A Transposable Element of Maize Emerges

A long-sought transposable element of maize has been isolated; it may permit the development of a gene transfer system for this crop plant

During the past few years transposable elements from bacteria, yeast, and the fruit fly have been isolated and cloned, but the elements of maize remained elusive even though Barbara McClintock of Cold Spring Harbor Laboratory discovered these bits of movable DNA in maize more than 30 years ago. That deficiency is now being overcome, according to W. James Peacock of CSIRO's Division of Plant Industry in Canberra, Australia.

Peacock told the Fifteenth Miami Winter Symposium, which was held in Miami Beach on 16 to 21 January, that he and his colleagues had cloned a maize Ds element when they were doing experiments aimed at developing a gene transfer system for this important crop plant. The Ti plasmid, which is now ready to apply as a gene transfer vector (see box on p. 830), may not be useful for maize and other grains because these plants are monocots, and *Agrobacterium tumefaciens*, the bacterium that transmits Ti plasmids, normally infects only dicotyledenous plants.

One of the requirements for a gene transfer system is a method for identifying those cells that have acquired the new gene. The Canberra group's approach is to use maize cells that lack the enzyme alcohol dehydrogenase (ADH). The enzyme-deficient cells cannot grow in the absence of oxygen, whereas cells that have the enzyme can grow anaerobically. The idea then is to transfer the *adh* gene for this enzyme together with some other desired gene into deficient plant cells and use the ability to grow anaerobically to select those cells that have acquired the transferred genes.

To transfer the *adh* gene, the Peacock group first had to clone it. They cloned the genes coding for both of the two types of ADH enzyme found in corn; these genes are designated *adh1* and *adh2*. They also had available a Ds-induced mutant of the *adh1* locus that was identified by John Osterman and Drew Schwartz of Indiana University. As described by McClintock, the Ds transposable element remains stationary in the *bronze* locus of maize unless a second element, designated Ac, is also present in the genome. Then the Ds element moves, causing a variety of chromosomal abnormalities, including insertions, rearrangements, deletions, and breakages. To obtain the mutant, Peacock says, Osterman and Schwartz used an Ac-positive strain and "just sat there and waited until the bus stopped at *adh1*."

The CSIRO workers then compared the DNA of the mutated *adh1* locus with that of the cloned normal gene. They found in the mutant an insertion of 402 base pairs (bp) of DNA that terminated in inverted repeats of 11 bp. The insert is bounded at each end by an 8-bp direct repeat of preexisting *adh1* DNA. In other words, the insert has the characteristic features of a transposable element.

A reversion of the mutant, which was also identified by Osterman and Schwartz, had lost the insert, the CSIRO workers found. "The insert goes out by a fairly precise excision, leaving the duplicated region," Peacock says.

Other investigators have found inserts that may also be Ds elements, although they are much longer than the relatively short one cloned by the Peacock group. For example, Mavis Shure, Susan Wessler, and Nina Fedoroff of the Carnegie Institution of Washington (Baltimore) have found a 2500-bp insert in the *waxy* locus, which codes for an enzyme needed for amylose synthesis. This insertion is excised in revertants of the mutation, Federoff says, although not precisely. The protein product is somewhat larger than normal.

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In previously published work,* Benjamin Burr and Frances Burr of Brookhaven National Laboratory reported that they found very large inserts in the *shrunken* locus of maize, which codes for the enzyme sucrose synthetase. The Burrs examined four Ds-induced mutants of the *shrunken* locus that had been isolated by McClintock. The inserts they describe are around 20,000 bp in length and differ from each other in structure, as determined by restriction mapping, even though they had a common genetic origin. The investigators hypothesize that the four inserts may be derived from one DNA sequence that underwent rearrangements.

Variation in length would not be unprecedented for a transposable element, although a variation as wide as 400 to 20,000 bp might be unusual. There are several parallels between the Ac-Ds element system of maize and the P element system of the fruit fly, which also has two types of elements (*Science*, 22 October 1982, p. 364). The smaller type of P element, which ranges in size from about 500 to 1500 bp, is apparently the equivalent of the Ds element. It moves about the fruit fly genome but only in the presence of the large, complete P element, which is about 3000 bp long and corresponds to the Ac element of maize. Fedoroff predicts, "Ds will probably turn out to be as variable in length as the P elements."

The smaller P elements are derived from the large one by the loss of interior sequences; all the elements terminate in the same inverted repeats of 31 bp. If the Ac-Ds system follows the same structural pattern as the P system, then the Ds elements of various lengths should terminate in the same 11 bp repeat as that seen in the *adh1* locus insert. And it should be possible to use the cloned Ds elements as probes to identify and clone the Ac element.

However, there still remains a puzzle concerning the nature of the Ds-induced mutations of the *shrunken* locus. Peter Starlinger's group at the University of Cologne has cloned the DNA of the *shrunken* locus of one of the same mutants studied by the Burrs. The mutant clone, Starlinger says, "contains a piece of DNA that is not present in the wild-type locus." But, according to Fedoroff, who is also studying the Ds-induced mutations of the *shrunken* locus and who has collaborated with the Starlinger group on some of the work, the alteration appears to be more complex than the simple, although large, insertions described by the Burrs. The mutation results, she explains, in a break in the DNA of the gene as well as a duplication of part of the broken gene and the newly introduced sequence adjacent to it.

When the mutation reverts to the wild type, breakage continues to occur. "Reversion to the wild type puts the gene back together, but keeps the duplicated

*B. Burr and F. A. Burr, *Cell* **29**, 977 (1982).

New Era for the Ti Plasmid in Gene Transfer

The Ti plasmid is now ready to apply to the genetic engineering of new traits into plants, according to reports presented by three groups of investigators at the Miami Winter Symposium. All three used the plasmid to introduce modified bacterial genes coding for antibiotic resistance into plant cells. The introduced genes were expressed, conferring antibiotic resistance on the recipient cells. The achievement is significant, not only because it shows that the products of foreign genes can be made in plant cells, but because it provides one of the prerequisites of a successful gene engineering system: an easy way of selecting those cells that carry transferred genes.

The Ti (for tumor-inducing) plasmid is a natural gene transfer vector. It is carried by *Agrobacterium tumefaciens*, a pathogenic bacterium that causes crown gall tumors in dicotyledonous plants. When the bacterium infects plants, it transfers a region of the Ti plasmid into the cells, where the transferred DNA becomes integrated into the plant genome.

The three groups, one from Mary-Dell Chilton's laboratory at Washington University, one at the Monsanto Company's St. Louis laboratory, and the third including investigators from the Max Planck Institute for Plant Breeding in Cologne and the State University of Ghent, Belgium, exploited these natural properties of the plasmid in introducing the antibiotic resistance genes.

The investigators first had to modify the genes so that they would be expressed, however. They all used similar approaches for this. As Robert Horsch of Monsanto told the Miami symposium, "The basic idea is to construct a chimeric gene that will function in plants."

The investigators fused control sequences from the nopaline synthase gene, a transferred Ti plasmid gene that is expressed in plant cells, with the protein-coding sequences of the bacterial genes. The Monsanto group and Michael Bevan, who is now at the Plant Breeding Institute in Cambridge, England, but who worked in Chilton's laboratory on these experiments, used the bacterial gene for kanamycin resistance. The European group, which is led by Jozef Schell and Marc van Montagu, incorporated the structural sequences of genes coding for methotrexate, kanamycin, or chloramphenicol resistance into their chimeric genes. Susceptible plant cells were then infected with Ti plasmids into which the chimeric genes had been inserted. As a result the cells became resistant to the appropriate antibiotics.

The apparent key to the success of these experiments was the use of genes with control sequences that are recognized by the plant cell machinery for gene expression. Previous efforts to transfer a variety of bacterial, yeast, and animal genes into plant cells did not lead to expression of the foreign genes, presumably because they were transferred with their own control sequences, which do not work in plant cells.

Whole plants were not regenerated in the current experiments because they used wild-type Ti plasmids that yield tumor cells. But it is possible to modify the DNA transferred by the plasmid so that it does not make the recipient cells tumorous. Investigators, including Chilton and Schell and van Montagu, have shown that normal plants can be regenerated from these cells.

The next step is to see whether a modified plasmid will transfer a chimeric gene for antibiotic resistance. If it does and if the gene is expressed, it should be possible to grow whole plants carrying the new gene. Other desirable genes may then be transferred along with that for antibiotic resistance. Moreover, the acquisition of antibiotic resistance, which is not a normal property of plant cells, should provide an easy way of identifying those cells that have acquired the foreign DNA, even though they do not behave like tumor cells. Schell says of the recent progress with the Ti plasmid, "I am somewhat sad. This is the end of the era of studying the Ti plasmid for itself. Now we can use it to transfer genes for genetic engineering and for studying gene regulation in plant cells."—**J.L.M.**

segment," Fedoroff says. The sequence of newly introduced DNA adjacent to the duplicated gene segment is the site of chromosome breakage in the revertants.

When that sequence is deleted, the developmental timing of the chromosome breakage is altered so that it no longer resembles the timing of Ds-induced breakage; this result suggests that the newly introduced sequence corresponds to at least part of the Ds element. This possibility is further strengthened by the finding in Starlinger's laboratory that the boundary between the newly introduced DNA and the gene DNA contains the same 11-bp sequence as that found by Peacock's group at the ends of the *adh1* insert.

Peacock hopes to use the Ds element to introduce new genes into the genome of maize. "It looks potentially useful as a vector," he notes, "although the insertion may not be completely random. The Ds terminus in this mutant is very homologous to the gene sequence at the point of insertion."

Gerald Rubin and Allan Spradling, who are also at Carnegie's Baltimore branch, have already used the P element system to introduce a foreign gene into the fruit fly. The transferred gene was expressed and corrected a genetic defect in the recipients, which could pass the transplanted gene to their progeny.

The cloned *adh1* gene can be inserted into the Ds element, and this hybrid DNA can be tested to see whether it will confer ADH enzyme activity on corn cells that lack the enzyme. The cells that will be used for this are protoplasts, plant cells that have been denuded of their cell walls and grown in culture.

So far it has not been possible to grow whole corn plants from protoplasts, a problem for investigators who want to genetically engineer new traits into maize. However, C. Edward Green of the University of Minnesota described some progress toward corn plant regeneration at the Miami meeting.

Meanwhile, other plants can be regenerated from their protoplasts. Horst Lörz of the CSIRO group has produced a mutant of one of these, *Nicotiana plumbaginifolia*, a close relative of tobacco, that lacks ADH activity. The CSIRO workers plan to try to transfer the maize *adh1* gene into this plant. The Ds element may not work in this species but other approaches to gene transfer are possible. For example, the Ti plasmid may be used as a vector for this plant. Peacock says of the current gene transfer effort, "We now have all the pieces, but we need to put them together."

—**JEAN L. MARX**