

# Plant Endoplasmic Reticulum And Seed Productivity (PER ASPERA)



ERA-NET for Coordinating  
Action in Plant Sciences



# PER ASPERA team



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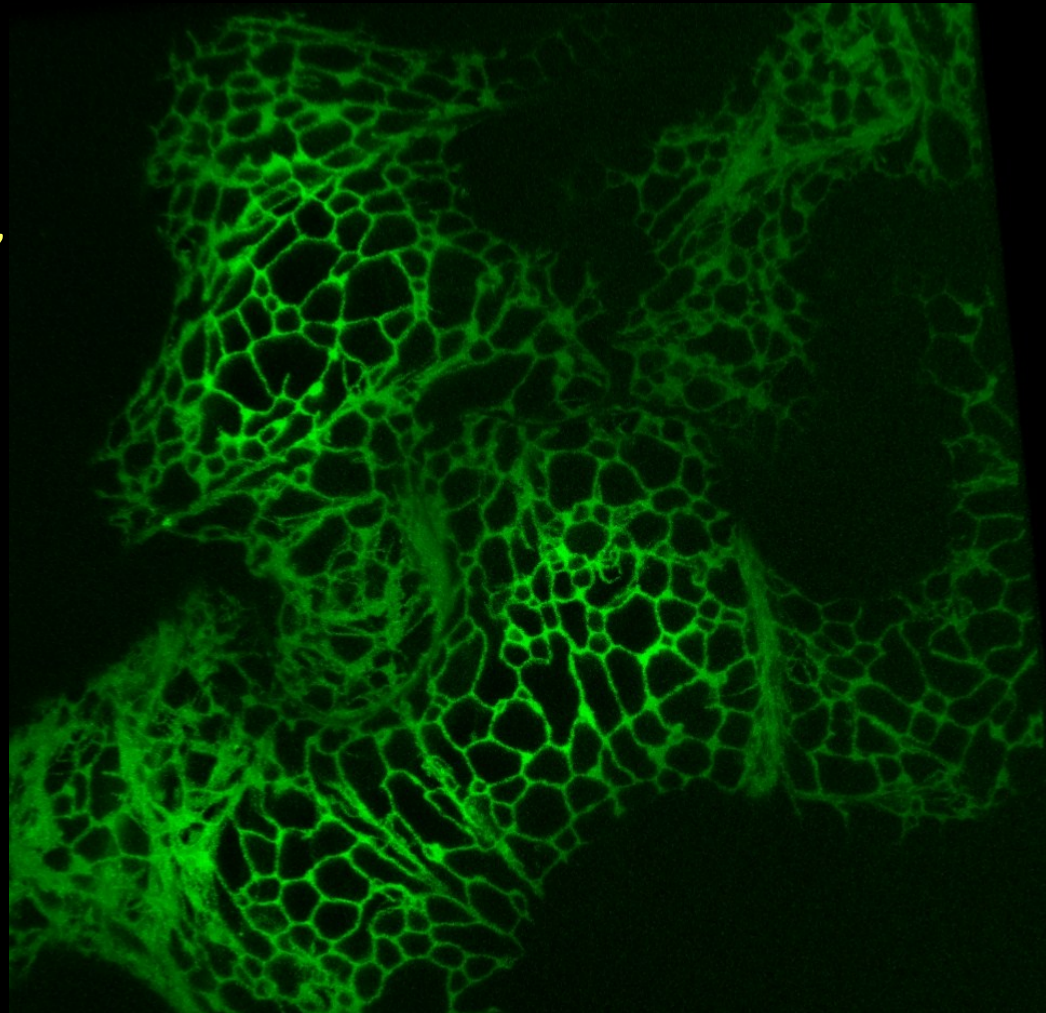
Verena Ibl



Patrick Moreau

# The endoplasmic reticulum

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



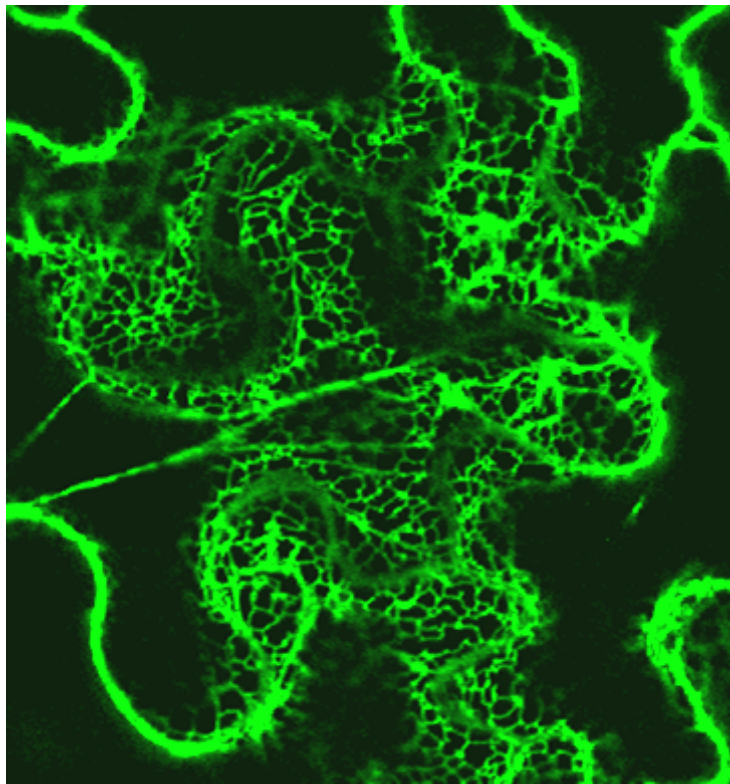
tobacco cells expressing GFP-HDEL

# AIMS

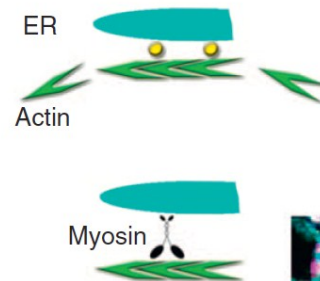
- 1) To unravel the machinery involved in shaping the plant ER network  
→ study known ER shaping proteins **and their interactors**
- 2) To analyse ER structure in depth : 3-D architecture
- 3) To study ER interactions/contacts with tonoplast and plasma membrane
- 4) To manipulate the biosynthetic capacity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



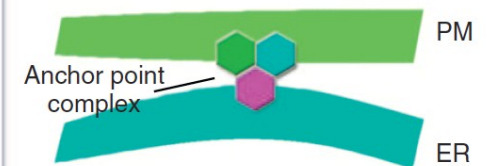
# ER structure



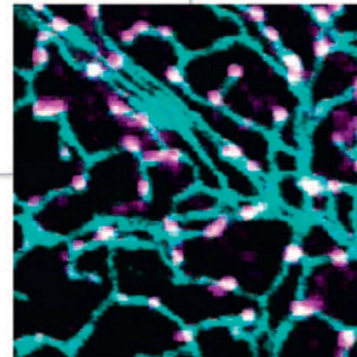
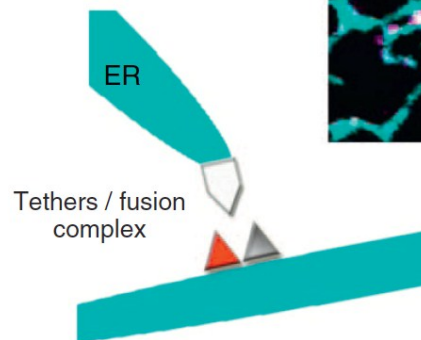
(a) Tubule growth / retraction



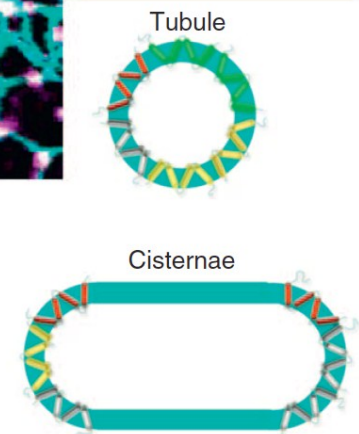
(b) Anchor points



(c) Tubule fusion and three way junction formation



(d) Tubulation versus cisternalisation

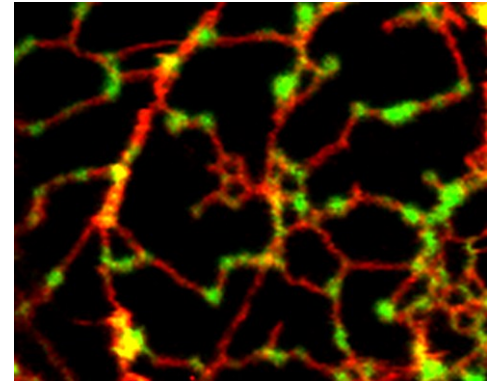
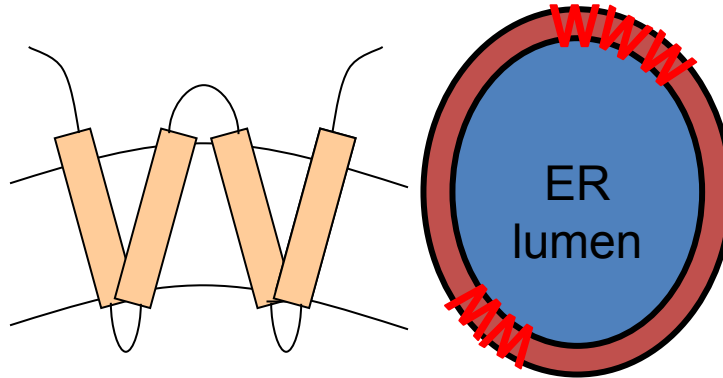


# 1. ER morphogens

## Reticulons

tubule formation

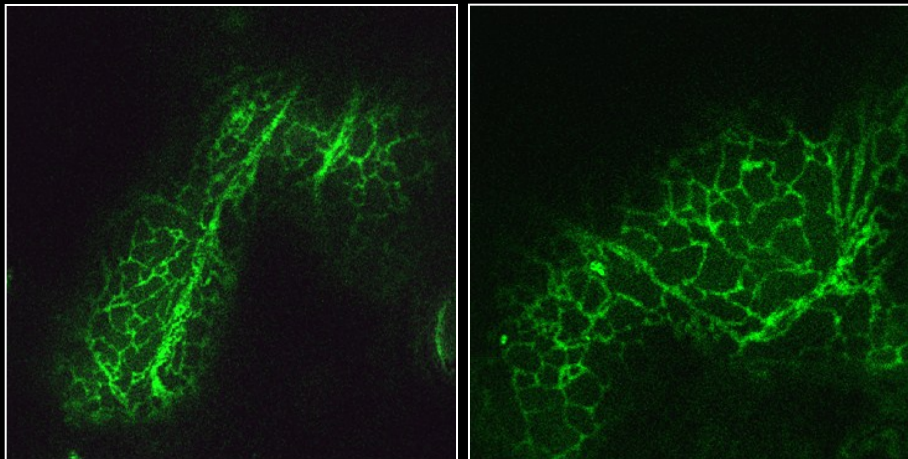
21 genes in Arabidopsis  
Cloned 15, fused to XFP



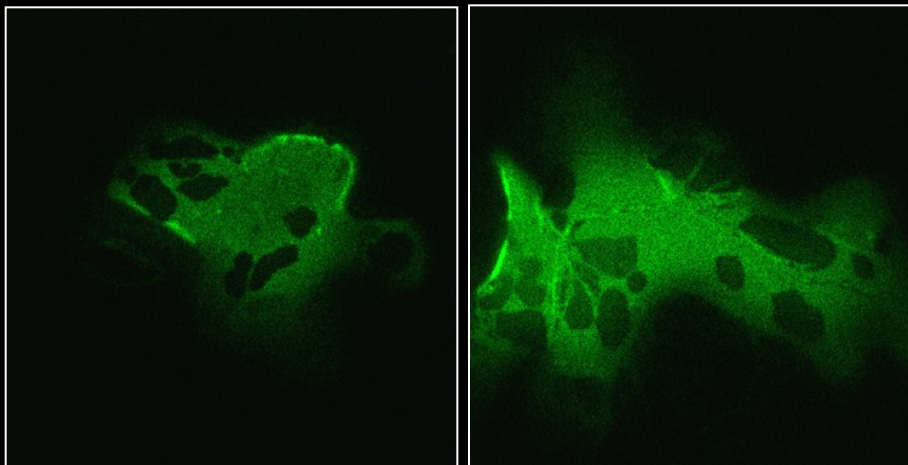
# RTN13 is sufficient to induce ER tubules *in vivo*

- Double mutant of phosphatidic acid hydrolase (*pah1pah2*)
- Major phenotype is swelling of the ER, which forms large sheets

wt

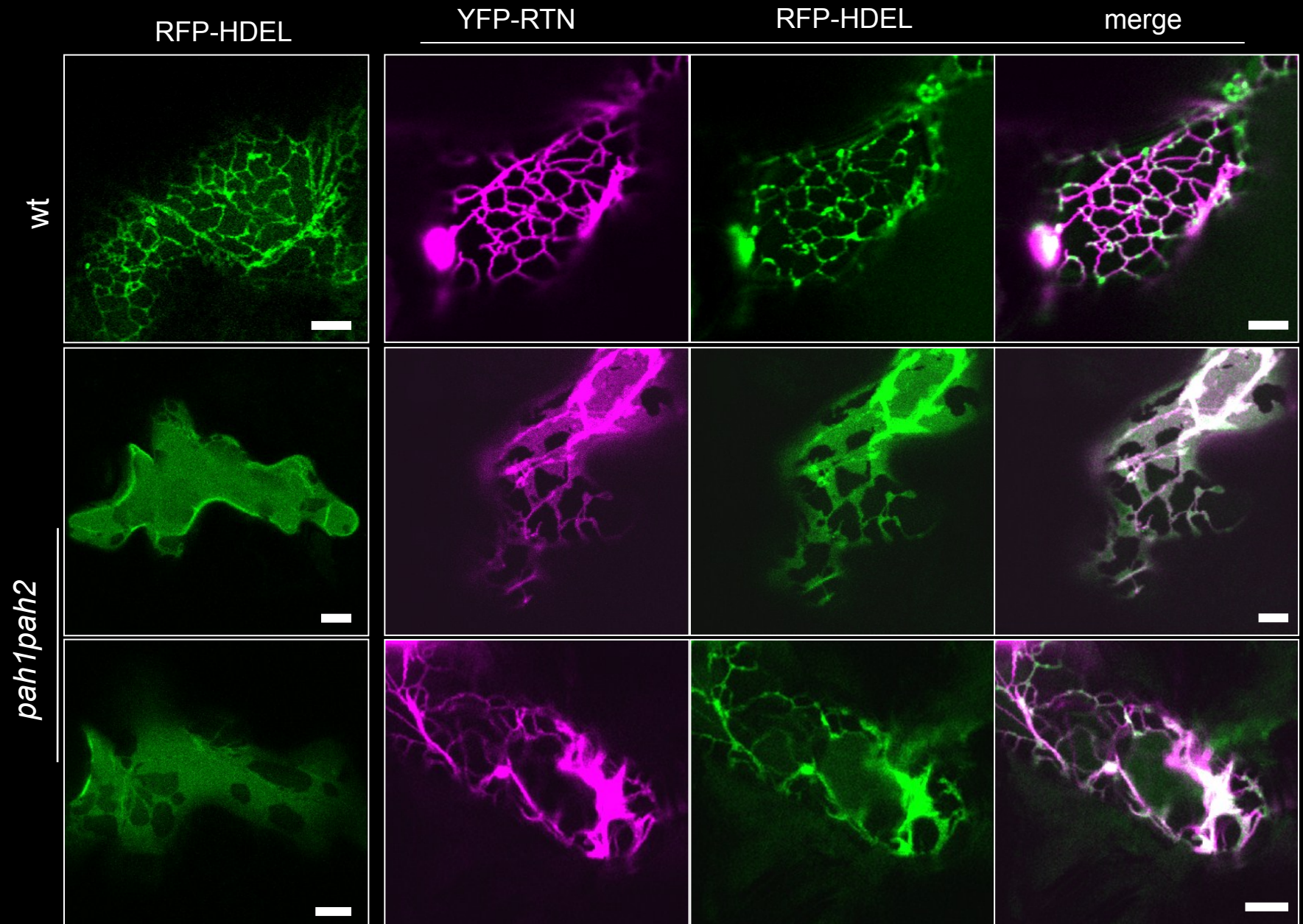


*pah1pah2*





# RTN13 is sufficient to induce ER tubules *in vivo*



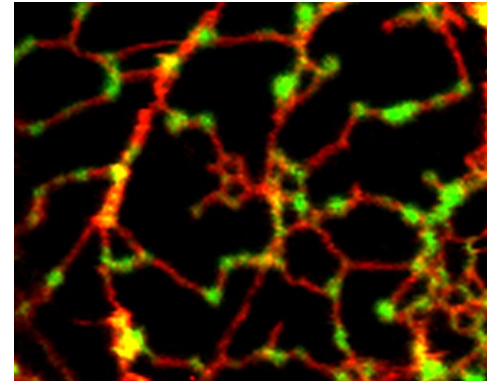
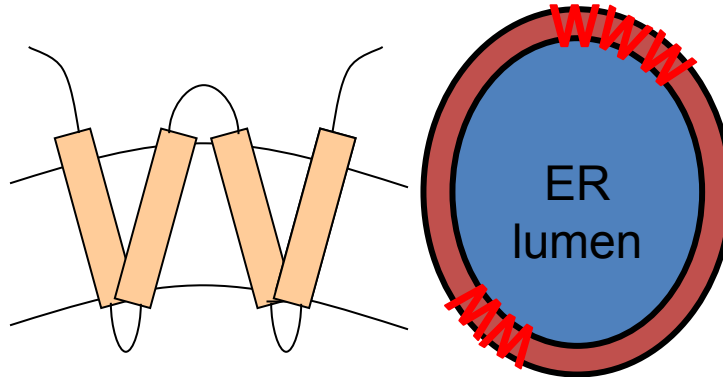


# 1. ER morphogens

## Reticulons

tubule formation

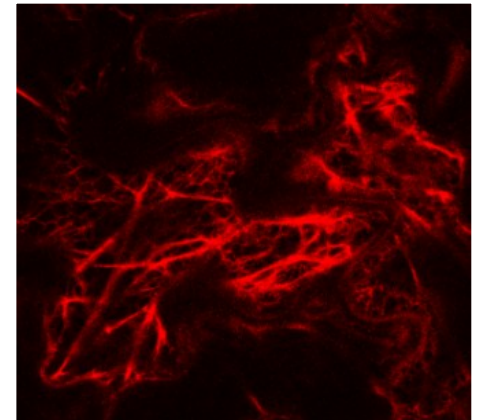
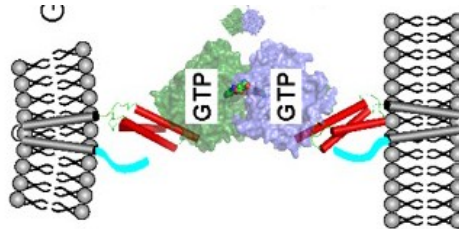
21 genes in Arabidopsis  
Cloned 15, fused to XFP



## Atlastins (RHD3)

tubule fusion

2 genes in Arabidopsis

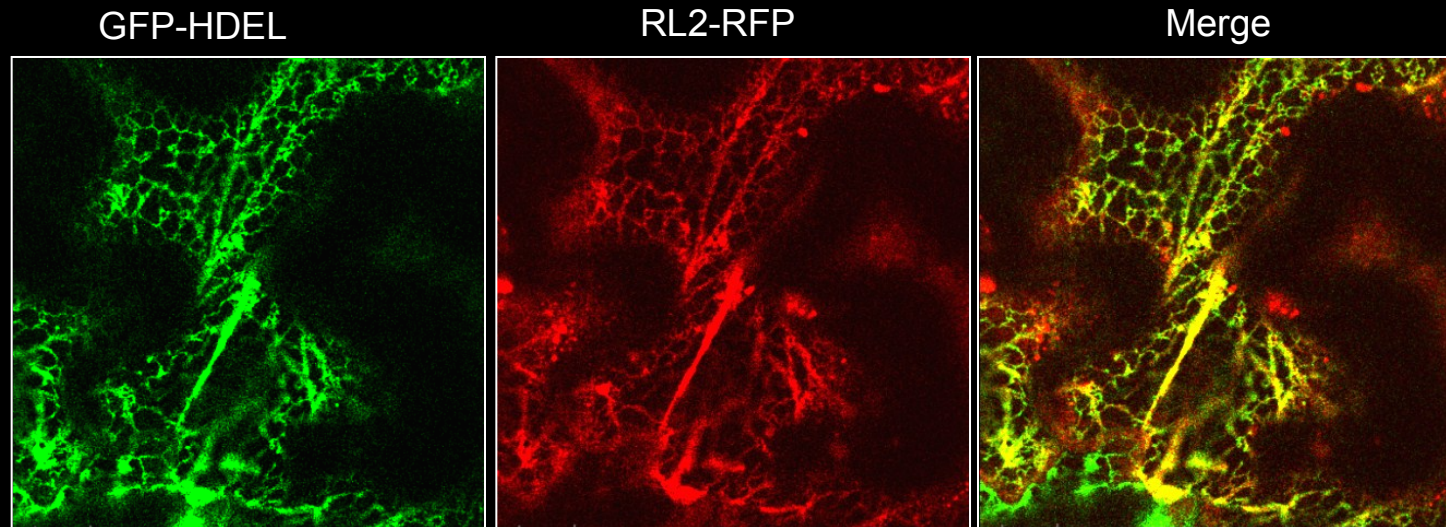


## LNPARK

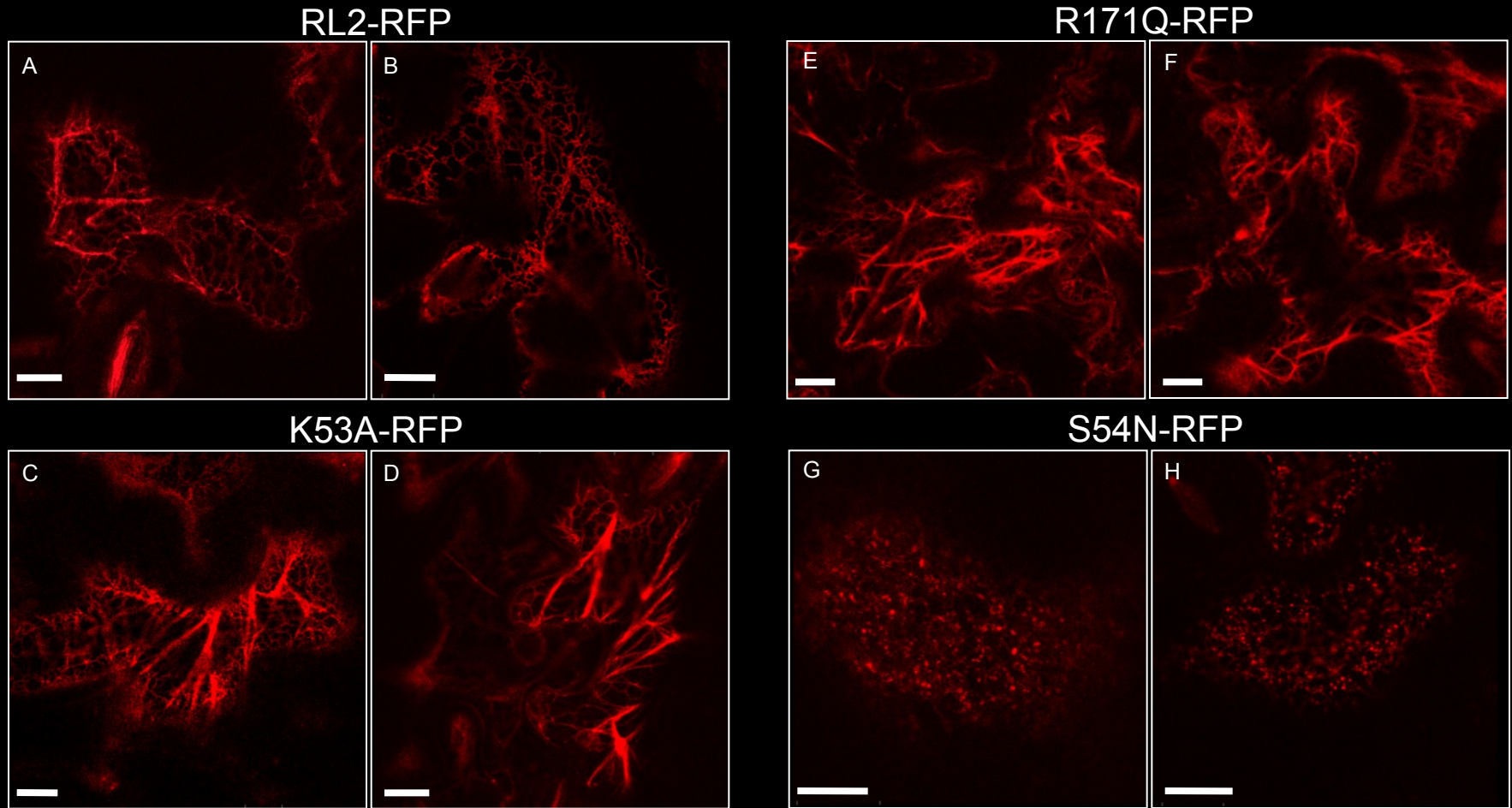
Located at 3-way junctions

2 genes in Arabidopsis

# RL2 is localized in the ER



# Mutations in the GTPase domain of RL2 affect ER architecture



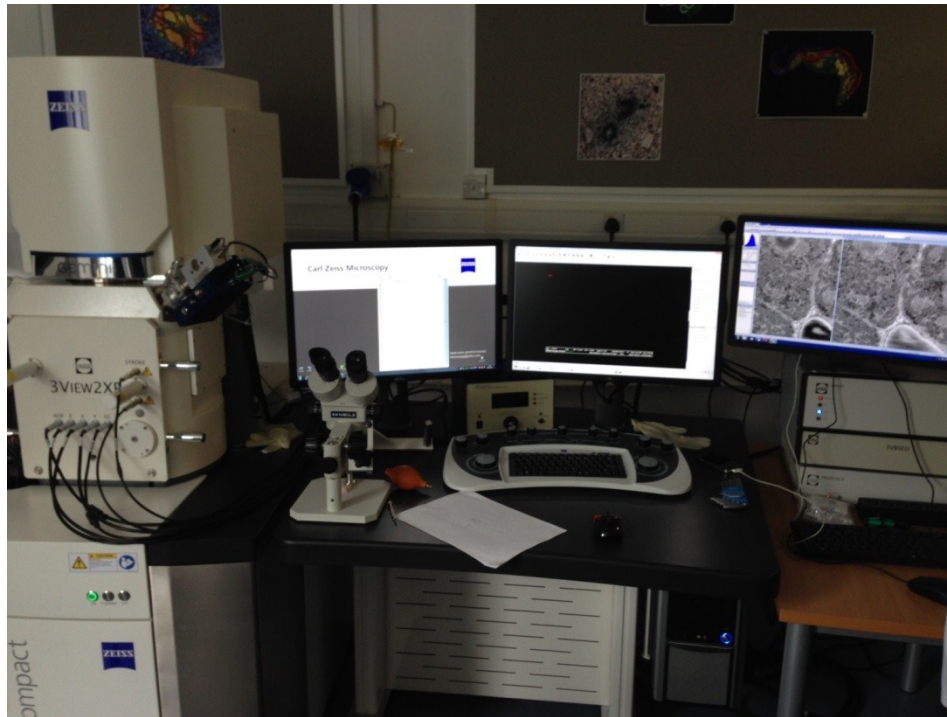


# 1. ER morphogens

- Map intracellular localisation / tissue distribution
- Assess effect of up/downregulation on ER morphology/architecture (aim 2)/biosynthetic capacity (aim 4)
- identify/validate interacting proteins
  - colP
  - yeast split ubiquitin
  - FRET-FLIM

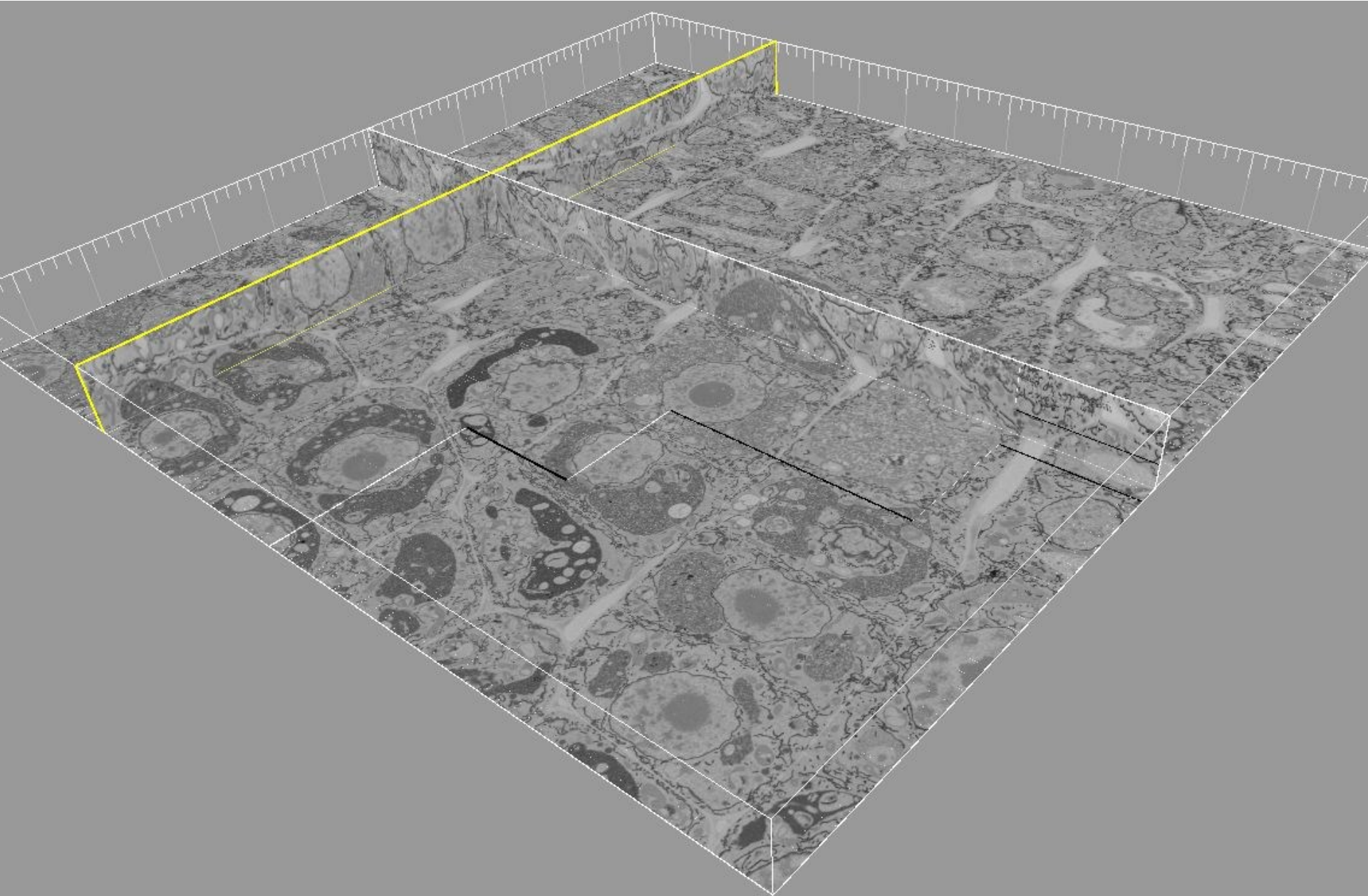
## 2. 3D ER architecture

### Serial Block Face Imaging – 3-View

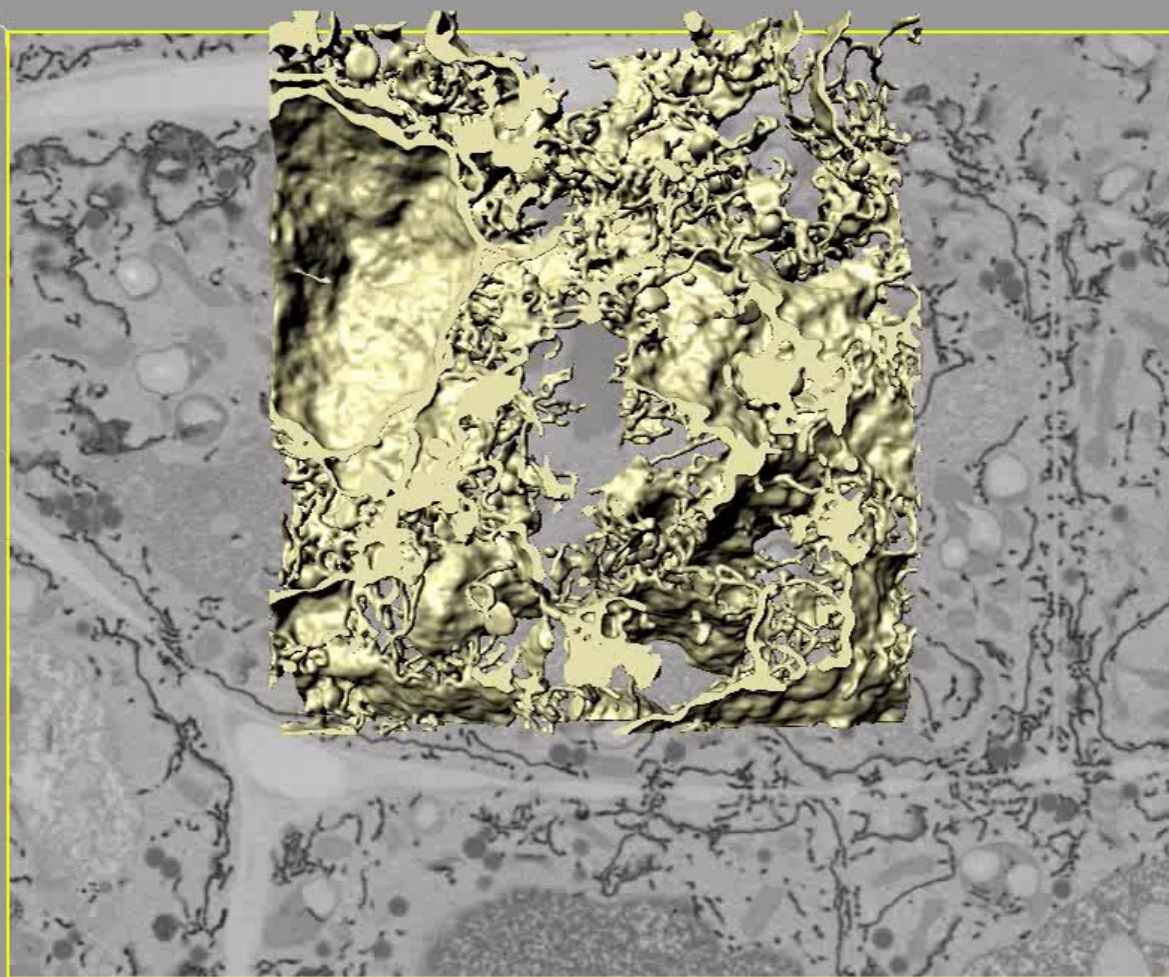


An ultramicrotome is inside the SEM chamber and slices a section off the block face before imaging.

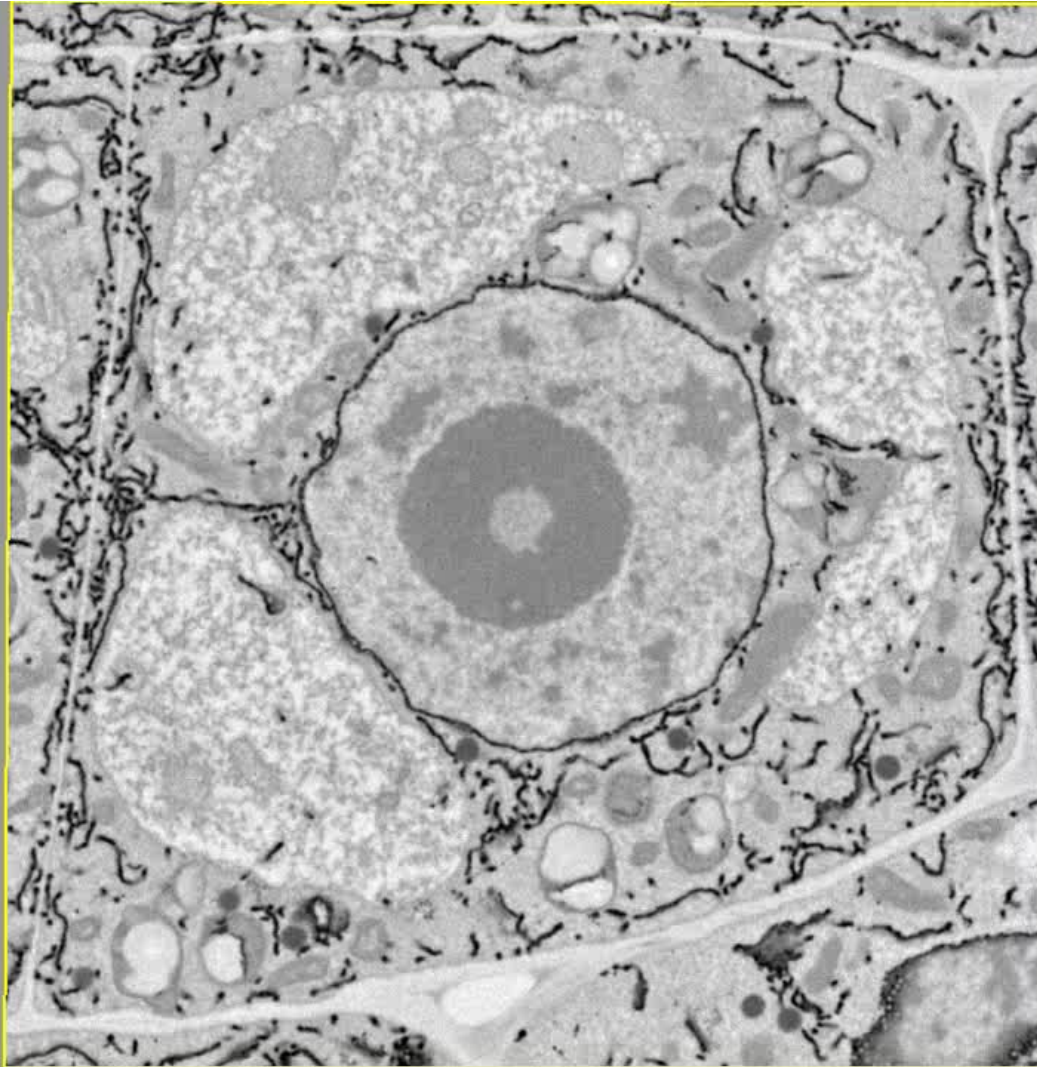
# High Resolution SEM of a resin block face







3000 nm

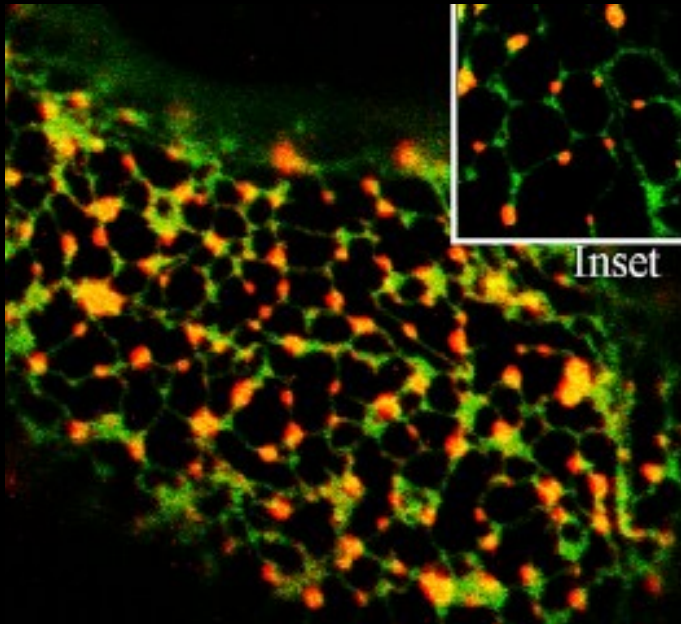


3000 nm

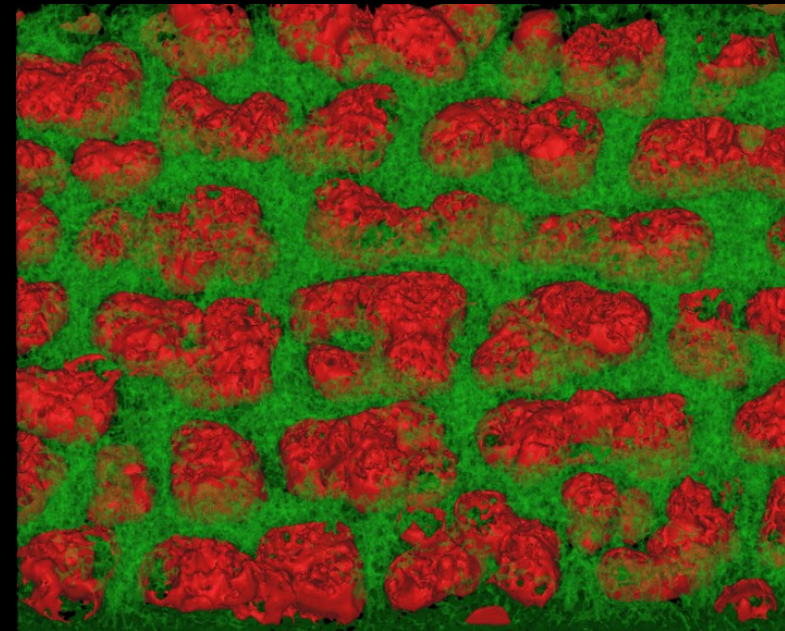


### 3. ER-organelle contact sites

- Identify key components of ER-PM contact sites
- Understand the role of the ER in protein storage vacuole biogenesis



ER and VAP27-YFP (plasma membrane contact sites)  
tobacco



ER and TIP3;1-YFP (protein storage vacuoles)  
Arabidopsis embryo

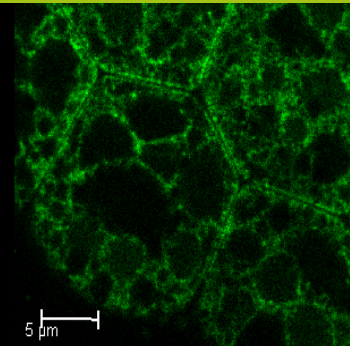
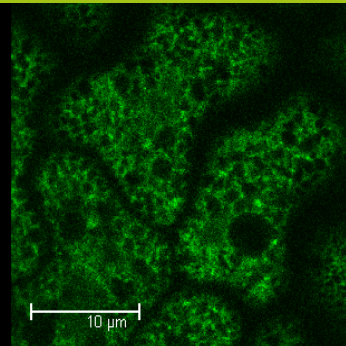


## 4. Manipulation of ER architecture: does it affect biosynthetic capacity?

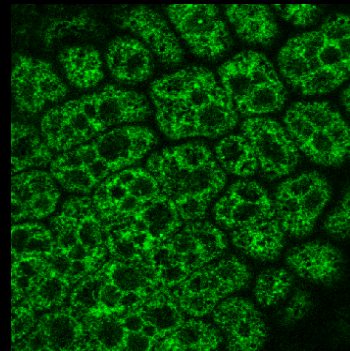
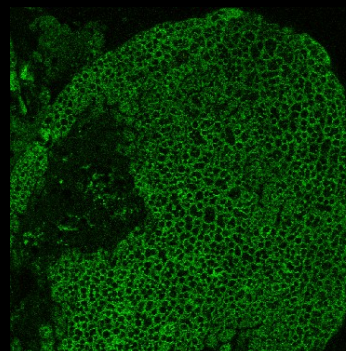
RTN1, RTN2 and  
RTN13 are  
expressed in seeds



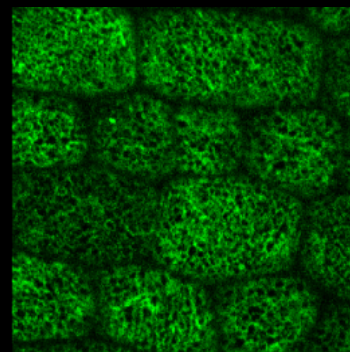
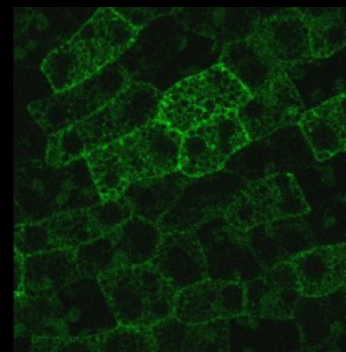
RTN1-YFP



RTN2-YFP



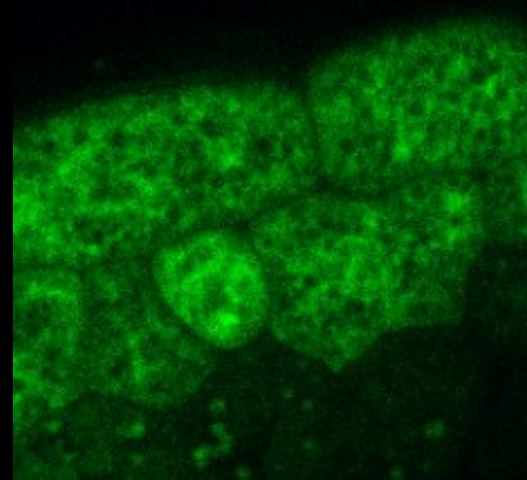
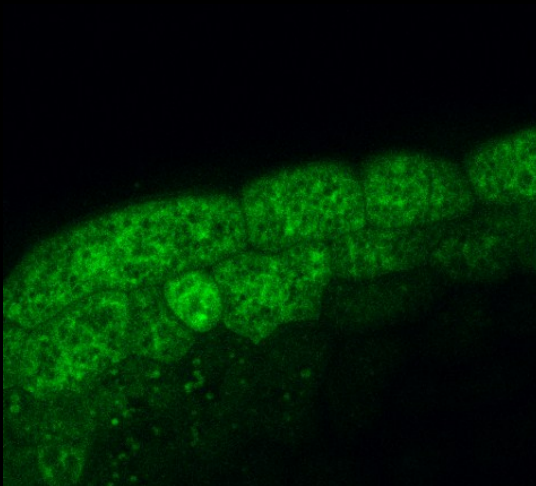
RTN13-YFP



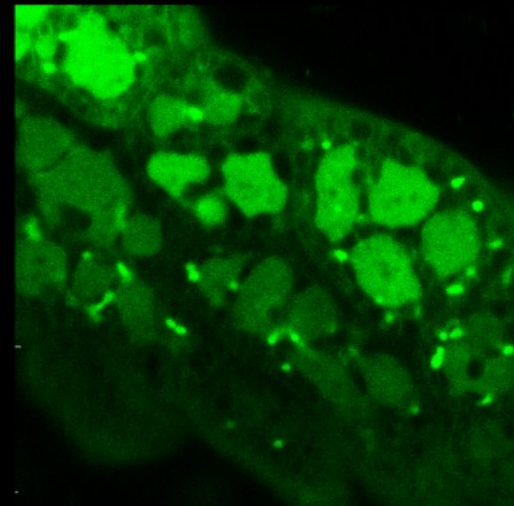
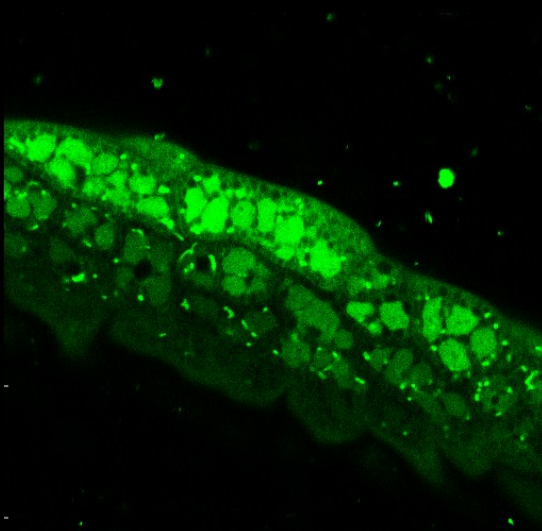
## 4. Manipulation of ER architecture: does it affect biosynthetic capacity?

downregulation of seed reticulons remodels the ER in Arabidopsis embryos

wt



*rtn13* x  
RNAi  
*rtn1/2*



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

- Modulation of RTN, atlastin, LNPARK
  - T-DNA, RNAi, amiRNA, overexpression
  - Effect on seed protein and lipid content/composition

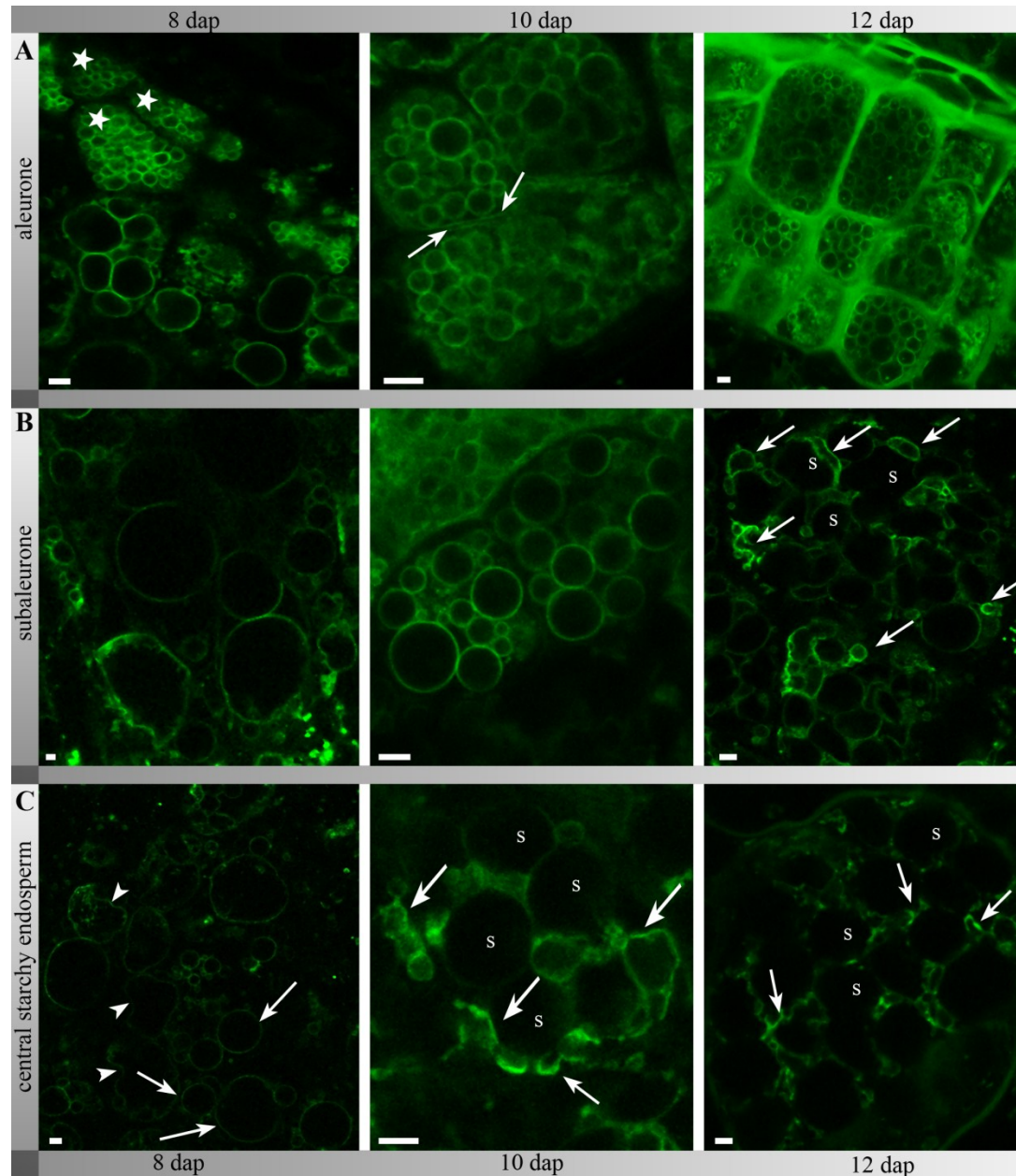


## 5. Studying the ER in cereals

- Barley as a model system for studying ER morphology and protein body biogenesis in cereals

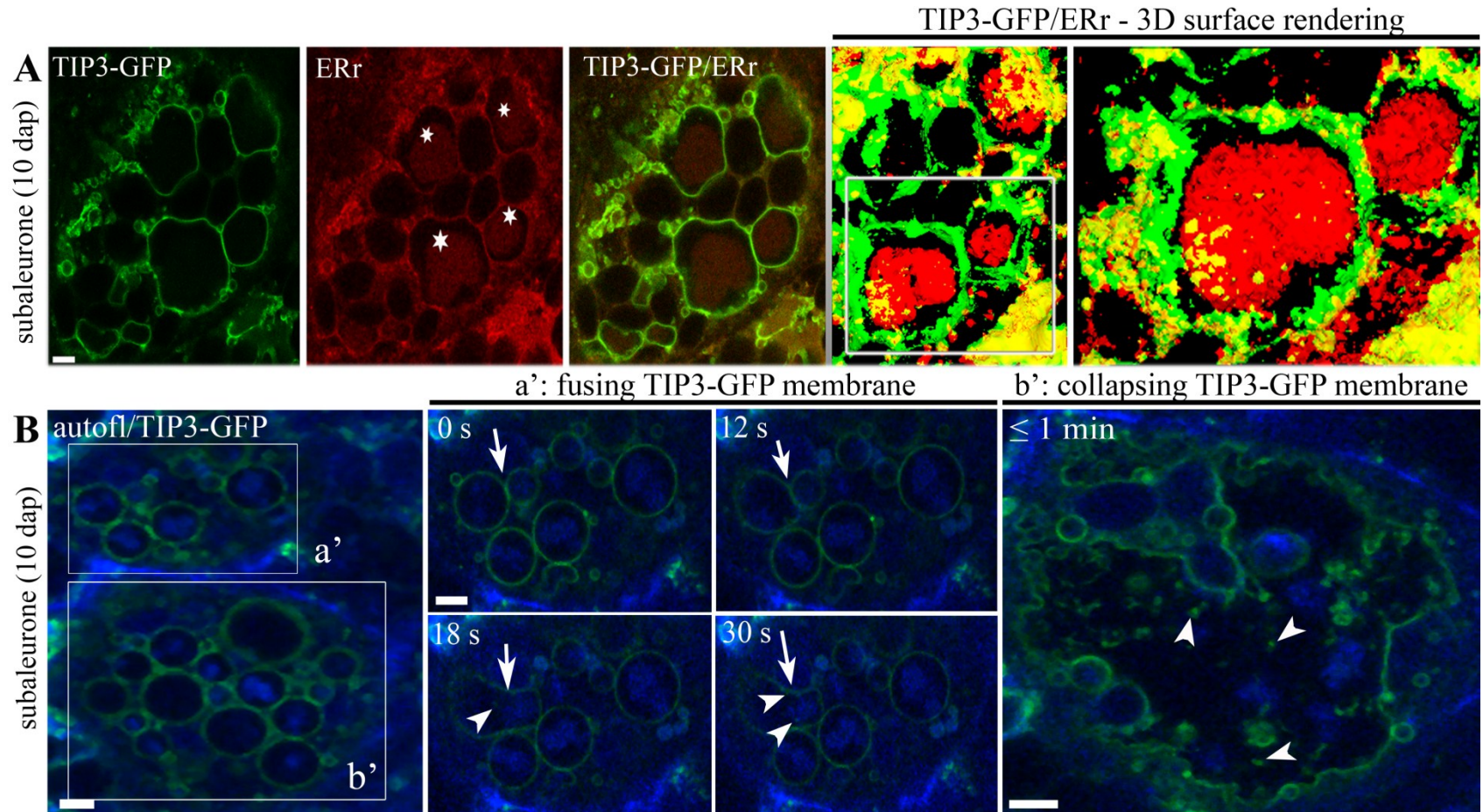


# TIP3-GFP-labeled PSV undergo morphological changes during endosperm development



Ibl et al., 2014

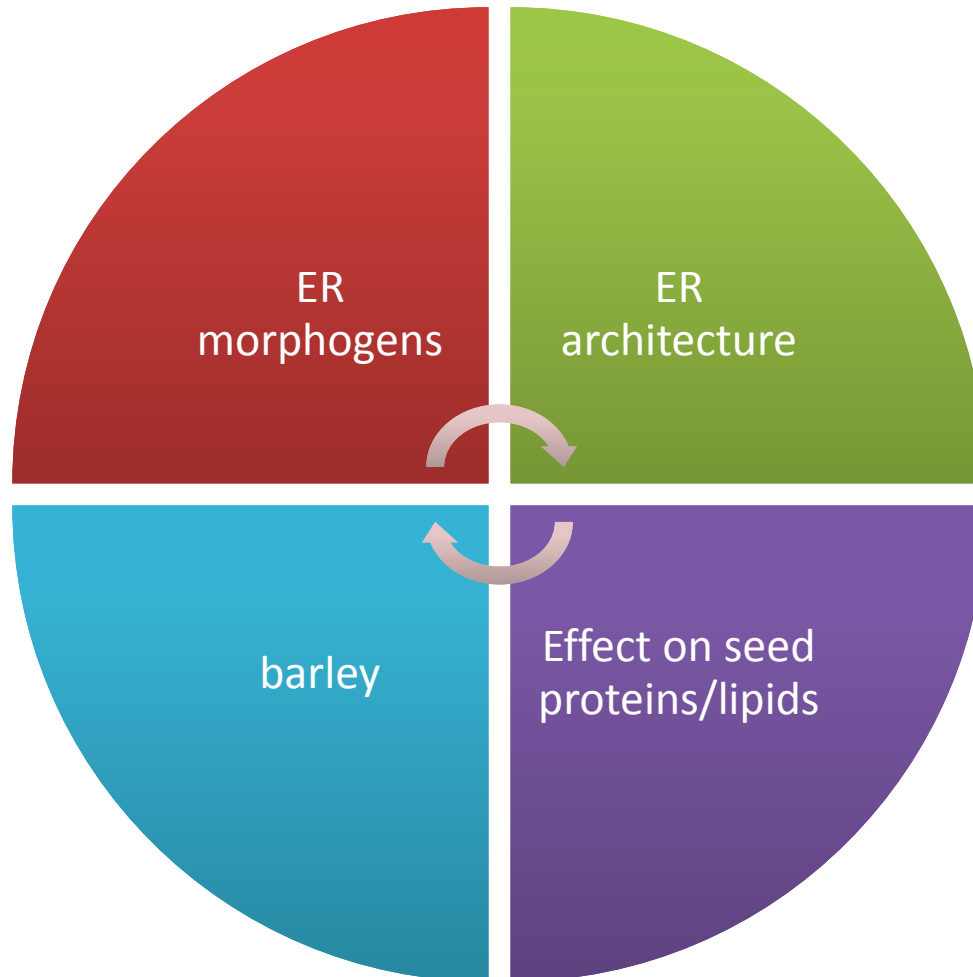
## TIP3-GFP-labeled PSVs contain protein bodies and are involved in fusion and rupture



Ibl et al., 2014



# Development loop



# Thank you!



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Action in Plant Sciences

