Lecture 11. Plant Transformation

I Elements of transformation

1. uptake of DNA into competent host cells

2. integration vs. transient expression

II methods of delivery

1. Agrobacterium-mediated transformation

- vacuum infiltration (floral dip)

- wounded explants

- Cultured cells (tobacco)

can't be applied to monocot, nor organelle

2. direct methods

-protoplast polyethyleneglycol (PEG) method

-protoplast electroporation

-protoplast microinjection

-particle bombardment

1. *Agrobacteria tumefaciens* mediated plant transformation

http://www.ndsu.nodak.edu/instruct/mcclean/plsc731/transgenic/transgenic1.htm

Agrobacteria: -carry Ti-plasmid

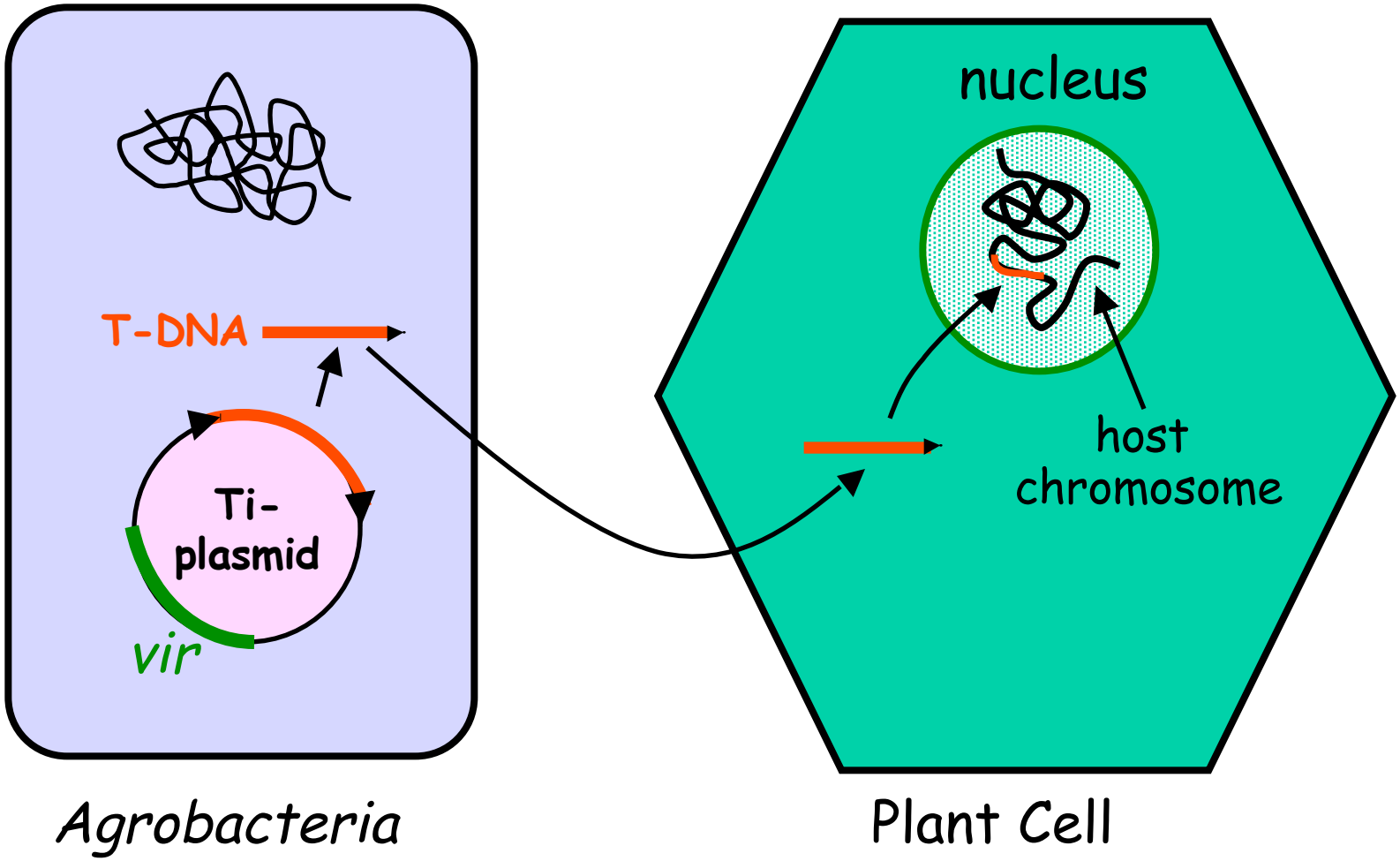
-induce tumor formation (crown gall tumor) in wound site of plants

-produce opines (nopaline or octopine)

Ti-plasmid has two parts:

- T-DNA the part of DNA that is exported into the plant cell and integrated into the plant genome
- *vir* region: encode proteins involved in this transfer, but stays within the bacteria

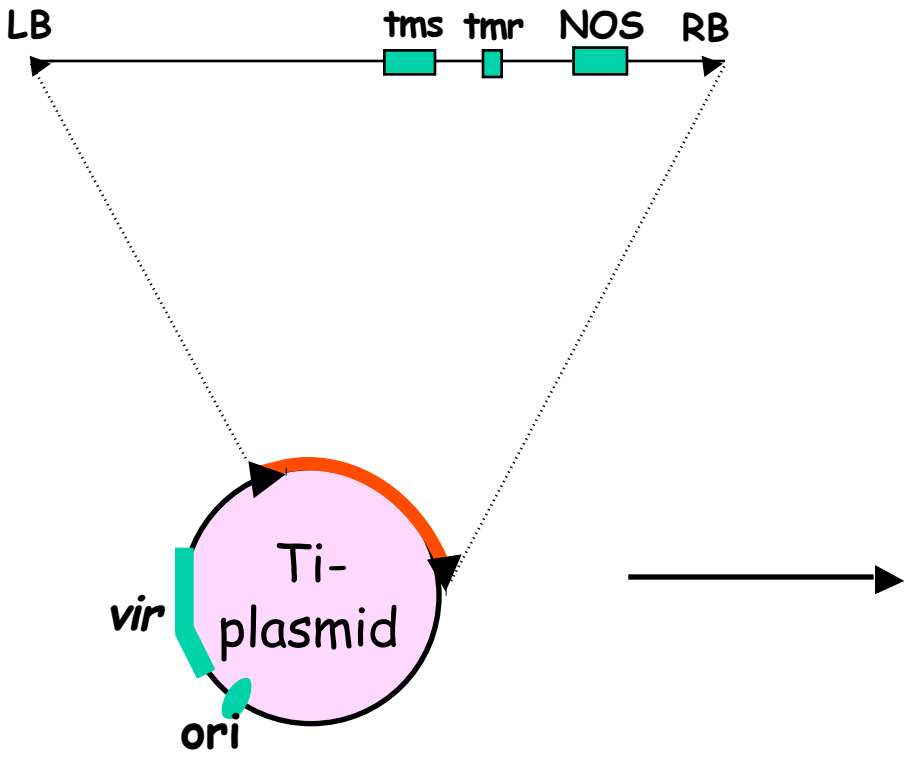
Principles of gene transfer from *Agrobacteria* into plant cells



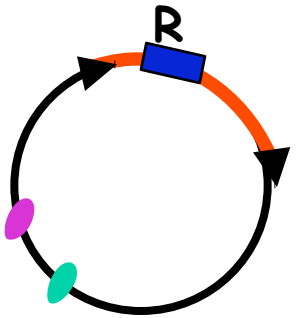
***vir*: *vir* region (vir = virulence)**

Ti: tumor-inducing plasmid

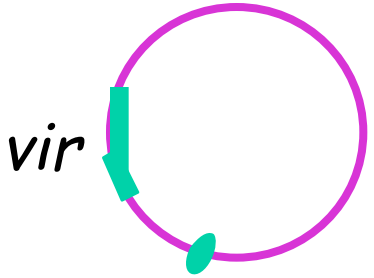
Binary gene vector for plant transformation



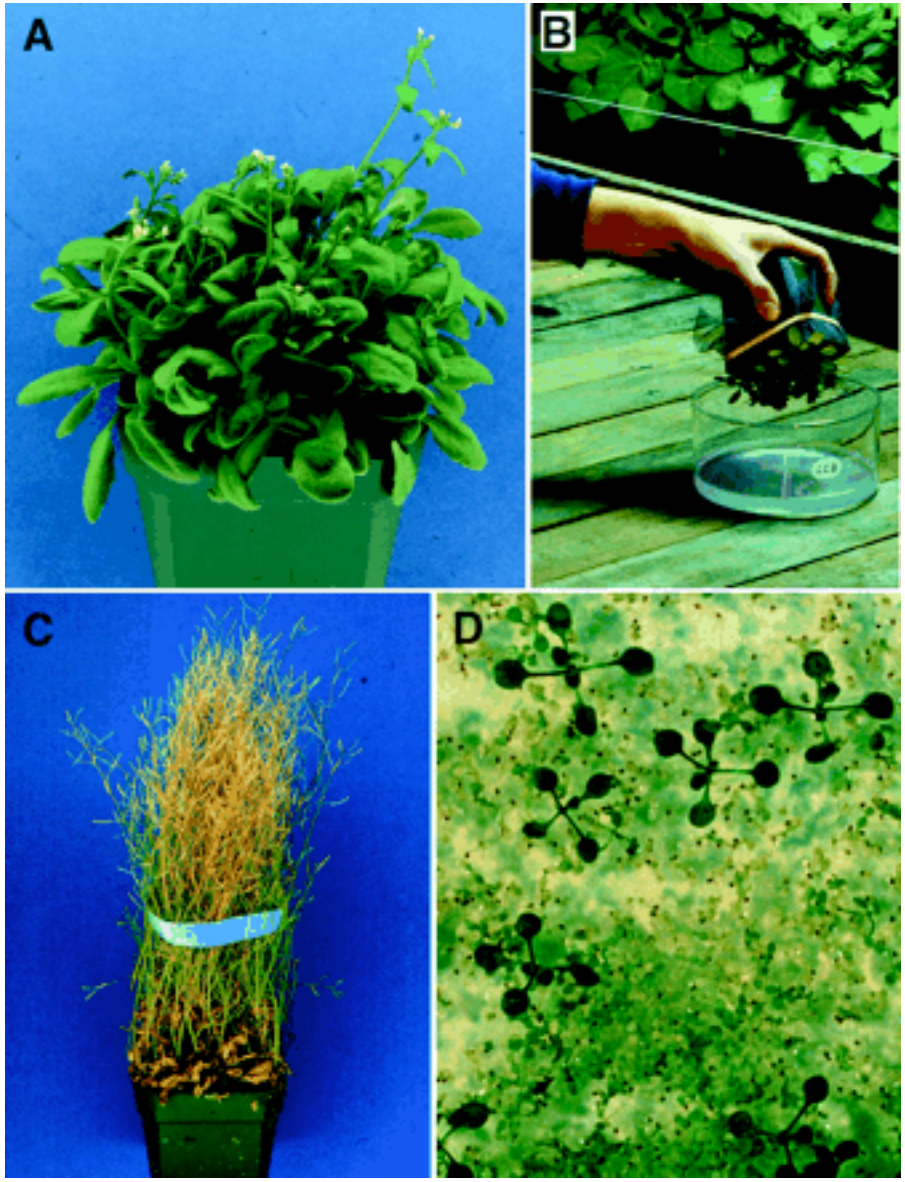
E. coli plasmid with T-DNA



Ti-plasmid without T-DNA



LB, RB: 25 bp repeat, left and right border
tms: tumor morphology shoot
tmr: tumor morphology root
ori: origin of replication
vir: virulence region



Floral Dip (Vacuum infiltration) for Arabidopsis

(A) Plants are grown to just flowering.
(B) Plants are dipped briefly in a suspension of *Agrobacterium*.
(C) Plants are grown until mature and then progeny seeds are harvested.
(D) Seeds are germinated on selective medium (e.g. containing kanamycin) to identify successfully transformed progeny.

A. Bent (2000). Plant physiology vol 124, pp 1540-1547.

Tomato transformation using wounded cotyledons
(http://www-ceprap.ucdavis.edu/Transformation/transform1.htm)

2. Biolistic transformation (particle bombardment)

DNA was bound to tiny metal particles, a gunpowder driven piston was fired at the target cell with a velocity of about 430 meters per second. Some of the cells that survived the bombardment incorporated the DNA into the genome.

-New devices use compressed gas to accelerate the particles

-tungsten or gold particles (0.5-5 mm)

Advantage: universal (all plant tissues, cells, or organelles)

Draw back: tissue damage, low efficiency (1-5%), size of the plasmid

Wheat biolistic transformation

(http://plantsciences.montana.edu/wheat-transformation/transformation1.htm)

3. Electroporation

The electric field causes holes to form in the plasma membrane allowing DNA to be taken up by the cell.

- -a high mortality rate (25-50% survival).
- -Alone, produces fair results. When coupled with PEG, transformation success can be dramatically increased.
- -has been achieved on a variety of species and tissue types.
- -drawback: requires protoplast regeneration which is difficult.

Transformation summary

Vector: viral-based or plasmid (Ti-plasmid or bacterial plasmid)

Delivery: *Agrobacterium*-mediated or physical delivery

Regeneration: from tissues/cells to calli, then to whole plants

Selection: antibiotic, herbicide

Characterization: integration vs transient expression

Summary of tools involved in transformation

Reporter genes

Name	Source	Substrate	Visual
GUS (β-glucuronidase)	**E.coli**	**5-bromo-4-chloro 3-indoyl-1-glucuronide (X-gluc)** →	**blue**
LUC (Luciferase)	**Firefly**	**luciferin & ATP** → **breakdown**	**emitting light**
GFP (Green Flourescent Protein)	**Jelly fish**	**none**	**emitting light**

Promoters

35S CaMV	constitutive
NOS	constitutive
Heatshock	inducible
CAB	light inducible
GAL4/GFP-mediated	specific tissues, organs

Selectable markers

Gene name	Resistant to	Type
NPT II (phosphotransferase II)	Kanamycin	Antibiotic
Gentamicin 3-N-aceytransferase	Gentamicin	Antibiotic
Aminoglycoside-3-adenyltransferase	Streptomycin	Antibiotic
Phospfinothricin acetyltransferase	Phosphinothricin	Herbicide
5-enolpyruvylshikamate-3-phosphate synthase (EPSP synthase)	Glyphosate	Herbicide
Acetplactate synthase (ALS)	Sulfonylureas	Herbicide