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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

genes via crown gall will lead to plants that retain and express new characteristics. In fact, similar demonstrations of T-DNA expression in normal plant tissues have now been accomplished in at least five other laboratories worldwide.

Because the abnormal growth of crown gall tissues is a major obstacle to crown-gall-mediated plant genetics, we have also investigated the unusual hormone metabolism associated with the disease to determine whether it is the cause of the abnormal growth. The fact that crown gall tissues differ from nontransformed plant tissues in being able to grow on a simple nutrient medium lacking auxin and cytokinin suggests that crown galls produce these hormones at elevated rates. Our own studies on cytokinins in a variety of crown galls indicate that these tissues generally overproduce cytokinins at levels ranging from 8- to 1,600-fold greater than normal. The predominant cytokinins in crown galls have been purified and identified as zeatin and ribosylzeatin, which are N6-substituted derivatives of adenine and adenosine, respectively. In addition, crown galls with extremely high total cytokinin contents contain glucose derivatives of both of these cytokinins.

Presumably, the hormone imbalance resulting from T-DNA expression in crown gall cells underlies the abnormal growth we observe. We have detected genes on the Ti plasmid affecting cytokinin biosynthesis by the pathogen, and C. I. Kado has shown that plasmid genes for auxin biosynthesis also exist. Identifying these genes and their products is a first step toward controlling them. Ultimately, we would like to have effective Ti plasmid vectors that introduce desirable genes into plants without also making tumors.

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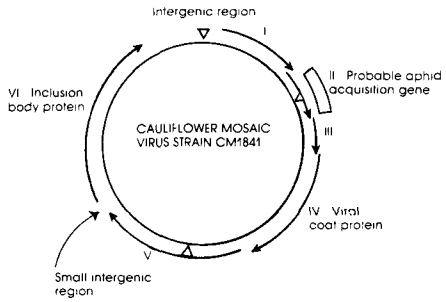

Physical map of circular chromosome of cauliflower mosaic virus. Genes defined by nucleotide sequence are indicated by arrowed lines I to VI. Box indicates naturally occurring deletion of most of region II (strain CM4-184). Open triangles indicate three single-stranded interruptions.


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Leaves from turnip plants infected with cauliflower mosaic virus. Leaf on left is infected with native, wild-type virus. Those on right are from plants infected with mutants produced by insertion of 12 additional nucleotide pairs into the chromosome of the same virus strain. Plants infected with the mutant on the right grow almost as rapidly as healthy plants.

DNA plant viruses

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A remarkably simple genetic system for study of DNA multiplication and gene expression in plants is provided by DNA plant viruses. These viruses have only a half-dozen or so genes that are believed to be regulated in the same way as other plant genes. The DNA replicates in nuclei and may be associated with nuclear proteins (histones) in the same way as plant genetic material. Thus, the virus provides a small-scale, readily manipulated model for gene expression.

Viruses reproduce in living tissue by supplying a few of their own functions, while filling most of their needs by parasitizing the host. Each viral particle contains a small loop of nucleic acid, the genetic component, enclothed in an outer shell of coat protein. The protein is shed soon after the particle enters the cell, and the DNA sets about reprogramming the cell to manufacture virus. In carrying out these changes, the virus interferes with normal cellular functions so that the cell becomes less well coordinated. The organism as a whole is affected and shows disease symptoms. Reductions in growth rate and leaf puckering and yellowing are common effects of the DNA viruses.

One DNA plant virus, the cauliflower mosaic virus, has received more attention than the others. Its biology has been intensively studied during the last few years, and the DNA from two strains of this virus has been completely sequenced. At Davis, for example, an isolate has been found to have 8,031 pairs of nucleotides in its circular chromosome. Nucleotides make up the language with which genetic information is expressed. This sequence of nucleotides and other information have been used to construct a physical map of the virus chromosome (see diagram). Of the half-dozen genes of this simple virus, one (gene II) is not essential for reproduction, enclothement in protein, or cell-to-cell movement. Recent work at Davis indicates that this nonessential gene is probably involved in insect transmission of the virus in nature. These dispensible regions of the virus chromosome provide sites for insertion of foreign DNA, which is carried into the plant and replicated along with the DNA of the infecting virus.

Another region of the cauliflower mosaic virus chromosome has been identified as being responsible for the severity of disease. This region is gene VI on the physical map. The other five genes appear to have little, if any, effect on symptom induction. A single change in gene VI can have a profound effect on disease expression: in one case, insertion of 12 base pairs at a particular location in gene VI almost abolished disease. Plants infected with this mutant of the virus show very mild symptoms and grow at the same rate as healthy plants. Information of this sort may enable the investigator to control disease expression, if portions of the virus chromosome are eventually used as a recombinant DNA vector for plants.

Cauliflower mosaic virus has been used as a vehicle to reproduce foreign DNA in plant cells and to carry this foreign DNA from cell to cell throughout the entire plant. However, not enough foreign DNA can be inserted into the virus chromosome to bring about useful transformations of plants. This limitation appears to be related to a low capacity of the virus particle to accommodate additional DNA. Assembly of DNA and coat protein to form virus particles seems to be necessary before the DNA will move from cell to cell in the plant. It does not appear, therefore, that the virus in its present form will be useful as a recombinant DNA vector. However, this virus will probably play an important role in defining the biological activity of those sequences involved in replication and expression of DNA in plants.

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