Agrobacterium tumefaciens as an agent of disease

Matthew A. Escobar[1] and Abhaya M. Dandekar[2]

[1]Department of Cell and Organism Biology, Lund University, Sölvegatan 35, Lund, SE-22362, Sweden
[2]Department of Pomology, University of California, 1 Shields Ave, Davis, CA 95616, USA

Twenty-six years ago it was found that the common soil bacterium *Agrobacterium tumefaciens* is capable of extraordinary feats of interkingdom genetic transfer. Since this discovery, *A. tumefaciens* has served as a model system for the study of type IV bacterial secretory systems, horizontal gene transfer and bacterial–plant signal exchange. It has also been modified for controlled genetic transformation of plants, a core technology of plant molecular biology. These areas have often overshadowed its role as a serious, widespread phytopathogen – the primary driver of the first 80 years of *Agrobacterium* research. Now, the diverse areas of *A. tumefaciens* research are again converging because new discoveries in transformation biology and the use of *A. tumefaciens* vectors are allowing the development of novel, effective biotechnology-based strategies for the control of crown gall disease.

Members of the genus *Agrobacterium* are ubiquitous components of the soil microflora, the vast majority of which are saprophytic, surviving primarily on decaying organic matter. However, several species of agrobacteria cause neoplastic diseases in plants, including *Agrobacterium rhizogenes* (hairy root disease), *Agrobacterium rubi* (cane gall disease), *Agrobacterium tumefaciens* (crown gall disease) and *Agrobacterium vitis* (crown gall of grape). During the past 26 years, it has become apparent that *Agrobacterium* pathogenesis is a unique and highly specialized process involving bacterium–plant interkingdom gene transfer [1]. Crown gall and hairy root have been described as a form of 'genetic colonization' [2] in which the transfer and expression of a suite of *Agrobacterium* genes in a plant cell causes uncontrolled cell proliferation and the synthesis of nutritive compounds that can be metabolized specifically by the infecting bacteria. Thus, infection effectively creates a new niche specifically suited to *Agrobacterium* survival. This article focuses specifically on crown gall, the most agriculturally significant disease caused by agrobacteria, and the state of current and future strategies for crown gall disease control.

Fridiano Cavara first identified a flagellate, bacilloid bacterium (termed *Bacillus ampelopsorae*) as the causal agent of crown gall of grape in 1897 [3]. This organism, now called *A. vitis*, causes the growth of neoplastic tumors on the stem and crown of grapevines and induces necrotic lesions on grape roots [4]. *A. vitis* can survive *in planta* in the intercellular spaces of grape tissue without causing disease but will initiate tumorigenesis upon tissue wounding (most commonly frost injury) [4]. Erwin Smith and C.O. Townsend [5] reported 10 years after the discovery of *A. vitis* that *Bacterium tumefaciens* (now *Agrobacterium tumefaciens*) was the causal agent of crown gall disease in Paris daisy. This organism is capable of inducing tumors at wound sites on the stems, crowns and roots of hundreds of dicots, although root necrosis is not characteristic of *A. tumefaciens*-mediated crown gall disease [6]. The pioneering work of Cavara and Smith and Townsend ushered in a century of study of *Agrobacterium* as an agent of disease, a model system of horizontal gene transfer and a tool for plant transformation (Table 1).

Although crown gall disease is not generally fatal unless infection occurs in young plants, crown gall related reduction in crop yield and/or vigor can be significant in many perennial horticultural crops [7], such as grape [8], apple [9] and cherry [10]. The decreased productivity of galled plants is probably caused by several factors, including decreased water and nutrient flow owing to damaged or constricted vasculature at the site of gall development, and significant water and nutrient allocation to the rapidly dividing but unproductive gall sink [11–13]. In addition, crown galls are sites for secondary infection by other phytopathogens (e.g. *Pseudomonas syringae* and *Armillarea mellea*) or pests (insect borers), and can increase plant susceptibility to abiotic stresses [6,8,12]. Finally, *in planta* populations of tumorigenic agrobacteria can negatively affect graft take, because tumor tissue developing at the graft union prevents fusion of stock and scion tissues [8].

Disease process – transformation and tumorigenesis

Agrobacterium pathogenesis requires two basic elements: (1) delivery of tumorigenic DNA into the plant genome (transformation); and (2) the resultant alteration of plant cell metabolism, resulting in cell proliferation and the synthesis of nutritive compounds that provide a selective advantage for *Agrobacterium* (tumorigenesis). The focus here is entirely on the gall-forming agrobacteria; see Ref. [14] for a review of *A. rhizogenes* and hairy root disease.

From chemotaxis to integration

A detailed treatment of *Agrobacterium* transformation is beyond the scope of this article but several reviews from

Corresponding author: Abhaya M. Dandekar (amdandekar@ucdavis.edu).

 1360-1385/03/$ - see front matter doi:10.1016/S1360-1385(03)00162-6

Table 1. Selected discoveries and insights in *Agrobacterium* biology

Year	Discovery	Refs
1853	First written report of crown gall disease.	[72]
1897	*Agrobacterium vitis* identified as the causal agent of crown gall in grape.	[3]
1907	*Agrobacterium tumefaciens* identified as causal agent of crown gall in Paris daisy (*Argyranthemum frutescens*).	[5]
1947	Sterile plant tumor tissue can proliferate indefinitely on hormone-free medium in culture. Tumor cells are proposed to be 'transformed' by an *Agrobacterium*-derived tumor-inducing principle (TIP).	[38]
1956	Unusual low molecular weight nitrogenous compounds (opines) are identified exclusively in tumor tissue.	[73]
1971	*Agrobacterium tumefaciens* loses virulence when grown at 37°C. The TIP can be transferred between virulent and avirulent *A. tumefaciens* strains.	[74,75]
1974	*Agrobacterium tumefaciens* virulence depends on the presence of a large 'tumor-inducing' (Ti) pasmid. The TIP is probably a component of the Ti plasmid.	[76]
1977	The T-DNA region of the Ti plasmid is present in the genome of crown gall tumor cells: the T-DNA is the TIP.	[1]
1980	The opine concept: the synthesis of opines by transformed cells creates an ecological niche for the infecting strain of *Agrobacterium*.	[34]
1983	First plant transformed with a recombinant gene using *Agrobacterium tumefaciens* as a vector.	[77]
1984	T-DNA oncogenes are identified that mediate overproduction of auxin and cytokinin.	[26,27]
1985	The *virA*/*virG* two-component regulatory system is identified as a central component of signal perception and transduction in *Agrobacterium* transformation.	[20]
1986–present	Further elucidation of the vir-gene-encoded T-DNA transfer process; identification of plant genes involved in *Agrobacterium tumefaciens* transformation; extension of *A. tumefaciens* host range for transformation of monocots; sequencing of the *A. tumefaciens* (C58) genome.	[16–18,47,78,79]

both bacterial [15,16] and plant [17,18] perspectives have recently been published. Briefly, amino acids, organic acids and sugars released from wounded plant cells act as chemoattractants to tumorigenic agrobacteria, which bind to plant cells in a polar orientation upon reaching the wound site [17,19]. Weak attachment to the plant cell is first achieved through synthesis of acetylated polysaccharides, followed by strong binding through the extrusion of cellulose fibrils [17]. Simultaneously, the vir regulon, a set of operons required for the transfer of virulent DNA, is activated by the VirA/VirG two-component regulatory system [20]. The presence of acidic extracellular conditions (pH 5.0–5.5), phenolic compounds and monosaccharides at a plant wound site directly or indirectly induce autophosphorylation of the transmembrane receptor kinase VirA [19]. Activated VirA transfers its phosphate to the cytoplasmic VirG protein, which then binds to the vir box enhancer elements in the promoters of the *virA*, *virB*, *virC*, *virD*, *virE* and *virG* operons, upregulating transcription [15]. Through the co-operative action of the VirD1 and VirD2 proteins, a single-stranded DNA fragment (the T-strand) is synthesized from one or more regions of the tumor-inducing (Ti) plasmid delimited by specific 25 nucleotide repeat sequences [19]. VirD2 remains covalently bound to the 5′ end of the T-strand, which is subsequently coated by the VirE2 single-stranded DNA binding protein, although it is unclear whether VirE2 associates with the T-strand in the bacterial cell or *in planta* [21].

The T-strand/Vir protein complex (T-complex) is exported from *Agrobacterium* to the plant cell cytoplasm through a type IV bacterial secretion apparatus encoded by the *virB* operon and *virD4* [21]. Both VirE2 and VirD2 possess nuclear localization sequences and interact with endogenous plant proteins thought to facilitate targeting of the T-complex to the nucleus, including an importin-α, a type 2C protein phosphatase and three cyclophilins (VirD2-interacting factors), and VIP1 and VIP2 (VirE2-interacting factors) [17,18]. The *Agrobacterium* transferred DNA (T-DNA) can then integrate into the plant cell genome through non-homologous recombination in a process that appears to require plant-encoded proteins (probably enzymes related to DNA repair or recombination) [22,23].

Forming a gall in planta

Genes present in the *Agrobacterium* T-DNA possess the *cis* motifs (e.g. TATA box, CAAT box, polyadenylation signal) required for expression in the eukaryotic plant host [24]. There are two general classes of genes in T-DNA: oncogenes and opine-related genes. The oncogenes alter phytohormone synthesis and sensitivity in the infected cell, thus generating the tumor phenotype. T-DNAs from octopine-type *A. tumefaciens* strains (e.g. 15955 and Ach5) contain five oncogenes: *iaaM*, *iaaH*, *ipt*, *6b* and *5* [25]. The tryptophan mono-oxygenase iaaM converts tryptophan into indole-3-acetamide, and the indoleacetamide hydrolase iaaH catalyzes the synthesis of the plant hormone indole acetic acid (IAA) from indole-3-acetamide (Fig. 1) [26]. The ipt gene product mediates the condensation of adenosine monophosphate with isopentenyl pyrophosphate (iPePP) and/or an unknown terpenoid, forming isopentenyl adenosine 5′ monophosphate (iPMP) or zeatin riboside-5′-monophosphate (ZMP) (Fig. 1) [27,28]. This is the rate-limiting step in cytokinin biosynthesis, and iPMP/ZMP are rapidly converted to *trans*-zeatin by plant-encoded enzymes. The massive accumulation of auxin and cytokinin brought about by the activities of the iaaM, iaaH and ipt enzymes is the primary driver of tumorigenesis. The secondary oncogenes *6b* (*tml*) and *5* are thought primarily to modify the effects of phytohormones in the cell. The 6b gene product is thought to alter hormone responsiveness, potentially by increasing sensitivity to auxins and decreasing sensitivity to cytokinins [29,30]. The biochemical nature of 6b activity has yet to be determined. The product of gene *5* of the T-DNA converts tryptophan into indole-3-lactate, which might act as an auxin antagonist by competing with IAA for auxin binding proteins [31,32].

The second class of T-DNA genes are involved in the synthesis of low molecular weight amino acid and sugar

phosphate derivatives called opines. More than 20 different opines have been identified in crown galls and hairy roots, but only a small subset of these are encoded by the T-DNA of any one *Agrobacterium* strain [33]. *Agrobacterium* strains possess Ti plasmid encoded opine uptake and catabolism genes corresponding to the particular opine species whose synthesis is directed by their resident T-DNA. Thus, opine production in transformed plant cells creates a distinct ecological niche for the infecting strain of *Agrobacterium* [34]. The type of opine(s) produced in infected tissues has traditionally been used to classify the infecting strain of *Agrobacterium* (e.g. octopine, nopaline and agropine-type strains), although these classifications are not always fully inclusive or mutually exclusive. Octopine-type T-DNAs possess four opine synthesis genes catalysing the production of octopine (*ocs*), agropine (*ags*) and mannopine (*mas1′*, *mas2′*) [25]. Correspondingly, octopine *Agrobacterium* strains have nearly 40 Ti-plasmid encoded genes related to octopine, agropine and mannopine uptake and use [25]. Chemically, opines are generally condensation products of amino acids, keto acids and sugars, and up to 7% of the dry weight of tumor tissue can be composed of opine [33]. Thus, although not directly involved in tumorigenesis, opines provide a growth substrate for *Agrobacterium* as well as encouraging conjugal Ti plasmid exchange and chemotaxis [35].

Current mechanisms of crown gall disease control

As with any plant disease, crown gall is a function of the environment, the pathogen and the plant host [12]. The absence of a favorable condition for any one of these elements precludes disease development, and various crown gall disease control measures have targeted each corner of this 'disease triangle' (Fig. 2).

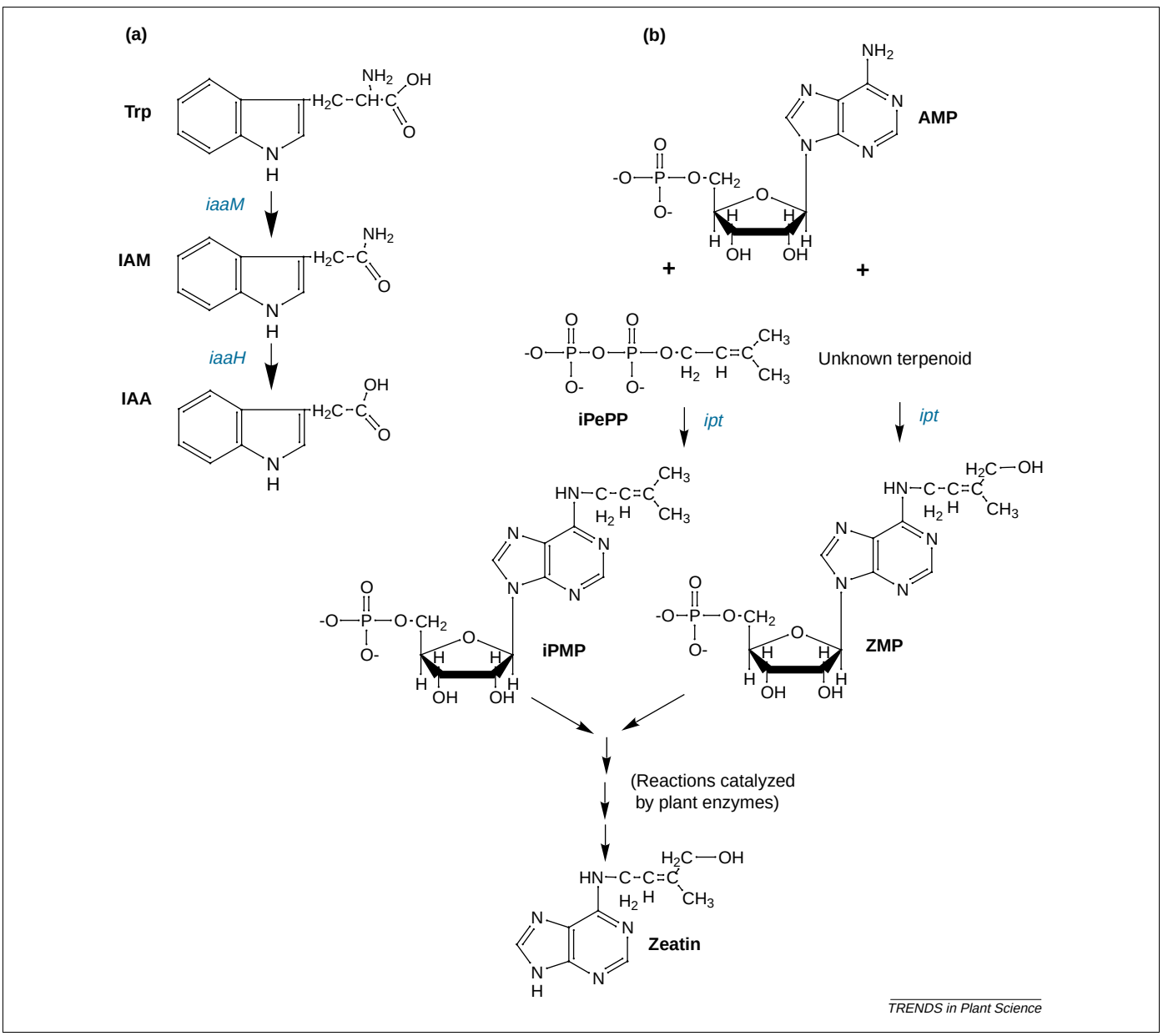

Fig. 1. *Agrobacterium tumefaciens* derived phytohormone biosynthesis pathways. (a) Auxin biosynthesis catalyzed by the *iaaM* and *iaaH* oncogenes. (b) Cytokinin biosynthesis catalyzed by the *ipt* oncogene. Adapted from [28,80].

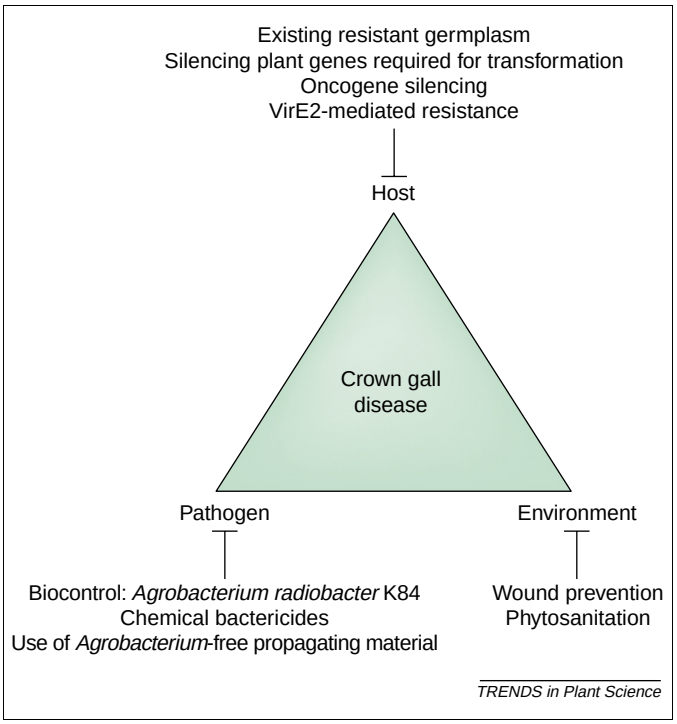

Fig. 2. A crown gall disease triangle. Permissive host, pathogen and environmental conditions are required for crown gall disease to develop. Various disease control strategies target specific corners of the disease triangle – generating resistance in the plant host, eliminating virulent *Agrobacterium tumefaciens* or preventing environmental conditions conducive to infection.

Environment

The primary controllable environmental requirement for the development of crown gall disease is a plant wound. Careful cultural practices that prevent unnecessary plant wounding can significantly reduce crown gall by denying *A. tumefaciens* an opportunity to introduce T-DNA into plant cells [12]. In addition, protection from subfreezing winter temperatures and control of chewing insects and nematodes can be crucial in preventing natural wounds that can act as sites of infection [4,12,36]. The soil environment can also play a major role in determining the incidence and severity crown gall disease. Smith *et al.* [6] prescribed the abandonment of highly infested soils, and intercropping with a non-susceptible host or soil fumigation can temporarily reduce soil populations of *Agrobacterium* [12,37]. The timely removal of infected plant material can also prevent the continued 'seeding' of soil with large populations of pathogenic *A. tumefaciens* derived from crown gall tissues.

Pathogen

Treatments designed to eliminate *Agrobacterium* directly must necessarily be exercised before infection, because disease development will progress independent of the causal agent following the initial transformation event [38]. In situations in which wounding is inevitable, such as grafting and transplanting, copper- or bleach-based bactericides can reduce *A. tumefaciens* populations on plant surfaces, minimizing disease [4]. However, biocontrol treatments using avirulent *Agrobacterium* strains that act as *A. tumefaciens* antagonists have proved to be the most effective means of controlling the crown gall pathogen. *Agrobacterium radiobacter* strain K84 and its plasmid-transfer-deficient derivative K1026 are the most widely used and best studied crown gall biocontrol agents, although several other *Agrobacterium* strains have been exploited for control of crown gall in grape [4]. Strain K84 possesses the 48 kb plasmid pAgK84, which encodes production of and immunity to the antibiotic agrocin 84 [39]. Agrocin 84 has potent bactericidal activity against *A. tumefaciens* strains harboring a nopaline-type Ti plasmid [40]. K84 also produces agrocin 434 and ALS 84, additional antibiotic compounds that probably expand the effective range of control beyond nopaline-type *A. tumefaciens* strains [39,41]. Still, pathogenic *A. tumefaciens* strains that are resistant to K84 biocontrol are not uncommon, so crown gall disease control by K84 is not universally effective [42–44]. The bactericidal treatments described above are essentially topical, so alternative measures to control *Agrobacterium* are often required in grape, which is commonly infected systemically by *A. vitis*. In this case, *A. vitis* free propagation material can be produced either by hot water treatment of dormant cuttings or through *in vitro* shoot tip culture [36].

Plant host

Although *A. tumefaciens* has perhaps the broadest host range of any plant pathogenic bacterium, the agricultural impact of crown gall disease is limited to a relatively small subset of horticultural crops [45,46]. Many cultivated monocots and legumes are not hosts for *A. tumefaciens*, although some of these recalcitrant plants (e.g. maize and rice) can be transformed by *Agrobacterium* vectors under controlled laboratory conditions [47]. The molecular bases of non-host resistance to *A. tumefaciens* are unknown, although the production of antimicrobial metabolites [48], a lack of *vir* gene inducers [49], inefficient T-DNA integration into the plant genome [50] and *Agrobacterium*-induced programmed cell death [51] have been proposed as potential mechanisms.

Among highly susceptible agricultural plant species, a great deal of effort has been focused on the identification and selection of crown-gall-resistant individuals or cultivars. Varying levels of disease susceptibility have been described in plum [52], peach [53], grape [54], aspen [55], rose [56] and others. Again, mechanisms of this genotypic and cultivar-level resistance are generally poorly understood. In the grapevine rootstock 'Glorie de Montpellier', resistance is manifested during or after T-DNA transfer, because *A. tumefaciens* proliferation, attachment and vir gene induction is comparable to susceptible varieties [54]. Crown gall resistance in aspen is negatively correlated with cytokinin sensitivity, suggesting that T-DNA-mediated phytohormone synthesis is insufficient to initiate tumorigenesis in resistant cultivars [55].

Nam *et al.* [57,58] identified several ecotypes and T-DNA-tagged mutants of *Arabidopsis* that are resistant to *Agrobacterium* transformation (RAT). In some RAT genotypes, resistance could be attributed to either reduced bacterial attachment or inefficient T-DNA integration [57,58]. T-DNA integration deficiency in the *RAT5* mutant was found to result from the inactivation of one copy of the histone *H2A-1* gene [59]. The inheritance of crown gall

resistance is highly variable because dominant, semi-dominant and recessive resistance traits have been identified among the different *Arabidopsis* RAT genotypes and among various agricultural species [57,58]. This again underlies the multitude of endogenous plant factors recruited by *Agrobacterium* during pathogenesis.

Inducible plant defenses such as the hypersensitive response (HR) and the oxidative burst are commonly mediated by 'gene for gene' interactions between plant resistance proteins and corresponding pathogen avirulence proteins [60,61]. Considering the diversity of crown gall disease resistance mechanisms, it is surprising that relatively few examples of induced resistance have been described in *A. tumefaciens*–plant interactions [57]. Co-inoculation experiments with *A. tumefaciens* and *Pseudomonas syringae* pv. *phaseolica* have shown that *Agrobacterium* can suppress induction of the HR in plants [62]. This HR inhibition was shown to depend on *A. tumefaciens* auxin synthesis [62]. In addition, *A. tumefaciens* can detoxify hydrogen peroxide, a primary component of the plant oxidative burst that has both direct germicidal activity and a signaling function in induced plant defense [63]. The *A. tumefaciens* catalase KatA, which converts hydrogen peroxide into oxygen and water, is required for virulence on kalanchoe [63]. Thus, it appears that *Agrobacterium* has developed several unique strategies to overcome induced plant defense mechanisms.

For many perennial crops, such as walnut and apple, available germplasm resources have not been satisfactory sources of crown gall disease resistance. Recently, several biotechnology strategies have been developed for the *de novo* generation of crown gall resistance in plants. These strategies generally interfere with the process of *A. tumefaciens* infection or disease development *in planta* without directly targeting the pathogen.

As discussed above, it is apparent that a suite of host plant genes is required for efficient *Agrobacterium* T-DNA transfer and integration [17,18]. Specific cell wall proteins (e.g. vitronectin-like proteins) are probably required for bacterial attachment, nuclear import machinery (e.g. importin-α and VIP1) is required for T-DNA subcellular trafficking and components of DNA repair and recombination pathways (potentially including histone H2A-1) appear to be required for T-DNA integration [17,18]. Post-transcriptional gene silencing (PTGS) of any of these plant genes could generate disease resistance by blocking the process of *Agrobacterium* transformation. Indeed, individual transgenic *Arabidopsis* plants expressing *VIP1* antisense RNA, *importin α1* antisense RNA or histone *H2A-1* antisense RNA display crown gall disease resistance [64,65] (S. Gelvin, pers. commun.). It must be realized, however, that molecular mechanisms exploited by *Agrobacterium* certainly serve other physiological functions in the plant, so silencing of these endogenous genes could have undesirable pleiotropic effects.

Another PTGS-mediated resistance strategy, oncogene silencing, interferes with crown gall disease development following T-DNA integration into the plant genome. As described previously, expression of the T-DNA-encoded oncogenes *iaaM*, *iaaH* and *ipt* in transformed plant cells causes rapid auxin and cytokinin synthesis, which initiates and maintains tumorigenesis. These oncogenes share high nucleotide sequence conservation (~90%) across all characterized *A. tumefaciens* strains [66]. *Arabidopsis*, tomato (*Lycopersicon esculentum*) and walnut (*Juglans regia*) plants transformed with self-complementary RNA constructs designed to initiate PTGS of *iaaM* and *ipt* demonstrated broad-spectrum crown gall resistance (Fig. 3) [66–68]. Resistance was correlated with a substantial decrease in *iaaM* and *ipt* mRNA *in planta* and an accumulation of *iaaM* and *ipt*-homologous small interfering RNAs [66,67]. Although oncogene-silenced plants display normal appearance and development, it remains to be seen whether any long-term developmental penalty is associated with constitutive activity of the PTGS pathway.

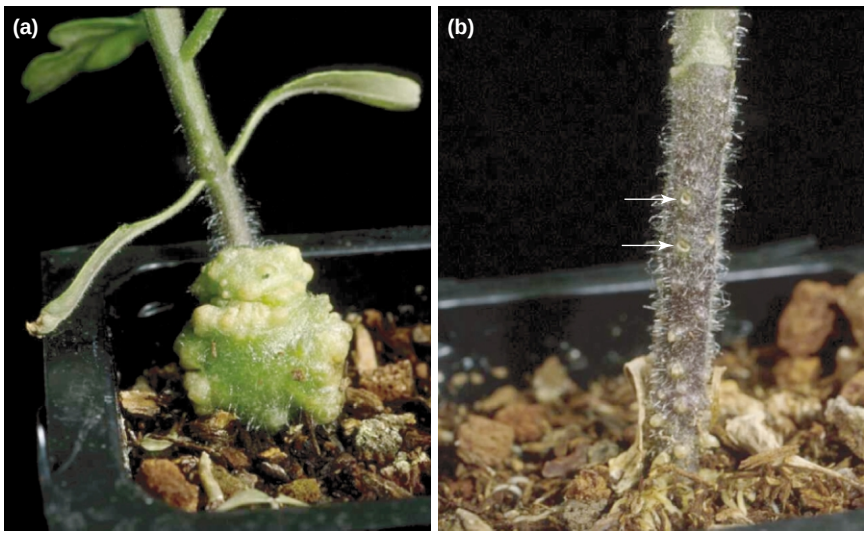

Fig. 3. Resistance to crown gall mediated by silencing of the T-DNA encoded *iaaM* and *ipt* oncogenes. Stems of tomato seedlings were inoculated with *Agrobacterium tumefaciens* and assayed for disease development five weeks after inoculation. (a) Wild-type plant displaying characteristic massive gall development and stunted growth. (b) An oncogene-silenced transgenic plant displaying normal growth and no gall development (two inoculation sites are visible on the central stem, indicated by arrows). Reproduced, with permission, from [81].

Finally, ectopic expression of a *virE2* deletion transgene *in planta* has been shown to confer resistance to crown gall disease in grape (*Vitis vinifera*) and tobacco (*Nicotiana tabaccum*) [36,69; United States patent #6172280]. The described deletion construct lacks 215 C-terminal amino acids, which eliminates *virE2*'s single-stranded-DNA binding domain. The precise mechanism of resistance has not been reported, but one possibility is that the mutant protein competes with wild-type virE2, titrating out virE2-interacting factors essential for transformation, such as VIP1 and VIP2 [64]. However, the recent demonstration that virE2 interacts with membrane lipids to form large, voltage-gated channels [70] begs the question: will constitutive expression of virE2 compromise plant physiology?

Conclusions

After over a century of study, crown gall disease continues to have a significant impact in orchards and vineyards worldwide. The ubiquity of *A. tumefaciens*, its effective mechanisms for evasion of plant defenses and the unique pathology of genetic colonization have made crown gall disease control especially challenging. The emergence of crown gall biocontrols, beginning almost 30 years ago with *A. radiobacter* K84, provided a new paradigm in disease control. Advances in our understanding of the transformation process *in planta* are now shifting the focus of disease control from *A. tumefaciens* to the susceptible plant host, as evidenced by the first generation of transgenic plants possessing *de novo* resistance to crown gall. As the genetic determinants of plant susceptibility to crown gall continue to be elucidated, it is likely that transformation competence might become a largely manipulatable factor in plants [59,71], with clear pathological applications in the field and biotechnological applications in the laboratory.

References

1 Chilton, M-D. *et al.* (1977) Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of crown gall tumorigenesis. *Cell* 11, 263–271
2 Schell, J. *et al.* (1979) Interactions and DNA transfer between *Agrobacterium tumefaciens*, the Ti plasmid, and the plant host. *Proc. R. Soc. London Ser B* 204, 251–266
3 Cavara, F. (1897) Tubercolosi della vite. Intorno alla eziologia de alcune malattie di piante coltivate. *Stazoni Sperimentale Agrarie Italiane* 30, 483–487
4 Burr, T.J. *et al.* (1998) Biology of *Agrobacterium vitis* and the development of disease control strategies. *Plant Dis.* 82, 1288–1297
5 Smith, E.F. and Townsend, C.O. (1907) A plant-tumor of bacterial origin. *Science* 24, 671–673
6 Smith, E.F. *et al.* (1911) Crown gall of plants: its cause and remedy. *US Dept Agric. Bull.* 213, 1–215
7 Kennedy, B.W. (1980) Estimates of U.S. crop losses to prokaryote plant pathogens. *Plant Dis.* 647, 674–676
8 Schroth, M.N. *et al.* (1988) Reduction in yield and vigor of grapevine caused by crown gall disease. *Plant Dis.* 72, 241–246
9 Riker, A.J. *et al.* (1959) Effects of crown gall and hairy root on the growth of apple trees. *Phytopathology* 49, 88–90
10 Lopatin, M.I. (1939) Influence of bacterial root canker on the development of the cherry tree in the orchard. *Plant Prot.* 18, 167–173
11 Klein, R.M. and Link, G.K.K. (1955) The etiology of crown gall. *Q. Rev. Biol.* 30, 207–277
12 Agrios, G.N. (1997) *Plant Pathology*, 4th edn, Academic Press
13 Aloni, R. *et al.* (1998) The *Never ripe* mutant provides evidence that tumor-induced ethylene controls the morphogenesis of *Agrobacterium tumefaciens*-induced crown galls on tomato stems. *Plant Physiol.* 117, 841–849
14 Nilsson, O. and Olsson, O. (1997) Getting to the root: the role of the *Agrobacterium rhizogenes rol* genes in the formation of hairy roots. *Physiol. Plant.* 100, 463–473
15 Rossi, L. *et al.* (1998) Role of virulence proteins of *Agrobacterium* in the plant. In *The Rhizobiaceae* (Spaink, H.P. *et al.*, eds), pp. 303–320, Kluwer Academic Publishers
16 Zupan, J. *et al.* (2000) The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights. *Plant J.* 23, 11–28
17 Gelvin, S.B. (2000) *Agrobacterium* and plant genes involved in T-DNA transfer and integration. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51, 223–256
18 Tzfira, T. and Citovsky, V. (2002) Partners-in-infection: host proteins involved in the transformation of plant cells by *Agrobacterium*. *Trends Cell Biol.* 12, 121–128
19 Winans, S.C. (1992) Two-way chemical signaling in *Agrobacterium*–plant interactions. *Microbiol. Rev.* 56, 12–31
20 Stachel, S.E. and Zambryski, P.C. (1985) VirA and VirG control the plant-induced activation of the T-DNA transfer process of *A. tumefaciens*. *Cell* 46, 325–333
21 Vergunst, A.C. *et al.* (2000) VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. *Science* 290, 979–982
22 Ziemienowicz, A. *et al.* (2000) Plant enzymes but not *Agrobacterium* VirD2 mediate T-DNA ligation *in vitro*. *Mol. Cell. Biol.* 20, 6317–6322
23 van Attikum, H. *et al.* (2001) Non-homologous end-joining proteins are required for *Agrobacterium* T-DNA integration. *EMBO J.* 20, 6550–6558
24 Barker, R.F. *et al.* (1983) Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. *Plant Mol. Biol.* 2, 335–350
25 Zhu, J. *et al.* (2000) The bases of crown gall tumorigenesis. *J. Bacteriol.* 182, 3885–3895
26 Klee, H. *et al.* (1984) Nucleotide sequence of the *tms* genes of the pTiA6NC octopine Ti plasmid: two gene products involved in plant tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A.* 81, 1728–1732
27 Lichtenstein, C. *et al.* (1984) Nucleotide sequence and transcript mapping of the *tmr* gene of the pTiA6NC octopine Ti plasmid: a bacterial gene involved in plant tumorigenesis. *J. Mol. Appl. Genet.* 2, 354–362
28 Åstot, C. *et al.* (2000) An alternative cytokinin biosynthesis pathway. *Proc. Natl. Acad. Sci. U. S. A.* 97, 14778–14783
29 Hooykaas, P.J.J. *et al.* (1988) The *Agrobacterium tumefaciens* T-DNA gene 6^b is an *onc* gene. *Plant Mol. Biol.* 11, 791–794
30 Gaudin, V. *et al.* (1994) Bacterial genes modifying hormonal balances in plants. *Plant Physiol. Biochem.* 32, 11–29
31 Hamill, J.D. (1993) Alterations in auxin and cytokinin metabolism of higher plants due to expression of specific genes from pathogenic bacteria: a review. *Aust. J. Plant Physiol.* 20, 405–423
32 Binns, A.N. and Costantino, P. (1998) The *Agrobacterium* oncogenes. In *The Rhizobiaceae* (Spaink, H.P. *et al.*, eds), pp. 251–266, Kluwer Academic Publishers
33 Dessaux, Y. *et al.* (1993) Chemistry and biochemistry of opines, chemical mediators of parasitism. *Phytochemistry* 34, 31–38
34 Guyon, P. *et al.* (1980) Agropine in 'null type' crown gall tumors: evidence for generality of the opine concept. *Proc. Natl. Acad. Sci. U. S. A.* 77, 2693–2697
35 Kim, K-S. *et al.* (2001) Intracellular accumulation of mannopine, an opine produced by crown gall tumors, transiently inhibits growth of *Agrobacterium tumefaciens*. *Mol. Plant–Microbe Interact.* 14, 793–803
36 Burr, T.J. and Otten, L. (1999) Crown gall of grape: biology and disease management. *Annu. Rev. Phytopahol.* 37, 53–80
37 Teviotdale, B.L. *et al.* (1985) Bark, fruit and foliage diseases. In *Walnut Orchard Management* (Ramos, D.E. *et al.*, eds), pp. 153–157, University of California Division of Agriculture and Natural Resources Publications
38 Braun, A.C. (1947) Thermal studies on the factors responsible for tumor initiation in crown-gall. *Am. J. Bot.* 34, 234–240
39 Penyalver, R. *et al.* (2000) Use of the genetically engineered *Agrobacterium* strain K1026 for biological control of crown gall. *Eur. J. Plant Pathol.* 106, 801–810

40 Ellis, J.G. *et al.* (1979) *Agrobacterium*: genetic studies on agrocin 84 production and the biological control of crown gall. *Physiol. Plant Pathol.* 15, 215–223
41 McClure, N.C. *et al.* (1998) Construction of a range of derivatives of the biological control strain *Agrobacterium rhizogenes* K84: a study of factors involved in biological control of crown gall disease. *Appl. Environ. Microbiol.* 64, 3977–3982
42 Moore, L.W. and Warren, G. (1979) *Agrobacterium radiobacter* strain 84 and biological control of crown gall. *Annu. Rev. Phytopathol.* 17, 163–179
43 Alconero, R. (1980) Crown gall of peaches from Maryland, South Carolina, and Tennessee and problems with biological control. *Plant Dis.* 64, 835–838
44 Burr, T.J. *et al.* (1993) Failure of *Agrobacterium radiobacter* strain K84 to control crown gall on raspberry. *HortScience* 28, 1017–1019
45 DeCleene, M. and DeLey, J. (1976) The host range of crown gall. *Bot. Rev.* 42, 389–466
46 Deng, W. and Nester, E.W. (1998) Determinants of host specificity of *Agrobacterium* and their function. In *The Rhizobiaceae* (Spaink, H.P. *et al.*, eds), pp. 321–338, Kluwer Academic Publishers
47 Nadolska-Orczyk, A. *et al.* (2000) *Agrobacterium*-mediated transformation of cereals: from technique development to its applications. *Acta Physiol. Plant.* 22, 77–78
48 Sahi, S.V. *et al.* (1990) Corn metabolites affect growth and virulence of *Agrobacterium tumefaciens*. *Proc. Natl. Acad. Sci. U. S. A.* 87, 3879–3883
49 Usami, S. *et al.* (1987) Absence in monocotyledonous plants of the diffusible plant factors inducing T-DNA circularization and vir gene expression in *Agrobacterium*. *Mol. Gen. Genet.* 209, 221–226
50 Narasimhulu, S.B. *et al.* (1996) Early transcription of *Agrobacterium* T-DNA genes in tobacco and maize. *Plant Cell* 8, 873–886
51 Hansen, G. (2000) Evidence for *Agrobacterium*-induced apoptosis in maize cells. *Mol. Plant–Microbe Interact.* 13, 649–657
52 Pierronnet, A. and Salesses, G. (1996) Behavior of *Prunus* cultivars and hybrids towards *Agrobacterium tumefaciens* estimated from hardwood cuttings. *Agronomie* 16, 247–256
53 Bliss, F.A. *et al.* (1999) Crown gall resistance in accessions of *Prunus* species. *HortScience* 34, 326–330
54 Sule, S. *et al.* (1994) Crown gall resistance of *Vitis* spp. and grapevine rootstocks. *Phytopathology* 84, 607–611
55 Beneddra, T. *et al.* (1996) Correlation between susceptibility to crown gall and sensitivity to cytokinin in aspen cultivars. *Phytopathology* 86, 225–231
56 Reynders-Aloisi, S. *et al.* (1998) Tolerance to crown gall differs among genotypes of rose rootstocks. *HortScience* 33, 296–297
57 Nam, J. *et al.* (1997) Differences in susceptibility of *Arabidopsis* ecotypes to crown gall disease may result from a deficiency in T-DNA integration. *Plant Cell* 9, 317–333
58 Nam, J. *et al.* (1999) Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium*. *Mol. Gen. Genet.* 261, 429–438
59 Mysore, K.S. *et al.* (2000) An *Arabidopsis* histone H2A mutant is deficient in *Agrobacterium* T-DNA integration. *Proc. Natl. Acad. Sci. U. S. A.* 97, 948–953
60 Flor, H.H. (1971) Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9, 275–296
61 Hammond-Kosack, K.E. and Jones, J.D.G. (1996) Resistance gene-dependent plant defense responses. *Plant Cell* 8, 1773–1791
62 Robinette, D. and Matthysse, A.G. (1990) Inhibition by *Agrobacterium tumefaciens* and *Pseudomonas savastanoi* of development of the hypersensitive response elicited by *Pseudomonas syringae* pv. *phaseolicola*. *J. Bacteriol.* 172, 5742–5749
63 Xu, X.Q. and Pan, S.Q. (2000) An *Agrobacterium* catalase is a virulence factor involved in tumorigenesis. *Mol. Microbiol.* 35, 407–414
64 Ward, D.V. *et al.* (2002) *Agrobacterium* VirE2 gets the VIP1 treatment in plant nuclear import. *Trends Plant Sci.* 7, 1–3
65 Zhu, Y. *et al.* (2003) Identification of Arabidopsis *rat* mutants. *Plant Physiol.* 132, 494–505
66 Escobar, M.A. *et al.* (2001) RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13437–13442
67 Escobar, M.A. *et al.* (2002) Silencing crown gall disease in walnut (*Juglans regia* L.). *Plant Sci.* 163, 591–597
68 Escobar, M.A. *et al.* (2003) Characterization of oncogene-silenced transgenic plants: implications for *Agrobacterium* biology and post-transcriptional gene silencing. *Mol. Plant Pathol.* 4, 57–65
69 Xue, B. *et al.* (1999) Transformation of five grape rootstocks with plant virus genes and a *virE2* gene from *Agrobacterium tumefaciens*. *In Vitro. Cell. Dev. Biol. Plant* 35, 226–231
70 Dumas, F. *et al.* (2001) An *Agrobacterium* VirE2 channel for transferred-DNA transport into plant cells. *Proc. Natl. Acad. Sci. U. S. A.* 98, 485–490
71 Tzfira, T. *et al.* (2002) Increasing plant susceptibility to *Agrobacterium* infection by overexpression of the *Arabidopsis* nuclear protein VIP1. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10435–10440
72 Fabre, E. and Dunal, F. (1853) Observations sur les maladies regantes de la vigne. *Bull. Soc. Cent. Agric. Dep. Herault.* 40, 46
73 Lioret, C. (1956) Sur la mise en evidence d'un acide amine non identifie particulier aux tissus de 'crown-gall'. *Bull. Soc. Fr. Physiol. Veg.* 2, 76
74 Hamilton, R.H. and Fall, M.Z. (1971) The loss of tumor-inducing ability in *Agrobacterium tumefaciens* by incubation at high temperature. *Experientia* 27, 229–230
75 Kerr, A. (1971) Acquisition of virulence by non-pathogenic isolates of *Agrobacterium radiobacter*. *Physiol. Plant Pathol.* 1, 241–246
76 Zaenen, I. *et al.* (1974) Supercoiled circular DNA in crown gall-inducing *Agrobacterium* strains. *J. Mol. Biol.* 86, 109–127
77 Zambryski, P. *et al.* (1983) Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity. *EMBO J.* 2, 2143–2150
78 Goodner, B. *et al.* (2001) Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science* 294, 2323–2328
79 Wood, D.W. *et al.* (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science* 294, 2317–2323
80 Wink, M. (1997) Special nitrogen metabolism. In *Plant Biochemistry* (Dey, P.M. and Harborne, J.B., eds), pp. 439–486, Academic Press
81 Jamison, J. (2001) Tomatoes silence crown gall disease. *Nat. Biotechnol.* 19, 1127

Five plant scientists chosen as new members by the National Academy of Sciences

Trends in Plant Science congratulates the following plant scientists who were recently elected as members of the National Academy of Sciences in recognition of their outstanding achievements in original research:

Anthony R. Cashmore, University of Pennsylvania, Philadelphia, PA, USA
Luis Herrera-Estrella, National Polytechnic Institute, Guanajuato, Mexico
June B. Nasrallah, Cornell University, Ithaca, NY, USA
Michael F. Thomashow, Michigan State University, East Lansing, MI, USA
James L. Van Etten, University of Nebraska, Lincoln, NE, USA