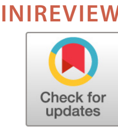
MINIREVIEW



Agrobacterium tumefaciens: a Transformative Agent for Fundamental Insights into Host-Microbe Interactions, Genome Biology, Chemical Signaling, and Cell Biology

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ABSTRACT *Agrobacterium tumefaciens* incites the formation of readily visible macroscopic structures known as crown galls on plant tissues that it infects. Records from biologists as early as the 17th century noted these unusual plant growths and began examining the basis for their formation. These studies eventually led to isolation of the infectious agent, *A. tumefaciens*, and decades of study revealed the remarkable mechanisms by which *A. tumefaciens* causes crown gall through stable horizontal genetic transfer to plants. This fundamental discovery generated a barrage of applications in the genetic manipulation of plants that is still under way. As a consequence of the intense study of *A. tumefaciens* and its role in plant disease, this pathogen was developed as a model for the study of critical processes that are shared by many bacteria, including host perception during pathogenesis, DNA transfer and toxin secretion, bacterial cell-cell communication, plasmid biology, and more recently, asymmetric cell biology and composite genome coordination and evolution. As such, studies of *A. tumefaciens* have had an outsized impact on diverse areas within microbiology and plant biology that extend far beyond its remarkable agricultural applications. In this review, we attempt to highlight the colorful history of *A. tumefaciens* as a study system, as well as current areas that are actively demonstrating its value and utility as a model microorganism.

KEYWORDS cell polarity, crown gall, gene regulation, genetic models, horizontal gene transfer, host-pathogen interactions, plant pathogens, plasmids, quorum sensing, secretion systems

Undoubtedly, one of the most fascinating and important areas of microbiology is the interaction of microorganisms with hosts. Indeed, the study of model bacterial pathogens has been an active component of microbial science from its earliest days, most notably, work on *Bacillus anthracis* and *Streptococcus pneumoniae* (1, 2). In studying these pathogenic bacteria and their host associations, many important biological phenomena have been discovered, including the elucidation of numerous processes that have broadly contributed to our fundamental understanding of biology. Among these, *Agrobacterium tumefaciens*, the causative agent of the plant neoplastic disease crown gall, is a prime example of how fundamental discoveries can result from comprehensively studying a pathogen. *A. tumefaciens* has a remarkable history of yielding important insights into microbial mechanisms of host interaction, as well as many core processes that are shared across different bacteria.

EARLY STUDIES OF *AGROBACTERIUM*

The earliest records reporting studies of plant crown galls are credited to Italian biologist Marcello Malpighi (of Malpighian tubule fame) (3). However, it was another Italian

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scientist, Fridiano Cavara who recognized that crown galls on grapevines could be correlated to the presence of a microbial isolate and that inoculation of this isolated "schizomycete" could induce tumors on young grapevines, and his observations were published in 1897 (4). It was not until 10 years later in 1907 that renowned U.S. plant pathologist Erwin F. Smith along with C. O. Townsend, applied bacteriological approaches to satisfy Koch's postulates for the etiologic agent of crown gall (5). Smith is often credited as first isolating and characterizing the pathogen. The range of basic observations made in this initial characterization that remain relevant today is startling. There are several excellent sources that document the remarkable history of *Agrobacterium* research (6, 7) and an entire volume that compiles and comments on many of the landmark discoveries that led to our current knowledge of *Agrobacterium* biology (8). The history of *A. tumefaciens* research is highlighted by a striking series of discoveries during the 20th century and now into the 21st century (Fig. 1, Table 1), and this delightfully complex bacterium continues to yield unexpected and illuminating new findings.

TAXONOMIC CHALLENGES

The taxonomic classification of *Agrobacterium* has had a storied and contentious history that often confounds scientists who do not study this bacterium, and even those who do (9). Smith and Townsend originally named the causative agent of crown gall *Bacterium tumefaciens* (5). The bacterium subsequently went through several different taxonomic designations until 1942, when the genus/species name *Agrobacterium tumefaciens* was proposed (10). Three issues are at the heart of the confusion over the name of the pathogen and its close relatives. First is that early classification schemes attempted to capture differences in pathogenicity and host range. For example, *Agrobacterium tumefaciens* (biovar 1) and *Agrobacterium rhizogenes* (biovar 2) were classically used to infer those that cause crown gall and hairy root diseases, respectively. The *Agrobacterium vitis* (biovar 3) and *Agrobacterium radiobacter* species names were used to describe those that cause crown gall disease on grapevine and strains that are not pathogenic, respectively. However, pathogenicity is dependent upon a plasmid that can be lost but can also mobilize broadly across taxonomic groups, making pathogenicity an unstable and horizontally acquirable trait that does not accurately reflect relatedness among strains. For example, members of biovar 2, reflecting a species-level phylogenetic group, can cause crown gall, hairy root disease, infect grapevine, or be non-pathogenic. The second issue is the amalgamation of some lineages of *Agrobacterium* and rhizobia that drive nitrogen fixation in legumes (also a mobilizable trait) into a genus and renaming them all *Rhizobium* (11, 12). Adoption of this naming nomenclature has met limited acceptance among both agrobacterial and rhizobial research communities. The third issue is related to the second in that effectiveness in communicating relationships among strains is undermined by repeated revisions to taxonomic classifications. Consequently, multiple schemes persist and are variably adopted or abandoned by researchers who contribute to the long history of publication on these bacteria (13). We do not tackle this topic here but recommend looking toward an invited review that will attempt to relate some classification schemes to the inferred evolutionary relationships among strains of *Agrobacterium* (Alexandra J. Weisberg, Yu Wu, Jeff H. Chang, Erh-Min Lai, Chih-Horng Kuo, in preparation). The association of arguably ephemeral properties such as pathogenicity with bacterial nomenclature understandably has important legacy effects on *Agrobacterium* taxonomy, and throughout this review we use *Agrobacterium tumefaciens* for simplicity.

A BURST OF BASIC RESEARCH PROPELLED APPLIED STUDIES

The initial work and much of the history of *A. tumefaciens* research was rightfully focused on its interaction with plant hosts and eventually its development as a tool to manipulate plants in profound ways. Following the isolation of *A. tumefaciens* and the establishment of its etiological relationship with crown gall disease, progress on the microorganism was initially relatively sparse, with just a few key labs contributing. The laboratory of Armin Braun at the Rockefeller Institute in the 1940s provided several critical

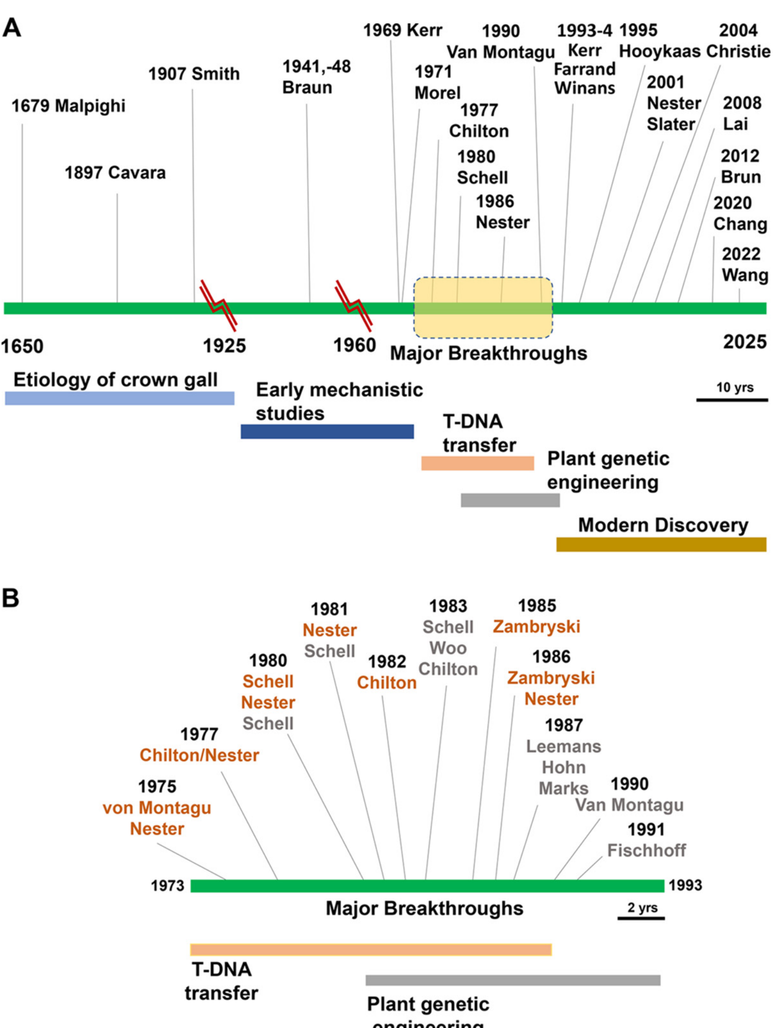

FIG 1 Timeline of publications reporting impactful *Agrobacterium* discoveries. (A) Timeline of selected discoveries arrayed linearly by year from 1650 to 2025. Lead scientists are indicated and usually match the corresponding author on the publications. Broad periods are indicated by the colored labeled bars below the timeline. "Major breakthroughs" refers to a very active period expanded in panel B. (B) Expanded view of discoveries that defined T-DNA transfer and harnessed it for use in plant transgenesis. Basic science discoveries are indicated by author names in orange text, and development of applications is indicated in silver text. All citations are referenced in Table 1.

insights into the formation of crown gall (14, 15) and, importantly, developed the concept of the "tumor-inducing principle" to describe the factor(s) that stimulates gall formation (Fig. 1A, Table 1). Certainly, Braun and his lab members must have been influenced by the seminal studies of Avery, Macleod, and McCarty (also performed at the Rockefeller Institute) on the "transforming principle" in *Streptococcus pneumoniae*, which produced the first evidence that DNA is the genetic material (1). The Braun group made the critical insight that, once formed, the neoplastic growth in plants could continue to be propagated in the absence of the infecting bacterium, implicating a genetic or heritable change (14, 16). The true nature of the tumor-inducing principle would require many more years to be revealed. Nonetheless, it is striking how in the cases of both Avery and Braun, and their colleagues, who were studying very different biological phenomena, they were also constructing the conceptual framework for horizontal gene transmission that would contribute so importantly to our understanding of heritability and evolution, enabling the dawn of molecular biology.

The late 1960s and early 1970s brought pivotal discoveries from laboratories in multiple countries about the host-microbe interactions that underlie crown gall. Initially,

TABLE 1 Selected major discoveries and innovations in *Agrobacterium* research

Yr	Discovery	Lead scientist(s)	Reference(s)
1679	Observation of crown galls on plants	Malpighi	3
1897	Isolation/reinoculation of pathogen	Cavara	4
1907	Etiological identification of pathogen	Smith	5
1941	Bacteria-free tumors	Braun	16
1948	Tumor-inducing principle	Braun	14
1969	Genetic transfer of virulence	Kerr	17
1971	Opine characterization	Morel	18
1975	Plasmid as the basis of virulence	von Montagu, Nester	19, 20
1977	Agrobacterial DNA in transformed plants	Chilton/Nester	21
1980	Tumor-inducing (Ti) plasmid genetic map	Schell	137
	Organization of T-DNA genes in plant tumors	Nester	138
	Ti plasmid for gene introduction in plants	Schell	139
1981	Fine structure genetic map of T-DNA in plants	Nester	140
	Mendelian transmission of transferred genes	Schell	141
1982	T-DNA border sequences and transfer	Chilton	142
1983	Expression of engineered genes in plants	Schell, Woo	143, 144
	Engineered mini-Ti plasmids	Chilton	145
1985	Plant phenolics induce virulence	Zambryski	27
1986	T-strand formation in early T-DNA transfer	Zambryski	33
	VirA-VirG two-component systems	Nester	28
1987	Engineered insect resistance	Leemans	146
	Gene transfer to corn	Hohn	147
	Gene transfer to *Arabidopsis*	Marks	148
1990	Nuclear targeting of the T-DNA	Van Montagu	149
1991	Transgene codon optimization	Fischhoff	150
1993–1994	Ti plasmid quorum sensing system	Kerr, Farrand, Winans	57–59
1995	T-DNA transfer to *Saccharomyces*	Hooykaas	23
2001	*A. tumefaciens* C58 genome sequence	Nester, Slater	86, 87
2004	Type IV secretion of T-DNA	Christie	37
2008	Type VI secretion in *A. tumefaciens*	Lai	38
2012	Polar budding in *A. tumefaciens*	Brun	74
2020	Ti plasmid diversity and evolution	Chang	49
2022	Multipartite replicon coordination	Wang	92

Australian plant pathologist Allen Kerr recognized that the ability to cause crown gall tumors was encoded on an element transmissible between bacteria (17). Next, crown gall tumors were found to produce unique metabolites called opines that are semiexclusive nutrients for *A. tumefaciens* (18). Finally, studies by several groups including those of Allan Kerr, Jeff Schell and Marc van Montagu in Belgium, and Eugene Nester in the United States, among others, demonstrated the relationship between crown gall disease and the episomal element, named the tumor-inducing plasmid (or Ti plasmid), as the genetic prerequisite for crown gall disease (17, 19, 20). Together, these discoveries led to the then radical hypothesis that *A. tumefaciens* transfers its own genetic material to plants. The groundbreaking studies of Mary-Dell Chilton in the Nester laboratory provided definitive proof of the transfer of agrobacterial DNA to plant tissues (21). Chilton's findings initiated a barrage of research within both academia and industry to understand the transferred DNA (T-DNA) and how it is generated, as well as to harness the power of interkingdom horizontal gene transmission (Fig. 1B). A barrage of brilliant work led to major advances in engineering the T-DNA and utilizing this natural process to transfer genes into plants. In several cases, the labs elucidating the basis for T-DNA transfer were the same ones propelling the applications for plant transgenesis, working in concert with applied scientists in the private sector. The burst of fundamental observations led to a wave of novel applications (Fig. 1B, Table 1). This research also yielded significant insights into plant cell biology and eventually provided transformation approaches for not only plants, but also fungal systems (22–24). It was a remarkable period of discovery and innovation and is an excellent example of basic research fueling applied science.

A MODEL FOR FUNDAMENTAL PROCESSES

The major breakthroughs on pathogenicity and interkingdom gene transfer propelled a sustained period of modern discoveries with *A. tumefaciens* that has continued to add crucial fundamental knowledge to inform molecular microbiology (Fig. 1A). Seminal work in *A. tumefaciens* has impacted our understanding of microbe-host and microbe-microbe interactions, particularly in the areas of secretion mechanisms and cell-cell communication. Furthermore, *A. tumefaciens* is emerging as a model system for bacterial cell biology with recent mechanistic insights related to polar growth, multipartite replicons, and plasmid biology. *A. tumefaciens* has been and remains a treasure trove for important, fundamental findings that have influenced the entire field of microbiology.

Host-microbe signaling and the two-component paradigm. For pathogenic microorganisms, we now take for granted the concept of interkingdom communication in which pathogens perceive and respond to host-released signals. *A. tumefaciens*-plant interactions are among the first where such host-released signals were identified, as was the signal transduction cascade that leads to virulence gene expression in response to these signals (25, 26). Early studies used transposon mutagenesis to disrupt genes required for *A. tumefaciens* virulence (*vir* genes), also fusing *lacZ* encoding β-galactosidase with the disrupted loci (Fig. 1B, Table 1). The mutants were often avirulent, but also, several of the *lacZ* fusions were inducible in the presence of plant extracts (25). Chemical characterization of the plant exudates using the *lacZ* fusions as a bioassay identified phenolic precursors of lignin, acetosyringone (AS), and related molecules, which are produced during the wound healing response of plants (27). It was also demonstrated that many of the *vir* genes required for T-DNA transfer are only significantly expressed in the presence of the plant signals (25). Subsequent genetic studies demonstrated that two of the *vir* genes, *virA* and *virG*, were required for *A. tumefaciens* to respond to inducing conditions (26). Sequence analysis of these loci showed first that VirG is similar to several proteins from *E. coli* now called response regulators, including NtrC, OmpR, and PhoB, and that VirA is similar to proteins now known as sensor kinases, NtrB, EnvZ, and PhoR (28, 29). These and other studies of VirA and VirG established the two-component signal transduction mechanism in *A. tumefaciens* but also contributed greatly to the establishment of this general model of environmental response in bacteria (30). Demonstration of phosphotransfer between VirA and VirG in response to acetosyringone not only reinforced findings from the *Escherichia coli* two-component systems, but also broadened the scope of these regulatory systems to include host perception by pathogens (31). The two-component regulatory paradigm is now recognized as one of the most pervasive and influential systems in microbiology.

Type IV secretion systems. The type IV secretion system (T4SS) is central to horizontal gene transfer, as this system is the apparatus used by conjugative plasmids and integrative conjugative elements to mediate transfer (32). The T4SS is also a protein secretion system used by pathogens important to human health. The T-DNA transfer process in agrobacteria is dependent on a T4SS and has long been a model for these systems. The *virB* operon on Ti plasmids encodes 11 proteins, all of which are involved in export of the T-DNA. It was reported by Stachel et al. in 1986 (Fig. 1B) that induction of virulence and T-DNA transfer created a single-stranded nick similar to that generated during the conjugation of bacterial plasmids (33). As DNA sequences from diverse plasmids emerged, and broad comparisons became possible, it was recognized that the *virB* gene products are similar to those of conjugative transfer systems (34, 35). Furthermore, secretion systems for bacterial toxins in unrelated systems, such as the *Bordetella pertussis* toxin liberation system (Ptl) were also found to be similar to the products of the *virB* genes (36). Several labs began focusing on the VirB proteins, and they rapidly became a model for the emergent type IV secretion systems. A large number of studies on the VirB T4SS from multiple research groups revealed many of the key mechanisms of T4SS, including an elegant study biochemically tracking the route of T-DNA through the VirB T4SS (Fig. 1A, Table 1) (37). This work propelled the *A. tumefaciens* VirB system to be the prototype for T4SS for many years, as more and more of these systems in mammalian pathogens were shown to export protein substrates (32). It is

now clear that the *A. tumefaciens* system independently secretes protein effectors and T-DNA into plant cells during infection.

Type VI secretion systems. The type VI secretion system (T6SS) was identified in *A. tumefaciens* strain C58 by Erh-Min Lai and colleagues during a screen for proteins secreted under conditions that induce virulence gene expression (Fig. 1A, Table 1) (38). T6SSs deploy toxic effector proteins to antagonize other bacteria (39). Studies of the T6SS of *A. tumefaciens* have yielded key discoveries about mechanisms of effector loading, such as the molecular features that determine specificity during engagement, and revealing a checkpoint for loading during T6SS activation (40–46). Studies have also revealed similarities and differences between T6SS and virulence gene regulation and provided a more complete view on the ecology of pathogenicity (46). Acidic pH, predicted to mimic the environment of the plant and rhizosphere, is crucial for inducing and activating the T6SS and virulence. Conversely, unlike virulence genes, sugars had no detectable effect, while phenolics attenuated T6SS activation. Overall, findings support a model predicting that the T6SS is activated when cells are near or associated with plants, but once virulence is induced, the T6SS is downregulated.

T6SS-associated effectors will cause self-intoxication, and cells must encode for immunity against each of their effectors (39, 47). Consequently, the activity and diversification of the T6SS is predicted to have a tremendous effect on the composition of bacterial communities (48). The *Agrobacterium* group has been deeply sequenced, and genomic data sets are valuable resources for understanding the natural variation of the T6SS (49). The presence of T6SS loci is variable across *Agrobacterium* taxa, but when present, genes encoding most of the structural and regulatory proteins are conserved in sequence and organization (50, 51). The presence/absence polymorphism was inferred to reflect recurrent loss, at various points in the history of these bacteria. Biovar 2, multiple genomospecies groups, and individual strains entirely lack a T6SS locus. Strains with T6SS-encoding loci are variable in the number and types of effector genes. The T6SSs in taxa that represent the diversity within the genus have different patterns of activation, suggesting that T6SSs of different strains or species-level groups are regulated under different environmental conditions (51). The T6SS of *Agrobacterium* is a model for understanding processes that have shaped its evolution and mechanistic diversification (52). Overall, the role of the T6SS in interbacterial competition among *Agrobacterium* cells is well supported, but its involvement directly or indirectly in virulence toward plants remains unresolved (43, 52).

Quorum sensing. *A. tumefaciens* has been a critical model system for understanding cell-cell communication, particularly via quorum sensing. These findings have their origin in the studies of Kerr and colleagues in the late 1960s (Fig. 1A, Table 1) on the conjugative transfer of the Ti plasmid between bacteria during plant infection (17). Others later found that Ti plasmid conjugation was stimulated by specific opines, called conjugal opines (53, 54). Alan Kerr and Lian-Hui Zhang isolated a small molecule that they called conjugation factor that was produced by *A. tumefaciens* grown in the presence of specific opines (55). Chemical characterization of the conjugation factor identified it as *N*-3-oxo-octanoyl-homoserine lactone (3-oxo-C8-HSL), similar to the so-called autoinducer (*N*-3-oxo-hexanoyl- homoserine lactone) that regulates bioluminescence in *Vibrio fischeri*, the symbiont of certain fishes and bobtail squid (Fig. 1A, Table 1) (56, 57). In parallel with this chemical characterization was the genetic identification of the TraR transcription factor from two different Ti plasmids and the evidence that it regulated conjugal transfer gene expression in response to 3-oxo-C8-HSL (58, 59). This inducer molecule was synthesized by an enzyme called TraI, also encoded on the Ti plasmid (59, 60). TraR and TraI were among the founding members of the larger LuxR-LuxI family of regulators. As with bioluminescence regulation by LuxR and its autoinducer synthase LuxI, TraI and TraR imparted population density-dependent expression control on their target genes and Ti plasmid conjugation (61, 62), and the general population density response was subsequently described as quorum sensing (63). Several other LuxR-LuxI type systems were also discovered in diverse bacteria in this same period, most prominently LasR-LasI from *Pseudomonas aeruginosa* (64), and acylated homoserine lactone (AHL) autoinducers were detected for multiple bacteria (65), resulting in a wave of research on quorum sensing that continues today.

In addition to its foundational role in expanding quorum sensing beyond the bioluminescent marine vibrios, *A. tumefaciens* has provided several major contributions to the mechanistic understanding of quorum sensing. These include TraI being the first LuxI-type protein for which there was *in vitro* evidence that AHLs could be synthesized from acylated-acyl carrier protein (ACP) and *S*-adenosylmethionine (AdoMet) precursors (66), the identification of antagonistic regulators such as TraM that function with TraR and TraI as an integral part of the quorum sensing mechanism (67, 68), and the first three-dimensional structure of a LuxR-type protein (69). The structure reported for TraR was associated with the AHL ligand, and double-stranded DNA, providing a wealth of biochemical insights for other LuxR-type proteins. These and other fundamental insights shaped the field of bacterial cell-cell communication and established a quorum sensing paradigm.

Polar growth. While most rod-shaped bacteria are assumed to elongate through growth in the lateral cell wall, it is becoming increasingly clear that asymmetric modes of peptidoglycan synthesis are prevalent among bacteria. It was in the early 1970s when Tamio Fujiwara and Sakuzo Fukui first proposed that *A. tumefaciens* grows unipolarly based upon striking morphologies of mutants and careful observations of microcolony formation (70, 71); however, it was not until the late 2000s that genome sequencing revealed that the entire clade of Rhizobiales, including *Agrobacterium* species, lacks the genes to encode the canonical elongation machinery (72). This opened up new research directions for using *A. tumefaciens* to answer the question, how do these bacteria maintain rod-like morphologies and elongate? Use of cell wall probes and timelapse microscopy to track *A. tumefaciens* cell growth clearly demonstrated that elongation is mediated by unipolar cell wall insertion (Fig. 1A, Table 1) (73, 74). As with more overtly asymmetric bacteria such as *Caulobacter* species, there are a number of extracellular polar structures such as a unipolar polysaccharide (UPP) adhesin and a tuft of polar flagella, that are integrated with the polar growth process (75, 76). These observations and processes have enabled *A. tumefaciens* to recently emerge as a model for mechanistic studies of unipolar growth in bacteria.

Systematic characterization of enzymes involved in cell wall biosynthesis and hydrolysis has resulted in several surprising findings that revealed diversification of these functional bacterial processes. First, unlike the proposed auxiliary function of penicillin binding protein 1a (PBP1a) in other rod-shaped bacteria, in *A. tumefaciens*, PBP1a is an essential enzyme required for polar growth (77). Second, while some LD-transpeptidases (LDTs) likely have broadly conserved roles in the modification of existing cell wall material, Rhizobiales-specific LDTs may function in peptidoglycan cross-linking during polar growth (77, 78). Third, cell wall hydrolytic enzymes have unique functions in establishing polarity to enable polar growth. While amidases and their regulators typically function in cell separation of many bacteria at the culmination of division, amidase AmiC and its regulator EnvC are required by *A. tumefaciens* to establish new poles following cell division (79). These observations suggested that AmiC-mediated modifications of the cell wall may serve as a signal for the recruitment of the polar growth machinery to the correct pole. Finally, scaffolding proteins such as PopZ and growth pole ring (GPR) protein reside at the growth pole and may function to facilitate the organization of specific growth pole proteins, including those important for cell wall and membrane biogenesis (80–85). The mechanism of polar growth in *A. tumefaciens* and other members of the Rhizobiales has evolved through the expansion, diversification, and altered regulation of the core cell wall synthesis machinery, and its further investigation has promise in providing a more comprehensive understanding of bacterial growth processes.

Multipartite replicons. *A. tumefaciens* has emerged as a model organism for understanding the evolution and mechanisms of DNA segregation in bacteria with multiple replicons. The genome of the type strain *A. tumefaciens* C58 consists of four replicons: a circular chromosome, a linear chromosome, and the pAtC58 and pTiC58 plasmids (86, 87). The circular chromosome contains an *oriC*-type origin of replication found in many bacteria chromosomes, whereas the other replicons contain *repABC* origins, typical of plasmids in this group of bacteria. Consequently, the linear chromosome is thought to have been a

plasmid into which gene transfer events from the circular chromosome occurred over evolution, enabling the transfer of some essential genes and an rRNA operon (86–88). These findings provided the foundation for a more generalizable model of genome evolution among bacteria with multiple replicons (88). It has been suggested that multipartite replicons may allow for acquisition of additional genetic material, enable faster genome duplication, and provide an advantage for microbes in changing environments, including host invasion (88–90). While the precise advantage for multipartite replicons remains unknown, the maintenance and management of multiple replicons pose some unique challenges.

In *A. tumefaciens*, the origin of replication of the circular chromosome is localized to the old pole of the cell, and during chromosomal replication the replicated origin migrates to the new pole (Fig. 1A, Table 1) (91–94). This polar localization is dependent on the ParABS chromosome segregation system, and docking of ParBI at the new poles is dependent on PopZ, while PodJ is indirectly responsible for docking of ParBI at the old poles (85, 91, 92). Like the circular chromosome, the origin of the linear chromosome is also dependent on the presence of PopZ and PodJ for efficient localization to cell poles (91, 93). The absence of GPR causes formation of spherical cells with origin location disrupted and a stochastic appearance (93). PodJ and GPR play indirect roles in localizing origins to the cell poles, while PopZ directly interacts with ParB and, to a lesser extent, RepB. Partition systems of bacterial replicons have sequences analogous to centromeres that are recognized by centromere-binding proteins. In *A. tumefaciens*, centromere clustering occurs independently of the polar organizing proteins (93). The circular and linear chromosomes have interarm interactions that depend on the SMC complex and require direct interaction of the centromere-binding ParB and RepB proteins (92, 93). These interactions result in a linear alignment of the chromosomes and may reduce chromosome entanglement, which if formed and not resolved can have severe fitness effects on cells. Indeed, disruption of this clustering pattern leads to loss of the linear chromosome and plasmids from progeny, indicating that coordination of centromere clustering is important for maintaining the integrity of multipartite genomes (93). These findings in *A. tumefaciens* suggest that centromeric clustering is a solution for ensuring that secondary replicons, such as virulence plasmids, are maintained in the absence of the selective forces that would otherwise promote their retention.

Plasmid biology. Plasmids innovate *Agrobacterium* spp. with several important traits. Pathogenicity is the most renowned and the one that has elevated the prominence of *A. tumefaciens* as a model and an indispensable biotechnology tool. Multiple excellent and detailed reviews on the mechanism of Ti plasmid-dependent genetic transformation of plants have been published (95–97). The oncogenic Ti plasmid encodes five core functions: (i) the *vir* gene products that are required for pathogenicity and T-DNA transfer, (ii) the T-DNA that is delivered to and integrates with the plant genome to cause crown gall disease and opine production, (iii) opine catabolic functions, (iv) the interbacterial conjugative transfer system, and (v) the plasmid replication functions. Several oncogenic plasmids have been "disarmed" by removing genes within the T-DNA and separating the disarmed T-DNA from the *vir* genes to make "binary" and "helper" vectors, respectively (98). Engineering the Ti plasmid was not trivial, as these plasmids are large and maintained at low copy numbers. The binary system is a foundational technology for plant transformation and remains central to both agricultural applications and basic research.

The study of *Agrobacterium* plasmids has also been illustrative for plasmid and bacterial evolution. While plasmids carry cargo genes that give their bacterial hosts a fitness benefit, there are costs for bearing plasmids, and advantages are realized only in certain environments (99). Studies of the Ti plasmid demonstrated that costs are context dependent, with higher costs measured under conditions depleted of resources and those that induced expression of *vir* genes (100). These observations explain why copy numbers of Ti plasmids and expression of *vir* genes are tightly regulated (101–103). In addition, observations were consistent with a fundamental theory of cooperation, which is that pathogenic cells bear costs to direct benefits to related cells, which in this case are

presumably those with related plasmids (104). This perspective of "taking one for the team" helps translate the understanding of molecular processes in single cells to understanding the evolution and ecology of populations.

Plasmids have traditionally been difficult to study because their sequences are challenging to resolve and analyze (105, 106). Conversely, because of the intensive work on oncogenic Ti and Ri plasmids (related to Ti plasmids, but they incite hairy root formation rather than galls, on plants), there is a depth of knowledge that positions them well as models for tackling the challenge of inferring plasmid evolution. Phylogenetic and network-based methods were used to analyze multiple scales of information and classify well over 100 oncogenic plasmids (Fig. 1A, Table 1) (49, 107). One of the major unexpected findings was that the oncogenic plasmids formed a limited number of types that could be related to each other and to a conceptional ancestral proto-oncogenic plasmid. Another major finding was that these plasmids are highly modular, with the most conserved regions being potential hot spots for homologous recombination, T-DNAs being extremely flexible, and virulence being conferred by *vir* genes mixed and matched from different plasmids or even distributed across plasmids (107). Nevertheless, despite the potential for tremendous diversity, structural organization of plasmids can constrain recombination such that certain events are more permissive, limiting most plasmids to the few extant types observed to date.

Agrobacterium spp. have another group of diverse nononcogenic plasmids often loosely referred to as "At" plasmids (108, 109). Although their existence has long been known, we still have little understanding of their roles in agrobacterial fitness. Catabolism may be one of the primary functions, as they can have homology to oncogenic plasmids, can catabolize opines, and can provide access to other nutrients (109–111). Regardless, nononcogenic and oncogenic plasmids can shape each other in multiple ways. Both classes can be simply viewed as members of a common pool of molecules that can recombine and diversify (107, 110). The members of this common pool can also influence signaling and stability across the two classes of plasmids (112, 113). It is also notable that these plasmids may have shaped the evolution of *Agrobacterium* genomes by capturing and shuffling genes across multiple large replicons. Plasmids have had a significant effect accessorizing *Agrobacterium* spp. with novel traits and shaping the structure and organization of their genomes.

FROM BASIC SCIENCE BACK TO APPLICATION: BIOCONTROL

Species of *Agrobacterium* are exemplary for demonstrating societal benefits of genetic engineering and biocontrol. Strain K84 of *Agrobacterium* was the first organism to be genetically engineered and commercialized for use as a live biocontrol product (114). K84 was discovered in 1970 from a plot with a history of crown gall (115). It is a nonpathogenic biovar 2 strain that lacks an oncogenic plasmid and, unlike others tested, consistently prevented crown gall disease following a 1:1 coinoculation with pathogenic strains, and was found to be a hyperparasite of *A. tumefaciens*. The value of K84 was recognized immediately and it emerged quickly as a highly successful preventative biocontrol strain (116). However, biocontrol is specific and reportedly effective against only strains that induce synthesis of the opine nopaline in crown galls (117). Furthermore, toxicity and immunity are mediated by pAgK84, a nononcogenic, nonconjugative but mobilizable plasmid, which raised concerns over potential for plasmid transmission causing breakdown of biocontrol (118–122). Concerns were mollified by deleting a small region to eliminate plasmid transfer (123). K1026, the strain carrying the engineered plasmid, is approved as a commercial biocontrol product in many countries and has no evidence for breakdown of plant protection.

Biocontrol is mediated by agrocin 84, a disubstituted adenine nucleotide and structural mimic of two molecules (124, 125). The protoxin is a mimic of a conjugative opine, agrocinopine A, and is consequently taken up by the cognate transporter (126, 127). The sugar moiety of agrocin 84, necessary for uptake but not toxicity, is subsequently released to yield a molecule that mimics leucyl-adenylate and interferes with translation by binding the catalytic domain of leucyl-tRNA synthase, an enzyme that catalyzes the transfer of

leucine to its cognate tRNA (128, 129). Cells carrying pAgK84 are resistant to agrocin 84 because the plasmid also encodes a novel variant of leucyl-tRNA synthase that is less sensitive to inhibition (130). The success of agrocin 84 in limiting infection by *A. tumefaciens* is exceptional and has helped make these bacteria potential sources for other forms of control. These include agrocin variants, secondary metabolites, and other compounds that have yet to be detailed at the genetic and molecular levels (131–134). Lastly, it has long been known that avirulent *Agrobacterium* cells can prevent disease presumably through competition and limiting attachment of pathogens to plant surfaces (135, 136). While this trait by itself may not be as useful for biocontrol, it makes nonpathogenic strains competitive for plants and contributes in important ways toward the effectiveness of other control mechanisms.

FUTURE OUTLOOK FOR THE *AGROBACTERIUM* MODEL SYSTEM

The purpose of this review is to highlight some of the rich history of *A. tumefaciens* as a model pathogenic bacterial system, and even so, there is a great deal of important biology and applications that we have been unable to cover. A model system allows researchers to investigate phenomena in detail and manipulate processes in ways that take advantage of the system's natural attributes and properties, to reveal new insights into general properties that are otherwise opaque. The interkingdom gene transfer from *A. tumefaciens* to plants certainly has warranted the intense level of attention it has received, and it is hard to argue with the scientific, societal, and economic impact its study and application have generated. Even with the tremendous and insightful research findings on *Agrobacterium*-plant interactions, there remains much to be learned. For example, how do molecular interactions with plant cells lead to nuclear import of T-DNA and integration of the T-DNA into the plant genome? These remain active areas of investigation and will yield new findings that have the potential to translate to innovations in genetic engineering. Beyond its utility as a genetic engineering tool, *Agrobacterium* research has arguably had as large an impact on our understanding of fundamental biological processes in bacteria, including but not limited to cell-cell communication, secretion, cell growth, and chromosome dynamics. As with all good model systems, there are many phenomena and insights that remain to be uncovered with the appropriate experiments, approaches, and perspective. *Agrobacterium* research continues to inform multiple areas of biology and expands in multiple new directions at the forefront of microbiology.

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