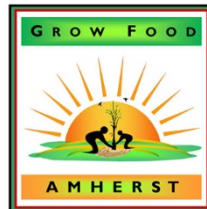


Inside the Science of Genetically Modified Plants

**Amherst Town Hall
April 15, 2014**

**Elizabeth Vierling
Professor of Biochemistry & Molecular Biology
UMass Amherst**

Co-Sponsored By:



Are you crazy?!?

In Amherst?!?

What I want to communicate

What is a **GENE**?

An introduction to DNA

The "Central Dogma"

How many genes are there in plants?

How do we put genes into plants?

Some history of how we got there.

What genes have I put in plants?

What is a "herbicide resistant" gene?

What is a *GENE*?

Let's start with DNA
and putting it in
context.

The four major Macromolecules of life:
(i.e. big stuff, e.g. relative to an atom)

Nucleic acids: DNA-Deoxyribonucleic acid
RNA-Ribonucleic acid
Store and transmit information

Proteins: Made of Amino acids (20 kinds) -
Workhorses of our cells/bodies

Lipids: Store energy (fat), lots of other functions

Carbohydrates: Store energy, other stuff too

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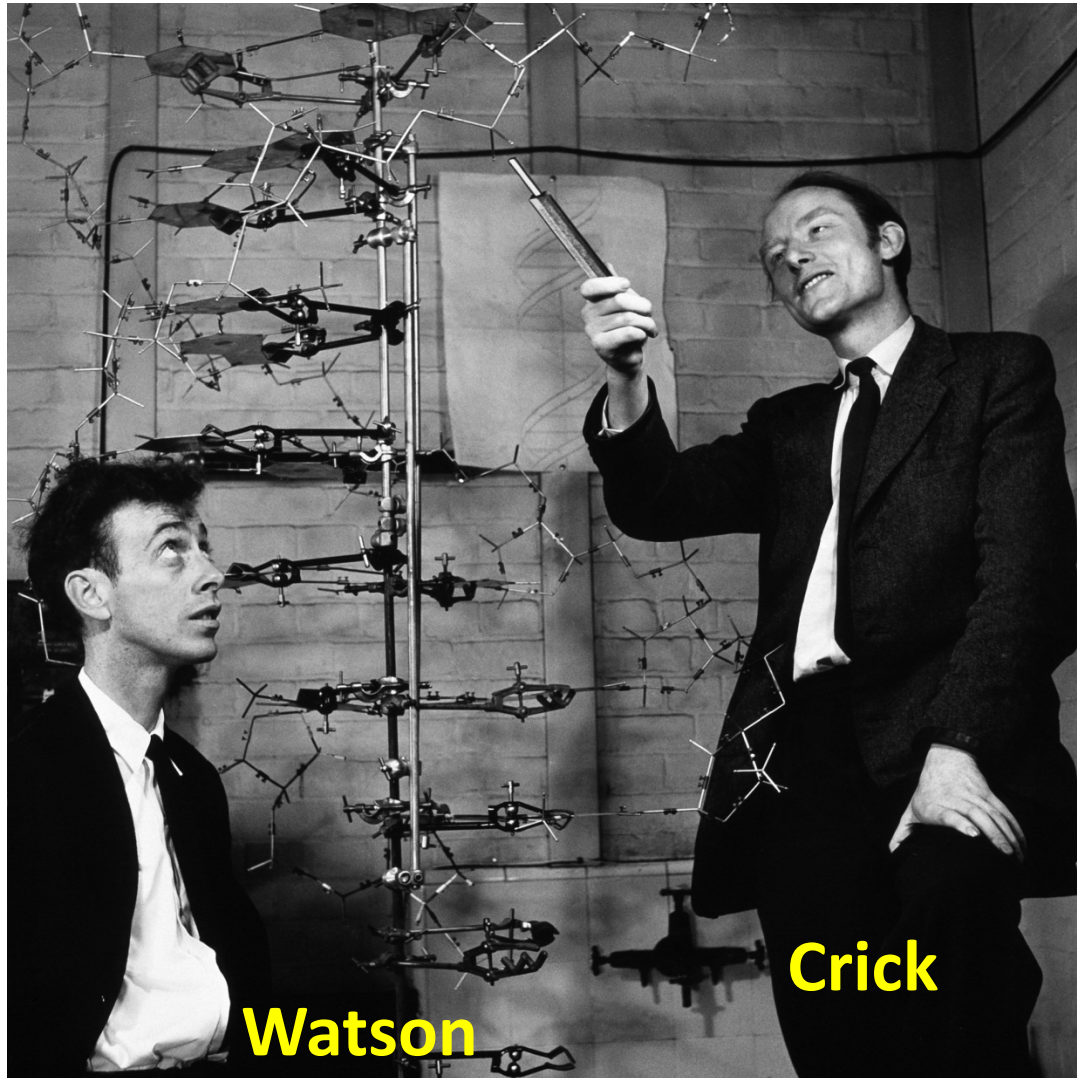
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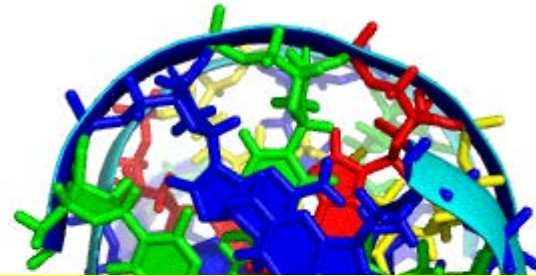
1953 - Defining the structure of DNA

James Watson and Francis Crick



DNA "Double Helix"

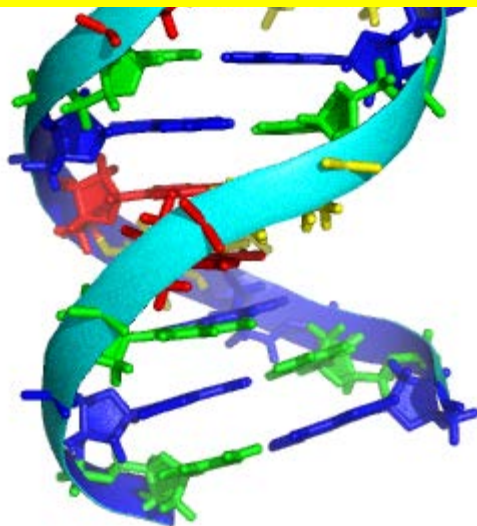
"Top" View



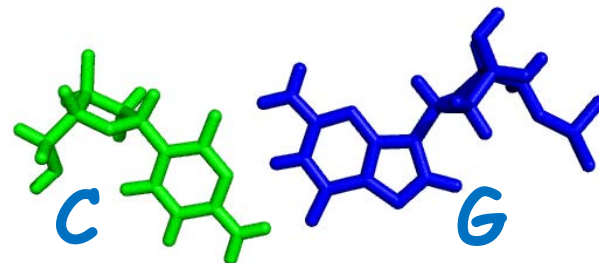
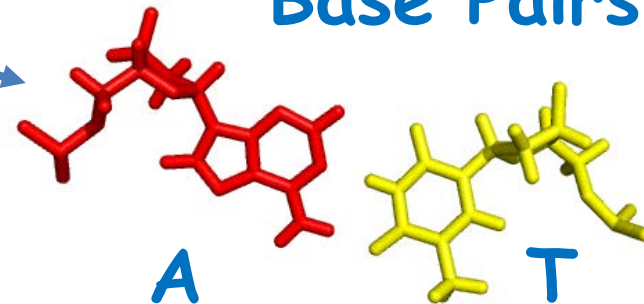
"Side" View



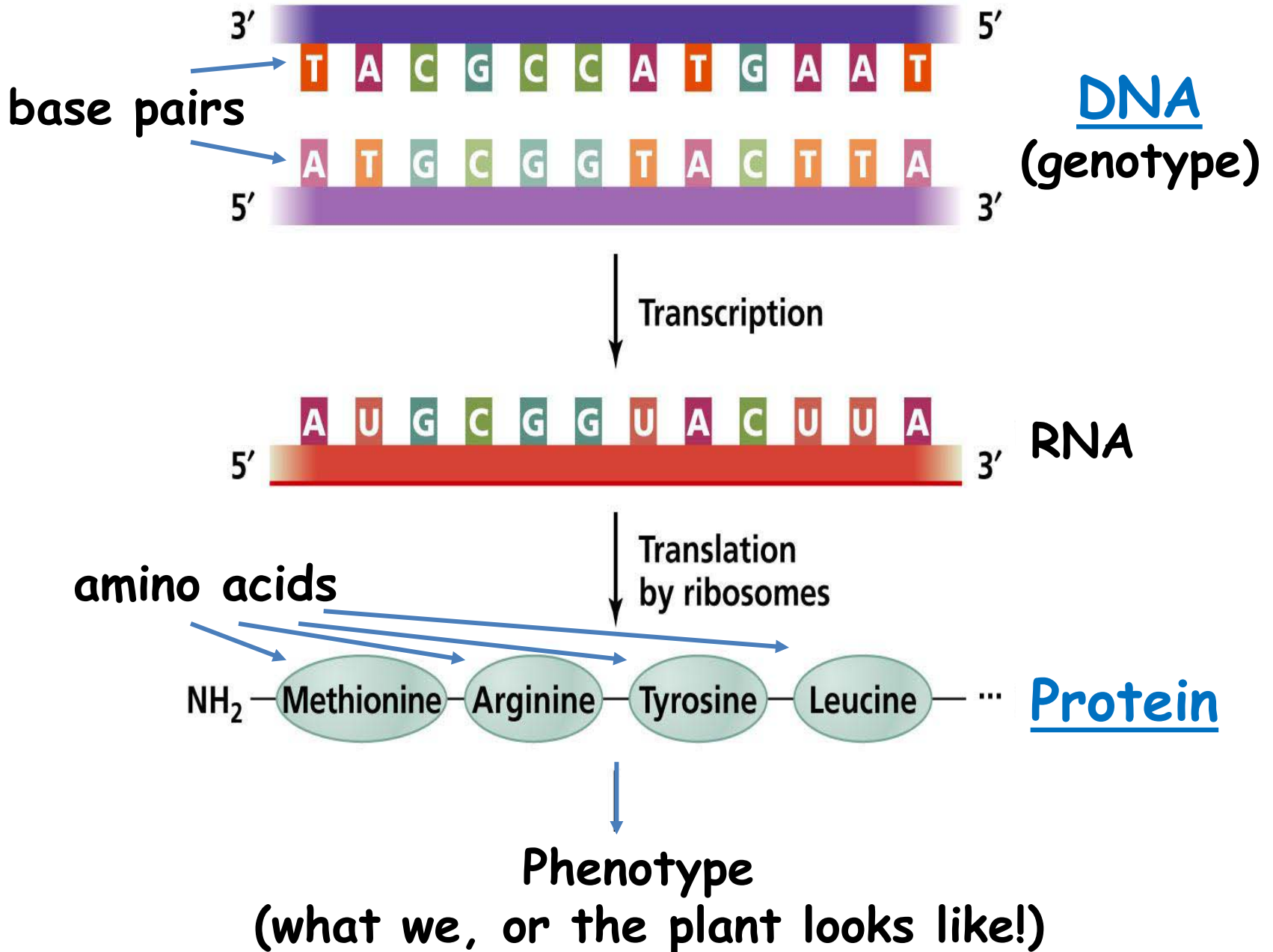
A **GENE** is a section of DNA with a specific sequence of "base pairs" that encodes the information required to make a protein



"Base Pairs"



From DNA to Protein - The Central Dogma



How much DNA is in each plant cell?

<u>Common name</u>	<u>Latin name</u>	<u>DNA amount</u> <u>Million "base pairs"</u>
Wheat	<i>Triticum aestivum</i>	15,966
Onion	<i>Allium cepa</i>	15,290
Carrot	<i>Daucus carota</i>	3,047

Plants and mammals, including humans, have on average about 25,000 to 35,000 GENES, i.e. Segments of DNA base pairs that specify unique PROTEINS.

Apple	<i>Malus X domestica</i>	743
Common bean	<i>Phaseolus vulgaris</i>	637
Cantaloupe	<i>Cucumis melo</i>	454
Grape	<i>Vitis vinifera</i>	483

So this brings us to the first fact about *GMO* plants:

GMO plants can be made using Recombinant DNA Technology with a GENE (a small piece of DNA that specifies a protein) from any organism, including even a highly related or even the same plant.

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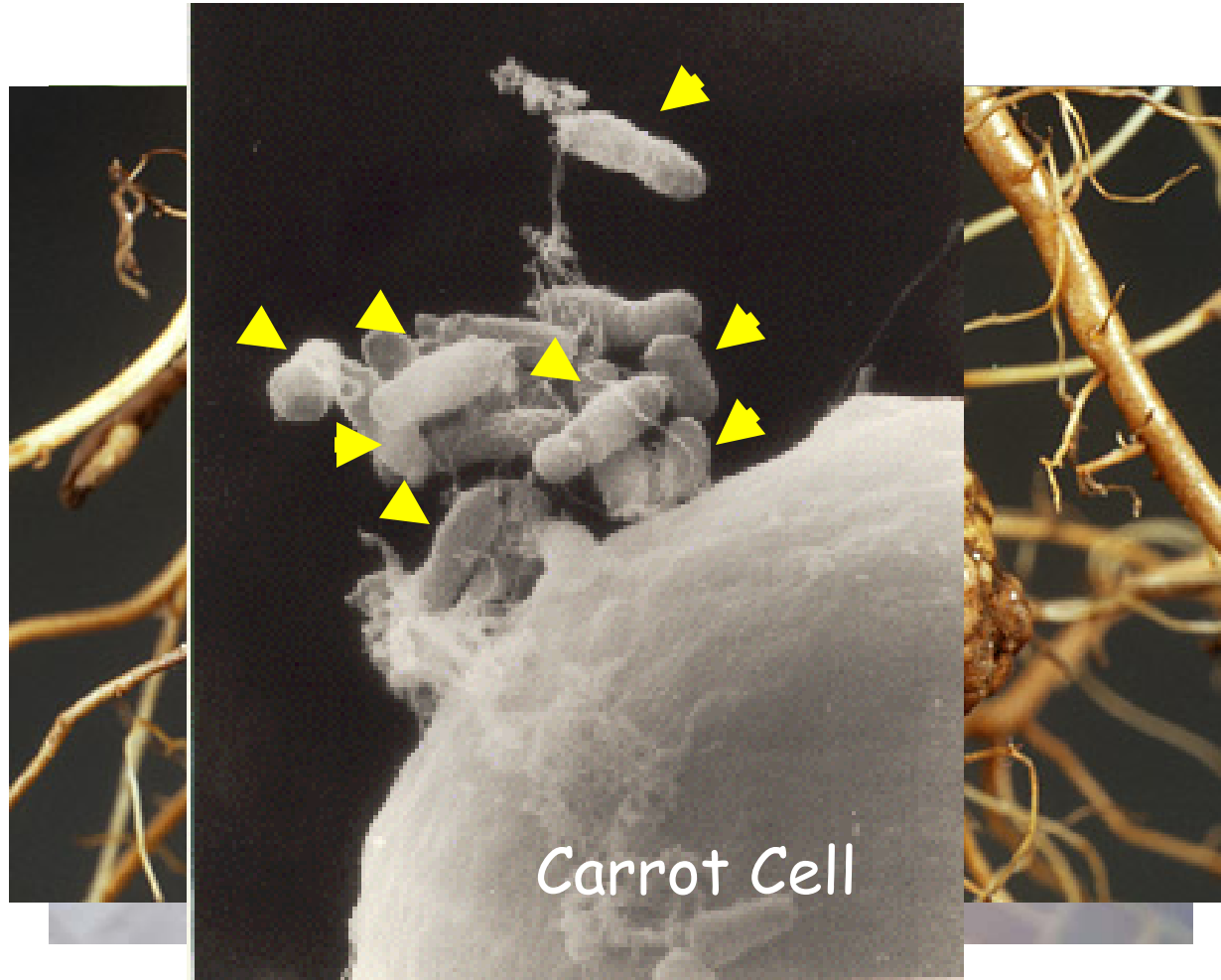
Some history of how we got there.

My research lab puts genes into plants.

What is a "herbicide resistant" gene?

A plant "disease" called "Crown Gall"

Caused by infection with a bacterium named "Agrobacterium"



Cell, Vol. 11, 263-271, June 1977, Copyright © 1977 by MIT

Stable Incorporation of Plasmid DNA into Higher Plant Cells: the Molecular Basis of Crown Gall Tumorigenesis

What does this mean?

The *Agrobacterium* actually transfers a small piece of its own DNA into the plant.

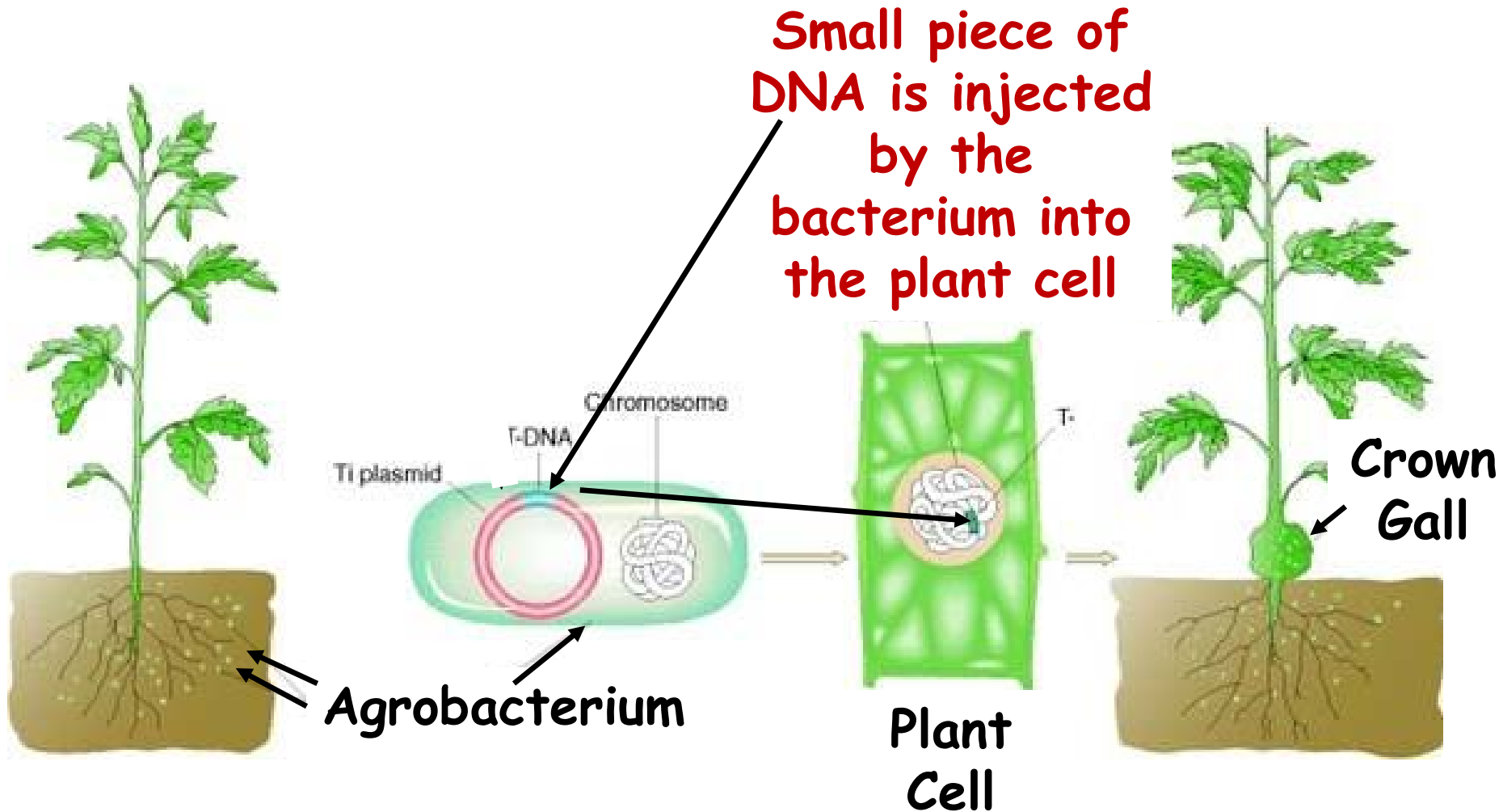
Cleverly, that piece of DNA produces proteins that generate plant hormones to make the cells grow and to make food for the bacterium, which then lives happily in its new home.

but not normal tobacco DNA. Treatment
DNA with DNAase abolished the accelera
determine whether all plasmid sequen
represented in tumor DNA, the labeled
DNA was separated into specific fragme
digestion with restriction endonuclease



Mary-Dell Chilton

How does *Agrobacterium* make "tumors"?



Agrobacterium can move genes into plants that the bacteria use to grow, so.....

We can use Agrobacterium to put any gene into plants.

Putting a GENE of choice into plants using Agrobacterium was first achieved in 1983

NATURE VOL. 303 19 MAY 1983

NATURE VOL. 303 19 MAY 1983

ARTICLES

209

Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector

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* Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

† Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG

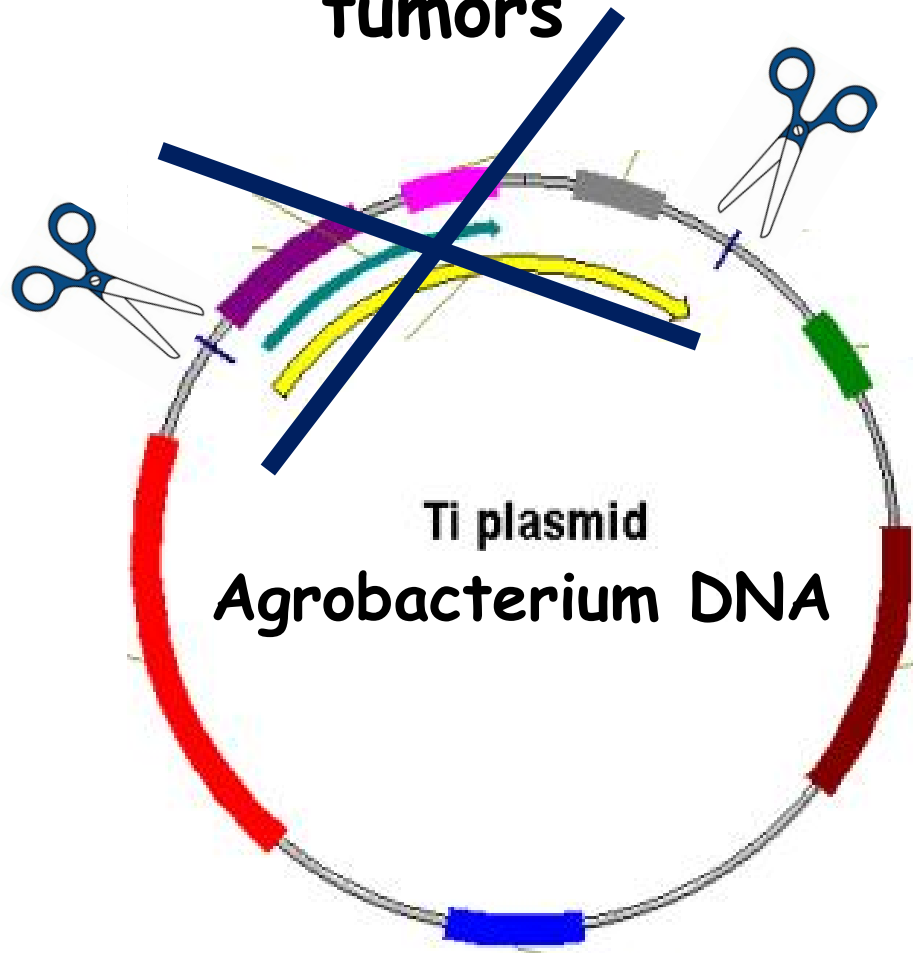
Foreign genes introduced into plant cells with Ti-plasmid vectors are not expressed. We have constructed an expression vector derived from the promoter sequence of nopaline synthase, and have inserted the coding sequences of the octopine synthase gene and a chloramphenicol acetyltransferase gene into this vector. These chimaeric genes are functionally expressed in plant cells after their transfer via a Ti-plasmid of Agrobacterium tumefaciens.

CROWN gall formation on dicotyledonous plants by *Agrobacterium tumefaciens* is the result of the transfer and covalent integration of a segment (called T-region) of the Ti-plasmid into the chromosomal DNA of plant cells (for reviews see refs 1–4). Insertion of foreign DNA sequences within the T-region of Ti-plasmids leads to their co-transfer and integration into the plant genome⁵. To date, inserts of up to 50 kilobases (kb)

from pTiT37 (refs 15, 16). Although both genes are encoded by plasmids of bacterial origin, they share more characteristics with eukaryotic genes than with prokaryotic genes. Both octopine and nopaline synthase genes, designated *ocs* and *nos* respectively, have a sequence similar to the so-called 'TATA' or 'Goldberg-Hogness' box¹⁷ in the 5' region upstream of the start of transcription, and a sequence 'AATAA' similar to the

We put new genes into plants in my lab:

Cut out the DNA
piece that causes
"tumors"

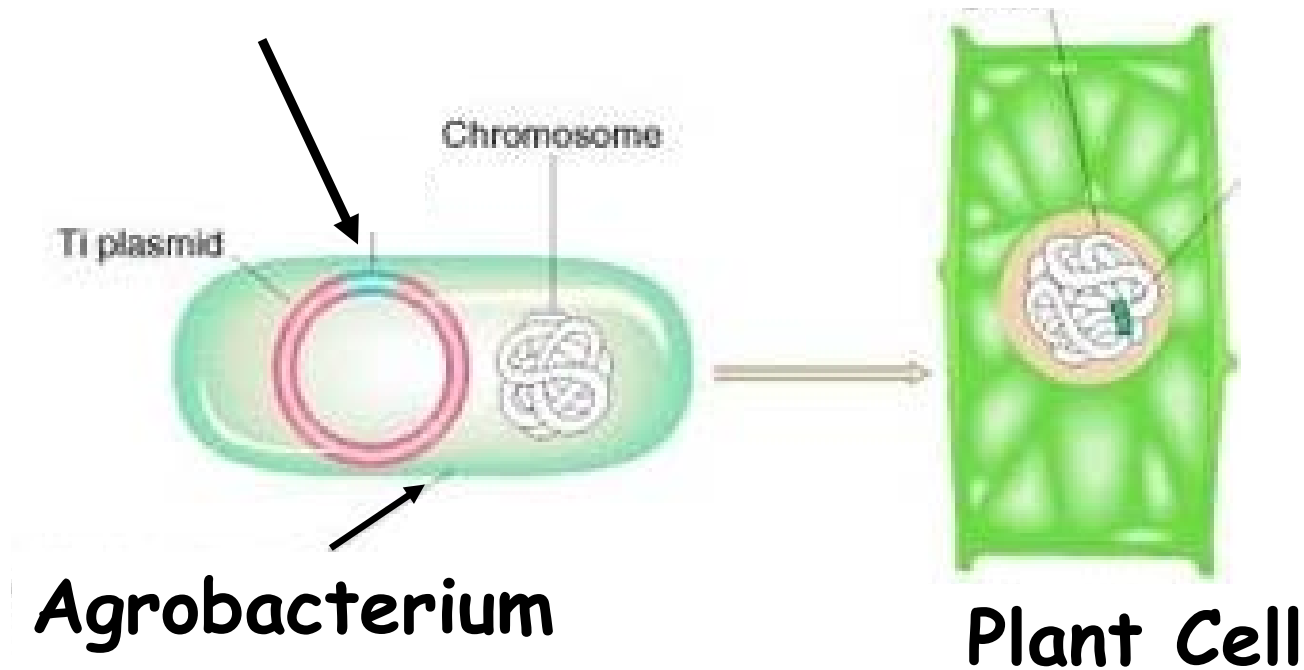


Replace that piece
with whatever DNA
we want to put into
the plant

Using Agrobacterium to make "Transgenic" Plants

We insert DNA with Our Favorite Gene into the bacterium

The bacterium puts it into the plant for us!



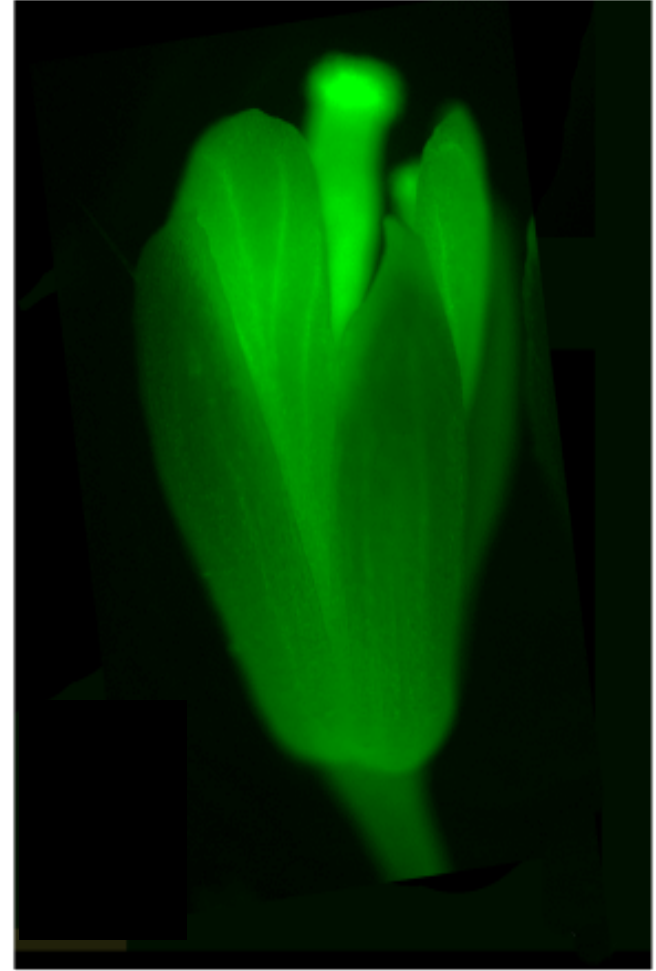
We use a small, easy to grow plant
"Mouse-eared cress" (*Arabidopsis thaliana*)



Mustard family
(Brassicaceae)

Related to Canola, Broccoli,
Cauliflower, Cabbage

Adding a Green Fluorescent "Tag" to Our Favorite Gene.



We can see where a protein is in the plant

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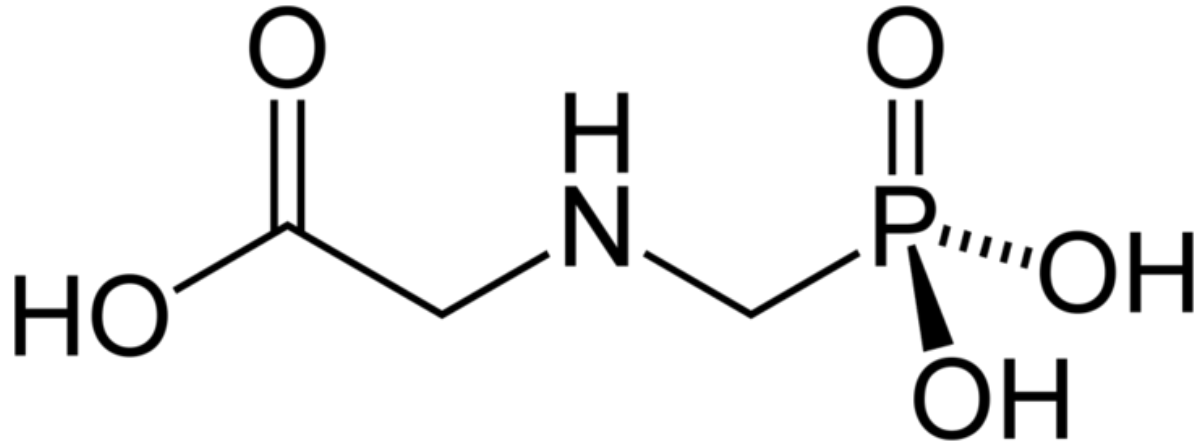
Resistance to the Herbicide "Roundup"

What is Roundup?

How much is used?

How have plants been made resistant to Roundup?

Glyphosate - Active ingredient of **Roundup**



Invented by Monsanto chemist John E. Franz in 1970

It is one of several different herbicides now used on crops.



Top Pesticides Used, by Percent of Corn Planted Acres Treated, 2010 Program States

Pesticide	Active Ingredient	Acres Treated (%)	Rate per Crop Year (Lbs/Acre)	Total Applied (Lbs)
Herbicide	Glyphosate isopropylamine salt			
	66	1.065	57,536,000	
Herbicide	Atrazine			
	61	1.034	51,129,000	
Herbicide	Acetochlor			
	25	1.398	27,921,000	

Glyphosate Inhibits A Protein Called:

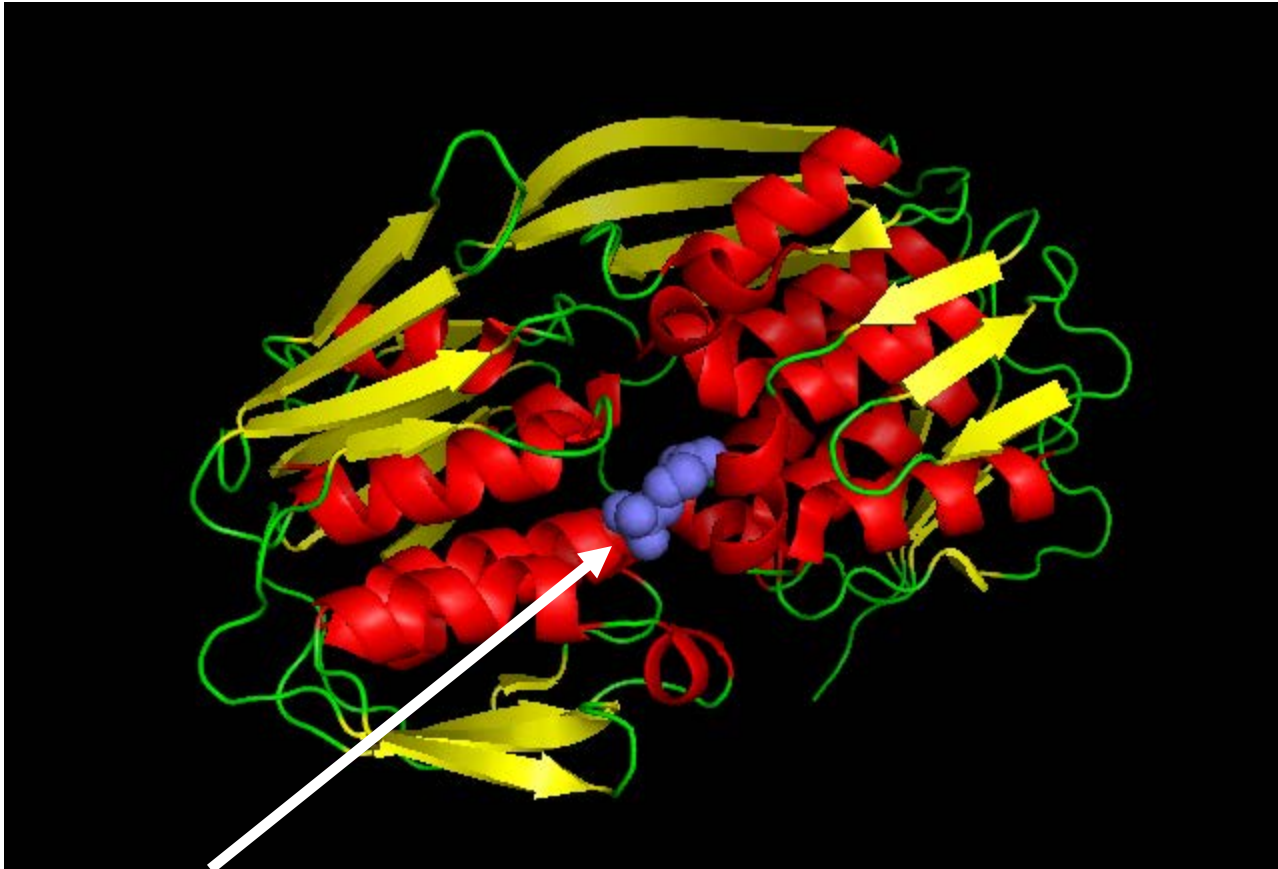
"EPSP Synthase"

This protein is found in bacteria, fungi, algae, some parasites and plants

EPSP Synthase is NOT found in animals including humans.

Its job is to make essential amino acids that must be obtained from an animal's diet.

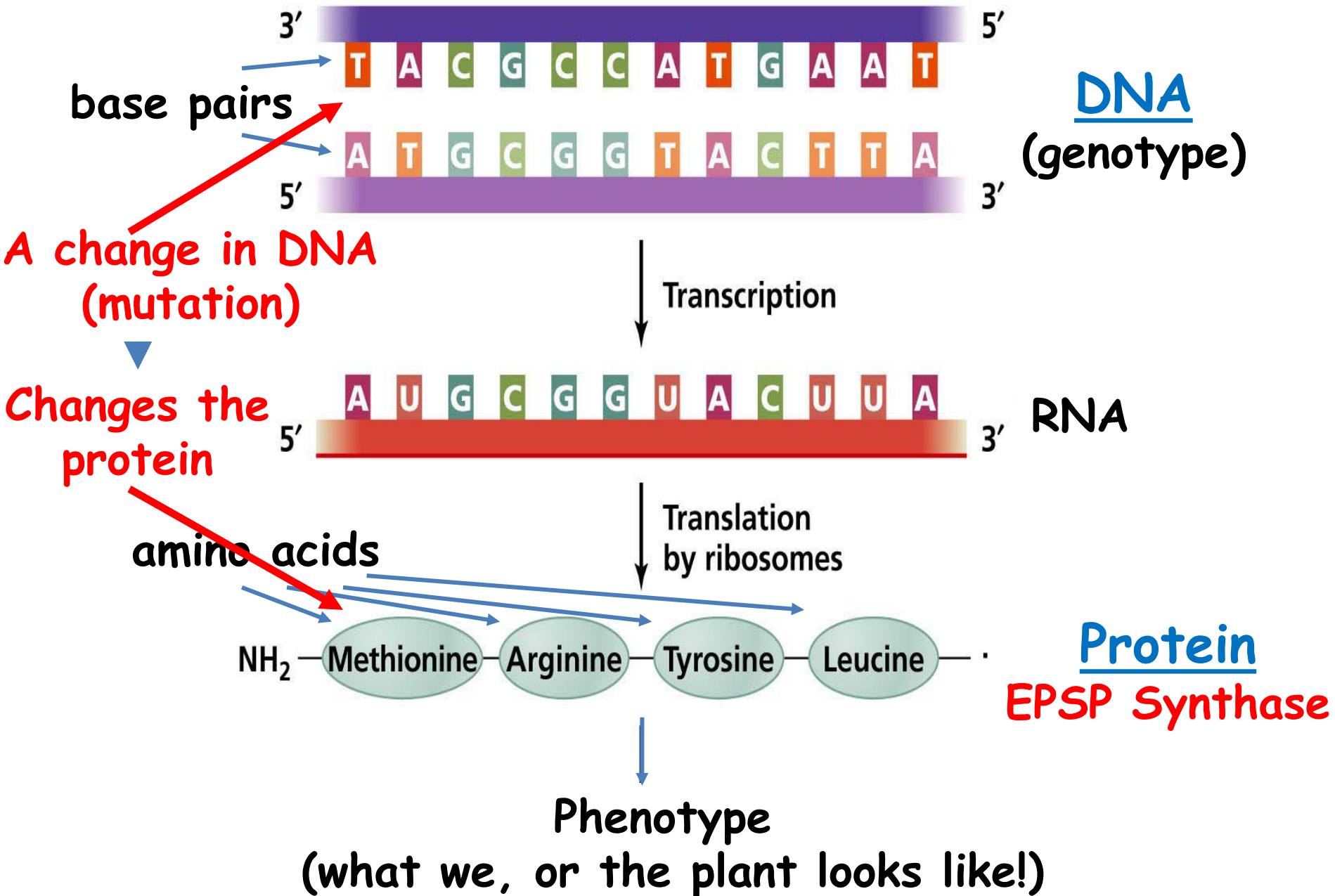
EPSP Synthase (the protein)



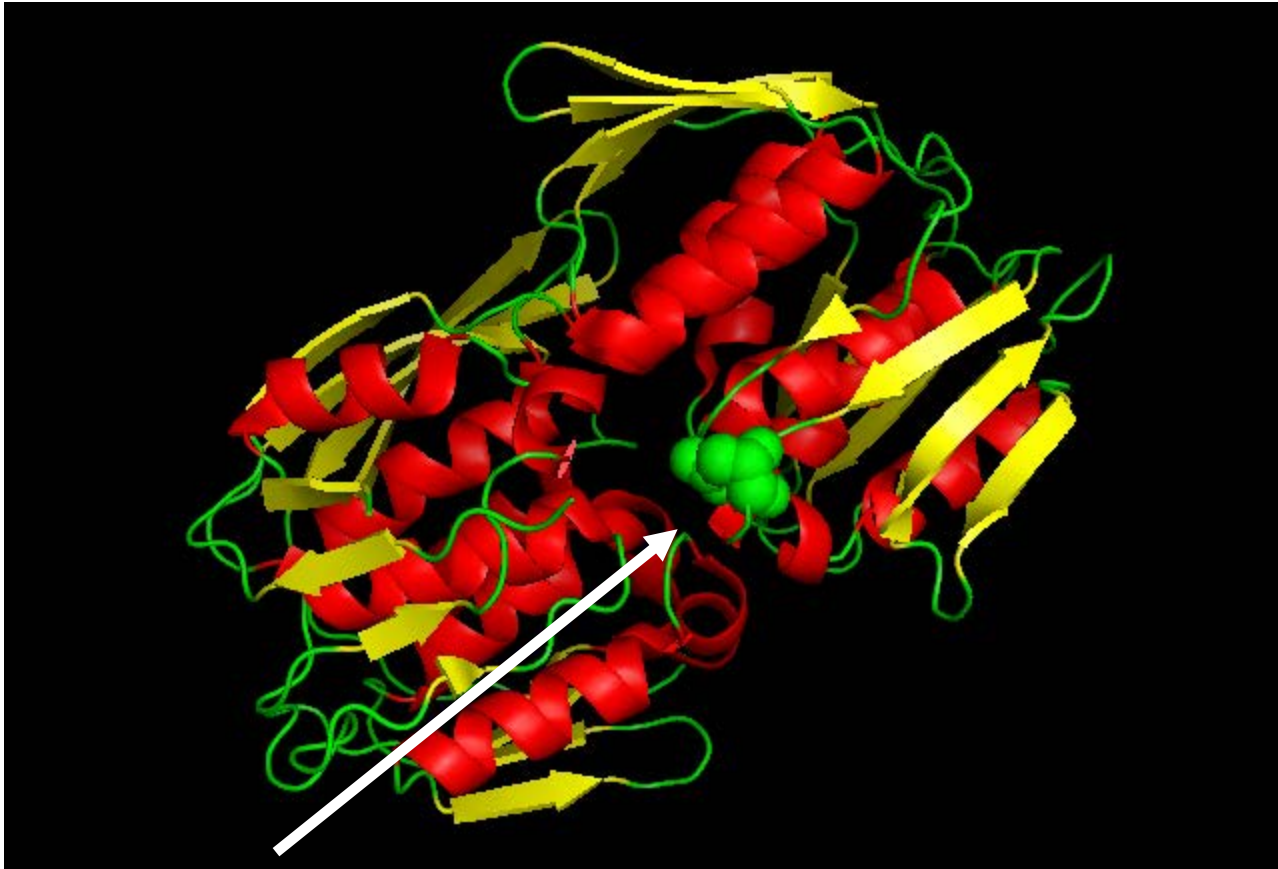
Glyphosate

If all plants have EPSP synthase,
how can we make plants resistant?

From DNA to Protein - The Central Dogma



EPSP Synthase (the protein)

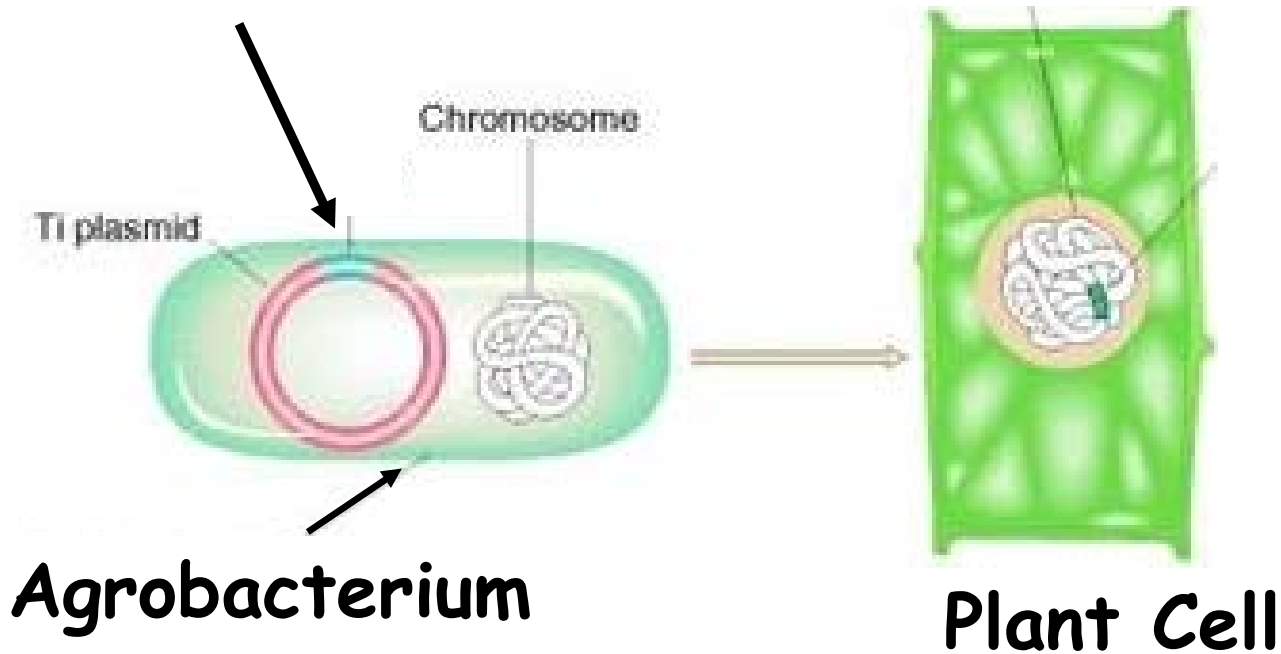


Changed EPSP synthase DNA, Changed Protein
Still functions in plant, but can't bind Roundup

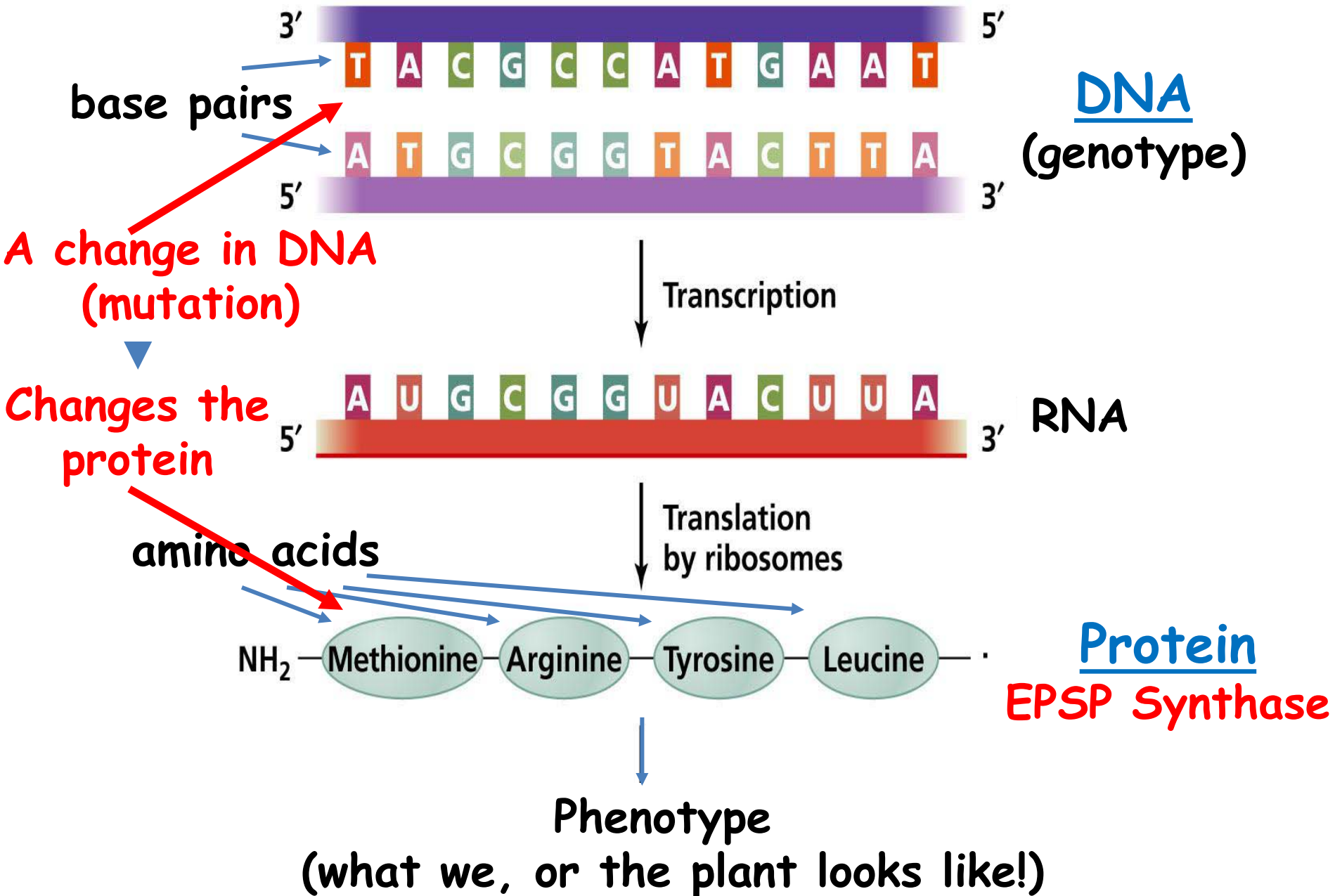
Make and EPSP synthase gene with altered base pairs

We insert DNA with EPSP synthase variant into the bacterium

The bacterium puts it into the plant - the plant is resistant to Roundup



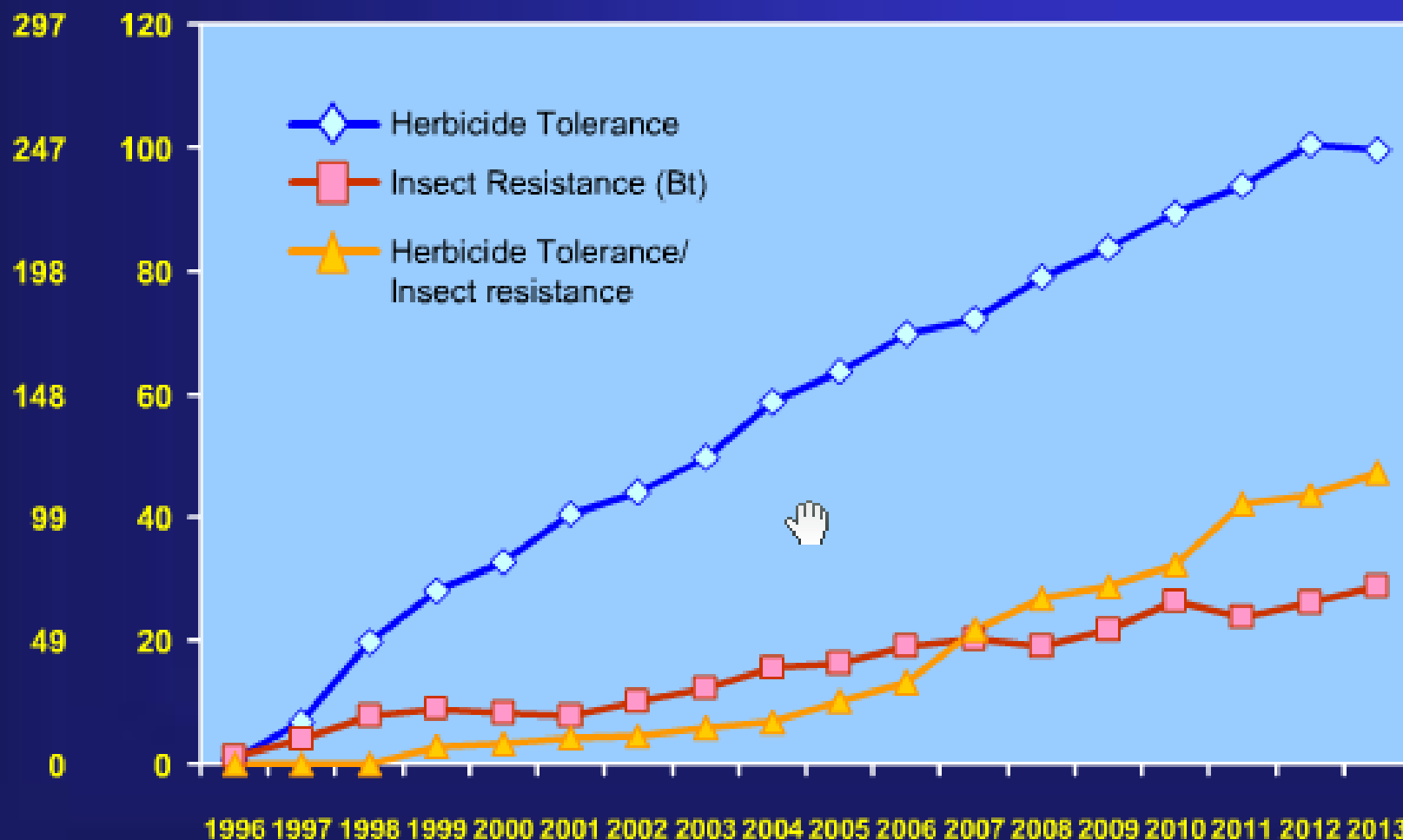
From DNA to Protein - The Central Dogma



Global Area of Biotech Crops, 1996 to 2013: By Trait (Million Hectares, Million Acres)



M Acres



GM Disease Resistant Papaya has replaced 80% of the Hawaiian Papaya crop

GM Crop Database

Database Product Description



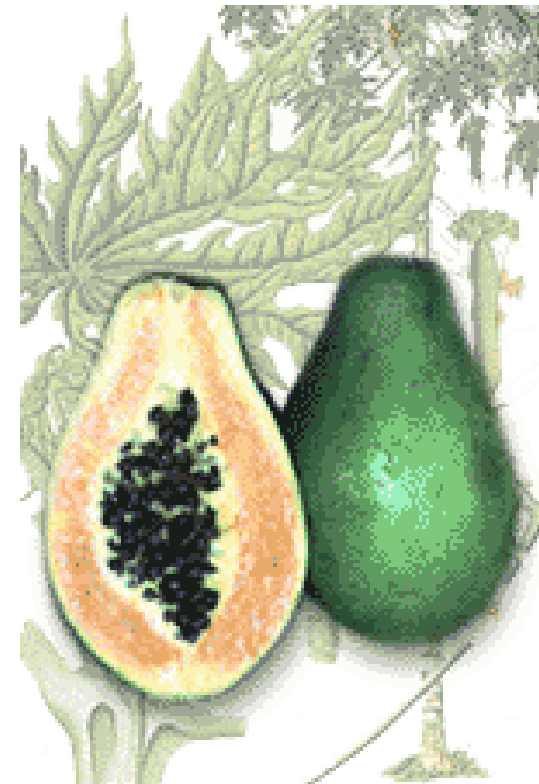
Show abstract



Print this page

UFL-X17CP-6 (X17-2)

Host Organism	<i>Carica papaya</i> L. (Papaya)
Trait	Resistance to viral infection, papaya ringspot virus (PRSV).
Trait Introduction	<i>Agrobacterium tumefaciens</i> -mediated plant transformation.
Proposed Use	Production of papaya for human consumption, either fresh or processed.
Company Information	University of Florida



http://cera-gmc.org/index.php?action=gm_crop_database

GM Example: Disease resistant banana by introduction of a gene from pepper



Resistant

Susceptible

Banana bacterial wilt is destroying plants in eastern Africa. Transgenic plants carrying a resistance gene from pepper are resistant to the disease

There has been, and continues to be significant research and discussion about the safety of *GMOs*, not only for human consumption, but also for the environment and the economy.

Some informative websites:



<http://www.biofortified.org/>



Center for
Environmental
Risk Assessment

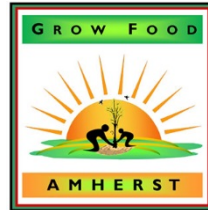
http://cera-gmc.org/index.php?action=about_us



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Thank you to my sponsors



Thanks to many members of my research lab over the last 28 years

Thank you for your attention!