

## Agrobacterium and Ti plasmids - a brief history

Agrobacterium tumefaciens - the causative agent of crown gall disease

Crown gall tumors:

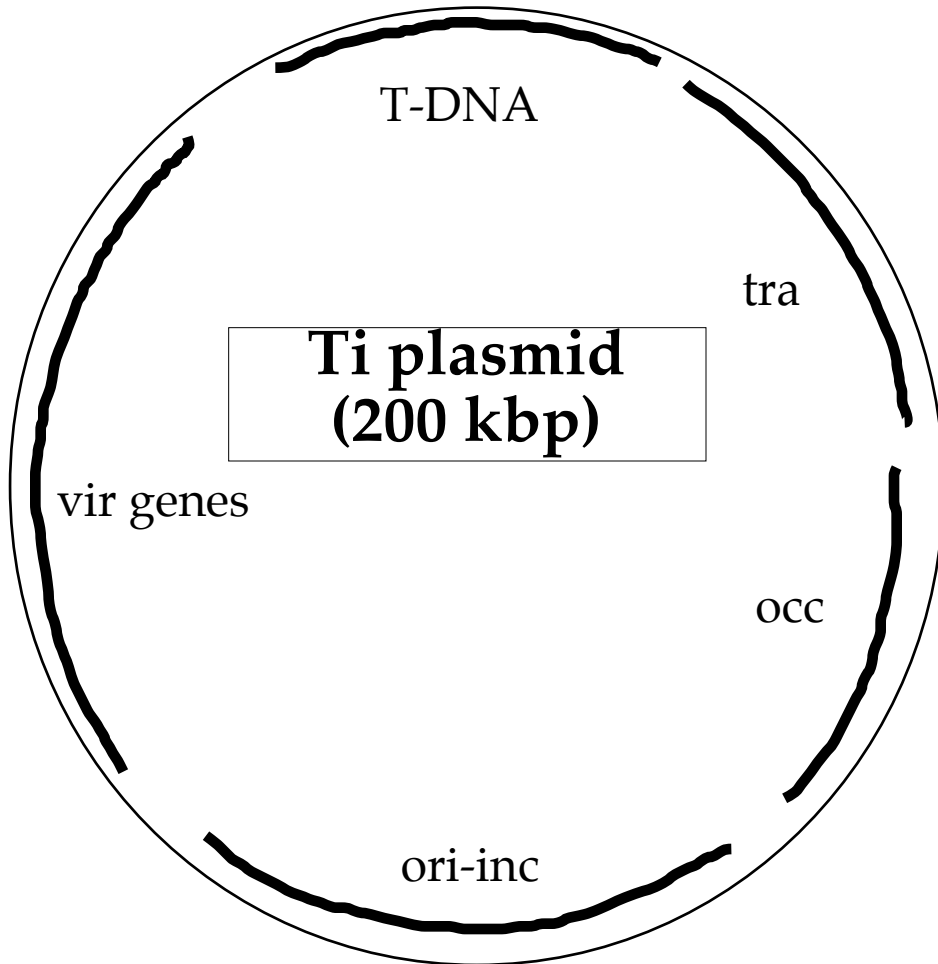
- Collections of cells growing in an undifferentiated, uncontrolled manner (a tumor)
- Occur usually at wound sites
- Can be grown axenically as callus cultures -> exposure to Agrobacterium produces a heritable (at least through mitosis) change in plant cells in the tumor
- Sterile crown gall cultures do not require phytohormones (contrast with normal plant cell cultures) -> crown gall cells make cytokinins and auxins
- Crown gall cells produce large amounts of opines (amino acid and/or sugar derivatives)

Tumor-causing ability (virulence) of *Agrobacterium* correlates with the presence of a large extrachromosomal element in the bacterium - the Ti plasmid

- All virulent bacteria have this plasmid
- Curing virulent strains of the plasmid eliminates virulence
- Transposon disruptions in the Ti plasmid alter or eliminate virulence

Transposons can be used to generate mutants, create a genetic map of the Ti plasmid

- Mobilize transposon (natural or tailored) from *E. coli* into *Agrobacterium* using a plasmid that replicates in *E. coli* but not *Agrobacterium* (takes advantage of the ability of episome or plasmid transfer systems to move plasmids between different species of gram-negative bacteria)
- Select for *Agrobacteria* that retain the selectable marker carried by the transposon
- Screen for virulence
- Test for chromosomal vs. extrachromosomal linkage



## T-DNAs of Ti plasmids

Transposon mutations in the T-DNA region change tumor morphology or abolish tumor growth

T-DNA-related sequences can be found in transformed plant cells that are free of *Agrobacterium*

T-DNA-related RNAs (that are polyadenylated) can be detected in axenically-grown tumors

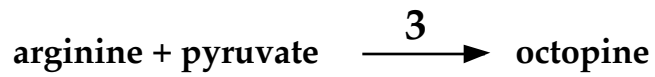
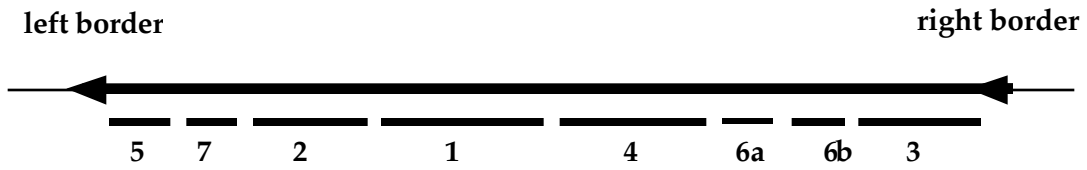
-> Transformed plant cells contain a part of the Ti plasmid (but not the entire plasmid)

the T-DNA contains eight potential genes - these are eukaryotic in nature (eukaryotic promoters, monocistronic, eukaryotic polyadenylation signals, eukaryotic translation mechanisms)

several T-DNA genes encode enzymes that can enable cytokinin and auxin production

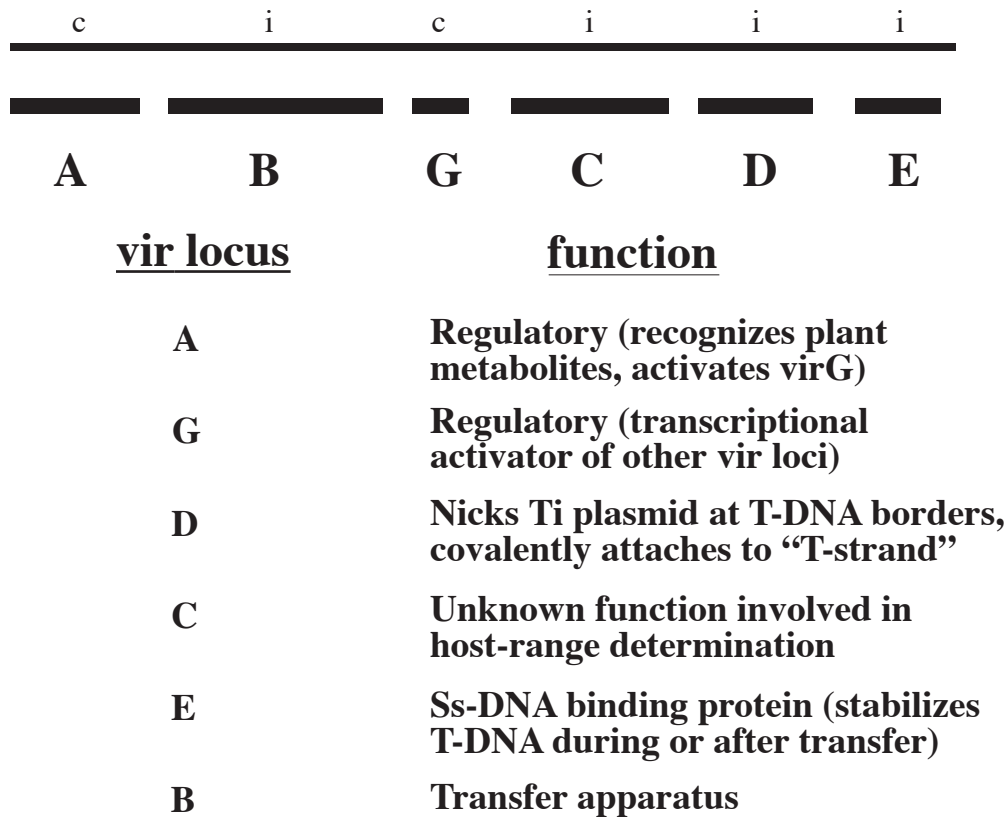
-> crown gall tumorigenesis is due to the "activation" of unregulated phytohormone synthesis in the transformed cells

# octopine TL-DNA



5, 6a, 6b, 7 - unknown functions

## the vir region of Ti plasmids



## Regulation of vir gene expression

Most vir genes are inducible, vir genes can be induced by exposure of *Agrobacterium* to exudates isolated from wounded plant cells

Many chemicals can induce vir gene expression; these are typified by phenolic compounds such as acetosyringone

Two vir loci are single genes and are expressed constitutively

Mutations in these (virA and virG) are pleiotropic - they affect expression of all other vir genes

VirA encodes a membrane-localized protein that resembles components of so-called two component sensor-regulator systems

The virA gene product can phosphorylate itself on a characteristic histidine residue, and can transfer this phosphate to an aspartate side chain of the virG gene product

VirG encodes a DNA binding protein that is an acceptor of phosphate from phosphorylated virA

The virG gene product can bind elements present in other vir gene promoters

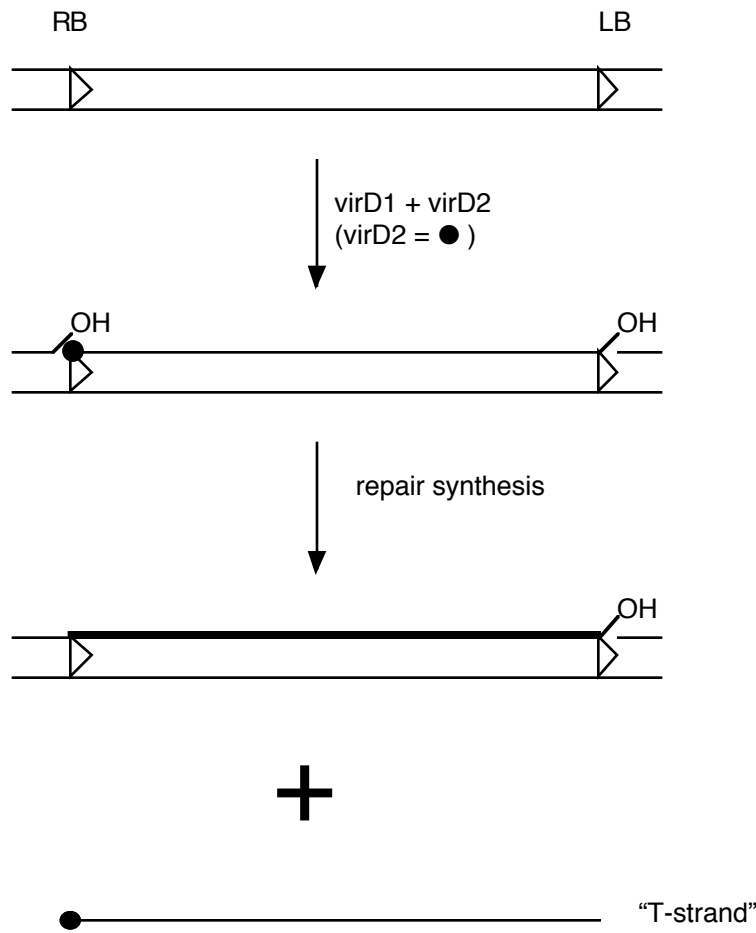
## forms of T-DNA that are found in Agrobacterium

- ds circles - found only in induced bacteria, not (apparently) in plant cells
- ds linear T-DNA - found only in induced bacteria, not (apparently) in plant cells
- ss linear T-DNA - found in bacteria and plant cells
- what is not found - Ti plasmids with evidence that T-DNA has been precisely deleted

## In vitro activities of virD proteins

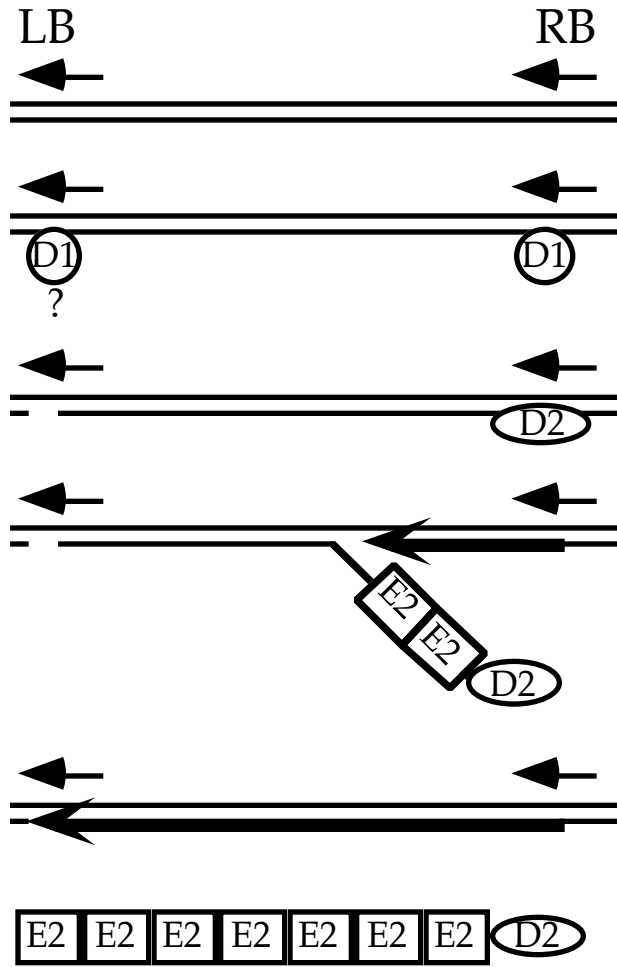
- virD1 + virD2 - recognize T-DNA borders with high specificity, introduce single-stranded nicks at borders
- virD2 alone can work on oligonucleotides containing the border
- virD1 as well is required for action on supercoiled circular DNA
- no reaction is seen with relaxed circles or linear ds molecules

# production of “T-strands” in Agrobacterium



## virE2 - where and how does it work?

- ss DNA binding protein
- can be found associated with T-strands in Agrobacterium extracts (co-immunoprecipitation)
- wt levels of T-strands accumulate in virE2 mutants (virE2 not needed for T-strand production or in vivo stabilization)
- virE2 mutants can transfer T-strands to plant cells (efficiency is lower) - no absolute requirement for transfer
- virE2 mutants can be complemented by expression of virE2 in plant cells - is the main site of virE2 function in the plant?
- virE2(+), T-DNA (-) + virE2(-), T-DNA (+) combination is virulent and tumorigenic - can virE2 be transferred from Agrobacterium to plant cell independent of T-DNA?



## Similarities between virB gene products and the transfer apparatus of F plasmids

Conjugation	function, location	Ti plasmid
Tra L	transglycosylase, E	VirB1
	cell-cell contact, E	VirB1*
TraM	pilin subunit, E	VirB2
TraA	unknown, E	VirB3
TraB	ATPase, transport, TM	VirB4
TraC	unknown, E	VirB5
TraD	pore former?, TM	VirB6
TraN	lipoprotein, covalent dimer with virB9, OM	VirB7
TraE	unknown, PIM	VirB8
TraO	nucleation center with VirB7, OM	VirB9
TraF	unknown, TM	VirB10
TraG	ATPase, transport, IM	VirB11
	ATPase, coupling of DNA processing with transport system, TM	VirD4

## Analogies between bacterial conjugation and T-DNA transfer

<u>Conjugation</u>	<u>Function</u>	<u>T-DNA transfer</u>
oriT	site of specific nicking	T-DNA borders
TraI/TraY endonuclease	site-specific endonuclease	VirD1/VirD2
TraD/TraM	DNA transfer (?)	?
?	mating signal	plant-synthesized phenolics
F-pilus (TraA,Q,L,E,K, B,V,C,W,U,F,H,G)	transfer machinery	VirB
TraG/TraN	stabilization of mating pair	?
TraS/TraT	prevention of non-productive mating	chv (?)
ssb	stabilization of T-strands	virE

**Conjugation, T-DNA transfer, and secretory systems involved in virulence in animal and human pathogens (*Bordetella pertussis*, *Legionella pneumophila*, *Rickettsia prowazekii*) are members of a large class of bacterial secretory systems (type IV systems)**