Microbial proteins in agricultural biotechnology

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Selective breeding vs. transgenics

- **Selective breeding: DNA mixing between organisms that can naturally interbreed: ie. individuals of SAME SPECIES**
- **Limitations of selective breeding: slow progress, hard to separate desirable/undesirable traits, species barriers**
- **Transgenic: an organism carrying foreign DNA, where 'Foreign' defined as derived from ANOTHER SPECIES**

~ 15,000 years

The power of selective breeding should not be underestimated !

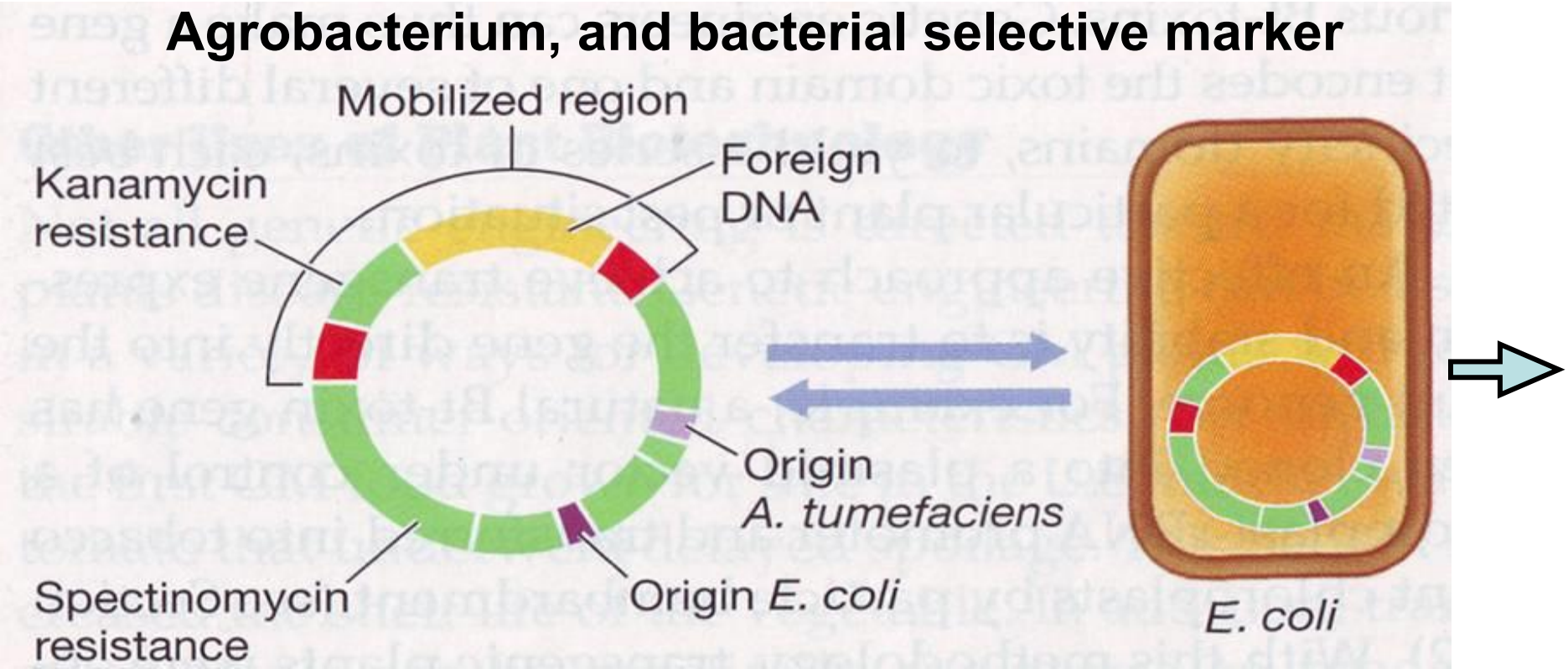
Possible benefits of transgenic plants and animals

- **Despite success of modern agriculture, serious problems still exist, or have been caused by intensive farming:**
  - **productivity doesn't keep pace with population growth**
  - **ecological damage caused by pesticides and fertilizers**
  - **drought and salinity → less arable land**

- **Can biotechnology provide solutions to these problems?**

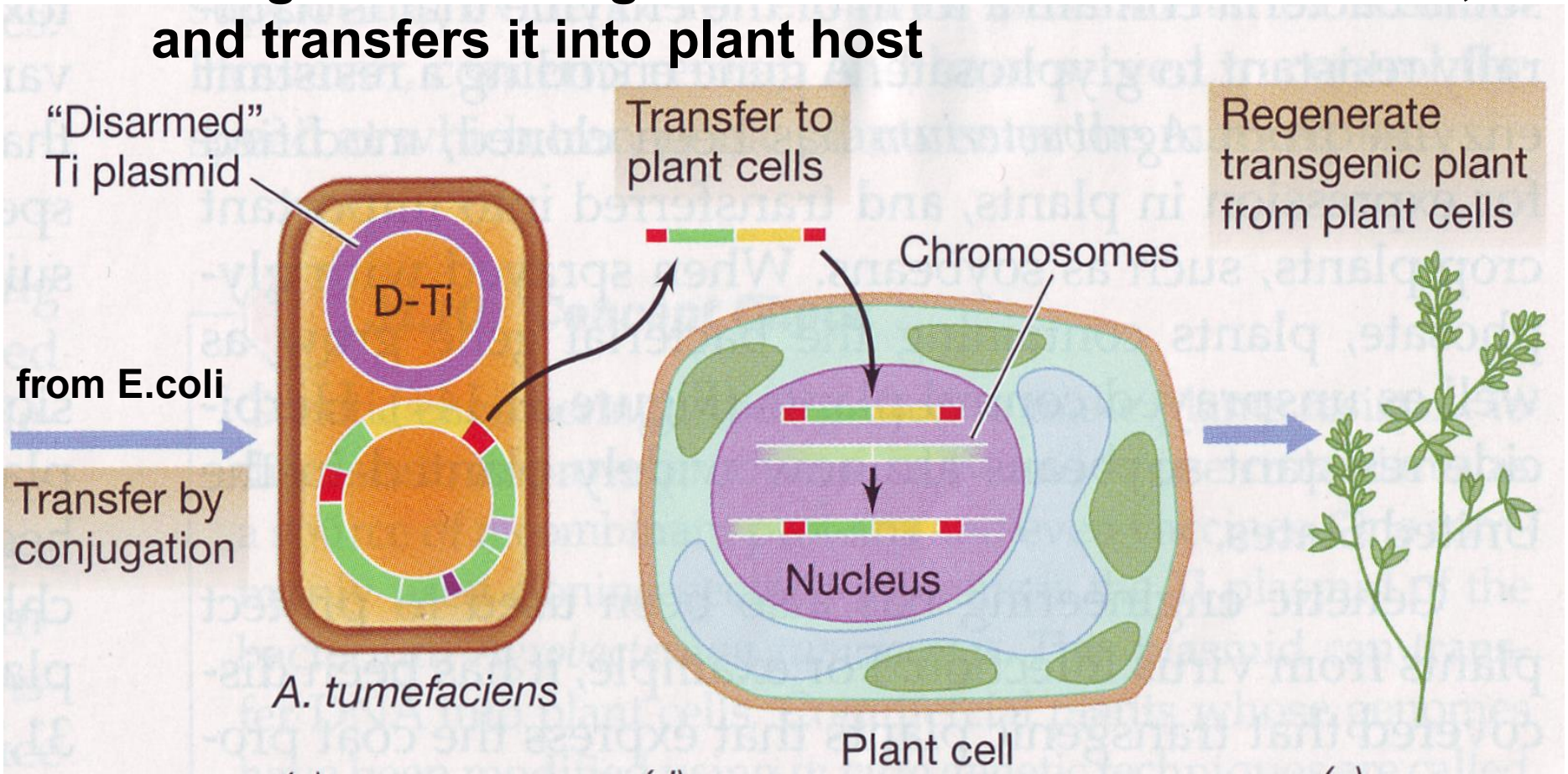
Plant transgenic methods – *Agrobacterium*

- **Engineering with *Agrobacterium* uses a 2-plasmid system**
- **First plasmid is an *E.coli/Agrobacterium* shuttle vector:**
  - **mobilized region: foreign DNA and plant selective marker flanked by recombinogenic ends of T-DNA**
  - **backbone: origins of replication for E.coli and Agrobacterium, and bacterial selective marker**



Plant transgenic methods – *Agrobacterium*

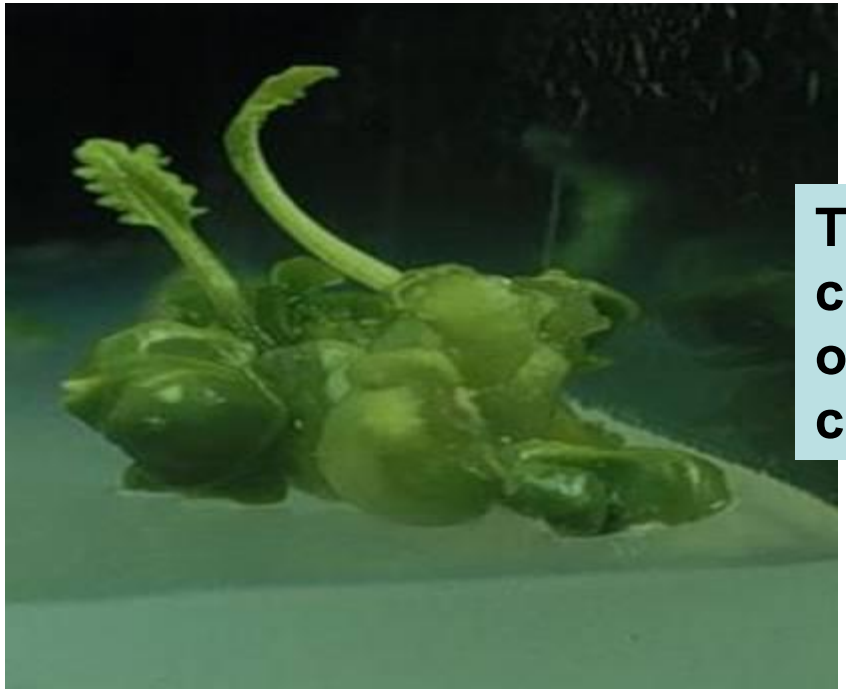
- **Second component is disarmed Ti plasmid (D-Ti):**
  - **lacks T-DNA and virulence functions (opine synthesis), but still has T-DNA transfer functions**
  - **recognises the engineered T-DNA on shuttle vector, and transfers it into plant host**



Plant transgenic methods – *Agrobacterium*

- **Integration of T-DNA into plant genome: illegitimate recombination mediated by DNA repair enyzmes**
- **Insertion site of T-DNA is semi-random; integration may cause deletions, inversions or duplications of target site**

→ *Agrobacterium*-mediated transformation suffers from some of the same disadvantages as animal transgenics

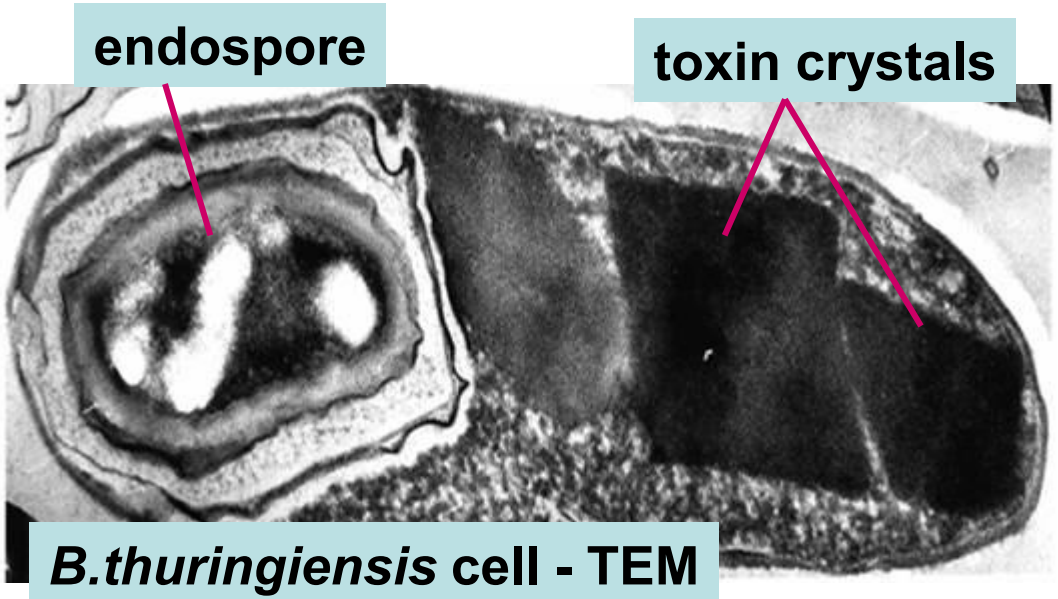


Transgenic plants initially grown as calluses (undifferentiated cell mass) on selective agar, then hormone addition causes tissue differentiation

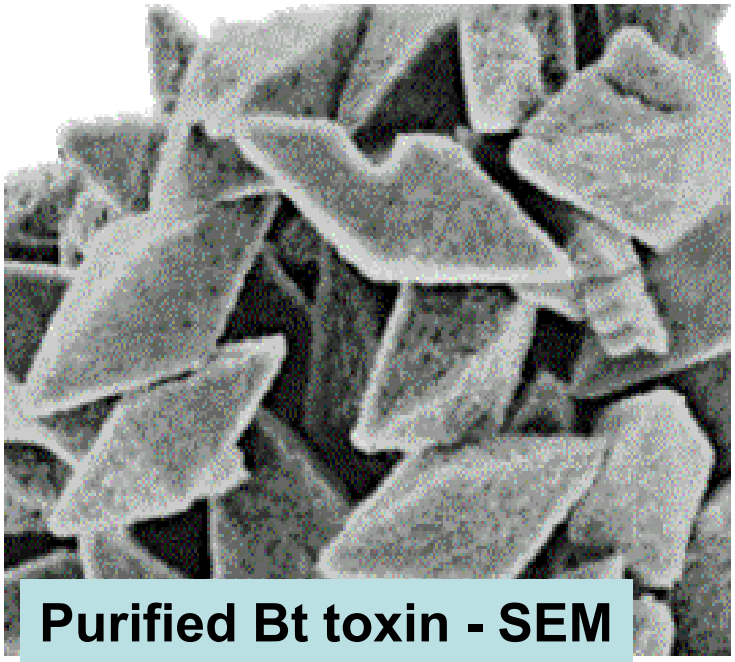
Microbial proteins in plants: Bt toxin

- ***Bacillus thuringiensis*** **(Bt): widely used biological control agent on diverse food and ornamental crops.**

- **Gram positive aerobic spore-forming bacteria**
  - **Produce potent insecticide (Bt toxin)**
  - **Protein toxin accumulates as crystal in cells**
  - **Toxin is highly specific to insects**



B.thuringiensis **cell - TEM**



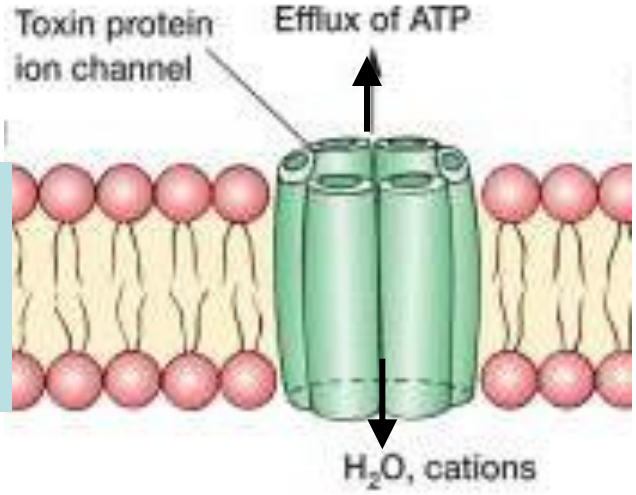
Purified Bt toxin - SEM

Microbial proteins in plants: Bt toxin

- **Bt toxin long used as 'natural' insecticide, acceptable to organic gardeners –spray contains Bt spores, cells, toxin**

- **Advantages:**
  - **specific to certain insect groups, especially beetles (*Coleoptera*) and moth/butterfly larvae (*Lepidoptera*)**
  - **biodegrades rapidly: proteins are natural molecules, unlike synthetic chemical insecticides**
  - **very low mammalian toxicity**

Hexamer of Bt toxin proteins form membrane channel → lysis of insect gut epithelial cells



Microbial proteins in plants: Bt toxin

- Agricultural crops engineered with Bt toxin genes (cotton, corn, potato, rice) now commercially available
- Diverse variants of Bt toxin used (eg. Cry1Ac, Cry2Ab etc.) depending on target pest and likelihood of resistance
- Advantages of Bt plants:
  - reduced usage of chemical pesticides
  - uniform expression in all tissues
  - reduced labour of insecticide application

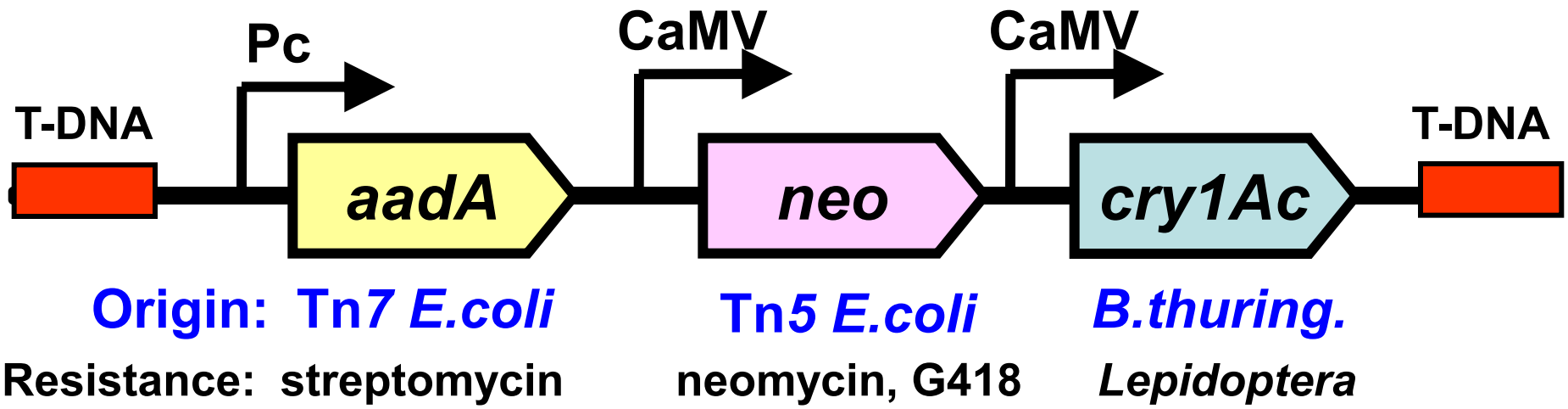
no boll-worm

boll-worm

Microbial proteins in plants: Bt toxin

Engineering of Bollgard cotton

- Wild-type cotton transformed via *Agrobacterium* with construct containing *cry1Ac* toxin and marker genes



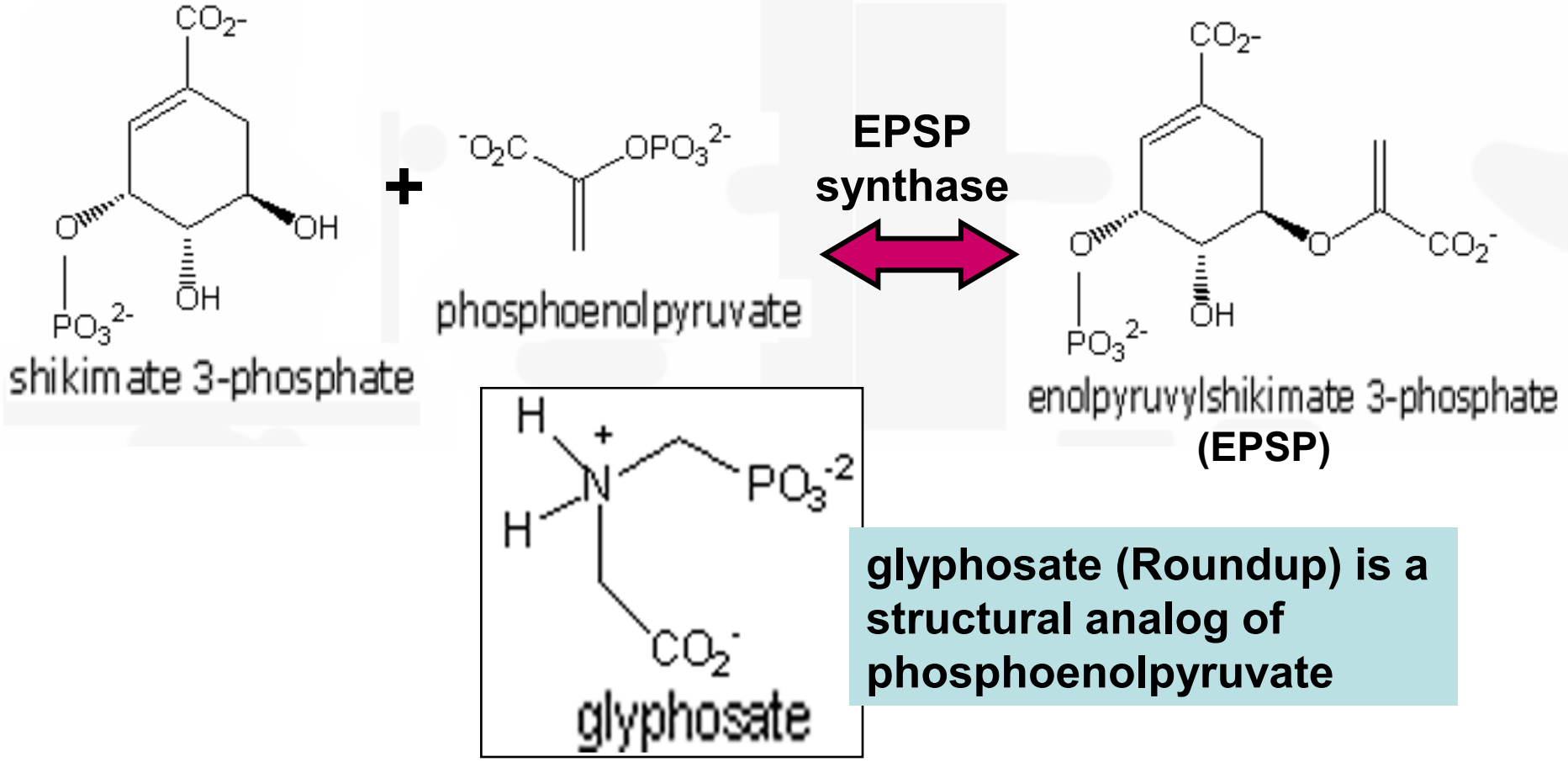
- *cry1Ac*: synthetic gene, codon usage modified by mutagenic PCR for efficient translation in plants

- CaMV: Cauliflower mosaic virus promoter – a strong, constitutive promoter recognised in many plants

- Pc: bacteria-only promoter → *aadA* silent in plants

Microbial proteins in plants: Glyphosate resistance

- **Glyphosate: broad-spectrum herbicide, inhibits plant biosynthesis of aromatic amino acids (EPSP synthase)**

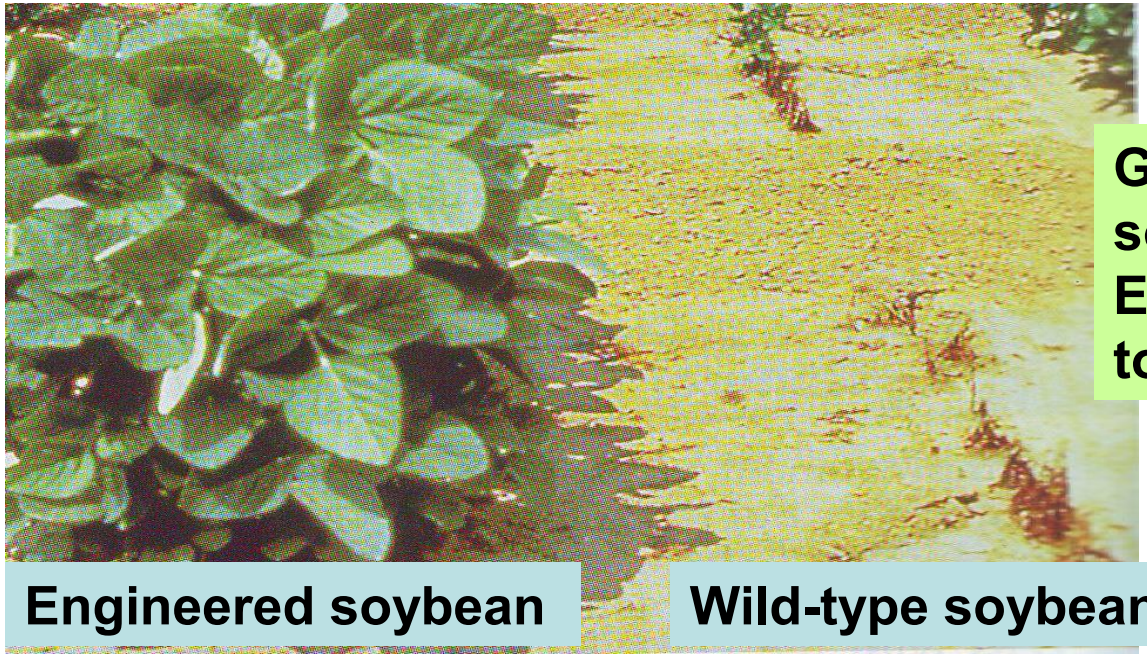


glyphosate (Roundup) is a structural analog of phosphoenolpyruvate

- **Glyphosate resistance is obtained by adding microbial EPSP synthase genes not inhibited by glyphosate**

Microbial proteins in plants: Glyphosate resistance

- **Benefits of glyphosate-resistant crops?**
- **Weed-control normally depends on selective herbicides and careful timing and spacing of herbicide application**
- **These approaches are cost- and labour- intensive**
- **With glyphosate-resistant crop, can apply a cheap and powerful herbicide to whole fields at any time**



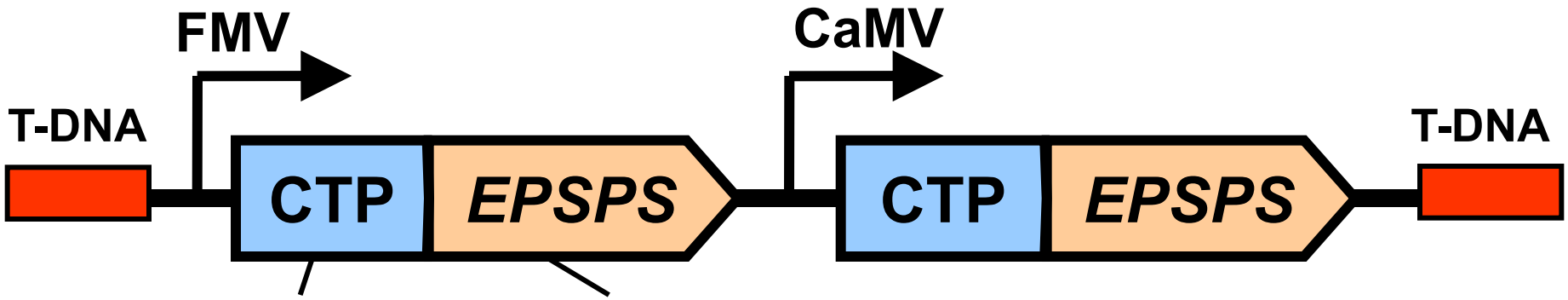
Brock

Glyphosate treatment of soybean with bacterial EPSP synthase compared to wild-type soybean

Microbial proteins in plants: Glyphosate resistance

Engineering of Roundup-Ready® cotton

- Wild-type cotton transformed via Agrobacterium with construct containing two copies of EPSP synthase genes



Origin: *Arabidopsis* *Agrobacterium*

Function: organelle targeting; glyphosate resistance

- FMV: Figwort mosaic virus promoter
- CTP: aromatic aa. biosynthesis occurs in the chloroplast
- Two copies of EPSPS: higher expression levels
- How to screen for transgenics? glyphosate resistance !

Human health risks of transgenic crops

- Toxicity or allegenicity
- Altered nutritional value
- Transfer of antibiotic resistance genes from transgenic plants to pathogenic bacteria

One GM plant that never made it to the marketplace – a brazil nut/soybean hybrid

'2S' protein from brazil nuts

+

Soybean host plant

Human health risks – resistance gene transfer?

- Resistance genes are likely to transfer from transgenic crops to bacteria, but some mitigating factors [ref 4.] :
  - resistance genes used in crops are already widespread in bacterial populations
  - estimated frequency low: 10^{-11} to 10^{-21} per recipient; compare to 10^{-1} to 10^{-8} for bacteria → bacteria transfer

★ spread of genes requires selection as well as transfer

Environmental risks of transgenic crops

- Toxicity to non-target organisms (Bt toxin)
- Increased selection pressure for resistant pests (Bt)
- Transgenic crop itself becomes a weed (Glyph-R)
- Gene transfer via pollen to related species
  - existing weeds become more problematic (Glyph-R)
  - could compromise integrity of non-GM crops

Bees can forage for pollen up to approx 5 km distant from their hive
→ implications for dispersal of transgenes in pollen?

Terminator technology for GMO control

- Complex set of further transgenes added to plants
  - protects environment from uncontrolled spread of transgenic plants by killing seeds
  - protects company patents by stopping farmers collecting seed for replanting next season

- Three-component system:
  - tetracyline repressor and operator
  - Cre recombinase / loxP targets
  - seed-specific expression of toxin

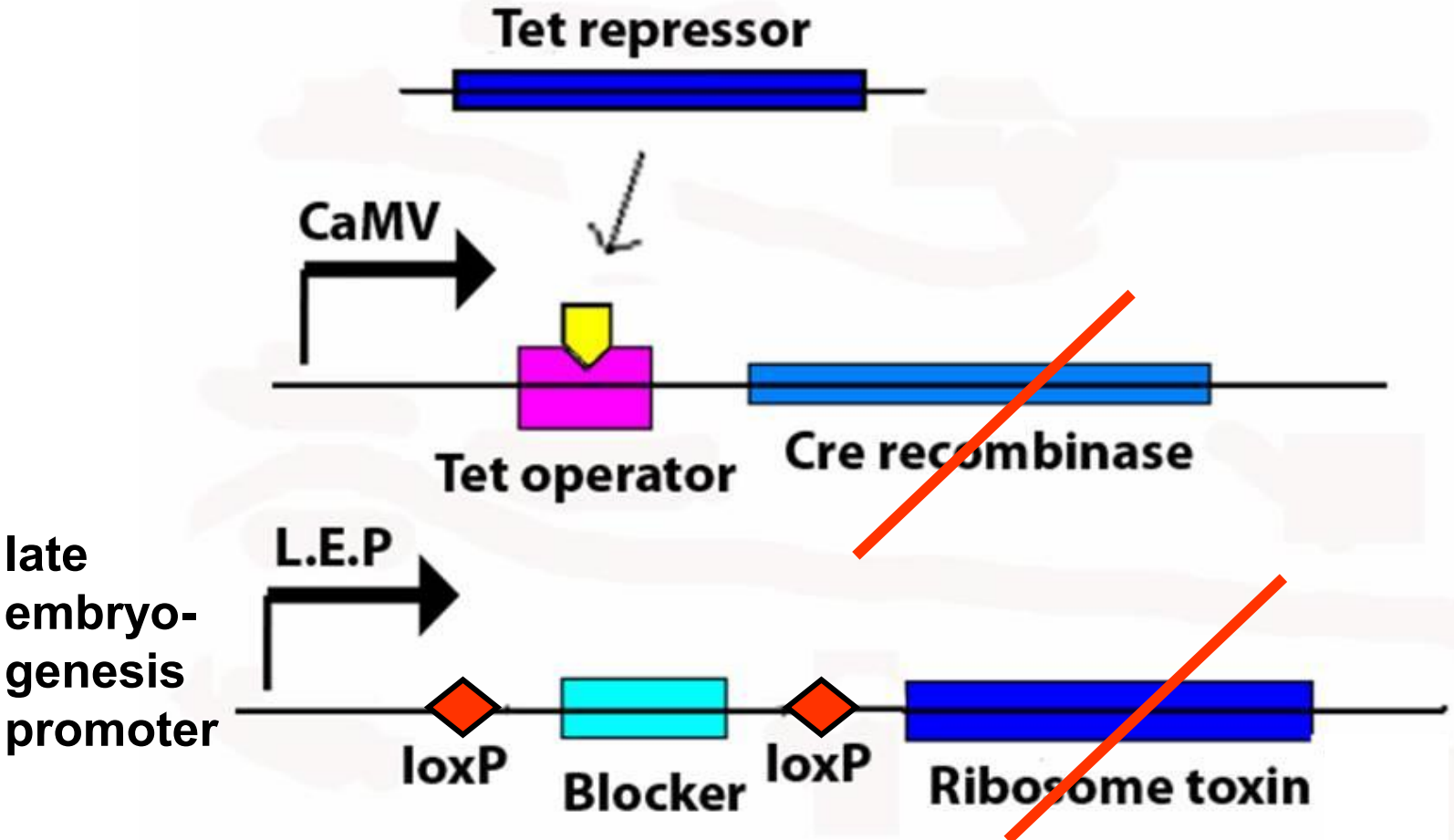
Terminator technology: During seed production

- **NO TETRACYCLINE ADDED**

→ Tet repressor binds Tet operator

→ Cre Recombinase NOT expressed

→ Blocker remains, toxin not expressed



Terminator technology: Before sale of seeds

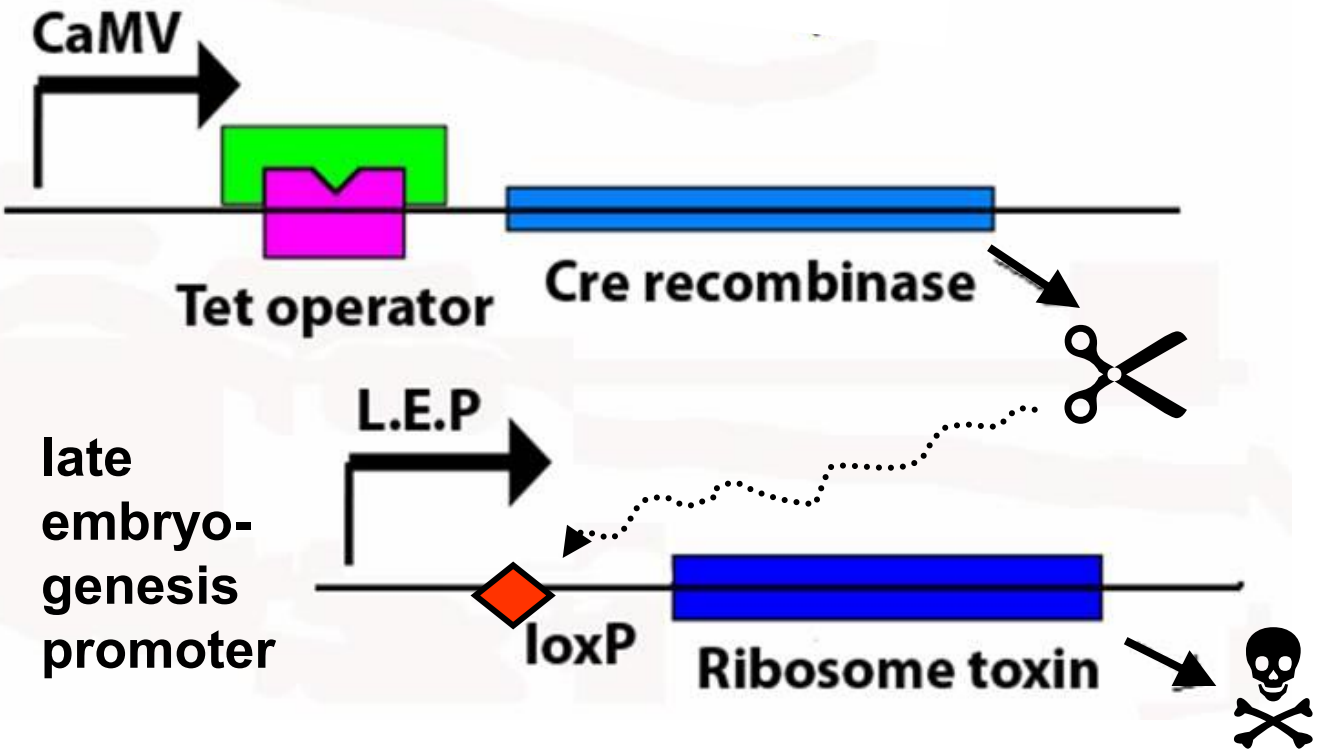
- **TREAT SEEDS WITH TETRACYCLINE**

→ Tet activates operator, repressor cant bind

→ Recombinase is expressed

→ Blocker DNA excised by Cre/lox system

→ Plant grows, toxin activated upon seed formation



Terminator technology: risks

- Adding more transgenes to control existing transgenes
  - terminator gene transfer kills seeds of other plants?
  - non-target effects of toxins used to kill seeds?

- Legal / ethical / social issues?
  - seed-saving by farmer becomes impossible/illegal
  - company ownership and control of crop species

Who really *owns* the plant / animal?
The company? The farmer? Nature?

References

- **Prescott, 6th ed. Chapter 42, Brock, 11th ed. Chapter 31**
- **Niemann and Kues (2003) "Application of transgenesis in livestock for agriculture and biomedicine" Animal Reproductive Science 79:291**
- **Christou et al (2006) "Recent developments and future prospects in insect pest control in transgenic crops" Trends in Plant Science 11:302**

Picture credits

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