Phytomer Growth

- Shoot meristem
- Lateral organ primordium
- Internode
- Node
- Node
- Lateral organ
- Axillary bud
- Phytomer
Phytomers vary depending on growth phase

vegetative → reproductive
Phase change modifies existing phytomers

Internode elongation

vegetative → reproductive
Many plants elongate stems after flowering

Grain crops show this growth habit

Lodging
The Green Revolution
variation in internode elongation
Internode growth in barley

Sreenivasulu and Schnurbusch, 2012
Intercalary meristems fuel internode growth

Unique to grasses, hornworts and horsetail ferns

Ruonala et al., 2008

Dicots

SAM
RM
RZ

p₁
p₂
p₃

peduncle

Intercalary Meristem

*Unique* to grasses, hornworts and horsetail ferns
Model of internode zonation

Testing the Model:

- Precision phenotyping
- Hormone networks
- Genome-wide analyses
- Mutant genetics

Maturation Zone
Expansion Zone
Division Zone
Intercalary meristem

growth
Development of internode zonation –
*A kinematic analysis*

Cell length

Mitotic Index

1 cm bin

1 mm bins

V. Patil et al, in prep
Proliferation and growth of DZ

Epidermal layer

Tissue section

Histone4 in situ

600μm

DZ

Mitotic Index

Days post-germination

d17

d24

CellProfiler
Coupled DZ and EZ

DZ restricted to base

Mitotic Index

Epidermal Cell Length (μm)

1cm bin

1.5cm

1cm bins
Consistent elongation in the EZ

Lignification profile

Epidermal Cell Length (μm)

1 cm bins
Three Phase Model

PHASE 1
Proliferation Dominant

PHASE 2
Proliferation + Expansion

spatial

temporal
Internode growth dynamics similar to leaves

PHASE 1

PHASE 2

PHASE 3

spatial
temporal

monocots

Nelissen et al (2016)
Dynamic Internode Transcriptome

- Cyclins, histones, Proliferation TFs
- Cell wall synthesis
- Cell wall extension
- Differentiation
- Carbohydrate metab.
- Lignification
- Photosynthesis

28 CLUSTERS
Regulatory Modules

- **MZ**: Suppress expansion
  - CUC2/BOP2

- **EZ**: Suppress proliferation
  - Tb1
  - ULT1

- **DZ**: Founder recruitment
  - WOX3
  - WOX2

- **Differentiation**
  - MND/PLA1
  - AS2
  - BLH4
  - BP, BLH6, KNAT7

- **Suppress stem cells**
  - AG, KAN4

- **Proliferation**
  - GRF5,4
  - AN3
  - ERF
  - ANT/AI
  - TCP

- **Proliferation duration**
  - Slows differentiation rate
Zeo alleles cause semi-dwarfism and dense spikes.
HvAP2 delays spike differentiation and limits internode growth

Bw

miR172

HvAP2

Zeo1.b

Culm Length (cm)

0 10 20 30 40 50 60

21 28 35 41 49
dpg

Bowman

Zeo1.b

miR172

HvAP2

slow

Houston and McKim et al (2013) PNAS
miR172 regulation of HvAP2 promotes internode phase transitions

HvAP2 does not control DZ size or density

miR172 down-regulation of HvAP2 promotes cell transfer rate to the EZ
The Dynamic Internode Transcriptome
Robust DETs between Bw and Zeo1.b

Overlap of S2-S3 Bw (S2-S3 Bw)

Overlap of S2-S3 Zeo1.b (S2-S3 Zeo)
Robust DETs between Bw and Zeo1.b

- WOX2, GRF5, AGL14, ERF1/10 (AGLs)
- ARF19
- Cell cycle/division
- JAZ, WRKY70/WRKY27/WRKY41
- BLH1, BLH6
- KNAT7
- LSH4
- BOP
- Expansins, MYB87

Jasmonate antagonises Gibberellin, proliferation and promotes lignification.
Conserved miRNA-target network regulates phase change

The phase change network, specifically AP2, controls developmental transitions through modulation of JA
HvAP2 underlies GWAS peak associated with internode length
Acknowledgments

University of Dundee

McKim Lab

Vrushali Patil
Hannah McDermott
Monika Zwirek
Jennifer Shoesmith
Nikoleta Tzioutziou
Trisha McAllister

James Hutton Institute
Robbie Waugh
Kelly Houston
Pete Hedley

Leibniz Institute of Plant Genetics and Crop Plant Research
Ahmad Alquudah
Thorsten Schnurbusch

BBSRC
The Royal Society of Edinburgh
The Royal Society
Semidwarfism associated with disruption of miR172-regulation of HvAPETALA2
Other Phase Change Factors in Barley?

- Identify and characterise HvAP2-likes and HvSPLs
  - 3 other HvAP2-likes
  - 8 HvSPLs regulated by miR172

**OVEREXPRESS (OX)**

- HvAP2-like or HvSPL
- GFP
- Mutated miRNA-binding site

**KNOCK-OUT (KO)**

- HvAP2-like and HvSPL
- CRISPR-Cas

Transgenic plant phenotype
*miR172 controls HvAP2 levels specifically after the reproductive transition*

**HvAP2 qPCR**

<table>
<thead>
<tr>
<th>Days post-germination</th>
<th>7 (veg)</th>
<th>14 aerial tissue</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>relative HvAP2 gen expression</td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
</tr>
</tbody>
</table>

* Zeo1.b

Bw

HvAP2

![Diagram](image)

miR172

vegetative

reproductive

Zeo1.b

HvAP2

![Diagram](image)
miR172 promotes spike phase progression

miR172 promotes spike phase progression by regulating spikelet initiation, spikelet differentiation, and internode elongation. The expression of HvAP2 is also affected, with development paused in the Zeo1.b background compared to the Bw background. The graph shows the relative HvAP2 gene expression over time, with the miR172 expression level indicated by the arrows and the development status marked by the labels 'development paused' and 'late differentiation reduced internode elongation.'
Development of internode zonation –
A kinematic analysis

Cell length

Mitotic Index

Epidermal Cell Length (μm)

Mitotic Index

Section from base (1 mm)
Phase 1 – Proliferation and genesis of DZ

Epidermal layer

Tissue section

Histone4 in situ

600μm

DZ

Mitotic Index

Days post-germination
Phase 2- Coupled DZ and EZ

Zonation restricts DZ to base

Mitotic Index

1mm bins from base

DZ

EZ

Epidermal Cell length (um)

lacuna

col

fl

node

node

EZ

DZ
Phase 2: DZ proximo-distal size is constant

Mitotic Index

Bins from base (1 mm)

15 cm internode

DZ

EZ

Mitotic Index

DZ

EZ

0 1 2 3 4 5 6

0 1 2 3 4 5 6 7

0 1 2 3 4 5 6

0 1 2 3 4 5 6 7

0 1 2 3 4 5 6

0 1 2 3 4 5 6 7

Bins from base (1 mm)
Phase 2: Consistent elongation in the EZ

Lignification profile

Epidermal Cell Length (μm)
Phase 2: Consistent elongation in the EZ
Phase 3: DZ shrinks and eliminated

Mitotic Index

Bins from base (1 mm)

15 cm

DZ

EZ

MZ

10 cm internode

1.5 cm internode

MZ

EZ

MZ

EZ

DZ

DZ

DZ

MZ

EZ

EZ

DZ

DZ

DZ

DZ

DZ

DZ
Altered phase timing effects plant architecture

MicroRNAs control phase change timing in plants and animals
Conserved miRNA-target network regulates phase change

vegetative  .........................................................→ reproductive
leaves, shoots  

APETALA2-like

miR172

miR156

SPLs

mRNA

miRNA

target mRNA
Rice Ideal Plant Architecture

Traditional

IPA – Ideal Plant Architecture

OsSPL14

miR156

OsSPL14

miR156

* 

Miura et al., 2010
Jiao et al, 2010
Agronomic traits are regulated by phase change

<table>
<thead>
<tr>
<th>Crop</th>
<th>Gene</th>
<th>Trait</th>
</tr>
</thead>
</table>
| Maize  | *TS6*  
         | *TGA*  
         | *LG*  
         | *GL15* 
         | *SID1* 
         | *IDS1* | Male fertility  
         | Kernel accessibility  
         | Leaf shape, branch angle  
         | Leaf wax  
         | Kernel number |
| Rice   | *OsSPL16*  
         | *OsSPL14* 
         | *OsLG* 
         | *SHAT1* 
         | *SNB*  
         | *IDS1* | Grain size  
         | IPA  
         | Panicle angle  
         | Grain retention  
         | Panicle branching  
         | Panicle branching |
| Wheat  | *Q–AP2*       | Height, grain retention                    |
| Barley | *UNKNOWN*     | *UNKNOWN*                                  |
Development of Plant Architecture
Phase Changes in Barley

What happens in barley???

vegetative .............................................→ reproductive

leaves, shoots       flowers, seeds

APETALA2-like

miR172

miR156

SPLs
Internode Patterning

barley

leaves
spike
internodes

Briggs, 1978

rice

Kende et al, 1998

maize

Scanlon, 1996; 2000
Internode Hormone Profile at 5cm

**Gibberellins**

- Concentration (ng/g DW)
- Bw 1, Bw 2, Bw 3, Bw 4, Bw 5
- GA53, GA51, GA44, GA34, GA19, GA8, GA4

**Cytokinins**

- Concentration (ng/g DW)
- Bw 1, Bw 2, Bw 3, Bw 4, Bw 5
- c-ZOG, t-ZOG, t-ZR, c-ZR, iPR, t-Z

**Auxins**

- Concentration (ng/g DW)
- Bw 1, Bw 2, Bw 3, Bw 4, Bw 5
- IAA, IAA-Asp

**ACC**

- Concentration (ng/g DW)
- Bw 1, Bw 2, Bw 3, Bw 4, Bw 5
- ACC
Integrated Model of Internode Growth

Differentiation
Suppress expansion
Slow differentiation rate

Expansion
Suppress proliferation
 Suppress stem cell identity

Proliferation
Founder recruitment

Patil et al in prep
Dynamic Internode Transcriptome

A

B

Patil et al in prep
Post-embryonic body plan development

Wheat  Tulip  Coconut  Corn  Teosinte
Consistent elongation in the EZ

Epidermal Cell Length (μm)

1 cm bins

DZ

MZ

EZ

5cm

10cm
Expansion dominant

DZ shrinks and eliminated

Mitotic Index

Bins from base (1 mm)

15 cm internode

10 cm internode

DZ  DZ  EZ  EZ

MZ

EZ

DZ

DZ

DZ
Expansion dominant

Epidermal Cell Length (μm)

1cm Bin

MZ

EZ

MZ

EZ
HvAP2 represses both proliferation and expansion
Zeo1.\( b \) is sensitive to Gibberellin (GA)

...but does not phenocopy a GA-deficient dwarf, \( sdw1.a \).
Semidwarfism caused by disruption of \textit{miR172}-regulation of \textit{HvAPETALALA2} (\textit{HvAP2})

Houston and McKim et al (2013) PNAS
Ancient regulatory network?

hornworts
Testing the model → Three Phase Model → Novel Regulators

Dynamic transcriptome Recapitulation of lateral organ programme

HvAP2 transition

miR172 regulation of HvAP2:
• promotes cell proliferation rate and transfer from DZ to EZ
• links lignification with zonation
• may promote GA signalling

Elevated HvAP2 =
↓ proliferative TFs,
↑ differentiation TFs,
↑ cell wall enzymes
↑ JA

why?

Putative regulators of IM activation

Comparative transcriptomics
Basis of Semi-dwarfism in *Zeol1.b*

- **Wild type**
  - Fewer cells
  - Smaller cells
  - Combination of both

- **Short internode**
  - Proliferation
  - Expansion

- **MZ**
- **EZ**
- **DZ**
Loss of miR172 regulation of HvAP2 uncouples lignification from stem zonation
Comparative Transcriptome of Peduncle Initials

Differentially Expressed Transcripts (DETs)

- 2485 down-regulated in Zeo1.b
- 261 up-regulated in Bw

Venn Diagram:
- Bw: 1776 transcripts
- Zeo1.b: 21594 transcripts
- Unique Bw: 709 transcripts
- Unique Zeo1.b: 68 transcripts
- Bw and Zeo1.b: 193 transcripts
DETs show Gene Ontology (GO) Enrichment for meristem function

- Stem cell differentiation
- Maintenance of meristem function
- Negative regulation of cell differentiation

- WUSCHEL-like homeoboxes = WOXs
- AGL TFs
- All downregulated in Zeo1.b