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Developmental regulation of tumor autonomy in plants

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Summary

Neoplastic diseases of plants have several proximal causes; for example, genetic transformation by plasmids of bacterial origin; transformation by double stranded RNA viruses; and, the combination of foreign genomes in interspecific plant hybrids. Regardless of proximal cause, autonomous growth in these diseases appears to result from the inappropriate production of growth factors by the tumor cells. There is evidence that genetic changes are neither necessary nor sufficient for expression of the tumor state in plants: autonomously growing cells can arise as a consequence of epigenetic changes. Tumor cells that contain foreign genes necessary for autonomous growth can differentiate, participate in organogenesis, and exhibit normal growth regulation. This suggests that even when transformation results from a genetic lesion, expression of the tumor state is ultimately regulated by epigenetic mechanisms of the type operating in normal development.

Introduction

As a result of neoplastic transformation, cells acquire the capacity for autonomous growth, which is then inherited by individual tumor cells. Plant neoplastic diseases provide experimental systems well suited for studying the transformation process since the physiological basis for autonomous growth is known and it is possible to establish the developmental and genetic potentialities of the tumor cell by regeneration of complete plants.

In this article I will briefly review the biology of plant tumors using crown gall disease as an example and then provide evidence to support the hypothesis that tumor transformation in plants is a form of inappropriate cell differentiation which is precisely regulated by the same types of mechanisms that operate in normal development.

Tumor inception in crown-gall

Crown-gall tumors result when cells conditioned by wounding from a wide variety of dicotyledonous plant species and certain gymnosperms are exposed to a tumor inducing principle (TIP) elaborated by virulent strains of *Agrobacterium tumefaciens*. The tumors which form at the site of inoculation are true tumors since the cells continue to express their neoplastic character in culture and when grafted onto the host plant in the absence of the inciting bacterium. The type of tumor obtained depends on the plant host, the site of inoculation, and the strain of bacterium used (Braun 1978). For example, *Kalanchoë* or tobacco plants inoculated with the B6 strain of bacterium form large, unorganized tumors that produce the opine octopine, whereas plants inoculated with the T37 strain form complex, highly organized tumors, known as teratomas, that produce another opine, nopaline. Both the pattern of development and type of opine made persist in cloned cell lines serially transferred in culture, indicating that these traits are inherited at the cellular level.

There are several lines of evidence indicating that TIP is a DNA sequence derived from a large, tumor-inducing plasmid (Ti) present in virulent strains of the crown-gall bacterium. First, during tumor inception a portion of this plasmid, T-DNA, is transferred to the host cell (Chilton *et al.* 1977) where it is integrated into one or more sites in the nuclear DNA (Yadav *et al.* 1980). Second, the type of opine produced by the tumor cell is specified by T-DNA (Bomhoff *et al.* 1976), which, in the case of octopine plasmids, for

example, codes for the key enzyme in the synthesis of octopine (Schröder *et al.* 1981; Murai and Kemp 1982). Third, mutations at specific sites in the T-DNA affect the growth and development of tumors elicited with the mutant strains of plasmid (Hooykaas *et al.* 1982; Garfinkel *et al.* 1981). Finally, reversion of tumor cells is accompanied by the loss of all, or a major portion, of the T-DNA (Yang *et al.* 1980; Yang and Simpson 1981). Thus it appears that the maintenance of the tumor state in crown-gall depends on the presence of Ti-plasmid-DNA sequences that are transferred from the bacterium to the plant cell during tumor inception.

Growth-promoting substances and tumor autonomy

Comparison of the nutritional requirements of normal and tumor cells in culture provides strong evidence that the autonomous growth of plant tumors involves an alteration in the capacity of the cell to produce growth factors. Several growth factors are required for proliferation of higher plant cells in culture. The most important ones are the auxins, which promote DNA synthesis, mitosis and cell enlargement, and cell-division factors, such as the cytokinins, which promote cell division. For exemple, pith cells of tobacco exhibit an absolute requirement for exogenous auxin and cytokinin for continuous growth on an otherwise complete medium (Jablonski and Skoog 1954). As a result of transformation, these cells acquire the capacity to grow without the added factors and produce auxin and cytokinin in quantities sufficient to support the growth of normal cells (Braun 1958). Similar changes, although less well-documented, are also found in tumors arising in tumor-prone genetic hybrids (Bayer 1982) and in wound tumor disease, which is caused by a double-stranded RNA virus (Black 1982). These findings lead to the important conclusion that, regardless of the proximal cause for transformation, autonomous growth results from changes in the production of substances necessary for the proliferation of normal cells (Braun 1958; Meins 1974).

The details of how production of growth factors is regulated in tumor cells and the relationship between growth and production of these factors is not known. The observation that crown-gall tumors on plants mimic the application of auxin has lead to the hypothesis that tumor autonomy results from the overproduction of auxin (Link and Eggers 1941). This seems unlikely. Tumor tissues *in planta* and in culture contain auxin at concentrations in the range found in rapidly growing normal tissues of the same plant species (Weiler and Spanier 1981; Pengelly and Meins 1981). There is growing evidence that different genes in the T-DNA affect auxin and cytokinin production by the tumor cell (Garfinkel *et al.* 1981; Hooykaas *et al.* 1982; Morris *et al.* 1982). It is not known, however, whether these genes code for enzymes needed for the synthesis of these factors or act indirectly by altering the expression of host genes for these enzymes.

Developmental regulation of growth autonomy

Insight into the relative contribution of foreign and host genes in the regulation of growth autonomy is provided by the observation that heritable changes in auxin and cytokinin requirement similar to those encountered in transformation also occur in normal development. For example, leaf cells of tobacco require exogenous cytokinin for rapid growth in culture, whereas stem-cortex cells do not (Meins and Lutz 1979). The fact that both states are inherited by individual cells implies that heritable changes in cytokinin requirement occurred sometime in the development of the two tissue types. Direct evidence that this can result in autonomous growth comes from studies of habituation (Meins 1982a).

Tissues from a variety of plant species in culture sometimes lose their requirement for exogenous auxin, or for both auxin and cytokinin. This process, known as habituation, is progressive and generates autonomously proliferating cells, which, in some cases, form transplantable tumors when grafted onto the host plant. Therefore, it appears that habituation is a form of spontaneous neoplastic transformation that occurs in culture.

In spite of their extreme phenotypic stability when serially propagated in culture, cytokinin-habituated cells can be induced to lose

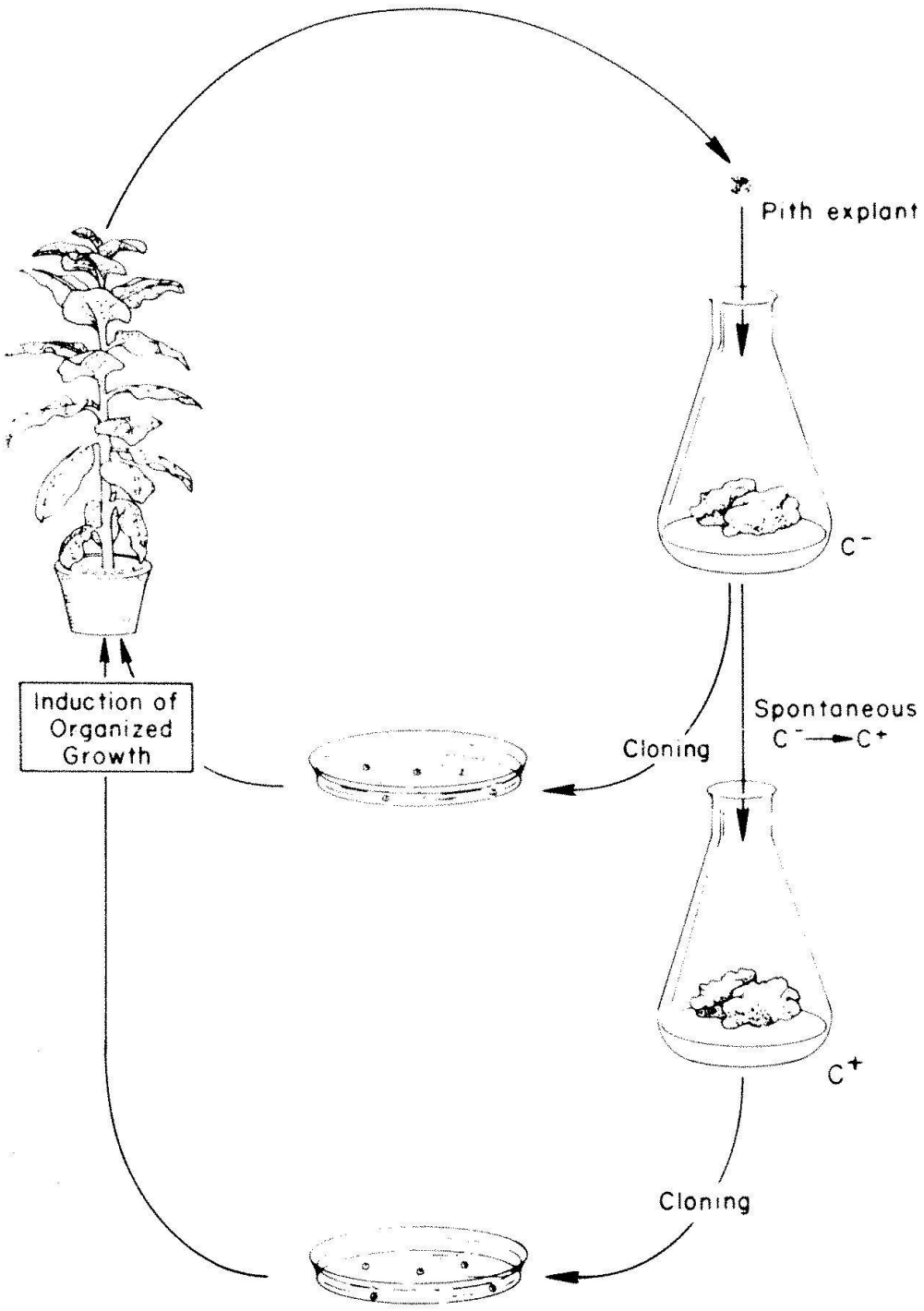

Fig. 1. Plant regeneration experiment showing that the cytokinin-habituated phenotype (C^+) reverts to the non-habituated phenotype (C^-) when cloned tobacco cells of pith origin are induced to form complete plants. From Meins 1974.

their autonomous character. Cloned lines of habituated tobacco cells, which are of pith origin, regularly form normal-looking, fertile tobacco plants when placed on a differentiation-inducing medium. Pith tissues from the regenerated plants exhibit the normal, non-habituated phenotype in culture (fig. 1). Results of this type obtained with many cloned lines and detailed measurements of the rates at which habituation is induced and reversed in culture indicate that habituation involves heritable, potentially reversible, epigenetic changes rather than classical mutations (Meins 1982a). Conversion of cells to the cytokinin-autotrophic state is a key event in both tumor transformation and habituation, which has an epigenetic basis. Therefore, neither somatic mutation nor the introduction of foreign genes need be invoked to account for the stability of the tumor state.

Functions essential for autonomous growth can also be switched on and off in tumor cells containing foreign genes (Braun 1959;

Braun and Wood 1976; Turgeon *et al.* 1976). When cloned, crown-gall teratoma tissues of tobacco are grafted onto the cut stem of tobacco plants from which the auxillary buds have been removed, they give rise to shoots which gradually become more normal in appearance. Some of these shoots eventually flower and set fertile seed. These seeds develop into normal tobacco plants, which have completely lost their tumorous character and, as expected, no longer contain readily detectable T-DNA sequences (Yang *et al.* 1980). Reversion with the loss of Ti-plasmid genes also occurs spontaneously in cultured teratoma tissues, but at a low incidence (Yang and Simpson 1981; Meins 1982a).

Of particular interest, however, are those cases in which tumor cells mimic the normal state but still retain T-DNA sequences. Leaves near the top of teratoma-derived shoots are perfectly normal in gross morphology, physiology and histology. Nevertheless these leaves still produce nopaline (Wood *et al.* 1978), contain T-DNA (Yang *et al.* 1980), and, when placed in culture on a basal medium that does not support the growth of normal leaf tissue, give rise to typical teratoma tissues. It may be argued that the teratoma-derived shoots consist predominantly of revertant cells and that teratomatous tissues arise from a few tumor cells that still retain T-DNA. There is strong evidence against this. Protoplasts from highly differentiated mesophyll cells isolated from leaves on teratoma-derived shoots regularly form teratomatous clones when cultured (Binns *et al.* 1981). Similar results are obtained with single, highly specialized, epidermal hair cells (Huff and Turgeon 1981). Thus, teratoma-derived shoots consist of tumor cells that differentiate, participate in organogenesis, and exhibit normal growth regulation while retaining their tumorous character in a covert form.

Suppression of the tumorous state also occurs in plants regenerated from tumors of genetic hybrids. Carlson *et al.* (1972) have fused protoplasts from two species of *Nicotiana* that give tumor-prone hybrid plants. The dikaryotic clones which result, like crown-gall tissues, do not require auxin and cytokinin for growth in culture. Plants regenerated from these clones are tumor prone; when wounded, typical teratomas arise at the wound site. This shows that cells genetically predisposed to grow autonomously do not express their tumor character in the intact plant, even though the cells retain this potential. Finally, there is a mutation of tobacco in which leaf cells exhibit the cytokinin-habituated phenotype in culture (Meins, 1982c). Plants regenerated from these cells develop normally and have normal appearing leaves, but the leaf tissue still expresses its autonomous character when placed in culture. Therefore, autonomous growth whether resulting from the incorporation of plasmid genes, mutation, or the hybridization of dissimilar genomes is phenotypically suppressed when plants or shoots are regenerated from the altered cells.

Conclusion

There is good evidence that the autonomous growth of plant tumor cells involves the inappropriate production of growth substances such as auxin and cytokinin. What is not clear, at present, is the molecular basis for these changes. It is still not known whether the primary lesion in different tumor diseases is the same or not, whether growth-factor requirement is regulated at the level of growth-factor production or growth-factor degradation, and how foreign genes regulate these processes.

Genetic events such as the integration of foreign genes, interspecific hybridization and somatic mutation appear to be neither necessary nor sufficient for the expression of growth autonomy. Autonomous tumor cells can arise by epigenetic changes that do not seem to involve permanent genetic changes. Studies of teratoma derived shoots and genetic hybrid tumors show that expression of the tumor state can be blocked even when transformation results from a genetic lesion. The picture that emerges is that transformation is a form of abnormal differentiation subject to regulation by the same types of epigenetic mechanisms involved in normal development.

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Updating note

Since this article was written in 1982, direct evidence has been obtained that genes in the T-DNA code for enzymes important in auxin and cytokinin production (Barry *et al.*, 1984; Inze *et al.*, 1983; Schroeder *et al.*, 1984). Comparison of the cytokinin and auxin contents of habituated and crown gall transformed cells has provided further support for the hypothesis that plant cells contain genes with functions similar to the oncogenic genes in the T-DNA (Pengelly and Meins, 1983; Hansen *et al.*, in press). An excellent review of the problem of tumor autonomy in plants has recently appeared (Binns, 1983).

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