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 de-
stroyed, rapidly growing, fully autonomous
tumors develop at the sites of inoculation (1). The
Ti plasmids responsible for the cellular trans-
formation in crown gall are circular double-
stranded DNAs that range in size from 95 to 156
megadaltons depending on the strain of the bac-
terium in which they are found (2–4). These plas-
mids not only confer oncogenic properties to viru-
 lent strains of crown gall bacteria and are impor-
tantly involved in determining the tumor phen-
type in transformed cells, but specify the synthesis
by tumor cells of certain unusual amino acid deriv-
atives 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. In-
cluded in the first group are those plasmids that
contain genetic information required not only for
the synthesis by transformed cells of octopine \( \text{N}^{2-}
[D-1-carboxyethyl]-L-\text{arginine} \) but for the utiliza-
tion by bacteria of octopine, but not of nopaline
\( \text{N}^{2-}[1,3\text{-dicarboxypropyl}]-L-\text{arginine} \), as the sole
source of carbon and nitrogen when grown in cul-
ture. Members of the second group of Ti plasmids
initiate tumors that synthesize nopaline and bac-
teria 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 utilizer,
whereas plasmids of the second group show a 58
to 97% homology with Ti plasmids carried by
strain C58, a nopaline utilizer. 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 bac-
terium but not a capacity to catabolize octopine or
nopaline; nor does it initiate tumors that synthe-
size either of these compounds. Recent studies
have shown that certain of these plasmids specify
the synthesis by transformed cells of an as yet in-
completely characterized opine, which has been
given the trivial name cryptopine (10). Other un-
usual 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 speci-
fic 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 esti-

mated that \( 8.6 \times 10^{10} \) 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 bac-
terial 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 com-
mon 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 re-
versible loss of oncogenic properties of the Ti plas-
mid DNA (18) is evidence of the etiological in-
volvement of this conserved region in the estab-
ishment and in the maintenance of the tumorous
state in the crown gall disease. Crown gall trans-
formations 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 reo-
type virus 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 capsomers. 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 es-

tablishment 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 sys-
temically 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 irrita-
tion 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