Transposons

- Short DNA sequence that have the ability to move from one location to other in genome.
- process is called Transposition
- Rare events
- Moderately repetitive DNA
- Human have no transposon but some eukaryotes have (plant and Drosophila).
- Most mobile genetic element in Bacteria are DNA.

- Mobile genetic element
- Insertion sequence
- Jumping gene
- Selfish DNA
- Junk DNA

Transposons
- Retrotransposons (RNA - Intermediate)
- DNA Transposons (DNA - Intermediate)

(Replicative Transposons eg - Eukaryotes) copy paste

(Conservative Transposons eg - Bacteria) cut paste

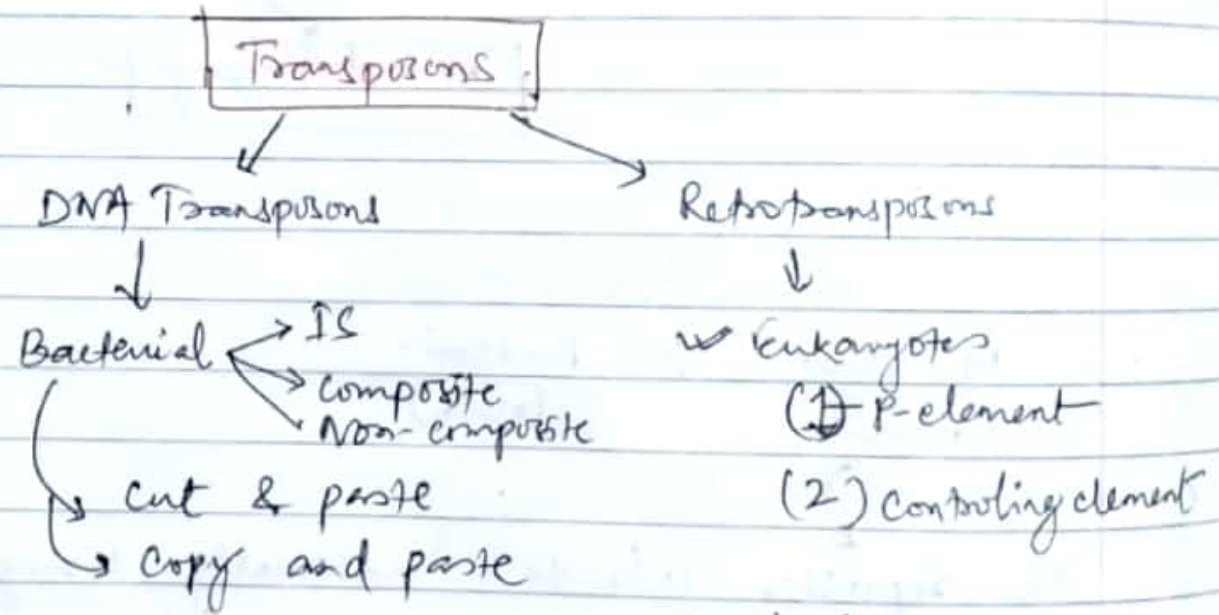
Due to lack of repressor, P-element are activated

↓

Transposition occur in germ line and cause the inactivation of many genes

↓

Resulted in sterile progeny
Hybride Dysgenesis



Long interspersed elements
short interspersed element

(i) DC Moves ⟶ when Ac is present
(ii) only DC ⟶ no migration
(iii) Ac can move both in presence and absence of DC (Due to Transposase enzyme)

∴ Ac = Autonomous
DC = Non autonomous (Its a mutated from of Ac)
↓
(No Transposase)

P-Element in Drosophila / Eukaryotic Transposons

Hyboride dysgenesis

→ P. Element ⟶ Transposons
- → Code for Transposase
- → Do not use RNA intermediate
- → DNA Transposons

Drosophila have 2 different strain :-
P - strain (paternal) < M / F
M - strain (Maternal) < M / F

Case 1:

M-Strain × P strain
(Male) (female)

↓

F1 - progeny
(Normal)

Case 2:

M- Strain × P- strain
(female) (Male)

↓

F1 - progeny (Sterile)

↓

Hyboride dysgenesis

Reason: P-element are causative agent of Hyboride dysgenesis

P-Strain contain: 30-40 copies of P-element but there element are absent in M-strain.

The transposition of this P-element at new site can cause inactivation of many genes.

case 1:

M-strain (male) x P-strain (female)

↓

→ Have P-element
→ Also contain repressor of transposition

↓

This keep transposon in silent state

↓

normal progeny

case 2:

M-strain (female) x P-strain (male)

↓

The repressor inherited maternally through egg cytoplasm

↓

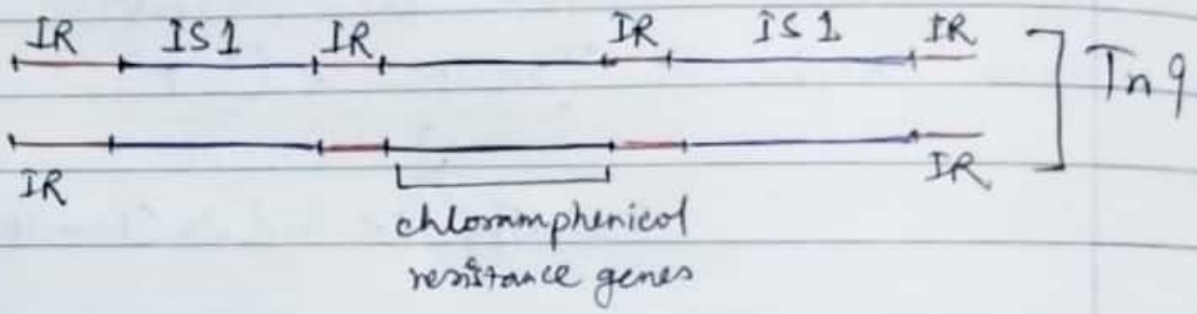
But egg from M-strain female lack a repressor protein that inhibit P-element transposition.

↓

Composite Transposons : (cT)

→ Larger than IS.
→ one or more protein coding genes.
→ have 2 IS elements
→ Composite transposons carry genes for antibiotic resistance.

Example: Tn 9 (chloramphenicol resistance), Tn 5
Tn 10 (Tetracycline resistance)



Non-Composite Transposons :

Example - Tn 3 & Tn 21

Mcclintock's Experiments : (Transposons in plants)

Dc (Dissociation)
Ac (Activator)
] 2 controling elements in maize

Bacterial - Transposons (DNA Transposons)

- IS element
- Composite transposons
- Non - composite transposons

IS element : (Insertion sequence element)

→ Most common

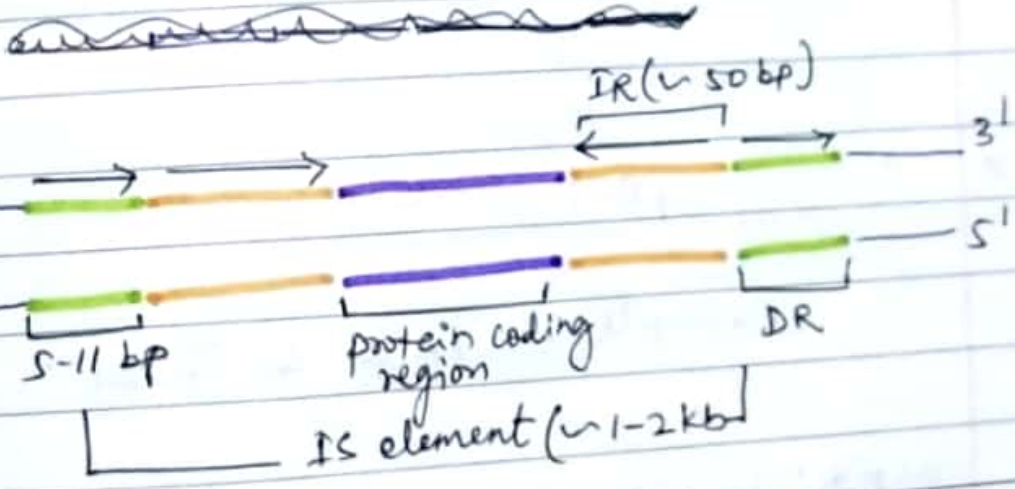
→ Have IR (Inverted Repeats) in opposite direction.

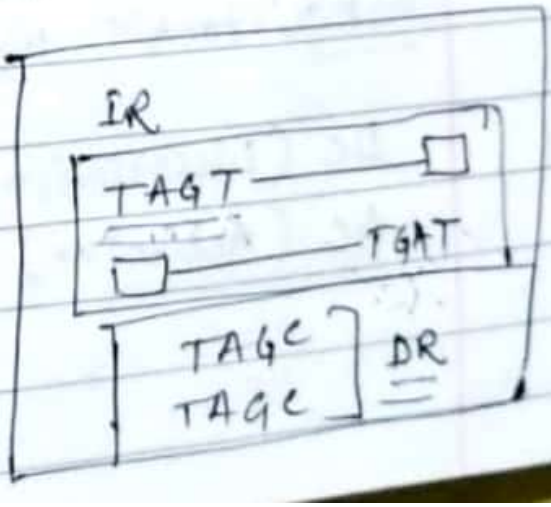
→ protein coding Region between IR which encode enzyme for transposition of IS.

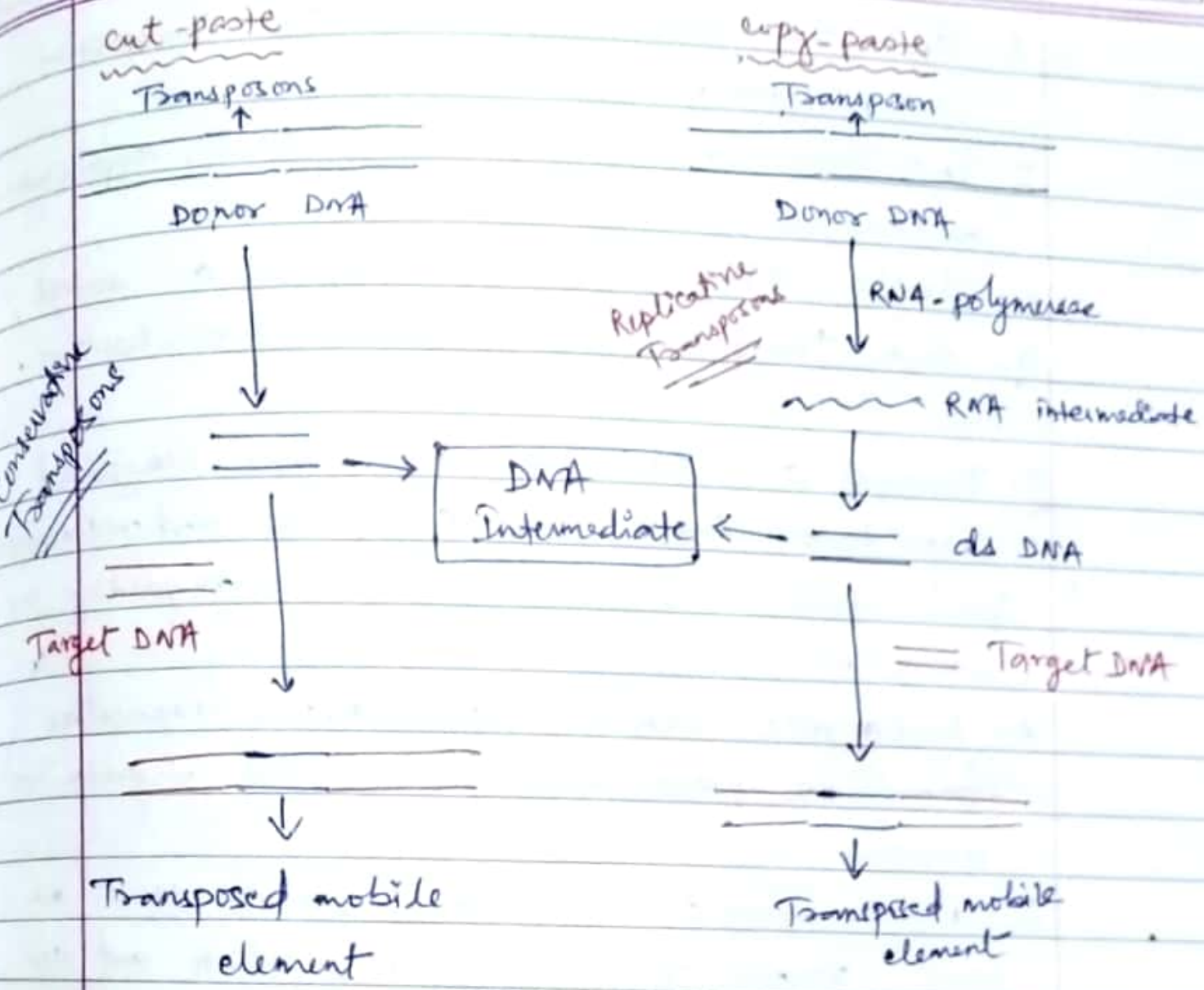
→ Enzyme = Transposase (Imp.)

↓

Identify IR and do Transposition.







Q. You have discovered a new transposon, TnX, and would like to identify its mode of replication. A heteroduplex of the TnX sequence is made with a few mismatches and introduced into bacteria. The newly transposed genomic loci are sequenced. You find that the sequence of the transposon matched exactly with one of its parent strands. This suggests that —

1. Tn X transposes by Conservative transposition mechanism.
2. Tn X transposes using a site-specific recombination mechanism.
3. Single-strands of the duplex are inserted.
4. Tn X transposes by replicative mechanism.

Q. Transposons can be primarily categorized into two types, DNA transposons and retrotransposons. Given below is some information regarding the above.

A. Eukaryotic DNA transposons excise themselves from one place in the genome and integrate into another site.

B. Retrotransposons are RNA sequences that are first reverse transcribed into cDNA and then integrate into the genome.

C. Retrotransposons move by a copy and paste mechanism through an RNA intermediate.

D. As DNA transposons move via a cut and paste mechanism, there can never be an increase in the copy number of a transposon.

Which of the statement(s) is/are true?

(1) A and C
(2) B and D
(3) B only
(4) D only.

Role as a genetic tool

- T-DNA region get replaced by any gene of interest and then targeted to plant cell for transformation.

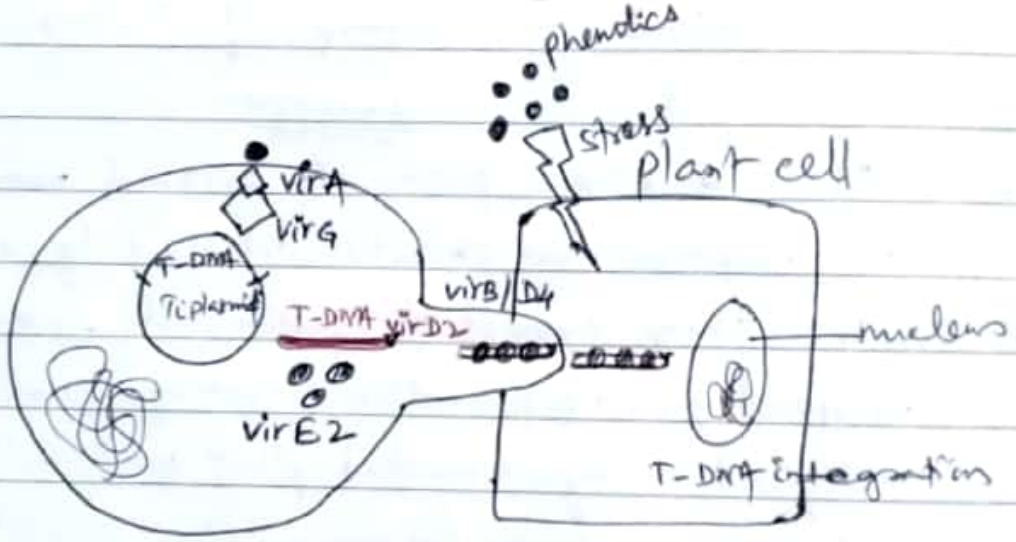
Note: Monocot are not good host for Agrobacterium.

There is a hypothetical belief that monocot are resistant to Agrobacterium because they do not produce phenolics that can induce virulence genes.

VirB/D4 : Type IV secretion system (mating pair formation apparatus), it's a pore channel formation T-DNA transfer from bacterium to plant cell.

VirD2 & VirE2 : Have NLS (Nuclear localization signal)

Mode of action / Transfer



Agrobacterium tumefaciens

Steps in transformation of plant cell.

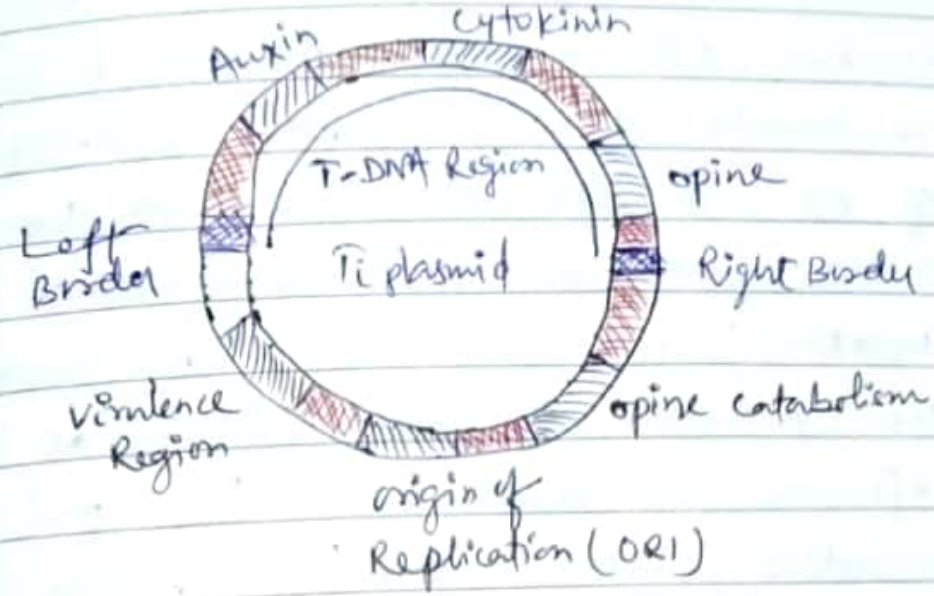
1. plant stress condition.
2. phenolics production
3. Signal to bacteria
4. Virulence system activation
5. Generation of T-DNA complex
6. T-DNA transfer
7. T-DNA integration in plant genome.

Genes	product	functions	
Ocs	octapine Synthase	octapine	synthesis
nos	Nopaline "	"	"
tms 1	Tryptophan-2-mono-oxygenase	Auxin	"
tms 2	Indoleacetamide hydrolase	"	"
tmr	Isopentyl transferase	cytokinin	"
ags	Agropine synthase	opine	"

Genes required for transfer of T-DNA

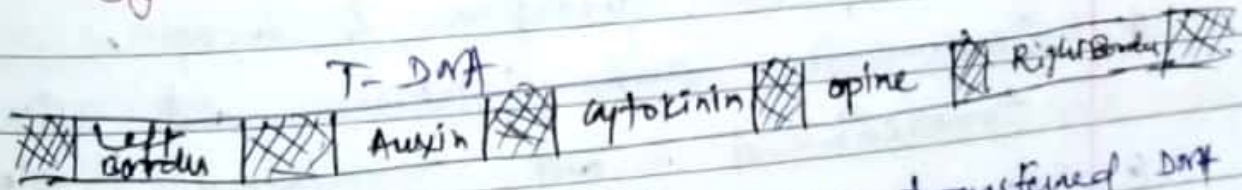
Genes	Function
VirA	It is a kinase protein in bacterial membrane. Act as receptor for phenolic released by wounded plant.
virA & virG	They expressed continuously. play role in responding to phenolics (acetosyringone) which released by wounded plant.
VirG	Activate other vir genes.
Vir D1 & vir D2	Endonuclease
vir D	vir D is responsible for virulence activity of bacterium.
virD2	prevent attack of exonuclease at 5' end of T-DNA play role in cutting phosphodiester bond.
VirE/E2	protect T-DNA against nuclease and target T-DNA to plant cell. it act as a single stranded binding protein.
Vir C	Stimulate transfer

Ti plasmid (Tumor inducing)



- Large size plasmid of 200kbp.
- The Ti plasmid is lost when Agrobacterium is grown above 28°C (curing of plasmid). Such cured bacteria do not induce crown galls.
- The modification of this plasmid is very important in the creation of ~~transgenic~~ plants.
- plasmid have T-DNA, right border, left border, vir genes, phytochrome region, origin of replication and opine region.

Organization of T-DNA



- The transfer DNA (T-DNA) is the transferred DNA of the tumor-inducing (Ti) plasmid of some species of bacteria. The size of T-DNA is between 15-30 kb.

- T-DNA contain genes for tumor induction.
- It has LB and RB. RB play a important role in transfer and integration of T-DNA. Absence of RB will terminate the T-DNA transfer.
- T-DNA carry genes for phytohormones (Auxin & cytokinin) and opine that are expressed in plant cell.
- Over production of these hormones at the site of infection is responsible for the proliferation of wounded cell in gall / tumor. These tumor can harbor a plenty of bacteria.
- opines are low molecular weight compounds found in plants crown gall tumors or hairy root tumors produced by parasitic bacteria of the genus Agrobacterium.
- The opines are used by the bacterium as an important source of nitrogen, Carbon and energy. opines are condensation product of —
  1. An amino acid & a keto acid
  2. Amino acid & sugar.
- Different types of opine may present — nopaline, octopine & agropin.
- Genes for auxin, cytokinin and opines are transcribed and translated in plant cell.
- Example - T-DNA genes in Ti plasmid.

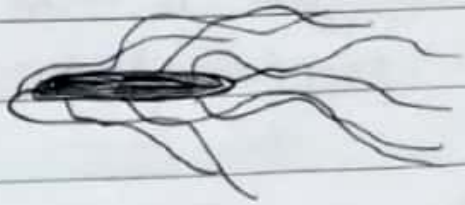
Agrobacterium tumefaciens

- A. tumefaciens is a rod-shaped, Gram-negative soil bacterium which infect dicot plants.
- Some common species of Agrobacterium are — A. tumefaciens and A. rhizogenes.
- It is consider as natural genetic engineer because they transform the plant.
- A. tumefaciens is a serious pathogen of walnuts, grape vines, stone fruits, nut trees, sugar beets, horse radish.
- A. tumefaciens grows optically at 28°C. The doubling time can range from 2.5 – 4 hrs depending on the media, culture format, grow aerobically, without forming endospores.
- The cells are rod-shaped and motile, having one to six peritrichous flagella.

A.  Monotrichous

B.  Lophotrichous

C.  Amphitrichous

D.  peritrichous

- To be virulent, the bacterium must contain a tumour-inducing plasmid (Ti plasmid or pTi), which contains the T-DNA and all the genes necessary to transfer it to the plant cell.
- Many strains of A. tumefaciens do not contain a pTi.
- The mechanism this bacterium uses to parasitize plant tissue involves the integration of some of its own DNA into the host genome resulting in tumors and changes in plant metabolism.
- A. tumefaciens, now used as a tool for engineering desired genes into plants.

Agrobacterium species

- A. tumefaciens has Ti (tumor inducing) plasmid, and that is responsible for tumor induction (crown gall tumor) in plant.
- A. tumefaciens is also known by the name A. radiobacter / Rhizobium radiobacter.
- A. rhizogenes has Ri (root inducing) plasmid, and that is responsible to cause hairy disease in plants. Ri plasmid is analogue to Ti plasmid.
- A. rubi - cause cane gall in sugar cane plant.
- A. vitis - gall in grapes plant.