

# Plant Endoplasmic Reticulum And Seed Productivity (PER ASPERA)



ERA-CAPS

ERA-NET for Coordinating  
Action in Plant Sciences



# PER ASPERA team



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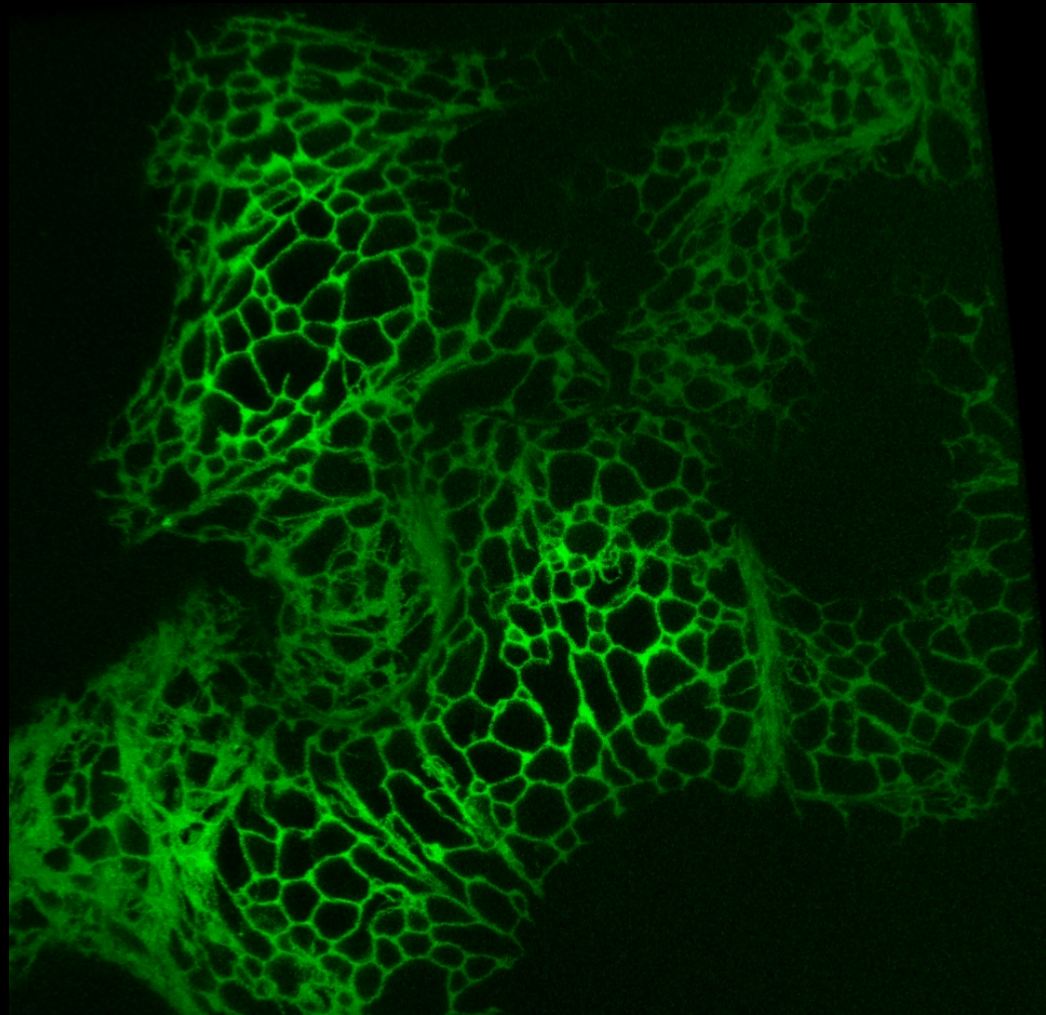


Patrick Moreau

Kick-off meeting:  
Vienna 17-11-14

# The endoplasmic reticulum

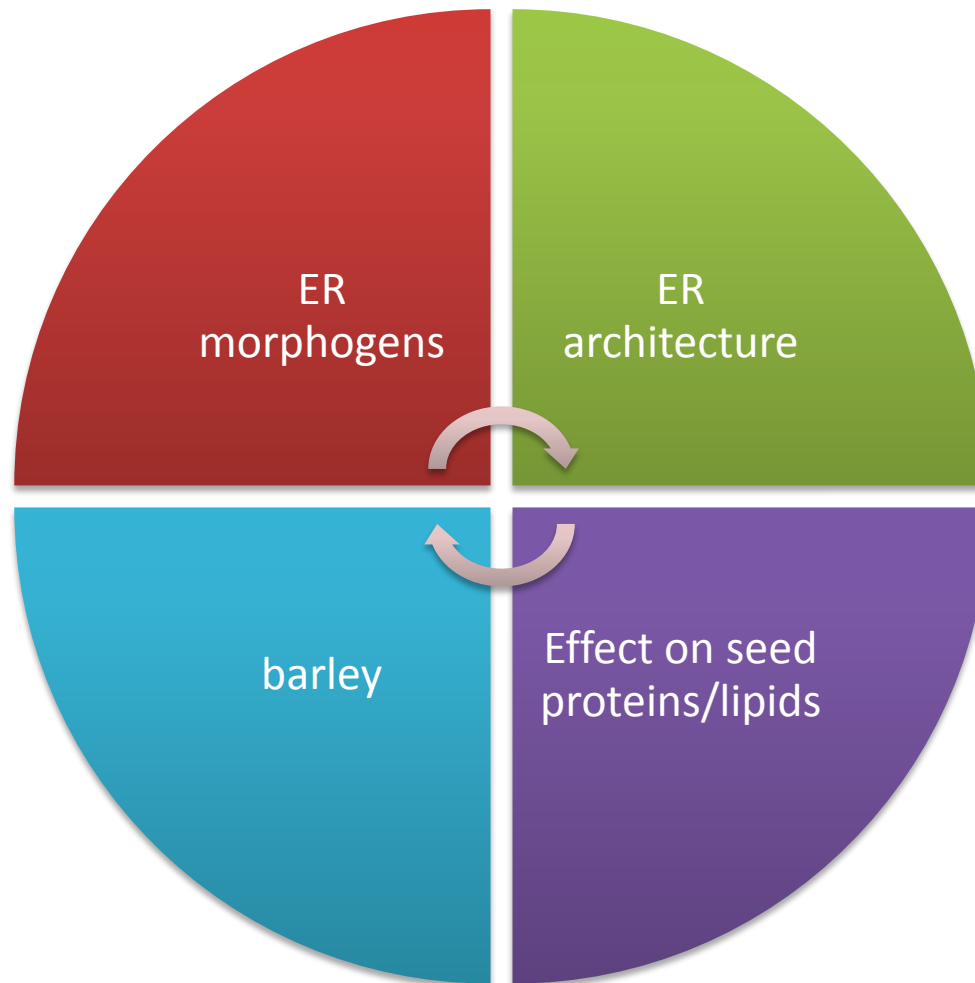
- Gateway of the secretory pathway
- Functions: secretory protein folding, glycosylation, quality control and export, lipid synthesis, biosynthetic scaffold
- The ER can store proteins in cereal seeds
- The ER can exist as membrane sheets or tubules
- **What determines ER structure?**
- **How does ER structure relate to its function?**



tobacco cells expressing GFP-HDEL

- 1) To unravel the machinery involved in shaping the plant ER network  
→ study known ER shaping proteins **and their interactors**
- 2) To analyse ER structure in depth : 3-D architecture
- 3) To study ER interactions/contacts with tonoplast and plasma membrane
- 4) To manipulate the biosynthetic capacity of the ER through manipulation of its morphology
- 5) To establish a cereal model (barley) to study ER structure/function

# Development loop



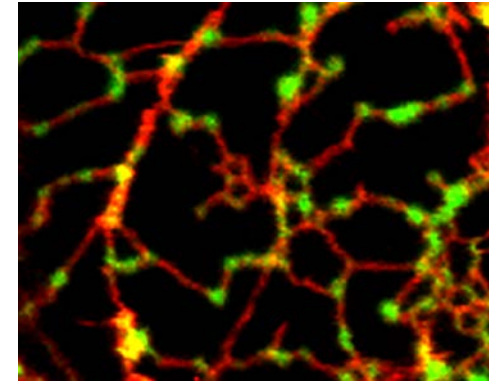
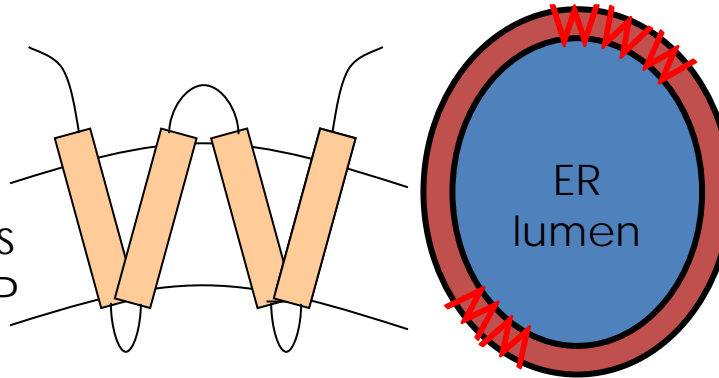


# 1. ER morphogens

## Reticulons

tubule formation

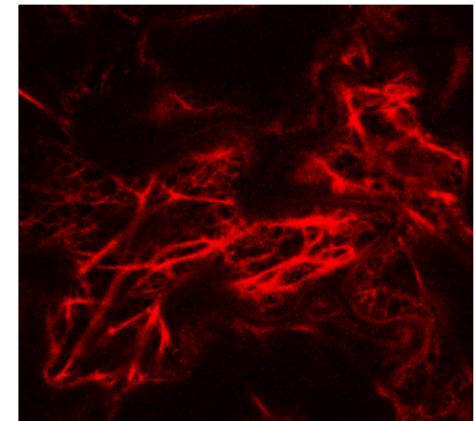
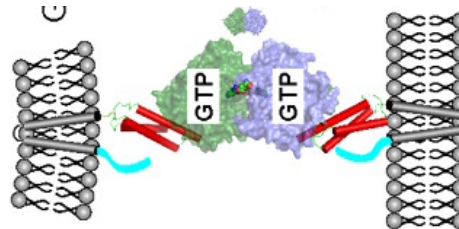
21 genes in Arabidopsis  
Cloned 15, fused to XFP



## Atlastins (RHD3)

tubule fusion

2 genes in Arabidopsis



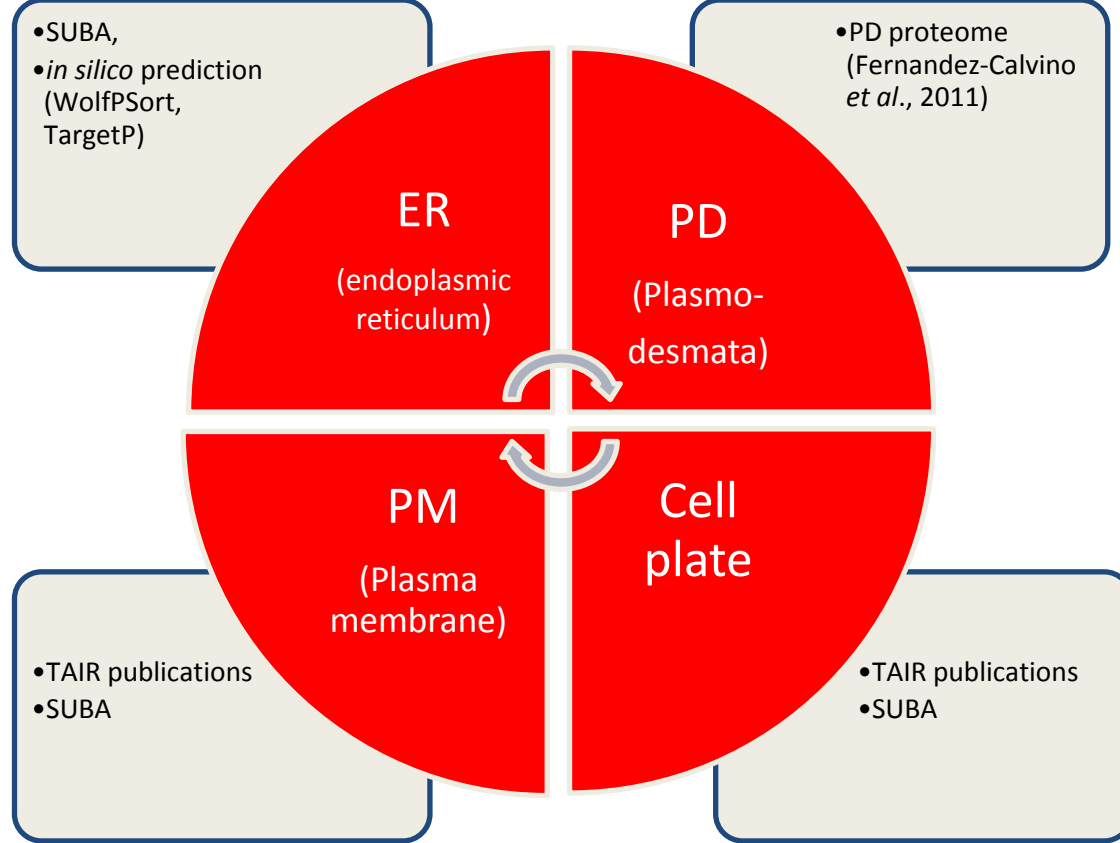
## LNPAK

Located at 3-way junctions

2 genes in Arabidopsis

# 1. ER morphogens

- Focus on reticulons:
  - Seed specific/enriched
  - associated with plasmodesmata
  - most abundant
- identify/validate reticulon - interacting proteins
  - coIP – Successful for RTN 1, 3, 6, 20, 13
  - FRET-FLIM – in progress for 20 candidates



## Expected RTN interactors

## Biosynthetic

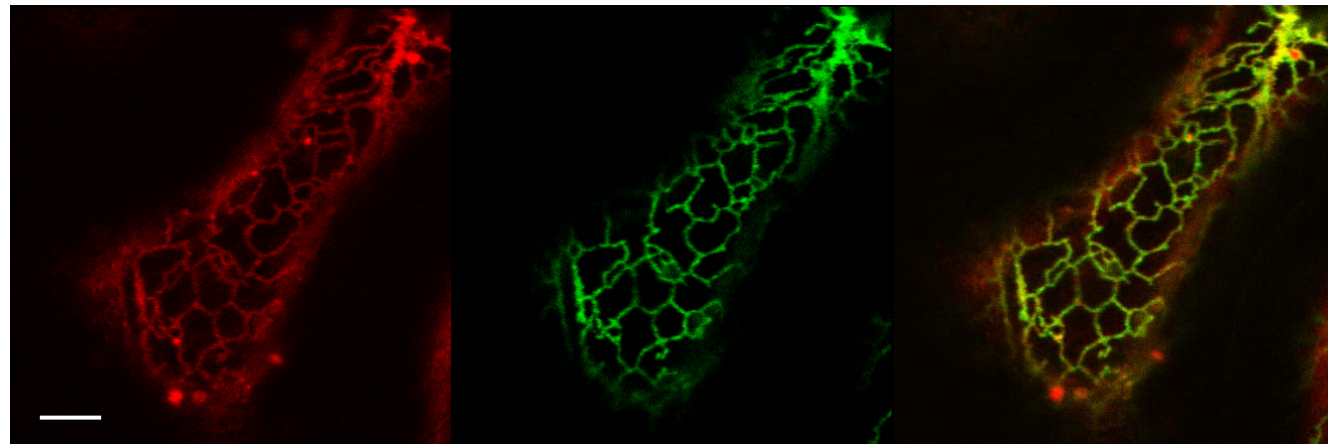
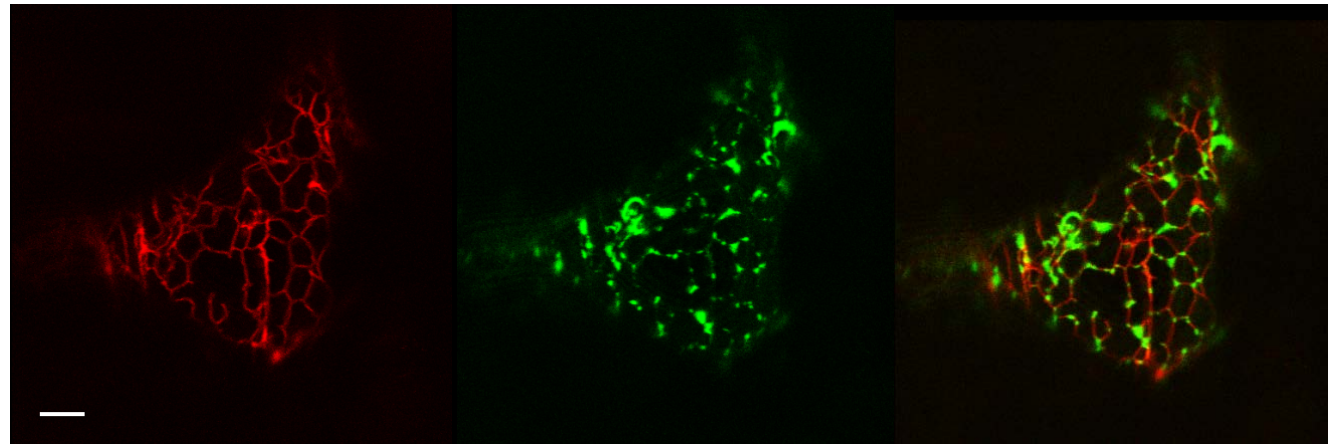
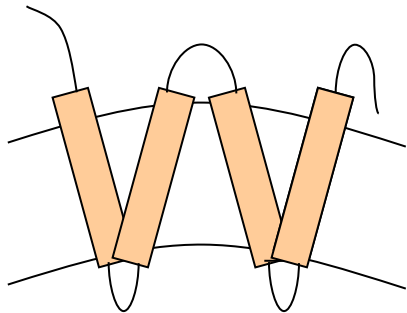
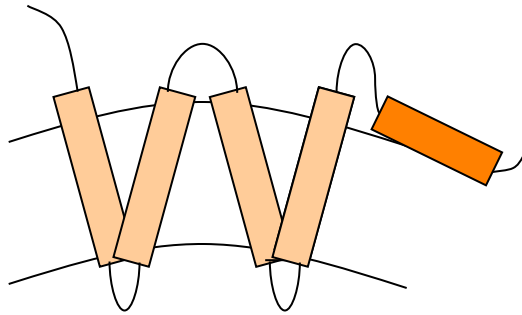
## Structural

- RTN3 and 6
- Vap27 (Wang *et al.* 2014 Curr.Opin.)
- RHD3 (Lee *et al.* 2013 New Phytol.)
- sterol biosynthesis
- glucosinolate biosynthesis
- P450, Cyb5
- reticulons
- remorins
- chaperones



# 1. ER morphogens

A putative amphipathic helix is necessary for RTN13 tubule-forming function

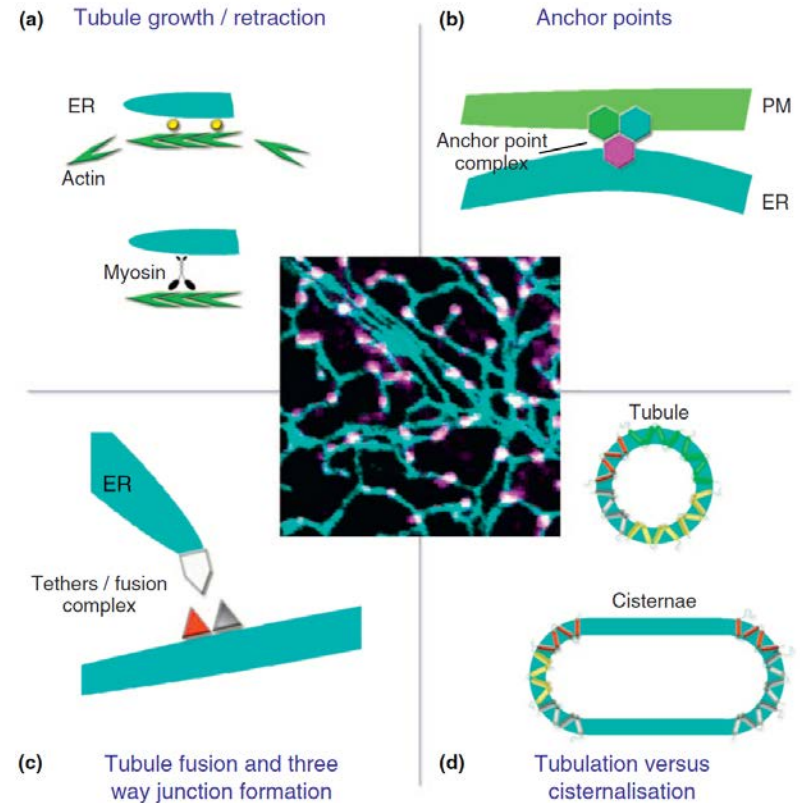
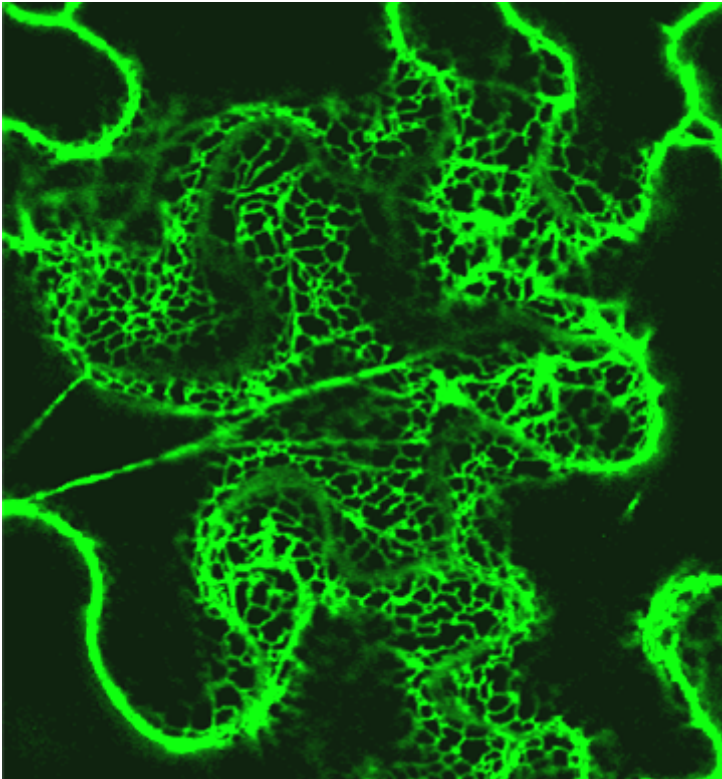


RTN13

ER lumen

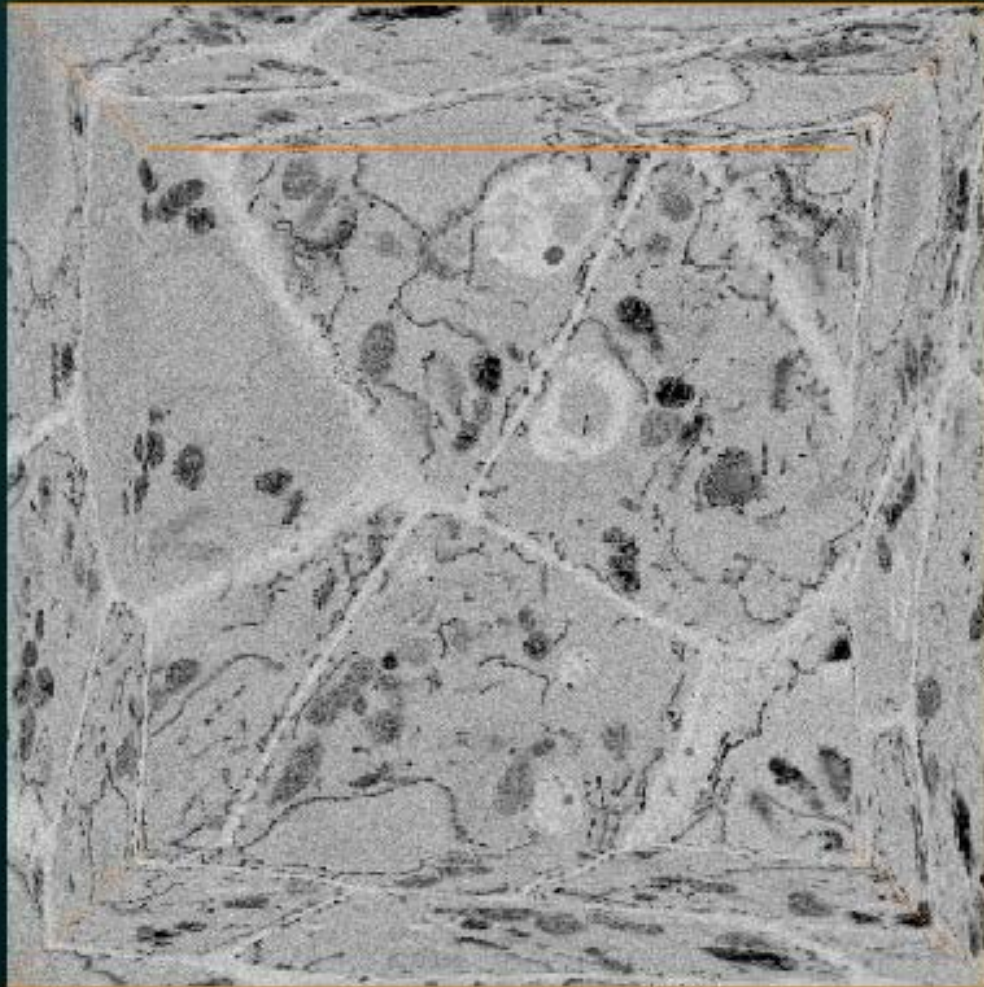
merge

## 2.ER structure



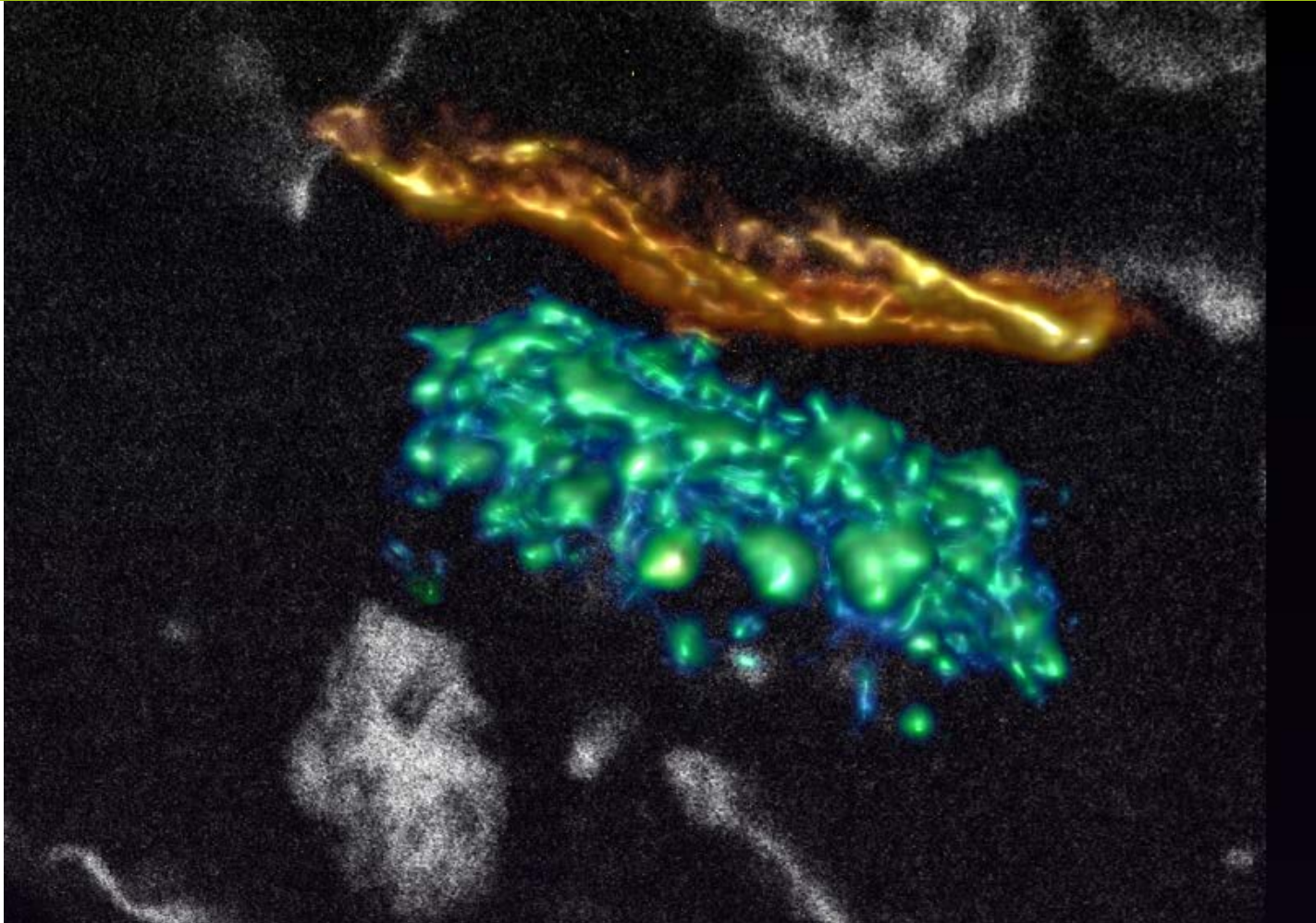
- Understand ER architecture  
by serial block face scanning EM – Gatan 3View

# 3View: whole cells



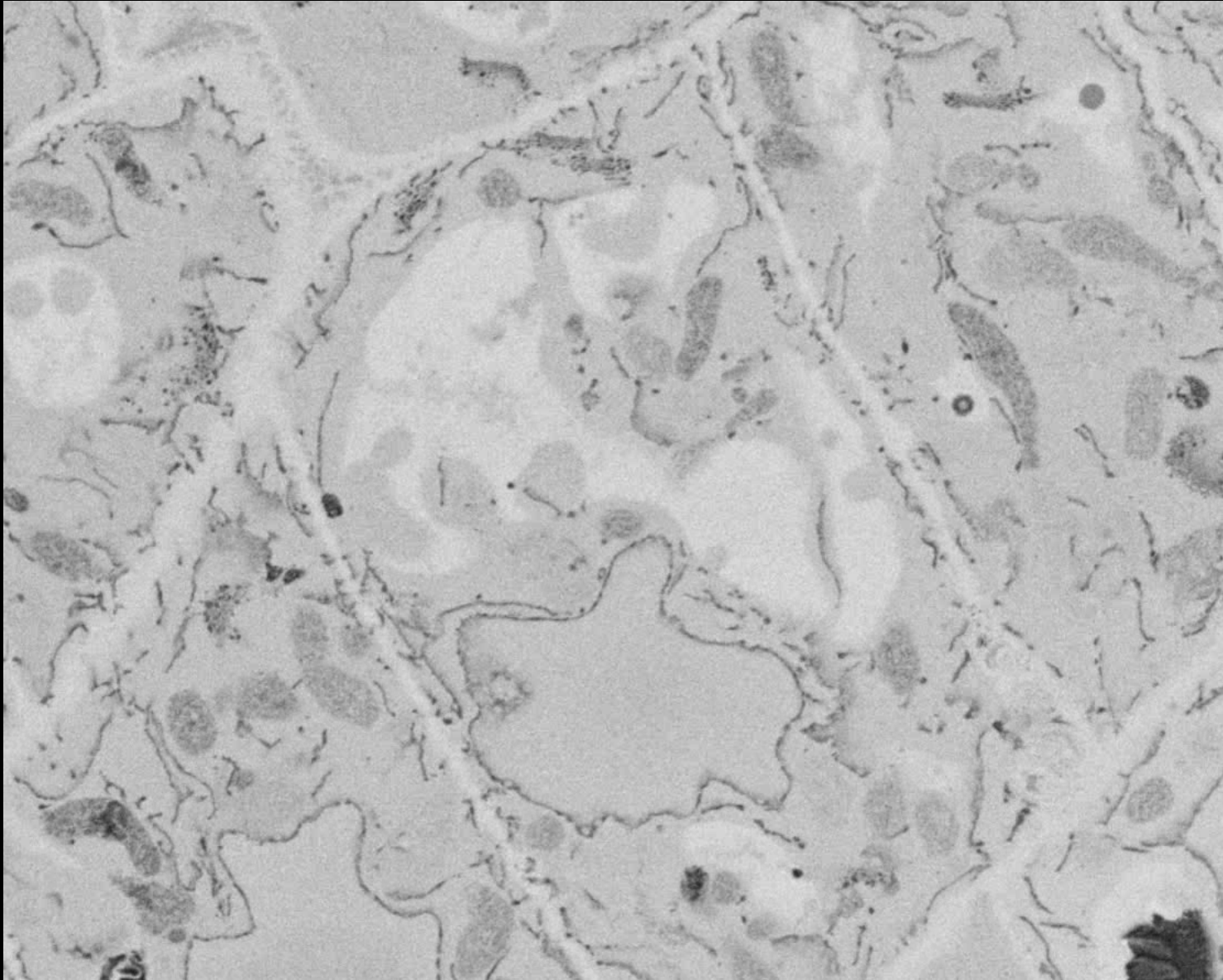


# 3-View: ER-Golgi interface



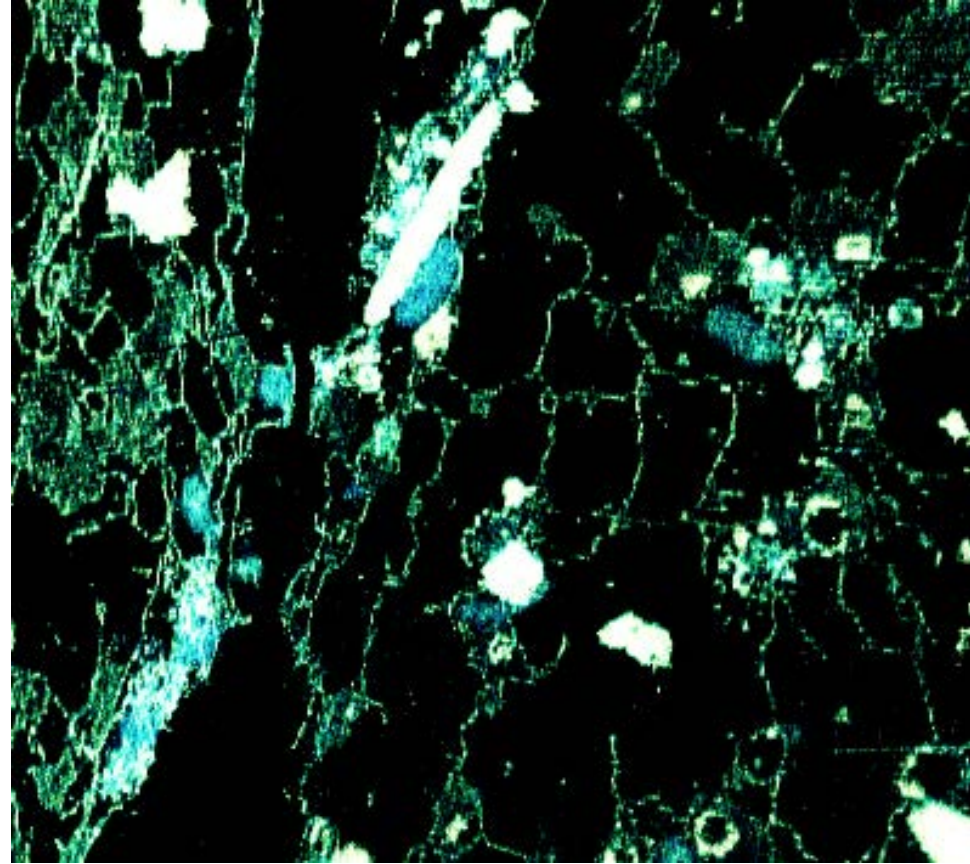
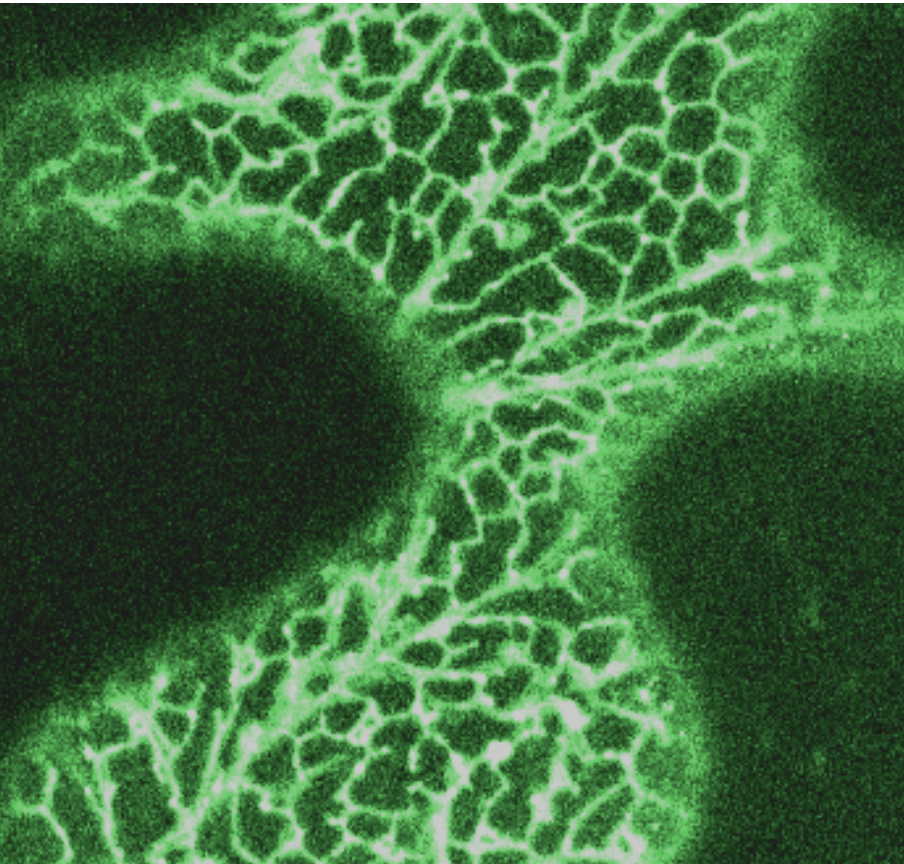
Arabidopsis root tip

## 3D structure - vacuoles





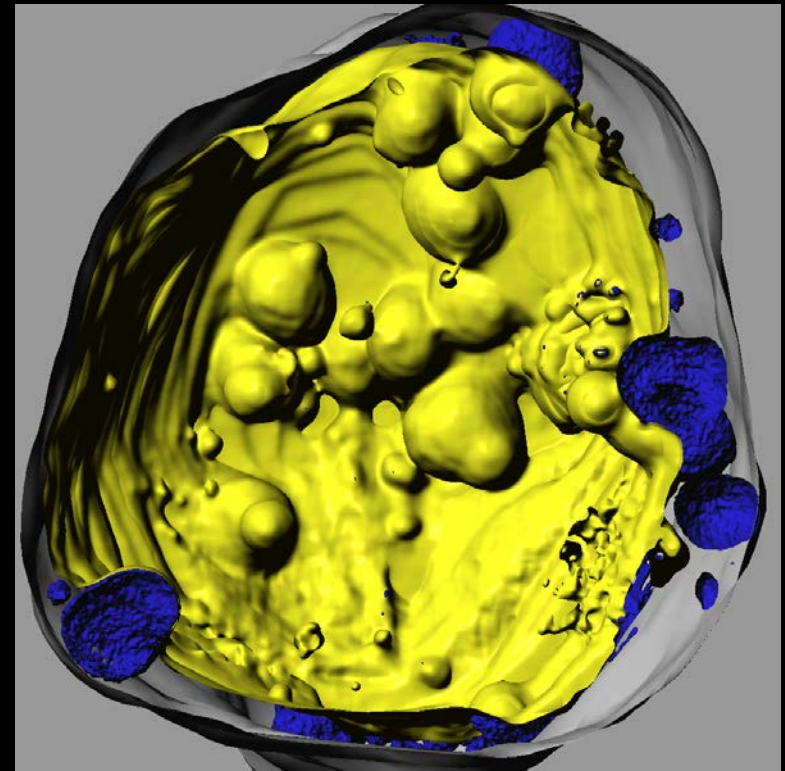
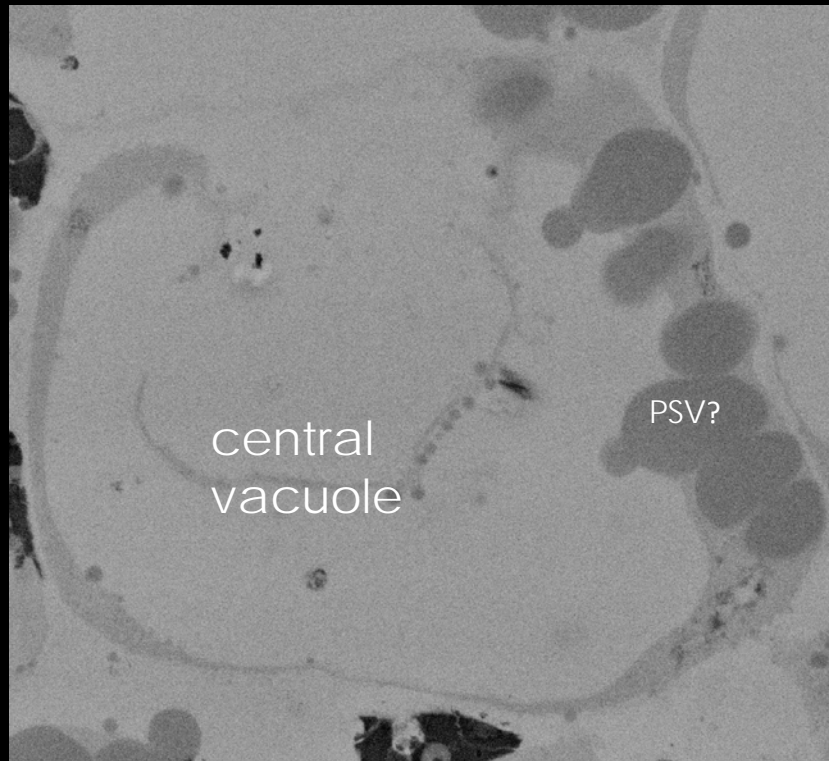
# 3View: cortical ER in leaves





### 3. ER-organelle contact sites

- Understand the role of the ER in protein storage vacuole biogenesis

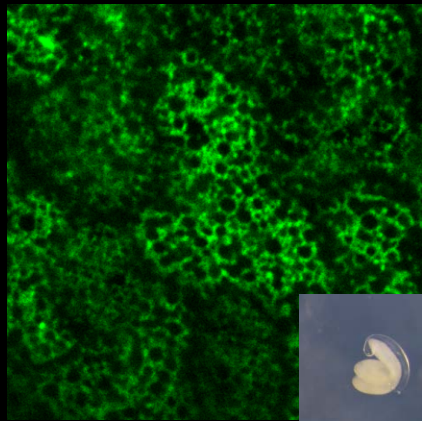


walking stick embryo – 3View

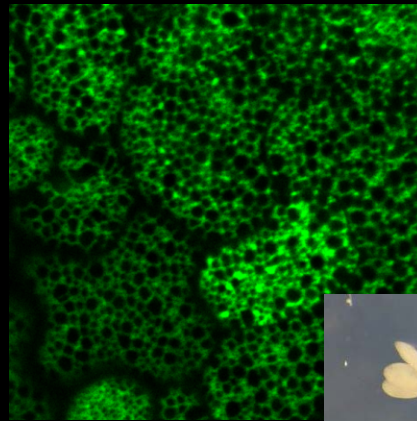
## 4. Manipulation of ER architecture: does it affect biosynthetic capacity?

- Assess effect of up/downregulation on ER morphology/architecture (aim 2)/biosynthetic capacity (aim 4)
  - single RTN1, 2, 3, 4, 6, 13, 20 T-DNA and amiRNA lines ready
  - Selected double and triple knockout lines – all will be ready by June 15
  - how to assess morphological changes?

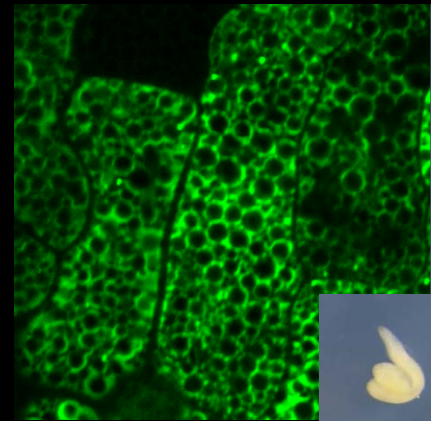
# ER morphology in Arabidopsis embryo cells changes during seed germination



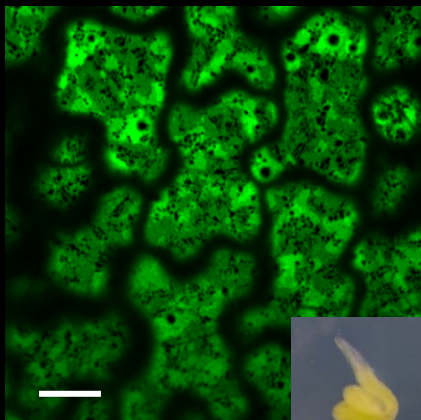
Day 1



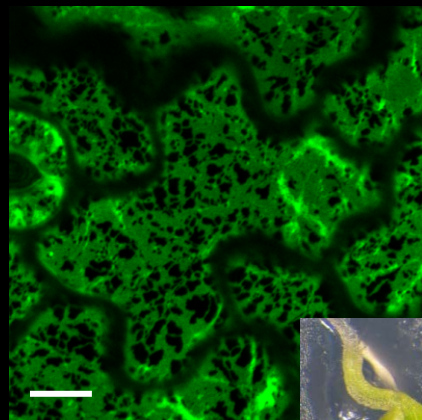
Day 2



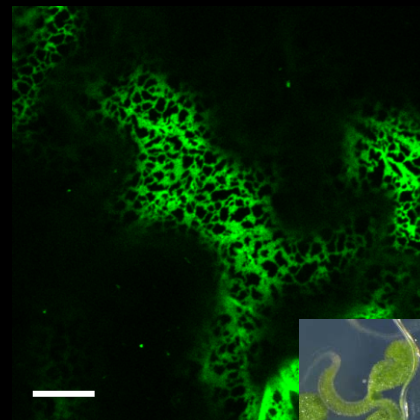
Day 3



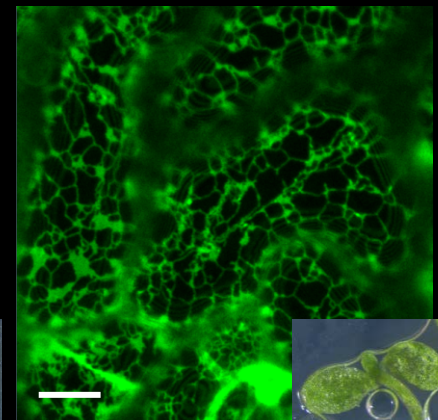
Day 4



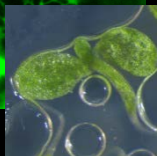
Day 5



Day 6



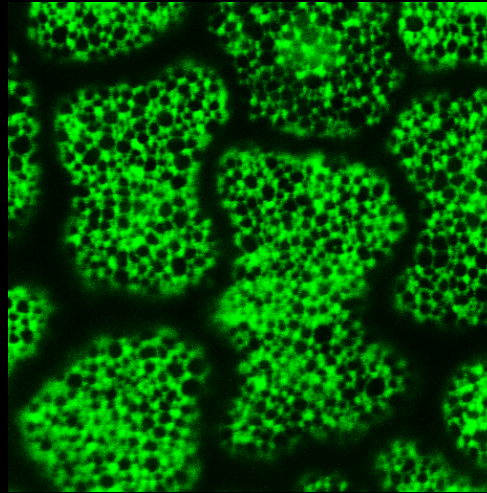
Day 7



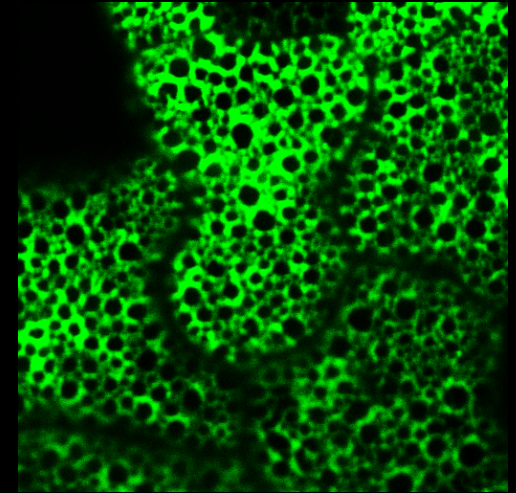


# ER morphology stages

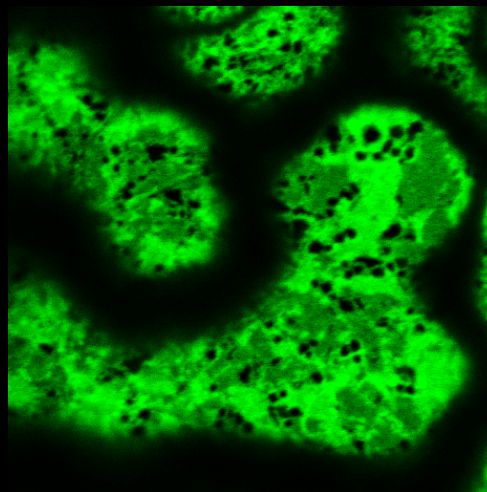
- Quantify the change in proportion of ER sheets vs tubules
- Use to compare various upcoming mutants



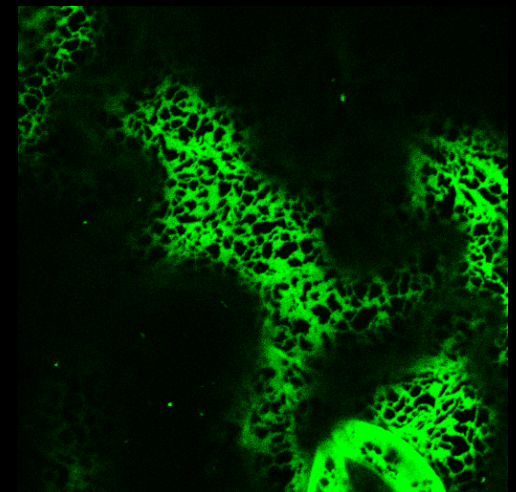
Stage 1



Stage 2



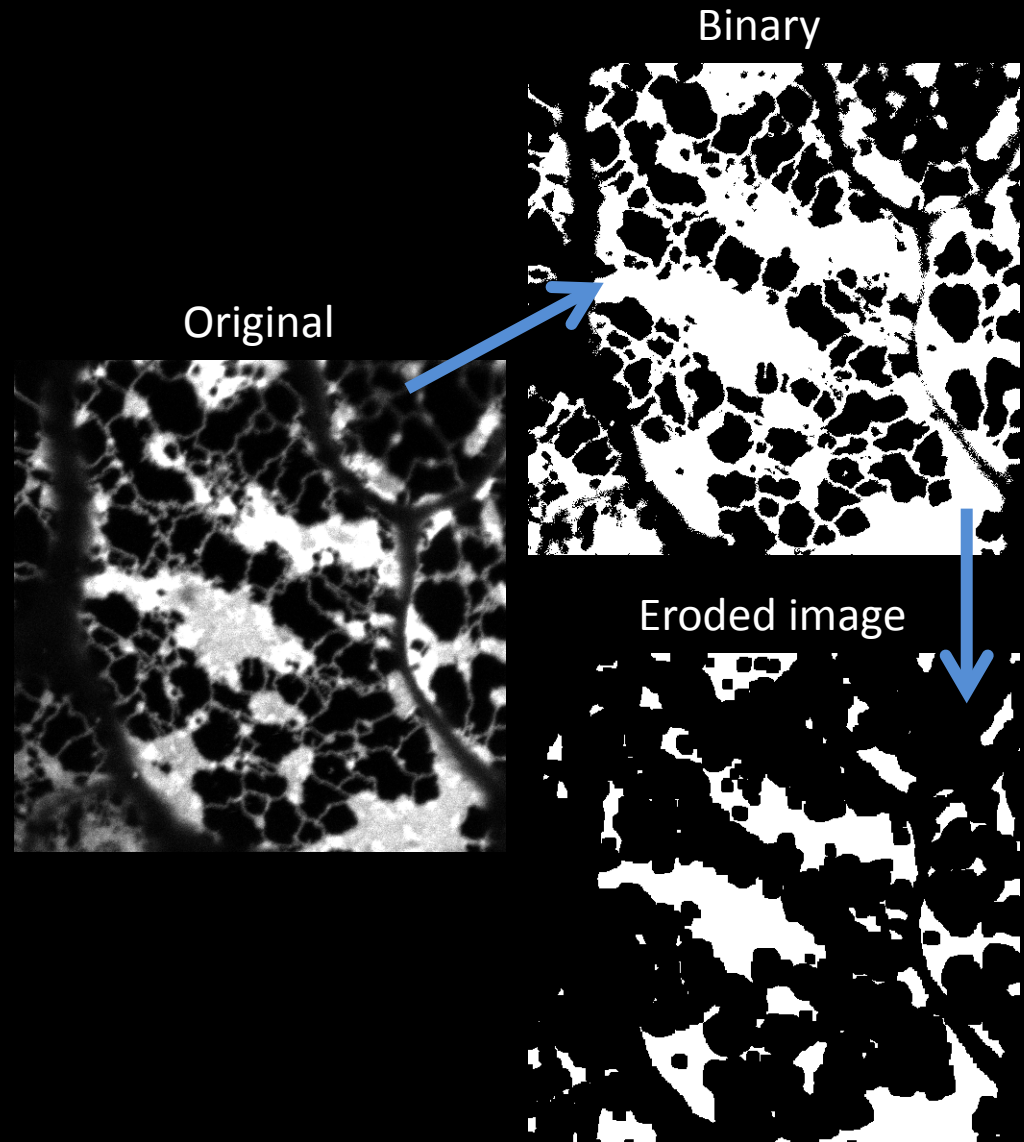
Stage 3



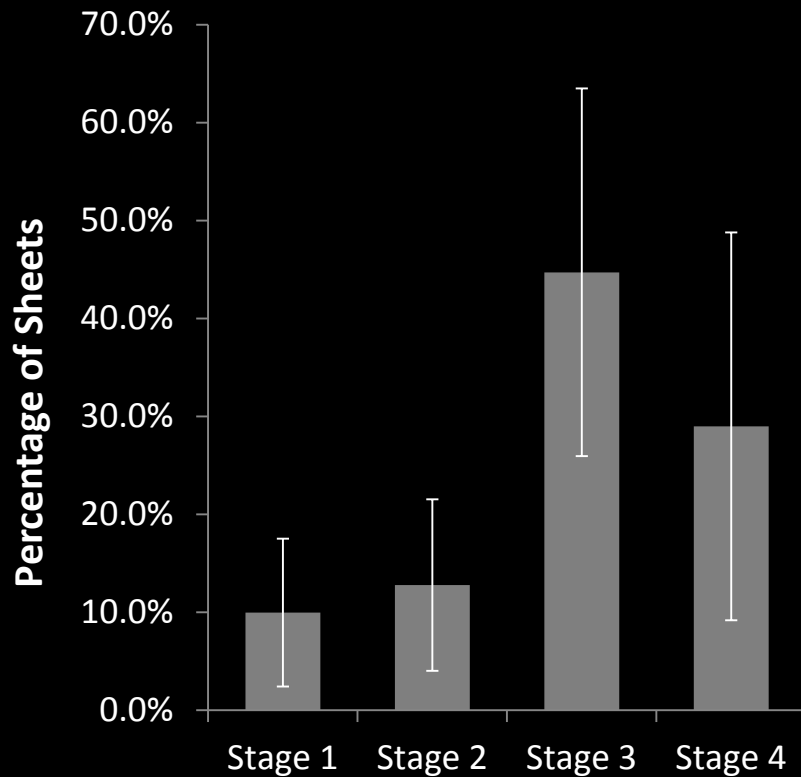
Stage 4

# Renyi-Entropy Thresholding

- Create a binary image with Renyi-Entropy thresholding
- Use the *erode* function to remove tubules



# Renyi Entropy Graph



- Average percentage of sheets from 10 cells

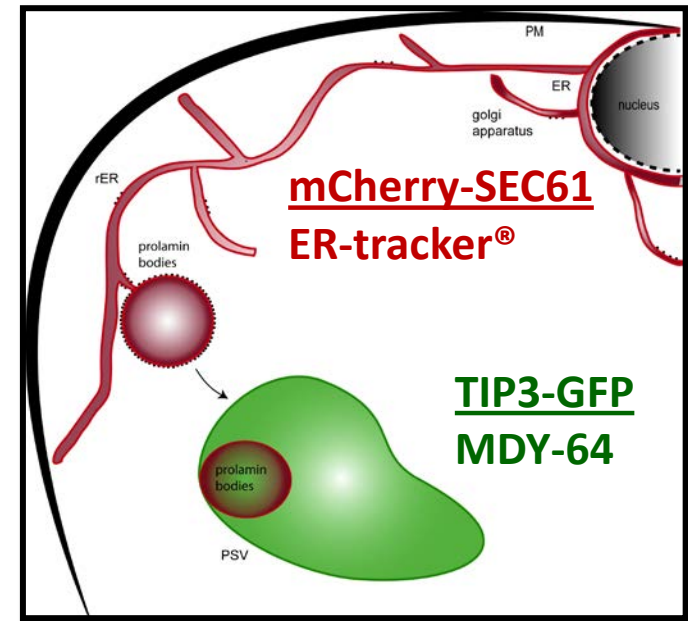
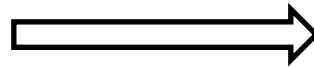
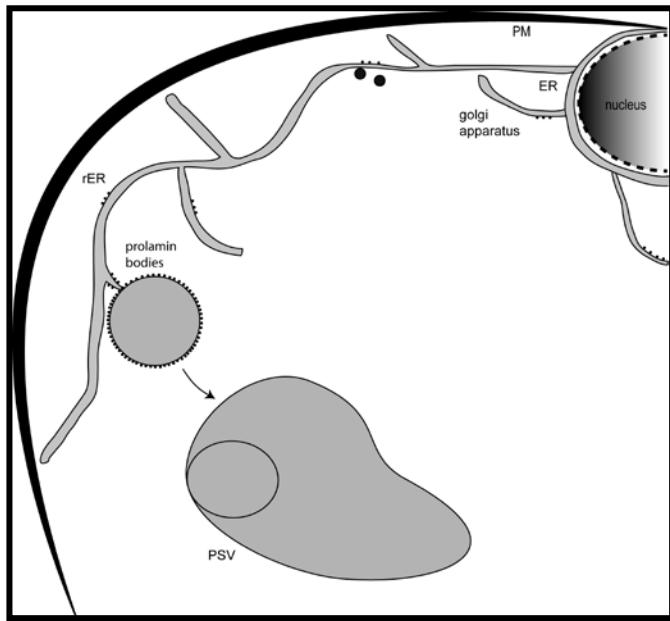


## 4. Manipulation of ER architecture: does it affect biosynthetic capacity?

- Assess effect of up/downregulation on ER morphology/architecture (aim 2)/biosynthetic capacity (aim 4)
  - single RTN1,2, 3, 4, 6, 13, 20 knockout lines ready
  - Selected double and triple knockout lines – all will be ready by June 15
  - how to assess morphological changes?
- Effect on seed protein and lipid content/composition
  - RTN20 T-DNA lines
  - seed RTN overexpressors

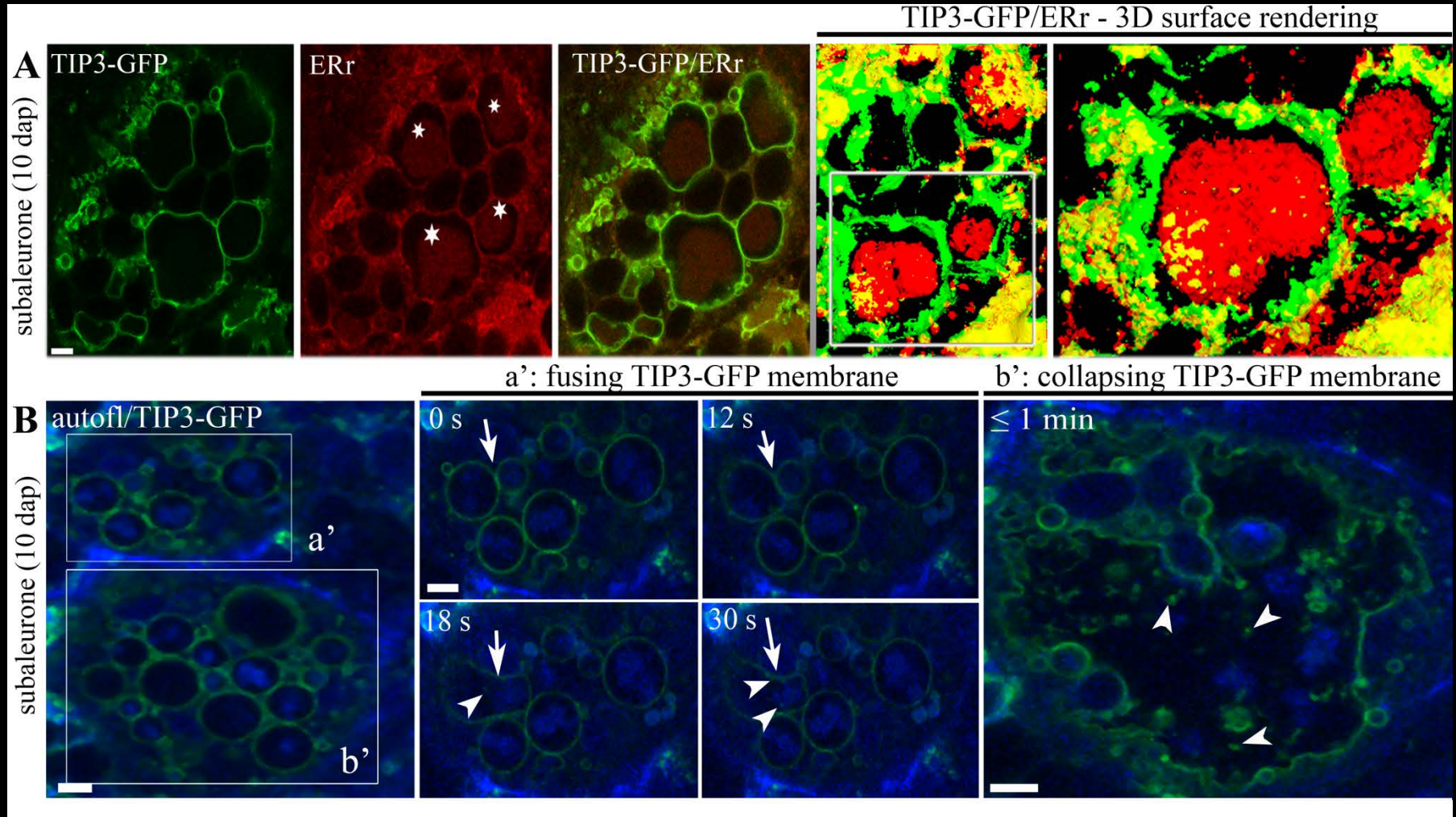
## 5. Studying the ER in cereals

- generated ER and PSV marker lines



- Transgenic marker lines of barley expressing further fluorescent marker proteins were initiated.

# TIP3-GFP-labeled PSVs contain protein bodies and are involved in fusion and rupture in barley endosperm



# Thank you!



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