T-DNA GENE-FUNCTIONS

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INTRODUCTION

The products of genes located on T-DNA, or Transferable DNA segments, of Ti and Ri plasmids from plant-pathogenic Agrobacteria are the immediate cause of the abnormal growth known as "Crown-gall's" and "Hairy-roots". (For recent review see Weising et al., 1988.) "T-DNA" segments from Ti and Ri plasmids are transferred from the bacterial pathogen to the nucleus of plant cells by a mechanism which is remarkably analogous to a bacterial conjugation system (see Zambryski, 1989). T-DNA's carry a number of genes that are transcribed and translated in plant cells. Genetic studies have demonstrated that the products of these T-DNA genes are responsible for the abnormal growth patterns of plant cells in "Crown-galls" (Ti-T-DNA) and "Hairy roots" (Ri-T-DNA). Two general categories of T-DNA linked genes have thus far been characterized:
a) oncogenes: the products of these genes are directly involved in causing the abnormal growth pattern and
b) opine synthase genes that code for enzymes involved in the synthesis, by transformed plant cells, of a number of organic compounds (opines) that can serve as C and N sources for the growth of free-living (or symbiotic?) Agrobacteria that are genetically endowed with the capacity to specifically catabolize defined opines.

In this short review we plan to discuss the function of some T-DNA linked genes involved in plant growth control that had not been previously understood.

T_L-DNA-GENES CARRIED BY Ti PLASMIDS

It has been documented that the major mechanism responsible for the proliferation of largely undifferentiated cells in Crown-gall tumors is the production by the transformed cells of abnormal levels of two of the major plant growth hormones: auxins and cytokinins. (For recent review see Zambryski et al., 1989). The abnormal production of auxins was

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shown to be the consequence of the activity of the T-DNA genes 1 (iaaM) and 2 (iaaH) (Inze et al., 1984; Schröder et al., 1984; Thomashow et al., 1984) which code respectively for a tryptophan 2-monooxygenase catalizing the formation of indol-3-acetamide from tryptophan and an indol-3-acetamide hydrolase, catalizing the conversion of indol-3-acetamide into indol-3-acetic acid. Similarly the abnormal production of cytokinin was shown to be controlled by the product of gene 4 (iptZ), which is an isopentenyl transferase which converts 5'AMP and isopentenylpyrophosphate into the active cytokinin isopentenyladenosine-5-monophosphate (Akiyoshi et al., 1984; Barry et al., 1984; Buchmann et al., 1985). One can readily demonstrate that the introduction in plant cells of a subsegment of T-DNA only carrying the genes iaaM, iaaH and ipt results in the formation of Crown-gall-like proliferations. Wild-type T-DNA's from many A. tumefaciens strains however carry some other genes in addition to these three essential oncogenes, such as gene 5 which is located to the left of genes iaaM and iaaH on the T_L-DNA genetic map and gene 6 which is located to the right of the iptZ gene. The elucidation of the function of gene 5 was not straightforward because mutant T-DNA's from which the function of gene 5 was eliminated by deletion or transposon insertion, are still capable of producing Crown-gall tumors essentially undistinguishable from W.T. Crown galls (Garfinkel et al., 1981; Leemans et al., 1982; Joos et al., 1983) and tobacco plants transgenic for gene 5 or for a chimeric gene, with the strong CaMV35S promoter driving its expression, exhibit a normal growth habit (i.e. no abnormal phenotype was observed that correlated with gene 5 expression). Two observations led to the elucidation of the function of this gene. 1) Koncz and Schell (1986) found that the natural promoter of gene 5 was activated by the presence of auxins and 2) transgenic tobacco plantlets that contain and express gene 5 are resistant to toxic levels of different exogeneously supplied auxins such as IAA, NAA and 2,4-D (our unpublished data). This phenotype was to some extent explained when it was found that gene 5 codes for an enzyme that catalizes the synthesis of indol-lactate (an auxin analogue). Apparently the synthesis of indol-lactate can counterbalance the toxic effect of exogeneously supplied auxins. In wild-type Crown-galls gene 5, induced by the presence of auxins synthesized by the products of the iaaM and iaaH genes, might protect the transformed cells from toxic effects resulting from the accumulation of auxins in Crown-gall tissue. In transgenic plants carrying a Gus gene driven by the promoter of gene 5, Gus (ß-glucuronidase) activity was observed primarily in phloem cells. The auxin induced function of the gene 5 promoter was shown to be mediated by a cis-regulatory element that was previously identified in auxin activated soybean and Arabidopsis genes. (5' CXAXCATCACAXXTXTGTCGGCXXC 3'). One would expect that an enzyme involved in the synthesis of an auxin analogue that can somehow block auxin-activity (in this case auxin-toxicity) would be detrimental to normal plant growth. However transgenic tobacco plants, overexpressing the gene 5 product and in which indol-lactate was detected, exhibit a normal growth phenotype except in the presence of toxic levels of auxins. This observation could be explained if one assumes that indol-lactate blocks an auxin-carrier or an auxin-receptor specifically involved in the movement or signal-transduction of exogeneously supplied auxins. This hypothesis is presently tested experimentally. The function of gene 6b appears to be similar and possibly reciprocal to that of gene 5. It was indeed observed (Spanier et al., 1989) that the activity of gene 6b reduces a concentration dependent activity of cytokinins. The molecular mechanism responsible for this effect has however not yet been elucidated.

rol GENES CARRIED BY T_L-DNA of Ri PLASMIDS

A. rhizogenes is the causative agent of the hairy root disease, consisting of adventitious roots growing at the site of bacterial infection. While the underlying mechanisms to transform plant cells are apparently identical for A. tumefaciens and A. rhizogenes, the latter one harbors genes on its T-DNA that use a different mechanism to alter the developmental faith of transformed cells. At least three genes (rolA, B and C) of the T_L-DNA are individually capable of stimulating root formation in competent plant tissues. The detailed action of these genes is unknown, but they probably affect the auxin sensitivity of transformed cells.

The idea that the rolB gene can enhance the sensitivity to auxins of rolB transformed cells stems from the observation that some plant tissues (e.g. kalanchoe leaves, carrot disks) that do not react to exogeneously added auxin, react by root formation if they are transformed by an active rolB gene, which by itself does not induce a morphogenetic reaction (White et al., 1985; Estramareix et al., 1986; Spena et al., 1987; Capone et al., 1989). To further investigate the mode of action of this gene it was cloned in a binary plant vector cassette and delivered via A. tumefaciens to plant cells. Its coding region was brought under the transcriptional control of the strong 35S RNA promoter of the cauliflower mosaic virus (P_{35S}-rolB) to study the consequences of overexpression of the rolB gene. Tobacco calli transgenic for the chimeric P_{35S}-rolB gene display an increased sensitivity towards auxins in tissue culture. These calli are necrotic when grown on MS medium containing 0.2 mg/l kinetin and 0.6 mg/l NAA, a medium usually required to support normal callus growth. Lowering the NAA concentration to 0.1 mg/l led to the disappearance of necrosis in P_{35S}-rol-B transgenic calli. Comparison of growth of P_{35S}-rolB calli to normal tobacco calli on media with varying concentrations of several substances with auxin activity (NAA, IAA, IBA, 2,4-D) revealed a 5-10 fold difference in the sensitivity towards the various auxins tested. Also rolB transgenic arabidopsis calli display a higher sensitivity towards auxin in tissue culture. Tobacco plants regenerated from P_{35S}-rolB transgenic calli display a number of phenotypic traits that are reminiscent of auxin-mediated effects (e.g. leaf necrosis). Interestingly Northern blot analysis has shown that the rolB gene, even when its transcription is controlled by the 35S promoter, is expressed at a rather low level in transgenic plants. Overexpression of this gene is probably lethal for plant cells, one might therefore select for transformants with a low level of rolB expression due to position effects. Further support for the viewpoint that the rolB gene product somehow controls auxin sensitivity comes from electrophysiological studies on the effect of NAA on the transmembrane electrical potential (Em) difference of tobacco mesophyll protoplasts. The Em variations have been measured as a function of auxin concentration. The NAA concentration inducing the maximum hyperpolarization gives an estimate of the sensitivity to auxin. These investigations revealed that protoplasts from rolB transgenic plants are 1000-fold more sensitive than normal protoplasts (Maurel et al., 1989). However, the increased sensitivity of rolB transgenic tobacco tissue towards auxin was not observed when the sensitivity of rolB transgenic seeds towards auxin was compared to that of seeds from normal tobacco plants. In either case inhibition of germination and growth was obtained at similar auxin concentrations in the medium. These results indicate that the putative auxin signal transduction chain that is somehow activated by the rolB

gene product, is not active or present at all developmental stages or in all tissues or cells.

In contrast tobacco calli containing and expressing the rolC gene under the control of the 35S promoter (P_{35S}-rolC) were less sensitive to auxin when compared to normal tobacco calli. Necrosis, as an indication of toxic auxin effects, was observed in these calli at auxin concentrations two to four fold higher than in control calli. Also plants regenerated from these calli display phenotypical traits that are indicative of a reduced auxin activity (e.g. reduced apical dominance). Seeds of P_{35S}-rolC transgenic plants were able to germinate and grow on media containing auxin concentrations toxic for control seeds. For example P_{35S}-rolC transgenic seedlings grow well on MS medium containing 5μM NAA, an auxin concentration inhibiting growth of control seedlings. The opposite reactions of rolC transformed plant tissues towards auxin might explain why the combined expression of these genes in transgenic plants can in part reverse the altered growth characteristics caused by the expression of either these genes separately (Schmülling et al., 1988) and why they work synergistically to induce root formation (Spena et al., 1987).

CONCLUSIONS

Ti and Ri plasmids of Agrobacteria have acquired, in their T-DNA's, a remarkable set of genes with which they modify plant cell growth and differentiation. In addition to genes involved in growth hormone synthesis, they also carry various genes involved in the modulation of hormone activity. It will be most fascinating to find out whether similar functions, involved in the modulation of plant growth hormone activity, are operative in non-transformed plant tissues.

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