Agrobacterium: Natural genetic engineer

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Agrobacterium is a genus of Gram-negative bacteria established by H. J. Conn that uses horizontal gene transfer to cause tumors in plants. *Agrobacterium tumefaciens* is the most commonly studied species in this genus. *Agrobacterium* is well known for its ability to transfer DNA between itself and plants, and for this reason it has become an important tool for genetic engineering. *A. tumefaciens* causes crown-gall disease in plants. The disease is characterised by a tumour-like growth or gall on the infected plant, often at the junction between the root and the shoot. Tumors are incited by the conjugative transfer of a DNA segment (T-DNA) from the bacterial tumour-inducing (Ti) plasmid. The closely related species, *A. rhizogenes*, induces root tumors, and carries the distinct Ri (root-inducing) plasmid. Overall, *Agrobacterium* can transfer T-DNA to a broad group of plants.Yet, individual *Agrobacterium* strains have a limited host range.

- The molecular basis for the strain-specific host range is unknown.
- Many monocot plants can be transformed (now), although they do not form crown gall tumors.
- Under lab conditions, T-DNA can be transferred to yeast, other fungi, and even animal and human cells.

2.Molecular Biology of *Agrobacterium* Infection

The process of infection by *Agrobacterium tumefaciens* culminates in the transfer of a small part of *p*Ti into the plant cell genome; this DNA sequence is called T-DNA.

- The infection process is governed by both chromosomal and plasmid-borne genes of *Agrobacterium tumefaciens*.
- Attachment of bacteria to plant cells begins the infection, governed by chromosomal virulence genes (*chv*); which are expressed constitutively.

2.1 The Ti Plasmid

- The Ti plasmid is a large conjugative plasmid or megaplasmid of about 200 *kb (range 150-250* kb).
- The ***pTi*** is unique bacterial plasmids in the following two respects***:***
  - They contain some genes, located within their T-DNA, which have regulatory sequences recognized by plant cells, while their remaining genes have prokaryotic regulatory sequences.
  - These plasmids naturally transfer a part of their DNA, the T-DNA, into the host plant genome, which makes *Agrobacterium* a natural genetic engineer.
- The Ti plasmids are classified into different **types** based on the type of opine, namely, octopine, nopaline, succinamopine and leucinopine, produced by their genes.
- The different Ti plasmids can be grouped into two general categories: octopine type and

nopaline type.

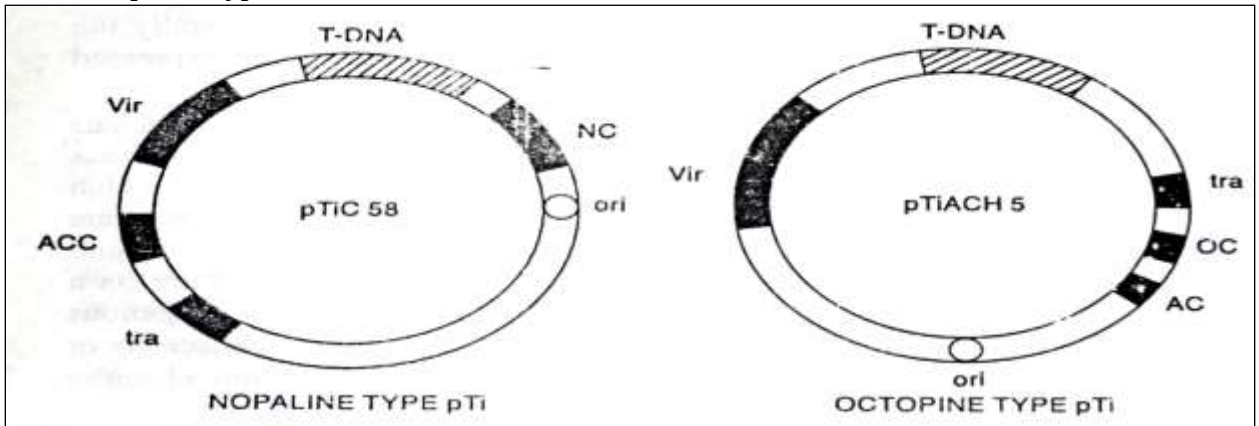


Fig. 8.2. The general organisation of nopaline type and octopine type Ti plasmids. *Vir*, virulence region; NC, nopaline catabolism; ACC, agrocinopine catabolism; *tra*, conjugative transfer; OC, octopine catabolism; AC, agropinu catabolism; *ori*, origin of replication in *Agrobacterium*.

2.2 Organization of T-DNA

- **T-DNA** (transferred DNA) is the 23 kb segment of Ti plasmids, which is transferred into the plant genome during ***Agrobacterium*** infection.
- T-DNA is defined on both its sides by a 24 bp direct repeat border sequence, and contains the genes for tumour/hairy root induction and those for opine biosynthesis.

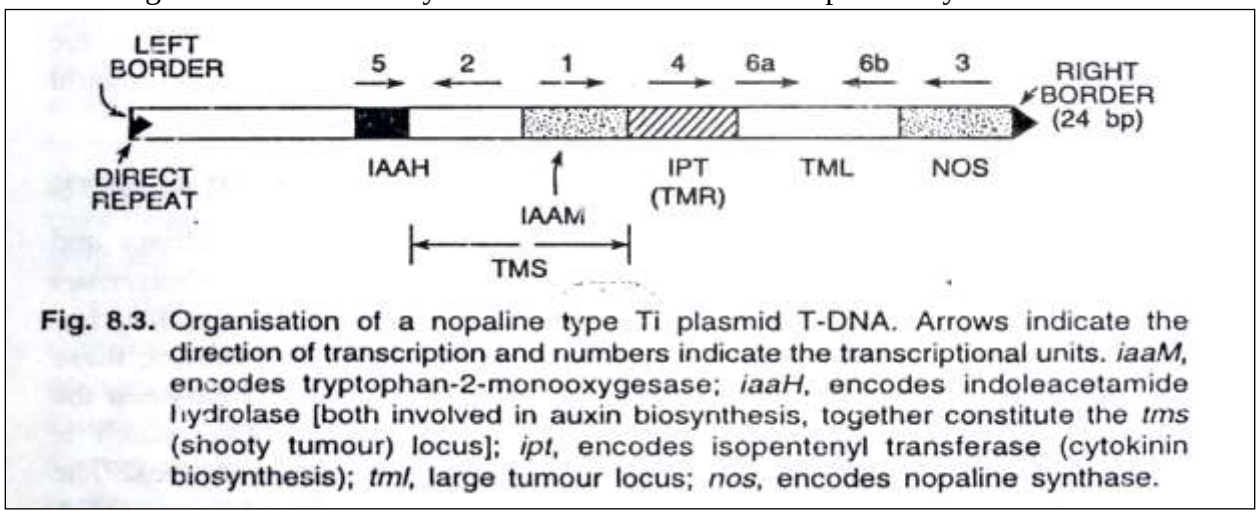


Fig. 8.3. Organisation of a nopaline type Ti plasmid T-DNA. Arrows indicate the direction of transcription and numbers indicate the transcriptional units. *iaaM*, encodes tryptophan-2-monooxygesase; *iaaH*, encodes indoleacetamide hydrolase [both involved in auxin biosynthesis, together constitute the *tms* (shooty tumour) locus]; *ipt*, encodes isopentenyl transferase (cytokinin biosynthesis); *tml*, large tumour locus; *nos*, encodes nopaline synthase.

- T-DNA carries genes involved in the synthesis of plant growth hormones (auxin, auxin synthesis; cyt, cytokinin synthesis) and the production of low molecular weight amino acid and sugar phosphate derivatives called opines (ocs, octopine; mas, mannopine; and ags, agropine).
- *Agrobacterium* are usually classified based on the type of opines specified by the bacterial T-DNA.
- All the genes present in T-DNA contain eukaryotic regulatory sequences. As a result, these genes are expressed only in plant cells, and they are not expressed in the *Agrobacterium.*

2.3 vir Region

- the genes present in T-DNA are not required for its transfer; only the 24 bp direct repeat

left and right borders of T-DNA are essential for the transfer.

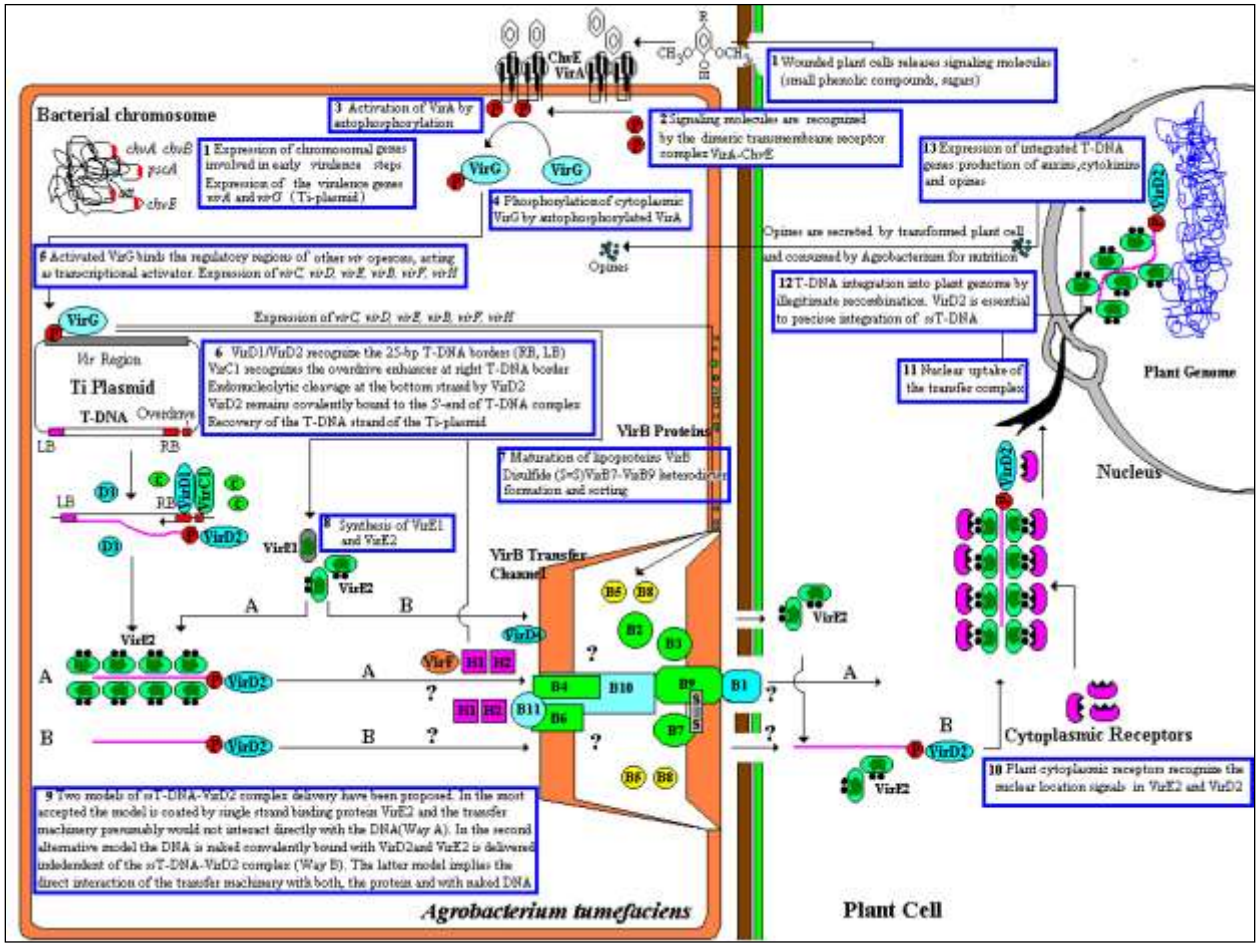
- Of the 8 *vir* operons, 4 operons, viz., *virA*, *virB*, *virD* and *virG*, are essential for virulence, while the remaining 4 operons play an accessory role.
- The operons *vir A* and *virG* are constitutive, encode one protein each, and are concerned with the regulation of all the *vir* operons.

Gene/Operon	**Function**
T-DNA	
iaaM (auxL tins I)*	Auxin biosynthesis; encodes enzyme tryptophan-2-mono-oxygenase, which converts tryptophan into indole-3-acctamide (IAM).
iaaH (aux2, tms2)	Auxin biosynthesis; encodes enzyme indole-3-acetamide hydrolase, which converts IAM into IAA (indole-3-acetic acid).
ipt (tmr, Cyt)	Cytokinin biosynthesis; encodes enzyme isopentenyl transferase, which catalyzes the formation of isopentenyl adenine.
Nos	Nopaline biosynthesis; encodes the enzyme nopaline synthase, which produces nopaline from arginine and pyruvic acid.
24 bp left and right border sequences	Site of endonuclease action during T-DNA transfer; the only sequences of T-DNA essential for its transfer.
***vir* Region**	
vir A(1)	Encodes a receptor for acetosyringone that functions as an autokinase; also phosphorylates VirG protein; *constitutive expression.*
***vir*B (11)**	Membrane proteins; possibly form a channel for T-DNA transport (conjugal tube formation); VirB 11 has ATPase activity.
***vir*C (2)**	Helicase; binds to the overdrive region just outside the right border; involved in unwinding of T-DNA.
***virD* (4)**	VirDl has topoisomerase activity; it binds to the right border of T-DNA; VirD2 is an endonuclease; it nicks the right border.
virE (2)	Single-strand binding proteins (SSBP); bind to T-DNA during its transfer.
***vir*F(l)**	Not well understood.
***virG* (l)**	DNA binding protein; probably forms dimer after phosphorylation by VirA, and induces the expression of all *vir* operons; constitutive expression.
***virH* (2)**	Not well known.

3.The genetic transformation process

The vir region, located on the Agrobacterium Ti plasmid, encodes most of the bacterial virulence (Vir) proteins used by the bacterium to produce its T-DNA and to deliver it into the plant cell. In wild-type Agrobacterium strains, the T-DNA region (defined by two 25 base pair direct repeats

termed left and right T-DNA borders) is located in cis to the vir region on a single Ti plasmid. In disarmed Agrobacterium strains, where the native T-DNA region has been removed from the Ti plasmid, a recombinant T-DNA region usually resides on a small,autonomous binary plasmid and functions in trans to the vir region.



The transformation process begins with the bacterium–plant attachment, followed by induction of the expression of the vir region by specific host signals. A single-stranded (ss) T-DNA molecule (T-strand) is then produced by the combined action of the bacterial VirD1 and VirD2 proteins. In bacterial cells, the T-DNA exists as a ssDNA–protein complex (immature T-complex) with one VirD2 molecule covalently attached to the 50 end of the T-strand. This complex, along with several other Vir proteins, is exported into the host cell by a VirB/D4 type IV secretion system, a step that requires interaction of the bacterial T pilus with at least one host-specific protein. Once inside the host-cell cytoplasm, the T-DNA is thought to exist as a mature T-complex (T-complex), in which the entire length of the T-strand molecule is coated with numerous VirE2 molecules. These molecules confer to the T-DNA the structure and protection needed for its travel to the host-cell nucleus. It is mainly during the last steps of the transformation process namely, transport through the cytoplasm, nuclear import , intranuclear transport , T-DNA uncoating and integration that the Agrobacterium utilizes various cellular mechanisms to accomplish the genetic transformation of its host.

4. Importance to Biotechnology & Genetically Modified Organisms

- Oncogenes and opine-creating genes can be removed from the Ti-Plasmid that is transferred to the plant cell by T-DNA.
- Scientists can insert any gene they want into the plasmid in place of the tumor causing genes and subsequently into the plant cell genome.
- Original problems existed in that *Agrobacterium tumefaciens* only affects dicotyledonous plants.
- Monocotyledon plants like corn are not very susceptible to the bacterial infection.
- By varying experimental materials, culture conditions, bacterial strains, etc. scientists have successfully used *A. tumefaciens* Gene Transfer to produce BT Corn .
- This method of gene transfer enables large DNA strands to be transferred into the plant cell without risk of rearrangement whereas other methods like the Gene Gun have trouble doing this.
- The vast majority of approved genetically engineered agriculture has been transformed by means of *Agrobacterium tumefaciens* Mediated Gene Transfer.

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