CHAPTER SIXTEEN

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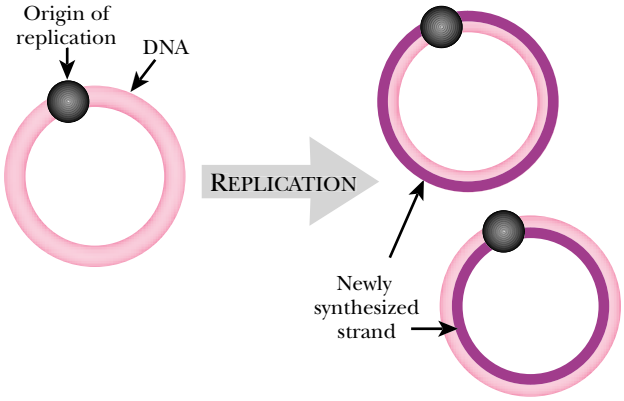


FIGURE 16.01 ***Plasmids are Self-Replicating Molecules of DNA***

Plasmids are most often rings of double-stranded DNA found inside cells but not attached to or associated with the chromosomal DNA. The plasmid carries its own origin of replication, thus it is considered a true replicon.

Plasmids as Replicons

Plasmids are autonomous self-replicating molecules of DNA (or very rarely RNA) (Fig. 16.01). They are not chromosomes, although they do reside inside living cells and carry genetic information. They are not regarded as part of the cell's genome for two reasons. First, a particular plasmid may be found in cells of different species and may move from one host species to another. Second, a plasmid may sometimes be present and sometimes absent from the cells of a particular host species. Thus, although plasmids carry genetic information that may be expressed, they are not a constant part of the cell's genetic make-up nor are they needed for cell growth and division under normal conditions.

Plasmids are "extra" self-replicating molecules of DNA that are found in many cells.

As discussed previously, **replicons** are self-replicating molecules of nucleic acid. Chromosomes, plasmids, virus genomes (both DNA and RNA) and viroids are all replicons. Strictly speaking, a replicon is defined by the possession of its own origin of replication where DNA (or RNA) synthesis is initiated. Thus, a replicon need not carry genes that encode the enzymes needed for its own replication, nor is it necessarily responsible for generating its own nucleotide precursors or energy. This means that plasmids and viruses are replicons, even though they rely on the host cell to provide energy, raw materials and many enzyme activities.

Plasmids may be regarded as living creatures in their own right. Just as worms wriggle through the soil and fish float in the sea, so plasmids proliferate inside their host cells. To a plasmid, the cell is its environment. So, although the plasmid is not alive in the same sense as a cell, neither is it merely part of the cell. In some ways plasmids are like domesticated viruses that have lost the ability to move from cell to cell killing as they go. Plasmids maintain some viral characteristics since the plasmid requires the host cell for replication enzymes, energy, and raw materials. Unlike viruses though, plasmids do not possess protein coats and since they cannot leave the cell they live in, they avoid damaging it. Viruses usually destroy the cell in which they replicate and are then released as virus particles to go in search of fresh victims. Plasmids replicate in step with their host cell (Fig. 16.02). When the cell divides, the plasmid divides and each daughter cell gets a copy of the plasmid.

Plasmids and viruses both rely on the host cell to provide energy and raw materials but plasmids do not damage the host cell.

It is easy to see how a virus that has lost the genes for its protein coat and/or for killing the host cell might evolve into a plasmid. Furthermore, certain genetic elements, such as P1 (see below), can switch between the two lifestyles and may live either as a plasmid or as a virus. It is also possible to imagine how a plasmid might pick up coat protein genes and/or killing functions and deregulate its DNA replication so evolving into a virus. Indeed, many plasmids possess host killing functions that they use to ensure that they are not lost by the host cell (see plasmid addiction, below). So which

plasmid Self-replicating genetic elements that are sometimes found in both prokaryotic and eukaryotic cells. They are not chromosomes nor part of the host cell's permanent genome. Most plasmids are circular molecules of double stranded DNA although rare linear plasmids and RNA plasmids are known

replicon Molecule of DNA or RNA that contains an origin of replication and can self-replicate

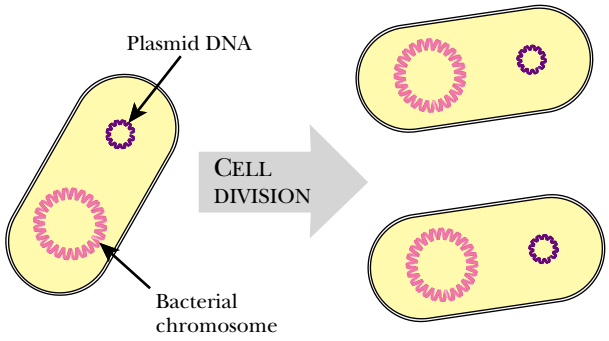


FIGURE 16.02 ***Plasmids Replicate in Step with the Host Cell***

When a bacterial cell is ready to divide, the replication machinery also duplicates the plasmid DNA. The two copies of the chromosome and two copies of the plasmid are then divided equally between the daughter cells. The replication of the plasmid does not harm the cell.

came first, plasmids or viruses? Here we have a true chicken and egg situation. Almost certainly some present day plasmids are derived from viruses and equally certainly some present day viruses are derived from plasmids. However, the ultimate origins of both kinds of element remain obscure.

General Properties of Plasmids

Most plasmids are circular, made of DNA, and much smaller than chromosomes.

Plasmids are usually circular molecules of DNA, although occasionally, plasmids that are linear or made of RNA exist. They may be found as single or multiple copies and may carry from half a dozen to several hundred genes. Plasmids can only multiply inside a host cell. Most plasmids inhabit bacteria, and indeed around 50% of bacteria found in the wild contain one or more plasmids. Plasmids are also found in higher organisms such as yeast and fungi. The 2μ circle of yeast (see below) is a well-known example that has been modified for use as a cloning vector.

Some plasmids are present in one or two copies per cell whereas others occur in multiple copies.

The **copy number** is the number of copies of the plasmid in each bacterial cell. For most plasmids it is one or two copies per chromosome but it may be as many as 50 or more for certain small plasmids such as the ColE plasmids. The number of copies influences the strength of plasmid-borne characteristics, especially antibiotic resistance. The more copies of the plasmid per cell, the more copies there will be of the antibiotic resistance genes, and therefore, higher the resulting level of antibiotic resistance.

The size of plasmids varies enormously. The F-plasmid of *E. coli* is fairly average in this respect and is about 1 percent the size of the *E. coli* chromosome. Most multicopy plasmids are much smaller (ColE plasmids are about 10 percent the size of the F-plasmid). Very large plasmids, up to 10 percent of the size of a chromosome, are sometimes found but they are difficult to work with and few have been properly characterized.

Plasmids carry genes for managing their own life cycles and some plasmids carry genes that affect the properties of the host cell. These properties vary greatly from plasmid to plasmid, the best known being resistance to various antibiotics. **Cryptic plasmids** are those that confer no identifiable phenotype on the host cell. Cryptic plasmids presumably carry genes whose characteristics are still unknown. A wide variety of plasmids, modified for different purposes, are used in molecular biology research and are often used to carry genes during genetic engineering.

The host range of plasmids varies widely. Some plasmids are restricted to a few closely related bacteria; for example, the F-plasmid only inhabits *E. coli* and related enteric bacteria like *Shigella* and *Salmonella*. Others have a wide host range; for example, plasmids of the P-family can live in hundreds of different species of bacteria. Although "P" is now usually regarded as standing for "promiscuous", due to their unusually wide host range, these plasmids were originally named after *Pseudomonas*, the bacterium in which they were discovered. They are often responsible for resistance to multiple antibiotics, including penicillins.

copy number The number of copies of a plasmid found within a single host cell
cryptic plasmid A plasmid that confers no identified characteristics or phenotypic properties

Plasmid or Chromosome?

When the genome of the Gram negative bacterium *Vibrio cholerae,* the causative agent of cholera, was sequenced it was found to consist of two circular chromosomes of 2,961,146 and 1,072,314 base pairs. Together this totals approximately 4 million base pairs and encodes about 3,900 proteins—about the same amount of genetic information as *E. coli*. Many genes that appear to have origins outside the enteric bacteria, as deduced from their different base composition, were found on the small chromosome. Many of these lack homology to characterized genes and are of unknown function. The small chromosome also carries an integron gene capture system (see Ch. 15) and host 'addiction' genes that are typically found on plasmids (see below). It seems likely that the "small chromosome" originated as a plasmid that has grown to its present size by accumulating genes from assorted external sources. The large chromosome carries almost all of the genes needed for vital cell functions such as protein, RNA and DNA synthesis as well as genes for pathogenicity.

Some plasmids can transfer themselves between bacterial cells and a few can also transfer chromosomal genes.

Certain plasmids can move themselves from one bacterial cell to another, a property known as **transferability**. Many medium sized plasmids, such as the F-type and P-type plasmids, can do this and are referred to as Tra^+ (transfer positive). Since plasmid transfer requires over 30 genes, only medium or large plasmids possess this ability. Very small plasmids, such as the ColE plasmids, simply do not have enough DNA to accommodate the genes needed. Nonetheless, many small plasmids, including the ColE plasmids, can be **mobilized** by self-transferable plasmids, i.e. they are Mob^+ (mobilization positive). However, not all transfer-negative plasmids can be mobilized. Some transferable plasmids (e.g. the F-plasmid) can also mobilize chromosomal genes. It was this observation that allowed the original development of bacterial genetics using *E. coli*. The mechanism of plasmid transfer and the conditions necessary for transfer of chromosomal genes are therefore discussed in Chapter 18, Bacterial Genetics.

Plasmid Families and Incompatibility

Plasmids are classified into families whose members share very similar replication genes.

Two different plasmids that belong to the same family cannot coexist in the same cell. This is known as **incompatibility**. Plasmids were originally classified by incompatibility and so plasmid families are often known as incompatibility groups and are designated by letters of the alphabet (F, P, I, X, etc.). Plasmids of the same incompatibility group have very similar DNA sequences in their replication genes, although the other genes they carry may be very different. It is quite possible to have two or more plasmids in the same cell as long as they belong to different families. So a P-type plasmid will happily share the same cell with a plasmid of the F-family (Fig. 16.03).

Occasional Plasmids are Linear or Made of RNA

Although most plasmids are circular molecules of DNA there are occasional exceptions. Linear plasmids of double-stranded DNA have been found in a variety of bacteria and in fungi and higher plants. The best-characterized linear plasmids are found in those few bacteria such as *Borrelia* and *Streptomyces* that also contain linear chromosomes (see Ch. 5). Linear DNA replicons in bacteria are not protected by

incompatibility The inability of two plasmids of the same family to co-exist in the same host cell
mobilizability Ability of a non-transferable plasmid to be moved from one host cell to another by a transferable plasmid
transferability Ability of a plasmid to move itself from one host cell to another

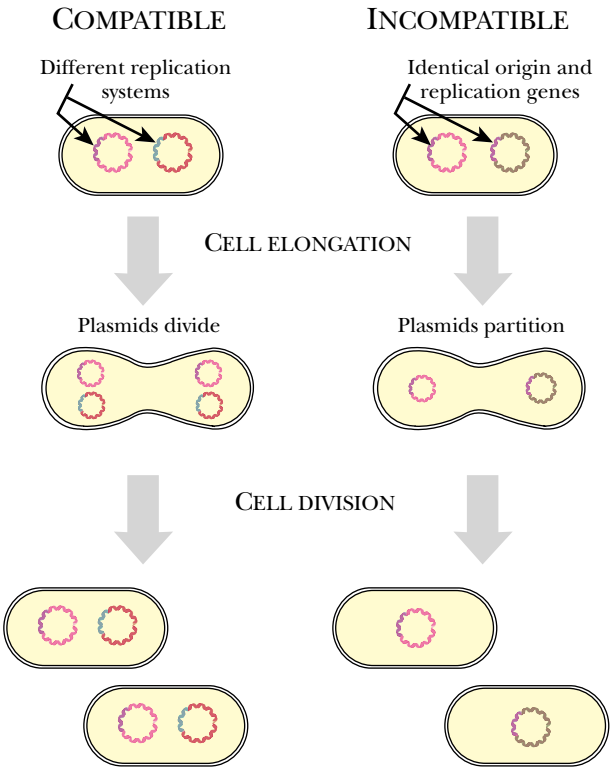


FIGURE 16.03 ***Plasmid Incompatibility***

Plasmids with different origins of replication and different replication genes are able to inhabit the same bacterial cell and are considered compatible (left). During cell division, both types of plasmid replicate; therefore, each daughter cell will inherit both plasmids, just like the mother cell. On the other hand, if two plasmids have identical origins and replication genes they are incompatible will not be replicated during cell division (right). Instead, the two plasmids are partitioned into different daughter cells. [Bacterial cells also contain circular chromosomes that divide in synchrony with cell division; however these have been omitted from this figure.]

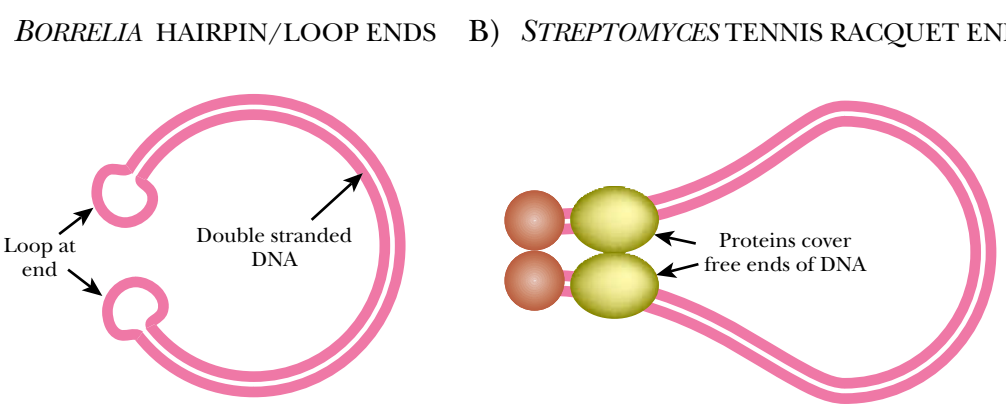


FIGURE 16.04 ***End Structures of Linear Plasmids***

A) Linear plasmids of *Borrelia* form hairpin loops at the ends. B) Linear plasmids of *Streptomyces* are coated with proteins that protect the DNA ends. If linear plasmids had exposed double-stranded ends, this might trigger recombination, repair, or degradation (Ch. 14).

Linear plasmids have special structures to protect the ends of the DNA.

telomeres like the linear chromosomes of eukaryotes. Instead a variety of individual adaptations protect the ends from endonucleases.

In *Borrelia* there are not actually any free DNA ends. Instead hairpin sequences of single-stranded DNA form loops at the ends of both linear plasmids and chromosomes (Fig. 16.04A). Some animal viruses, such as the iridovirus that causes African swine fever, have similar structures. Different species of *Borrelia* cause Lyme's disease and relapsing fever. Their linear plasmids appear to encode both hemolysins that damage blood cells and surface proteins that protect the bacteria from the host immune system. Thus, as is true of many other infectious bacteria, the virulence factors of *Borrelia* are also largely plasmid borne.

The linear plasmids of *Streptomyces* are indeed genuine linear DNA molecules with free ends. They have inverted repeats at the ends of the DNA that are held

together by proteins. In addition, special protective proteins are covalently attached to the 5′-ends of the DNA. The net result is a tennis racket structure (Fig. 16.04B). The DNA of adenovirus, most linear eukaryotic plasmids and some bacterial viruses show similar structures.

Linear plasmids are also found among eukaryotes. The fungus *Flammulina velutipes,* commonly known as the enoki mushroom, has two very small linear plasmids within its mitochondria. The dairy yeast, *Kluyveromyces lactis,* has a linear plasmid that normally replicates in the cytoplasm. However, on occasion the plasmid relocates to the nucleus where it replicates as a circle. Circularization is due to site specific recombination involving the inverted repeats at the ends of the linear form of the plasmid. The physiological role of these plasmids is obscure.

RNA plasmids are rare and most are poorly characterized. Examples are known from plants, fungi and even animals. Some strains of the yeast, *Saccharomyces cerevisiae*, contain linear RNA plasmids. Similar RNA plasmids are found in the mitochondria of some varieties of maize plants. RNA plasmids are found as both single-stranded and double-stranded forms and replicate in a manner similar to certain RNA viruses. The RNA plasmid encodes RNA-dependent RNA polymerase that directs its own synthesis. Unlike RNA viruses, RNA plasmids do not contain genes for coat proteins. Sequence comparisons suggest that these RNA plasmids may have evolved from RNA viruses that have taken up permanent residence after losing the ability to move from cell to cell as virus particles.

Plasmid DNA Replicates by Two Alternative Methods

Most plasmids undergo bidirectional replication like bacterial chromosomes.

Typical plasmids made of circular dsDNA use two alternative mechanisms for replicating their DNA. Most plasmids replicate like miniature bacterial chromosomes (see Ch. 5 for details of chromosome replication). They have an origin of replication where the DNA opens and replication begins. Then two replication forks move around the circular plasmid DNA in opposite directions until they meet (Fig. 16.05). A few very tiny plasmids have only one replication fork that moves around the circle until it gets back to the origin.

Some plasmids and many viruses use the rolling circle mechanism for replication.

The other replication mechanism is **rolling circle replication**, which is used by some plasmids and quite a few viruses. At the origin of replication, one strand of the double stranded DNA molecule is nicked (Fig. 16.06). The other, still circular, strand starts to roll away from the broken strand. This results in two single stranded regions of DNA, one belonging to the broken strand and one that is part of the circular strand. DNA is now synthesized starting at the end of the broken strand, which is therefore elongated (Fig. 16.06). The circular strand is used as a template and the gap left where the two original strands rolled apart is filled in. This process of rolling and filling in continues. Eventually the original broken strand is completely unrolled and the circular strand is fully paired with a newly made strand of DNA. We now have a single strand of DNA, equal in length to the original DNA circle, dangling loose.

What happens next varies, depending on the circumstances. For simple plasmid replication, the unrolled old strand is used as a template to make a complementary strand. This double-stranded region is cut free and circularized to give a second copy of the plasmid.

Transferable plasmids use the rolling circle mechanism during transfer but bidirectional replication when dividing in step with the host cell.

Some plasmids, such as the F-plasmid of *E. coli*, can transfer themselves from one bacterium to another. Such plasmids have two separate origins of replication. They divide by bi-directional replication (also known as vegetative replication) when their host cell divides, but use the rolling circle mechanism when they move from one cell to another during conjugation (see Ch. 18). Bi-directional replication starts at *oriV*, the origin of vegetative replication, which is at a different site on the plasmid from *oriT*,

rolling circle replication Mechanism of replicating double stranded circular DNA that starts by nicking and unrolling one strand and using the other, still circular, strand as a template for DNA synthesis. Used by some plasmids and viruses

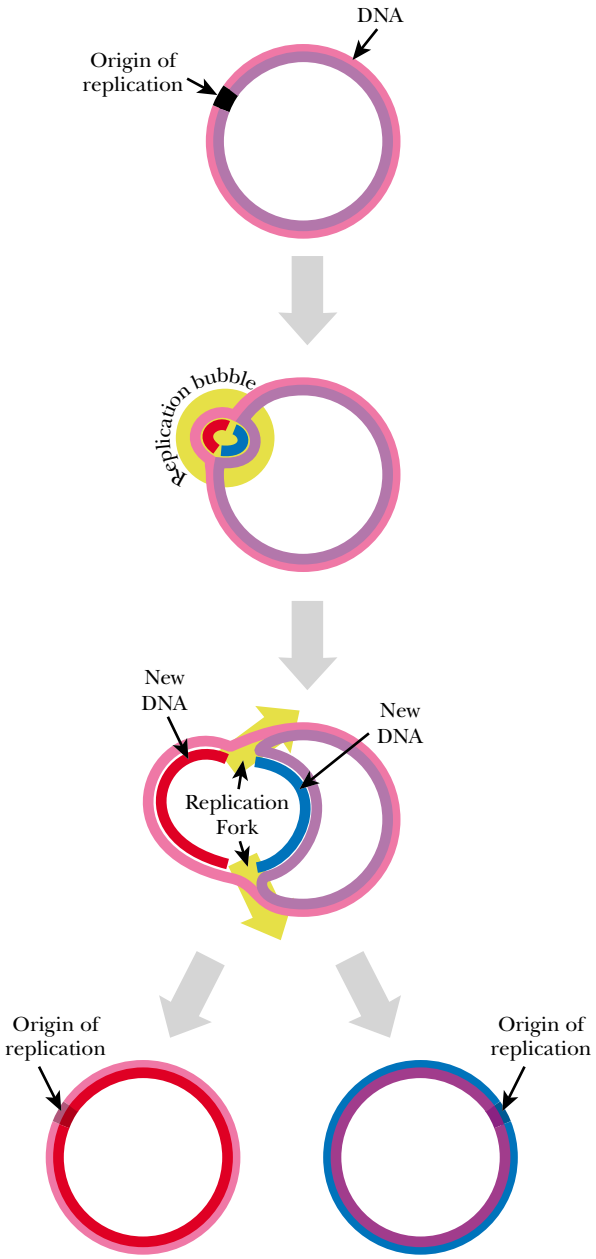


FIGURE 16.05 ***Bi-Directional Plasmid Replication***

For some circular plasmids, replication enzymes recognize the origin of replication, unwind the DNA, and start synthesis of two new strands of DNA, one in each direction. The net result is a replication bubble. As the new strands are synthesized, two distinct replication forks keep moving around the circle until they meet on the opposite side. When both DNA circles are complete, two distinct plasmids are produced.

the origin used during transfer. All plasmids must have a vegetative origin since they must all divide to survive. But only those plasmids that can transfer themselves have a special transfer origin.

The relationship between certain plasmids and viruses is illustrated by their DNA replication mechanisms. Rolling circle replication is not only used by transferable plasmids but also by many viruses (Fig. 16.07). Some manufacture many double stranded molecules of virus DNA. These viruses use the dangling strand as a template to synthesize a new strand of DNA. They just keep rolling and synthesizing and end up with a long linear double stranded DNA many times the length of the original DNA circle. This is chopped into unit genome lengths and packaged into virus particles. (Some of these viruses convert the DNA into circles before packaging, whereas others package linear DNA and only circularize their DNA after infecting a new cell when it is time to replicate again.)

Other viruses contain single stranded DNA. These viruses leave the dangling strand unpaired. They continue rolling and end up with a long linear single-stranded

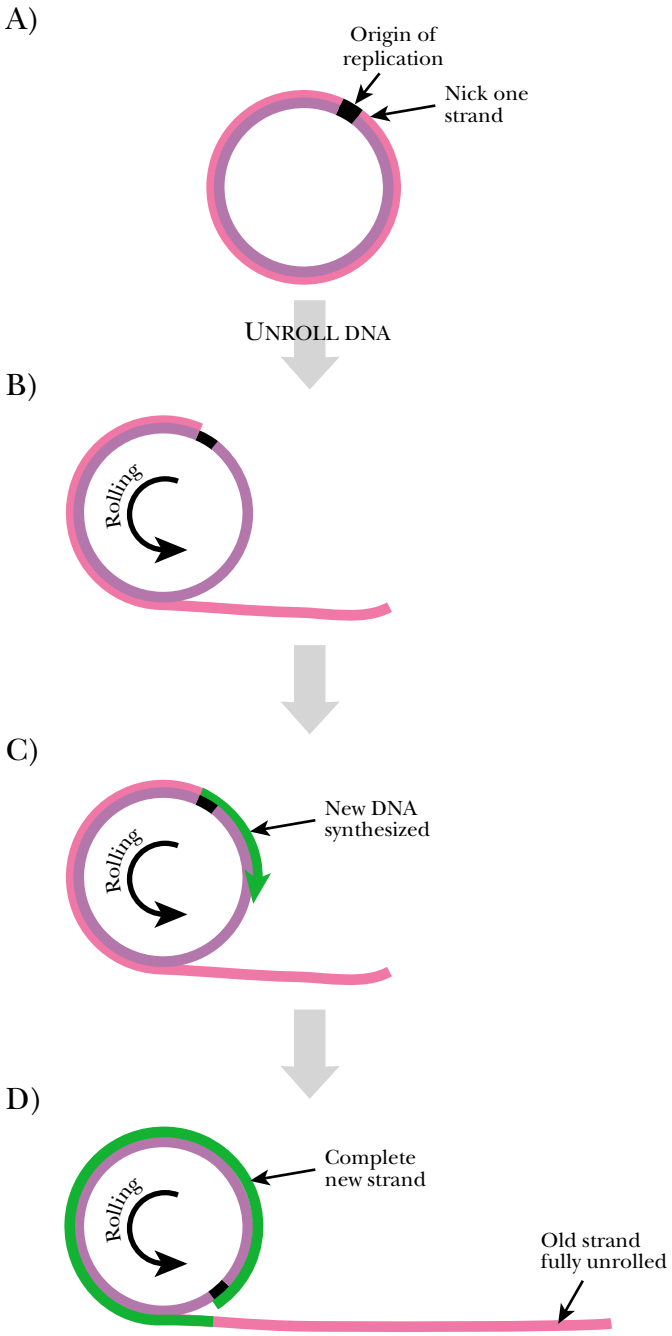


FIGURE 16.06 ***Rolling Circle Replication***

During rolling circle replication, one strand of the plasmid DNA is nicked, and the broken strand (pink) separates from the circular strand (purple). The gap left by the separation is filled in with new DNA starting at the origin of replication (green strand). The newly synthesized DNA keeps displacing the linear strand until the circular strand is completely replicated. The linear single-stranded piece is fully "unrolled" in the process.

DNA (Fig. 16.07B). This is cut into unit genome lengths and packaged as before. When these viruses infect a new cell, they synthesize the opposite strand, so converting their single strand to a double stranded DNA molecule.

Control of Copy Number by Antisense RNA

Antisense RNA is involved in regulating the copy number of many plasmids.

Both single-copy and multicopy plasmids regulate their copy number carefully. However, the regulatory mechanisms differ significantly for the two groups. High copy number plasmids limit the initiation of plasmid replication once the number of plasmids in the cell reaches a certain level. These plasmids are sometimes said to have "relaxed" copy number control. In contrast, single copy plasmids have "stringent" copy number control as their division is more tightly regulated and they replicate only once

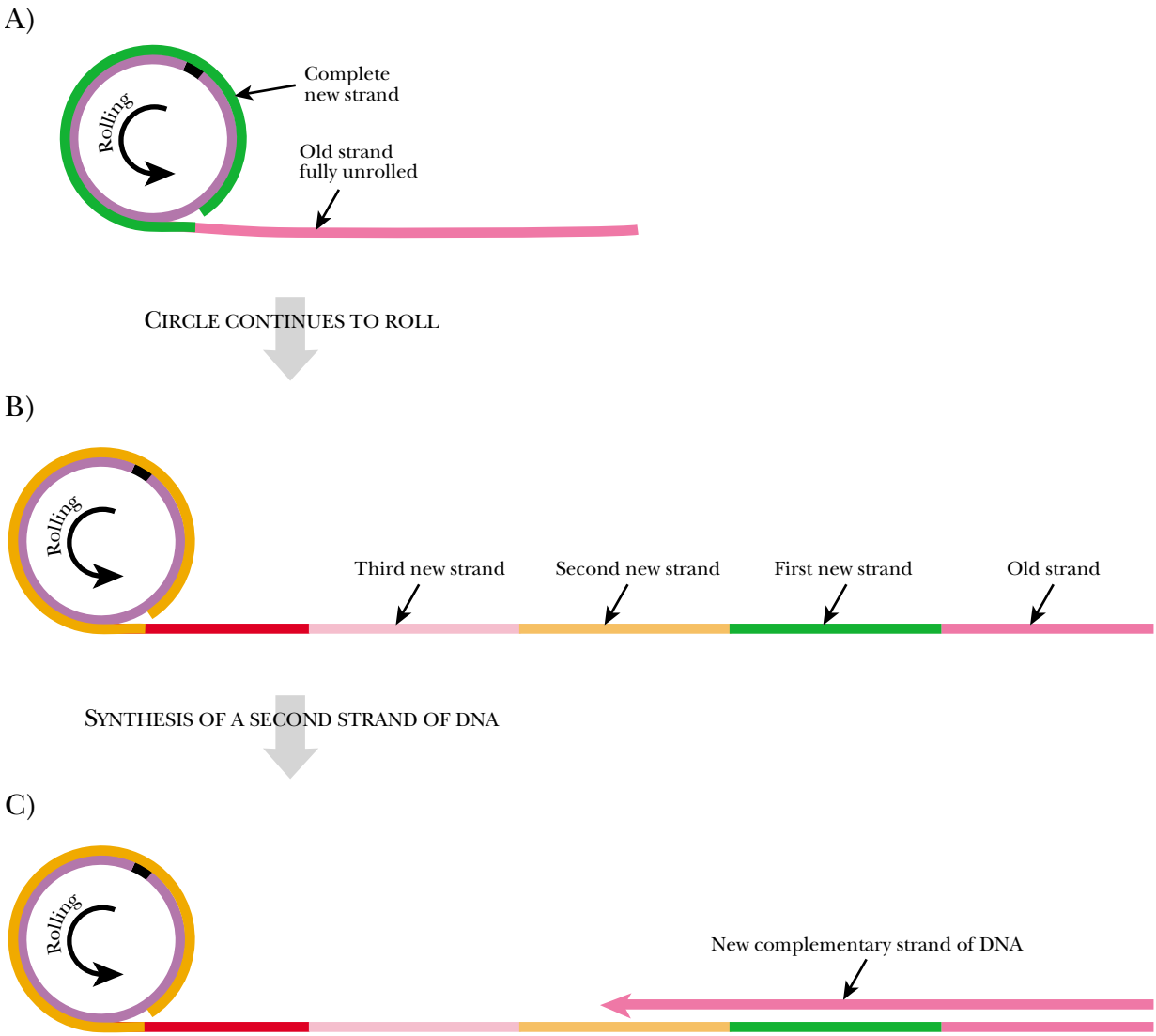


FIGURE 16.07 ***Viruses may Use Rolling Circle Replication***

Rolling circle replication occurs as described in the previous figure (A), but the replication continues around the circular DNA (purple) for many rounds (B). In some viruses, the long single-stranded piece of DNA is cut and packaged into virus particles as single-stranded DNA. In other viruses, a complementary strand is synthesized, giving double-stranded DNA (C). The double-stranded segments are then cut and packaged as single genome units.

during the cell cycle. The regulation of replication is much better understood for multicopy plasmids than for single-copy plasmids.

The most interesting aspect of copy number regulation is the involvement of **antisense RNA** to control the initiation of plasmid replication. The details are best investigated for the multicopy plasmid ColE1, but the principle of using antisense RNA applies to single-copy plasmids also.

Initiation of ColE1 replication starts with the transcription of an RNA molecule of 555 bases that can act as a primer for DNA synthesis. This pre-primer RNA (sometimes called RNAII) is cleaved by **ribonuclease H** to generate a primer with a free 3′-OH group, which can be used by DNA polymerase I (Fig. 16.08).

antisense RNA An RNA molecule that is complementary to messenger RNA or another functional RNA molecule
ribonuclease H A ribonuclease of bacterial cells that is specific for RNA-DNA hybrids

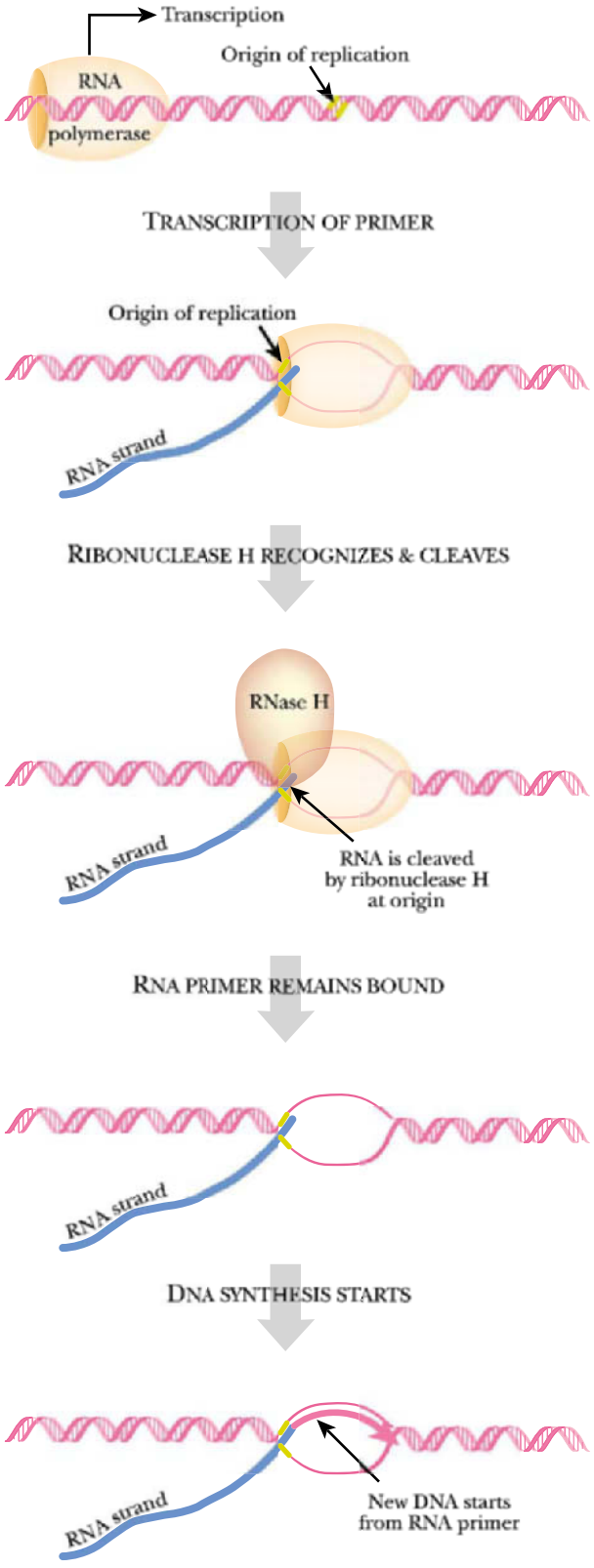


FIGURE 16.08 ***Priming of ColE1 Plasmid Replication***

RNA polymerase synthesizes a strand of RNA (RNAII) near the origin of replication. RNAII (blue strand) is recognized and cleaved by ribonuclease H. The free 3′-OH created by the cleavage primes the synthesis of DNA at the origin. The ColE1 plasmid is then replicated.

If ribonuclease H fails to cleave the pre-primer RNAII, no free 3′-end is made and replication cannot proceed. Ribonuclease H is specific for RNA-DNA hybrids. Consequently, when an antisense RNA, known as RNAI, binds to pre-primer RNAII, this prevents cleavage (Fig. 16.09). Both RNAII and RNAI are transcribed from the same region of DNA but in opposite directions. RNAI is 108 bases long and is encoded

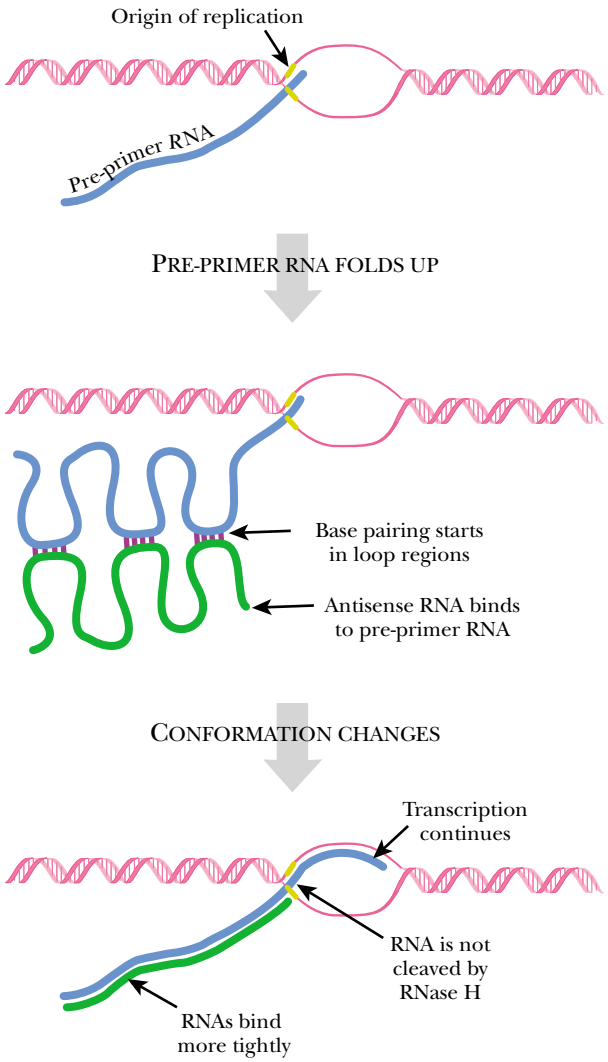


FIGURE 16.09 ***Antisense RNA Prevents Primer Formation***

A second transcript, RNAI, is also made from the same region of ColE1 as RNAII. The RNA I (green) is transcribed from the opposite DNA strand and is therefore complementary to RNAII. The complementary regions of RNAII and RNAI start to base pair, forming a region of bubbles. Eventually, the entire sequence aligns and a double stranded RNA molecule is formed. Since ribonuclease H only recognizes DNA-RNA hybrid molecules, no cut is made and RNAII transcription continues. DNA synthesis fails to start and the ColE1 plasmid is not replicated.

by the opposite strand from RNAII. RNAI is complementary to the 5′-end of RNAII.

The copy number is determined by the relative strengths of binding of RNAII to the DNA at the origin of replication and of RNAI to RNAII. Mutations affecting either of these interactions will change the copy number. The Rom protein, also encoded by a gene on ColE1, increases binding between RNAI and RNAII. If the gene for Rom protein is inactivated, the copy number rises, but is still controlled.

Plasmid Addiction and Host Killing Functions

Large plasmids often make toxins that kill the host cell if, and only if, it loses the plasmid DNA.

Many larger plasmids possess genes whose function is to ensure that the host cell does not lose the plasmid. These "plasmid addiction" systems kill the bacterial cell if the plasmid is lost, so only cells that retain the plasmid survive. The details vary but the scheme involves two components that are made by the plasmid. One is lethal to the host cell and the other is the antidote. The toxin is long-lived and the antidote is short-lived. As long as the plasmid is present it continues to synthesize the antidote. If the plasmid is lost, the antidote decays but the stable toxin survives longer and kills the cell. Many plasmids actually have two or more different systems to decrease the chances of the host cell surviving after losing the plasmid.

The protein-based host killing operon of the F-plasmid consists of two genes, *ccdAB* that are expressed to give two proteins, CcdA and CcdB. CcdA is the antidote

and is readily degraded by host cell proteases. As long as the plasmid is present it constantly makes a fresh supply of CcdA, which binds to CcdB and blocks its action. Consequently, the cell survives. CcdB is the toxin and in the absence of CcdA it kills the cell by inhibiting DNA gyrase and generating double-stranded breaks in the bacterial chromosome. Other plasmids have similar systems.

Both the F-plasmid and some R-plasmids also have a system where the antidote is an antisense RNA that prevents translation of the host-killing protein. This type of system was first found in plasmid R1. Here the Hok (Host Killing) protein is not translated as long as the Sok antisense RNA is present. Sok RNA binds to *hok* mRNA and prevents ribosome binding, which in turn promotes degradation of the mRNA by ribonuclease III. Sok RNA decays relatively rapidly. If the plasmid is lost Sok RNA decays and the *hok* mRNA is free to be translated. Hok protein then damages the cell membrane and kills the cell.

Many Plasmids Help their Host Cells

If plasmids are not an essential part of the cell's genome, why do cells allow them to persist? Some plasmids are indeed useless and, as discussed above, some plasmids possess special mechanisms to protect their own survival at the expense of the host cell. Nonetheless, most plasmids do in fact provide useful properties to their host cells. In principle any gene can be plasmid borne and plasmids are indeed widely used in genetic engineering to move genes between organisms. In practice certain properties are widespread among naturally occurring plasmids. A selection of these are given in Table 16.01.

Many plasmids carry genes that are beneficial to their host cells, but only under certain environmental conditions.

Plasmids often carry genes for resistance to antibiotics. This protects bacteria both from human medicine and from antibiotics produced naturally in the soil. Plasmids with genes for resistance to toxic heavy metals such as mercury, lead or cadmium protect bacteria from industrial pollution and from natural deposits of toxic mineral. Other plasmids provide genes that allow bacteria to grow by breaking down various industrial chemicals, including herbicides, or the components of petroleum. From the human perspective, such bacteria may be a nuisance or may be useful in cleaning up oil spills or other chemical pollution. Finally, some plasmids provide virulence or colonization factors needed by infectious bacteria to invade their victims and survive the countermeasures taken by the host immune system.

Antibiotic Resistance Plasmids

R-plasmids make bacteria resistant to antibiotics.

Plasmids were first discovered in Japan just after World War II, inhabiting the bacterium *Shigella*, which causes dysentery. The type of dysentery due to bacteria was originally treated with sulfonamides, the earliest type of antibiotic. Suddenly, strains of *Shigella* appeared that were resistant to sulfonamide treatment. The genes for resistance to sulfonamide proved to reside on plasmids, rather than the bacterial chromosome. Plasmids that confer antibiotic resistance are called **R-plasmids** or **R-factors** (Fig. 16.10).

Worse, the plasmids carrying the sulfonamide resistance genes were able to transfer copies of themselves from one bacterial cell to another. Consequently, the sulfonamide resistance spread rapidly from *Shigella* to *Shigella*. Although the resistance plasmid allowed the *Shigella* to survive, transferable antibiotic resistance is highly dangerous from the human medical viewpoint. By 1953, the year Watson and Crick discovered the double helix, 80 percent of the dysentery-causing *Shigella* in Japan had become resistant to sulfonamides. By 1960, 10% of the *Shigella* in Japan was resistant to four antibiotics, sulfonamides, chloramphenicol, tetracycline and streptomycin, and

R-plasmid or R-factor Plasmid that carries genes for antibiotic resistance

TABLE 16.01 Properties Conferred by Naturally Occurring Plasmids

Resistance and Defense

Antibiotic resistance against aminoglycosides, β-lactams, chloramphenicol, sulfonamides, trimethoprim, fusidic acid, tetracyclines, macrolides, fosfomycin
Resistance to many heavy metal ions including Ni, Co, Pb, Cd, Cr, Bi, Sb, Zn, Cu and Ag
Resistance to mercury and organomercury compounds
Resistance to toxic anions such as arsenate, arsenite, borate, chromate, selenate, tellurite, etc
Resistance to intercalating agents such as acridines and ethidium bromide
Protection against radiation damage by UV and X-rays
Restriction systems that degrade bacteriophage DNA
Resistance to certain bacteriophages

Aggression and Virulence

Synthesis of bacteriocins
Synthesis of antibiotics
Crown gall tumors and hairy root disease in plants caused by *Agrobacterium*
Nodule formation by *Rhizobium* on roots of legumes
R-body synthesis due to plasmids of *Caedibacter* symbionts in killer *Paramecium*
Virulence factors of many pathogenic bacteria, including toxin synthesis, protection against immune system and attachment proteins

Metabolic Pathways

Degradation of sugars e.g. lactose (in *Salmonella*), raffinose, sucrose
Degradation of aliphatic and aromatic hydrocarbons and their derivatives such as octane, toluene, benzoic acid, camphor
Degradation of halogenated hydrocarbons such as polychlorinated biphenyls
Degradation of proteins
Synthesis of hydrogen sulfide
Denitrification in *Alcaligenes*
Pigment synthesis in *Erwinia*

Miscellaneous

Transport of citrate in *E. coli*
Transport of iron
Gas vacuole production in *Halobacterium*

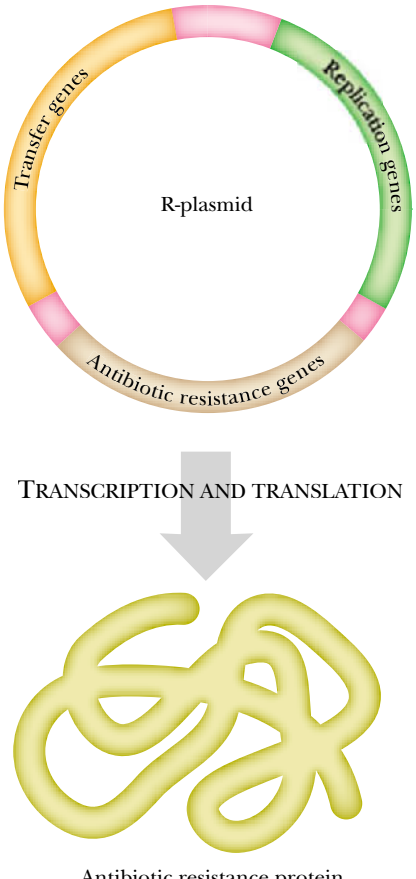


FIGURE 16.10 ***Antibiotic Resistance Plasmids***

Plasmids carry genes for replicating their DNA, transferring themselves from one host cell to another, and genes for a variety of phenotypes. Many plasmids carry genes that confer antibiotic resistance on the host cell when the genes are expressed.

by 1970 this had risen to over 30%. These strains often carry resistance genes for different antibiotics on one single plasmid. Today, the transfer of multiple antibiotic resistance plasmids between bacteria has become a major clinical problem. Patients with infections after surgery, with severe burns, or with immuno-compromised systems are at highest risk to antibiotic resistant infections.

Soil bacteria (e.g. *Streptomyces*) or fungi (e.g. *Penicillium*) produce antibiotics as a natural part of there physiology. Consequently, R-plasmids were in existence before the clinical use of antibiotics by humans, but they have spread far and wide since wide scale use of antibiotics started. A major factor in R-plasmid spread is the practice of feeding animals (e.g. pigs and chickens) antibiotics to prevent illnesses that reduce yield. Recently, some countries have banned the use of human antibiotics in animal feed and there has been a major decline in the frequency of R^+ bacteria carried by farm animals.

Most R-plasmids are of moderate to large size and present in 1–2 copies per host cell. Most are self-transmissible at a low frequency, although de-repressed mutants showing high transfer frequency are sometimes found. The original F-plasmid is such a "naturally-occurring mutant". R-plasmids belong to a wide range of incompatibility groups. Many carry resistances to one or more antibiotics and/or toxic heavy metals and may also carry genes for colicins, virulence factors etc.

Mechanisms of Antibiotic Resistance

Plasmid borne resistance mechanisms usually inactivate or expel the antibiotic, rather than altering vital cell components.

Antibiotic resistant mutants of bacteria may be easily isolated in the laboratory. However, the mechanism of resistance in such chromosomal mutants is usually quite distinct from that of plasmid-borne resistance. The chromosomal mutations usually alter the cell component that is the target of antibiotic action, which often causes detrimental side effects. Plasmid-borne resistance generally avoids altering vital cell components. Instead the antibiotic may be inactivated or pumped out of the cell. Occasionally plasmids do provide an altered (but still functional) target component. Several of the resistance genes originally found on plasmids have been used in genetic engineering. Antibiotic resistance allows scientists to screen for cells that contain a plasmid, and kill all the cells that do not (see Ch. 22). Chloramphenicol, kanamycin/neomycin, tetracycline and ampicillin resistance genes are the most widely used in laboratories.

Resistance to Beta-Lactam Antibiotics

Penicillin and its relatives are the most widely used family of antibiotics.

The **β-lactam** family includes the **penicillins** and **cephalosporins** and is the best-known and most widely used group of antibiotics. All contain the β-lactam structure, a four-membered ring containing an amide group, which reacts with the active site of enzymes involved in building the bacterial cell wall. Cross-linking of the peptidoglycan is prevented, so causing disintegration of the cell wall and death of the bacteria. Since peptidoglycan is unique to bacteria, penicillins and cephalosporins have almost no side effects in humans, apart from occasional allergies.

Penicillin and related antibiotics are destroyed by the enzyme beta-lactamase.

Resistance plasmids carry a gene encoding the enzyme, **β-lactamase**, which destroys the antibiotic by opening the β-lactam ring (Fig. 16.11). Most β-lactamases prefer either penicillins or cephalosporins, though a few attack both antibiotics equally well. Resistance to **ampicillin**, a popular type of penicillin, is widely used in molecular

ampicillin A widely used antibiotic of the penicillin group
beta-lactams or β-lactams Family of antibiotics that inhibit cross-linking of the peptidoglycan of the bacterial cell wall; includes penicillins and cephalosporins
beta-lactamase or β-lactamase Enzyme that inactivates β-lactam antibiotics such as ampicillin by cleaving the lactam ring
cephalosporins Group of antibiotics of the β-lactam type that inhibit cross-linking of the peptidoglycan of the bacterial cell wall
penicillins Group of antibiotics of the β-lactam type that inhibit cross-linking of the peptidoglycan of the bacterial cell wall

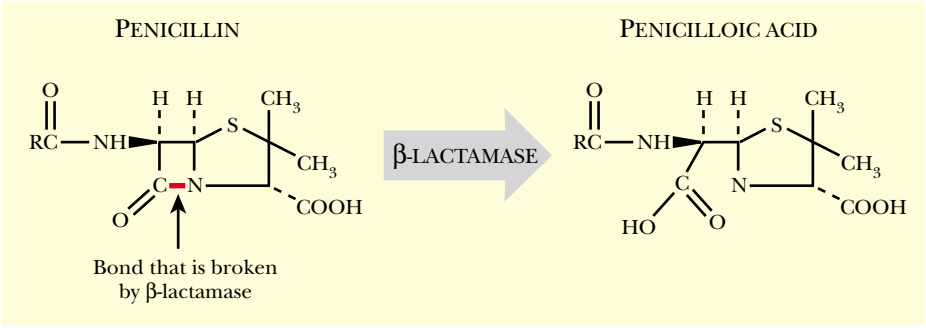


FIGURE 16.11 ***Inactivation of Penicillin by β-Lactamase***

Penicillin is an antibiotic that attacks the cell wall of bacteria, preventing the cells from growing or dividing. The antibiotic has a four-membered β-lactam ring that binds to the active site of the enzymes that assemble the cell wall. The enzyme β-lactamase cleaves the *β*-lactam ring of penicillin (red bond). The penicillin is inactivated.

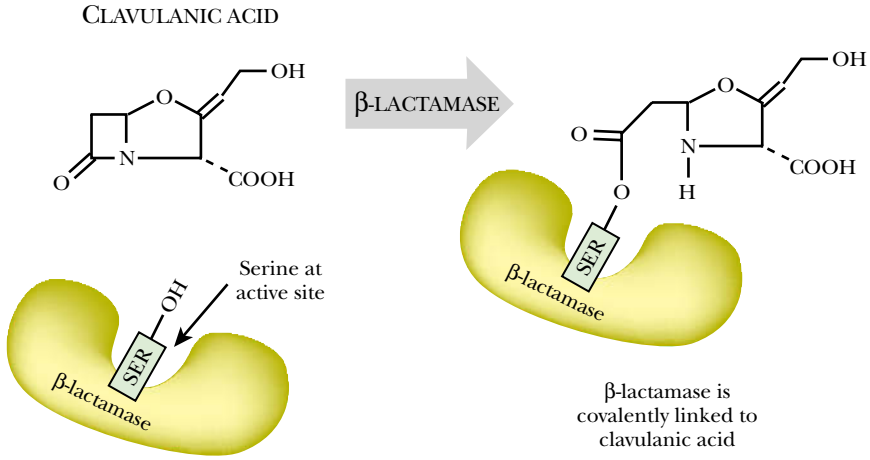


FIGURE 16.12 ***Inactivation of β-Lactamase by Clavulanic acid***

In order to inactivate β-lactamase, analogs of penicillin such as clavulanic acid are added along with the antibiotic. Clavulanic acid has a four-membered ring similar to penicillin. Consequently, β-lactamase will bind and cleave this ring. When this happens, clavulanic acid is covalently bound to β-lactamase rendering it useless against penicillin. Added penicillin can now kill the bacteria, even though they contain the resistance genes.

biology, especially for selecting plasmids during cloning procedures (see Ch. 22). The same gene is referred to as either *amp* (for ampicillin) or ***bla*** for β-lactamase. Certain strains of *Pseudomonas* carrying the R-plasmid RPl that encodes a high activity, broad-spectrum β-lactamase can actually grow on ampicillin as sole carbon and energy source!

A vast number of penicillin and cephalosporin derivatives have been made by the pharmaceutical industry. Some of these are much less susceptible to breakdown by β-lactamase. Their development has in turn led to the emergence of altered and improved β-lactamases among bacteria carrying R-plasmids. Another approach is to administer a mixture of a β-lactam antibiotic plus a β-lactam analog that inhibits β-lactamase. **Clavulanic acid** and its derivatives bind to β-lactamases and react forming a covalent bond to the amino acids in the active site that kills the enzyme (Fig. 16.12).

Resistance to Chloramphenicol

Chloramphenicol, streptomycin and kanamycin are all antibiotics that inhibit protein synthesis by binding to the bacterial ribosomes. The difference in mechanism between resistance due to chromosomal mutations as opposed to plasmid-borne genes is espe-

***bla* gene** Gene encoding β-lactamase thereby providing resistance to ampicillin. Same as *amp* gene
clavulanic acid And its derivatives bind to β-lactamases and react forming a covalent bond to the protein that kills the enzyme

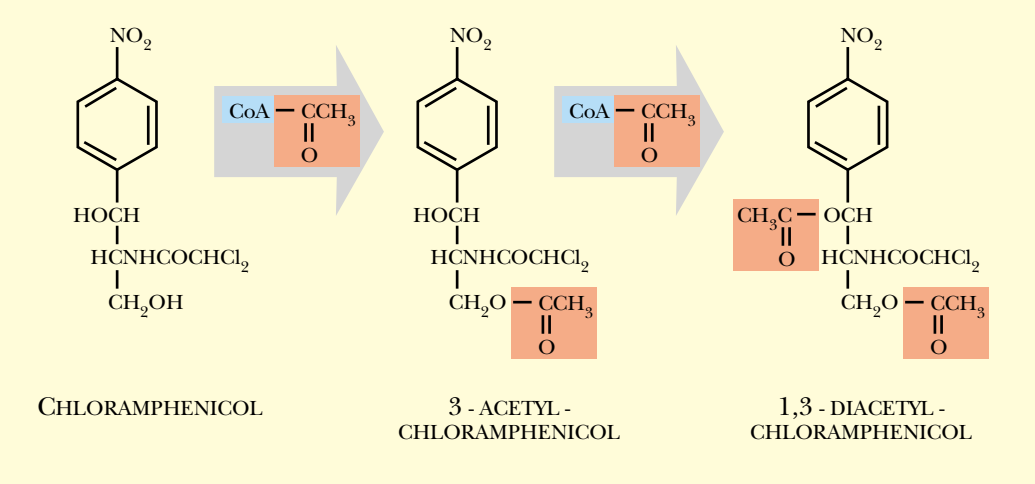


FIGURE 16.13 ***Inactivation of Chloramphenicol***

The side chain of chloramphenicol has two –OH groups that are important for binding to the bacterial ribosomes. Chloramphenicol acetyl transferase, produced by R-plasmids, catalyzes the addition of two acetyl groups to chloramphenicol. The enzyme uses acetyl-CoA as a source for the acetyl groups. The resulting 1,3-diacetyl-chloramphenicol can no longer bind to the ribosomes.

Chloramphenicol is inactivated by addition of acetyl groups.

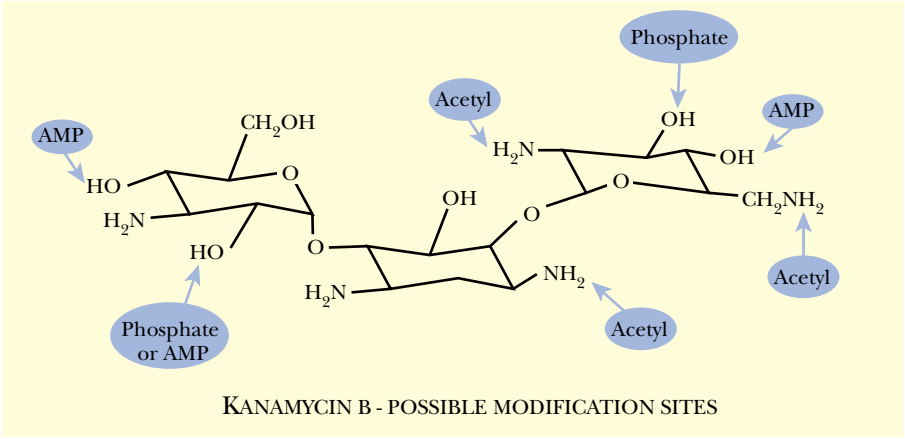
cially notable for these antibiotics. Chromosomal mutants usually have altered ribosomes that no longer bind the antibiotic. Not surprisingly such mutations often cause slower or less accurate protein synthesis and the cells grow poorly. In contrast, plasmid-borne resistance to these antibiotics usually involves chemical attack on the antibiotic itself by specific enzymes encoded by the plasmid.

Chloramphenicol binds to the 23S rRNA of the large subunit of the bacterial ribosome and inhibits the peptidyl transferase reaction (see Ch. 8). R plasmids protect the bacteria by producing the enzyme, **chloramphenicol acetyl transferase (CAT)**. **CAT** transfers two acetyl groups from acetyl CoA to the side chain of chloramphenicol. This prevents binding of the antibiotic to the 23S rRNA (Fig. 16.13). Replacement of the terminal -OH of chloramphenicol with fluorine results in non-modifiable yet still antibacterially active derivatives. There are two major groups of chloramphenicol acetyl transferase, one from gram-positive and one from gram-negative bacteria. The two groups differ greatly from each other except for the chloramphenicol-binding region.

Resistance to Aminoglycosides

The **aminoglycoside** family of antibiotics includes **streptomycin**, **kanamycin**, **neomycin**, tobramycin, gentamycin and a host of others. Aminoglycosides consist of three (sometimes more) sugar rings, at least one of which (and usually two or three) has amino groups attached. They inhibit protein synthesis by binding to the small subunit of the ribosome (see Ch. 8). Plasmid-borne resistance is due to inactivation of the antibiotics. Several alternatives exist, including modification by phosphorylation of -OH groups, adenylation (i.e. addition of AMP) of -OH groups or acetylation of -NH_2 groups. ATP

aminoglycosides Family of antibiotics that inhibit protein synthesis by binding to the small subunit of the ribosome; includes streptomycin, kanamycin, neomycin, tobramycin, gentamycin and many others
chloramphenicol Antibiotic that binds to 23S rRNA and inhibits protein synthesis
CAT Chloramphenicol acetyl transferase
chloramphenicol acetyl transferase (CAT) Enzyme that inactivates chloramphenicol by adding acetyl groups
kanamycin Antibiotic of the aminoglycoside family that inhibits protein synthesis
neomycin Antibiotic of the aminoglycoside family that inhibits protein synthesis
streptomycin Antibiotic of the aminoglycoside family that inhibits protein synthesis



KANAMYCIN B - POSSIBLE MODIFICATION SITES

FIGURE 16.14 ***Inactivation of Aminoglycoside Antibiotics***

Much like chloramphenicol, members of the aminoglycoside family are inactivated by modification. One member, kanamycin B, can be modified by a variety of covalent modifications, such as phosphorylation, acetylation, or adenylation. A variety of bacterial enzymes make these modifications to prevent kanamycin B from attaching to the small ribosomal subunit.

Aminoglycoside antibiotics are inactivated by addition of phosphate, AMP, or acetyl groups.

is used as a source of phosphate and AMP groups, whereas acetyl-CoA is the acetyl donor (Fig. 16.14).

Modified aminoglycosides no longer inhibit their ribosomal target sites. There are many different aminoglycosides and a correspondingly wide range of modifying enzymes. The ***npt*** gene (**neomycin phosphotransferase**) is the most widely used and provides resistance to both kanamyin and the closely related neomycin. Aminoglycosides are made by bacteria of the *Streptomyces* group, which are mostly found in soil. These organisms need to protect themselves against the antibiotics they produce. Probably, therefore, the aminoglycoside modifying enzymes came originally from the same *Streptomyces* strains that make these antibiotics.

Amikacin is a more recent derivative of kanamycin A in which the amino group on the middle ring that gets acetylated is blocked with a hydroxybutyrate group. This made amikacin resistant to all modifying enzymes except one obscure N-acetyl transferase. However, evolution moves on and an enzyme that phosphorylates amikacin has already appeared in some bacterial strains!

Resistance to Tetracycline

Tetracycline resistance is due to energy-driven export of the antibiotic.

Tetracycline binds to the 16S rRNA of the small subunit and also inhibits protein synthesis. However, the mechanism of resistance is quite different from chloramphenicol and aminoglycosides. Rather than inactivating tetracycline by modification, R-plasmids produce proteins that pump the antibiotic out of the bacteria. Tetracycline actually binds to both prokaryotic and eukaryotic ribosomes. Bacteria are more sensitive than animal cells because tetracyclines are actively taken up by bacterial cells, but not by eukaryotic cells. In fact, eukaryotic cells actively export tetracyclines. In tetracycline resistant bacteria, the antibiotic is actively taken into the cell, but then pumped out again. As there is no similarity between tetracycline and any known transportable nutrients, the purpose of the bacterial transport system that takes up tetracycline and its mechanism of operation are still baffling. However, the Tet resistance protein is part of a large family of sugar transporter proteins.

Plasmid-encoded tetracycline resistance is typically two level. A basal constitutive level of resistance protects by 5–10 fold relative to sensitive bacteria. In addition, exposure to tetracycline induces a second higher resistance level. Both resistance levels are due to production of proteins that are found in the cytoplasmic membrane and actively expel tetracycline from the cell. Tetracycline enters the cell as the protonated form by an active transport system. Inside the cell it binds Mg^{2+}. The Tet resistance protein uses energy to expel the Tet-Mg^{2+} complex by proton antiport (Fig. 16.15).

neomycin phosphotransferase Enzyme that inactivates the antibiotics kanamycin and neomycin by adding a phosphate group
***npt* gene** Gene for neomycin phosphotransferase. Provides resistance against the antibiotics kanamycin and neomycin
tetracycline Antibiotic that binds to 16S ribosomal RNA and inhibits protein synthesis

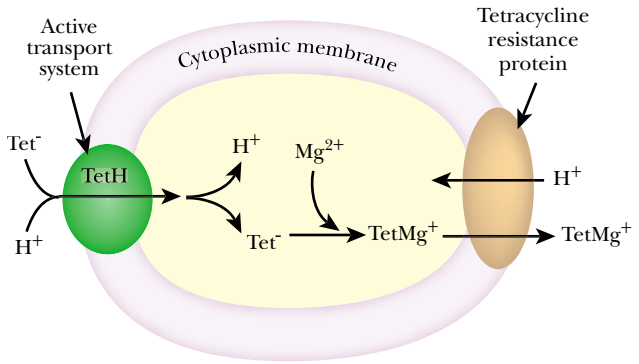


FIGURE 16.15 ***Expulsion of Tetracycline from Resistant Bacteria***

The bacterial chromosome contains the gene for TetH, a protein that takes tetracycline from the environment and actively pumps the antibiotic and a proton into the cell. Once inside the cell, tetracycline complexes with Mg^+, and may bind to the ribosome. In bacterial cells with an R-plasmid for tetracycline, another transport protein, called the tetracycline resistant protein, is manufactured. This protein allows a proton to enter the cell to produce energy for export of the Tet-Mg^+ complex.

Resistance to Sulfonamides and Trimethoprim

Resistance to trimethoprim and sulfonamides is due to replacement of the target enzyme.

Both sulfonamides and trimethoprim are both antagonists of the vitamin folic acid. The reduced form of folate, tetrahydrofolate, is used as a cofactor by enzymes that synthesize methionine, adenine, thymine and other metabolites whose synthesis involves adding a one carbon fragment. **Sulfonamides** are wholly synthetic antibiotics and are analogs of *p*-aminobenzoic acid (Fig. 16.16), a precursor of the vitamin folic acid. Sulfonamides inhibit dihydropteroate synthetase, an enzyme in the synthetic pathway for folate. **Trimethoprim** is an analog of the pterin ring portion of tetrahydrofolate. It inhibits dihydrofolate reductase, the bacterial enzyme that converts dihydrofolate to tetrahydrofolate. Animal cells rely on folate in their food and so these antibiotics are effective against bacteria that normally manufacture their own tetrahydofolate.

Plasmid mediated resistance to both sulfonamides and trimethoprim involves synthesis of folic acid biosynthetic enzymes that no longer bind the antibiotic. R-plasmid encoded dihydropteroate synthetase has the same affinity for *p*-aminobenzoic acid as the chromosomal enzyme but is resistant to sulfonamides. Similarly, R-plasmid encoded dihydrofolate reductase is resistant to trimethoprim. Sulfonamides plus trimethoprim are often used in combination for double blockade of the folate pathway. As a result, sulfonamide and trimethoprim resistance are often found together on the same R-plasmid.

Plasmids may Provide Aggressive Characters

The first plasmids drew attention because they provided their host bacteria with resistance to antibiotics. Other plasmids protect bacteria against heavy metal toxicity. However, many plasmids are known that confer aggressive, rather than defensive properties. These may be sub-divided into two broad groups. Bacteriocin plasmids encode toxic proteins used by certain strains of bacteria to kill related bacteria. **Virulence plasmids** carry genes for a variety of characters deployed by bacteria that infect higher organisms, both plants and animals, including humans.

sulfonamides Synthetic antibiotics that are analogs of *p*-aminobenzoic acid, a precursor of the vitamin folic acid. Sulfonamides inhibit dihydropteroate synthetase
trimethoprim Antibiotic that is an analog of the pterin ring portion of the folate cofactor. It inhibits dihydrofolate reductase
virulence plasmid Plasmid that carries genes for virulence factors that play a role in bacterial infection

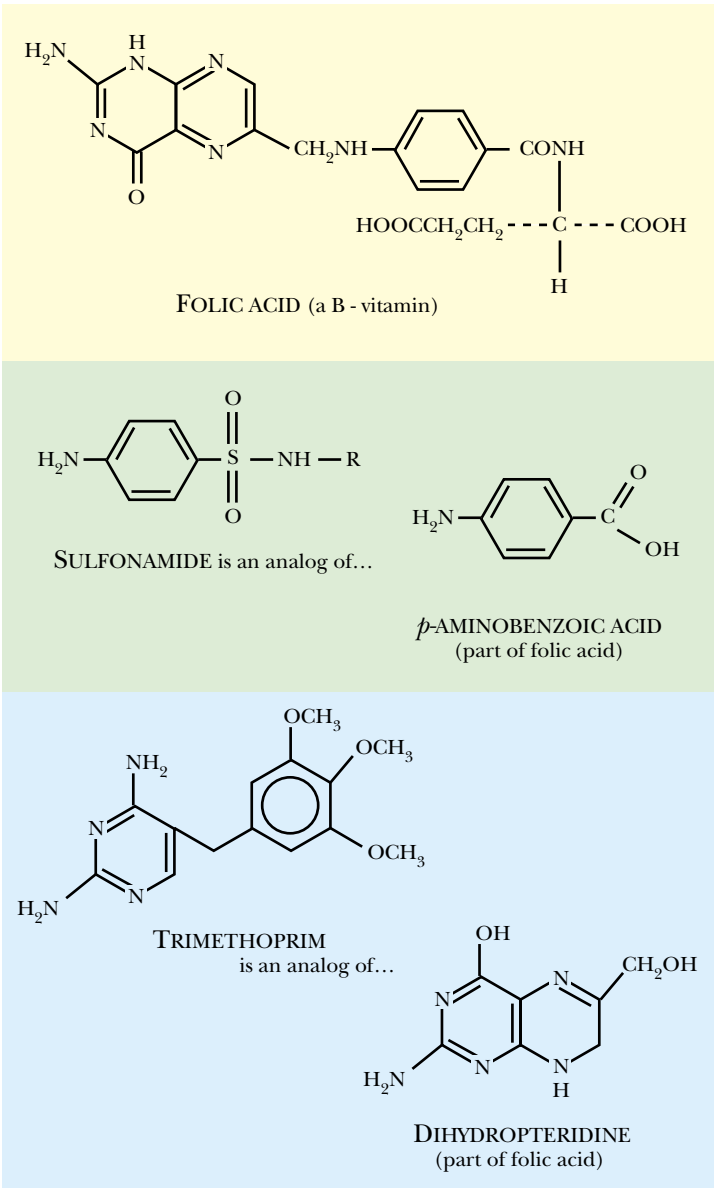


FIGURE 16.16
Trimethoprim, Sulfonamides and the Folate Cofactor

Bacterial cells make folic acid, whereas, animal cells do not. The antibiotic sulfonamide is an analog of the *p*-aminobenzoic acid portion of folic acid. Trimethoprim is an analog of the dihydropteridine portion of folic acid. Both trimethoprim and sulfonamide bind to the biosynthetic enzymes and prevent synthesis of folic acid from its precursors.

Many bacteria make toxic proteins—bacteriocins—to kill closely related bacteria that compete for the same resources.

Bacteriocins are usually encoded on plasmids. These provided the starting point for many genetic engineering vectors.

Generally speaking, bacteria are most likely to attack their close relatives. The reason is that the more closely related they are, the more likely two strains of bacteria will compete for the same resources. Proteins made by bacteria to kill their relatives are known generally as **bacteriocins**. Particular bacteriocins are named after the species that makes them. So, for example, many strains of *Escherichia coli* deploy a wide variety of **colicins**, intended to kill other strains of the same species. [Since most work has been done on *E. coli* bacteriocins from other bacteria are often referred to as colicins although this is not strictly correct.] Surveys suggest that 10–15% of enteric bacteria make bacteriocins.

On several occasions, *Yersinia pestis*, the bacterium that causes Black Death (bubonic plague), has wiped out a third of the human population of Europe, and probably most of Africa and Asia. The virulence factors required for infection are carried on a series of plasmids (see below). As if this was not enough, *Yersinia pestis* also makes bacteriocins, called pesticins in this case, designed to kill competing strains of its own species.

bacteriocin Toxic protein made by bacteria to kill closely related bacteria
colicin Toxic protein or bacteriocin made by *Escherichia coli* to kill closely related bacteria

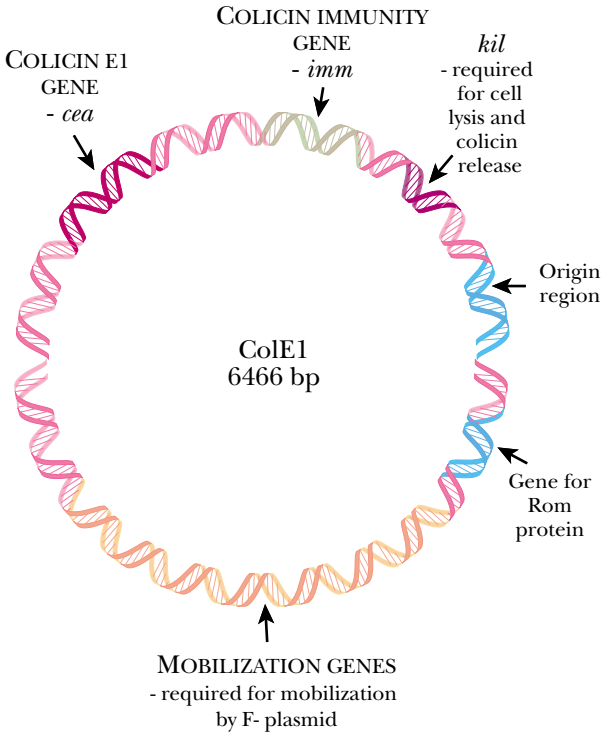


FIGURE 16.17 *ColE1 is an Example of a Colicin Plasmid*

The ColE1 plasmid of *E. coli* carries genes for colicin E1 (*cea*), immunity to colicin E1 (*imm*) and the *kil* gene, required for liberation of colicin from the producer cell. The Rom gene is involved in copy number control as discussed above. ColE1 is the basis for many plasmids used in genetic engineering. The mobilization genes allow ColE1 to be transferred from cell to cell during conjugation mediated by the F plasmid.

The ability to make bacteriocins is usually due to the presence of a plasmid in the producer cell. The best known examples are the three related **ColE plasmids** of *E. coli*, ColE1 (Fig. 16.17), ColE2 and ColE3. These are small plasmids that exist in 50 or more copies per cell and have been used to derive many of the cloning vectors used in genetic engineering (see Ch. 22). These cloning vectors have the actual colicin genes removed. A variety of other colicin plasmids also occur, including the ColI and ColV plasmids. These are large single-copy plasmids and are usually transferable from one strain of *E. coli* to another. Many ColI and ColV plasmids also carry genes for antibiotic resistance.

Most Colicins Kill by One of Two Different Mechanisms

The two most popular modes of action for bacteriocins are:
a) damaging the cell membrane or
b) destroying nucleic acids.

The Col plasmids allow the strains of *E. coli* that possess them to kill other related bacteria. There are two basic approaches to this. The first is to damage the victim's cell membrane. A gene on the ColE1 plasmid encodes the colicin E1 protein that inserts itself through the membrane of the target cell and creates a channel allowing vital cell contents, including essential ions to leak out and protons to flood into the cell (Fig. 16.18). The influx of protons collapses the proton motive force. The energy derived from the proton motive force drives the production of ATP and the uptake of many nutrients, without which the bacteria quickly die. A single molecule of colicin E1 that penetrates the membrane is enough to kill the target cell. Colicin I and colicin V operate by a similar mechanism.

Colicin M and Pesticin A1122 destroy the peptidoglycan of the cell wall rather than puncturing the cytoplasmic membrane. These colicins need to penetrate only as far as the outer surface of the cytoplasmic membrane, i.e. the site of peptidoglycan assembly. Without the peptidoglycan, the bacterial cell loses shape and eventually bursts. Pesticin A1122 is made by *Yersinia pestis* and kills *Y. pseudotuberculosis, Y. enterocolitica*, plasmid free *Y. pestis* and many strains of *E. coli* (although curiously not *E. coli* K12).

ColE plasmid Small multicopy plasmid that carries genes for colicins of the E group. Used as the basis of many widely used cloning vectors

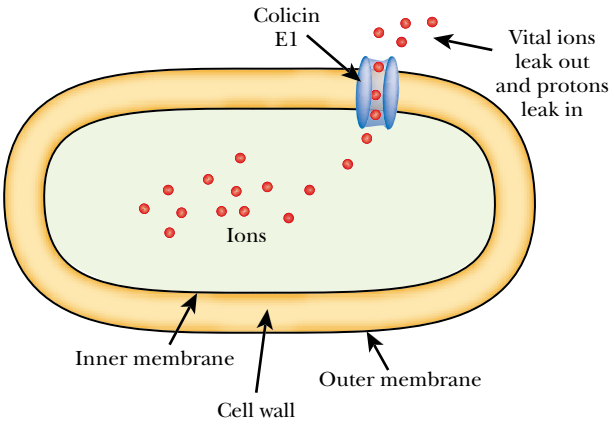


FIGURE 16.18 ***Some Colicins Damage the Cell Membrane***

When colicin E1 protein attacks a bacterial cell, it punctures a hole through the outer membrane, cell wall, and inner membrane. The hole allows protons to leak into the bacteria and vital ions to leak out. A single channel abolishes energy generation.

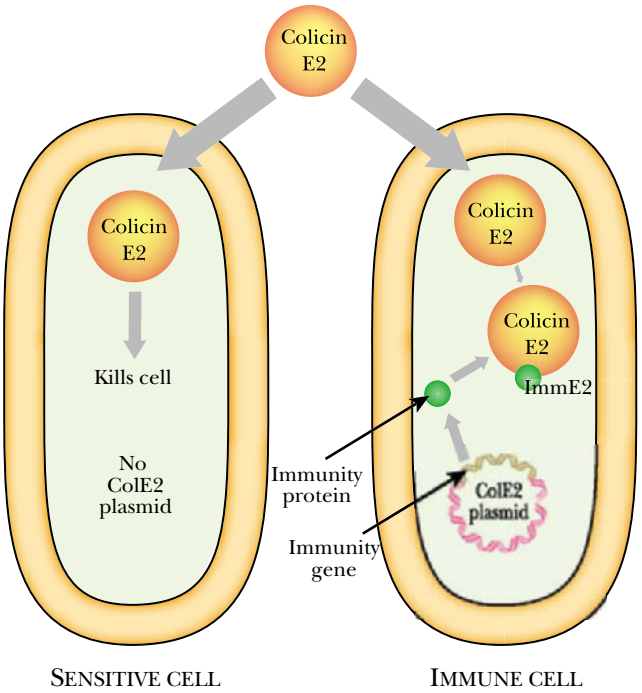


FIGURE 16.19 ***Colicin Immunity System***

In order to protect itself, a colicin-making cell also produces an immunity protein (right). This protein is also encoded by the colicin plasmid. It blocks the active site of the colicin thus preventing the cell from killing itself. The immunity protein is specific and only inhibits one type of colicin. If a cell lacks immunity protein, the colicin is able to kill the cell (left).

The second approach is to degrade the nucleic acids of the victim. The ColE2 and ColE3 plasmids both encode nucleases, enzymes that degrade nucleic acids. The colicin E2 and E3 proteins are very similar over their N-terminal 75% and as a result they share the same receptor on the surface of sensitive bacteria. They differ in the C-terminus and have different nucleic acid targets. Colicin E2 is a deoxyribonuclease that cuts up the chromosome of the target cell. Colicin E3 is a ribonuclease that snips the 16S rRNA of the small ribosomal subunit at a specific sequence, releasing a fragment of 49 nucleotides from the 3′ end. This abolishes protein synthesis and though much more specific than colicin E2, is just as lethal. Again, a single colicin molecule that enters the victim is enough to kill the target cell.

Bacteria are Immune to their own Colicins

Those bacterial cells producing a particular colicin are immune to their own brand, but not to other brands of colicin. Immunity is due to specific **immunity proteins** that bind to the corresponding colicin proteins and cover their active sites (Fig. 16.19). For

immunity protein Protein that provides immunity. In particular bacteriocin immunity proteins bind to the corresponding bacteriocins and render them harmless

Bacteria that make bacteriocins also make immunity proteins to protect themselves.

example, the ColE2 plasmid carries genes for both colicin E2 and a soluble immunity protein that binds colicin E2. This immunity protein does not protect against any other colicin, including the closely related colicin E3. Immunity to membrane active colicins is due to a plasmid-encoded inner membrane protein that block the colicin from forming a pore in the host cell. For example, the Ia immunity protein protects membranes against colicin Ia but not against the closely related colicin Ib even though colicins Ia and Ib share the same receptor, have the same mode of action, and have extensive sequence homology. Although the immune systems of animals are much more complex, the concept of immunity is based on the ability of immune system proteins to recognize and neutralize specific alien or hostile molecules.

Colicin Synthesis and Release

In a population of ColE plasmid-carrying bacteria, most cells do not produce colicin. Every now and then an occasional cell goes into production and manufactures large amounts of colicin. It then bursts and releases the colicin into the medium. This kills the producer cell. Note that the burst and release mechanism kills the producer cell, not the colicin. All sensitive bacteria in the area are wiped out, but those with the ColE plasmid have immunity protein and survive.

Bacteriocin production is often a suicidal process.

About 1 in 10,000 cells actually produce colicin in each generation. Thus release of colicin E is a communal action in the sense that a small minority of producer cells sacrifice themselves so that their relatives carrying the same ColE plasmid can takeover the habitat. Colicin E production involves expression of two plasmid genes, *cea* (colicin protein) and *kil* (lysis protein). LexA, the repressor of the SOS DNA repair system (Chapter 14), normally represses these genes. Thus colicin production is induced by DNA damage and those cells that sacrifice themselves were probably injured anyway. Note that many lysogenic bacteriophage are also induced by DNA damage monitored via the SOS system (see Ch. 17).

Not all colicins are produced by the suicidal mechanism. Many colicins made by large single-copy plasmids (e.g. colicin V, colicin I) are apparently made continuously in smaller amounts. These colicins tend to remain attached to the surface of the producer cell rather than being released as freely soluble proteins, like the E colicins. When the producer bumps into a sensitive bacterium the colicin may be transferred, with lethal results.

Virulence Plasmids

Virulence plasmids help bacteria infect humans, animals or even plants, by a variety of mechanisms. Some **virulence factors** are toxins that damage or kill animal cells, others help bacteria to attach to and invade animal cells (Fig. 16.20), whereas yet others protect bacteria against retaliation by the immune system.

Many pathogenic bacteria carry genes for virulence on plasmids or other mobile genetic elements.

Although most strains of *Escherichia coli* are harmless, occasional rogue strains cause disease. These pathogenic *E. coli* generally rely on plasmid-borne virulence factors. Wide ranges of toxins are found in different pathogenic *E. coli* strains, including heat-labile **enterotoxin** (resembles **choleratoxin**), heat-stable enterotoxin, **hemolysin** (lyses red blood cells) and Shiga-like toxin (similar to the toxin of dysentery-causing *Shigella*). There is a similar variety of **adhesins** or "**colonization factors**",

adhesin Protein that enables bacteria to attach themselves to the surface of animal cells. Same as colonization factor
choleratoxin Type of toxin made by *Vibrio cholerae* the cholera bacterium
colonization factor Protein that enables bacteria to attach themselves to the surface of animal cells. Same as adhesin
enterotoxins Types of toxin made by enteric bacteria including some pathogenic strains of *E. coli*
hemolysin Type of toxin that lyses red blood cells
virulence factors Proteins that promote virulence in infectious bacteria. Include toxins, adhesins and proteins protecting bacteria from the immune system

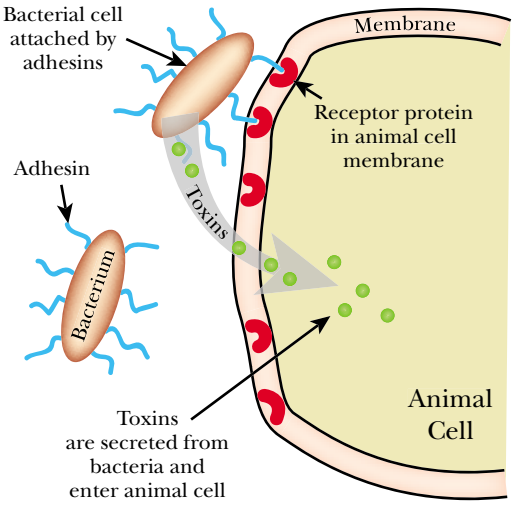


FIGURE 16.20 ***Toxins and Adhesins***

Bacteria are able to attack animal cells by attaching to the cellular membrane and releasing toxins. The bacteria contain plasmids that encode adhesins, which are protein filaments able to recognize and attach to cell-surface receptors found on animal cells. One attached, the bacteria secrete toxins, which can penetrate the animal cell membrane and kill the cell.

proteins that enable bacteria to stick to the surface of animal cells. Adhesins form filaments that vary in length and thickness, but generally resemble pili. Consequently the symptoms and severity of infection by *E. coli* vary greatly.

Other enteric bacteria, such as *Salmonella typhi* (typhoid) and *Yersinia pestis* (bubonic plague) cause severe infections. They also carry virulence plasmids. In *Salmonella* the majority of the virulence genes are on the chromosome, but a handful are plasmid-borne. In contrast, in *Yersinia* several plasmids carry the bulk of the virulence genes. In addition to toxins and adhesins, these "professional" pathogens possess more sophisticated virulence factors that protect against host defenses. Although plasmids have been investigated most intensively in enteric bacteria, it is clear that virulence in many other bacteria often depends on at least some plasmid-borne genes.

Ti-Plasmids are Transferred from Bacteria to Plants

Although the F-plasmid of *E. coli* is limited in its host range to a few enteric bacteria, it can actually promote DNA transfer between *E. coli* and yeast! Similarly, broad host range plasmids of the IncP, IncQ and IncW incompatibility groups can mobilize DNA from gram-negative bacteria into both gram-positive bacteria and yeast. In both cases, the range of species in which the plasmid can survive and replicate is much smaller than the range of species to which DNA may be transferred. Therefore, many plasmids are degraded or destroyed after they are transferred to an incompatible cell. Some DNA mobilized in this manner may survive if it is recombined with the host chromosome or resident plasmids.

The Ti-plasmids can mediate transfer of DNA from bacteria to plant cells.

The greatest versatility in plasmid transfer is shown by the highly specialized **Ti-plasmids** (Ti = tumor-inducing) that allow certain bacteria to insert DNA into the nucleus of plant cells. The Ti-plasmid is carried by soil bacteria of the *Agrobacterium* group, in particular *A. tumefaciens*, and confers the ability to infect plants and produce tumors, inside which the bacteria grow and divide happily. This results in tumor-like swellings on the stems of infected plants, a condition known as **"crown gall disease"**. The related Ri-plasmid is carried by *Agrobacterium rhizogenes,* which infects roots and causes hairy root disease.

Agrobacterium is attracted by chemicals, such as acetosyringone, which are released by wounded plants. It then enters via the wound and transfers a portion of

crown gall Type of tumor formed on plants due to infection by *Agrobacterium* carrying a Ti-plasmid
Ti-plasmid Tumor-inducing plasmid. Plasmid that is carried by soil bacteria of the *Agrobacterium* group and confers the ability to infect plants and produce tumors

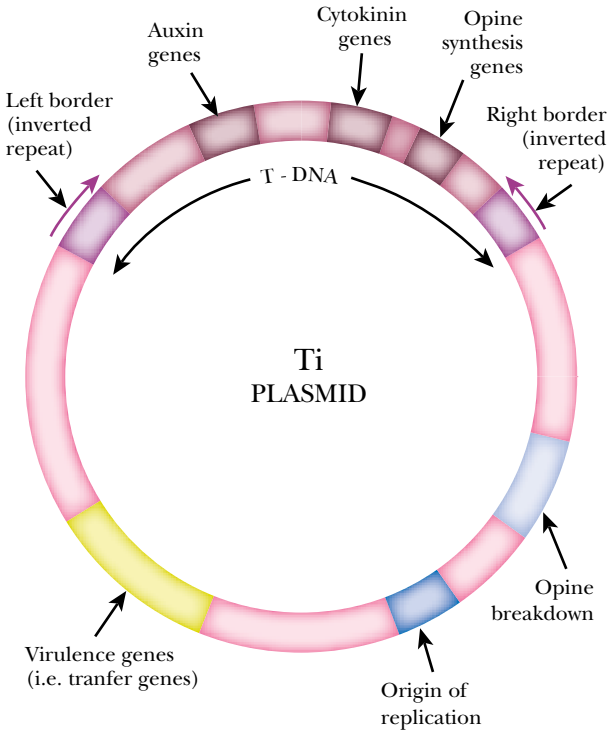


FIGURE 16.21 ***Structure of the Ti-Plasmid***

The Ti plasmid of *Agrobacterium* has several regions. The T-DNA region is flanked by two inverted repeats and contains genes for auxin and cytokinin, which induce the plant cells to grow, and genes for opine synthesis, a carbon source for *Agrobacterium*. This region is transferred into the plant cell by the expression of the transfer genes found on the other part of the Ti plasmid. The Ti plasmid also has an origin of replication and genes for opine breakdown.

Bacteria carrying Ti-plasmids infect plants and cause the formation of tumors.

the Ti plasmid into the plant cell by a mechanism similar to bacterial conjugation. A slight abrasion that is trivial to the health of the plant is of course sufficient for the entry of a microorganism. The result is a crown gall tumor that provides a home for the *Agrobacterium* at the expense of the plant.

The Ti-plasmid consists of several regions (Fig. 16.21), but only one segment, the **T-DNA** (tumor-DNA), is actually transferred into the plant cell, where it enters the nucleus. The T-DNA is flanked by 25 bp inverted repeats. Any DNA included within these repeats will be transferred into the plant cell. Consequently, Ti-plasmids have been widely used in the genetic engineering of plants. The virulence genes on the plasmid are responsible for cell-to-cell contact and transfer of the T-DNA but do not themselves enter the plant cells.

Only part of the Ti-plasmid enters the plant cell, where it integrates into the plant chromosomes.

Acetosyringone, which attracts the bacteria to the wounded plant, also induces the virulence genes, thus facilitating the transfer of the T-DNA region (Fig. 16.22). Acetosyringone binds to VirA protein in the *Agrobacterium* membrane. This activates VirG, which in turn switches on the other *vir* genes, including *virD* and *virE*. VirD makes a single-stranded nick in the Ti-plasmid at the left border of the T-DNA and the T-DNA unwinds from the cut site. The single-stranded T-DNA is bound by VirE protein and unwinding stops at the right border. The Ti-plasmid then replicates by a rolling circle mechanism as the single stranded T-DNA region enters the plant cell. Overall this results in DNA transfer from the bacteria into the plant cells. The mechanism resembles bacterial conjugation and the "virulence" genes of the Ti-plasmid are equivalent to the *tra* genes of other plasmids. The T-DNA then integrates at random into a chromosome in the plant cell nucleus.

Once inserted, the genes in the T-DNA are switched on. The enzymes they encode synthesize two plant hormones, **auxin** and **cytokinin**. Auxin makes plant cells grow bigger and cytokinin makes them divide. When this happens rapidly in the absence of normal cell differentiation, the result is a tumor (Fig. 16.23 and Fig. 16.24).

auxin Plant hormone that induces plant cells to grow bigger
cytokinin Plant hormone that induces plant cells to divide
T-DNA (tumor-DNA) Region of the Ti-plasmid that is transferred into the plant cell nucleus

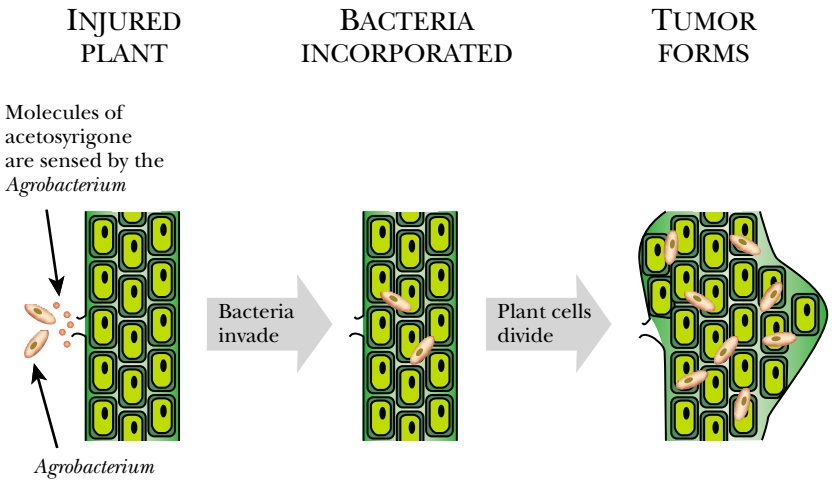


FIGURE 16.22 ***Formation of Tumor by Agrobacterium***

Agrobacterium are attracted to an injured region of a plant by sensing molecules of acetyosyrigone. The bacteria enter the plant through the open wound, and begin colonizing the area. The plant cells are stimulated to divide and a tumor forms around the bacteria.

FIGURE 16.23 ***Crown Gall Tumor Caused by* Agrobacterium**

A crown gall tumor formed by *Agrobacterium* is shown on a tree trunk. © E. R. Degginger, Photo Researchers Inc.

The T-DNA also carries genes that subvert the plant cell into making opines. These are unusual nutrient molecules that are made at the expense of the plant cell but can only be used by bacteria that carry special genes for opine breakdown. The genes for opine degradation are found on the part of the Ti-plasmid that does not enter the plant cell. So the *Agrobacterium* can grow by using the opines but the plant cannot use them. Other bacteria that might infect the plant are also excluded as they do not have the opine breakdown genes either.

Modified Ti-plasmids are widely used in the genetic engineering of plants.

Modified Ti-plasmids are widely used in the genetic engineering of plants. The genes for plant hormones and opine synthesis are removed and the genes to be trans-

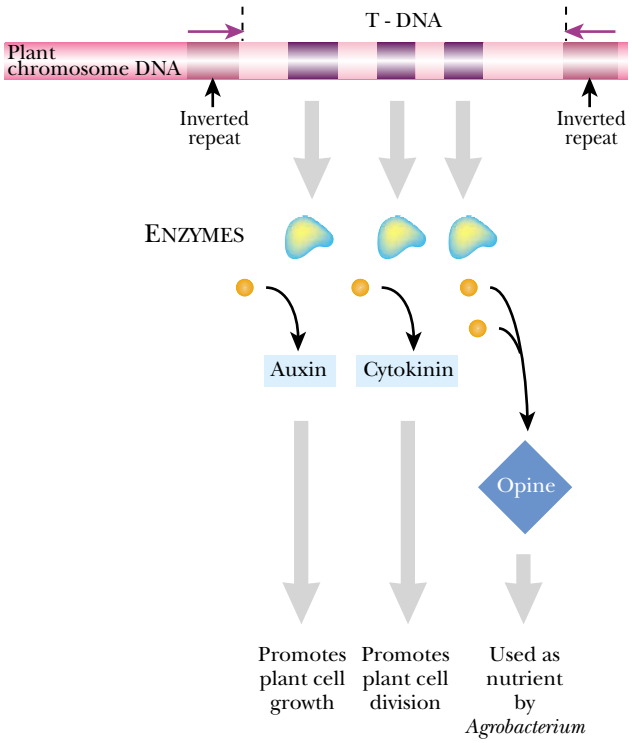


FIGURE 16.24 ***Expression of Genes on T-DNA***

Survival of *Agrobacteria* in the plant requires space for them to grow and a carbon source to provide energy. The genes of the T-DNA region trick the plant cell into providing these factors. The genes for auxin and cytokinin are growth factors that induce the plant cells to grow at the site of infection, providing the space. The opine is a carbon source for the bacteria, providing a constant food supply.

ferred into the plant are inserted in their place. In practice, *Agrobacterium* carrying an engineered Ti-plasmid is used to transfer genes of interest into plants using plant tissue culture.

In addition to inserting external genes into plants the Ti-plasmid system may be used for analysis of plant gene function. Insertion of T-DNA into the plant chromosome may disrupt a plant gene if insertion occurs into the coding sequence (or essential regulatory sequences). The model plant, *Arabidopsis thaliana*, has been used to generate a set of gene knockouts by random insertion of T-DNA. The locations of nearly 90,000 such insertions has been determined (as of 2003). These include insertions into about 22,000 of the estimated 29,500 genes of *Arabidopsis*. These insertions may be used to investigate the functions of the inactivated genes by comparing the knockout mutants with the parental wild type plant.

The 2-Micron Plasmid of Yeast

Many yeast strains contain a small multicopy plasmid, the "2 micron circle".

Plasmids are found in higher organisms, although they are less common than in bacteria. The yeast, *Saccharomyces cerevisiae*, has been used as a model organism for the investigation of eukaryotic molecular biology. Most strains of yeast harbor a plasmid known as the **2μ circle** or **2μ plasmid**. This is a circular molecule consisting of 6318 bp of double-stranded DNA. It is present at 50–100 copies per haploid genome and is located in the nucleus of the yeast cell, where it is bound by histones and forms nucleosomes like chromosomal DNA. The 2μ plasmid has been widely used in genetic engineering as the basis for multicopy eukaryotic cloning vectors. Similar plasmids are found in other species of yeast.

Flippase catalyses inversion of the DNA located between its recognition sites.

The 2μ plasmid contains two perfect inverted repeats of 599 bp that separate the plasmid into two regions of 2774 and 2346 bp respectively (Fig. 16.25). The plasmid encoded Flp protein (**Flp recombinase or "flippase"**) catalyzes recombination between

2 micron plasmid See 2μ plasmid
2μ circle Same as 2μ plasmid
2μ plasmid (or 2μ circle) A multicopy plasmid found in the yeast, *Saccharomyces cerevisiae*, whose derivatives are widely used as vectors
Flp recombinase (or flippase) Enzyme encoded by the 2μ plasmid of yeast that catalyzes recombination between inverted repeats (FRT sites)

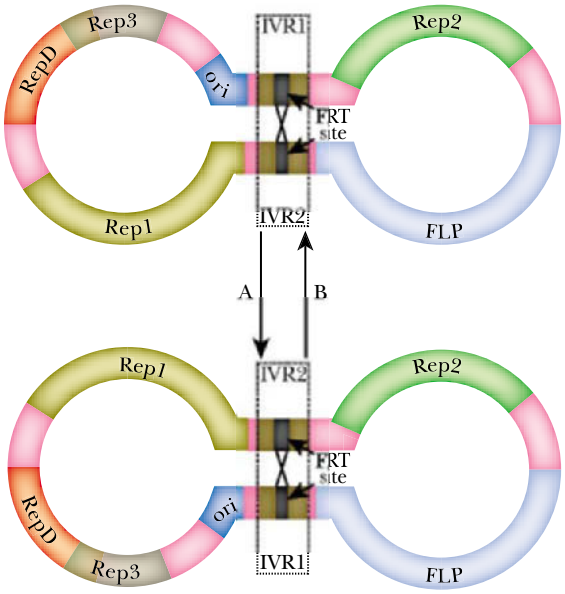


FIGURE 16.25 ***The 2μ Plasmid of Yeast***

Two alternate forms of the 2μ plasmid are inter-converted by recombination. The plasmid has two inverted repeats (IVR1 and IVR2), which can align. The enzyme, Flp recombinase, recognizes the FRT sites (flip recombination target) and makes a crossover that inverts one half of the plasmid relative to the other. Notice the top plasmid has origin (*ori*) close to the Rep2 sequence, whereas, the bottom plasmid has the origin on the other side (close to the *FLP* gene). The Rep1 and Rep2 proteins regulate both the *FLP* gene and the replication of the plasmid itself.

the inverted repeats. Flp recognizes a 48 bp target site (**Flp recombination target, or FRT site**) located within the inverted repeats. The result is the inversion of one half of the plasmid relative to the other. The two forms of the plasmid are found in roughly equal proportions. The Rep1 and Rep2 proteins regulate the expression of the *FLP* gene and also bind to the origin of replication (*ori*) and the *REP3* DNA sequence.

The Flp recombinase is used in genetic engineering to control the expression of a variety of genes by inverting segments of DNA. Flp is functional in bacteria, plants and animals provided the correct recognition sites are present. In addition to the inversion reaction, Flp recombinase will promote site-specific insertion and deletion reactions of segments flanked by **FRT sites**. The Flp/FRT system is similar to the widely used Cre/*loxP* recombinase system of bacterial virus P1.

Certain DNA Molecules may Behave as Viruses or Plasmids

P1 combines the properties of plasmid and virus and can choose either lifestyle depending on the circumstances.

There are several similarities between the behavior of plasmids and viruses. In fact, some circles of DNA can choose to live either as a plasmid or as a virus. The bacterial virus P1 is a good example. It can indeed behave as a virus, in which case it destroys the bacterial cell, replicates by rolling circle mode and manufactures large numbers of virus particles to infect more bacterial cells. This is known as **lytic growth** since the host cells are "lysed" (derived from the Greek for broken).

Alternatively, P1 can choose to live as a plasmid and divide in step with the host cell. In this case, the circular P1 DNA uses bi-directional replication like a typical plasmid (Fig. 16.26). Each descendant of the infected bacterial cell gets a single copy of P1 DNA. The cell is unharmed and no virus particles are made. This state is known as **lysogeny** and a host cell containing such a virus in its plasmid mode is called a **lysogen**.

Changing conditions may stimulate a lysogenic virus to return to destructive virus mode. This tends to happen if the host cell is injured, in particular if there is severe

Flp recombination target (or FRT site) Recognition site for Flp recombinase
FRT site Flp recombination target, the recognition site for Flp recombinase
lysogen Host cell containing a lysogenic virus
lysogeny State in which a virus replicates its genome in step with the host cell without making virus particles or destroying the host cell. Same as latency, but generally used to describe bacterial viruses
lytic growth Growth of virus resulting in death of cell and release of many virus particles

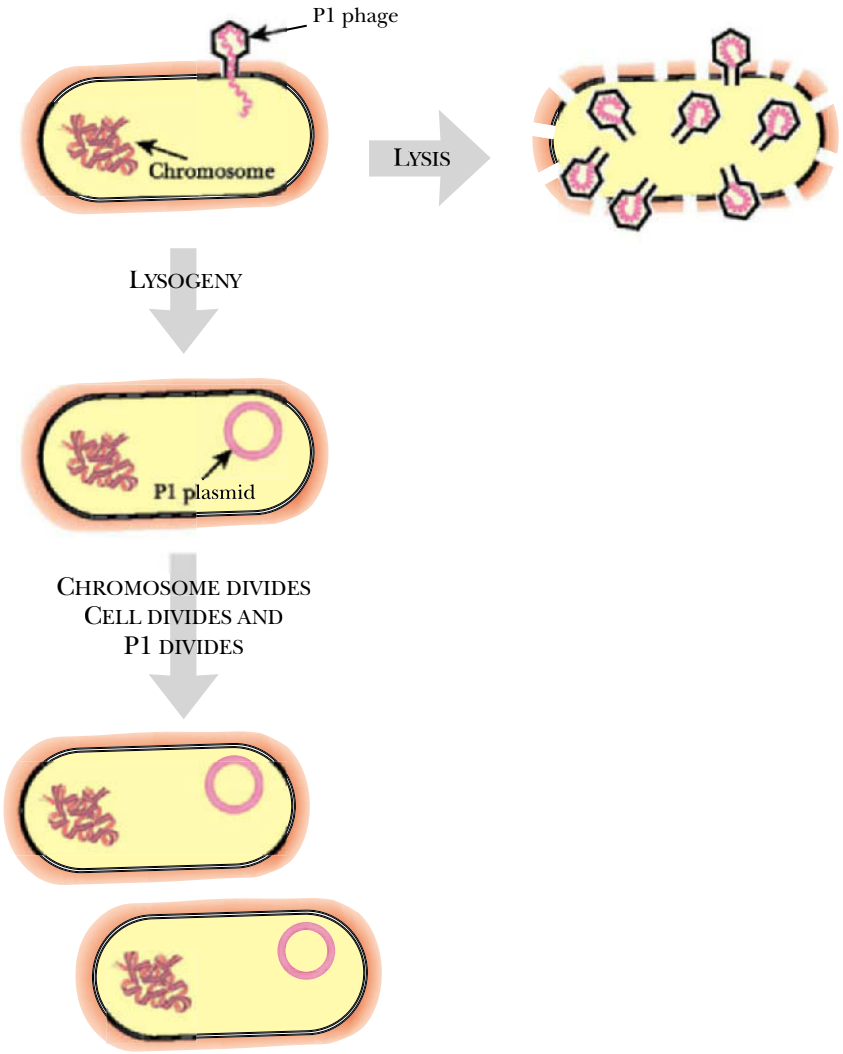


FIGURE 16.26 ***Lysis versus Lysogeny***

Some plasmids, such as the P1 plasmid of bacteria, have a dual personality. P1 can exist in a lysogenic state as a plasmid, using bi-directional replication to divide when the host cell divides. P1 can also grow as a virus and destroy the cell. During such lytic growth, P1 divides by the rolling circle mechanism, creating a large number of copies. It then packages genome-sized units into new virus particles and lyses the bacterial cell.

damage to the host cell DNA. The virus decides to make as many virus particles as possible before the cell dies. If, on the other hand, the host cell is growing and dividing in a healthy manner, the virus will most likely decide to lie dormant and divide in step with its host. Further aspects of virus behavior are covered in the following chapter, Ch. 17.