GENETIC AND BIOCHEMICAL STUDIES ON THE SUPPRESSION OF AND A RECOVERY FROM THE TUMOROUS STATE IN HIGHER PLANTS

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SUMMARY

Four neoplastic diseases of plants: crown gall, which is caused by Ti plasmid DNA; Black’s wound tumor disease by an RNA virus; the Kostoff genetic tumors by chromosomal imbalance; and habituation, which results from a spontaneous activation of select biosynthetic systems, have been analyzed and compared. It has been found that both the development of a capacity for autonomous growth and the nature of the heritable cellular change that underlies tumorigenesis are similar in the four instances. All develop a capacity for autonomous growth as a result of the persistent activation of select biosynthetic systems, the products of which are concerned with cell growth and division. That the persistent activation of these biosynthetic systems does not involve heritable changes of an irreversible type is indicated by the finding that a reversal of the neoplastic state occurred in three of the test systems. Since the tumor cells in these instances were found to remain totipotent the results suggest that whether the normal or tumor phenotype is expressed is determined by how the genetic information is regulated in a cell. Regulation appears to be accomplished in part through positive feedback control mechanisms. Foreign genetic information could act either in a regulatory manner to persistently activate normal biosynthetic systems or it could code for one or more essential but normally limiting substance(s) and thus replace a substance(s) that in the case of the Kostoff tumors or habituation is specified by host cell genes, or it could do both. In either case, the foreign genetic information can be regulated in much the same manner as are the host cell genes to give rise to either the normal or tumor phenotype.

Key words: plant tumors; autonomy; totipotent; epigenetic; regulation.

If insight is to be gained into the problems of the persistent but potentially reversible suppression of, and a recovery from, the tumorous state in higher plant species it would appear necessary to characterize first the specific substances and regulatory mechanisms that underlie the neoplastic state. The nature of the heritable cellular change that is ultimately responsible for the abnormal and autonomous proliferation of tumor cells should also be determined. In order to develop these two distinct but related aspects of the tumor problem, four neoplastic diseases of plants, each of which is initiated in a different and quite distinct way and all of which have their counterparts in animal pathology, will be briefly described and analyzed. A comparative study of diversely initiated tumors would appear necessary if a synthesis leading conceptually to a unified whole is to be achieved.

The most thoroughly studied and perhaps the best understood of the neoplastic diseases of plants is the crown gall disease. This disease is widespread in nature and plant species that belong to at least 142 genera found in 61 widely separated families of dicotyledonous angiosperms and gymnosperms have been found to be susceptible to it. The crown gall disease is now believed to be caused by large genetic elements that have been identified as Ti plasmids and are transmitted to susceptible plant cells by virulent strains of the crown gall bacterium, Agrobacterium tumefaciens. It has been found possible to kill the incit-
ing bacteria selectively by thermal treatment and if the bacteria are allowed to act on the host cells for as short a period as 3 to 4 days before being destroyed, rapidly growing, fully autonomous tumors develop at the sites of inoculation (1). The Ti plasmids responsible for the cellular transformation in crown gall are circular double-stranded DNAs that range in size from 95 to 156 megadaltons depending on the strain of the bacterium in which they are found (2-4). These plasmids not only confer oncogenic properties to virulent strains of crown gall bacteria and are importantly involved in determining the tumor phenotype in transformed cells, but specify the synthesis by tumor cells of certain unusual amino acid derivatives that are collectively known as the opines (5-8).

Ti plasmids present in different strains of the crown gall bacterium have been found to fall into essentially three genetically distinct groups. Included in the first group are those plasmids that contain genetic information required not only for the synthesis by transformed cells of octopine (N²-[D-l-carboxyethyl]-L-arginine) but for the utilization by bacteria of octopine, but not of nopaline (N²-[1,3-dicarboxypropyl]-L-arginine), as the sole source of carbon and nitrogen when grown in culture. Members of the second group of Ti plasmids initiate tumors that synthesize nopaline and bacteria that carry these plasmids are able to utilize nopaline, but not octopine, as the sole sources of carbon and nitrogen when grown in culture. Ti plasmids of the first group show a high degree of homology (58 to 100%) with Ti plasmids obtained from bacterial strain A6, an octopine utilizator, whereas plasmids of the second group show a 58 to 97% homology with Ti plasmids carried by strain C58, a nopaline utilizator. Homology was found to be 30% or less between plasmids of the two groups (9). There is, in addition, a third type of Ti plasmid that confers virulence to the bacterium but not a capacity to catabolize octopine or nopaline; nor does it initiate tumors that synthesize either of these compounds. Recent studies have shown that certain of these plasmids specify the synthesis by transformed cells of an as yet incompletely characterized opine, which has been given the trivial name cryptopine (10). Other unusual amino acid derivatives have been reported to be present in crown gall tumor tissues initiated by certain strains of the bacteria (11-13). Since the opines appear to be found only in crown gall tumor cells and not in normal plant cells or other types of plant tumor cells they may serve as specific biochemical markers for crown gall tumor cells.

Multiple copies of a fragment of Ti plasmid DNA have not only been found to be present in crown gall tumor cells but to be, in part at least, transcribed in these cells (14,15). It has been estimated that 8.6 x 10⁶ daltons, which amounts to about 7% of the total Ti plasmid DNA, is stably incorporated and replicates in cells of a tobacco tumor line induced by the octopine-utilizing bacterial strain B6-806 (16). Ti plasmid DNA, as it exists in a transformed plant cell, has now in part been mapped and a highly conserved region common to both octopine- and nopaline-type plasmids has been identified (17,18). Results obtained with the use of genetic engineering methods in which it was found that the insertion of plasmid RP4 DNA into the conserved region resulted in the reversible loss of oncogenic properties of the Ti plasmid DNA (18) is evidence of the etiological involvement of this conserved region in the establishment and in the maintenance of the tumorous state in the crown gall disease. Crown gall transformations have their counterparts in animal pathology in the transformations that result from the activities of the two small DNA-containing oncogenic viruses, polyoma and SV40.

A second neoplastic disease of plants that will be dealt with very briefly is Black’s wound tumor disease (19). This disease is caused by an RNA-containing virus. The wound tumor virus is a reovirus composed of 78% protein and 22% RNA. The RNA consists of 12 double-strand components, which have a total molecular weight of 16 million. The viral genome is enclosed in a membrane that supports 32 capsomeres. An RNA polymerase has been found to be associated with the viral particle. Although the several RNA components present in the viral genome have been isolated and studied, no one has as yet been identified as being specifically involved in the establishment and maintenance of the tumorous state.

Some 45 plant species belonging to 20 families have been found to be susceptible to the wound tumor virus. Although many plant species are systemically infected by the virus, overt tumors arise in only a few such as, for example, sweet clover and Rumex plants, and then only at sites of irritation such as those found at wound sites, at points where lateral roots emerge or in regions where hormones dissolved in lanolin are applied locally.

A third class of neoplastic diseases of plants are those that have a genetic basis, and no external