PLANT TUMOR REVERSAL ASSOCIATED WITH THE LOSS OF FOREIGN DNA

FUN-MEI YANG, ALICE L. MONTOYA, EUGENE W. NESTER, AND MILTON P. GORDON

Department of Microbiology and Immunology (F. M. Y., A. L. M., E. W. N.) and Department of Biochemistry (M. P. G.), University of Washington, Seattle, Washington 98195

SUMMARY

Transformation of plant tissues into crown gall tumors has been associated with the transfer of a portion of a tumor-inducing plasmid (Ti-plasmid) into plant DNA. Various laboratories have regenerated normal-appearing plants from a number of crown gall tumors. This study investigates the fate of the foreign DNA in a series of tissues derived from various parts of a plant regenerated from the tumor BT-37 by Braun and his co-workers. It was found that all the foreign DNA sequences were lost from tissues that had lost all their tumorous traits; whereas the plasmid DNA sequences were still present in tissues that appeared normal but still exhibited tumorous traits when returned to tissue culture media. From these studies it would appear that the presence of the Ti-plasmid sequences in the plant DNA is required for the maintenance of the transformed state.

Key words: tumorigenesis; crown gall; reversion; regeneration; foreign DNA sequences.

Plants have a number of conceptual and methodological advantages for the study of transformation in higher eukaryotic cells. Foremost among these is the fact that intact plants can be generated from single cells. Thus, in studies of the reversion of the transformed state, a single transformed plant cell can be reverted to produce an intact, fully functional plant. Such studies have been carried out using plant tumors generated by infection of susceptible plants with various strains of Agrobacterium tumefaciens. The transformed tissues, in contrast to normal tissues, grow in vitro in the absence of the two major types of phytohormones, auxins and cytokinins. The transformation induced by Agrobacterium can be divided into two general types. The first are disorganized tumors (Fig. 1) that have no characteristic morphology; the only structural features present appear to be the rudiments of a vascular system. The second type of transformed tissue is the teratoma (Fig. 1). These cultures produce abnormal shoots and leaves and turn green in the light. Many teratoma cultures are also distinguished by their tendency to burrow into the supporting agar. The transformation of plant tissue into tumors is associated with the transfer of part of the tumor-inducing plasmid "Ti plasmid" into plant DNA (1,2). The plasmid DNA present in the tumor tissue, "T-DNA," is covalently linked to the genome of the plant (M. Thomashow et al., in preparation). The T-DNA is thought to code for enzymes that synthesize the abnormal amino acids (opines) octopine, together with agropine, or nopaline depending on the strain of Agrobacterium that incites the tumor (3).

Both disorganized and teratoma tumors have been reverted to apparently normal tissues. In a recent investigation, Sacristan and Melchers (4) cloned single cells from tumors induced on Samsun tobacco by A. tumefaciens strain 542. The first single cell platings were done 10 weeks after infection. Only 3 out of 341 clones were truly phytohormone independent. This surprisingly low number of transformed cells in tumor callus may reflect either a tendency of the procedures used to exclude tumor cells systematically, or it may indicate that only a small number of transformed cells are present in young tumors. All three of the clones generated plants upon suitable
manipulation, and portions of the young leaves of these plants required auxin and kinetin for growth in vitro. Attempts to induce growth on phytohormone-free medium by growing explants on auxin-containing medium for one passage were unsuccessful in contrast to the behavior of Braun's teratoma tissues (see below).

The second well-established reversal of a disorganized tumor has an interesting history. A number of laboratories around the world have obtained material that was thought to be normal tissue of *Nicotiana tabacum* var. White Burley that had become habituated to phytohormone-independent growth (5). Complete plants were obtained from single cells of this habituated tissue as early as 1966 (6). Subsequently, Sacristan and Melchers (4) found that 41% of phytohormone-autotrophic clones obtained from this tissue were able to generate shoots and leaves and 20 of these also rooted. This reversal occurred in “habituated” material, which had been in culture in Melcher's laboratory for at least a decade. Recent investigations in our laboratory have shown that the material considered to be habituated in fact contained T-DNA and is, undoubtedly, a crown gall tumor (Yang et al., manuscript in preparation).

Recently, Scott (7) has obtained some interesting results with unorganized tumors formed on White Burley by *A. tumefaciens* strain B6. The original tumors occasionally formed outgrowths that showed greater degrees of organization. Two types of cultures were obtained. One type formed small leaflike structures with green peripheries. The opine content of this tissue was similar to that of the parent tissue. A second, teratoma type of tissue also formed spontaneously. This tissue produced clusters of green leafy shoots. The opine content of the latter tissue was strikingly different from the parent in that the teratoma did not contain detectable amounts of agropine. The parent tissue in this experiment was not cloned. Therefore, the results could be interpreted either as a complex interaction between normal cells and cells that differ in the amount of incorporated T-DNA or a partial reversal of an unorganized crown gall tumor.

The formation of teratoma crown gall tumors has also been reversed in a more deliberate manner by Professor Armin Braun and his associates (8–10). Nopaline-synthesizing teratoma tumors generated by *A. tumefaciens* strain T37 on Havana tobacco were grafted to the cut tips of Turkish tobacco plants from which side shoots

![Fig. 1. Crown gall tumors. Right. A disorganized tumor cultured axenically on Linsmaier and Skoog media (11) without phytohormones. This tumor was induced on *Nicotiana tabacum* var. Xanthi by an octopine-utilizing strain of *Agrobacterium tumefaciens*. Left. A teratoma tumor growing axenically on Linsmaier and Skoog media without phytohormones. This tumor was induced by a nopaline-utilizing strain of *Agrobacterium tumefaciens* on *Nicotiana tabacum* var. Xanthi.](image)