Crown Gall Tumors

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Glossary

Auxin A plant growth regulator known to promote plant cell expansion and involved in most of the developmental regulation in plants, in association with other plant growth regulators, such as cytokinins. The most common natural form of auxin is indole-3-acetic acid (IAA).
Cytokinin Comprised of a group of plant growth regulators (e.g., kinetin), known to promote cell division, in conjunction with auxin.
Neoplastic growth A mass of uncontrollably proliferating cells not coordinated with the surrounding normal tissue.
Opines Low-molecular-weight molecules composed of an amino acid and a keto acid or a sugar.
T-DNA Transferred DNA; a segment of the *Agrobacterium* Ti-plasmid delimited by two 25 bp sequences that is transferred from *Agrobacterium* to its host cell genome.
Ti-plasmid Tumor-inducing plasmid; a large plasmid present in virulent *Agrobacterium* strains containing the T-DNA as well as the genes required for the virulence of *Agrobacterium* (i.e., transfer of the T-DNA to the host plant cell).

Historical Background

The crown gall disease (Figure 1) was described in biblical times on trees and grapevines as galls and nodules. The first scientific description of galls on grapevines was reported in France by Fabre and Dunal. The causal agent of crown gall was first isolated in 1895 from galls on grapevines in Naples, Italy, by Cavara, who cultured the bacterium on agar medium and showed it to cause the tumor disease that he called 'Tubercolosi della vite'. In the United States, George G. Hedgcock in 1904 isolated bacteria that produced white colonies on agar medium and caused the same galls as those from which he isolated the microorganism. In 1907, Erwin F. Smith and C. O. Townsend designated the bacterium as *Bacterium tumefaciens* and showed that this white colony-producing bacterium elicits tumors in chrysanthemum, marguerite daisy, tobacco, tomato, potato, and sugar beets, and on peach roots. Smith continued exploring the range of plants susceptible and 'immune' to the crown gall disease. By 1920, numerous reports appeared describing the crown gall disease on fruit trees, primarily on apple trees and stone fruit trees. The original name of the organism was changed from *B. tumefaciens* to *Phytomonas tumefaciens* and subsequently to *Agrobacterium tumefaciens*. Interestingly, however, the galls initially studied by Cavara were probably caused by the closely related *Agrobacterium vitis*, rather than *A. tumefaciens*. Between 1930 and 1950, a number of investigators sought to identify the oncogenic material produced by *A. tumefaciens*. There were lengthy debates on whether the bacterium itself or a 'tumor-inducing-principle' causes the crown gall disease. Plant tissue culture studies provided evidence that the tumor tissue remained in a transformed state in the absence of bacteria. The transforming agent was subsequently sought with a number of studies directed toward the physiological and biochemical differences between the crown tumor and its surrounding healthy tissues, and between *A. tumefaciens* and other tumor-causing bacteria such as *Pseudomonas savastanoi* (now called *Pseudomonas syringae* pv. *savastanoi*). Avirulent strains were found when *A. tumefaciens* was cultured at 37 °C or when treated with ethidium bromide, suggesting that an extrachromosomal element is required for virulence. In support of this notion, *Agrobacterium radiobacter*, a naturally occurring avirulent relative of *A. tumefaciens*, was shown to be converted to the virulent form when mixed with the virulent strain and inoculated on plants. The direct analysis of *A. tumefaciens* and *A. radiobacter* revealed the presence of a large virulence-conferring plasmid, termed the tumor-inducing plasmid (Ti-plasmid), in *A. tumefaciens*. Though *A. radiobacter* also contained large plasmids, it is remarkable that the early work concluded correctly that it is the plasmid in *A. tumefaciens* that conferred virulence. Subsequent DNA hybridization studies in the late 1970s and the early 1980s confirmed the original hypothesis that genetic elements, termed the transferred (T) DNA, were transferred from *A. tumefaciens* into the plant chromosomes. The transmission of genetic material across kingdom boundaries by *A. tumefaciens* is the first *bona fide* case in evolutionary biology of active horizontal gene transfer between living organisms of different kingdoms, that is, from prokarya to eukarya. The research on *A. tumefaciens* gave rise to the modern technology of plant genetic engineering whereby any segment of DNA placed within the transferred DNA (T-DNA) region of the Ti plasmid can be transferred to and expressed in plants. Besides the crown gall disease caused by *A. tumefaciens*, several other plant diseases are caused by closely related bacterial strains/species and result in different cell/tissue proliferation symptoms; these diseases are elicited by the respective pathogens via very similar mechanisms, except for the nature of the transferred genes (e.g., cane galls caused in grape by *Agrobacterium vitis* or hairy roots caused by *Agrobacterium rhizogenes*).

Horizontal Transmission of T-DNA Genes

A. tumefaciens is a natural genetic engineer, uniquely equipped with horizontally transfer foreign genes into plants and genetically transform plant cells into 'biofactories' that benefit and enhance the survival of the *A. tumefaciens* cells by producing

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Figure 1 Crown gall tumor developing on the trunk of a tree.

opines, the major carbon and nitrogen sources for the bacterium. *A. tumefaciens* has a very broad host range, capable of causing crown tumors in a wide variety of plants, which include mostly dicotyledonous, but also some monocotyledonous species. The sensitivity of different plant species, and of different plant tissues, to *A. tumefaciens* varies considerably. For example, members of the *Solanaceae* such as *Datura stramonium* (Jimson weed) are 50-fold more sensitive than members of the *Crassulaceae* such as *Kalanchoe daigremontiana*.

Activities of *A. tumefaciens* T-DNA Genes

Genes contained within the T-DNA region are transferred to and expressed in the transformed host cell, resulting in the visible symptoms of *A. tumefaciens* infection (crown gall disease) and the production of opines (Table 1). Like many 'effector' proteins from pathogenic bacteria that are exported to the host cell, genes contained in the T-DNA often harbor functionalities specific for eukaryotic cells, such as TATA and CAAT boxes, and polyadenylation signals, allowing them to utilize the transcription machinery of the host cell. The origin of these eukaryotic elements has not been elucidated, and they may have been acquired by convergent evolution or result from ancient acquisition from a eukaryote.

Oncogenes

By analogy to animal oncogenes, the T-DNA genes involved in the uncontrolled cell division that results in crown gall tumors are also called oncogenes, although the mechanism of tumor induction is different between the animal and plant oncogenes. The involvement of protein products of five T-DNA genes in crown gall development has been clearly demonstrated. Tryptophan-2-monooxygenase (*iaa*M) and indoleacetimide hydrolase (*iaa*H) catalyze the synthesis of auxin, whereas indole-3-lactate synthase transforms tryptophan to indole-3-lactate, which probably acts as an auxin antagonist. Isopentenyltransferase (*ipt*) catalyzes the rate-limiting step in the cytokinin biosynthesis pathway. The product of gene *6b* stimulates plant growth regulator-independent cell division *in vitro* and induces abnormal cell growth and morphological alterations and ectopic expression of various genes, including genes related to cell division, in planta. 6b is a nuclear protein that interacts with various nuclear proteins of the host, and it may act as a histone chaperone and also interfere with the host microRNA (miRNA) pathways. Although other T-DNA genes have not been implicated directly in tumor induction or development, at least some of them may also contribute to these processes. Most of these genes harbor a RolB/C domain (initially identified in the *rolB* and *rolC* genes of *A. rhizogenes* T-DNA), which is thought to confer a glucosidase activity that releases auxin (RolB) or cytokinin (RolC) from glyco-conjugates, although this activity has not been demonstrated in crown gall tumors. Collectively, products of those genes induce massive accumulation of auxins and cytokinins and reprogram the cells in which they are expressed to trigger the cell proliferation that forms the crown gall tumor.

Opine Synthesis Genes

Also contained in the T-DNA are genes encoding enzymes involved in the production of unusual amino acid derivatives composed of a basic amino acid, such as arginine, and an organic acid, such as pyruvic acid or 2-ketoglutaric acid, to form octopine and nopaline, respectively. Additional genes on the T-DNA encode products that form disaccharides linked by a phosphate bond. These sugar phosphates are known as agrocinopines. Collectively, these compounds are termed 'opines'. Opines are secreted by the producing cells of the tumor and utilized by *Agrobacterium* as major carbon and nitrogen sources. Both the type of opine produced by the crown gall cells as a result of *Agrobacterium* infection and the type of opine that *Agrobacterium* cells can consume depend on the type of Ti plasmid that resides in the cell. This is because the Ti plasmid possesses both the specific oncogenes and the genes needed to take up and catabolize a specific opine. Thus, crown gall tumors serve as specialized ecological niches for *A. tumefaciens*.

Crown Gall Tumor Development

Under the influence of expressed T-DNA genes, and the subsequent increase in auxin and cytokinin levels, the transformed plant tissue undergoes uncontrolled cell division that results in the crown gall. The newly formed tissue presents remarkable features that differentiate it from the surrounding tissue. Recent studies have demonstrated the changes that occur in the transformed tissue at molecular and biochemical levels. Formation of the crown gall tumor is an extreme developmental change that requires increased transport and metabolic fluxes achieved via genome-wide effects. For example, the concentrations of many anions, sugars, and amino acids are higher in tumors than that in normal cells, which correlate with changes in expression of

Table 1 Genes encoded by the T-DNA of the *A. tumefaciens* nopaline strain C58

Atu #	*Protein product (gene name)*	*Function*	*Homology*
6000	Agrocinopine synthase	Opine synthesis	
6001	5 protein	Unknown	RolB/RolC family
6002	C protein	Auxin sensitivity[b]	
6003	C′ protein	Unknown	RolB/RolC family
6004	D protein	Unknown	RolB/RolC family
6005	E protein	Unknown	RolB/RolC family
6006	Isopentenyltransferase[a]		
6007	Mannopine synthase	Opine synthesis	
6008	Agrocinopine synthase	Opine synthesis	
6009	Indole-3-lactate synthase	Auxin metabolism	
6010	Indoleacetimide hydrolase (*iaa*H)	Auxin metabolism	
6011	Tryptohane-2- monooxygenase (*iaa*M)	Auxin metabolism	
6012	Isopentenyltransferase (*ipt*)	Cytokinin metabolism	
6013	6a protein	Unknown	RolB/RolC family
6014	6b protein	Histone chaperone, RNAi	RolB/RolC family
6015	D-Nopaline dehydrogenase	Opine synthesis	

RNAi, RNA interference

[a]Pseudogene (nonfunctional gene, which is not expressed).

[b]Unpublished data, B. Lacroix and V. citovsky

specific enzymes and solute transporters. Similar to many animal tumors, crown gall tumors and their interface with the surrounding tissues are characterized by strong vascularization; vascular bundles consisting of both phloem and xylem ensure connection between tumors and the rest of the host plant, thus enhancing water and solute transport. Crown galls become nutritional sinks that depend for nutrients and water on the plant on which they develop. Indeed, tumors produce carbon and nitrogen heterotrophically (mostly from glucose and amino acids) and gain energy largely anaerobically. Whereas plant defense reaction pathways are activated during early *Agrobacterium* infection and crown gall tumor development, usually no extensive necrosis is observed. It seems that host defense, which involves mostly salicylic acid- and ethylene-dependent pathways, is offset by hormonal changes, which are likely auxin-dependent, caused by the tumor growth. Furthermore, although *Agrobacterium* infection initially elicits RNA silencing, which represents the host defense against foreign DNA, this defense response is suppressed in the tumors, probably due to the high levels of auxin and cytokinin which reprogram the transformed differentiated cell to an undifferentiated dividing cell.

Spread and Control of Crown Gall Disease

Crown gall disease is spread primarily through infected stock. Secondary spread originates through cultivation practices. Soil surrounding the crown gall tumors becomes infested with *A. tumefaciens* cells and can serve as reservoir of this pathogen. Selective media designed to culture *A. tumefaciens* from soil are used to monitor the presence of this bacterium in orchards. Many fruit and nut trees are highly susceptible to *A. tumefaciens*. The disease is most severe on young trees since crown gall tumors on their roots and small trunks restrict the flow of water and nutrients. As with many animal tumors, unless caught very early in tumorigenesis, surgical excision of crown gall tumors from the infected plants is ineffective in controlling the disease. Prophylactic measures using antagonistic soil-borne bacteria such as *A. radiobacter*, which harbors the plasmid pAgK84 encoding the antibiotic bacteriocin K84, have been successful only for certain strains of *A. tumefaciens*. Strain specificity of the biological control agent, therefore, limits its use to the sensitive pathogen strains. Other prophylactic strategies include maintaining propagation nurseries free of crown gall-affected plants and sanitary culturing practices. The future of crown gall control in agronomically important plants, however, lies in the use of genetic engineering technologies to produce *Agrobacterium*-resistant lines of fruit and nut trees, including grapevines and canes.

See also: Agrobacterium; Horizontal Gene Transfer; *Agrobacterium* and Ti Plasmids; Transfer of Genetic Information from *Agrobacterium Tumefaciens* to Plants.

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