

Gene exchange by design

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Gene transfer from bacteria to plants was thought to be limited to the bacterial genus *Agrobacterium*. But other bacterial groups also contain species capable of interkingdom genetic exchange.

All organisms must be able to transfer genetic information from parent to progeny — without this ‘vertical’ gene transmission, species would simply die out. But this is not the only mechanism for transmitting genes from one organism to another. ‘Horizontal’ gene transfer has long been recognized as a major factor in evolution, and there are numerous likely examples of past horizontal gene transfer events. In terms of ongoing processes, bacterial conjugation (gene transmission from one bacterium to another through direct physical contact) is the most commonly cited example. The horizontal transfer of genetic material between species from different phylogenetic kingdoms — from bacteria to plants, for instance — is also possible, but had previously been documented only for members of the bacterial genus *Agrobacterium*. Now, on page 629 of this issue, Broothaerts *et al.*¹ show that several bacterial species outside this genus are also capable of interkingdom ‘sex’.

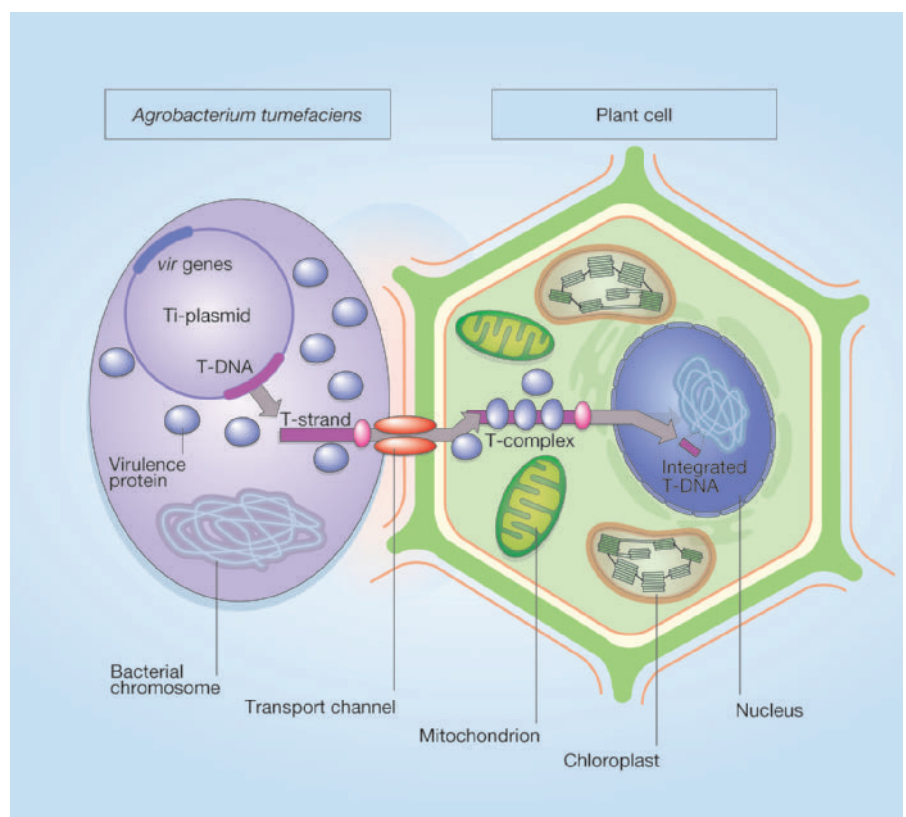
Agrobacterium tumefaciens is a pathogenic bacterium that causes crown gall disease (Fig. 1), a cancerous disorder that affects a range of plant species². Along with the related species *A. rhizogenes*, *A. vitis* and *A. rubi*, *A. tumefaciens* incites tumours by transferring a portion of a large extrachromosomal DNA element (the tumour-inducing, or Ti, plasmid) to its plant hosts. Once within the plant cell, this transferred DNA (the T-DNA) targets the nucleus, where it eventually integrates into the host genome. The expression of T-DNA-encoded oncogenes results in the plant overproducing or becoming overly sensitive to hormones, leading to loss of cell-growth control and to tumour formation³.

This ability of *Agrobacterium* species to transfer DNA to plants has been exploited by scientists for genetic-engineering purposes; oncogenes can be deleted from the bacterial T-DNA and substituted with any gene of interest (Fig. 2). Once transferred, these ‘transgenes’ can endow plants with new characteristics, including herbicide or pathogen resistance, altered growth or nutritional qualities, or the ability to produce drugs or edible vaccines — antigens that can direct immunity against pathogens in humans or animals that ingest the plants. Under laboratory conditions, *Agrobacterium* can also genetically transform fungal and animal cells^{4–6}.



Figure 1 Crown gall tumour on an elm tree. Most crown gall tumours form at or below the surface of the soil, but aerial tumours can also occur, as here. (Courtesy of Jer-Ming Hu, National Taiwan University, Taipei.)

Figure 2 How *Agrobacterium* genetically transforms plants. *Agrobacterium* contains a tumour-inducing (Ti) plasmid, which includes virulence (*vir*) genes and a transferred-DNA (T-DNA) region. Genes of interest can be inserted into the T-DNA. Wounded plant cells produce phenolic defence compounds, which can trigger the expression of the *Agrobacterium vir* genes. The encoded virulence (*Vir*) proteins process the T-DNA region from the Ti-plasmid, producing a ‘T-strand’. After the bacterium attaches to a plant cell, the T-strand and several types of *Vir* proteins are transferred to the plant through a transport channel. Inside the plant cell, the *Vir* proteins interact with the T-strand, forming a T-complex. This complex targets the nucleus, allowing the T-DNA to integrate into the plant genome and express the encoded genes.



Quantum optics

Cheat detection

Alice and Bob are getting divorced, but who keeps the dog? They could toss a coin for it, but as they now live apart, how can Bob be sure, if Alice tosses, that she won't lie about the result?

In most cryptographic problems of communication between two parties, the challenge is to eliminate third-party eavesdropping. But in the 'coin-tossing protocol', which is relevant to real-world situations such as remote signing of contracts, the issue is a lack of trust between the communicants. Can quantum

cryptographic methods stamp out cheating? Yes, they can, say G. Molina-Terriza *et al.* (*Phys. Rev. Lett.* **94**, 040501; 2005).

The authors have staged a quantum-optical enactment of a protocol that works as follows. The result is decided by many throws — the best of 100, say. Alice tosses each coin and encodes the outcome in the angular-momentum quantum state of a photon in an entangled pair. This encoding determines the quantum state of the other photon in the pair, which has been sent to

Bob. But Bob cannot determine the state of his photon until Alice sends him the information on her own photon — in essence, until she tells him how the coin fell. So he bets on the toss, and then Alice sends him a signal encoding the 'actual' outcome, which Bob can verify by performing a measurement on his own photon.

Because of its probabilistic nature, some such measurements will fail — Alice won't be able to encode the true outcome. But the crucial point is that if Alice is



systematically cheating, this shows up as a higher than average rate of 'failures', and Alice can do nothing to suppress this tell-tale signature of dishonesty. **Philip Ball**

Agrobacterium-mediated genetic transformation has become a staple for basic plant research, as well as a principal means of generating transgenic plants for the agricultural biotechnology industry. However, at least for commercialization purposes, a tangled patent thicket complicates *Agrobacterium*-based plant-transformation technology. For this reason — as well as for more fundamental reasons of scientific interest — it would be interesting to determine whether other species of bacteria could, like *Agrobacterium*, serve as 'plant genetic engineers'. Although several previous papers have indicated that other bacterial species could incite plant tumours when engineered to carry a Ti-plasmid⁷⁻⁹, molecular evidence for the actual transfer of DNA from these bacteria to plants has been lacking. Broothaerts *et al.*¹ now provide this evidence.

In their study, the authors used *Rhizobium* species NGR234, *Sinorhizobium meliloti* and *Mesorhizobium loti* — representatives of two different bacterial families. They first introduced a 'disarmed' Ti-plasmid that lacks a T-DNA region into these bacteria. This Ti-plasmid contained virulence genes, the protein products of which are necessary to act on a T-DNA and transfer it to plants (Fig. 2). Then Broothaerts *et al.* introduced a second plasmid containing a T-DNA region into these non-*Agrobacterium* species and into a disarmed *Agrobacterium* strain. This T-DNA contained a gene that encodes resistance to the antibiotic hygromycin, as well as *gusA*, a gene that directs the expression of the marker enzyme β -glucuronidase. To safeguard against misinterpretation of the data should the bacterial strains be mixed up, the T-DNAs introduced into *Agrobacterium* and non-*Agrobacterium* species contained different molecular 'tags'.

The authors used these bacteria to infect several different plant species, representing two major higher-plant groupings: species

from the dicots (which are highly susceptible to *Agrobacterium*-mediated transformation) included tobacco and the thale cress *Arabidopsis*; from the monocots (which are considerably less susceptible), the authors used rice. The generation of plants that express β -glucuronidase and are resistant to hygromycin indicated successful transformation by all bacterial species tested. This was confirmed by DNA-blot analysis and by the sequencing of T-DNA-plant-DNA junctions recovered from plants transformed with non-*Agrobacterium* species. Moreover, the junctions from these plants resembled those previously characterized as a result of *Agrobacterium*-mediated transformation. Therefore, the processing, transfer and integration of T-DNAs from the different bacterial species probably occurred by the same mechanisms.

Broothaerts and colleagues' results also suggest that non-*Agrobacterium* species are capable of the full range of genetic transformation mechanisms shown by their *Agrobacterium* counterparts. The transformation of *Arabidopsis* involved a 'flower dip' protocol, which targets female reproductive (germ-line) cells¹⁰. However, tobacco and rice were transformed via somatic tissues — leaves and callus cells, respectively — which probably involves a different mechanism¹¹.

The authors further found that although non-*Agrobacterium* bacterial species could be engineered to genetically transform several plant species, the transformation efficiency was relatively low, ranging from less than 1% to almost 40% of that of *Agrobacterium*-mediated transformation, depending on the species and transformation assays used. Broothaerts *et al.* note, however, that alterations in transformation conditions (the age and type of plant tissue inoculated, for instance) could enhance these frequencies. The low frequencies of *Agrobacterium*-mediated transformation of many 'recalcitrant' plant species have been

substantially increased through such manipulations over the past 20 years.

Broothaerts and colleagues' findings¹ will no doubt have implications for plant science and biotechnology. The ability of several bacterial species to transfer genes to plants also reinforces the possibility that such horizontal gene flow may have contributed to plant evolution. It is already known that the genomes of some plant species contain remnants of what were probably ancient transformation events by *A. rhizogenes*^{12,13}. In some instances, the *Agrobacterium*-derived transgenes are now expressed in a regulated manner in the host plant¹⁴, hinting that these genes have become an important part of the plant's genetic make-up. We may find that transgenes from other bacteria have likewise been assimilated by plants. Our understanding of the importance of horizontal gene flow in the evolution of higher organisms should be enhanced by knowledge of the mechanism of such transmission, and of the range of organisms that can participate in these events. ■

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