Crown Gall Disease: Nature as Genetic Engineer

Not very many people know it, but plants get a form of cancer called crown gall disease. This condition is characterized by the formation of tumors, or galls, that usually appear near the plant crown, which is the region of the plant where the stem and root merge. For many years crown gall disease has been studied both because it is an economically important disease of plants and because it is a model for animal cancers. The results obtained from these studies have generally supported the hypothesis that plant and animal cancers have many common characteristics.

Recently, another interesting finding about the plant tumors has come to light. They appear to be the result of a natural form of genetic engineering involving the transfer of DNA from bacteria to plant cells. This has suggested to many investigators that it may be possible to adapt this transfer process in order to introduce desirable genetic traits—such as the ability to fix nitrogen—into plants.

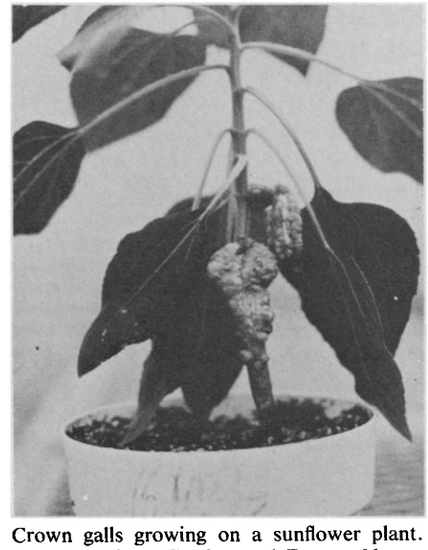
The bacterium *Agrobacterium tumefaciens* was identified as the cause of crown gall disease more than 70 years ago, but the manner in which the organism produces the disease remained a mystery for a long time. Only in the past few years did evidence suggesting a DNA transfer begin to accumulate. Among the milestones on the path leading to the current findings was a discovery in the 1940's by Armin Braun and his colleagues at the Rockefeller Institute (now University). They showed that once the bacteria induce the formation of plant tumor cells, the continued presence of the bacteria is not required for tumor growth. Tumor tissue will grow in laboratory dishes in the absence of bacteria, for example. And these bacteria-free tumors will continue to grow after they are grafted back onto healthy plants.

These results implied that *A. tumefaciens* causes permanent alterations in infected plant cells with the result that they acquire such characteristics of tumor cells as uncontrolled growth. Braun proposed that the bacteria effect these changes by producing a tumor-inducing principle (TIP) that they transmit to the plant cells.

At the time Braun made his suggestion the chemical nature of the gene was still being debated. Once it was learned that genes were made of DNA, the possibility arose that TIP might also be DNA, because a transfer of genetic material could explain how the plant cells become permanently altered. However, early efforts to identify bacterial DNA, or other bacterial components, for that matter, in the plant tumor cells failed.

Then, in 1974, Jeff Schell, Marc Van Montagu, and their colleagues at the University of Ghent, Belgium, produced evidence suggesting that large plasmids found in many strains of *A. tumefaciens* are responsible for the tumor-producing properties of the bacteria. (Plasmids are circular DNA molecules located outside the bacterial chromosome. They are known to carry a number of genes, such as the genes for antibiotic resistance.)

Further research in Ghent and in the laboratories of Milton Gordon and Eugene Nester at the University of Washington, Robbert Schilperoort at the University of Leiden, The Netherlands, and Allen Kerr at the University of Adelaide, Australia, supported this hypothesis. The investigators showed that infective strains of *A. tumefaciens* that lost the plasmid also lost the ability to cause tumors. Conversely, when the plasmids were introduced into noninfective strains, these strains gained the ability to cause crown gall disease. Although these experiments did not provide information about how the plasmids, now called Ti (for tumor-inducing) plasmids, act in conferring the ability to produce tumors on the bacteria, they raised the possibility that plasmid DNA could be Braun's TIP.



Crown galls growing on a sunflower plant. [Source: Milton Gordon and Eugene Nester of the University of Washington]

Plasmid exchange between different bacterial strains and even between different bacterial species is a well-known natural phenomenon. But the suggestion that bacterial Ti plasmids or portions of them might be entering and functioning in plant cells was highly controversial. There are many barriers to such a happening. They include the plant cell walls and membranes, and the plant enzymes that can destroy foreign DNA. In addition, the control of gene expression in simple nonnucleated bacterial cells is generally thought to differ from that in more complex nucleated plant or animal cells. Even if the plasmid DNA were to enter the plant cells and survive, there were doubts that it could function in the alien plant environment.

Nevertheless, there is general agreement that plasmid DNA does enter infected cells. Mary-Dell Chilton of the University of Washington, with Gordon and Nester, has detected plasmid DNA in cells that have been transformed to the tumorous state by *A. tumefaciens* infection. Normal plant cells do not contain the plasmid DNA segments.

Acquisition of the plasmid DNA is thought to be the cause of the transformation of the plant cells. In experiments performed in Seattle and Ghent, mapping of the plasmid DNA has shown that all the Ti plasmids studied thus far have a number of DNA segments in common. Chilton, Gordon, and Nester have shown that one of these common segments is part of the plasmid DNA transferred to plant cells. In addition, Schell and his colleagues have found that mutations in this common region destroy the tumor-inducing properties of the plasmids. Thus, the production of plant tumors by the acquisition of plasmid DNA might be analogous to the production of some animal tumors as a result of the uptake of nucleic acids from tumor viruses.

Another line of evidence indicating that bacterial DNA is transferred to plant cells and also expressed there originated in observations by researchers at the Institut National de la Recherche Agronomique in Versailles, France. Jacques Tempé and Annik Petit, in collaboration with the late George Morel, found that crown galls induced by some strains of *A. tumefaciens* synthesize an unusual amino acid called octopine, whereas tumors induced by other strains produce a different "opine" called nopaline. These compounds are not produced by normal

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mature plant cells. Moreover, the Versailles workers showed that the bacterial strains that induce octopine-producing tumors can use octopine, but not nopaline, as their sole source of energy, whereas the reverse is true for strains that induce nopaline-producing tumors. The work suggested that the bacteria transfer the genes coding for the opine-synthesizing enzymes to the plant cells where the genes direct the synthesis of the enzyme proteins. There are alternative explanations for the effect, however. The bacteria might only activate plant genes that are normally turned off in uninfected cells.

The question of whether the genes for opine synthesis are of bacterial or plant origin is an important one. If they are bacterial genes, then the plant's synthetic machinery must be capable of translating the information of a foreign DNA into the structure of a functioning enzyme protein. There are indications that this is possible. The Seattle and Leiden groups and John Kemp and his colleagues at the University of Wisconsin have detected RNA copies of the plasmid DNA in transformed plant cells. Thus, at least the first step of gene expression appears to be carried out by the plant cells. But this does not necessarily mean that the final steps of the process are completed and the foreign proteins synthesized. Synthesis of the foreign proteins is a requirement that must be met if the Ti plasmids are ever to be used as vehicles for genetic engineering.

Although some investigators still have reservations about the matter, there is evidence that the genes coding for the enzymes needed for opine synthesis are carried on the Ti plasmids of *A. tumefaciens*. For example, Schell and his colleagues have produced a number of mutant plasmids by inserting transposons into the plasmid DNA. (Transposons are segments of DNA that can reversibly insert themselves into target DNA at many different sites.)

One of the mutant plasmids lost the ability to induce nopaline synthesis in plant cells but retained its ability to transform the cells. The Ghent workers showed that the transposon was inserted in the Ti plasmid in a region adjacent to the common segment that is found in transformed plant cells. Moreover, they also identified the transposon in cells transformed by the mutant plasmid. Thus, Schell concludes that the plasmid DNA entering the plants contains a minimum of two independent sets of genes. One set includes the genes required for transformation of the cells; these are the genes in the common segment. The other set of genes codes for the enzymes required for opine synthesis.

All this means that *A. tumefaciens* practices an unusual form of parasitism. Nester calls it "genetic parasitism," and Schell characterizes it as "genetic colonization." By transferring a bit of its DNA into infected cells, the bacteria induce the plant to synthesize a compound that the plant cannot use but which provides the bacteria with a source of energy and building blocks for growth.

Although most of the evidence indicates that the genes for opine synthesis are plasmid genes, there are still some dissenters. For example, James Lippincott and Barbara Lippincott of Northwestern University have suggested that the genes are plant genes activated as a result of uptake of the plasmid DNA. The Lippincotts have identified octopine in embryonic bean cells. They propose that the genes for octopine synthesis are turned off during development and then turned back on by the plasmid DNA.

This proposal agrees with their hypothesis that the tumorous state of plant cells represents a return to the embryonic or undifferentiated condition. (Similar suggestions have been made for animal tumor cells.) The Lippincotts have also found that the cell walls of transformed plant cells lose their ability to bind *A. tumefaciens*, which is the first step of infection by the bacterium. In this regard the transformed cells resemble embryonic cells but not normal differentiated cells. Other investigators have not been able to duplicate the Lippincotts' finding of octopine in embryonic cells, however.

One of the major reasons for the current interest in Ti plasmids is the possibility that they may be useful as vectors for transferring desirable genes into plant cells. These might be genes conferring resistance to pests or disease, for example, or the ability to fix nigrogen and allow plants to make their own nitrogen fertilizer. Schell's group has already shown that the Ti plasmid will transfer the transposon DNA it carries into plant cells, this being the first demonstration that the plasmid can be used to introduce a specific set of genes into the cells.

The plasmid would not be of much value for genetic engineering of plants if only tumor cells resulted, however. But there is evidence from Braun's laboratory that plants, which appear to have normal structures, can be regenerated from some kinds of plant tumor tissue. Both the Seattle and Ghent groups have shown that cells from these apparently normal plants still carry Ti plasmid DNA and synthesize nopaline.

Another approach to using Ti plasmids for genetic engineering is to introduce the plasmid DNA into plant protoplasts, plant cells from which the outer cell wall has been digested away. Under the appropriate conditions, whole plants can be regenerated from some kinds of protoplasts. A number of investigators, including Clarence Kado and his colleagues at the University of California at Davis, Edward Cocking of the University of Nottingham, England, and Toshiyuki Nagata of the University of Tokyo, are finding that protoplasts will take up Ti plasmid DNA. Kado says that the protoplasts contain RNA copies of plasmid DNA, a finding that shows that at least the first steps in gene expression can be carried out.

Finally, it may be possible to engineer Ti plasmids that can infect plant cells without transforming them. Studies of the nature of the genes and gene products involved in bacterial binding, plasmid DNA transfer, and the other steps required for transformation may help in this regard.

Many problems will have to be solved before Ti plasmids can be used for introducing desirable genes into plants, however. For example, studies of the plants regenerated from tumor tissue indicate that the plasmid DNA they carry is lost during seed production. This could mean that the use of Ti plasmids for genetic engineering of plants will have to be limited to those plants that can be reproduced by such means as cuttings. Another limitation may be imposed by the fact that *A. tumefaciens* rarely infects monocotyledons (plants with embryos having only one cotyledon or seed leaf). Although many of our most important crop plants, including corn and wheat, are monocotyledons, investigators think that Ti plasmids may eventually play an important role in the genetic improvement of plants.

—JEAN L. MARX

Hammond to Develop New AAAS Magazine

Allen L. Hammond, originator and editor of Research News, is leaving the section to develop a new popular magazine of science for the AAAS. In his 8 years of service, Research News has grown from an occasional and experimental section to an award-winning feature of the magazine. He will continue to serve *Science* as contributing editor.