Plant engineering

More prospects for Ti plasmids

from M.H. Drummond

AGROBACTERIUM TUMEFACIENS causes crown gall tumours in some gymnosperms and most dicotyledonous angiosperms[1] by inserting its Ti plasmids into the nuclear genome of the infected plant; and agrobacterial Ti plasmids are now well established as vectors for introducing foreign genetic material into a wide range of higher plants[2,3]. This may ultimately make possible useful additions to the genomes of crop plants. Unfortunately the prospect for using Ti-based vectors in monocotyledonous plants has been gloomy because *A. tumefaciens* is not a pathogen for them, a matter of considerable regret since the monocots include the grass crops, which feed the greater part of mankind. On page 763 of this issue, however, Hooykaas-Van Slogteren *et al.* indicate that Ti plasmid DNA enters the cells of at least some monocot species, but is simply not oncogenic[4]. Vectors based on Ti plasmids may thus be useful in monocots after all.

The observation made by the Dutch group concerns the presence in plant tissue of opines. These metabolites are absent from normal tissue but are synthesised in large amounts in crown gall tissue by synthases encoded in T-DNA, the Ti plasmid segment that is integrated into the plant genome. The best known opines, octopine and nopaline, characterize the two principal varieties of Ti plasmid. Ti plasmid sequences outside the T-DNA segment encode oxidases that catabolize opines in the bacterium. Thus the plasmid both diverts the biosynthetic resources of the plant and enables its bacterial carrier to exploit the resulting opine pool as a very specific carbon and nitrogen source. This strategem has been termed genetic colonization. The ecological niche it creates is further enlarged by multiplication of opine-producing cells into a crown gall tumour. This is brought about by other T-DNA genes which perturb hormone metabolism in the plant cell, raising the level of both auxins and cytokinins[5]. The Ti plasmid vectors developed for genetic engineering can be 'disarmed' by deleting, or otherwise mutating, their oncogenic genes, in order to permit differentiation of engineered tissue into entire plants[3,6].

The Dutch group find that when certain ornamental monocots are infected with virulent strains of *A. tumefaciens,* opine synthesis ensues in the absence of any sustained neoplastic growth. Opine synthase genes are known to be under the control of eukaryotic regulatory sequences[7]. The remote possibility that they are being expressed by the bacterial cell in this system is excluded by the observation that no octopine synthesis is detectble when T-DNA transfer is blocked by a plasmid mutation, *virB*, outside the T-DNA[8]. The obvious interpretation is that T-DNA does enter the monocot cell, but fails to bring about oncogenesis. If so, Ti plasmids, far from being unsuitable for the genetic engineering of monocots, can be considered well-suited because already disarmed, although it remains to be seen whether the Dutch group's observations can be extended to the important grass crops.

The definitive experiment, demonstrating the presence of T-DNA sequences in monocot tissues by nucleic acid hybridization, remains to be carried out. Southern blotting is now used routinely to characterize DNA sequences inserted into dicot genomes[2,3,9], but this is more problematical for monocots because of difficulties in the culture of large amounts of transformed tissue without significant contamination by normal cells. In dicots the hormone autotrophy conferred by T-DNA facilitates the selection and growth of transformed tissue, and in many species the establishment of tissue culture lines from single cells is quite straightforward. This is not yet the case for monocots but other means of deriving monocot tissue enriched for cells containing inserted DNA should be feasible. For example, a Ti vector encoding neomycin phosphotransferase such as that constructed by Bevan *et al.*[9] could be used in combination with G418 drug selection in tissue culture. Neomycin resistance is expressed from the *nos* (nopaline synthase) promoter in this construct, which underlines the importance for monocot genetic engineering of the present demonstration that synthase genes are transcribed in the monocot genetic background.

If T-DNA is integrated into the monocot genome in its entirety, its failure to induce tumorigenesis must be accounted for. There are two obvious possibilities: either the oncogenic genes are not properly expressed, or their protein products fail to disrupt control of cell division. If the oncogenic genes are not transcribed, then comparing their regulatory regions with the *nos* or *ocs* (octopine synthase) promoters might bring to light differences between monocot and dicot promoter sequences. However, the Dutch group seem to favour the second possibility.

The work has one further interesting aspect. By illustrating nicely that oncogenesis is not an essential part of genetic colonization, it raises the question of whether other procaryotes have evolved similar stratagems for exploiting plant or animal hosts. To discover any that were a tenth as useful as Ti plasmids would be very exciting, but in the absence of any obvious pathogenic effect, how does one start looking? □

1. Elliot, C. *Manual of Bacterial Plant Pathogens* 2nd edn (Chronica Botanica, Waltham, 1951).
2. Herrera-Estrella, L. *et al. Nature* **310**, 115 (1984).
3. Barton, K.A. *et al. Cell* **32**, 1033 (1983).
4. Hooykaas-Van Slogteren, G.M.S., Hooykaas, P.J.J. & Schilperoort, R.A. *Nature* **311**, 763 (1984).
5. Akiyoshi, D.E. *et al. Proc. natn. Acad. Sci.* U.S.A. **80**, 407 (1983).
6. Zambryski, P. *et al. EMBO J.* **2**, 2143 (1983).
7. De Greve, H. *et al. J. molec. appl. Genet.* **1**, 499 (1983).
8. Hoekema, A. *et al. Nature* **303**, 171 (1983).
9. Bevan, M.W., Flavell, R.B .& Chilton, M-D. *Nature* **304**, 184 (1983).

M.H. Drummond is in the Agricultural and Food Research Council Unit of Nitrogen Fixation, University of Sussex, Brighton BN1 9RQ.

DNA recombination

Resolution of intermediates

from Nancy L. Craig

HOLLIDAY structures[1] are thought to join DNA duplexes by a reciprocal single-strand exchange and are thought to be intermediates in various pathways of genetic recombination (Fig. 1). They have been visualized by electron miscroscopy, but biochemical evidence in support of their role in recombination has been hard to come by. Now, proteins encoded by *Escherichia coli* phages are beginning to provide information about breakage and rejoining reactions that can mediate the formation and resolution of Holiday structures. Kemper *et al.*[2] and de Massy *et al.*[3] have characterized phage endonucleases that can specifically cleave Holliday structures *in vitro*, probably reflecting an important step in the resolution of Holliday intermediates in homologous recombination. And on page 721 of this issue, Hsu and Landy show that Holliday structures can be resolved *in vitro* to recombinant duplexes by the site-specific recombination machinery of phage lambda, providing strong evidence that Holliday structures are intermediates in this recombination pathway[4].

Integration of phage lambda into the *E. coli* chromosome occurs by reciprocal combination between *att*P(POP′), a ~240-base pair (bp) specific site in the phage chromosome, and *att*B(BOB′), a ~25-bp site in the bacterial chromosome[5] (see Fig. 2). The products of integrative recombination are the hybrid sites *att*L(BOP′) and *att*R(POB′) which flank the integrated prophage. Prophage excision occurs by reciprocal recombination between *att*L and *att*R, regenerating *att*P and *att*B. Each *att* site contains the same