

INTRODUCTION TO

# BIOTECHNOLOGY

# GY BIOTECHNOLOGY

# TECHNOLOGY BI

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# TECHNOLOGY B

# BIOTECHNOLOGY

William J. Thieman and Michael A. Palladino

THIRD EDITION

# CHAPTER 6

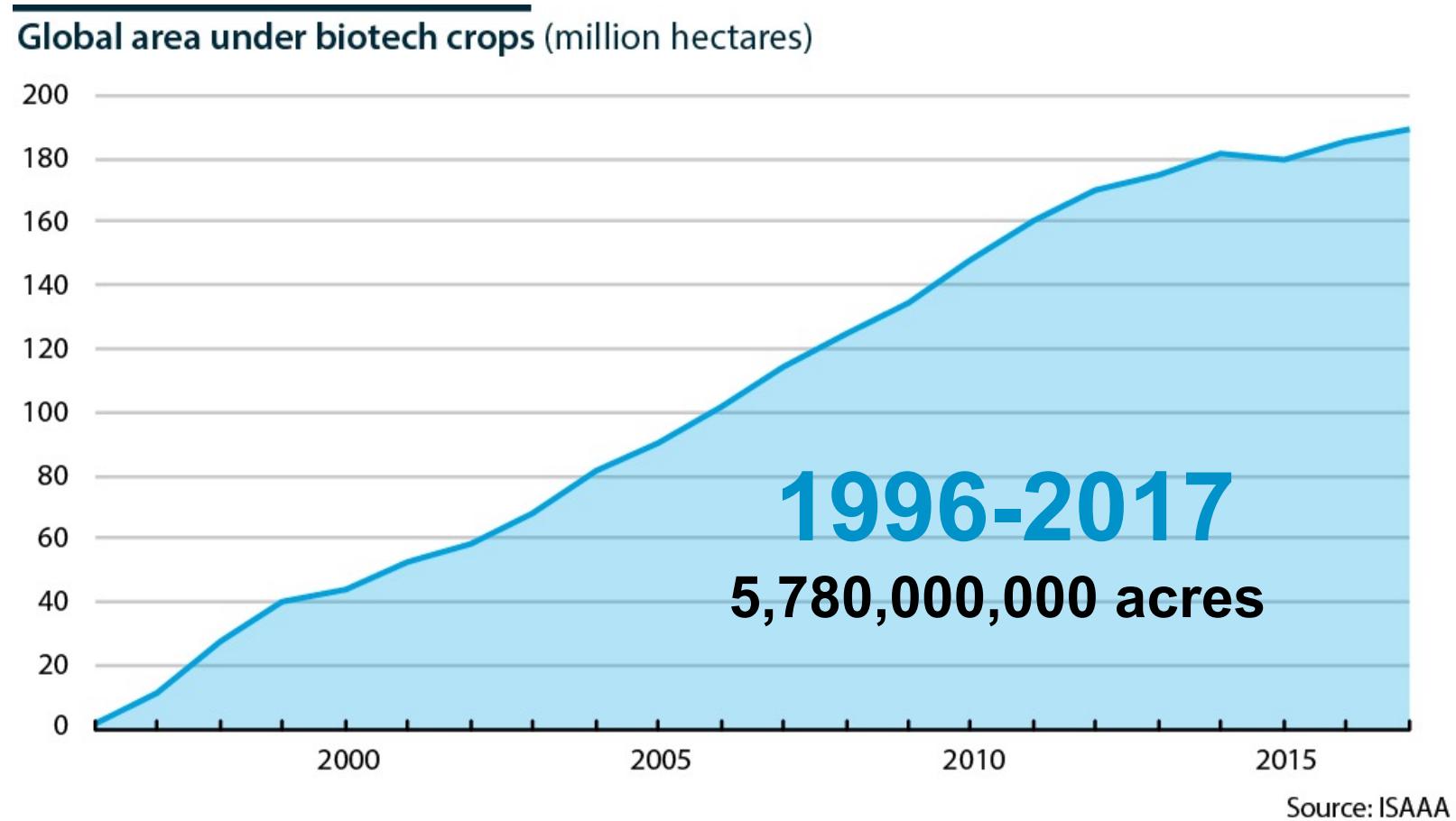
## Plant Biotechnology

PowerPoint® Lecture by:  
Lisa Werner  
Pima Community College

## 6.1 The Future of Agriculture: Plant Transgenics

- The world population has nearly doubled in the past 40 years, while arable land has only increased by 10%
- Improved crop breeding through traditional methods has allowed us to feed so many people
- Recently, development of new, more productive crops has been accelerated by direct transfer of genes

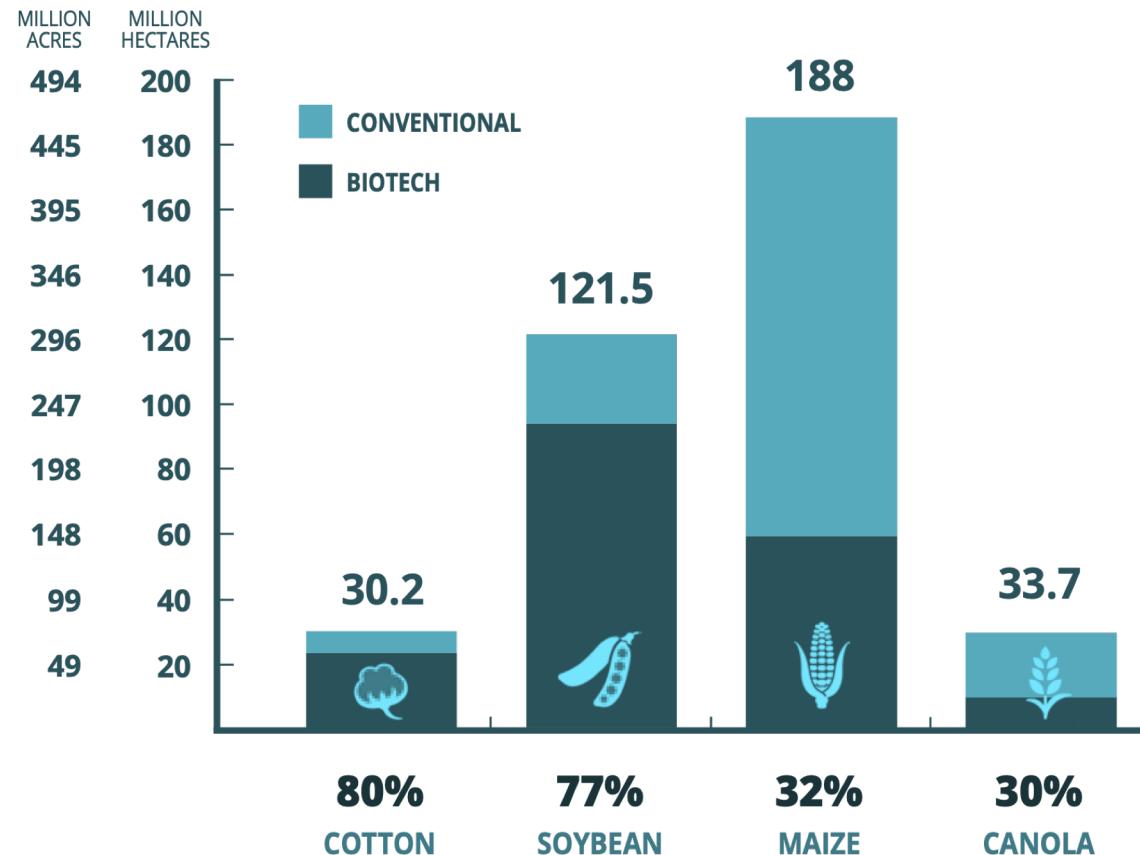
# 6.1 The Future of Agriculture: Plant Transgenics



## 6.1 The Future of Agriculture: Plant Transgenics

- **Plant Transgenesis** – the direct transfer of genes to plants
  - Development of plant vaccines, plants that produce their own pesticides and are resistant to herbicides
- By 2008, 13.3 million farmers in 25 countries planted transgenic crops
  - 90% in developing countries
- By 2009, a significant portion of several key crops world wide, were transgenic
  - 70% of soybeans, 40% of corn, 10% of cotton
- Focus of considerable controversy

# 6.1 The Future of Agriculture: Plant Transgenics



**FIGURE 3. GLOBAL ADOPTION RATES (%) FOR TOP 4 BIOTECH CROPS (MILLION HECTARES)**

Source: ISAAA, 2017

## **6.2 Methods Used in Plant Transgenesis**

- **Conventional Selective Breeding and Hybridization**
- Cloning
  - Protoplast fusion
  - Leaf fragment technique
  - Gene guns
  - Chloroplast engineering
  - Antisense technology

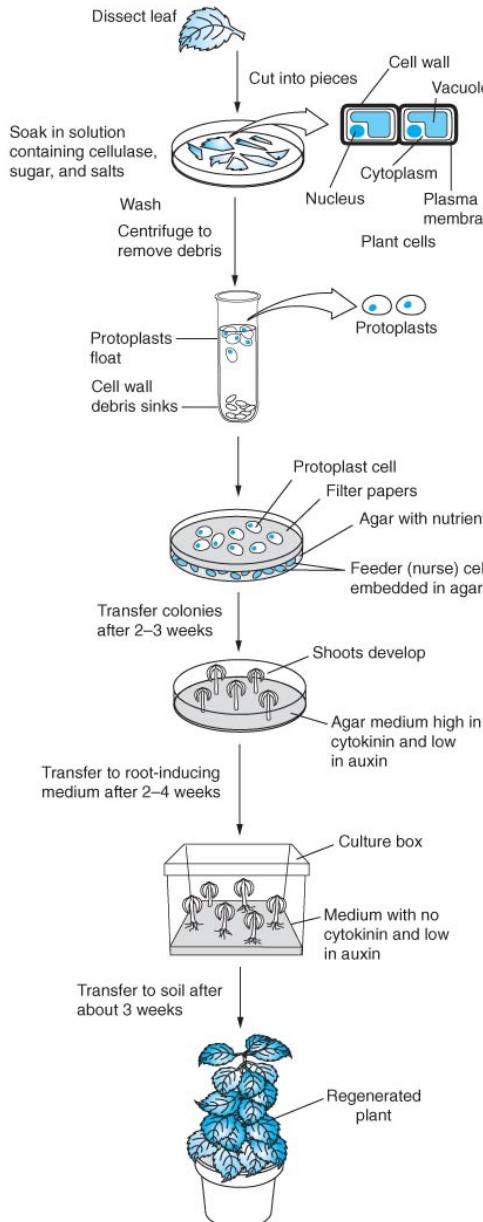
## 6.2 Methods Used in Plant Transgenesis

- **Conventional Selective Breeding and Hybridization**
  - Sexual cross between two lines and repeated backcrossing between hybrid offspring and parent
    - Can take years
  - Polyploid plants (multiple chromosome sets greater than normal)
    - Increases desirable traits, especially size
    - Whole chromosomes can be transferred rather than single genes

## 6.2 Methods Used in Plant Transgenesis

- **Cloning** – growing plants from a single cell
  - **Protoplast fusion** is the fusion of two protoplast cells from different species
    - Protoplast cell is a callus cell whose cell wall has been dissolved by the enzyme cellulase
    - Fusion of the two protoplast cells creates a cell that can grow into a hybrid plant
    - Examples include broccoflower

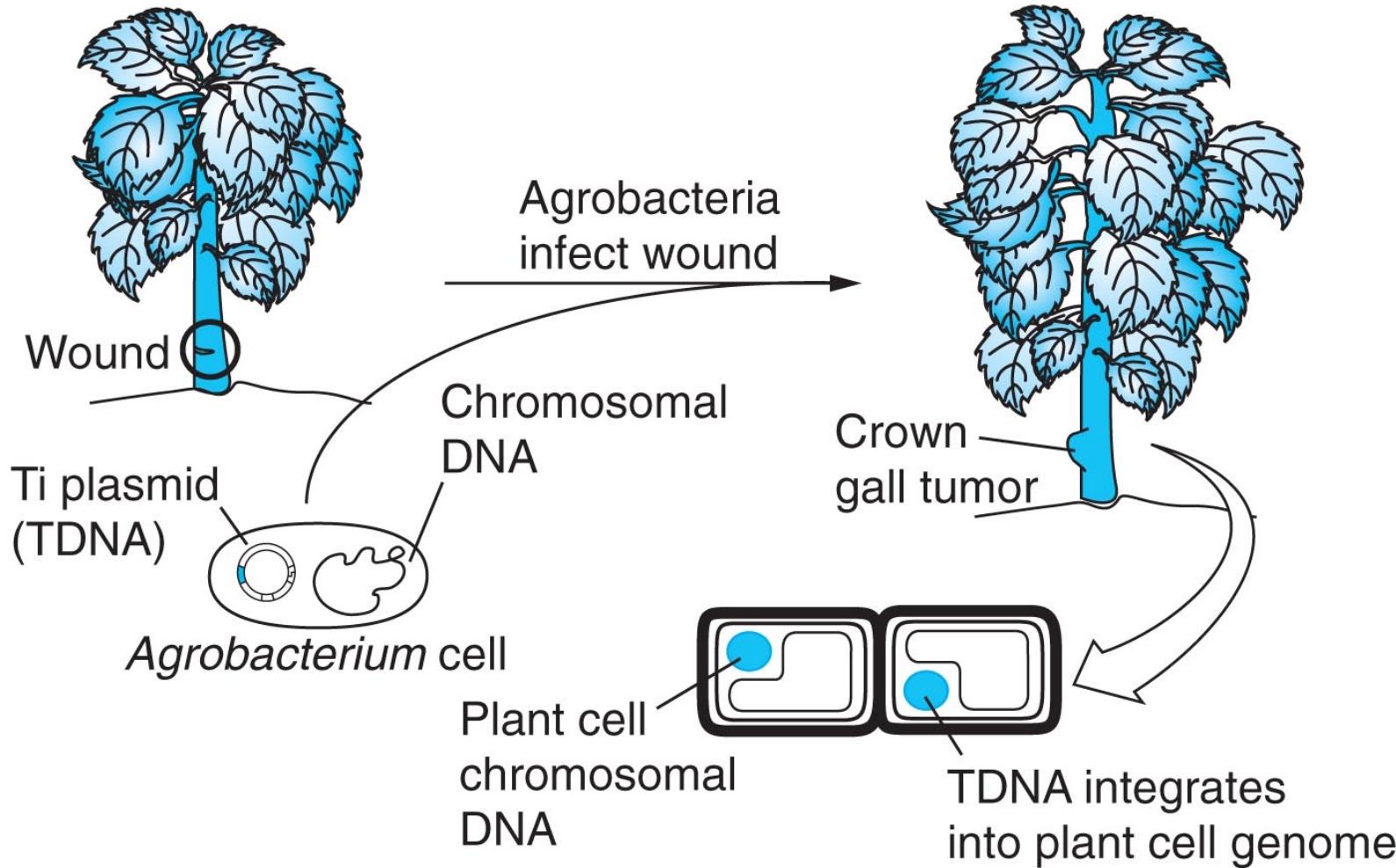
## 6.2 Methods Used in Plant Transgenesis



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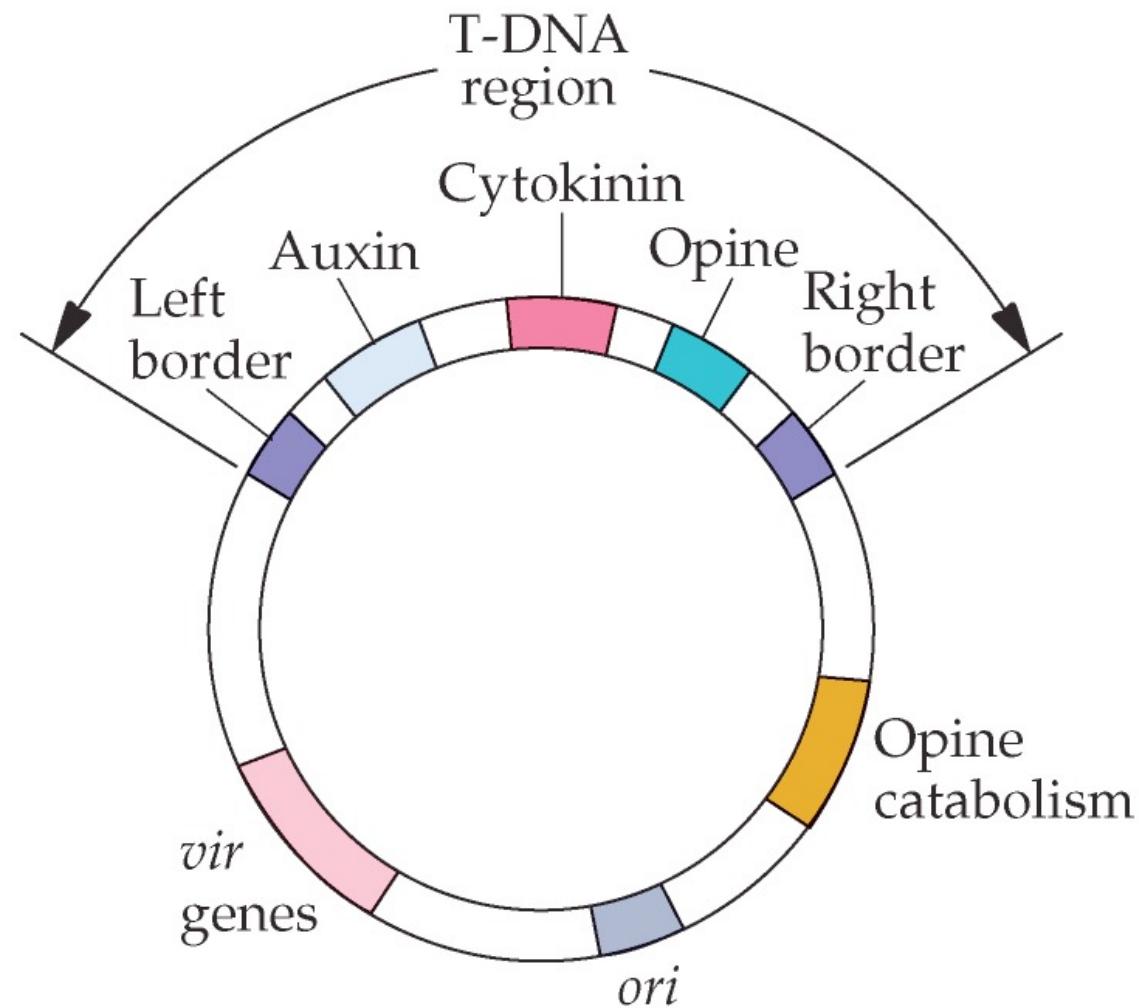
- **Cloning**
  - **Leaf fragment technique**
    - Small discs are cut from leaf
    - Cultured in a medium containing genetically modified *Agrobacter* (*Agrobacterium tumefaciens*)
      - A soil bacterium that infects plants
      - Bacterium contains a plasmid, the Ti plasmid, that can be genetically modified
      - DNA from the Ti plasmid integrates with DNA of the host cell
    - Leaf discs are treated with plant hormones to stimulate shoot and root development

## 6.2 Methods Used in Plant Transgenesis

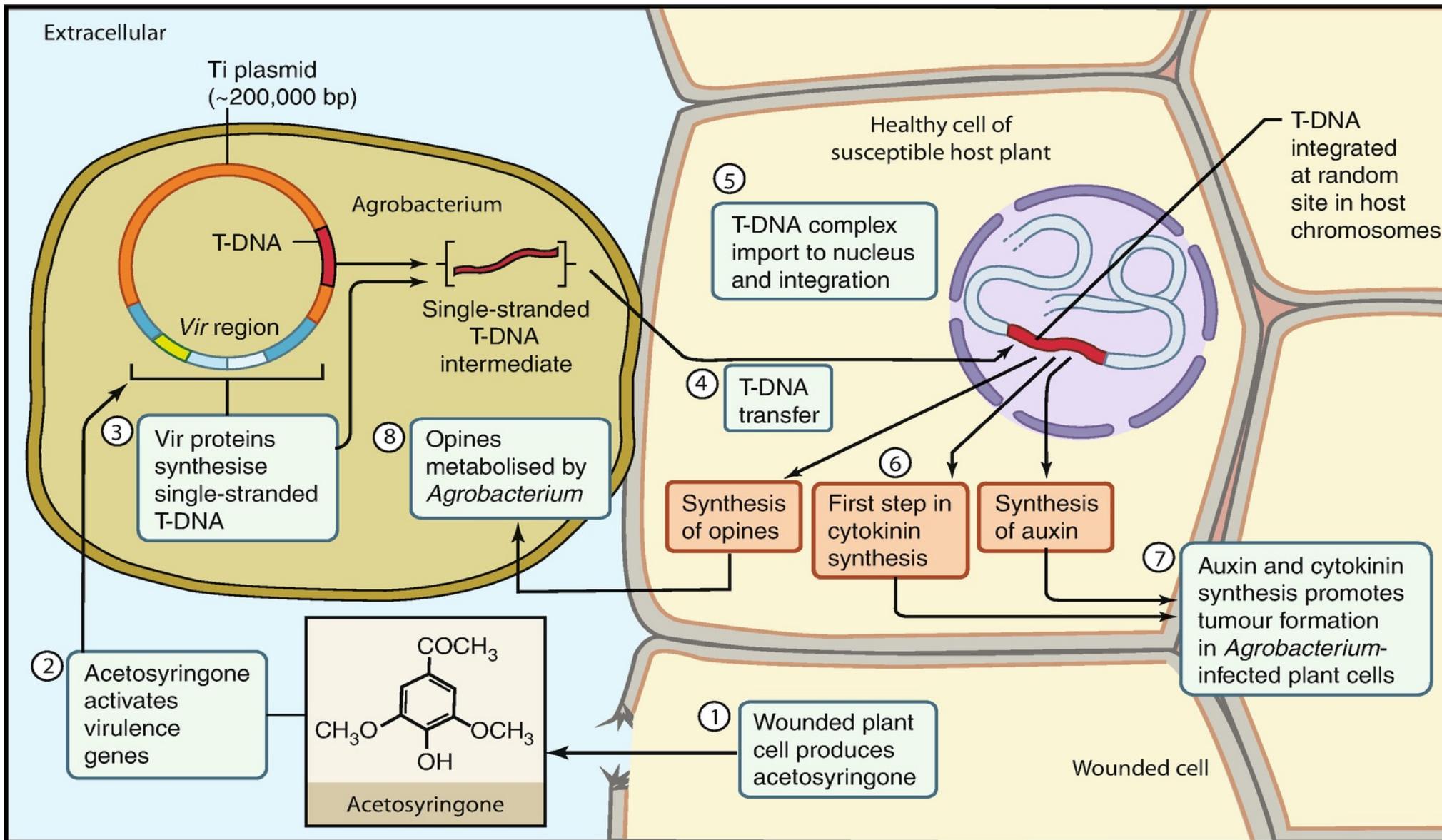


Palcurar et al. (2011) Physiological and Molecular Plant Pathology

# Ti plasmid

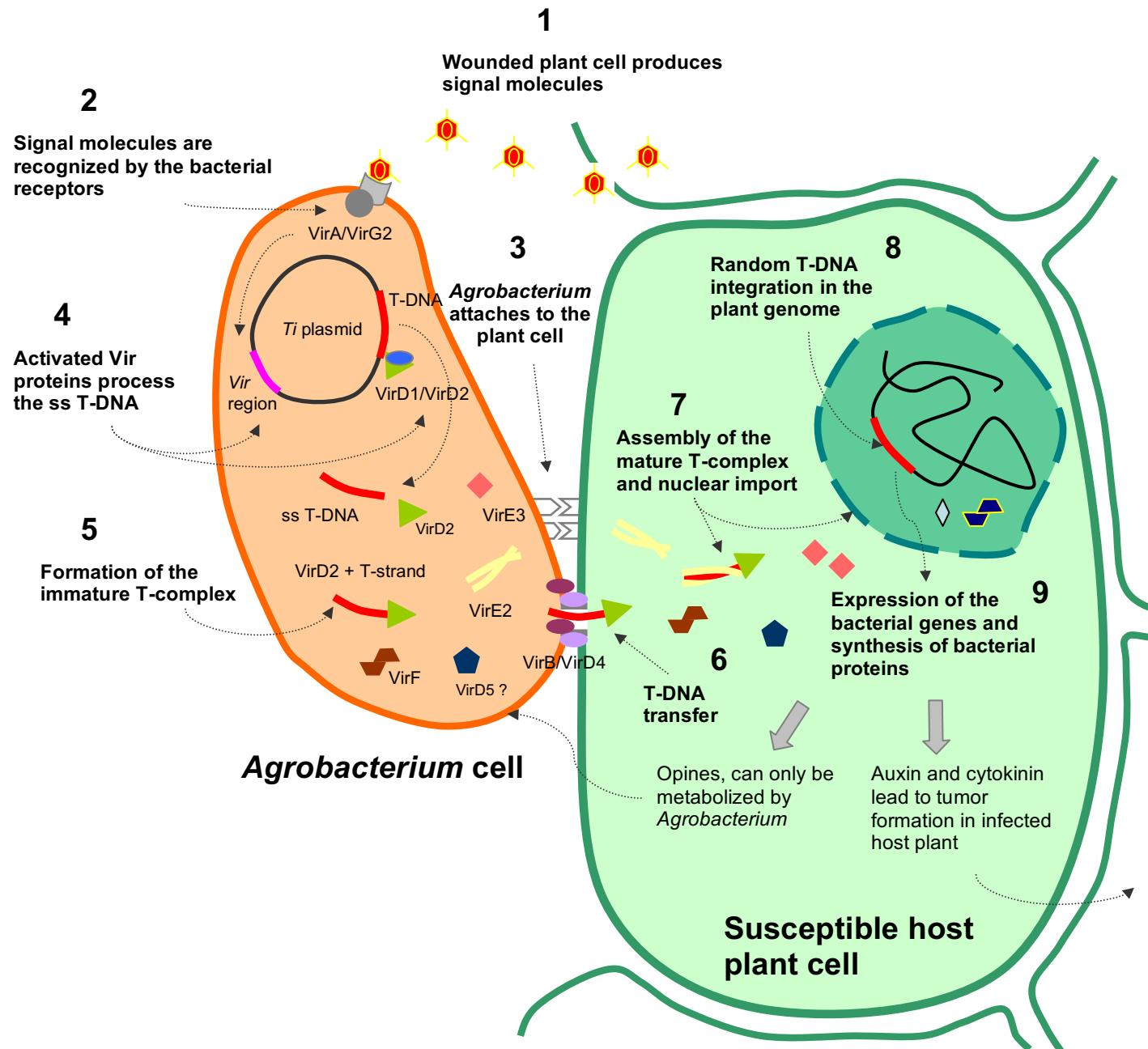


# Infection of a plant cell by *Agrobacterium tumefaciens*

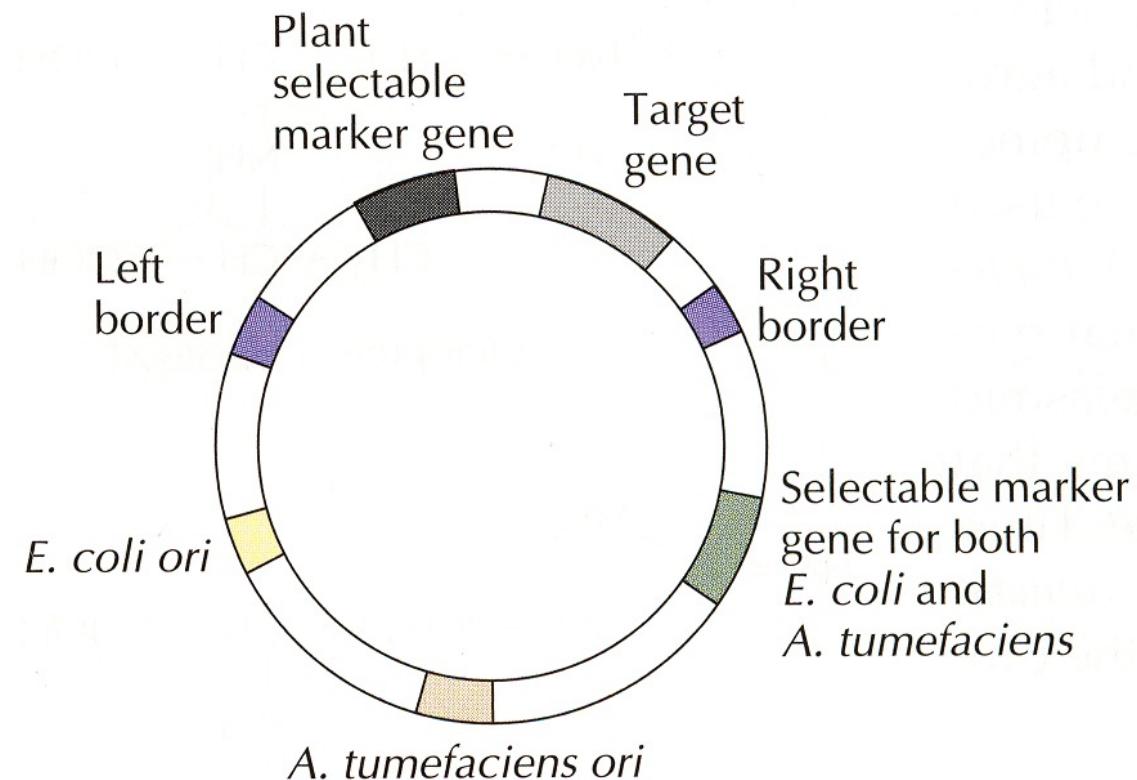


## Function of *vir* genes

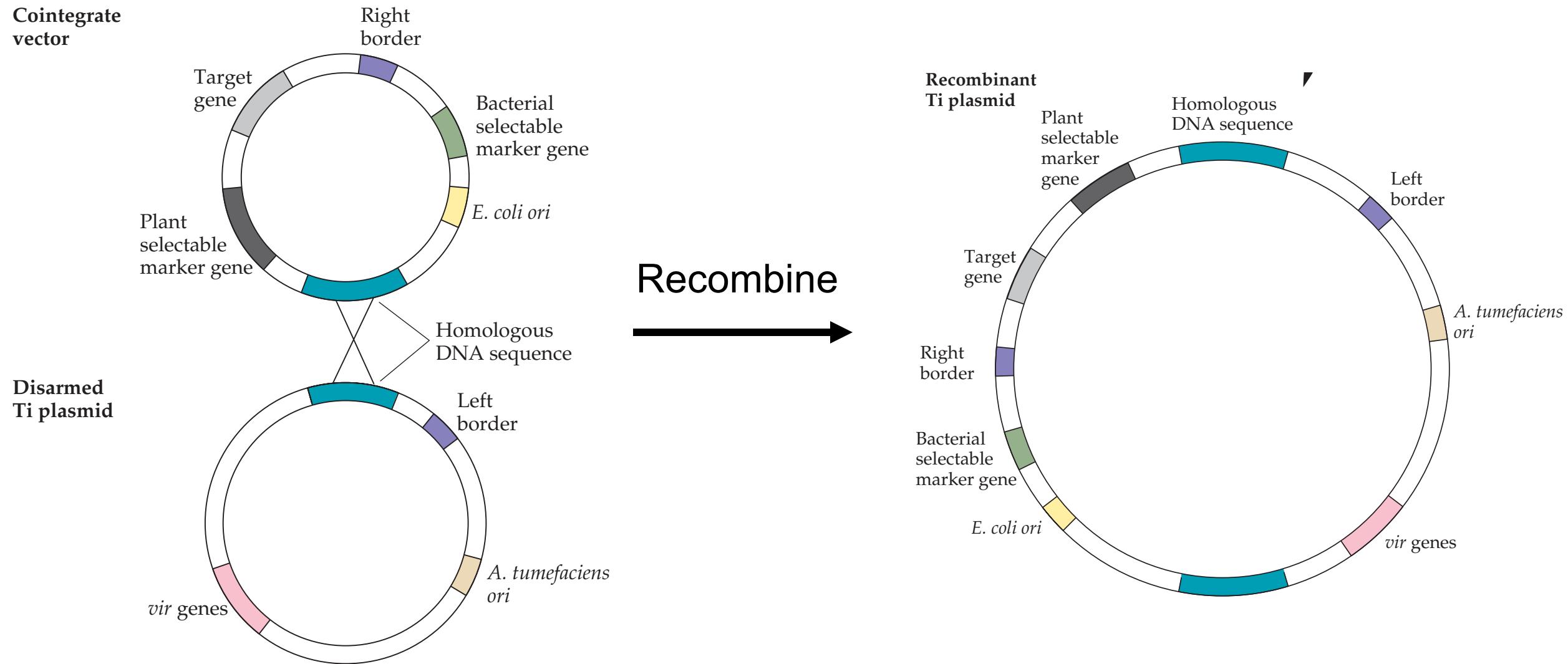
- ***virA***: transports acetosyringone into bacterium, activates *virG* post-translationally (by phosphorylation)
- ***virG***: promotes transcription of other *vir* genes
- ***virD2***: endonuclease/integrase that cuts T-DNA at the borders but only on one strand.
- ***virE2***: can form channels in membranes
- ***virE1***: chaperone for *virE2*
- ***virD2 & virE2***: also have NLSs, gets T-DNA to the nucleus of plant cell
- ***virB***: operon of 11 proteins, gets T-DNA through bacterial membranes



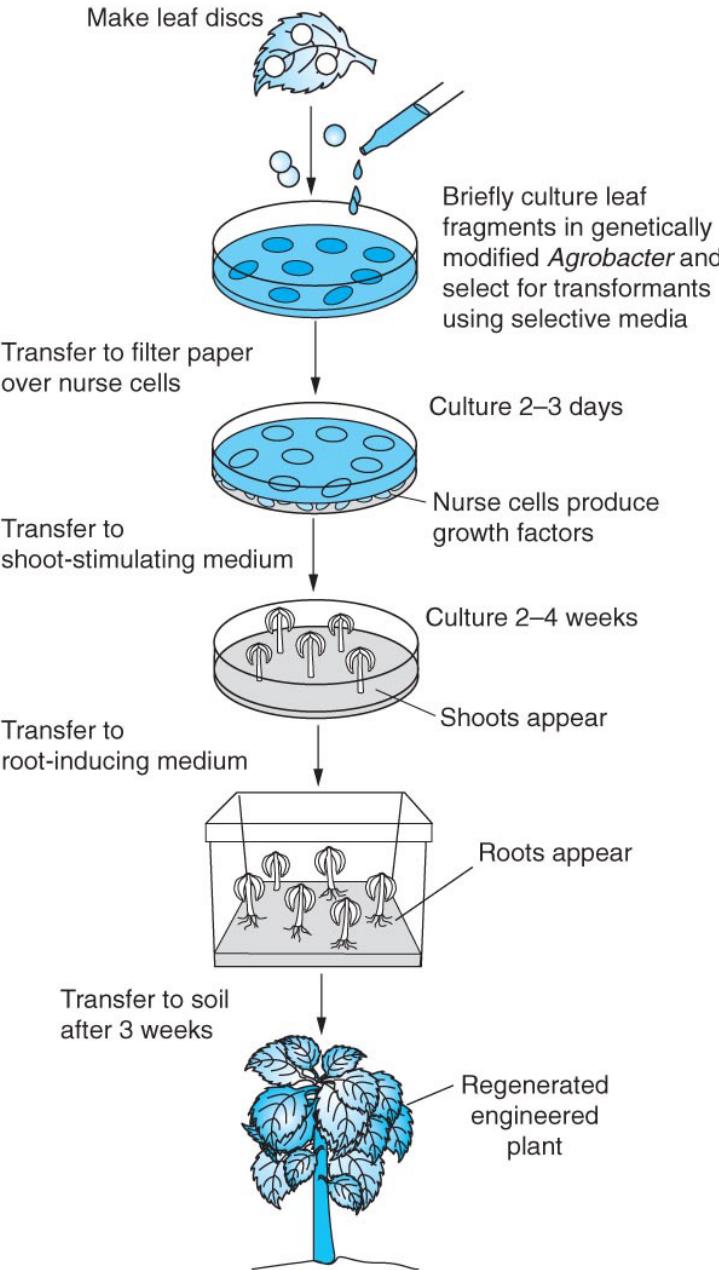
**The binary Ti plasmid system involves using a small T-DNA plasmid (Shown) and a disarmed Ti plasmid (not shown) in *A. tumefaciens***



# The co-integration plasmid system involves using a small T-DNA plasmid and a disarmed Ti plasmid in *A. tumefaciens*



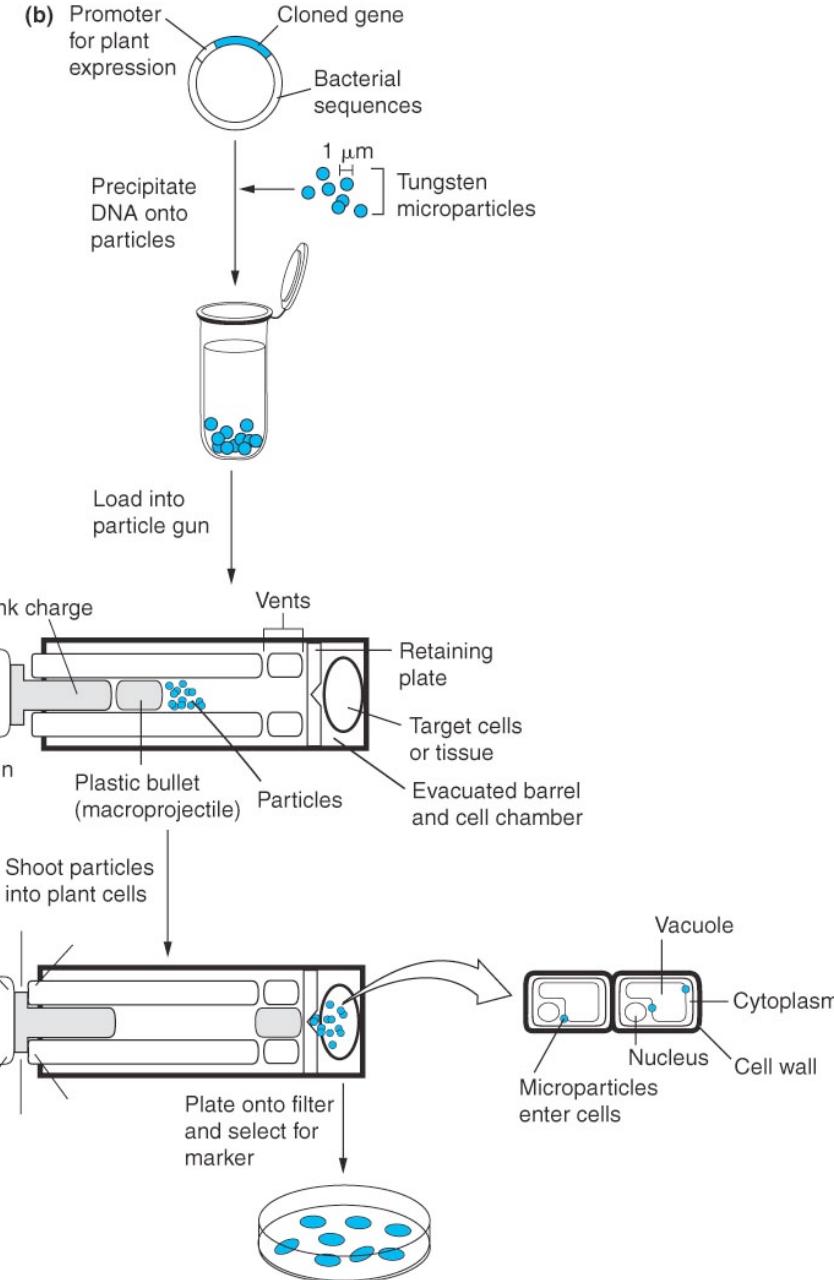
## 6.2 Methods Used in Plant Transgenesis



## 6.2 Methods Used in Plant Transgenesis

- **Cloning**
  - **Gene Guns**
    - Used to blast tiny metal beads coated with DNA into an embryonic plant cell
    - Aimed at the nucleus or the chloroplast
    - Use marker genes to distinguish genetically transformed cells
      - Antibiotic resistance
    - Technique is useful in plants that are resistant to *Agrobacter*

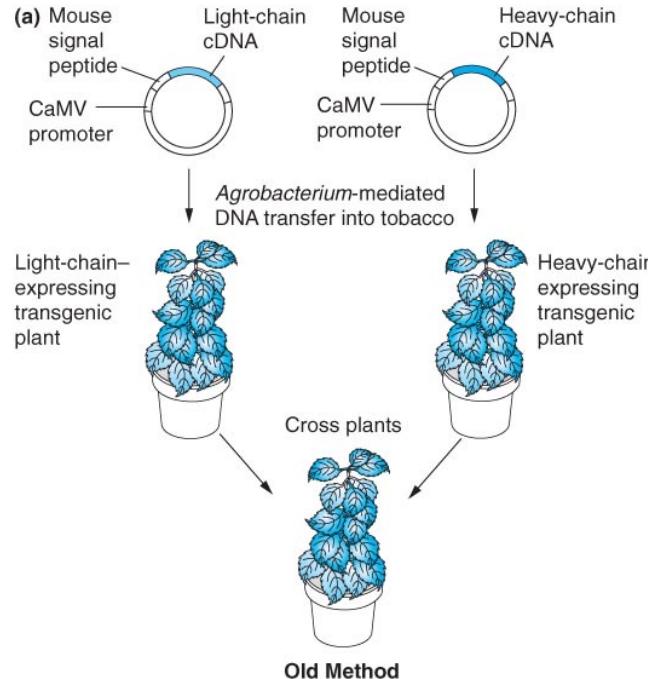
## 6.2 Methods Used in Plant Transgenesis



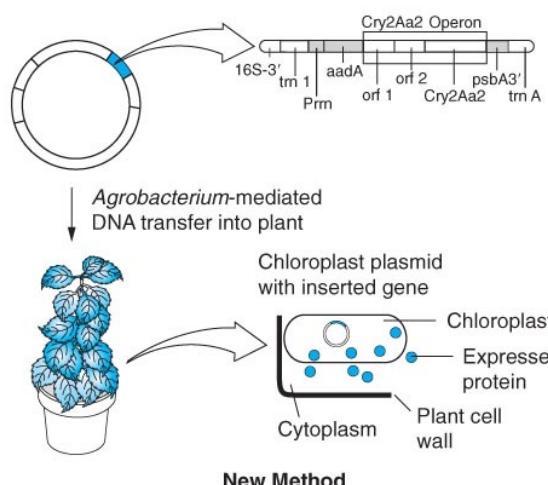
## 6.2 Methods Used in Plant Transgenesis

- **Cloning**
  - **Chloroplast engineering**
    - DNA in chloroplast can accept several new genes at once
    - High percentage of genes will remain active
    - DNA in chloroplast is completely separate from DNA released in pollen
      - no chance that transformed genes will be carried on wind to distant crops

## 6.2 Methods Used in Plant Transgenesis



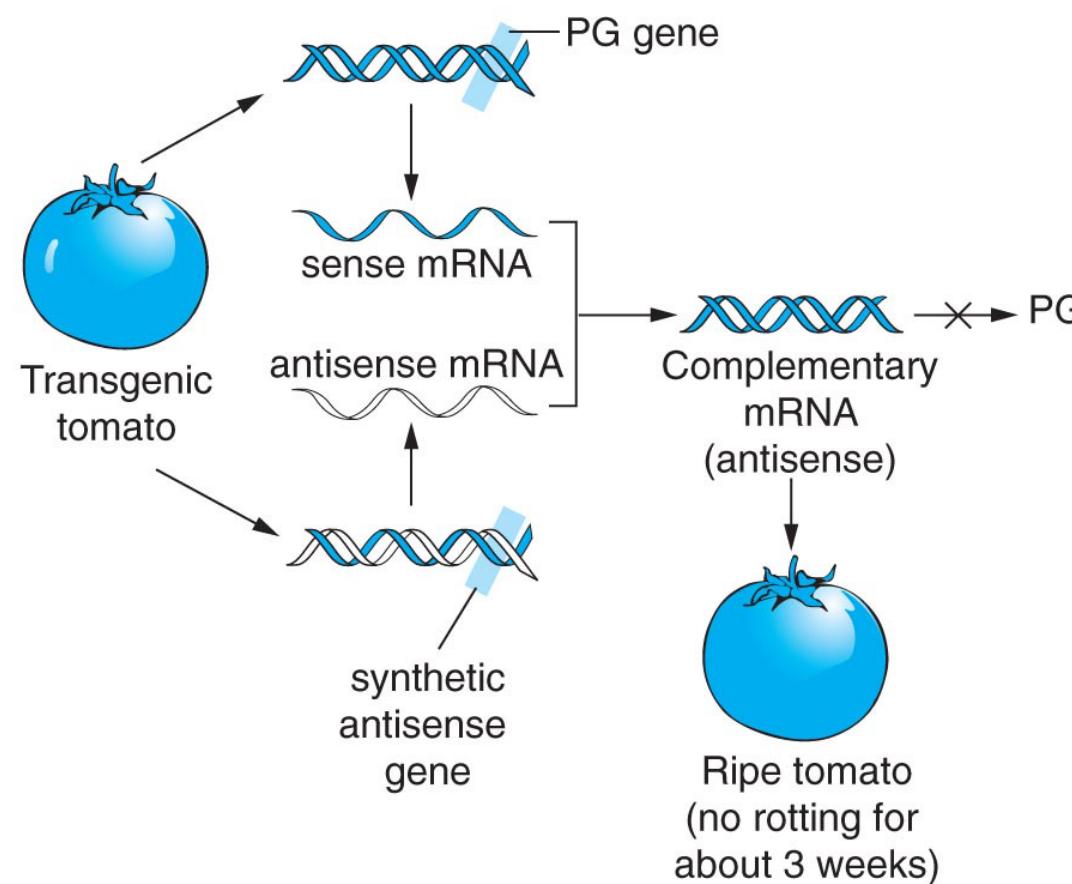
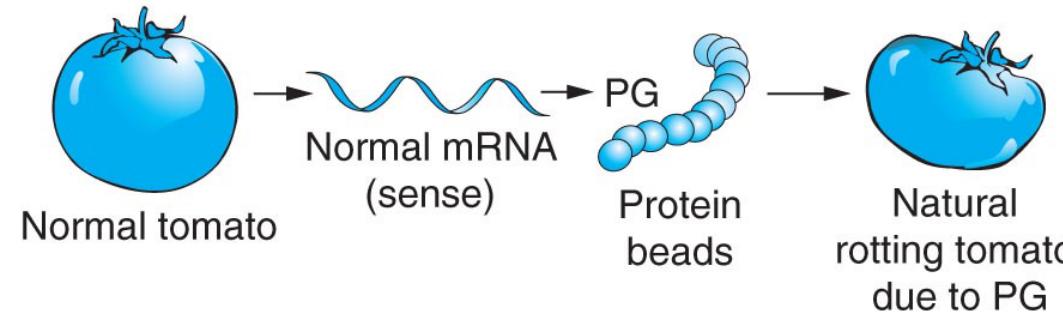
(b) Engineered Ti plasmid from *Agrobacter*



## 6.2 Methods Used in Plant Transgenesis

- **Cloning**
  - **Antisense technology**
    - Process of inserting a complementary copy of a gene into a cell
    - Gene encodes an mRNA molecule called an antisense molecule
    - Antisense molecule binds to normal mRNA (sense molecule) and inactivates it
    - Example is *Flavr Savr* tomato

## 6.2 Methods Used in Plant Transgenesis



# Applications of CRISPR–Cas in agriculture and plant biotechnology

- Gene targeting technology in plants relies on HDR, which enables precise genome editing. However, the low editing efficiency achieved with HDR has limited its application in plants.
- Deaminase-mediated base editing and reverse transcriptase-mediated prime editing technologies are alternative genome editing technologies
  - These technologies do not involve DSB formation
  - Do not require donor DNA
  - These CRISPR–Cas-based tools induce precise sequence editing and are more efficient than HDR in plants.

# **Applications of CRISPR–Cas in agriculture and plant biotechnology**

- CRISPR– Cas-based molecular platforms used for precise genome editing:
  1. Cytosine base editing (CBE)
  2. Adenine base editing (ABE)
  3. Dual base editing, saturated targeted endogenous mutagenesis editor (STEME)
  4. CBE-based precise DNA deletion. The APOBEC–Cas9 fusion-induced deletion system (AFID)
  5. Prime editing

# Applications of CRISPR–Cas in agriculture and plant biotechnology

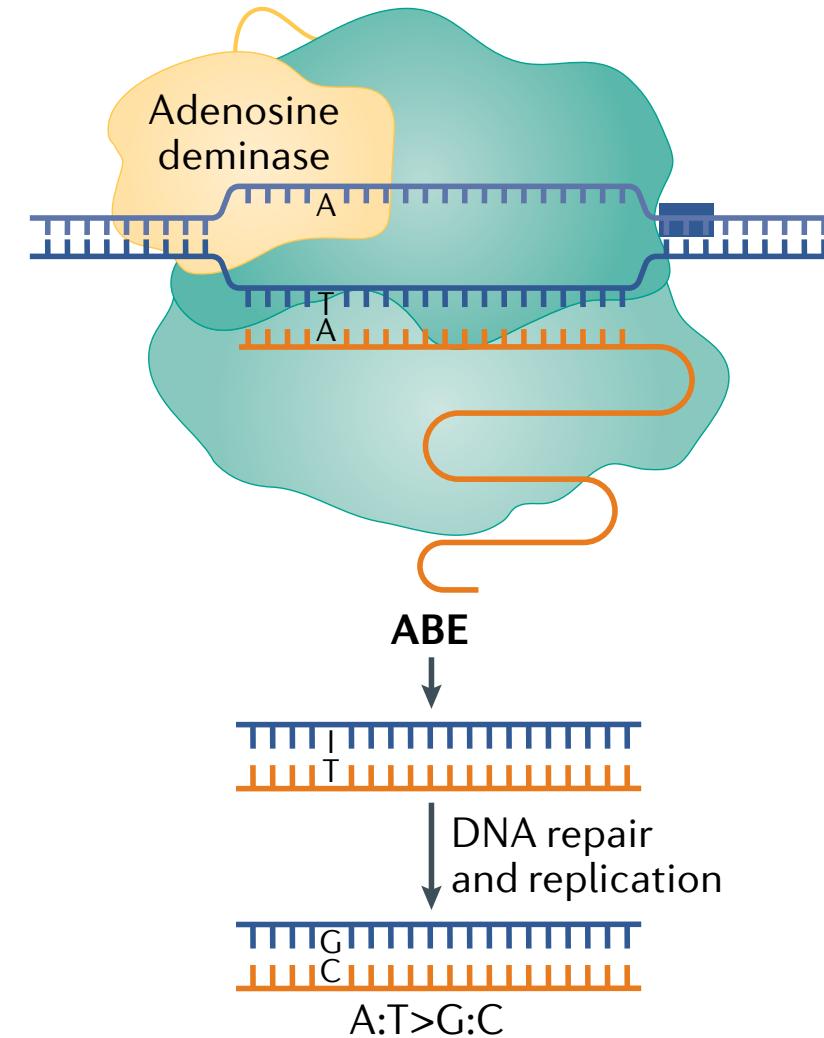
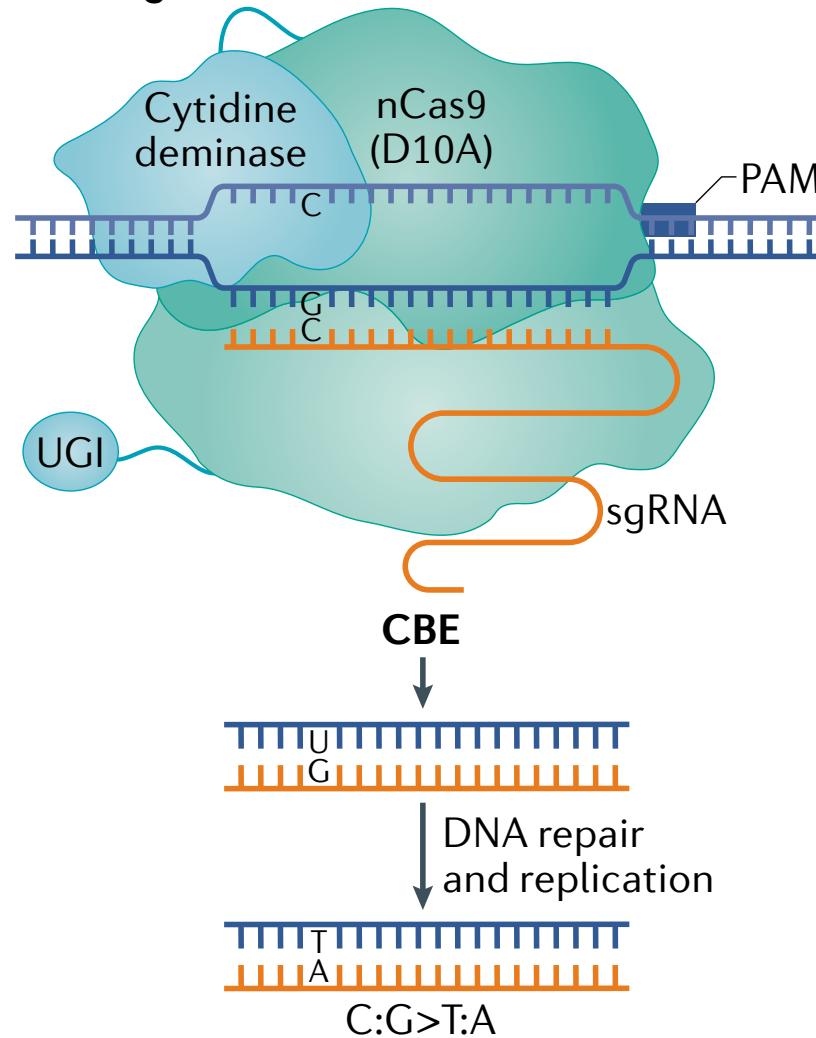
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# Cytosine base editing (CBE)

- CBE is composed of:
  1. Cas9 nickase (nCas9): catalytically defective (Mutation D10A) Cas9 variants that cut only one strand of the target DNA
  2. Cytidine deaminase: deaminates cytidines to uridines in the non-target strand
  3. Uracil DNA glycosylase (UDG) inhibitor (UGI): prevents UDG from deaminating cytidines to apyrimidinic (AP) sites
- CBE introduces C:G>T:A base transitions directly into DNA sites targeted by single guide RNA (sgRNA)
- When nCas9 (D10A) induces a nick on the target strand, the DNA mismatch repair pathway is activated and preferentially resolves the U:G mismatch into the desired U:A, and following DNA replication a T:A product, thereby generating a C:G>T:A base transition.

# Cytosine base editing (CBE) & Adenine base editing (ABE)

Base editing

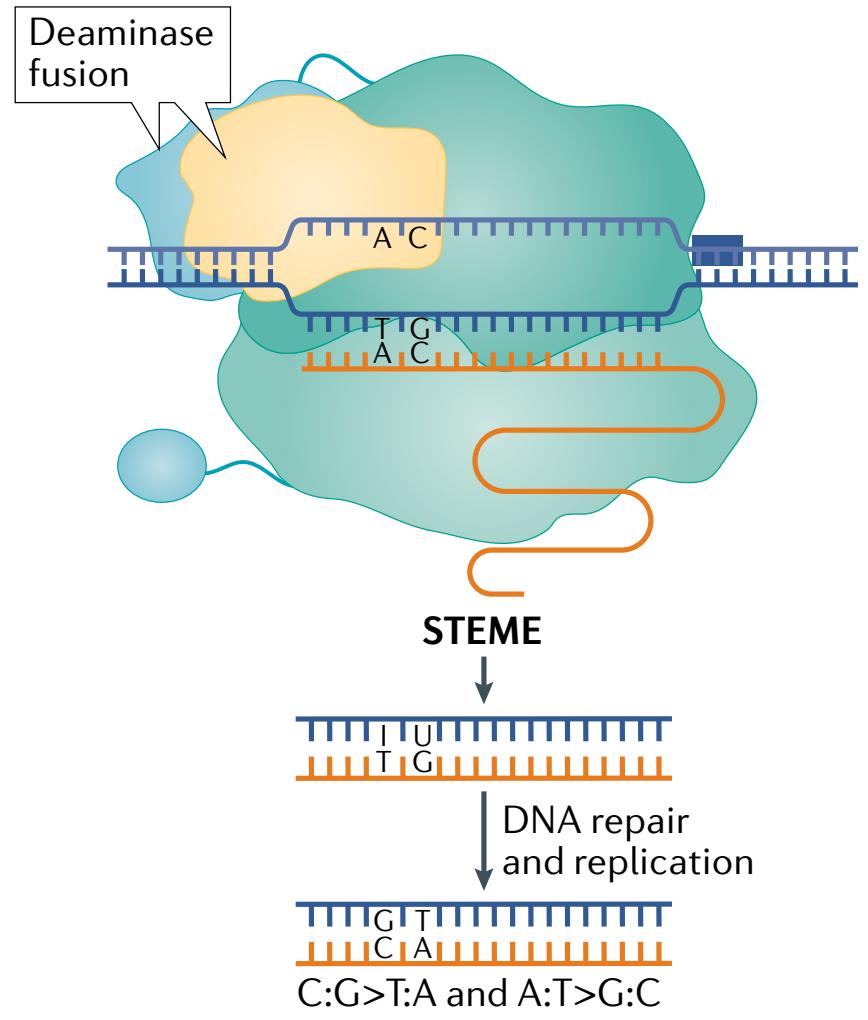


# Adenine base editing (ABE)

- ABEs expand base editing to include A:T>G:C substitutions using
  1. Cas9 nickase (nCas9): catalytically defective (Mutation D10A) Cas9 variants that cut only one strand of the target DNA
  2. Adenosine deaminase: deaminates adenosines to inosines, which are recognized as guanosines by DNA polymerase during DNA repair and replication.
- Although there is no natural adenosine deaminase for deaminating ssDNA, such an enzyme has been evolved from *Escherichia coli* tRNA-specific adenosine deaminase (ecTadA)

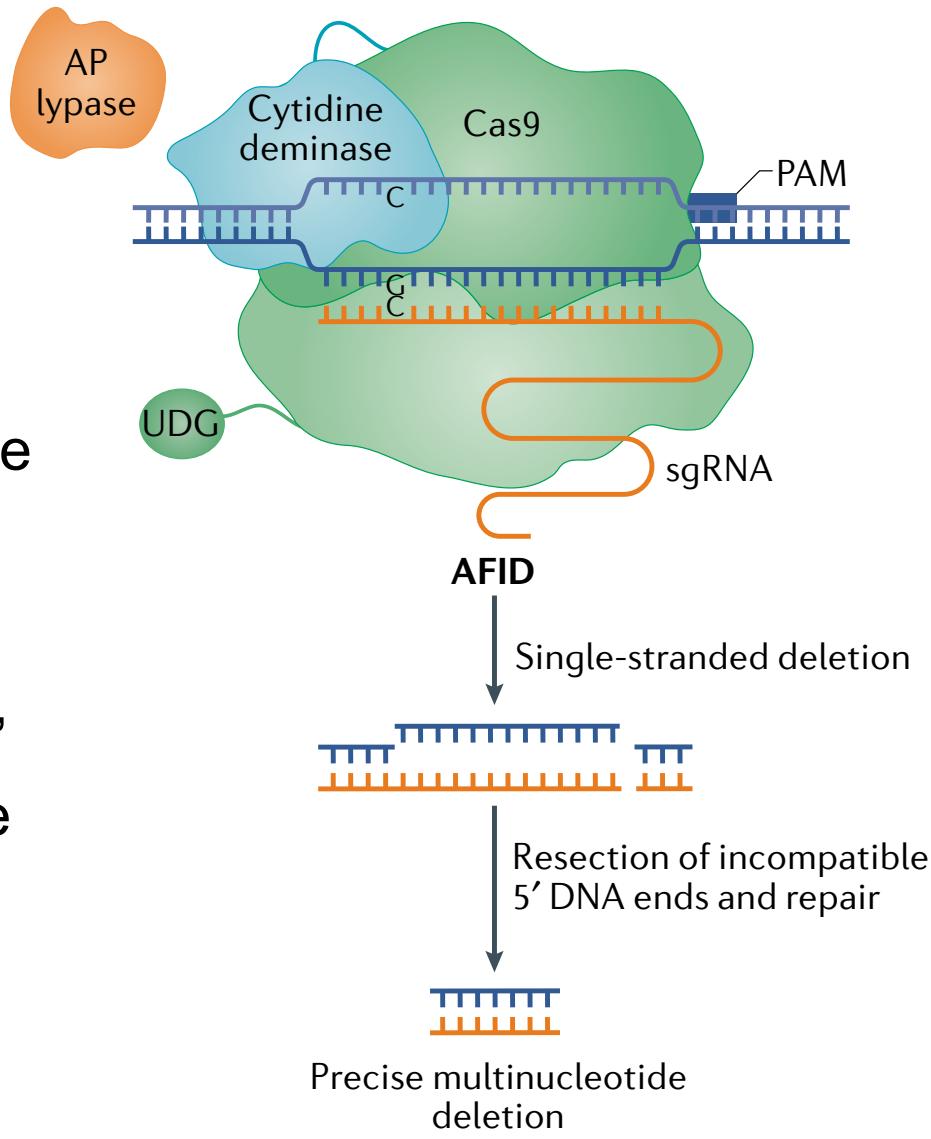
# Dual base editing (saturated targeted endogenous mutagenesis editor, STEME)

- A cytosine and adenine dual base editor has been created to simultaneously perform C:G>T:A and A:T>G:C editing using a single sgRNA19 and the following proteins:
  1. Cytidine deaminase
  2. Adenosine deaminase (ecTadA–ecTadA\*)
  3. nCas9 (D10A)
  4. UGI fusion
- The STEME system deaminates cytidines to uridine and adenosines to inosines in the editing window of the protospacer, and these are then copied by DNA repair and replication
- These dual base editors facilitate directed evolution of endogenous plant genes *in situ*.
- STEME might also be used to change cis elements in regulatory regions and genome-wide screening in a high-throughput manner in plants.



# CBE-based precise DNA deletion

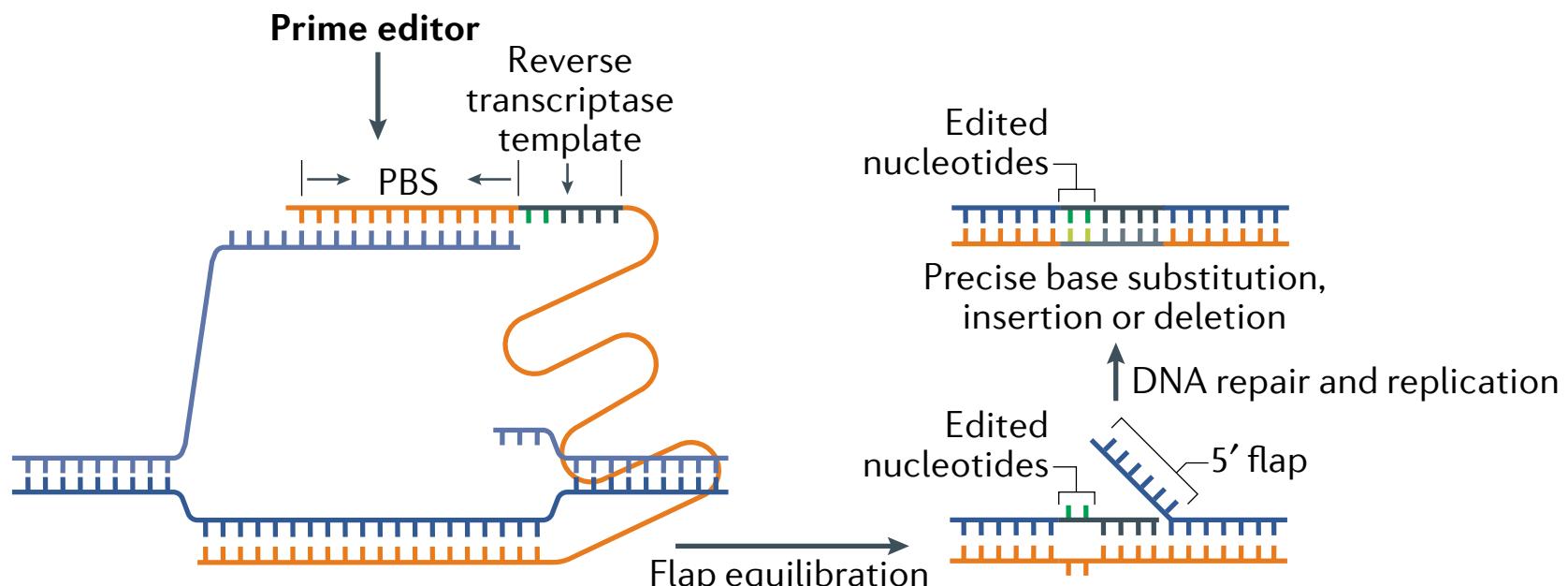
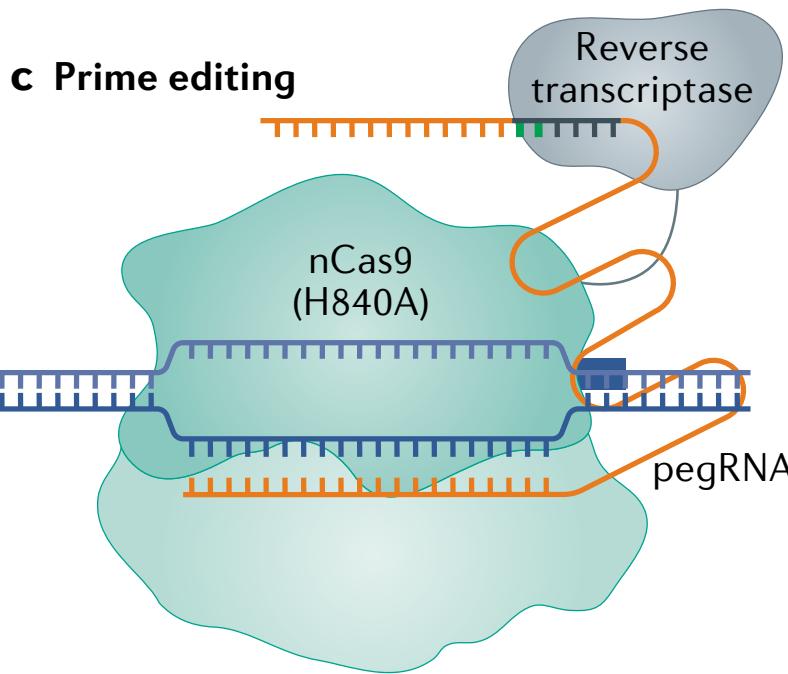
- Using the following proteins:
  1. Cas9
  2. Cytidine deminase
  3. Uracil DNA glycosylase
  4. Apyrimidinic lyase
- In CBEs, uridine generated by deaminating cytidines are preserved by the UGI, which inhibits the activity of the cellular UDG.
- The opposite situation, in which UDG is overexpressed, should trigger base excision repair and lead to excision of the uridines and generation of AP sites, which can be nicked by AP lyases
- The combination of such a nick with the formation nearby of a DSB by Cas9 should produce a specified and precise deletion between the deaminated cytidine and the Cas9 cleavage site



# Prime editing

- This technology can produce all 12 kinds of base substitutions, precise insertions of up to 44 bp, deletions of up to 80 bp and combinations of these edits.
- Prime editor uses a
  1. nCas9 (H840A): can cut the non-target strand
  2. Reverse transcriptase
  3. Prime editing guide RNA (pegRNA)
- The pegRNA is composed of a reverse transcriptase template and a primer-binding site at the 3' end of the sgRNA.
- The reverse transcriptase template contains the genetic information for the desired mutations, and the primer-binding site pairs with the nCas9 (H840A)-nicked ssDNA strand, thereby priming reverse transcription and incorporating the genetic information from the reverse transcriptase template into the genome
- This is then followed by equilibration between the edited 3' flap and the unedited 5' flap, ligation and repair, which generate the desired edit.
- As prime editor generates base substitutions and short insertions and deletions at a relatively wide range of positions

# Prime editing



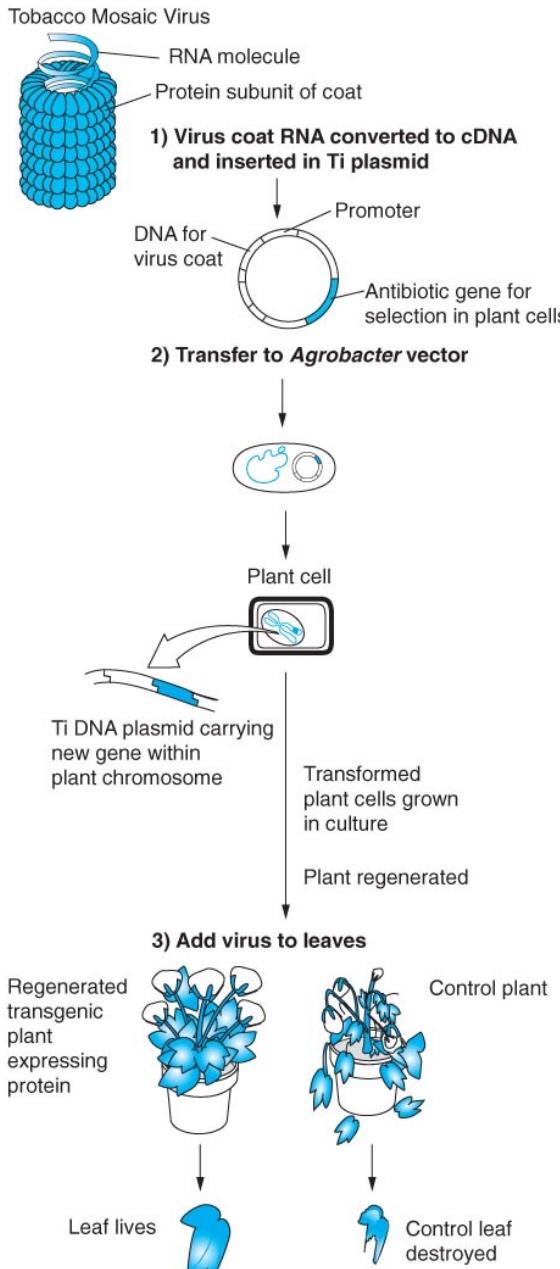
## 6.3 Practical Applications in the Field

- Vaccines for plants
- Genetic pesticides
- Herbicide resistance
- Enhanced nutrition
- The future: from pharmaceuticals to fuel

## 6.3 Practical Applications in the Field

- Vaccines for Plants
  - Vaccine is encoded in a plant's DNA
  - For example, a gene from Tobacco Mosaic Virus (TMV) inserted into tobacco plants
    - Protein produced from the viral gene stimulates the plant's immune system
    - Plant is invulnerable to virus

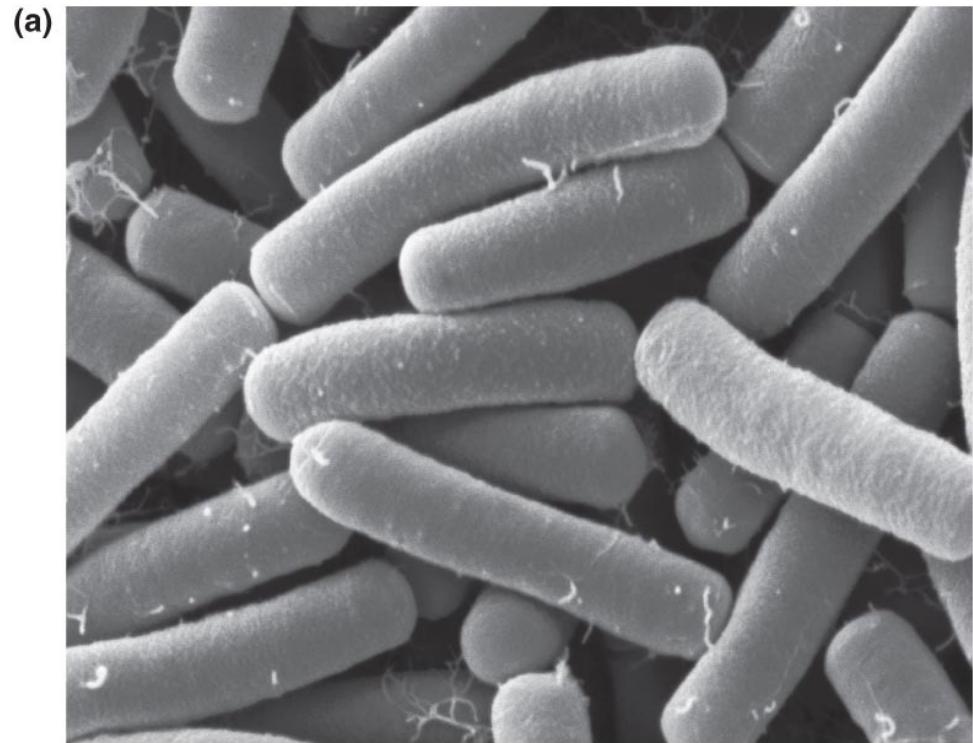
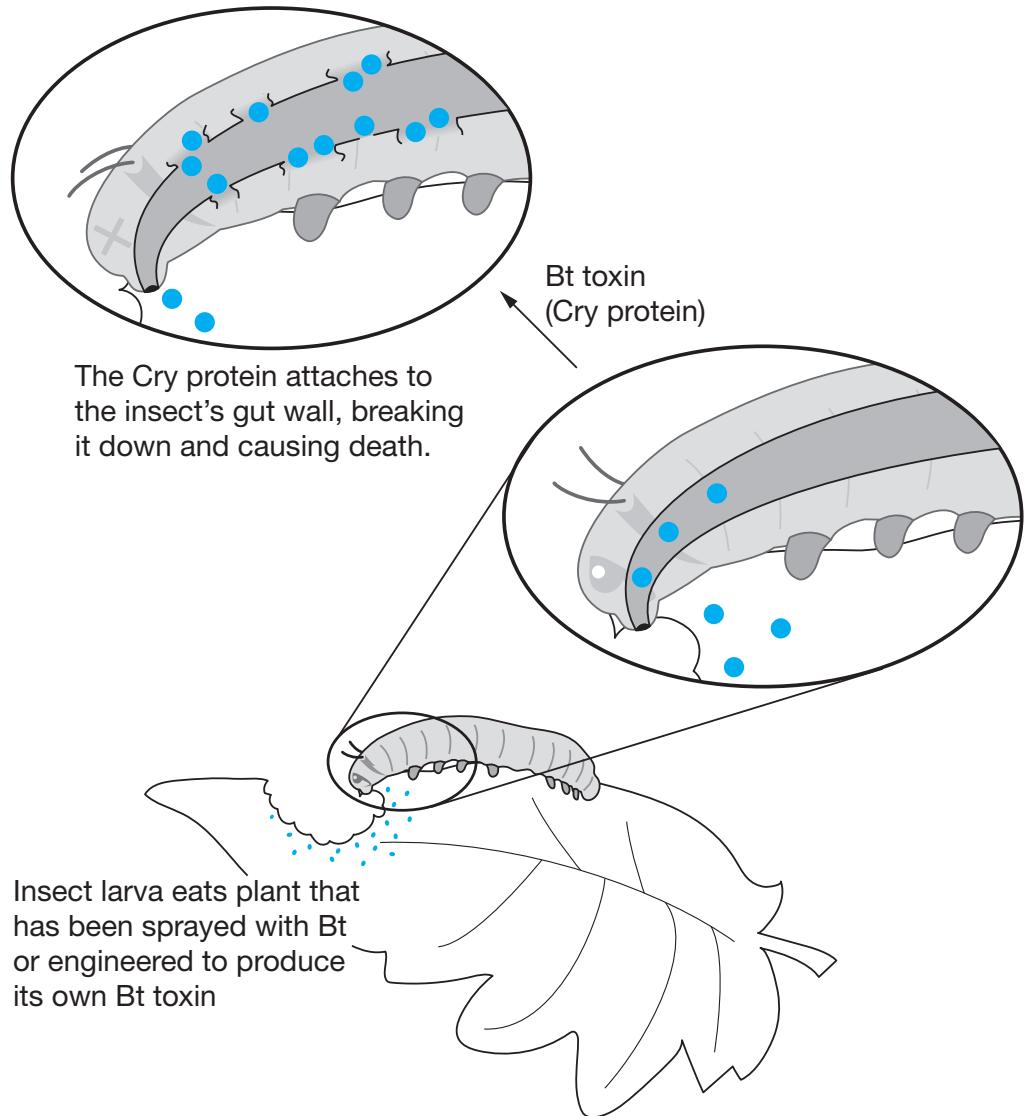
## 6.3 Practical Applications in the Field



## 6.3 Practical Applications in the Field

- Genetic Pesticides
  - *Bacillus thuringiensis* (Bt) is a bacterium that produces a protein that kills harmful insects and their larvae
  - It has been used as a natural pesticide for over 50 years
  - Bt genes can be inserted into a plant's DNA
    - Creates a built-in defense against certain insects
  - Controversy surrounding Monarch butterflies

## 6.3 Practical Applications in the Field



## 6.3 Practical Applications in the Field

- Safe Storage
  - Millions of dollars are lost every year to insect infestations of crops during storage
  - Transgenic corn that expresses avidin is highly resistant to pests during storage
    - Avidin blocks the availability of biotin, a vitamin required by insects to grow

## 6.3 Practical Applications in the Field

- Herbicide Resistance
  - Traditional weed killers kill desirable plants along with the weeds.
  - Can genetically engineer crops to be resistant to common herbicides
  - Allows farmers to control weeds with chemicals that are milder and more environmentally friendly than typical herbicides

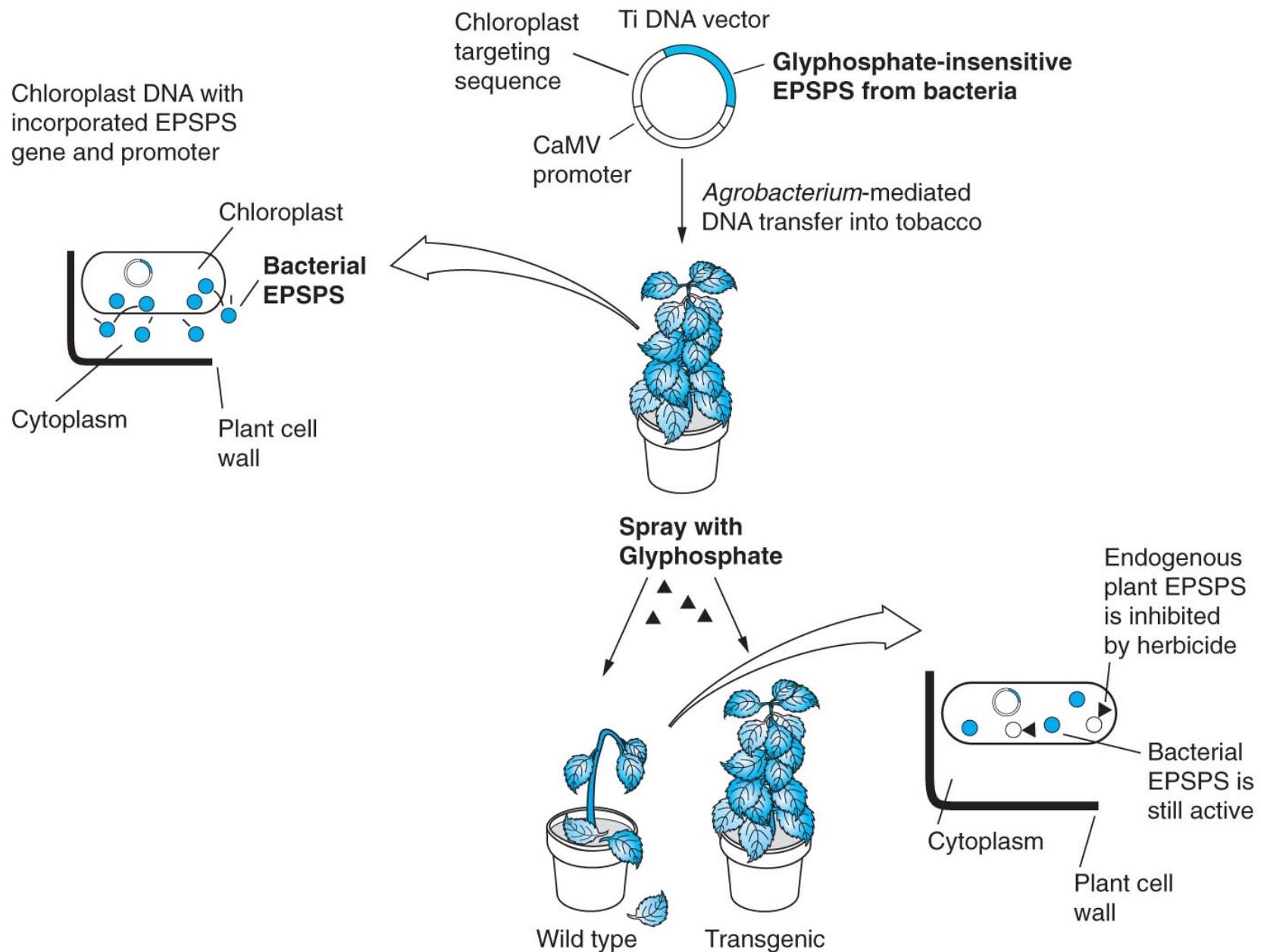
## 6.3 Practical Applications in the Field

- Herbicide Resistance
  - One example is resistance to glyphosate, which blocks the enzyme EPSPS, which functions in a key biochemical pathway
  - Crops that transgenically produce an alternative enzyme not affected by glyphosate have been developed
  - Most soybeans grown today contain herbicide resistance genes
  - Unfortunately, glyphosate resistant weeds have evolved

## 6.3 Practical Applications in the Field

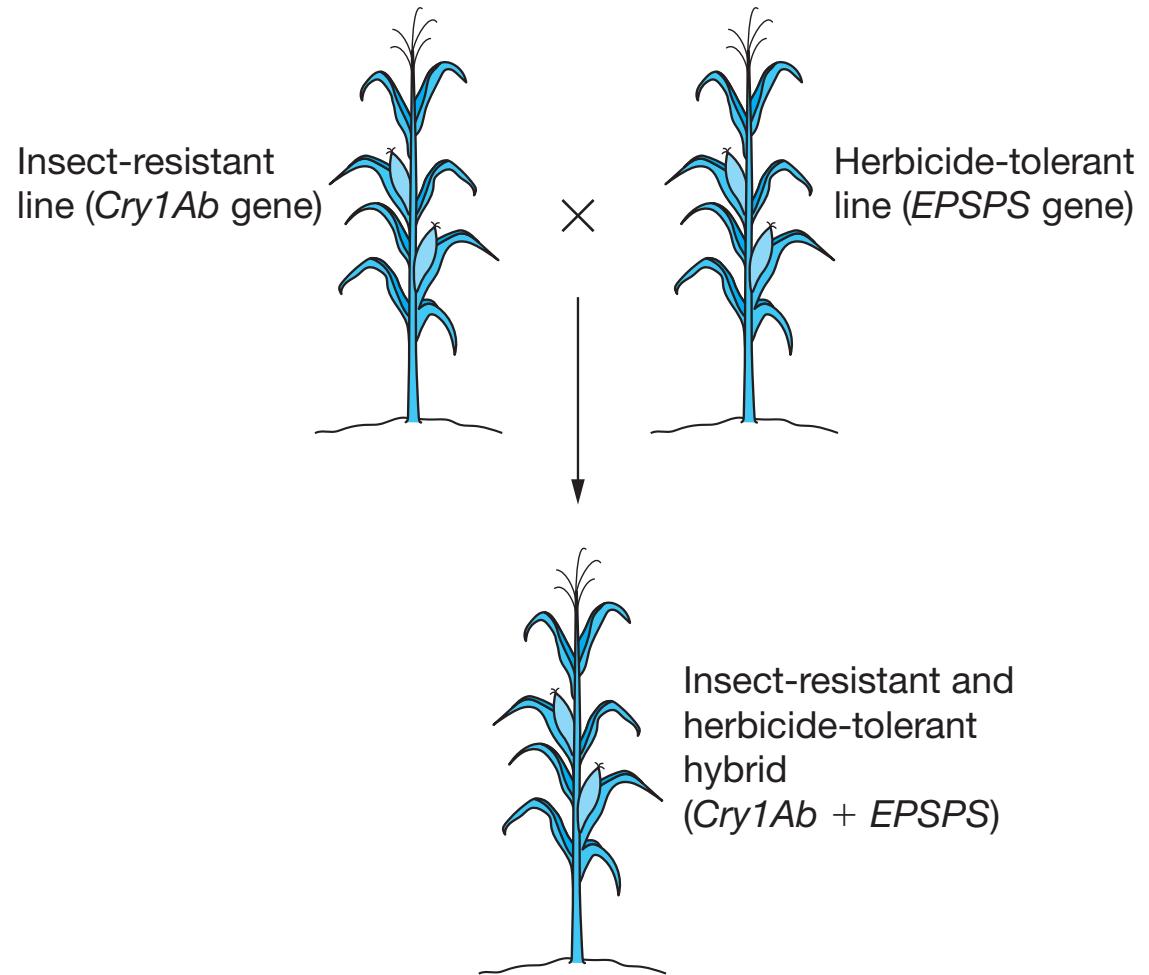
- Glufosinate is a naturally occurring broad-spectrum herbicide produced by *Streptomyces* soil bacteria
- Works by interfering with the synthesis of glutamine and with ammonia detoxification.
- Plants have been engineered to resist this herbicide by using two genes first isolated from *Streptomyces* bacteria:
  1. "bialaphos resistance" or "bar" gene
  2. "phosphinothricin acetyltransferase" or "pat" gene
- Crops that are resistant to the herbicide glufosinate have been engineered for resistance to multiple herbicides, permitting growers to use a mixed group of two to four different chemicals that combat herbicide resistance.

## 6.3 Practical Applications in the Field



# Gene Stacking

- Gene stacking
  - For both conventional breeders and genetic engineers, the goal is often to move more than one desired gene into a plant
  - Such combinations of two or more inserted genes are called gene stacks.
  - A common way to stack genes is to cross parental lines that each have one of the genes, then select offspring that inherit both. This process is called hybrid stacking.



## 6.3 Practical Applications in the Field

- Enhanced Nutrition
  - Golden rice has been engineered to contain large amounts of beta carotene, which the body converts to vitamin A
  - However, as of 2011, no farmers have planted golden rice due to concerns voiced by environmental organizations

## 6.3 Practical Applications in the Field

- The Future of Plant Biotechnology in Pharmacology
  - Engineered crops could be used as miniature factories for producing pharmaceutical proteins and industrial chemicals (called “biopharming”)
  - Used to grow medicines
    - Inexpensive edible vaccines that do not require refrigeration
    - "Molecular pharming" of phytochemicals that produce chemicals useful to human health
      - Phytochemicals, antibodies, blood products, cytokines, growth factors, hormones and recombinant enzymes.

## 6.3 Practical Applications in the Field

- The Future of Plant Biotechnology: Fuels
  - Biofuels are fuels produced from biological products, such as plants
  - The need for alternatives to fossil fuels is increasing
  - However, it takes 7 gallons of gasoline to produce 10 gallons of kernel corn ethanol
- In the future, want to convert plant wastes, such as husks and stems, to sugars that can be converted to ethanol
- Algae may be the next alternative to petroleum