PROTOPLAST TRANSFORMATION

A NEW ROUTE TO VIRUS RESISTANCE IN PLANTS

ST. LOUIS, Mo.—Scientists at Washington University and Monsanto have engineered tobacco and tomato plants that show significant resistance to tobacco mosaic virus (TMV). Roger Beachy of Washington University made this announcement—noting that these are the first virus-resistant plants ever produced via recombinant DNA techniques—at a recent meeting of the American Society of Agronomy. He says transformed plants could be ready for field testing this spring, and some field-ready strains could be developed within the year.

This work exemplifies one of the real powers of *in vitro* gene manipulation: the relative ease with which it allows scientists to approach the molecular basis of empirical observations, often with direct practical applications. The experiments—conducted in collaboration with Patricia Abel and Richard Nelson of Washington University and Robert Fraley and Stephen Rogers of Monsanto (which funded the research)—were based on observations made in the late 1960s that infection of some plants with a mild strain of TMV affords protection from subsequent challenge by a virulent form of the virus. Known as cross-protection, this technique has been used with some success to protect certain tomato species from devastating viral infections.

The first explanation of cross-protection at a molecular level was proposed by Robert Fulton (now emeritus professor, University of Wisconsin, Madison), who suggested in a 1982 *Virology* paper that the coat protein of the pre-infecting virus re-encapsidates the RNA of the viral superinfecting strain, thereby rendering it noninfectious. Elaborating on this intriguing possibility, Beachy and his co-workers proceeded to construct a DNA copy of the 6,400-nucleotide RNA genome of the tobacco mosaic virus. After removing a fragment encoding the capsid protein from the 3′ end, they attached promoter and transport sequences. Then, using the Monsanto Split End Vector (SEV) system (see Fraley et al., *Bio/Technology* **3**:629–635, July '85), they integrated the construct into the nuclear DNA of tobacco and tomato protoplasts. When the transformed cells were regenerated into whole plants and infected with large amounts of virulent TMV, the results were quite convincing: all 150 of the transformed plants showed delayed symptoms of infection. Some, in fact, remained completely symptom free.

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Leaves of plants that have been infected with tobacco mosaic virus for 10 days. *Left:* A systemically infected leaf from a control plant (transformed but not expressing the TMV coat protein gene) shows characteristic signs of viral infection. *Right:* A leaf from a transformed plant in which a functional coat protein gene has been introduced into the nuclear DNA.

Although Beachy has shown that the transformed cells synthesize capsid protein, this does not necessarily mean that the observed resistance involves re-encapsidation or even active protein synthesis. Milton Zaitlin (Cornell University, Ithaca, NY) has demonstrated that infection of tobacco plants with nucleic acid from a TMV mutant deficient in encapsidation still confers cross-protection. Cross-protection is also seen with potato spindle tuber viroids, which do not synthesize any capsid proteins.

However, plausible mechanisms involving only RNA are not hard to imagine. Since Beachy's construct contains the replicase binding site of TMV, it is possible that the RNA produced in transformed cells serves to titrate the replication enzyme of the superinfecting virus. Alternatively, it might interfere directly with synthesis of replicative intermediates or with virus maturation. Explanations involving activation of host defense mechanisms are also possible.

The obvious next steps are to define the molecular basis of the resistance, and to examine the related questions of specificity and dose-responses on which the agronomic utility of the system depends.

The potential value of a system to instill virus resistance in plants is staggering. In the U.S. alone, viruses can destroy an estimated $50 million worth of tomatoes, $95 million of wheat, and $30 million of potatoes in a single year. Ron Thompson, Monsanto's director of business development, will not reveal which crop and virus combinations the company hopes to commercialize. He notes that the research team still must confirm that the system truly works, and then must determine the range of crops and viruses for which it is applicable. Monsanto maintains marketing rights to results of the collaboration with Washington University, and is in the process of determining what crops it might develop itself, and where it will turn to licensing or joint venture relationships.

Even though Thompson declines to elaborate on which crops Monsanto will stress, some choices seem obvious. According to George Kidd, advanced science consultant at L. William Teweles (Milwaukee, WI), the most promising markets are grain diseases, including barley mosaic virus, wheat viruses, and corn mosaic virus. These large-acreage crops make up a major portion of the estimated $1.5 billion to $2 billion in crop value that are lost to viruses in the U.S. each year. Kidd points out, however, that because farmers never "see" the yield lost to viruses—unless the fields are devastated—such figures do not represent true market figures for a potential virus-resistant seed. Rather, such a product will have to compete—presumably favorably—with non-improved seed already on the market. **—Harvey Bialy and Arthur Klausner**