Université Grenoble Alpes

PLANT-Int Master 1st year

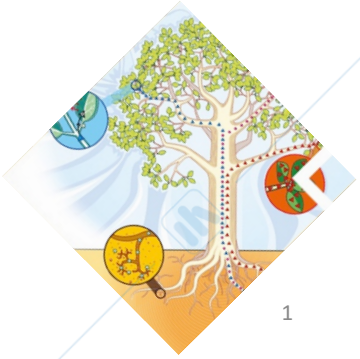
2022-2023

INTRODUCTION TO PLANT DEVELOPMENT AND SIGNALING

Monday 5th Sept, 2pm, Room E302

Coord.: Christel.Carles@univ-grenoble-alpes.fr
Gabrielle.Tichtinsky@univ-grenoble-alpes.fr

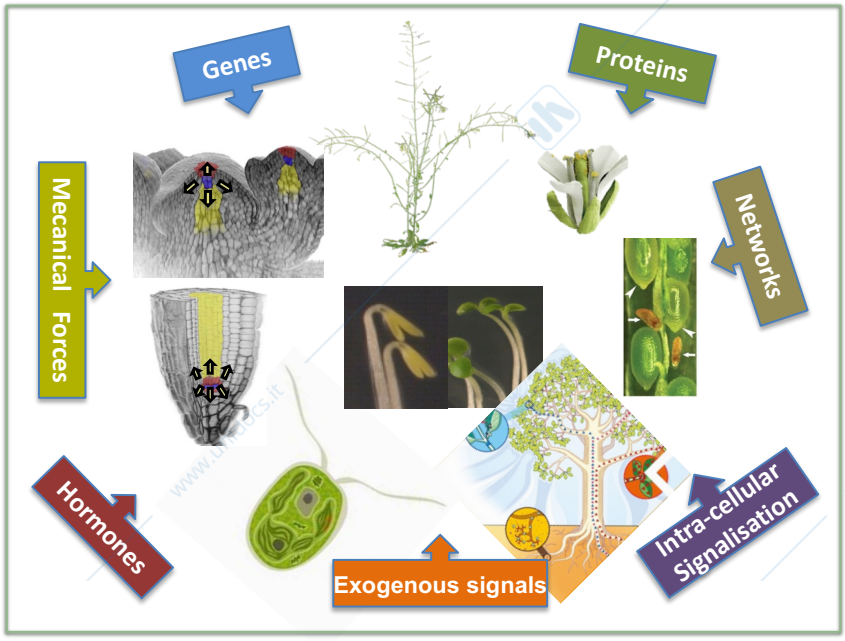
Plant and Cell and Physiology lab
CEA-CNRS-INRA-UGA
Grenoble



INTRODUCTION TO PLANT DEVELOPMENT AND SIGNALING

Objectives

- **Comprehend the genetic, cellular and molecular principles** of plant development and signaling.
- **Search, in articles, key elements** that inform on advances in a precise field (vs state-of-the-art).



Content

17 Lectures *(25,5h)*
⇒ *Written Exam, 2h (counts 50%)*

9 Tutorials *(18h)*
- 3 Approaches & Data Analyses
- 6 Paper Analyses
  ⇒ *Oral Presentations (counts 50%)*

Lab Visit: Wednesday 8th November
⇒ *Research Workshops, 1pm to 6pm*

Teaching team

Local Professors & Researchers of the Plant Cell and Physiology lab (LPCV)

INTRODUCTION TO PLANT DEVELOPMENT AND SIGNALING

***Skills to be developed**, both in written and oral exercises*

- **Working autonomously and in collaborative groups and networks**
- **Participating actively to peers' oral presentations**, asking questions
- **Putting a scientific question in the context of the state-of-the-art**
- **Searching in primary articles, for key/pertinent elements** that inform on advances in a precise scientific field
- **Analysing and presenting a scientific dataset** made of experimental results
- **Understanding the experimental protocol** behind a result, a methodology
- **Proposing an approach and methodologies** to answer a scientific question

INTRODUCTION TO PLANT DEVELOPMENT AND SIGNALING

Topics

- **Approaches and tools** to study plant development and signalling
- Gametogenesis, Fertilization and Self-Incompatibility
- Early **embryogenesis**: establishment of the apico-basal axis
- Late embryogenesis: ABA, dessiccation tolerance and dormancy
- Function of the **Meristems:** Root, Shoot, Flower, Cambium
- **Phyllotaxis** and organ **growth** (auxins, CKs, GAs, Ethylene…)
- Flowering transition (vernalisation, photoperiod, autonomous pathway)
- **Photo/Skotosignaling** and morphogenesis
- **Retrograde signalling** and plant development
- Photo perception and photosynthesis (**Phototropins**)
- **Abiotic stress responses** (nutrients, genotoxic agents, phytoremediation)

Next semester at UNIMI:

PLANT DEVELOPMENT — Fabio Fornara & Aureliano Bombarely

PLANT SIGNALING — Alex Costa

Detailed program: Lectures

LECTURES (1,5h each) Room E302 (Biology buildings)		
Approaches & tools for studying plant development & signaling: part1	Mon 05/09, 14h-15h30	CM1 **CC**
Approaches & tools for studying plant development & signaling: part2	Mon 05/09, 15h45-17h15	CM2 **CC**
Gametogenesis, Fertilization and Self-Incompatibility	Mon 12/09, 8h-9h30	CM3 **GT**
Early embryogenesis, establishment of the apico-basal axis	Fri 16/09, 9h45-11h15	CM4 **GT**
Development from meristems: Shoot apical meristem and cambium	Tues 20/09, 10h15-11h45	CM5 **CC**
Development from meristems: Root apical meristem	Tues 20/09, 13h30-15h	CM6 **GT**
Late Embryogenesis and seed development: storage, desiccation tolerance, dormancy, ABA	Mon 26/09, 13h30-15h	CM7 **CC**
Flowering transition (vernalisation, photoperiod, autonomous pathway)	Wed 28/09, 9h-10h30	CM8 **RB**
Development from meristems: The determinate Floral meristem	Wed 05/10, 13h30-15h	CM9 **CC**
Flower morphogenesis (ABCE model, symmetries)	Wed 05/10, 15h15-16h45	CM10 **CC**
Phyllotaxis, organ initiation, growth and polarities: part 1	Wed 12/10, 13h30-15h	CM11 **KF**
Phyllotaxis, organ initiation, growth and polarities: part 2	Wed 12/10, 15h15-16h45	CM12 **KF**
Photo/Skotosignaling and morphogenesis (degradosome, COP1)	Fri 14/10, 8h-9h30	CM13 **RB**
Retrograde signalling from organelles, for development and environmental response	Fri 14/10, 9h45-11h15	CM14 **RB**
Temperature sensing	Mon 17/10, 13h30-15h	CM15 **CZ**
Plant perception of metals: nutrition, stress and phytoremediation	Mon 17/10, 15h15-16h45	CM16 **JB**
Algal Photoreceptors: Cellular Functions and Impact on Physiology	Thur 20/10, 14h	CM17 **DP**
Total lectures	**17**	**25,5h**

Teachers: Robert Blanvillain (**RB**), Jacques Bourguignon (**JB**), Christel Carles (**CC**), Kateryna Fal (**KF**), Dimitris Petroutsos (**DP**), Gabrielle Tichtinsky (**GT**), Gilles Vachon (**GV**), Chloé Zubiéta (**CZ**)

Detailed program: TD, Tutorials, Lab visit

TD (1,5h each) Room E302 (Biology buildings)		
TD 1: Molecular Cloning for protein sub-cellular visualization	Tues 13/09, 9h-10h30	**CC**
TD 2: Analysis of a transcription factor activity	Tues 13/09, 10h45-12h15	**CC**
TD 3: Analysis of protein-protein interactions	Mon 19/09, 13h30-15h	**CC**
Total TD	3	4,5 h

TUTORIALS (1,5h each, a week ahead of presentation) & 6 PRESENTATIONS (1,5h), 2 students expected / slot		
Tutorial paper assignments	Mon 19/09, 15h15-16h45	**CC**
Slot 1 for meeting w/ tutor (preparation to paper presentation)	Tue 8/11, 15h15-16h45 (CEA?)	**GT & CC**
Slot 2 for meeting w/ tutor (preparation to paper presentation)	Wed 9/11, 15h15-16h45 (CEA?)	**RB & DP**
Slot 3 for meeting w/ tutor (preparation to paper presentation)	Wed 9/11, 15h15-16h45 (CEA?)	**KF & CZ**
Pres 1&2	Tue 15/11, 13h30-15h	**GT**
Pres 3&4	Tue 15/11, 15h15-16h45	**CC**
Pres 5&6	Wed 16/11, 13h30-15h	**RB**
Pres 7&8	Wed 16/11, 15h15-16h45	**DP**
Pres 9&10	Fri 18/11, 8h-9h30	**KF**
Pres 11&12	Fri 18/11, 9h45-11h15	**CZ**
Total Tutos/Pres		15h

LAB VISIT, LPCV-CEA (week 46): Wednesday 9th November 2022, 13h-18h.		5h
Workshops to be detailed		**RB, CC, GT, DP**
Total TD, Tuto, Lab visit		**24,5h**

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Detailed program: TD methodology & techniques

TD 1: Molecular Cloning for sub-cellular visualization of a protein of interest
- Design primers to screen a cDNA library
- Clone a DNA fragment for *in situ* expression in translational fusion to GFP
- Methods to follow the sub-cellular localization (transient and stable transformations)

TD 2: Analysis of a transcription factor activity
- GR/DEX, CHX treatments for assessment of direct targeting
- EMSA, ChIP for DNA binding (mention Y1H)
- Dual Luciferase assay for transcriptional activation

TD 3: Analysis of protein-protein interactions
- Y2H, BiFC
- co-IP from recombinant proteins or crude extract from plants
- Mention some *in vitro* techniques (gel filtration after affinity co-purification, HTRF…)

Detailed program: Tutorials & paper presentations

TUTORIALS (1,5h each, a week ahead of presentation) & 6 PRESENTATIONS (1,5h), 2 students expected / slot		
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Pres 9&10	Fri 18/11, 8h-9h30	**KF**
Pres 11&12	Fri 18/11, 9h45-11h15	**CZ**
Total Tutos/Pres		15h

Organisation for the paper studies:

- Designation of students & questioning pairs
- Papers are sent to the students by the teacher 2 weeks in advance and a 1h30-slot for tutorial is agreed on (ideally 1 week ahead of the presentation); 30 min per student
- Tutorials aim at promoting discussions with an expert of the field, who can answer questions and help the student to get a critical sight on the study
- Presentations: 20 min + 7 min questions by student pair + 7 min questions by tutor

PLANT-Int Master 1st year

2022-2023

Approaches and tools for studying plant development and signaling

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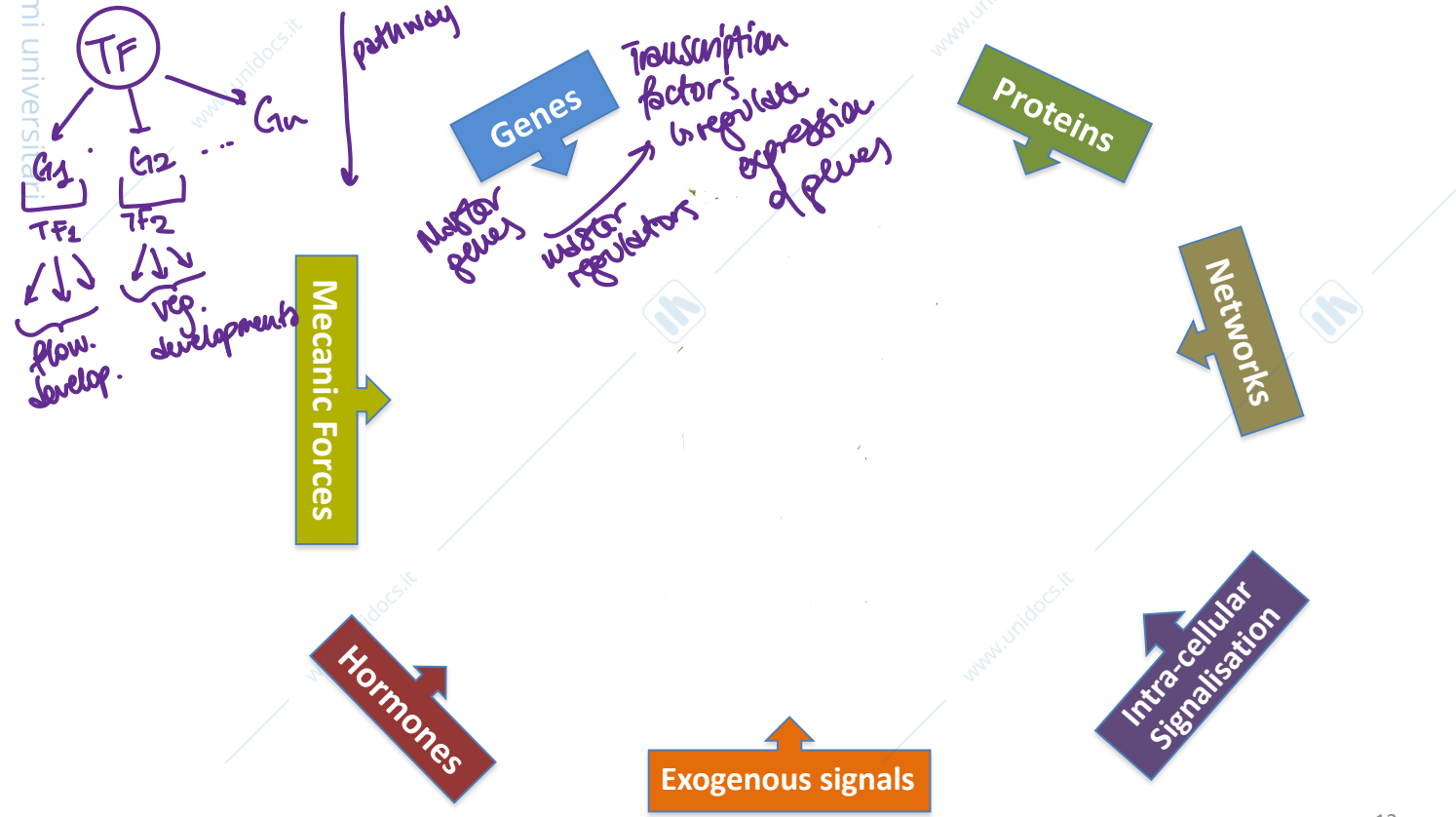
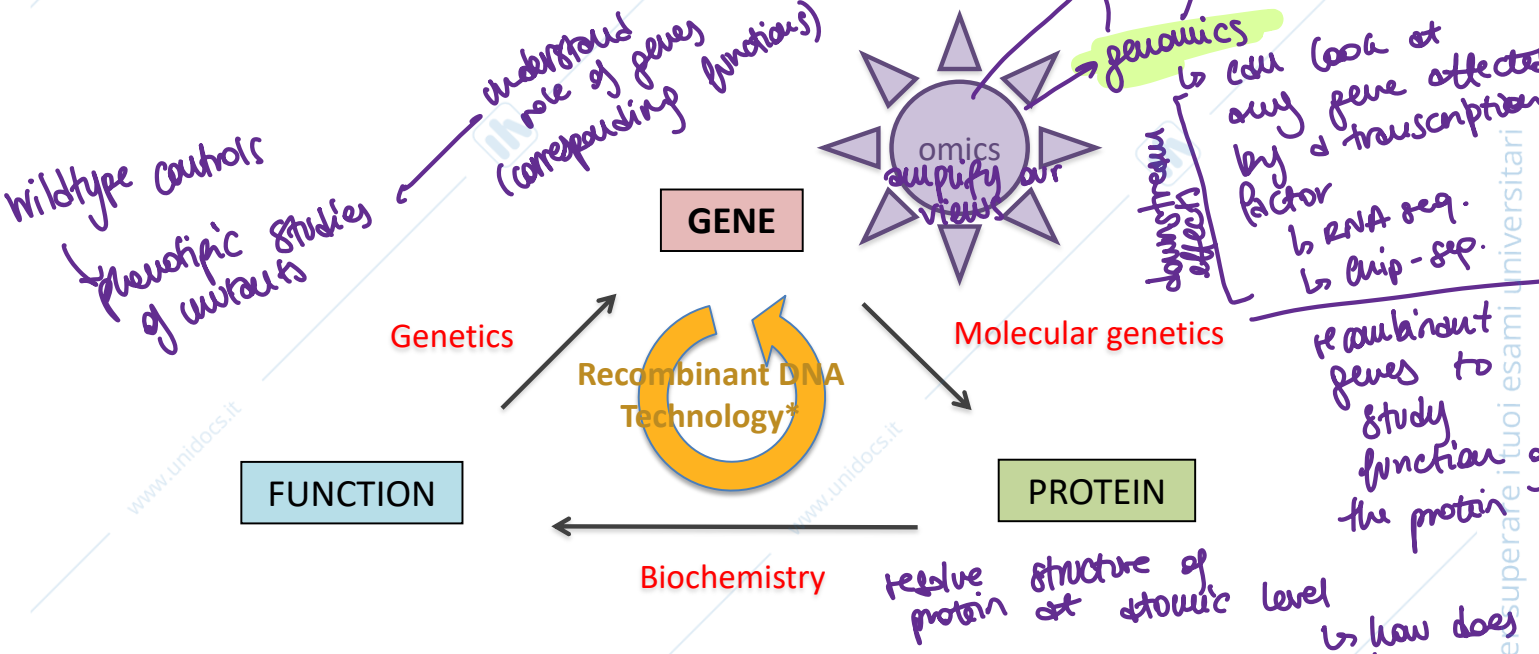
The regulators of plant development and signaling



Our starting point: the gene

To decrypt the molecular factors that regulate plant development and signaling



The unit of information = the GENE

→ made of DNA, transmitted through heredity
→ gives the information (DNA > RNA > Proteins)
→ seats in genomes with many others

* cf. Mol Gen Epigen Cell lecture

How do we decipher regulation of development and signaling?

➔ The developmental geneticist approach:

1. **Detailed description of the developmental/signaling process in the control (WT)**

2. ***Direct* genetics approach:**
   1. Selection of loss or gain of function lines (e.g. mutants) with developmental/signaling defects
   2. Description of the development of the selected lines
   3. Identification of the responsible gene and fonction

3. ***Reverse* genetics approach:**
   1. Identification of candidate genes
   2. Creation of / screen for loss or gain of function lines (e.g. transgenic plants)
   3. Analyses of developmental/signaling phenotypes

4. **Analysis of interactions between factors functioning in the same process**

5. **Modelisation of networks**

↳ how does the double mutant look? → interactions (antagonistic, synergistic ...)

The technological revolution and "omics" era improved the developmental geneticist approach

- **Fully sequenced genomes, full length cDNAs**
  - ➔ **Model plants: «** experimental » and crops
- **Mutant collections** (T-DNA, transposon, TILLING…)
- **Transgenesis tool, reporter genes, RNA interference** (RNAi)
- **Genome editing tools** (TALE, CRISPR/Cas9)
- **Arrays: DNA chips**
- **Next generation sequencing (genomics, transcriptomics, epigenomics)**
- **Proteomics** → protein complexes identification
- **Metabolomics**
- **Microscopy:** Confocal, light sheet, tomography, Atomic Force (AFM)…

I. Plant model organisms

At the beginning were:

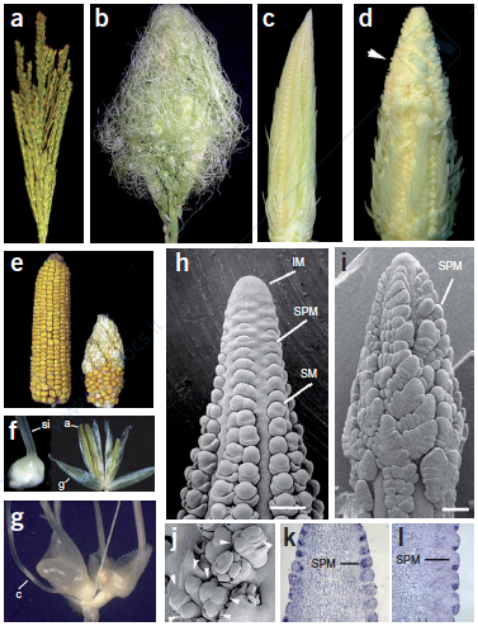
Arabidopsis - dicot, brassica

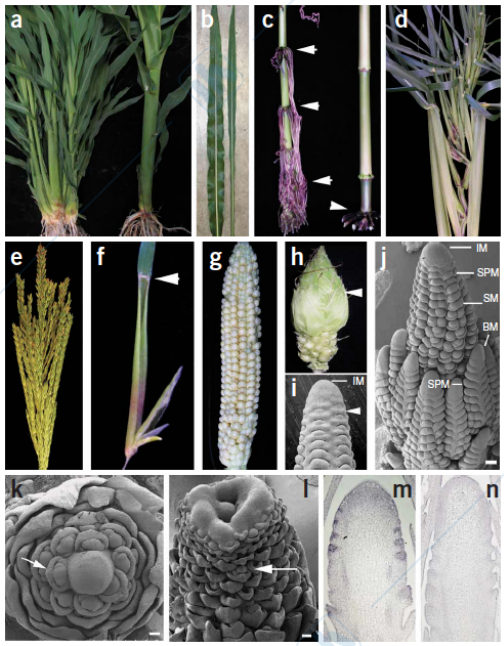
maize

nice

I. Plant model organisms

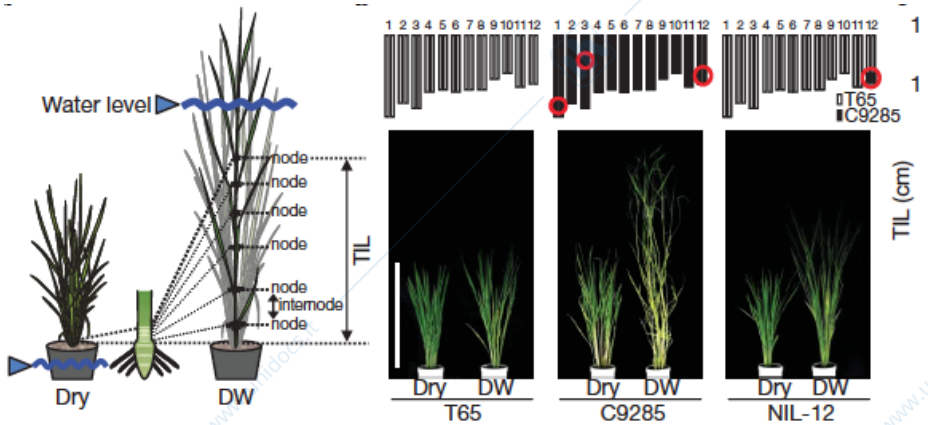
Corn:





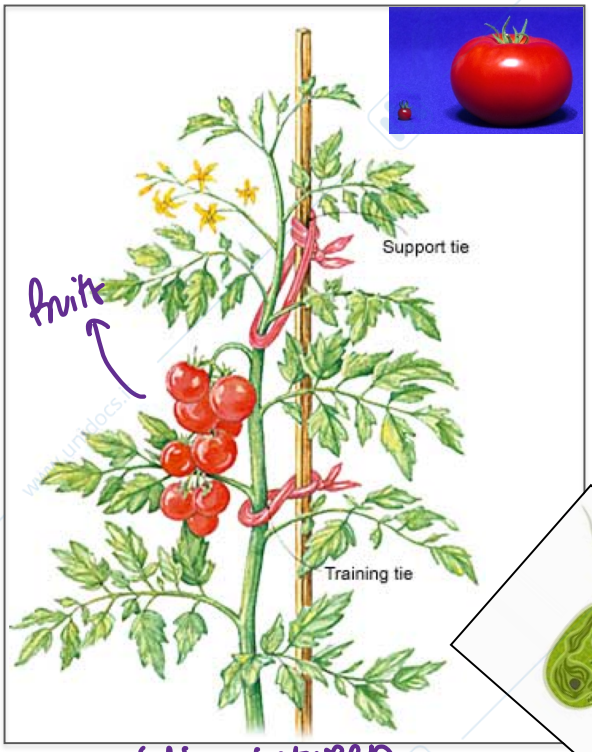
I. Plant model organisms

Rice:



I. Plant model organisms

But also:



fruits

symmetry

Brachipodium
small genome

(crispr)
homologous recombination
Physcomitrella patens
Easy up

cotyledons

no clear correlation between genome size & complexity of the species
Is intergenic regions (non coding)

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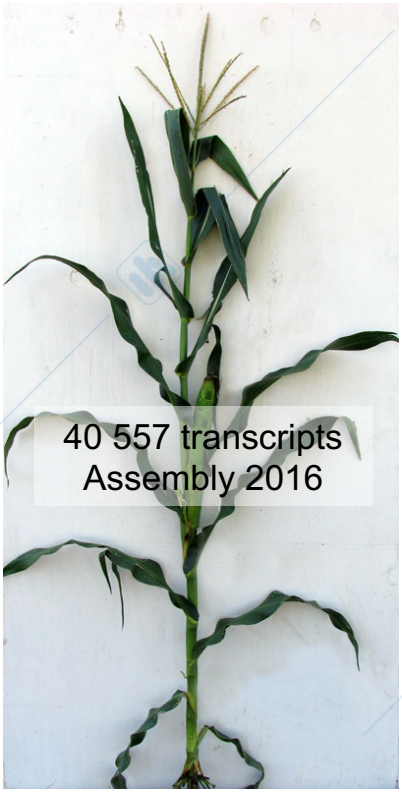
I. Plant model organisms

The run to whole genome sequencing

125 Mb
sequenced in 2000



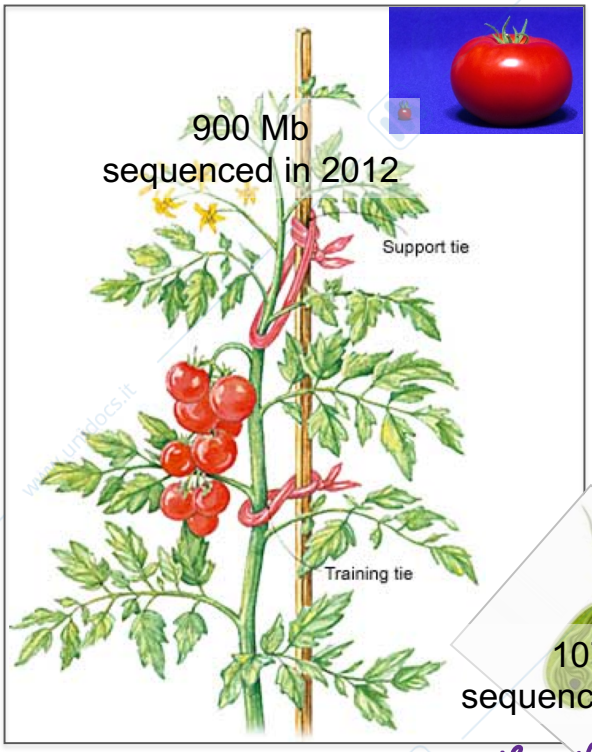
40 557 transcripts
Assembly 2016

372 Mb
sequenced in 2002

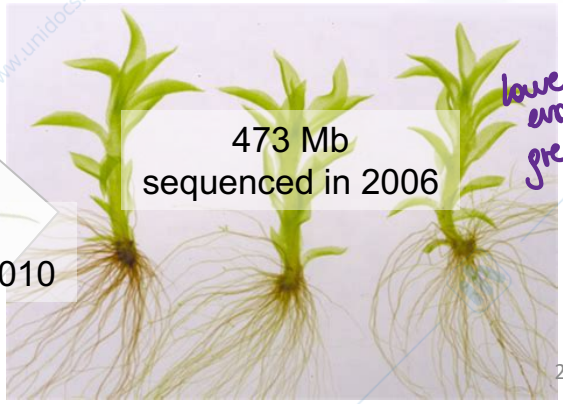
20

I. Plant model organisms

But also (from crops to algeae):



self-sufficient unicellular



lower in the evolutionary tree, greater genome

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Plant model organisms

Plant Latin Name/ Common Name	Image	Reason for Use as a Model/ Link to General Information	Year of Genome Sequence/ Link to Genetic Database
Arabidopsis thaliana Mouse-ear Cress or Thale Cress		Small genome, easily grown in lab, self-fertile (does not require a pollinator) About *Arabidopsis*	2000 (first plant to have its genome sequenced) Summary of Arabidopsis
Brachypodium distachyon Purple False Brome		Small genome, small physical size, self-fertile, grass family, Monocot About *Brachypodium*	2010 Summary of *Brachypodium* genome
Lotus japonicus Japanese common name: Miyakogusa		Nitrogen fixer, different symbiotic relationship from *Medicago* About *Lotus japonicus*	In progress in Japan Current Genome Database
Medicago truncatula Barrel Clover		Nitrogen fixer, different symbiotic relationship from *Lotus*, small genome and physical size About *Medicago*	In progress internationally Current Genome Database

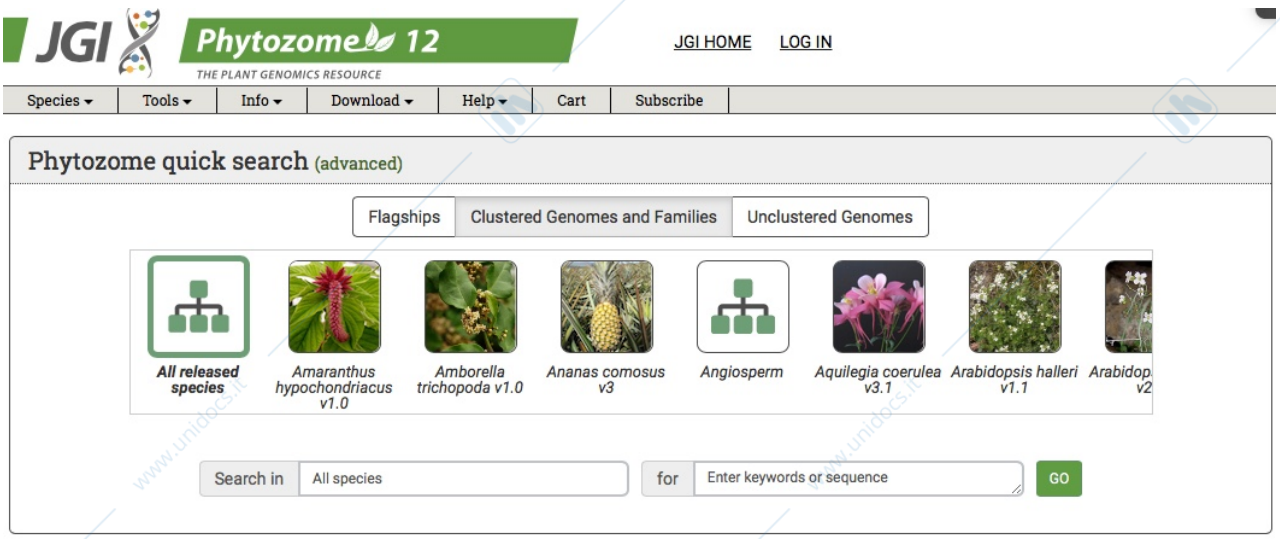
Plant model organisms

Picea abies Norway Spruce		Conifer, used for somatic embryogenesis About Conifer genetic sequencing projects	In progress in Sweden Genome Reseach Project
Selaginella moellendorffii Spikemoss		Smallest genome of any plant species, lycophyte–a primitive vascular plant About Spikemoss	2008 *Selaginella* Genome Database
Populus trichocarpa Western Balsam Poplar		Tree, important in paper manufacturing About Western Balsam Poplar	2006 (first tree genome sequenced) *Populus trichocarpa* Genome Database
Physcomitrella patens Moss		Non-vascular plant, used for investigating tip growth and cell polarity About *Physcomitrella*	2006 *Physcomitrella* Genome Database
Chlamydomonas reinhardtii Green alga		Single-celled alga. Study of its flagellar system has been important in revealing the basis of some human ciliary diseases. About *Chlamydomonas*	2010 *Chlamydomonas* Genome Database

Plant model organisms

Phytozome: Plant Comparative Genomics portal of the Department of Energy's Joint Genome Institute (JGI)

Provides a hub for accessing, visualizing and analyzing plant genomes (sequenced at JGI and elsewhere).
Phytozomev12.1.6 hosted 93 assembled and annotated genomes, from 82 Viridiplantae species.
More than half were sequenced, assembled and/or annotated with JGI Plant Science program resources.



https://phytozome.jgi.doe.gov/pz/portal.html

Plant model organisms

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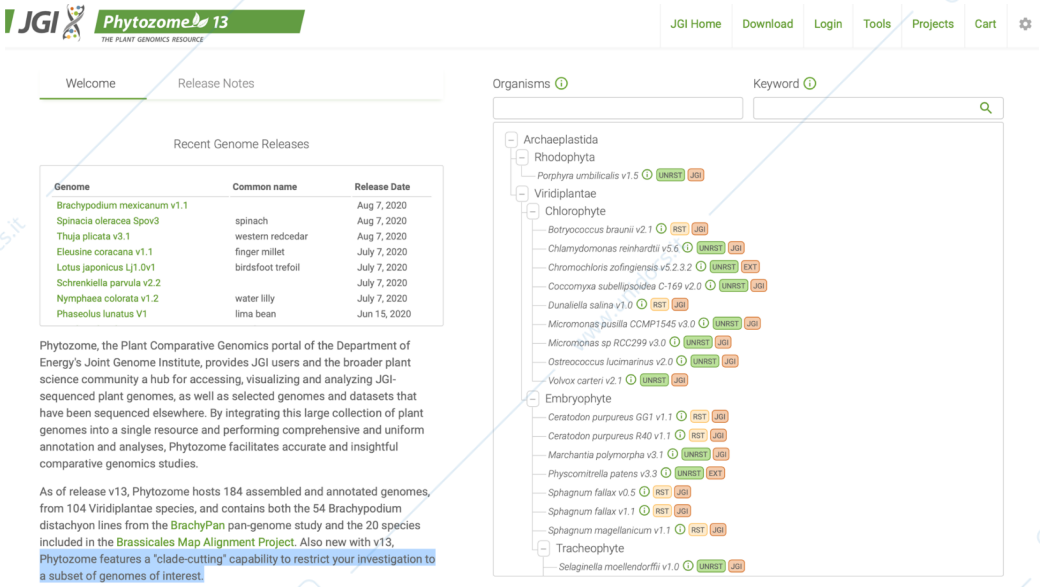
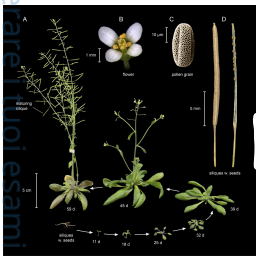
Provides a hub for accessing, visualizing and analyzing plant genomes (sequenced at JGI and elsewhere).
Phytozomev13 hosts 261 assembled and annotated genomes, from 129 Archaeplastida species.
Contains the 54 Brachypodium distachyon lines from the BrachyPan pan-genome study and the 20 species included in the Brassicales Map Alignment Project.



https://phytozome-next.jgi.doe.gov

Phytozome features a "clade-cutting" capability to restrict your investigation to a subset of genomes of interest.



Arabidopsis *thaliana*, model plant for the Angiosperms

plants that produce true flowers

complex structures w/ both sexes + other organs that protect the reprod. ones (flower sepals)

- **Small Cruciferae (= Brassicaceae family)** needs < 10cm² soil surface, 25cm height

  Cruciferae: older name, meaning "cross-bearing", describes the 4 petals of mustard flowers, reminiscent of a cross

- **Short generation time** (8 weeks)
- Can be grown *in vitro* , growth chamber or greenhouse
- **Self-fertilizing species**
- Well known genetics, **genome saturated with mutant lines**
- **Small genome** (1,2 x 10^8 pb) all annotated and sequenced, diploid
- Approximately 27 000 genes, ie an average of 1 gene each 4-5kb
- **Easy mutagenesis**: chemical, irradiation, T-DNA insertion or transposons
- **Easy gene transfer**, via *Agrobacterium*
- Numerous ressources available on line (databases, seed collections…)

can cross by opening the flower bud

stam higher than pistil → self pollination

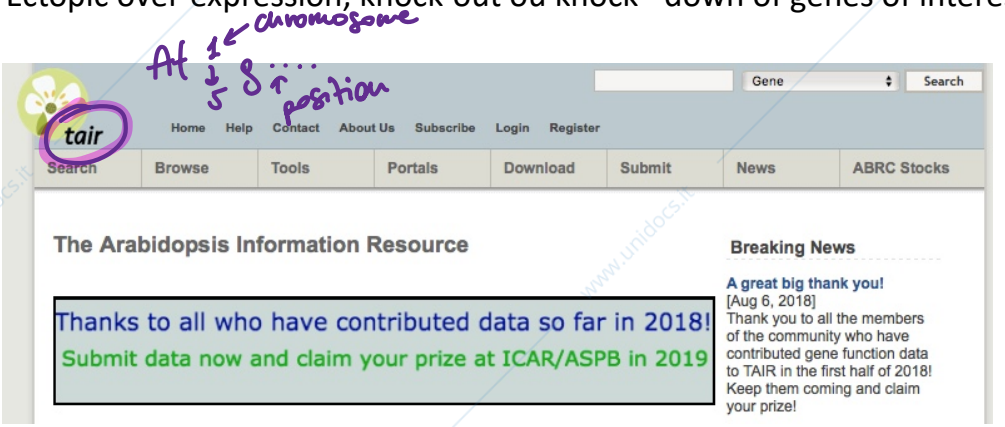
no risk for outcrossing → when the flower opens it has already self-fert.

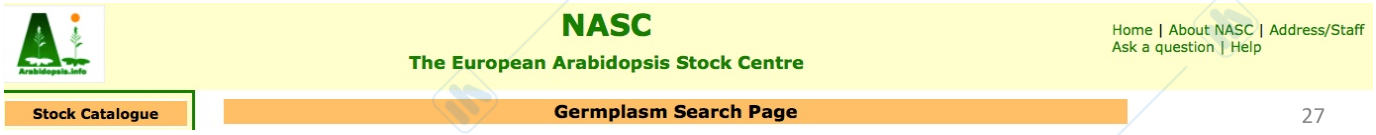
model organism
- ✓ small surface needed to grow
- ✓ short gen time
- ✓ small genome

insertion of t-DNA

Methods and resources in *Arabidopsis thaliana*

- ✧ Mutant screen : lots of accessible collections, with gene-based classification
- ✧ Expression of reporter transgenes under specific promoter control
- ✧ Inducible lines (expression, protein subcellular localisation)
- ✧ Ectopic over-expression, knock-out ou knock –down of genes of interest





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Other methods and resources in crops (e.g. maize)

- ✧ Mutant screen : lots of accessible collections (EMS, radiation, or Transposon-induced)
- ✧ Cell ablation and analysis of induced phenotypes
- ✧ Construction of chimeres (drafting, spontanous mutagenesis)
- ✧ Construction of genetic chimeres by transgenesis

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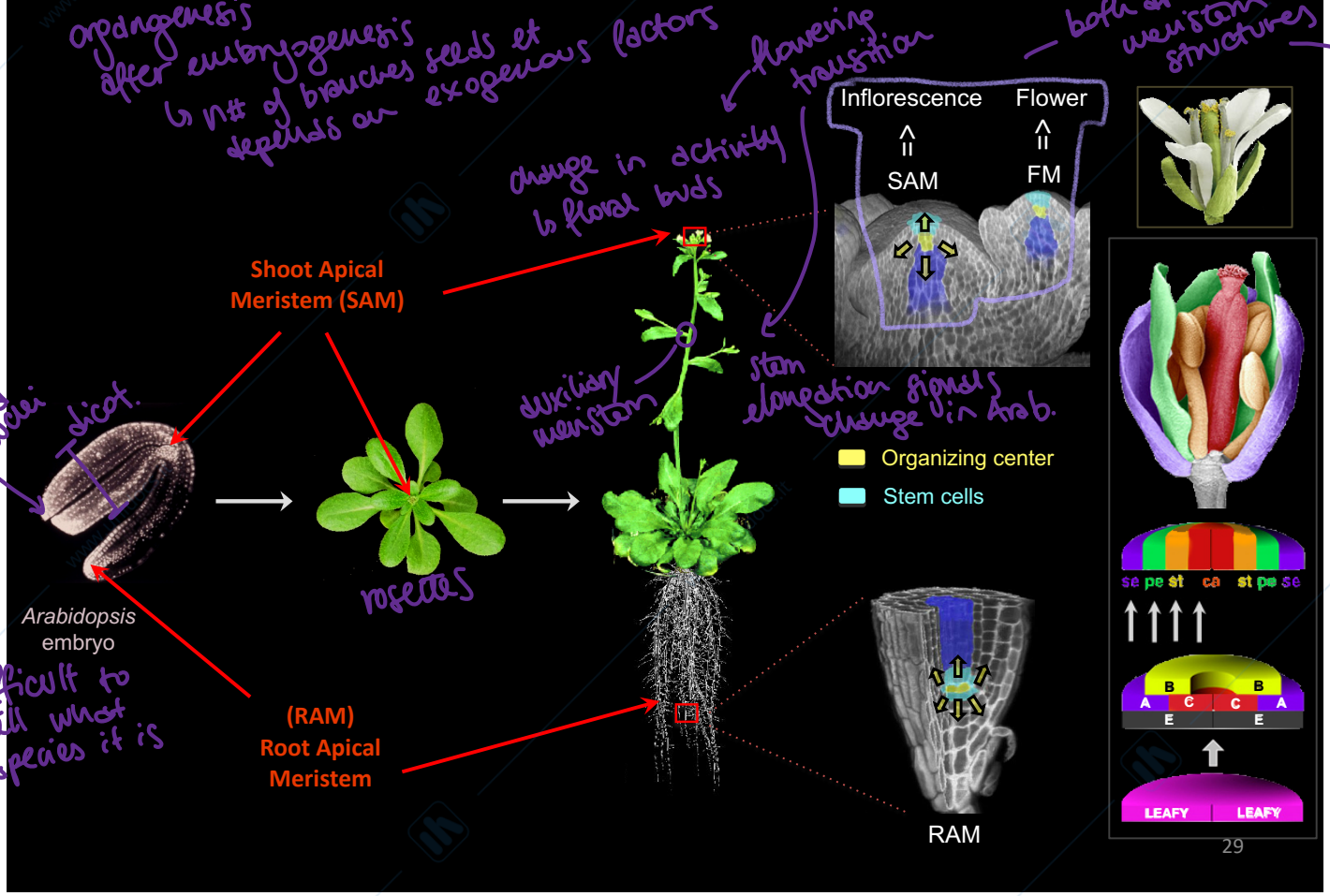
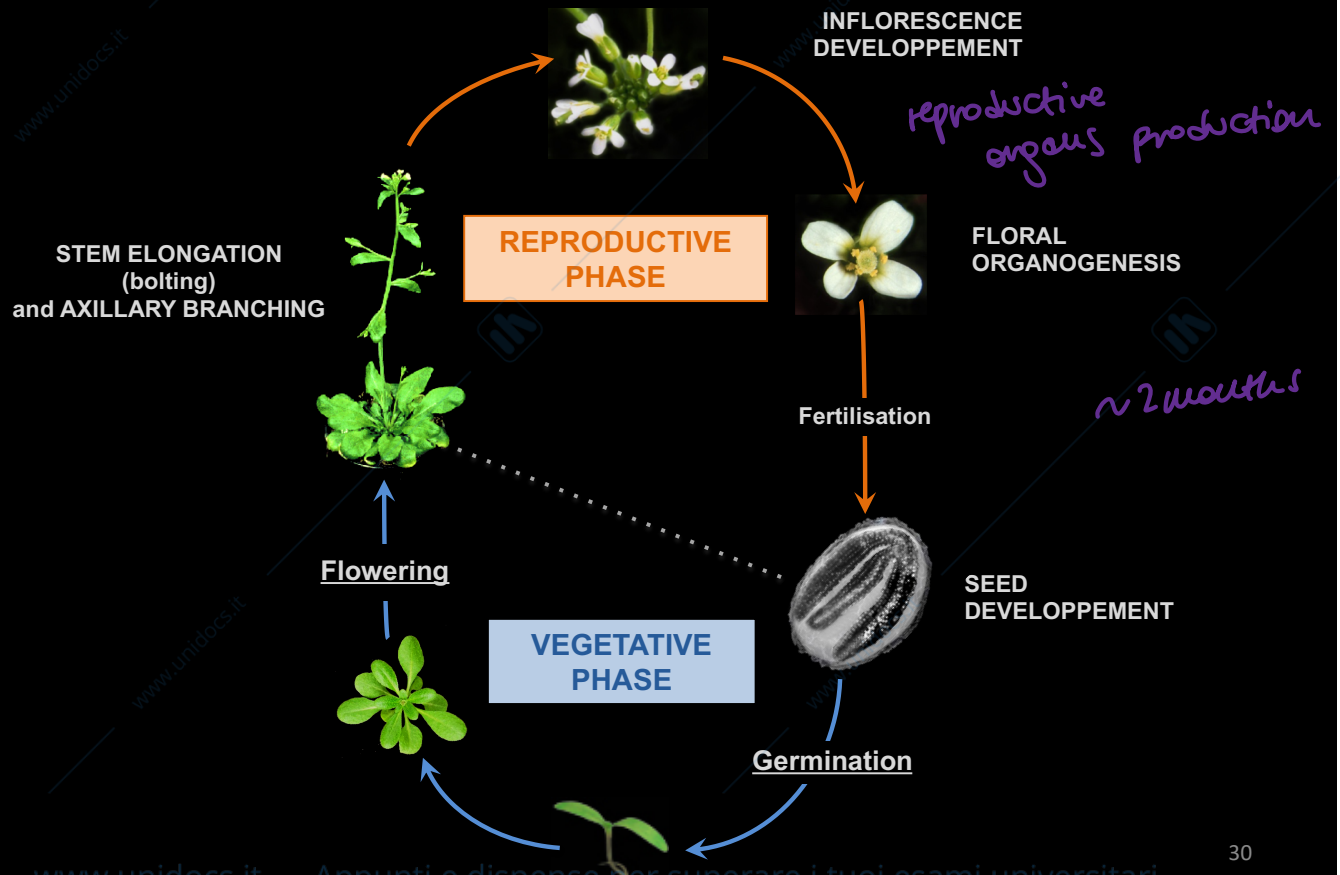
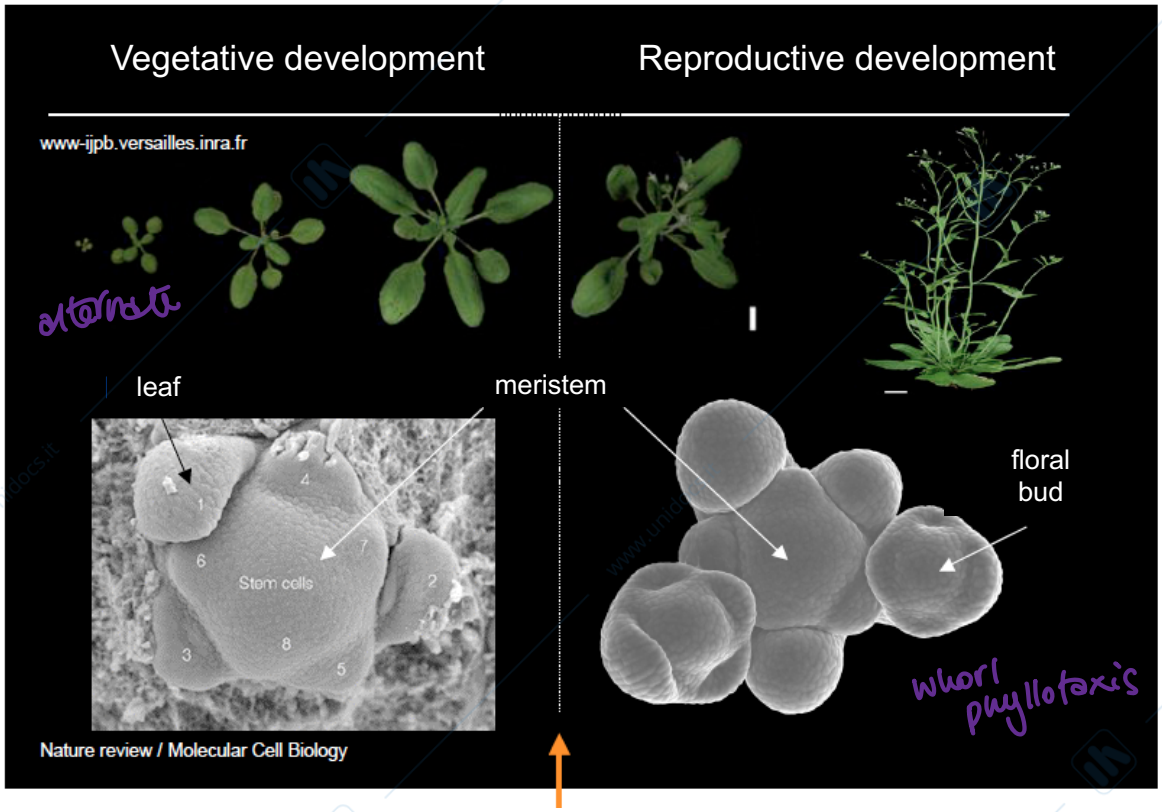
How *Arabidopsis thaliana* develops



Developmental cycle of the *Arabidopsis thaliana* model plant



Phase transition in *Arabidopsis thaliana*

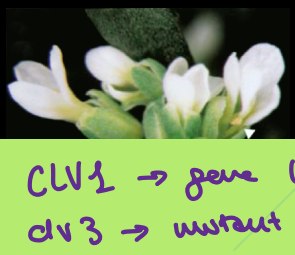


scanning electron microscopy

Floral transition corresponds to a change in meristematic activity

Arabidopsis mutant plants affected in stem cell maintenance

stm-11
shoot meristem less



clv3-2
clavata

CLV1 → gene (big caps)
clv3 → mutant (small caps)

recessive

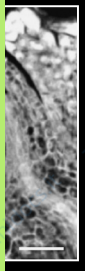
CLV1 / CLV1 × clv1 / clv1
clv3 / clv3 × CLV3 / CLV3
♂ ♀

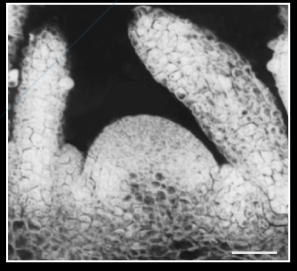
↓

CLV1 / clv3 ← phenotype

[alleles on test] (CLV1 / clv1, CLV3 / clv3)

test to see how the phenotype is influenced by the genes





mutants : italics small caps + number of allele
stm-11 ↳ 11th mutation found
gene : caps italics STM
protein : caps STM
clv1-2, clv1-1, clv1-3 -> allelic mutant
Allelism test

Stem cells

II. Approaches and tools for Plant Functional analyses

- **Transient expression** → next slide

- **Transgenesis**
  - Insertion lines
  - Expression / complementation analyses
  - Activation tagging
  - Enhancer trap

- **Mutagenesis**
  - Forward genetics
  - Reverse genetics

    Tilling: Targeting Induced Local Lesions IN Genomes

- **Genome (and epigenome) editing**
  - ZFP, TALE
  - CRISPR/Cas9

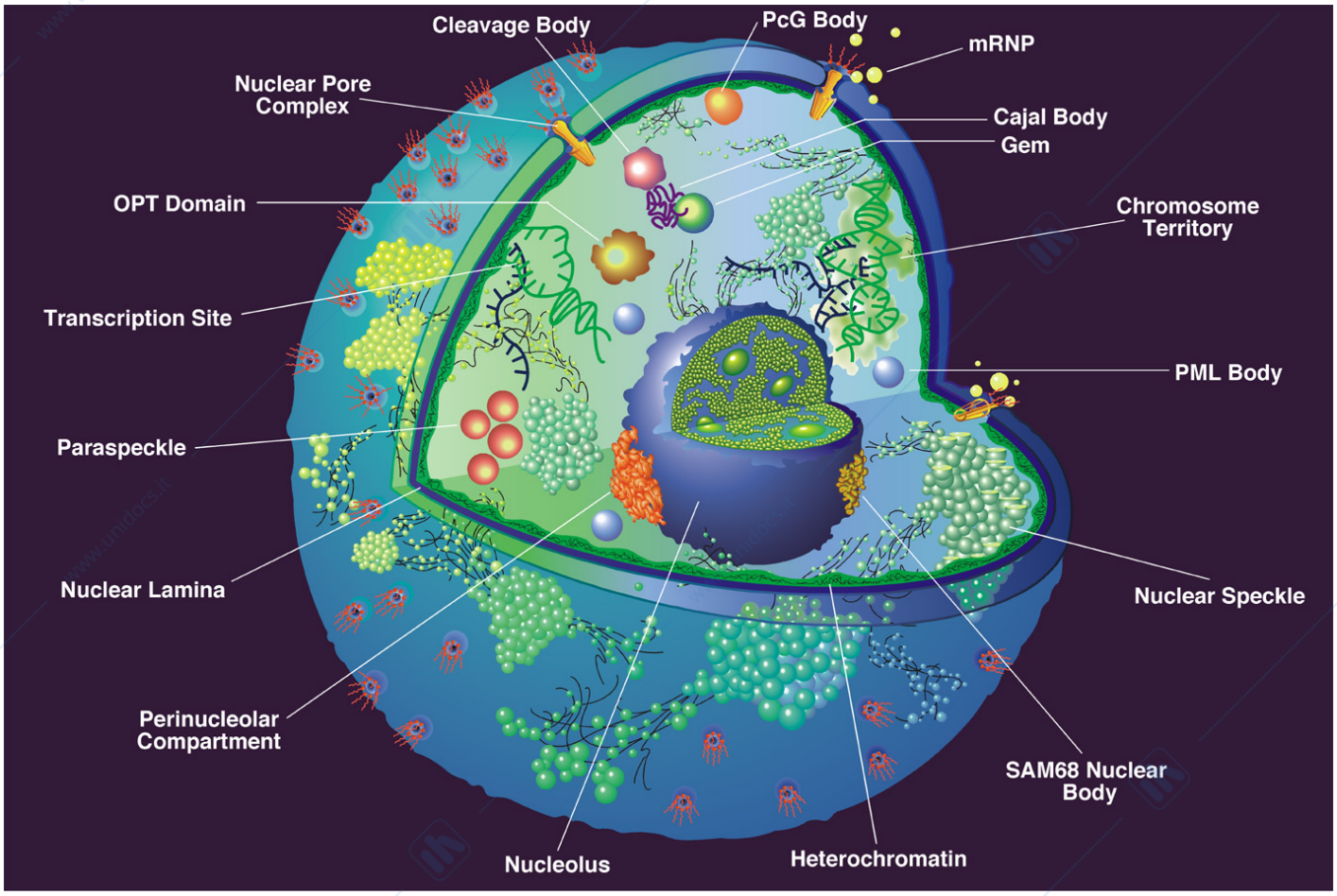
Transient vs Stable Transformation (e.g. for gene expression)

Deliver **DNA** into cells that **will:**

- **not integrate into genomes or into the germline → Transient**
- **Integrate into genomes or into the germline → Stable**

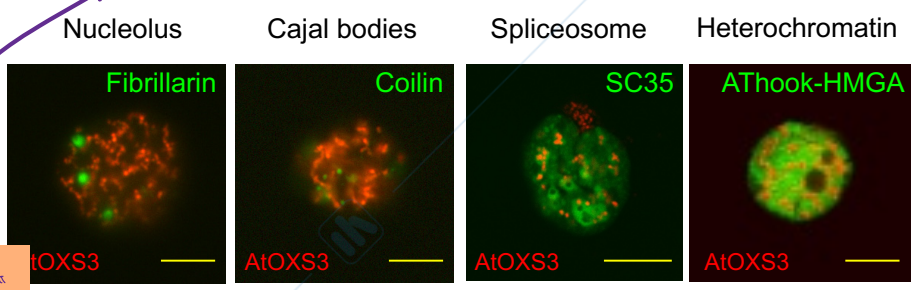
- ✧ Particle bombardment
- ✧ Protoplast PEG transfection
- ✧ Agrobacterium-infiltration
- ...

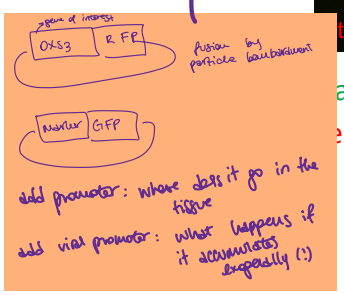
Example of gene expression application: Nuclear Sub-localisation



Transformation for protein localization and co-localization within the nucleus

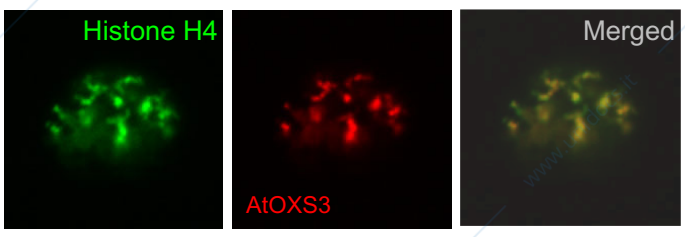
Transient expression in onion epidermis cells, after particle bombardment





arker: *Fibrillarin-GFP*

ene of interest: *AtOXS3-RFP*



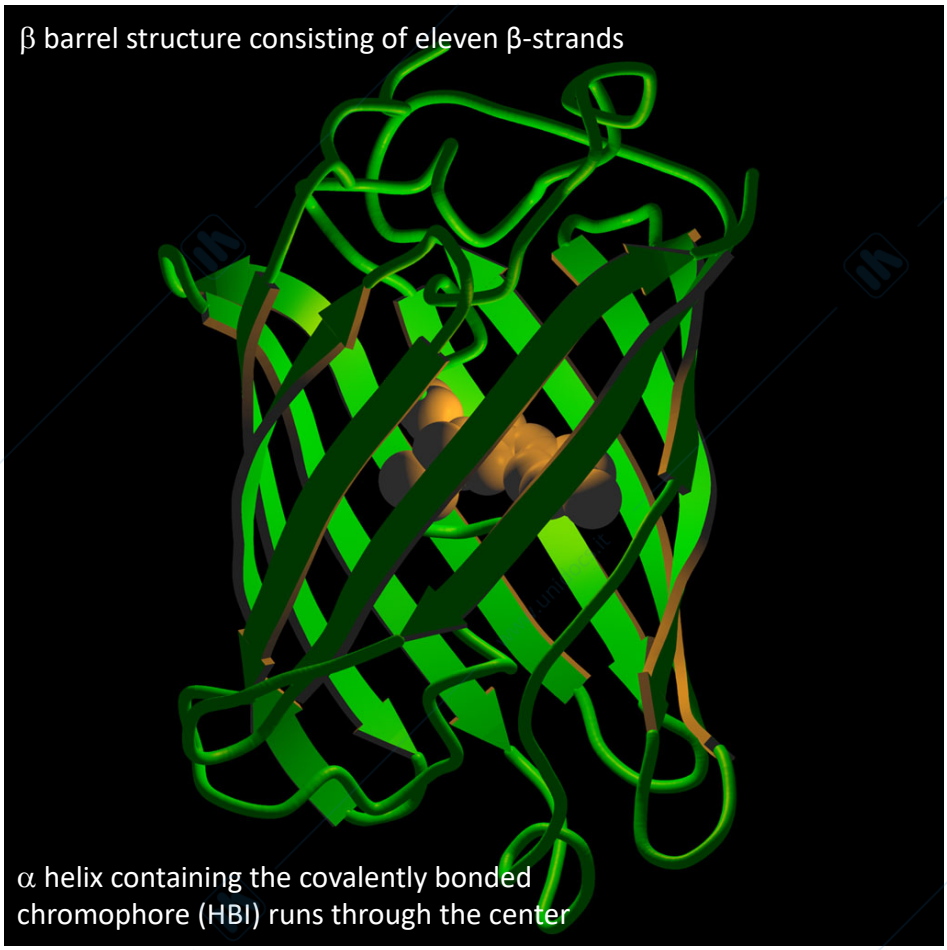
colocalisation of protein

GFP: Green Fluorescent Protein

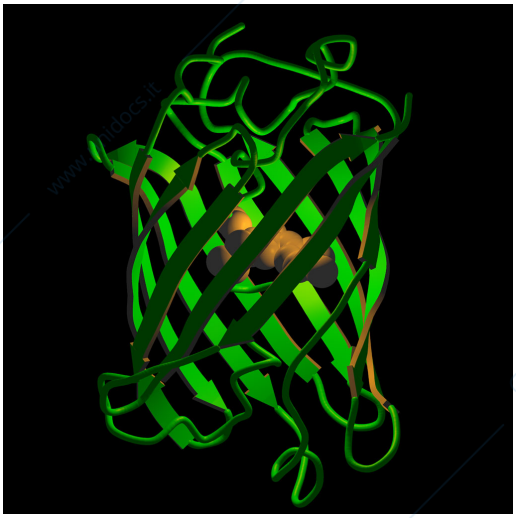
RFP: Red Fluorescent Protein

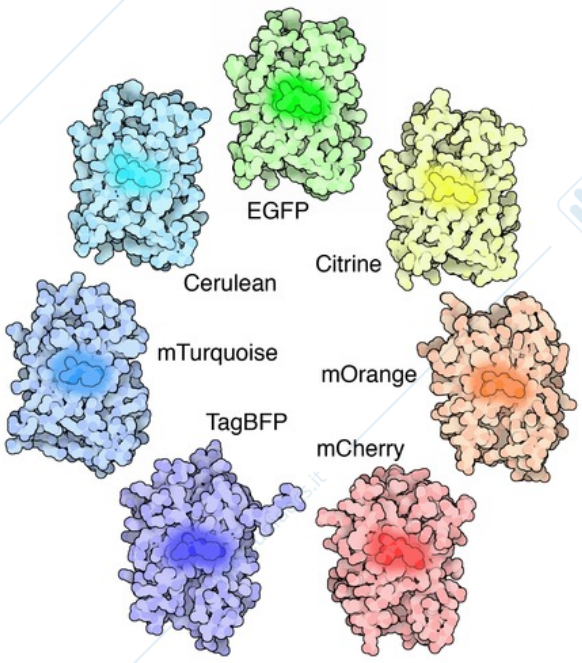
Courtesy: R. Blanvillain

Green Fluorescent Protein (GFP)

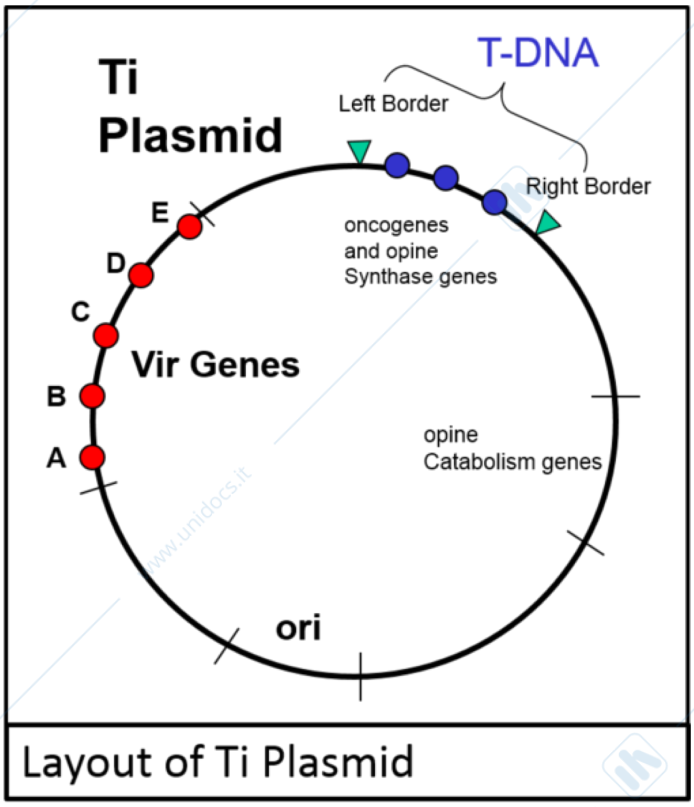


GFP derivatives (variable chromophores inside the barrel)





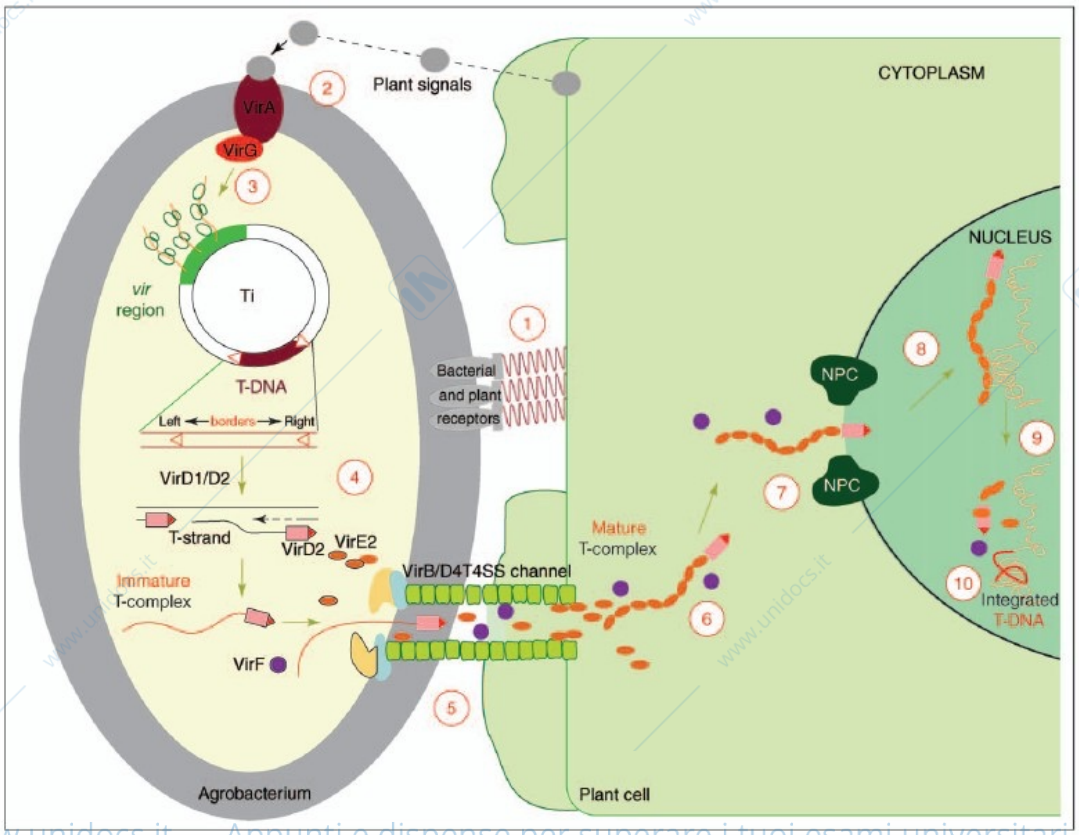
Agrobacterium tumefaciens causes crown gall disease (tumor)



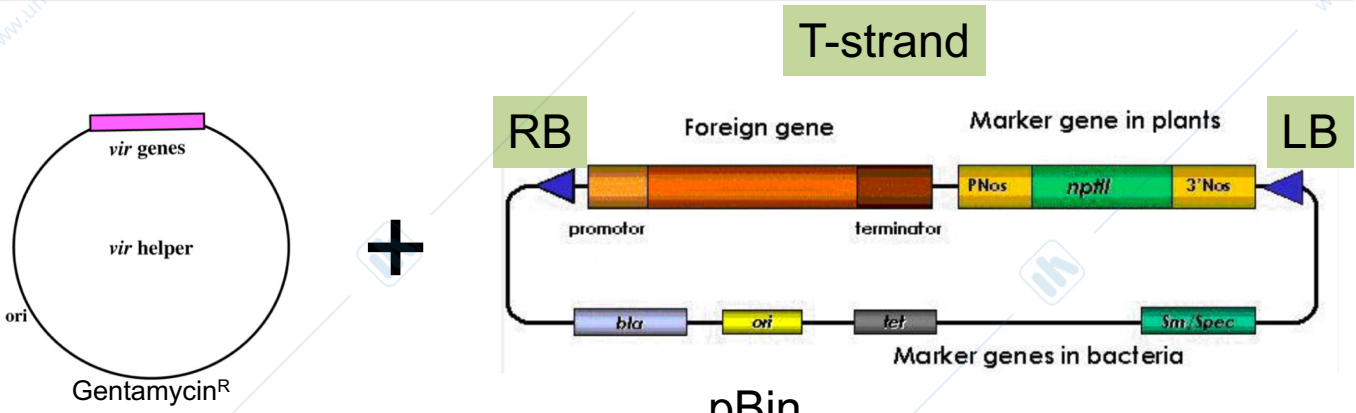
Layout of Ti Plasmid

Agrobacterium-mediated mutagenesis/transgenesis

Single strand T-DNA is transfered to the plant cell and integrated into the nuclear DNA

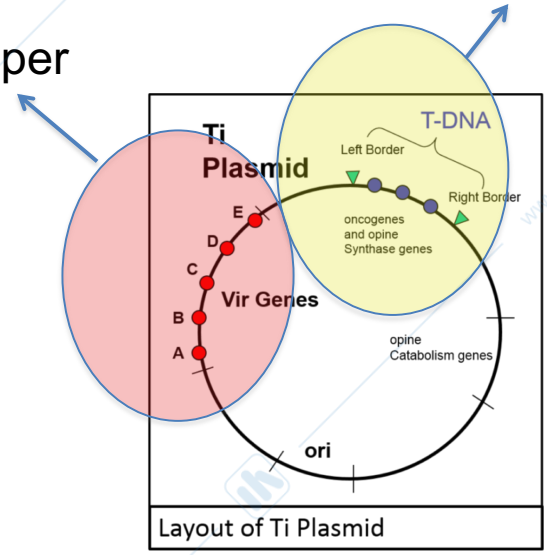


Binary vector system

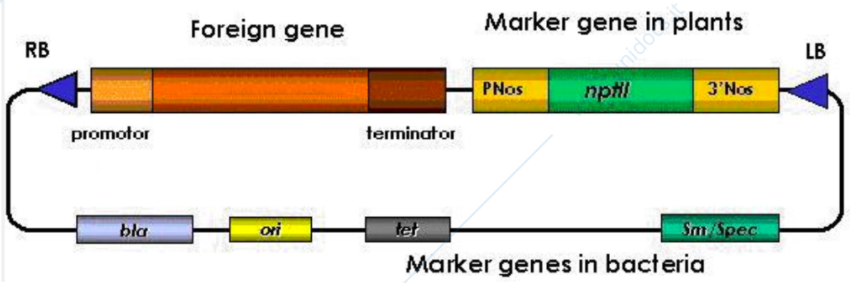


pBin

pHelper



Expression lines

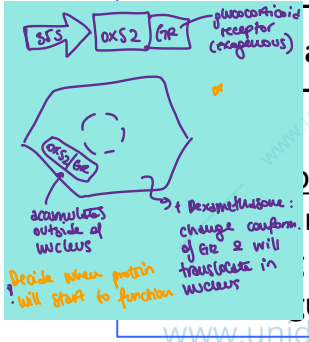


FLC repressor for flowering → mutant for loss of function / overexpression → find function

Expressing a Gene of interest

Choice of promoter

- ✧**strong ubiquitous** promoter (CaMV35S) (cauliflower mosaic virus)
  - → over-expression / functional complementation (WT / mutant background)
- ✧its **own gene promoter**
  - → functional complementation
- ✧**al or inducible promoter** (specific promoter, AlcR, EstR) (cond. put gene somewhere else and see if just that allows change)
  - → Controlled, customized expression



onal fusion ? (cytochemical test – more sensitive – enzymatic assay)

- **rker** (GFP, GUS, …) → follow protein *in cyto* / *in situ*
- (HA, cMyc, …) → IP
- **ulatory module** (e.g. GR) → inducible activity

IND.: useful to see what happens at one point (e.g. after flowering)

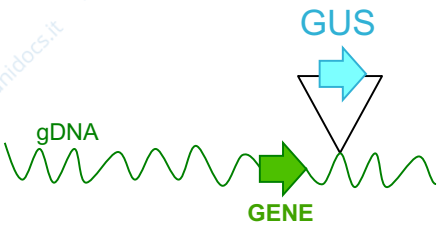
Promoter studies using reporter genes

Exemple : GUS transcriptional fusion

looking just at activity of promoter

Promoter trap / Enhancer trap

to discover promoter

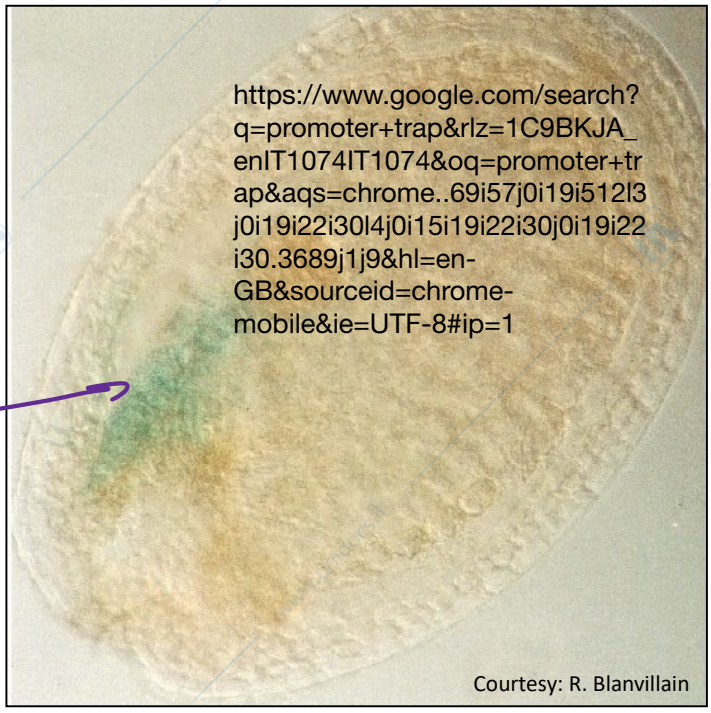


no promoter → only coding sequence

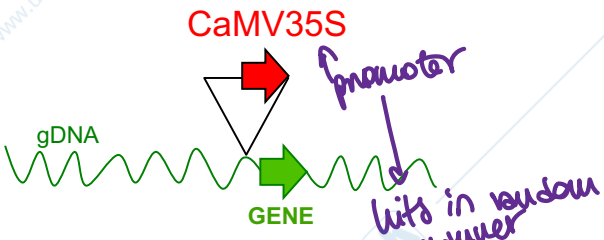
↓ find new promoters for the tissue

→ find killer peptides

https://pubmed.ncbi.nlm.nih.gov/18370002/

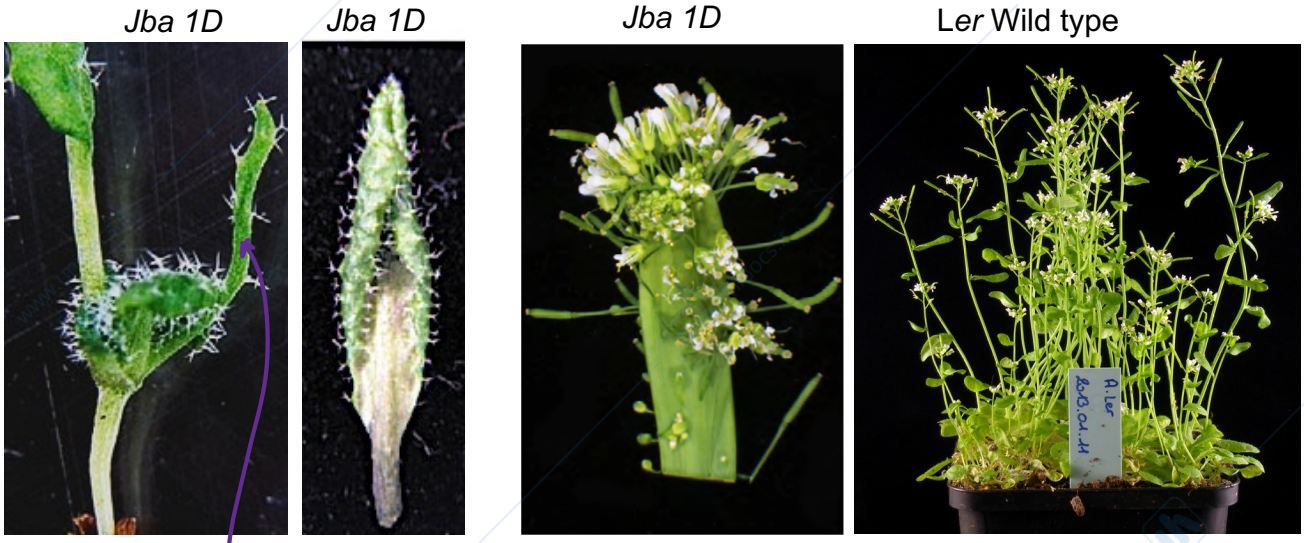


Activation tagging



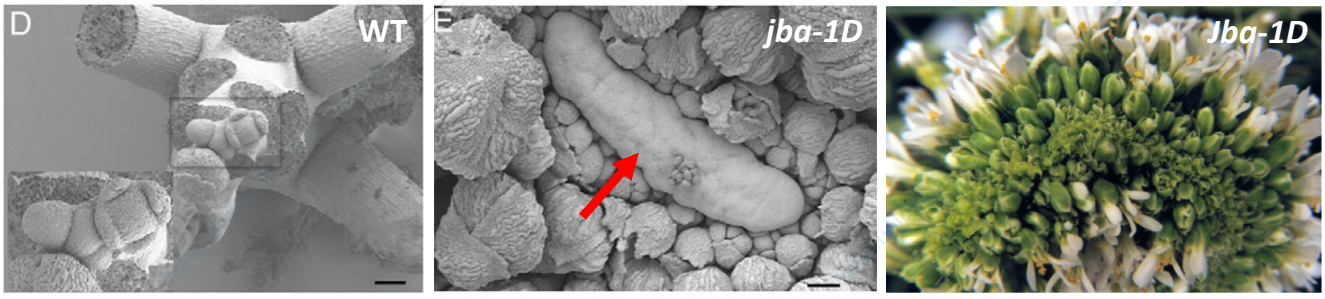
micro RNA - sequences out or in genes
- complementary to mRNA
to control of gene expression
eg boundaries → upper/lower cell

https://www.nature.com/articles/nrg1341



lose polarity → no faces of blade → no shape

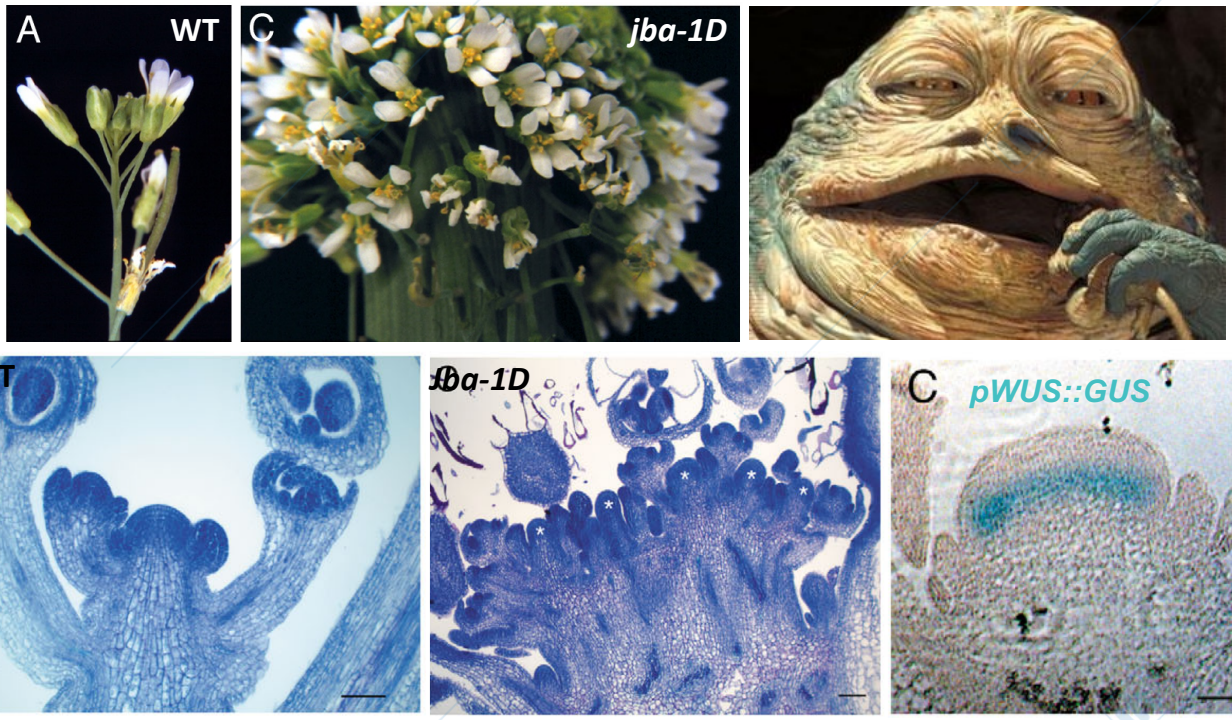
The *JABBA* (*JBA*) micro RNA (*miR*)



Williams *et al*, Dev 2005

https://pubmed.ncbi.nlm.nih.gov/16033795/

The *JABBA* (*JBA*) micro RNA (*miR*)



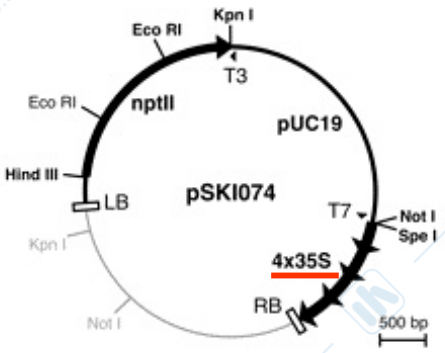
Williams *et al*, Dev 2005

jba-1D: an activation tagging Arabidopsis mutant

Plant Physiol, April 2000, Vol. 122, pp. 1003-1014

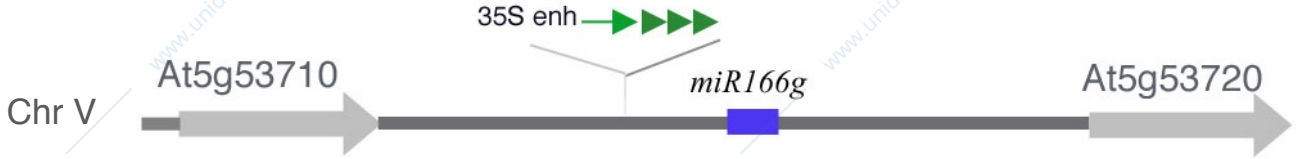
Activation Tagging in Arabidopsis

Detlef Weigel,* Ji Hoon Ahn,2 Miguel A. Blázquez,2 Justin O. Borevitz,2 **Sioux K. Christensen**,2 Christian Fankhauser,2 Cristina Ferrándiz,2 Igor Kardailsky,2 3 Elizabeth J. Malancharuvil,2 Michael M. Neff,2 4 Jasmine Thuy Nguyen,2 5 Shusei Sato,2 Zhi-Yong Wang,2 Yiji Xia,2 Richard A. Dixon, Maria J. Harrison, Chris J. Lamb,6 Martin F. Yanofsky, and Joanne Chory



jba-1D Insertion Site

JBA = MIR166g



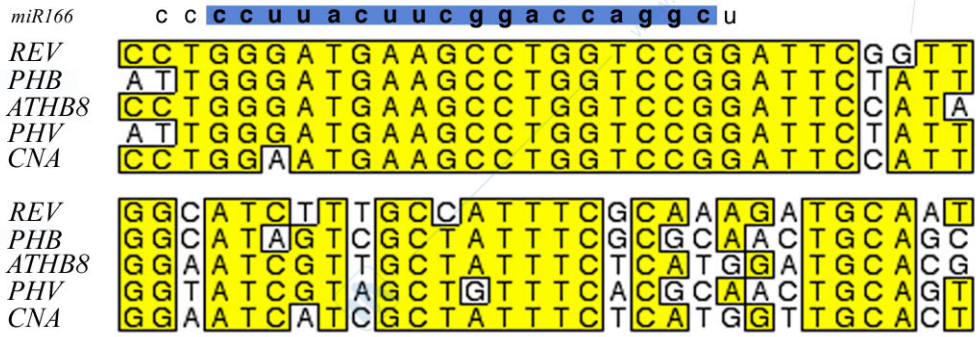
The *miR166* gene family

* The miRNA length is 21 nt: **TCGGACCAGGCTTCATTCCCC**

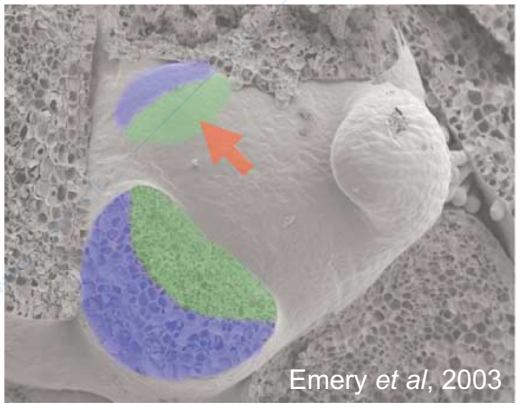
MIR name	Precursor size	chromosome position	
MIR166a 3'	136	2	4.7 kb upstream of At2g46690 (a)
MIR166b 3'	112	3	3.5 kb upstream of At3g61900 (a)
MIR166c 3'	108	5	10 kb downstream of At5g08690 (s)
MIR166d 3'	101	5	22 kb downstream of At5g08740 (a)
MIR166e 3'	135	5	2.6 kb downstream of At5g41910 (a)
MIR166f 3'	91	5	1.1 kb downstream of At5g43600 (s)
***MIR166g* 3'**	**123**	**5**	**1.5 kb upstream of At5g63720 (s)**

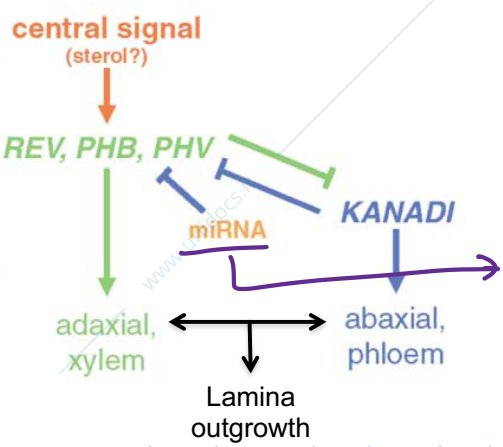
All found in a non coding region

miR166 targets the class III HD-ZIP genes



35S landed close to this → overexpressed → no differentiation of upper & lower





buffer levels of genes from adaxial to abaxial & vice versa

Mutagenesis

Physical or Chemical mutagenesis

✧ γ-irradiation
- small deletions (1-16 bp)
- large deletions (9.4-129.7 kbp),
- single-base substitutions,
- inversions.

✧ X-irradiation
- Mostly large deletions

} delete sequences

✧ Ethyl methanesulfonate (EMS)
- alkylating agent G > O^6-ethylG
- O^6-ethylG pairs with Thymine leading to G/C to A/T transitions
- STOP codons or AA substitutions
- 10% of EMS lesions are deletions or other chromosomal rearrangements

} change aminoacids

Agrobacterium-mediated mutagenesis

Forward genetics

From mutant phenotype to the gene identification

✧ Mutant screens (historically, EMS or irradiated-induced)

✧ Second site mutagenesis (mutation modifiers)
- enhancer screen
- suppressor screen

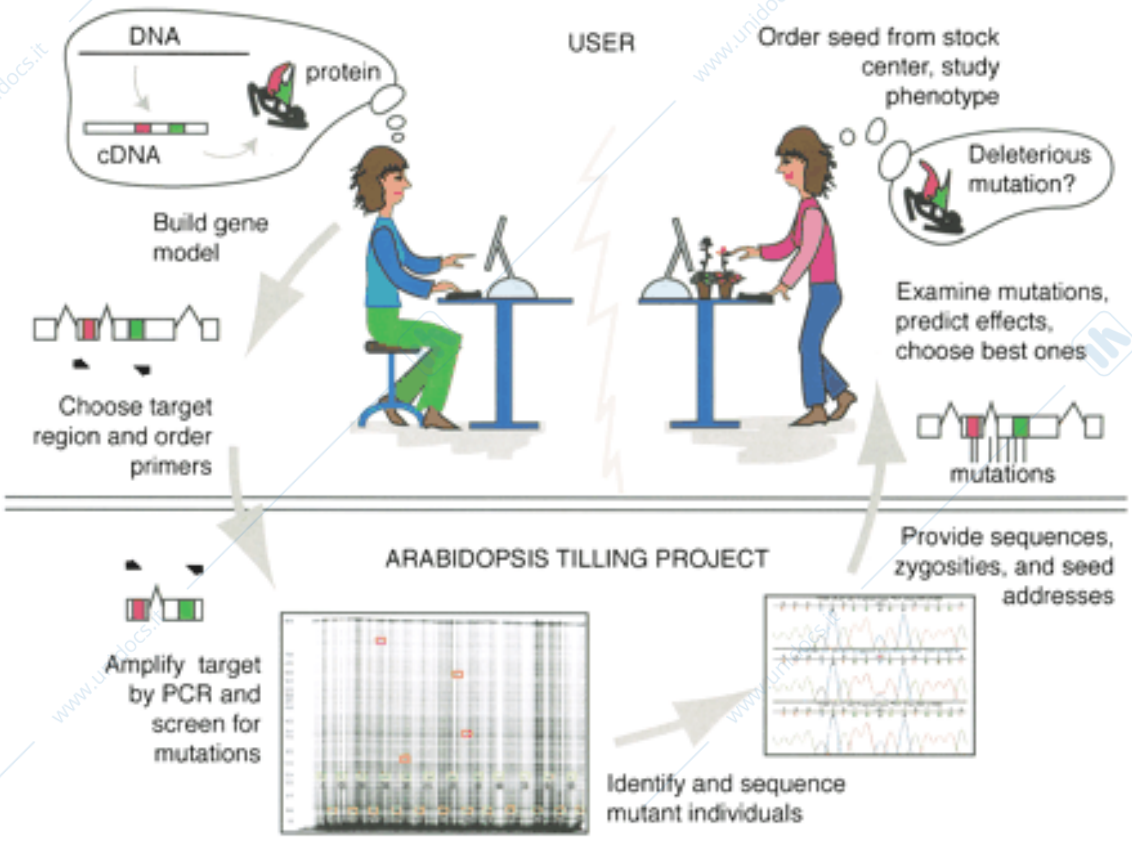
→ know which gene is effected by mutation
↓
what interacts with this mutation

Reverse genetics

From gene of interest to its function identification

- ✧ TILLING – EMS collections
- ✧ Insertional collections (T-DNA)

Targeting Induced Local Lesions IN Genomes (TILLING)



Targeting Induced Local Lesions IN Genomes (TILLING)

Step 1 **Mutagenesis** (e.g. EMS)
Large mutant collection

Step 2 **Mismatch cleavage analysis**

PCR Fluo-primer design in the region of interest
Amplification test on the collection
Heteroduplex formation (« bubble » at the mismatch)
Cel 1 digest (single strand cut)
Flow electrophoresis (sequencher machine)

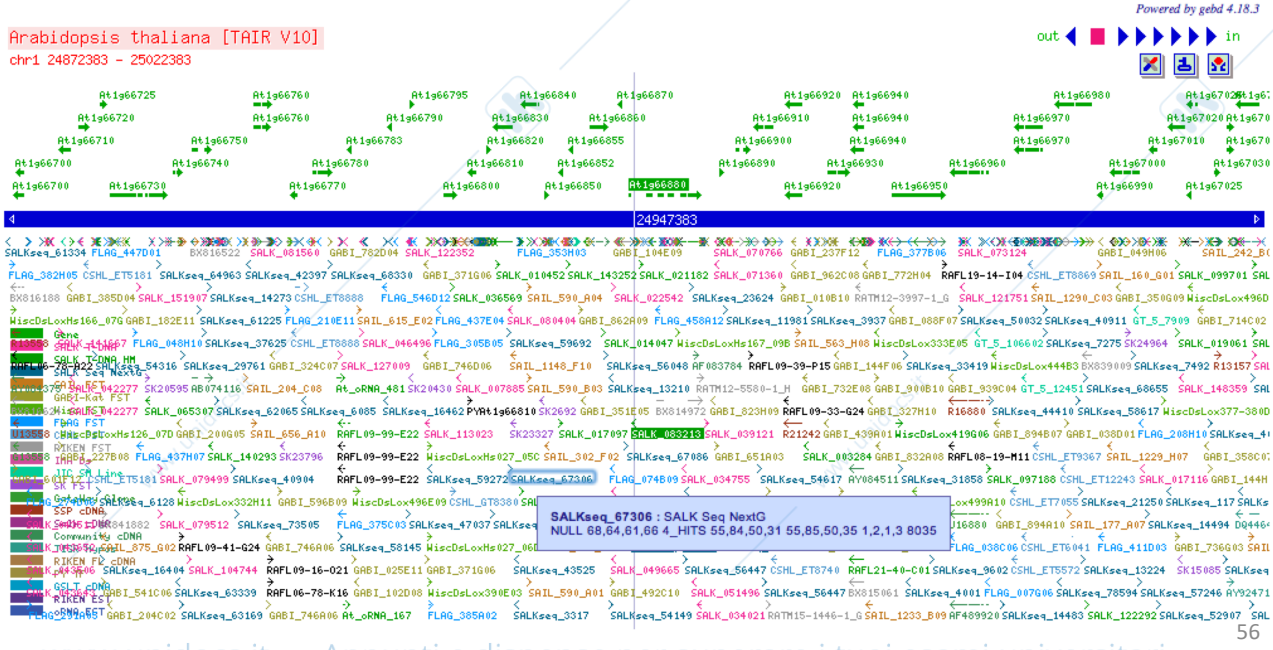
Step 3 **Confirmations**

Sample sequencing
Mutation purification (how to ? → Backcrosses)
Complementation

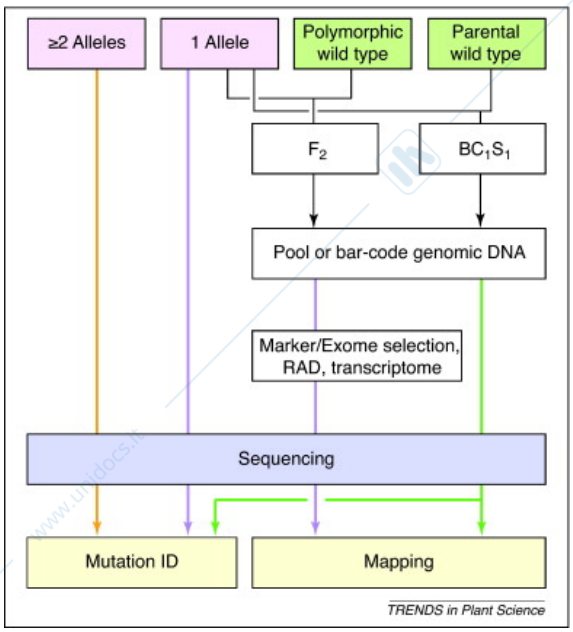
T-DNA collections

http://signal.salk.edu/cgi-bin/tdnaexpress

T-DNA Express: Arabidopsis Gene Mapping Tool (Feb. 7, 2014)

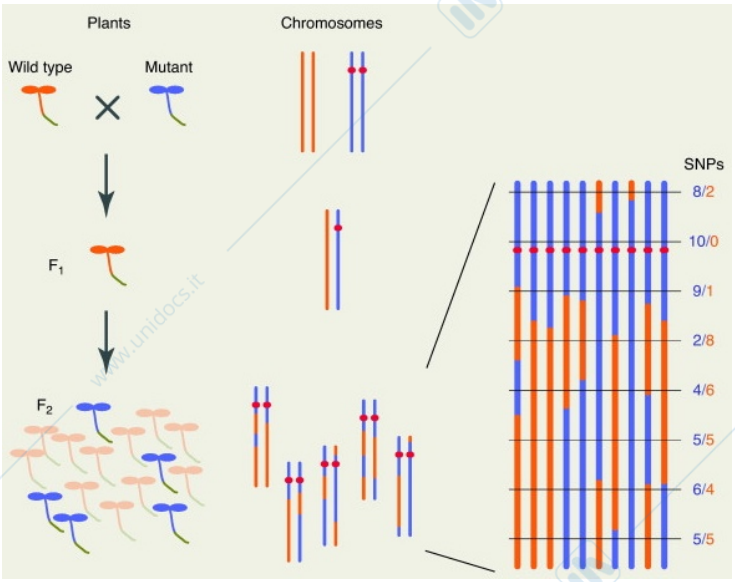


NGS-based mutation mapping



Takes advantage of the **polymorphism between ecotypes**

e.g. Col vs Ler in *Arabidopsis thaliana*



homologous recombination isn't possible in all species

57

specific sequence that we want

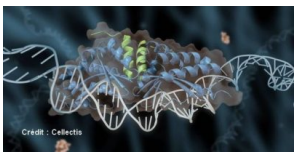
Genome Editing:
different technologies to guide the effector to the DNA target

↳ we cannot control where tDNA ends up → make many lines w/ mutations

↓ low chance of having insertion all in the same place

Meganucleases
animals

Recognize long DNA sequences (>12 bp)
Induce double-strand break → mistake in repair to make mutants



ZFPs
= Zinc Finger Nucleases

Hybrid Proteins (Zinc finger + Restriction Enz):
- Recognize a 3bp specific DNA sequence
- Fok I cut DNA (isolated from *Flavobacterium okeanokoites)*

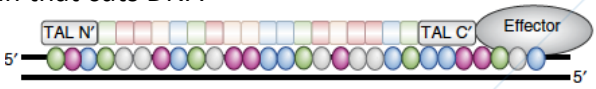
can put many together to fusion protein
↳ Fok cuts & can add mutations

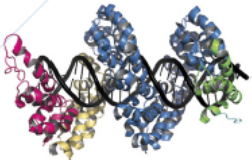
clump w/ specific binding site } ↑ rare to hit where we want

TALEs
= trans-activator effectors

before

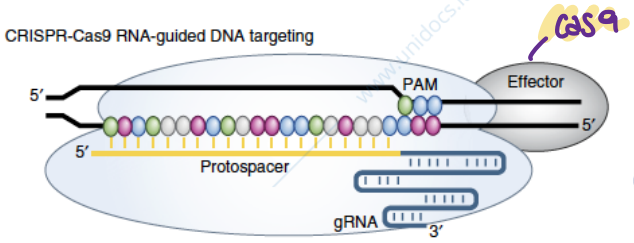
Artificial enzymes (TAL domain from *Xanthomonas*)
- TAL domain recognizes a specific DNA sequence
- Catalytic domain that cuts DNA

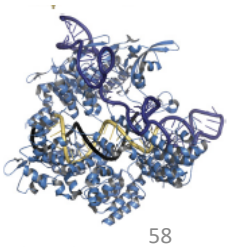




CRISPR-Cas9

Targets DNA with an RNA





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Advantage of the CRISPR-Cas9 technique:

Specific recognition of the target DNA by a RNA that can be customized (depending on the sequence of the DNA to be targeted)

and not on a protein-DNA interaction, needing a specific DNA sequence to be present in the target.

✓ The guide RNA is simple to synthesize

✓ The guide RNA and RNA encoding Cas9 can be injected directly in cells (embryos).

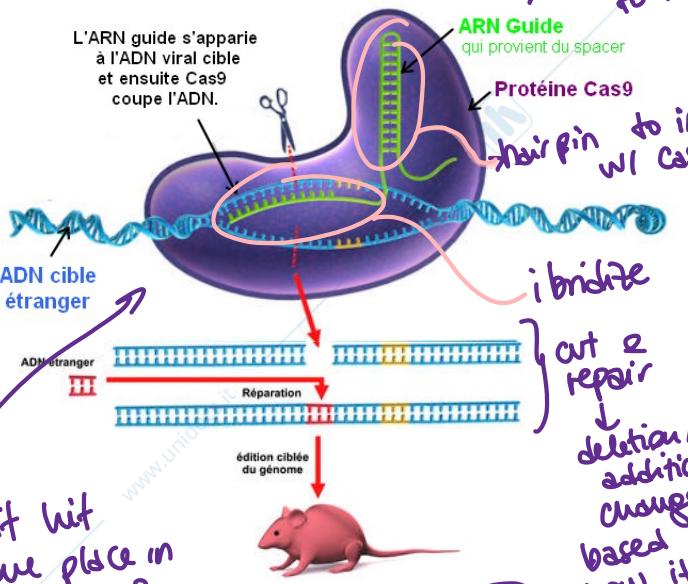
→ faster, simpler, cheaper

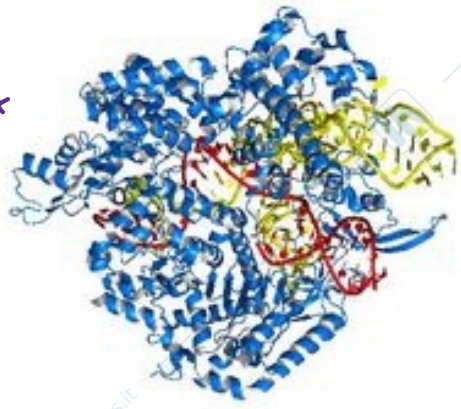
CRISPR-Cas9: The new DNA scissors

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat

Cas9: CRISPR associated protein 9

Bacterial origin





3D structure of the CRISPR-Cas9 complex : **Cas9** enzyme coupled to its **guide RNA** and its **target DNA**

↳ deep sequencing ↘ no → backcross to remove off-targets

CRISPR-Cas9

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat
Cas9: CRISPR associated protein 9


NEWS FEATURE

RIDING THE CRISPR WAVE

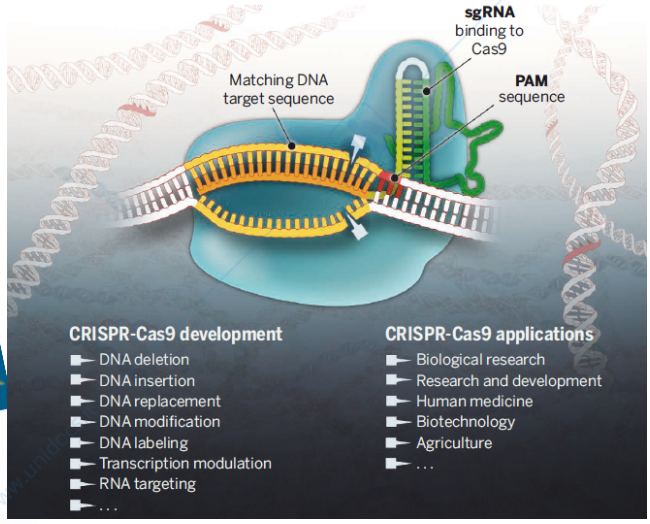
Biologists are embracing the power of gene-editing tools to explore genomes.

BY HEIDI LEDFORD

Whenever a paper about CRISPR-Cas9 hits the press, the staff at Addgene quickly find out. The non-profit company is where study authors often deposit molecular tools that they used in their work, and where other scientists immediately turn to get them. "We get calls within minutes of a hot paper publishing," says Joanne Kamens, executive director of the company in Cambridge, Massachusetts.

Addgene's phones have been ringing a lot since early 2013, when researchers first reported[1,2] that they had used the CRISPR–Cas9 system to slice the genome in human cells at sites of their choosing. "It was all hands on deck," Kamens says. Since then, molecular biologists have rushed to adopt the technique.

CRISPR EVERYWHERE
A Nature special issue
nature.com/crisp

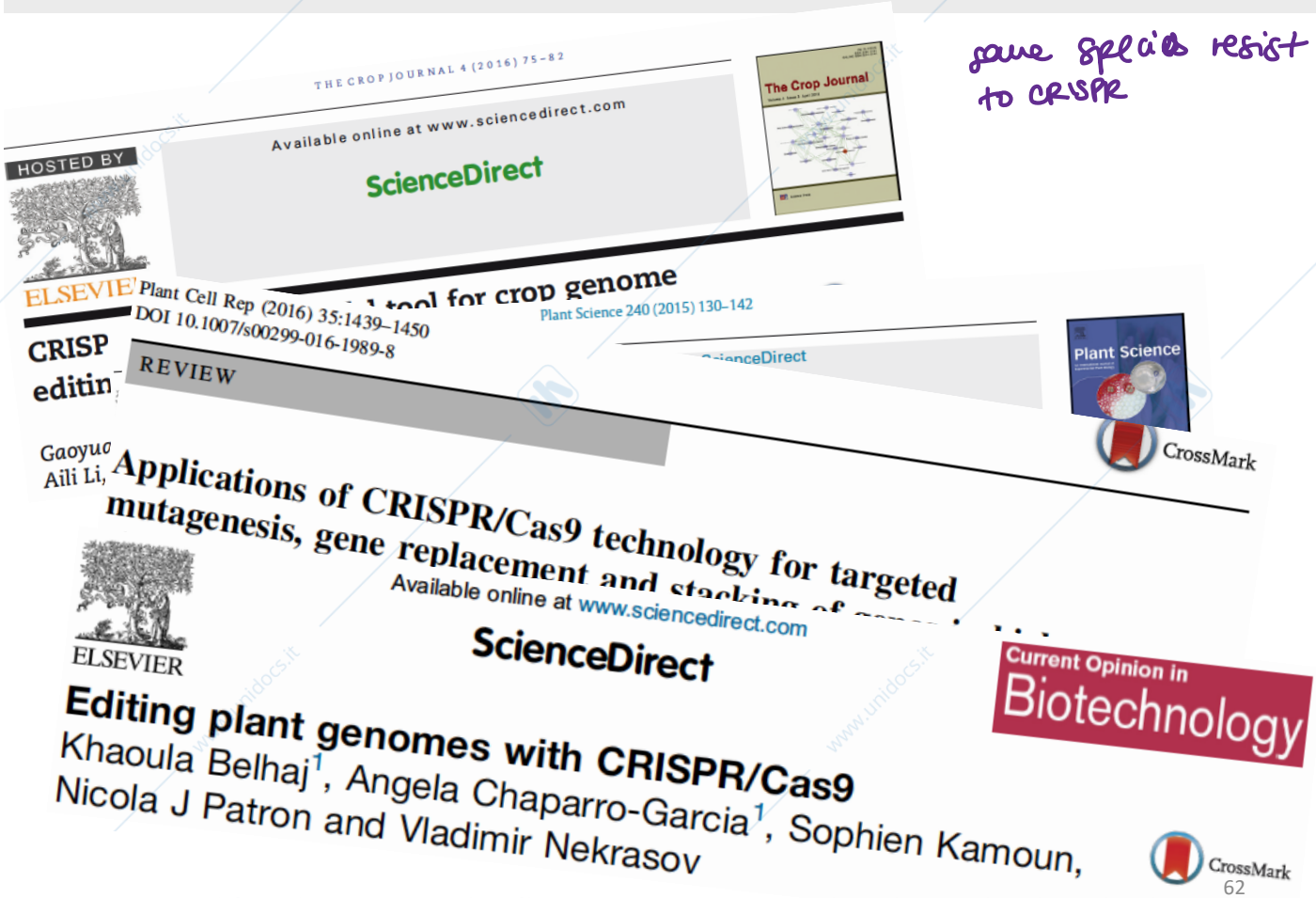


sgRNA: « single guide RNA »

PAM: « Protospacer Adjacent Motif »

The CRISPR/Cas9 revolution (in plants as well)

some species resist to CRISPR


THE CROP JOURNAL 4 (2016) 75–82

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Plant Cell Rep (2016) 35:1439–1450
DOI 10.1007/s00299-016-1989-8

Plant Science 240 (2015) 130–142

Plant Science

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REVIEW

Gaoyuan Aili Li, **Applications of CRISPR/Cas9 technology for targeted mutagenesis, gene replacement and stacking of**

Available online at www.sciencedirect.com

ScienceDirect

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Current Opinion in Biotechnology

Editing plant genomes with CRISPR/Cas9

Khaoula Belhaj[1], Angela Chaparro-Garcia[1], Sophien Kamoun, Nicola J Patron and Vladimir Nekrasov

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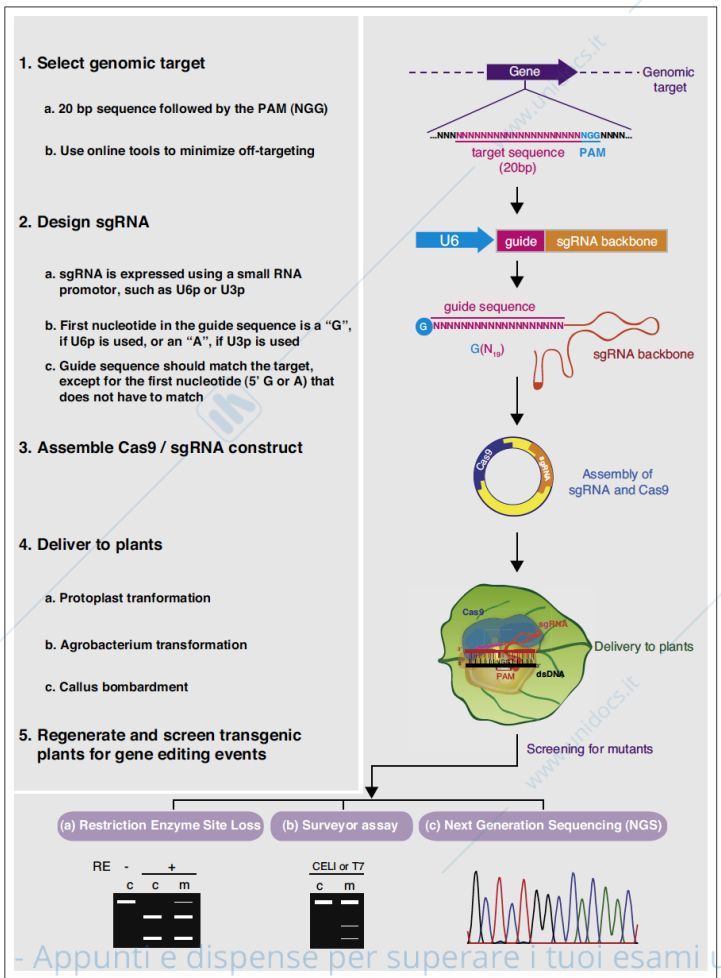
CRISPR-Cas9 publications (on crops)

Arabidopsis
→ transgenic editing
to express CRISPR
then if it
work back cross
to remove
transgenic
sequence

Corn	Targeted mutagenesis	Liang et al. 2014
Rice	Targeted mutagenesis	Belhaj et al. 2013
Sorghum	Targeted gene modification	Jiang et al. 2013b
Sweet orange	Targeted genome editing	Jia and Wang 2014
Tobacco	Targeted mutagenesis	Belhaj et al. 2013
Wheat	Targeted mutagenesis	Upadhyay et al. 2013, Yanpeng et al. 2014
Potato	Targeted mutagenesis	Shaohui et al., 2015
Soybean	Gene editing	Yupeng et al., 2015

Pipeline for generating a CRISPR/Cas9-mutagenised plant line



A « non-GMO » CRISPR/Cas9-edited maize line

nature COMMUNICATIONS

ARTICLE

Received 29 Aug 2016 | Accepted 16 Sep 2016 | Published 16 Nov 2016

DOI: 10.1038/ncomms13274 OPEN

Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes

Sergei Svitashev[1], Christine Schwartz[1], Brian Lenderts[1], Joshua K. Young[1] & A. Mark Cigan[1,†]

Targeted DNA double-strand breaks have been shown to significantly increase the frequency and precision of genome editing. In the past two decades, several double-strand break technologies have been developed. CRISPR-Cas9 has quickly become the technology of choice for genome editing due to its simplicity, efficiency and versatility. Currently, genome editing in plants primarily relies on delivering double-strand break reagents in the form of DNA vectors. Here we report biolistic delivery of pre-assembled Cas9-gRNA ribonucleoproteins into maize embryo cells and regeneration of plants with both mutated and edited alleles. Using this method of delivery, we also demonstrate DNA- and selectable marker-free gene mutagenesis in maize and recovery of plants with mutated alleles at high frequencies. These results open new opportunities to accelerate breeding practices in a wide variety of crop species.

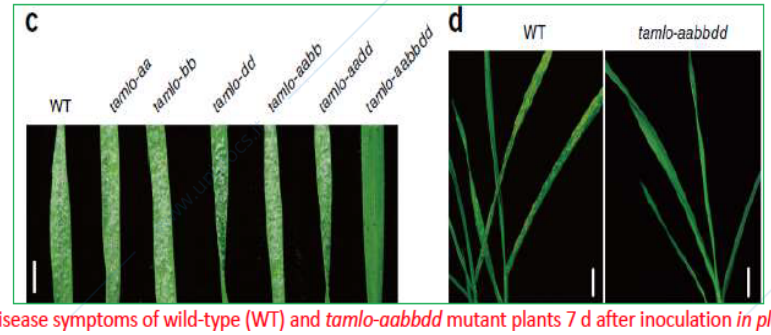
Simple injection of a Ribonucleo-proteic cocktail is sufficient !

This works in:
- plants: e.g. maize embryo
- animals: e.g. mouse and… human embryos

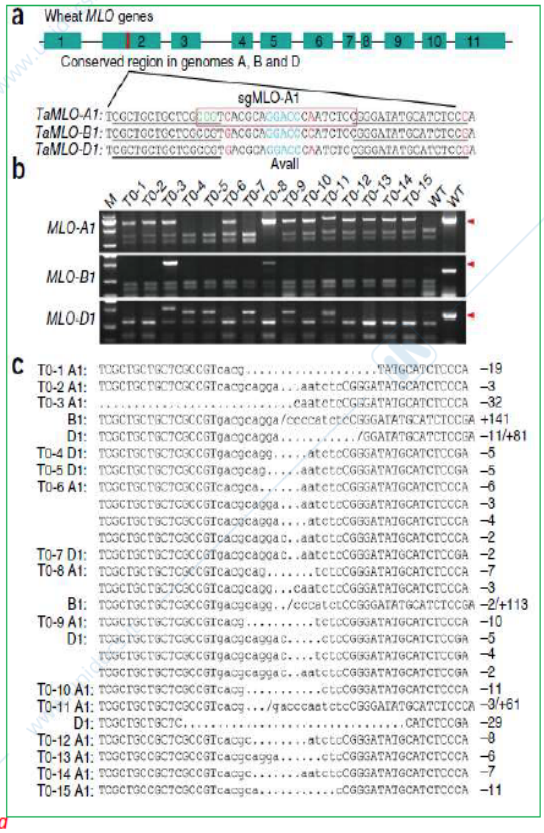
No T-DNA / transgene required !

Examples of application of CRISPR-Cas9 in plants: Resistance to pests (1)

Micrographs of microcolony formation of *Bgt* on the surfaces of leaves



Disease symptoms of wild-type (WT) and *tamlo-aabbdd* mutant plants 7 d after inoculation *in planta*

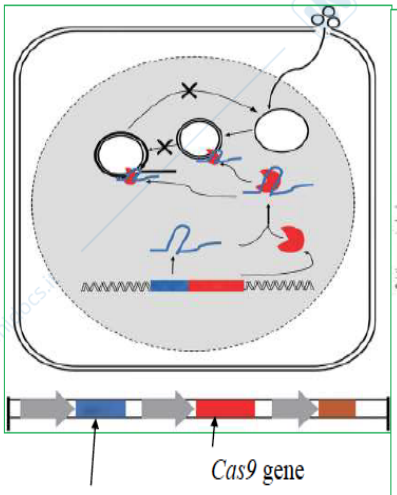


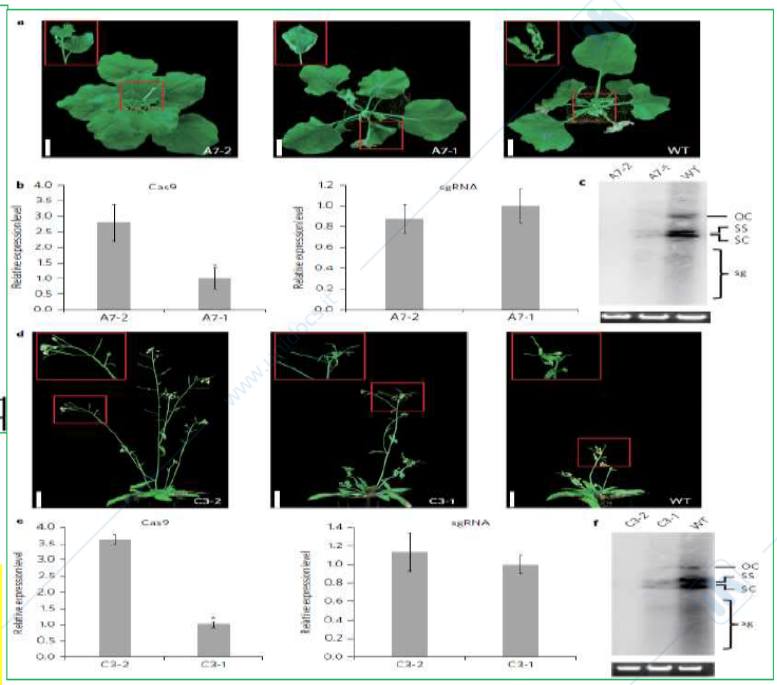
Examples of application of CRISPR-Cas9 in plants: Resistance to pests (2)

nature plants
BRIEF COMMUNICATION
PUBLISHED: 28 SEPTEMBER 2015 | ARTICLE NUMBER: 15144 | DOI: 10.1038/NPLANTS.2015.144

Establishing a CRISPR–Cas-like immune system conferring DNA virus resistance in plants

Xiang Ji[1,2†], Huawei Zhang[1†], Yi Zhang[1,2], Yanpeng Wang[1,2] and Caixia Gao[1*]





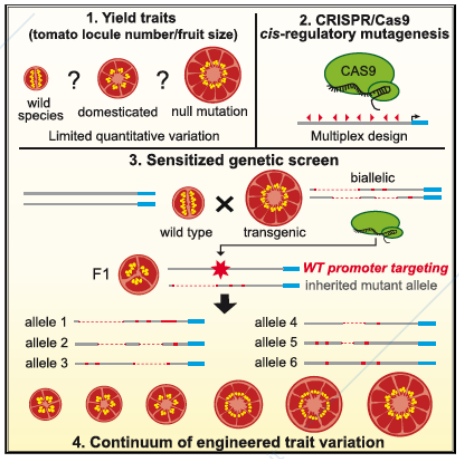
Transgenic *N. benthamiana* and Arabidopsis plants develop resistance to **beet severe curly top virus** (BSCTV)

Ex of application of CRISPR-Cas9 in plants: Tomato trait improvement

Cell
Oct 2017
Resource

Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing

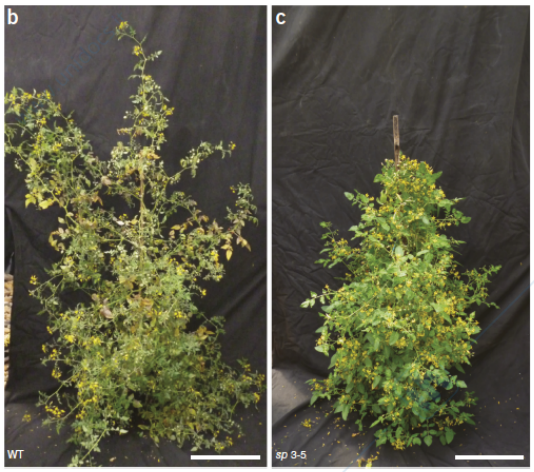
Graphical Abstract



Authors
Daniel Rodríguez-Leal, Zachary H. Lemmon, Jarrett Man, Madelaine E. Bartlett, Zachary B. Lippman

Correspondence
lippman@cshl.edu

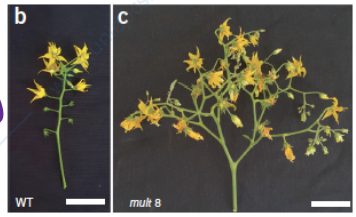
In Brief
In this article, we discuss how to use the CRISPR/Cas9 genome editing approach to dissect the biology of quantitative trait loci.



nature biotechnology
Nature Biotech, Oct 2018

De novo domestication of wild tomato using genome editing

Agustin Zsögön[1,7], Tomáš Čermák[2,6,7], Emmanuel Rezende Naves[1], Marcela Morato Notini[3], Kai H Edel[4], Stefan Weinl[4], Luciano Freschi[5], Daniel F Voytas[2], Jörg Kudla[4] & Lázaro Eustáquio Pereira Peres[3]



proof CRISPR Cas is useful to create varieties much faster than with traditional methods (introgression of

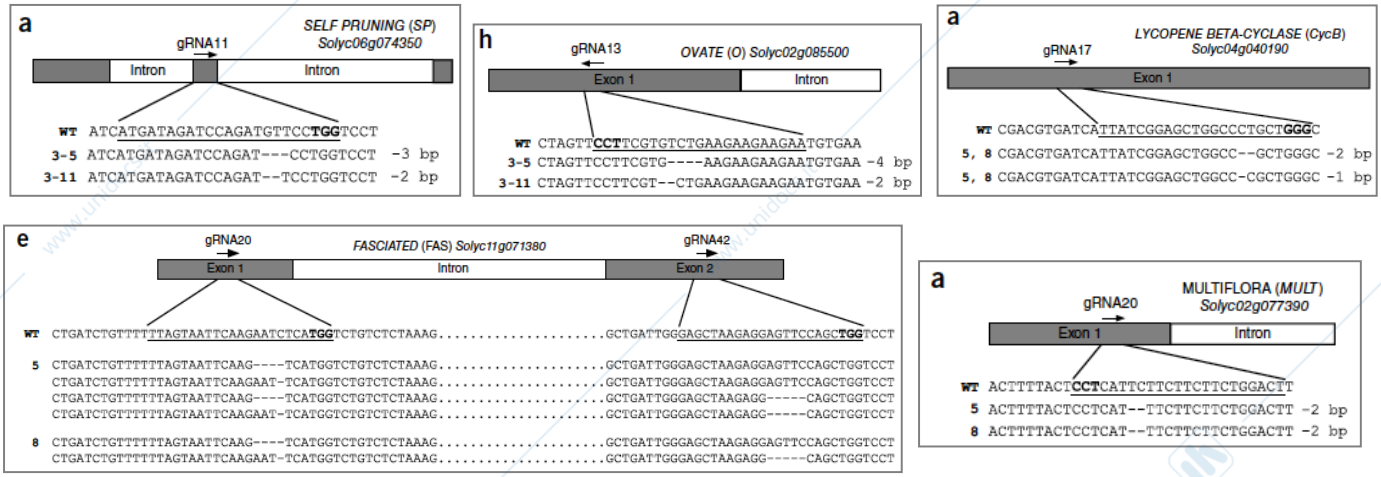
knows function of genes responsible

The Tomato domestication journey: Dramatically fastened with the CRISPR/Cas technology

De novo domestication of wild tomato using genome editing

nature biotechnology

Agustin Zsögön[1,7], Tomáš Čermák[2,6,7], Emmanuel Rezende Naves[1], Marcela Morato Notini[3], Kai H Edel[4], Stefan Weinl[4], Luciano Freschi[5], Daniel F Voytas[2], Jörg Kudla[4] & Lázaro Eustáquio Pereira Peres[3]

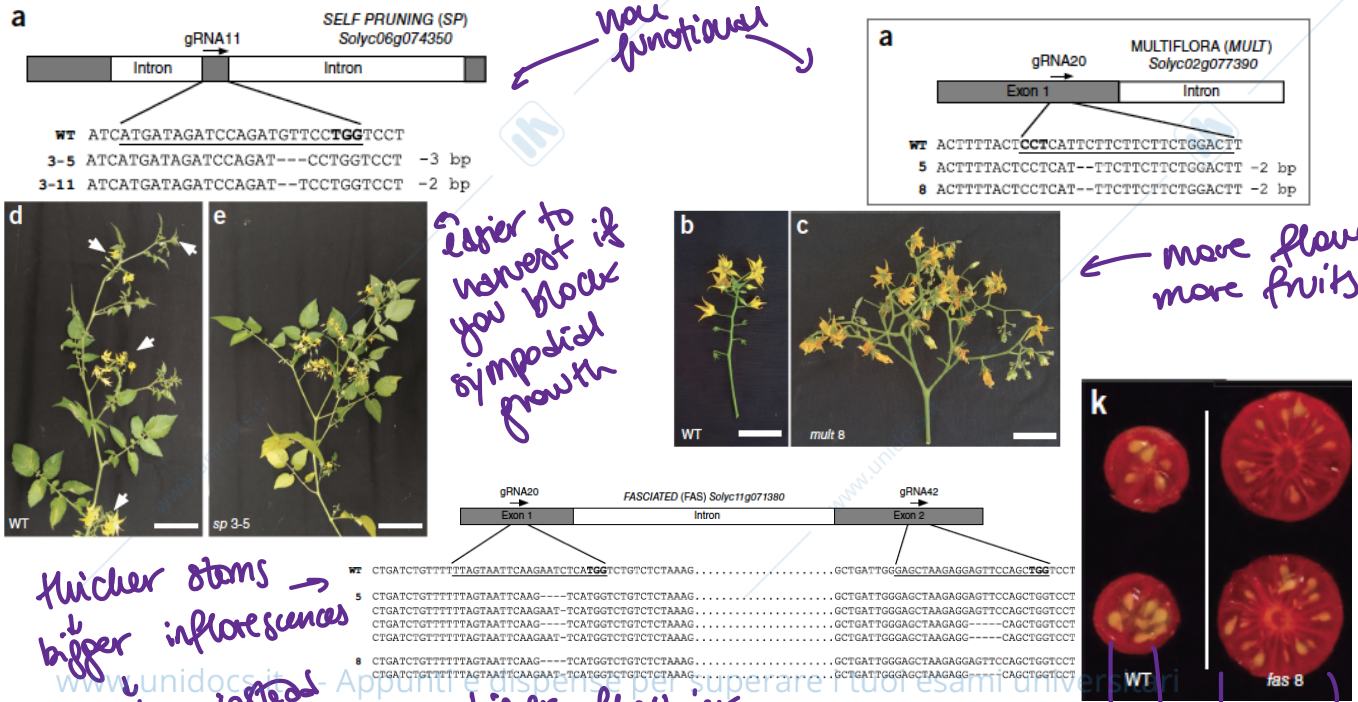


Zsögön et al, Nat Biotech 2018

growth of tomatoes by sympodium

The Tomato domestication journey: Dramatically fastened with the CRISPR/Cas technology

« Compared with the wild parent, our engineered lines have a **threefold increase in fruit size** and a **tenfold increase in fruit number**. Notably, **fruit lycopen accumulation is improved by 500%** compared with the widely cultivated S. lycopersicum. Our results pave the way for molecular breeding programs to exploit the genetic diversity present in wild plants. »



retrace domestication of the tomato

Genome and epigenome Editing:

The CRISPR/Cas9 power

homology-directed repair (HDR)

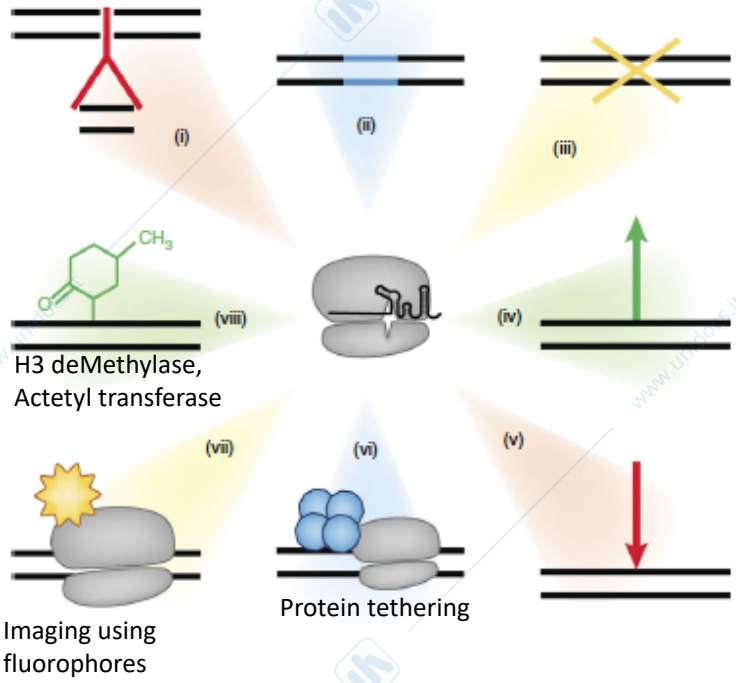
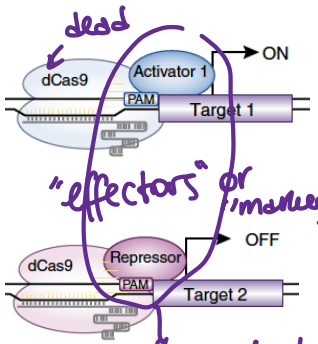


Figure 5 Genome editing redefined. This figure illustrates the range of applications based on CRISPR–Cas9 technologies. (i) Deletions (using HDR with a template in which a deletion is engineered). (ii) Insertions (by providing a HDR template carrying a designer sequence). (iii) Knockouts (using NHEJ-mediated DSB repair). (iv) Transcriptional activation (CRISPRa, using dCas9 tethered to a transcriptional activator, such as VP64). (v) Transcriptional repression (CRISPRi, using dCas9, potentially fused to a transcriptional repressor such as KRAB). (vi) Fusion protein delivery (by direct or indirect recruiting of an effector molecule of interest, through fusion, tethering, or by the use of guides carrying protein-binding DNA sequences of interest). (vii) Imaging (using fluorophores). (viii) Epigenetic state alteration (using either epigenetic repressors such as the LSD1 histone demethylase for interaction with distal enhancers, or epigenetic activation using the p300 histone acetyltransferase).



still has ability to go to gene of interest but doesn't edit the gene

↳ eg GFP to see fluorescence in nucleus

↳ useful for chromatin dynamics

The CRISPR/Cas9 to dCas9 revolution

→ Using dCas9, researchers can specifically mark or modify a region of interest, in a living system (cell, tissue, organism) for dynamics studies of induced chromatin changes

Editing the epigenome: technologies for programmable transcription and epigenetic modulation Nature Methods 2016

Pratiksha I Thakore[1,2], Joshua B Black[1,2], Isaac B Hilton[1,2] & Charles A Gersbach[1–3]

Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers Nature Biotech 2015

Isaac B Hilton[1,2], Anthony M D'Ippolito[2,3], Christopher M Vockley[2,4], Pratiksha I Thakore[1,2], Gregory E Crawford[2,5], Timothy E Reddy[2,6] & Charles A Gersbach[1,2,7]

International Journal of *Molecular Sciences* MDPI

Review

Chromatin Manipulation and Editing: Challenges, New Technologies and Their Use in Plants

Kateryna Fal [1,†], Denisa Tomkova [2,†], Gilles Vachon [1], Marie-Edith Chabouté [2], Alexandre Berr [2,*] and Cristel C. Carles [1,*]

III. How to get more resolutive to tackle precise functions?

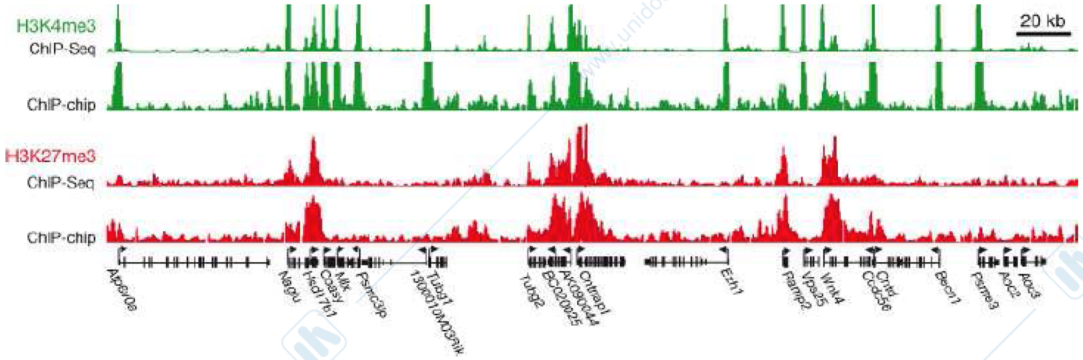
The modern challenge in plants

August 2007 | doi:10.1038/nature06008 nature

ARTICLES

Genome-wide maps of chromatin state in pluripotent and lineage-committed cells

Tarjei S. Mikkelsen[1,2], Manching Ku[1,4], David B. Jaffe[1], Biju Issac[1,4], Erez Lieberman[1,2], Georgia Giannoukos[1], Pablo Alvarez[1], William Brockman[1], Tae-Kyung Kim[5], Richard P. Koche[1,2,4], William Lee[1], Eric Mendenhall[1,4], Aisling O'Donovan[4], Aviva Presser[1], Carsten Russ[1], Xiaohui Xie[1], Alexander Meissner[3], Marius Wernig[3], Rudolf Jaenisch[3], Chad Nusbaum[1], Eric S. Lander[1,3]* & Bradley E. Bernstein[1,4,6]*



73

III. How to get more resolutive?

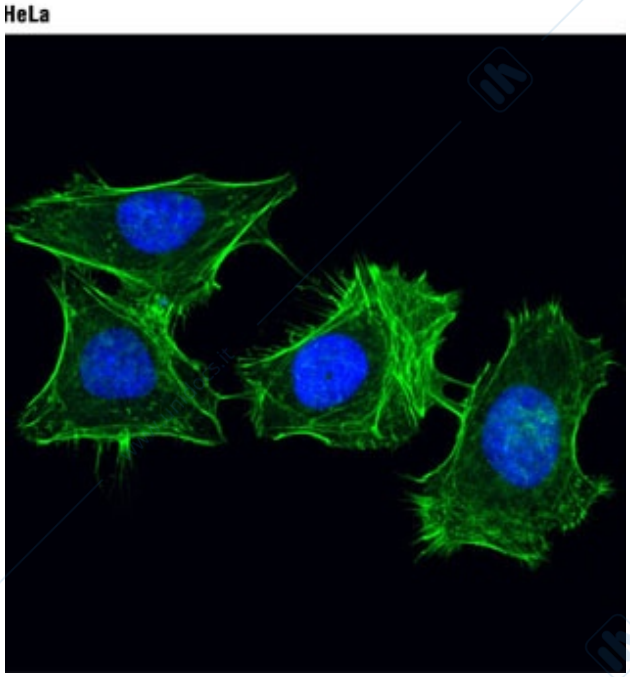
From tissue/cell-type specific to single cell analyses

- Fluorescence activated cell sorting
- Affinity purification for isolation of specific cell-type nuclei
- Single cell manipulation

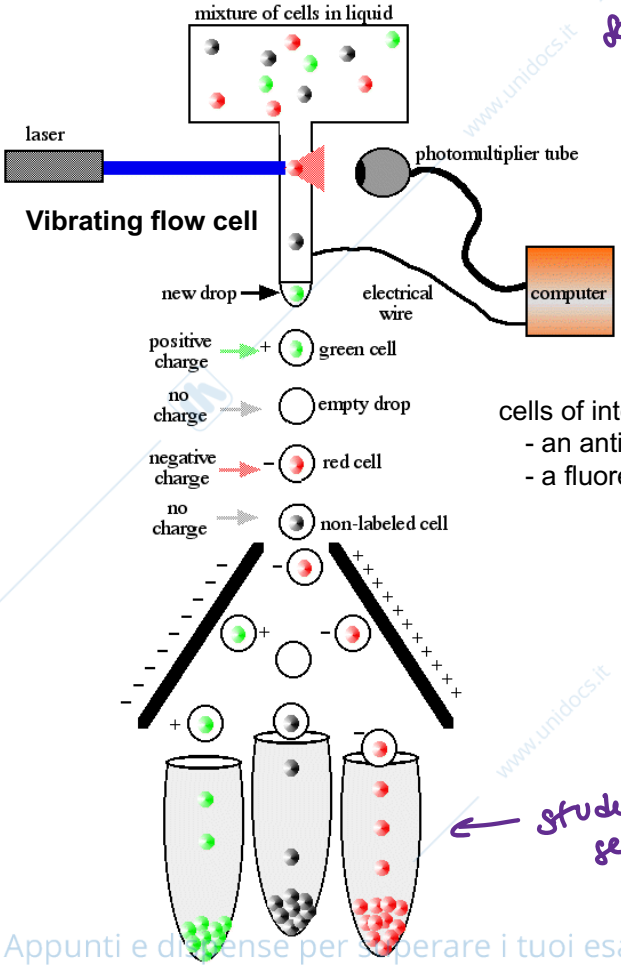
Sub-cellular fractionation

- Nucleo-cytoplasmic fractionation
- Chloroplast purification, chloroplast compartment purification
- Mitochondria-chloroplast contacts

Immunofluorescence



Fluorescence activated cell sorting

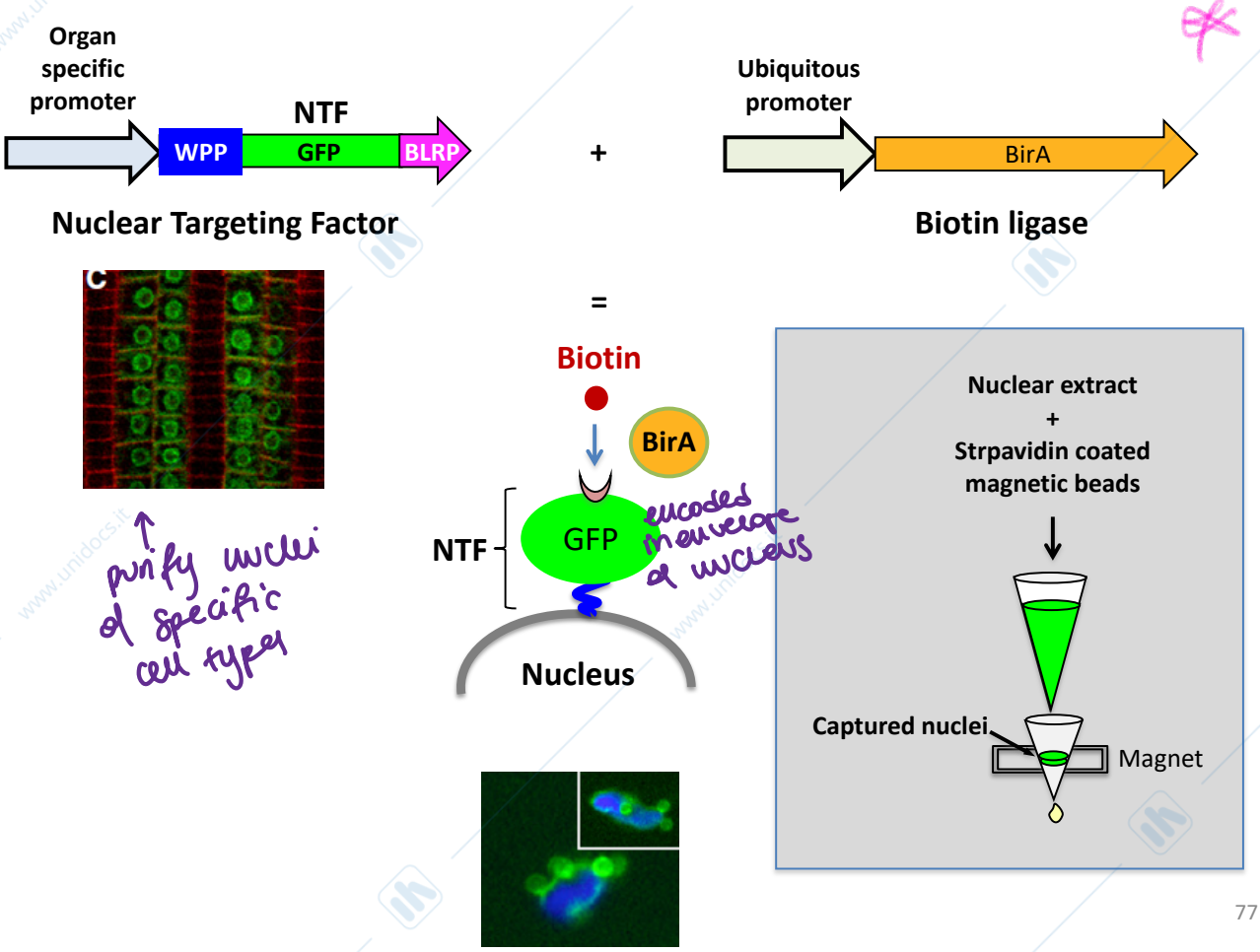


sort cells based on fluorescence

cells of interest are tagged with
- an antibody linked to a fluorescent dye
- a fluorescent protein GFP / RFP

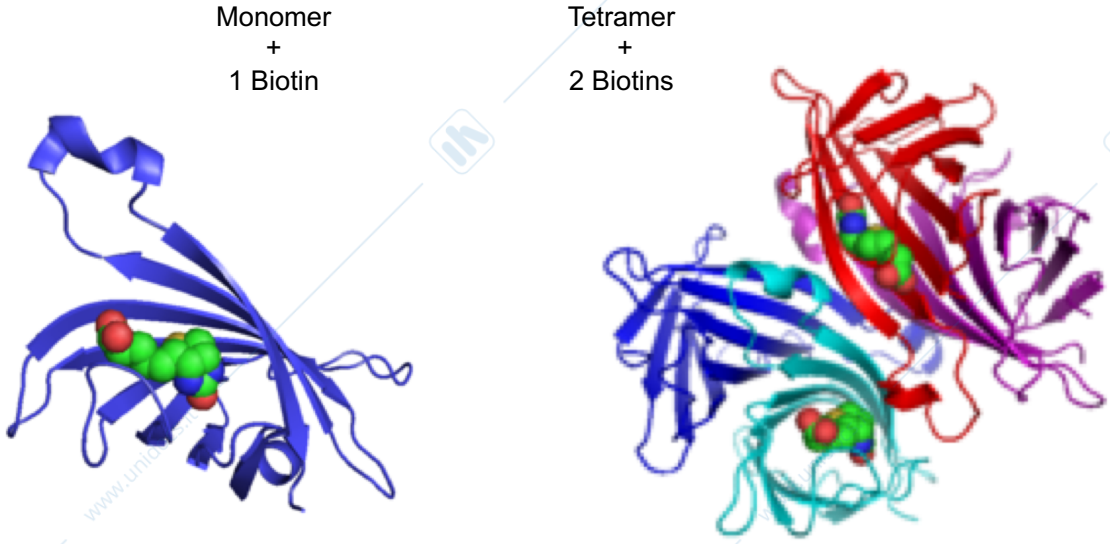
← study cells seperately

INTACT = Isolation of Nuclei Tagged in Specific Cell Types

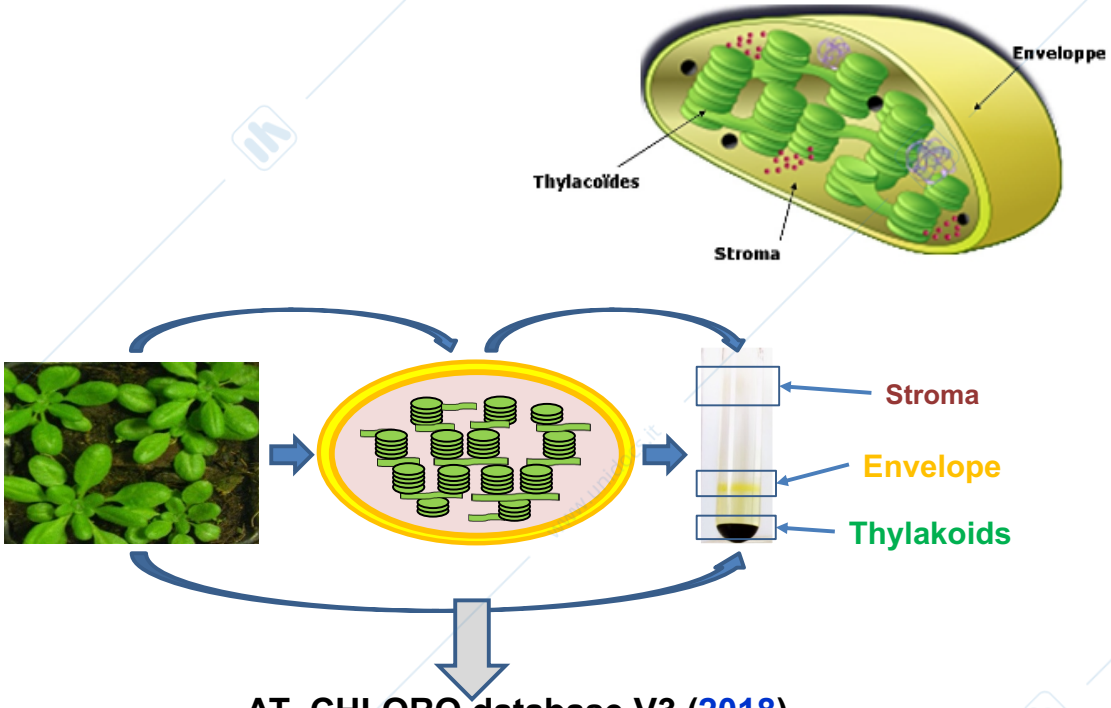


Affinity purification

Streptavidin high affinity for Biotin
(Vitamin B7)



Separation of chloroplast components



AT_CHLORO database V3 (2018)

http://at-chloro.prabi.fr/at_chloro/

IV. How to think as a plant developmental biologist

1. Modeling / Simplifying

2. The Dynamics

How to think as a plant developmental biologist

1. Modeling / Simplifying

Example of the flower development:

1. **Description**
2. **Mutagenesis (several thousands lines)**
3. **Selection ofmutants (dozens of mutants)**
4. **Proposition of the ABC model**

(Meyerowitz et al, 1991)

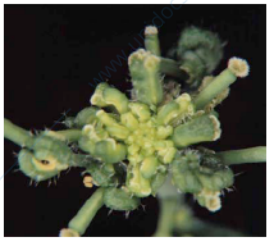
wt

se pe st ca

pi-1 (ap3)

se se ca ca

ap2-2 (ap1)



ca st st ca

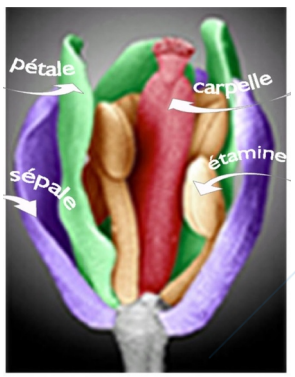
ag-3

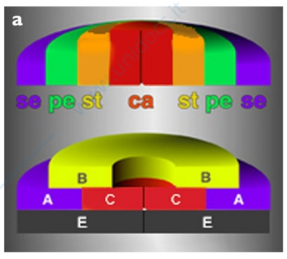
se pe pe se(n)

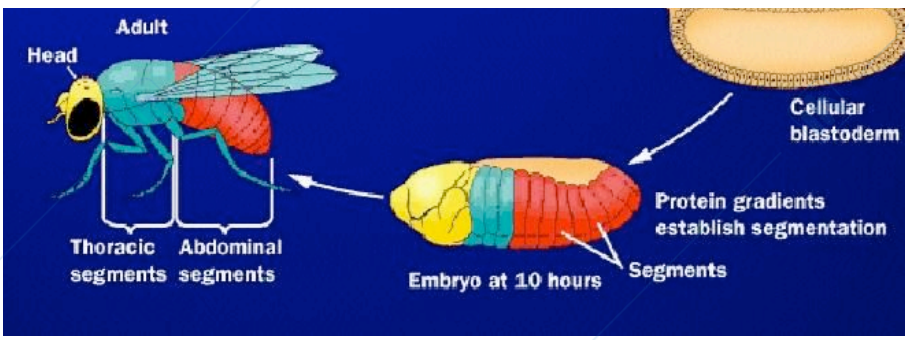
How to think as a plant developmental biologist

1. Modeling / Simplifying & comparing

Similarities between flower ABC and body plan establishment in Drosophila larva







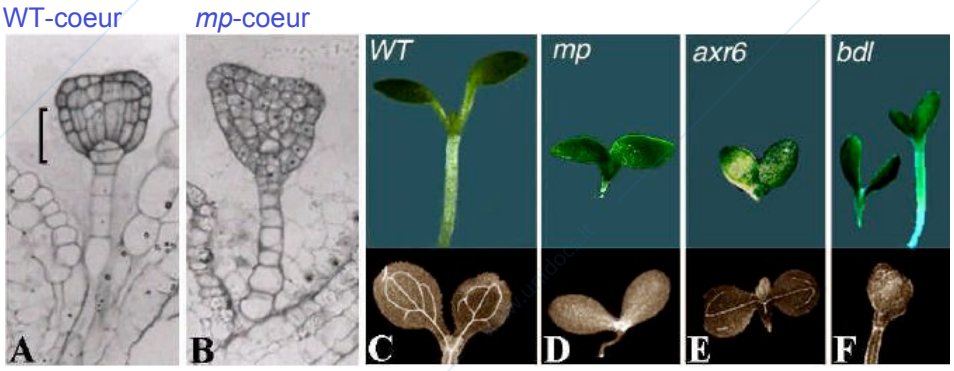
Homeotic genes: loss- and gain-of-function lines → segment identities are affected

Mutations in these genes cause replacement of an organ by another one (e.g.: ANTENNAPEDIA)

How to think as a plant developmental biologist

2. The dynamics

Example of embryo development : auxin signaling for sets-up the apico-basal axis



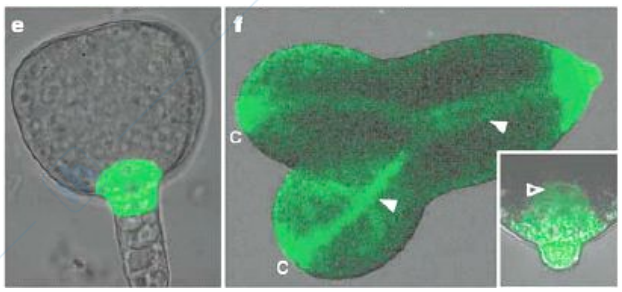
Mutations in MP, AXR6 or BDL (invovled in auxin signal transduction) affects the root pole of the embryo

(Berleth and Chatfield, 2002, *The Arabidopsis book*, eds. C.R. Somerville and E.M. Meyerowitz, American Society of Plant Biologists, Rockville, MD, doi/10.1199/tab.0009, http://www.aspb.org/publications/arabidopsis/).

How to think as a plant developmental biologist

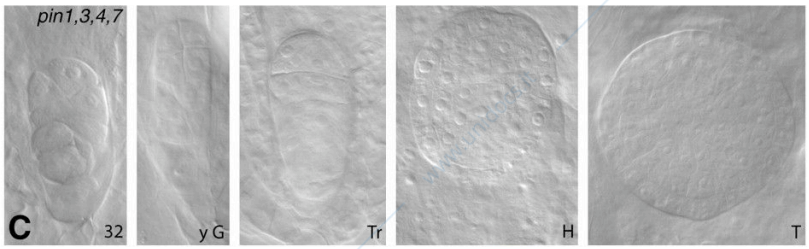
2. The dynamics

Embryons d'Arabidopsis
WT + DR5::GFP
Concentration de l'auxine au pôle racinaire



Polar transport of auxin leads to auxin wells that drive organogenesis (root, but also aerial organs)

Embryons d'Arabidopsis quadruple mutant *pin1 pin3 pin4 pin7* incapable de mettre en place le pôle racinaire



Friml *et al.*, 2003, *Nature* 426:147-153