Ti Plasmids as Gene Carriers

When suitably modified, tumor-inducing plasmids can transfer genes into plant cells from which normal plants may be regenerated

A few years ago researchers discovered that *Agrobacterium tumefaciens* causes crown gall tumors in plants by transferring a piece of DNA from a bacterial plasmid (called the Ti for tumor-inducing plasmid) into plant cells. If the bacterium could use the plasmid to genetically engineer tumorous changes in plants, researchers postulated, they might be able to use it to transfer beneficial genes into plants.

Since then, much effort has been directed at developing the Ti plasmid as a gene carrier. At a recent symposium* on genetic engineering in agriculture, Jozef (Jeff) Schell of the Max Planck Institute for Plant Breeding in Cologne, West Germany, and the State University of Ghent, Belgium, described how the Ti plasmid can be modified to put active genes into the cells of whole plants. "Any DNA sequence can now be transferred without further ado," Schell concluded. In addition, the Ti plasmid is turning out to be a useful tool for probing the control of cell development in plants.

Transferring new genes into plant cells is not likely to be of much practical value if the cells also become tumorous as a result of acquiring the new DNA. To circumvent this problem, Schell, Marc van Montagu, and their colleagues set out to determine if they could separate the tumorigenic activities of the Ti plasmid from its gene-transferring capabilities. They found that this is possible.

Crown gall tumors consist of a mass of apparently similar, undifferentiated cells from which normal shoots and roots do not develop. "One of the reasons you have a tumor," Schell explains, "is that the plasmid DNA actively prevents differentiation."

Many of the undifferentiated tumor cells carry plasmid DNA in their genomes. The plasmid sequences do not enter the cellular DNA at any specific location, Schell says, and they do not undergo major rearrangements when they integrate. Other cells of the tumor do not carry plasmid DNA, however. Its presence in some, but not all, of the tumor cells implies that it specifies the formation of some diffusible substance (or substances) that can block differentiation in nearby cells.

One Ti plasmid studied by the Schell group transfers seven genes, collectively called T DNA, into plant cells. When Schell and his colleagues systematically introduced mutations into each of the seven genes of the T DNA, they found that five of them were involved in blocking differentiation. Knocking out one particular gene allowed cells not carrying the T DNA to differentiate into normal roots. According to Schell, "Plants may have a relatively simple coordinated program for differentiation. The whole developmental pattern for root formation can be controlled negatively by one gene." Mutations in either of two additional genes permitted the differentation into shoots of cells that do not carry T DNA. And, finally, if two more genes were mutated, even cells that contain T DNA developed normally.

The segments of the Ti plasmid that are required for DNA integration are short sequences to the immediate left and right of the T region, Schell says. When all five of the genes involved in suppressing differentiation were mutated, T DNA could still enter tobacco cells. Another gene of the T region, this one coding for the enzyme opine synthase, retained its activity and directed the synthesis of its protein product in the cells. Opine synthase is not a normal constituent of plant cells.

Moreover, tobacco cells that were infected by the altered T DNA could be regenerated into whole plants, whose tissue also produced the plasmid-specific enzyme. The progeny of these plants inherited both the T DNA and the ability to make opine synthase. Schell says, "The new gene was transmitted through both the pollen and the egg without loss." Sexual transmission of an introduced gene is a prerequisite if genetic engineering of new traits is to succeed.

The expression of the opine synthase gene was under the control of its own regulatory sequences, which are apparently different from those of bacteria and of animals. In an early experiment, the Schell group introduced a bacterial gene that confers resistance to the drug methotrexate into a Ti plasmid and showed that cells that were infected by the plasmid acquired methotrexate resistance. This suggested that the regulatory sequences of the bacterial gene could be used by plant cells to turn on expression of the gene.

Additional work has shown that this result may have been a fluke, however. Other bacterial genes that were put into Ti plasmids were not expressed. Nor was an interferon gene. The researchers tried this gene in the plasmid, not because they hoped to use plant cells as a source of commercial quantities of interferon, which is potentially valuable as an antiviral agent or as a drug for cancer chemotherapy, but because they wanted to see if animal regulatory sequences would work with plant synthetic machinery. They did not.

Several other investigators are also trying to use Ti plasmids to introduce foreign genes into plant cells. For example, at Beltsville, John Kemp of the Agrigenetics Corporation of Madison, Wisconsin, described an attempt to introduce a gene coding for the bean protein phaseolin into sunflower plants. The experiment, which was performed in collaboration with Timothy Hall of the University of Wisconsin at Madison, succeeded in that the transferred gene directed the synthesis of messenger RNA in the resulting tumor cells. The protein was not made, however, and the tumor cells did not regenerate into whole sunflower plants. Modifications of the Ti plasmid, such as those described by Schell, ought to help overcome these obstacles, at least for plants that are easy to regenerate from cultured cells.

The main problem limiting the potential use of Ti plasmids in genetic engineering is the restricted specificity of the transmitting bacterium. *Agrobacterium tumefaciens* infects only dicotyledonous plants. Unfortunately, many important crop plants, including the cereals, are monocotyledonous.

Other approaches, such as introducing the recombinant plasmid directly into cultured plant cells, may be possible, although this will require that whole plants be regenerated from the cultured cells. Currently, many plants, especially the cereals, are proving refractory to attempts to get them to regenerate from single cells. Most investigators think this problem will eventually be overcome, however.—JEAN L. MARX

*The symposium, "Genetic Engineering: Applications to Agriculture," was held on 16 to 19 May at the Agricultural Research Center of the U.S. Department of Agriculture in Beltsville, Maryland.

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