Liam Dolan

Evolution and development of the earliest land plant rooting systems

Kavli Institute of Theoretical Physics,
Santa Barbara, 13th August 2019
Plants colonised the land 500 million years ago

Earth history (billion years)

- Meristems
- Shoots
- Rooting structures
- Leaves
Colonization of the land was followed by a radiation in morphological diversity
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*
Rhizoids develop on the lower sides of bilaterally symmetric axes segments of *Nothia aphylla*.
Tips of rhizoids observed in *Nothia aphylla*

Hetherington and Dolan unpublished
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

(Tree after Nishiyama, 2003)
Filamentous rooting cells develop at the interface between land plants and the soil.

Filamentous rooting structures

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

(Tree after Nishiyama, 2003)
Tip-growing cells deposit new cell surface material at the apex

Mulvey and Dolan unpublished
Marchantia polymorpha

**Good for genetic analysis:**
- Haploid
- Transformable
- Mutagenesis: T-DNA, UV
- CRISPR/Cas9
- Genome assembly

- Rapid life cycle
- Small size
- Separate sexes
- 10 µm spores
- Rhizoids

Honkanen et al (2016) *Current Biology*
Phenotypes of mutants that define genes controlling rhizoid development

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

Honkanen et al (2016) *Current Biology*
Insertions into the MpRSL1 coding sequence of two rhizoidless mutants

Wild type

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

Mprs/1-2

Mprs/1-1

Proust H et al (2016) *Current Biology*
Insertions in 5’ region of MpRSL1 gene in mutants with dorsal rhizoids
Expression of Mp*RSL1* gene is higher in dorsal rhizoid mutants than wild type.

Proust et al 2016 *Current Biology*
MpRSL1 is necessary and sufficient for rhizoid development in *Marchantia*.

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

Proust et al (2016) *Current Biology*
RSL genes are necessary and sufficient for rhizoid development in the moss Physcomitrella patens.

Loss of function

Wild type  Pprsl1 Pprsl2

Gain of function

A  Wild type  B  35S:PpRSL1  35S:PpRSL2

Jang et al (2011) Development
RSL genes are necessary and sufficient for root hair development in angiosperms

Loss of function in *A. thaliana*  

Gain of function in *Brachypodium distachyon*

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Proust et al (2016) *Current Biology*

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.

Proust et al (2016) Current Biology
2. Evolution of a novel patterning mechanism among liverworts (genetics)

Different negative regulatory mechanisms evolved in different lineages (repressors are not conserved)
Mpfew rhizoids\textsubscript{1} (Mpfrh1) mutants develop few rhizoids

Four of the six rhizoidless mutants had mutations in the same gene, Mp\textit{FRH1}
T-DNA inserts upstream of 1.2 kb transcript in four \textit{Mpfrh1} mutants

Data from Mario Arteaga-Vazquez

\textit{MpFRH1} miRNA
\textit{MpFRH1} miRNA*

Honkanen et al (2018) \textit{eLife}
miRNA processing in flowering plants

DICER-LIKE1 (DCL1) endoribonuclease

mRNA transcript cleavage or translational inhibition

Modified from: Chapman & Carrington 2007 Specialization and evolution of endogenous small RNA pathways
MpFRH1 miRNA is less abundant in weak Mpdicerlike one MpFRH1^GOF double than in MpFRH1^GOF single mutant.
Reduced MpDICERLIKE ONE function restores rhizoid formation in MpFRH1\textsuperscript{GOF} background

Streubel and Dolan \textit{unpublished}
$Mp_{dcl1}^{dm}$ suppresses the $Mp_{Frh1}^{GOF}$ phenotype

![Box plot showing rhizoid length comparison between different conditions](image)

Streubel and Dolan *unpublished*
Mphst suppresses the MpFrh1\textsuperscript{GOF} phenotype

Streubel and Dolan \textit{unpublished}
MpRSL1 is target of the 21 nt MpFRH1 miRNA

Honkanen et al (2018) eLife
MpFRH1 negatively regulates MpRSL1 expression

Rhizoid $\rightarrow$ MpRSL1 $\rightarrow$ MpFRH1microRNA

Honkanen et al (2018) eLife
MpRSL1 positively regulates MpFRH1 expression

Growth or pattern?

Honkanen et al. (2018) eLife
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

$\text{MpFRH1}^{GOF}$ plants transformed with an $\text{MpFRH1}$-resistant $\text{MpRSL1}$ develop rhizoids.

MpFRH1 evolved among the liverworts
Evolution of mechanism regulating filamentous rooting structure development

(Tree after Nishiyama 2003)
Does MpFRH1 miRNA – MpRSL1mRNA interaction function in patterning?

Development of rhizoids on wild type gemmae (vegetative propagules)

Bar = 50 µm

Percentage cells in each size-class of rhizoid cluster

<table>
<thead>
<tr>
<th>Size-Class</th>
<th>TAK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-cell</td>
<td>65.4</td>
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<tr>
<td>2-cell</td>
<td>20.2</td>
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<tr>
<td>3-cell</td>
<td>8.7</td>
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<tr>
<td>4-cell</td>
<td>2.9</td>
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<tr>
<td>5-cell</td>
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<tr>
<td>6-cell</td>
<td>1.9</td>
</tr>
<tr>
<td>7-cell</td>
<td>-</td>
</tr>
<tr>
<td>8-cell</td>
<td>-</td>
</tr>
<tr>
<td>9-cell</td>
<td>-</td>
</tr>
<tr>
<td>&gt;10-cell</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
</tr>
</tbody>
</table>

Thamm and Dolan, unpublished
Supernumerary rhizoids develop where \( \text{MpRSL1} \) is ectopically expressed at high levels.

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

<table>
<thead>
<tr>
<th></th>
<th>1-cell</th>
<th>2-cell</th>
<th>3-cell</th>
<th>4-cell</th>
<th>5-cell</th>
<th>6-cell</th>
<th>7-cell</th>
<th>8-cell</th>
<th>9-cell &gt;10-celln</th>
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</thead>
<tbody>
<tr>
<td><strong>MpRSL1(^{\text{GOF}})</strong></td>
<td>58.6</td>
<td>14.1</td>
<td>13.1</td>
<td>5.1</td>
<td>3.0</td>
<td>1.0</td>
<td>2.0</td>
<td>-</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>TAK2</strong></td>
<td>65.4</td>
<td>20.2</td>
<td>8.7</td>
<td>2.9</td>
<td>1.0</td>
<td>1.9</td>
<td>-</td>
<td>-</td>
<td>104</td>
</tr>
</tbody>
</table>

Thamm and Dolan, unpublished
Rhizoids do not develop on plants that do not express \textit{MpRSL1}

Does \textit{MpFRH1} repress rhizoid growth and differentiation?

Does \textit{MpFRH1} repress rhizoid cell identity during the patterning of epidermal cells?

Thamm and Dolan, unpublished
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

Thamm and Dolan, unpublished
Deletion of the MpFRH1 miRNA from the genome using CRISPR/Cas9

Thamm and Dolan, unpublished
Larger clusters of rhizoids develop in \textit{Mpfrh1} loss of function mutants

![Image showing percentage cells in each size-class of rhizoid cluster](Thamm and Dolan, unpublished)
Wild type MpFRH1 gene rescues Mpfrh1\textsuperscript{LOF27} phenotype

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

Bar = 100 µm

Thamm and Dolan, unpublished
Arrangement of cells in rhizoid cell clusters is defective in Mpfrh1 loss of function mutants.

Cell number per cluster

1 2

3

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)

Thamm and Dolan, unpublished
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 \textit{Mpfrh1} mutants than in wild type – five-cell clusters

Linear cluster (CSI = 1.6)

Non-linear cluster (CSI = 2.8)

Thamm and Dolan, unpublished
Rhizoid cells in wild type clusters have one or two neighbors; Cells in Mpfrh1 mutants have more neighbors than wild type

<table>
<thead>
<tr>
<th>genotype</th>
<th>3-cell cluster</th>
<th>4-cell cluster</th>
<th>5-cell cluster</th>
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<tbody>
<tr>
<td>TAK1</td>
<td>1.33 ±0</td>
<td>1.50 ±0</td>
<td>1.60 ±0</td>
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<tr>
<td>TAK2</td>
<td>1.33 ±0</td>
<td>1.50 ±0</td>
<td>1.60 ±0</td>
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<tr>
<td>Mpfrh1\textsc{LOF7}</td>
<td>1.40 ±0.21</td>
<td>1.73 ±0.37</td>
<td>1.71 ±0.3</td>
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<td>Mpfrh1\textsc{LOF11}</td>
<td>1.56 ±0.34</td>
<td>1.70 ±0.45</td>
<td>2.00 ±0.57</td>
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</tbody>
</table>

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

Thamm and Dolan, unpublished
Directionality: MpFRH1 miRNA represses 2-D cluster formation

MpFRH1 miRNA
1-D – linear clusters

No MpFRH1 miRNA
2-D planar clusters

Bar = 50 µm
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

Honkanen et al (2018) *eLife*
Model: MpFRH1 miRNA regulates pattern of rhizoid cell development
Activator (A) and repressor (R) regulate rhizoid pattern

Tak-2

Wild type

Excess Activator

Loss of Repressor

Loss of Activator

Excess Repressor

Scale bar = 100 µm

Thamm and Dolan, unpublished
**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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Predicted function</th>
<th>Arabidopsis homolog</th>
<th>Mutant phenotype</th>
<th>Mutant alleles</th>
<th>mRNA in root hairs</th>
<th>Root hair development</th>
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<td><strong>CELL WALL BIOSYNTHESIS AND INTEGRITY SENSING</strong></td>
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<td>MpCSLD1</td>
<td>cellulose synthase-like class D protein</td>
<td>At3G03050 (CSLD3)</td>
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<td>At3G03050 (CSLD3)</td>
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<td>MpPTI</td>
<td>Pti-like serine/threonine kinase</td>
<td>At2G30740</td>
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<td>GDP-mannose pyrophosphorylase</td>
<td>At2G39770 (GMP1)</td>
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<td>embryo lethal</td>
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<td>rhamnose biosynthesis</td>
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<td>CrRLK1L family receptor-like kinase</td>
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<td>MpPI4Ka</td>
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<td>At1G49340</td>
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<td>Rab guanine nucleotide exchange factor</td>
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<td>MpSPI</td>
<td>WD-40 repeat protein</td>
<td>At1G03060 (SPI)</td>
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<td>MpSR1</td>
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<td>At3G61480</td>
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<td>MpWDL</td>
<td>predicted microtubule binding protein / TPX2 domain containing protein</td>
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<td>MpXI</td>
<td>Class XI myosin</td>
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</table>

Honkanen et al (2016) Current Biology