Gene cloning

The idea that genes reside on chromosomes was proposed by **W. Sutton** in 1903.

Avery, MacLeod, and McCarty in 1944, and of Hershey and Chase in 1952, that anyone believed that **deoxyribonucleic acid (DNA)** is the genetic material.

What exactly is gene cloning? The easiest way to answer this question is to follow through the steps in a gene cloning experiment (Figure)

1. A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a vector, to produce a recombinant DNA molecule.
2. The vector transports the gene into a host cell, which is usually a bacterium, although other types of living cell can be used.
3. Within the host cell the vector multiplies, producing numerous identical copies, not only of itself but also of the gene that it carries.
4. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place.
5. After a large number of cell divisions, a colony, or clone, of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule. The gene carried by the recombinant molecule is now said to be cloned.

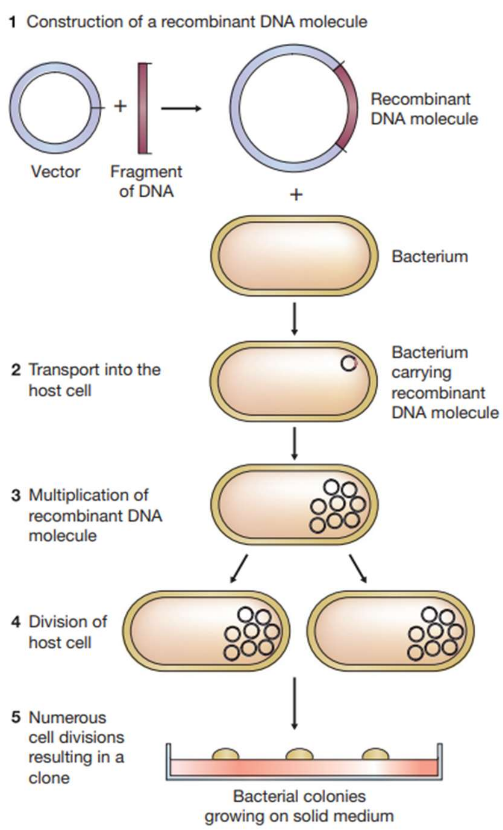

Figure: Cloning allows individual fragments of DNA to be purified.

Vectors for Gene Cloning: Plasmids and Bacteriophages

A DNA molecule needs to display several features to be able to act as a vector for gene cloning. Most importantly, 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 vector also needs to be relatively small, ideally less than 10 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, namely plasmids and bacteriophage chromosomes.

1. **Plasmids**

Plasmids are circular molecules of DNA that lead an independent existence in the bacterial cell (Figure 1.1). Plasmids almost always carry one or more genes, and often these genes are responsible for 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 1.2). Most plasmids possess at least one DNA sequence that can act as an origin of replication, so they are able to multiply within the cell independently of the main bacterial chromosome (Figure 1.3).

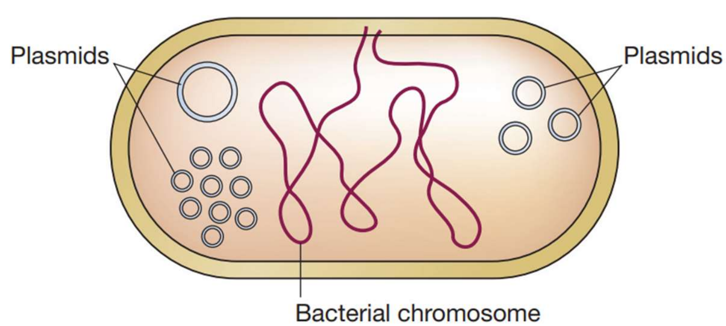

Figure 1.1: Plasmids: independent genetic elements found in bacterial cells.

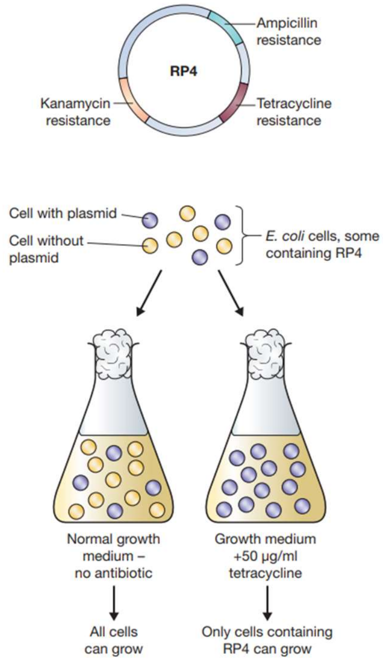

Figure 1.2 The use of antibiotic resistance as a selectable marker for a plasmid. RP4 (top) carries genes for resistance to ampicillin, tetracycline, and kanamycin. Only those E. coli cells that contain RP4 (or a related plasmid) are able to survive and grow in a medium that contains toxic amounts of one or more of these antibiotics.

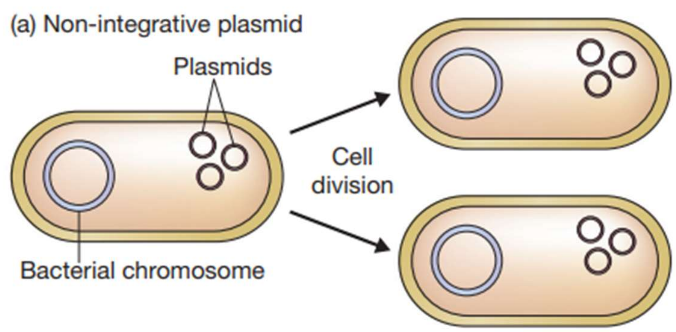

Figure 1.3 Replication strategies for a non-integrative plasmid

Plasmid classification

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

- **Fertility or F plasmids** carry only *tra* genes and have no characteristic beyond the ability to promote conjugal transfer of plasmids. A well-known example is the F plasmid of E. coli.
- **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. An example is RP4, which is commonly found in Pseudomonas, but also occurs in many other bacteria.
- **Col plasmids** code for colicins, proteins that kill other bacteria. An example is ColE1 of E. coli.
- **Degradative plasmids** allow the host bacterium to metabolize unusual molecules such as toluene and salicylic acid, an example being TOL of Pseudomonas putida.
- **Virulence plasmids** confer pathogenicity on the host bacterium; these include the Ti plasmids of Agrobacterium tumefaciens, which induce crown gall disease on dicotyledonous plants.

2. 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 ribonucleic acid (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 1.4).

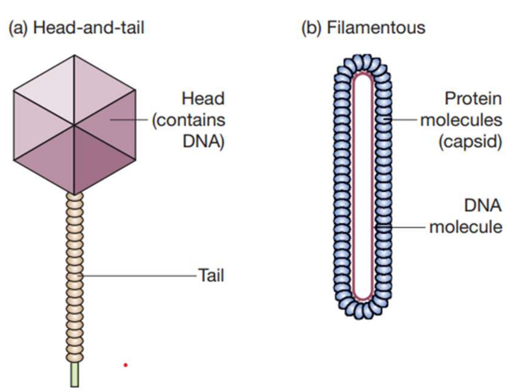

Figure 1.4: The two main types of phage structure. (a) Head-and□tail (e.g., λ). (b) Filamentous (e.g., M

The phage infection cycle

The general pattern of infection, which is the same for all types of phage, is a three-step process (Figure 1.5):

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 in 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.

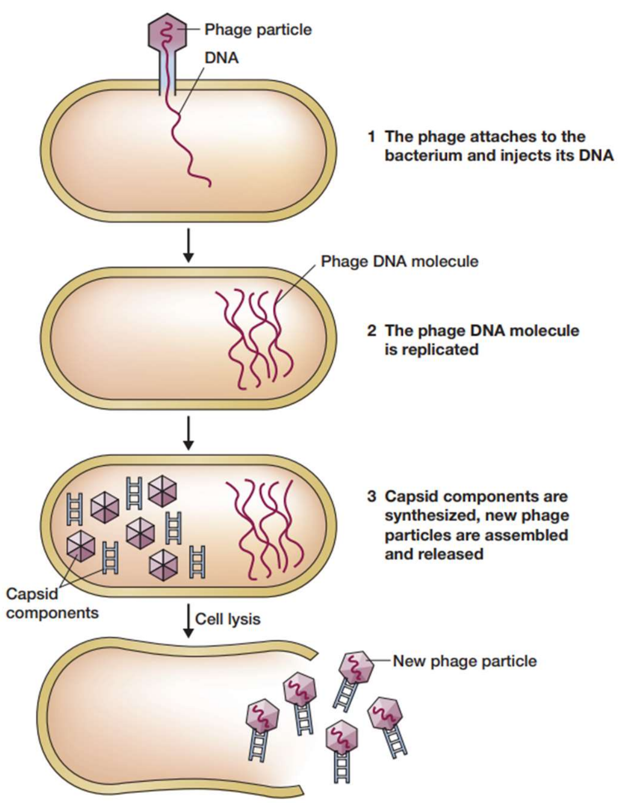

Figure 1.5 The general pattern of infection of a bacterial cell by a bacteriophage

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 (Figure 1.6). The integrated form of the phage DNA (called the prophage) is quiescent, and a bacterium (referred to as a lysogen) that carries a prophage is usually physiologically indistinguishable from an uninfected cell. 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 lambda (λ), a typical lysogenic phage of this type, is shown in Figure 1.7. 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 1.8 shows the M13 infection cycle. Although there are many different varieties of bacteriophage, only λ and M13 have found a major role as cloning vectors.

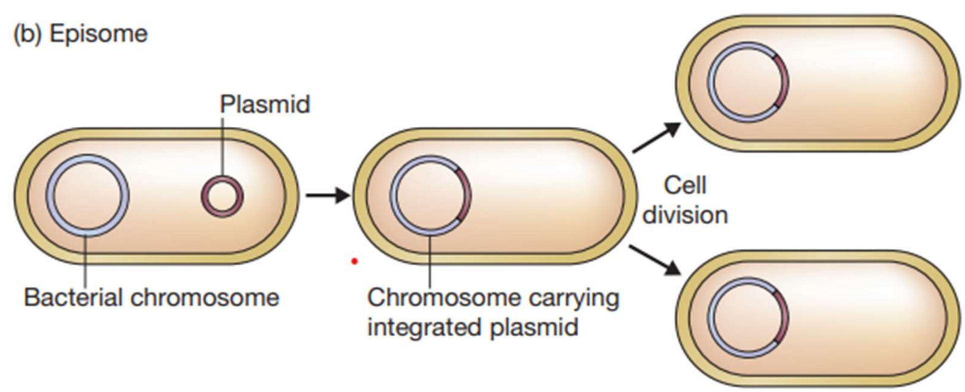

Figure 1.6 Replication strategies for an episome.

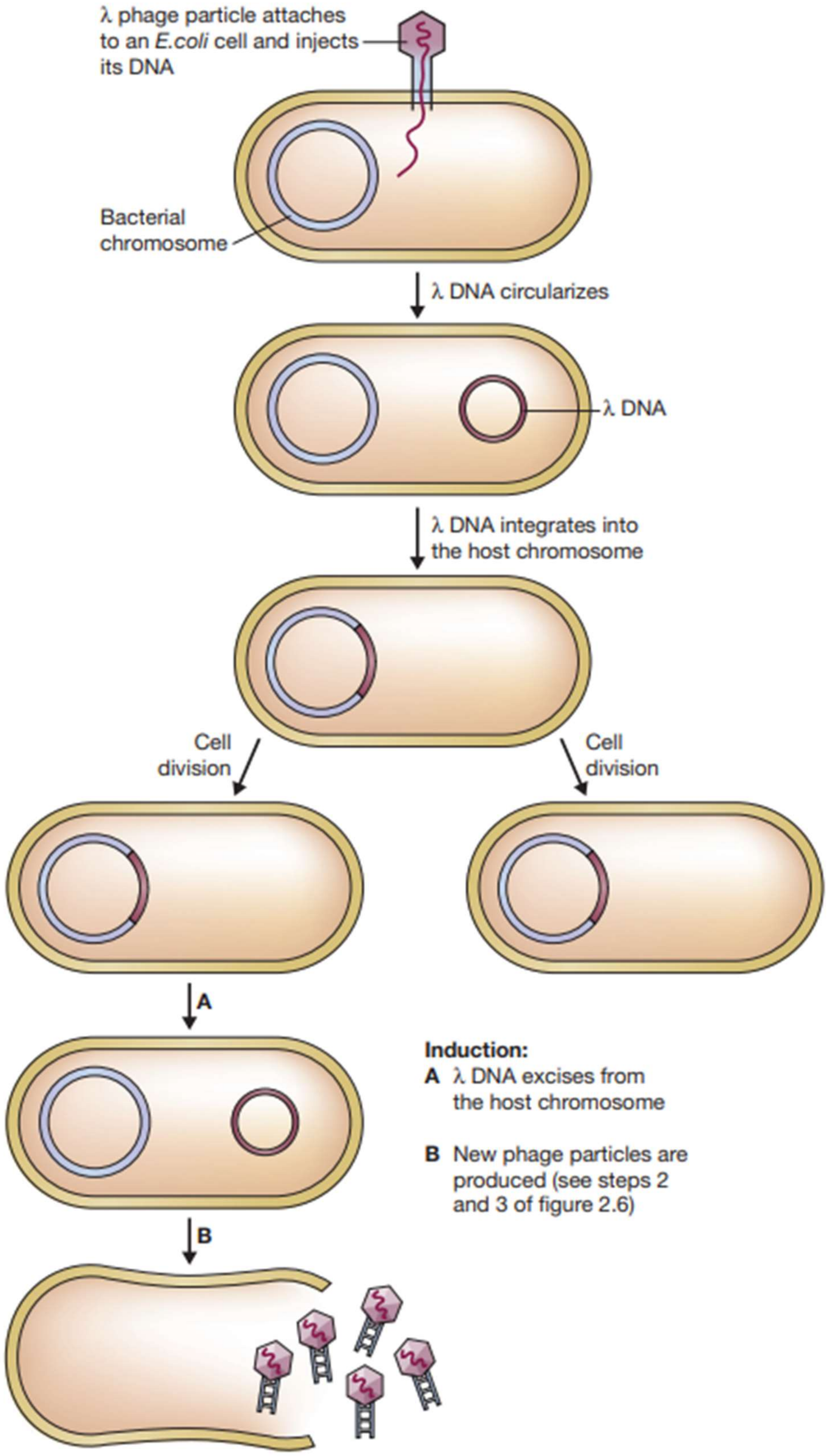

Figure 1.7 The lysogenic infection cycle of bacteriophage λ.

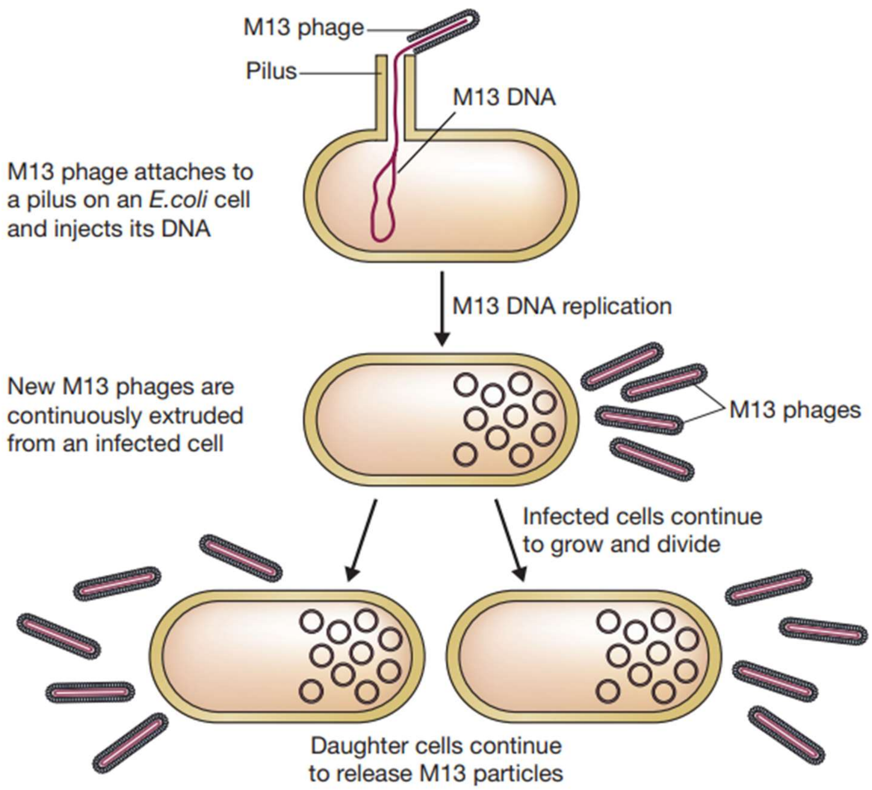

Figure 1.8 The infection cycle of bacteriophage M13.