Although the field of kinase inhibitors is coming into its own in the clinic, many questions remain. So far, the best targets are kinases activated by mutation, and there is less evidence that an overexpressed but wild-type kinase is a clinically useful target for a small molecule, at least in the case of EGFR in lung cancer[9,10], and FLT3 in acute myeloid leukemia[11]. Whereas mutated protein kinases are common in myeloproliferative diseases such as chronic myelogenous leukemia and in other hematopoeitic malignancies, the incidence of mutations in solid tumors is low so far, except in melanoma (B-RAF)[12]. However, recent reports of mutations in PI3K in breast cancer[13] reinforce the notion that we probably need to look for mutations in all kinases in all types of human tumors and premalignant lesions.

In the clinic, we need more and better inhibitors against these targets, and we urgently need to understand and develop strategies to reduce the emergence of drug-resistant clones. Combining kinase inhibitors with drugs that block other oncogene-dependent pathways should improve tumor cell kill and reduce the rate of drug resistance. Similarly, having multiple kinase inhibitors against the same target would be of value. Precise methods to measure competition among drugs, such as the one described by Fabian *et al.*, should accelerate these efforts.

1. Fabian, M.A. *et al. Nat. Biotechnol.* **23**, 329–336 (2005).
2. O'Brien, S.G. *et al. New Eng. J. Med.* **348**, 994–1004 (2003).
3. Buchdunger, E. *et al. J. Pharmacol. Exp. Ther.* **295**, 139–145 (2000).
4. Demetri, GD. *et al. N. Engl. J. Med.* **347**, 472–480 (2002).
5. Cools, J. *et al. N. Engl. J. Med.* **348**,1201–1214 (2003).
6. Shah, N.P. *et al. Science* **305**, 399–401 (2004).
7. Weisberg, E. *et al. Cancer Cell*, **7**, 129–141 (2005).
8. Wang, Z. *et al. Science* **304**, 1164–1166 (2004)
9. Paez, J.G. *et al. Science* **304**, 1497–500 (2004).
10. Lynch, T.J. *et al. N. Engl. J. Med.* **350**, 2129–2139 (2004).
11. Stone, R.M. *et al. Blood* **105**, 54–60 (2005).
12. Davies, H. *et al. Nature* **417**, 949–954 (2002).
13. Samuels, Y. *et al. Science* **304**, 554 (2004).

© 2005 Nature Publishing Group http://www.nature.com/naturebiotechnology

Adding diversity to plant transformation

Mary-Dell Chilton

Alternatives to *Agrobacterium* may circumvent patents on plant transformation technologies.

The production of genetically modified crops that could alleviate hunger and malnutrition in developing countries may be impeded by patents on the DNA components or on the techniques for introducing DNA into the plant genome and regenerating plants. Currently, the most widely used method of gene transfer into plant cells employs a reformed plant pathogen called *Agrobacterium*, which has a natural ability to put foreign genes into plant cells. In an effort to circumvent technology patents involving this bacterium, Broothaerts *et al.*[1] have recently described alternative bacteria that do the work of *Agrobacterium*, albeit at lower efficiencies. The researchers, based at the Center for the Application of Molecular Biology to International Agriculture (CAMBIA), are offering their technology under an 'open source' license arrangement (http://www.bios.net)[2] to address the patent issue.

Mary-Dell Chilton is at Syngenta, Research Triangle Park, North Carolina 27709, USA.
e-mail: marydell.chilton@syngenta.com

Methods for introducing new genes into plant cells fall into two categories: the 'natural,' biological approach, using *Agrobacterium*, and the 'physical' approach, using more violent methods such as the 'gene gun' or electroporation. With the natural approach, one or a few copies of a predictable linear piece of DNA, called T-DNA, often containing only the gene(s) of interest, are neatly inserted into plant DNA. Physical methods are generally less tidy, shattering the DNA and inserting a veritable puzzle of pieces into the plant genome. Both approaches have been included in inventions, with patent claims to gene parts as well as delivery tools.

The title of the paper by Broothaerts *et al.* captured my attention immediately: "Gene transfer to plants by diverse species of bacteria" seems to herald an end to the cosmic loneliness of *Agrobacterium,* heretofore the only microbe known to have evolved a means to transfer genes into plants. The outward and visible sign of its activity in nature is the formation of a gall at the site of infection in the plant. The gene transfer process itself is mainly the handiwork of virulence genes on a family of giant extrachromosomal circular plasmids called Ti (Tumor-inducing) plasmids. Virulence genes deliver a specific part of the plasmid, T-DNA (Transferred DNA), to the plant cell nucleus. The T-DNA genes cause the plant cells to synthesize auxin and cytokinin, phytohormones that make the plant cells grow into a gall, or tumor.

Transfer of Ti plasmids into other bacteria had previously been undertaken to test whether the plasmid could confer tumor-inducing ability more widely. Introduction of a Ti plasmid into unrelated bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* failed to make them oncogenic on plants, but Ti plasmids did confer tumor-inducing ability on several of *Agrobacterium*'s siblings in the family Rhizobiaceae: *Rhizobium trifolii*, *Rhizobium leguminosarum* and *Phyllobacterium myrsinacearum*[3].

As a taxonomical footnote, *Agrobacterium, Rhizobium* and *Phyllobacterium* have been classified as three distinct genera within the family Rhizobiaceae. Some taxonomists have published a proposal to designate *Agrobacterium* spp. as *Rhizobium* spp., whereas others have argued, based on a number of taxonomic criteria, that *Agrobacterium* be retained as a separate genus. Most researchers working with *Agrobacterium* retain the original name, partly because of observable taxonomic differences and partly because the new nomenclature would generate confusion in an enormous body of literature.

By the mid-1980s we had learned much about how the Ti plasmid works[4]. We found that T-DNA is defined by left and right 'border' sequences, and that the remainder of T-DNA was not functional in the transfer process (**Fig. 1**). Thus, T-DNA could be 'disarmed' by removing its oncogenes and placing genes of scientific interest between T-DNA borders. T-DNA and virulence genes were found to operate equally well when placed on separate plasmids. Studies of T-DNA transfer to plant cells became easier with the advent of selectable and screenable markers for identifying transformed plant cells. The DNA sequence and functions of virulence genes showed that the mechanism of T-DNA transfer to plant cells is similar to conjugational transfer of plasmids between bacteria[5].

Broothaerts *et al.* have now taken the next logical step in studying the role of the bacterium in T-DNA transfer to plants. Their work provides a clear demonstration that an *ex Agrobacterium*, Ti-plasmid-derived gene

© 2005 Nature Publishing Group http://www.nature.com/naturebiotechnology

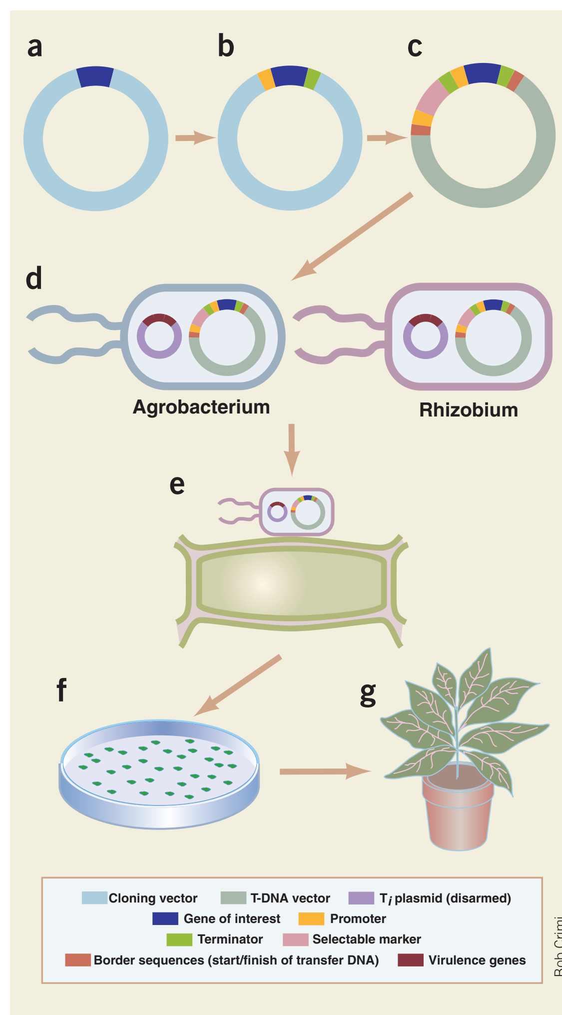

Figure 1 Intellectual property landscape along the route to a transgenic plant. Patents of competitors may intervene at any step—the technology proposed by CAMBIA is illustrated at the fourth step. (**a**) Discover a beneficial protein and isolate or synthesize its gene (gene of interest (GOI)) and amplify it on a cloning vector. (**b**) Attach a promoter and terminator to the GOI to form an expression cassette that functions in the crop of interest. (**c**) Splice the GOI expression cassette into a transfer-DNA (T-DNA) vector with a selectable marker that works in the crop plant. The border sequences—left and right—mark the start and finish of the T-DNA. (**d**) Introduce the T-DNA vector into a bacterium (*Agrobacterium* or *Rhizobium* as proposed by CAMBIA) that contains a disarmed T_i plasmid with the full repertoire of virulence genes needed for gene transfer to plants. (**e**) Cocultivate the bacterium with susceptible cells from the crop plant. (**f**) Select for cells that contain the T-DNA (using the selectable marker). (**g**) Regenerate cells to fertile plants and test 50–100 independent transformants for activity of the GOI and stability of expression. Follow up by backcrossing chosen transformant with breeding line for several generations to assure plant quality, and test for expression of the GOI at each generation.

transfer system (that is, virulence genes) can indeed deliver T-DNA genes to plant cells. Consistent with earlier findings, the transformations worked best with bacteria most closely related to *Agrobacterium*.

The authors put suitable plasmids into four bacterial strains: *Agrobacterium*, *Rhizobium* spp. NGR234 (a wide host range nodulator), *Sinorhizobium meliloti* (an alfalfa nodulator) and *Mesorhizobium loti*. The non-*Agrobacterium* strains in their study produced many transgenic tobacco plants, one transgenic rice plant and a few transgenic *Arabidopsis thaliana*. Southern blot analysis evidence is presented for T-DNA integration into tobacco transformed by all three non-*Agrobacteria*, and for *A. thaliana* and one rice plant transformed by *Sinorhizobium*. For several transformants, the authors cloned out and sequenced the T-DNA/plant DNA junctions to identify the flanking DNA in the plant genome. I am left with no doubt that they have indeed transformed several plants with plasmids introduced into bacteria that are not (called) *Agrobacterium* strains.

What is the efficiency of these noncanonical transformation systems? It depends on one's criterion. Transgenic tobacco plants were regenerated from cells transformed by all three non-*Agrobacterium* strains. The tobacco transformation efficiency ranged from 1–20% that of *Agrobacterium* based on numbers of β-glucuronidase positive foci. A less sensitive estimate of efficiency is percentage of leaf discs transformed, which ranged from 10–30% (compared with 95–100% for *Agrobacterium*). For rice and *A. thaliana* transformation, the authors present details for only *Sinorhizobium*. On rice, this strain produced β-glucuronidase activity in 0.6% of infected calli (compared with 50–80% for *Agrobacterium*) and yielded exactly one transgenic rice plant. *A. thaliana* transformation by this bacterium gave a frequency of 0.01%, tenfold below the frequency for *Agrobacterium*, yielding six seedlings. Two seedlings analyzed by Southern blot are clearly transgenic. The work of Broothaerts *et al.* is convincing and thorough, and the large number of experiments reported indicates that they have worked very hard to get these somewhat uncooperative bacteria to perform.

I have some reservations about the ultimate impact of this work, despite its technical soundness. As a practical matter, the idea that these recalcitrant bacteria may be harnessed in real projects to transform recalcitrant crop plants may be unrealistic. The transformation frequency of *Sinorhizobium* is substantially lower than that of *Agrobacterium* for the two 'easiest' dicot plants (tobacco and *A. thaliana*) and the one easiest monocot plant (rice) studied here. The one bacterial strain that works at a moderate frequency, *Rhizobium*, is by the authors' own admission "clearly closely related" to *Agrobacterium*. This admission calls into question whether this curiously "wide host range *Rhizobium*" strain is really distinct from an *Agrobacterium* strain, especially in view of the ongoing taxonomic debate on nomenclature. Thus it appears 'safer,' from the intellectual property point of view, to focus on *Sinorhizobium* or *Mesorhizobium* and put up with their lower transformation efficiencies.

Notwithstanding this concern, I certainly applaud the intention of Broothaerts *et al.* to provide a cost-effective plant transformation technology for ethically desirable projects declined by industry as insufficiently profitable. There is also good reason to hope that patent holders, having little to lose and much goodwill to gain, will offer contributions of intellectual property for such projects. For example, in the case of Golden Rice, which is genetically modified to produce provitamin A, contributions of intellectual property, in the form of seeds and lines, and of related technology have allowed the dissemination of this modified crop for research and agricultural purposes (see http://www.syngenta.com for more information on this project). Contributions of this type will still be needed even if the non-*Agrobacteria* described by Broothaerts *et al.* can be harnessed successfully for transformation of crop plants, because in the end, the transforming bacterium, whatever its Latin name, is but a small piece of the technology needed to produce a transgenic plant.

1. Broothaerts, W. *et al. Nature* **433**, 629–633 (2005).
2. Dennis, C. *Nature* **431**, 494 (2004).
3. Hooykaas, P.J.J. & Schilperoort, R.A. *Plant Mol. Biol.* **19**, 15–38 (1992).
4. Hansen, G. & Chilton, M.-D. in *Plant Biotechnology* (eds. Hammond, J., McGarvey, P. & Yusibov, V.) 21–57 (Springer, New York, 1999)
5. Zupan, J., Muth, T.R., Draper, O. & Zambryski, P. *Plant J.* **23**, 11–28 (2000).