In search of horizontal gene transfer

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Critics of agricultural biotechnology have often been dismissed as modern-day Luddites. However, these critics have an understandable concern, based on a detail of the technology that has been nearly unanimously adopted for introducing foreign genes into plants. This method relies on cointroducing, along with the gene to impart a desired characteristic, another gene that confers antibiotic resistance. Culturing the transformed cells in antibiotic-containing growth media allows selection and regeneration of only those cells that have incorporated the transgenes. The antibiotic resistance genes used in genetic engineering are, in fact, normal bacterial genes that have been adapted for expression in plants. However, the prospect of releasing these antibiotic resistance genes into the environment in large quantities has raised fears that they could be taken up by native bacteria, thereby posing a risk to public health. Industry and the FDA have responded to these concerns by asserting that genes that have been transferred into plants cannot be reincorporated into bacteria[1].

When industry first began making such guarantees in the 1980s, I was exploring the idea (and trying to convince my colleagues) that horizontal gene transfer between kingdoms could be a natural process. This idea was based on the numerous demonstrations that bacteria, fungi, and animal cells readily take up and express foreign DNA. I argued that this could reflect natural processes that facilitate horizontal gene transfer. And if this speculation was proven, the debate over the safety of recombinant DNA technology would have to be recast.

Since then, research has focused primarily on the question of whether or not the genes in these plants will move to bacteria in the environment, rather than on whether or not the genes pose any danger if they do move. The FDA assertion that horizontal gene transfer does not occur between transgenic plants and microorganisms has stimulated a number of investigations, centering on two types of experiments, neither of which have provided evidence for such gene transfer. In the first, transgenic plant materials were fed to mice and the coliform bacteria isolated from their feces screened for the presence of antibiotic resistance genes originating from the plants. After eight trials, none could be recovered (M. Syvanen, unpublished data). In the second approach, two labs examined bacteria that cause spoilage of vegetables and asked whether, after growth on transgenic plants, the bacteria had been transformed with transgenic DNA. In both cases the bacterium *Erwinia chrysanthemum* was grown on a variety of vegetables, but no transformants were detected (ref. 2, M. Syvanen, unpublished data).

Other studies have used experiments in which the likelihood of gene transfer occurring was maximized, in order to determine a lower frequency for horizontal gene transfer, or possibly, a barrier to it. Using this approach, researchers were able to detect horizontal gene transfer between a naturally occurring *Acinetobacter* species selected for the study based on its inherently high transformation frequency[3,4]. These authors used a high-frequency recombinational repair assay between a neomycin phosphotransferase gene (*npt*) with an internal deletion carried by the bacterium, and an intact *npt* gene in transgenic plants. If the bacteria were transformed with the intact *npt* gene from the plants, neomycin resistance would be restored. Using this system, with its high transformation rates and recombination rates, they were able to demonstrate horizontal gene transfer from transgenic plants to bacteria; in one trial using an optimized protocol, 5μg of transgenic potato DNA yielded 100 transformants. It would not be hard to imagine that this frequency could be observed in a container storing tons of spoiling transgenic vegetables.

In another set of experiments, the fate of plasmid DNA was monitored after being fed to mice[5,6]. Most DNA was degraded rapidly, but a fraction remained in a high molecular weight form for many hours and was even detected in the feces. Transformation of gut bacteria was not detected. The biggest surprise, however, was finding intact plasmid DNA in both mouse lymphocytes and the fetuses of pregnant females after feeding.

Although naturally occurring horizontal transfer has not yet been demonstrated, the search has been on for new vector systems that do not rely on the use of antibiotic resistance genes. In this issue (p. 916), Chua and colleagues describe such a system, based on a selectable marker that eliminates the need for any bacterial antibiotic resistance gene. Their vector imparts inducible expression of a cytokinin biosynthesis hormone that allows shoot regeneration from transformed plant cells—an essential step in converting such cells to whole plants. In contrast, with vectors containing antibiotic resistance genes, transformed plant cells are selected with antibiotics prior to the regeneration step. Chua and colleagues showed that their system, in which about 50% of the shoots regenerated from transformed cells carry genes from the vector, thus appears to be effective for plant transformation. Although there have been previous attempts to use cytokinin expression in selecting for transgenic plants, Chua's system appears to be more practical and should have broader applicability.

Unfortunately, the development of new transformation systems does not solve the problem of antibiotic resistance genes in transgenic plants already approved for use in the US. The investment of time and money in these products makes it unlikely that they will be withdrawn from the market without some evidence that they are dangerous. And the refusal of Europe to accept some of this produce is threatening to strain economic relations between Europe and the US.

In summary, no one has been able to show that native bacteria will take up antibiotic resistance genes when exposed to transgenic plant material under natural conditions. But the experiments with pure culture and with purified DNA demonstrate the existence of mechanisms that would make such an event possible. If and when experiments prove that horizontal transfer occurs, the implications will be debatable. In such a scenario, some vectors might, indeed, create an unacceptable risk. The case of neomycin phosphotransferase (the most common antibiotic resistance gene used in transgenic plants) is, however, more ambiguous. This gene is already ubiquitous, so it is far from clear that its presence in genetically engineered plants will add substantially to the existing danger. If horizontal transfer of genes from plants to bacteria is found to occur naturally, perhaps the most significant outcome would be the reshaping of our evolutionary paradigms.

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