Agrobacterium-mediated gene transfer

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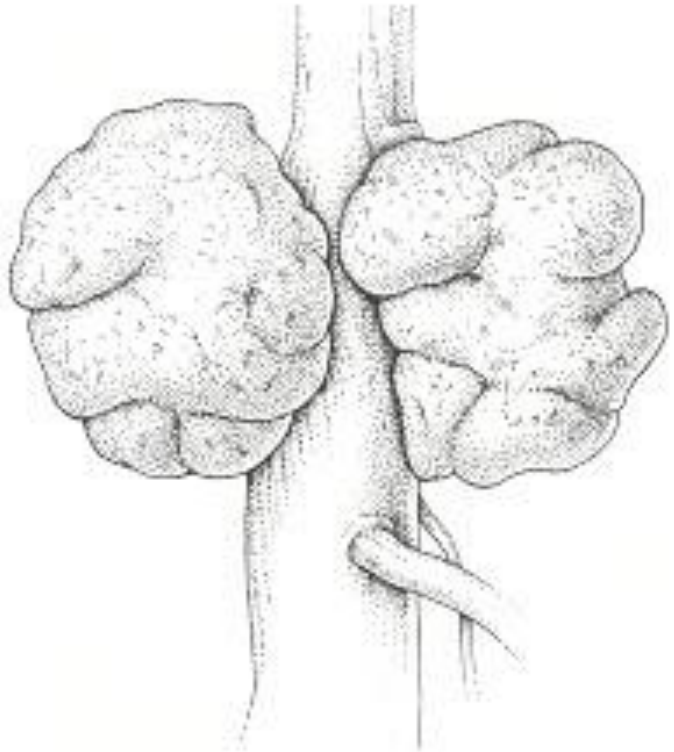
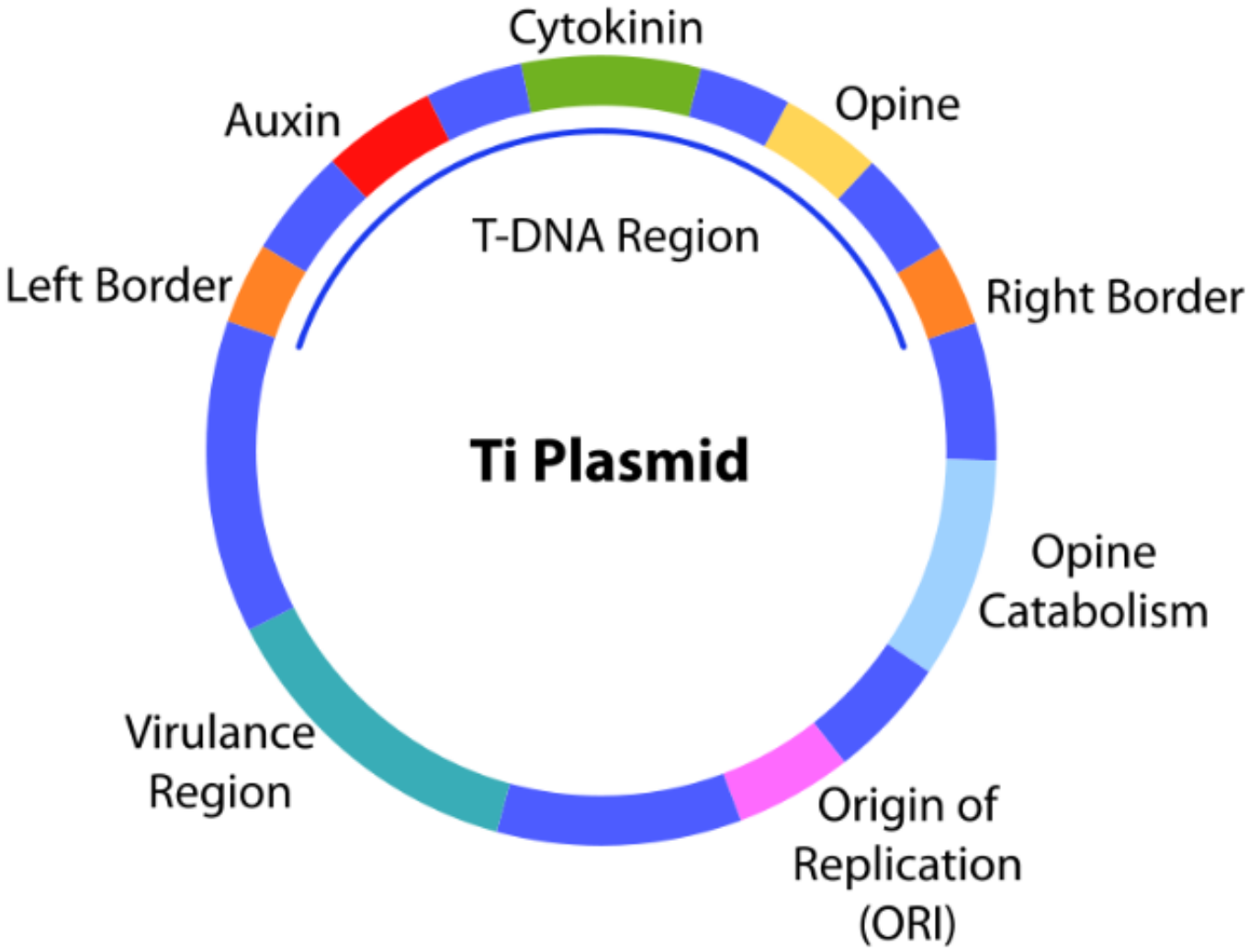
Agrobacterium tumefaciens

- *Agrobacterium tumefaciens* (synonym *Agrobacterium radiobacter*) is a rod-shaped, Gram-negative soil bacterium, the causal agent of **crown gall** disease in over 140 species of dicots.



https://www.ncbi.nlm.nih.gov/books/NBK22390/figure/A843/

- *A. tumefaciens* harbors a plasmid called Ti plasmid, is capable of transferring a particular DNA segment (T-DNA) of the tumour-inducing (Ti) plasmid into the nucleus of infected cells where it is subsequently stable integrated into the host genome and transcribed, causing the crown gall disease



https://en.wikipedia.org/wiki/Transfer_DNA

- T-DNA contains eight or so genes that are expressed in the plant cell and are responsible for the cancerous properties of the transformed cells. These genes also direct synthesis of unusual compounds, called opines, that the bacteria use as nutrients.
- Genes in the virulence region are grouped into the operons *vir A,B,C,D,E,F,G*, which code for the enzymes responsible for mediating transduction of T-DNA to plant cells

T-DNA genes

- The T-DNA contains two types of genes:
- the tumor inducing genes,
- encoding for enzymes involved in the synthesis of auxins and cytokinins which are responsible for tumour formation;
- and the Opine synthase genes that is genes encoding for the synthesis of opines,
- a product resulted from condensation between amino acids and sugars, which are produced and excreted by the crown gall cells and consume by *A. tumefaciens* as carbon and nitrogen sources.
- Outside the T-DNA, are located the genes for the opine catabolism, the genes involved in the process of T-DNA transfer from the bacterium to the plant cell and for the bacterium-bacterium plasmid conjugative transfer genes

Borders Left and right

- The T-DNA is bordered by 25-base-pair(bp) direct repeats (DR) on each end. Transfer is initiated at the right border and terminated at the left border and requires the *vir* genes of the Ti plasmid.

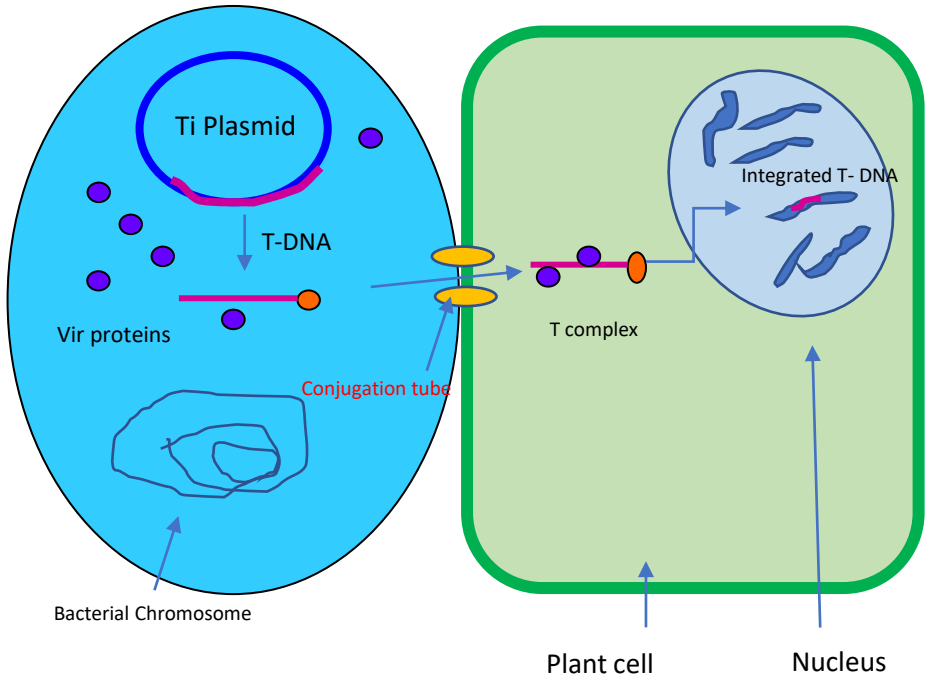
Mechanism

- The infection process of T-DNA into the host cell and integration into its nucleus involve multiple steps. First, the bacteria multiply in the wound sap before infection and then attach to the plant cell walls. The bacterial virulence genes expression of approximately 10 operons is activated by perception of phenolic compounds such as acetosyringone emitted by wounded plant tissue and follows cell-cell contact.
- Then pseudoconjugataion takes place and translocation of T-DNA from *Agrobacterium* to cytoplasm of host cell, transmission of T-DNA along with associated proteins (called **T-complex**) to the host cell nucleus followed by disassembly of the T-complex, stable integration of T-DNA into host plant genome, and expression of the transferred genes.

'Lacroix B, Citovsky V (2013). "The roles of bacterial and host plant factors in Agrobacterium-mediated genetic transformation". *The International Journal of Developmental Biology*. **57** (6–8): 467–81. doi:10.1387/ijdb.130199bl. ISSN 0214-6282. PMID 24166430'

- The T-DNA fragment is flanked by 25-bp direct repeats, which act as a *cis* element signal for the transfer apparatus.
- The transfer is mediated by the co-operative action of proteins encoded by genes determined in the Ti plasmid virulence region (*vir* genes) and in the bacterial chromosome.
- The *vir* gene products nick the T-DNA region at its left border (LB) and right border (RB) and then transfer T-DNA into plant cells. The 30 kb virulence (*vir*) region is essential for the T-DNA transfer

- Wounded plant cells produce phenolic defence compounds, which can trigger the expression of the *Agrobacterium vir* genes.
- The activation of *vir* genes carries out the generation of single-stranded (ss) molecules representing the copy of the T-DNA strand **'T-strand'** . Any DNA placed between T-DNA borders will be transferred to the plant cell, as single strand DNA, and integrated into plant genome.


Ti Plasmid
T-DNA
Vir proteins
Conjugation tube
Bacterial Chromosome
T complex
Integrated T- DNA
Plant cell
Nucleus

- After the bacterium attaches to a plant cell, the T-strand and several types of Vir proteins are transferred to the plant through **a transport channel**.
- The transferring vehicle to the plant nucleus is a **ssT-DNA-protein complex**.
- Inside the plant cell, the **Vir proteins interact with the T-strand**, **forming a T-complex**. This complex targets the nucleus, allowing the T-DNA to integrate into the plant genome and express the encoded genes.

- The final step of T-DNA transfer is its integration into plant genome. It is considered that the integration occurs by illegitimate recombination

So overall

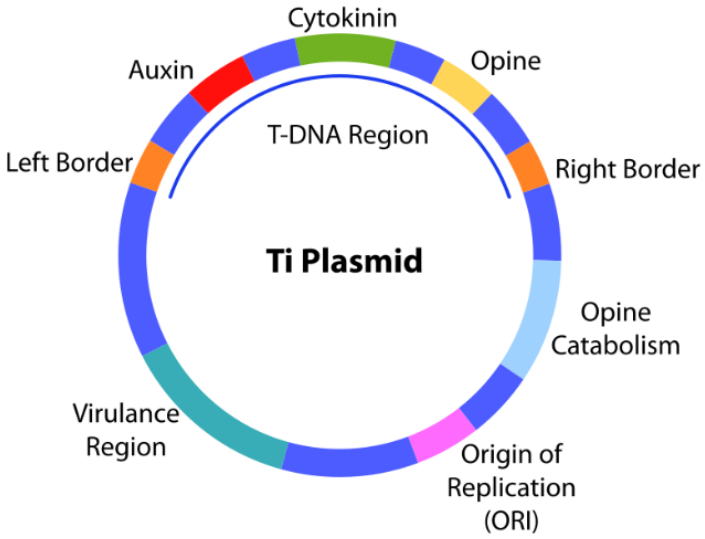
- The process of gene transfer from *Agrobacterium tumefaciens* to plant cells requires these essential steps: **(1)** bacterial colonisation **(2)** induction of bacterial virulence system, **(3)** Generation of T-DNA transfer complex **(4)** T-DNA transfer and **(5)** integration of T-DNA into plant genome

- The tumour formation is a transformation process of plant cells resulted from transfer and integration of T-DNA and the subsequent expression of T-DNA genes.
- Any foreign DNA placed between the T-DNA borders can be transferred to plant cell, no matter where it comes from.

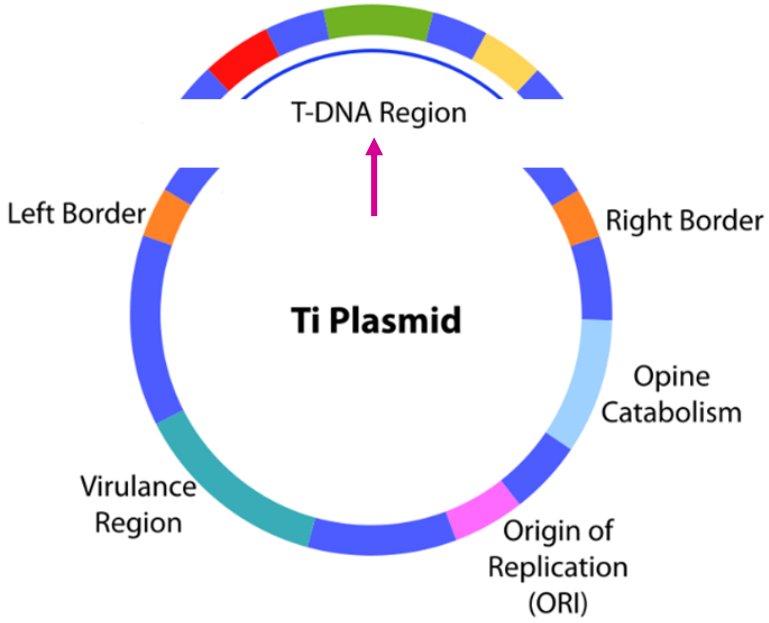
Disarming Ti-plasmid

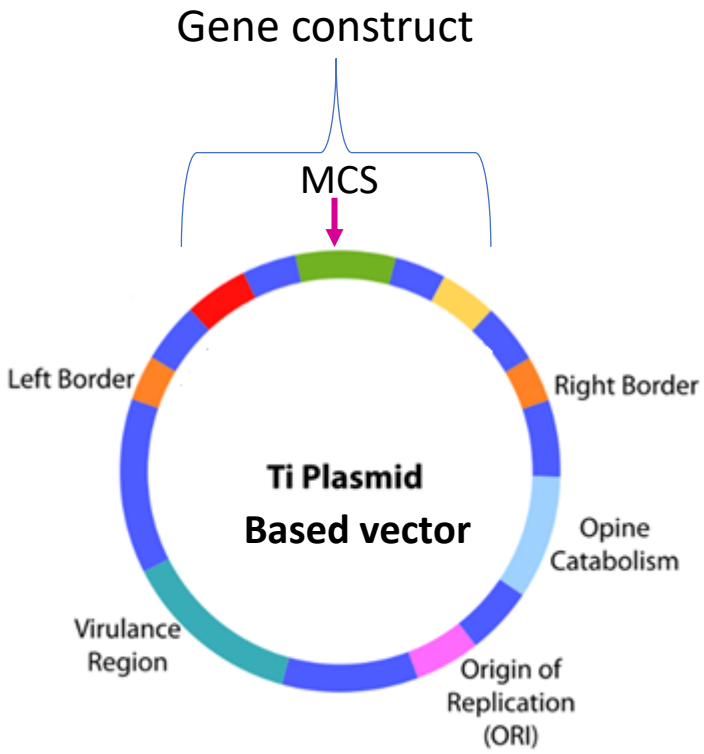
- To ensure transformation without tumorigenicity, Ti plasmid, which lacks T-DNA but retains the entire *vir* region are constructed.
- This process of removing the tumor causing region or the T-DNA of Ti plasmid is called disarming

Ti Plasmid



Disarmed Ti Plasmid





Co-integrated Vectors

- **Co-integrated vectors** or **hybrid Ti plasmids**, these vectors were among the first types of modified and engineered Ti plasmids devised for *Agrobacterium* -mediated transformation, but are not widely used today.
- These vectors are constructed by homologous recombination of a bacterial plasmid with the T-DNA region of an endogenous Ti plasmid in *Agrobacterium*. Integration of the two plasmids requires a region of homology present in both.

- A resulting **co-integrated plasmid** assembled by *in vitro* manipulation normally contains:
- the *vir* genes,
- the left and right T-DNA borders,
- an exogenous DNA sequence between the two T-DNA borders, and
- plant and bacterial selectable markers.

Binary Vectors

- **Binary vectors** are cloning vectors which are able to replicate in both *E.coli* and *Agrobacterium tumefaciens*,
- The discovery that the *vir* genes do not need to be in the same plasmid with a T-DNA region to lead its transfer and insertion into the plant genome led to the construction of a system for plant transformation where the T-DNA region and the *vir* region are on separate plasmids.

- In the binary vector system, the two different plasmids employed are:
- 1. a **wide-host-range small replicon**, which has an origin of replication (ori) that permits the maintenance of the plasmid in a wide range of bacteria including *E. coli* and *Agrobacterium*. This plasmid typically contains:
  - foreign DNA in place of T-DNA,
  - the left and right T-DNA borders (or at least the right T-border),
  - markers for selection and maintenance in both *E. coli* and *A. tumefaciens*,
  - a selectable marker for plants.
- The plasmid is said to be "disarmed", since its tumor-inducing genes located in the T-DNA have been removed.

- **2.Helper vectors**
  These are small plasmids maintained in *E. coli* that contain transfer (*tra*) and mobilization (*mob*) genes, which allow the transfer of the conjugation-deficient intermediate vectors into *Agrobacterium*.

- 2. a **helper Ti plasmid**, harbored in *A. tumefaciens*, which lacks the entire T-DNA region but contains an intact *vir* region.
- In general, the transformation procedure is as follows:
- the recombinant small replicon is transferred via bacterial conjugation or direct transfer to
  A. tumefaciens harboring a helper Ti plasmid,
- the plant cells are co-cultivated with the *Agrobacterium*, to allow transfer of recombinant T-DNA into the plant genome, and transformed plant cells are selected under appropriate conditions.

Three vectors are necessary in this system:

- **Disarmed *Agrobacterium* Ti plasmids**
  In these Ti plasmids, the oncogenes located in the T-DNA region have been replaced by exogenous DNA.

- **Intermediate vectors**

  These are small pBR322-based plasmids *(E. coli* vectors) containing a T-DNA region. Intermediate vectors are replicated in *E.coli* and are transferred into *Agrobacterium* by conjugation. They cannot replicate in *A. tumefaciens* and therefore, carry DNA segments homologous to the disarmed T-DNA to permit recombination to form a co-integrated T-DNA structure.

Resource

- The agrobacterium tumefaciens gene transfer to plant cell, Gustavo A. de la Riva
- Free web resources