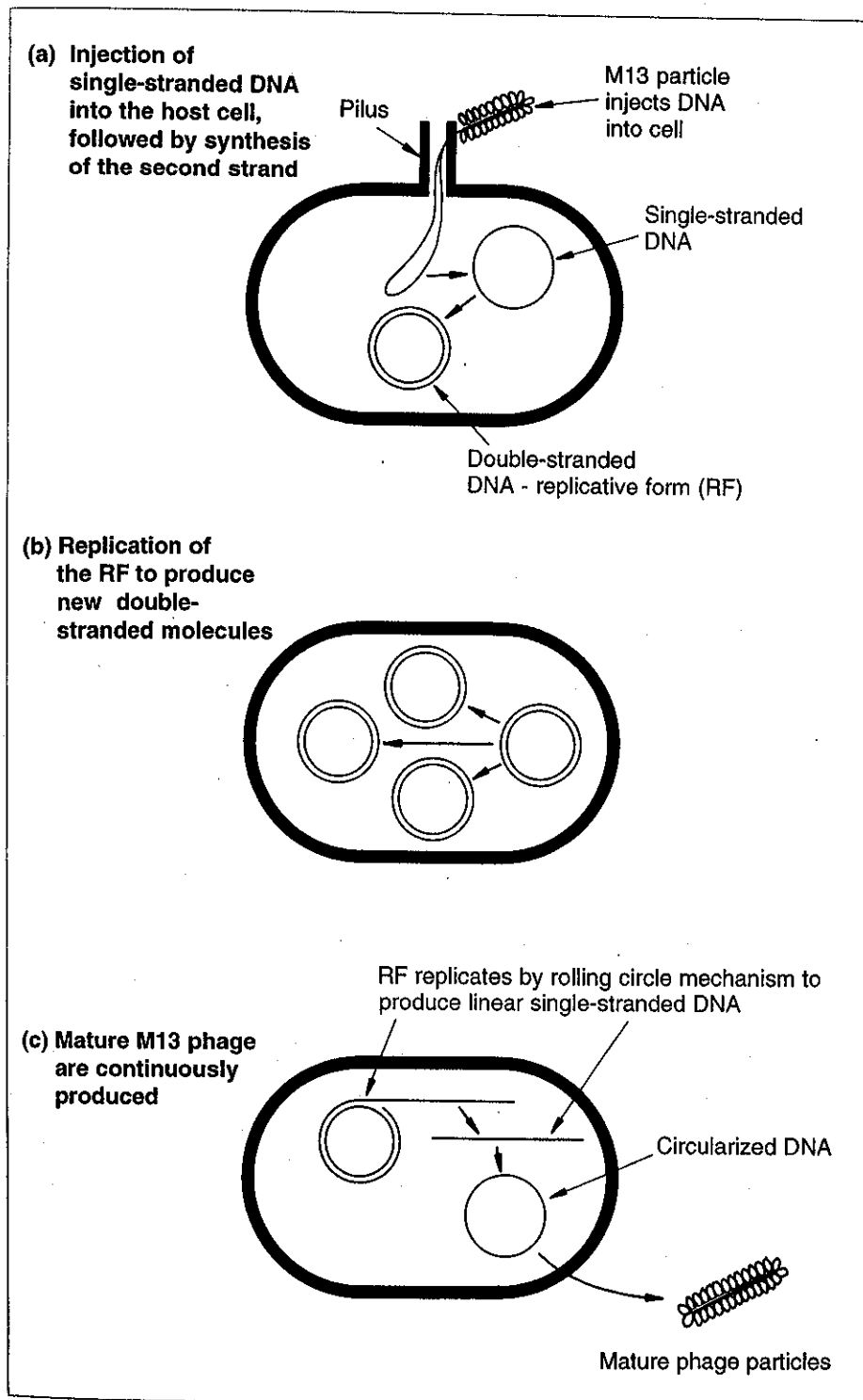


Figure 2.11 The M13 infection cycle showing the different types of DNA replication that occur. (a) After infection the single-stranded M13 DNA molecule is converted into the double-stranded replicative form (RF). (b) The RF replicates to produce multiple copies of itself. (c) Single-stranded molecules are synthesized by rolling circle replication and used in the assembly of new M13 particles.

that we stated was desirable for a potential vector. In addition, the double-stranded **replicative form** of the M13 genome behaves very much like a plasmid and can be treated as such for experimental purposes. It is easily prepared from a culture of infected *E. coli* cells (p. 36) and can be reintroduced by **transfection** (p. 99).

Most importantly, genes cloned with an M13-based vector can

be obtained in the form of single-stranded DNA. Single-stranded versions of cloned genes are useful for several techniques, notably DNA sequencing and *in vitro* mutagenesis (pp. 193 and 223). Using an M13 vector is an easy and reliable way of obtaining single-stranded DNA for this type of work.

2.2.3 Viruses as cloning vehicles for other organisms

Most living organisms are infected by viruses and it is not surprising that great interest has been shown in the possibility that viruses might be used as cloning vehicles for higher organisms. This is especially important when it is remembered that plasmids are not commonly found in organisms other than bacteria and yeast (p. 18).

In fact viruses have considerable potential as cloning vehicles for animal cells. Mammalian viruses such as **simian virus 40 (SV40)** and **adenoviruses**, and the insect **baculoviruses**, are the ones that have received most attention so far, but others are also being studied. These are discussed more fully in Chapter 7.

FURTHER READING

Dale, J. W. (1994) *Molecular Genetics of Bacteria*, 2nd edn, Wiley, Chichester, UK – provides a detailed description of plasmids and bacteriophages.