

Transgenic Plants: From Basic Research to Agricultural Applications

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What I want to communicate

What is my bias?

What are the major “transgenic crops” today?

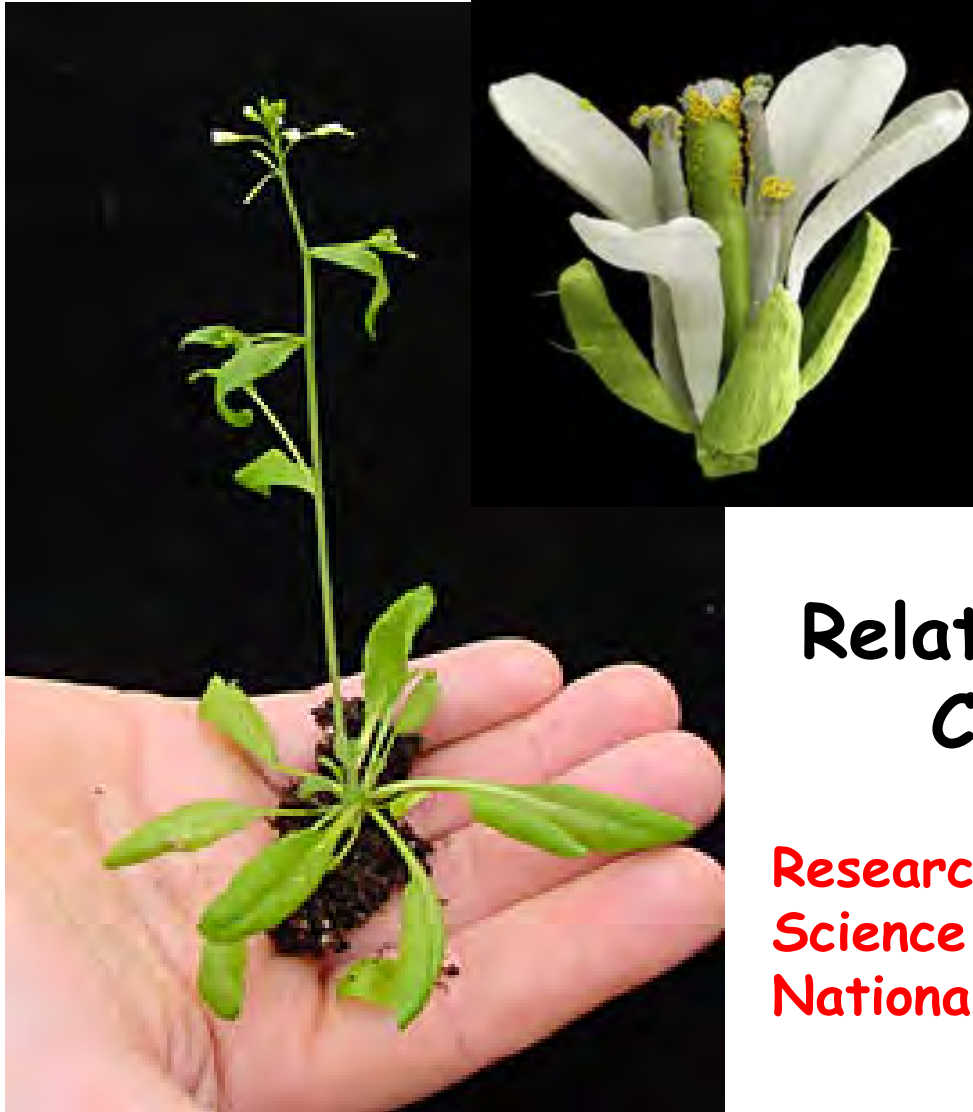
How do we add to, or modify plant genes?

Will new technologies “replace” *GMO* plants?

What do we need to do next?

NOT ALL *GMOs* ARE THE SAME!

I use *GMO* "technology" to put genes into plants for basic research and discovery.



"Mouse-eared cress"
(*Arabidopsis thaliana*)

Mustard family
(Brassicaceae)

Related to Canola, Broccoli,
Cauliflower, Cabbage

Research support: the National
Science Foundation, USDA, DOE,
National Institutes of Health

What is my bias?

- Use knowledge-based processes to understand potential risks and rewards of new (and old) technologies
- Protect the right for farmers to farm in different sustainable ways (and make a living), and for consumers to choose foods of their preference
- Concern: Over-heated rhetoric and "Fake News" obscures both the risks and rewards of GMOs.
 - GMOs will double yields and solve all agricultural problems!!!
 - GMOs will kill you, or at least make you sick, and besides...it's MONSANTO (buy organic, GMO free) !!!

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“Biotech” crops currently grown

Countries listed in order of number of acres

Country	Biotech Crops
USA*	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash, apples, potatoes
Brazil*	Soybean, maize, cotton
Argentina*	Soybean, maize, cotton
India*	Cotton
Canada*	Canola, maize, soybean, sugar beet

Table 1. Global Area of Biotech Crops in 2016: by Country (Million Hectares)**

Rank	Country	Area (Million Hectares)	Biotech Crops
1	USA*	72.9	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash, potato
2	Brazil*	49.1	Soybean, maize, cotton
3	Argentina*	23.8	Soybean, maize, cotton
4	Canada*	11.6	Canola, maize, soybean, sugar beet, alfalfa
5	India*	10.8	Cotton
6	Paraguay*	3.6	Soybean, maize, cotton
7	Pakistan*	2.9	Cotton
8	China*	2.8	Cotton, papaya, poplar
9	South Africa*	2.7	Maize, soybean, cotton
10	Uruguay*	1.3	Soybean, maize
11	Bolivia*	1.2	Soybean
12	Australia*	0.9	Cotton, canola
13	Philippines*	0.8	Maize
14	Myanmar*	0.3	Cotton
15	Spain*	0.1	Maize
16	Sudan*	0.1	Cotton
17	Mexico*	0.1	Cotton, soybean

Table 1. Global Area of Biotech Crops in 2016: by Country (Million Hectares)**

Rank	Country	Area (Million Hectares)	Biotech Crops
18	Colombia*	0.1	Cotton, maize
19	Vietnam	<0.1	Maize
20	Honduras	<0.1	Maize
21	Chile	<0.1	Maize
22	Portugal	<0.1	Maize
23	Bangladesh	<0.1	Brinjal/Eggplant
24	Costa Rica	<0.1	Cotton, soybean, pineapple
25	Slovakia	<0.1	Maize
26	Czech Republic	<0.1	Maize
	Total	185.1	

*18 biotech mega-countries growing 50,000 hectares, or more, of biotech crops

**Rounded-off to the nearest hundred thousand.

The EU has authorized GM Food crops



EUROPEAN COMMISSION

European Commission > Food Safety > Plants > GMOs > New

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Genetically Modified Organisms

EU Register of authorised GMOs

Search the register for products containing GMOs e.g. if you type 'cotton', you will get a list of all products containing cotton in their description..

This search covers the EU GMOs register (Regulation EC 1829/2003) and the products subject to EC decisions on withdrawal from the market.

Keyword(s) :

Registered / Withdrawn :

Category :

http://ec.europa.eu/food/dyna/gm_register/index_en.cfm/

"Biotech" crops currently grown

Countries listed in order of number of acres

Country	Biotech Crops
USA*	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash, apples, potatoes
Brazil*	Soybean, maize, cotton
Argentina*	Soybean, maize, cotton
India*	Cotton
Canada*	Canola, maize, soybean, sugar beet

Note: There is currently NO "GMO" rice, wheat, peanut

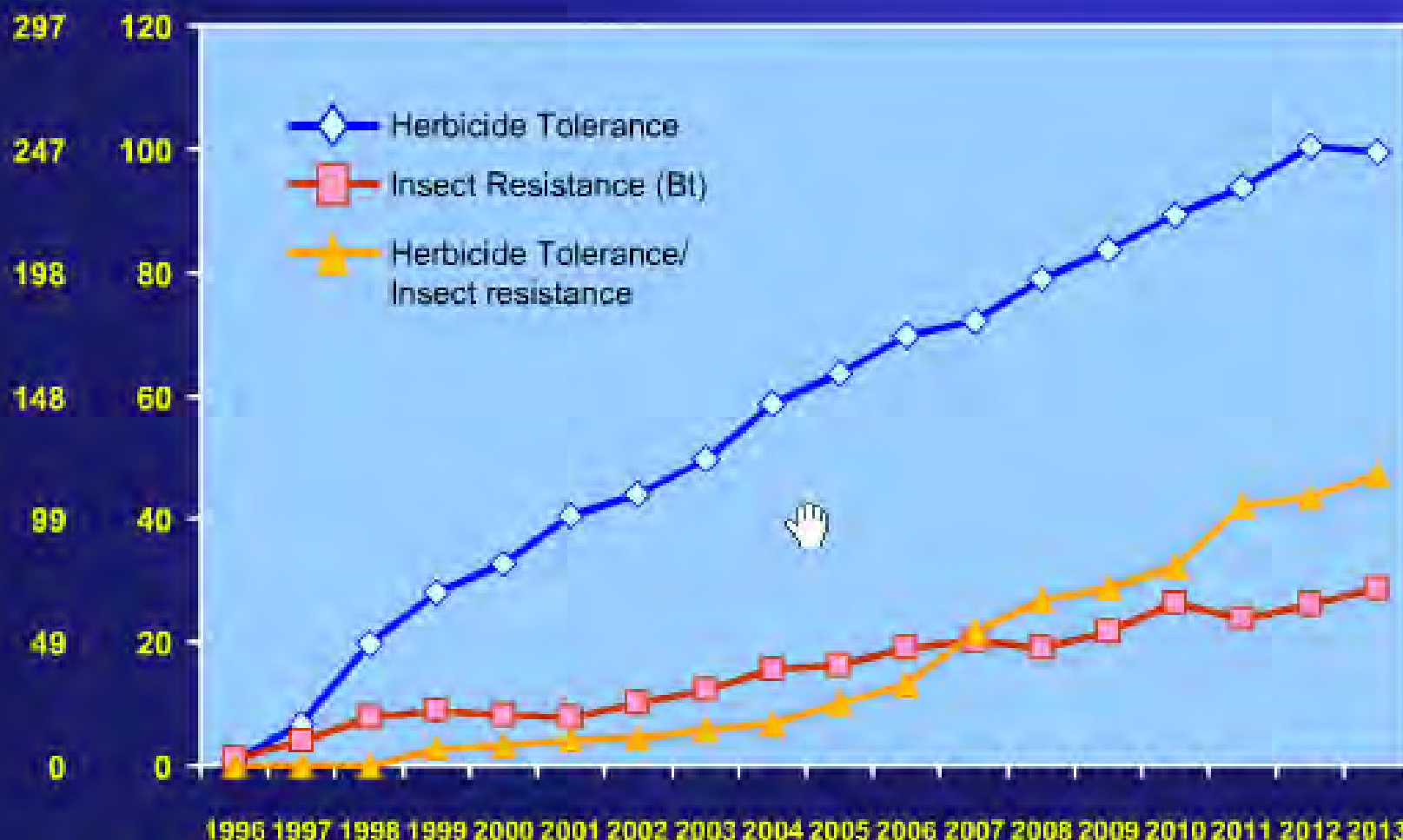
Major genes currently in transgenic crops

- **Herbicide tolerance (HT):** Corn, soybean, canola, cotton, sugar beet, alfalfa, others
“Roundup Ready”, and others
Gene: EPSP synthase, and others
- **Insect Resistance (Bt):** Corn, Cotton
“*Bacillus thuringiensis* toxin”
Gene: Bt toxin (many types)

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

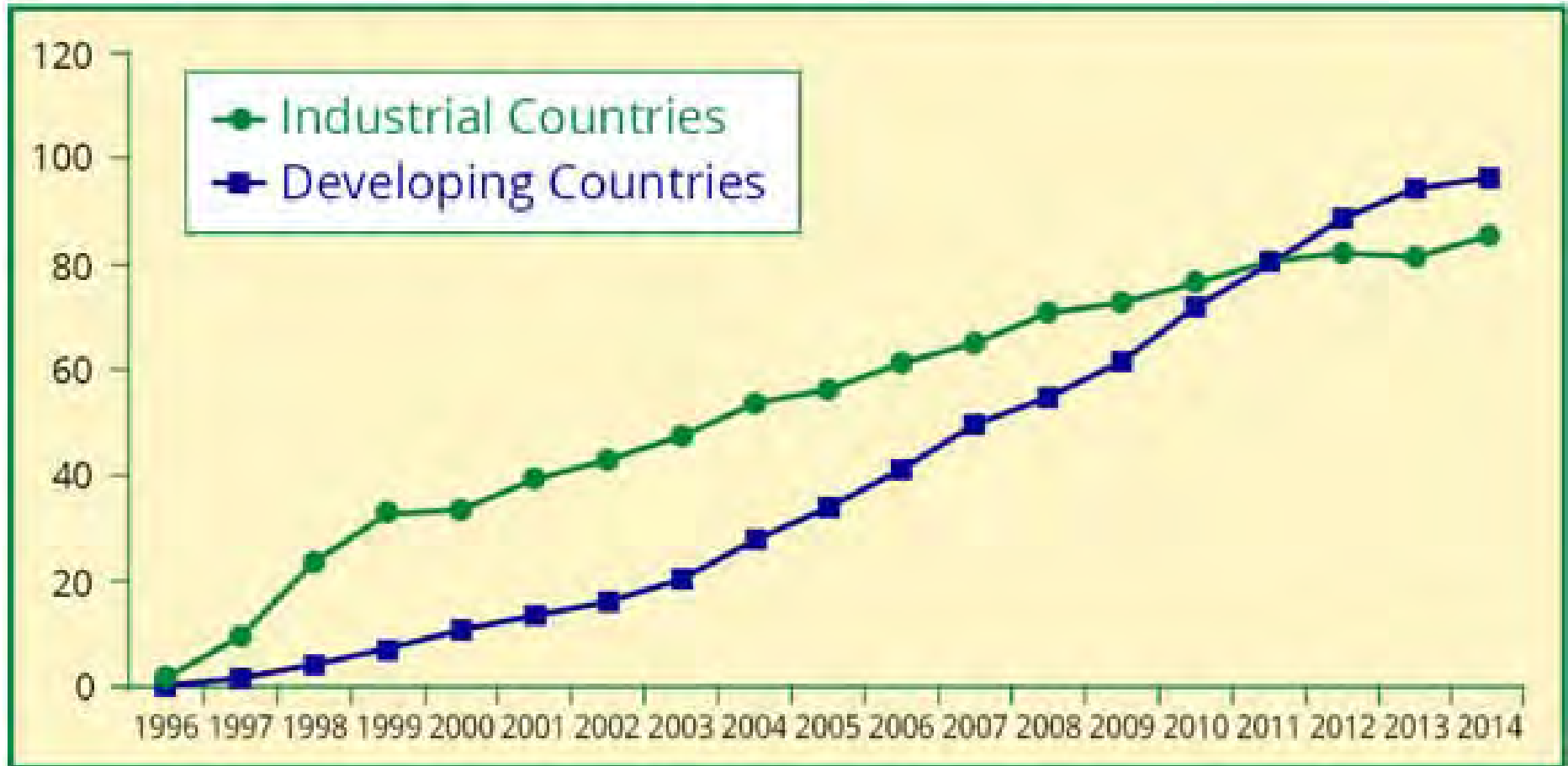


M Acres



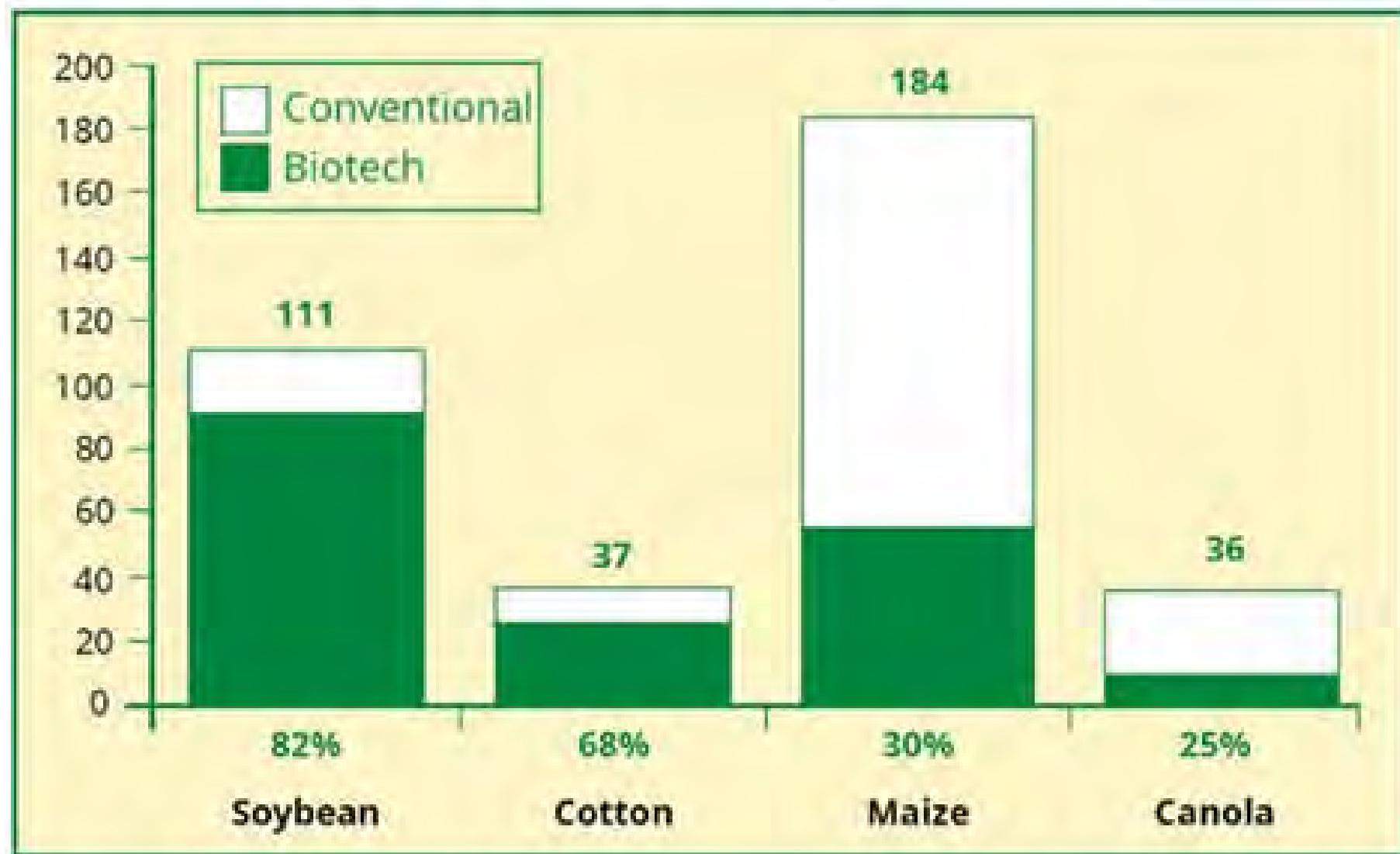
Source: Clive James, 2013

Figure 2. Global Area of Biotech Crops, 1996 to 2014: Industrial and Developing Countries (Million Hectares)



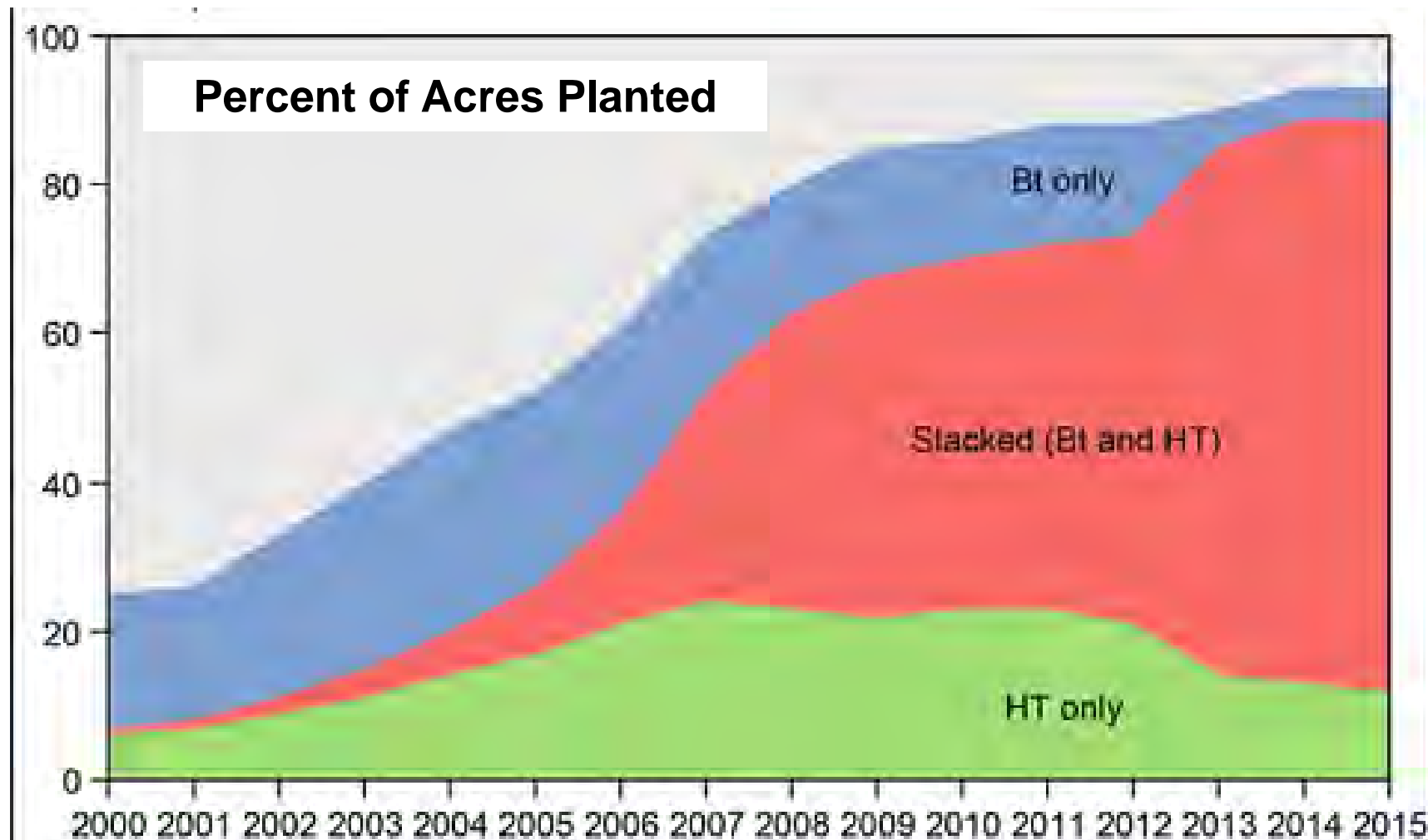
Source: Clive James, 2014.

Figure 3. Biotech Crop Area as % of Global Area of Principal Crops, 2014 (Million Hectares)



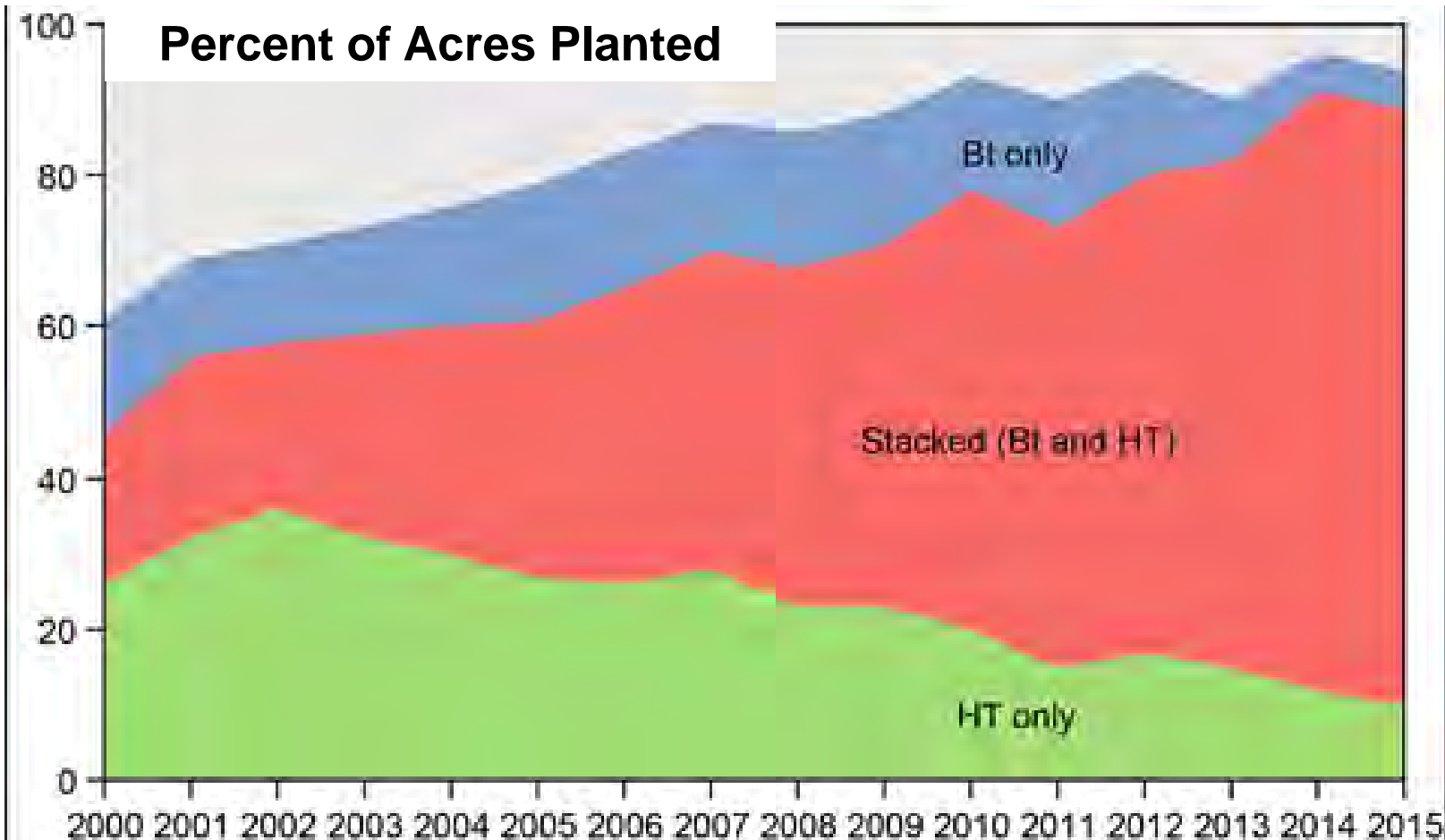
Source: Clive James, 2014.

Adoption of Genetically Engineered Corn in the US



Source: USDA, Economic Research Service using data from USDA, National Agricultural Statistics Service, *June Agricultural Survey*.

Adoption of Genetically Engineered Cotton in the US



Source: USDA, Economic Research Service using data from USDA, National Agricultural Statistics Service, *June Agricultural Survey*.

What I want to communicate

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How do we put genes into plants?

Will new technologies “replace” *GMO* plants?

What do we need to do next?

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

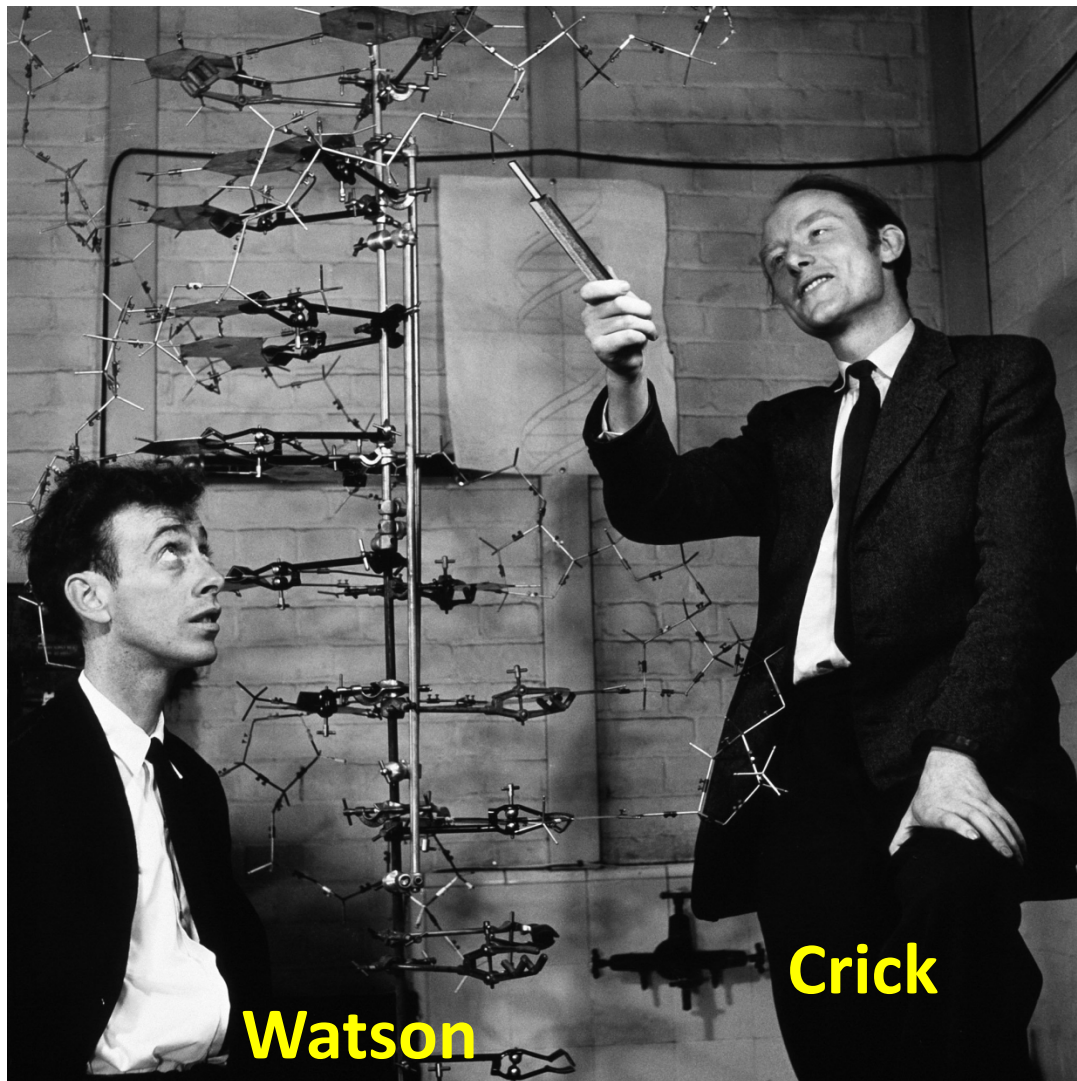
Proteins: Made of Amino acids (20 kinds) -
Workhorses of our cells/bodies

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

Carbohydrates: Store energy, other stuff too

1953 - Defining the structure of DNA

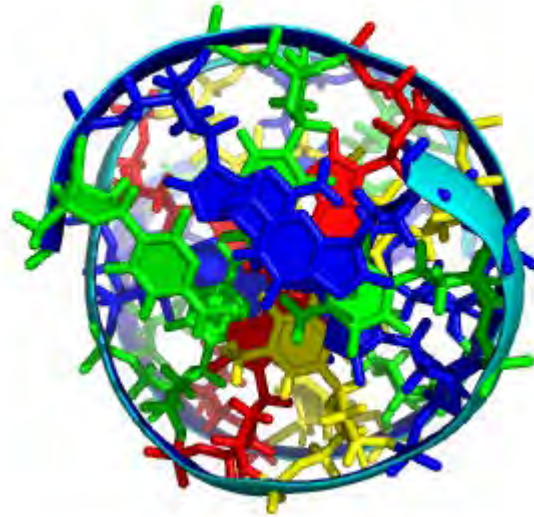
James Watson and Francis Crick



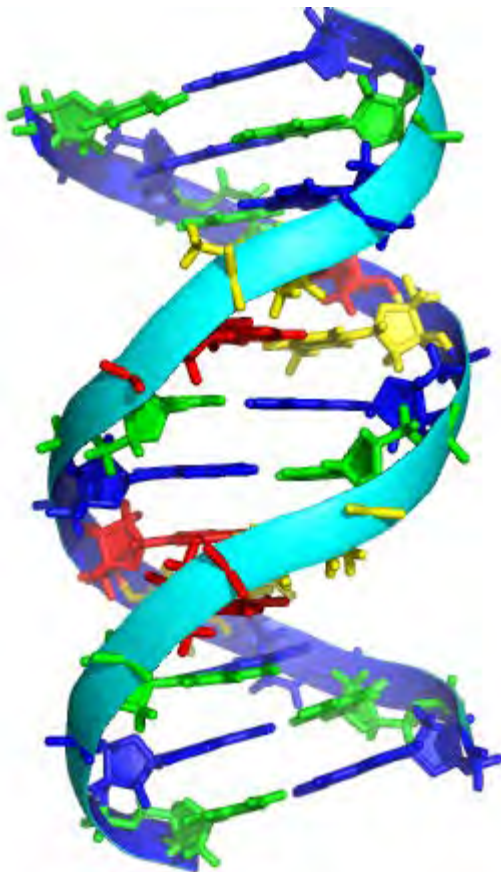
A *GENE* is a section of DNA with a specific sequence of “base pairs” that encodes the information required to make a protein

DNA "Double Helix"

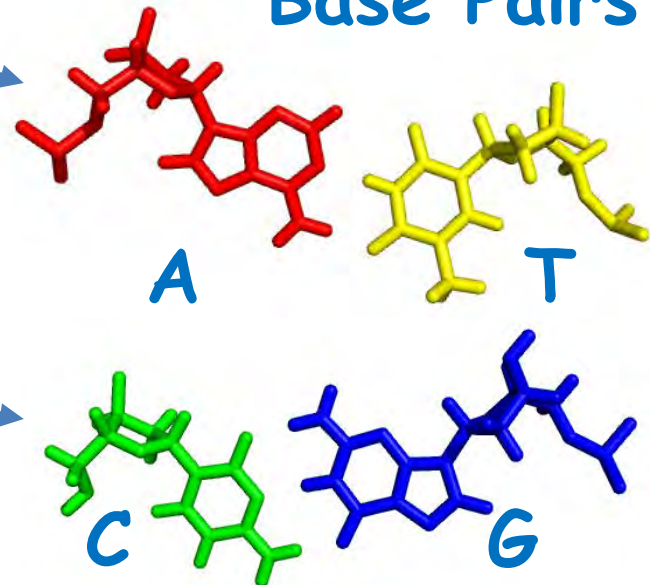
"Top" View



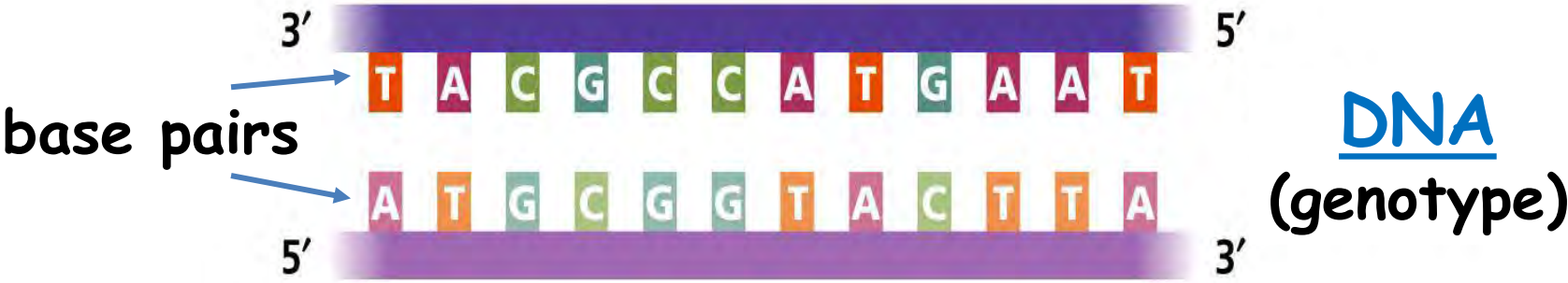
"Side" View



"Base Pairs"



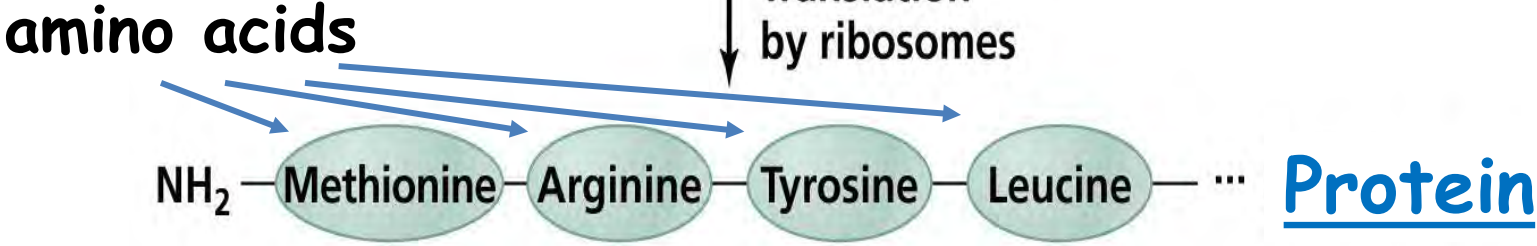
From DNA to Protein - The Central Dogma



Transcription



Translation by ribosomes



Phenotype
(what we, or the plant looks like!)

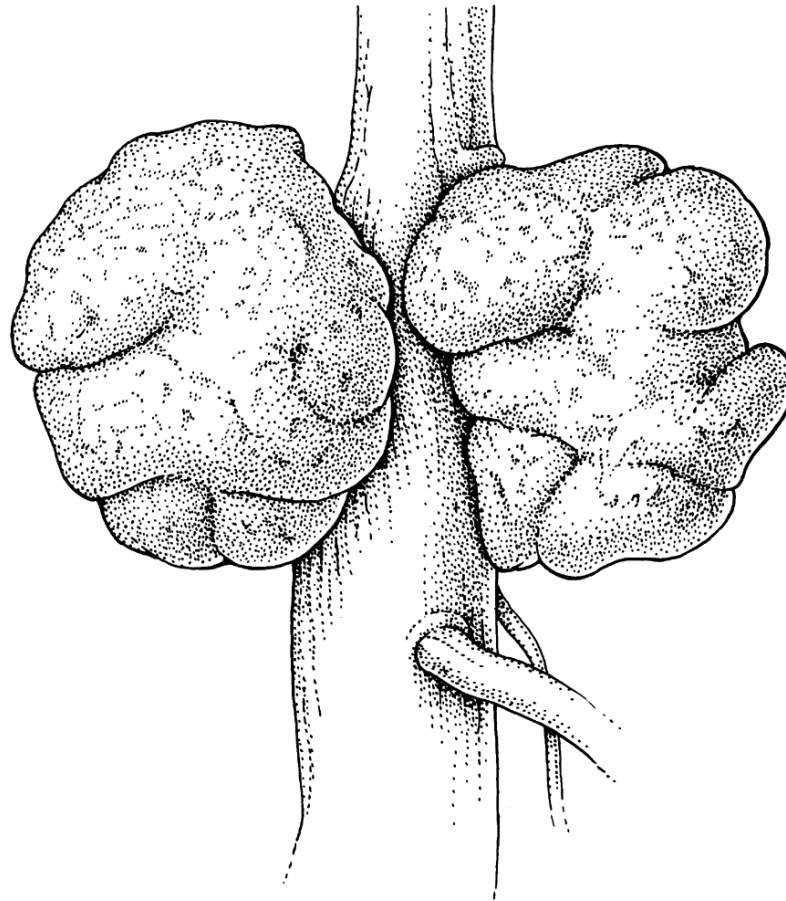
How much DNA is in each plant cell?

<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
Garden pea	<i>Pisum sativum</i>	3,947
Corn	<i>Zea mays</i>	2,292
Asparragus	<i>Asparagus officinalis</i>	1,308
Tomato	<i>Lycopersicum esculentum</i>	907
Sugarbeet	<i>Beta vulgaris</i>	758
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

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.

A plant "disease" called "Crown Gall"

Caused by infection with a bacterium named "Agrobacterium"



A plant "disease" called "Crown Gall"

Caused by infection with a bacterium
named "Agrobacterium"



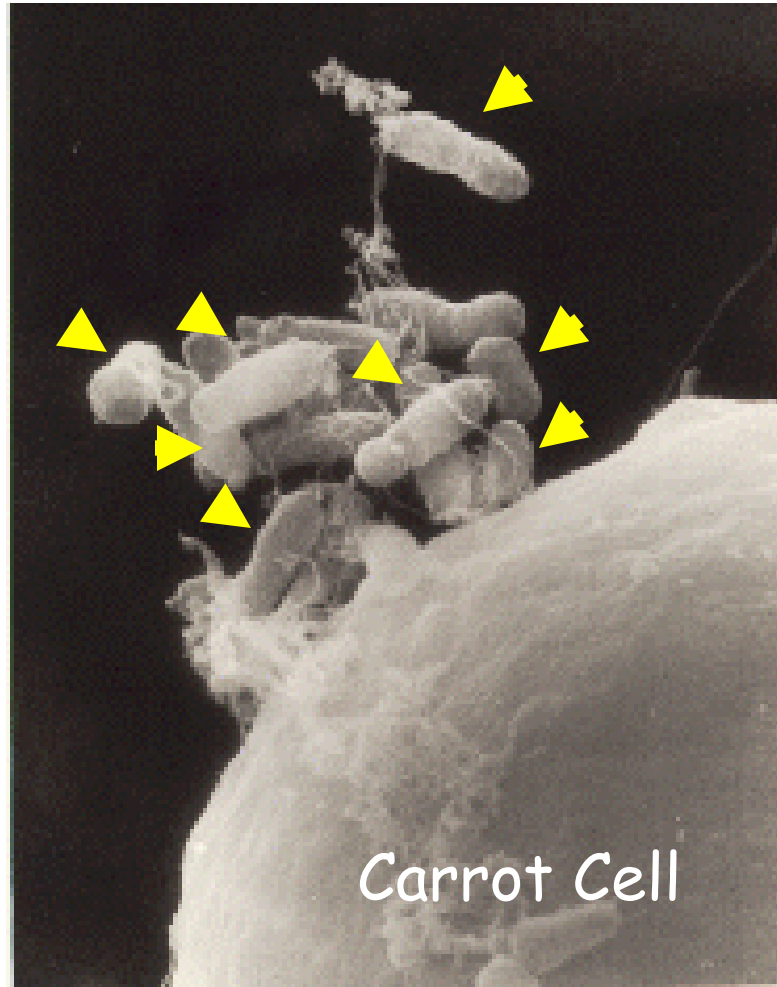
A plant "disease" called "Crown Gall"

Caused by infection with a bacterium named "Agrobacterium"



A plant "disease" called "Crown Gall"

Caused by infection with a bacterium named "Agrobacterium"



▲ bacterial cell

Carrot Cell

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

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J. Merlo,* Daniela Sciaky,* Alice L. Mont
Milton P. Gordon† and Eugene W. Nester

*Department of Microbiology and Immunology
†Department of Biochemistry,
University of Washington,
Seattle, Washington 98195

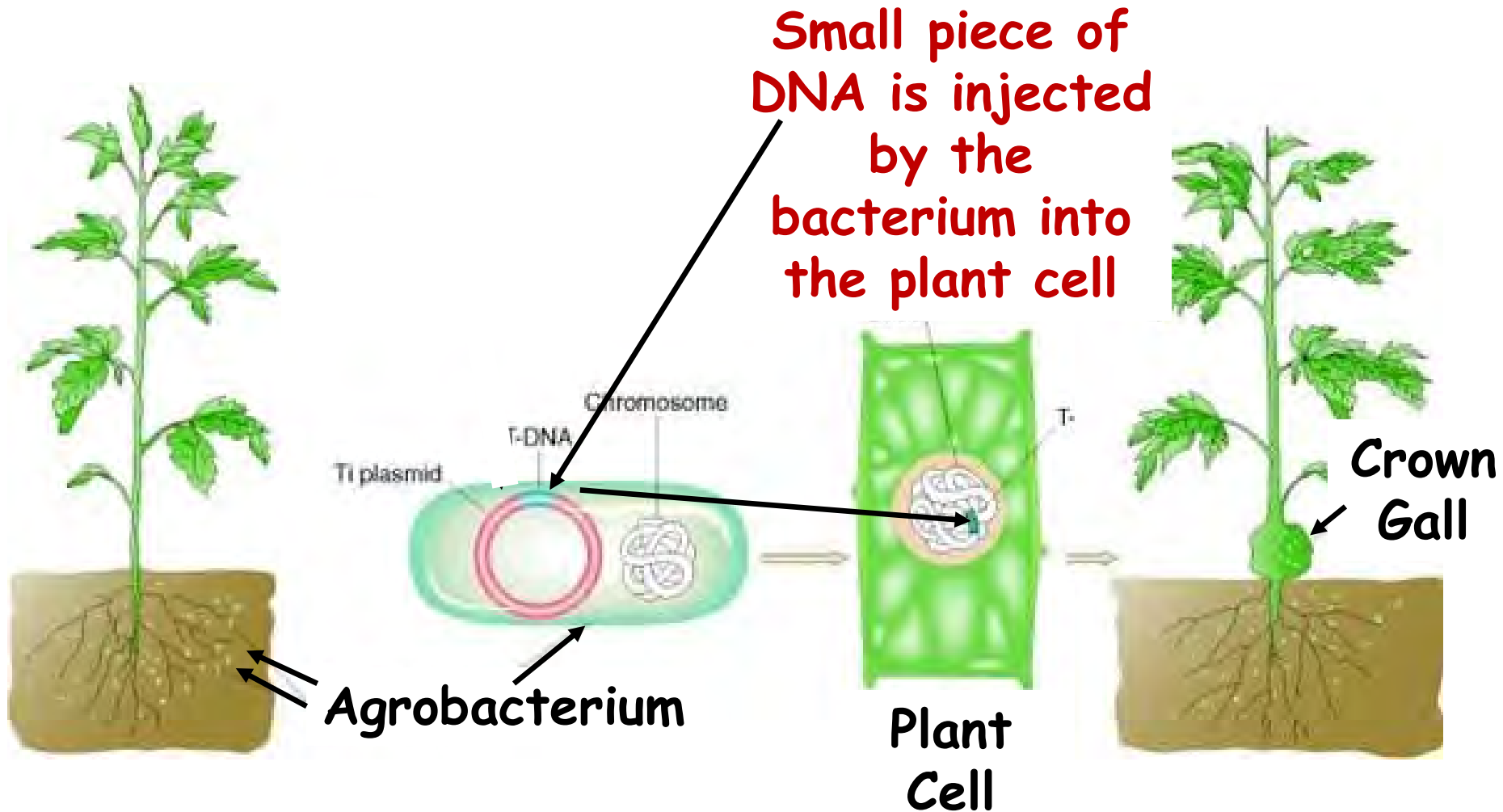
Summary

Evidence is presented that crown gall tumors caused by the incorporation of part of a viral plasmid carried by the inciting bacterium *Agrobacterium tumefaciens*. The rate of reassociation of labeled plasmid DNA was slightly accelerated in the presence of tobacco crown gall tumor DNA but not normal tobacco DNA. Treatment of tumor DNA with DNAase abolished the acceleration. To determine whether all plasmid sequences were represented in tumor DNA, the labeled plasmid DNA was separated into specific fragments by 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

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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

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 4803–4807, August 1983
Genetics

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Vol. 80, pp. 4803–4807, August 1983
Genetics

Expression of bacterial genes in plant cells

(plant protoplasts/transformation/foreign DNA/antibiotic resistance/selectable markers)

ROBERT T. FRALEY, STEPHEN G. ROGERS, ROBERT B. HORSCH, PATRICIA R. SANDERS, JEFFERY S. FLICK, STEVEN P. ADAMS, MICHAEL L. BITTNER, LESLIE A. BRAND, CYNTHIA L. FINK, JOYCE S. FRY, GERALD R. GALLUPPI, SARAH B. GOLDBERG, NANCY L. HOFFMANN, AND SHERRY C. WOO

Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, Missouri 63167

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Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, Missouri 63167