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Evolution and development of the earliest land plant  
rooting systems

Kavli Institute of Theoretical Physics,  
Santa Barbara, 13<sup>th</sup> August 2019

# Plants colonised the land 500 million years ago

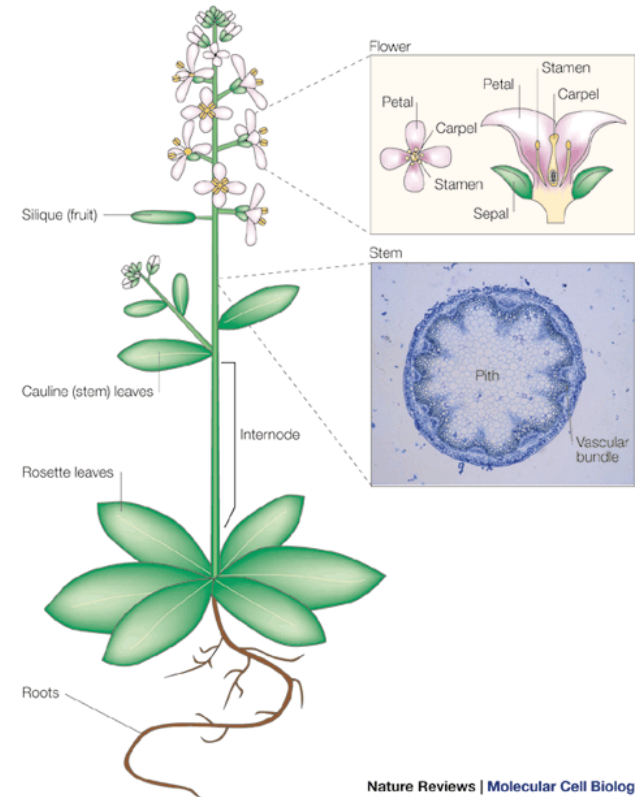
Earth history (billion years)

4.5 3.5 2.5 1.5 0.5 0



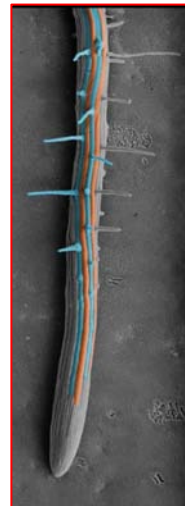
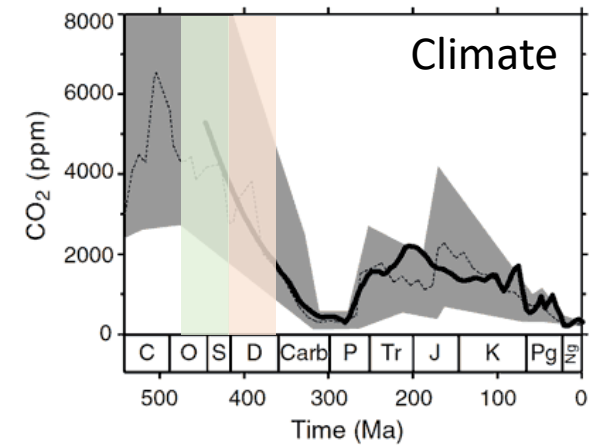
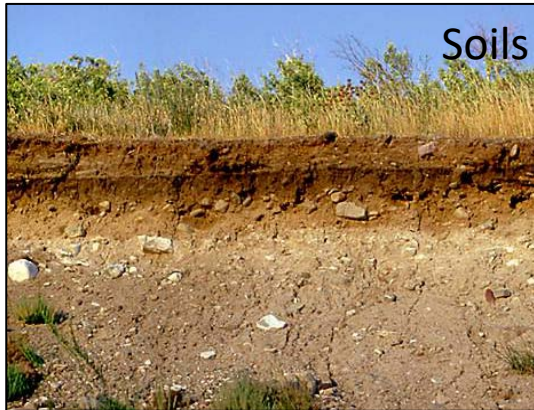
- Meristems
- Shoots
- Rooting structures
- Leaves

# Colonization of the land was followed by a radiation in morphological diversity



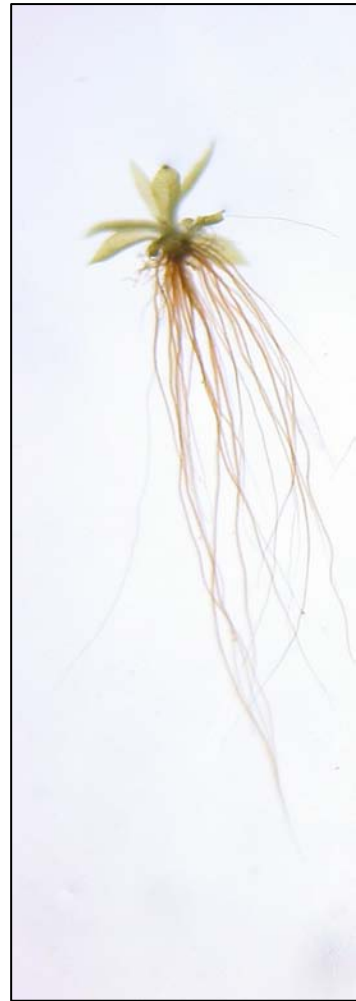
- Cellular diversity
- Meristems
- Shoots
- Rooting structures
- Leaves

# The evolution of land plants dramatically changed the Earth system



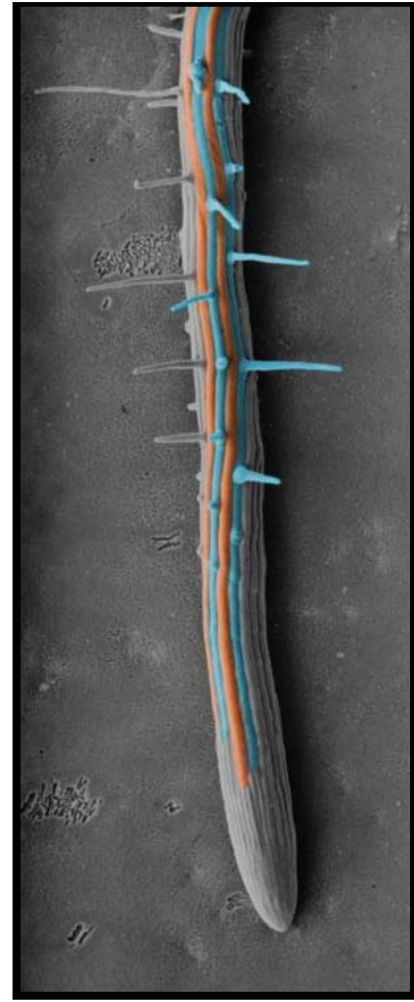
# Two types of rooting structure among land plants

Rhizoids



Non vascular plants

Axes

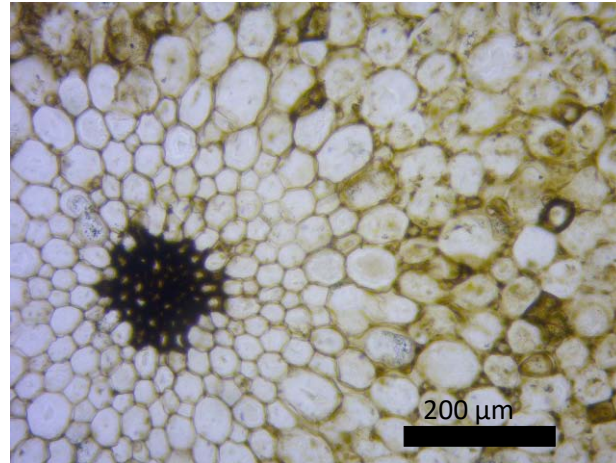
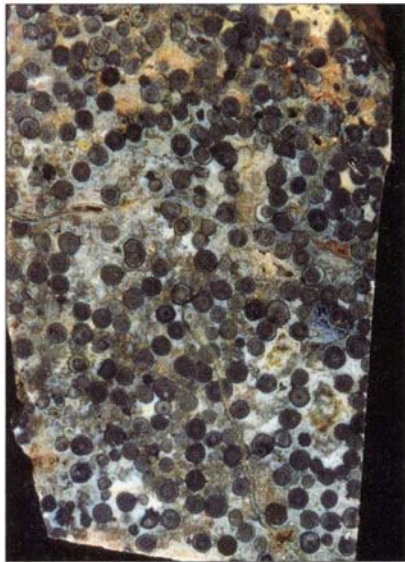


Vascular plants

# 407 million year old Rhynie chert preserves an entire ecosystem *in situ*



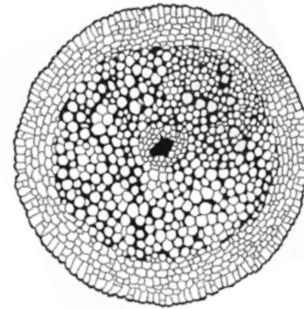
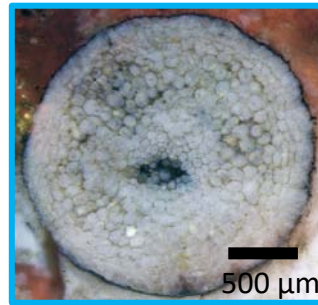
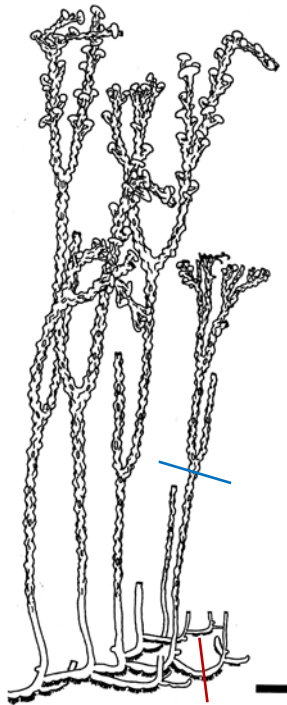
1 cm



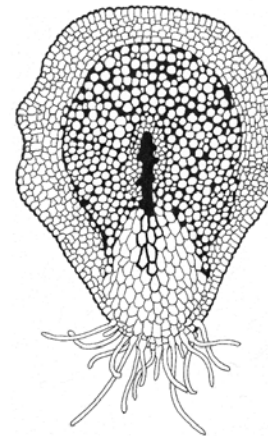
200 μm



# Rhizoids develop on the lower sides of bilaterally symmetric axes segments of *Nothia aphylla*



Orthotropic  
(vertical) axis



Plagiotropic  
(horizontal)  
rhizoidal  
sporophyte  
axis

# Tips of rhizoids observed in *Nothia aphylla*

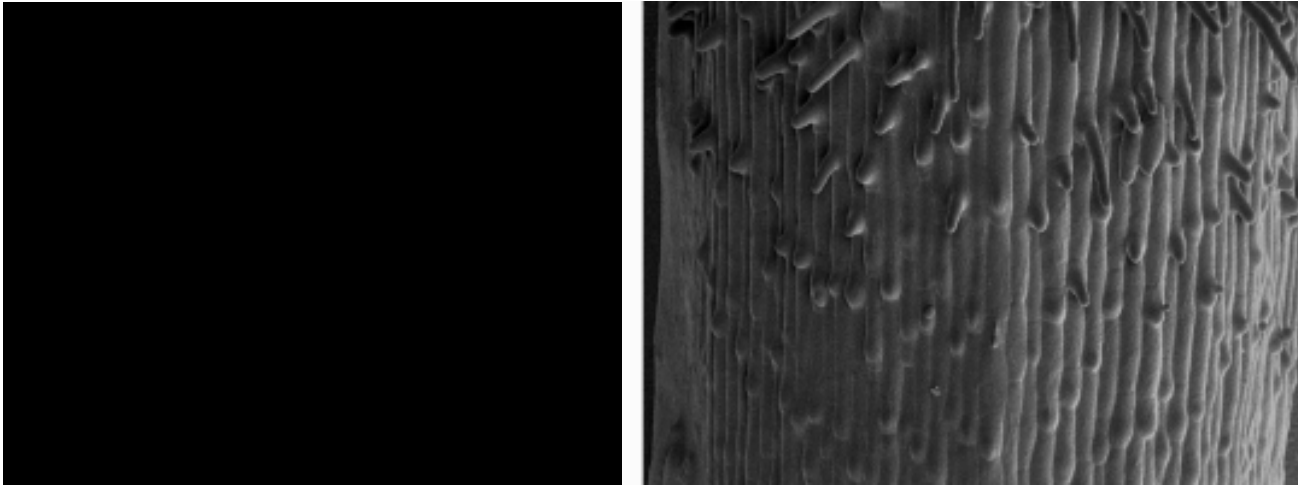




# Outline

1. Genetic mechanism that regulated the development of the rooting structures in the last common ancestor of the land plants
2. Evolution of a novel patterning mechanism among liverworts

1. Genetic mechanism that regulated the development of the first rooting structures (comparative developmental genetics)



Identification of positive regulator of rooting structure development in land plant common ancestor (activator is conserved)

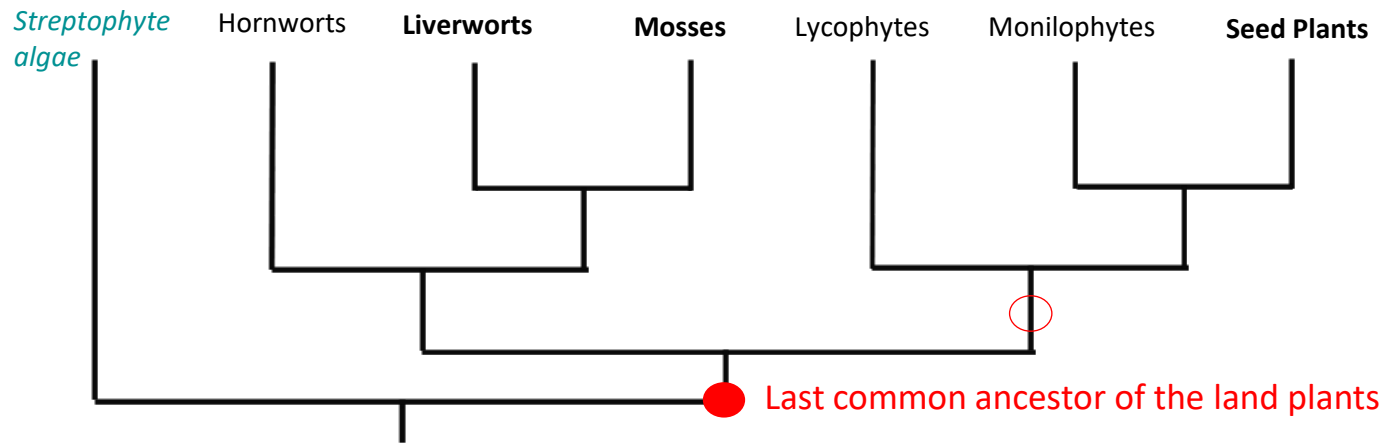
# Two monophyletic groups of land plants; vascular and non-vascular



Non-vascular



Vascular



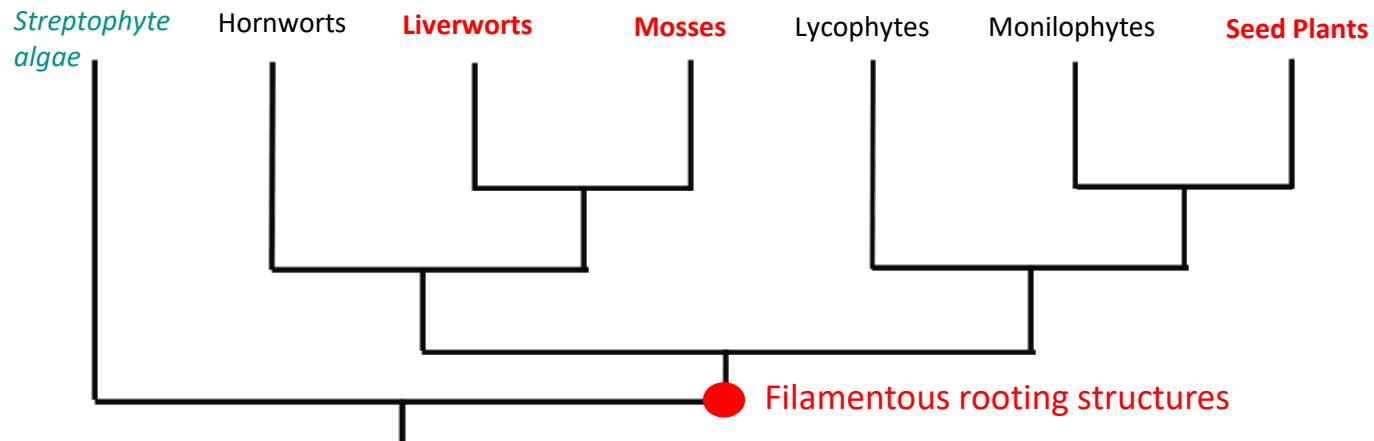
# Filamentous rooting cells develop at the interface between land plants and the soil



Non-vascular

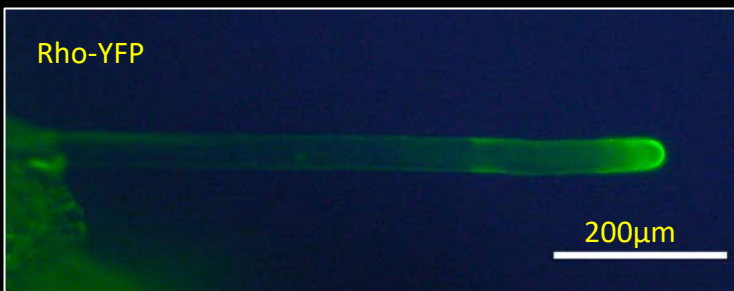
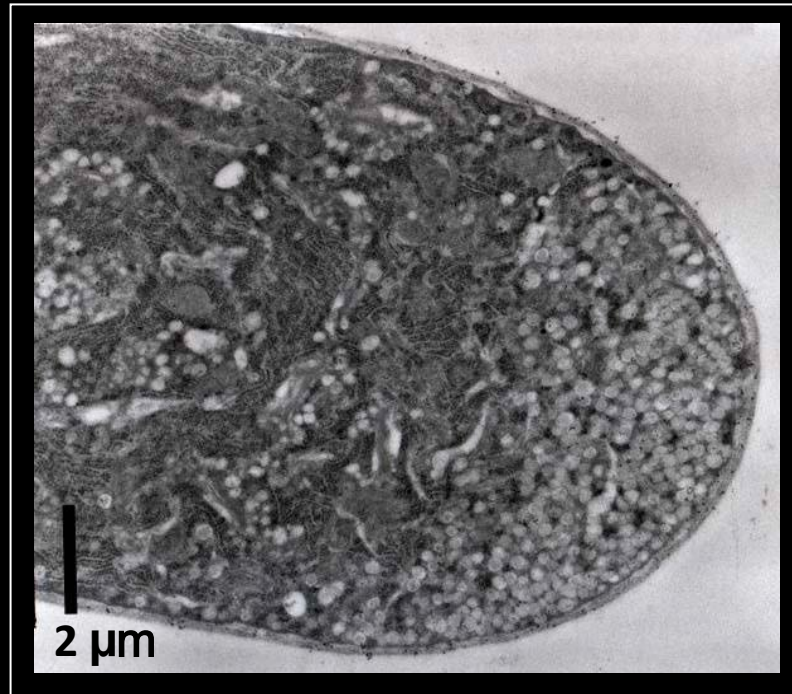


Vascular



What mechanism controlled the development rooting structures in the common ancestor of extant land plants?

# Tip-growing cells deposit new cell surface material at the apex



# *Marchantia polymorpha*



## **Good for genetic analysis:**

- Haploid
- Transformable
- Mutagenesis: T-DNA, UV
- CRISPR/Cas9
- Genome assembly
- Rapid life cycle
- Small size
- Separate sexes
- 10  $\mu\text{m}$  spores
- Rhizoids



# Phenotypes of mutants that define genes controlling rhizoid development

Wild type

6 rhizoidless



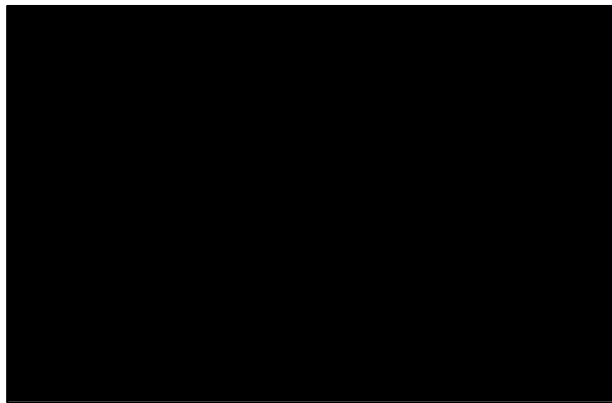
20 ectopic rhizoids

## Defective rhizoid development screen

- **336,000** T-DNA transformed lines were screened (rare phenotypes)
- **329** defective rhizoid mutant were identified
- **89** were T-DNA tagged
- Defined the function of **36 genes**
- **20%** had not previously been characterised in plants



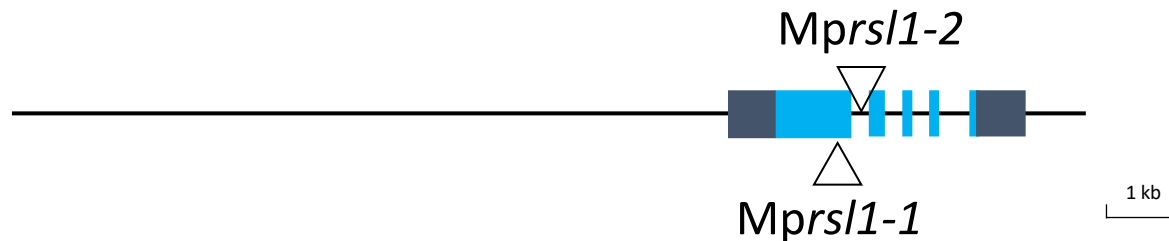
# Insertions into the *MpRSL1* coding sequence of two rhizoidless mutants



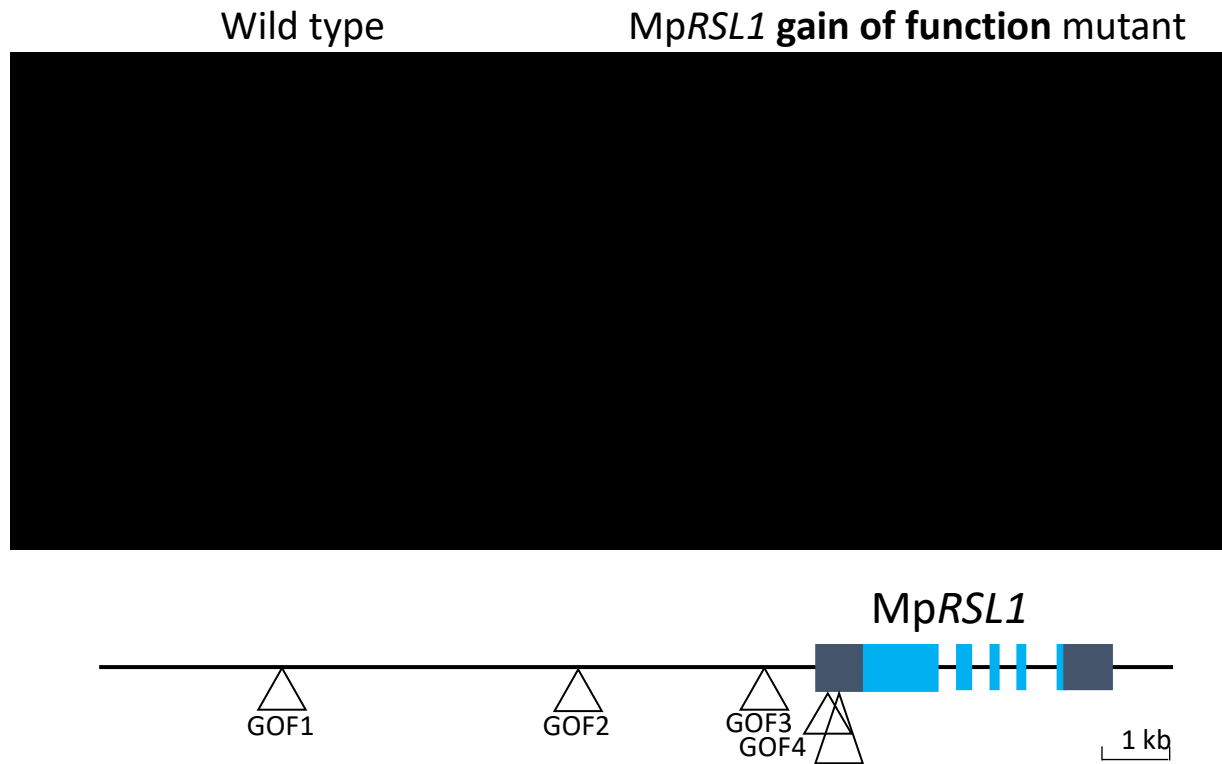
Wild type



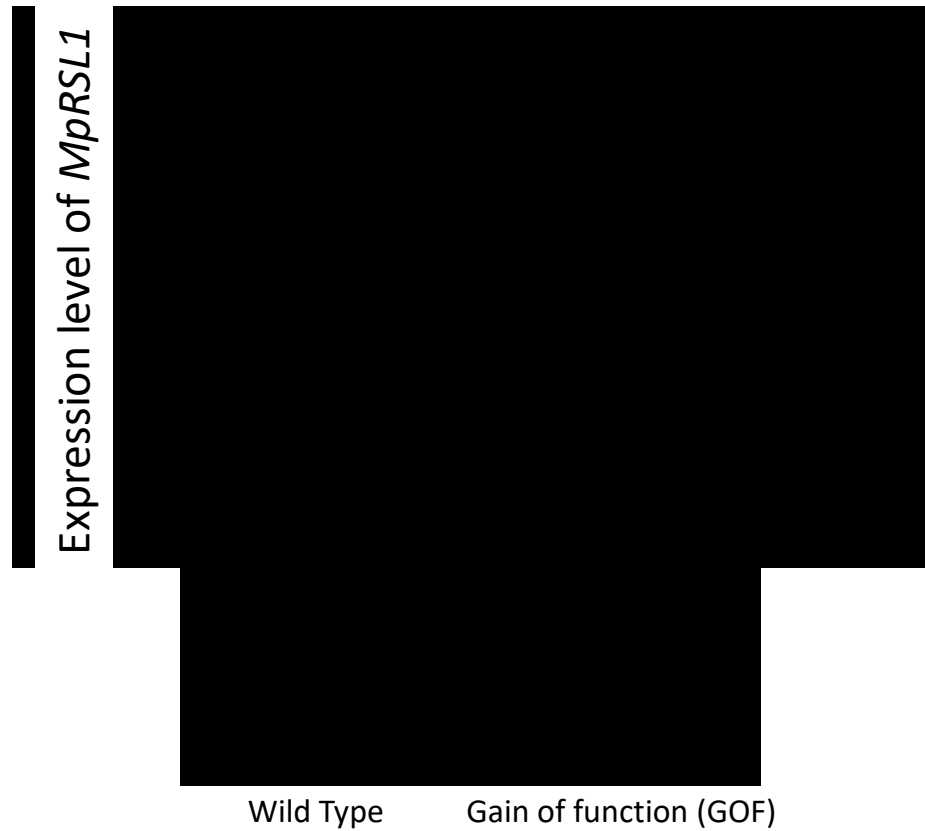
*Mprs/1* **loss of function** mutant  
(no *MpRSL1* expression detected)



# Insertions in 5' region of *MpRSL1* gene in mutants with dorsal rhizoids



Expression of *MpRSL1* gene is higher in dorsal rhizoid mutants than wild type



# MpRSL1 is necessary and sufficient for rhizoid development in *Marchantia*

Wild type

Mprs1-1<sup>loss of function</sup> (2 alleles)

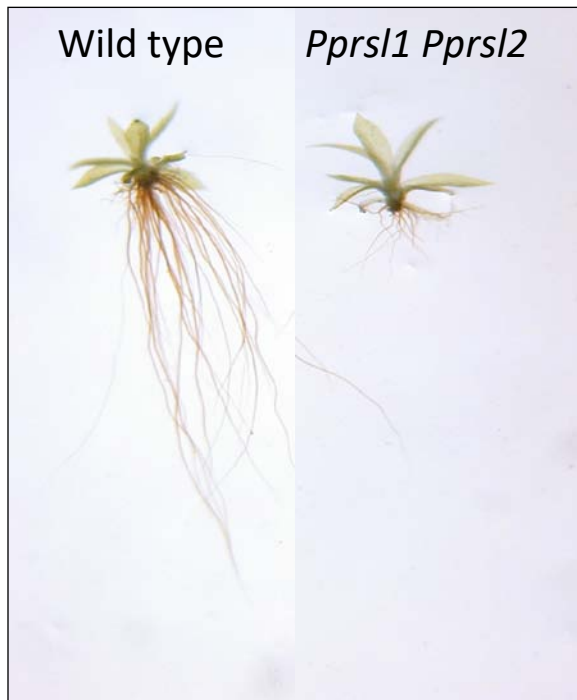


MpRSL1<sup>GAIN OF FUNCTION</sup> (20 alleles)

RSL1 is a **class VIIIc** basic helix loop helix transcription factor protein found in all major land plant clades

# *RSL* genes are necessary and sufficient for rhizoid development in the moss *Physcomitrella patens*

## Loss of function

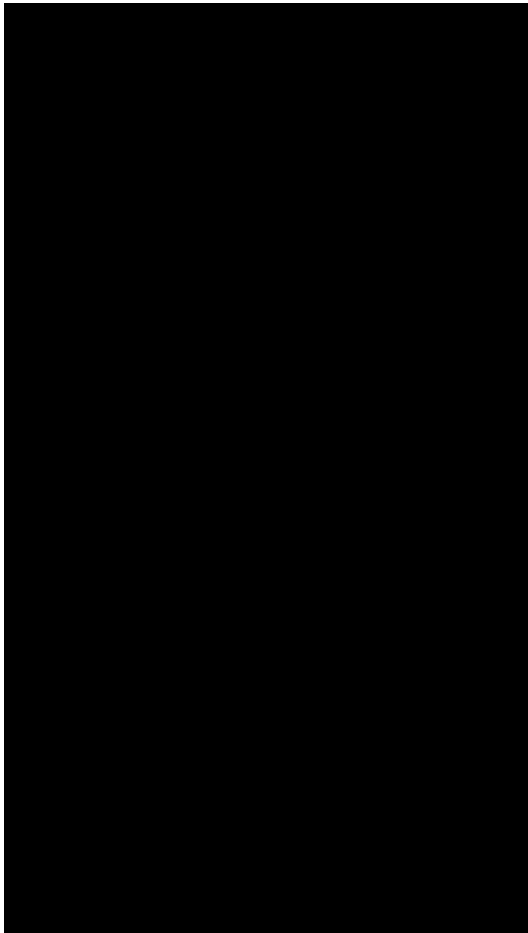


## Gain of function

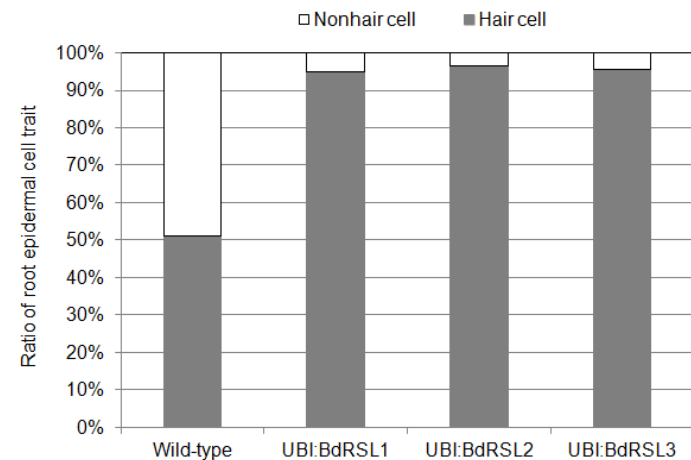
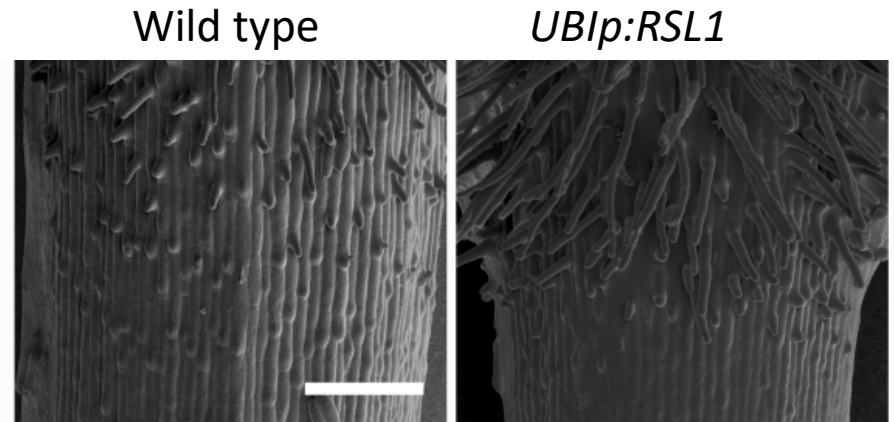


# *RSL* genes are necessary and sufficient for root hair development in angiosperms

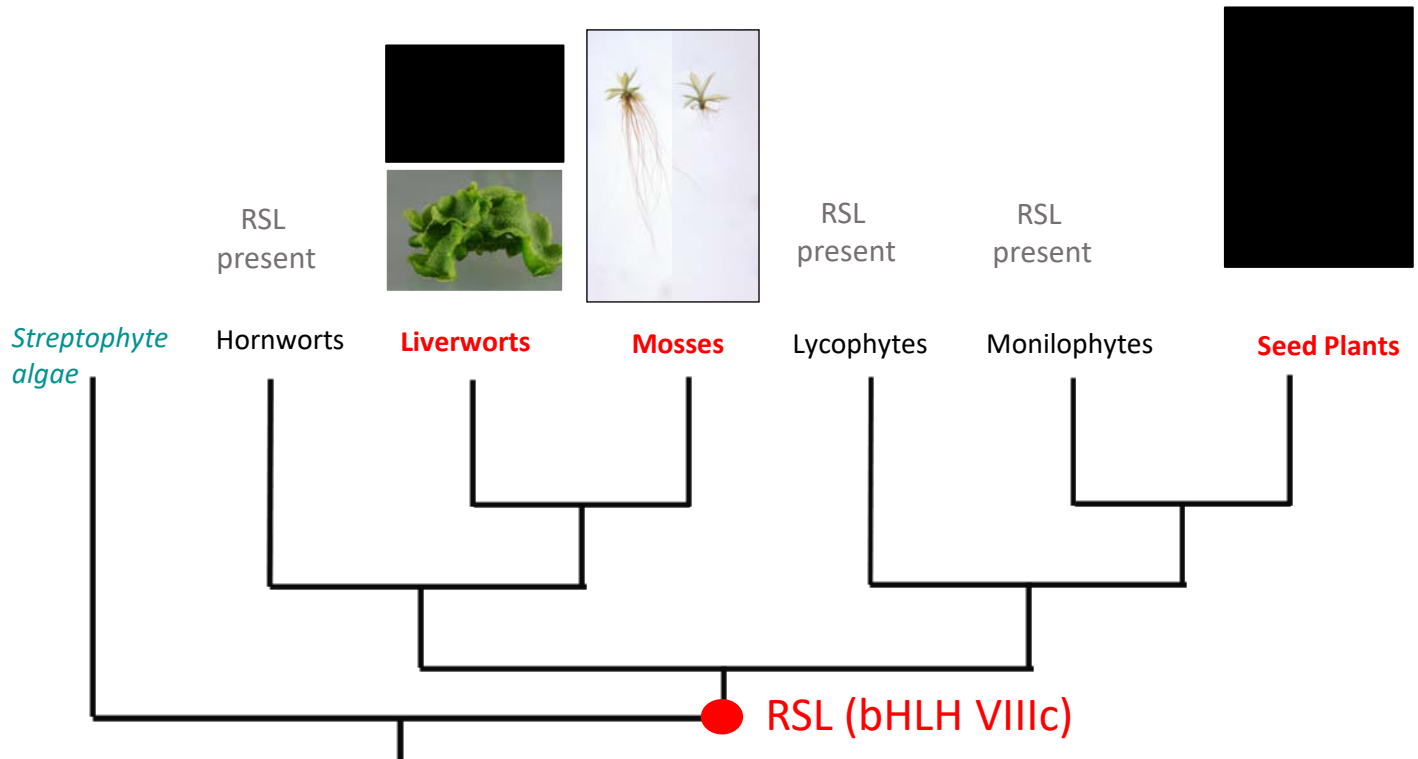
Loss of function in *A. thaliana*



Gain of function in *Brachypodium distachyon*

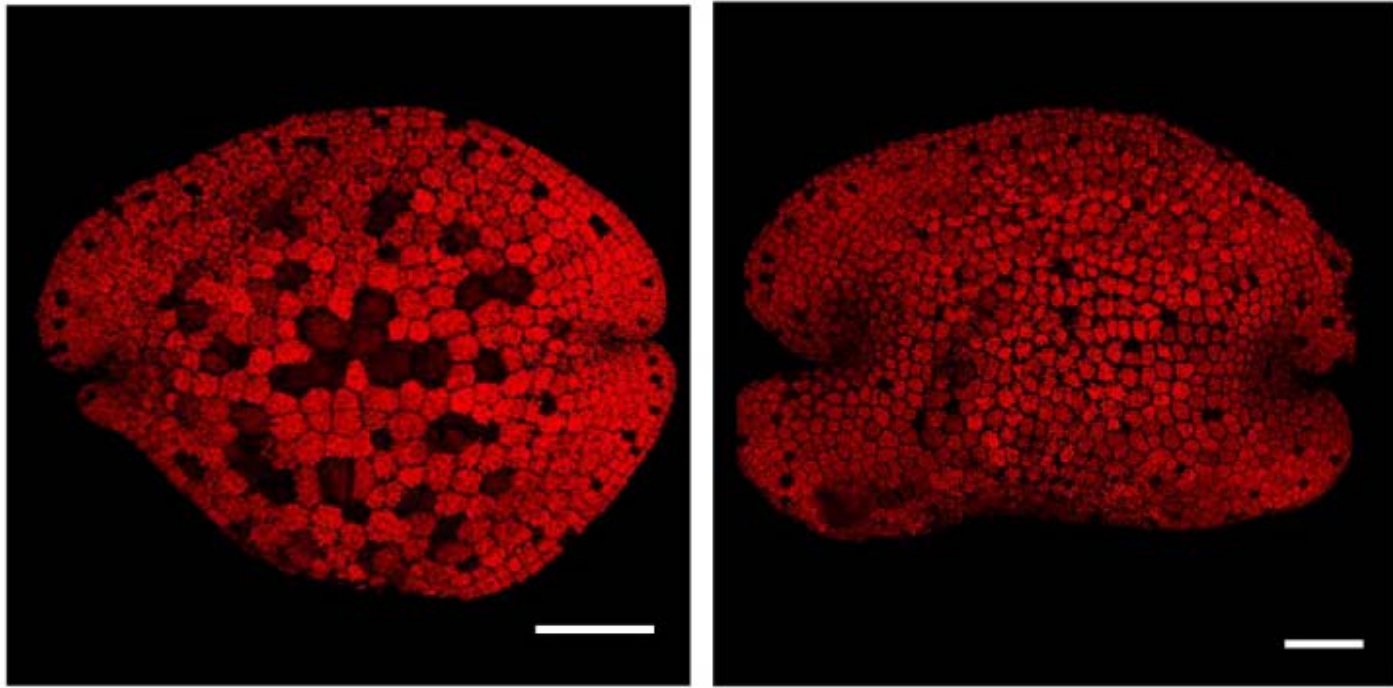


*RSL* genes regulate rhizoid development in *Marchantia*, *Physcomitrella* and root hair development in *Arabidopsis*, *Oryza*, *Brachypodium*



RSL (VIIIc bHLH ) proteins positively regulated the development of rhizoids in the common ancestor of the land plants

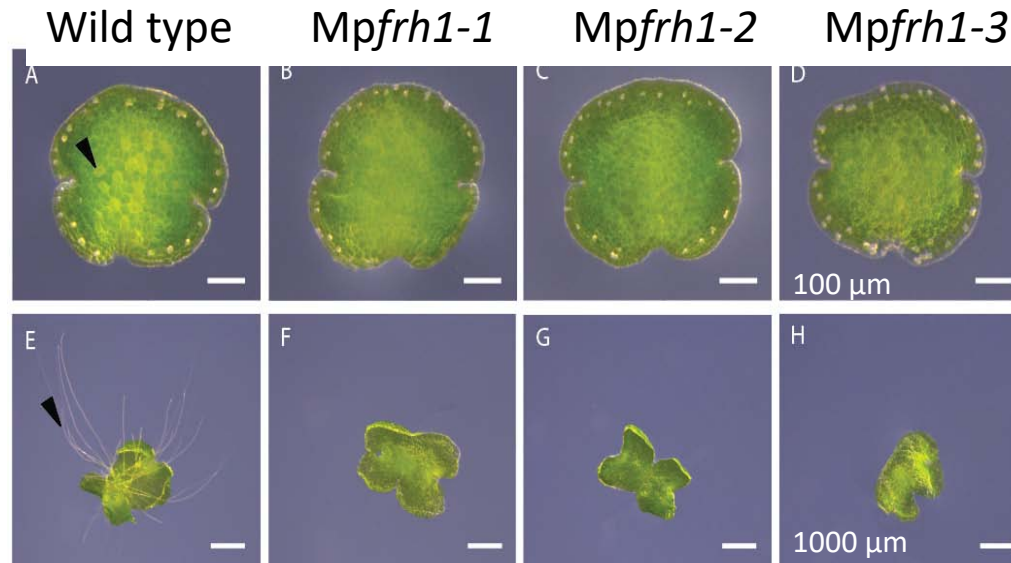
## 2. Evolution of a novel patterning mechanism among liverworts (genetics)



Different negative regulatory mechanisms evolved in different lineages (repressors are not conserved)

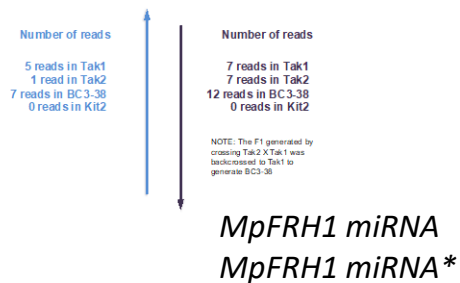
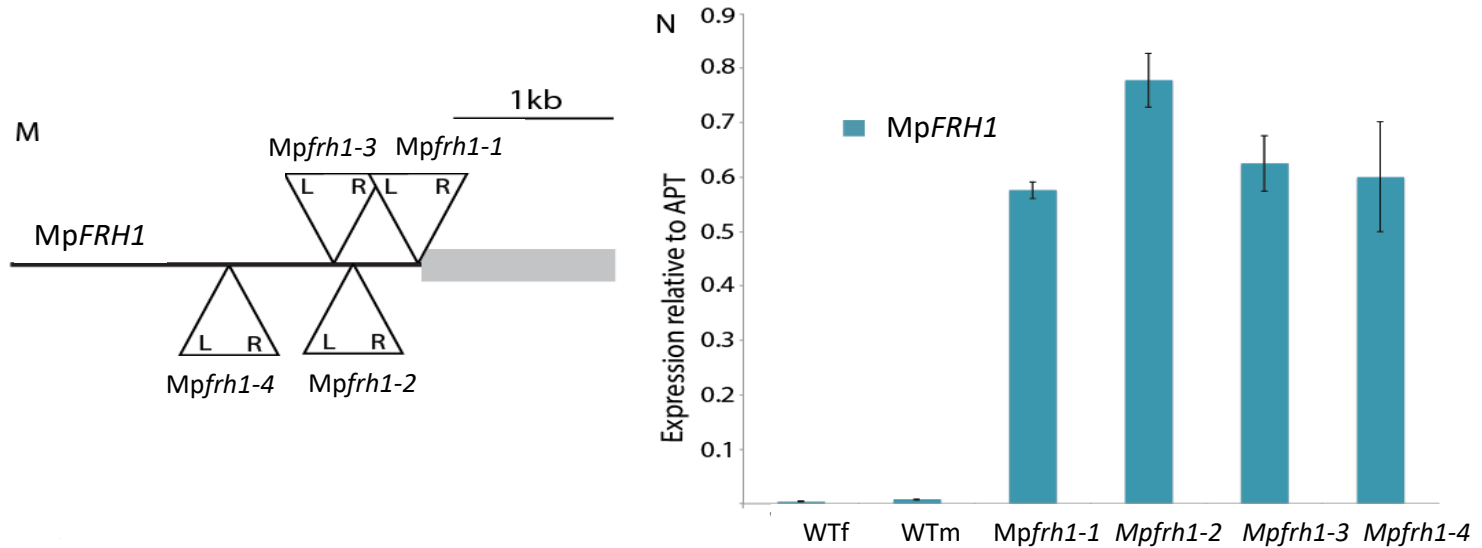


# Mp*few rhizoids1* (Mp*frh1*) mutants develop few rhizoids

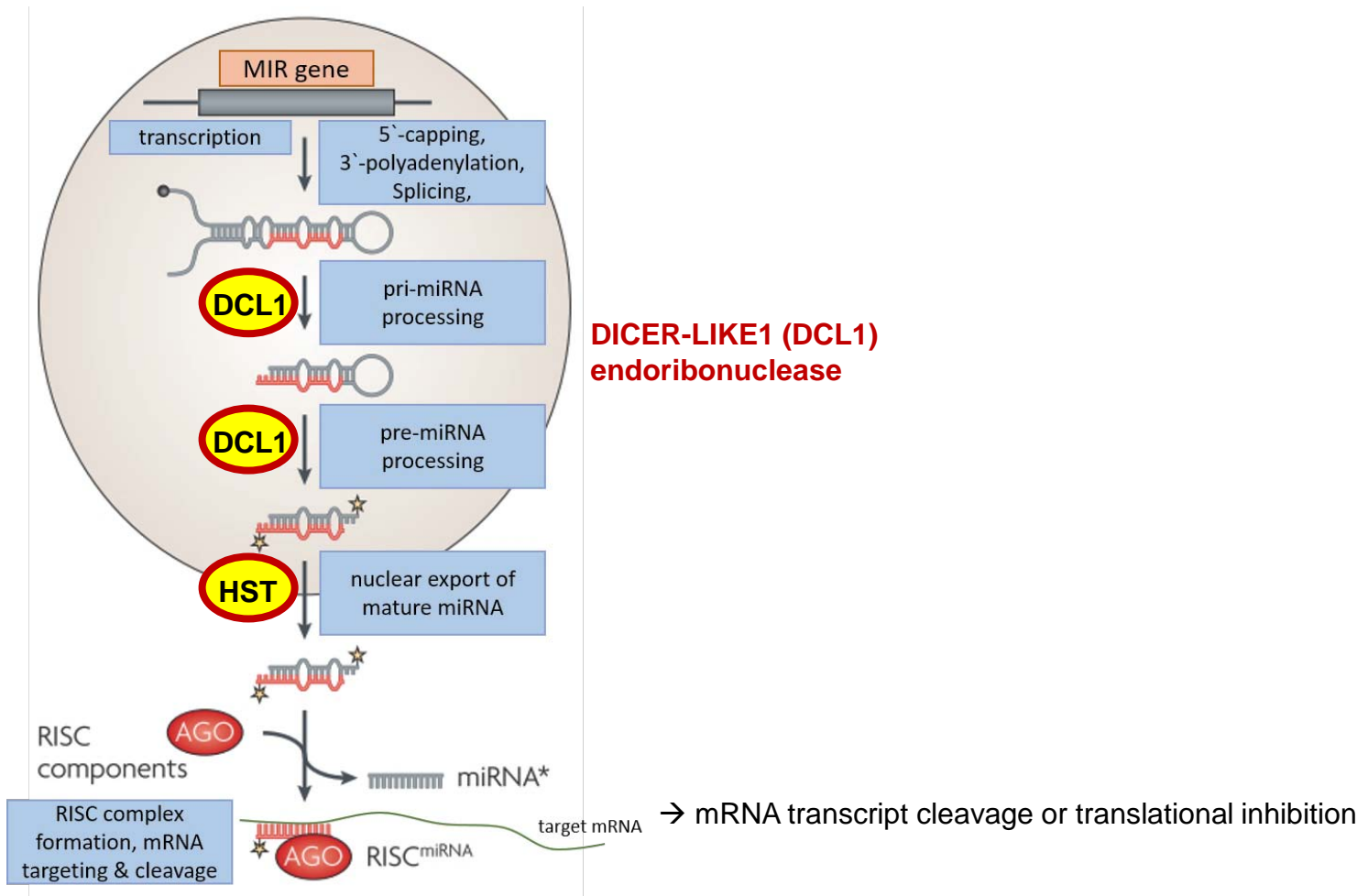


Four of the six rhizoidless mutants had mutations in the same gene, Mp*FRH1*

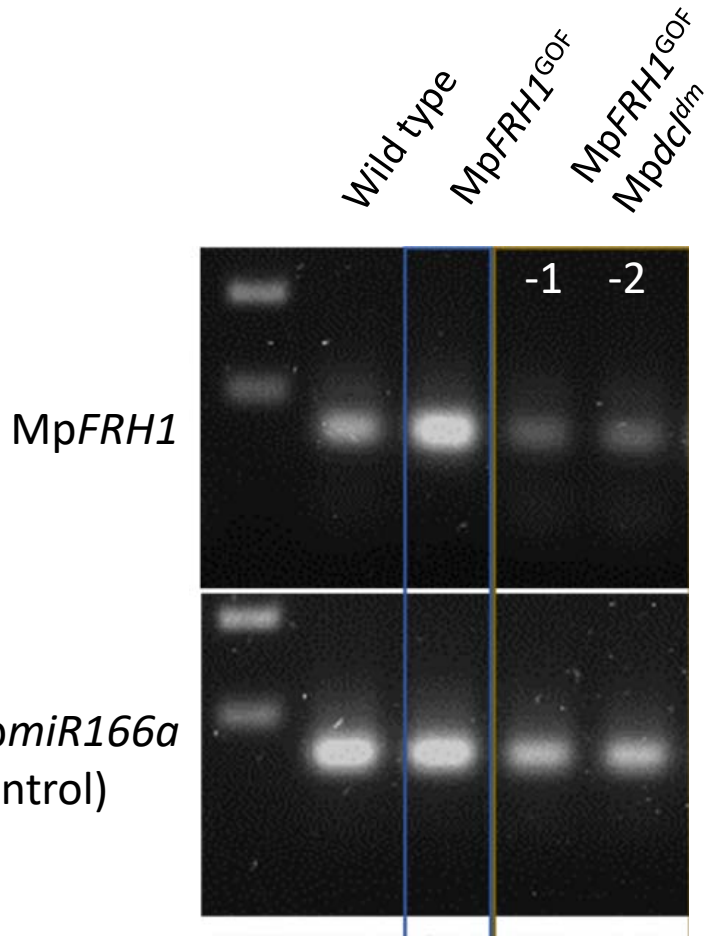
# T-DNA inserts upstream of 1.2 kb transcript in four *Mpfrh1* mutants



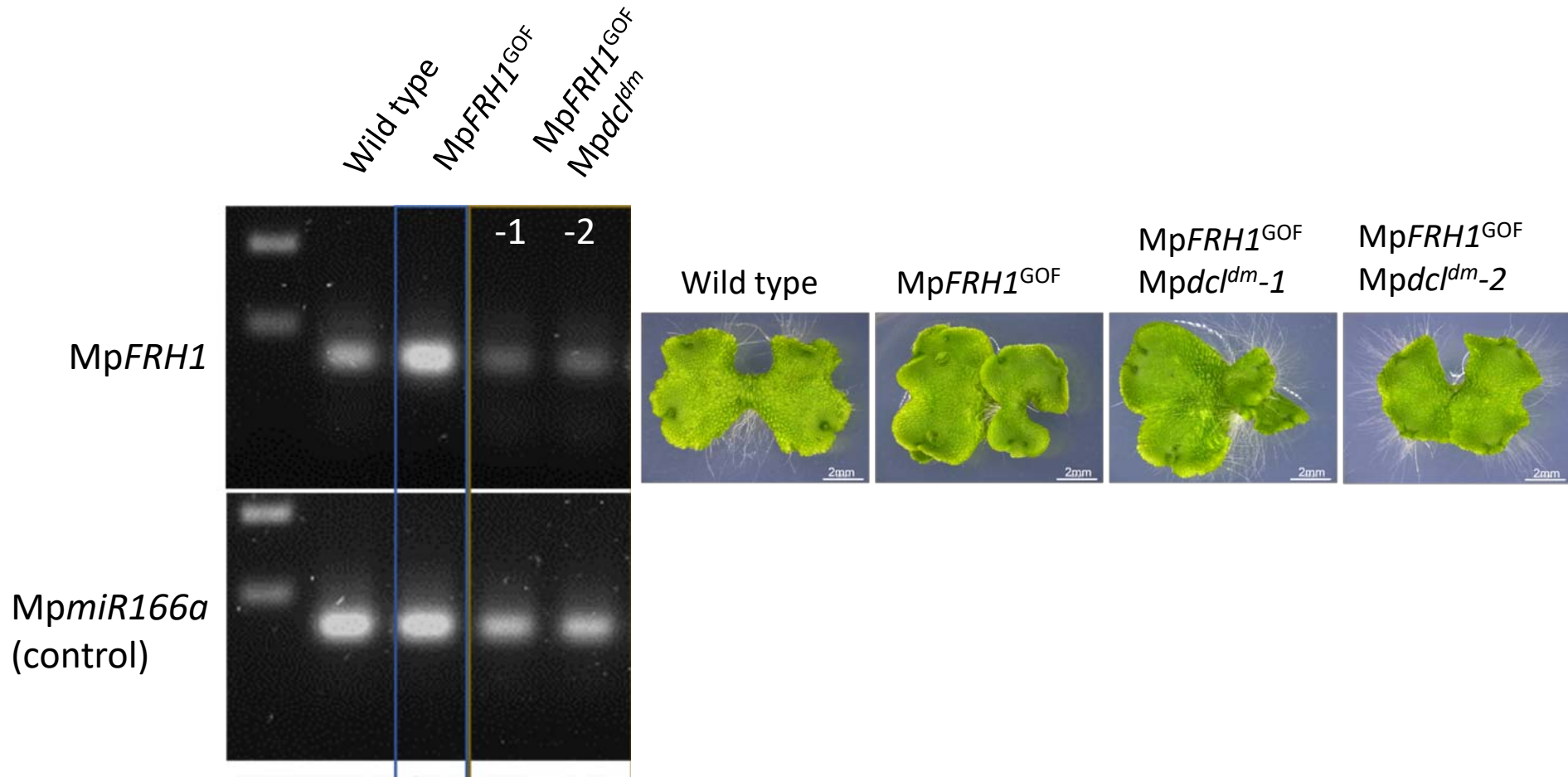
# miRNA processing in flowering plants



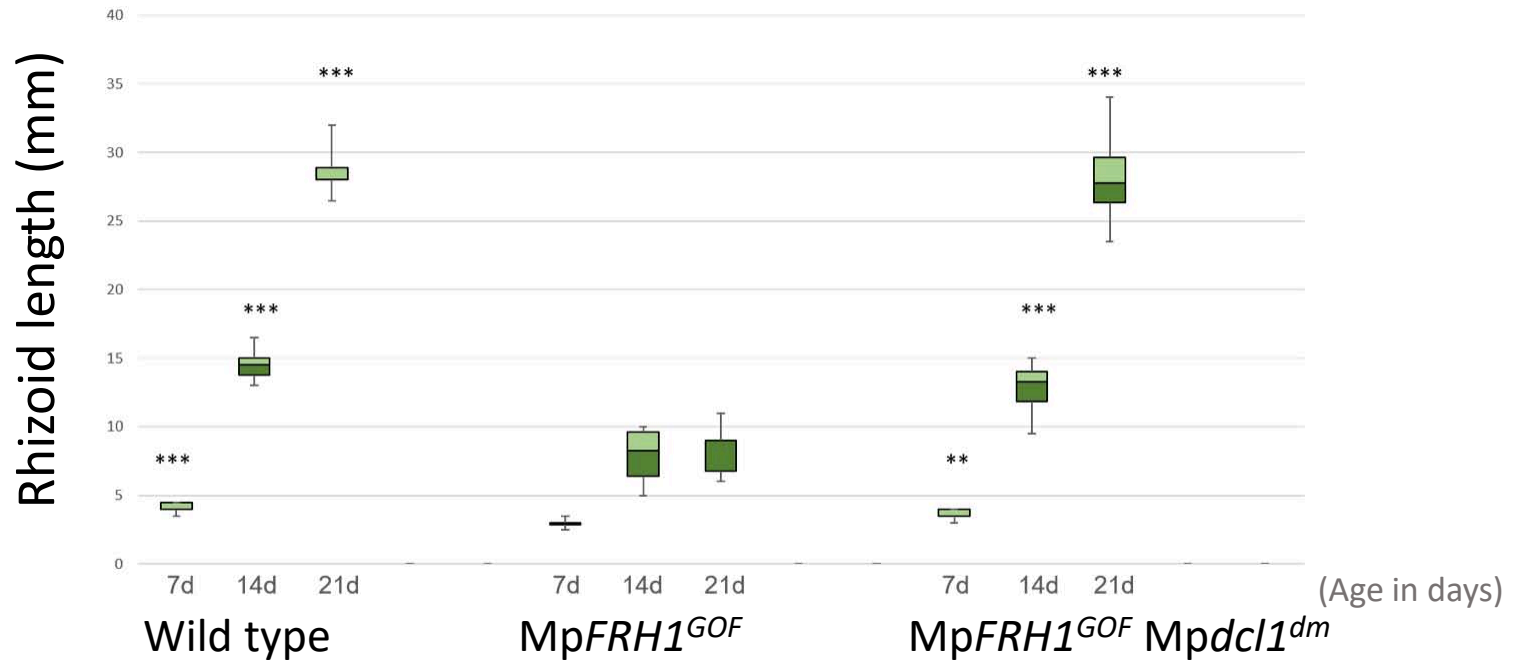
MpFRH1 miRNA is less abundant in weak Mp*dicerlike one*  
MpFRH1<sup>GOF</sup> double than in MpFRH1<sup>GOF</sup> single mutant



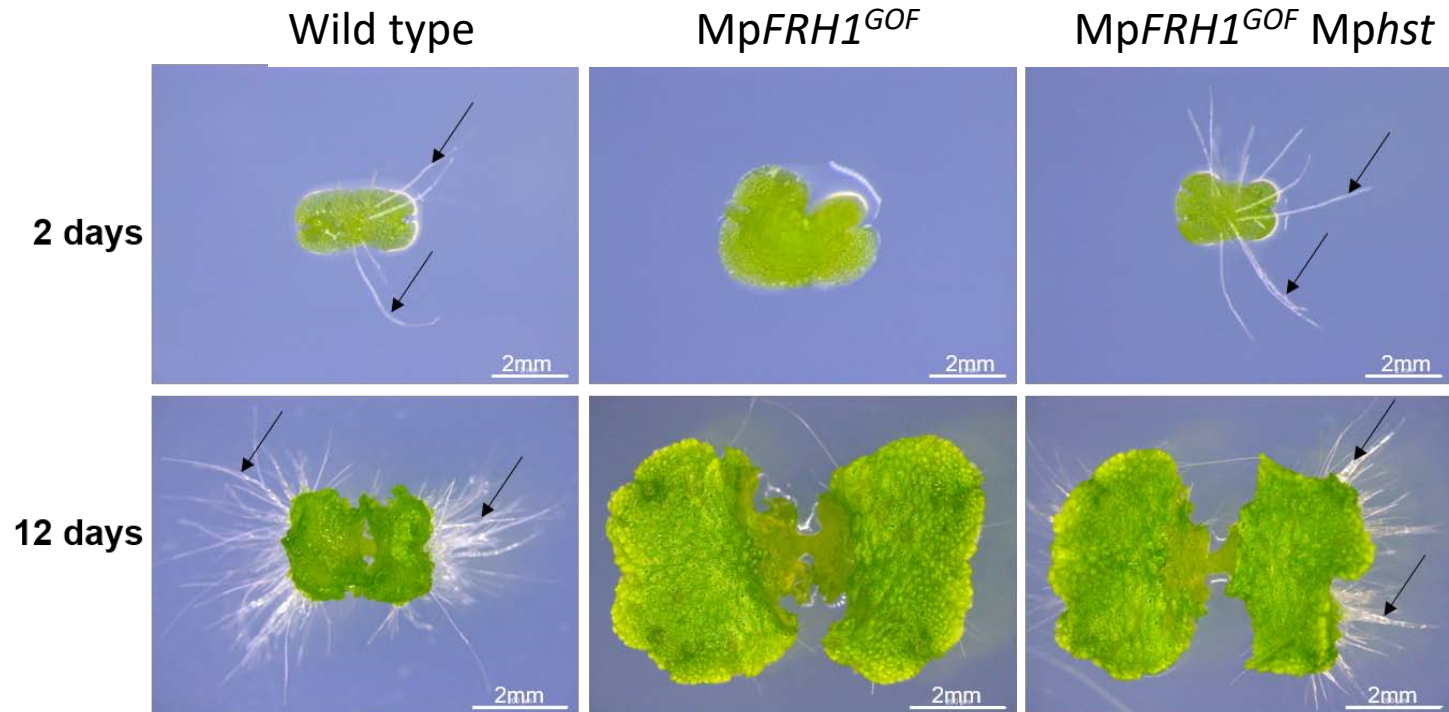
# Reduced Mp*DICERLIKE ONE* function restores rhizoid formation in Mp*FRH1*<sup>GOF</sup> background



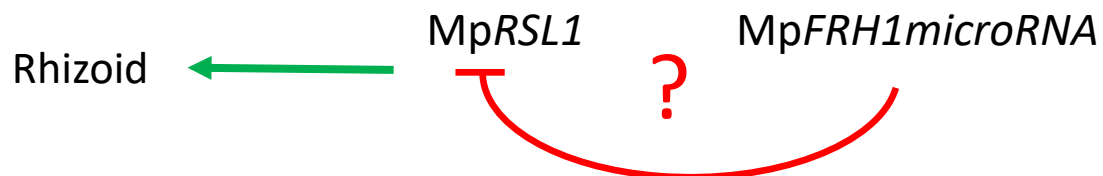
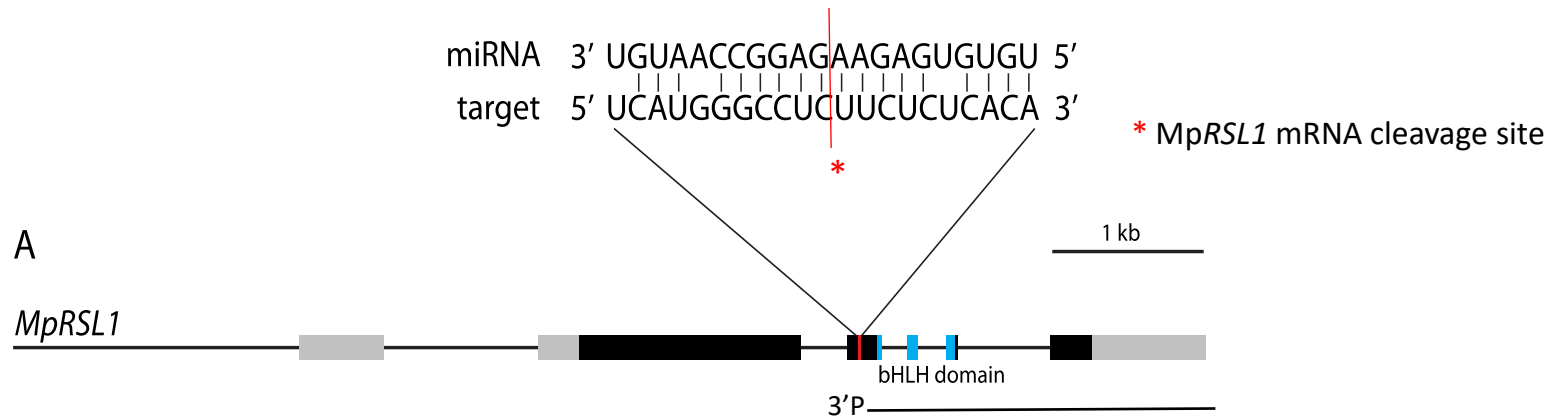
# Mpdcl1<sup>dm</sup> suppresses the MpFrh1<sup>GOF</sup> phenotype



# Mphst suppresses the MpFrh1<sup>GOF</sup> phenotype

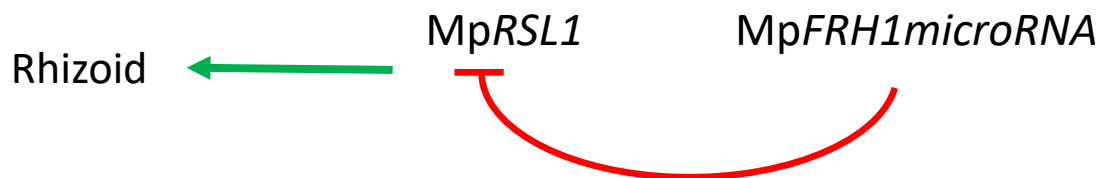
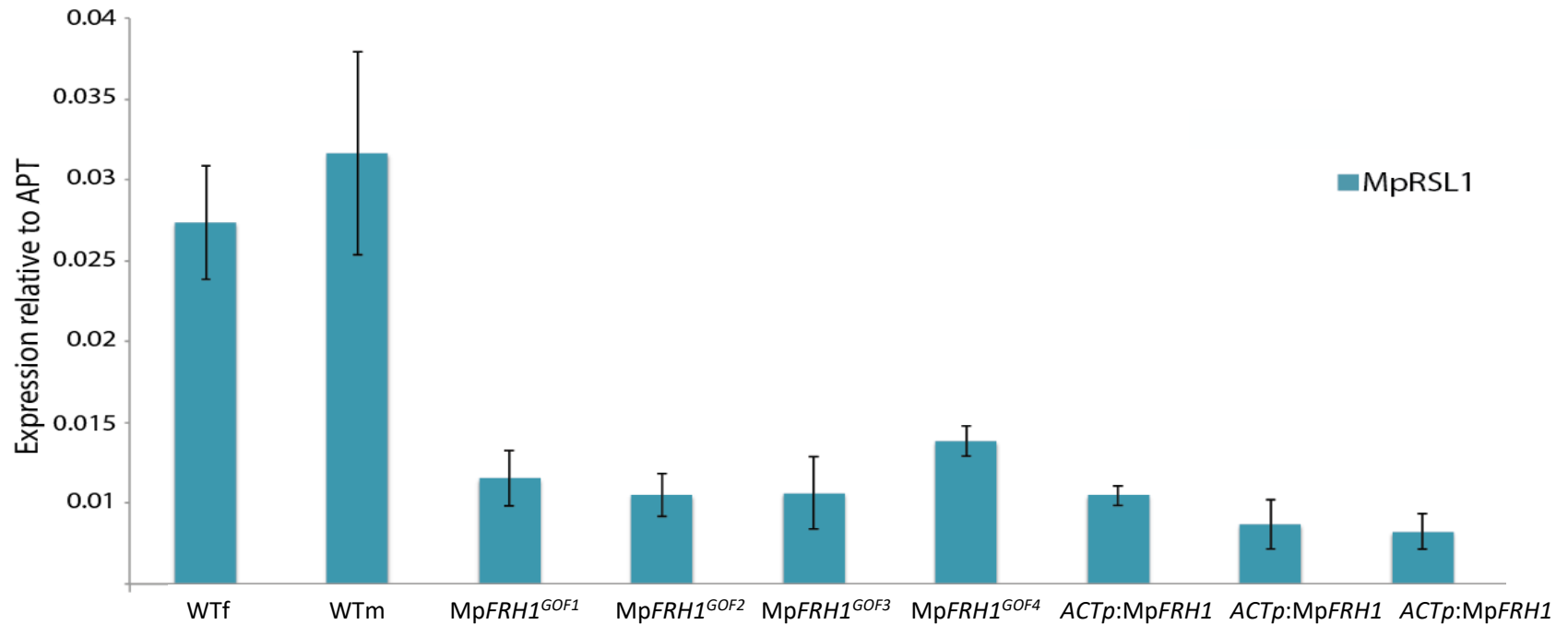


# MpRSL1 is target of the 21 nt MpFRH1 miRNA

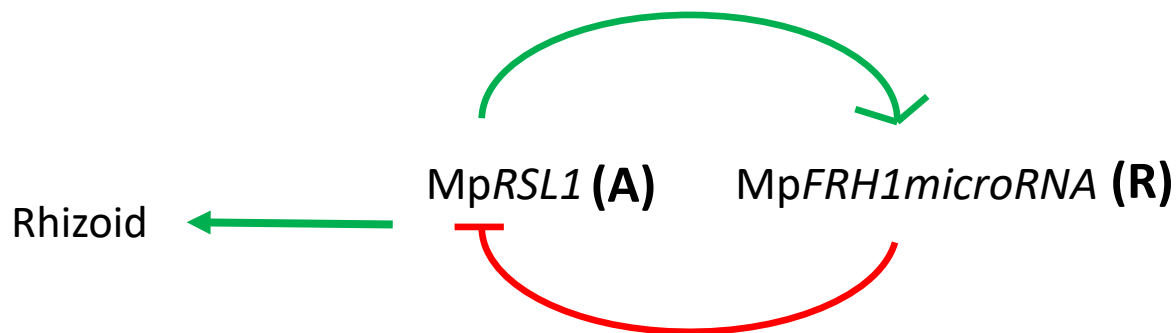
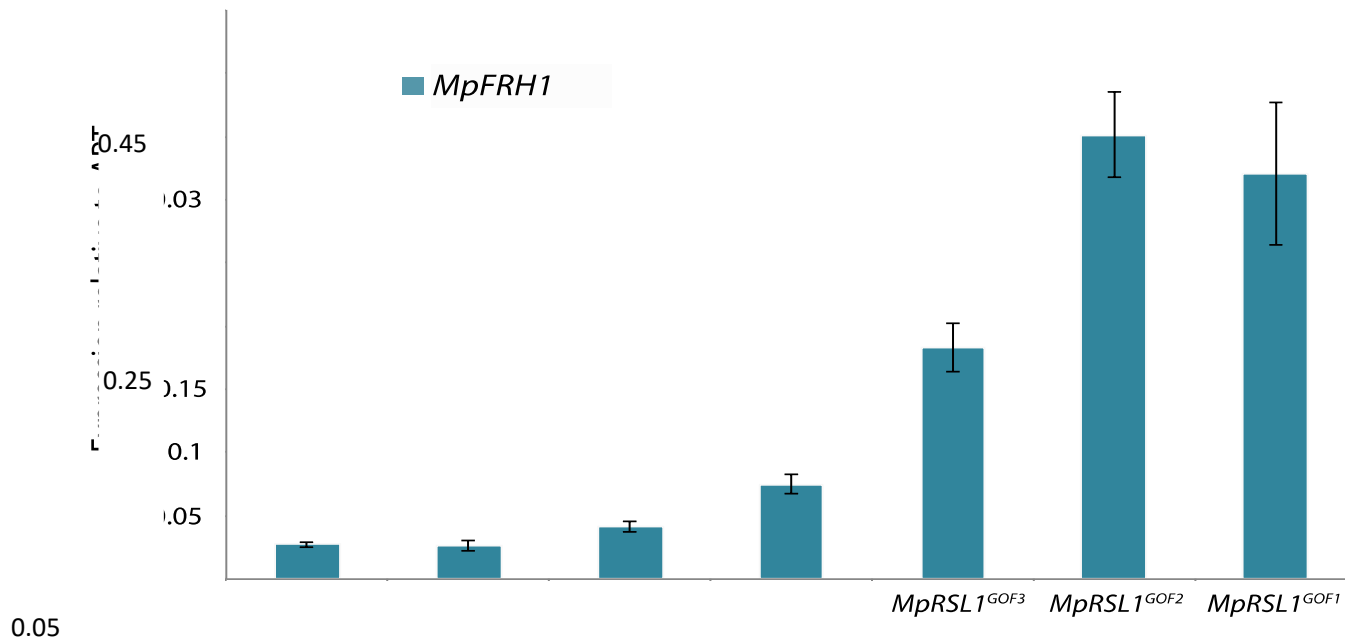




# MpFRH1 negatively regulates MpRSL1 expression

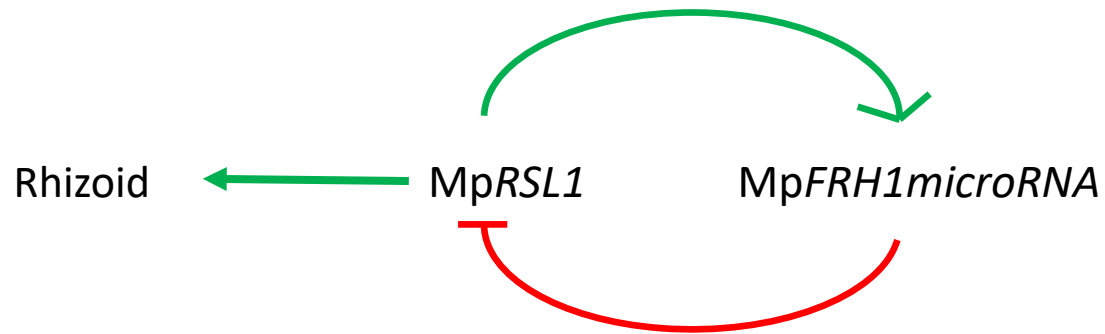


# MpRSL1 positively regulates MpFRH1 expression

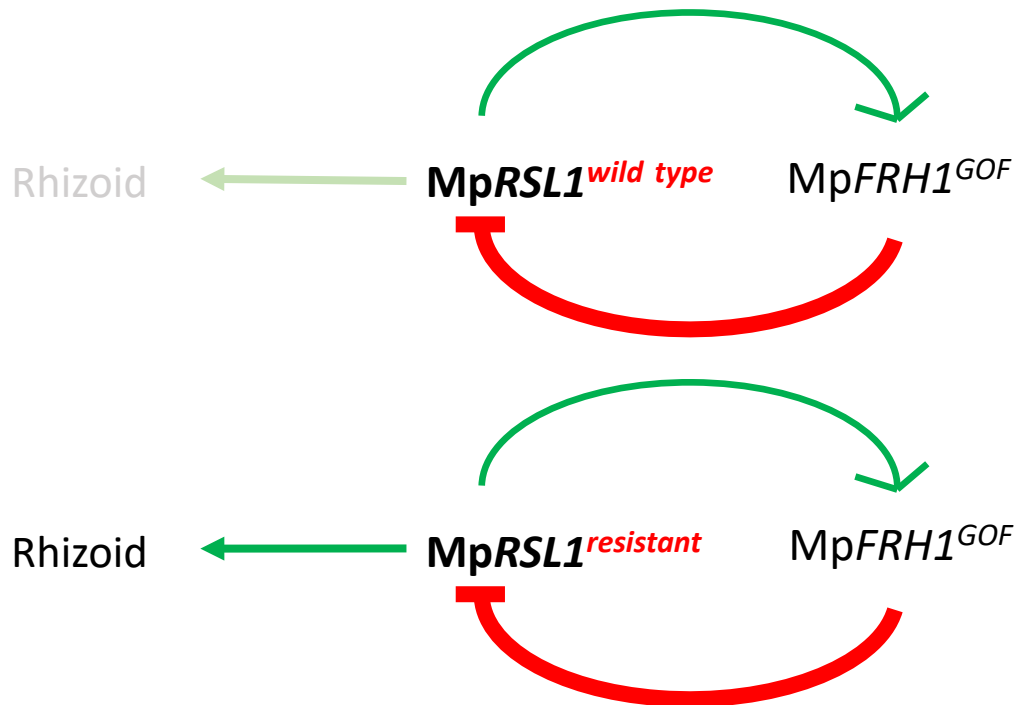


Growth or pattern?

# Regulatory loop with feedback

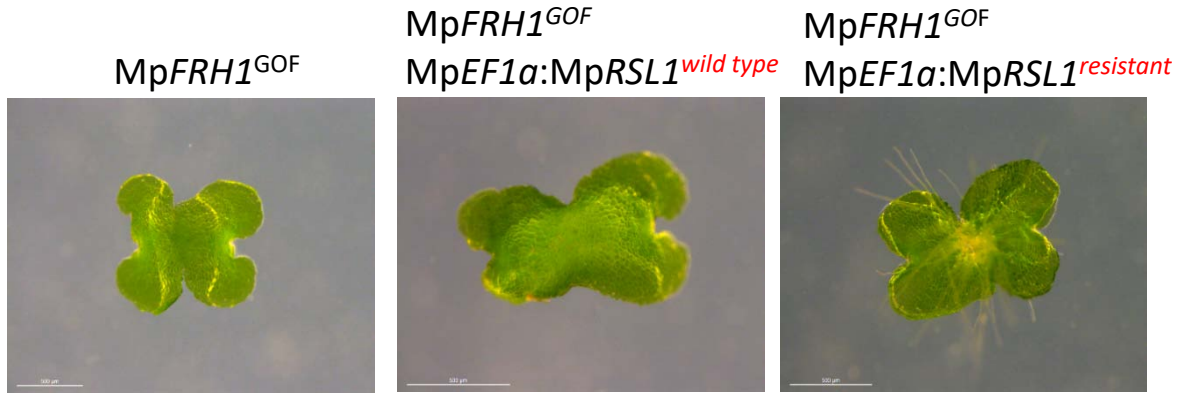


# Regulatory loop with feedback: test

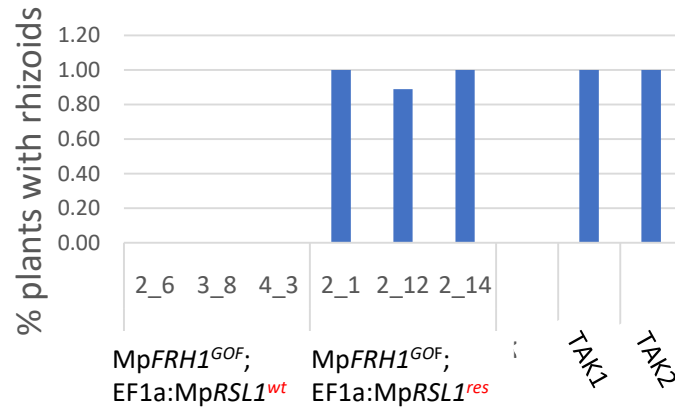


TCA TGG GCC TCT TCT CTC ACA Wild type MpRSL1  
TCG TGG GCC AGT AGT TTG ACA Resistant MpRSL1

# MpFRH1<sup>GOF</sup> plants transformed with an MpFRH1-resistant MpRSL1 develop rhizoids

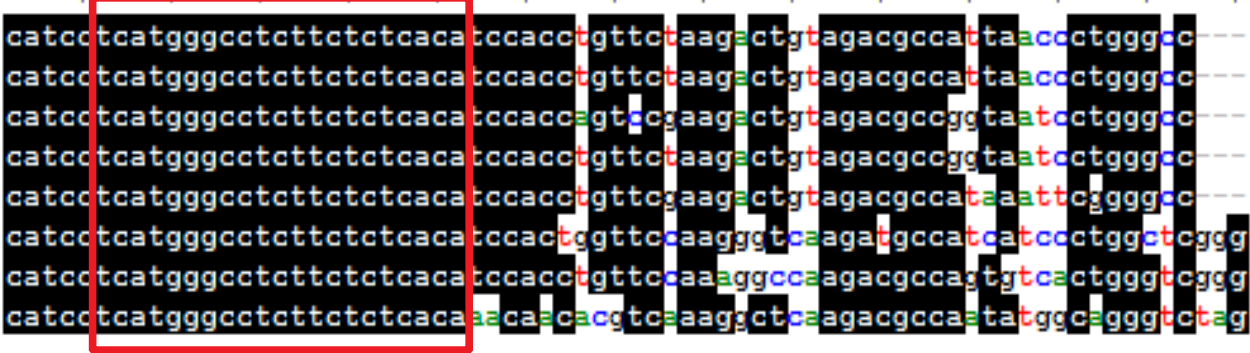


Rhizoid formation after 3 days

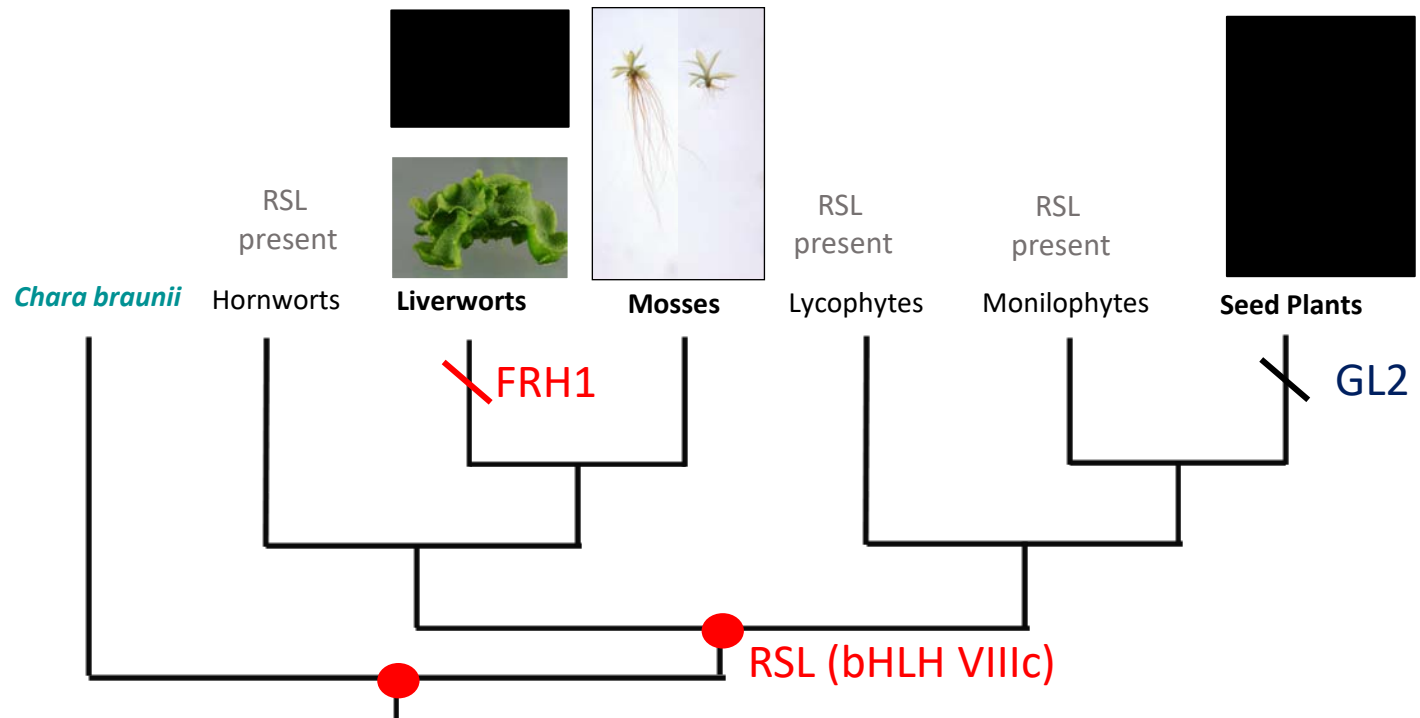


# MpFRH1 evolved among the liverworts

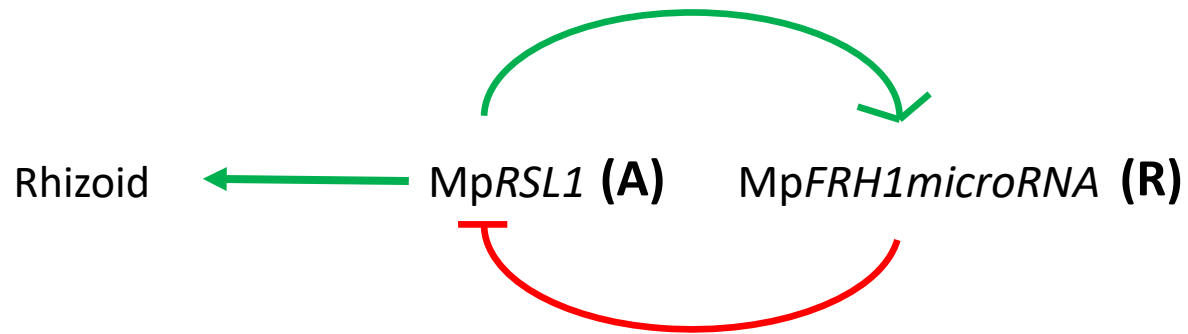
sida



# Evolution of mechanism regulating filamentous rooting structure development



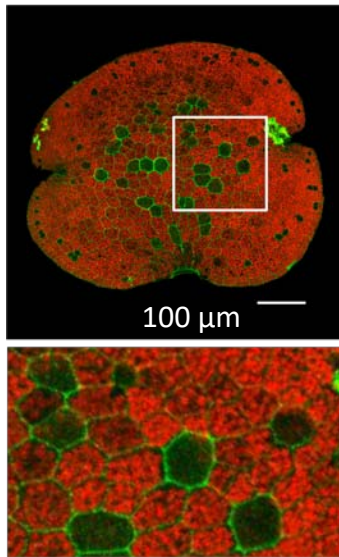
Does MpFRH1 miRNA – MpRSL1mRNA interaction function in patterning?



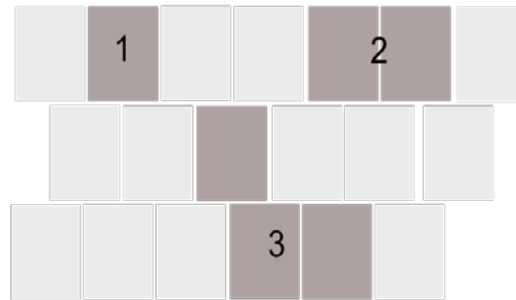


# Development of rhizoids on wild type gemmae (vegetative propagules)

Tak-2



Rhizoid cells per cluster

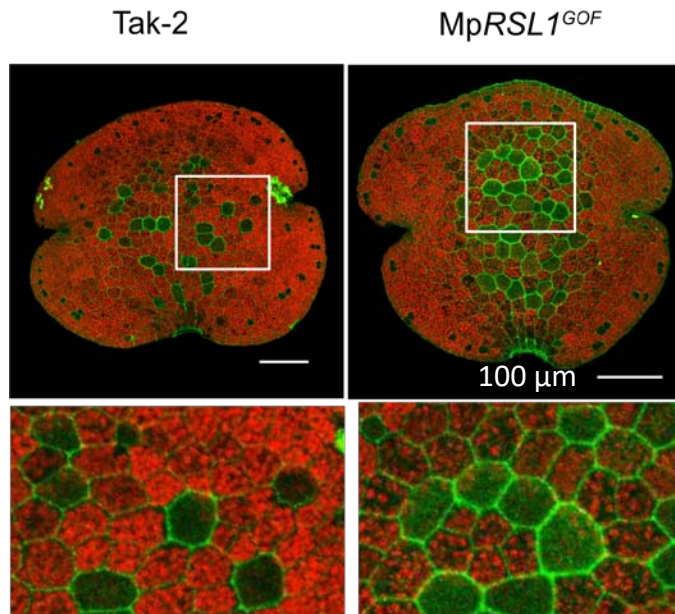


Bar = 50 μm

**Percentage cells in each size-class of rhizoid cluster**

|      | 1-cell | 2-cell | 3-cell | 4-cell | 5-cell | 6-cell | 7-cell | 8-cell | 9-cell | >10-cell | n   |
|------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------|-----|
| TAK2 | 65.4   | 20.2   | 8.7    | 2.9    | 1.0    | 1.9    | -      | -      | -      | -        | 104 |

# Supernumerary rhizoids develop where *MpRSL1* is ectopically expressed at high levels

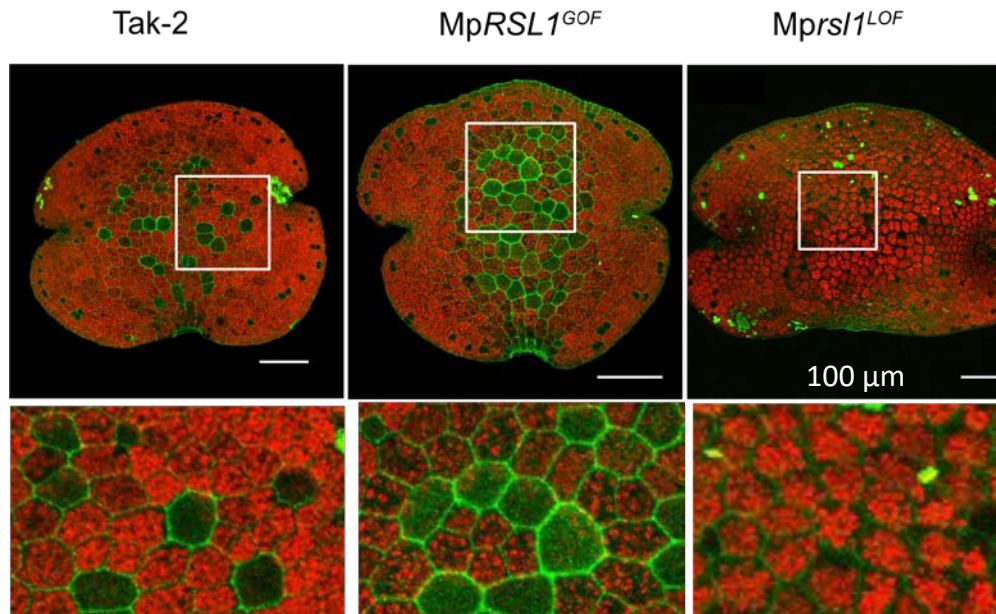


**Percentage cells in each size-class of rhizoid cluster**

Bar = 50 μm

|                              | <b>1-cell</b> | <b>2-cell</b> | <b>3-cell</b> | <b>4-cell</b> | <b>5-cell</b> | <b>6-cell</b> | <b>7-cell</b> | <b>8-cell</b> | <b>9-cell</b> | <b>&gt;10-cell</b> | <b>n</b> |
|------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------------|----------|
| <i>MpRSL1</i> <sup>GOF</sup> | 58.6          | 14.1          | 13.1          | 5.1           | 3.0           | 1.0           | 2.0           | -             | -             | 3.0                | 99       |
| TAK2                         | 65.4          | 20.2          | 8.7           | 2.9           | 1.0           | 1.9           | -             | -             | -             | -                  | 104      |

# Rhizoids do not develop on plants that do not express *MpRSL1*

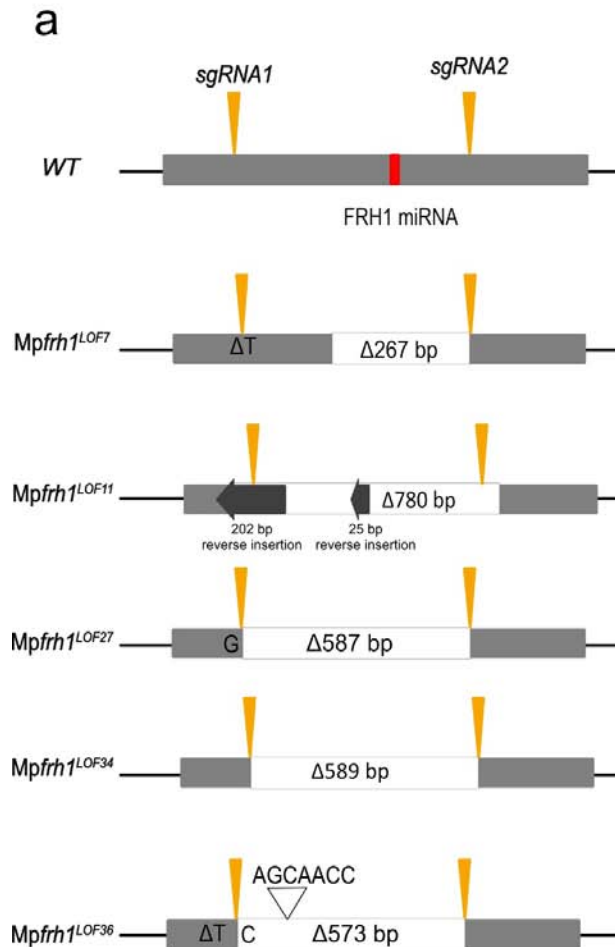


Bar = 50 μm

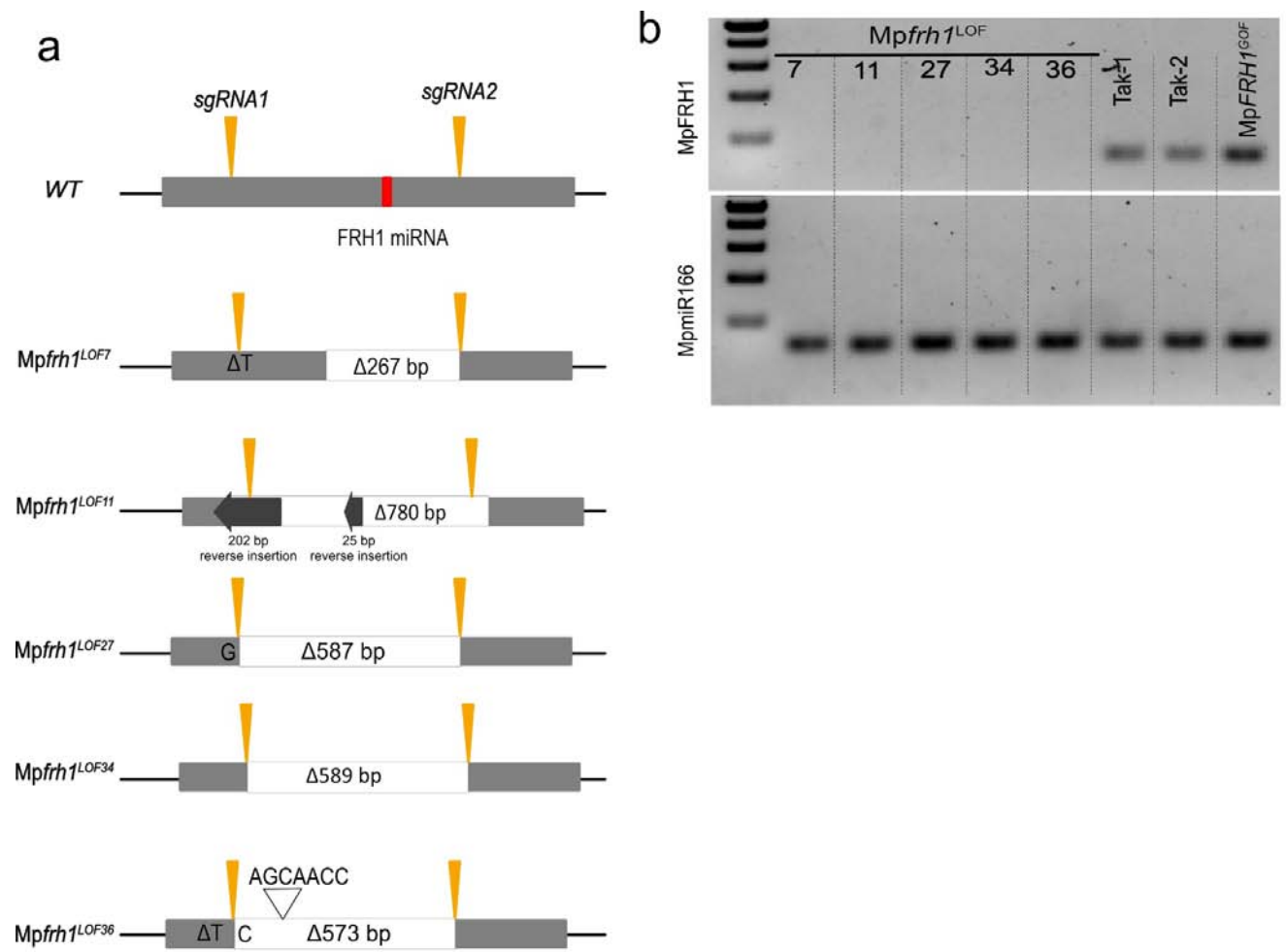
Does *MpFRH1* repress rhizoid growth and differentiation?

Does *MpFRH1* repress rhizoid cell identity during the patterning of epidermal cells?

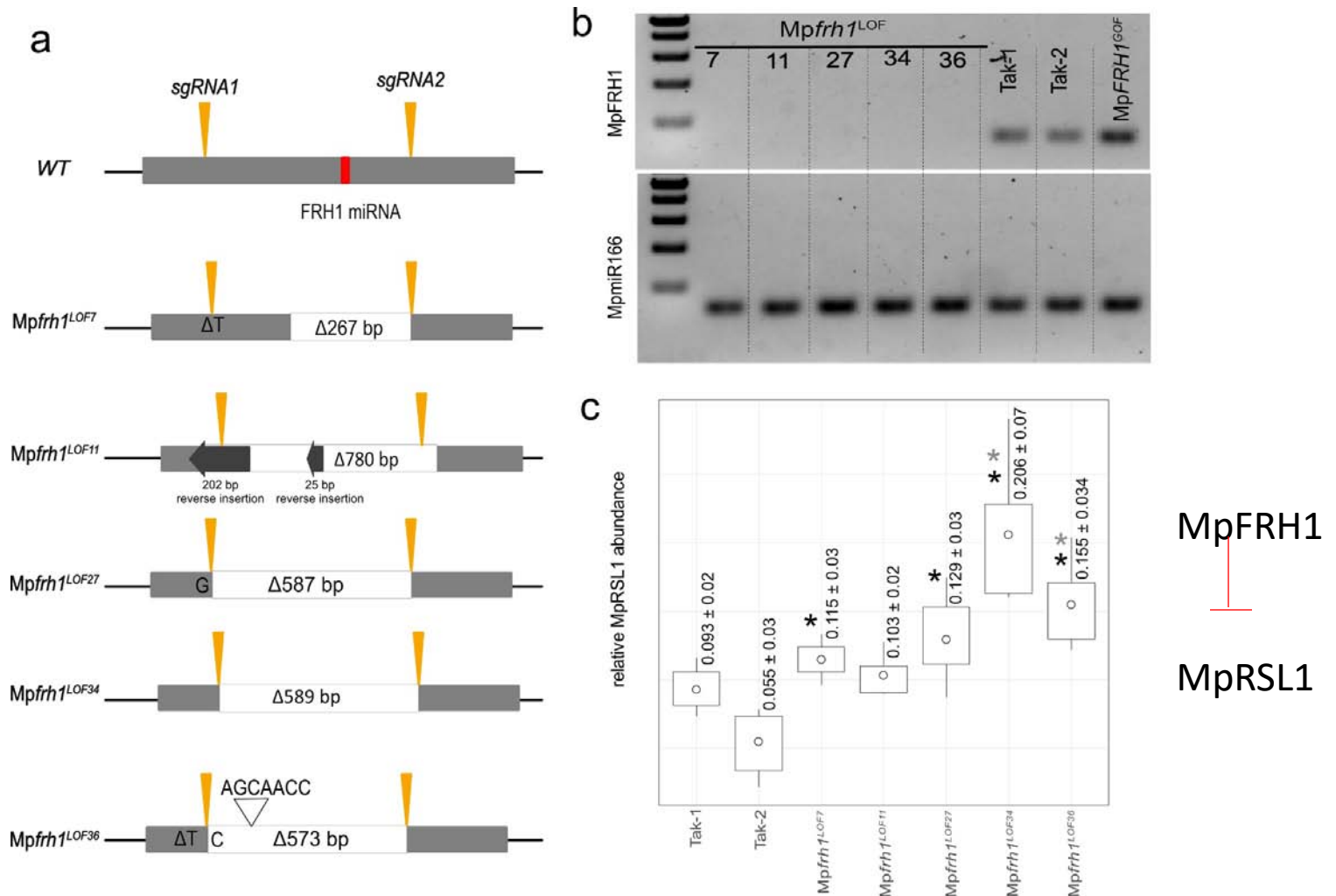
# Deletion of the FRH1 miRNA from the genome – an *Mpfrh1* loss of function mutant



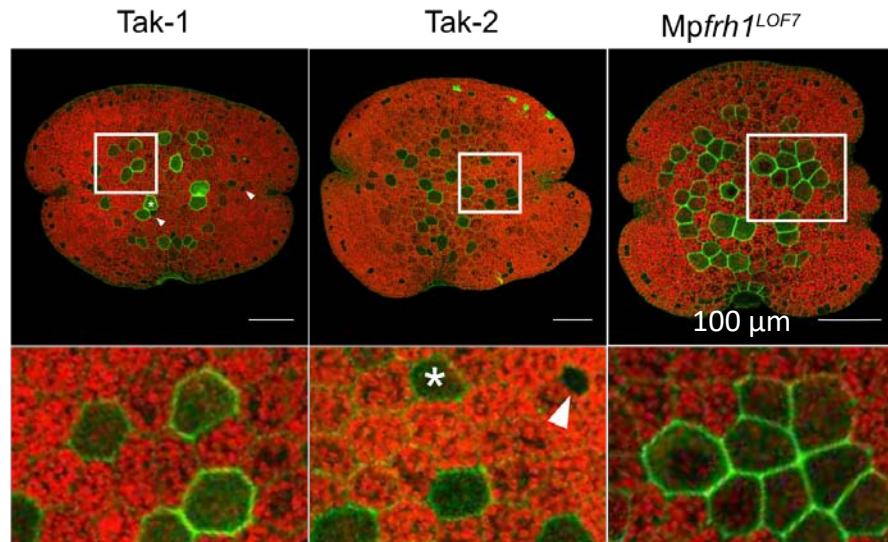
# MpFRH1 miRNA is not detectable in the *Mpfrh1* loss of function mutant



# Deletion of the MpFRH1 miRNA from the genome using CRISPR/Cas9



# Larger clusters of rhizoids develop in *Mpfrh1* loss of function mutants

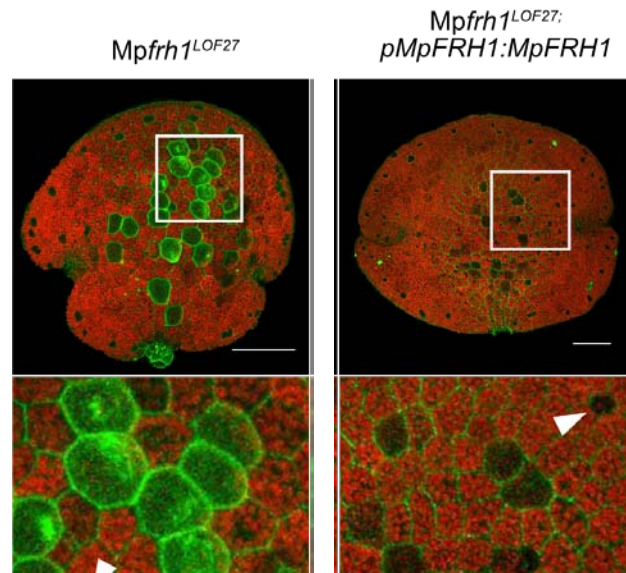


Bar = 50 µm

**Percentage cells in each size-class of rhizoid cluster**

|                               | 1-cell | 2-cell | 3-cell | 4-cell | 5-cell | 6-cell | 7-cell | 8-cell | 9-cell | >10-cell | n   |
|-------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------|-----|
| <i>Mpfrh1</i> <sup>LOF7</sup> | 47.1   | 23.2   | 12.3   | 9.0    | 4.5    | 1.3    | -      | 0.6    | 1.9    | -        | 155 |
| TAK1                          | 63.6   | 25.0   | 6.8    | 3.0    | 0.8    | 0.8    | -      | -      | -      | -        | 132 |
| TAK2                          | 65.4   | 20.2   | 8.7    | 2.9    | 1.0    | 1.9    | -      | -      | -      | -        | 104 |

# Wild type MpFRH1 gene rescues Mpfrh1<sup>LOF27</sup> phenotype



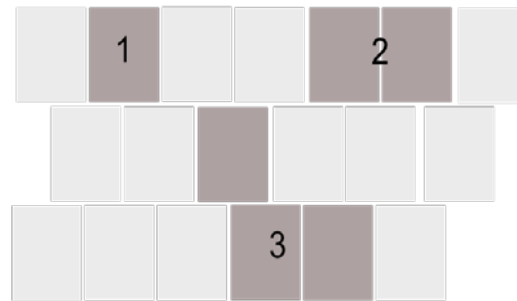
Bar = 100  $\mu$ m

- Patterning defects are due to defective MpFRH1 function in Mpfrh1 loss of function mutants
- Seven-rhizoid cell cluster never observed in wild type



Arrangement of cells in rhizoid cell clusters is defective in *Mpfrh1* loss of function mutants

Cell number  
per cluster

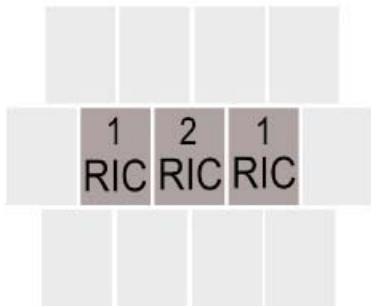


Arrangement  
of cells  
in clusters

# Classification of clusters by the average number of rhizoid cell neighbors

**Clustering shape index (CSI): average number of rhizoid neighbors per cell in a cluster**

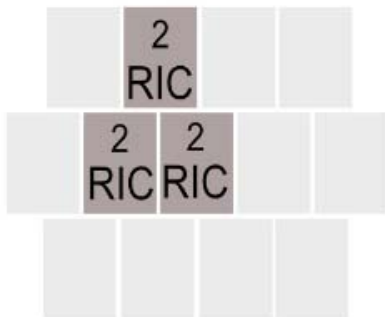
Linear cluster



Linear cluster: 1.33 neighbouring RICs

$$(1 + 2 + 1)/3 = 1.33$$

Non-linear cluster

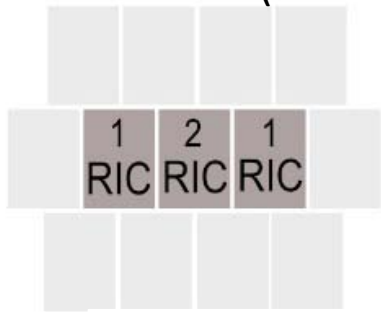


Non-linear cluster: 2 neighbouring cells

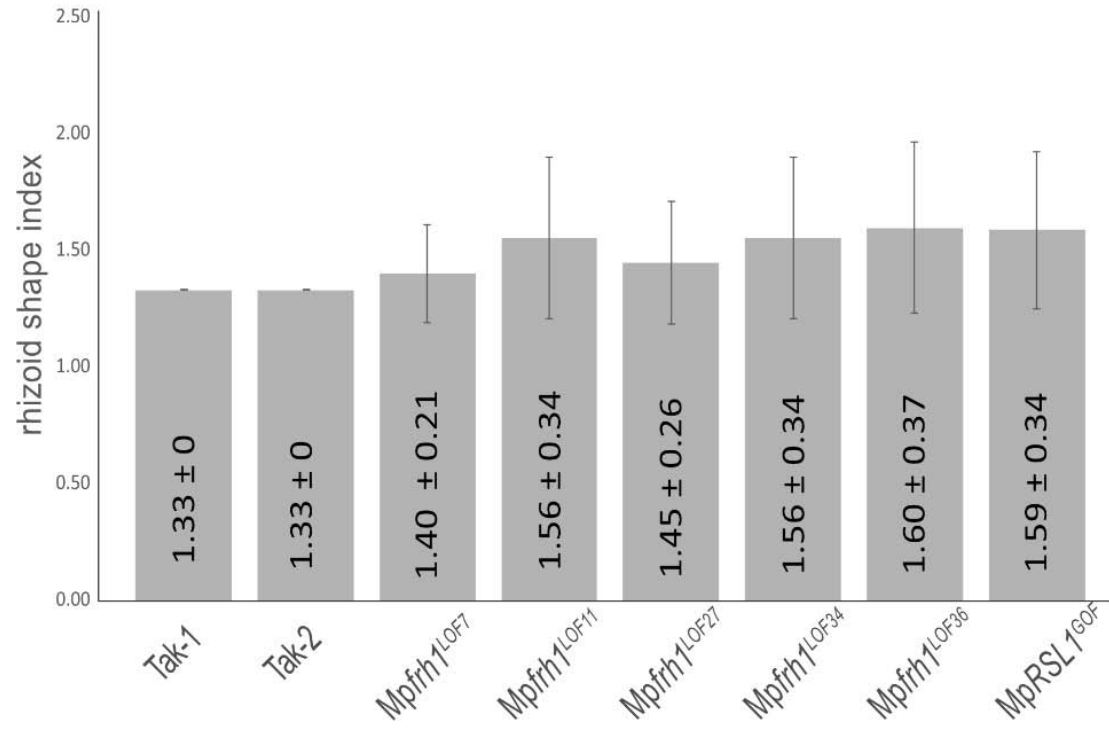
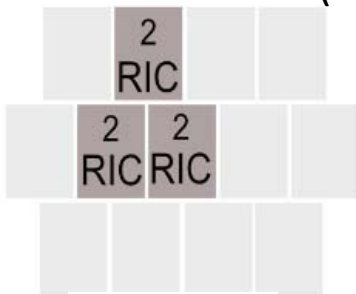
$$(2 + 2 + 2)/3 = 2$$

# Higher variability of rhizoid cell neighbor number in *Mpfrh1* mutants than in wild type – three cell clusters

Linear cluster (CSI = 1.33)

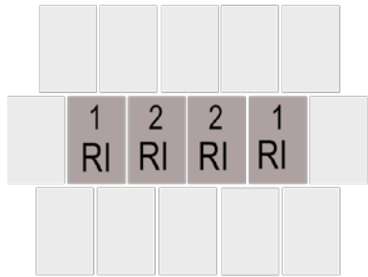


Non-linear cluster (CSI = 2)

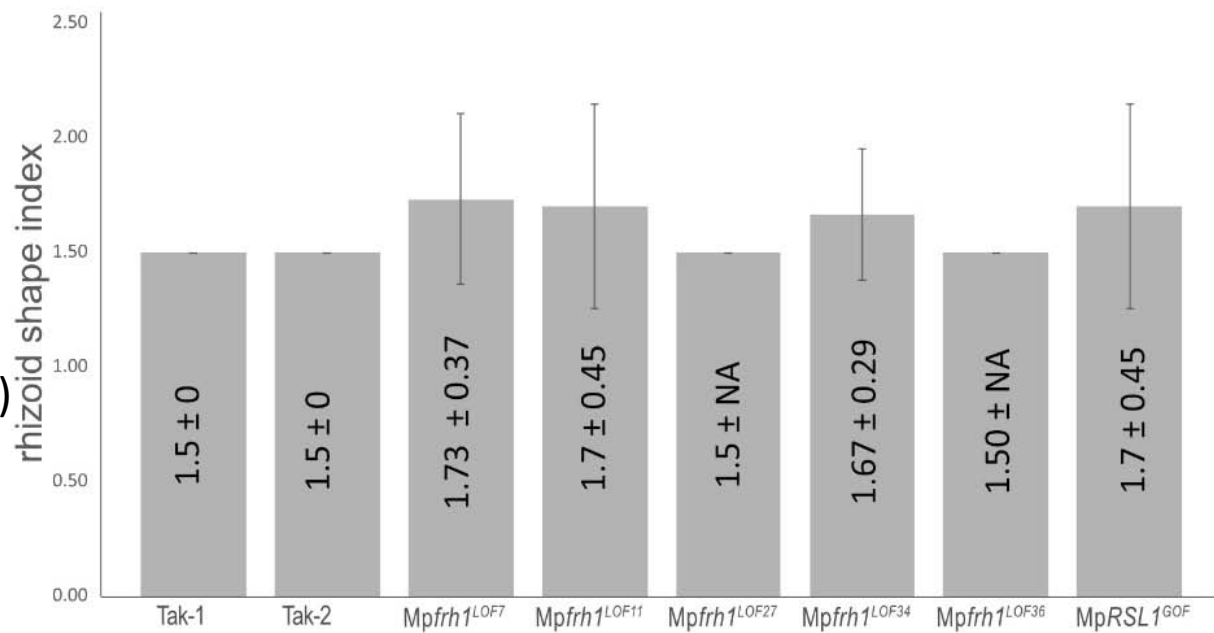
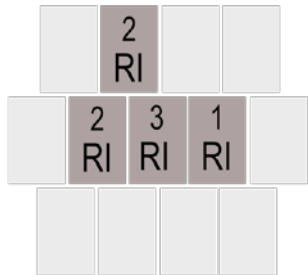


# Higher variability of rhizoid cell neighbor number in *Mpfrh1* mutants than in wild type – four-cell clusters

Linear cluster (CSI = 1.5)



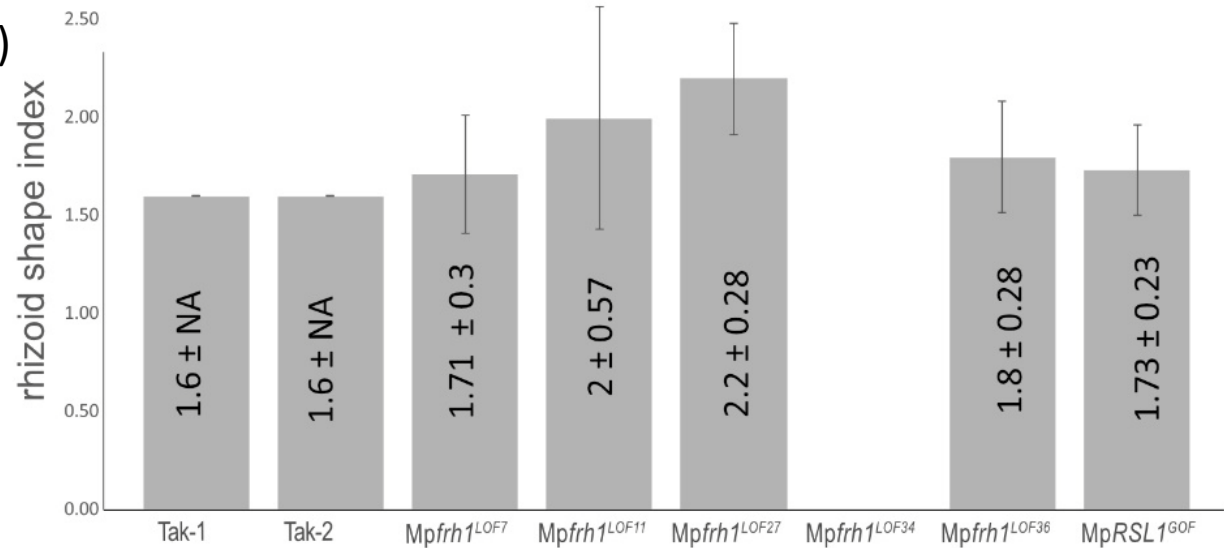
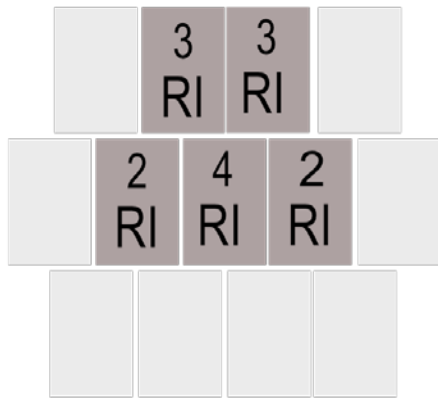
Non-linear cluster (CSI = 2)



# Higher variability of rhizoid neighbor number in *Mpfrh1* mutants than in wild type – five-cell clusters

Linear cluster (CSI = 1.6)

Non-linear cluster (CSI = 2.8)



Rhizoid cells in wild type clusters have one or two neighbors;  
Cells in *Mpfrh1* mutants have more neighbors than wild type

| genotype                       | 3-cell cluster    | 4-cell cluster    | 5-cell cluster    |
|--------------------------------|-------------------|-------------------|-------------------|
| TAK1                           | 1.33 ±0           | 1.50 ±0           | 1.60 ±0           |
| TAK2                           | 1.33 ±0           | 1.50 ±0           | 1.60 ±0           |
| <i>Mpfrh1</i> <sup>LOF7</sup>  | <b>1.40 ±0.21</b> | <b>1.73 ±0.37</b> | <b>1.71 ±0.3</b>  |
| <i>Mpfrh1</i> <sup>LOF11</sup> | <b>1.56 ±0.34</b> | <b>1.70 ±0.45</b> | <b>2.00 ±0.57</b> |

Wild type forms clusters in one dimension and mutant clusters are two dimensional

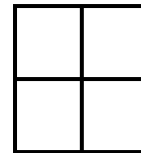
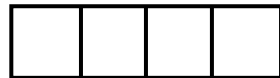
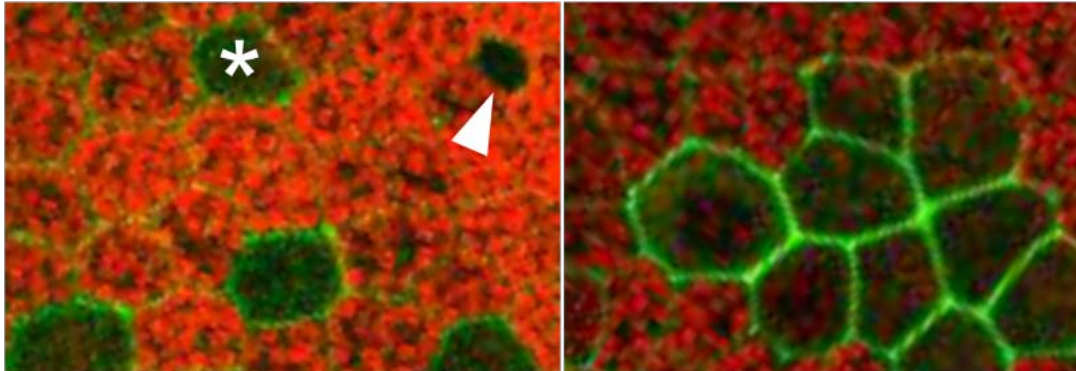
# Directionality: MpFRH1 miRNA represses 2-D cluster formation

MpFRH1 miRNA

No MpFRH1 miRNA

1-D – linear clusters

2-D planar clusters



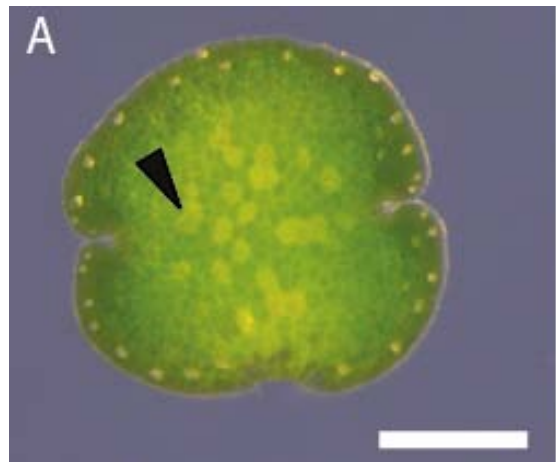
Bar = 50  $\mu$ m

# Cell arrangement in clusters - summary

- Wild type rhizoid clusters always form one-dimensional linear arrangements
- *Mpfrh1* mutants form two-dimensional arrangements
- Suggests that MpFRH1 miRNA imposes linear (one dimensional) cluster development
- Directionality of MpFRH1 miRNA action

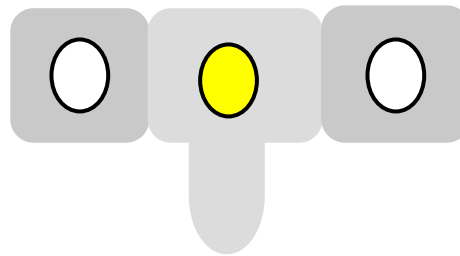


# MpFRH1 promoter is active in rhizoid precursor cells

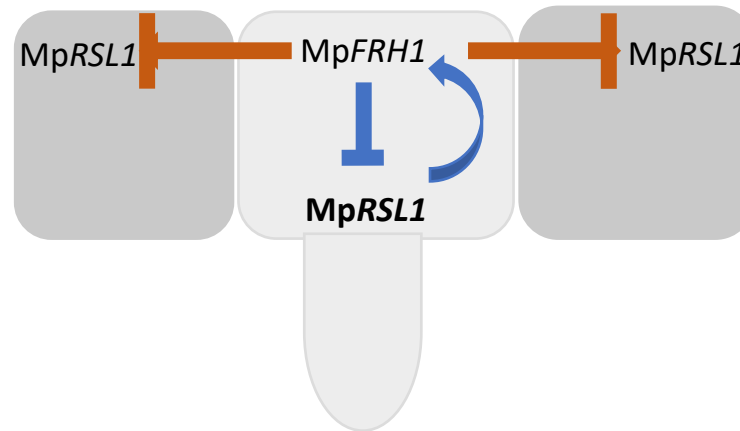


MpFRH1<sub>pro</sub>:3xYFP

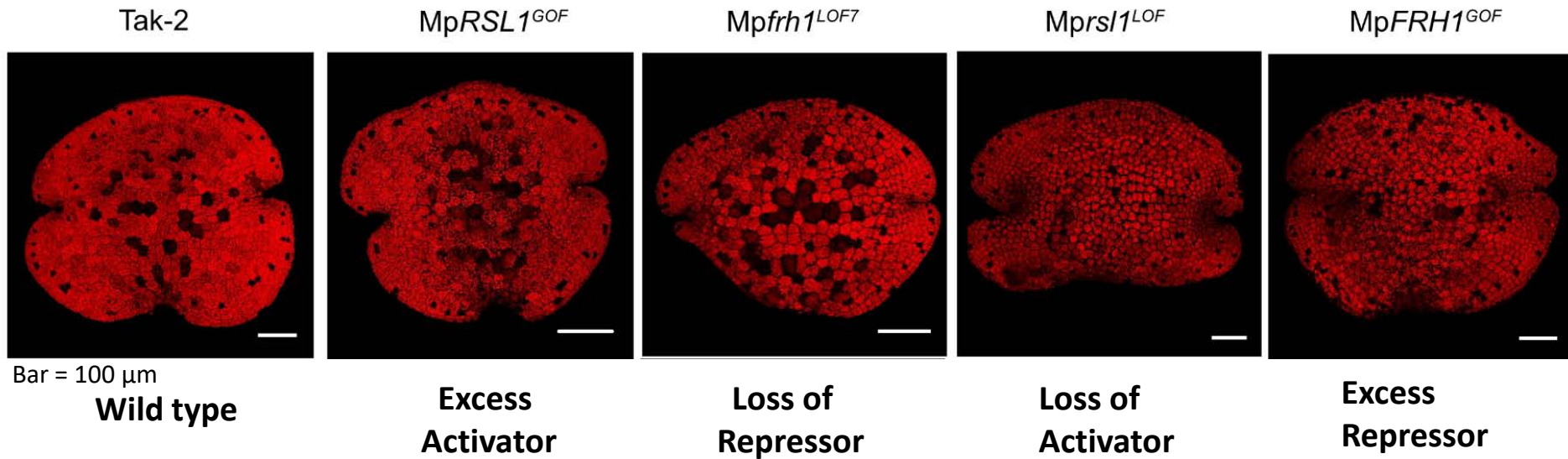
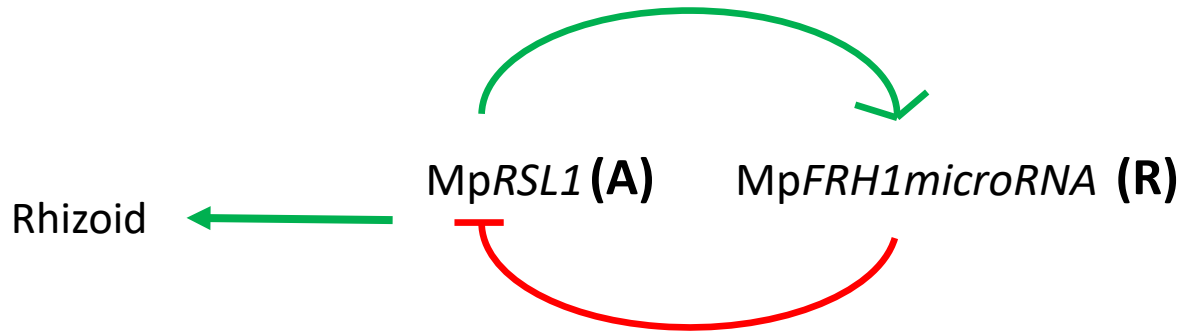
MpFRH1<sub>pro</sub>:3xYFP



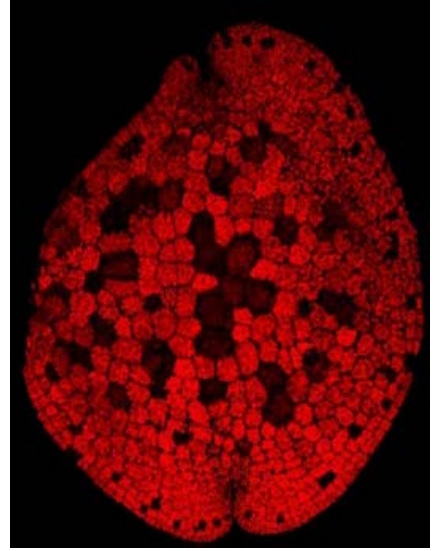
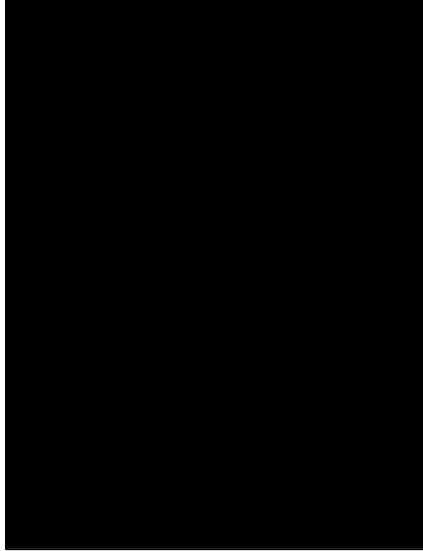
# Model: MpFRH1 miRNA regulates pattern of rhizoid cell development



# Activator (A) and repressor (R) regulate rhizoid pattern

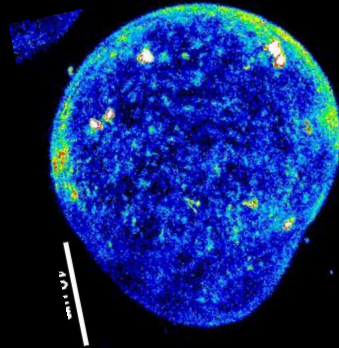


Bar = 100  $\mu$ m



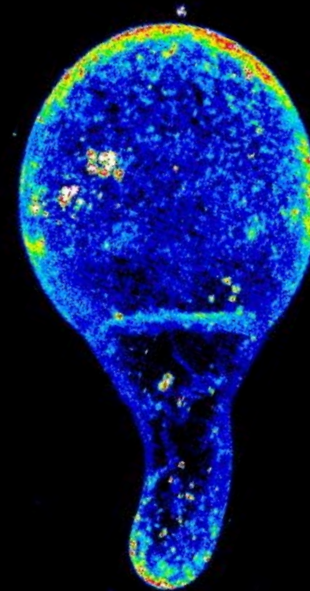
### Current members:

Sarah Atrill  
Sam Caygill  
Alex Casey  
Chloe Casey  
Sandy Hetherington\*  
Hugh Mulvey  
Radka Slovak  
Susanna Streubel\*  
Anna Thamm\*  
Reka Toth



### Recent members:

Clemence Bonnot  
Holger Breuninger  
Bruno Catarino  
Clement Champion  
Suvi Honkanen\*  
Victor Jones  
Chulmin Kim  
Giulia Morieri  
Hélène Proust\*  
Thomas Tam  
Natsuki Tanaka



### Collaborators:

Mario Arteaga  
Chris Berry  
Henrik Buschmann  
Kimitsune Ishizaki  
Paul Kenrick  
Hans Kerp  
Takayuki Kochi  
Tomoaki Nishiyama  
Stefan Rensing  
Hidetoshi Sakayama  
Steve Kelly

### Fossils:

British Geological Survey, Hunterian Museum, Glasgow, Manchester Museum, Oxford University Herbaria, Oxford Museum of Natural History, Natural History Museum London, University of Münster, University of St Andrews



# Screen defined 33 genes required for tip growth in rhizoid

| Gene  | Predicted function   | Arabidopsis homolog         | Mutant phenotype    | Mutant alleles | mRNA in root hairs | Root hair development |
|---|--|-----------------------------|---------------------|----------------|--------------------|-----------------------|
| <b>CELL WALL BIOSYNTHESIS AND INTEGRITY SENSING</b> |  |                             |                     |                |                    |                       |
| MpCSLD1   | cellulose synthase-like class D protein                                      | At3G03050 ( <i>CSLD3</i> )  | very short rhizoids | 3              | yes                | yes                   |
| MpCSLD2   | cellulose synthase-like class D protein                                      | At3G03050 ( <i>CSLD3</i> )  | short rhizoids      | 5              | yes                | yes                   |
| MpPTI   | PTI-like serine/threonine kinase   | At2G30740                   | short rhizoids      | 2              | yes                | yes                   |
| MpXUT1  | xyloglucan-specific galacturonosyltransferase                                | At5G41250                   | very short rhizoids | 3              | yes                | yes                   |
| MpGMP   | GDP-mannose pyrophosphorylase  | At2G39770 ( <i>GMP1</i> )   | very short rhizoids | 1              |                    | embryo lethal         |
| MpRHM1  | rhamnose biosynthesis  | At1G78570 ( <i>RHM1</i> )   | short rhizoids      | 1              | yes                | yes                   |
| MpTHE   | <i>CrRLK1L</i> family receptor-like kinase                                   | At5G54380 ( <i>THE1</i> )   | very short rhizoids | 1              | yes                | yes                   |
| <b>VESICLE TRANSPORT AND CYTOSKELETON</b>           |  |                             |                     |                |                    |                       |
| MpPI4Ka   | 1-phosphatidylinositol 4-kinase alpha  | At1G49340                   | very short rhizoids | 6              |                    |                       |
| MpSCD   | Rab guanine nucleotide exchange factor                                       | At1G49040 ( <i>SCD1</i> )   | short rhizoids      | 2              | yes                | yes                   |
| MpSPI   | WD-40 repeat protein   | At1G03060 ( <i>SPI</i> )    | short rhizoids      | 3              | yes                | yes                   |
| MpSRI1  | Rab guanine nucleotide exchange factor, similar to <i>S. Cerevisiae RIC1</i> | At3G61480                   | short rhizoids      | 3              | yes                |                       |
| MpWDL   | predicted microtubule binding protein / TPX2 domain containing protein       | At2G35880                   | curly rhizoids      | 3              | yes                |                       |
| MpXI  | Class XI myosin  | At3G12130                   | short rhizoids      | 5              | yes                | yes                   |
| MpAP5M  | AP-5 complex subunit mu  | At2G20790                   | short rhizoids      | 1              | yes                |                       |
| MpREN   | Pleckstrin homology (PH) domain / RhoGAP domain-containing protein           | At5G12150                   | curly rhizoids      | 1              | yes                |                       |
| MpSRI2  | calcium binding EF-hand family protein, similar to <i>S. Cerevisiae PAN1</i> | At1G21630                   | very short rhizoids | 1              | yes                |                       |
| MpZWI   | calmodulin binding /microtubule motor  | AT5G65930                   | short rhizoids      | 1              | yes                |                       |
| <b>OTHERS/UNKNOWN FUNCTION</b>                      |  |                             |                     |                |                    |                       |
| MpALBA  | alba-like DNA/RNA-binding protein  | At1G76010                   | short rhizoids      | 5              |                    |                       |
| MpEMB2756   | DUF616 containing protein, ceramidase  | AT1G34550                   | short/few rhizoids  | 2              |                    |                       |
| MpEXL1  | Exordium-like  | AT4G08950                   | short rhizoids      | 2              |                    |                       |
| MpFBA1  | fructose-bisphosphate aldolase   | At4G38970 ( <i>FBA2</i> )   | short rhizoids      | 4              |                    |                       |
| MpGATA1   | Class A GATA zinc finger transcription factor                                | At5G25830 ( <i>GATA12</i> ) | short rhizoids      | 2              | yes                |                       |
| MpIRE   | AGC-kinase   | At5G62310 ( <i>IRE</i> )    | very short rhizoids | 1*             | yes                | yes                   |
| MpSRI3  | unknown protein, ceramide metabolic process                                  | AT5G42660                   | short rhizoids      | 2              |                    |                       |
| MpTMT   | tonoplast monosaccharide transporter   | At3G51490 ( <i>TMT3</i> )   | short rhizoids      | 2              | yes                |                       |
| MpACLB-2  | ATP citrate lyase subunit B  | AT5G49460                   | short rhizoids      | 1              |                    |                       |
| MpCPR   | Regulator of expression of pathogenesis-related (PR) genes                   | AT5G64930                   | short rhizoids      | 1              |                    |                       |
| MpGDPD  | glycerophosphodiester phosphodiesterase                                      | AT3G02040                   | short rhizoids      | 1              | yes                | yes                   |
| MpGDPDL   | glycerophosphodiester phosphodiesterase-like                                 | AT3G20520                   | few rhizoids        | 1              | yes                | yes                   |
| MpPRPL  | Plastid ribosomal protein large subunit                                      | AT1G07320                   | very short rhizoids | 1              |                    |                       |
| MpSRI4  | unknown protein  | At2G41830                   | short rhizoids      | 1              |                    |                       |
| MpTZP1  | Zinc knuckle (CCHC-type) family protein                                      | AT5G49400                   | short rhizoids      | 1              |                    |                       |
| MpXF  | squalene monooxygenase   | At1G58440                   | short rhizoids      | 1              |                    | yes                   |