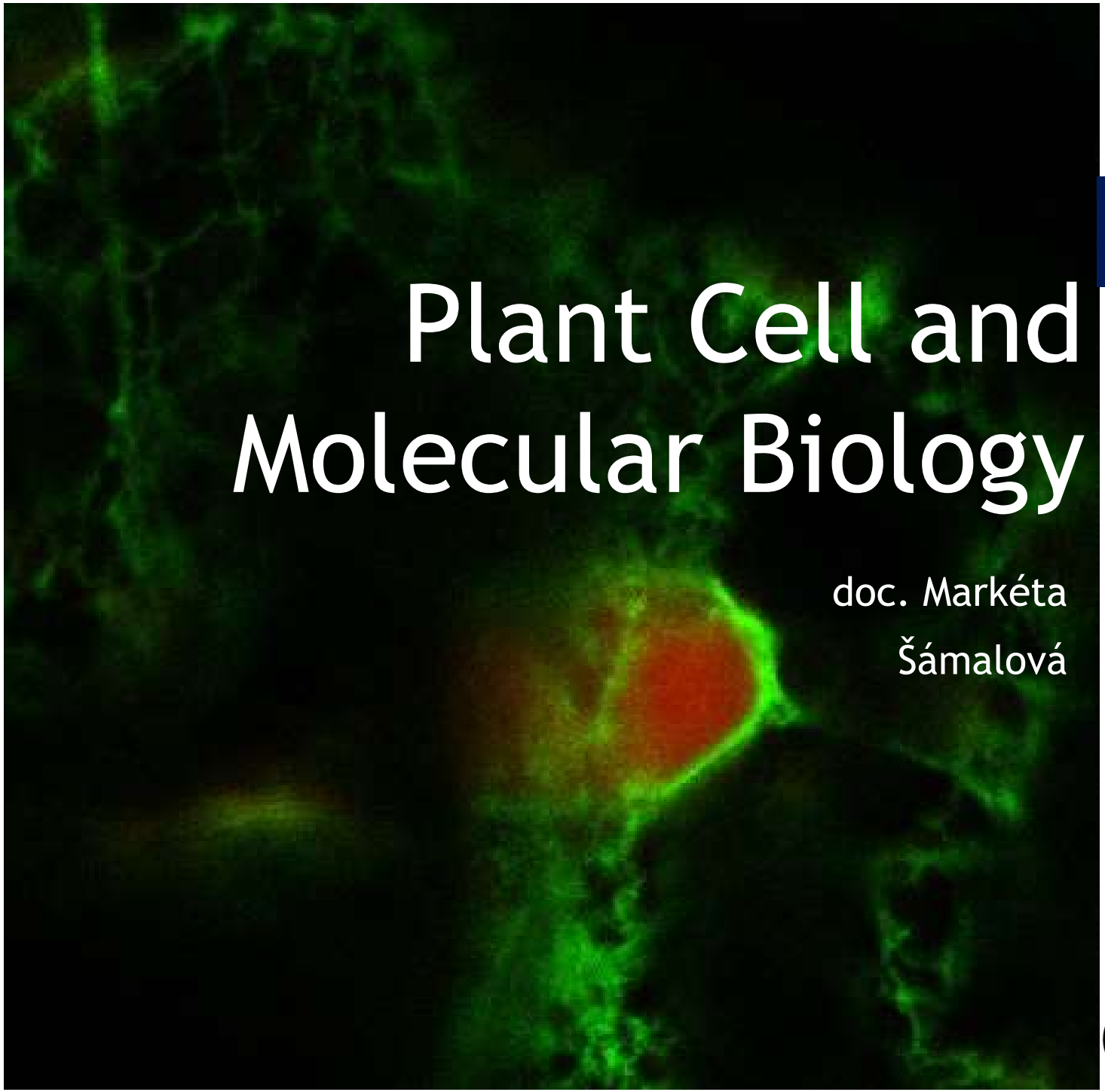

Plant Cell and Molecular Biology

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UNIVERSITY OF OXFORD

SCIENTIA PLANTARUM 1621 OXONIENSIS

Institut Pasteur

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cost

CEITEC

Oddělení experimentální biologie rostlin

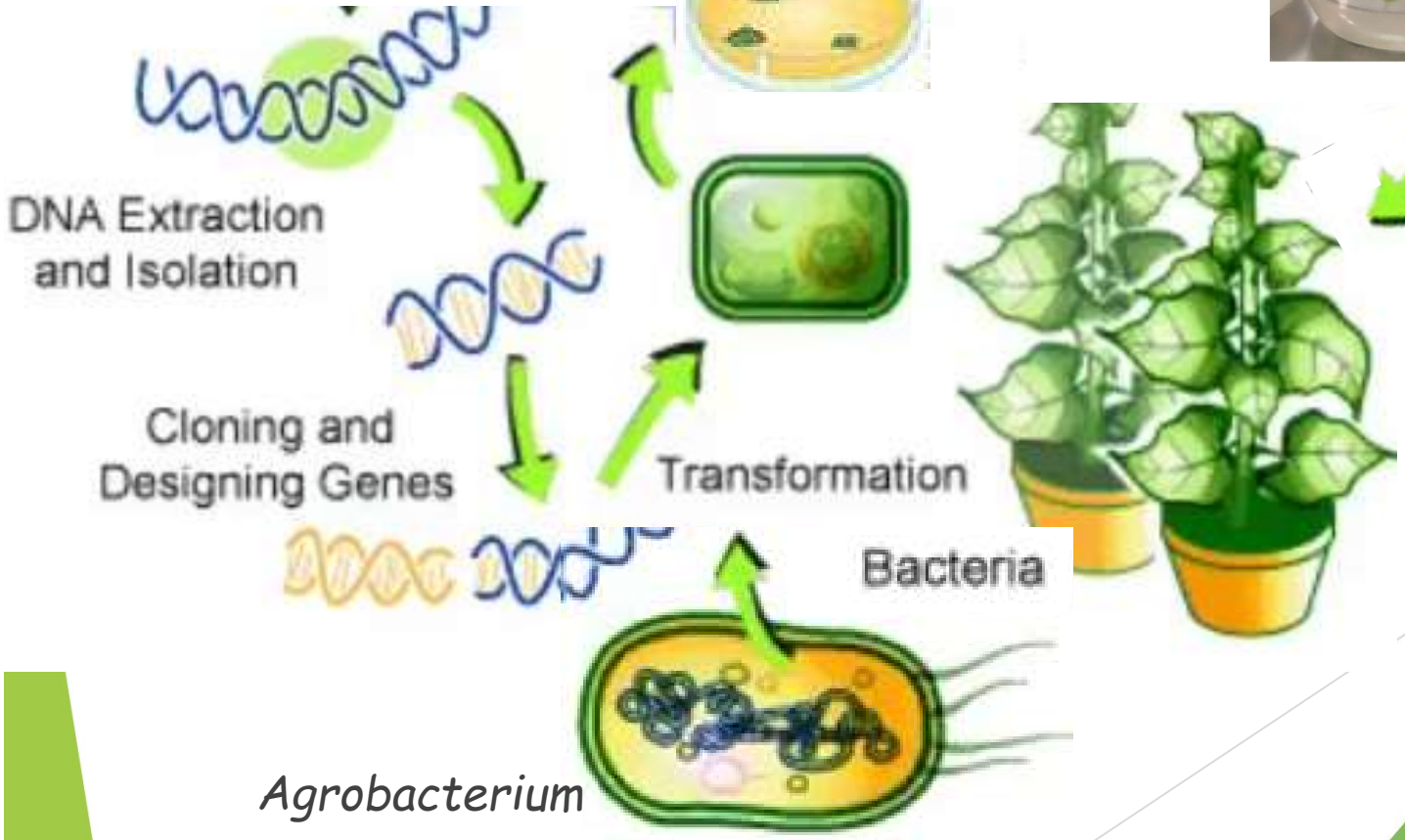
OUTLINE of the talk

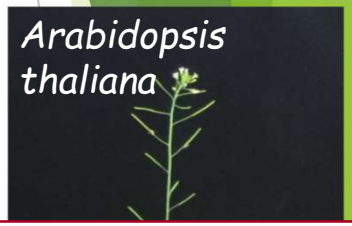
- How to make a genetically modified plant?
  - Tobacco, rice
  - *Arabidopsis thaliana*
- How to regulate (trans)gene expression?
  - The pOp6/LhGR system
  - CRISPR/Cas9
- Transient gene expression
- Fluorescent proteins
- Plant endomembrane system
- Plant cell wall
  - Expansins & (a)biotic stresses
- Fungal cell wall
  - *Magnaporthe oryzea* – a model organism

How to make a genetically modified or genome-edited plant?

Transformation

- **Tissue cultures**
  - tobacco
  - rice





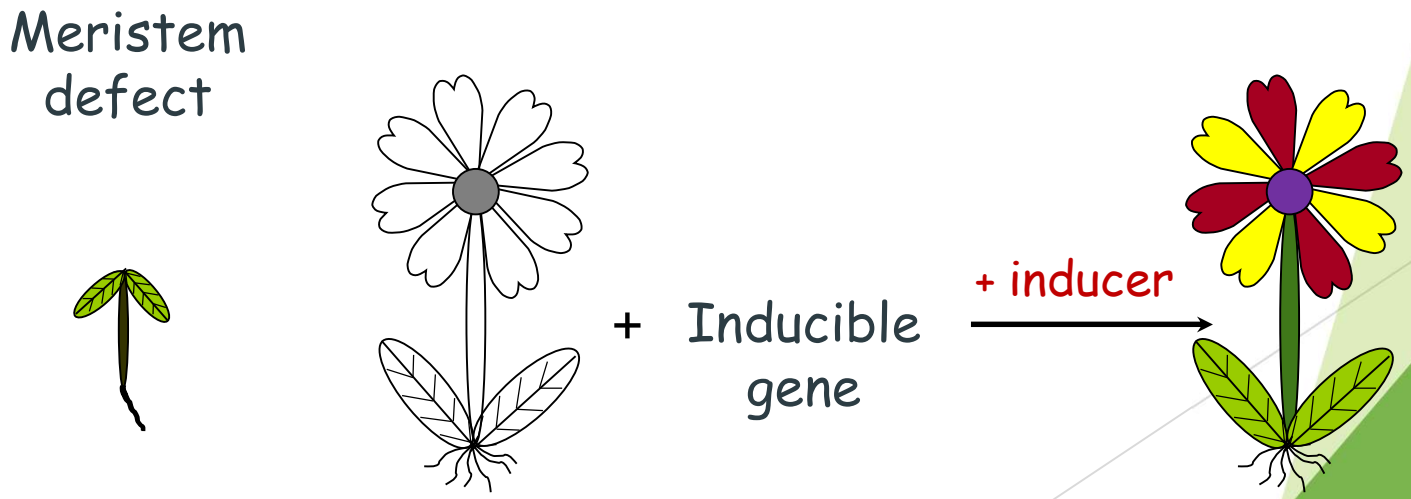
- **Floral dip**

Arabidopsis

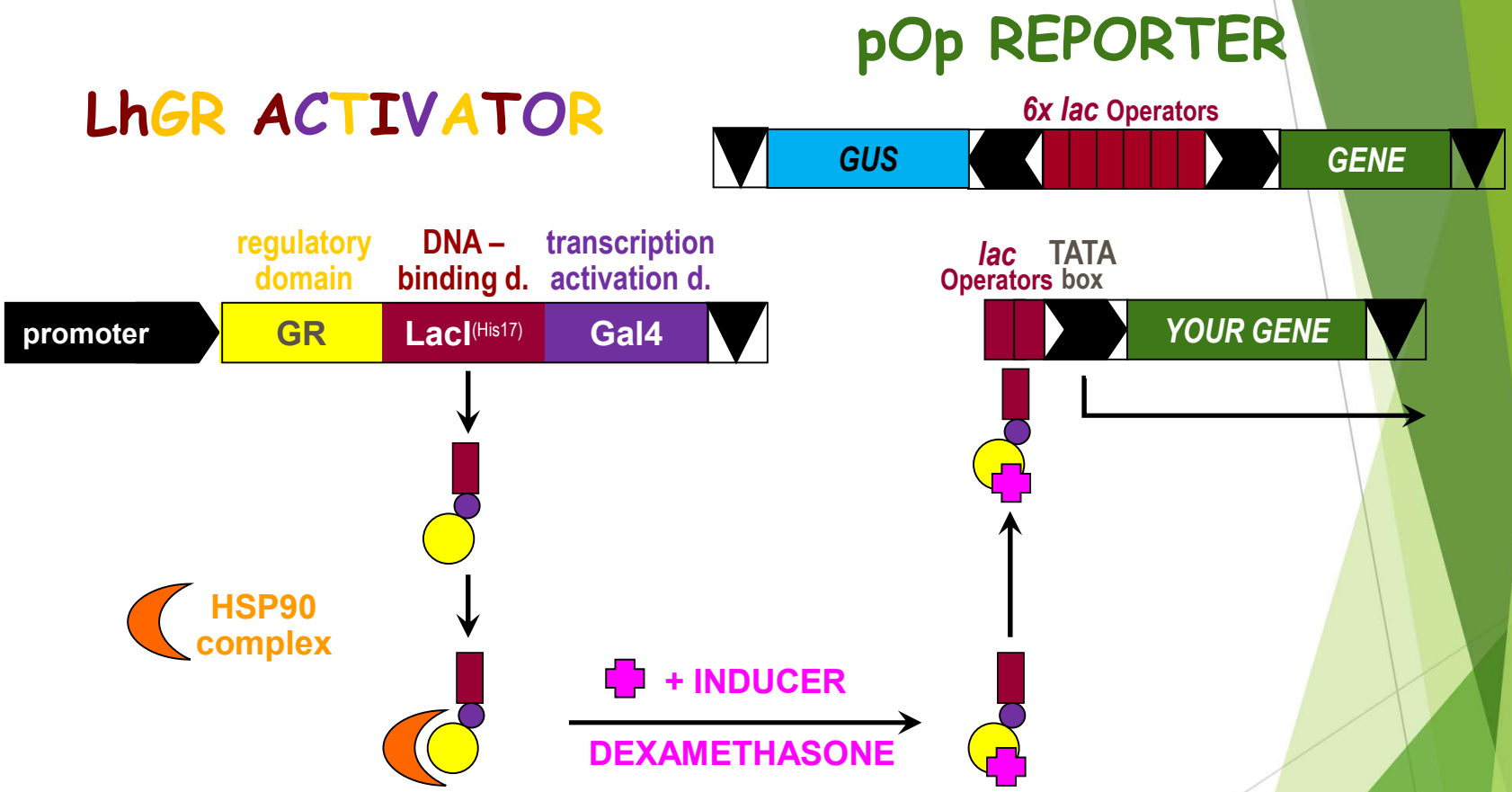
How to regulate (trans)gene expression?

Chemically inducible gene expression systems in plants

- regulate (trans)gene expression at a particular developmental stage and for a specific duration using chemical inducers.
- **Expression can be SWITCHED ON or OFF using chemical inducers.**
  - Gene overexpression, knock-down expression by amiRNAs, knock-out gene by combining the system with CRISPR/Cas9 (Gehrke *et al.*, 2023)
- Essential for expression of gene products that interfere with regeneration, growth or reproduction…



The chemically inducible transcription activation system pOp/LhGR



- Developed in the laboratory of Dr Ian MOORE
- Use world-wide today… an "ideal" inducible system

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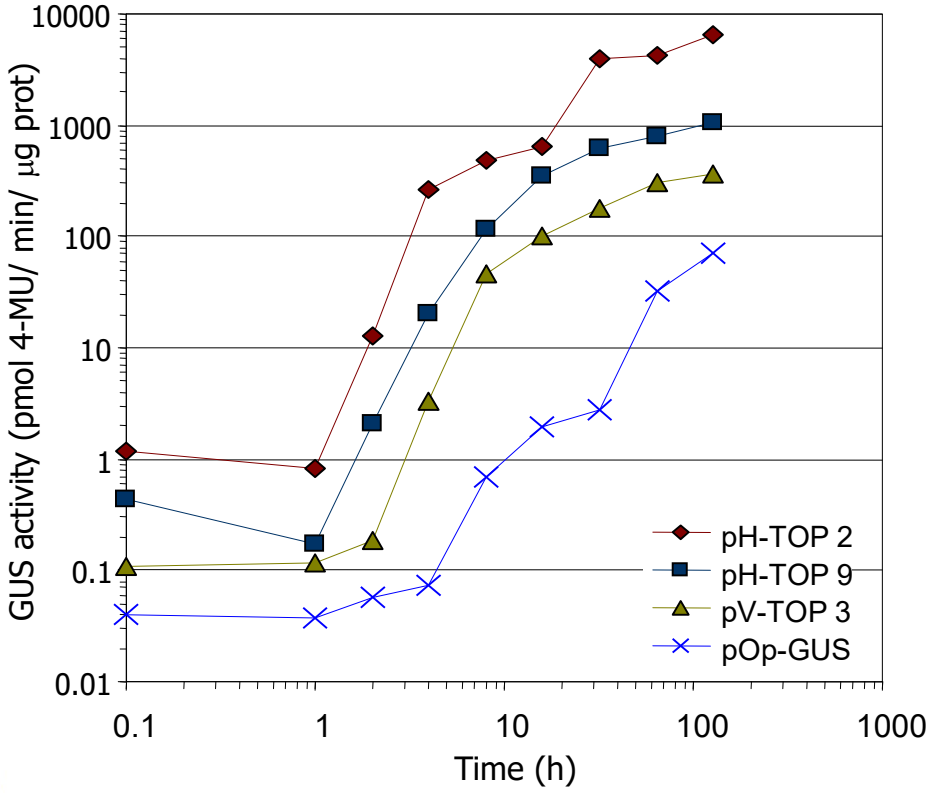
An ideal inducible system

- High induced expression (e.g. 1000x or more).
- No uninduced expression (not leaky).
- Rapid uptake and wide distribution of inducer.
- No toxicity, no physiological effects in plants.
- Convenient application by a number of methods.
- Functional in several plant species.

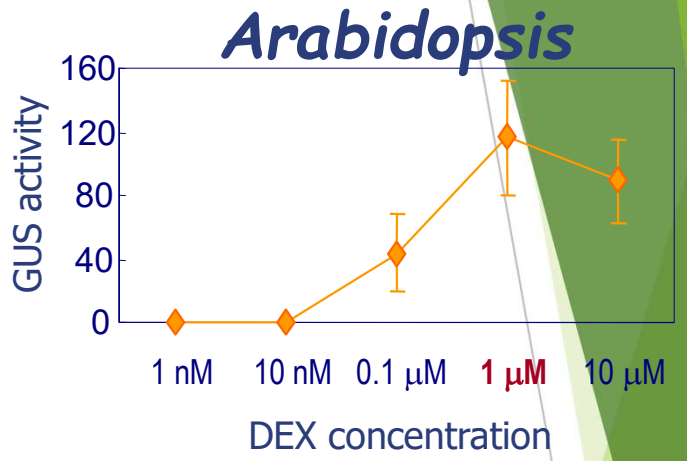
Depend on the type of application, the gene being expressed and the plant species!

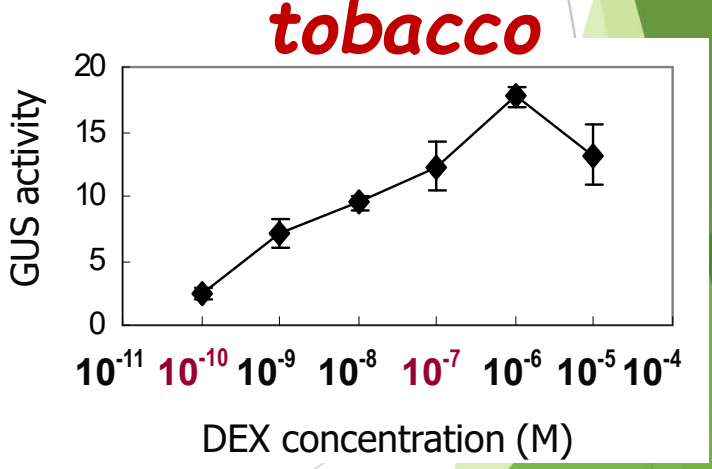
The pOp6/LhGR is highly inducible, fast & v. sensitive

- 10,000-fold induction of GUS activity (log scale !)



- Increase of GUS activity in 2h!

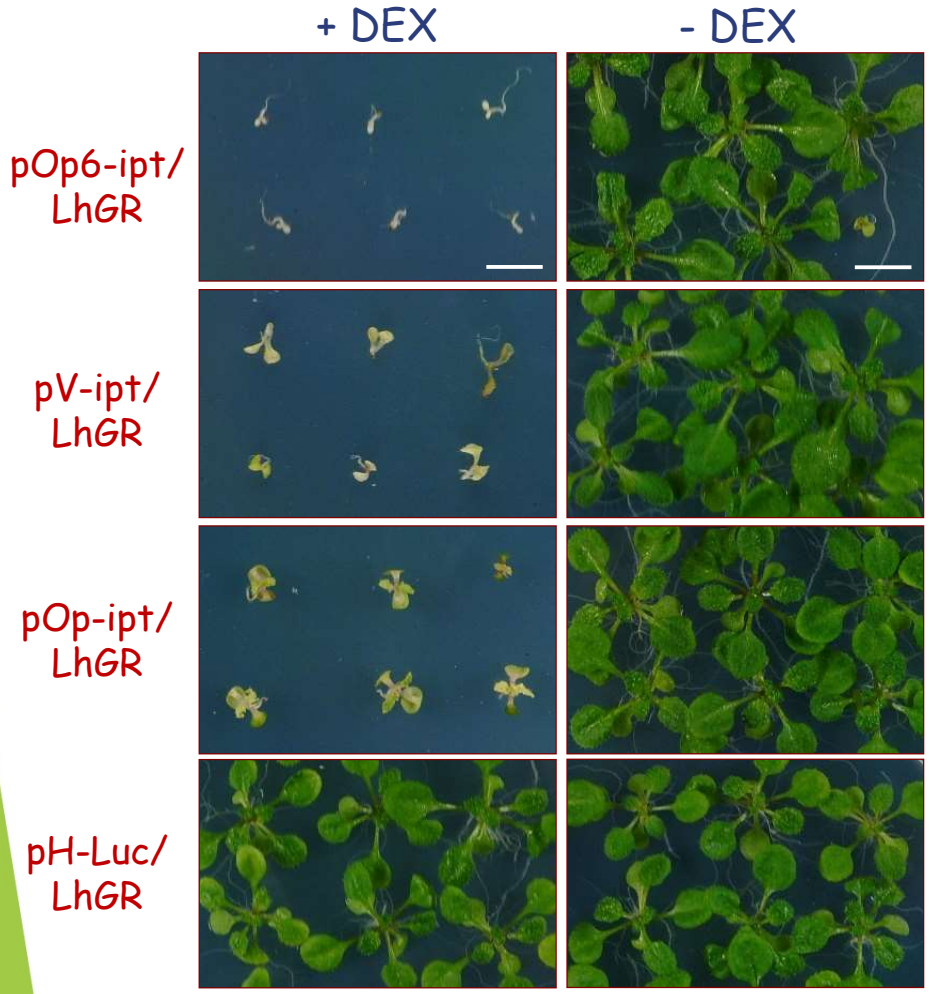




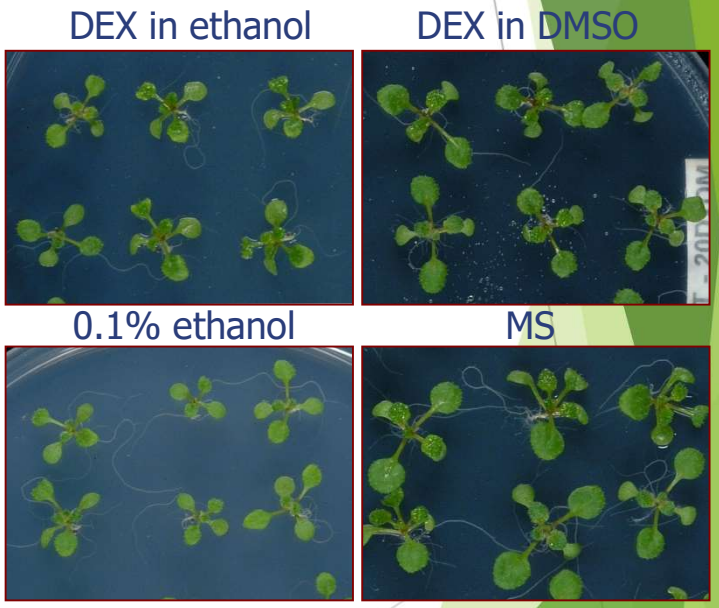
- The most sensitive system for tobacco!

The pOp6/LhGR system is tightly regulated & not toxic!

- Basal expression levels tested with *ipt* gene
  - from *Agrobacterium* (cytokinin biosynthesis)
  - physiologically strong transgene

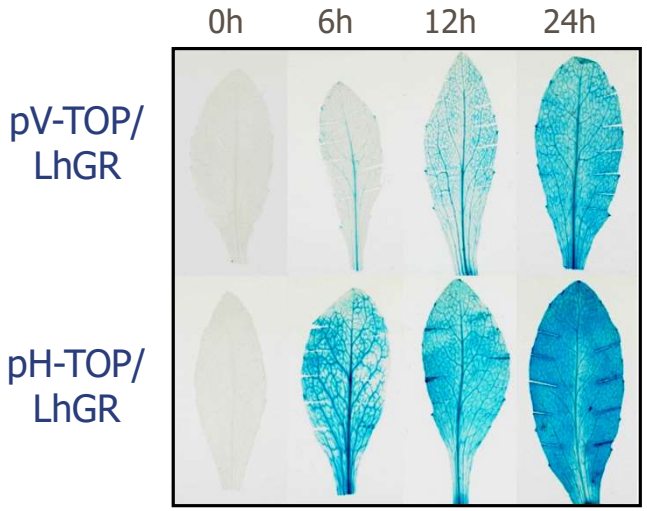


- **neither DEX nor LhGR affects endogenous processes in plants ... though ethanol does!**

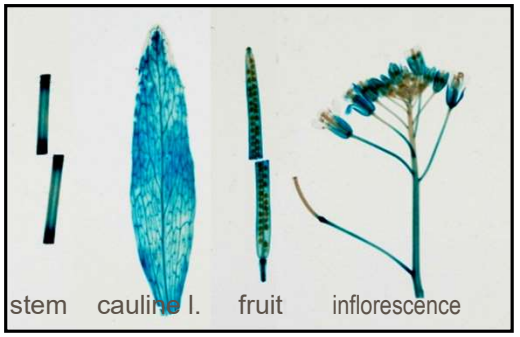


Arabidopsis seedlings were grown on plates in the presence or absence of 10 μM DEX.

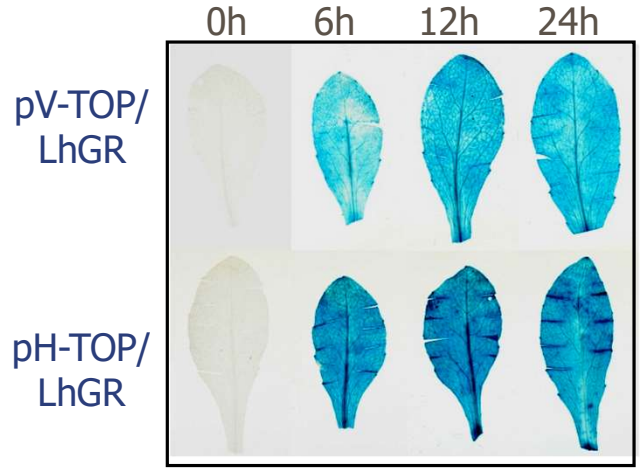
The pOp6/LhGR system is inducible by various methods



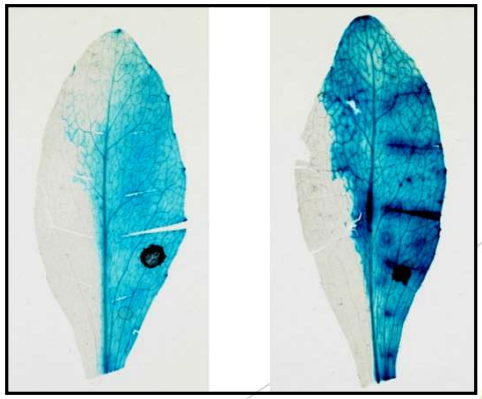
Watering plants with DEX



DEX distribution through tissues (24h after watering).

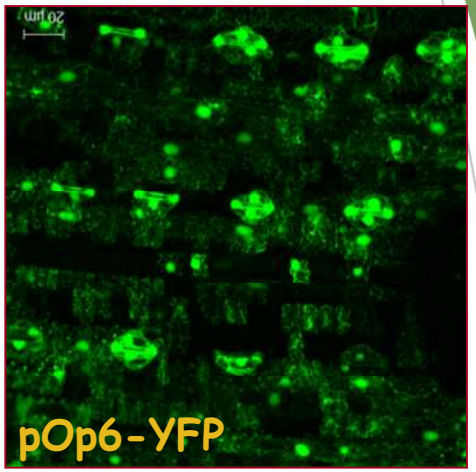
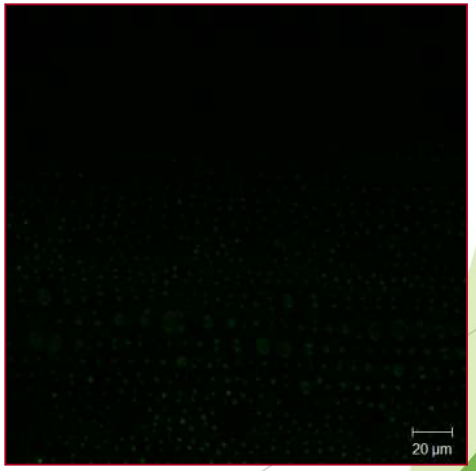


Painting plants with DEX



A leaf half painted with DEX

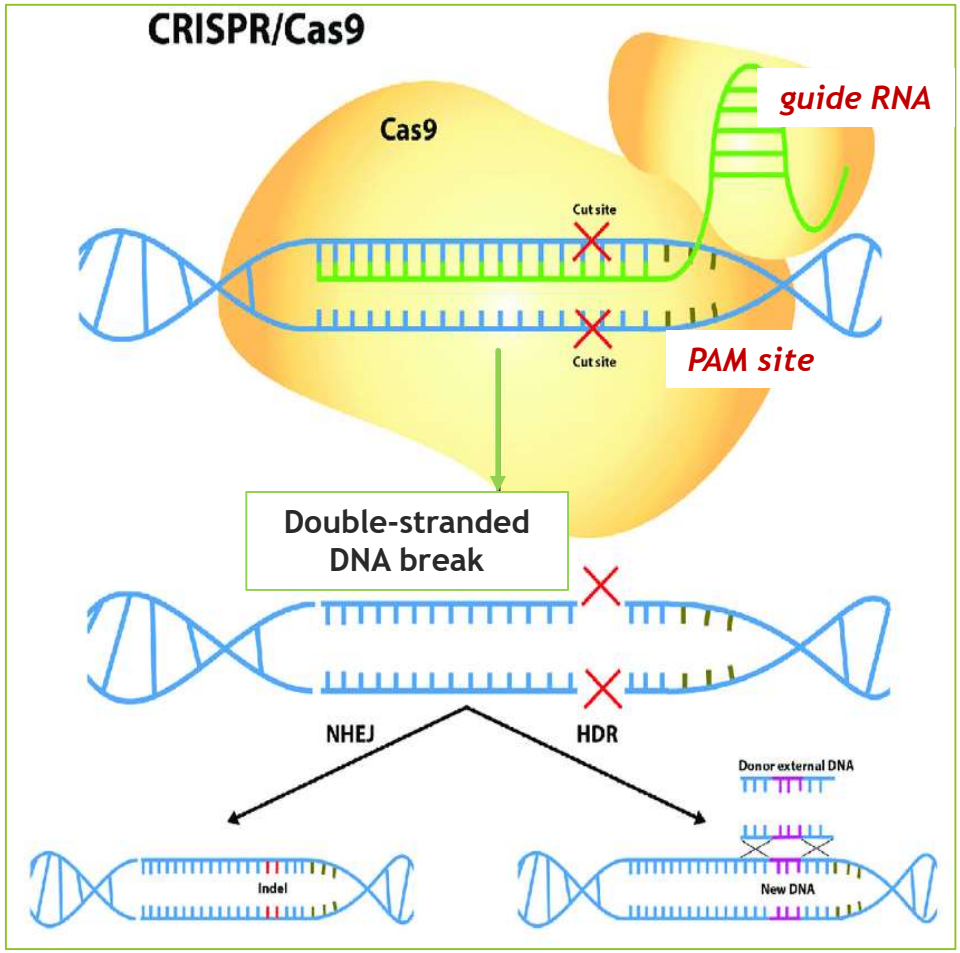
The pOp6/LhGR system is functional in several species

	Arabidopsis (Craft, Samalova *et al.*, 2005)	***Tobacco*** (Samalova *et al.*, 2005)	***Rice*** (Samalova & Moore, 2021)
+ DEX			
- DEX			

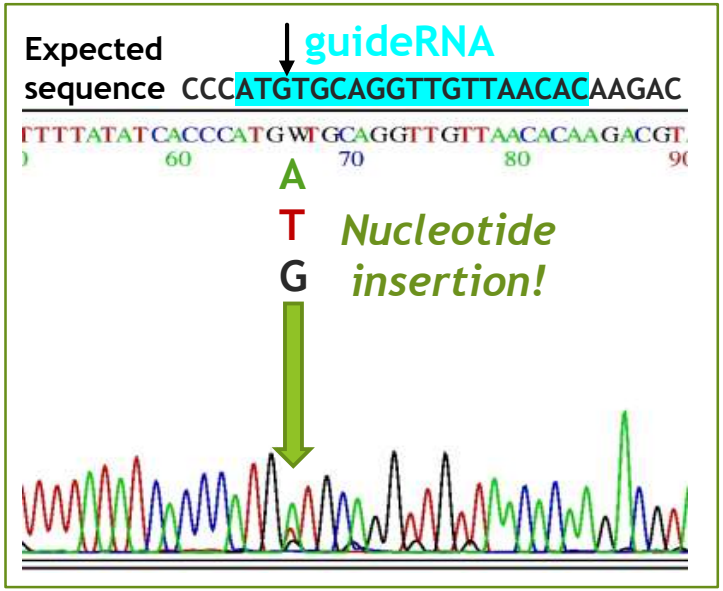
- Maize, potato, tomato, *Cardamine hirsuta*, citrus…
- Detailed step-by-step protocols in Samalova *et al.*, 2019

CRISPR/Cas9 bacterial system adapted to edit the genome of various species ~ *"genetic scissors"*

- The ability of Cas9 (nuclease) **to target a specific site** of genomic DNA using gRNA
  - **2020 Nobel Prize in chemistry** awarded to E. Charpentier a J. Doudna



- **Genome-edited organism**
  - **Changes in the open reading frame (ORF) generate a stop codon! Creating "knock-out" (KO mutant)**



CRISPR: *Clustered Regularly Interspaced Short Palindromic Repeats*

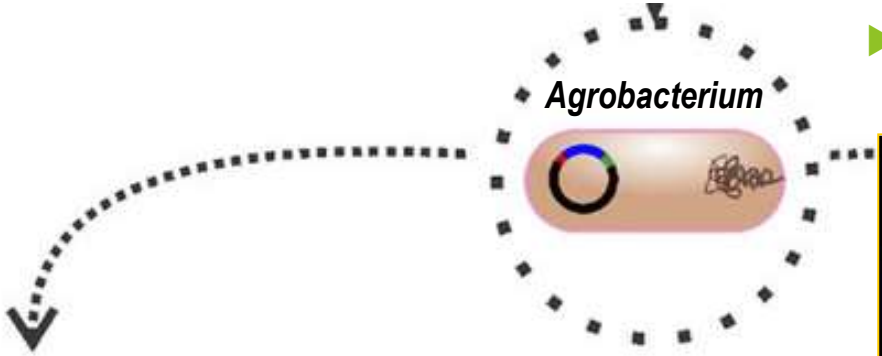
PAM: *Protospacer Adjacent Motifs*

https://www.youtube.com/watch?v=4YKFw2KZA5o&ab_channel=naturevideo

Transient gene expression and fluorescent proteins

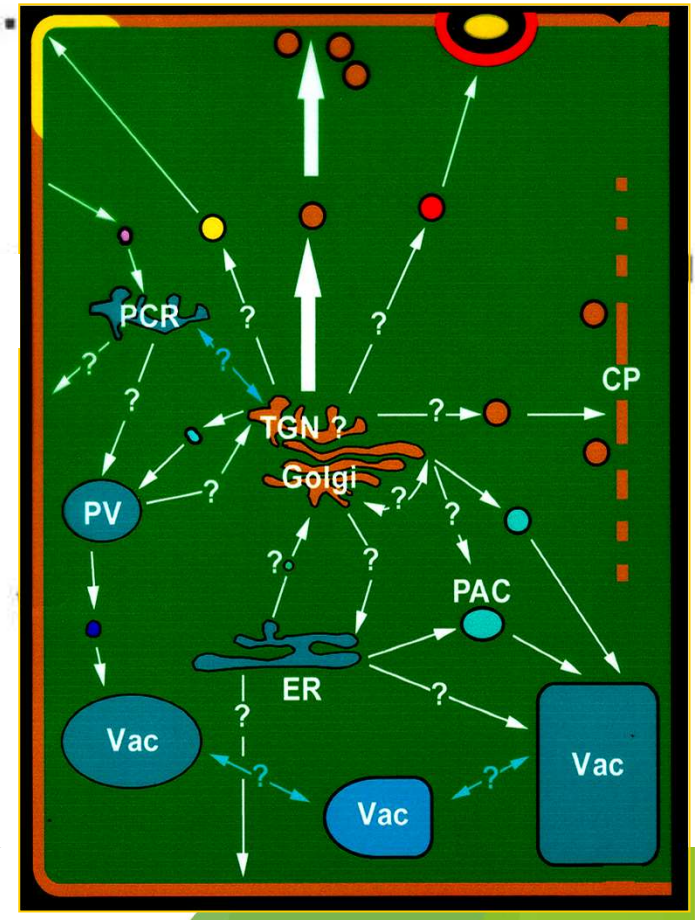
Transient gene expression assay

- ***AGROINFILTRATION method***
- *Agrobacterium* infiltrated into tobacco plants



3. TRANSIENT GENE EXPRESSION ASSAY

- e.g. to study plant endomembrane trafficking



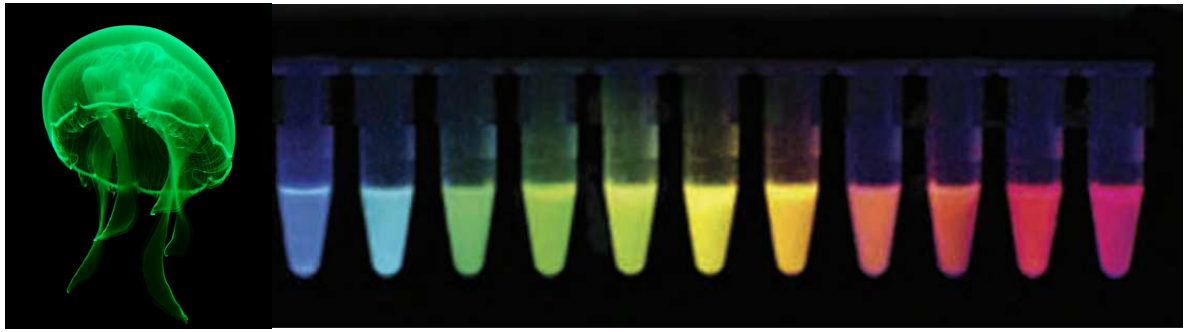
Use of fluorescent proteins (FP) in cell biology

- Protein localization, protein-protein interactions…

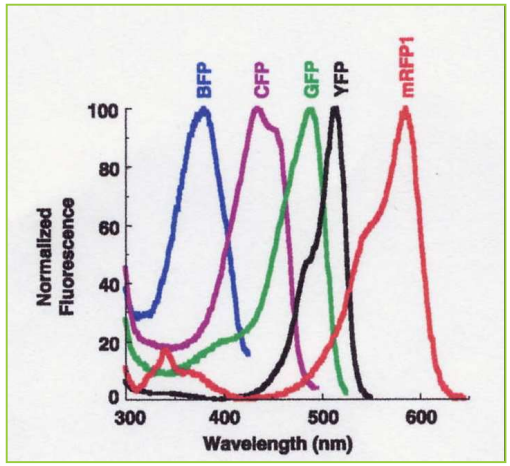
GFP ~ green FP from jellyfish *Aequorea victoria*

YFP – yellow FP mutant variant of GFP

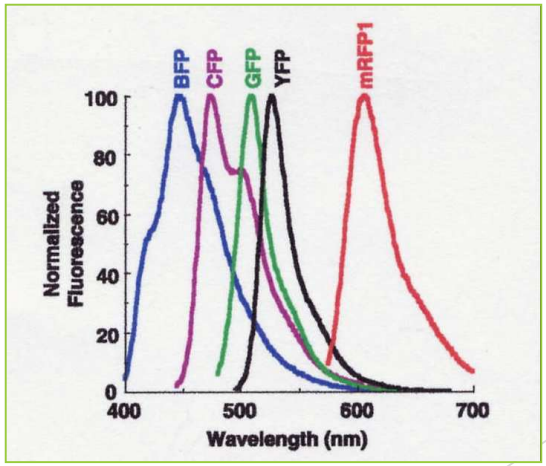
mRFP1 – monomeric red FP from *Discosoma* coral



- **Excitation spectra**

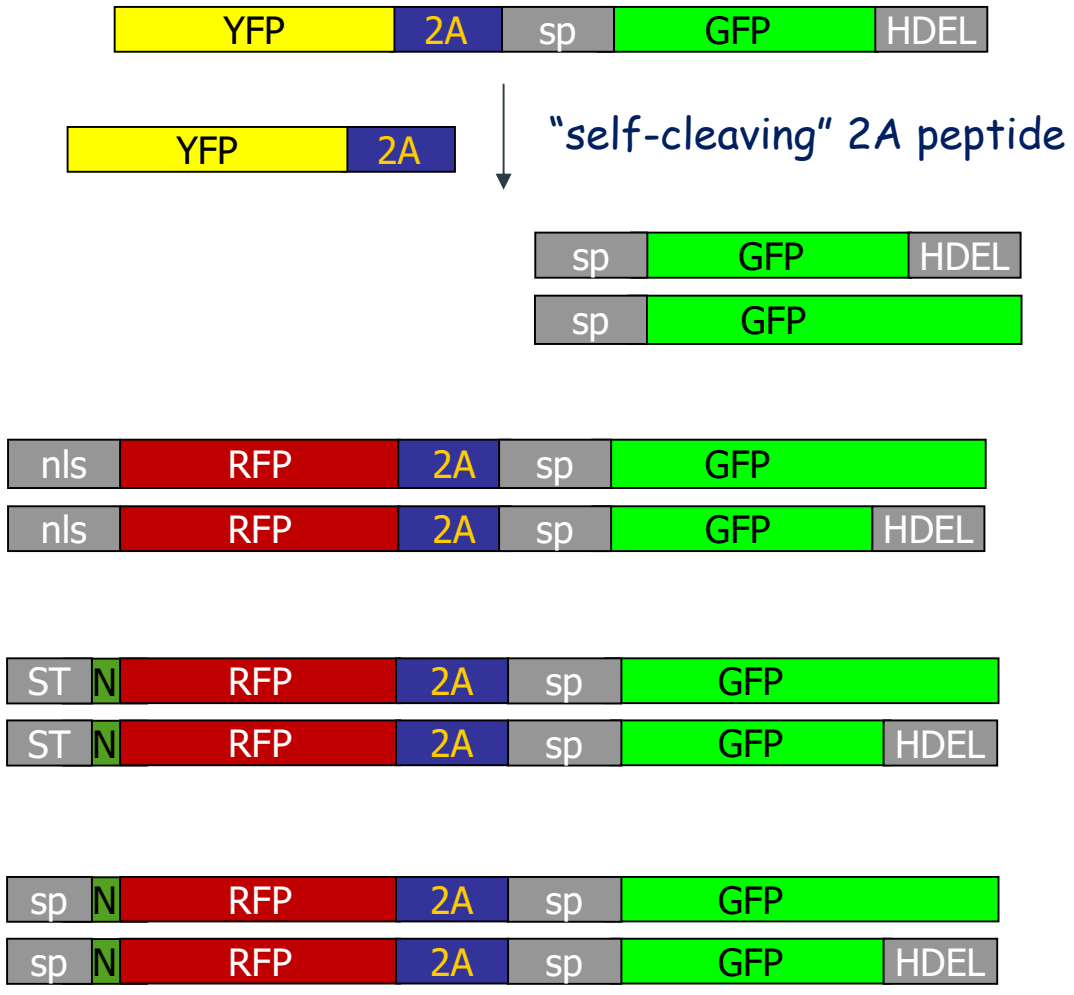


- **Emission spectra**



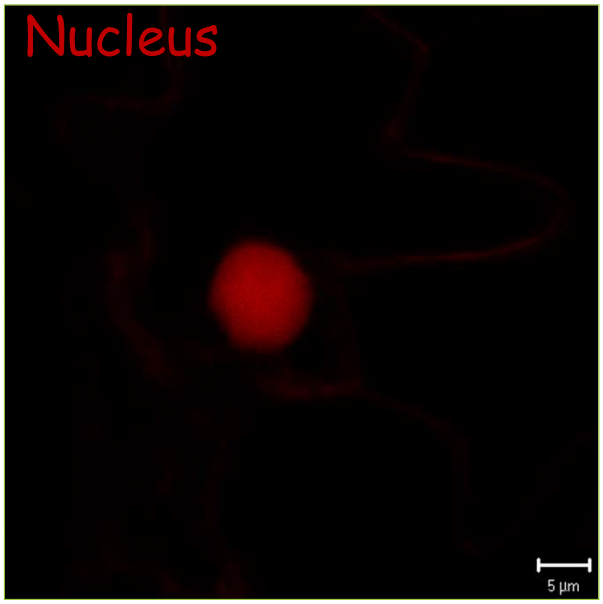
- **CLSM ~ confocal laser scanning microscope**
- Generates optical slices through live specimens.

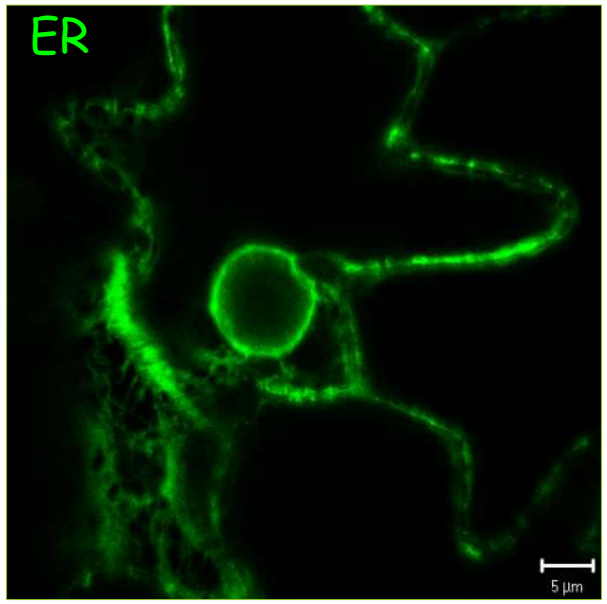
Targeting fluorescent fusion proteins into different cell compartments (Samalova *et al.*, 2006)

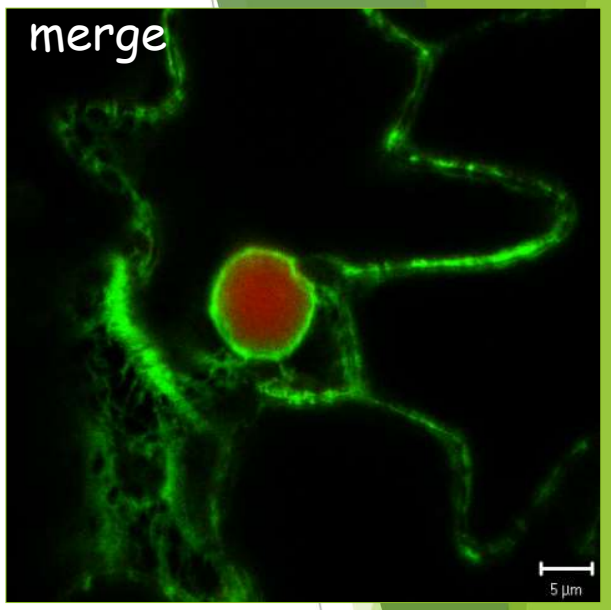


Location		
Cytoplasm	Endoplasmic reticulum (ER)	
	Cell wall	
Nucleus	Cell wall	
	ER	
Golgi app.	Cell wall	
	ER	
Vacuole	Cell wall	
	ER	

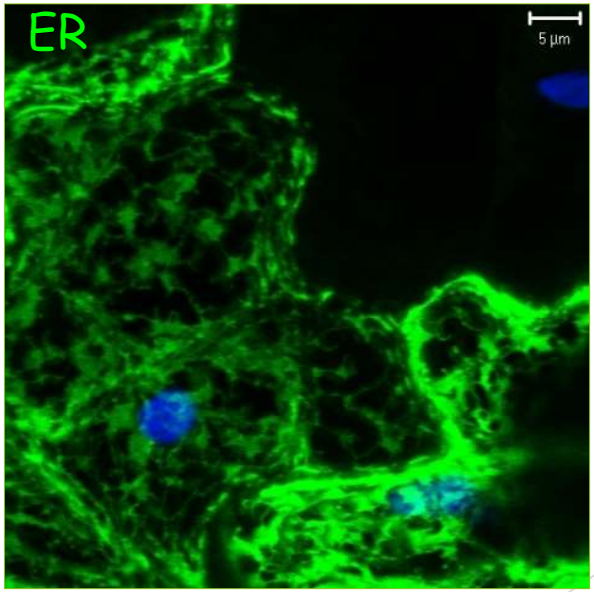

nls
RFP
2A
sp
GFP
HDEL

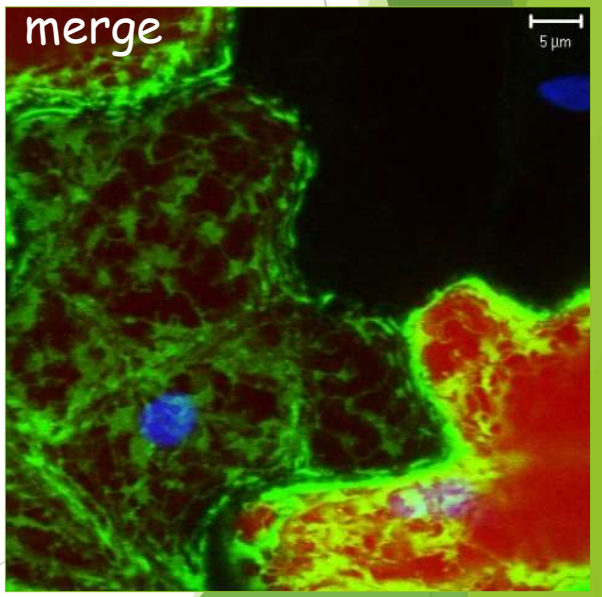

Nucleus
5 µm


ER
5 µm


merge
5 µm

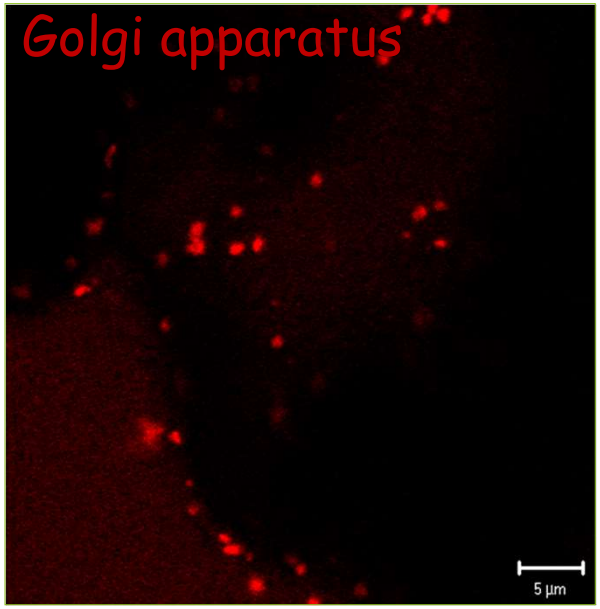

Vacuoles
5 µm

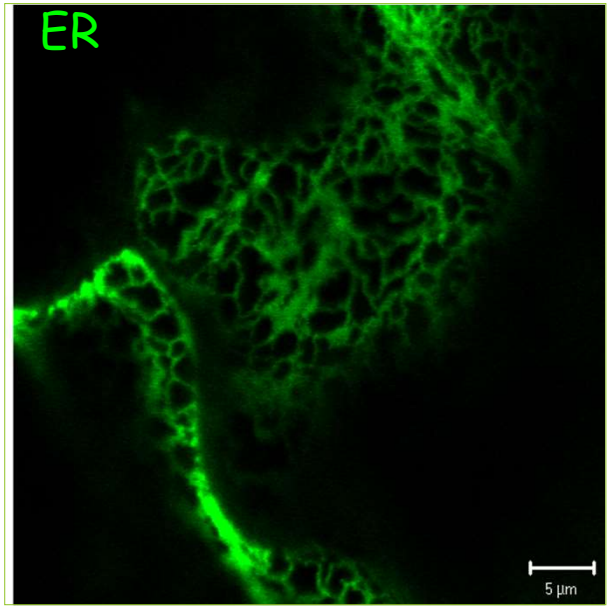

ER
5 µm

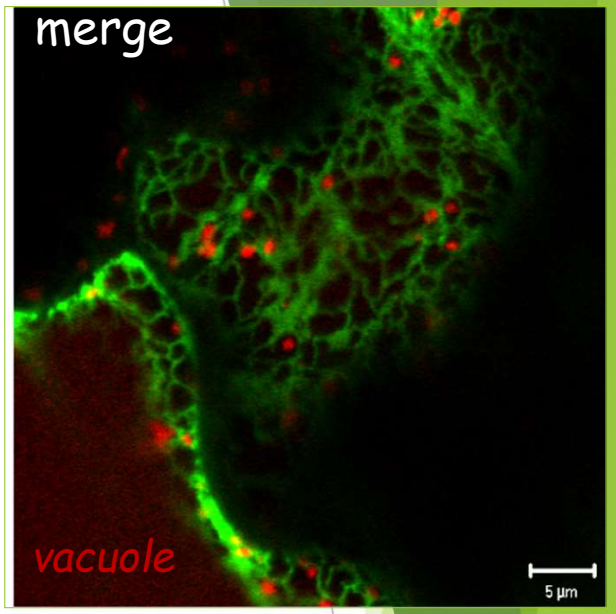

merge
5 µm

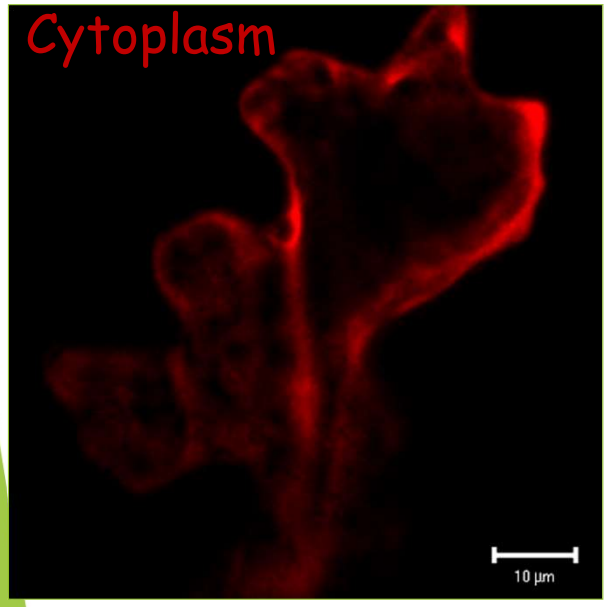

sp
N
RFP
2A
sp
GFP
HDEL

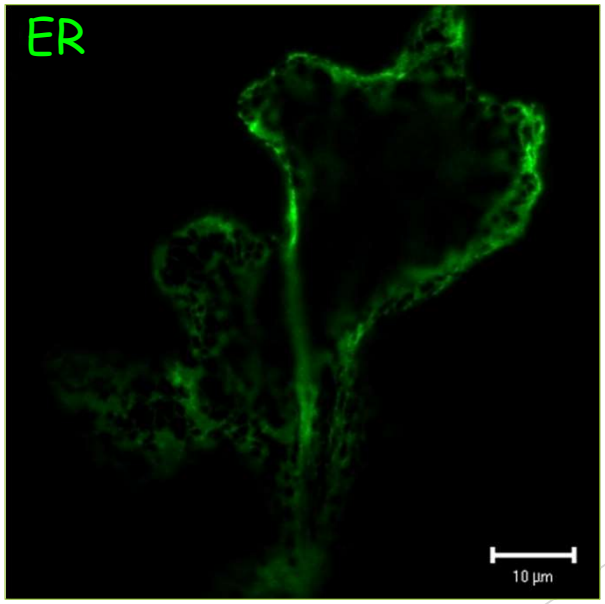

ST
N
RFP
2A
sp
GFP
HDEL

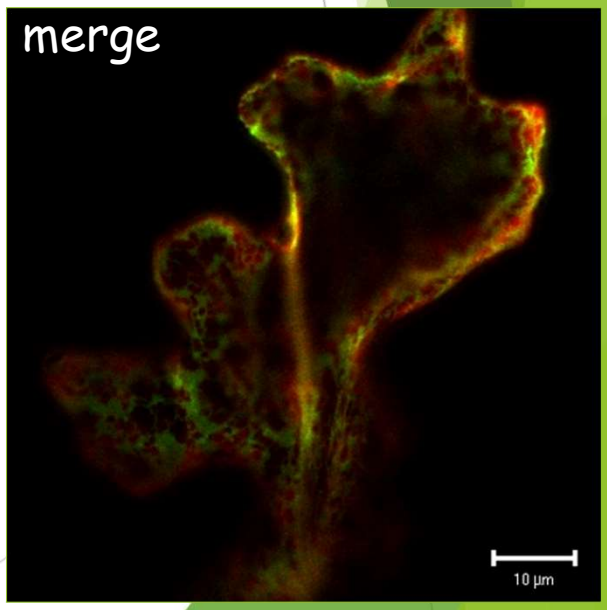

Golgi apparatus
5 µm


ER
5 µm


merge
vacuole
5 µm

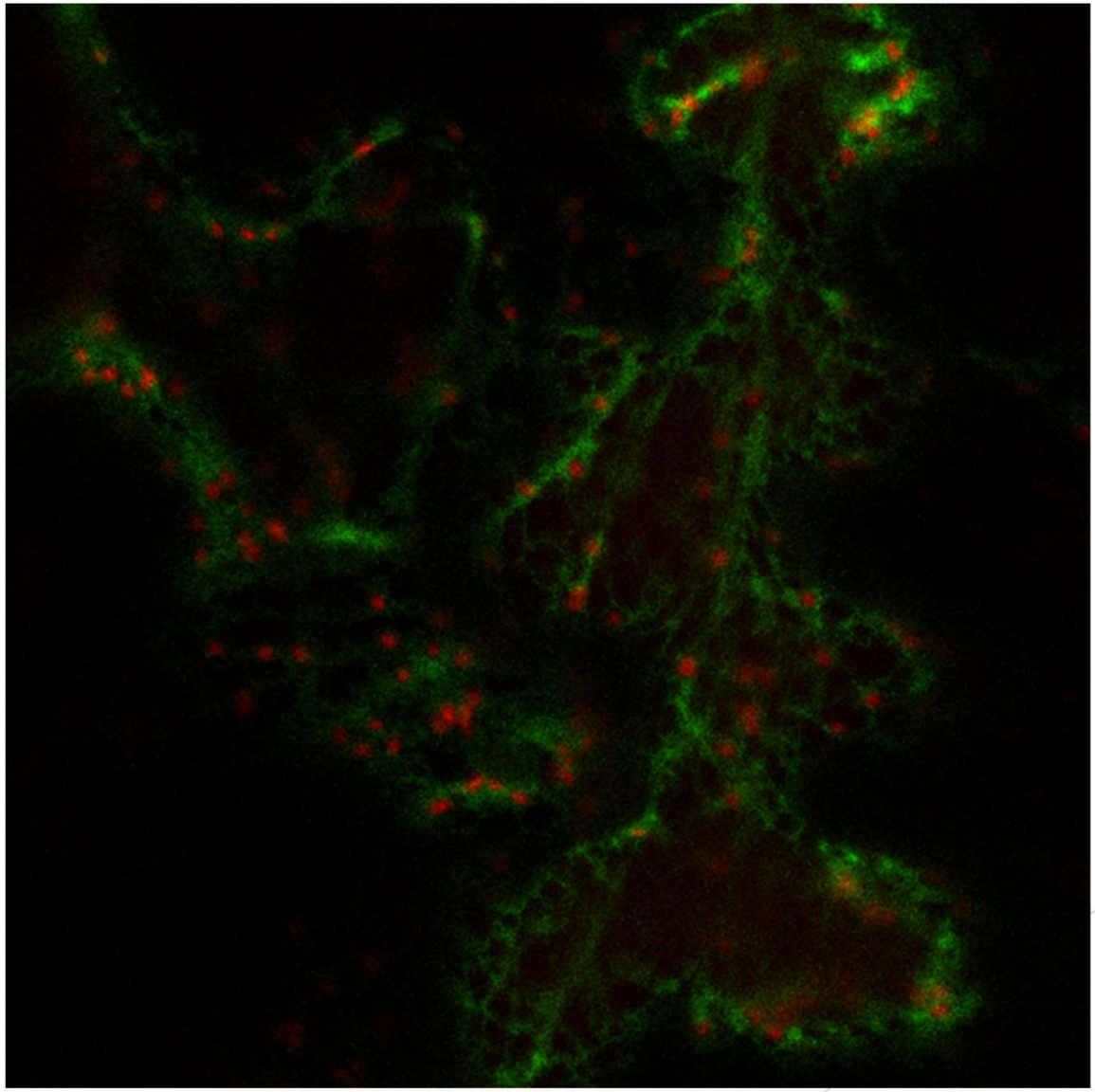

Cytoplasm
10 µm


ER
10 µm


merge
10 µm


YFP
2A
sp
GFP
HDEL

The Golgi apparatus moving along the ER network in living tobacco cells….

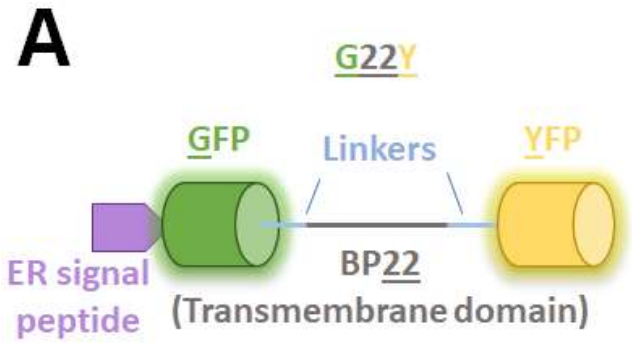


PLANTS ARE MOVING!

Create your own compartment :)

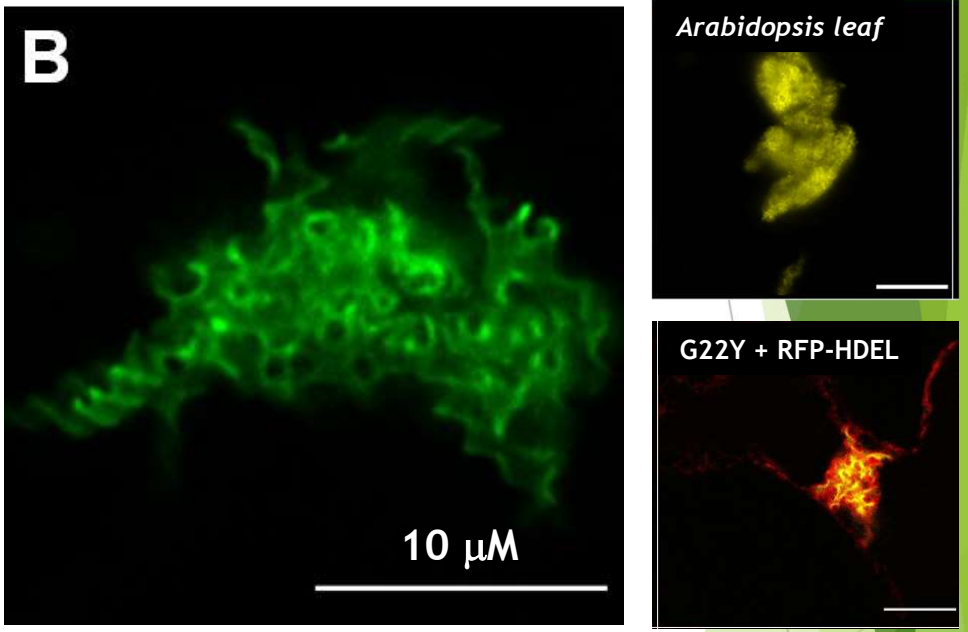
A tool for plant synthetic biology

- substantial expansion of the endomembrane system in each cell of the plant (Sandor, Samalova *et al.*, 2024)



OSER

Samalosome

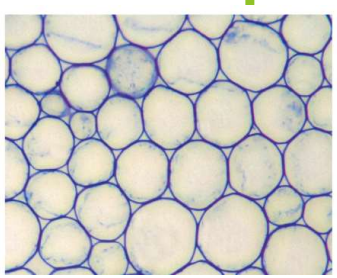


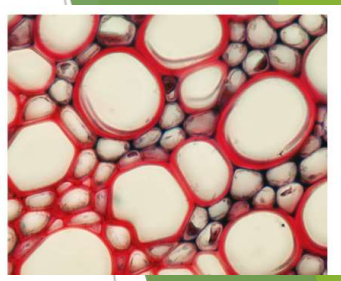
- ***Organised Smooth Endoplasmic Reticulum***
- Potential applications of the synthetic compartment for the metabolic engineering of plants, e.g. recombinant or toxic proteins.
- No detrimental effects in plants!

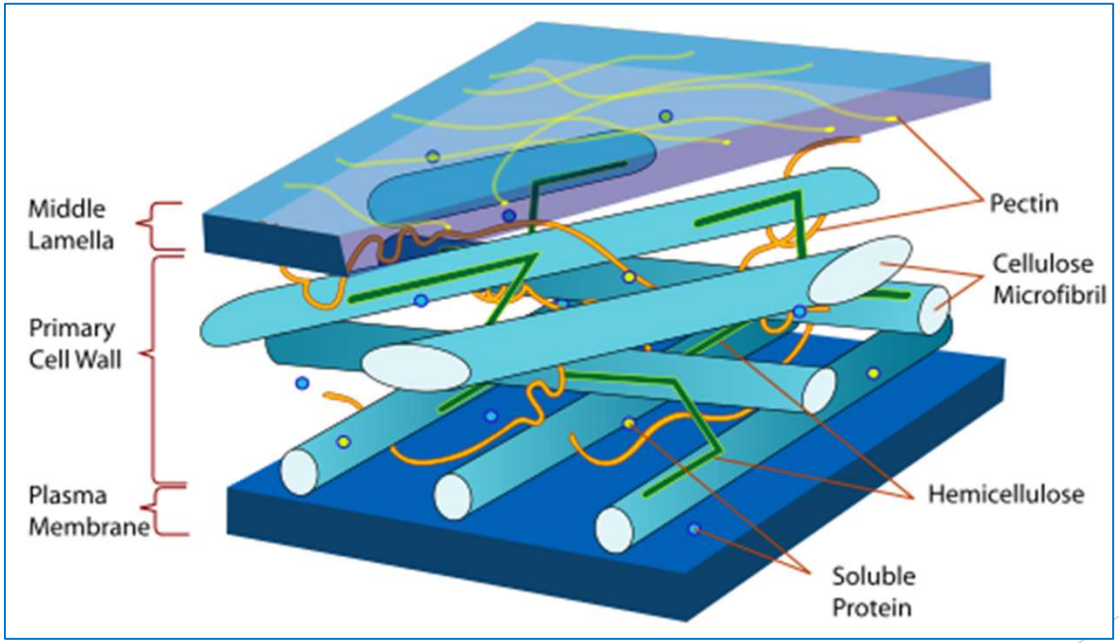
Plant cell wall (CW)

CW is crucial for plant growth & development

- shapes the plant body
- movement of solutes and nutrients
- protects plants from the environment
- intercellular communication (Wolf *et al.*, 2012)
  - **Cellulose is the most abundant biopolymer on Earth!**





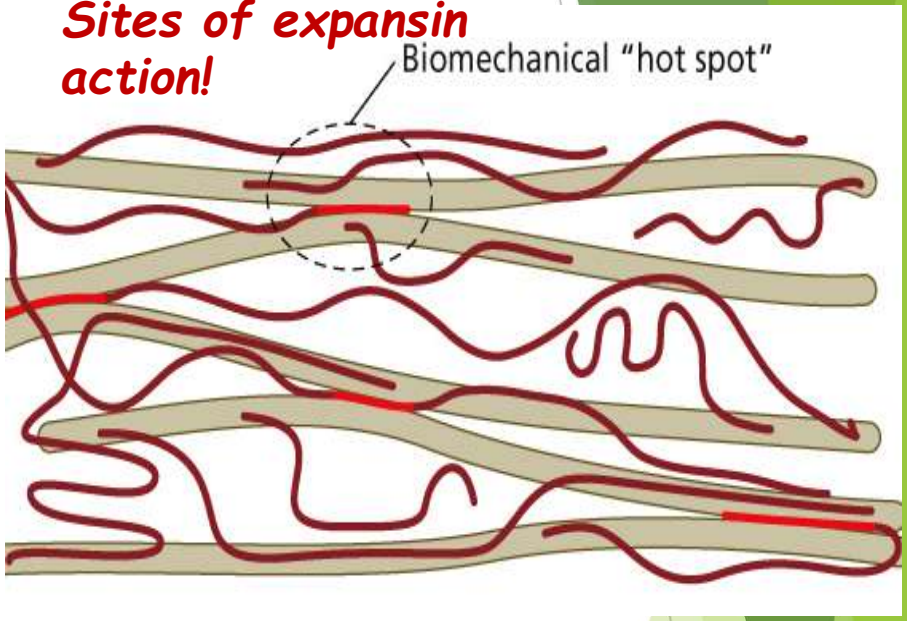


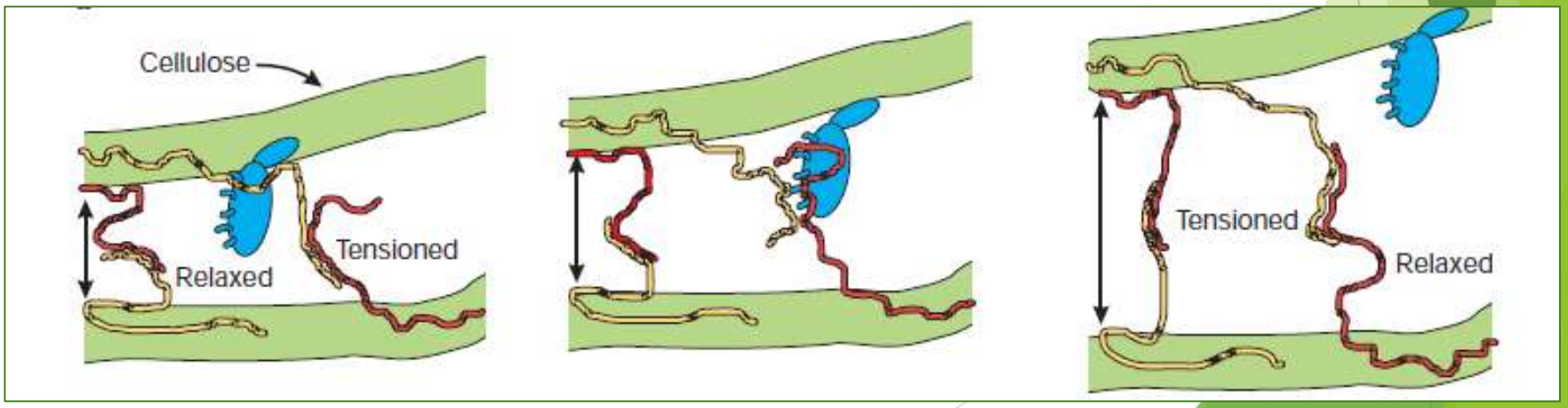
- *Load-bearing cellulose microfibrils*
- *embedded into viscoelastic matrix of hemicellulose and pectins.*

How do plant cells grow?

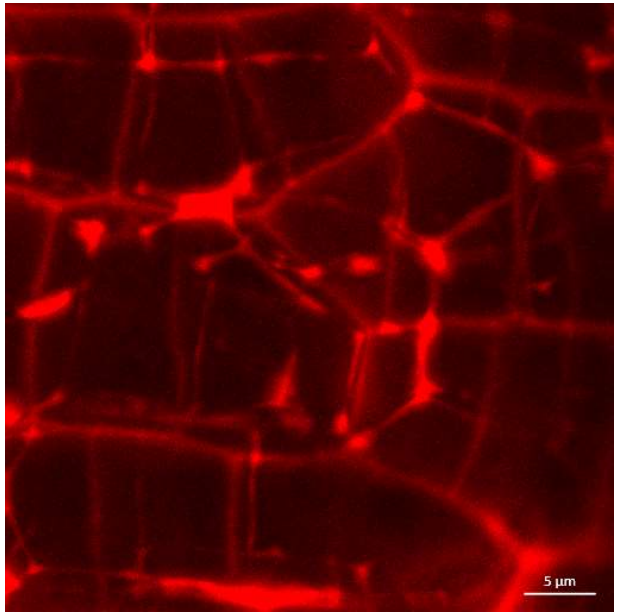
- **Plant CW combine strength with extensibility …**
- **Wall extensibility may be controlled at limited regions, 'biomechanical hotspots'** (Cosgrove, 2014; 2018).
- **EXPANSINs are small proteins** that disrupt the non-covalent bonds between CW polysaccharides, thus relaxing wall stresses and allowing turgor-driven **cell expansion** (Cosgrove, 2000).

Sites of expansin action!

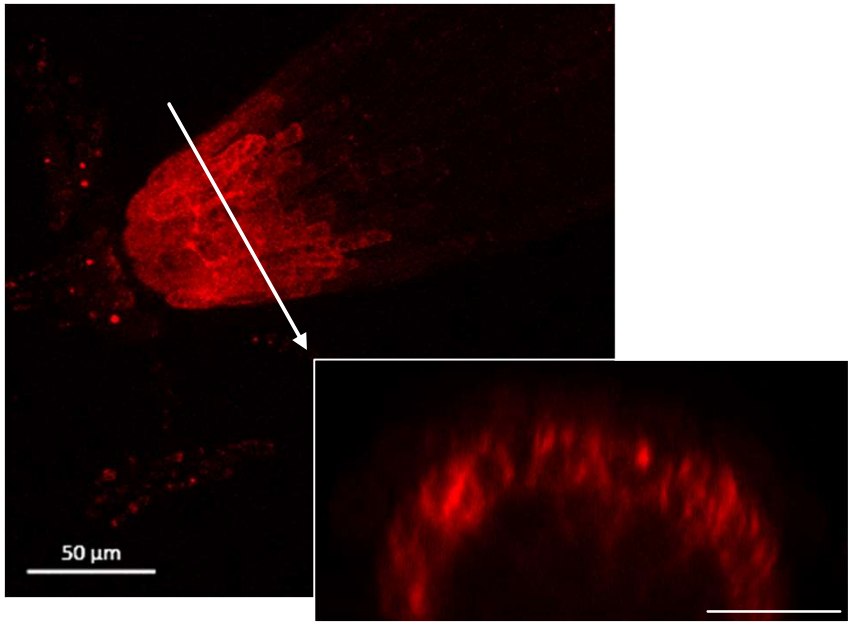


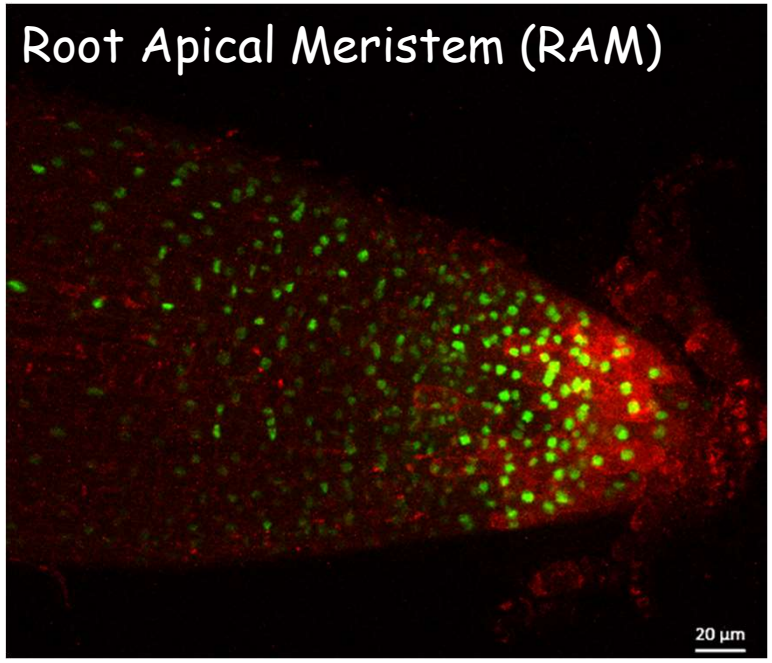


EXPANSINS are localized in the cell wall

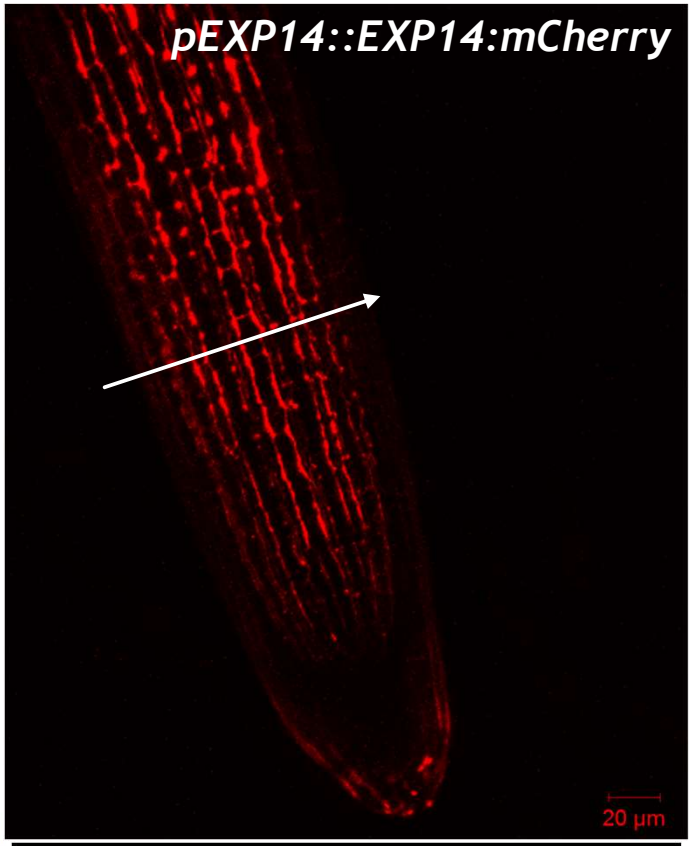


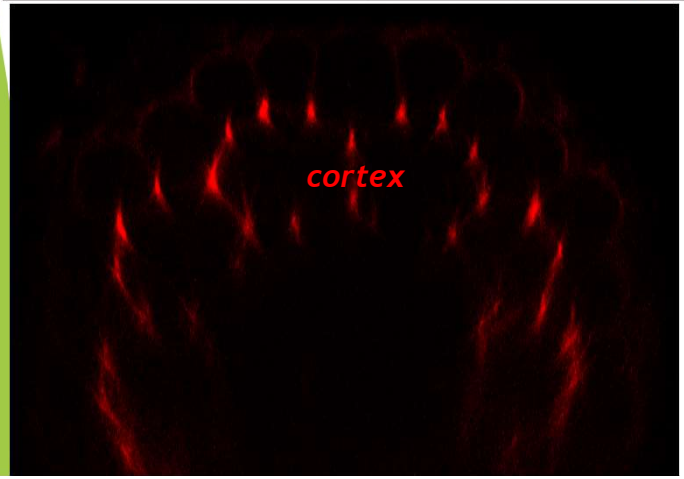
- EXPANSINs localised to the CW *in vivo* for the first time! (Samalova *et al.*, 2024)
  - Use of mCherry (RFP) instead of pH sensitive GFP



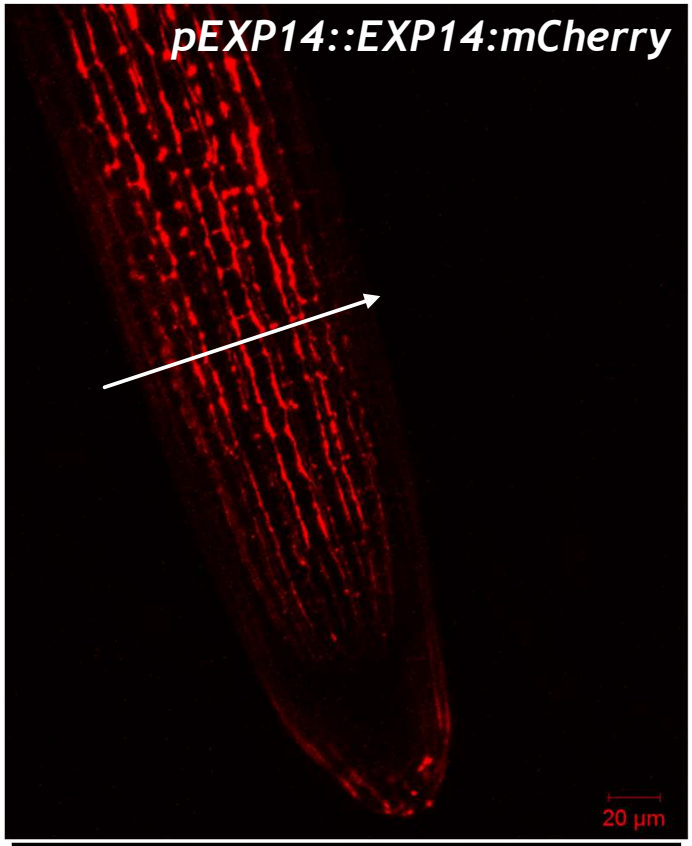


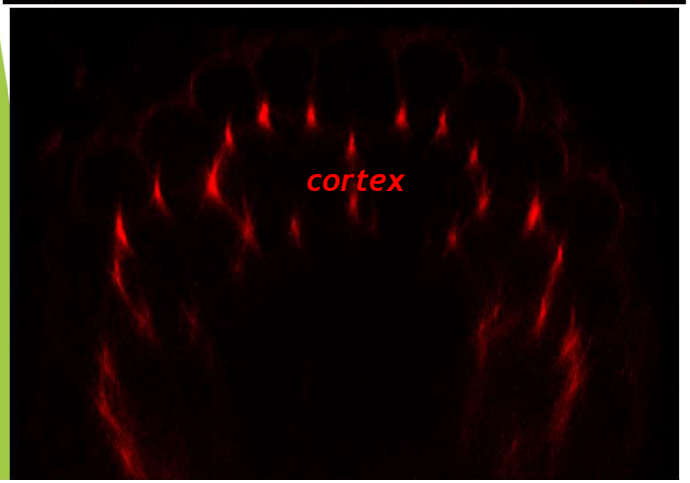
EXPANSINS are localized into various root tissues





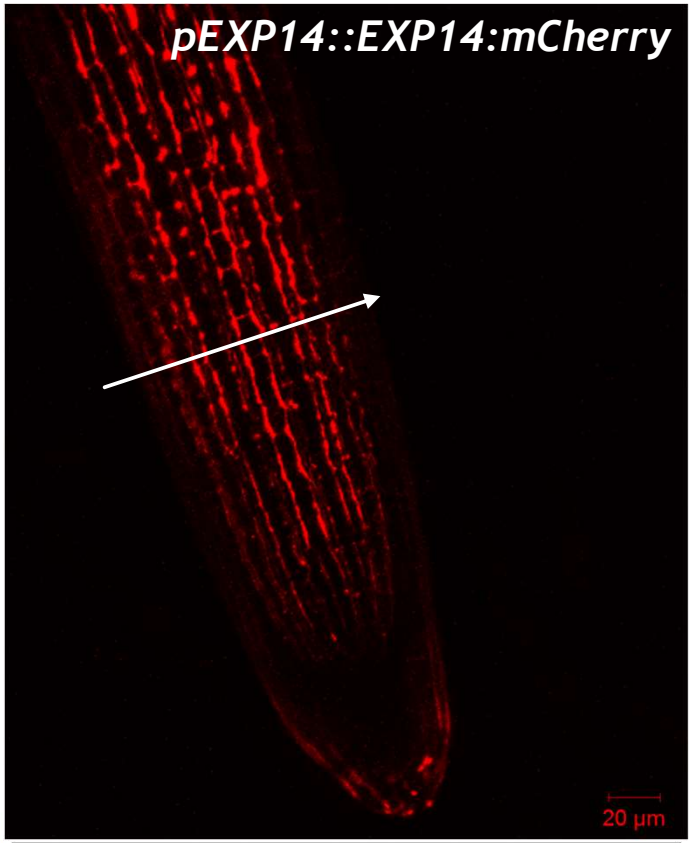
EXPANSINS are localized into various root tissues

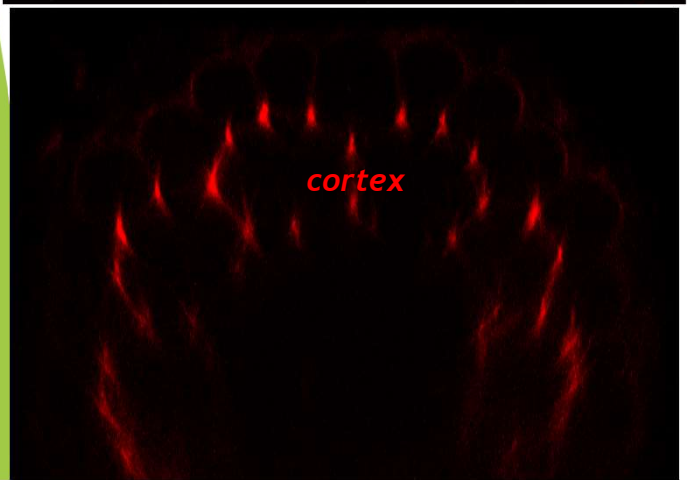


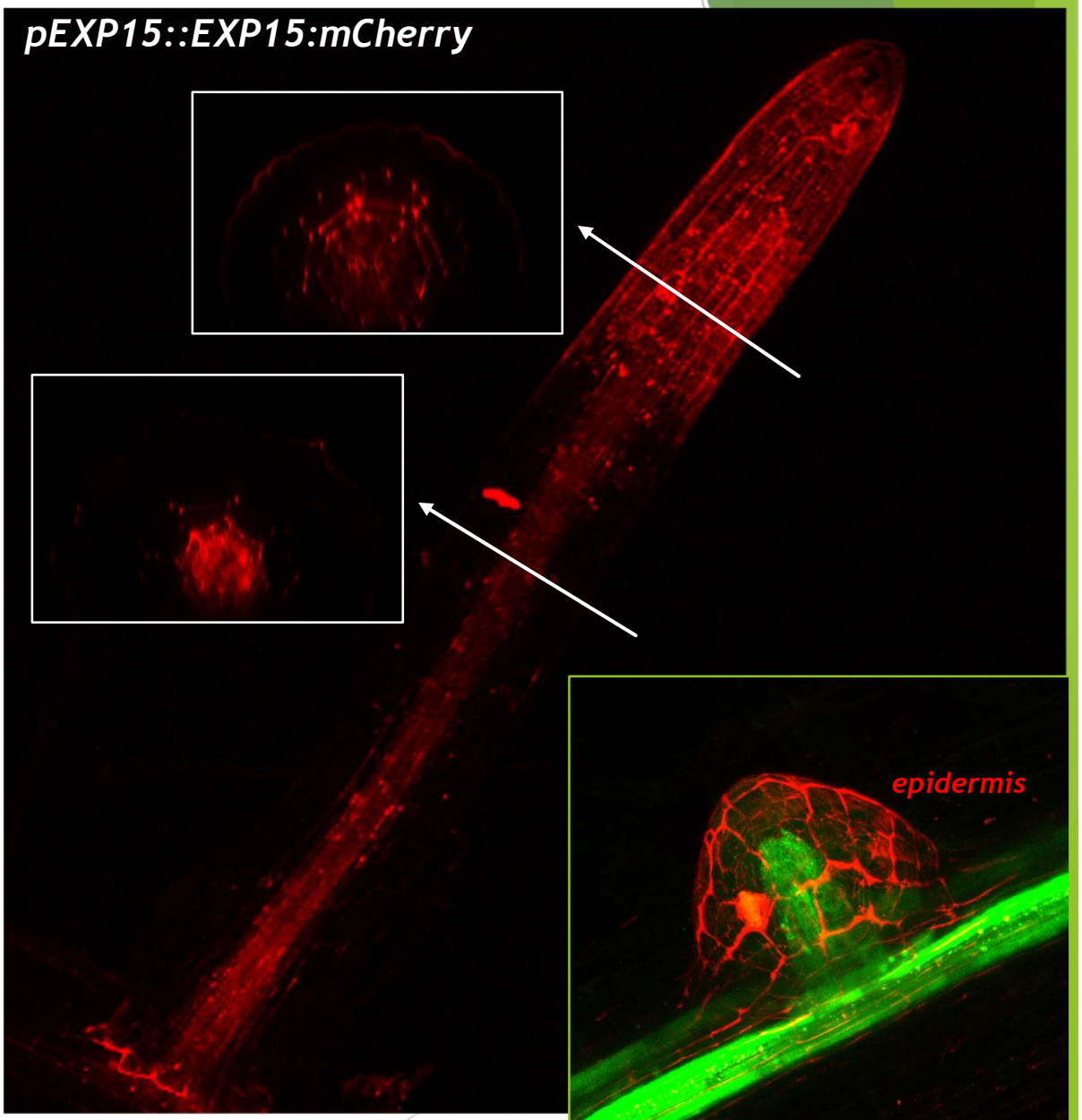


- 3D projection of Z-stack (combined optical slices) taken by a confocal microscope.

EXPANSINS are localized into various root tissues



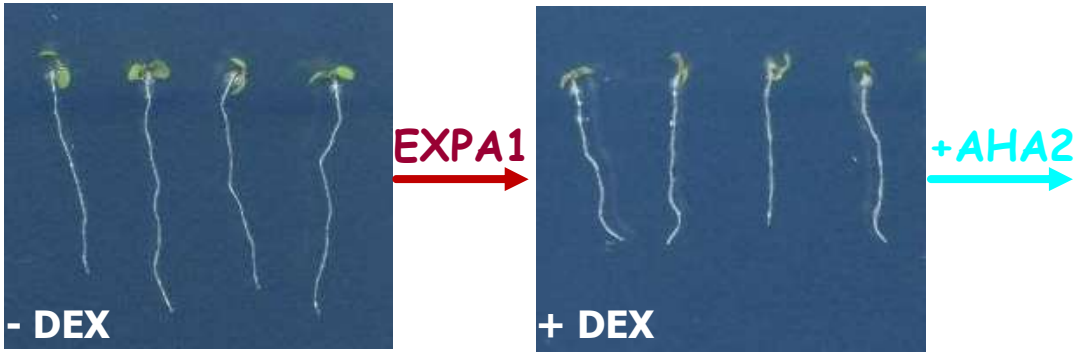


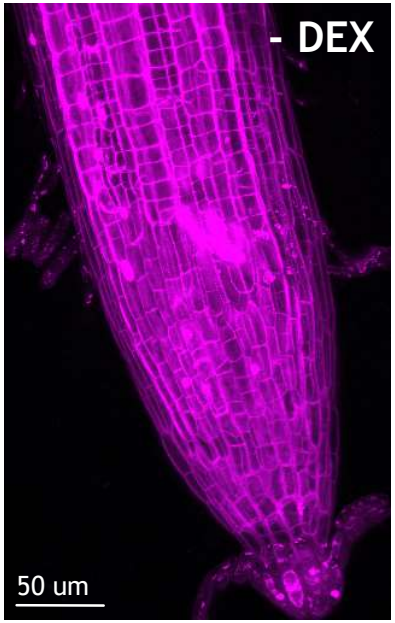


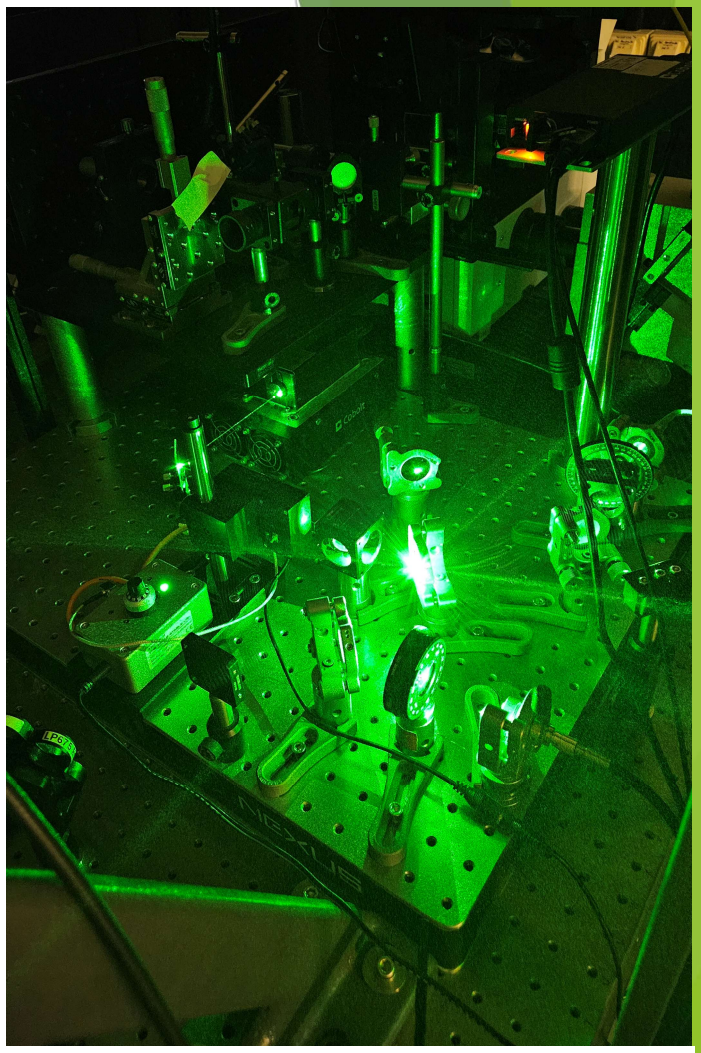
Overexpression of *EXPA1* makes the plants smaller by "stiffening" cell walls

- Changes in biomechanical properties of CWs.

pRPS5A>>EXPA1





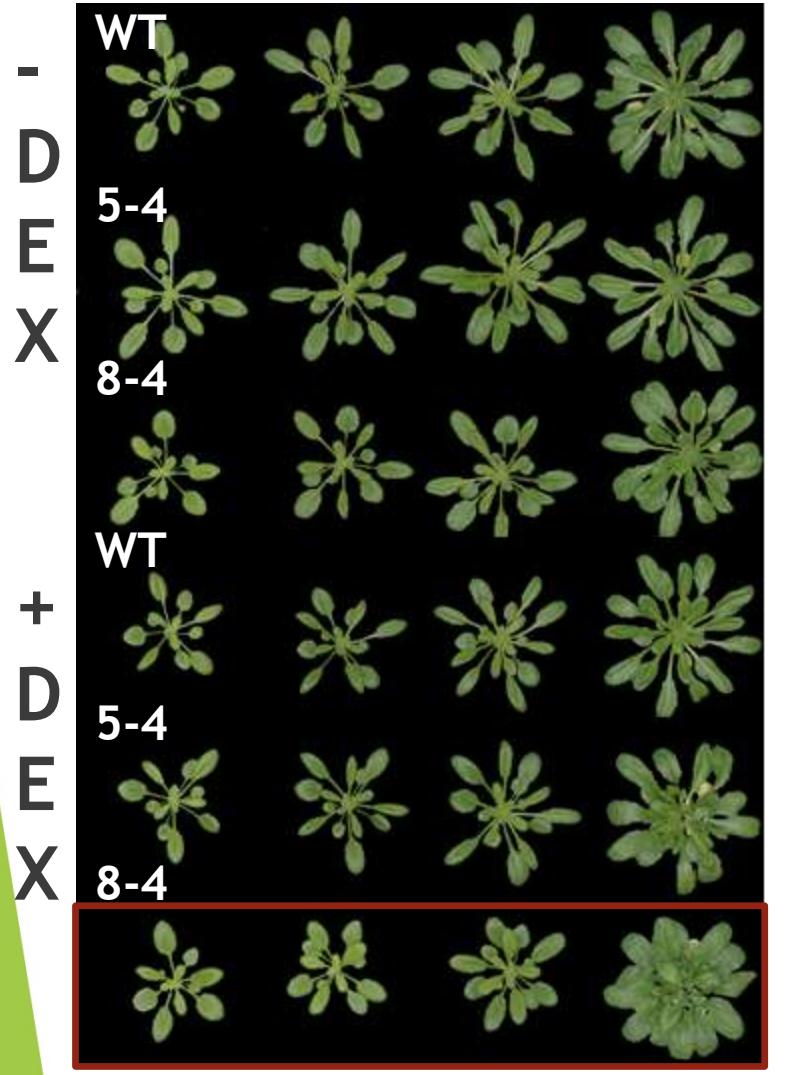


nature photonics

Keshmiri, Cikes, Samalova *et al.* 2024

Brillouin light scattering anisotropy microscopy for imaging the viscoelastic anisotropy in living cells

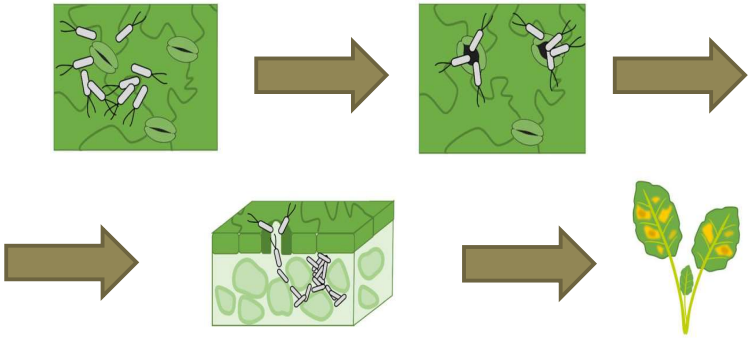
Overexpression of *EXPA1* leads to smaller, compact plants that are more resistant to (a)biotic stresses



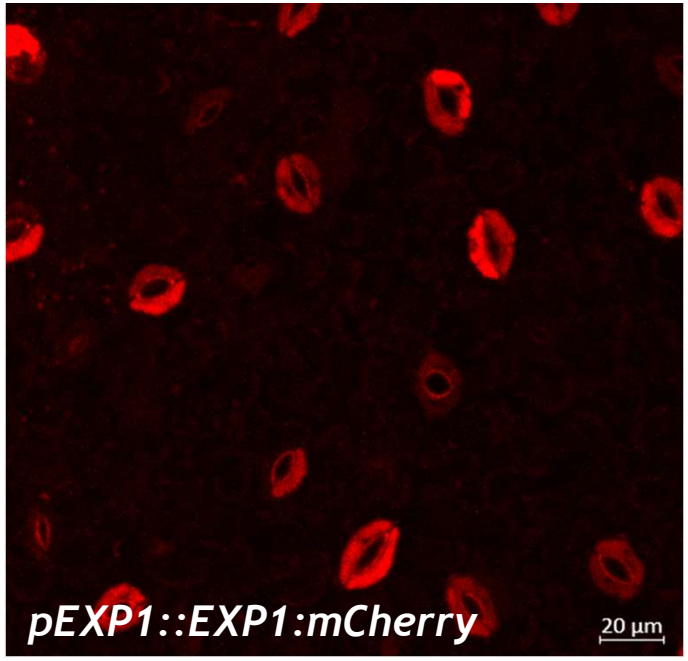
- ***Exploring a role of EXPANSINs under stress:***

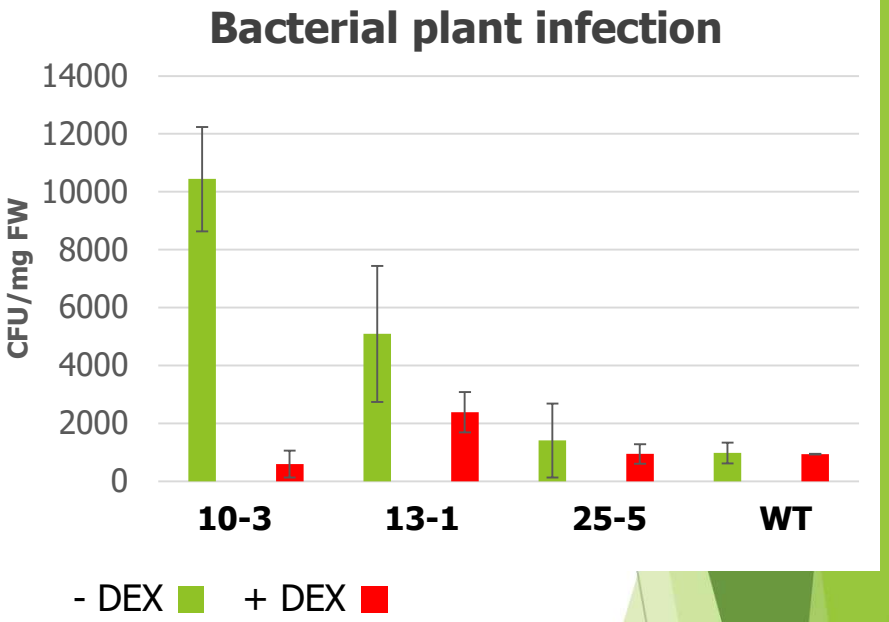
Plants overexpressing *EXPA1* are more resistant to bacteria *Pseudomonas syringae*

- P. s. is an agressive bacterial pathogen.
- Entres plants thought stomata!



***EXPA1* localizes in stomata!**





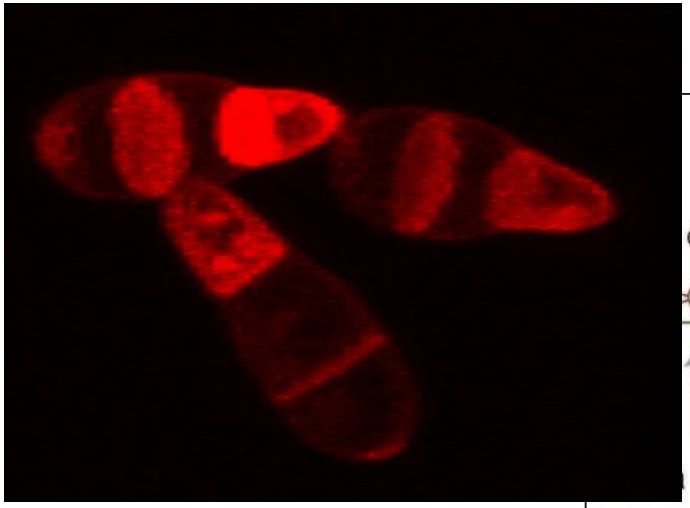
Not only plant cells have the CW…

Magnaporthe oryzae the most devastating pathogen of rice!

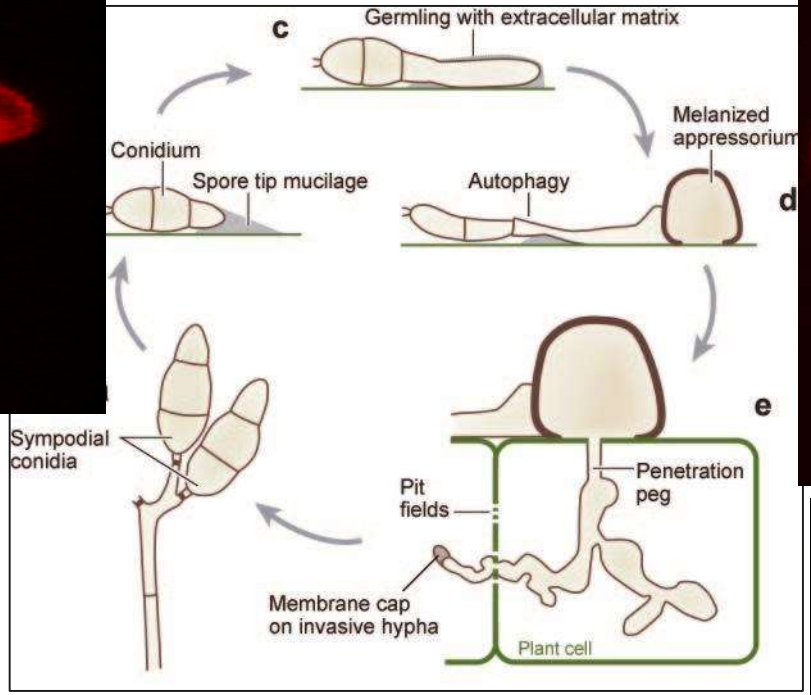
- **Model organism for plant pathogens: 1st sequenced** (Dean *et al.*, 2005)
- Hemibiotrophic filamentous *Ascomycete* fungus causing **rice blast**!
- Haploid, short (asexual) life cycle, gene deletions by homologous recombination.

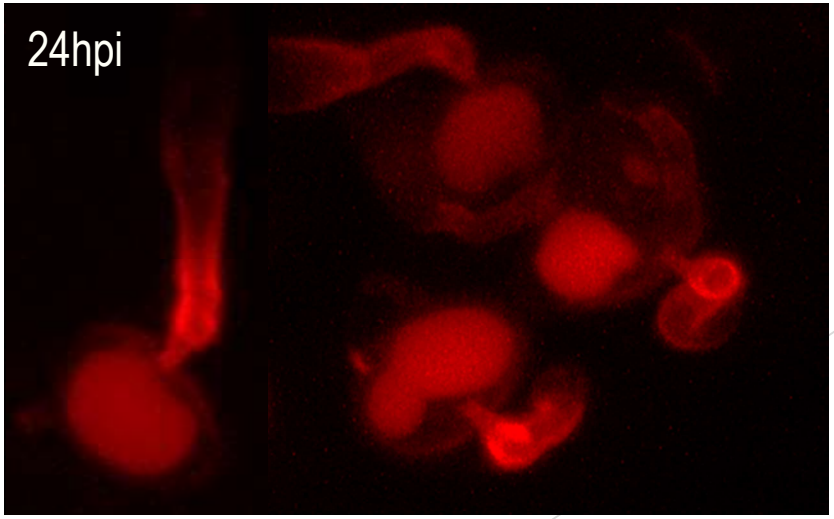
➢ Food security & climate change

Magnaporthe oryzae asexual life-cycle



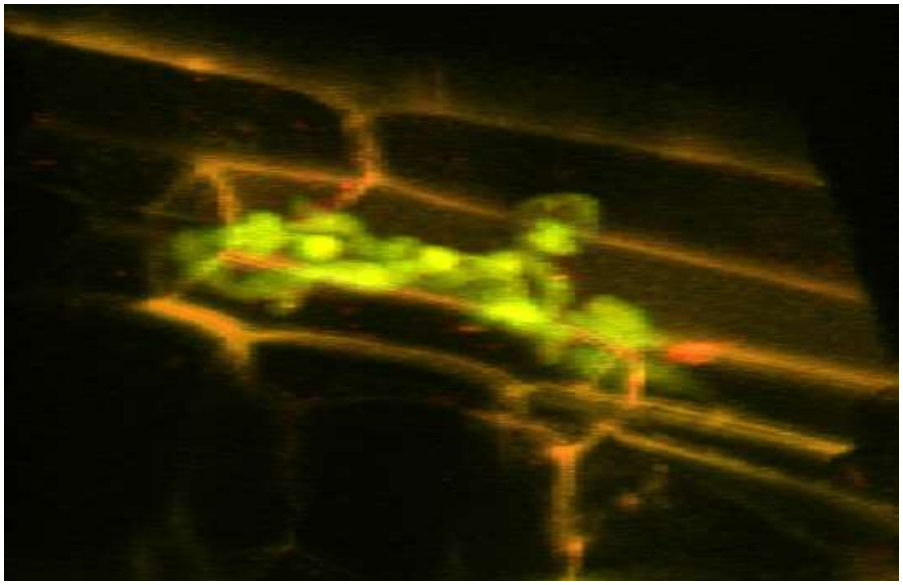
pGEL3::mCherry:GEL3

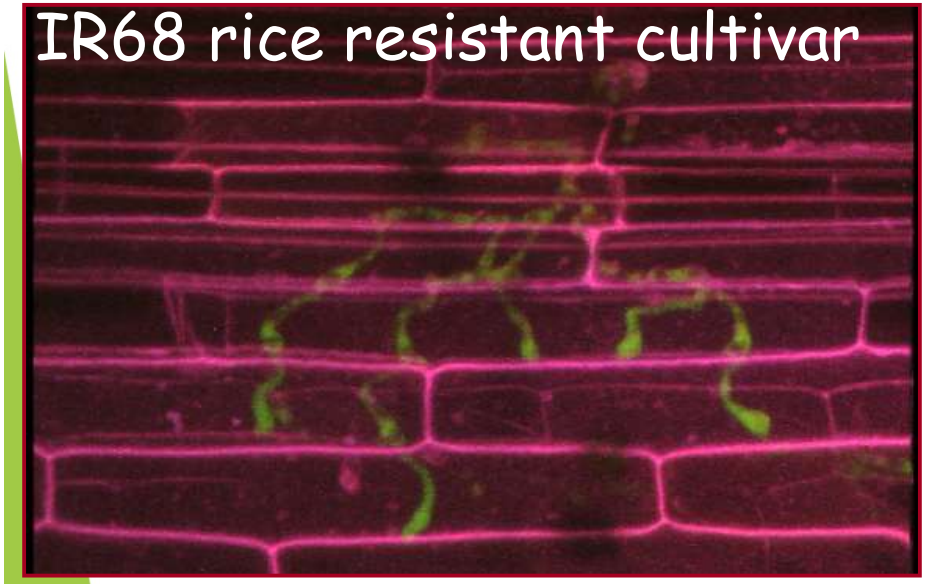


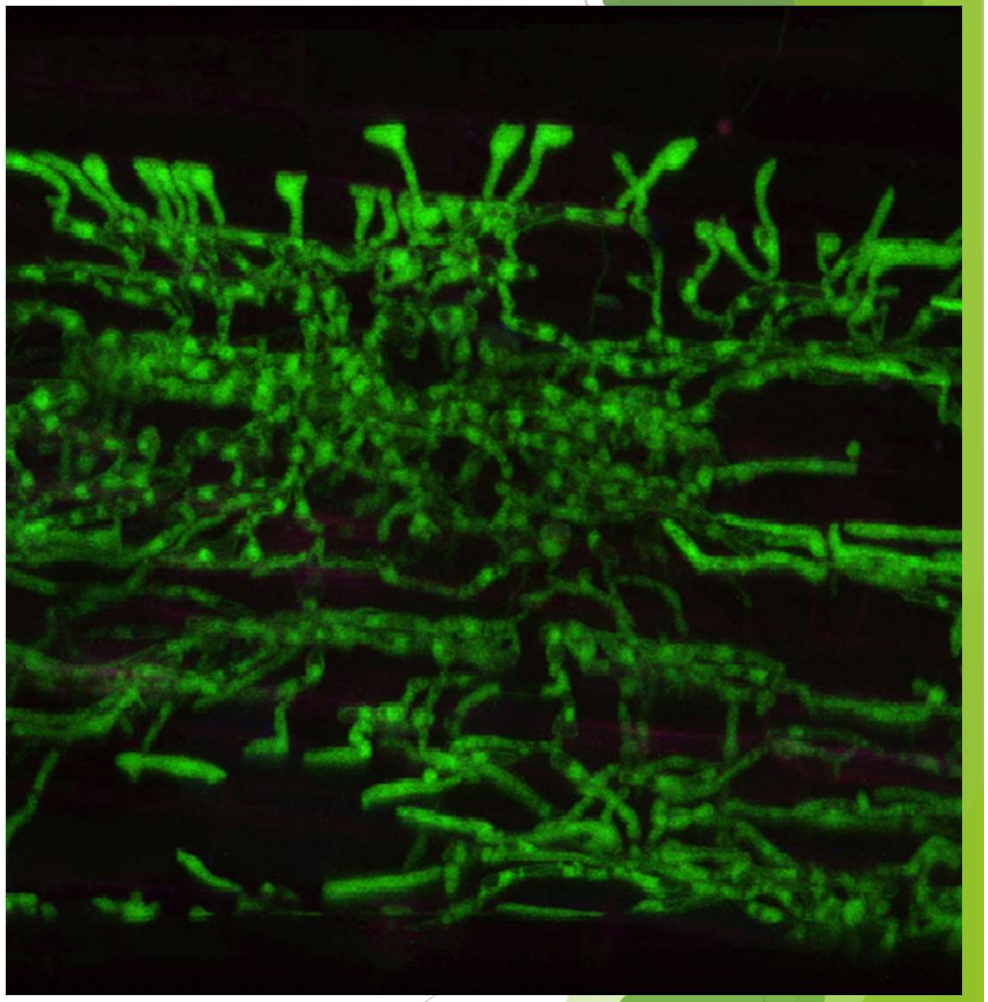


Samalova *et al.*, 2017

ROS toxicity alone is NOT sufficient to kill *Magnaporthe oryzae* in resistant rice!



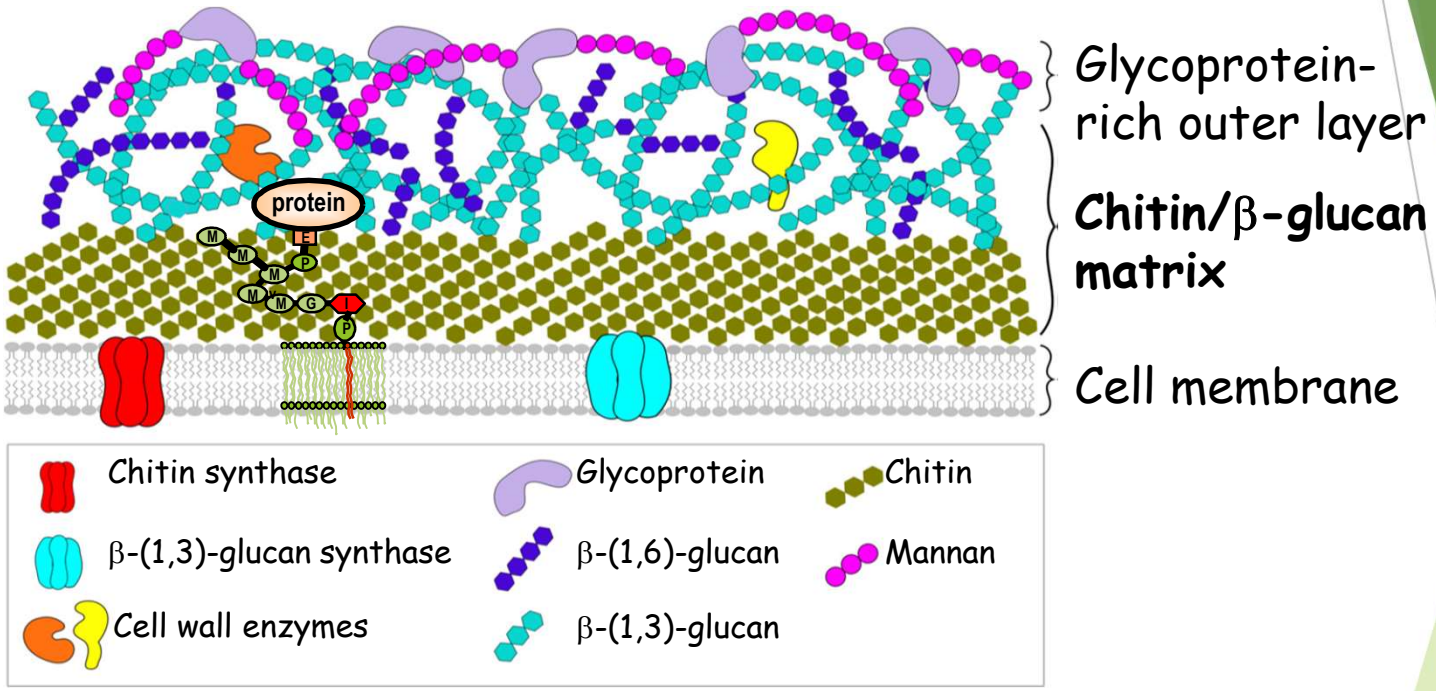




- **Exploring redox state in susceptible & resistant** (Samalova *et al.*, 2013; 2014)

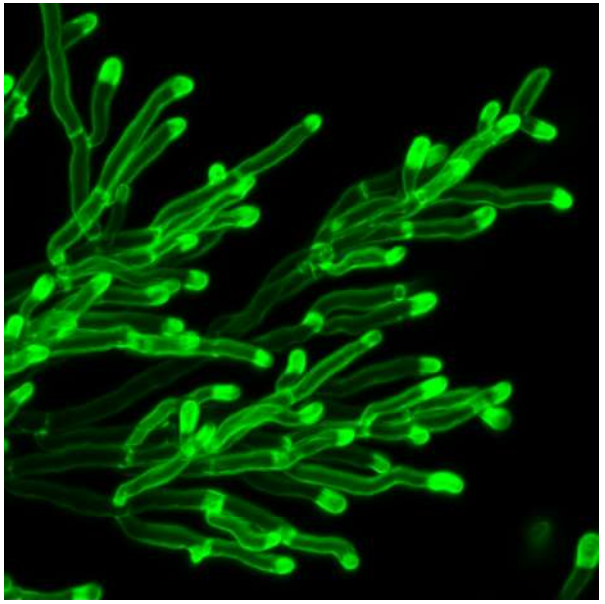
Unique composition of the fungal cell wall

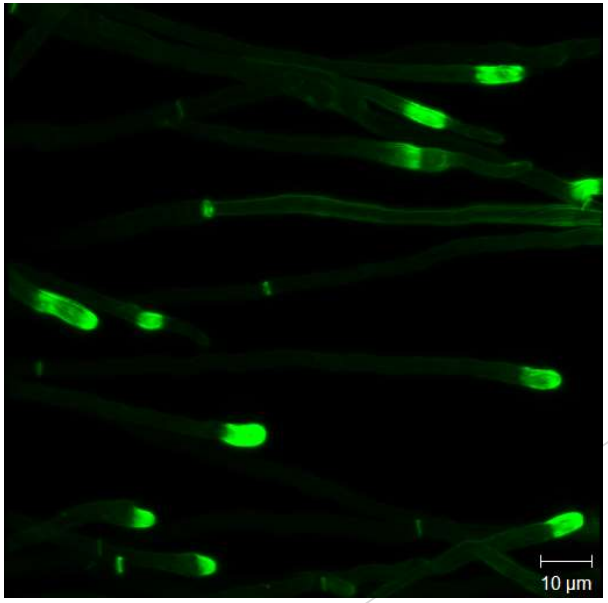
- *makes it an ideal target for the development of **fungicides!***



- **GPI** (GlycosylPhosphatidylInositol) **Anchored Proteins** = **GAP**
  - Cell wall modifying enzymes
  - e.g. **G**lucan **El**ongation (**Gel**) proteins elongating ß-1,3-glucan chains

*Triple*Δ*gel1* Δ*gel3*Δ*gel4 KO* has reduced mycelial growth, hyper branching phenotype and **is non-pathogenic!!!**





Unique composition of the fungal cell wall

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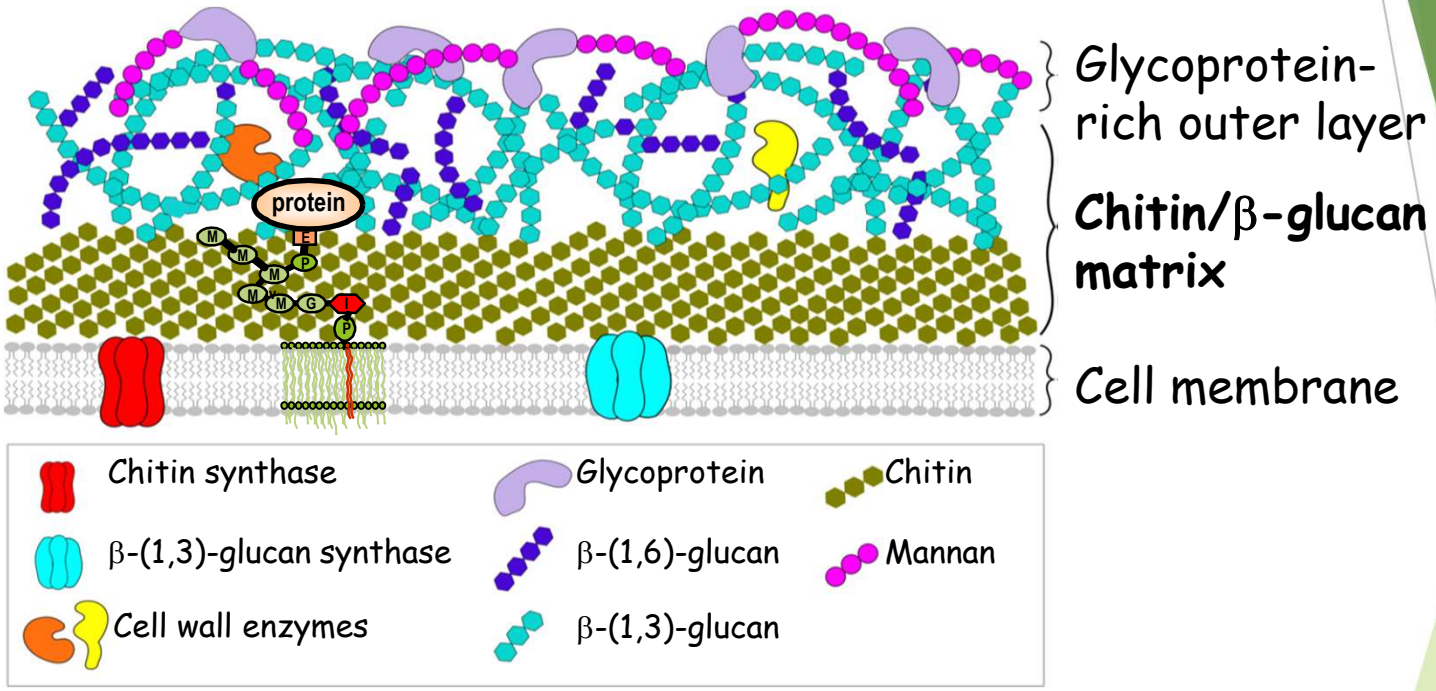
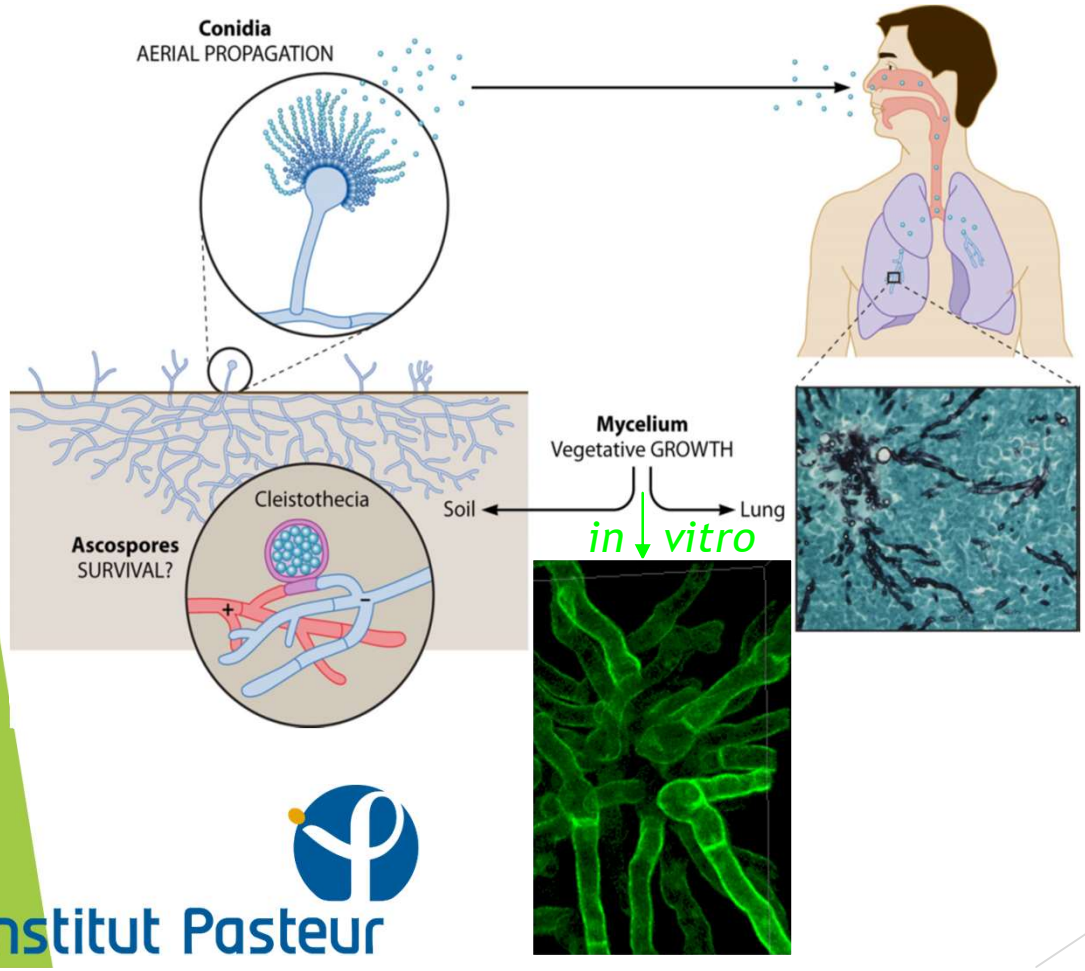
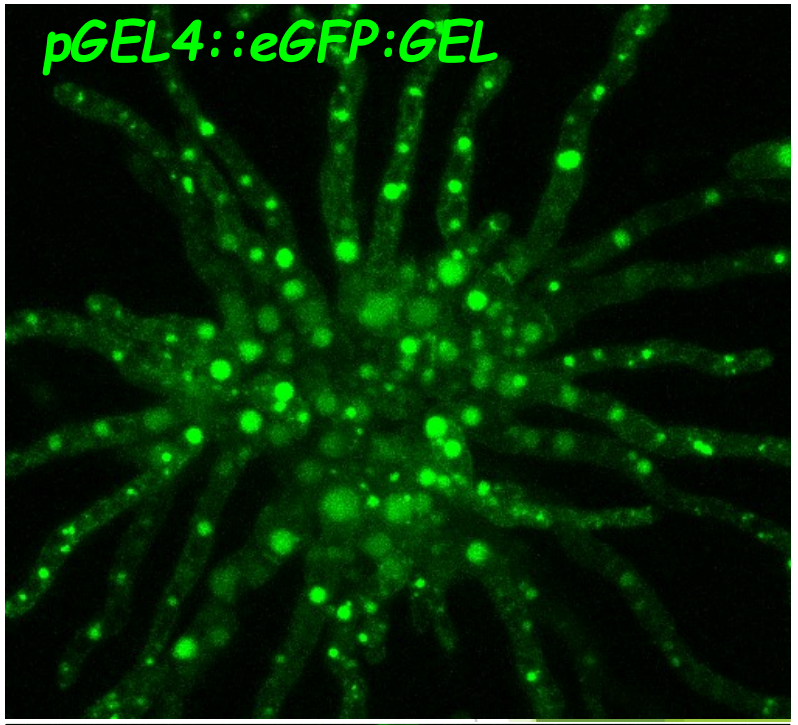


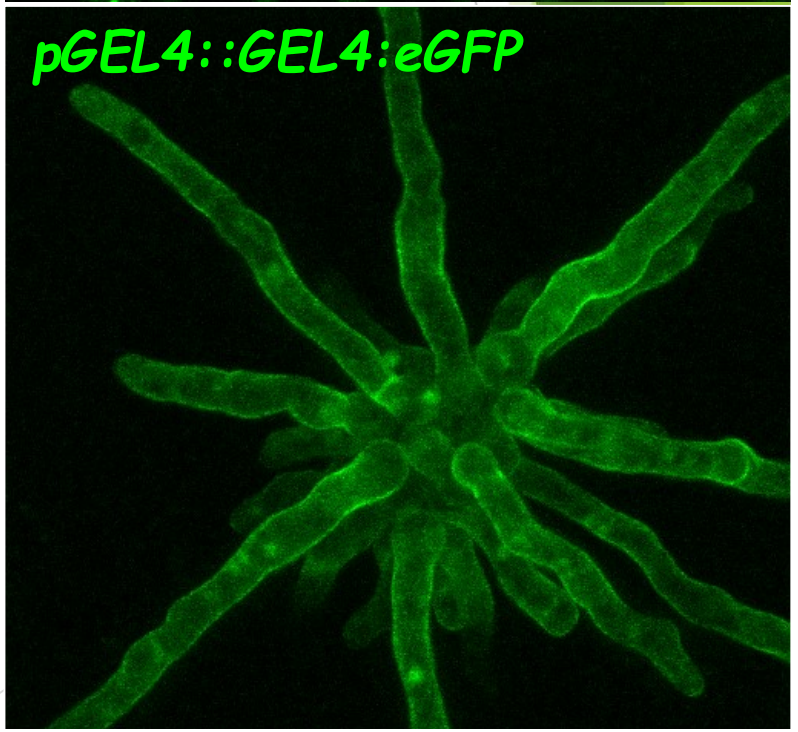
- **GPI** (GlycosylPhosphatidylInositol) **Anchored Proteins** = **GAP**
  - Cell wall modifying enzymes
  - e.g. **G**lucan **El**ongation (**Gel**) proteins elongating ß-1,3-glucan chains

Aspergillus fumigatus is a fungal saprotroph BUT opportunistic human pathogen!

- Causes aspergillosis in immunocompromised patients…. deadly

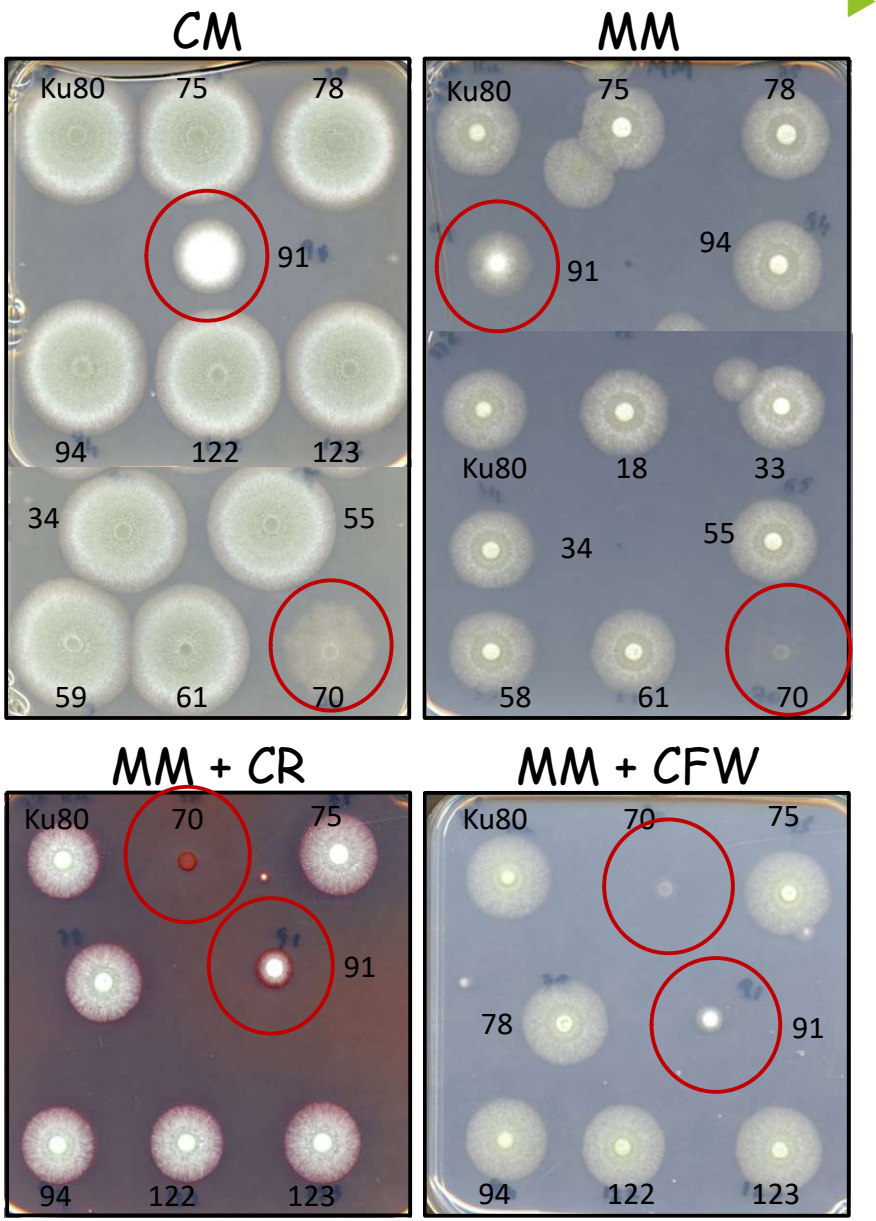




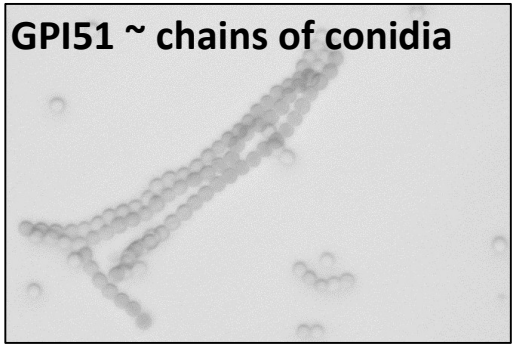


Institut Pasteur

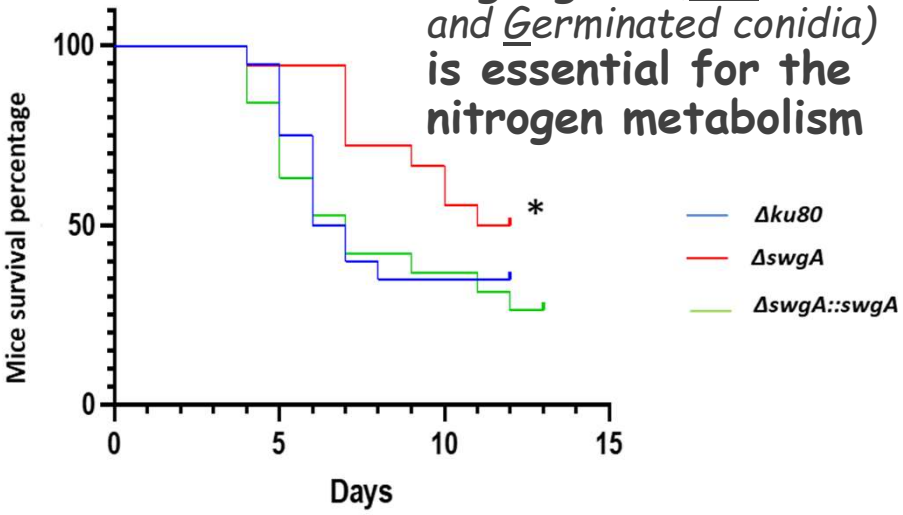
How to knock-out 132 genes in one summer . . .



- **Single KOs of all *GAP* proteins!!!**
  - Growth defects /phenotype
  - Spore phenotype



- ***swgA gene*** *(SWollen and Germinated conidia)* **is essential for the nitrogen metabolism**



- Samalova *et al.*, 2020, 2023