PLANT BIOTECH

EXPANDING AGROBACTERIUM'S HOST RANGE NOW POSSIBLE?

NEW YORK—Plant genetic engineers have long been hampered by the paucity of suitable vectors to transfer foreign DNA into plants. To date, there has been only one, the Ti (tumor-inducing) plasmid of the bacterium *Agrobacterium tumefaciens*. Although research scientists have been very successful in exploiting the Ti plasmid's transfer functions (while eliminating its tumorigenic capabilities), it still suffers from a major drawback: a decided preference to transfer DNA to dicotyledonous plants (tobacco, tomato, and alfalfa) rather than the more agronomically important monocot field crops, such as corn and wheat.

But the recent discovery by scientists at BioTechnica International (Cambridge, MA) that Agrobacterium can transfer plasmids other than Ti opens the possibility that its host range can be extended. Vicky Buchanan-Wollaston, Joan E. Passiatore, and Frank Cannon (*Nature* **328:**172, 1987) have shown that the mobilization functions of a small, wide-host-range bacterial plasmid (RSF1010) can effectively substitute for the Ti plasmid's 25 base pair direct repeat T-DNA borders (until now considered essential for transfer to occur). Cannon and co-workers have found that if the host Agrobacterium contains a helper plasmid to supply the *vir* (virulence) functions (which effect DNA transfer by initiating a site-specific cleavage at each end of the border sequences), then RSF1010's *ori*T (origin of transfer) and *mob* (mobilization) genes are able to transfer an incorporated drug marker (kanamycin resistance) to tobacco plant leaf pieces. The leaf pieces, growing on medium containing kanamycin, regenerated calli that formed kanamycin-resistant plants.

Robb Fraley, director of plant science research at Monsanto Agricultural Co. (St. Louis, MO), says that these results are not unexpected; the researchers are just replacing a couple of T-DNA functions with other genes. Clarence I. Kado, a professor of plant pathology at the University of California (Davis), feels, however, that the research "puts everything up in the air again for the labs studying the T-DNA borders. It means they will have to reconsider whether the borders are really important as specific recognition sites. They may be part of a specific site rather than the site itself. If *ori*T and *mob* are all that is necessary for transfer, then this opens the possibility that many different Ti-compatible plasmids will work."

In fact, it is the mechanism of T-DNA transfer itself that is of most interest to molecular biologists. There is already some evidence (see *Nature* **322:**706, 1986) that it is similar to that for bacterial conjugation, in which one strand of a double-stranded plasmid (the F factor) is transferred unidirectionally. The F plasmid is first nicked by a site-specific endonuclease at *ori*T. One of the two strands is transferred as a DNA-protein complex to the recipient bacterium; subsequent DNA synthesis in both donor and recipient restores the plasmids' double-stranded configuration. In *A. tumefaciens*, there is also a site-specific endonuclease cleavage, producing a strand-specific copy of the T-DNA that may exist as a transferrable protein-DNA complex.

Luca Comai, a senior principal scientist at Calgene (Davis, CA), says that these data do suggest that the origin of the T-DNA transfer system is related to plasmid transfer. "If [BioTechnica's] system is not an isolated event, then it tells us a lot about transfer itself and about how it evolved." But, as Kado cautions, "If one makes an analogy between this transfer system and the F system, one must be careful. The F system is the only single-stranded transfer system known; it could be the exception rather than the rule."

Cannon, BioTechnica's vice president for agricultural genetics, says that the research group has used this system to transfer antibiotic resistance to tomato, potato, and alfalfa, as well as tobacco. In all cases, regenerated plants exhibit kanamycin resistance. He adds that there is nothing unique about their particular Agrobacterium strain. It is rather the general process (for which BioTechnica is seeking patent protection) that will allow the genetic improvement of crops which have so far proved intractable.

Calgene's Comai, however, feels that the results do not really provide a "handle" for genetic engineering; they probably will not improve the efficiency of plant transformation in the near future. "The exciting thing," he concludes, "is that these results might help unravel the mechanisms of [plasmid] transfer."

—Jennifer Van Brunt