Ti and Ri Plasmids

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Contents

Abstract *Agrobacterium* species harboring tumor-inducing (Ti) or hairy root-inducing (Ri) plasmids cause crown gall or hairy root diseases, respectively. These natural plasmids provide the basis for vectors to construct transgenic plants. The plasmids are approximately 200 kbp in size. Complete sequence analysis indicates that the pathogenic plasmids contain gene clusters for DNA replication, virulence, T-DNA, opine utilization, and conjugation. T-DNA genes have lower G + C content, which is presumably suitable for expression in host plant cells. Besides these genes, each plasmid contains a large number of unique genes. Even plasmids of the same opine type differ considerably in gene content and have highly chimeric structures. The plasmids seem to interact with each other and with plasmids of other members of the *Rhizobiaceae* and are likely to shuffle genes for infection between

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Ti and Ri plasmids. Plasmid stability genes are discussed, which are important for plasmid evolution and construction of useful strains.

1 Introduction

A group of *Agrobacterium* species are the causative agent of crown gall disease and hairy root disease on dicot plants (Fig. 1). On the positive side from the point of view of biotechnology and molecular biology, the species with the infection system are also the most reliable tool for introducing chimeric DNA into plants. *A. tumefaciens*, *A. rhizogenes*, and *A. vitis*, also termed *Agrobacterium* biovar 1, biovar 2, and biovar 3, respectively, are the three major pathogenic *Agrobacterium* species. Recent bacterial classification schemes use the genus *Rhizobium* in place of the genus *Agrobacterium* (Young et al. 2003), since the strains are indistinguishable from *Rhizobium* strains except for their pathogenic and the nitrogen-fixing characteristics. In fact, the pathogenic strain ATCC11325, which is the type strain of *A. rhizogenes*, contains a symbiotic plasmid (Velázquez et al. 2005). In this paper, however, we use the genus *Agrobacterium* to highlight the pathogenic species. Most genes essential for pathogenicity are found on the large plasmids, approximately 200 kbp, called tumor-inducing (Ti) or root-inducing (Ri) plasmids. Introduction of the plasmids

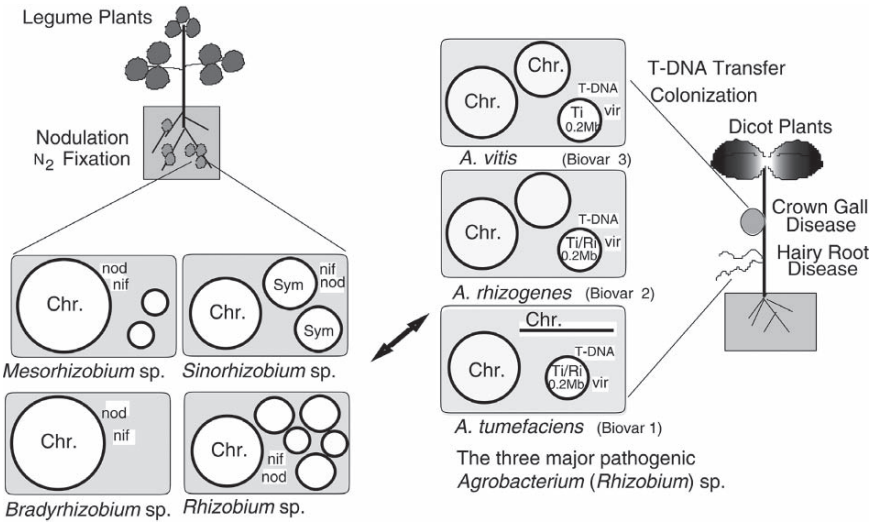

Fig. 1 Association of symbiotic and pathogenic strains of the order *Rhizobiales* with plants. The double arrow indicates that gene transfer between the pathogenic and nitrogen fixing bacteria takes place. The evolutionary relationships between the symbiotic plasmids are observable in the plasmid sequences. See text for details. Genomic structures of the species are based on Kaneko et al. (2000, 2002), González et al. (2006), Suzuki et al. (2001), Urbanczyk et al. (2003), and Tanaka et al. (2006)

into nonpathogenic *Agrobacterium* strains and *Rhizobium* strains makes them pathogenic (Klein and Klein 1953, Hooykaas et al. 1977). Better understanding of the plasmids should give insights useful for controlling the spread of the diseases and also improve plant and fungal transformation technology (Lacroix et al. 2006).

Ti and Ri plasmids are classified by organic compounds termed opines, which are produced by infected plant cells and delivered to the pathogen as nutrients. Opine synthesis is directed by the genes on the T-DNA portion of the plasmids in plant cells. The complete sequences of two nopaline-type plasmids, pTi-SAKURA and pTiC58; a mikimopine-type plasmid pRi1724; a cucumopine-type plasmid pRi2659; an agropine/mannopine-type Ti plasmid, pTiBo542; and a composite assembly of five octopine-type plasmids, pTiA6NC, pTi15955, pTiAch5, pTiR10, and pTiB6S3, have been reported (Suzuki et al. 2000, Goodner et al. 2001, Wood et al. 2001, Moriguchi et al. 2001, Mankin et al. 2007, Zhu et al. 2000). Table 1 lists the general information for the six plasmids. In this paper, we describe the structural and functional properties of Ti and Ri plasmids based on these plasmid sequences as well as several partially sequenced plasmids. For a general review on Ti plasmids, refer to the article by Christie (2004a). For a general review with a biotechnological viewpoint, refer to the article by Gelvin (2003). More focused reviews are cited below in the sections dealing with specific subjects.

Table 1 List of Ti and Ri plasmids whose complete nucleotide sequences are available in the public DNA databases

Plasmid	*inc* group	Disease	Opine production[a]	Host range	Length (bp)	Accession number	Key reference
pTiC58	Rh-1	Crown gall	Nopaline Agrocinopine	Broad	214,234	AE007871	Goodner et al. (2001) Wood et al. (2001)
pTi-SAKURA	Rh-1	Crown gall	Nopaline Agrocinopine	Broad	206,479	AB016260	Suzuki et al. (2000)
Octopine type pTi[b]	Rh-1	Crown gall	Octopine agropine mannopine	Broad	194,140	AF242881	Zhu et al. (2000)
pTiBo542	Rh-2	Crown gall	Succinamopine Agropine Mannopine	Broad	244,978	DQ058764	Oger et al.'s data in the DNA database
pRi1724	(Rh-3)	Hairy root	Mikimopine	Broad	217,594	AP002086	Moriguchi et al. (2001)
pRi2659	(Rh-3)	Hairy root	Cucumopine	Broad	202,297[c]	EU186381	Mankin et al. (2007)
						EF433766	Collier et al.'s data in the DNA database

[a] The opine type follows the opine(s) marked with underline
[b] Composite sequence from five well-conserved plasmids
[c] Calculated for this paper by assembling two nucleotide sequences from the two groups

2 Chimeric Structure

The overall G + C content of these plasmids is about 56%, which is slightly lower than that of *A. tumefaciens* total genomic DNA (Goodner et al. 2001, Wood et al. 2001). Ti and Ri plasmids consist of many segments with different G + C content (see Fig. 2 for example). A few segments are rich in A and T. T-DNA is especially abundant in A and T. Generally, functionally related genes are clustered in a segment with an even G + C content (Fig. 2). The data suggest a chimeric structure comprising segments from different sources. As illustrated in Fig. 3, there are five clusters common among Ti and Ri plasmids: (1) T-DNA, which is transferred to the host plants; (2) the virulence gene (*vir*) region, which directs the recognition of plant phenolic compounds, processing, and transfer of T-DNA; (3) the replication gene (*rep*) region, which is required for the plasmid replication; (4) *tra* and (5) *trb* regions, which direct conjugal transfer of the plasmid. Two or more regions that direct uptake and catabolism of a respective opine are present in each plasmid. An exception to the functional clustering is the *tra* and *trb* regions in Ti plasmids. These two regions are separated from each other by more than 60 kb in nopaline- and octopine- type plasmids (Suzuki et al. 2000; Zhu et al. 2000). We termed the

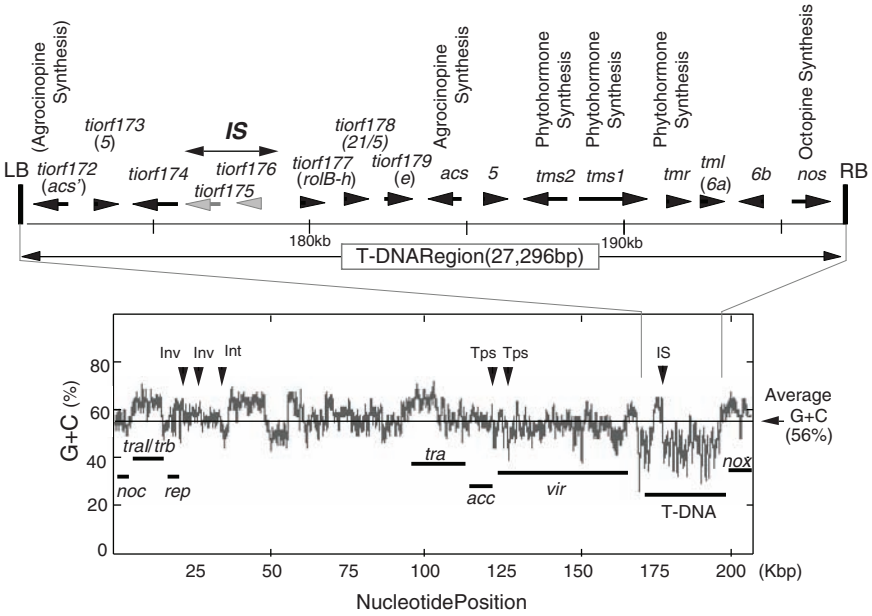

Fig. 2 Genetic organization and G + C distribution of the nopaline type plasmid pTi-SAKURA. *Lower panel*: G + C content (%) determined for a sliding window size of 400 bp. Functionally defined gene clusters are indicated by *thick horizontal bars*. *Arrowheads* indicate sequences for invertase (Inv), integrase (Int), transposase (Tps), and insertion sequence (IS). *Upper panel*: An exploded view of the T-DNA. Arrows give the size and direction of transcription of genes. See text for details

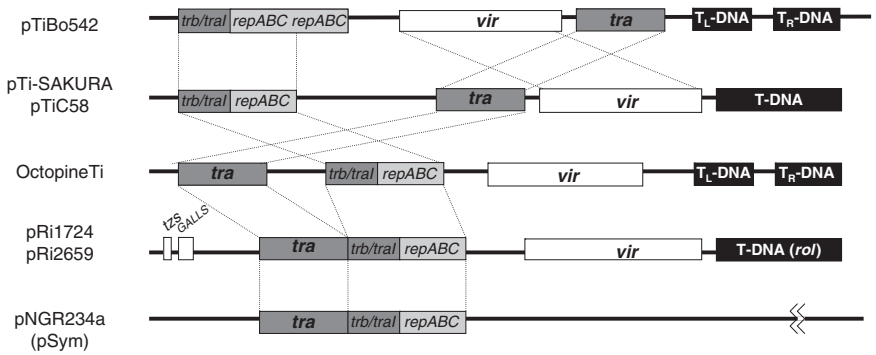

Fig. 3 Gene clusters commonly observable among Ti and Ri plasmids. Each box represents a gene cluster or a set of clusters. Functionally related segments are indicated by a box with the same shading level. The diagram is based on published sequence data and annotations (see Table 1 for references and the database accession numbers). A symbiotic plasmid pNGR234a (536,165 bp; accession No. NC000914) was included as a reference replicon

large region the large variable region (VAR), since it is highly variable even between the two nopaline-type Ti plasmids (Suzuki et al. 2000; Goodner et al. 2001). The VAR region contains many genes with unknown function. Phylogenetic trees based on a *trb* gene and the *oriV* sequence indicate a closer relationship of Ri plasmids to the symbiotic plasmid pNGR234a. However, a tree based on a *vir* gene indicates that pRiA4b is closer to the nopaline type plasmids than to pRi1724 (see reviews by Yoshida et al. 2003, 2004). These data together with the G + C values indicate that the plasmids have exchanged and shuffled genes, including those for virulence.

As illustrated in Fig. 1, strains in the order *Rhizobiales* have various types of genome constitutions. The nitrogen-fixing symbiosis system genes are observable in many genera either on plasmids or on chromosomes. Contrarily, the T-DNA/*vir* pathogenesis system has been found only on plasmids so far. Neither fragments of *vir* genes nor T-DNA genes were found on chromosomes. It would be interesting to survey chromosomal sequences for the presence of the pathogenesis gene system.

3 T-DNA

T-DNA is a DNA segment transferable to host cells and integrated into the host plant genomic DNA. Nopaline-type Ti plasmids and pRi1724 contain a single T-DNA region, whereas the agropine-type plasmid, pRiA4b, and octopine-type Ti plasmids contain two T-DNA regions (Suzuki et al. 2000, Goodner et al. 2001, Wood et al. 2001, Moriguchi et al. 2001, Zhu et al. 2000). The size of each T-DNA region(s) in a plasmid is around 20 kbp: 15,098 bp in pRi1724 and 27,296 bp in pTi-SAKURA. The two extreme ends of a T-DNA region are defined by 25-bp direct repeats, called right

border (RB) and left border (LB) sequences (Yadav et al. 1982). RB is essential for T-DNA transfer and is the initiation site for DNA processing. LB is dispensable for T-DNA transfer but is the termination site for single-strand DNA formation, which is initiated at RB. As shown in Fig. 2, the T-DNA coding regions have a G + C content of about 50% (Suzuki et al. 2000, Hattori et al. 2000, Zhu et al. 2000, Moriguchi et al. 2001), and the intergenic portions have less. The relatively low G + C content is likely suitable for efficient expression of T-DNA genes in plant cells. A portion in nopaline-type T-DNA exhibits high G + C content and contains an insertion sequence (Hattori et al. 2000, Suzuki et al. 2000).

Ti plasmids contain three T-DNA genes that direct synthesis of the plant hormones, cytokinin and auxin. Production of the two phytohormones in plants directed by the T-DNA genes primarily causes the tumor formation (for a review see Zambryski et al. 1989). Enzymes coded by *tmsA* and *tmsB* (also designated *iaaM* and *iaaH*, respectively) direct the conversion of tryptophan to indoleacetic acid. The *tmr* (*ipt*) gene directs production of cytokinin and *tml* (also designated gene *6b*) enhances cell division (Hooykaas et al. 1988). Gene *6b* from strain AKE10 can induce plant cell division even in media without phytohormones (Wabiko and Minemura 1996). The 6b protein localizes to the plant nucleus by interacting with NtSIP1 (Kitakura et al. 2002). The *rol* genes, *rolA*, *rolB*, and *rolC*, and *orf13* are conserved among Ri plasmid T-DNA regions. The *rolA* and *rolB* genes play primary roles in adventitious root induction (Zambryski et al. 1989) by a mechanism other than the production of phytohormones. Similar to the 6b protein of Ti T-DNA, RolB protein of pRi1724 is localized in the nucleus and interacts with 14-3-3-like proteins (Nt14-3-3) (Moriuchi et al. 2004). The 6b and Rol proteins are supposed to affect expression of plant genes that regulate plant cell division and morphology. Terakura et al. (2007) indicated that 6b protein binds specifically to histone H3 and has a histone–chaperon like activity, suggesting a relationship between alterations in nucleosome structure and the expression of growth-regulating genes.

Several genes that direct the synthesis of a different opine are observable in T-DNA. Two genes *nos* and *acs* in nopaline-type Ti plasmids encode nopaline synthase and agrocinopine synthase, respectively (Hattori et al. 2000). Two genes, *ocs* and *ags* in octopine-type Ti plasmids encode octopine synthase and agropine synthase, respectively. In addition, two genes, *mas1* and *mas2* in octopine-type plasmids direct synthesis of mannopines (Zhu et al. 2000). The T-DNA of pRi1724 harbors a mikimopine synthase gene (*mis*) and an unknown opine synthesis gene (Moriguchi et al. 2001). The opines are produced by expression of the genes in the infected plants. The product opines support bacterial growth as nutrients. The phenomenon is called "genetic colonization." An agrocinopine-like compound is presumed to be an ancestral opine in the evolution of the T-DNA, because a homolog or fragment of the *acs* gene is present close to the right of LB of the T-DNA in most Ti and Ri plasmids (Paulus and Otten 1993; see also references for the complete nucleotide sequences given in Table 1) as shown, for example, in the ORF map in Fig. 2.

Expression of the T-DNA genes is repressed in *Agrobacterium* cells. However, the *ipt* gene is derepressed in a *ros* mutant *A. tumefaciens* cells. The *ros* gene codes

for a zinc finger motif-containing protein that binds to the 40-bp *ros*-box sequence in the operators of *ipt*, *virC/D* and the succinoglycan synthesis operon (for more details, see a review by Kado (2002)). It remains to be elucidated whether there are additional genes that repress T-DNA genes in bacterial cells.

4 Virulence Genes

In addition to T-DNA, virulence (*vir*) gene regions are essential for pathogenicity. The essential genes are coded in the core *vir* region consisting of four operons, *virB*, *virC*, *virD*, and *virE*, and two regulatory genes, *virA* and *virG*. The genes form a large cluster about 30 kbp in size. Both the nucleotide sequence of the genes in the core *vir* gene region and the order of the genes are well conserved among Ti and Ri plasmids. For more details on host recognition and DNA transfer, refer to the article by McCullen and Binns (2006).

4.1 Regulation by VirA and VirG

The two-component-system proteins, VirA and VirG, regulate virulence gene expression (Stachel and Zambryski 1986). The *vir* regulon is induced by low molecular weight phenolic compounds, such as acetosyringone, which are released from the host plant. The induction requires weakly acidic pH (pH 5.0–5.5) and moderate temperature (below 28°C) optimally in the presence of monosaccharides. The phenolics are detected by the transmembrane sensor kinase VirA, which phosphorylates the response regulator VirG (Fig. 4). Phosphorylated VirG binds to sequences called *vir* boxes upstream of *vir* regulon promoters and positively regulates *vir* gene expression (Roitsch et al. 1990). A periplasmic sugar binding protein, ChvE (Cangelosi et al. 1990, Shimoda et al. 1993), which is encoded by a chromosomal gene, associates with VirA for induction of *vir* genes.

4.2 vir Operon Genes for Processing and Transfer of T-DNA

T-DNA is processed by the action of the *vir* gene products (Fig. 4). The RB sequence is nicked by VirD2 in the presence of VirD1, and simultaneously VirD2 is linked covalently to the 5′ end of the T-DNA strand (Scheiffele et al. 1995). The VirD2-bound strand is then cleaved at the left border end and released from the plasmid. VirC1 binds to a 24-bp sequence called overdrive (Peralta et al. 1986), which lies adjacent to RB and is required for efficient transfer (van Haaren et al. 1987, Toro et al. 1989). The single-stranded T-DNA covalently linked with VirD2 is called T-complex. The opposite strand remains in the plasmid, and DNA polymerase fills in

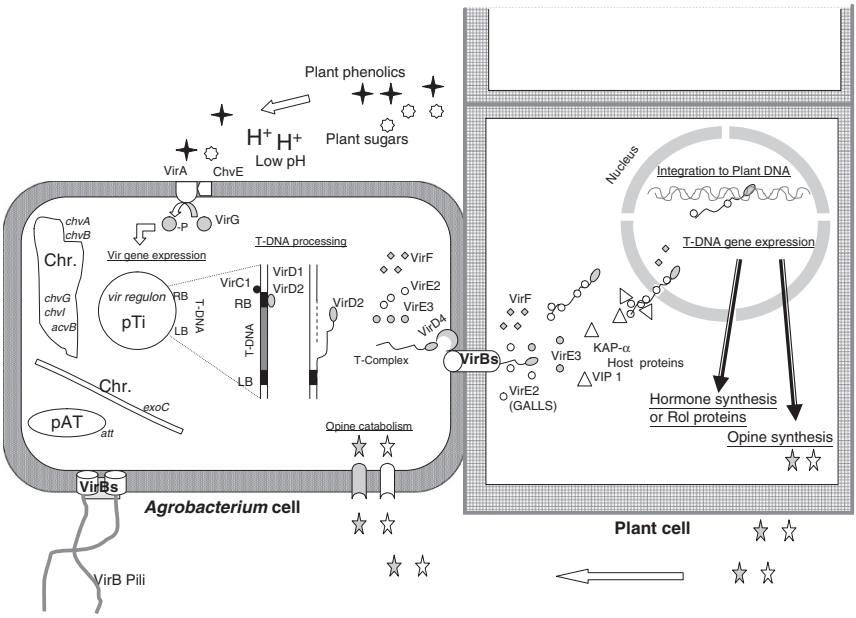

Fig. 4 Molecular mechanism of the interaction between pathogenic *Agrobacterium* cells and plant cells. See text for details

the single stranded portion on the plasmid. Ri plasmids contain 8-bp repeats, named TSS (T-DNA transfer stimulator sequence) (Hansen et al. 1992, Moriguchi et al. 2001), similar to overdrive. However, the function of TSS remains to be elucidated.

Proteins for the T-DNA transfer channel apparatus (see reviews by Christie 2004b, Schröder and Lanka 2005) are encoded by the *virB* operon, which contains 11 genes. Pili containing VirB2 as the major subunit form on the bacterial cell surface under conditions suitable for *vir* gene induction (Fullner et al. 1996). A coupling protein, VirD4, mediates the association of the T-complex with the VirB apparatus. The T-complex and several proteins including a single-stranded DNA binding protein, VirE2, are transferred to the cytoplasm of host plant cells via the VirB apparatus. VirE1 is a chaperone for VirE2 (Sundberg et al. 1996, Deng et al. 1999). In the plant cell cytoplasm, VirE2 covers the T-complex DNA and protects the single-stranded T-DNA. Import of the VirE2/T-complex into the plant nucleus through nuclear pores is mediated by nuclear localization signals (NLSs) in VirE2 and VirD2 (Citovsky et al. 1992, Howard et al. 1992). VirE2-interacting plant proteins were surveyed by yeast two hybrid screen, and termed as VirE2-interacting proteins (VIPs). A plant protein, VIP1, associates with VirE2 in the complex and helps its entry into the plant nucleus (Tzfira et al. 2001). Another plant protein, VIP2, is required for T-DNA integration in plants (Anand et al. 2007).

The two genes *virE1* and *virE2* are absent in pRiA4, pRi1724, and pRi2659 (Aoyama et al. 1989, Moriguchi et al. 2001, Mankin et al. 2007), although they are essential for Ti plasmid-directed tumorigenesis. The GALLS gene is present in the Ri plasmids and substitutes for *virE2* (Hodges et al. 2004, 2006). The GALLS protein does not resemble VirE2 and the gene is not located near the core *vir* region, but it has a NLS and is transferred from the bacteria to plant cells in the same manner as VirE2.

4.3 Auxiliary vir Genes

Several *vir* genes such as *virF*, *virJ*, and *virH* are not always essential for tumorigenesis. Presence and location of the auxiliary *vir* genes are variable depending on the plasmid. They are usually located near or within the core *vir* region and inducible by phenolics (Hattori et al. 2001). The host plant determinant gene *virF* is contained in octopine-type plasmids and many but not all nopaline-type plasmids (Schrammeijer et al. 1998). pTiC58, pTi-SAKURA, pTiBo542, and pRi1724 harbor a *virF* or *virF*-related gene. VirF is exported to host plant cells via the VirB apparatus, and then destabilizes the complex between ViE2 and VIP1 in the plant nucleus (Tzfira et al. 2004). Eventually, this VirF function enhances T-DNA integration in some plant species. Octopine-type plasmids and pTiBo542 have *virJ*, whereas nopaline-type plasmids and the two Ri plasmids, pRi1724 and pRi2659, do not. A chromosomal gene, *acvB* (Wirawan et al. 1993), is functionally equivalent to *virJ* (Pan et al. 1995). Thus, *virJ* is not necessary for tumorigenesis in the wild-type chromosomal background. Two genes *virH1* and *virH2* (also named *pinF1* and *pinF2*, respectively) encode cytochrome P450 family proteins, which have been supposed to detoxify plant substances. Actually, VirH2 converts the toxic inducer phenolic substance, ferrulic acid, to a less toxic noninducer, caffeic acid (Kalogeraki et al. 1999). VirH2 presumably supports infection and subsequent colonization in plants rich in ferrulic acid. Nopaline-type plasmids and the octopine-type plasmid contain both *virH* genes.

Nopaline-type plasmids contain *tzs* (trans-zeatin synthesis) gene (Suzuki et al. 2000, Goodner et al. 2001, Wood et al. 2001), which encodes a cytokinin biosynthetic prenyl transferase. The phytohormone is produced and released by the bacterium before and during infection. In plant transformation, pretreatment with auxin and cytokinin increases the transformation frequency, and auxin treatment suppresses silencing of gene expression (Dunoyer et al. 2006). Hormone production by enzymes encoded by *tzs* and hormone genes of T-DNA ensure infection and efficient expression of opine synthesis genes. The *tzs* gene is not present in octopine-type Ti plasmids (Zhu et al. 2000), whereas pRi1724 and pRi2659 contain *tzs* in the VAR region (Moriguchi et al. 2000, Mankin et al. 2007) as illustrated in Fig. 3. *GALLS* and *tzs* in the two Ri plasmids are located close to each other, but are far away (60 and 34 kbp in the two plasmids, respectively) from the core *vir* region. pTiBo542 harbors *virH1*, *virH2*, and *virJ*, but not *tzs*.

5 Opine Utilization Genes

In addition to the genes for opine synthesis, Ti and Ri plasmids encode genes for utilization of opines. Three operons, *noc*, *nox*, and *acc*, are responsible for uptake and catabolism of nopaline and agrocinopine in nopaline-type plasmids (Hattori et al. 2000). More than 40 genes in octopine-type Ti plasmids are devoted to growth on opines, including octopine, agropine, and mannopine (Zhu et al. 2000). The opine utilization genes differ from plasmid to plasmid.

6 Replication and Stability Genes

6.1 repABC Genes

Ti plasmids are stably maintained at a low copy number comparable to chromosomal DNA copy number (Suzuki et al. 2001). Three genes, *repA*, *repB*, and *repC*, are sufficient for the replication and copy number control (Tabata et al. 1989). The *repC* gene is essential for replication, while *repA* and *repB* are required for stable plasmid inheritance. The copy number of pTiC58 is increased by binding of a conjugational regulatory protein TraR to a region called *tra*-box upstream from *repA* (Li and Farrand 2000). The *repABC* type replicators are commonly contained in large plasmids in members of the family *Rhizobiaceae*. In *A. tumefacience*, strain C58, three replicons, pTiC58, a cryptic plasmid pAtC58 (543 kbp), and a linear chromosome (2,075 kbp) harbor *repABC* loci of their own (Goodner et al. 2001, Wood et al. 2001). As shown in Table 1, octopine-type and nopaline-type Ti plasmids belong to the same incompatibility group, IncRh-1. pTiBo542 and a vitopine-type plasmid pTiS4 belong to different groups, IncRh-2 and IncRh-4, respectively (Szegedi et al. 1996). Ti plasmids are compatible with Ri plasmids, which belong to group IncRh-3.

6.2 Incompatibility and Stability Enhancing Genes

It is known that many nopaline-type Ti plasmids are resistant to the exclusion pressure by incompatible plasmids. They fuse with incompatible plasmids upon encountering them in a cell, indicating that the nopaline-type plasmids are highly stable (Hooykaas et al. 1980). Most Ti plasmids are hard to cure. These characteristics cause serious difficulty in genetic manipulation of strains. However, there has been no report about stability-enhancing genes in Ti and Ri plasmids. In pTi-SAKURA, a locus containing two genes, *tiorf24* and *tiorf25*, enhances incompatibility of plasmids and increases stability of unstable plasmids (Yamamoto et al. 2007). Our additional data suggest that the plasmid stabilization mechanism is the toxin–antitoxin (TA)

addiction system, in which Tiorf24 and Tiorf25 are antitoxin and toxin, respectively. Tiorf25 protein contains a PIN domain, which is also found in the VapC protein encoded by the *vapBC* operon. Another locus pTi-SAKURA also helps to stabilize the plasmid (Yamamoto, personal communication). When Ti plasmids enter a cell that already contains an incompatible Ti plasmid, the resident Ti plasmid is usually expelled. However, a plasmid with increased stability is not always expelled but amalgamates with the incoming plasmid to form a larger plasmid. The resultant large co-integrate plasmids are likely to reduce their size subsequently to around 200 kbp, because all pathogenic strains so far examined posses a Ti and/or a Ri plasmid of this size. During this process, both regions that provide a selection advantage and also maintain stability determine which portions of the parental plasmids remain in the new plasmid.

Uraji et al. (2002) proposed a simple method to prepare Ti plasmid-less cells using a small *repABC* plasmid. The small plasmid remains in the resulting Ti plasmid-less cells and is easily removable when equipped with a counter-selectable marker gene, such as *sacB*. The Ti plasmid-less cells can then accept Ti plasmids with high efficiency and without formation of fusion plasmids. This method is applicable to many pathogenic strains, and thereby can help construction of new and useful strains for biotechnology (Tanaka et al. unpublished data).

7 Conjugation Genes

Ti plasmids harbor a *tra* gene region and a *traI*/*trb* operon. The *tra* gene region contains an origin of transfer (*oriT*) and DNA precessing genes, while the *traI*/*trb* operon codes for components for formation of conjugal pili and mating pairs. TraI is an enzyme to synthesize *N*-acyl-homoserine lactone, which is the quorum-sensing signaling molecule for cell–cell communication (for a review see White and Winans 2007). Special opines induce the expression of the *tra* and *traI*/*trb* genes: octopine for octopine-type plasmids, agrocinopine A for nopaline type, and agrocinopine C and D for agropine-type plasmids (Dessaux et al. 1992). The *traI*/*trb* operon is located far away from the *tra* region in Ti plasmids, whereas the two regions are neighboring in Ri plasmids (Fig. 3), similar to those in several Sym plasmids such as pNGR234a.

8 Perspectives

In addition to the genes on Ti and/or Ri plasmids, a number of chromosomal genes are necessary for the pathogenicity of *Agrobacterium* species. Those chromosomal genes, such as the above mentioned *acvB*, *chvE*, and *ros* genes, are unevenly located on chromosomal replicon(s) (Suzuki et al. 2001, 2004). There has been some controversy about the involvement of an auxiliary plasmid pAtC58 in pathogenicity.

Nair et al. (2003) reexamined this and found that the plasmid is not necessary for but has a positive effect on *vir* gene induction. Evolutionary relationships between the chromosomal gene, auxiliary plasmid(s), and pathogenesis and between pathogenesis and symbiosis remain to be elucidated. In nitrogen-fixing strains of the order *Rhizobiales*, the nitrogen-fixing genes (*nif*) and nodulation genes (*nod*) are located on plasmids in some species and on a chromosomal replicon in others (see Fig. 1). In contrast to the *nif* and *nod* genes, the *vir* genes and T-DNA have been found exclusively on plasmids. This raises interesting questions regarding the physiological significance of the location of genes within a complex genome.

From a biotechnological viewpoint, *Agrobacterium* pathogenic strains and plasmids are important resources for developing new and useful strains. Sequencing more Ti and Ri plasmids would advance the engineering strategy aimed at removing parts of the T-DNA portion (armless), which is applicable at least to a group of closely related plasmids. Studies on their variability in general in addition to the molecular genetic analysis might help to solve problems of transformation-recalcitrant plants and to widen the range of hosts. Recently, we determined rDNA partial sequences and analyzed their variability among strains in the three pathogenic species (Bautista-Zapanta et al. 2007). It is hoped that these data will be useful both for future bioengineering efforts as well as for the tracking and prevention of *Agrobacterium*-associated plant diseases.

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