Frontiers of Modern Genetics

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University of the Third Age (U3A)
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What will you learn? (hopefully)

1) Quick ‘catch-up’ on cells, genetics and DNA (central dogma)
2) Special advances in modern genetics
3) Impact and future needs

Fundamental Questions:

How does GENETICS function?

How does it affect us directly?

Examples and approximations will be given

What is GENETICS:

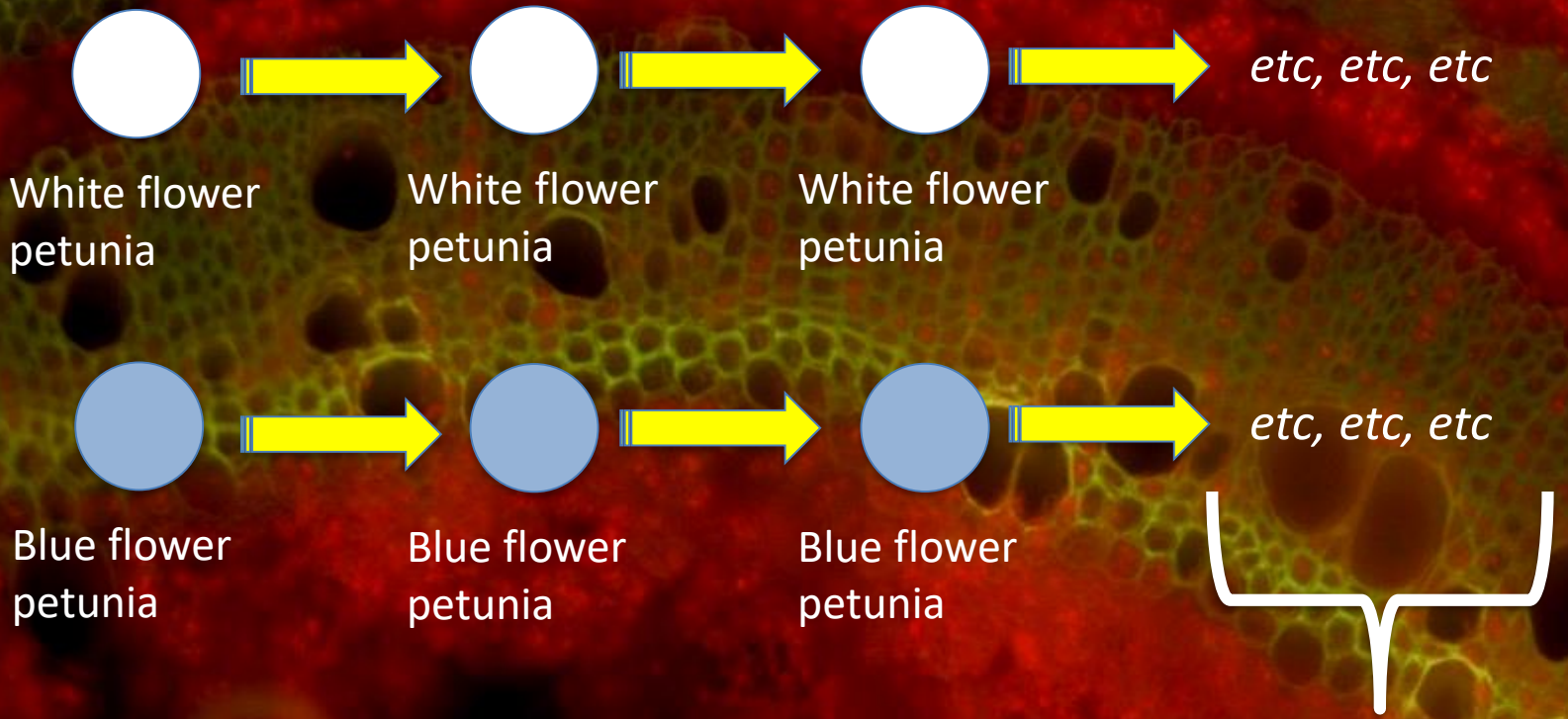
The science / study of inheritance of TRAITS in both higher organisms (humans, other animals, plants, fungi, algae), viruses and bacteria

What is MODERN GENETICS:

Analysis of inheritance and gene expression viewed from either a WHOLISTIC or MOLECULAR perspective
(or **their combination)**

Quickly some BASIC GENETICS:

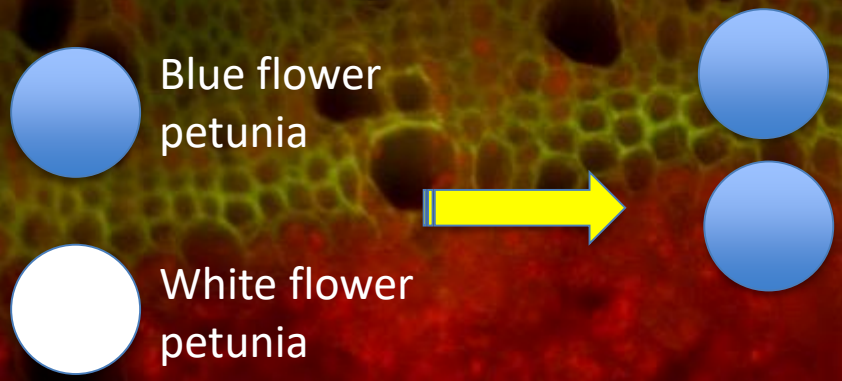
2) Traits of a species are inherited



Quickly some BASIC GENETICS:

2) Traits of a species are inherited

-- what, if HYBRIDISED/crossed ????



Hybrid called ‘F1’ (filia 1)

*The ‘blue’ trait is dominant; ‘white’ is recessive

*Non-function in ‘white’ is suggested

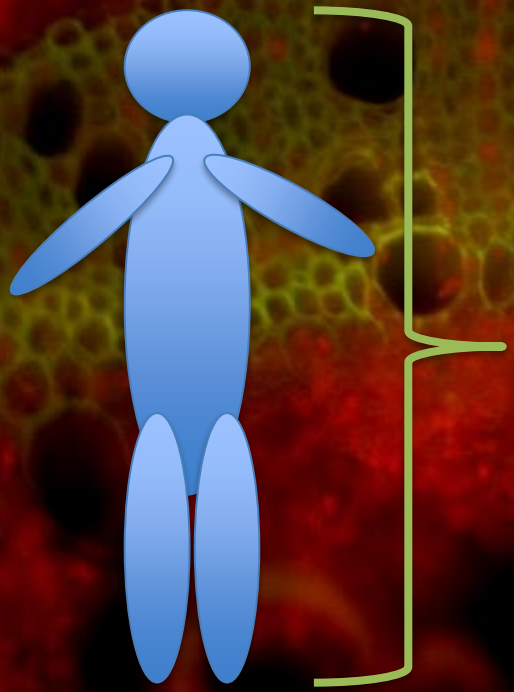
Quickly some BASIC GENETICS:

2) Traits of a species are inherited …….
and segregate, as if '**PARTICULAR**'

Gregor Mendel Law of Inheritance

3) Humans, animals and plants are made up of CELLS

Bacteria are commonly SINGLE cells

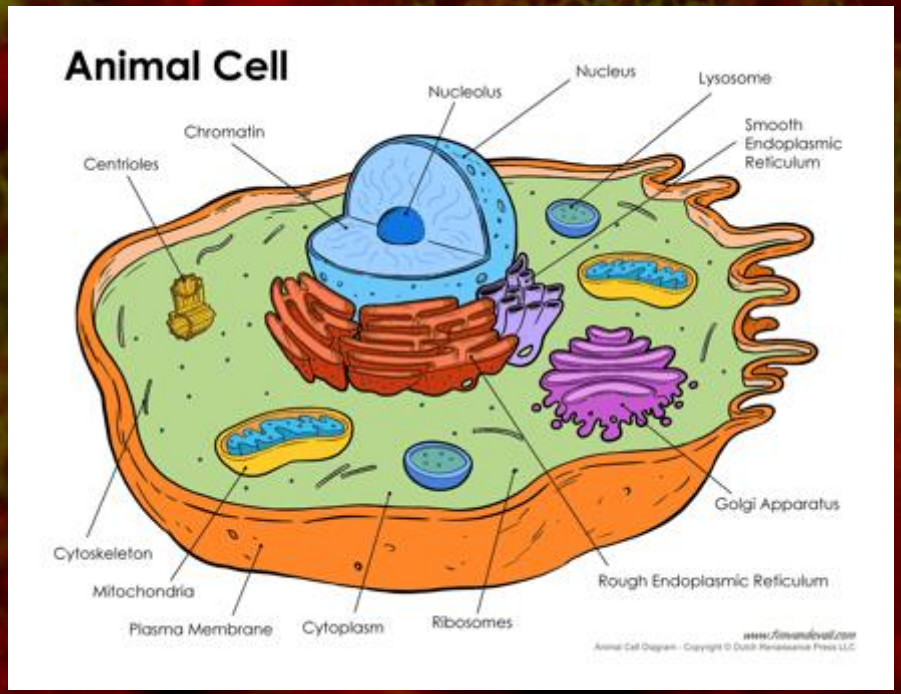


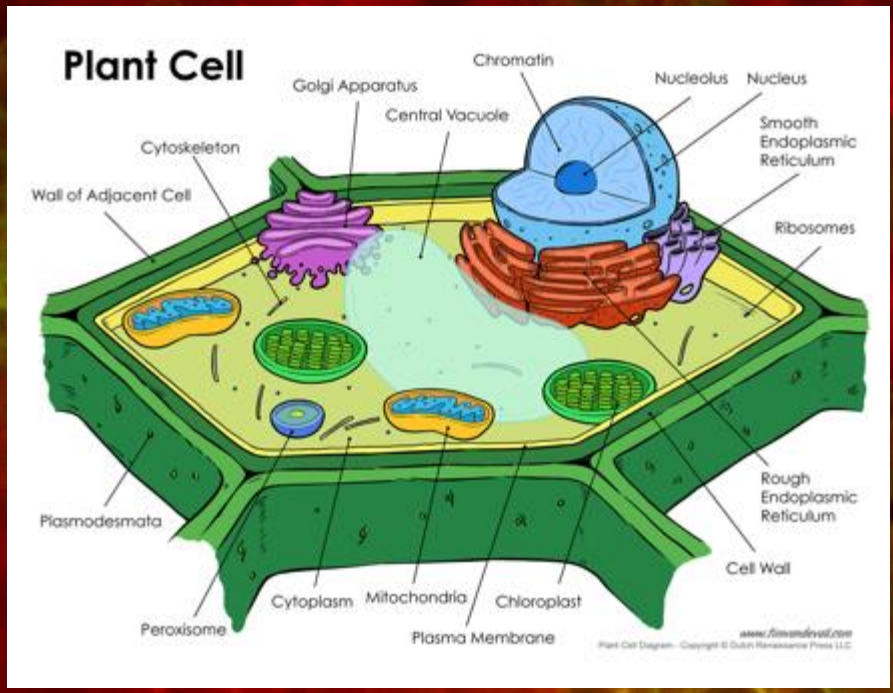
About
10 trillion
cells
(10,000,000,000,000)

and some CELL BIOLOGY:

4) Cells have a common design

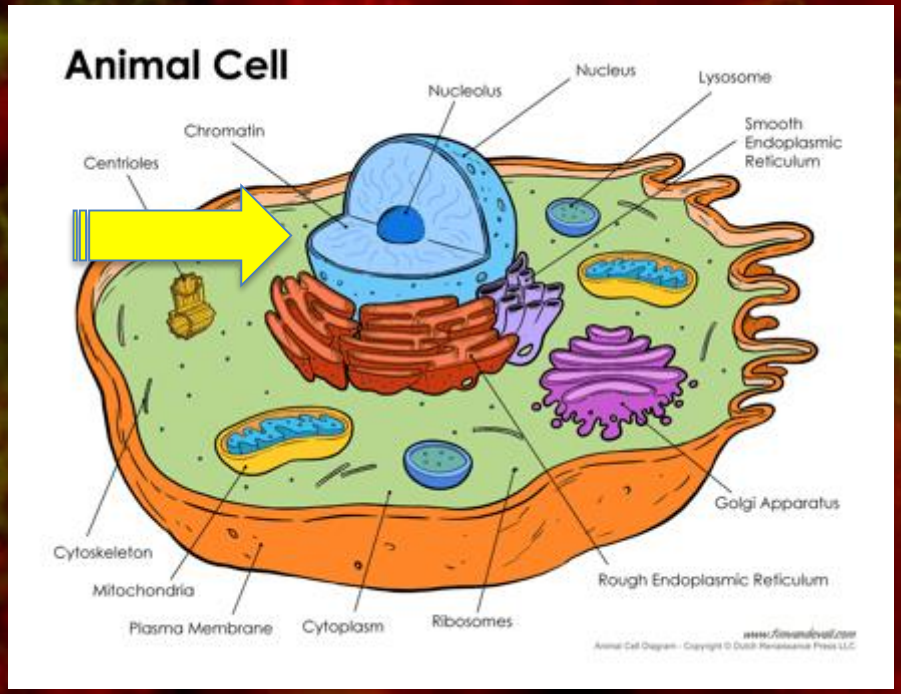
(some minor differences depending on function and origin)

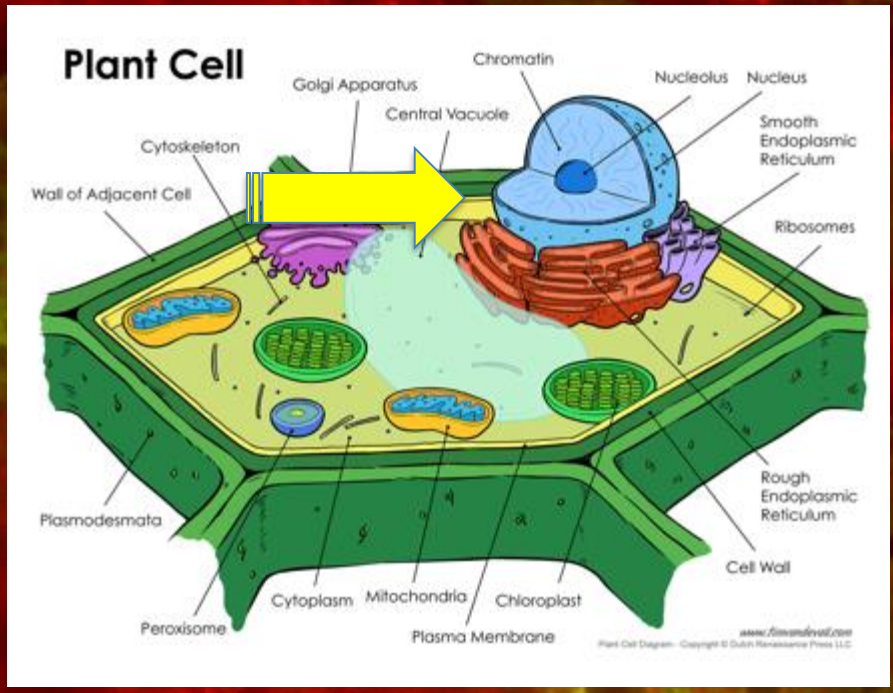




4) the nucleus stores genetic material (DNA)

(note: small DNA also found in mitochondria and chloroplasts)

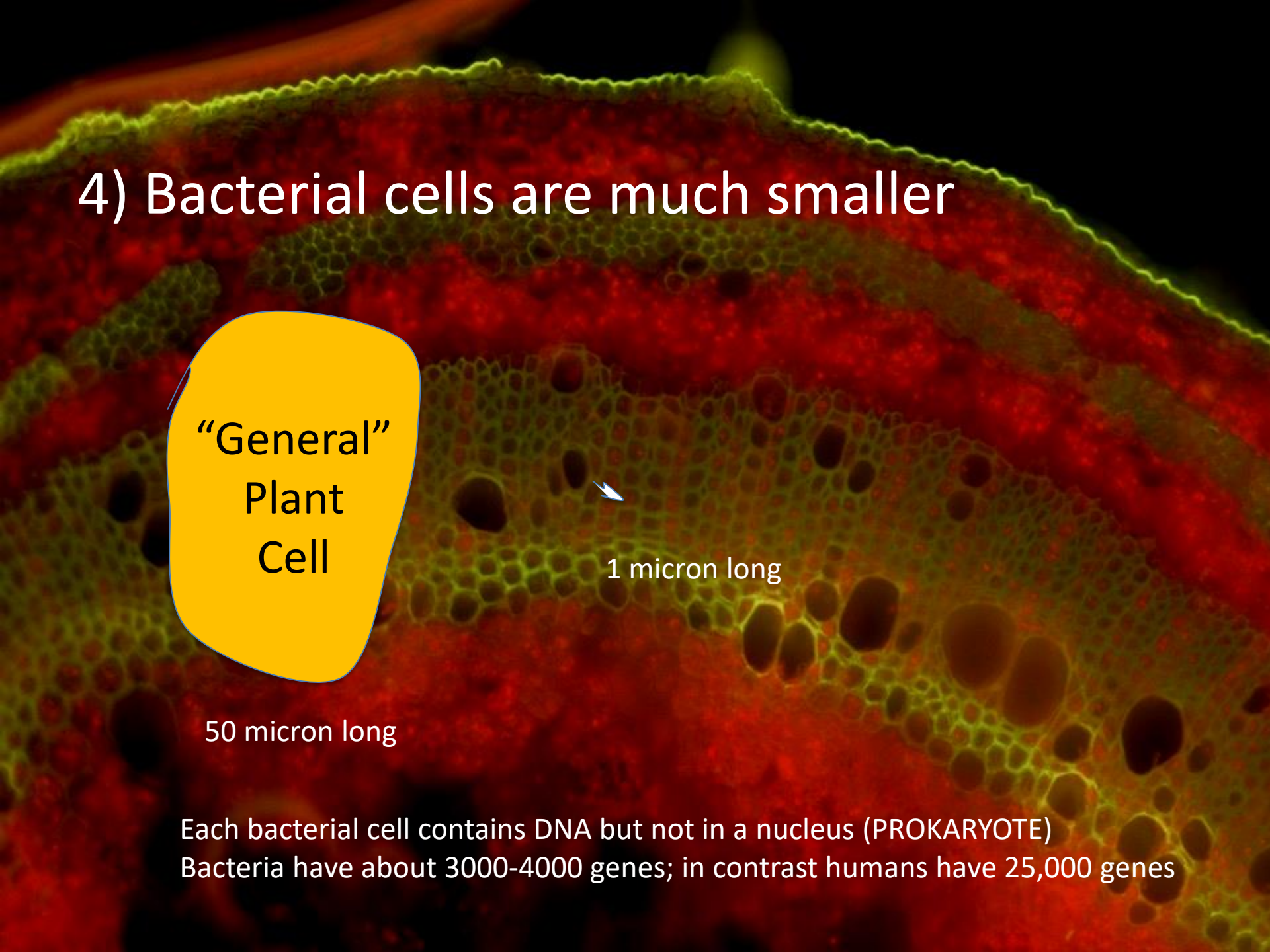




About 3 to 12 microns long

About 20 to 50 microns long

4) Bacterial cells are much smaller

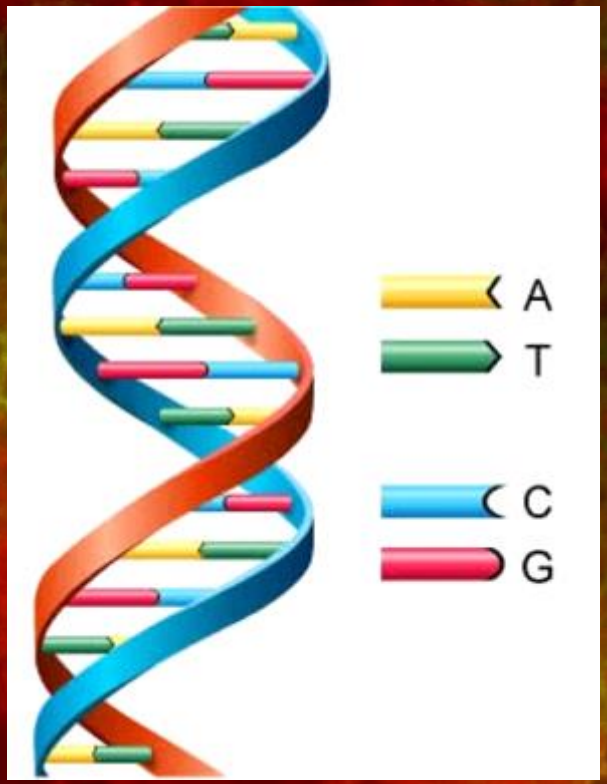


Each bacterial cell contains DNA but not in a nucleus (PROKARYOTE)
Bacteria have about 3000-4000 genes; in contrast humans have 25,000 genes

The molecular basis of inheritance:

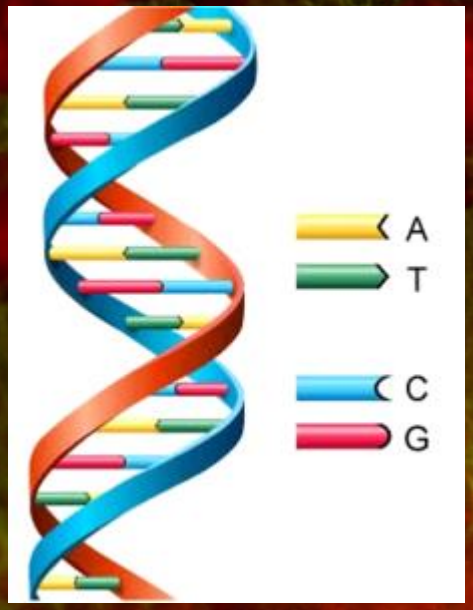
5) DNA: deoxy-ribo-nucleic acid (double helix)

(note: chemically the same in ALL organisms; bacteria to humans!!)



Structure discovered by
James Watson and Francis Crick (1953)

5) DNA: deoxy-ribo-nucleic acid make-up



Sugar: deoxy-ribose

Phosphate: PO_4^-

These make the sugar-phosphate backbones (**strong** coupling (covalent))

Nucleotides:

Adenine A

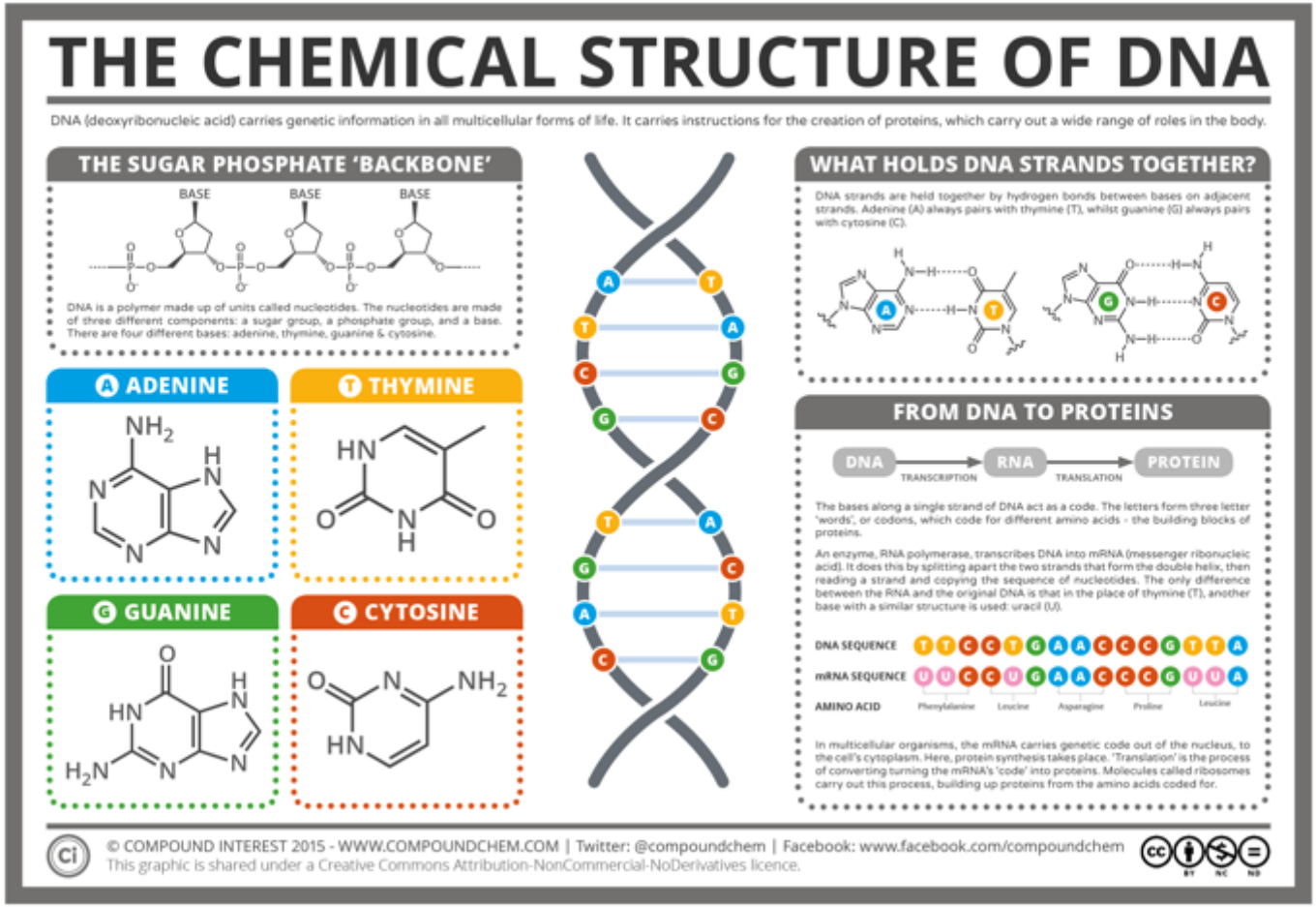
Guanine G

Thymine T

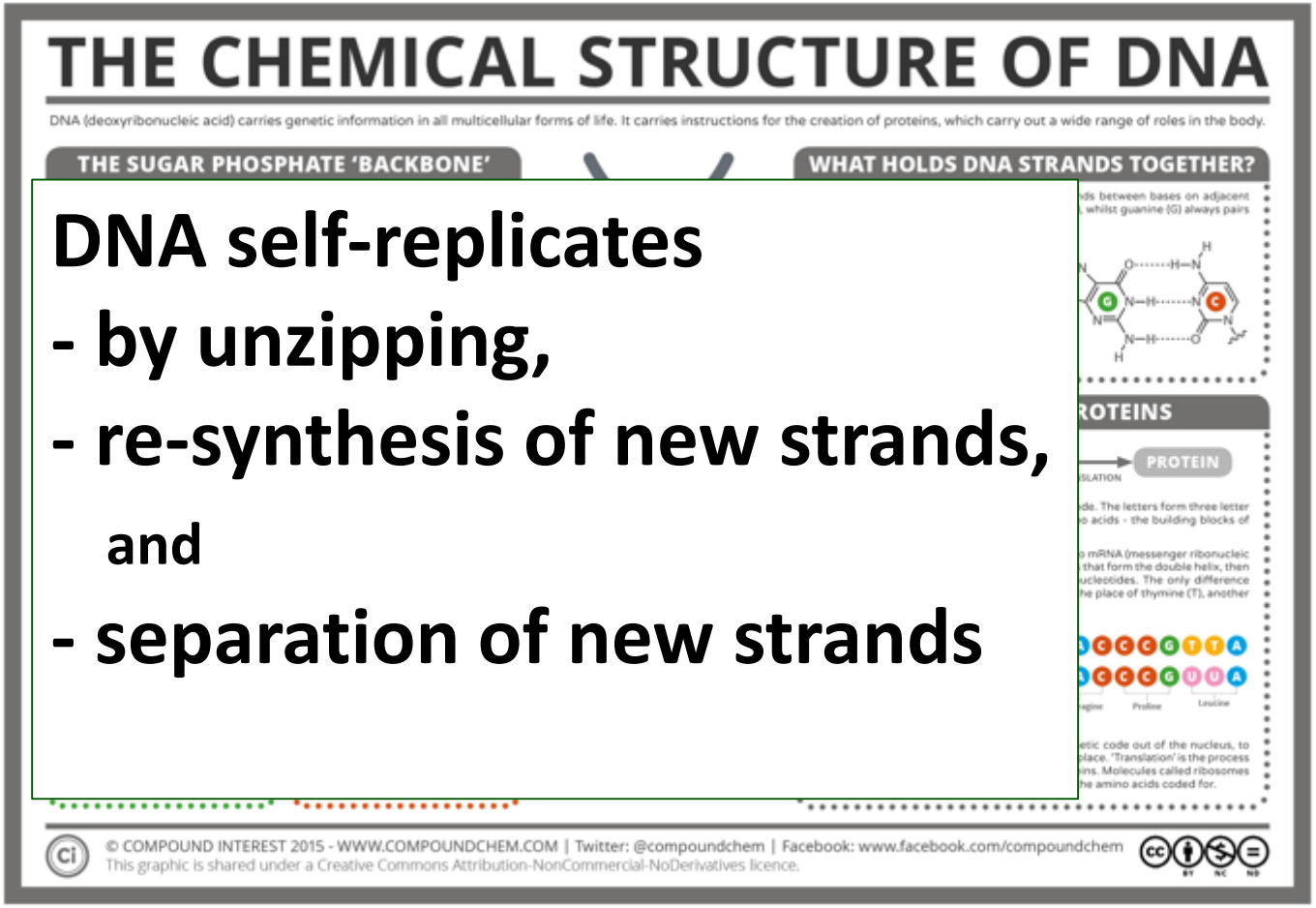
Cytosine C

- Double Helix
- A and T bind, G and C bind (ionic; **weak**)

5) DNA: deoxy-ribo-nucleic acid make-up



5) DNA: deoxy-ribo-nucleic acid



DNA self-replicates

- by unzipping,

- re-synthesis of new strands,

and

- separation of new strands

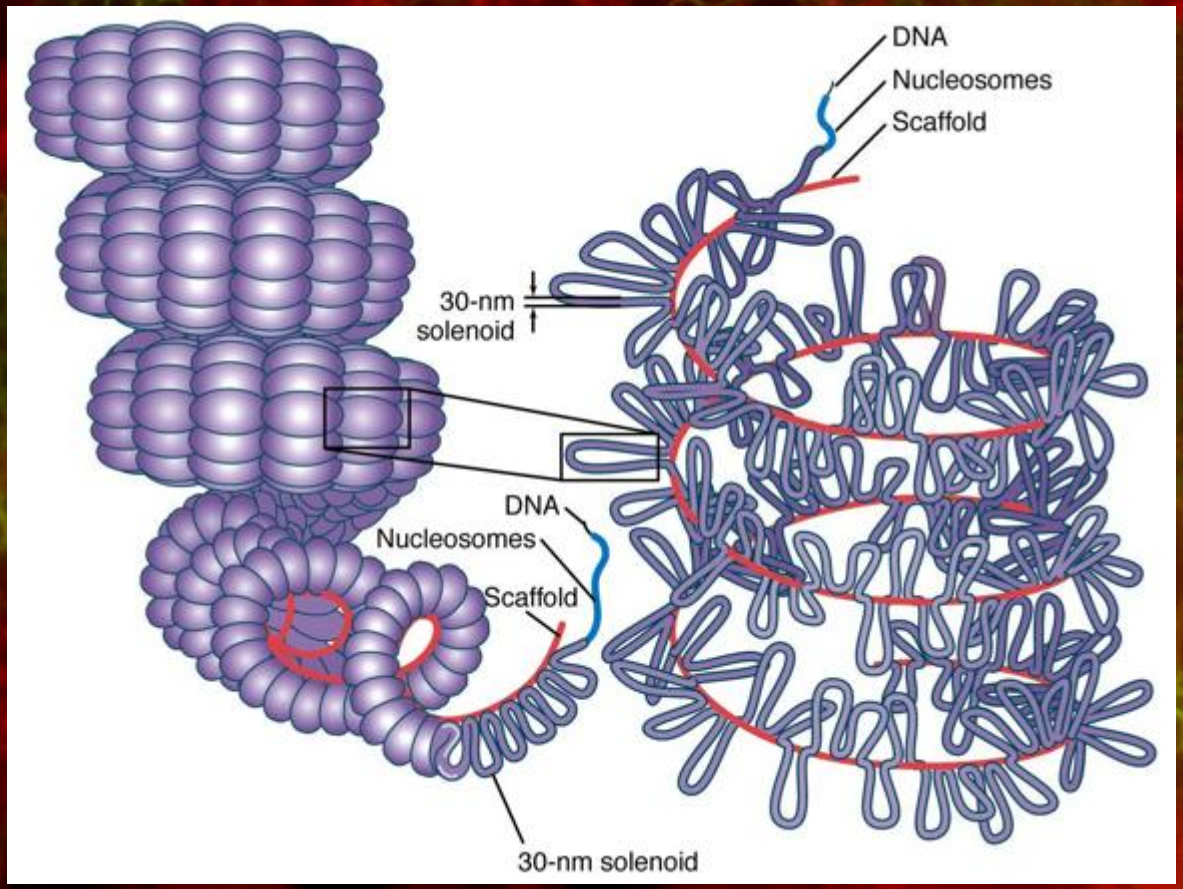
6) DNA is kept in CHROMOSOMES

(note: different sizes and DNA per chromosome.
'Chromo'-'some' means: 'coloured' …. 'body')

Metaphase chromosome; coiled DNA

Human cells each have 46 chromosomes*; 23 from mum, 23 from dad
Soybean has 40 chromosomes; 20 from the pollen, 20 from egg

7) VERY large amounts of DNA are coiled up as solenoid structures



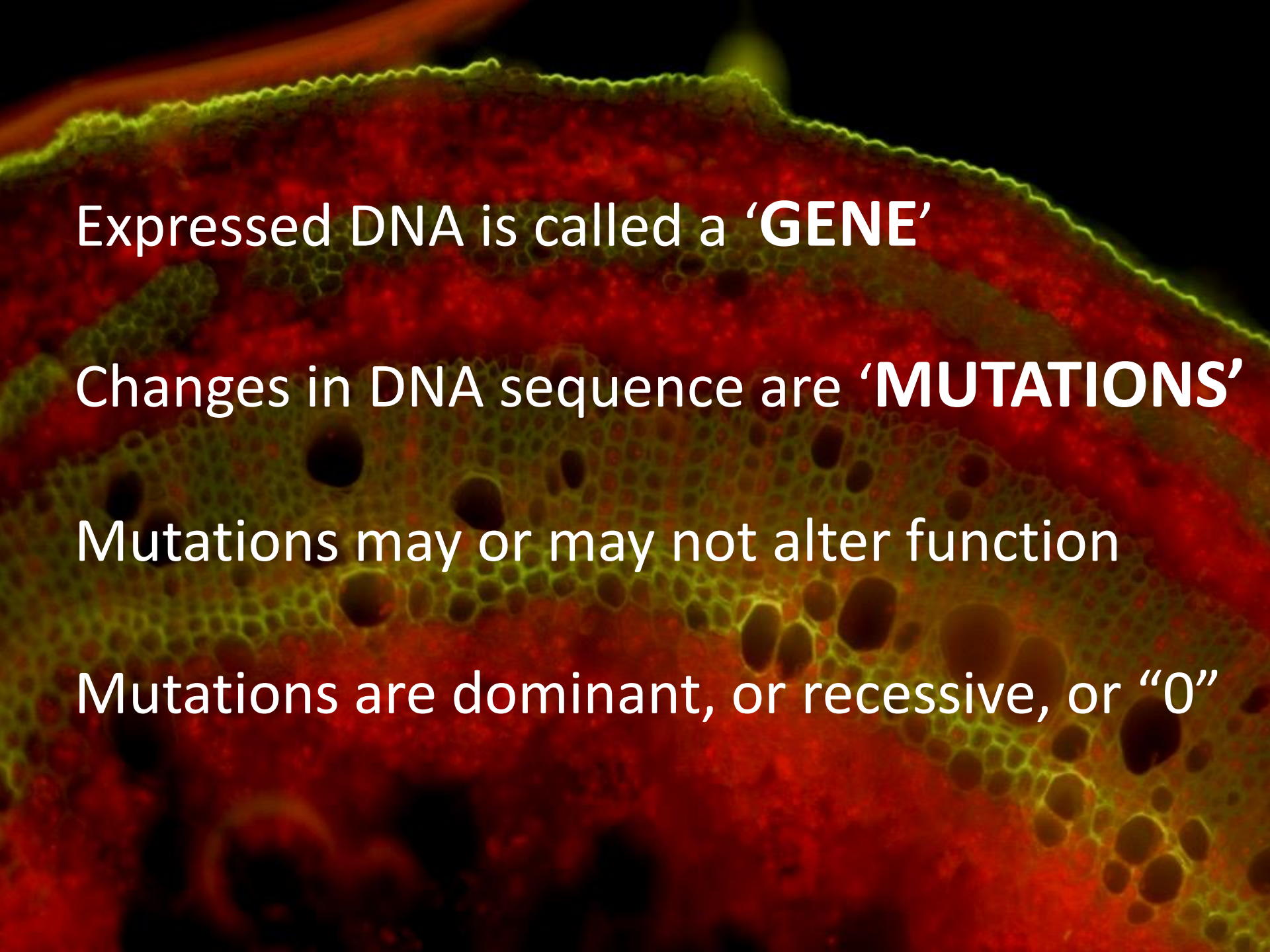
8) The CENTRAL DOGMA

DNA sequence (..ATGGGGCCCTTATAG...)
is read in TRIPLET fashion (*e.g.*, ATG x n).

The product is RNA (ribonucleic acid)

RNA is functional by itself (rRNA, tRNA, miRNA)
or
is TRANSLATED to make PROTEINS

DNA → RNA → protein → function in cell


Expressed DNA is called a '**GENE**'
Changes in DNA sequence are '**MUTATIONS**'
Mutations may or may not alter function
Mutations are dominant, or recessive, or "0"

DNA sequencing:

- developed in early 1970
- (2 D electrophoresis)

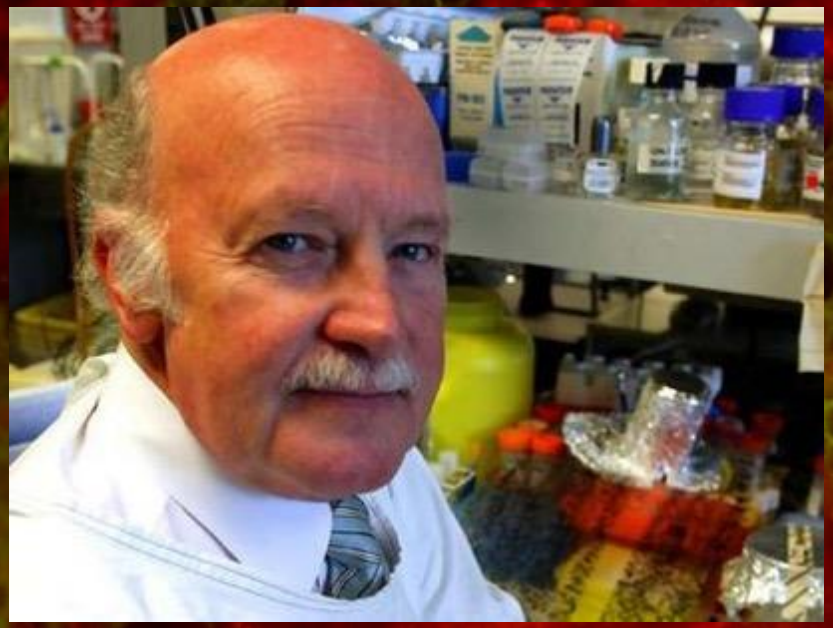
(Aussie example: Professor John Shine)

'Shine-Dalgarno' sequence in bacterial promoters' AUG start codon

ACCUCCUUA 3'

These 9 nucleotides took over 3 years (one PhD study) to discover

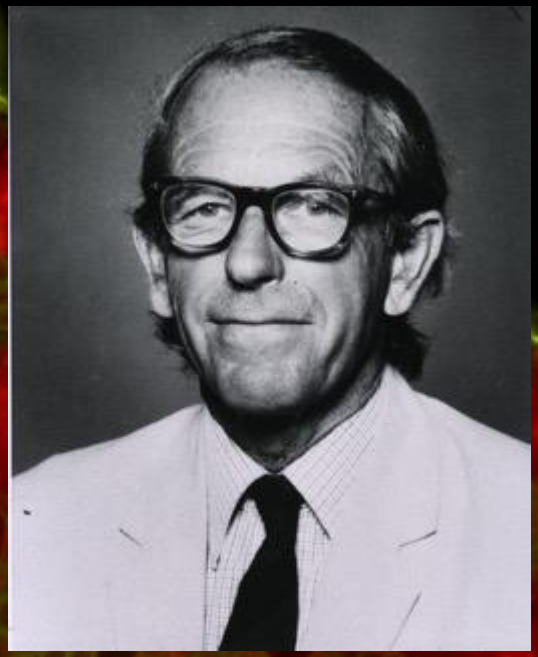
John Shine (ANU, Genentech, ANU, Sydney):



Genentech USA) made profits from synthetic genes and their products. Prof. Shine (now retired) donated $10 million to the Australian Academy

DNA sequencing:

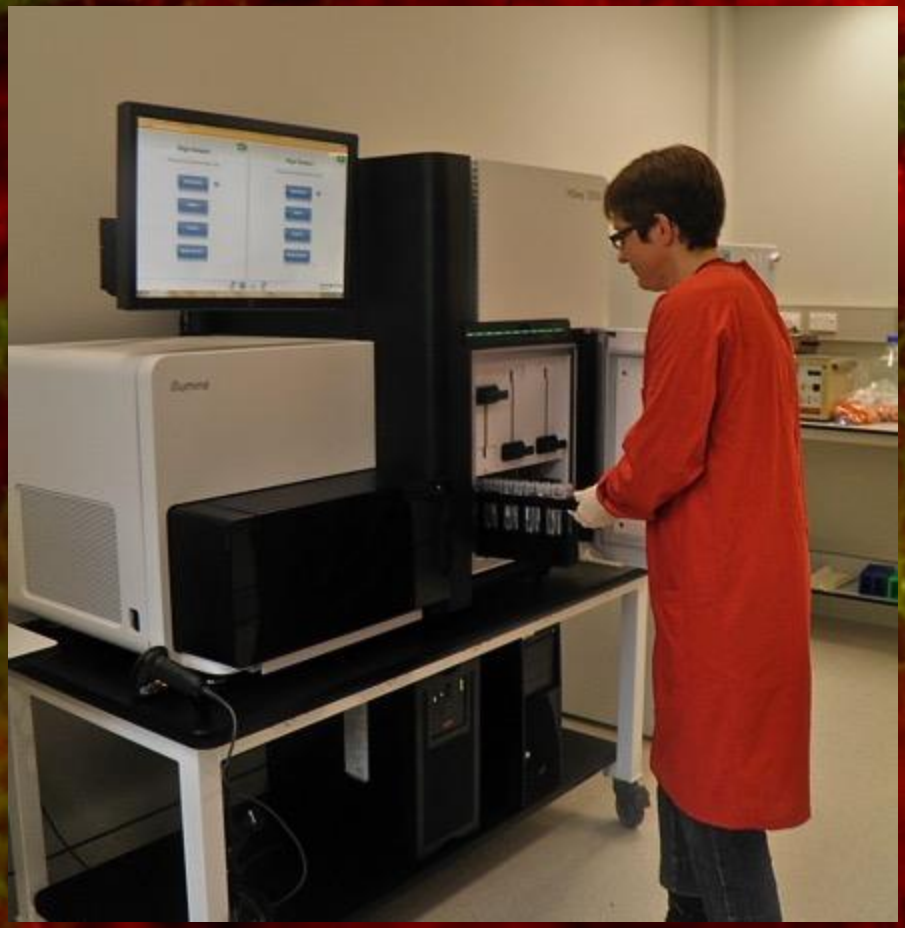
DNA sequencing gel



Professor Fred Sanger

DNA sequencing machines

DNA sequencing instruments:



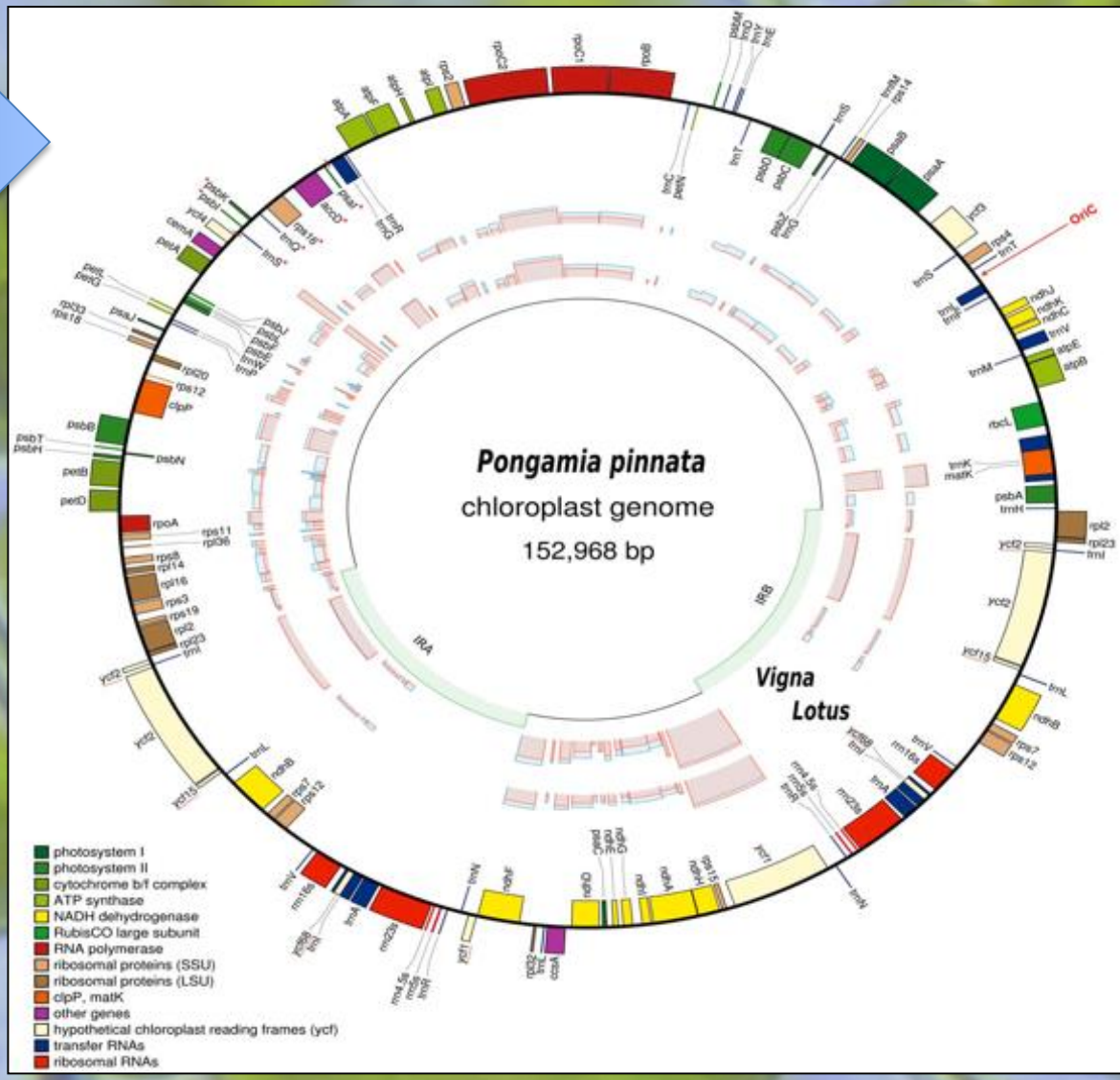
ILLUMINA sequencer

PACBio sequencer

Specific example: tree chloroplast (only small; 153,000 base pairs)

about 50

about 100



Chloroplasts are the site of PHOTOSYNTHESIS (capture of CO_2 gas to make sugar and O_2 gas)

DNA sequencing:

Total DNA (called 'GENOME') of 1000s of species has been obtained.
Animals, humans, plants, viruses, bacteria, fungi

Lays the basis for DNA Profiling, DNA fingerprinting, DNA forensics

Now: about $1-2,000 to have YOUR OWN DNA sequenced. Disease diagnosis.

DNA sequencing:

*Genomes are large (millions of base pairs)
*Most DNA is 'non-functional'
*Some is repeated 100,000 times (*e.g.*, ATAATn)
*Higher organisms have about 15,000 to 40,000 genes
*Some species are polyploid (multiple sets of chromosomes, and thus extra genes (duplicated)

Idealised Human genome: 6,469,660,000 bp (diploid)
Soybean genome: 2,300,000,000 bp (diploid)
Wheat genome: 160,000,000,000 bp (hexaploid)
Escherichia coli genome: 4,600,000 bp (4288 genes)

RNA sequencing (RNAseq):

- Gives a ‘TRANSCRIPTOME’
- Near complete sequence of ALL RNA
- Identifies, via BIOINFORMATICS, the expression of 30 - 100,000 genes in a tissue

Reverse Genetics:

Forwards Genetics goes from altered phenotype to DNA analysis

Reverse Genetics is the ‘reverse’, *i.e.*, DNA to phenotype

Reverse Genetics:

To do so, we need to isolate DNA that is responsible for a phenotype.

This involves ‘positional cloning’ also called ‘Chromosome walking’

Reverse Genetics:
‘Chromosome walking’

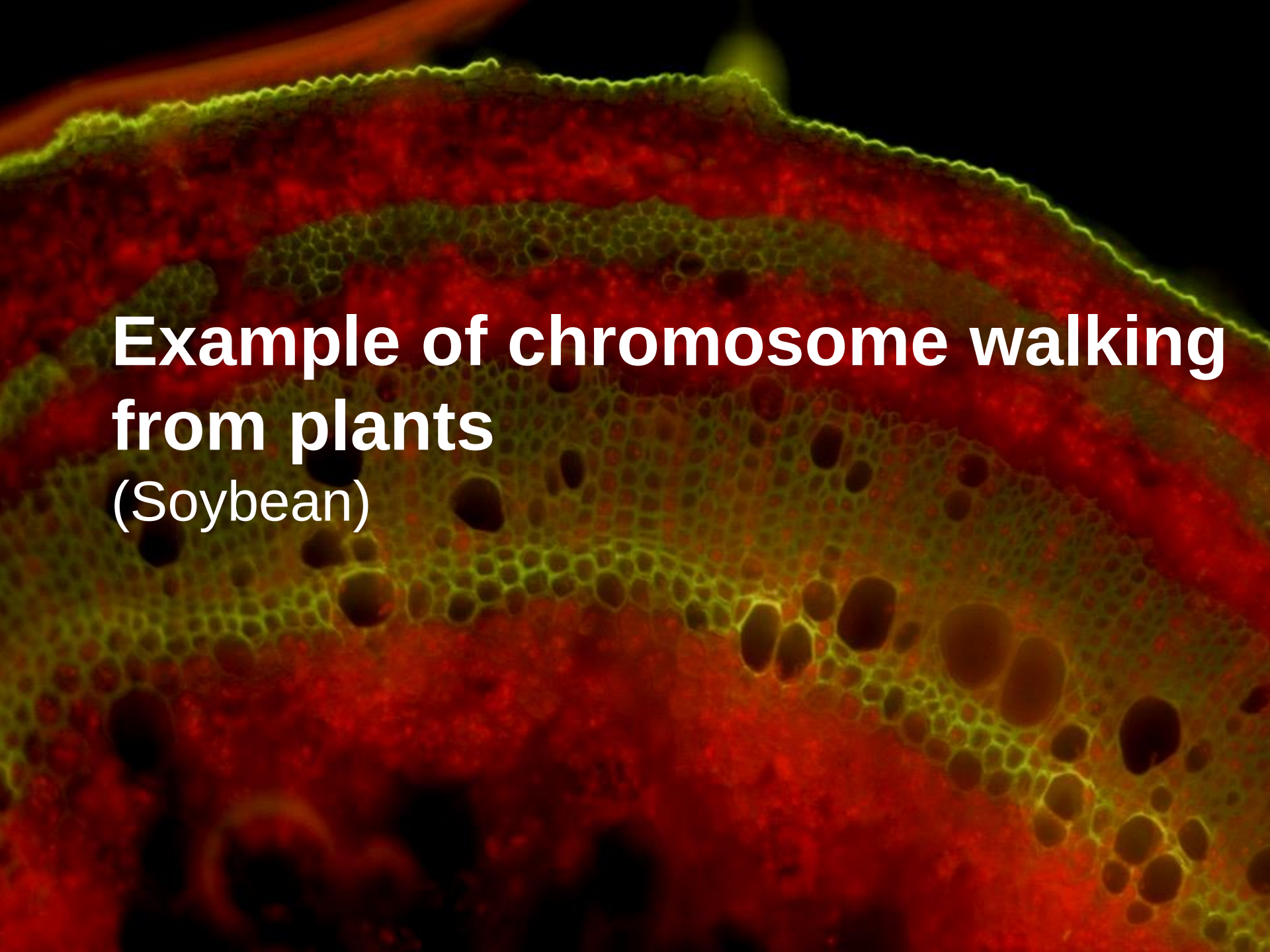
1) Mutant phenotype

2) DNA marker maps

3) Positional cloning

4) Gene identification

5) Mutant correction by gene transfer



Example of chromosome walking from plants

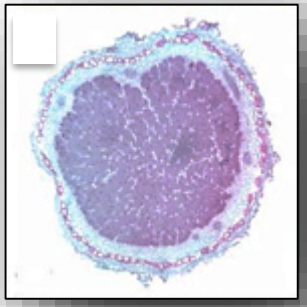
(Soybean)

Root Systems with Nodules

Pea
(*Pisum sativum*)

Soybean
(Glycine max)

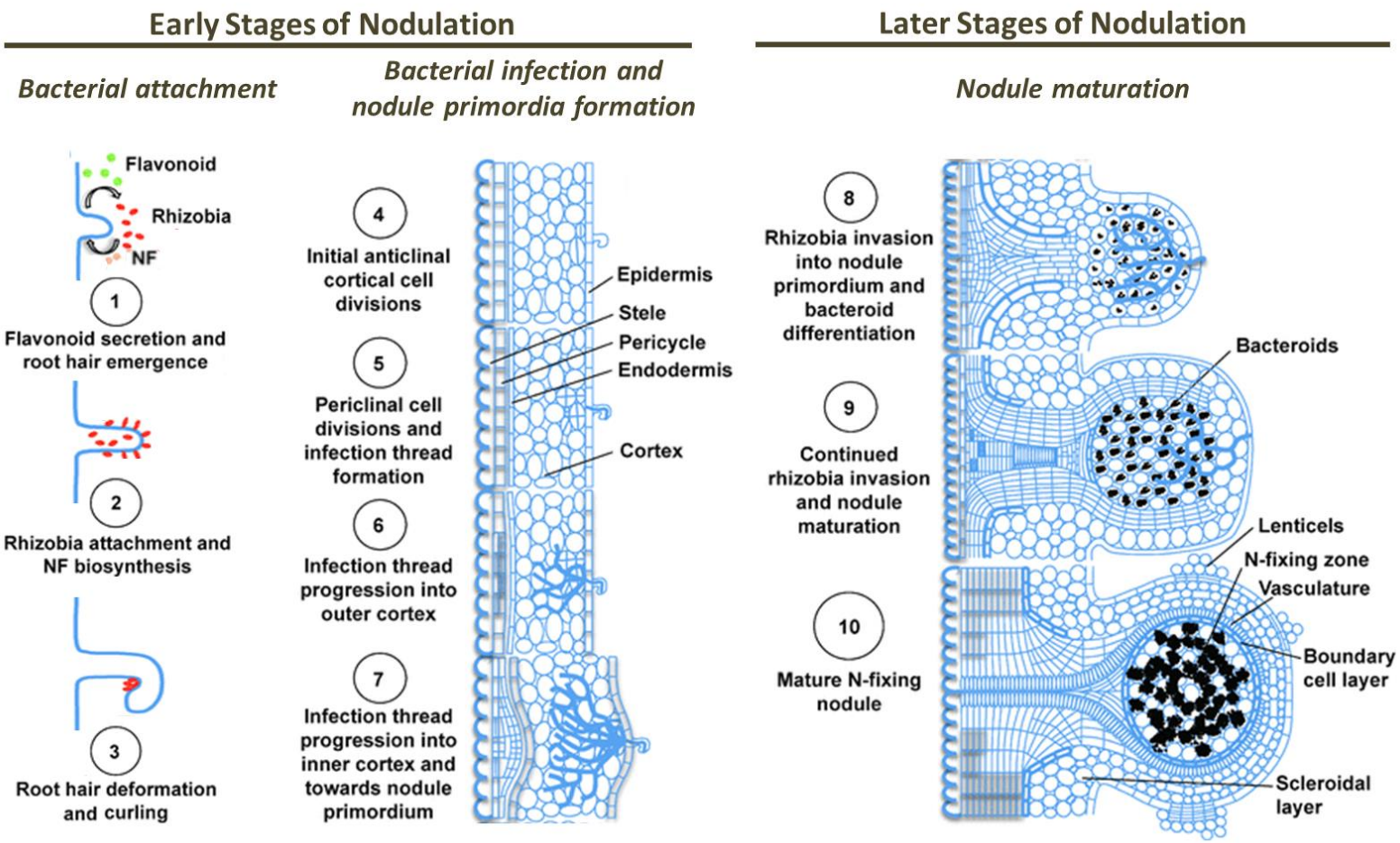
A soybean nodule (determinate)



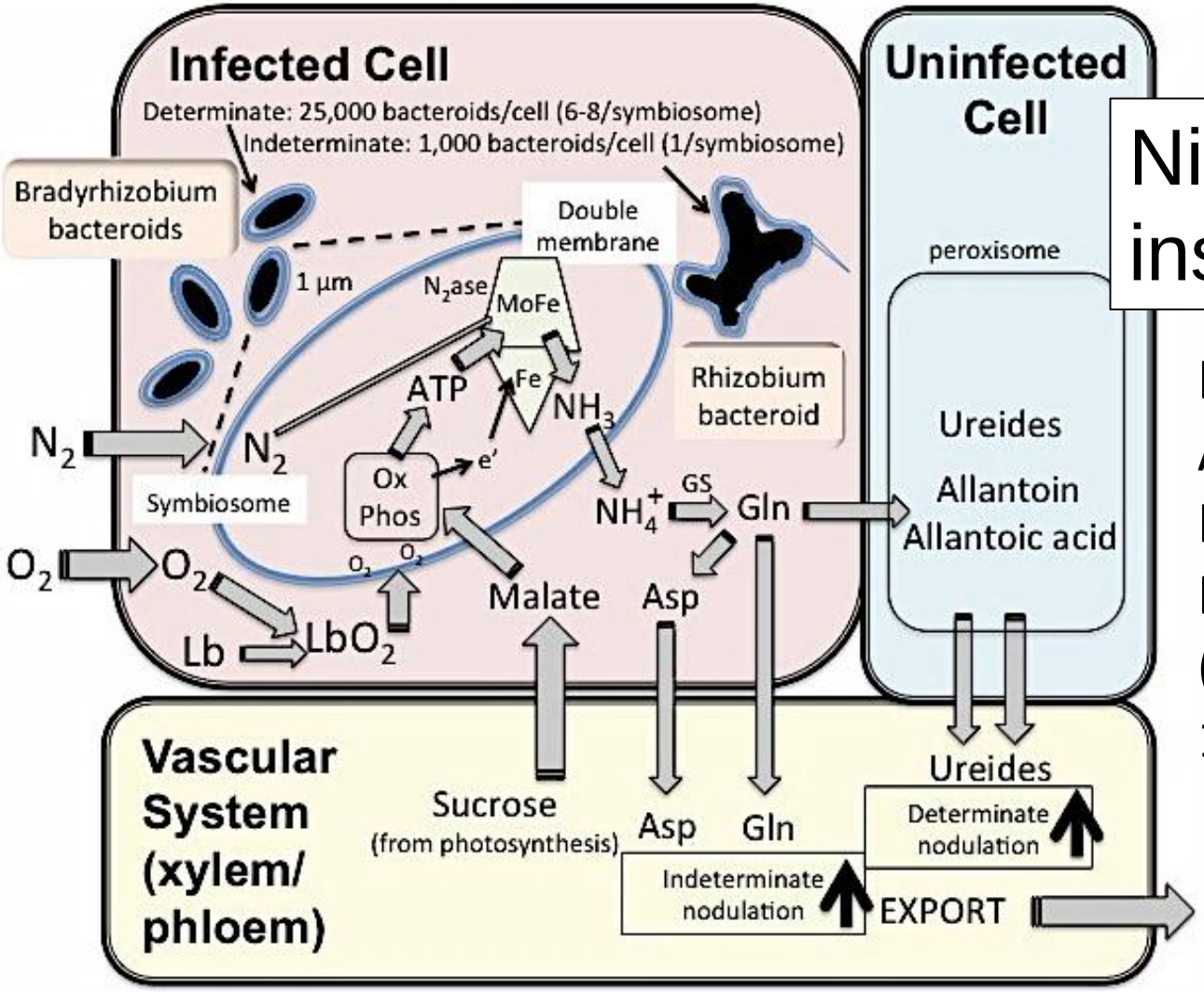
95% of legumes nodulate.
18,000 species in total

SOYBEAN Nodule DEVELOPMENT

** morphological changes associated with nodulation **



Modified from Ferguson et al. (2010) JIPB



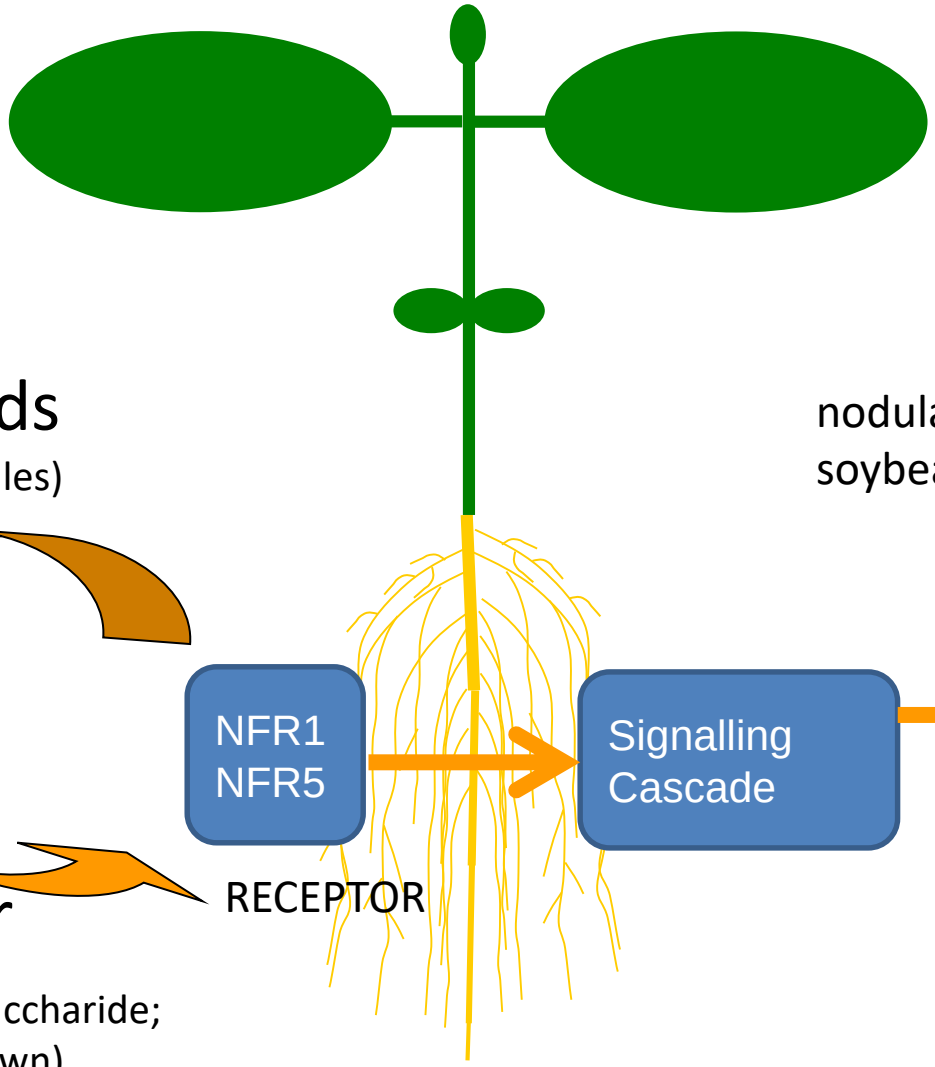
Nitrogen fixation inside the nodule

Leghemoglobin
Ammonia assimilation
Iron protein (Fe)
Molybdenum-Iron (Fe-Mo) protein
16 ATP per mole N

$$N_2 + 8H^+ + 8e- + 16\ ATP \leftrightarrow 2NH_3 + H_2 + 16\ ADP$$

Biswas and Gresshoff (2014) *Int. J. Mol. Sci.* 15:7380-7397

Nodule Development Pathway



Flavonoids
(simple molecules)

Nod factor
(chito-lipo-oligosaccharide; complex, but known)

RECEPTOR

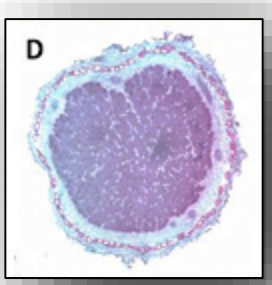
NFR1
NFR5

Signalling Cascade

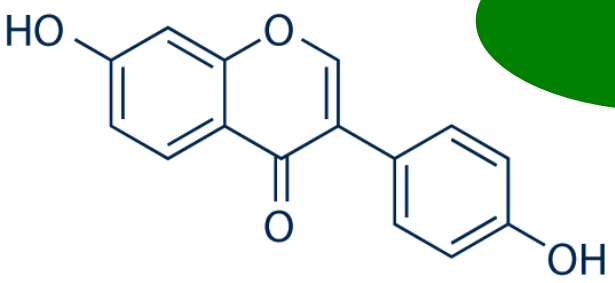
Nodulation

nodulating soybean root

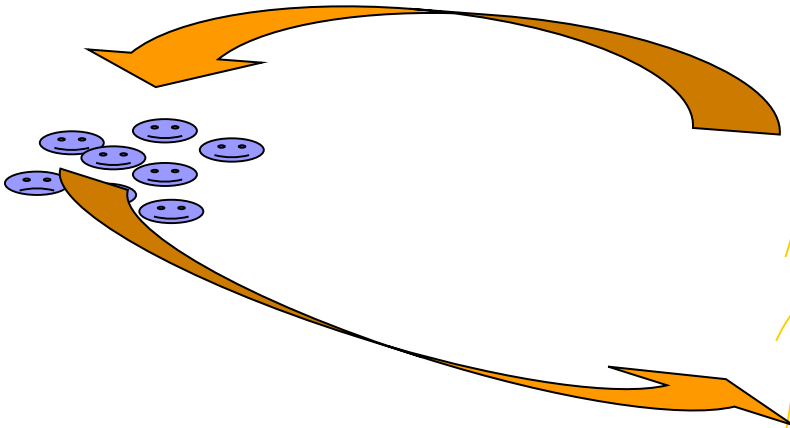
nodule Section
(3 mm)

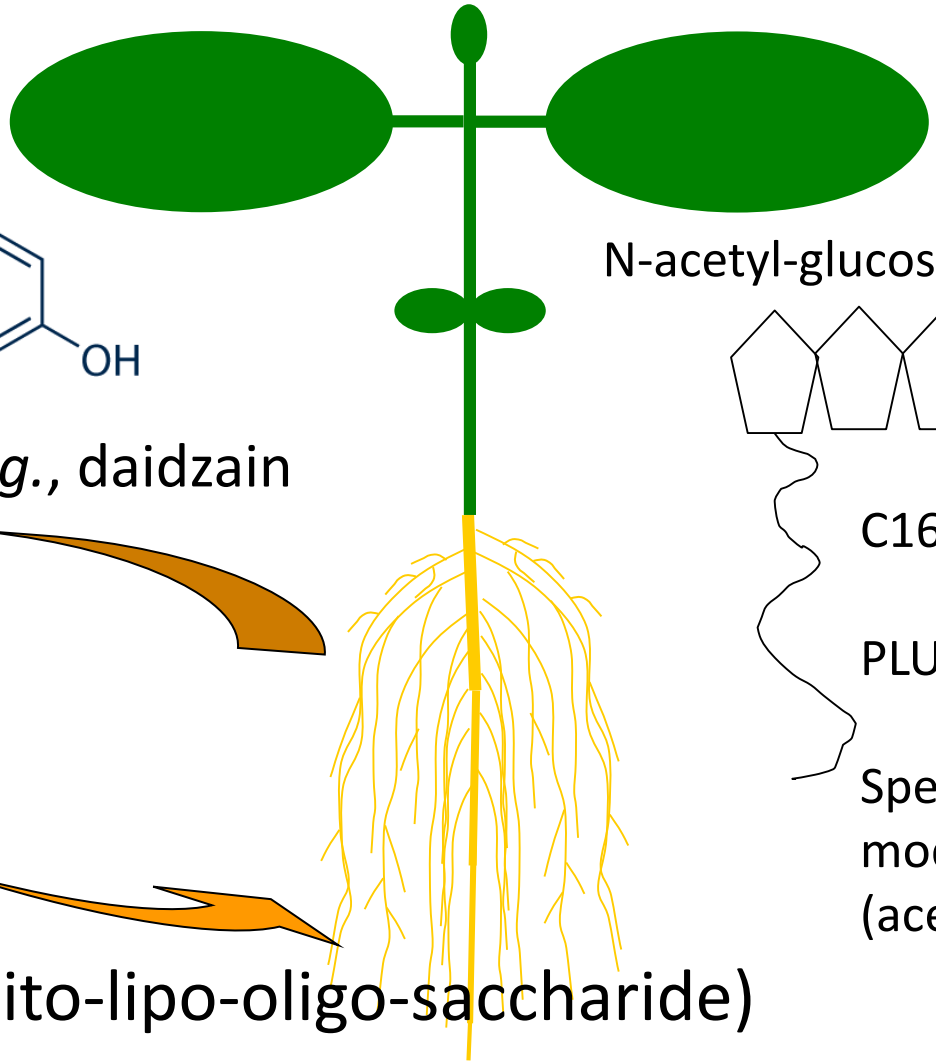


Nodule Development Pathway

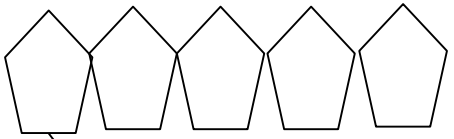


Flavonoids *e.g.*, daidzain





N-acetyl-glucosamine (5x)



C16 or C18 fatty acid

PLUS

Species-specific modification (acetyl, sulphate)

Nod factor (chito-lipo-oligo-saccharide)

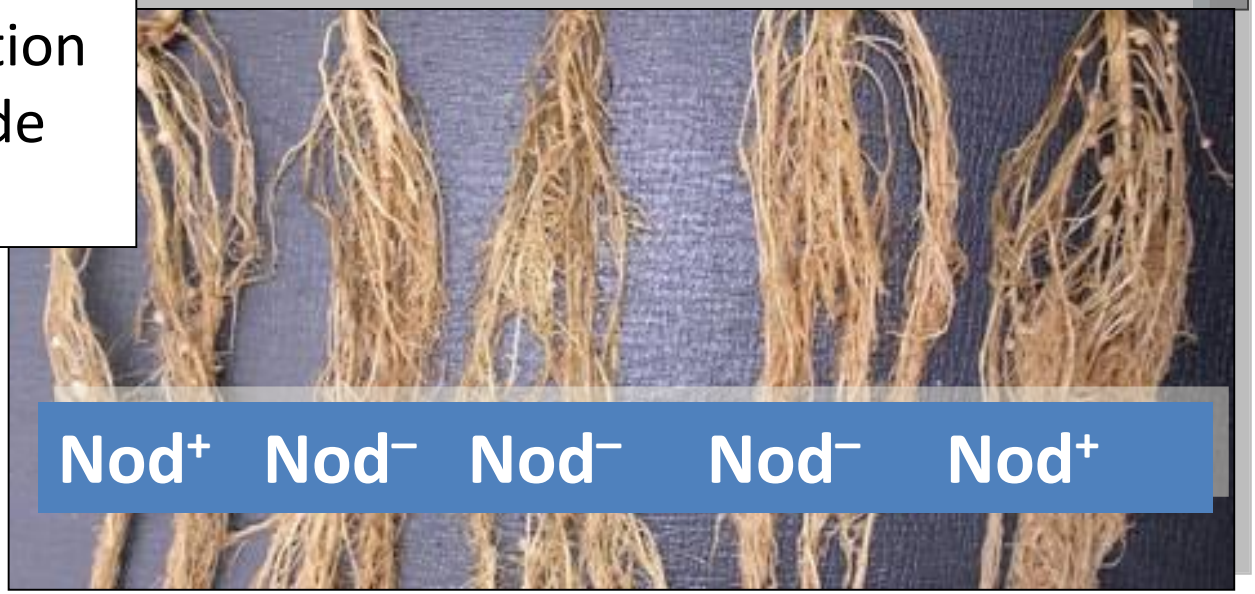
How to isolate (and thus characterise) The genes controlling nodulation in legumes

1) Isolate Non-Nodulating Mutants of Soybean

To Detect the Receptor for the Nod-Factor

Non-Nodulation Mutants of Soybean

Non-Nodulation mutants made with EMS



Non-Nodulation Mutants of Soybean

1) *nod49* (*rj1*) and *nod139* (*nn5*) lack nodules
2) *nod139* has no 'Rhizobium'-response
3) *nod49* develops subepidermal CCDs
4) *nod49* and *nod139* are root-controlled
5) mutant phenotype determined by recessive alleles

Cloning of *nod49* in Soybean

the plant journal

SEB Society for Experimental Biology

The Plant Journal (2011) 65, 39–50 doi: 10.1111/j.1365-313X.2010.04398.x

Nodulation factor receptor kinase 1α controls nodule organ number in soybean (*Glycine max* L. Merr)

Arief Indrasumunar[1,2], Iain Searle[3,4], Meng-Han Lin[1], Attila Kereszt[1,5], Artem Men[1,6], Bernard J. Carroll[1,3] and Peter M. Gresshoff[1,*]

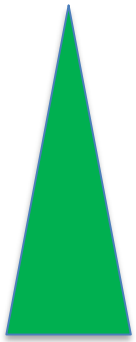
[1]ARC Centre of Excellence for Integrative Legume Research, The University of Queensland, Brisbane St Lucia, QLD 4072, Australia,

Cloning of '*Nodulation*' trait in Soybean

(Indrasumunar et al (2011) Plant Journal 65: 39-50)

Mutant nod49

Wild type *Glycine soja*



Wild type *G. soja*:
-Nodulates
-has slightly different DNA in places (called **MOLECULAR MARKERS** or **Polymorphisms**)

Crossed and then selfed: Non-nodulation segregates at 1 to 3 in F2 (Mendel ratio !!!)

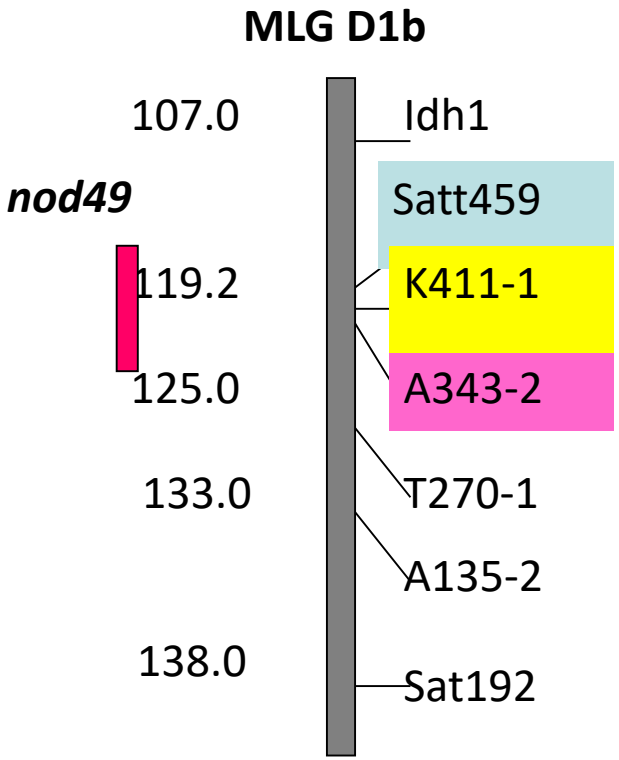
Look at segregation of Molecular Markers and you get a map of each chromosome

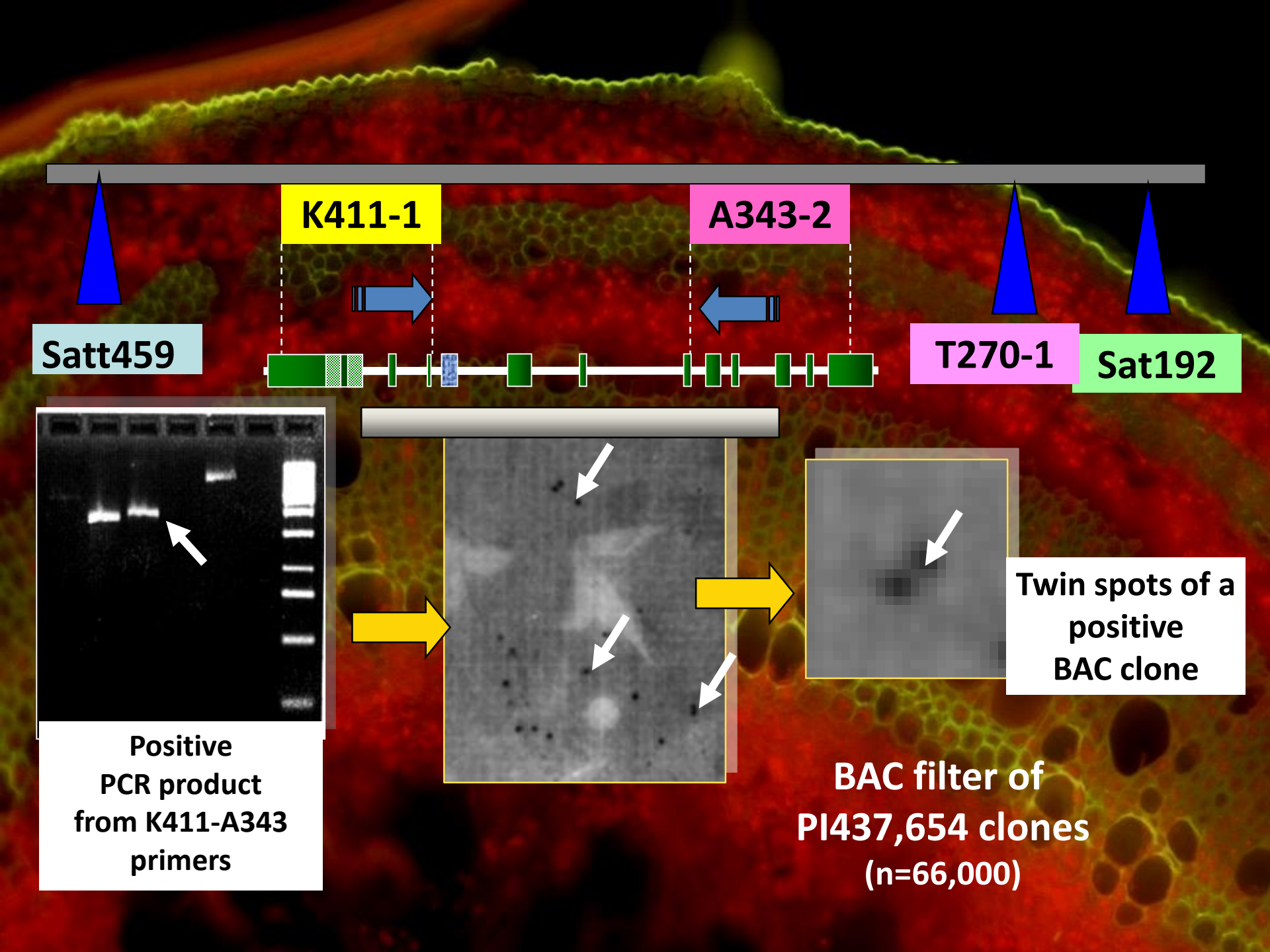
Look which Molecular Markers moved along with the non-nodulation trait

Cloning of *nod49* in Soybean

(Indrasumunar et al (2011) Plant Journal 65: 39-50)

MAPPING of *nod49*

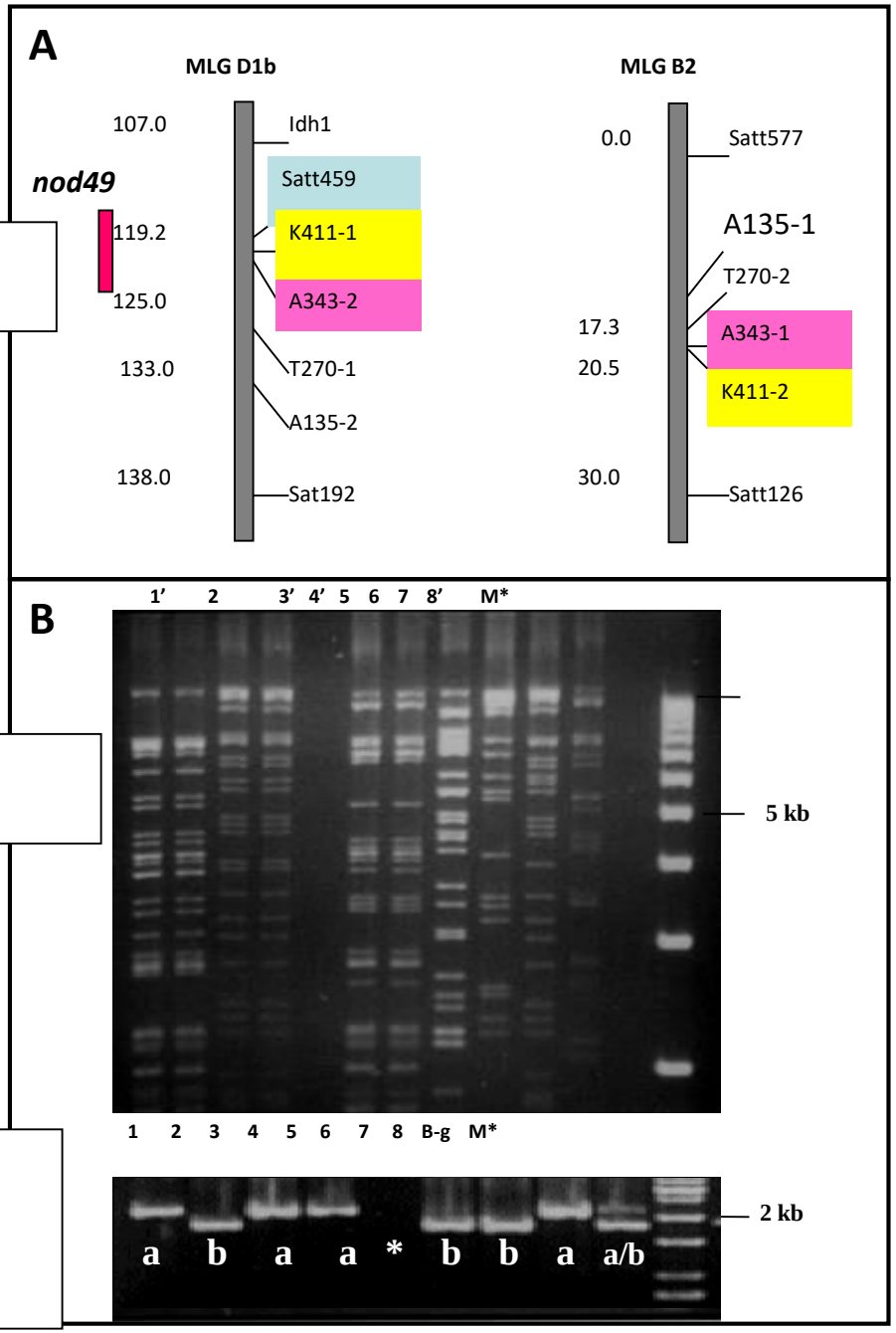



K411-1
A343-2
Satt459
T270-1
Sat192
Positive
PCR product
from K411-A343
primers
Twin spots of a
positive
BAC clone
BAC filter of
PI437,654 clones
(n=66,000)

MAPPING

BAC CLONING

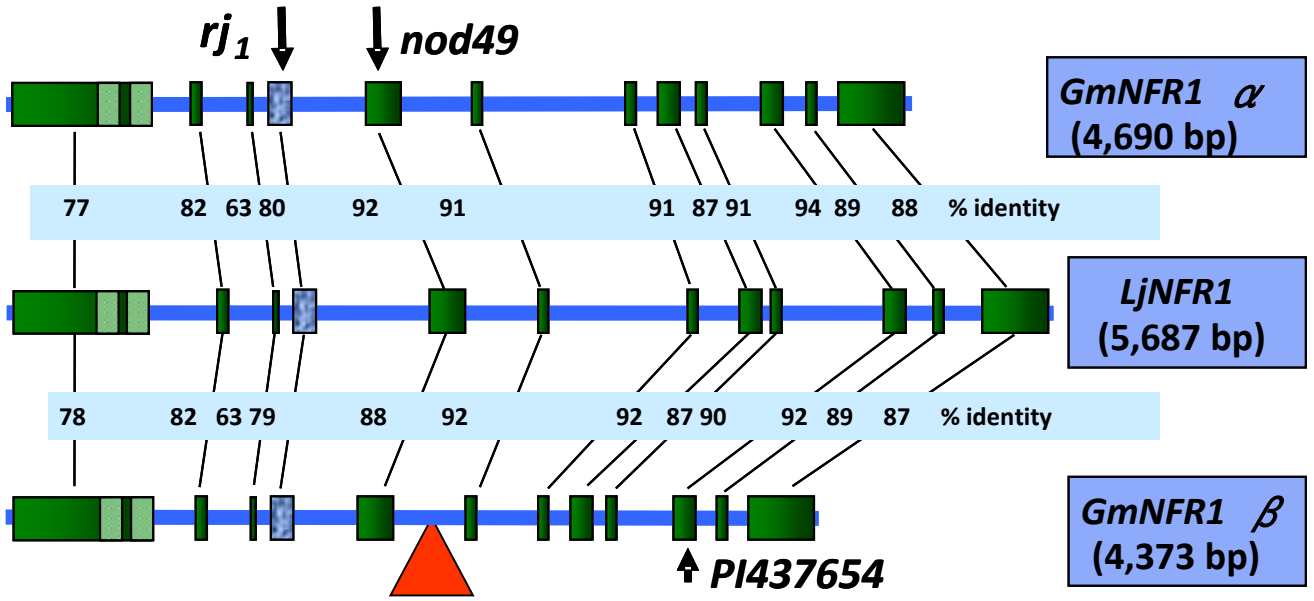
GENE ISOLATION



Identified two regions (*i.e.*, two genes)

Identified a small (α) and a large (β) gene

Performed Chromosome Walking



- TWO genes sequenced: *GmNFR1α* and *GmNFR1β*
- Two mutant alleles identified in *GmNFR1α* (nod49 and rj1)
- *GmNFR1β* has an insertion in intron & is non-functional
- *GmNFR1β* makes a normal transcript (ORF)
- mRNA for *GmNFR1β* about 1-5% of *GmNFR1α*

Complementation using chimeric plants

PROTOCOL

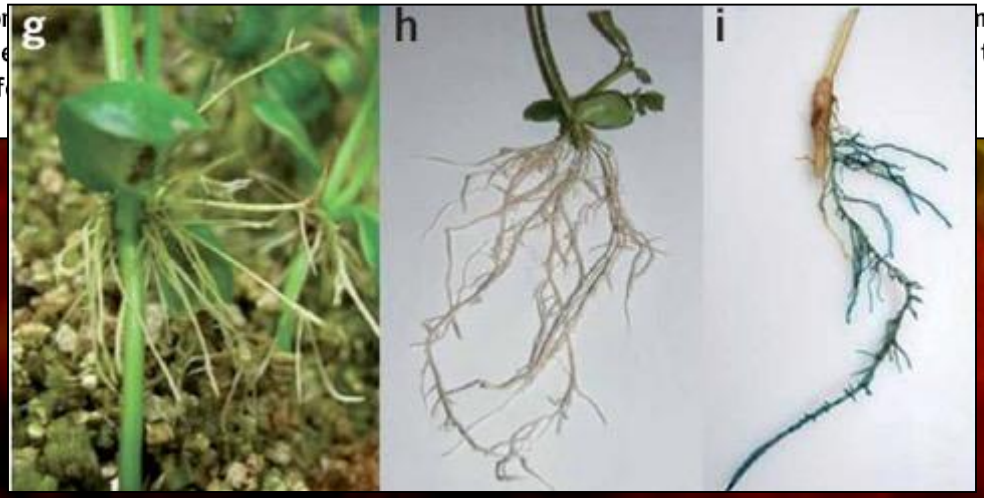
Agrobacterium rhizogenes-mediated transformation of soybean to study root biology

Attila Kereszt[1,2], Dongxue Li[1,2], Arief Indrasumunar[1], Cuc DT Nguyen[1], Sureeporn Nontachaiyapoom[1], Mark Kinkema[1] & Peter M Gresshoff[1]

[1]ARC Centre of Excellence for Integrative Legume Research, The University of Queensland, St Lucia, Queensland 4072, Australia. [2]These authors contributed equally to this work. Correspondence should be addressed to P.M.G. (p.gresshoff@uq.edu.au).

Published online 19 April 2007; doi:10.1038/nprot.2007.141

This protocol is used to induce transgenic roots on soybean to study the function of genes required in biological processes of the root. Young seedlings with unfolded cotyledons are infected at the cotyledonary node and/or hypocotyl with *Agrobacterium rhizogenes* carrying the gene co nidity. When the emerged hairy roots can support the this method, almost 100% of the infected plants f



Gene Discovery ‘Everywhere’

1) The “Positional Cloning” approach works in all higher organisms

2) Diverse variations exist (synthetic gene probes from protein)

3) Candidate genes from one species help to detect genes in others

NB: genes in humans are nearly the same in all other mammals, even plants. Many physiological/biochemical functions are shared across species groups, even biological kingdoms !!!

Take-home messages

So much more needs to be discovered.

Sadly so many problems are not close to being solved.

Detailed knowledge of gene function and expression in specific cells or tissues, and their networking, is helping to dissect the complexity of biology (‘macro’ and ‘micro’)


Thank you

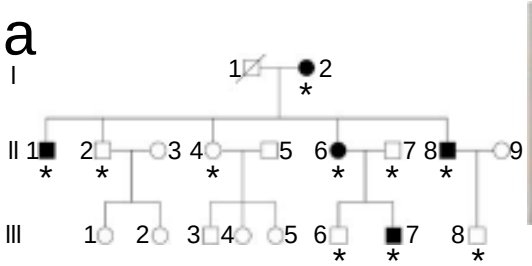
Modern Genetics: gene discovery

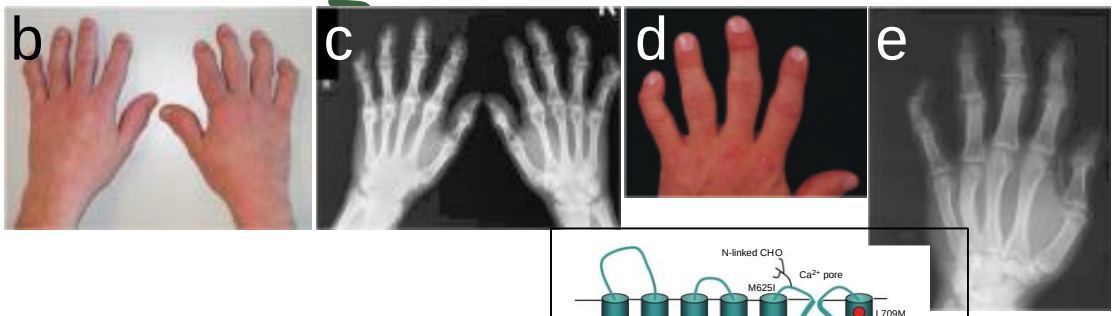
Mutations in *TRPV4* cause an inherited arthropathy of hands and feet

Shireen R Lamandé[1,2], Yuan Yuan[3], Irma L Gresshoff[1,2], Lynn Rowley[1], Daniele Belluoccio[1], Kumara Kaluarachchi[1], Christopher B Little[4], Elke Botzenhart[5], Klaus Zerres[5], David J Amor[1,2,6], William G Cole[7], Ravi Savarirayan[1,2,6], Peter McIntyre[3] & John F Bateman[1,8]

Nature Genetics

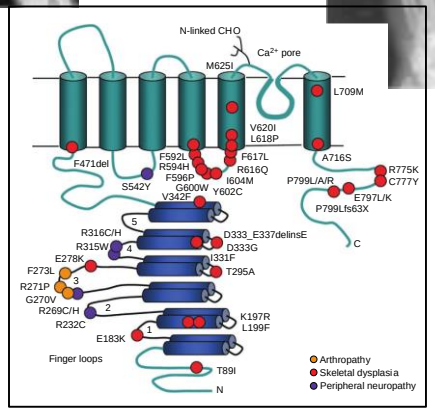
(University of Melbourne research team)





Inheritance (dominant)

The PHENOTYPE



The protein structure