Crown Galls Tumors

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Abstract

Crown gall is one of several plant tumor diseases typified by a non-self-limiting tissue overgrowth usually on the roots and bottom portions of stems of mainly woody plants. The appearance of tumors is rough on the surface with semi-soft, smooth, spongy inner layers of tissue (Fig. 1). With age, the tumors are easily dislodged and their outer layers are friable. Unlike other tumor diseases, crown gall is the result of genetic transformation caused by *Agrobacterium tumefaciens*, a gram-negative, rod-shaped bacterium that resides in soil preferably on the surface of roots. Unique among bacteria is the ability of *A. tumefaciens* to transmit tumor-forming genes (oncogenes) into its host plant cell, culminating in the integration of the oncogenes into the plant chromosomes at one or more sites. The products of the integrated oncogenes modify plant growth regulators (cytokinin and auxin) synthesis and sensitivity, causing the abnormal proliferation of the transformed cells.

Glossary

Auxin a plant growth regulator known to promote plant cell expansion, and is 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 cytokinins are 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 amino acid and a keto-acid or 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, 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 (**Fig. 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. 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 that from which he isolated the organism. In 1907, Erwin F. Smith and C.O. Townsend designated the bacterium as *Bacterium tumefaciens* and showed that the white colony producing bacterium causes tumors in chrysanthemum, marguerite daisy, tobacco, tomato, potato, sugar beets, and peach roots. Smith continued exploring the range of susceptible and 'immune' plants 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 *Bacterium tumefaciens* to *Phytomonas tumefaciens* and subsequently to *Agrobacterium tumefaciens*. Between 1930 and 1950, several investigators sought to identify the oncogenic material produced by *A. tumefaciens*. There were lengthy debates on whether the bacterium itself or a 'tumor-inducing

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

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 several 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, *A. 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, called the Ti (for tumor-inducing) plasmid (see Ti Plasmids). Though *A. radiobacter* also contained large plasmids, it is remarkable that the early work concluded correctly that the plasmid in *A. tumefaciens* conferred virulence. Subsequent DNA hybridization studies in the late 1970 s and early 1980 s confirmed the original hypothesis that genetic elements 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 (*Prokarya* to *Eukarya*). The research on *A. tumefaciens* gave rise to the modern technology of plant genetic engineering, whereby any segment of DNA placed in the T-DNA can be transferred into and expressed in plants. Besides the crown gall disease caused by *A. tumefaciens*, several other diseases are caused by closely related bacterial strain/species and result in different cell/tissue proliferation symptoms, with a very similar mechanism except for the nature of the transferred genes (e.g. cane galls caused in grape by *A. vitis*, or hairy roots caused by *A. rhizogenes*).

Horizontal Transmission of T-DNA Genes

In essence, *A. tumefaciens* is a natural genetic engineer, uniquely equipped to horizontally transfer foreign genes into plants and genetically transform plant cells into cells that benefit and enhance the survival of the *A. tumefaciens* cells. Experimentally, *A. tumefaciens* was found to have a very broad host range, capable of causing crown tumors in a wide variety of plants, including some monocotyledons. The sensitivity of plant species, and 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 *K. daigremontiana*.

Activities of *Agrobacterium tumefaciens* T-DNA Genes

Genes contained in the T-DNA are 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 translocated

Table 1 Genes encoded by the T-DNA of the *Agrobacterium 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	
6003	C' protein	unknown	RolB/RolC family
6004	D protein	unknown	RolB/RolC family
6005	E protein	unknown	RolB/RolC family
6006	Isopentenyl transferase[a]		
6007	Mannopine synthase	Opine synthesis	
6008	Agrocinopine synthase	Opine synthesis	
6009	Indole-3-lactate synthase	Auxin metabolism	
6010	*iaa*H, Indole acetimide hydrolase	Auxin metabolism	
6011	*iaa*M, Tryptohane-2- monooxygenase	Auxin metabolism	
6012	*ipt*, Isopentenyl transferase	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).

or expressed in the host cell, proteins encoded by T-DNA genes harbor eukaryotic specific features (e.g. subcellular localization sequences, domain interacting with eukaryotic machineries such as protein degradation), resulting in their activities in the host cells. Their origin is not elucidated, and those features may have been acquired by convergent evolution or result from ancient acquisition from a eukaryotic organism. Untranscribed sequences (*cis* motifs such as TATA and CAAT boxes, and polyadenylation signal) are also compatible with host cell transcription machinery and may be subject to transcriptional regulation in the host cell.

Oncogenes

By analogy with animal oncogenes, the T-DNA genes involved in the uncontrolled cell division that results in the crown gall (tumor) are also called oncogenes, although the mechanism of tumor induction is different. The involvement of five T-DNA genes has been demonstrated. *iaa*M (tryptophane-2-monooxygenase) and *iaa*H (indole acetamide hydrolase) catalyze the synthesis of auxin, whereas the indole-3-lactate synthase transforms tryptophane to indole-3-lactate, which probably acts as an auxin antagonist. *ipt* (isopentenyl transferase), catalyzes the rate-limiting step in the cytokinin biosynthesis pathway. The protein C (Atu6002 in the *A. tumefaciens* strain C58) was shown to interfere with the host plant response to auxin. The product of gene 6b also plays a role in tumor formation. It stimulates plant growth regulator-independent cell division in vitro and induces abnormal cell growth and morphological alterations in plants, and ectopic expression of various genes (including genes related to cell division). 6b is a nuclear protein interacting with various plant nuclear proteins, it may act as a histone chaperone, but also interferes with host miRNA pathways. Other T-DNA genes are potentially involved in tumor induction or development, most of them harboring a RolB/C domain. The RolB/C domain (Root loci, initially identified in the RolB and RolC genes in *Agrobacterium rhizogenes* T-DNA) is thought to confer a glucosidase activity, releasing auxin (RolB) or cytokinin (RolC) from glycoconjugates, although this activity is not demonstrated in crown gall tumors. Collectively, products of those genes induce a massive accumulation of auxin and cytokinin 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 unusual compounds are called 'opines.' The type of opines consumed by *A. tumefaciens* depends on the type of Ti plasmid that resides in the organism. The Ti plasmid possesses the genes needed to take up and catabolize a specific opine, which is used by *Agrobacterium* as a source of nitrogen and carbon. Thus, the type of opine utilized is defined by the type of Ti plasmid present in the bacterial cell, and 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 proliferation that forms the crown gall. The newly formed tissue presents remarkable features that differentiate it from the surrounding tissue. Recent studies have shown the modification occurring in transformed tissue, at the

molecular and biochemical levels. The formation of crown gall tumor represents extreme developmental changes that require increased transport and metabolic fluxes, via a genome-wide effect resulting in an adaptation of transport and metabolism. Globally, the concentrations of anions, sugars, and amino acids are higher in tumors, which correlates with changes in the expression of specific enzymes and solute transporters. Consistently, tumors and their interface with host surrounding tissues are characterized by a strong vascularization; vascular bundles consisting of phloem and xylem ensure the connection between tumors and the rest of the host plant, thus enhancing water and solute transport. Crown gall tumors become nutritional sinks, depending on the plant on which they are developing for nutrients and water. Indeed, tumors produce C and N heterotrophically (mostly from glucose and amino acids) and gain energy mostly anaerobically. Whereas plant defense reaction pathways are activated during early host plant infection and crown gall tumor development, usually no extensive necrosis is observed. It seems that plant defense from the host (involving mostly salicylic acid and ethylene pathways) is balanced by tumor growth induced by *Agrobacterium* (likely via the action of auxin). It was also shown that in tumors, RNA silencing (usually induced in the presence of foreign DNA) is induced in the first stage of *Agrobacterium*-host interaction and then repressed in the tumors, probably due to the high levels of auxin and cytokinin, reprogramming the transformed cell to a status of an undifferentiated dividing cell.

Dissemination 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 diseased tissues become infested with *A. tumefaciens* cells and can serve as a reservoir of the 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 tumor growths on their roots and small trunks restrict the flow of water and nutrients. Whereas the crown gall disease is not usually fatal for the infected plants, it results in significant losses in crop yield and vigor. Unless caught very early in tumorigenesis, mechanical elimination of crown gall tumors from infected material is a relatively fruitless way to control the disease. Prophylactic measures using antagonistic soil-borne bacteria such as *A. radiobacter*, harboring the plasmid pAgK84 encoding for the antibiotic bacteriocin K84, have proven successful in certain cases where the antagonist inhibits the growth of the *A. tumefaciens* strain. Strain specificity of the biological control agent, therefore, limits its use to *A. tumefaciens* strains that are sensitive to the antagonist. Other prophylactic strategies include maintaining clean propagation nurseries free of crown gall diseased plants, and sanitary cultural practices. The recent rise of genetically engineered crop technology has opened the way for developing crown gall resistant lines of fruit and nut trees, including grapevines and canes.

Further Reading

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