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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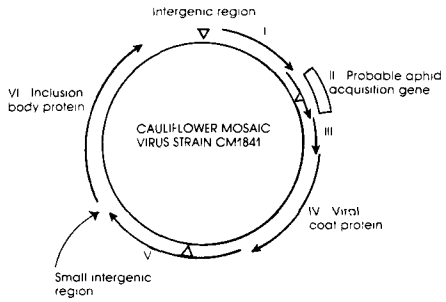

Physical map of circular chromosome of cauliflower mosaic virus. Genes defined by nucleotide sequence are indicated by arrowed lines I to VI. Box indicates naturally occurring deletion of most of region II (strain CM4-184). Open triangles indicate three single-stranded interruptions.


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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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