

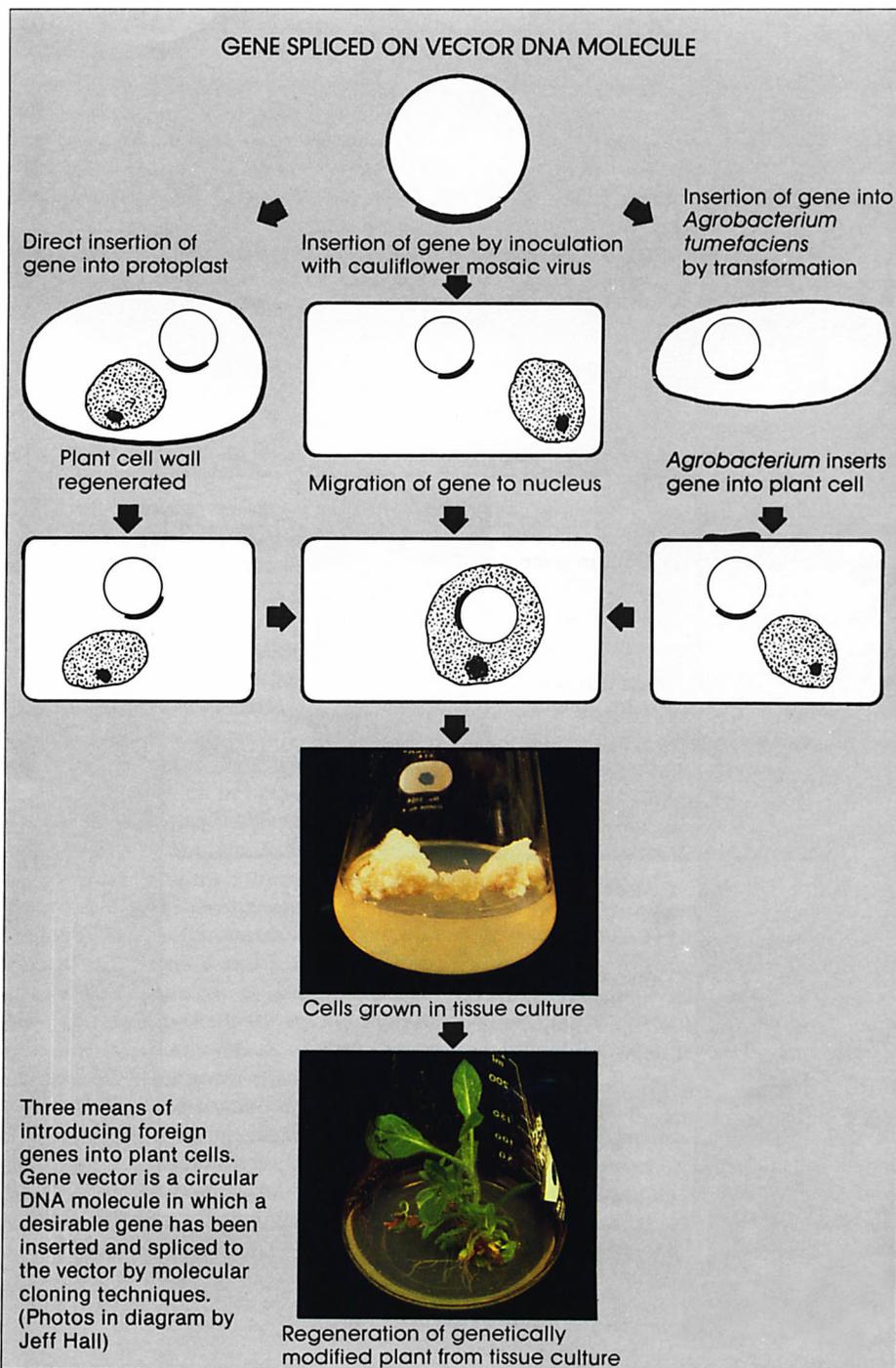
Crossing natural barriers to genetic manipulations

Clarence I. Kado, Professor, Plant Pathology,
Davis Crown Gall Research Group

The potential application of genetic engineering to agriculture has been predicted to be limitless. Such enthusiasm, however, should be expressed judiciously, because much information is needed before we can apply the methods to the agricultural industry. The potentials of this technology are apparent, but several important natural barriers need to be crossed before progress can be achieved. A primary limitation is the need to find an efficient means of introducing foreign genes (or DNA) into plant cells. Since 1978, U.C., Davis, scientists have devised several ways by which this can be accomplished. Foreign genes may be introduced as purified DNA directly into plant cells through the use of protoplasts (plant cells freed of their rigid cell wall material). Or these genes can be spliced to another DNA molecule, which serves as a vector. These gene vectors may be plant DNA viruses, bacterial and yeast plasmids, plant organelle DNA, and transposition elements from lower and higher cells.

In the first case, foreign genes are spliced to a bacterial plasmid (a DNA molecule capable of autonomous replication in a bacterial cell), and the hybrid plasmid (now consisting of the foreign gene joined to the plasmid) is amplified in the bacterial cell under controlled conditions as the bacterium multiplies; millions of copies of the gene are manufactured in these bacteria. When these amplified hybrid plasmid molecules are isolated and purified, they may be introduced directly into protoplasts, which in turn are regenerated into a plant.

The second approach for gene insertion is by means of a plant virus containing DNA as its chromosome. For example, a gene may be spliced onto the DNA of cauliflower mosaic virus. This virus infects primarily plants of the mustard family (Cruciferae), causing mosaic symptoms. Since it is the most thoroughly studied plant DNA virus, plant pathologists have investigated its potential use as a vector of foreign genes. In recent studies, several unexpected problems have arisen. One is that only small genes or a portion of a





The bacterium that causes crown gall in potato and other plants has potential as a vector for transfer of desirable traits from one plant to another.

gene can be spliced to the virus vector. Also, the added gene affects the cell-to-cell movement (invasiveness) of the virus, since the virus can no longer mature. Moreover, the virus is mainly limited to Cruciferae as host plants and therefore the prospects of infecting other major crops are narrow. Nevertheless, in-depth studies on this virus will help provide important information on means to overcome these natural obstacles.

Another promising gene vector is the Ti plasmid harbored in the crown gall bacterium *Agrobacterium tumefaciens*. Ti plasmids are very large, autonomously replicating, circular DNA molecules about 30 times larger than the DNA of cauliflower mosaic virus. This plasmid carries genes that cause crown gall tumors in many plants, representing more than 90 plant families. Although the mechanism by which these tumors occur is not well understood, *A. tumefaciens* naturally introduces the Ti plasmid DNA into plant cells during infection. A portion of this plasmid, known as the T-DNA, which carries genes for synthesis of unusual amino acids (opines) and phosphorylated sugars, is incorporated into the nuclear DNA of the plant cell. Thus, during infection of a wound, the crown gall bacterium "genetically colonizes" the plant by converting normal cells into tumor cells that are directed to produce the opines and phosphorylated sugars, which are assimilated by the infecting bacterium.

With the knowledge that *A. tumefaciens* inserts a piece of its genetic material into plants, Davis plant pathologists and other workers have isolated the portion of the Ti plasmid that is transferred into plant cells. Foreign genes that confer resistance to certain antibiotics, such as methotrixate and chloramphenicol, and that direct the synthesis of seed proteins have now been inserted in the T-DNA on the Ti plasmid. *A. tumefaciens*

cells carrying this hybrid plasmid are allowed to insert the chimeric T-DNA into tobacco, petunia, cowpea, and sunflower cells by natural infection. Although such genes have been transferred to plants, these genes are not stably maintained, particularly during meiosis (chromosome reduction, division, and segregation); however, the genes seem to be more stable in crop and ornamental plants that are vegetatively propagated.

As with cauliflower mosaic virus, there are several limiting factors. The Ti plasmid carries genes that cause tumors in plants, and it will need to be "disarmed." Once disarmed, an efficient means of selecting transformed cells will have to be developed. Also, most plants susceptible to crown gall have not been regenerated successfully from cell culture, an essential step in the development of useful plants. Finally, stability of the T-DNA in transformed plants needs to be enhanced.

Other potential gene vectors being studied are transposition elements and organelle DNA. Well-defined segments of DNA that can insert themselves in random locations along a chromosome are known as transposition elements (or "transposons"). Organelles such as chloroplasts and mitochondria carry DNA with characteristics much like plasmids. The most promising aspect of these studies is that of harnessing existing plant DNAs as vehicles of genes that can be attached to them.

In all of these examples of gene vectors, the pressing problem has been to find a suitable gene capable of conferring the desirable trait(s) on a plant. To solve this problem further studies are required on gene maintenance in plant cells. Two of the promising gene vectors are reported in the articles that follow.

The Ti plasmid

John W. Einset

Crown gall, a bacterial disease of dicots and gymnosperms, is characterized by tumorous overgrowths on infected plants. Because the disease involves gene transfer from a bacterium to a plant cell and subsequent expression of new characteristics, crown gall has tremendous potential as a vector for genetic manipulation of important agricultural crops.

Under natural conditions, *Agrobacterium tumefaciens* cells in the soil enter plant tissues through wounds and attach themselves to

specific sites on the cell walls. A circle of DNA in the pathogen known as the Ti plasmid then mobilizes the transfer of a piece of DNA into the plant cell, where it becomes attached to the plant's nuclear DNA. The subsequent expression of this implanted DNA, the T-DNA, results in proliferation of a tumor and production of unusual compounds known as opines, which are used by *A. tumefaciens* as food sources.

Scientists have already demonstrated that this natural mechanism of DNA transfer can be harnessed as a vector for foreign genes: Ti plasmids can be genetically engineered to mobilize genes other than those normally transferred during the disease process. Unfortunately, no one has yet obtained clear-cut evidence for expression of these foreign genes in crown gall cells. For this reason, the next step will be to develop methods for regeneration of crown gall cells into normal-appearing plants that retain and express beneficial foreign genes.

In our research, we have used crown galls of tobacco plants to answer fundamental questions about the physiology of the disease. Two questions pertaining to crown gall genetics have been of particular concern: (1) Can T-DNA genes be retained and expressed in structurally normal tissues? (2) What is the hormonal basis for the abnormal growth associated with crown gall?

Experiments related to these questions have utilized crown gall tissue cultures maintained on a semisolid nutrient medium consisting of mineral salts, vitamins, and sucrose. We derived these bacteria-free tissue cultures about four years ago from tumors on tobacco plants representing seven strains of *A. tumefaciens*. Because we conduct individual experiments on a variety of types of crown galls, we feel justified in making general conclusions about the physiology of the disease.

Under appropriate conditions of temperature, lighting, and hormone concentrations, unorganized crown galls can be regenerated into normal tobacco plantlets with discernible leaves, stems, and roots. In most cases, unfortunately, these plants seem to be free of all tumor characteristics. If however, one uses crown galls produced by a specific strain of *A. tumefaciens*, one can regenerate plantlets retaining crown gall characteristics, such as the nonrequirement for hormones (auxin and cytokinin) by explants in tissue cultures and the ability to produce radioactive opines from radioactively labeled arginine fed to the tissues.

These observations demonstrate that the abnormal growth of crown galls can be overcome without affecting other tumor-borne characteristics. They also give us confidence that future attempts to introduce foreign