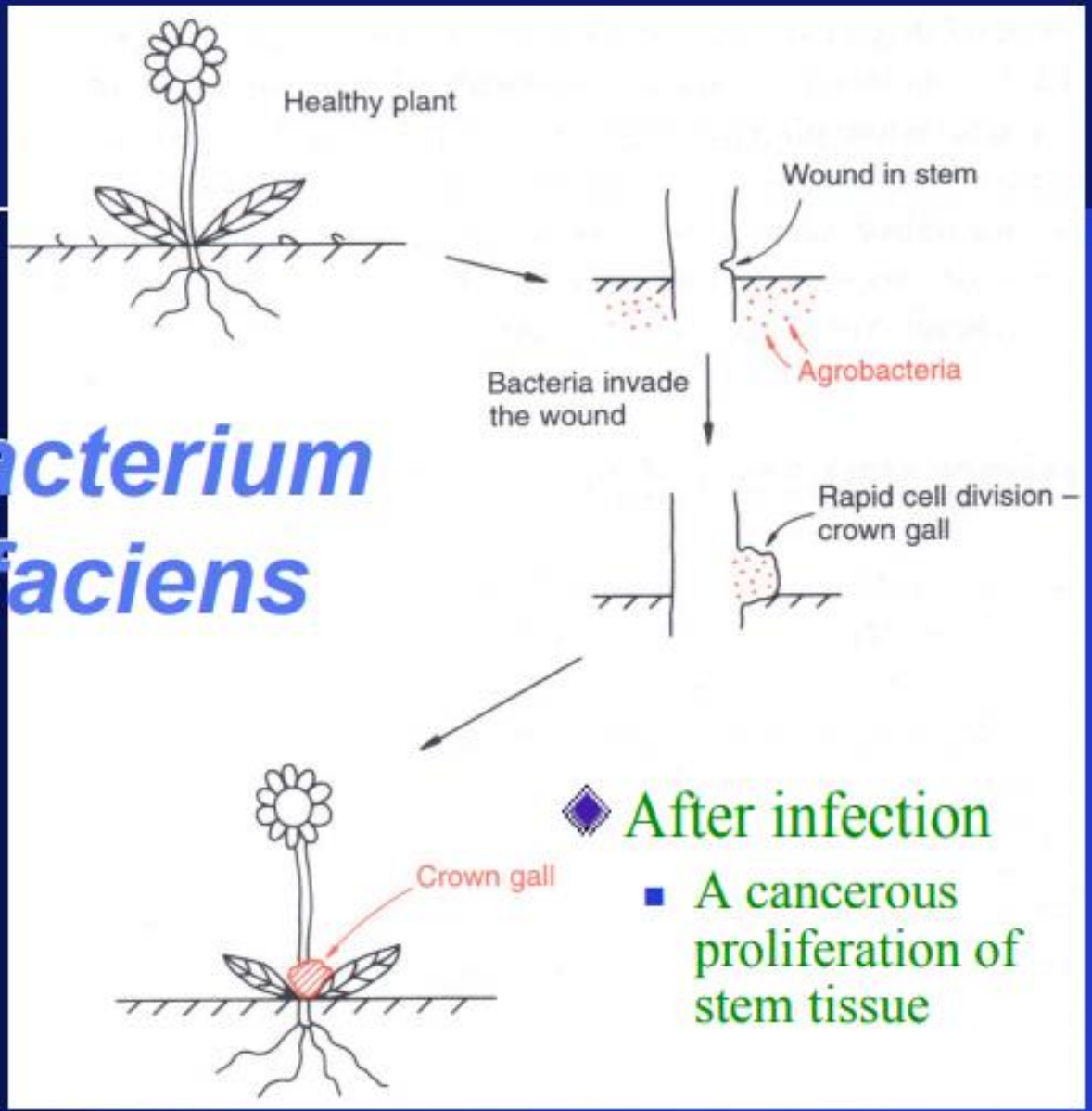
Cloning Vectors for Plants

7.2 Cloning vectors for higher plants

- **Cloning vectors for higher plants were developed in the 1980s**
  - **Genetically modified (GM) crops**
  - That are in headlines today

- **Three types of vectors system**
  - Vectors based on naturally occurring plasmids of *Agrobacterium*.
  - Direct gene transfer using DNA fragments not attached to a plant cloning vector
  - Vectors based on plant viruses

Agrobacterium tumefaciens



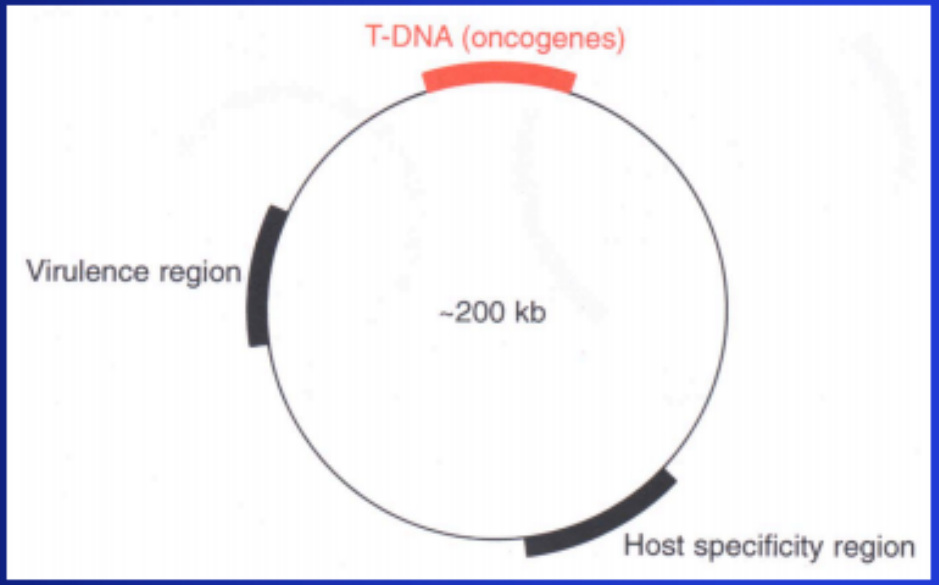
- After infection
  - A cancerous proliferation of stem tissue

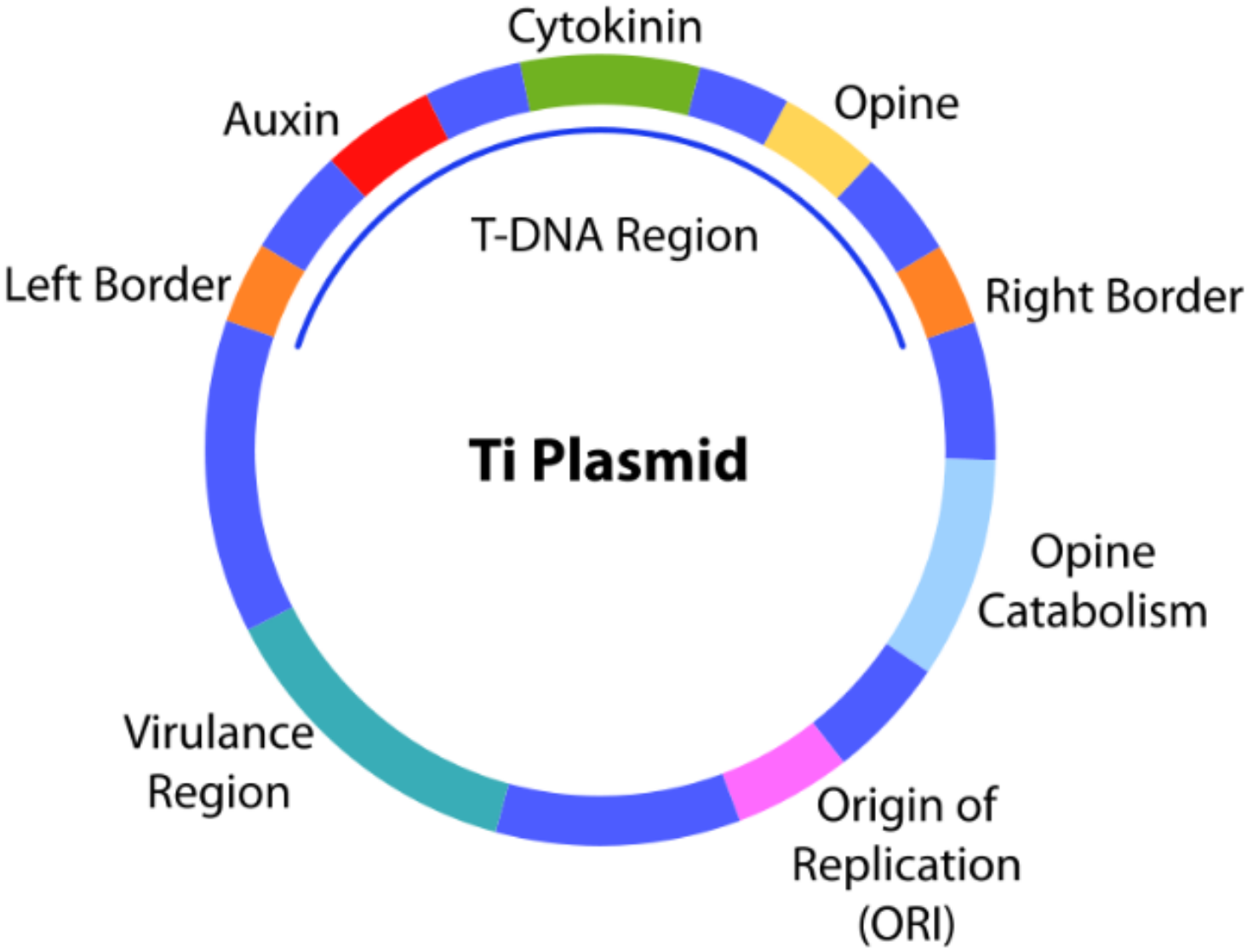
Figure 7.10 The Ti plasmid & its intergration into the plant chromosomal DNA after *A. tumefacients* infection

(a) A Ti plasmid

- Ability to cause crown gall disease is associated with the presence of Ti (tumor inducing) plasmid within bacterial cell

A large plasmid





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Virulence region

- Genes in the virulence region are grouped into the operons *virABCDEFG*, which code for the enzymes responsible for mediating transduction of T-DNA to plant cells.[22]
- · *virA* codes for a receptor which reacts to the presence of phenolic which leak out of damaged plant tissues.[24]
- · *virB* encodes proteins which produce a pore/pilus-like structure.[23]
- · *virC* binds the overdrive sequence, a T-DNA transfer enhancer.[23]
- · *virD1* and *virD2* produce endonucleases which target the direct repeat borders of the T-DNA segment,
- · *vir E* Binds to T-strand protecting it from nuclease attack, and intercalates with lipids to form channels in the plant membranes through which the T-complex passes, [23] beginning with the right border.[24]
- · *virG* (TRANSCRIPTIONAL FACTOR) activates vir-gene expression after binding to a consensus sequence,[23] once it has been phosphorylated by *virA*.[24]

Figure 7.10 (b) Integration of T-DNA into plant genome

- After infection
  - Part of Ti plasmid is integrated into plant chromosome
  - T-DNA: 15-30 kb in size, eight or so genes

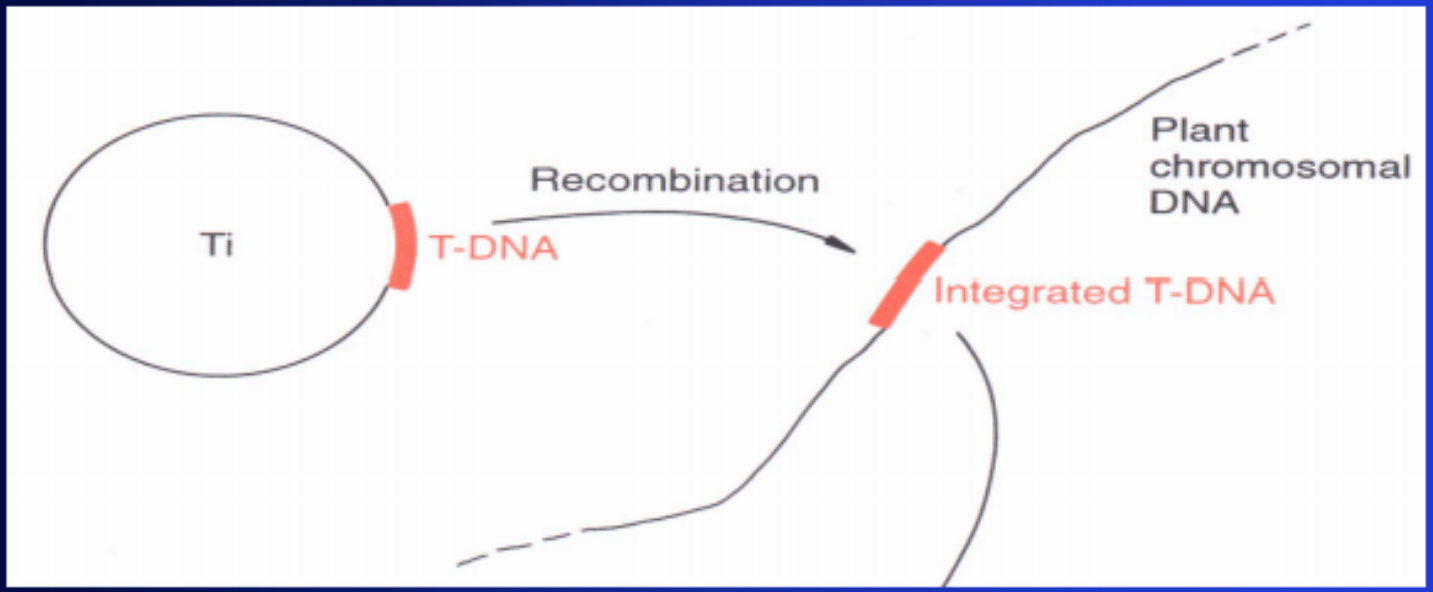
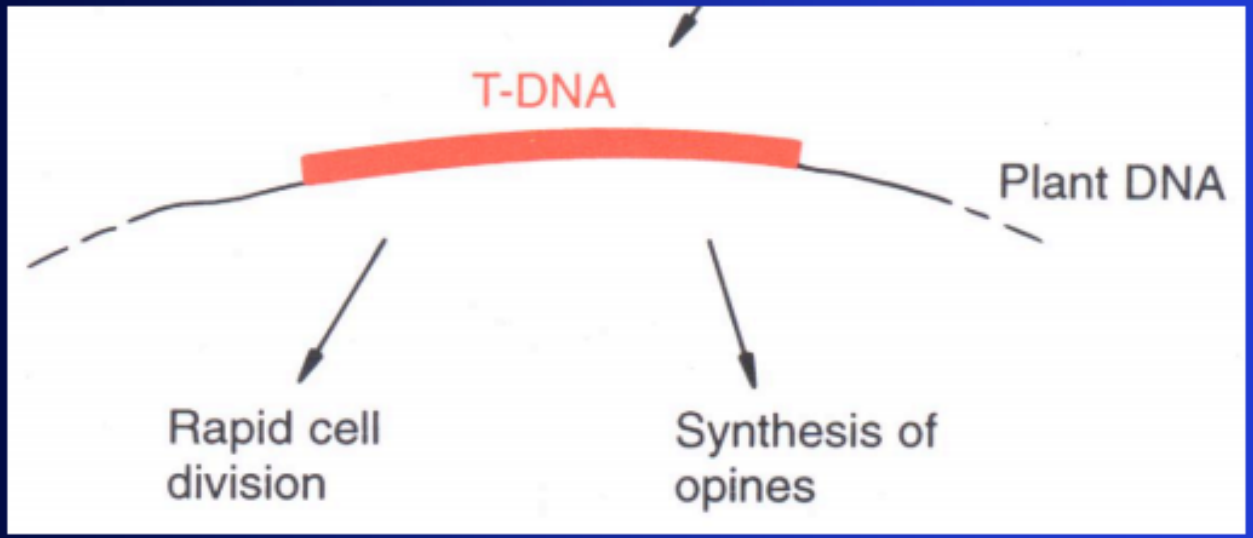


Figure 7.10 (c) Expression of T-DNA gene

- Responsible for cancerous properties of transformed cells
- Direct synthesis of opines: nutrients of bacteria



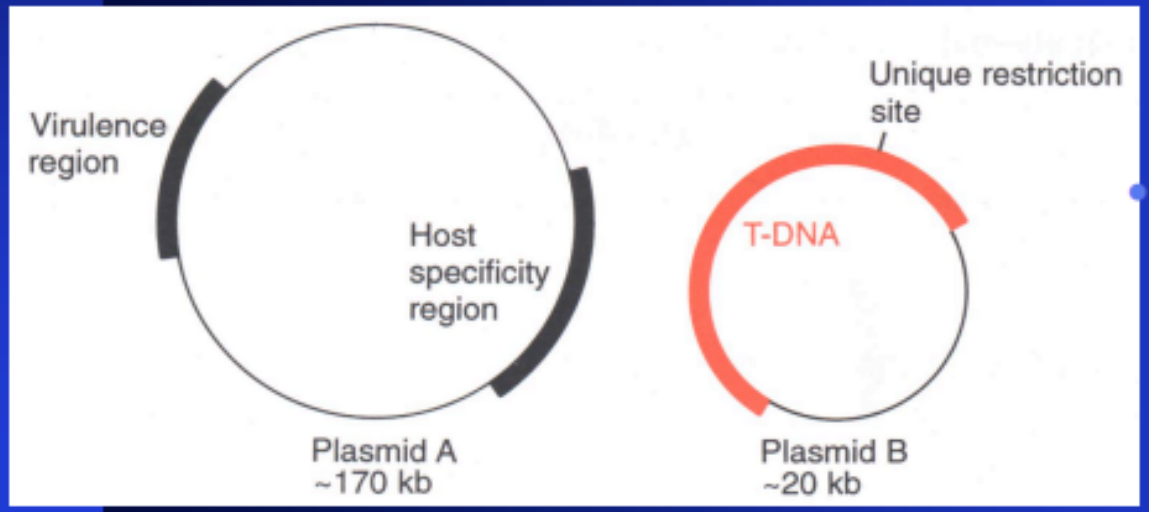
Using the Ti plasmid to introduce new genes into a plant cell

- The large size of Ti plasmid makes its manipulation very difficult
  - A unique restriction site is an imposibility

1) The binary vector strategy
2) The cointegration strategy

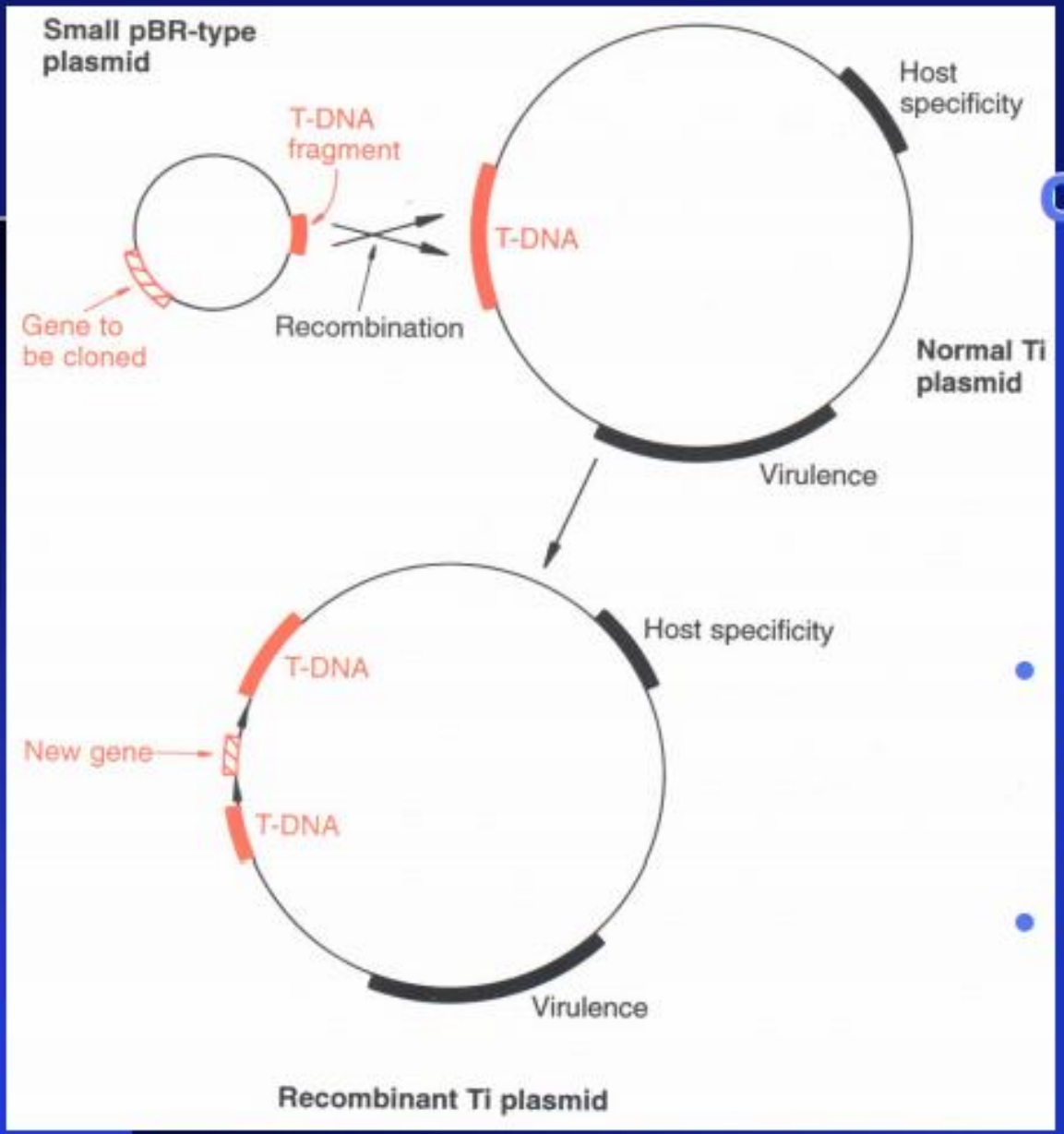
The binary vector strategy

Figure 7.11 Plasmids A & B complement each other when present together in the same *A. tumefaciens* cell. The T-DNA carried by plasmid B is transferred to the plant chromosome DNA by proteins coded by genes carried by plasmid A.



The T-DNA plasmid (B) is small enough to have a unique restriction site

The cointegration strategy



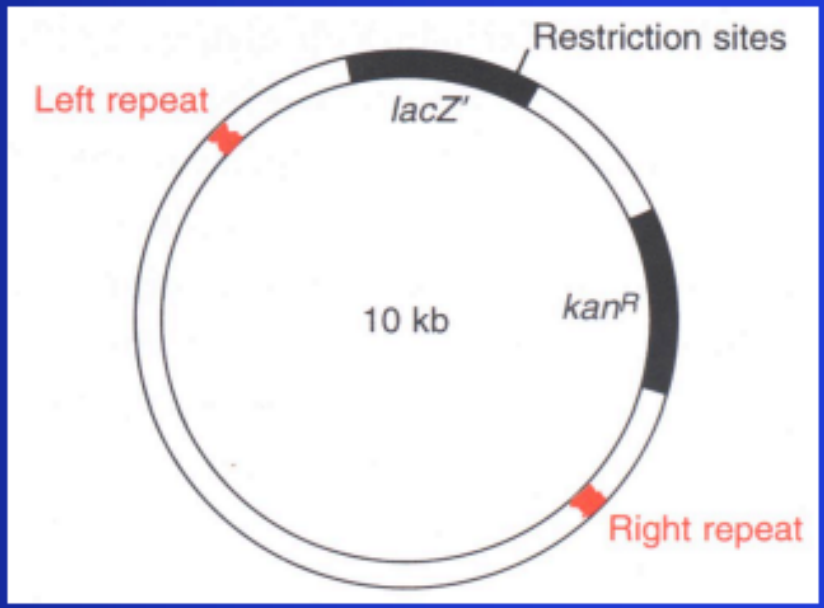
- A new plasmid carrying a small portion of T-DNA
- Recombination

The "disarmed" Ti vectors:
transformed cells do not display cancerous properties

- All cancer genes lie in T-DNA & are not needed for infection process
- T-DNA involved in infection
  - Two 25 bp repeat sequence at left & right borders of region integrated into plant DNA
- Disarmed: remove all cancer genes

Figure 7.14 The binary Ti vector pBIN19

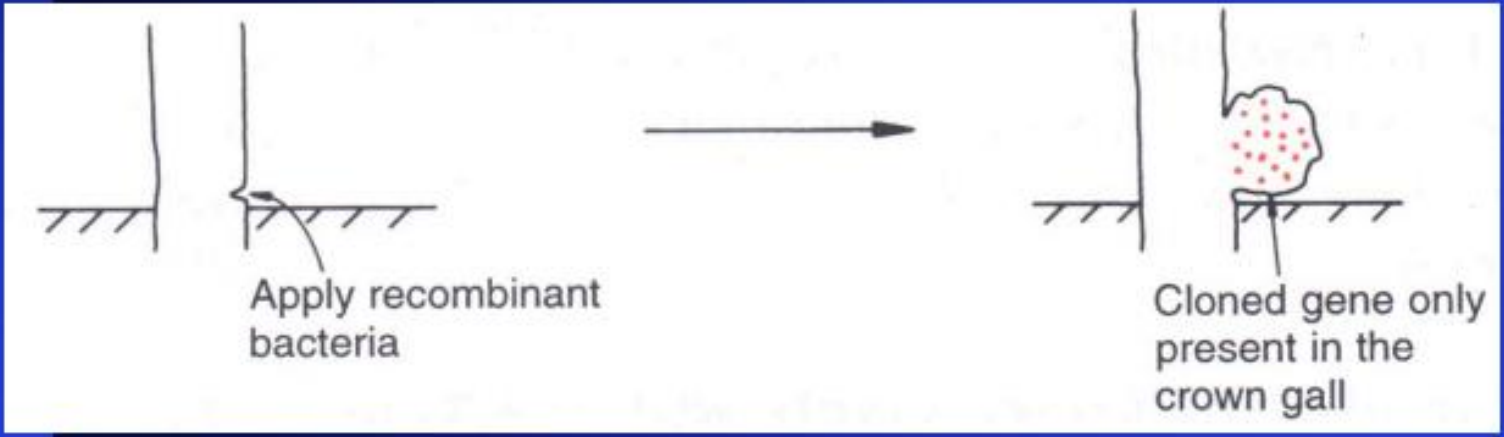
- A number of disarmed Ti vectors are now available
  - A shuttle vector
  - Initial manipulations are carried out in *E. coli*
  - Then, correct recombinant pBIN19 being transferred to *A. tumefaciens* & thence into plant
- kan^R=kanmycin resistance gene

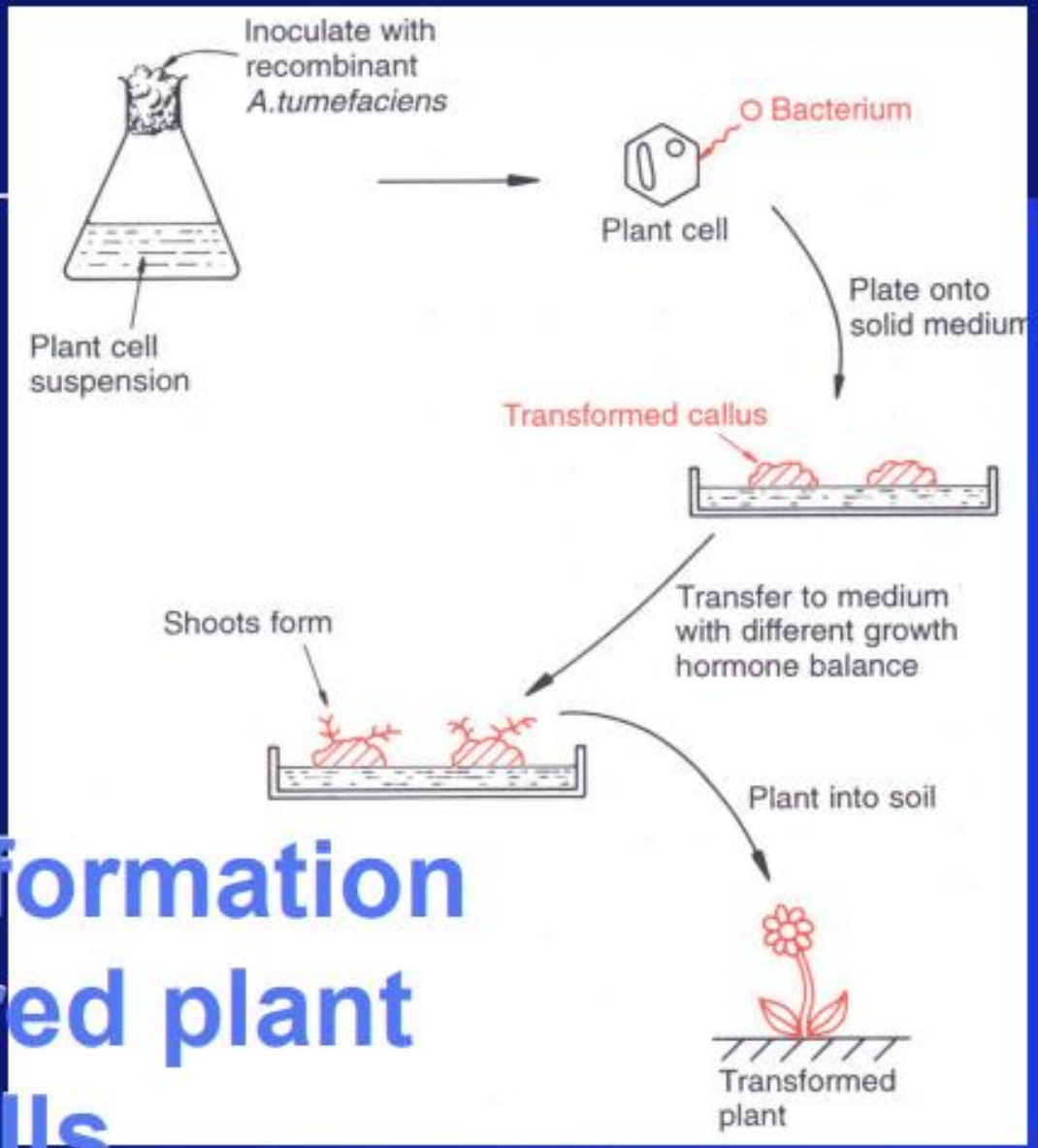


Production of transformed plants with the Ti plasmid

(a) Wound infection by recombinant *A. tumefaciens*

- By infection of a wound in stem
- Only cells in resulting crown gall will possess cloned gene
- Little value to biotechnologist





(b) Transformation of cultured plant cells

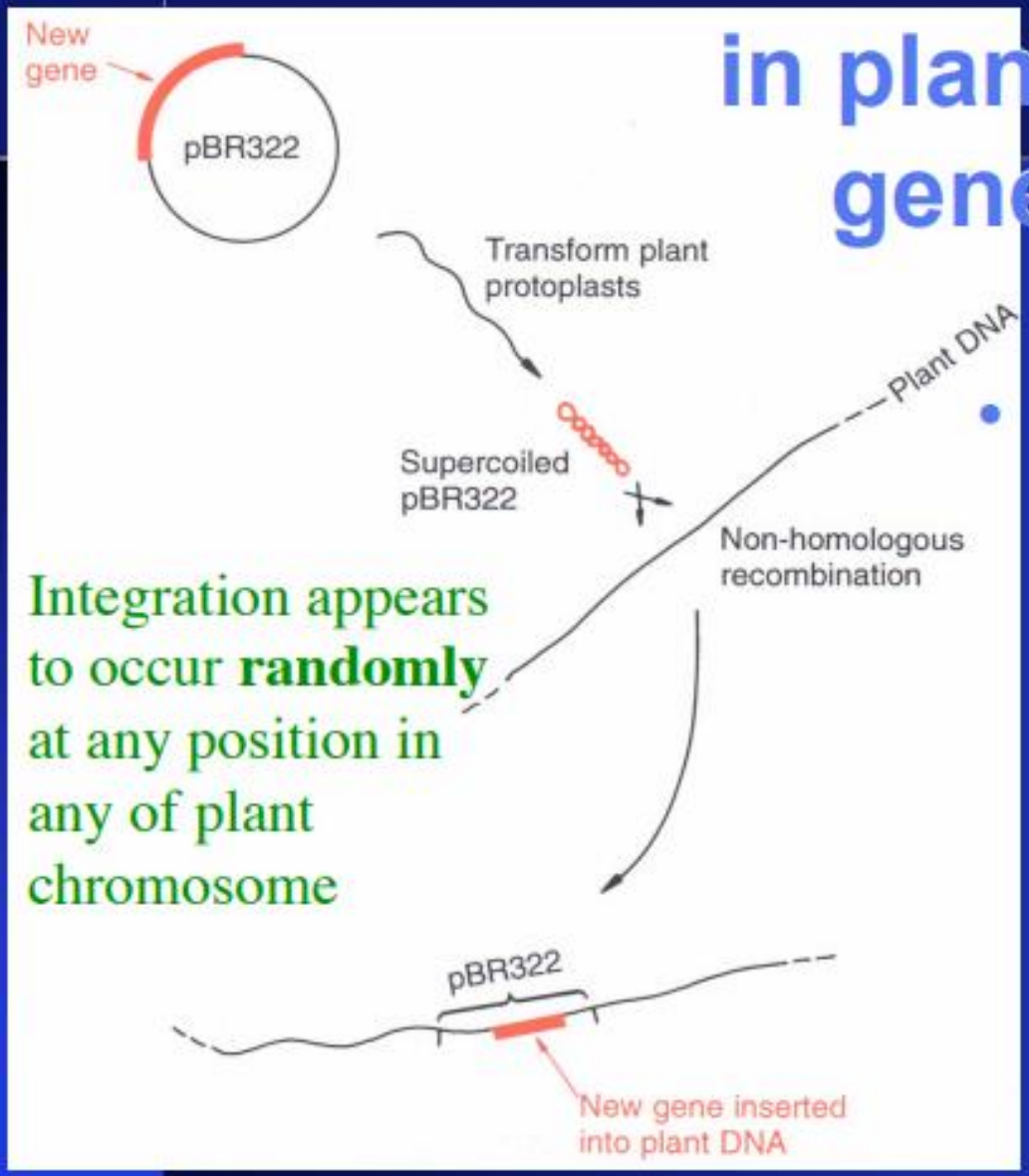
The Ri plasmid

- The Ri plasmid of *Agrobacterium rhizogenes*
- Main difference between Ri & Ti plasmids
  - Transfer of T-DNA from an Ri plasmid to a plant results in hairy root disease
  - Massive proliferation of a highly breached root system
- Growing transformed roots at highly density in liquid culture
  - Obtaining large amounts of protein

Limitations of cloning with Agrobacterium plasmids

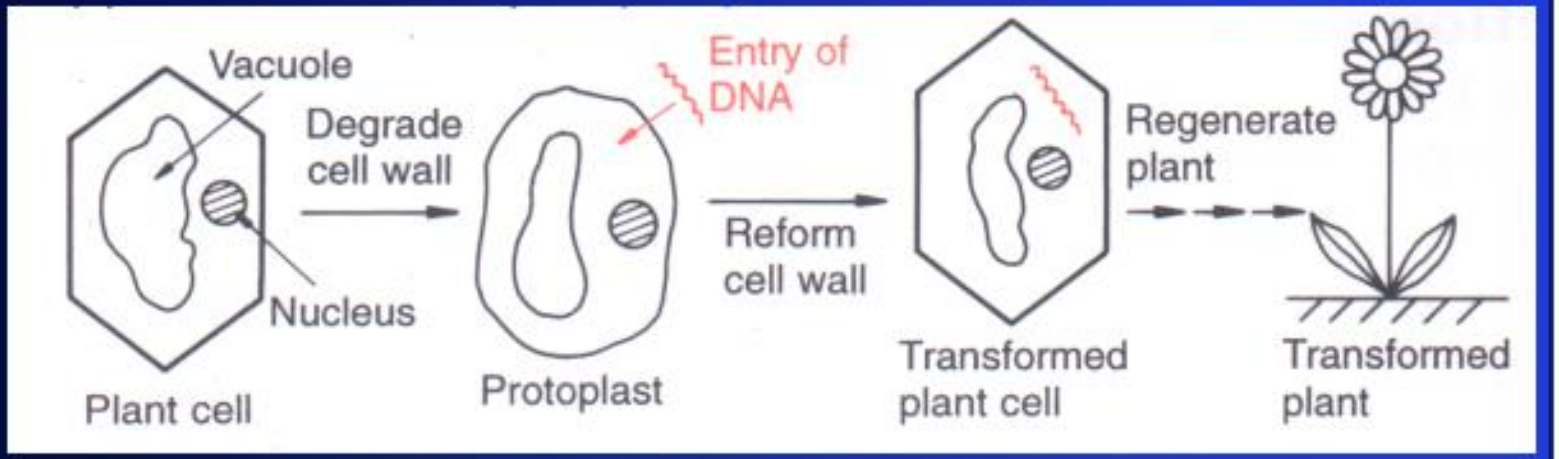
- In nature *A. tumefaciens* & *A. rhizogenes* infect only dicotyledonous plants
  - Monocots are outside of normal host range
  - OK: tomato, tobacco, potato, peas & beans
  - No: wheat, barley, rice & maize
- Bombardment with microprojectiles
  - Introduce plasmid DNA directly into plant embryos
  - Successful with maize & several other important monocots
  - Figure 5.15 (b)

7.2.2 Cloning genes in plants by direct gene transfer



- A supercoiled plasmid
  - Unable to replicate in a plant cell on its own
  - Become integrated by recombination into one of plant chromosomes
  - The recombination event is poorly understood

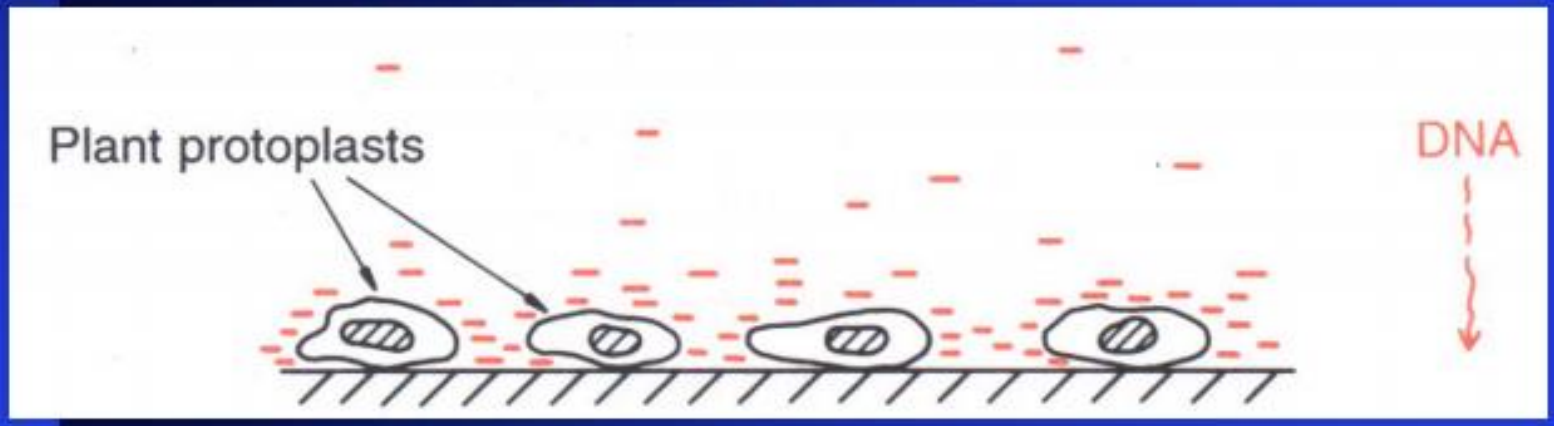
Strategies for introducing new DNA into plant cells



- Figure 5.14 (b) Transformation of plant protoplasts
- Protoplasts:
  - Enzyme that degrade yeast, fungal & plant cell walls are available

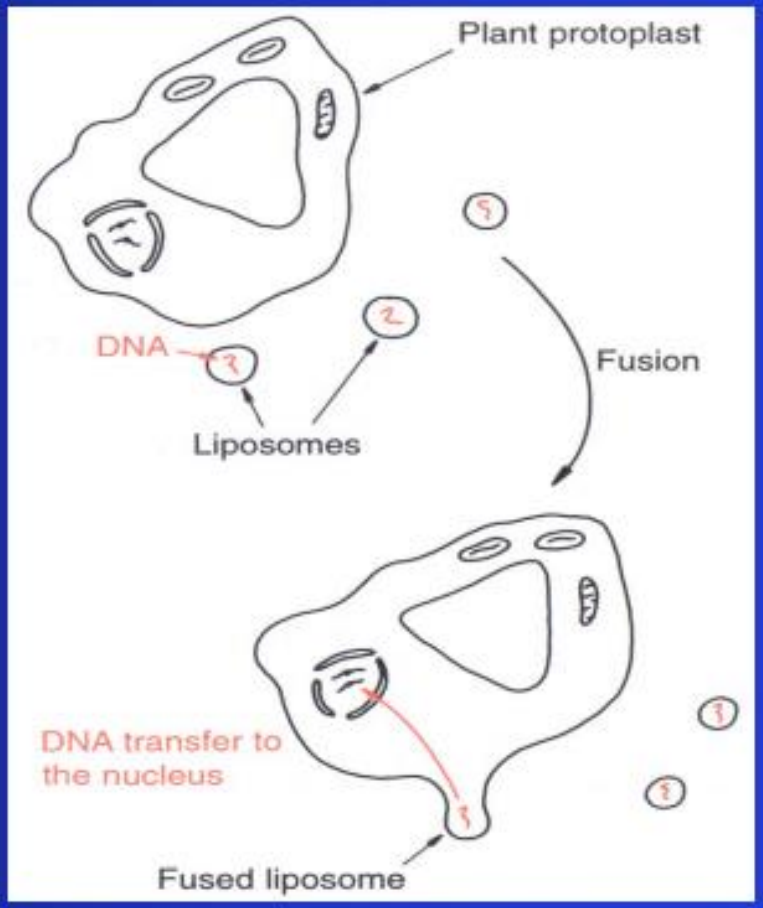
(a) precipitation of DNA onto surfaces of protoplasts

- Resuspending protoplasts in a viscous solution of polyethylene glycol
  - a polymeric, negatively charged compound
  - precipitate DNA onto surfaces of protoplasts
  - induce uptake by endocytosis



(b) fusion of protoplasts with DNA-containing liposomes

- Protoplast can also be fused with DNA-containing liposomes
- Intact cells can be vigorously shaken with DNA-coated silica needles
  - Which penetrate the cell wall and transfer the DNA into the interior



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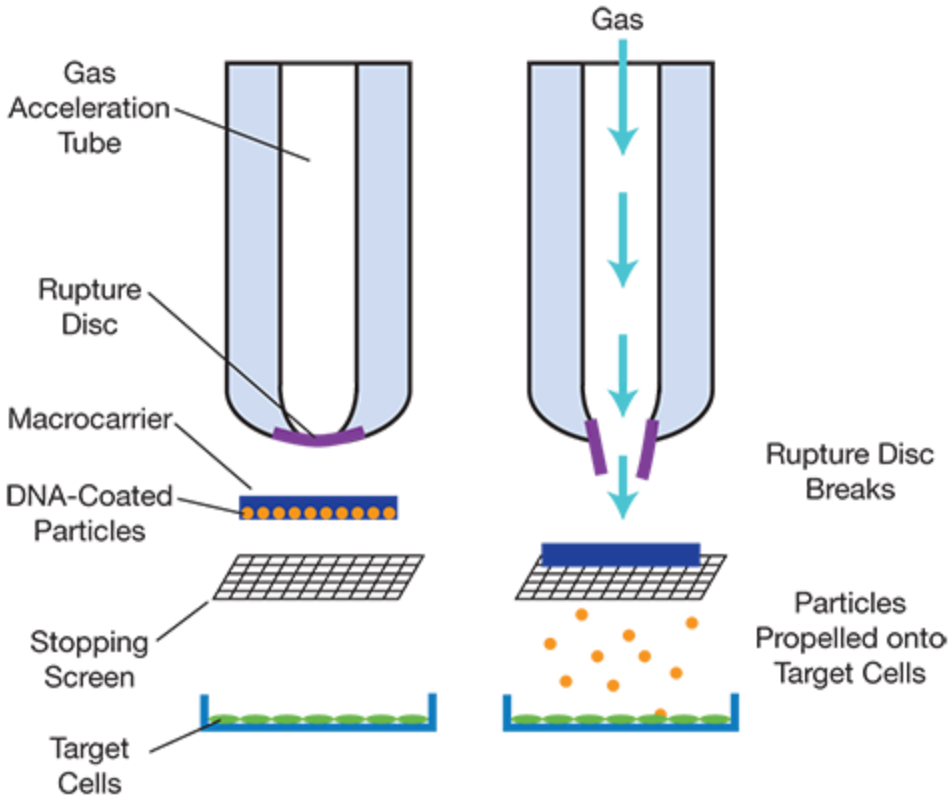
Biolistic

- In biolistic delivery, genes are coated and dehydrated onto heavy-metal particles, such as gold or tungsten. High-pressure helium pulses accelerate the particles, propelling them into plant cells at high velocities (Figure 2). Typically, the epidermal tissue of plant cells is targeted. Depending on the experimental parameters, DNA can pass through both the plant cell wall and plasma membrane, and can also penetrate into the nucleus *(2)*. Helium gas pressure, net particle size, and dosing frequency are critical experimental parameters that determine the penetration efficiency, toxicity, and overall gene transfer levels in plants.



Attempts to use plant virusses as cloning vectors

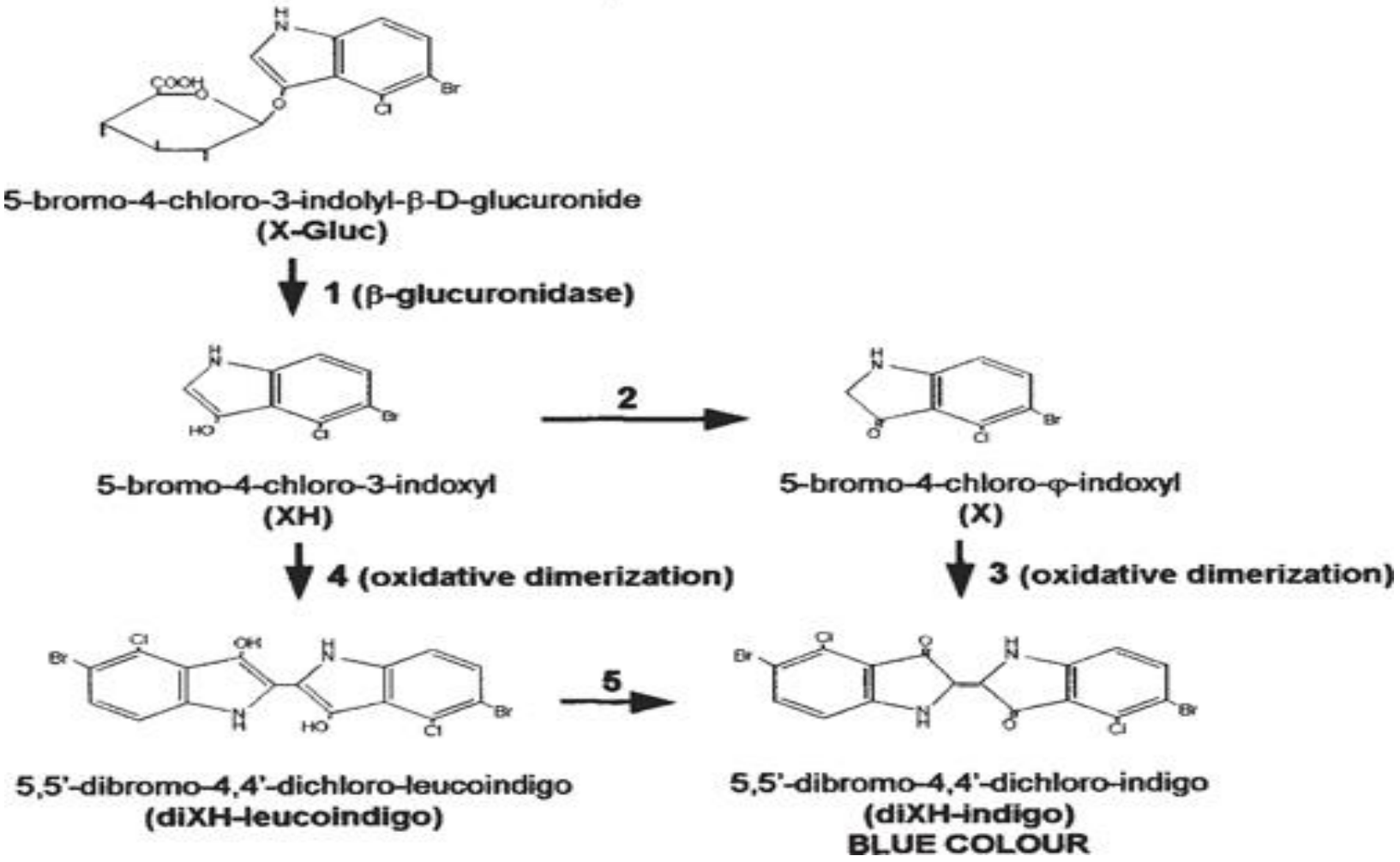
- One Problem
  - Vast majority of plant viruses have genome not of DNA but of RNA
  - RNA viruses are nor so useful as potential cloning vectors
  - Manipulations with RNA are rather more difficult to carry out
- Only two classes of DNA virus infect higher plants
  - Caulimovirus vector & Geminivirus vectors
  - Neither is ideally suited for gene cloning

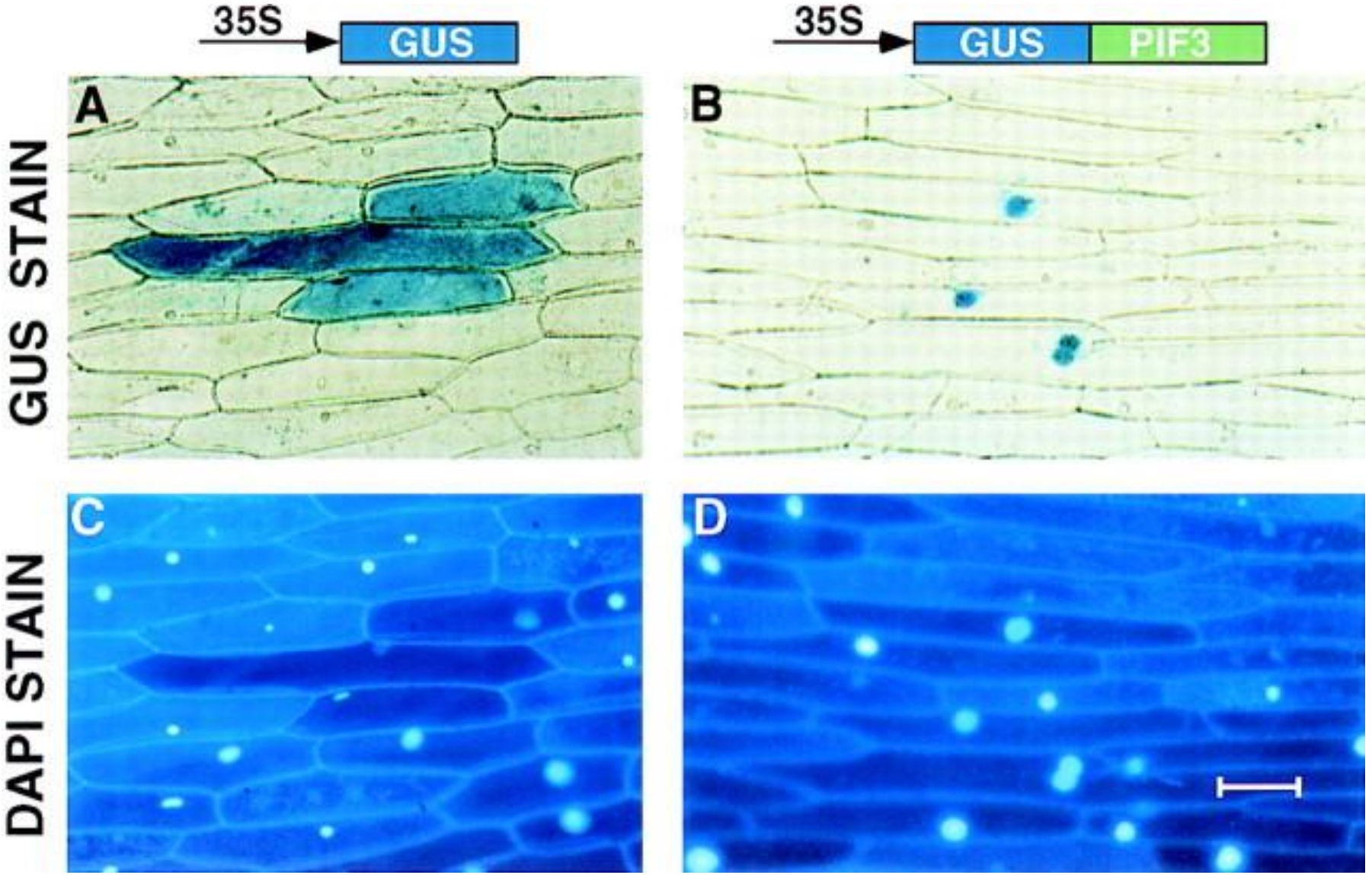
Caulimovisrus- size, narrow host range, Geminivirus- rearrangement or deletion of added DNA, not stable

Various selectable markers and reporter genes commonly used in transgenic plants

Gene	Enzymes encoded	Substrate	Gene source	Reference
Selectable markers				
bar	Phosphinothricin acetyl transferase	Phosphinothricin	*Streptomyces hygroscopicus*	(175)
BADH	Betaine aldehyde dehydrogenase	Betaine aldehyde	*Spinacia oleracea*	(176)
bxn	Bromoxynil nitrilase	Oxynils	*Klebsiella pneumonia*	(177)
cat	Chloramphenicol acetyl transferase	Chloramphenicol	*Escherichia coli* Tn5	(178)
dhfr	Dihydrofolate reductase	Methotrexate	*Candida albicans*	(179)
EPSPS	5-Enolpyruvyl shikimate-3-phosphate synthase	Glyphosate	*Petunia ×hybrida*	(180)
gox	Glyphosate oxidoreductase	Glyphosate	*Ochrobactrum anthropi*	(181)
hpt II	Hygromycin phospho-transferase II	Hygromycin B	*E. coli*	(182)
ManA	Phosphomannose isomerase	D-Mannose	*E. coli*	(183)
npt II	Neomycin phosphotrans-ferase II	Kanamycin	*E. coli* Tn5	(184)
xylA	Xylose isomerase	D-Xylose	*Streptomyces rubignosus*	(185)
Reporter genes				
uidA/GUS	β-Glucuronidase	X-gluc	*E. coli*	(186)
gfp	Geen fluorescent protein		*Aequorea victoria*	(187)
lacZ	Galactosidase	X-gal	*E. coli*	(188)
luc	Luciferase	Luciferin	*Photinus pyralis*	(189)
	Oxalate oxidase	Oxalic acid	*Triticum aestivum*	(190)

GUS Assay


5-bromo-4-chloro-3-indolyl-β-D-glucuronide
(X-Gluc)
1 (β-glucuronidase)
5-bromo-4-chloro-3-indoxyl
(XH)
2
5-bromo-4-chloro-φ-indoxyl
(X)
4 (oxidative dimerization)
3 (oxidative dimerization)
5
5,5'-dibromo-4,4'-dichloro-leucoindigo
(diXH-leucoindigo)
5,5'-dibromo-4,4'-dichloro-indigo
(diXH-indigo)
BLUE COLOUR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