Hormone signaling during the development of the clubroot disease in Arabidopsis thaliana roots.
Arabidopsis thaliana as a model system for clubroot research

Arabidopsis roots in different disease classes

disease progression
Plant hormones are associated with club development.

- **Leaf**: Cytokinin signaling
- **Shoot**: Auxin transport
- **Root**: Monitoring cytokinin and auxin responsiveness

- **Auxin**
  - Hypertrophy
  - Cell division
  - Retention in infected cells by flavonoids
  - Biosynthesis

- **Cytokinin**
  - Degradation
  - Biosynthesis

- **Photosynthesis assimilates**
  - Assimilates
  - Photosynthesis

- **Sucrose**
  - Transport
  - Sugar

- **Invertase**
  - Available sugar
  - Nutrition

- **P.b.**
  - Phytophthora brassicae
  - Biosynthesis

- **ARR5::GUS**
  - DR5::GUS
Auxin signaling and target gene regulation

taget genes

auxin receptors

transcriptional repressors

transcriptional activators

auxin homeostasis

PM

acidification

cell wall loosening

H^+

H^+-ATPase

K^+-channel

water uptake

apoplast

nucleus

SCF^{TIR}\text{-complex}

degradation 26S proteasome

transcriptional activation

repression of transcription

Aux/IAAs

ARFs

Aux/RE

translation

GH3

ER

IAA-AA

IAA

IAA

IAA
The SCF<sup>TIR</sup> pathway for auxin signaling

- **Aux/IAA** (repressor)
- **Auxin**
- **ARF** (transcription factor)
- **AuxRE** (target gene)

**SCF<sup>TIR1</sup>-complex**
- Ubiquitin ligase
- Auxin receptor
- Ubiquitination and degradation of repressor by 26S proteasome

Expression of auxin responsive genes
Expression of the TIR1 family in clubroots

Sabine Bergmann
TIR receptor mutants are more susceptible

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>$10^4$ spores ml$^{-1}$</th>
<th>$10^5$ spores ml$^{-1}$</th>
<th>$10^6$ spores ml$^{-1}$</th>
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<tr>
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<td><img src="image" alt="Col-0 10^6" /></td>
</tr>
</tbody>
</table>

Sabine Bergmann
Expression analysis of transcriptional repressors and activators in clubroots

- genes were chosen according to microarray data  
  Siemens et al. (2006)

- ARF5 $\rightarrow$ monopteros (MP)  
  Hardtke et al. (2004) Development

- ARF7 positive regulator of lateral root formation  
  Okushima et al. (2007) Plant Cell

- ARF8 $\rightarrow$ positive regulator of GH3.5!  
  Gutierrez et al. (2012) Plant Cell
The constitutive repressor mutant *axr2-1 (iaa7)* can help to find other target genes of the pathway.

Comparison microarray with *iaa7* and microarray with *P. brassicae*

Microarray data Nakamura et al. (2006)
Siemens et al. (2006)
Auxin conjugation is upregulated


\[
\text{IAA concentration (µg / g fr wt)}
\]

control roots 24 dpi
infected roots 24 dpi

Endogenous auxin

GH3.5

IAA

shikimate

GH3.2
GH3.4
GH3.3
GH3.6
GH3.9
GH3.17

GH3.12

GH3.11

tryptophan

chorismate

SA

IAA conjugates

SA conjugates

JA conjugates

linolenic acid

inactivation

activation
Differential regulation of selected \textit{AtGH3} genes

**A**

<table>
<thead>
<tr>
<th>GH3.2</th>
<th>GH3.3</th>
<th>GH3.4</th>
<th>GH3.5</th>
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<th>GH3.11</th>
<th>GH3.13</th>
<th>GH3.14</th>
<th>GH3.17</th>
<th>GH3.20</th>
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</table>

Relative level of expression (compared to control)

**B**

<table>
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<tr>
<th>Days after inoculation</th>
<th>P. brassicae actin relative expression</th>
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<tbody>
<tr>
<td>7</td>
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</tr>
<tr>
<td>10</td>
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<td>4.0</td>
</tr>
</tbody>
</table>

Winter et al., bar.utoronto.ca

Cornelia Horn
Linda Jahn
Stefanie Mucha
The auxin response element in the GH3 promoter is necessary for upregulation in clubroots.

GUS lines obtained from Claus Schwechheimer (TU München)

pGH3-2::GUS

mpGH3-2::GUS
GH3 double knockouts are slightly more susceptible than wild type

\[ \text{Disease Index} \]

\[ \text{Shoot Index (\% of wild type)} \]

\[ \text{Inoculum density (spores ml}^{-1}\text{)} \]

\[ \text{WT, gh3.5, gh3.17, gh3.5,3.17} \]

double mutants obtained from Paul Staswick, University of Nebraska

Linda Jahn
Auxin binding protein 1 expression is upregulated in clubroots

Relative transcript induction

Days after inoculation

14 dai c i 21 dai c i

collaboration Bianka Steffens (University Kiel) pABP1::GUS Sabine Bergmann
Potassium channels are upregulated by clubroot

- data from microarray analysis  
  Siemens et al. (2006)
- channels depicted here are expressed in the Arabidopsis hypocotyl  
  Fuchs et al. (2006)
- all belong to cyclic nucleotide binding / inward rectifier potassium channels
Expansins: Target genes for auxin action?

Several Expansins are upregulated by auxins and during clubroot

collaboration Florian Grundler (University Bonn)  Sabine Marschollek
Auxin signaling and target gene regulation

- Target genes
- Auxin receptors
- Transcriptional repressors
- Transcriptional activators
- Auxin homeostasis
- Auxin too high
- Higher susceptibility
- Larger galls
- Growth inhibition
- Resistance
- Cell elongation
- Expansins
- Potassium channels

?
Thanks to:

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