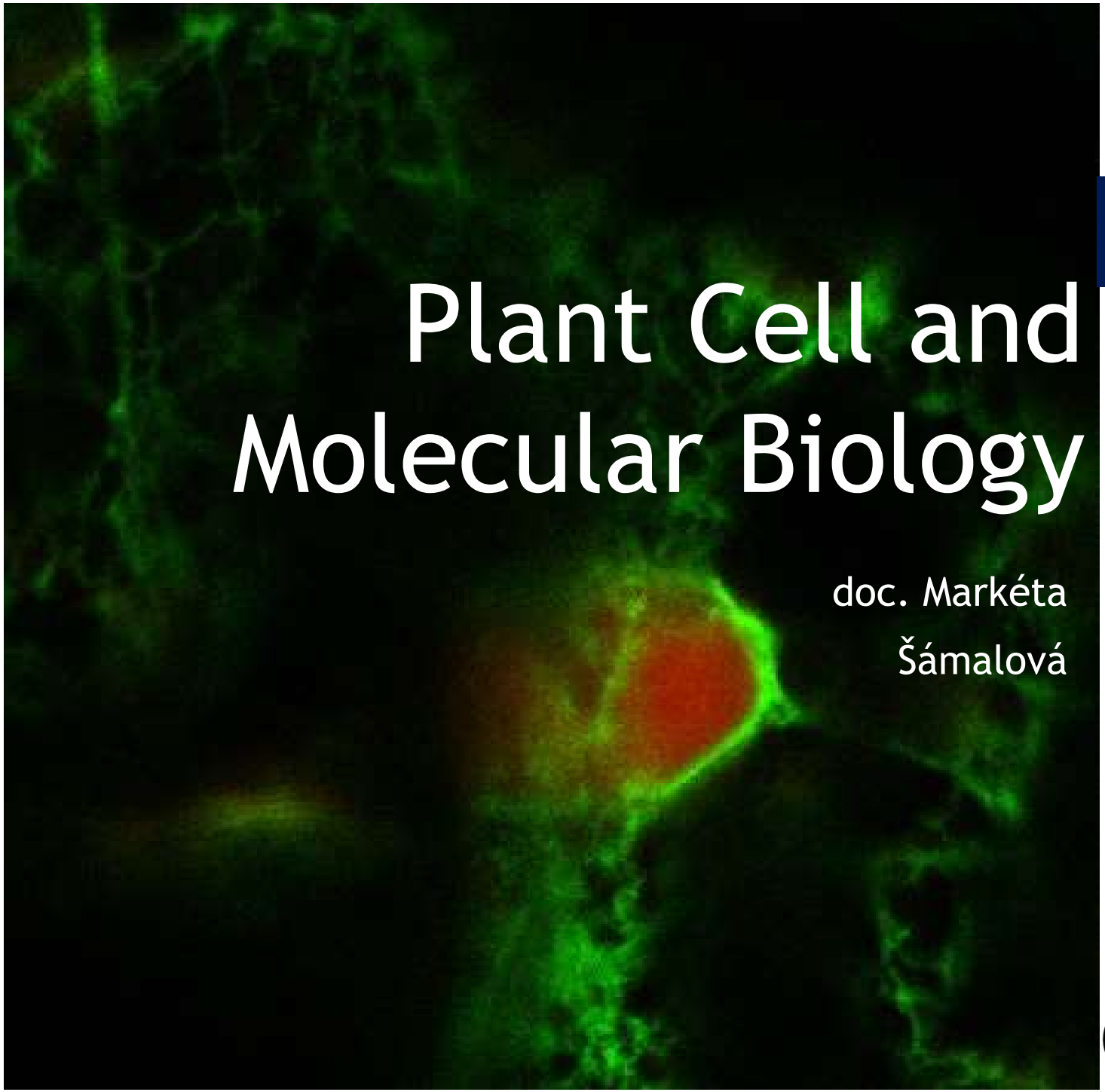

# Plant Cell and Molecular Biology

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cost

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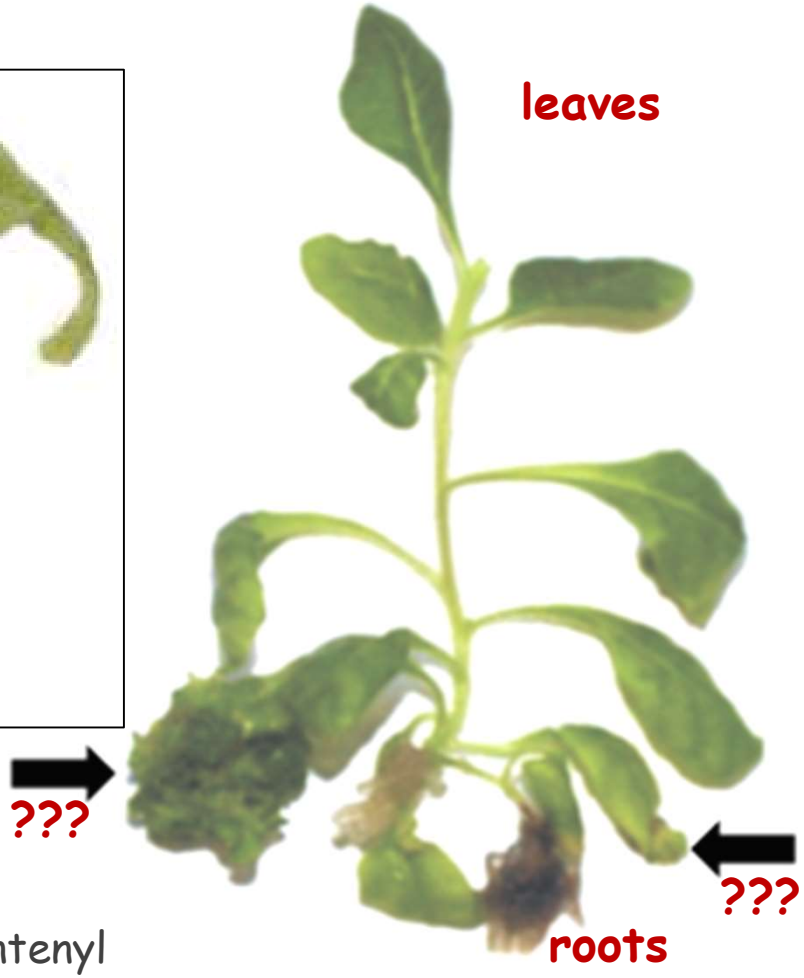
Oddělení experimentální biologie rostlin

# My 1st transgenic tobacco plant that I made (in the last century…:)

- Re-generated *in vitro* using tissue cultures




- Ectopic meristems developing into shoot-like structures…
- Introduced *IPT* gene encoding isopentenyl transferase which catalysis the first step in cytokinin hormone biosynthesis…

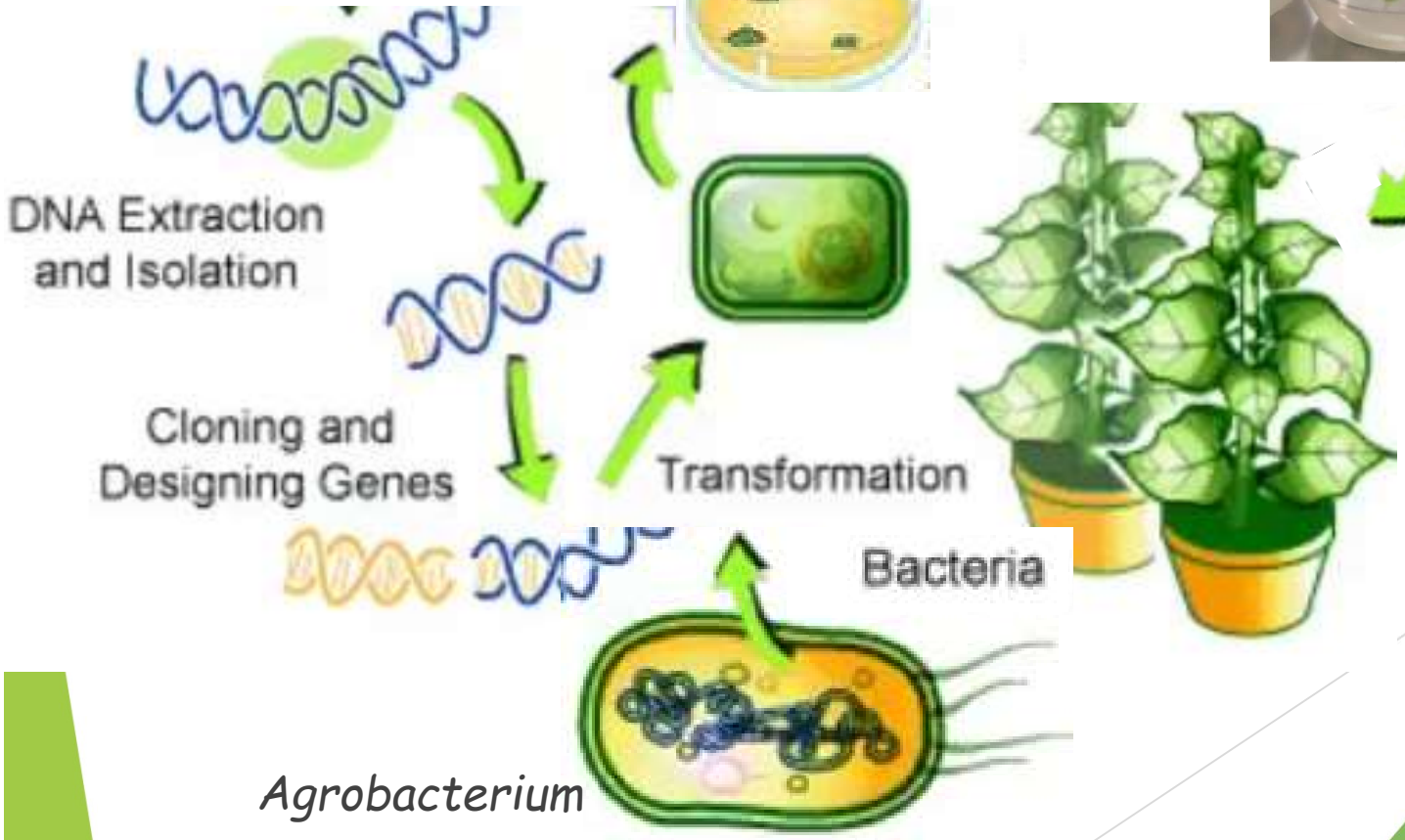
# OUTLINE of the talk

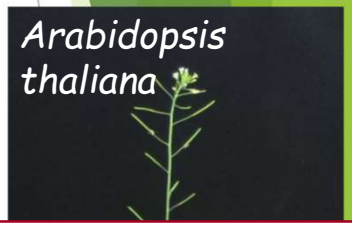
- How to make a genetically modified plant?
  - Tobacco, rice
  - *Arabidopsis thaliana*
- How to regulate (trans)gene expression?
  - The pOp6/LhGR system
  - CRISPR/Cas9
- Transient gene expression
- Fluorescent proteins
- Plant endomembrane system
- Plant cell wall
  - Expansins & (a)biotic stresses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


# How to make a genetically modified/ genome-edited plant?

# Transformation

- **Tissue cultures**
  - tobacco
  - rice



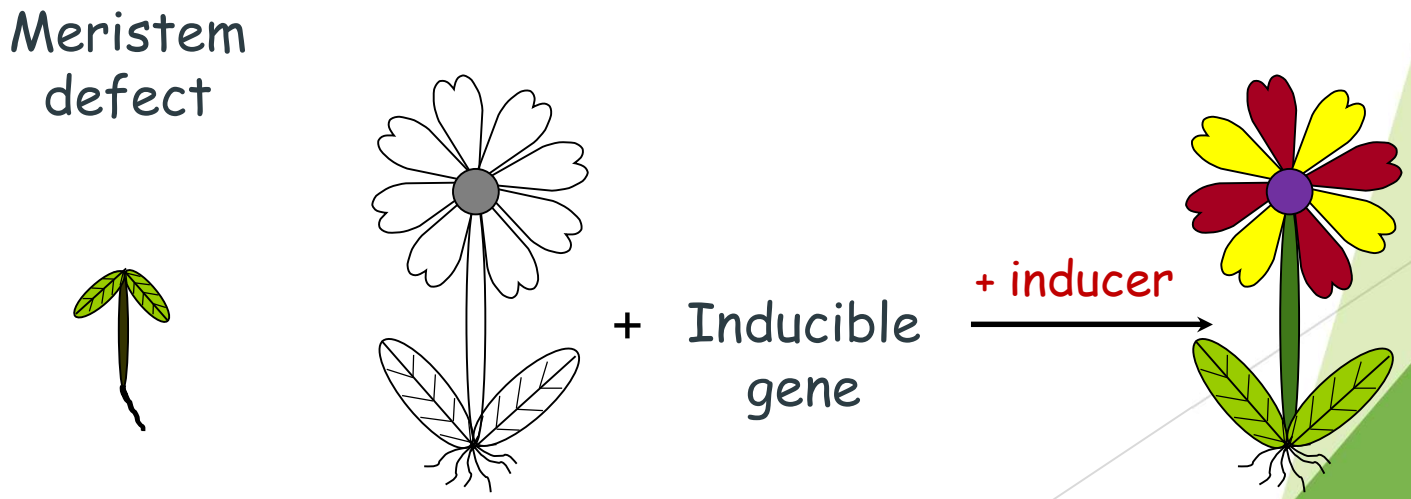

- **Floral dip**

*Arabidopsis*

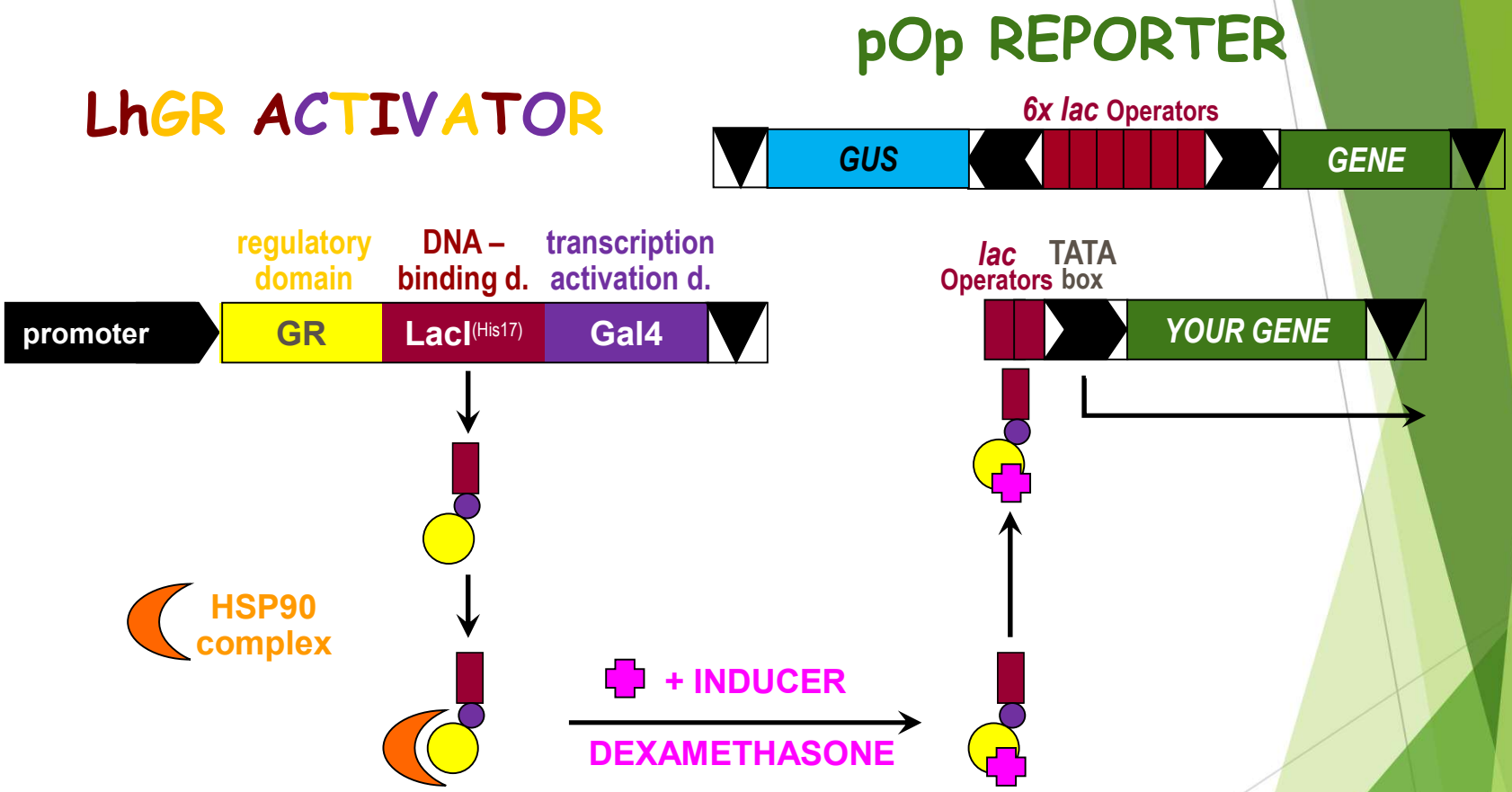
# How to regulate (trans)gene expression?

# 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

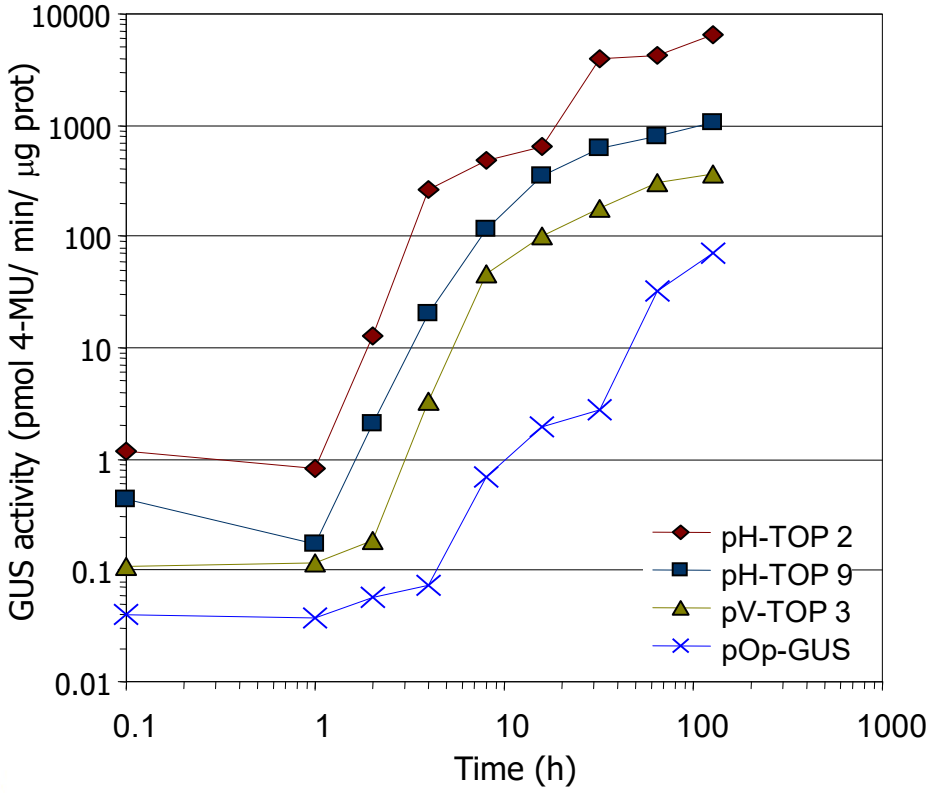



- Developed in the laboratory of Dr Ian MOORE
- Use world-wide today… an "ideal" inducible system

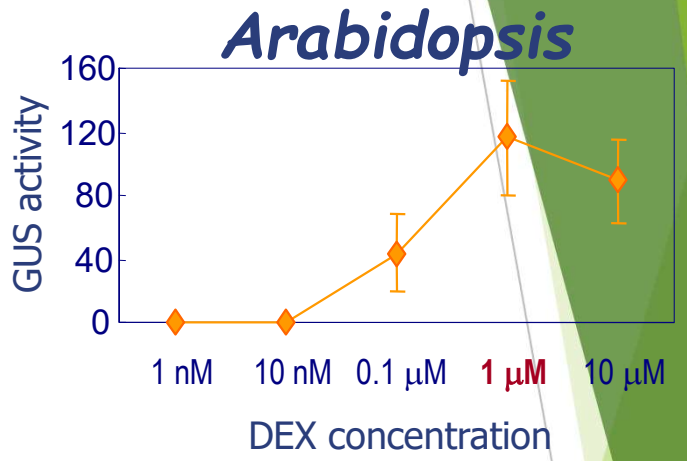
UNIVERSITY OF OXFORD

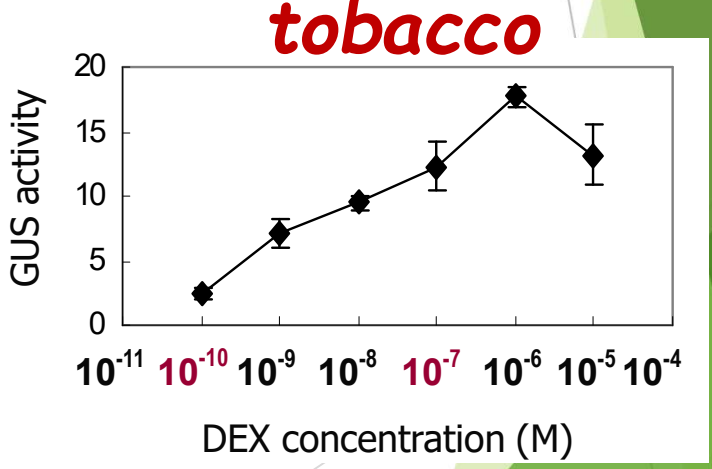
# The pOp6/LhGR is highly inducible, fast & v. sensitive

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


- Increase of GUS activity in 2h!

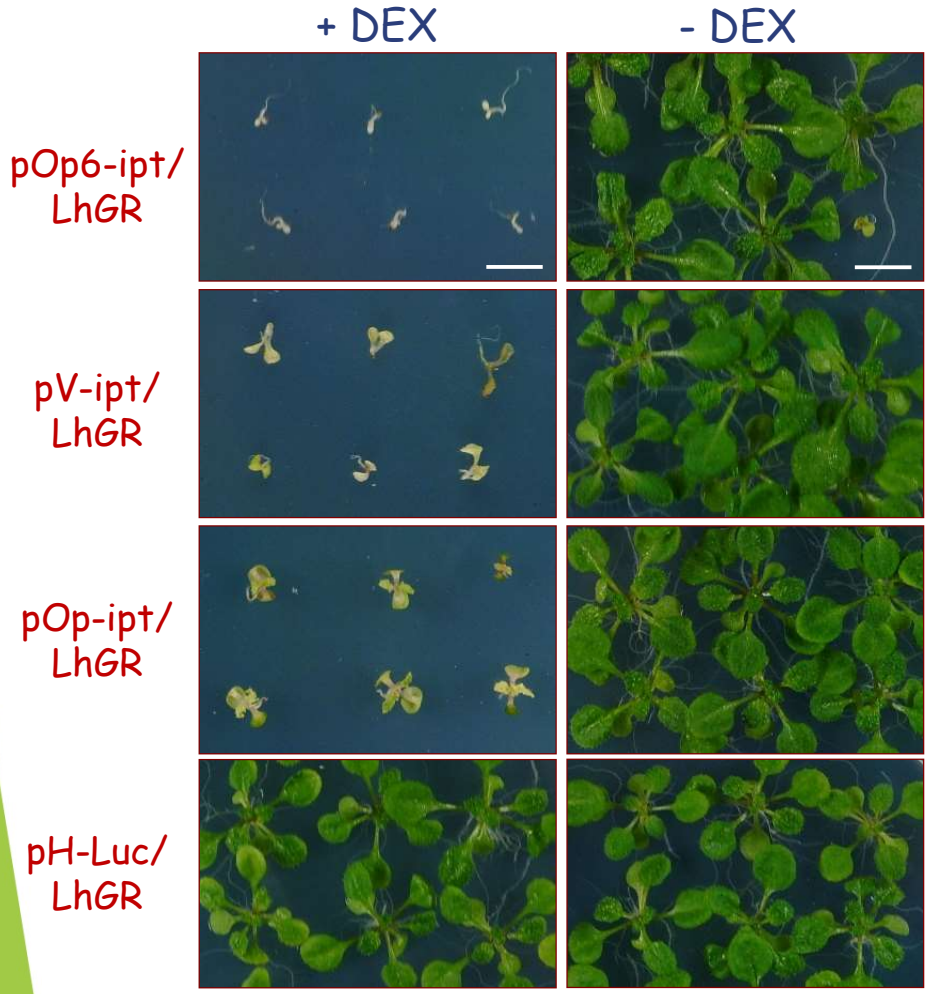


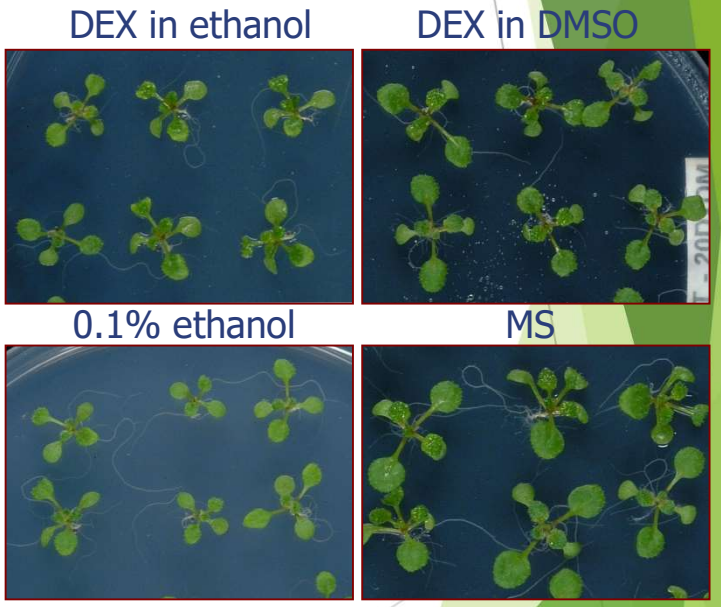

- The most sensitive system for tobacco!

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

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

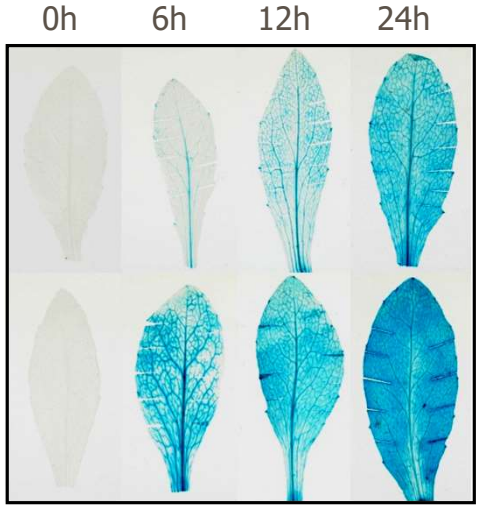



- **neither DEX nor LhGR affects endogenous processes in plants ...**
  **though ethanol does!**

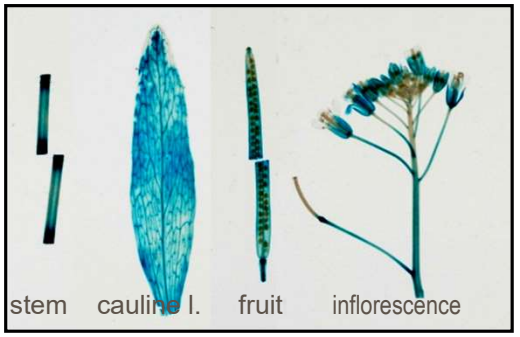



*Arabidopsis* seedlings were grown on plates in the presence or absence of 10 μM DEX.

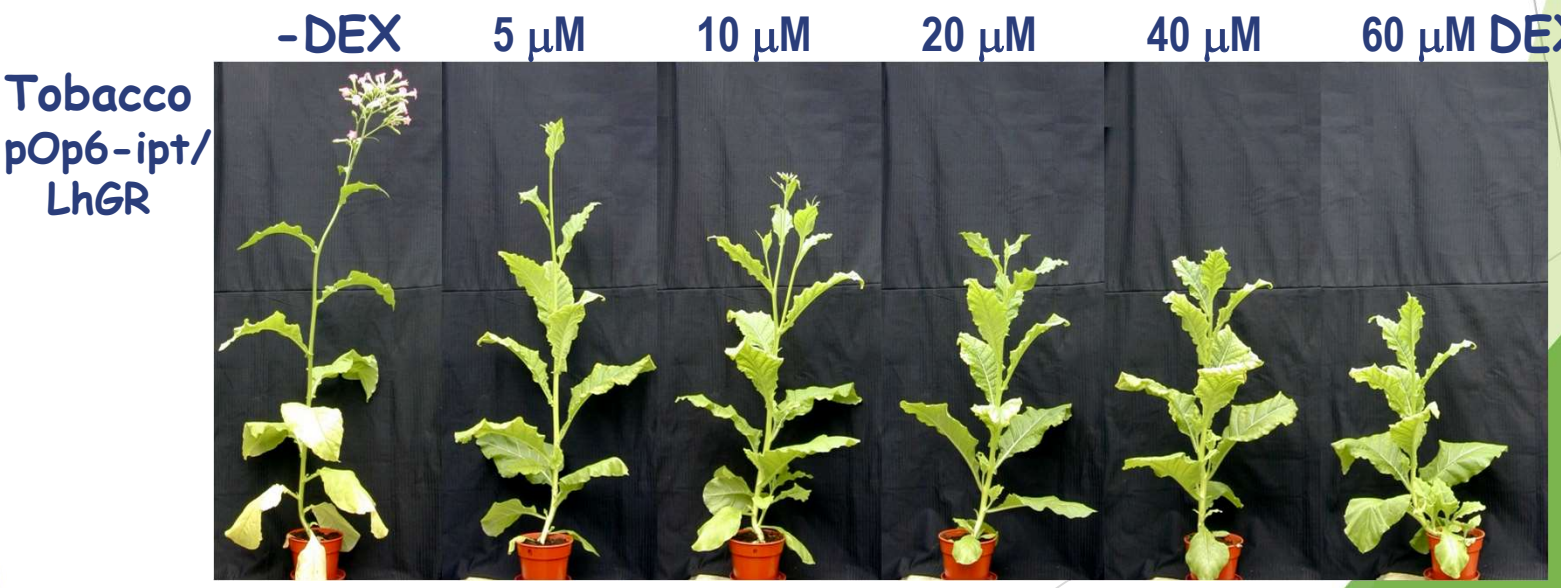
# The pOp6/LhGR system is inducible by various methods




## Watering plants with DEX

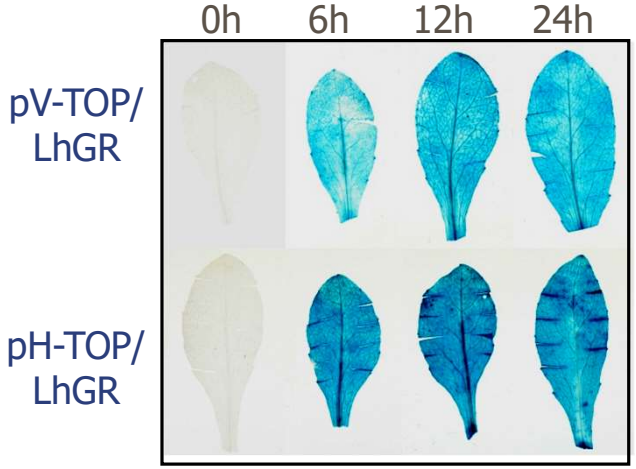



*DEX distribution through tissues (24h after watering).*

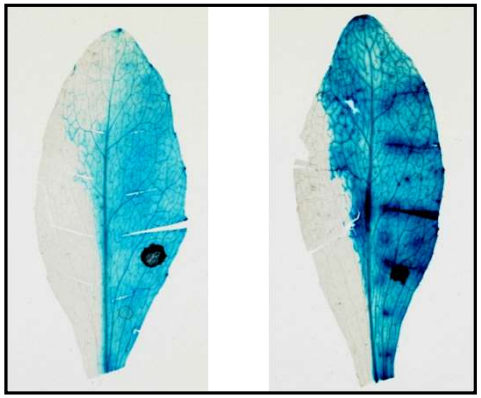


# The pOp6/LhGR system is inducible by various methods

## Painting plants with DEX

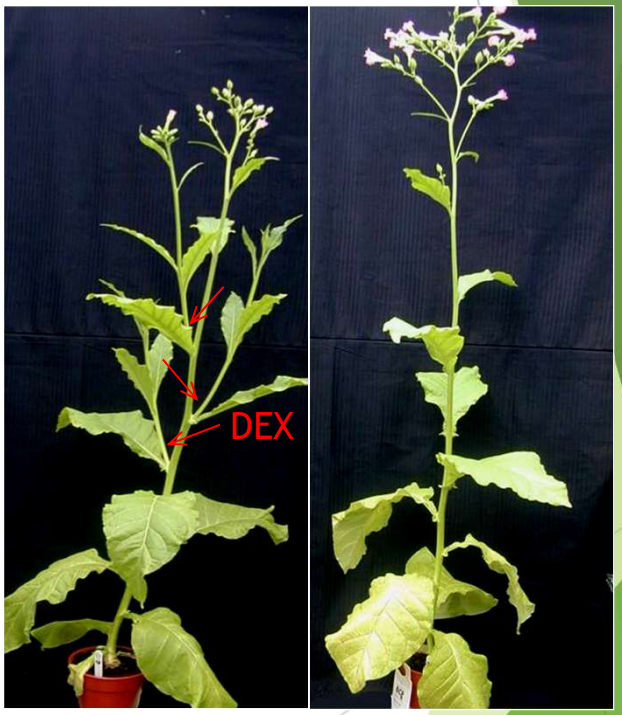



A leaf half painted with DEX



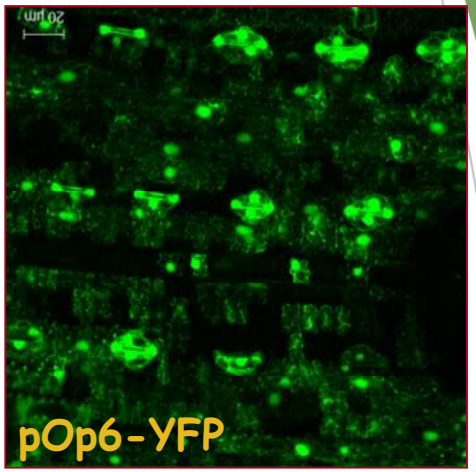
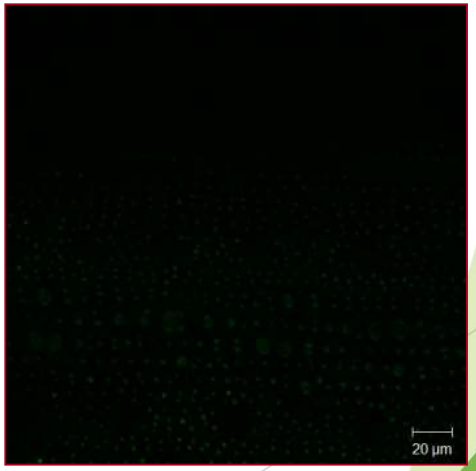

application on axillary buds

Control




Tobacco
pOp6-ipt/LhGR

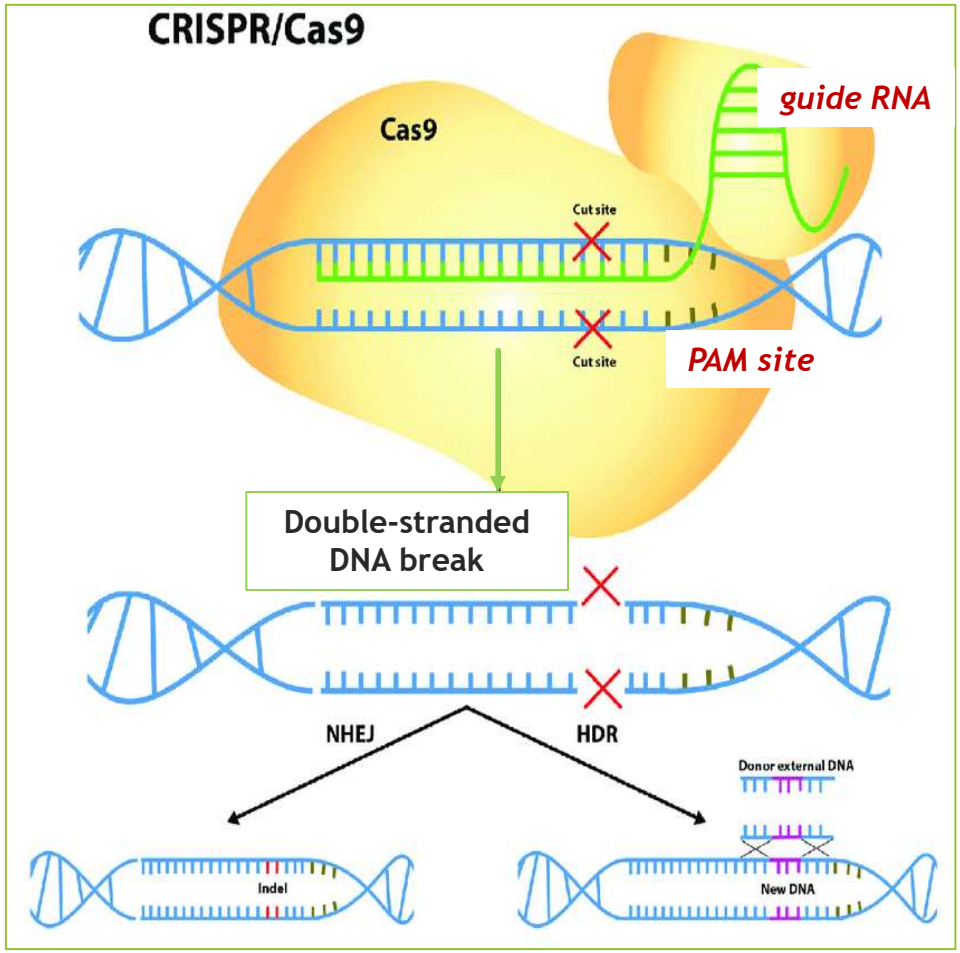
# The pOp6/LhGR system is functional in several species

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

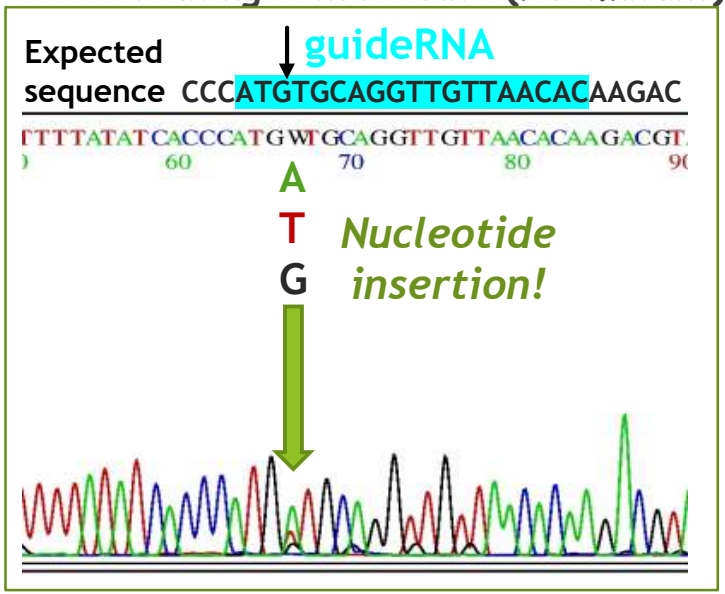
- Maize, potato, tomato, *Cardamine hirsuta*, citrus…
- Detailed step-by-step protocols in Samalova *et al.*, 2019

# 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*

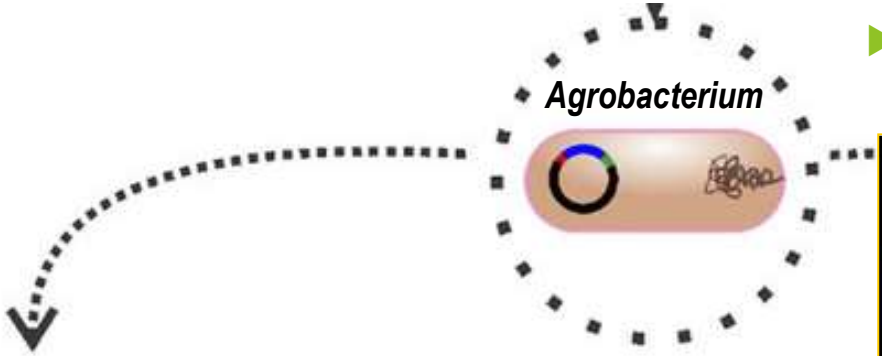
***PAM:*** *Protospacer Adjacent Motifs*

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

# Transient gene expression and fluorescent proteins

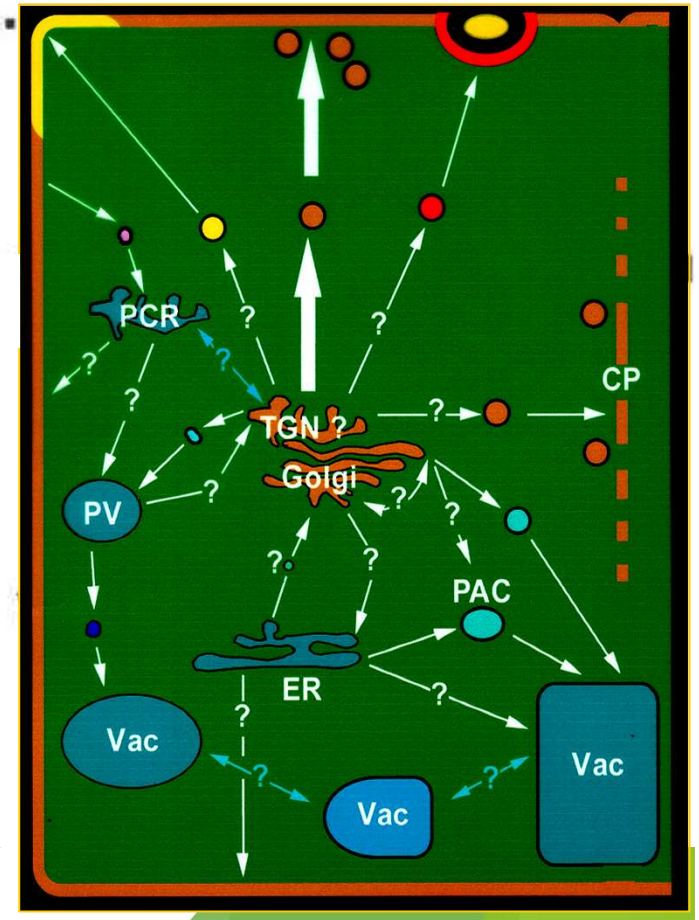
# Transient gene expression assay

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




3. TRANSIENT GENE EXPRESSION ASSAY

- e.g. to study plant endomembrane trafficking



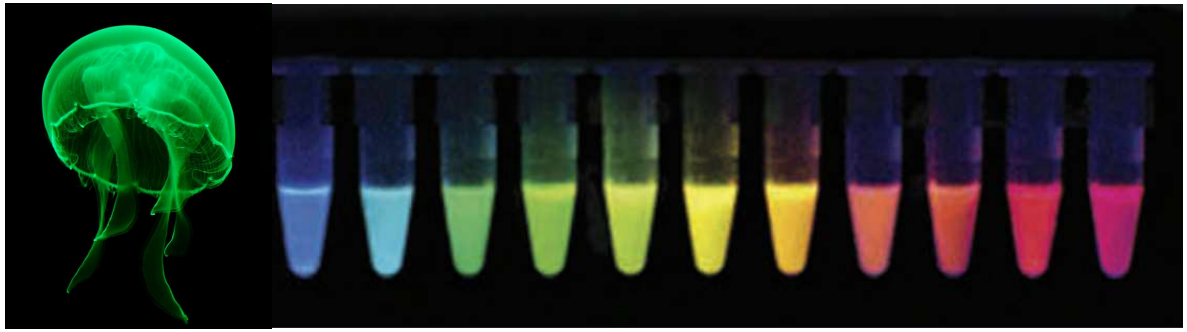
# Use of fluorescent proteins (FP) in cell biology

- Protein localization, protein-protein interactions…

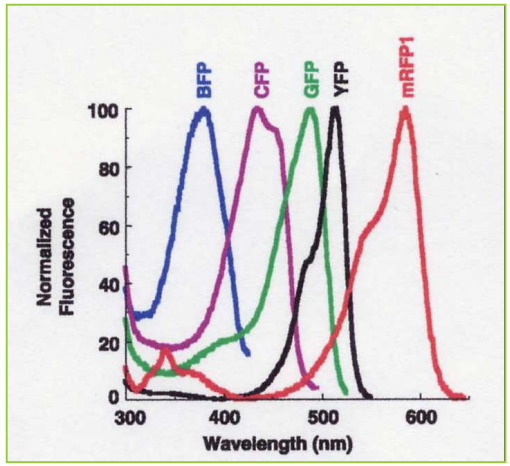
GFP ~ green FP from jellyfish *Aequorea victoria*

YFP – yellow FP mutant variant of GFP

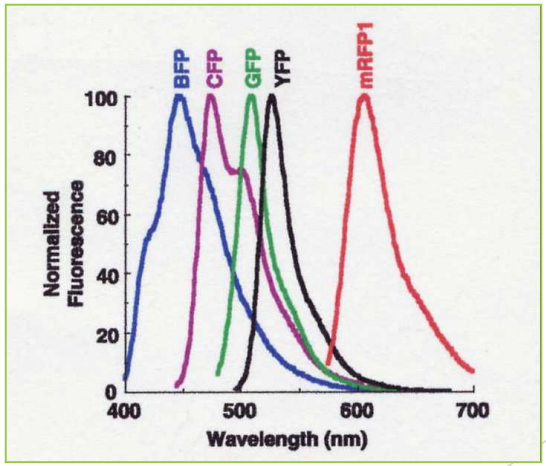
mRFP1 – monomeric red FP from *Discosoma* coral



- **Excitation spectra**

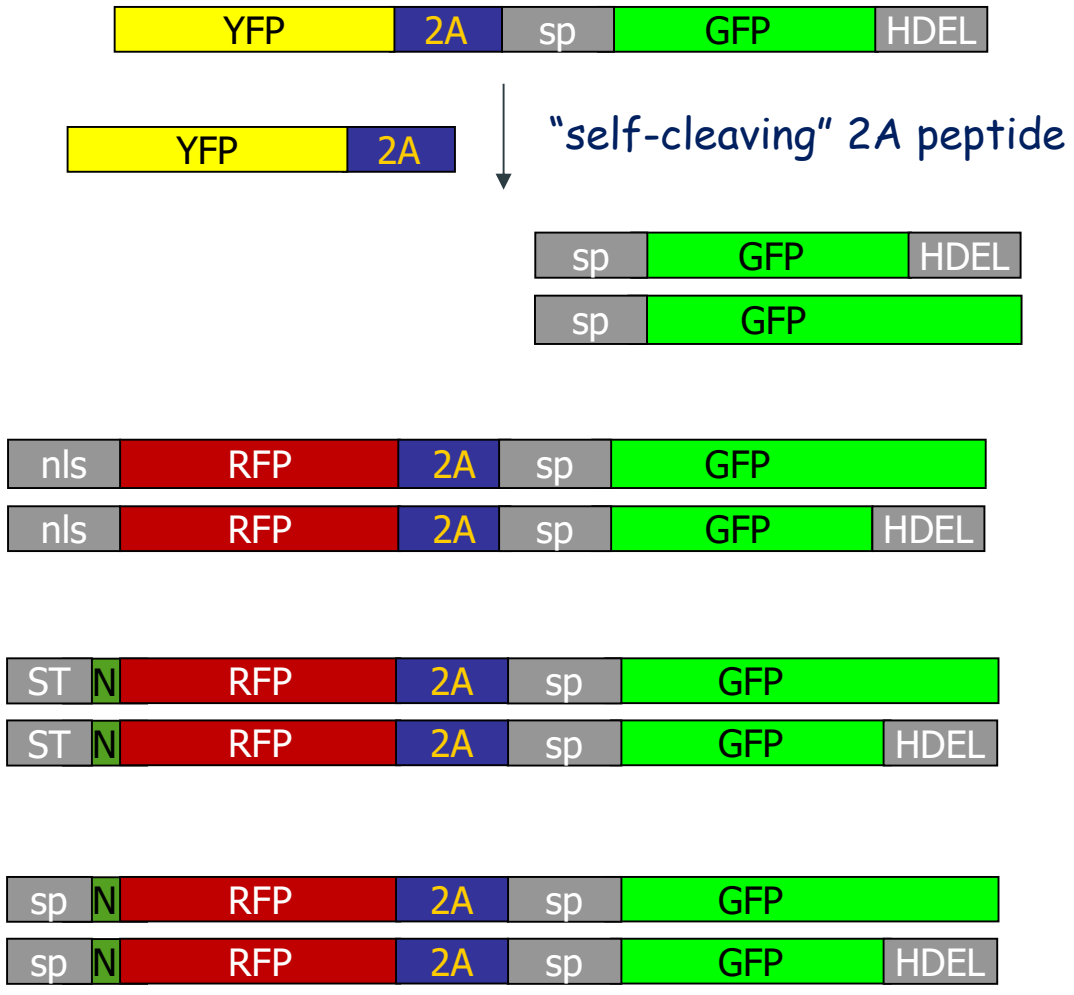



- **Emission spectra**



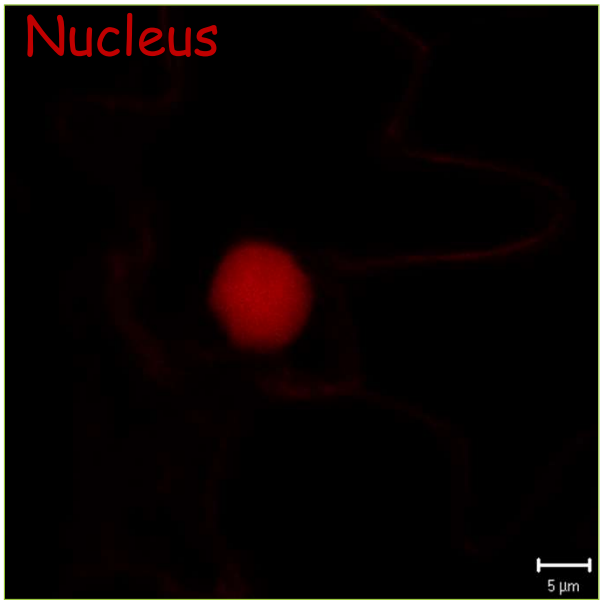

- **CLSM ~ confocal laser scanning microscope**
- Generates optical slices through live specimens.

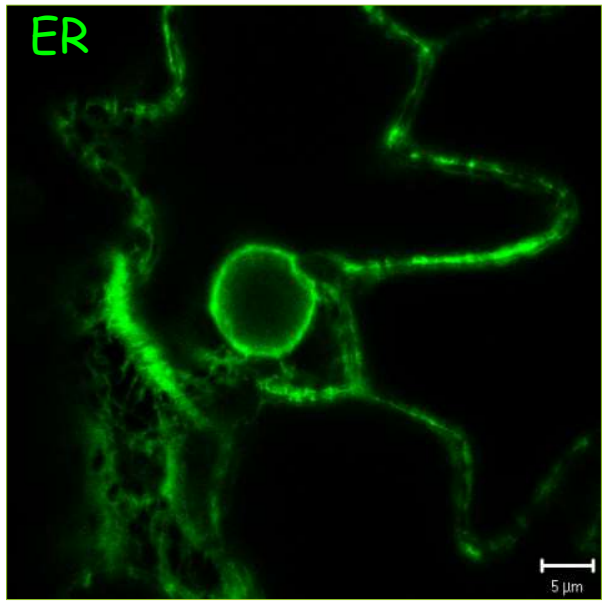
# Targeting fluorescent fusion proteins into different cell compartments (Samalova *et al.*, 2006)

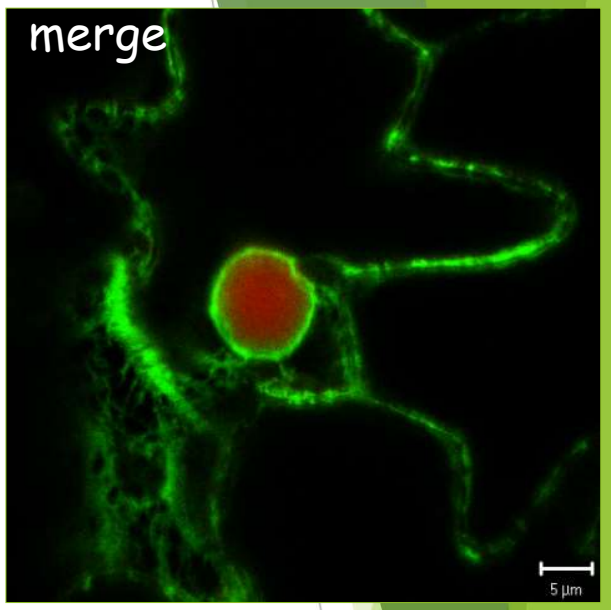



| Location | | |
|---|---|---|
| Cytoplasm | Endoplasmic reticulum (ER) | |
| | Cell wall | |
| Nucleus | Cell wall | ER |
| Golgi app. | Cell wall | ER |
| Vacuole | Cell wall | ER |

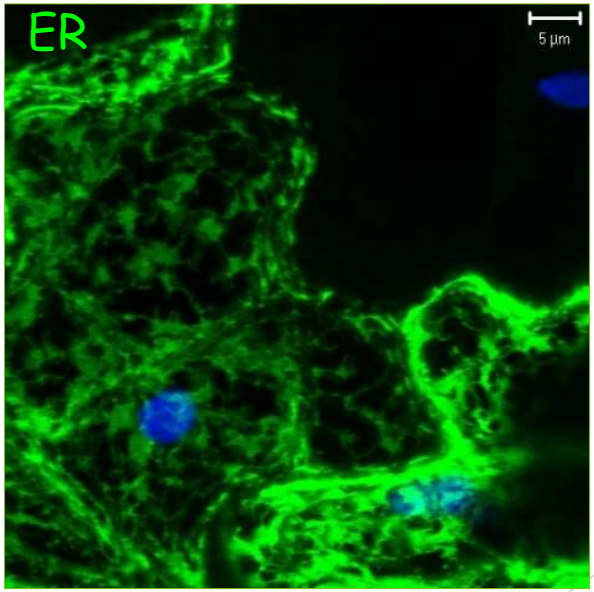

nls
RFP
2A
sp
GFP
HDEL

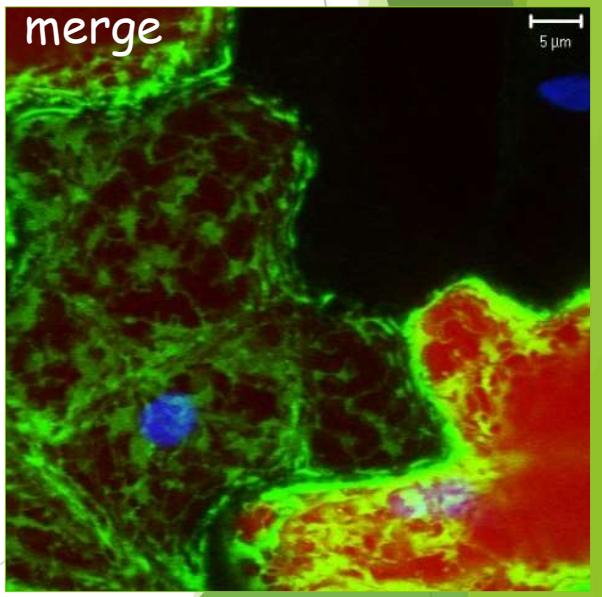

Nucleus
5 µm


ER
5 µm


merge
5 µm

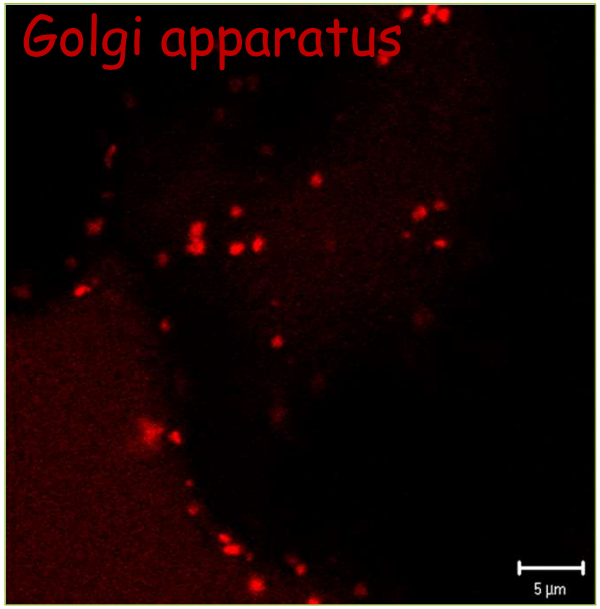

Vacuoles
5 µm

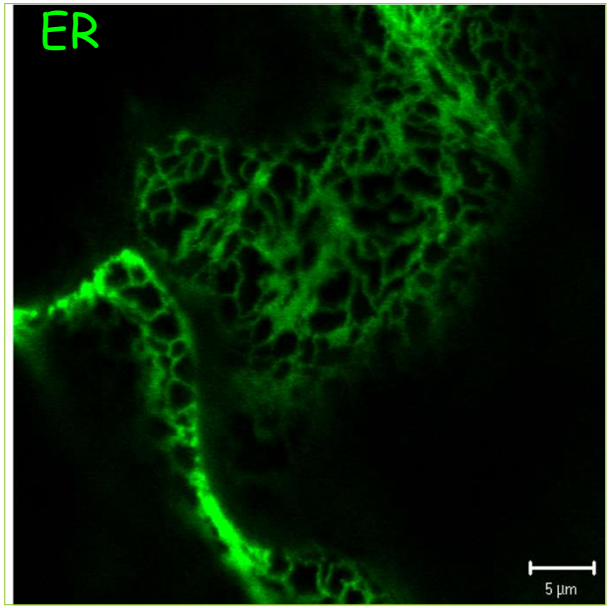

ER
5 µm

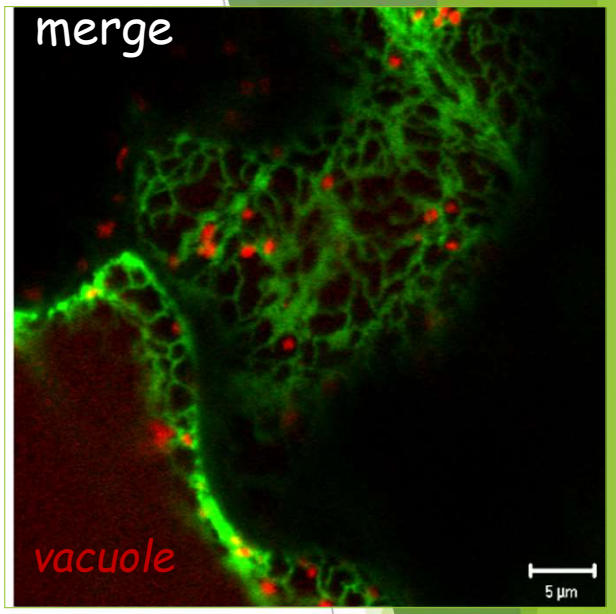

merge
5 µm

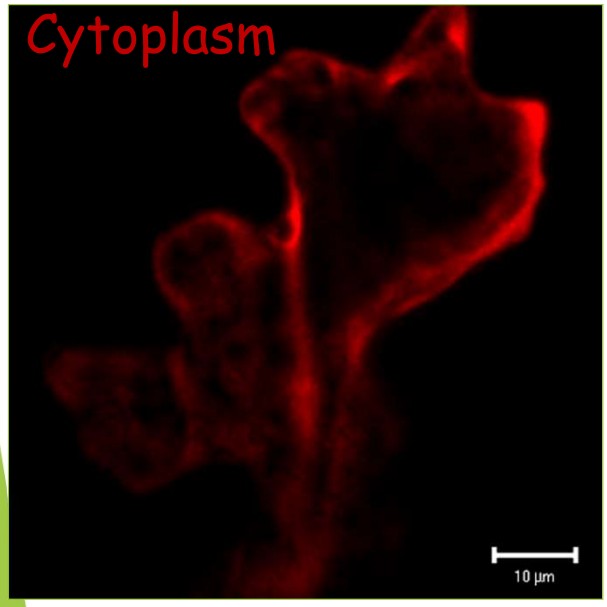

sp
N
RFP
2A
sp
GFP
HDEL

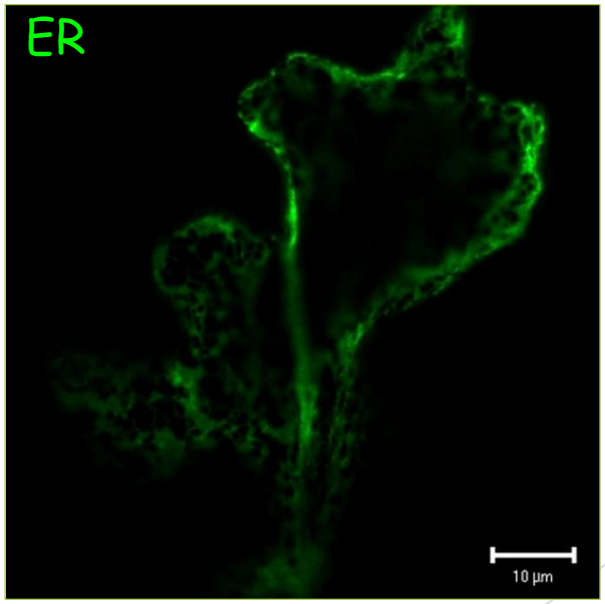

ST
N
RFP
2A
sp
GFP
HDEL

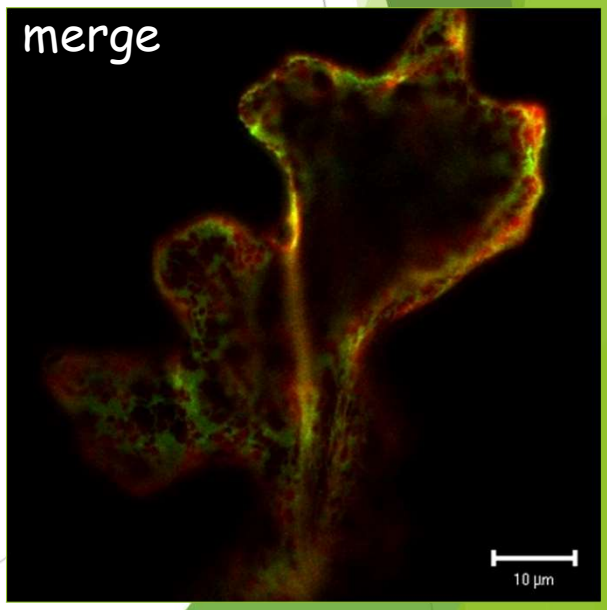

Golgi apparatus
5 µm


ER
5 µm


merge
vacuole
5 µm

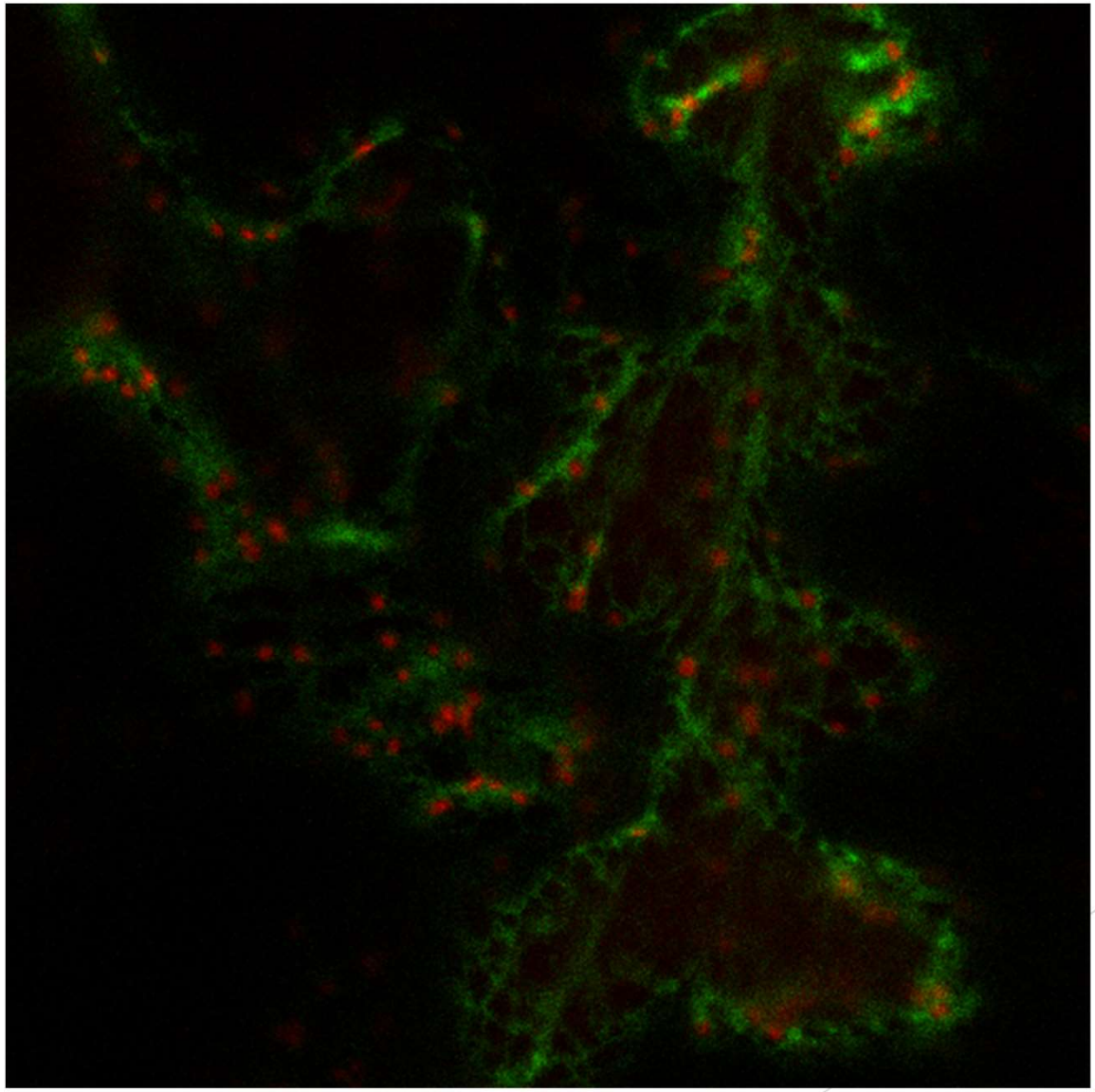

Cytoplasm
10 µm


ER
10 µm


merge
10 µm


YFP
2A
sp
GFP
HDEL

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

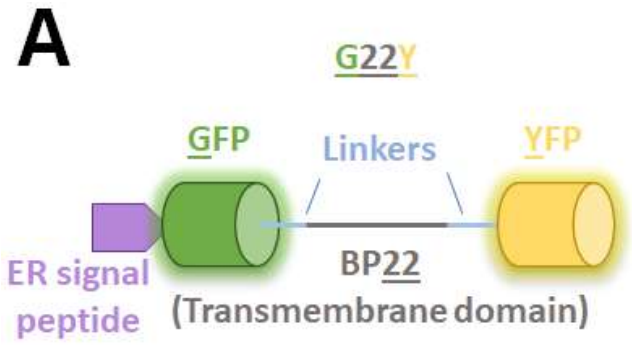


PLANTS ARE MOVING!

# Create your own compartment :)

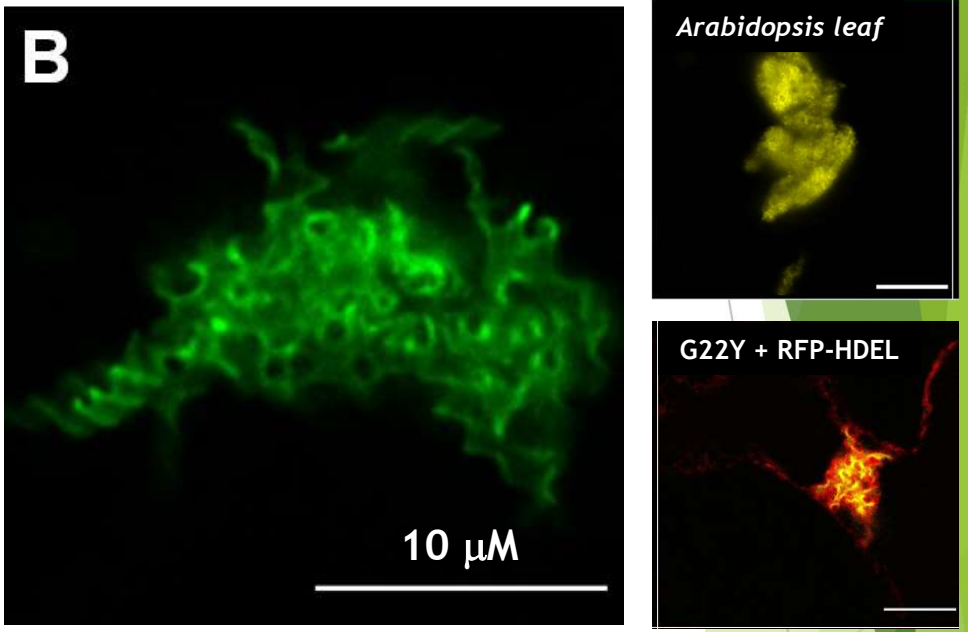
# A tool for plant synthetic biology

- substantial expansion of the endomembrane system in each cell of the plant (Sandor, Samalova *et al.*, 2023)




**OSER**

***Samalosome***

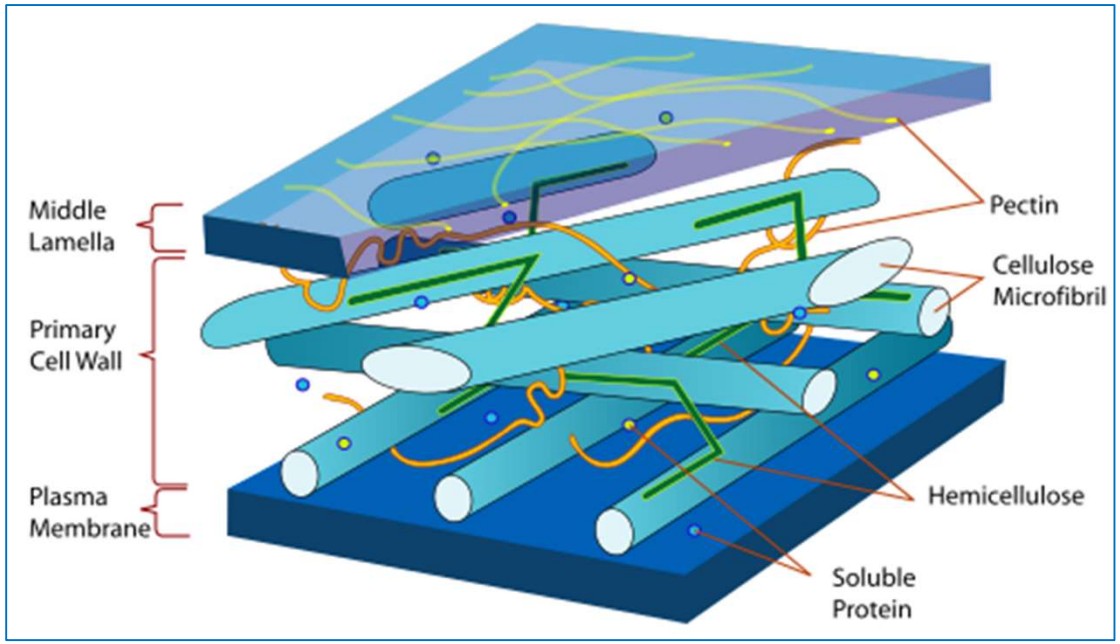



- ***Organised Smooth Endoplasmic Reticulum***
- Potential applications of the synthetic compartment for the metabolic engineering of plants, e.g. recombinant or toxic proteins.
- No detrimental effects in plants!

# Plant cell wall (CW)

# CW is crucial for plant growth and development

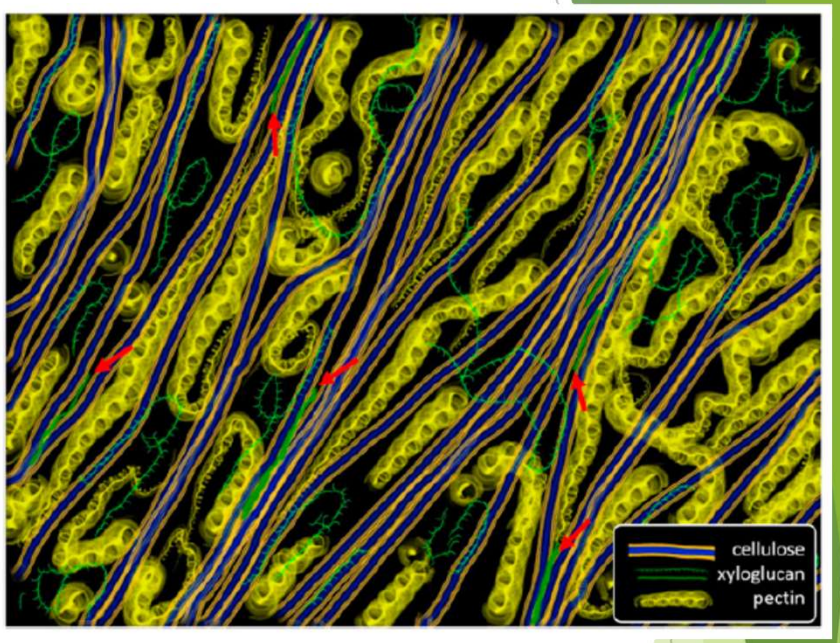
- shapes the plant body
- movement of solutes and nutrients
- protects plants from the environment
- intercellular communication (Wolf *et al.*, 2012)
  - **Cellulose is the most abundant biopolymer on Earth!**

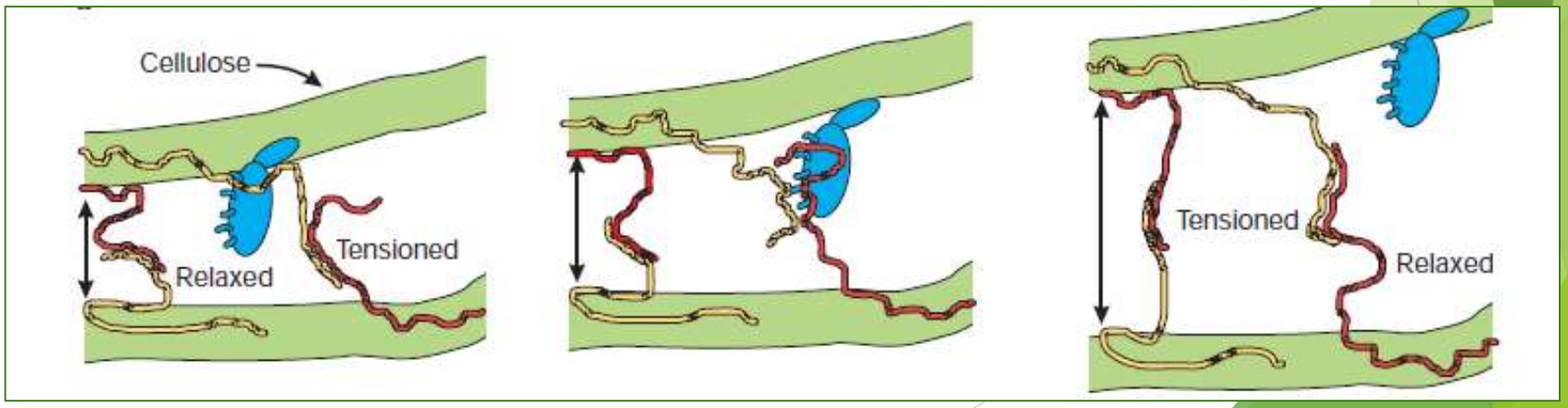



- *Load-bearing cellulose microfibrils*
- *embedded into viscoelastic matrix of hemicellulose and pectins.*

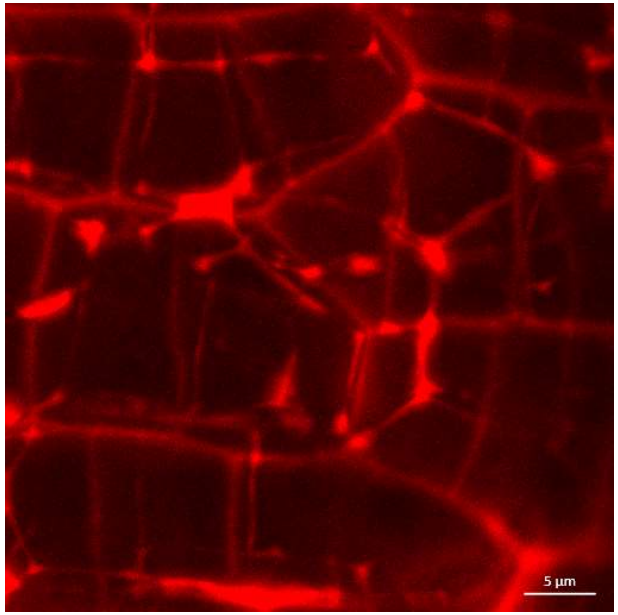
# Plant CWs combine strength with extensibility

- **Wall extensibility may be controlled at limited regions, 'biomechanical hotspots'** (Cosgrove, 2014; 2018).
- **EXPANSINs** discovered as the most pH-responsive substance in the CW (McQueen-Mason *et al.*, 1992).
- They do not have a hydrolytic activity
- but disrupt the non-covalent bonds between CW polysaccharides, thus relaxing wall stresses and allowing turgor-driven cell expansion (Cosgrove, 2000).



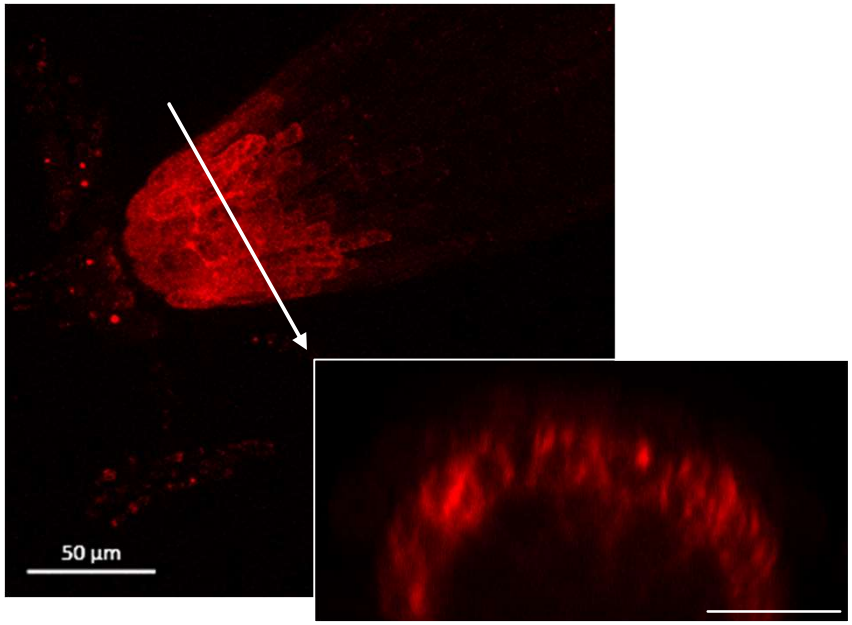



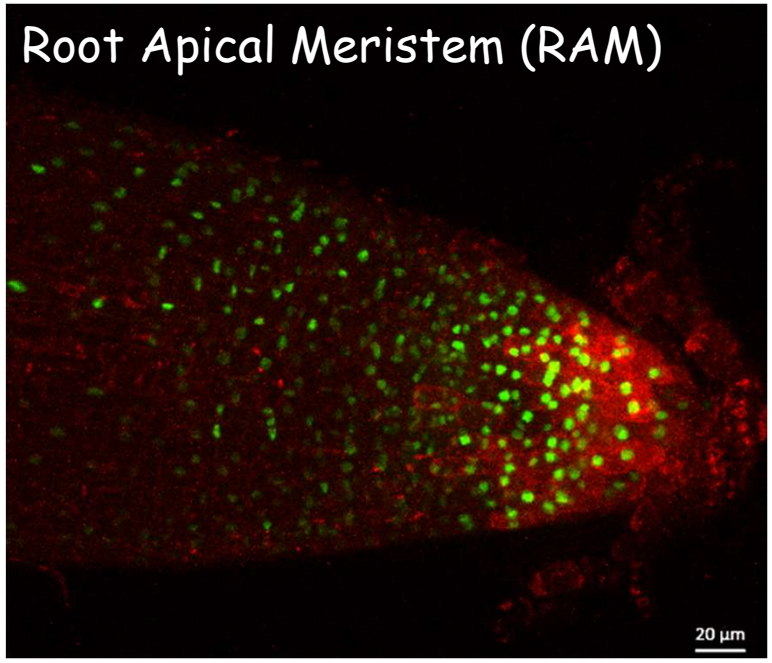
# EXPANSINS are localized in the cell wall



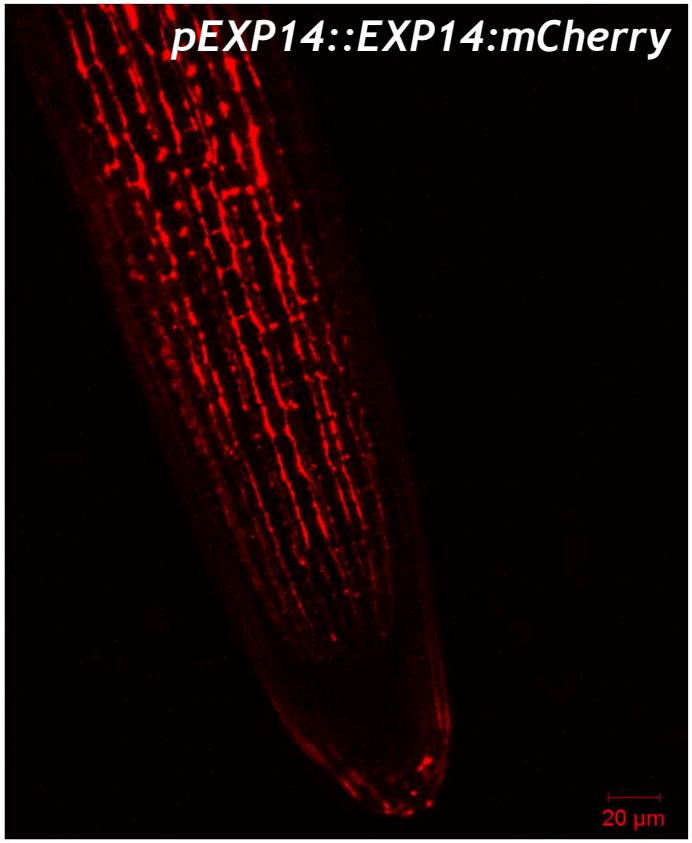


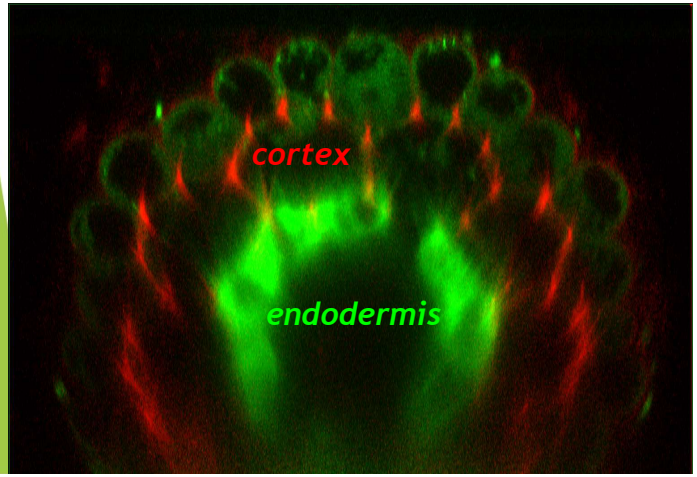
- EXPANSINs localised to the CW *in vivo* for the first time! (Samalova *et al.*, 2023)
  - Use of mCherry (RFP) instead of pH sensitive GFP

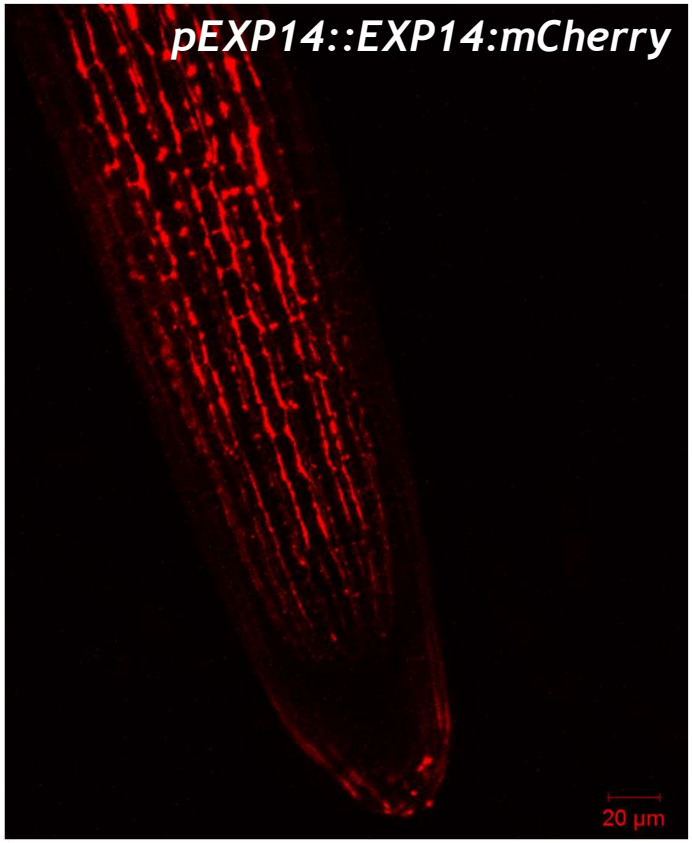



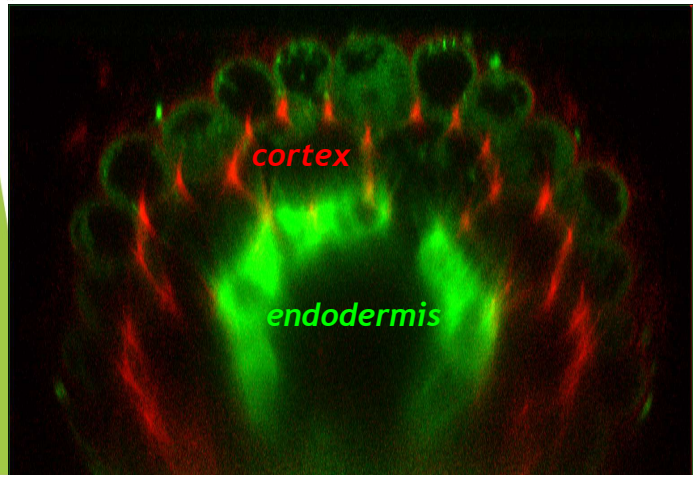
# EXPANSINS are localized into various root tissues



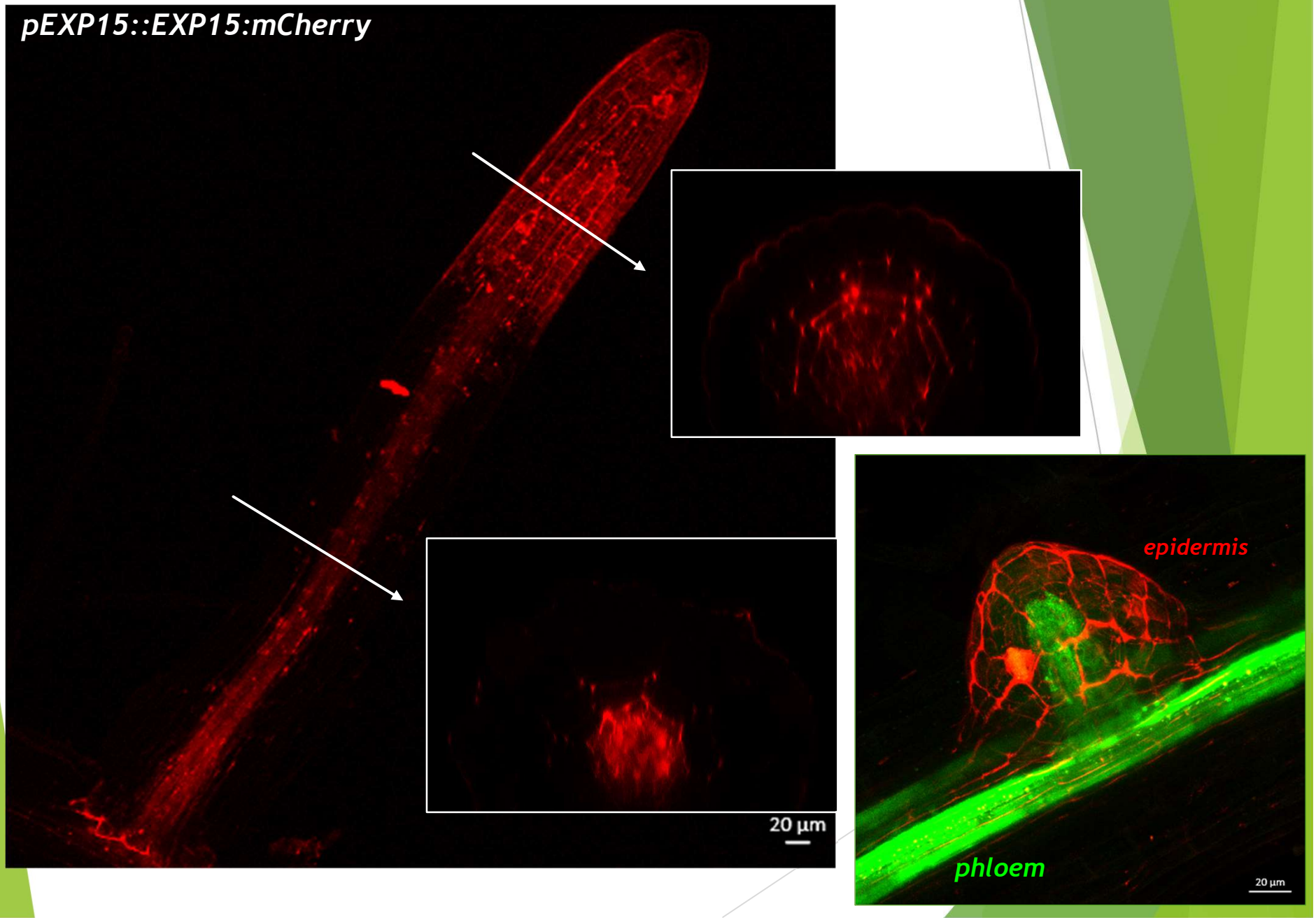
# EXPANSINS are localized into various root tissues



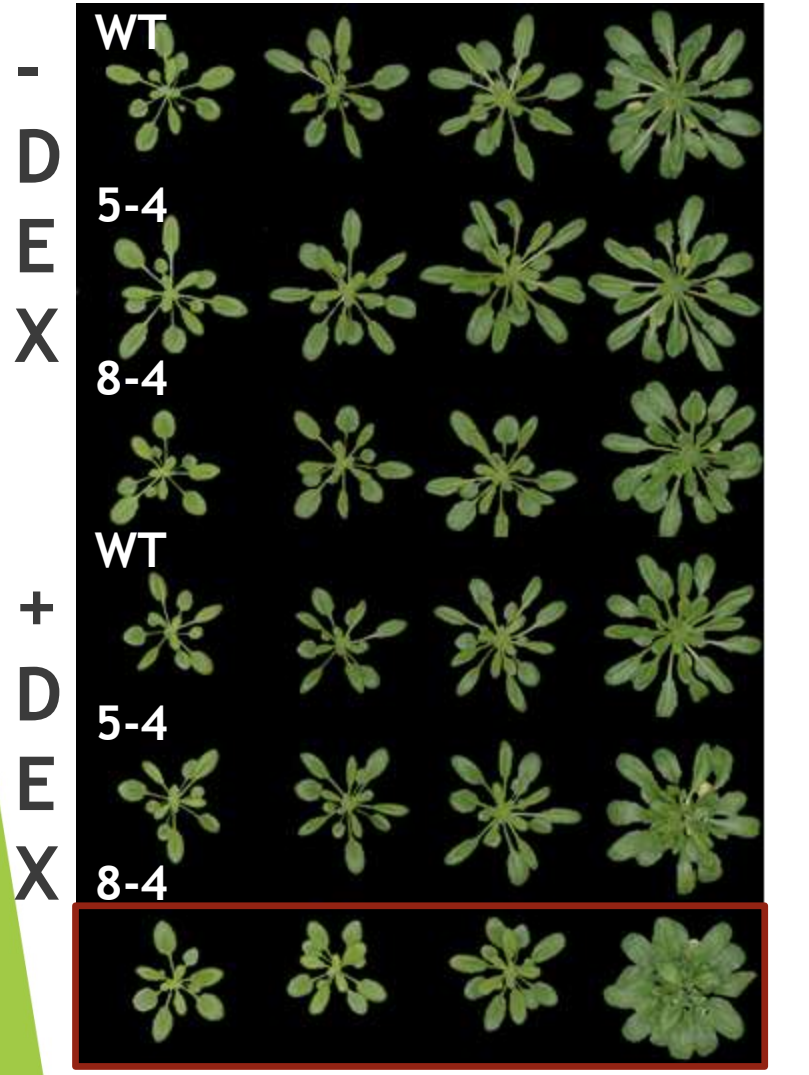




- 3D projection of Z-stack (combined optical slices) taken by a confocal microscope.

# EXPANSINS are localized into various root tissues



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



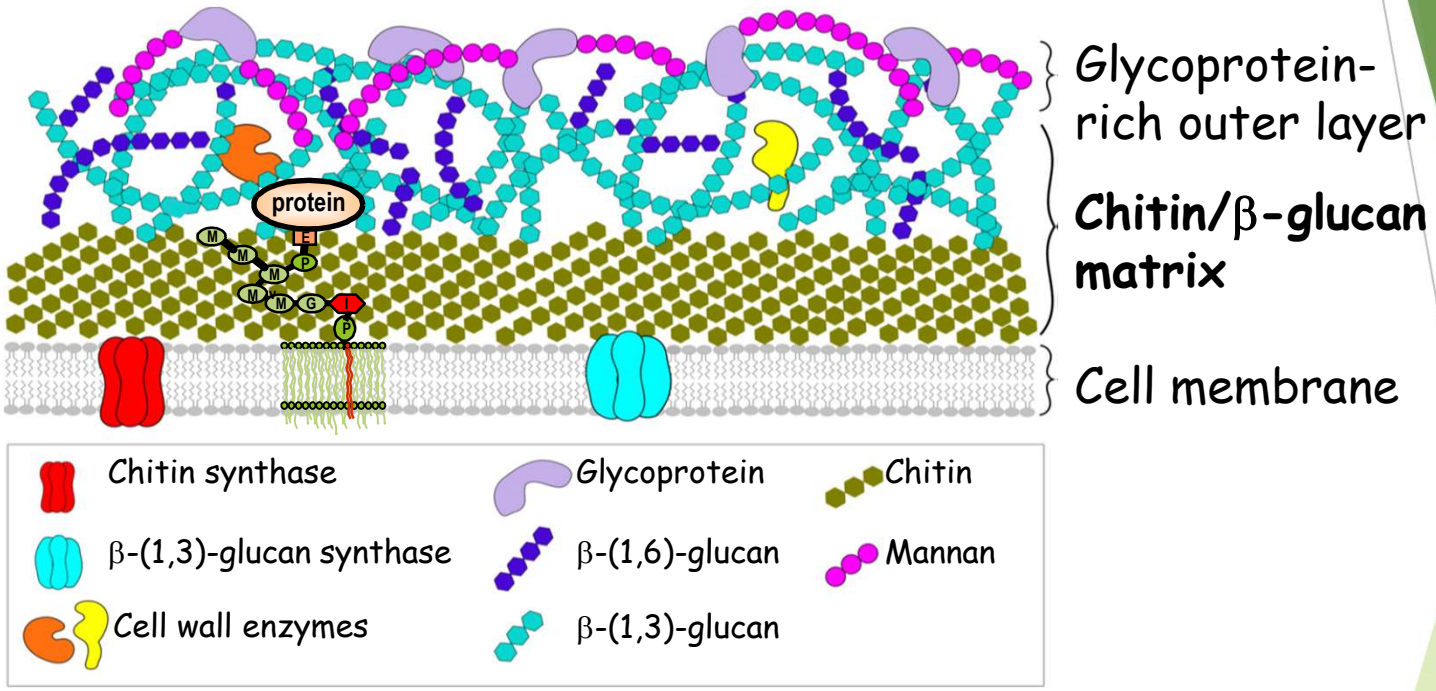


- ***Exploring a role of EXPANSINs under stress:***

# Not only plant cells have the CW…

# Unique composition of the fungal cell wall

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



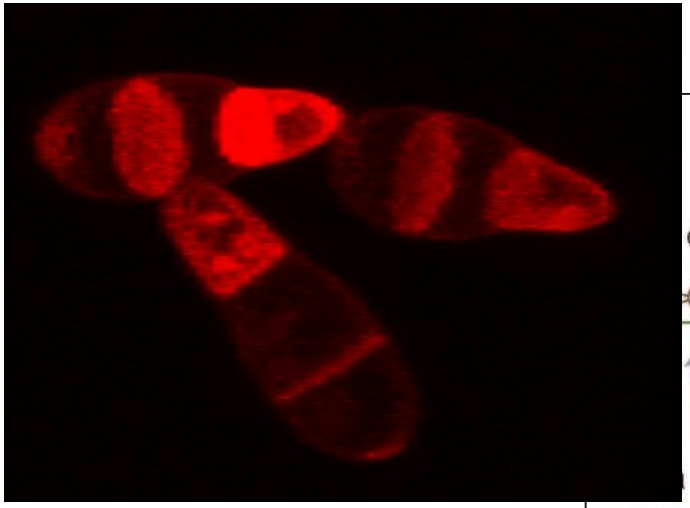

- **GPI** (GlycosylPhosphatidylInositol) **Anchored Proteins** = **GAP**
  - Cell wall modifying enzymes
  - e.g. **G**lucan **El**ongation (**Gel**) proteins elongating ß-1,3-glucan chains

## *Magnaporthe oryzae* the most devastating pathogen of rice!

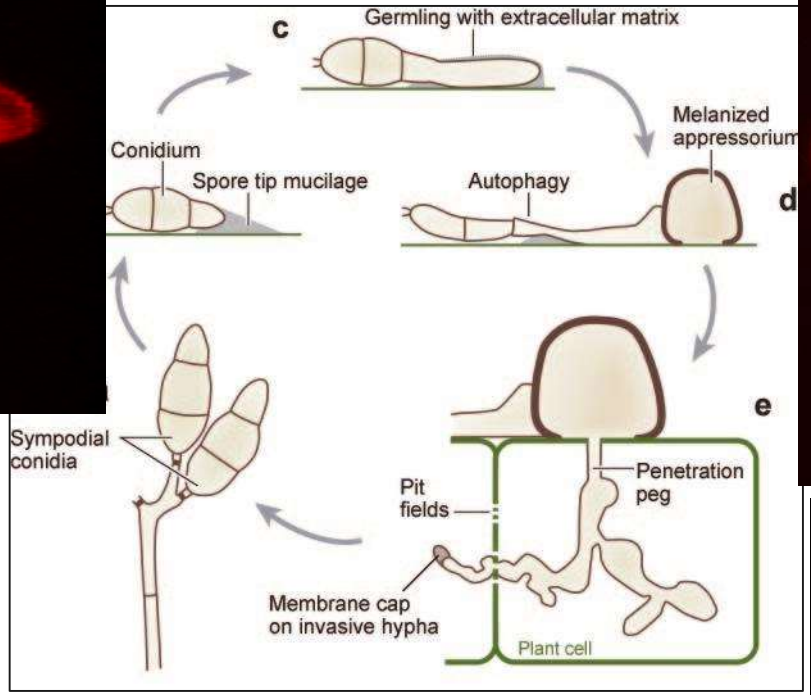
- **Model organism for plant pathogens: 1st sequenced** (Dean *et al.*, 2005)
- Hemibiotrophic filamentous *Ascomycete* fungus causing **rice blast**!
- Haploid, short (asexual) life cycle, gene deletions by homologous recombination.

### ➢ Food security & climate change

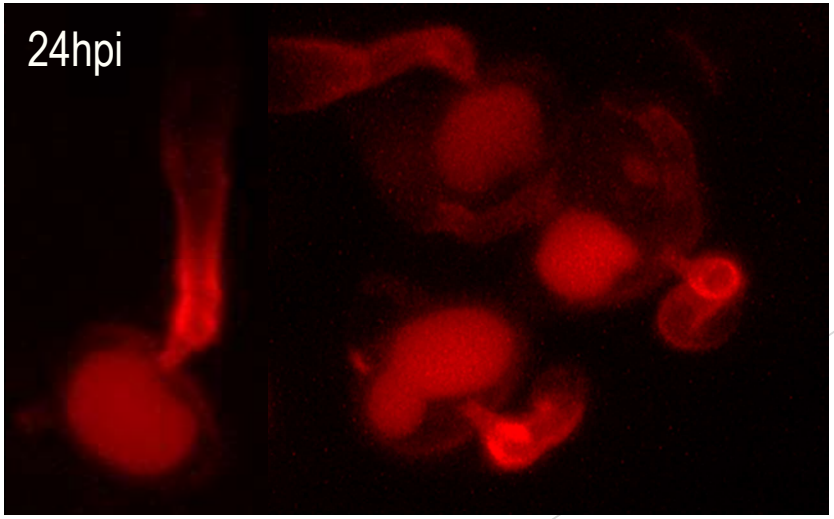
# *Magnaporthe oryzae* asexual life-cycle



**pGEL3::mCherry:GEL3**



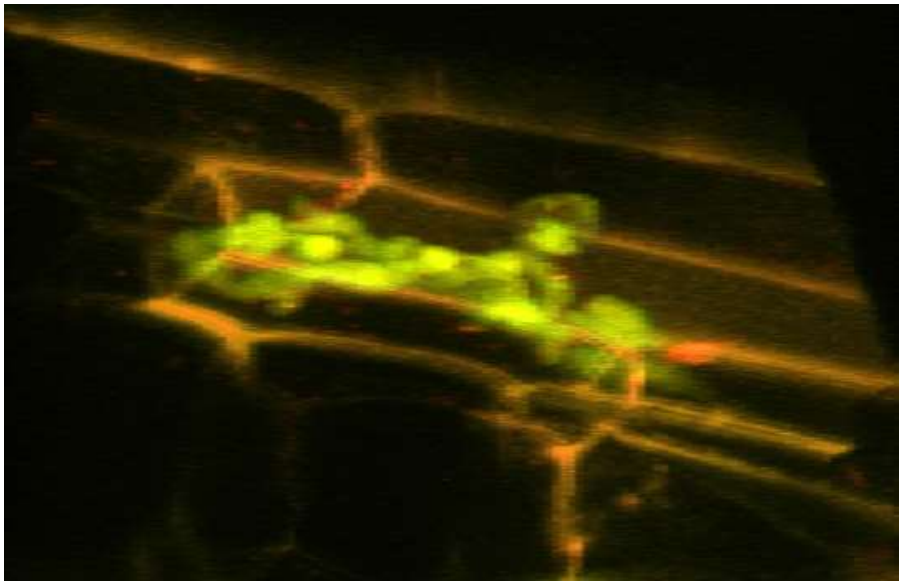


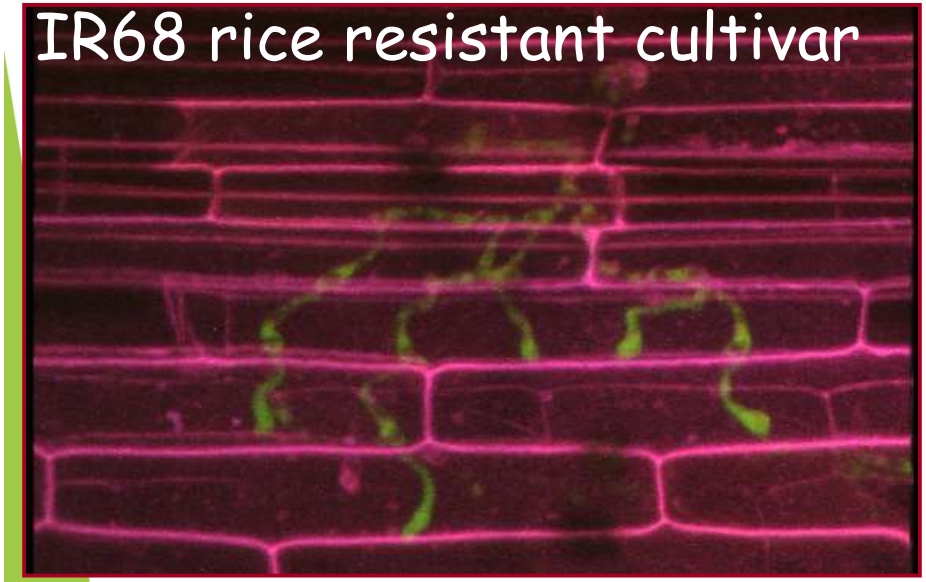



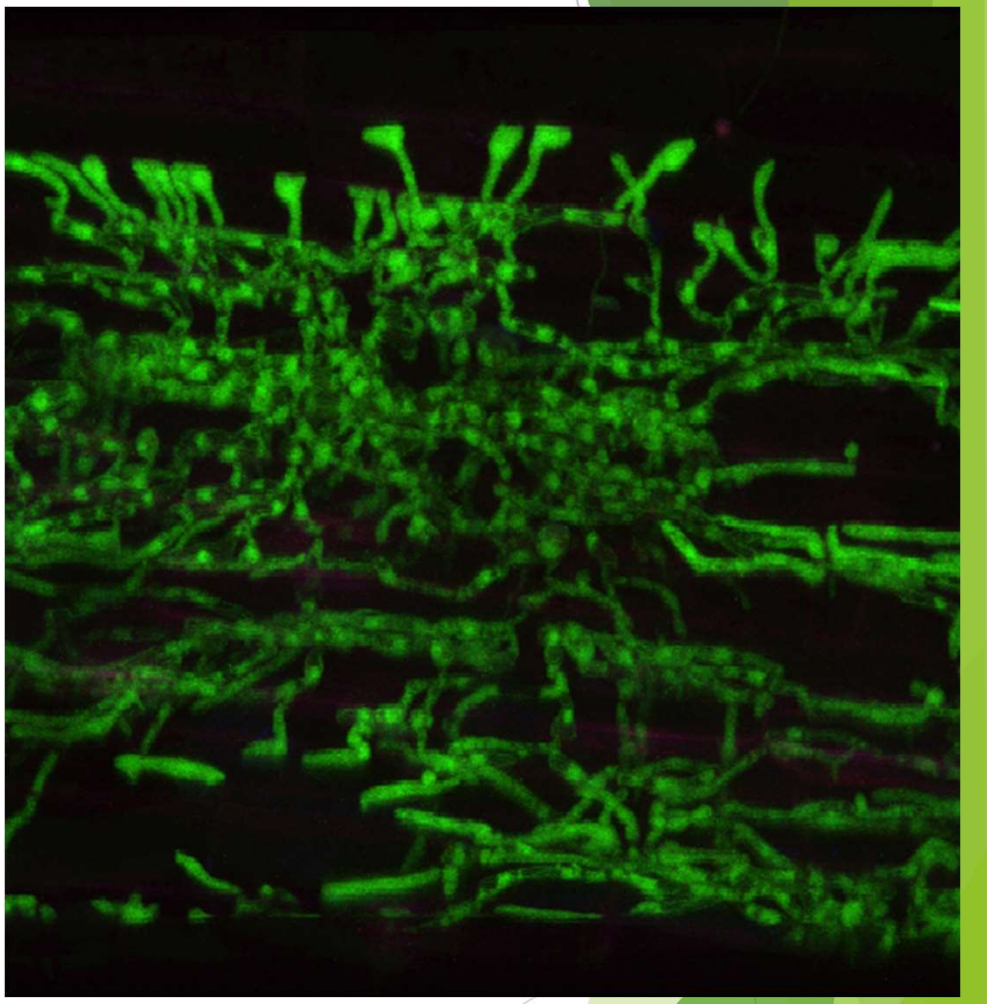


Samalova *et al.*, 2017

# Exploring redox state in susceptible & resistant rice

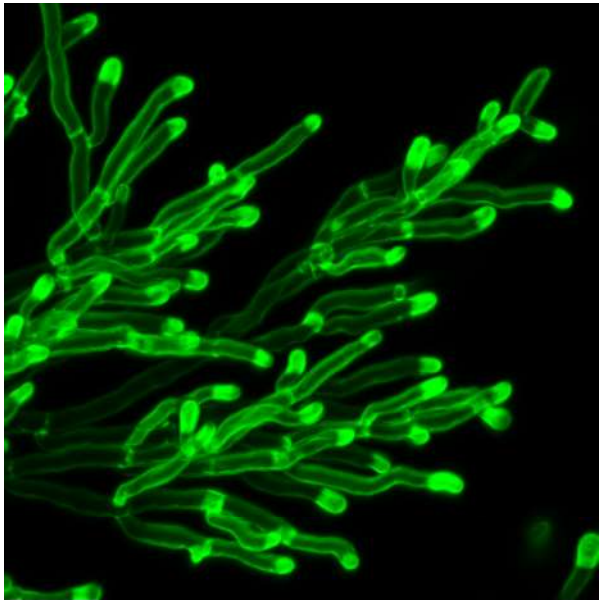




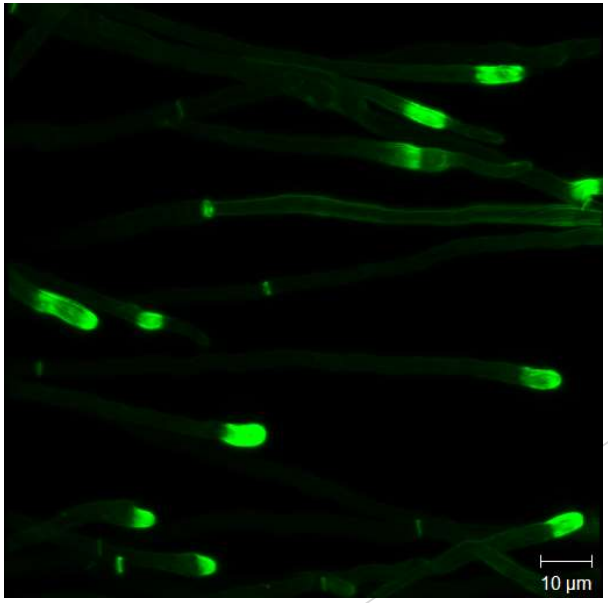



- ROS toxicity alone is NOT sufficient to kill *Magnaporthe oryzae* in resistant rice! (Samalova *et al.*, 2013; 2014)

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

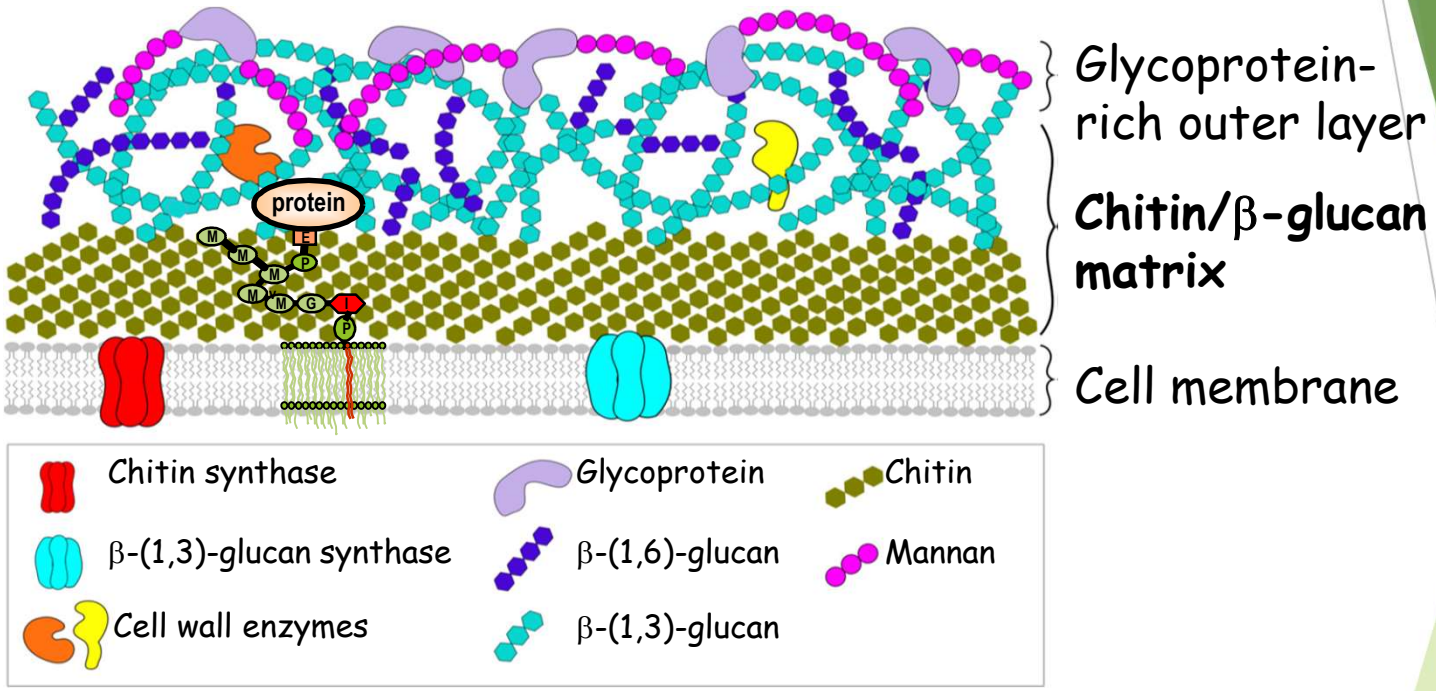








# Unique composition of the fungal cell wall

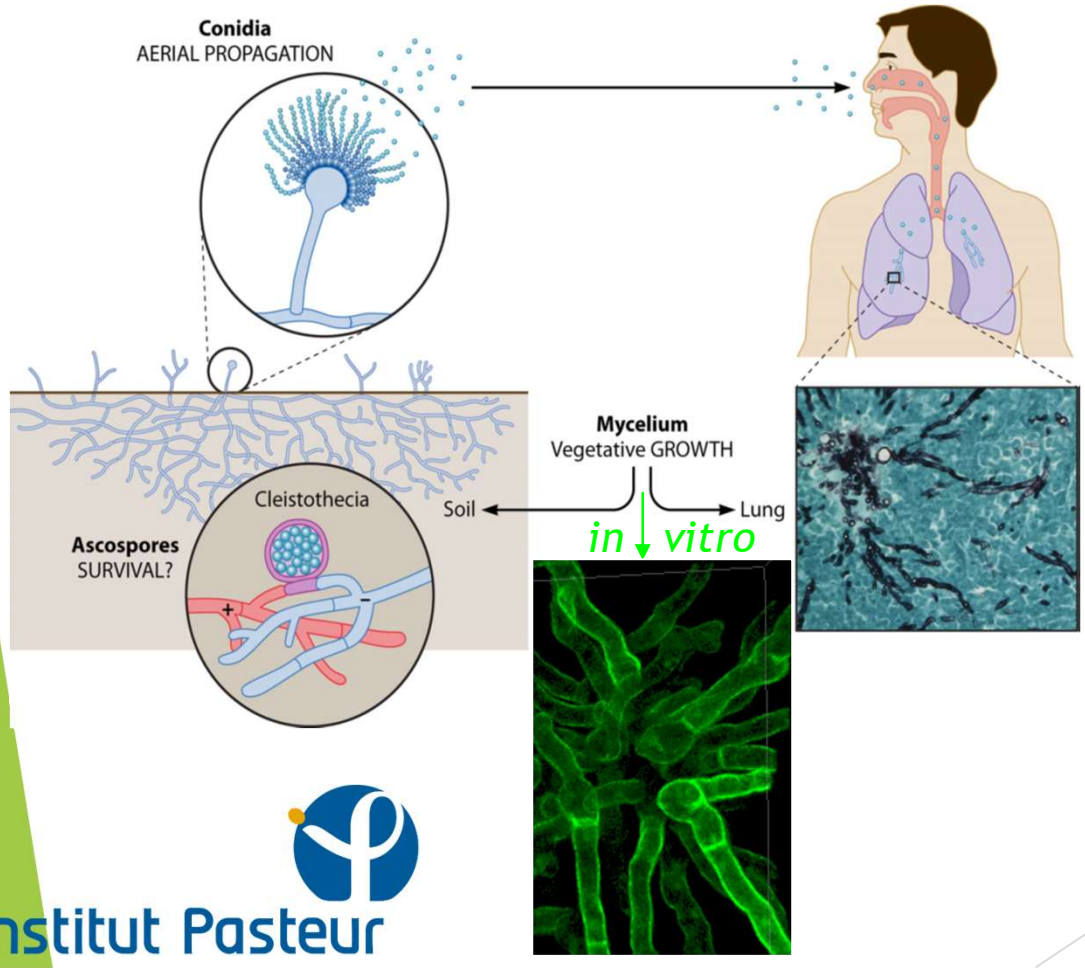
- *makes it an ideal target for the development of **fungicides!***

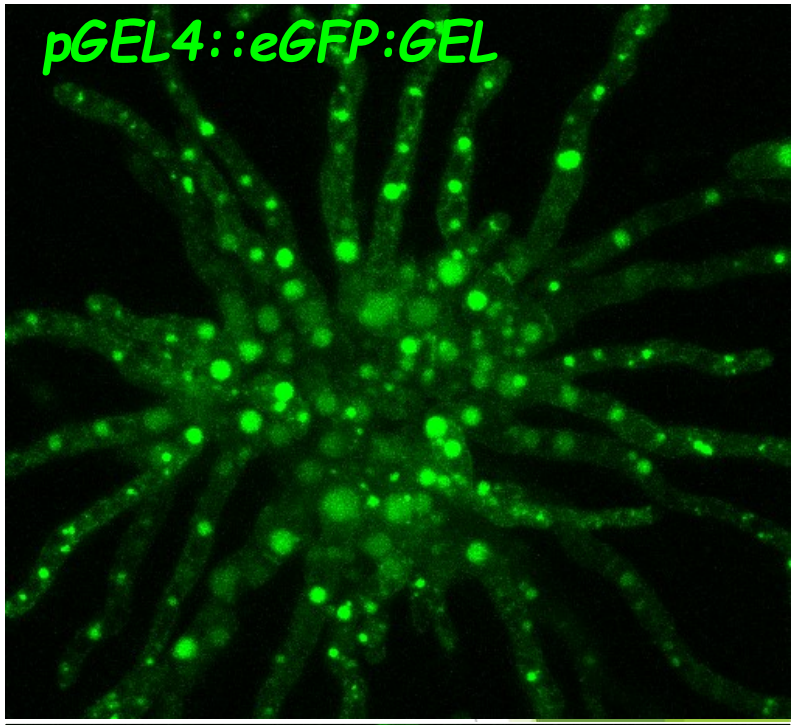


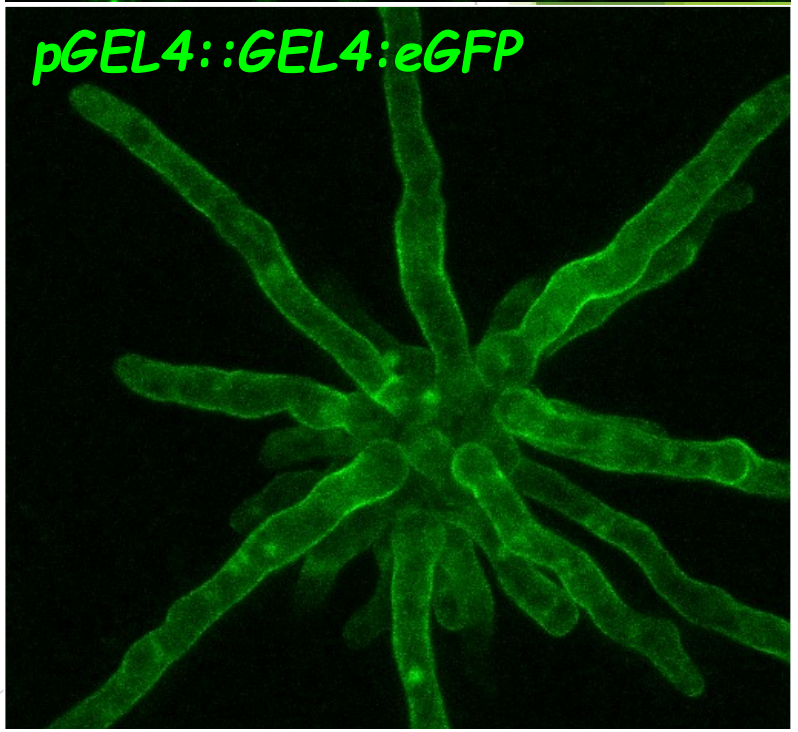

- **GPI** (GlycosylPhosphatidylInositol) **Anchored Proteins** = **GAP**
  - Cell wall modifying enzymes
  - e.g. **G**lucan **El**ongation (**Gel**) proteins elongating ß-1,3-glucan chains

# *Aspergillus fumigatus* is a fungal saprotroph BUT opportunistic human pathogen!

- Causes aspergillosis in immunocompromised patients…. deadly

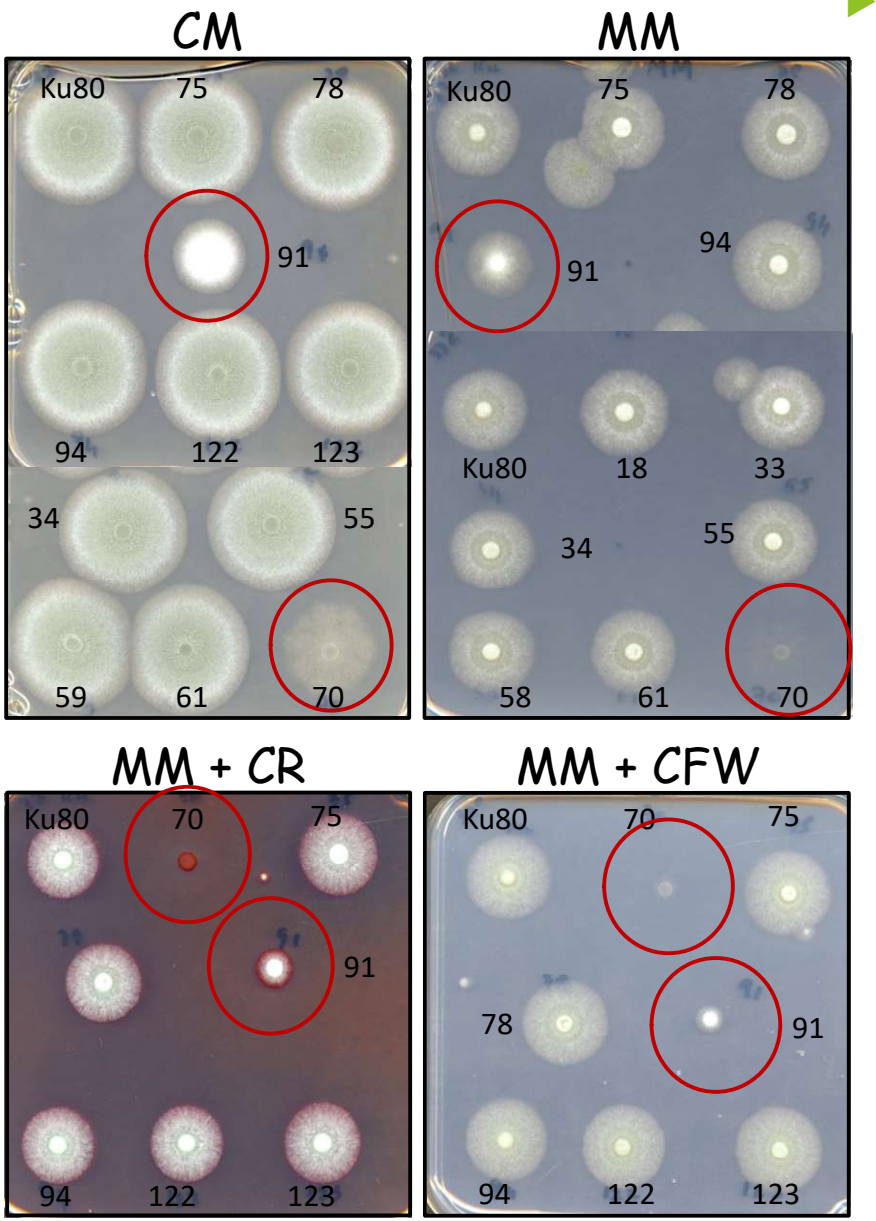


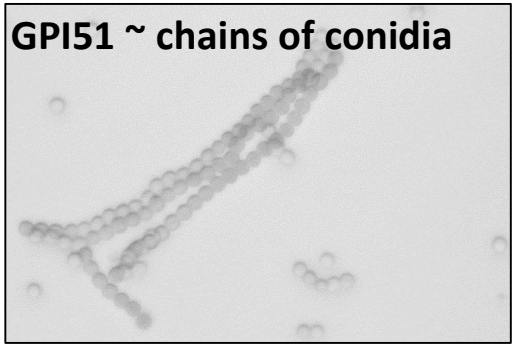




Institut Pasteur

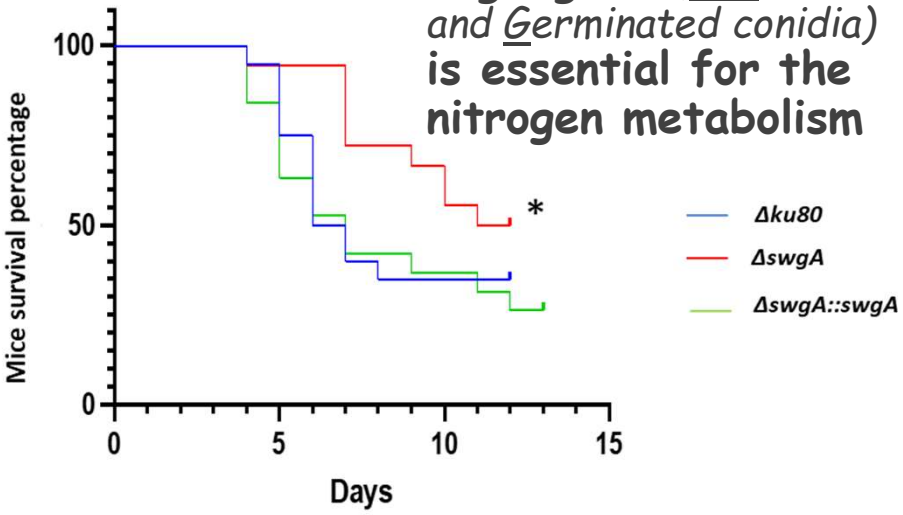
# How to knock-out 132 genes in one summer . . .




- **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

# Thank you for your attention!

# Acknowledgement

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