



FIGURE 6.12

Transfer of T-DNA by the binary vector system. The foreign DNA is cloned into the middle of T-DNA in the plasmid, shown at the top, which contains one or more origins of replication that function in both *E. coli* and *A. tumefaciens*. In the particular vector shown, the original T-DNA sequence has been replaced by a multiple cloning site (MCS), followed by a terminator sequence functional in plants, located between the left border (LB) and right border (RB) sequences. The plasmid also contains an antibiotic resistance gene (in this case, an aminoglycoside phosphoryltransferase, *aph*, to make possible selection of the plasmid-containing cells on aminoglycoside-containing plates). This composite plasmid is introduced into *A. tumefaciens* cells that contain another plasmid, shown at the bottom. This larger plasmid, a derivative of the Ti plasmid, contains only the *vir* region and the origin of replication and is totally devoid of the T-DNA region. Because the two plasmids have no common sequences, there is no recombination and cointegrate formation. Nevertheless, the products of the *vir* genes on the larger plasmid can mediate the transfer, into plants, of the T-DNA sequence of the other plasmid.

Redrawn based on artwork from the first edition (1995), published by W.H. Freeman.

the usual *in vitro* methods is difficult because of its large size. Consideration of these points prompted the development of the binary plasmid approach.

Use of Binary Vectors

This method takes advantage of the fact that the *vir* genes on one plasmid can catalyze the excision and transfer of a T-DNA sequence located on another plasmid; that is, these genes can act in *trans*. The binary plasmid approach consists of cloning the DNA fragment of interest into the T-DNA sequence of a plasmid vector with a broad host range that is capable of replicating in both *E. coli* and *A. tumefaciens*. The plasmid DNA is then introduced into *A. tumefaciens* cells that contain a “disarmed” Ti plasmid with *vir* genes but no T-DNA sequence. The *vir* genes of the disarmed plasmid effect the transfer of the T-DNA from the other plasmid without the formation of a cointegrate intermediate (Figure 6.12). In this method, only the piece of DNA that had been inserted between the left and right borders of the smaller plasmid is transferred into plants, allowing more precise control of the process.

Both of these methods require several modifications in the Ti plasmid. In the Ti plasmid used for the cointegrate method, tumor-producing genes are either inactivated or removed; otherwise, transformed plant cells would become tumors, not healthy “transgenic plants.” In addition, a pBR sequence is inserted into the T-DNA region, as shown in Figure 6.11. In the Ti plasmid used for the binary plasmid strategy, the entire T-DNA region is removed (see Figure 6.12); otherwise, T-DNA from the Ti plasmid would compete with the injection of T-DNA from the smaller plasmid.

General Considerations

Regardless of which strategy is used, the site of insertion of the foreign gene is usually sandwiched between a promoter sequence that functions effectively in plants and a terminator sequence. The 35S protein promoter from the cauliflower mosaic virus (CaMV) was popular in the early experiments because of its reliably high expression in a variety of plants. However, CaMV promoter drives the expression of foreign genes in any plant tissue, a situation that may be unnecessary or unwanted. In more recent attempts, promoters that drive tissue-specific expressions were also used. For example, the promoter for a rice seed protein is expected to drive expression of cloned genes only in rice grains; therefore, it was used for the expression of introduced protein genes for the modification of amino acid content in rice. A popular terminator sequence is the one for nopaline synthetase. (An effective terminator ensures that the 3′-ends of mRNA are processed and polyadenylated so that the mRNA achieves a reasonable degree of stability [see Chapter 3].)

In addition, the region to be introduced into plants must contain a good marker so that plant cells that have received and integrated the foreign genes can be recognized easily. β -Glucuronidase is a marker whose activity can be detected readily in plant tissue. Even more useful are markers that enable