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Appressorium

THE BREAKTHROUGH IN *DIKARYA*

Alexander Demoor
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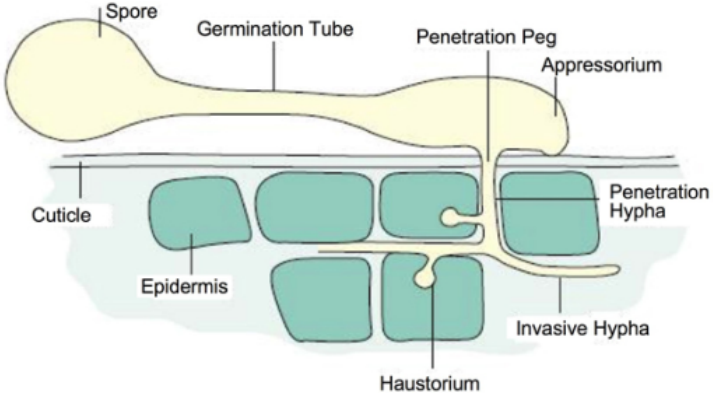
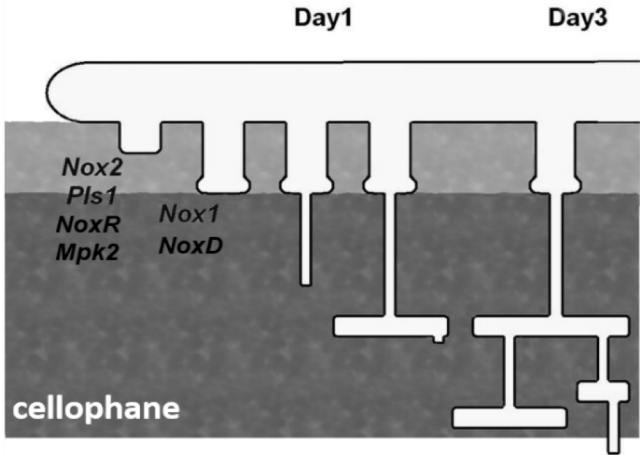
What's an appressorium?

- Emmett & Parbery, 1975: "All structures adhering to host surfaces to achieve penetration, regardless of morphology"



***M. oryzae* appressorium**

Meng *et al.*, 2009

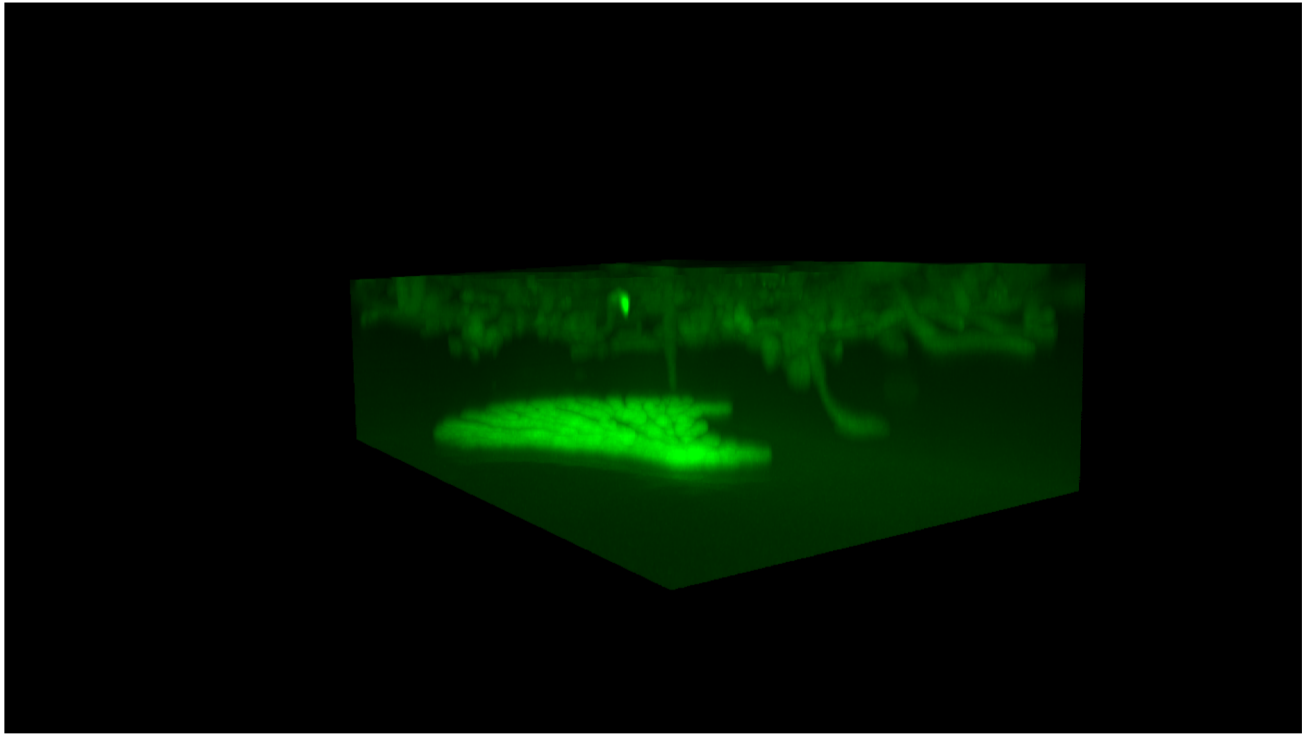


***P. anserina* appressorium**

Brun *et al.*, 2009

Podospora's appressorium

- *Podospora anserina* tagged with cytoplasmic GFP



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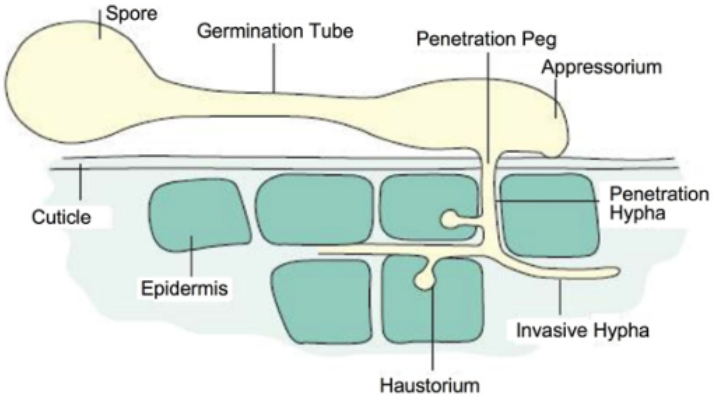

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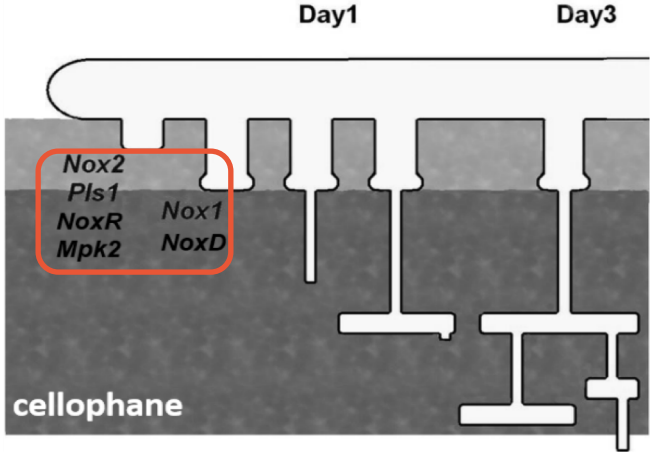


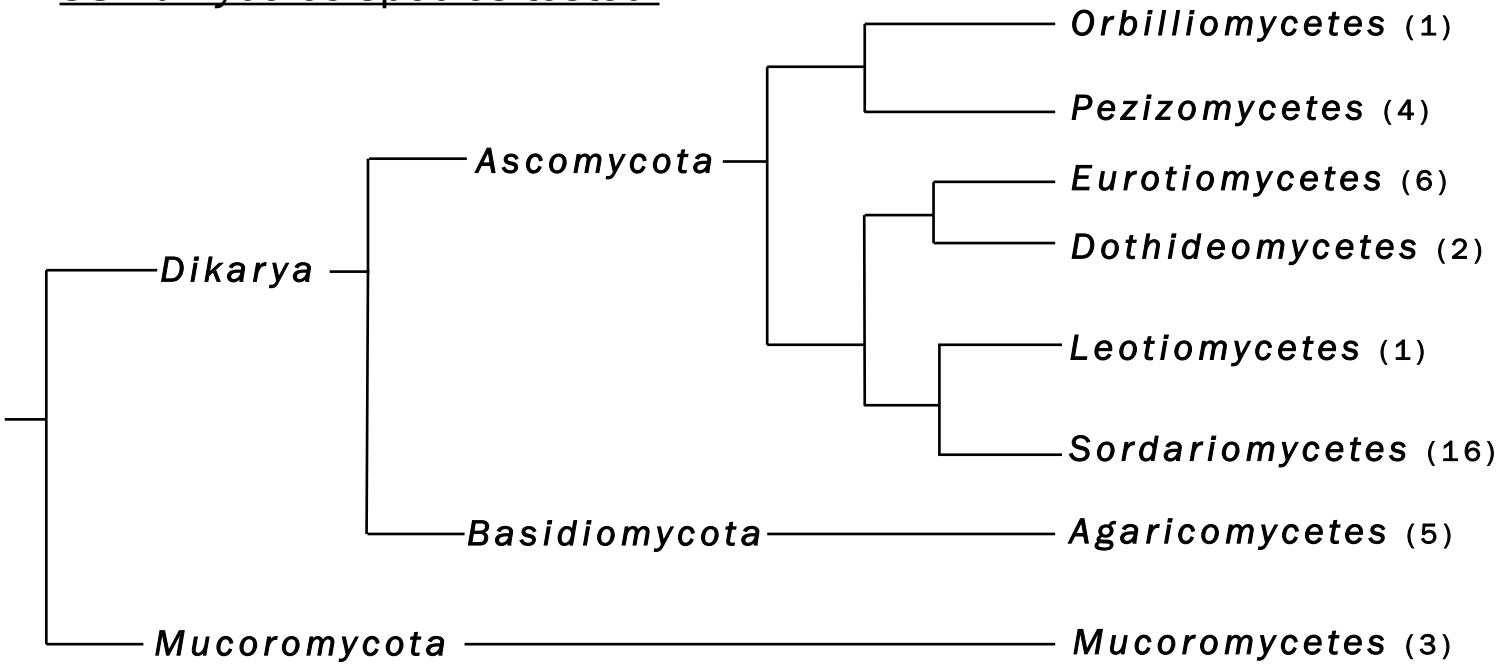
***P. anserina* appressorium**

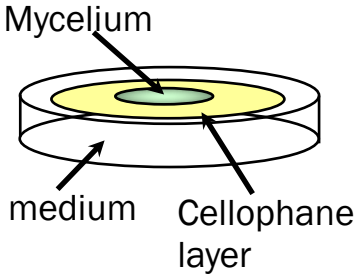
Brun *et al.*, 2009

- The appressorium is not exclusive to pathogenic species!
- Homologous structures
- Is this structure widespread among saprotrophic *Eumycetes*?

What about other species?

- 38 *Eumycetes* species tested:





- 24/38 (63%) species tested develop an appressorium in our conditions
- Appressorium development is widespread among saprotrophic *Dikarya*

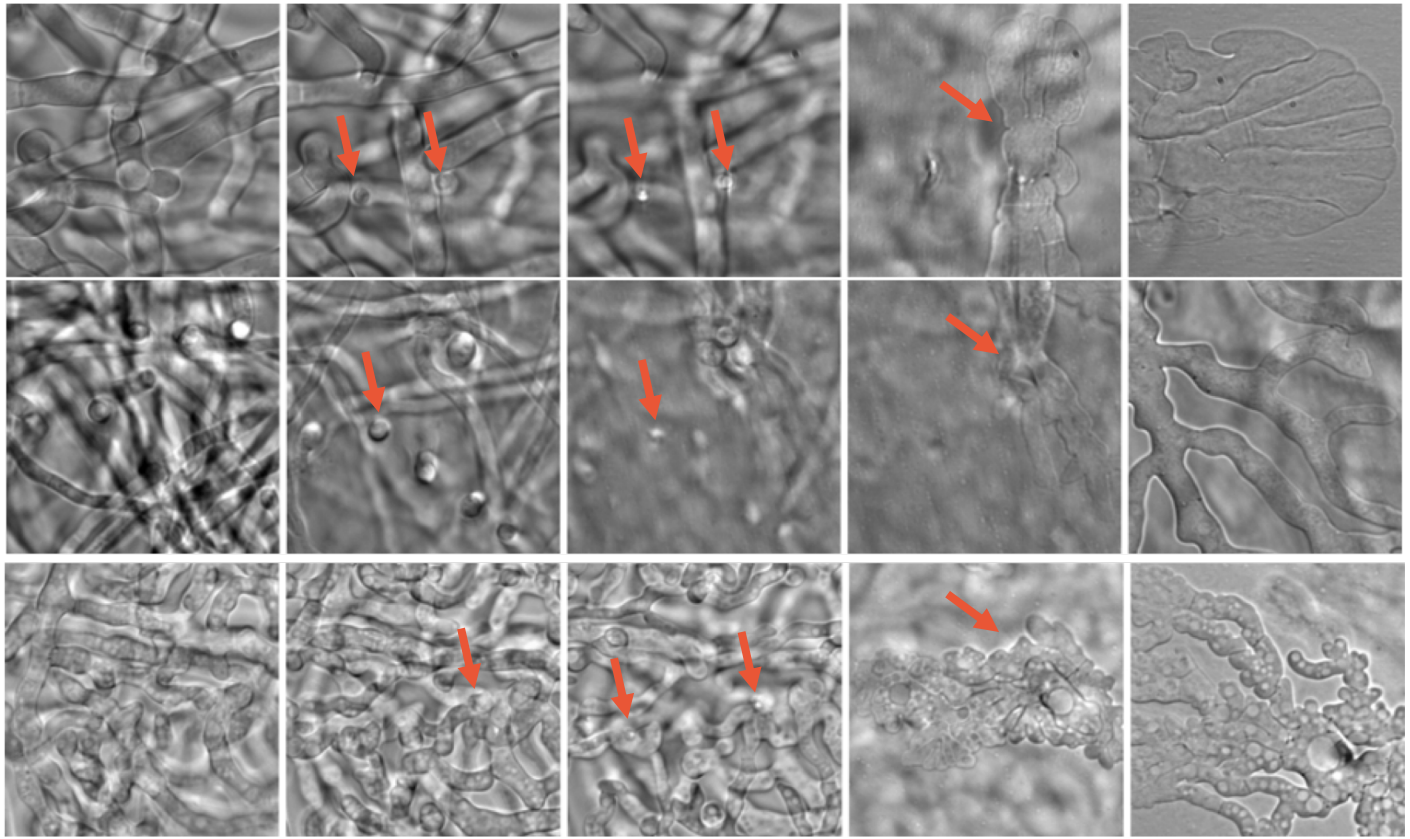
Demoor *et al.*, 2019

Some species of interest develop an appressorium

Trichoderma reesei
Hypocreales

Neurospora crassa
Sordariales

Sordaria macrospora
Sordariales



Demoor *et al.*, 2019

A breakthrough in *Eumycetes*?

- The appressorium is an adhesion and penetration structure encountered in numerous fungal species: pathogenic/symbiotic/saprotrophic

- The appressorium is an ancestral feature among *Dikarya*

- What about other *Eumycetes*?
  - Could some *Mucoromycetes* actually develop an appressorium?
  - Some *Glomeromycota* species can differentiate appressoria
  - Test other *Eumycetes* species

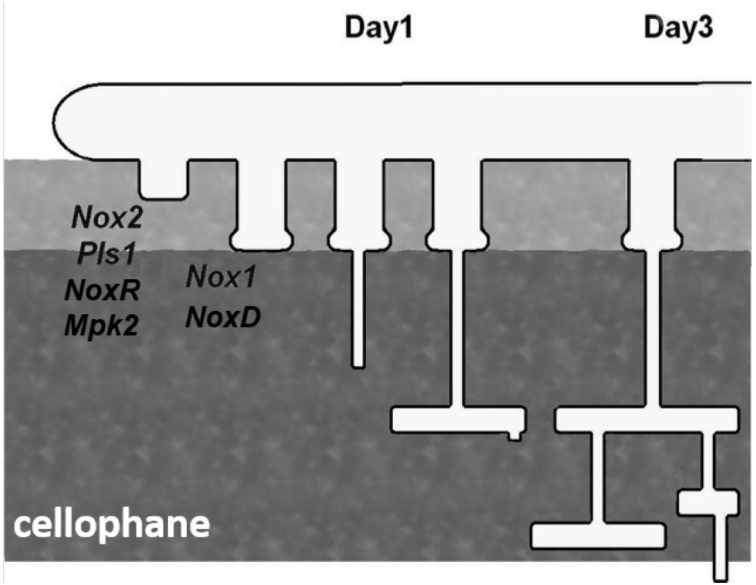
    ↳ When did the appressorium appear during the evolution?

- What about the genetic program of appressorium development?

Podospora anserina: a genetic model

- *Ascomycota*
- Genome sequenced: 35 Mb, 7 chromosomes
- Non-pathogenic
- Easily cultured in the lab
- Fast growing: 7mm per day
- One-week sexual reproduction cycle
- Easy molecular genetics studies

How to study its genetic program?



Aim: Identify the genes involved in this mechanism

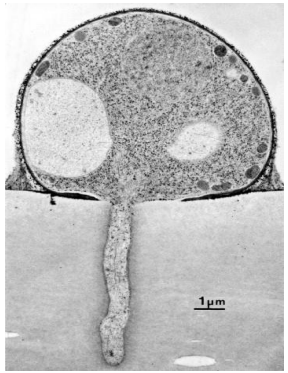
→ Screening for suppressors restored for penetration

↳ No new gene identified → New strategy

How to study its genetic program?

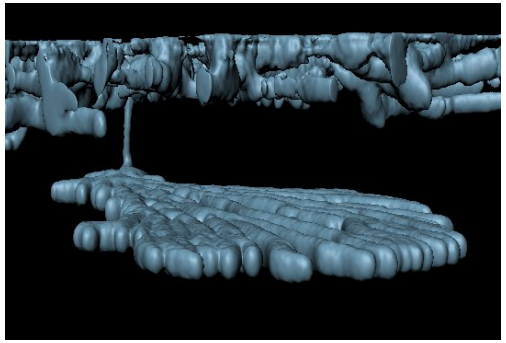
Pls1, Nox2 and Mpk2

Appressorium differentiation



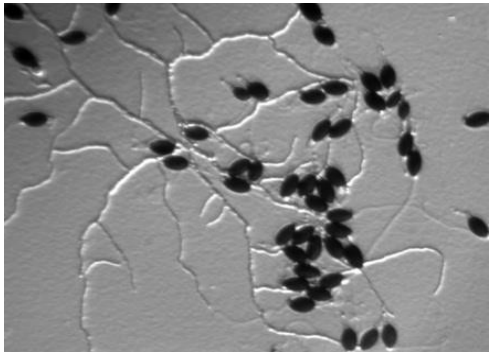
M. oryzae

Appressorium differentiation



P. anserina

Ascospore germination



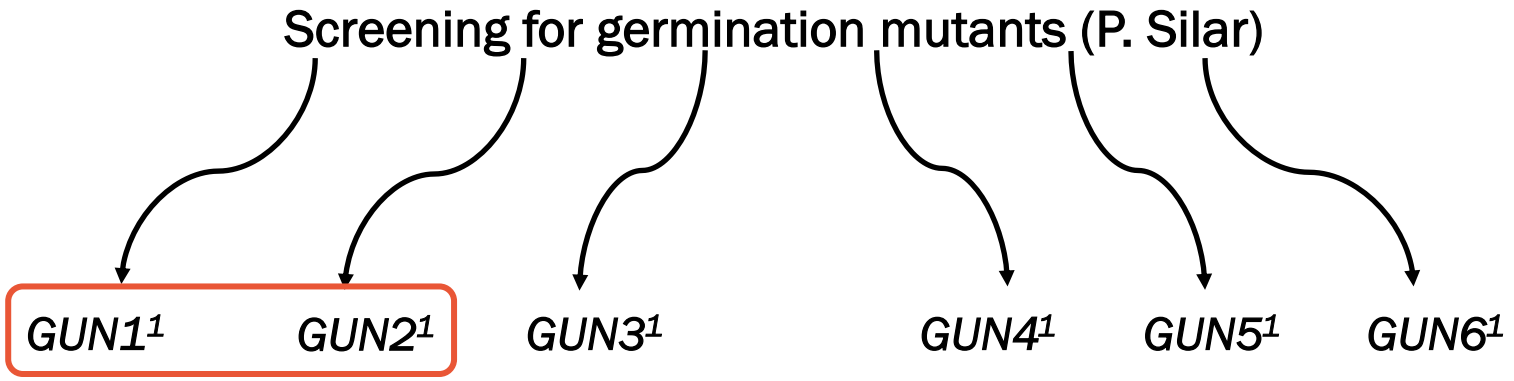
P. anserina

➢ Share common regulating elements

Lambou *et al.*, 2008

The AGADFUN project

- Ascospore Germination and Appressorium Development in FUNgi
- **Combined study**: Study germination mutants to identify genes involved in both ascospore germination and appressorium development



- 6 *Germination UNcontrolled* (*GUN*) mutants sequenced; 1 candidate gene for each
- Unravel the regulation pathways of both processes

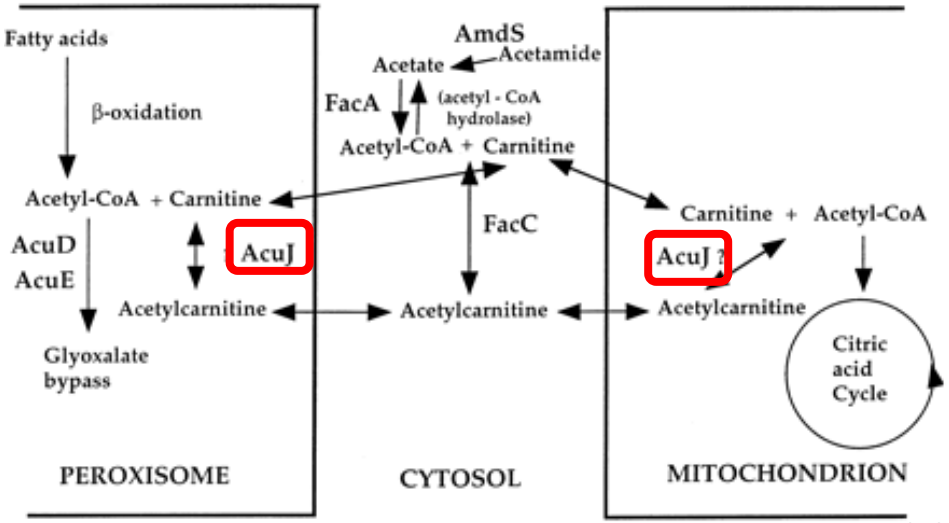
The *GUN2*[1] mutant

- *GUN2*: Transcription factor (Gal4 family)
- Never studied in filamentous fungi
- Involved in:
  - The control of germination: *ΔGUN2* germinates spontaneously
  - The appressorium formation: *ΔGUN2* has a delay in appressorium formation
- Transcriptomics analysis of *ΔGUN2* during germination: in progress

→ Identification of new actors of both pathways

The *GUN1*[1] mutant

- Point mutation in *Pa_6_1340* (*GUN1*) which encodes a perox/mito Carnitin Acetyl-transferase (CAT)



A. nidulans

Stemple *et al.*, 2010

GUN1=AcuJ

- Identified in *M. oryzae* as virulence factor (*Pth2;* Bhambra *et al., 2006*)
  - Validates our approach

Study of the *GUN1*[1] mutant

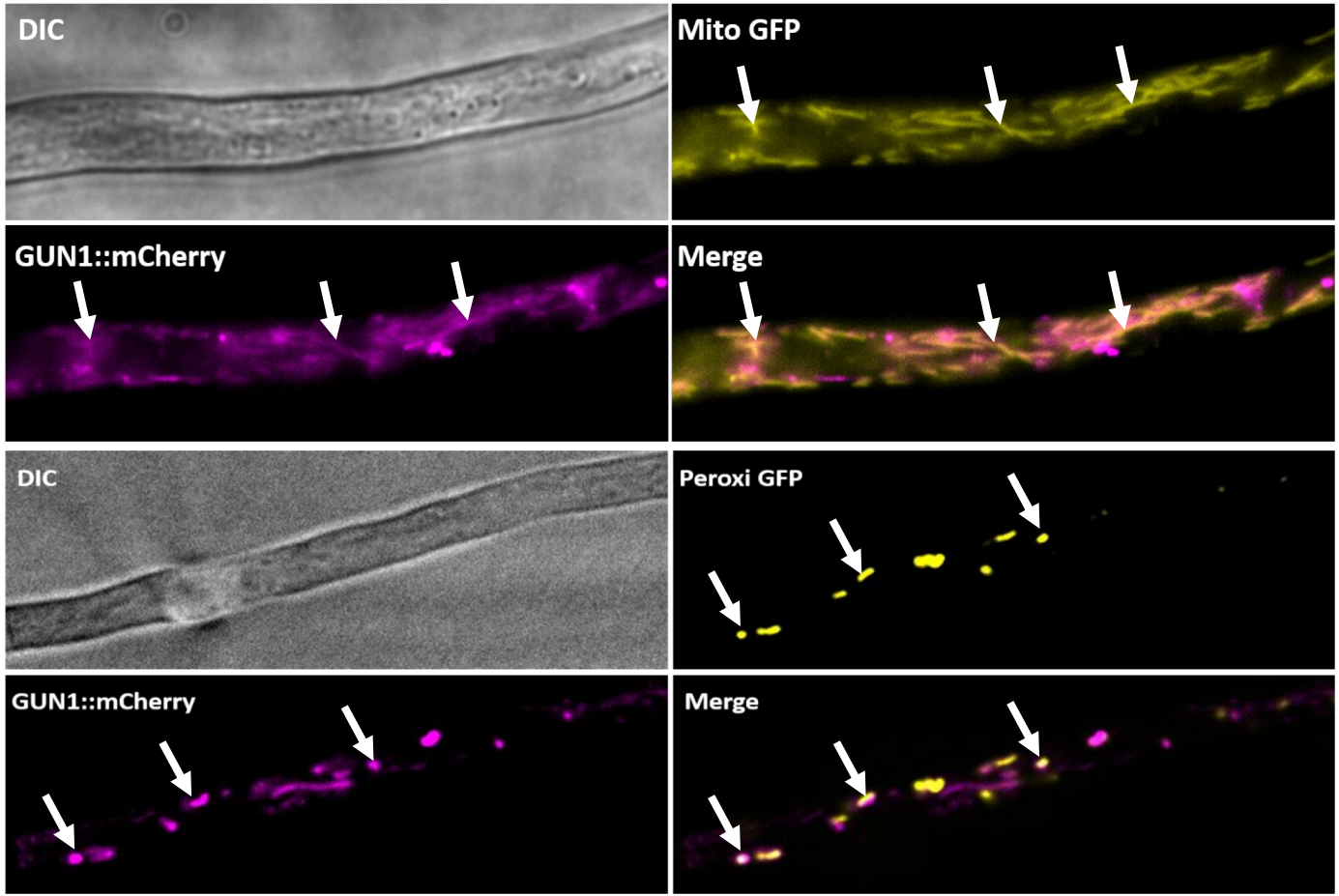
- *GUN1* is involved in:
  - The control of germination: *ΔGUN1* does not germinate
  - The setting up of appressorium: *ΔGUN1* has a delay of appressorium formation

- Tagging of the GUN1 protein:

The *GUN1*[1] mutant

GUN1::mCherry::AKI, mito-GFP

GUN1::mCherry::AKI, peroxi-GFP



Study of the *GUN1*[1] mutant

- *GUN1* is involved in:
  - The control of germination: *ΔGUN1* does not germinate
  - The setting up of appressorium: *ΔGUN1* has a delay of appressorium formation

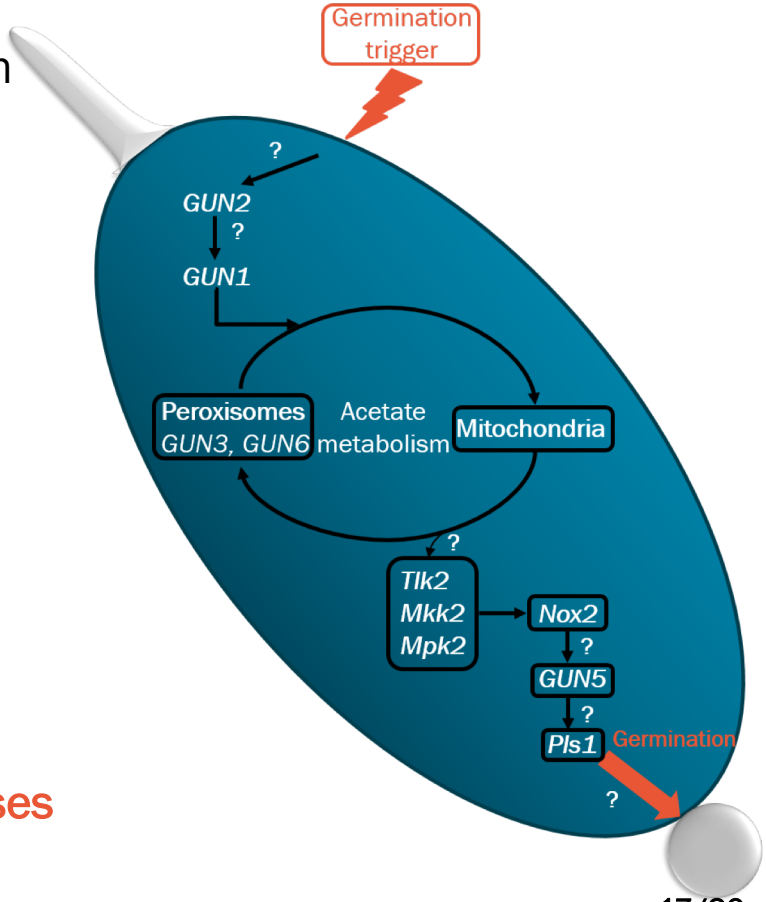
- Tagging of the GUN1 protein:
  - Both peroxisomal and mitochondrial
  - Results for the mutant protein in progress: different localization?

- Epistasis studies place *GUN1* upstream of Mpk2 and Pls1/Nox2 in the pathway

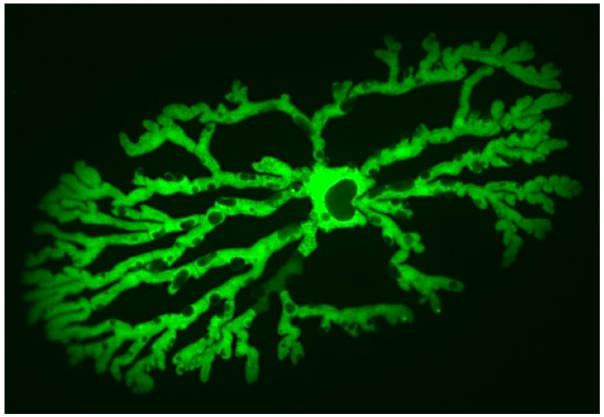
- Study of *GUN1*[1] mutant: Understand the role of the mitochondria and peroxisomes in ascospore germination and appressorium development

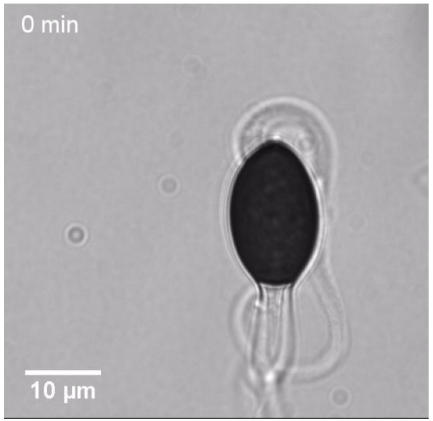
The *GUN* mutants

- Study both ascospore germination and appressorium development
- This strategy allowed to find a gene involved in pathogenicity
- Unravel their regulation pathways
- Candidate genes for other *GUN* mutants also connected to the acetate metabolism
- Promising strategy to find new actors of both processes



Take home message





1. The appressorium refers to all fungal mechanical penetration structures
2. It is widespread among *Dikarya*
3. *Podospora anserina* is a model species allowing us to find new regulators in these processes
4. Combined study of both ascospore germination and appressorium development
5. Find new actors of both pathways

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Thank you for your attention