MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 5, No. 4, pp. 279-287, 1992

Current Review

Evolution of Agrobacteria and Their Ti Plasmids—A Review

Léon Otten, Jean Canaday, Jean-Claude Gérard, Pascal Fournier, Philippe Crouzet, and François Paulus

Institut de Biologie Moléculaire des Plantes du C.N.R.S., 12 Rue du Général Zimmer, 67084, Strasbourg, France
Received 4 December 1991. Accepted 21 February 1992.

The genus *Agrobacterium* is well-known for its remarkable and unique capacity to transfer DNA into plant cells. The transferred DNA (T-DNA) is part of a large plasmid, the tumor- or root-inducing plasmid (pTi or pRi; in this paper we will use the general name pTi for both pTi and pRi, except where distinction is necessary); its transfer during infection is due to the activity of the plant-inducible virulence (*vir*) genes located on the Ti plasmid. The T-DNA is integrated into the plant nuclear DNA and subsequent expression of the T-DNA genes leads to the formation of tumors (crown galls) or roots (hairy root disease) and to the production of low molecular weight molecules called opines, which are used by the bacterium for its growth. All known Ti plasmids carry genes coding for opine uptake and catabolism (Petit *et al.* 1983). Several opines induce the conjugative transfer of the Ti plasmids (Petit and Tempe 1983) and thereby amplify opine induction and utilization. The Ti plasmids can therefore be considered as compact and genetically largely independent packages of genes encoding sophisticated systems of metabolic parasitism. During the last 15 yr, many molecular details of the T-DNA transfer process and of T-DNA gene function have been elucidated. These aspects will not be detailed here, as they have been reviewed on several occasions (Hooykaas and Schilperoort 1984; Morris 1986; Ream 1989; Zambryski *et al.* 1989). In this review we would like to summarize what is known about the evolution of the Ti plasmids and the *Agrobacterium* chromosome.

Agrobacterium is a member of the alpha subdivision of the class Proteobacteria. It shares this position with *Rhizobium, Phyllobacterium,* and the *Rickettsiae* (Holmes and Roberts 1981; Weisburg *et al.* 1985; Young *et al.* 1991). 16S RNA data have yielded a phylogenetic tree for *Agrobacterium* and its relatives (Young *et al.* 1991). From data on host evolution it has been estimated that the *Agrobacterium-Rhizobium* divergence occurred some 250 Mio yr ago (Ochman and Wilson 1987). *Agrobacterium* has been divided into five species: *radiobacter, tumefaciens, rhizogenes, rubi,* and *vitis* (Ophel and Kerr 1990). Because these epithets refer to Ti plasmid-encoded properties and because Ti plasmids may be lost or acquired by conjugation (Petit *et al.* 1978), it is preferable to use a classification based on chromosomal characteristics. Several systems have been proposed (White 1972; Kersters *et al.* 1973; Kerr and Panagopoulos 1977; Kerr and Brisbane 1983). The scheme of Kerr and Panagopoulos has become generally accepted; it divides *Agrobacterium* into three biovars or biotypes. Uncertainties remain with regard to the taxonomical position of certain isolates (for example: Holmes and Roberts 1981; Zoz *et al.* 1986; Bouzar and Moore 1987).

Most of the basic crown gall and hairy root studies have been done with three model strains: The biovar I strains C58 (a nopaline strain) and A6 (an octopine strain, the Ti plasmid of which is very similar to pTiB6S3, pTiAch5, pTiR10, and pTi15955), and the biovar II *A. rhizogenes* strain A4 (pRiA4 is very similar to pRi1855, pRiHR1, and pRi15834). However, various other strains with quite different types of Ti plasmid have been described (Sciaky *et al.* 1978; White and Nester 1980; Thomashow *et al.* 1981; Costantino *et al.* 1981; Perry and Kado 1982; Knauf *et al.* 1983; Buchholtz and Thomashow 1984; Unger *et al.* 1985; Blundy *et al.* 1986; Komari *et al.* 1986; Bouzar and Moore 1987; Brevet and Tempe 1988; Wabiko *et al.* 1989). The evolutionary links between strains or Ti plasmids remain largely obscure. Recently, we have described a group of related biovar III strains (now called *Agrobacterium vitis* [Ophel and Kerr 1990]) and reconstructed the evolutionary history of the TA regions of the corresponding Ti plasmids (Huss *et al.* 1989; Bonnard *et al.* 1989a, 1989b, 1991; Paulus *et al.* 1989a, 1989b, 1991a, 1991b, 1991c). Here we summarize these results and relate them to what is known about Ti plasmid evolution in general. Finally, we discuss future areas of research that may improve our knowledge of the origin and evolution of present-day *Agrobacterium* strains and their Ti plasmids.

Ti plasmids are mosaic structures. Homology studies have shown that Ti plasmids are composed of homologous and nonhomologous sequences; they can therefore be considered as evolutionary mosaics. pTiA6 and pTiC58 have been compared in detail. Overall homology is only 30%, but detailed analysis revealed four regions of about 80–85% DNA homology embedded in nonhomologous regions (Engler *et al.* 1981). Thus, the octopine and nopaline Ti plasmids did not derive from a common ancestor by gradual accumulation of small nucleotide changes. Most likely some of the homologous and nonhomologous pTi sequences were acquired by horizontal gene transfer, as several pTiA6 and pTiC58 sequences have also been found on other Ti plasmids and even in other bacterial species. A number of such promiscuous Ti sequences are listed in Table 1.

The mosaic structures of Ti plasmids have important consequences for evolutionary studies. Ti plasmid phylogenies cannot be derived without delimiting the areas of common evolutionary origin, and a reconstruction of

the large-scale events. A method that uses overall levels of DNA similarity like quantitative restriction fragment analysis would not discriminate between large changes brought about slowly by numerous small nucleotide changes and large changes that took place in a single step such as insertions or deletions. Also, it is difficult to propose a Ti phylogeny from the study of selected regions: On the basis of *vir* gene sequences it has been proposed (Hirayama *et al.* 1988) that pTiC58 is more closely related to pRiA4 than it is to pTiAch5. Although this conclusion may be true for the *vir* region, it may be different for other Ti plasmid regions. Indeed, if different Ti plasmids are composed of segments with different levels of relatedness, the overall relatedness of the composite structures can no longer be expressed in percentages of nucleotide or amino acid homology but must include a description of the order and time of occurrence of the different assembly events.

Table 1. Ti and Ri plasmid genes found in different sequence environments[a]

Organism	pTi type	Strain	Region	Reference
Auxin (*iaaM* and *iaaH*) genes				
At	o	Ach5	TL	Barker *et al.* 1983
At	o/c	Tm4-Hm1	TA	Bonnard *et al.* 1991
At	n	C58	T	(Joos *et al.* 1983)
At	o/c	Tm4-Ag162	TB	(Knauf *et al.* 1984; Yanofsky *et al.* 1985; Huss *et al.* 1989)
At	v	S4	T2	(Canaday, unpublished)
Ar	a/m	A4	TR	Camilleri *et al.* 1991
Ar[b]	a/m	A4	TL	Slightom *et al.* 1986; Levesque *et al.* 1988
Ar[b]	m	8196	T	Hansen *et al.* 1991
Psav[c]	...	...	...	Yamada *et al.* 1985
Bj[d]	...	...	...	Sekine *et al.* 1989
Cytokinin (*ipt*, *tzs*, *ptz*) genes				
At	o	Ach5	TL	Heidekamp *et al.* 1983
At	o/c	Tm4	TA	Bonnard *et al.* 1989a
At	n	C58-T37	T	Goldberg *et al.* 1984
At	n	C58-T37	*vir*	Beaty *et al.* 1986; Akiyoshi *et al.* 1985
At[e]	s	Bo542	TL	Strabala *et al.* 1989
At	v	S4	T3	(Canaday, unpublished)
Psav	...	...	...	Powell and Morris 1986
Psol	...	...	...	Akiyoshi *et al.* 1989
Mannopine synthesis genes (*mas1'*, *mas2'*)				
At	o	Ach5	TR	Barker *et al.* 1983
At	s	Bo542	TR	(Hood *et al.* 1986)
Ar	a/m	A4	TR	Bouchez and Tourneur 1991
Ar	m	8196	T	Hansen *et al.* 1991
Agrocinopine synthase (*ags*) gene				
At	o/c	Tm4	TA	(Paulus, unpublished)
At	n	C58	T	(Joos *et al.* 1983)
Ar	a/m	A4	TL	Slightom *et al.* 1986
rolB				
Ar	a/m	A4	TL	Slightom *et al.* 1986
Ar	a/m	A4	TR	Bouchez and Camilleri 1990
Cucumopine synthase (*cus*) gene				
At	o/c	Tm4	TB	(Paulus *et al.* 1989a)
Ar	c	2659	T	Brevet and Tempé 1988
Common T-DNA (5-*iaaH*-*iaaM*-*ipt*-6a-6b)				
At	o	Ach5	TL	Barker *et al.* 1983
At[f]	o/c	Tm4	TA	(Paulus *et al.* 1991c)
At	n	C58	T	(Joos *et al.* 1983; Willmitzer *et al.* 1983)
At[g]	s	AT181	T	(Chilton *et al.* 1984; Blundy *et al.* 1986)

[a] Abbreviations are: At: *Agrobacterium tumefaciens*; Ar: *Agrobacterium rhizogenes*; Psav: *Pseudomonas syringae* pv. *savastanoi*; Psol: *Pseudomonas syringae* pv. *solanacearum*; Bj: *Bradyrhizobium japonicum*. o, octopine; n, nopaline; c, cucumopine; a, agropine; m, mannopine; s, succinamopine; v, vitopine. References refer to sequence data; if sequence data are not available, references are given in parentheses.
[b] ORF8 (see text).
[c] *iaaM* and *iaaH* in direct orientation, in the other cases in opposite orientation.
[d] Only *iaaH* gene sequenced.
[e] This T-DNA may resemble the pTiAch5 TL DNA.
[f] 6a gene deleted.
[g] 6b gene deleted.

Evolutionary origin of T-DNA genes. In spite of the fact that the T-DNA genes are carried by a bacterial plasmid, they are expressed in plant cells and contain plant cell-specific expression signals (Zambryski *et al.* 1989; Ream 1989). It may therefore be thought that the T-DNA genes are of plant origin. This model would require a hypothetical plant-*Agrobacterium* DNA transfer mechanism for which no evidence exists. Instead, it seems likely (Yamada *et al.* 1985; Morris 1986; Schell 1986) that at least some T-DNA genes are of bacterial origin since *iaaM*, *iaaH*, and *ipt* also occur in other plant-associated bacteria where they are under control of prokaryotic expression signals (Table 1). According to this idea, bacterially expressed hormone genes became part of a T-DNA region and chance insertions close to plant promoters or unspecific low-level expression from plantlike promoter sequences could have led to sufficient plant growth stimulation to initiate a selection process towards eukaryotic promoter sequences. Whether the other T-DNA genes have bacterial counterparts remains unknown.

Some untransformed *Nicotiana* species carry a nuclear DNA fragment that is homologous to the central part of the *A. rhizogenes* TL DNA and contains the *rolB, C,* and *D* genes (White *et al.* 1983; Furner *et al.* 1986). The lack of this fragment in other *Nicotiana* species and its inverted repeat structure which resembles similar structures found for certain T-DNAs (Jorgensen *et al.* 1987) suggest that it was acquired through a rare transformation event, which led to the formation of a fertile, transformed regenerant (Furner *et al.* 1986). The structure, evolution, and possible function of this fragment merit further study. Moreover, this unique example of interkingdom lateral gene transfer links Ti plasmid evolution to the evolution of higher plants and thus provides us with a geological time scale for *Agrobacterium* evolution. Such a scale is normally lacking for bacterial genes due to the absence of a fossil record (Ochman and Wilson 1987).

Evolution of Ti plasmids. The Ti plasmids consist of a number of integrated functional components: origin of replication and incompatibility region, conjugative transfer genes (regulated by the conjugative opines), virulence genes, opine catabolism genes, and T-DNA genes. Presently, only few clues exist about the evolutionary history of the different regions and the ways they were combined. Little is known about the origins of replication of Ti plasmids. Do all Ti plasmids derive from a common replicon, or have typical Ti plasmid functions become associated with originally unrelated plasmids? Plasmids that belong to the same incompatibility group (like pTiC58 and pTiA6, Hooykaas and Schilperoort 1984) may share the same ancestor, whereas Ti plasmids belonging to other groups (like pRiA4 [Hooykaas and Schilperoort 1984] and pTiAg162 [Knauf

et al. 1984]) may be less related. Sequence data for various Ti plasmid origins of replication (already available for pRiA4, Nishiguchi *et al.* 1987) could help resolve this question. The Ti plasmid virulence system has most probably been derived from a bacterial conjugation system (Stachel and Zambryski 1986; Buchanan-Wollaston 1987; Zambryski *et al.* 1989; Ream 1989; Ziegelin *et al.* 1991; Pansegrau and Lanka 1991; Waters *et al.* 1991). All known T-region borders have a common consensus structure and the *vir* genes which products act on these sequences are also related (Hirayama *et al.* 1988; Rogowski *et al.* 1990). Ti-specific *vir* genes (*virF* in pTiAch5, Hooykaas *et al.* 1984; Otten *et al.* 1985; *tzs* in pTiAch5, Beaty *et al.* 1986; Akiyoshi *et al.* 1985) may have been added to an older, common *vir* region. The T-regions seem to be composed of a limited number of T-DNA genes that are combined in different ways. For example, genes for auxin and agrocinopine synthesis have been found on several different Ti plasmids (see Table 1). The assembly pathways of the different T-DNA genes into T-regions remains to be elucidated. The anabolic and catabolic opine genes can be expected to evolve in concert. Homology between the anabolic and catabolic mannopine cyclase gene (Hong *et al.* 1990) suggests that this particular combination arose by gene duplication, but this has not been found for other opine genes.

Although the mosaic Ti plasmid structures probably arose by horizontal DNA transfer, the transition areas from homologous to nonhomologous DNA do not reveal any particular structure that indicates how DNA transfer and integration into the Ti plasmid may have taken place. In one case a transpositional mechanism has been proposed. Yamada *et al.* (1986) noted that the *iaa* genes of octopine Ti plasmids like pTiAch5 and *P. syringae* subsp. *savastanoi* were both linked to an IS*51*-like element (truncated in *Agrobacterium*, and at some distance from the *iaa* genes) and proposed that the *Agrobacterium iaa* genes were inserted into a pTiAch5 precursor as part of an IS*51*-*iaa*-IS*51* transposon. If this happened only once, the different *Agrobacterium iaa* genes (Table 1) would be expected to be surrounded by the same sequences belonging to the originally transposed fragment. This is not the case, but changes due to subsequent DNA rearrangements cannot be excluded. It is striking that several other Ti plasmids contain IS*51*-like elements close to *iaa* genes (Table 2), but in none of these cases do the sequences in between resemble each other.

In cases where homologous regions can be clearly delimited, the phylogenies of individual genes or regions may be reconstructed by sequence comparisons. The *iaaM* and *iaaH* genes found in different strains (Table 1) may be taken as an example. The *iaa* genes of the "common T-DNAs" of octopine, octopine/cucumopine (o/c), nopaline, and succinamopine Ti plasmids are strongly homologous. The *iaa* genes of the agropine Ri plasmid and those of the o/c TB region occur in a different sequence context and are less related to the *iaa* genes of the octopine/nopaline group. Even less homologous are the *iaa* genes from pTiS4 (J. Canaday, unpublished). *iaaH* and *iaaM* genes have also been found in *Pseudomonas* but differ from the *Agrobacterium* genes in several respects: The *iaaM* gene is shorter at the 5′ end and the *iaaM* and *iaaH* genes are oriented in the same, rather than opposite direction. *Bradyrhizobium* also carries an *iaaH* gene (Sekine *et al.* 1989). ORF8 of *A. rhizogenes* strain A4 is a puzzling *iaaM*-like hybrid gene coding for a protein of which the N-terminal end is homologous to the products of gene 5 and *rolB* (Levesque *et al.* 1988; Bouchez 1990). A phylogenetic tree of the *iaa* genes should therefore not only use percentages of sequence homologies but must also incorporate data on changes in gene orientation and *iaaM* gene size. GC constraints may differ between strains (Bouchez and Tourneur 1991; Hirayama *et al.* 1988) and may therefore complicate sequence comparisons, especially when genes can be shuttled between strains with different GC contents. The possibility of codon usage differences between strains also remains to be investigated. It would be interesting to compare phylogenies based on defined Ti plasmid genes or regions with phylogenies based on (supposedly) stable chromosomal loci like 16S RNA genes (Young *et al.* 1991). Discrepancies between the two trees could indicate lateral gene transfer.

Several Ti plasmid-encoded proteins are distantly related to proteins from other organisms: Octopine and nopaline synthase are related to various dehydrogenases (Monneuse and Rouzé 1987), VirA and VirG to several two-component systems of *Escherichia coli* (reviewed in Stock *et al.* 1990), VirB4 to TraG of the IncP plasmid RP4 (Ziegelin *et al.* 1991), VirB11 to DNA and protein transport systems in other bacteria (Christie *et al.* 1989; Dums *et al.* 1991) and to KilB (a Tra protein of RK2, Motallebi-Veshareh *et al.* 1991), VirD2 to TraI of RP4 (Pansegrau and Lanka 1991), PinF to cytochrome-P-450 (Kanemoto *et al.* 1989). The products of the opine catabolism genes *occQ, occM, occP,* and *occJ* show homology to the family of osmotic shock-sensitive permeases (Valdivia *et al.* 1991). One study groups several T-DNA genes from *A. tumefaciens* and *A. rhizogenes* into families on the basis of weak amino acid homologies and postulates a number of gene duplication

Table 2. IS elements in or around T-regions[a]

IS element	Organism	Strain	Location	Reference
IS*66*	At	A66	TL	Machida *et al.* 1984
	At	A66	vir	Machida *et al.* 1984
	Rf	···	···	Ramakrishnan *et al.* 1986
IS*51*	Psav	···	···	Yamada *et al.* 1986
	At	Ach5	TC	Yamada *et al.* 1986
IS*868*	At	AB3	TA	Paulus *et al.* 1991b
	At	o/c	TB	Paulus *et al.* 1991b
	At	S4	pTiS4	Canaday, unpublished
IS*866*	At	Tm4	TA	Bonnard *et al.* 1989b
IS*867*	At	o/c	TA	Paulus *et al.* 1989b
	At	o/c	TB	Paulus *et al.* 1989b
	At	S4	pTiS4	Canaday, unpublished
IS*427*	At	T37	pTiT37	De Meirsman *et al.* 1991
IS*869*	At	AB3	TA	Paulus *et al.* 1991c
	At	nop-III	chrom	Paulus *et al.* 1991c
IS*426*	At	T37	T	Vanderleyden *et al.* 1986
	At	Ach5	TC	De Meirsman *et al.* 1987
IS*Rm1*	Rm	···	···	Martinez *et al.* 1990

[a] Abbreviations: At: *Agrobacterium tumefaciens*; Rf: *Rhizobium fredii*, Rm: *Rhizobium meliloti*; Psav: *Pseudomonas syringae* pv. *savastanoi*. Bold Face: IS elements chosen are representative for a given family. References refer to sequence data (if available).

events followed by sequence divergence (Levesque *et al.* 1988).

Finally, Ti plasmidlike functions have been found outside *Agrobacterium*: Some *Rhizobium* strains possess an opine-like system (Murphy *et al.* 1987), and various bacteria are able to utilize opines (Beaulieu *et al.* 1988; Bell *et al.* 1990; Nautiyal *et al.* 1991; Beauchamp *et al.* 1991). The corresponding genes have not yet been identified. In this respect it is interesting to note that the agrocinopine utilization systems of some agrobacteria show no detectable DNA homology (Hayman and Farrand 1990) and may therefore be of independent evolutionary origin.

Occurrence of IS elements in *Agrobacterium*. Part of the Ti plasmid variability is due to the activity of insertion elements. IS elements and repeated DNA elements of unknown origin are a conspicuous feature of Rhizobiaceae (Flores *et al.* 1987; Martinez *et al.* 1990). Several IS elements have been found in or close to the T-regions of various Ti plasmids (Table 2). Additional IS sequences are suspected in the TA region of Hml (Paulus *et al.* 1991c), TA region of NW233 (L. Otten, unpublished), o/c TB region (P. Fournier, unpublished), several areas outside the o/c T-regions (F. Paulus and L. Otten, unpublished), pTiAch5 TC region (also found in *R. leguminosarum,* Yun *et al.* 1987) and to the immediate left of the pTiAch5 TL region (L. Otten, unpublished). The *A. rhizogenes* TL DNA and the pSym plasmid of *Bradyrhizobium japonicum* share a common sequence (Krishnan and Pueppke 1991). The vitopine Ti plasmid pTiS4 (J.-C. Gérard, unpublished) contains a large number of repeated sequences, thereby recalling the structure of some pSym plasmids (de Lourdes Girard *et al.* 1991). Insertion elements are known to produce deletions, rearrangements, amplifications, and changes in gene expression. In the case of octopine/cucumopine strains it could be shown that they have played an important role in the evolution of the Ti plasmids.

Evolution of octopine/cucumopine Ti plasmids. Recent studies of a group of octopine/cucumopine plasmids of *Agrobacterium vitis* strains have uncovered some new aspects of Ti plasmid evolution. The TA- and TB-regions of five subclasses of o/c Ti plasmids, represented by pTiTm4, pTiHml, pTiAB3, pTiAg57, and pTiNW233 (Huss *et al.* 1989; Paulus *et al.* 1989a, 1991a, 1991b, 1991c; L. Otten *et al.,* unpublished) have different structures. Remarkably, the differences are nearly entirely due to IS elements (some of them still putative): Nucleotide sequences outside the IS elements are more than 99.7% identical. The different TA-regions can be derived from a common ancestor by invoking only a few molecular events (Fig. 1). In this model, an ancestor TA region separated into three lineages by insertion of one of three different IS elements: IS*866* for Tm4, IS-x for Hml, and IS*868* for the AB3/NW233/Ag57 group. AB3, NW233, and Ag57 diverged from each other by insertion of IS*869*, IS-y, and IS*867*, respectively. Sequence and restriction site conservation shows that the various insertions happened recently. O/c strains are specifically associated with grapevine, possibly due to their ability to degrade tartrate (Szegedi 1985) or to the presence of an *Agrobacterium vitis*-specific polygalacturonase (Rodriguez-Palenzuela *et al.* 1991). We have therefore proposed that the recent radiative evolution of o/c strains is related to the development of viticulture or to the technique of grapevine root-stock grafting. Large-scale grafting was introduced at the end of the 19th century because of the *Phylloxera* epidemic and is considered to be a major factor in the spread of the crown gall disease (Burr and Katz 1983; Burr *et al.* 1987; Jäger 1988). The role of grafting could be tested by a study of *Agrobacterium* strains from grapevine areas where grafting has never been practiced or by the study of strains that infect wild *Vitis* species.

Several of the TA-associated IS elements also occur outside the Ti plasmid. The distribution of IS*867* and IS*866* has been studied in detail (Paulus *et al.* 1989b) and can be summarized as follows (Fig. 2):

IS*867*. All Ti plasmids of the 46 o/c isolates studied so far carry two IS*867* copies, one close to the TA region, the other within the TB region. These copies were therefore part of the o/c ancestor plasmid. Because none of the copies outside the o/c Ti plasmid are common to all strains, it is possible that the ancestor strain only contained the two pTi-located copies and that its descendants underwent separation into three lineages before they started to accumulate additional IS*867* copies outside the Ti plasmid. Within a given lineage, the IS*867* element transposed up to four times within a short period (too short for DNA sequence divergence of the TA region). Transfer of the o/c Ti plasmid between different strains (as described for other *Agrobac-*

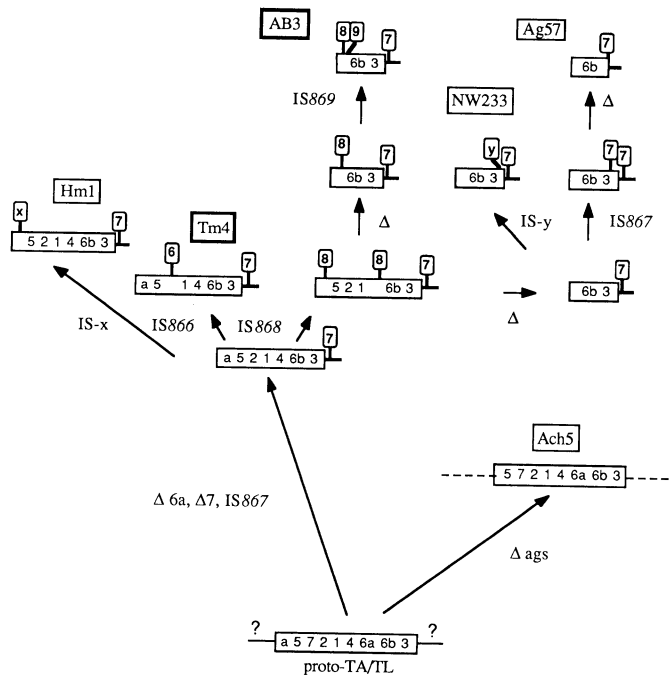

Fig. 1. Evolution of the TA/TL region. The symbols within the T-regions denote: a, agrocinopine synthase gene; 1, *iaaM*; 2, *iaaH*; 3, octopine synthase (*ocs*); 4, *ipt*; 5, 6a, 6b, and 7, genes 5, 6a, 6b, and 7 (Willmitzer *et al.* 1983). IS elements are indicated as tags with the following symbols: 6, IS*866*; 7, IS*867*; 8, IS*868*; 9, IS*869*; the putative IS elements are indicated by x (IS-x) and y (IS-y). Δ, deletion. T-regions are not drawn to scale. For each T-region type a representative strain is shown, Tm4 and AB3 represent frequent TA types; Hml, NW233, and Ag57 rare types. Dashed line: pTiAch5-specific sequences. The question marks (?) denote uncertainty regarding the nature of the sequences surrounding the proto-TA/TL region.

terium strains, [Petit *et al.* 1978] and found in three exceptional cases for small TA o/c Ti plasmids [Paulus *et al.* 1989a]) would obviously complicate this model. A study of the occurrence of IS*866*, IS*867*, IS*868*, IS*869*, and IS-x in different *Agrobacterium vitis* strains indicates, however, that plasmids found in such strains do not appear to move between different chromosomal backgrounds.

IS*866*. In many o/c isolates, the TA-*iaaH* gene is interrupted by a copy of IS*866*. Such strains contain in most cases additional IS*866* copies (up to five) outside the Ti plasmid. As in the case of IS*867*, the distribution pattern of IS*866* suggests that the first copy of this element was introduced on the Ti plasmid, and then spread to other sites by replicative transposition (Bonnard *et al.* 1989b; Paulus *et al.* 1989b).

The influence of the TA IS elements on the functional properties of the strains is unknown. They do not seem to reduce tumor formation on grapevine: Functional studies of pTiTm4 (Huss *et al.* 1990) and reconstruction of the TA-*iaaH gene* (Paulus *et al.* 1991c) have shown that five of the six known pTiTm4 tumor genes can be deleted without loss of tumorigenicity. This suggests that the o/c ancestor Ti plasmid was originally associated with a more demanding host plant. When a strain carrying this plasmid became established on grapevine, nonessential T-DNA genes may have served as selectively neutral target sites for IS elements. In spite of this, some strains appear to be more frequent than others: Our collection (with isolates from Germany, Hungary, France, South Africa, Australia, Greece, Russia, United States, and Spain) contains many strains of the Tm4 type, whereas Hm1 and Ag57 are unique. Several hypothetical intermediate TA structures (Fig. 1) have not been found so far. Differences in frequency may be explained by coselection of certain TA structures with unknown favorable mutations or by chance factors like a particular geographical distribution of clonally infected grapevine material resulting from human trade. The origin of the various IS elements, the way in which they were introduced into the recipient strains, and the regulation of their transposition deserve further study.

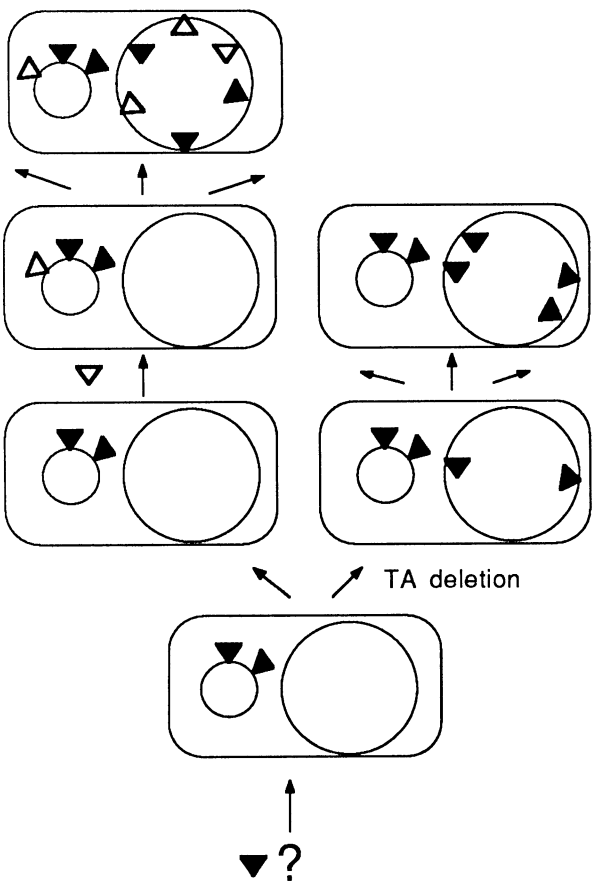

Fig. 2. Model of IS*867* and IS*866* transposition in Ti plasmids and chromosomes of o/c strains. The original strain contained two, Ti plasmid-borne IS*867* copies (of unknown origin, as indicated by the question mark) and its Ti plasmid had a large TA region. In one line of evolution (on the right) the TA region underwent an internal deletion (see also Fig. 1). IS*867* subsequently multiplied into different positions outside the Ti plasmid, as symbolized by the three arrows. In a second line (on the left) the TA region was interrupted by IS*866*, and both IS*867* and IS*866* subsequently multiplied to different positions outside the Ti plasmid. Black triangles: IS*867*; white triangles: IS*866*.

Chromosomal evolution. Although *Agrobacterium* strains can be grouped in three biovars on the basis of chromosomal properties that are supposedly stable, i.e., not subjected to horizontal gene transfer, nothing is known about the evolutionary relationships of the different strain types. Our analysis of the distribution of IS*866* and IS*867* elements in the chromosomes of o/c strains (Paulus *et al.* 1989b) indicates that the phylogenetic relationships between TA regions may be extrapolated to the corresponding chromosomes. O/c chromosomes show a high degree of polymorphism (L. Otten, unpublished) as also found for *Rhizobium* (Martinez *et al.* 1990). In contrast to this, the octopine Ti plasmids of the biovar I strains have been found to be conserved (Sciaky *et al.* 1978). For reasons that remain to be established, such plasmids may have been less vulnerable to modification by IS elements.

It remains to be established whether IS elements play the same important role in chromosomal evolution as they do in the case of the TA region. Chromosomal diversity in *Rhizobium* has been attributed to IS elements and has led some workers to conclude that *Rhizobium* taxonomy might be faced "with an unpredictable huge number of genomes" (Martinez *et al.* 1990). The same will almost certainly be true for *Agrobacterium*.

Perspectives. The study of the evolution of Ti plasmids and of the *Agrobacterium* genome is still in its infancy. The analysis of the TA region of the o/c Ti plasmids has yielded a detailed picture of the evolution of this DNA fragment and has demonstrated the importance of IS elements. Further studies should establish whether other o/c Ti regions have a similar IS density and are subject to the same loss of original sequences as the TA region. Several IS elements present on the o/c TA regions may have been introduced from other strains. Because o/c strains occur exclusively on grapevine it should be possible to determine whether other grapevine-associated bacteria contain similar elements and are able to donate them to *Agrobacterium*. As more o/c isolates from grapevine become available, additional forms of o/c Ti plasmids will probably be found and can extend the existing evolutionary

scheme. The study of other strains may show whether our results can be generalized to other Ti plasmids; it may be that the o/c Ti plasmids constitute a particular case of rapid divergence due to the association of one particular Ti plasmid type with one particular host; other Ti plasmids may diverge more slowly or be less exposed to insertion elements.

A challenging task for the future is to follow the evolution of natural *Agrobacterium* populations. Molecular analysis of large numbers of isolates may yield information on the frequency of occurrence and geographical distribution of particular strain types. Such studies will require the development of rapid analytical methods, such as PCR techniques and the use of specific probes to recognize the variants. Descriptive studies may be complemented by experimental approaches, for example by release of genetically marked strains under controlled conditions resembling the natural environment. The chance of detecting genetic changes within a reasonable time span will depend on the frequency of these changes, their fixation in the population, and the sensitivity of the detection method. If the o/c strains started their evolution only 100 years ago and if the number of five different IS elements (in 50 different o/c isolates) inserted in the TA region (average size about 10 kb) can be extrapolated to the entire *Agrobacterium* genome (at least 5,000 kb, Burkhardt *et al.* 1987), 50 randomly isolated descendants of a released strain may together accumulate as many as 25 new IS elements within 1 yr. Methods for rapid inspection of large parts of the genome may detect such changes and need to be developed. Especially interesting is the question of how populations develop within crown gall tumors and whether certain conditions will accelerate their evolution. Grapevine might be an excellent model plant to study such problems because the naturally infecting strains are now well-known (Paulus *et al.* 1989a). Coinfections with couples of isolates may show whether a correlation exists between frequency of occurrence in nature and competitiveness under experimental conditions and may lead to the identification of factors involved in selection. Nopaline and vitopine strains of *Agrobacterium vitis* deserve more study, as they occur ubiquitously, but do not seem to show the same variability in their T-regions as the o/c strains (Paulus *et al.* 1989a). Is this equally true for the rest of their genomes, or do functional constraints prevent modification of the nopaline and vitopine T-regions?

The evolutionary relationships within and between the three biovars could be established by comparative studies of chromosomal sequences. If lateral gene transfer is a common phenomenon, the choice of those sequences that should yield a phylogenetic tree may be difficult. The extent of lateral gene transfer may be determined by detailed analysis of a large number of different chromosomal sequences, which should include supposedly stable sequences like origins of replication or ribosomal genes. Reconstruction of the events within the chosen regions (nucleotide sequence changes and large-scale events) may yield a phylogenetic tree for *Agrobacterium* chromosomes. As in the case of the o/c TA region, the success of this enterprise will depend on the discovery of a sufficient number of related structures that retain the traces of the past.

ACKNOWLEDGMENTS

J. C. Gérard and P. Fournier were supported by a grant from the French Ministry for Research and Technology.

LITERATURE CITED

Akiyoshi, D. E., Regier, D. A., Jen, G., and Gordon, M. P. 1985. Cloning and nucleotide sequence of the *tzs* gene from *Agrobacterium tumefaciens* strain T37. Nucleic Acids Res. 13:2773-2788.

Akiyoshi, D. E., Regier, D. A., and Gordon, M. P. 1989. Nucleotide sequence of the *tzs* gene from *Pseudomonas solanacearum* strain K60. Nucleic Acids Res. 17:8886.

Barker, R. F., Idler, K. B., Thompson, D. V., and Kemp, J. D. 1983. Nucleotide sequence of the T-region from *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. Plant Mol. Biol. 2:335-350.

Beaty, J. S., Powell, G. K., Lica, L., Regier, D. A., MacDonald, E. M. S., Hommes, N. G., and Morris, R. O. 1986. *Tzs*, a nopaline Ti plasmid gene from *Agrobacterium tumefaciens* associated with trans-zeatin biosynthesis. Mol. Gen. Genet. 203:274-280.

Beauchamp, C. J., Klöpper, J. W., Lifshitz, R., Dion, P., and Antoun, H. 1991. Frequent occurrence of the ability to utilize octopine in rhizobacteria. Can. J. Microbiol. 37:158-164.

Beaulieu, C., Gill, S., Miville, L., and Dion, P. 1988. Genetic regions of *Pseudomonas aureofaciens* strains 211 involved in nopaline catabolism. Can. J. Microbiol. 34:843-849.

Bell, C. R., Cummings, N. E., Canfield, M. E., and Moore, L. W. 1990. Competition of octopine-catabolizing *Pseudomonas* spp. and *Agrobacterium tumefaciens* for octopine in chemostats. Appl. Environ. Microbiol. 56:2840-2846.

Blundy, K. S., White, J., Firmin, J. L., and Hepburn, A. G. 1986. Characterisation of the T-region of the SAP-type Ti-plasmid pTiAT 181: Identification of a gene involved in SAP synthesis. Mol. Gen. Genet. 202:62-67.

Bonnard, G., Tinland, B., Paulus, F., Szegedi, E., and Otten, L. 1989a. Nucleotide sequence, evolutionary origin and biological role of a rearranged cytokinin gene isolated from a wide host range biotype III *Agrobacterium* strain. Mol. Gen. Genet. 216:428-438.

Bonnard, G., Vincent, F., and Otten, L. 1989b. Sequence and distribution of IS*866*, a novel T-region-associated insertion sequence from *Agrobacterium tumefaciens*. Plasmid 22:70-81.

Bonnard, G., Vincent, F., and Otten, L. 1991. Sequence of the *Agrobacterium tumefaciens* biotype III auxin genes. Plant Mol. Biol. 16:733-738.

Bouchez, D. 1990. L'ADN transféré du plasmide Ri d'*Agrobacterium rhizogenes*: Aspects structuraux et fonctionnels de quelques fonctions portées par le TR-DNA du plasmide à agropine pRiA4. Ph.D. thesis. University of Paris-Sud, Paris.

Bouchez, D., and Camilleri, C. 1990. Identification of a putative *rolB* gene on the TR-DNA of the *Agrobacterium rhizogenes* A4 Ri plasmid. Plant Mol. Biol. 14:617-619.

Bouchez, D., and Tourneur, J. 1991. Organisation of the agropine synthesis region of the T-DNA of the Ri plasmid from *Agrobacterium rhizogenes*. Plasmid 25:27-39.

Bouzar, H., and Moore, L. W. 1987. Isolation of different *Agrobacterium* biovars from a natural oak savanna and tallgrass prairie. Appl. Environ. Microbiol. 53:717-721.

Brevet, J., and Tempé, J. 1988. Homology mapping of T-DNA regions on three *Agrobacterium rhizogenes* Ri plasmids by heteroduplex studies. Plasmid 19:75-83.

Buchanan-Wollaston, V., Passiatore, J. E., and Cannon, F. 1987. The *mob* and *oriT* mobilization functions of a bacterial plasmid promote its transfer to plants. Nature 328:172-175.

Buchholtz, W. B., and Thomashow, M. F. 1984. Comparison of T-DNA complements of *Agrobacterium tumefaciens* tumor-inducing plasmids with limited and wide host ranges. J. Bacteriol. 160:319-326.

Burkhardt, B., Schillik, D., and Pühler, A. 1987. Physical characterization of *Rhizobium meliloti* megaplasmids. Plasmid 17:13-25.

Burr, T. J., and Katz, B. H. 1983. Isolation of biovar 3 from grapevine galls and sap and from vineyard soil. Phytopathology 73:163-165.

Burr, T. J., Katz, B. H., and Bishop, A. L. 1987. Populations of *Agrobacterium* in vineyard and nonvineyard soils and grape roots in vineyards and nurseries. Plant Dis. 71:617-620.

Camilleri, C., and Jouanin, L. 1991. The TR-DNA region carrying the auxin synthesis genes of the *Agrobacterium rhizogenes* agropine-type

plasmid pRiA4: Nucleotide sequence analysis and introduction into tobacco plants. Mol. Plant-Microbe Interact. 4:155-162.
Chilton, W. S., Tempé, J., Matzke, M., and Chilton, M-D. 1984. Succinamopine: A new crown gall opine. J. Bacteriol. 157:357-362.
Christie, P. J., Ward, J. E., Gordon, M. P., and Nester, E. W. 1989. A gene required for transfer of T-DNA to plants encodes an ATPase with autophosphorylating activity. Proc. Natl. Acad. Sci. USA 86:9677-9681.
Costantino, P., Mauro, M. L., Micheli, G., Risuleo, G., Hooykaas, P. J. J., and Schilperoort, R. A. 1981. Fingerprinting and sequence homology of plasmids from different virulent strains of *Agrobacterium rhizogenes*. Plasmid 5:170-182.
de Lourdes Girard, M., Flores, M., Brom, S., Romero, D., Palacios, R., and Davila, G. 1991. Structural complexity of the symbiotic plasmid of *Rhizobium leguminosarum* bv. *phaseoli*. J. Bacteriol. 173:2411-2419.
De Meirsman, C., Desair, J., Vanderleyden, J., van Gol, A. P., and Jen, J. G. 1987. Similarities between nucleotide sequences of insertion elements of *Agrobacterium tumefaciens* and *Pseudomonas savastanoi* in relation to *Agrobacterium tumefaciens* TC-DNA. Nucleic Acids Res. 15:10591.
De Meirsman, C., van Soom, C., Verreth, C., van Gol, J., and Vanderleyden, J. 1991. Nucleotide sequence of IS*427* and its target sites in *Agrobacterium tumefaciens* T37. Plasmid 21:129-137.
Dums, F., Maxwell Dow, J., and Daniels, M. J. 1991. Structural characterization of protein secretion genes of the bacterial phytopathogen *Xanthomonas campestris* pathovar *campestris*: Relatedness to secretion systems of other gram-negative bacteria. Mol. Gen. Genet. 229:357-364.
Engler, G., Depicker, A., Maenhout, R., Villaroel, R., van Montagu, M., and Schell, J. 1981. Physical mapping of DNA base sequence homologies between an octopine and nopaline Ti plasmid of *Agrobacterium tumefaciens*. J. Mol. Biol. 152:183-208.
Flores, M., Gonzalez, V., Brom, S., Martinez, E., Pinero, D., Romero, D., Davila, G., and Palacios, R. 1987. Reiterated sequences in *Rhizobium* and *Agrobacterium* spp. J. Bacteriol. 169:5782-5788.
Furner, I. J., Huffman, G. A., Amasino, R. M., Garfinkel, D. J., Gordon, M. P., and Nester, E. W. 1986. An *Agrobacterium* transformation in the evolution of the genus *Nicotiana*. Nature 319:422-427.
Goldberg, S. B., Flick, J. S., and Rogers, S. G. 1984. Nucleotide sequence of the *tmr* locus of the *Agrobacterium tumefaciens* pTiT37 T-DNA. Nucleic Acids Res. 12:4665-4677.
Hansen, G., Lartibe, M., Vaubert, D., Tempé, J., Biermann, B. J., Montoya, A. L., Chilton, M-D., and Brevet, J. 1991. *Agrobacterium rhizogenes* pRi8196 T-DNA: Mapping and DNA sequence of functions involved in mannopine synthesis and hairy root differentiation. Proc. Natl. Acad. Sci. USA 88:7763-7767.
Hayman, G. T., and Farrand, S. K. 1990. *Agrobacterium* plasmids encode structurally and functionally different loci for catabolism of agrocinopine-type opines. Mol. Gen. Genet. 223:465-473.
Heidekamp, F., Dirkse, W. G., Kille, J., and van Ormondt, H. 1983. Nucleotide sequence of the *Agrobacterium tumefaciens* octopine Ti plasmid-encoded tmr gene. Nucleic Acids Res. 11:6211-6223.
Hirayama, T., Muranaka, T., Ohkawa, H., and Oka, A. 1988. Organization and characterization of the *virCD genes* from *Agrobacterium rhizogenes*. Mol. Gen. Genet. 213:229-237.
Holmes, B., and Roberts, P. 1981. The classification, identification and nomenclature of *Agrobacteria*. J. Appl. Bacteriol. 50:443-467.
Hong, S. B., Dessaux, Y., Guyon, P., Tempé, J., and Farrand, S. K. 1990. Relatedness between an opine catabolic gene and a T-DNA opine biosynthetic gene. Phytopathology 80:1037.
Hood, E. E., Scott-Chilton, W., Chilton, M-D., and Fraley, R. T. 1986. T-DNA and opine synthetic loci in tumors incited by *Agrobacterium tumefaciens* A281 on soybean and alfalfa plants. J. Bacteriol. 168:1283-1290.
Hooykaas, P. J. J., Hofker, M., den Dulk-Ras, H., and Schilperoort, R. A. 1984. A comparison of virulence determinants in an octopine Ti plasmid, a nopaline Ti plasmid and an Ri plasmid by complementation analysis of *Agrobacterium tumefaciens* mutants. Plasmid 11:195-205.
Hooykaas, P. J. J., and Schilperoort, R. A. 1984. The molecular genetics of Crown gall tumorigenesis. Adv. Genet. 22:209-283.
Huss, B., Bonnard, G., and Otten, L. 1989. Isolation and functional analysis of a set of auxin genes with a low root-inducing activity from an *Agrobocterium tumefaciens* biotype III strain. Plant Mol. Biol. 12:271-283.
Huss, B., Tinland, B., Paulus, F., Walter, B., and Otten, L. 1990. Functional analysis of a complex oncogene arrangement in biotype III *Agrobacterium tumefaciens* strains. Plant Mol. Biol. 14:173-186.
Jäger, J. 1988. Untersuchungen zur Epidemiologie und Bekämpfung von *Agrobacterium tumefaciens* (Smith & Townsend) Conn, dem Erreger der Mauke (crown gall) an Reben. Ph. D. thesis. University of Kaiserslautern.
Joos, H., Inzé, D., Caplan, A., Sörmann, M., van Montagu, M., and Schell, J. 1983. Genetic analysis of T-DNA transcripts in nopaline crown gall cells. Cell 32:1057-1067.
Jorgensen, R., Snyder, C., and Jones, J. 1987. T-DNA is organized predominantly in inverted repeat structures in plants transformed with *Agrobacterium tumefaciens* C58 derivatives. Mol. Gen. Genet. 207:471-477.
Kanemoto, R. H., Powell, A. T., Akiyoshi, D. E., Regier, D. A., Kerstetter, R. A., Nester, E. W., Hawes, M. C., and Gordon, M. P. 1989. Nucleotide sequence and analysis of the plant-inducible locus *pinF* from *Agrobacterium tumefaciens*. J. Bacteriol. 171:2506-2512.
Kerr, A., and Panagopoulos, C. G. 1977. Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. Phytopathol. Z. 90:172-179.
Kerr, A., and Brisbane, P. G. 1983. Agrobacterium. Pages 27-43 in: Plant Bacterial Diseases. P. C. Fahy and G. J. Presley, eds. Academic Press Australia, Sydney.
Kersters, K., De Ley, J., Sneath, P. H. A., and Sackin, M. 1973. Numerical taxonomic analysis of *Agrobacterium*. J. Gen. Microbiol. 78:227-239.
Knauf, V. C., Panagopoulos, C. G., and Nester, E. W. 1983. Comparison of Ti plasmids from three different biotypes of *Agrobacterium tumefaciens* isolated from grapevines. J. Bacteriol. 153:1535-1542.
Knauf, V. C., Yanofsky, A. M., Montoya, A., and Nester, E. W. 1984. Physical and functional map of an *Agrobacterium tumefaciens* tumor-inducing plasmid that confers a narrow host range. J. Bacteriol. 160:564-568.
Komari, T., Halperin, W., and Nester, E. W. 1986. Physical and functional map of supervirulent *Agrobacterium tumefaciens* tumor-inducing plasmid pTiBo542. J. Bacteriol. 166:88-94.
Krishnan, H., and Pueppke, S. G. 1991. Repetitive sequences with homology to *Bradyrhizobium japonicum* DNA and the T-DNA of *Agrobacterium rhizogenes* are closely linked to *nodABC* of *Rhizobium fredii* USDA 257. Mol. Plant Microbe Interact. 4:521-529.
Levesque, H., Delepelaire, P., Rouzé, P., Slightom, J., and Tepfer, D. 1988. Common origin of the central portions of the Ri TL-DNA of *Agrobacterium rhizogenes* and the Ti T-DNAs of *Agrobacterium tumefaciens*. Plant Mol. Biol. 11:731-744.
Machida, Y., Sakurai, M., Kiyokawa, S., Ubasawa, A., Suzuki, Y., and Ikeda, J-E. 1984. Nucleotide sequence of the insertion sequence found in the T-DNA region of mutant Ti plasmid pTiA66 and distribution of its homologues in octopine Ti plasmid. Proc. Natl. Acad. Sci. USA 81:7495-7499.
Martinez, E., Romero, D., and Palacios, R. 1990. The Rhizobium genome. Pages 59-93 in: Critical Reviews in Plant Sciences.
Monneuse, M-O., and Rouzé, P. 1987. Sequence comparisons between *Agrobacterium tumefaciens* T-DNA-encoded octopine and nopaline dehydrogenases and other nucleotide-requiring enzymes: Structural and evolutionary implications. J. Mol. Evol. 25:46-57.
Morris, R. O. 1986. Genes specifying auxin and cytokinin biosynthesis in phytopathogens. Annu. Rev. Plant Physiol. 37:509-538.
Motellabi-Veshareh, M., Jagura-Burdzy, G., Ross Williams, D., and Thomas, C. M. 1991. Proceedings of the Fallen Leaf Lake Conference on promiscuous plasmids in Gram-negative and -positive bacteria. Plasmid 25:225-250.
Murphy, P., Heyke, N., Banfalvi, Z., Tate, M. E., de Bruijn, F., Kondorosi, A., Tempé, J., and Schell, J. 1987. Genes for the catabolism and synthesis of an opine-like compound in *Rhizobium meliloti* are closely linked and on the Sym plasmid. Proc. Natl. Acad. Sci. USA 84:493-497.
Nautiyal, C. S., Dion, P., and Chilton, W. S. 1991. Mannopine and mannopinic acid as substrates for *Arthrobacter* sp. strain MBA209 and *Pseudomonas putida* NA513. J. Bacteriol. 173:2833-2841.
Nishiguchi, R., Takanami, M., and Oka, Atsuhiro. 1987. Characterization and sequence determination of the replicator region in the hairy-root-inducing plasmid pRiA4b. Mol. Gen. Genet. 206:1-8.
Ochman, H., and Wilson, A. C. 1987. Evolution in bacteria: Evidence for a universal substitution rate in cellular genomes. J. Mol. Evol.

26:74-86.
Ophel, K., and Kerr, A. 1990. *Agrobacterium vitis*—new species for strains of *Agrobacterium* biovar 3 from grapevine. Int. J. Syst. Bacteriol. 40:236-241.
Otten, L., Piotrowiak, G., Hooykaas, P. J. J., Dubois, M., Szegedi, E., and Schell, J. 1985. Identification of an *Agrobacterium tumefaciens* pTiB6S3 *vir* region fragment that enhances the virulence of pTiC58. Mol. Gen. Genet. 199:189-193.
Pansegrau, W., and Lanka, E. 1991. Common sequence motifs in DNA relaxases and nick regions from a variety of DNA transfer systems. Nucleic Acids Res. 19:3455.
Paulus, F., Huss, B., Bonnard, G., Ridé, M., Szegedi, E., Tempé, J., Petit, A., and Otten, L. 1989a. Molecular systematics of biotype III Ti plasmids of *Agrobacterium tumefaciens*. Mol. Plant-Microbe-Interact. 2:64-74.
Paulus, F., Ridé, M., and Otten, L. 1989b. Distribution of two *Agrobacterium tumefaciens* insertion elements in natural isolates: Evidence for stable association between Ti plasmids and their bacterial hosts. Mol. Gen. Genet. 219:145-152.
Paulus, F., Huss, B., Tinland, B., Herrmann, A., Canaday, J., and Otten, L. 1991a. Role of T-region borders in *Agrobacterium* host range. Mol. Plant-Microbe Interact. 4:163-172.
Paulus, F., Canaday, J., and Otten, L. 1991b. Limited host range Ti plasmids: Recent origin from wide host range Ti plasmids and involvement of a novel IS element, IS*868*. Mol. Plant-Microbe Interact. 4:190-197.
Paulus, F., Canaday, J., Vincent, F., Bonnard, G., Kares, C., and Otten, L. 1991c. Sequence of the *iaa* and *ipt* region of different *Agrobacterium tumefaciens* biotype III octopine strains: Reconstruction of octopine Ti plasmid evolution. Plant Mol. Biol. 16:601-614.
Perry, K. L., and Kado, C. I. 1982. Characteristics of Ti plasmids from broad-host range and ecologically specific biotype 2 and 3 strains of *Agrobacterium tumefaciens*. J. Bacteriol. 151:343-350.
Petit, A., Tempé, J., Kerr, A., Holsters, M., van Montagu, M., and Schell, J. 1978. Substrate induction of conjugative activity of *Agrobacterium tumefaciens* Ti plasmids. Nature 271:570-571.
Petit, A., and Tempé, J. 1983. La piste des opines. Pages 14-32 in: Molecular Genetics of the Bacteria-Plant Interaction. A. Pühler, ed. Springer Verlag, Berlin.
Petit, A., David, C., Ellis, G. J., Guyon, P. G., Casse-Delbart, F., and Tempé, J. 1983. Further extension of the opine concept: Plasmids in *Agrobacterium rhizogenes* cooperate for opine degradation. Mol. Gen. Genet. 190:204-214.
Powell, G. K., and Morris, R. O. 1986. Nucleotide sequence and expression of a *Pseudomonas savastanoi* cytokinin biosynthetic gene: Homology with *Agrobacterium tumefaciens tmr* and *tzs* loci. Nucleic Acids Res. 14:2555-2565.
Ramakrishnan, N., Prakash, R. K., and Atherly, A. G. 1986. Conservation of IS*66* homologue of octopine Ti plasmid DNA in *Rhizobium fredii* plasmid DNA. Plant Mol. Biol. 7:177-188.
Ream, W. 1989. *Agrobacterium tumefaciens* and interkingdom genetic exchange. Annu. Rev. Phytopathol. 27:583-618.
Rodriguez-Palenzuela, P., Burr, T. J., and Collmer, A. 1991. Polygalacturonase is a virulence factor in *Agrobacterium tumefaciens* biovar 3. J. Bacteriol. 173:6547-6552.
Rogowski, P. M., Powell, B. S., Shirasu, K., Lin, T. S., Morel, P., Zyprian, E. M., Steck, T. R., and Kado, C. I. 1990. Molecular characterization of the *vir* regulon of *Agrobacterium tumefaciens*: Complete nucleotide sequence and gene organization of the 28.63 kbp regulon as a single unit. Plasmid 23:85-106.
Schell, J. 1986. The T-DNA genes of *Agrobacterium* plasmids appear to be of a complex evolutionary origin. Pages 193-211 in: Genetics, Development and Evolution. J. P. Gustafson, G. L. Stebbins, and F. J. Ayala, eds. Plenum Press, New York.
Sciaky, D., Montoya, A. L., and Chilton, M. D. 1978. Fingerprints of *Agrobacterium* Ti plasmids. Plasmid 1:238-253.
Sekine, M., Watanabe, K., and Syono, K. 1989. Nucleotide sequence of a gene for indole-3-acetamide hydrolase from *Bradyrhizobium japonicum*. Nucleic Acids Res. 17:6400.
Slightom, J. L., Durand-Tardif, M., Jouanin, L., and Tepfer, D. 1986. Nucleotide sequence analysis of TL-DNA of *Agrobacterium rhizogenes* agropine type plasmid. Identification of open reading frames. J. Biol. Chem. 261:108-121.
Stachel, S. E., and Zambryski, P. C. 1986. *Agrobacterium tumefaciens* and the susceptible plant cell: A novel adaptation of extracellular recognition and DNA conjugation. Cell 47:155-157.
Stock, J. B., Stock, A. M., and Mottonen, J. M. 1990. Signal transduction in bacteria. Nature 344:395-400.
Strabala, T. J., Bednarek, S. Y., Bertoni, G., and Amasino, R. M. 1989. Isolation and characterization of an *ipt* gene from the Ti plasmid Bo542. Mol. Gen. Genet. 216:388-394.
Szegedi, E. 1985. Host range and specific L(+)-tartrate utilization of biotype 3 of *Agrobacterium tumefaciens*. Acta Phytopathol. Hung. 20:17-22.
Thomashow, M. F., Knauf, V. C., and Nester, E. W. 1981. Relationship between limited and wide host range octopine-type Ti plasmids of *Agrobacterium tumefaciens*. J. Bacteriol. 146:484-493.
Unger, L., Ziegler, S. F., Huffmann, G. A., Knauf, V. C., Peet, R., Moore, L. W., Gordon, M. P., and Nester, E. W. 1985. New class of limited-host-range *Agrobacterium* mega-tumor-inducing plasmids lacking homology to the transferred DNA of a wide-host-range, tumor-inducing plasmid. J. Bacteriol. 164:723-730.
Valdivia, R. H., Wang, L., and Winans, S. C. 1991. Characterization of a putative periplasmic transport system for octopine accumulation encoded by *Agrobacterium tumefaciens* Ti plasmid pTiA6. J. Bacteriol. 173:6398-6405.
Vanderleyden, J., Desair, J., De Meirsman, C., Michiels, K., van Goll, A. P., Chilton, M-D., and Jen, G. C. 1986. Nucleotide sequence of an insertion sequence (IS) element identified in the T-DNA region of a spontaneous variant of the Ti plasmid pTiT37. Nucleic Acids Res. 14:6699-6709.
Wabiko, H., Kagaya, M., Kodama, I., Masuda, K., Kodama, Y., Yamamoto, H., Shibano, Y., and Sano, H. 1989. Isolation and characterization of diverse nopaline type Ti plasmids of *Agrobacterium tumefaciens* from Japan. Arch. Microbiol. 152:119-124.
Waters, V. L., Hirata, K. H., Pansegrau, W., Lanka, E., and Guiney, D. G. 1991. Sequence identity in the nick regions of IncP plasmid transfer origins and T-DNA borders of *Agrobacterium* Ti plasmids. Proc. Natl. Acad. Sci. USA 88:1456-1460.
Weisburg, W. G., Woese, C. R., Dobson, M. E., and Weiss, E. 1985. A common origin of *Rickettsiae* and certain plant pathogens. Science 230:556-558.
White, L. O. 1972. The taxonomy of the crown-gall organism *Agrobacterium tumefaciens* and its relationship to rhizobia and other agrobacteria. J. Gen. Microbiol. 72:565-574.
White, F. F., and Nester, E. W. 1980. Relationship of plasmids responsible for hairy root and crown gall tumorigenicity. J. Bacteriol. 144:710-720.
White, F. F., Garfinkel, D. J., Huffmann, G. A., Gordon, M. P., and Nester, E. W. 1983. Sequences homologous to *Agrobacterium rhizogenes* T-DNA in the genomes of uninfected plants. Nature 301:348-350.
Willmitzer, L., Dhaese, P., Schreier, P. H., Schmalenbach, W., van Montagu, M., and Schell, J. 1983. Size, location and polarity of T-DNA-encoded transcripts in nopaline crown gall tumors: Evidence for common transcripts present in both octopine and nopaline tumors. Cell 32:1045-1056.
Yamada, T., Palm, C. J., Brooks, B., and Kosuge, T. 1985. Nucleotide sequences of the *Pseudomonas savastanoi* indoleacetic acid genes show homology with *Agrobacterium tumefaciens* T-DNA. Proc. Natl. Acad. Sci. USA 83:6522-6526.
Yamada, T., Lee, P-D., and Kosuge, T. 1986. Insertion sequence elements of *Pseudomonas savastanoi*: Nucleotide sequence and homology with *Agrobacterium tumefaciens* transfer DNA. Proc. Natl. Acad. Sci. USA 83:8263-8267.
Yanofsky, M., Lowe, B., Montoya, A., Rubin, R., Krul, W., Gordon, M. P., and Nester, E. W. 1985. Molecular and genetic analysis of factors controlling host range in *Agrobacterium tumefaciens*. Mol. Gen. Genet. 201:237-246.
Young, J. P. W., Downer, H. L., and Eardly, B. D. 1991. Phylogeny of the phototrophic *Rhizobium* strain BTAil by polymerase chain reaction-based sequencing of a 16S rRNA gene segment. J. Bacteriol. 173:2271-2277.
Yun, A. C., Hadley, R. G., and Szalay, A. A. 1987. A plasmid sequence from *Rhizobium leguminosarum* 300 contains homology to sequences near the octopine TL-DNA right border. Mol. Gen. Genet. 209:580-584.
Zambryski, P., Tempé, J., and Schell, J. 1989. Transfer and function

of T-DNA genes from *Agrobacterium* Ti and Ri plasmids in plants. Cell 56:193-201.

Ziegelin, G., Pansegrau, W., Strack, B., Balzer, D., Kröger, M., Kruft, V., and Lanka, E. 1991. Nucleotide sequence and organization of genes flanking the transfer origin of promiscuous plasmid RP4. DNA Sequence 1:303-327.

Zoz, N. N., Avdienko, I. D., Lemanova, N. B., Ovadis, M. I., Sultanova, O. D., Sacharova, M. N., Poglazov, A. B., Pleshakova, R. J., Kmel, J. A., and Chernin, L. S. 1986. Biochemical and genetic characteristics of *Agrobacterium tumefaciens* strains isolated on grapevine in Moldavia. Molekularnaya Genetika, Mikrobiologia i Virusologia 2:22-27.