Plant Defense against Pathogens

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Plant Defense against Pathogens

Background introduction (~10 min)

Architecture of plant innate immunity (~15 min)

Molecular basis of plant disease resistance (~60 min)
  Diversity and conservation of plant R genes
  Recognition of pathogens by R genes: the guard hypothesis
  R protein regulation and signal initiation
  Signaling pathways of plant resistance

A case study: Broad-spectrum resistance by RPW8 (10 min)
Food for:

Human
Animals
Insects
Micro-organisms
  Viruses
  Bacteria
  Fungi
  Nematodes
  ....
Plants face challenges from various herbivores and microbes at all stages and in all organs.
Microbes

Harmful (pathogens)
Beneficial (symbionts)
**Pathogens**

**Biotrophic** *(co-survive)*

**Necrotrophic** *(kill)*

**Hemi-biotrophic** *(Let alive first and then kill)*
Plants are resistant to most potential pathogens in most times !!!

Because plants have evolved defense systems to protect themselves !!!
Plant defenses systems

Physical barriers
(trichomes, leaf hairs, wax, thick cell walls, cuticles etc)

Chemical defenses
• **Preformed** antimicrobial compounds such as phytoalexins;
• **Induced** reactions such as expression of **PR** (pathogenesis-related) proteins and **HR** (hypersensitive response, a localized host cell death at the site of infection)
Overall goal of research in this field

Understand and utilize the mechanism of plant natural resistance to maximize plant yield and quality with minimum use of pesticides
The Architecture of Plant innate Immunity

Nonhost and Host resistance

Basal and $R$-gene mediated resistance

Broad-spectrum and Race-specific resistance

Local and Systemic acquired resistance
Nonhost Resistance vs Host-(specific) Resistance

Nonhost Resistance

- Acts at special level
- A major contribution to safety of plants
- More evolutionary ancient
- Believed to be multi-layered (therefore complicated)
- (Part of it) activated upon recognition of general features of pathogens called PAMPs (pathogen-associated molecular patterns) such as LPS (lipopolysaccharides) and Flagellin of bacteria, chitin of fungus etc
- Not much known, getting more attention
Nonhost Resistance vs Host-(specific) Resistance

Host Resistance

• Acts under the species level (some cultivars are resistant, some are susceptible)

• More recently evolved (after the breakdown of nonhost resistance)

• Controlled by polymorphic host genes, i.e. $R$ (resistance) genes in the case of bio- and hemi-biotrophs,

• Major focus of studies (economic importance and genetic simplicity)
Basal Resistance vs $R$-gene-mediated resistance

Basal Resistance

- Part of nonhost resistance that remains operative even in susceptible plants to limit pathogen growth
- Could be targets of pathogen effectors
- May share signaling components with $R$-gene resistance
- Neglected before but getting more and more attention now

![Images of leaf samples: RPW8, Col-0 wt, Col-0 pad4]
Basal Resistance vs $R$ gene-mediated resistance

$R$ gene-mediated Resistance

- Activated upon recognition of **Avr** (avirulence factor, i.e. a pathogen effector that elicits resistance)
- Often associated with **HR** (in the case of biotrophs)
- **$R$-resistance consists of local resistance and SAR** (systemic acquired resistance)

- Normally **race-specific** but could be **broad-spectrum**

- Extensively studied & tremendous progress made

Main focus of this lecture
Molecular mechanisms of \( R \)-mediated disease resistance
Cellular signaling of plant defense responses

Ion fluxes
- $\text{Ca}^{++}$
- $\text{K}^+$
- Anion channels

Kinase activation of NADPH oxidase complex

$\text{O}_2$

ROI production

Cell wall buttressing

ROI Perception

SA accumulation

Activation of latent cytoplasmic transcription factors

Expression of defense-related genes

Synthesis of antimicrobial compounds

Cell death

Local response

Systemic acquired resistance
Step 1  Plant-Pathogen Recognition

The "gene-for-gene" model (Flor, 1940s, 1970s)

<table>
<thead>
<tr>
<th></th>
<th>$R$</th>
<th>$-R$</th>
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<tbody>
<tr>
<td>$Avr$</td>
<td>Resistance</td>
<td>Disease</td>
</tr>
<tr>
<td>$-Avr$</td>
<td>Disease</td>
<td>Disease</td>
</tr>
</tbody>
</table>

The "gene-for-gene" model is based on the genetic interaction between plants and pathogens and predicts that $R$ genes may function as receptors to recognize the cognate pathogen-derived ligands (elicitor) encoded by $Avr$ genes.
Step 1  **Plant-Pathogen Recognition**

The “gene-for-gene” model

HR cell death, *PR* gene expression and other defence responses
### Table 1. A complete list of isolated plant disease resistance genes.

<table>
<thead>
<tr>
<th>Year</th>
<th>Gene</th>
<th>Plant</th>
<th>Pathogen</th>
<th>Protein</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>1992</td>
<td>Hml</td>
<td>Maize</td>
<td>Hemileia vapans hominis</td>
<td>eR</td>
<td>(Johal and Briggs 1992)</td>
</tr>
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<td>1993</td>
<td>Pvt</td>
<td>Tomato</td>
<td>Pseudomonas syringae pv. tomato (avpT2)</td>
<td>Kinase</td>
<td>(Martin et al. 1993)</td>
</tr>
<tr>
<td>1994</td>
<td>RPS2</td>
<td>Arabidopsis</td>
<td>Pseudomonas syringae pv. maculicola (avpT1)</td>
<td>CC-NBS-LRR</td>
<td>(Bert et al. 1994)</td>
</tr>
<tr>
<td>1994</td>
<td>N</td>
<td>Tobacco</td>
<td>Mosaic virus</td>
<td>CC-NBS-LRR</td>
<td>(Gandolfo et al. 1994)</td>
</tr>
<tr>
<td>1995</td>
<td>CI-9</td>
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<td>Cladosporium fulvum (Avr9)</td>
<td>RLP</td>
<td>(Jones et al. 1995)</td>
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<td>RP11</td>
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<td>Pseudomonas syringae pv. maculicola (avpRpi1)</td>
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<td>(Grant et al. 1995)</td>
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<tr>
<td>1997</td>
<td>Rs1</td>
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<td>Xanthomonas oryzae pv. oryzae</td>
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<td>(Sung et al. 1997)</td>
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<tr>
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<td>Flax</td>
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<td>(Pennell et al. 1997)</td>
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<td>Mo</td>
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<td>1998</td>
<td>Bs2</td>
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<td>(Tao et al. 1998)</td>
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<td>Blumeria graminis sp.</td>
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<td>(Hartman et al. 2001)</td>
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<td>Mlt6</td>
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<td>Ve1</td>
<td>Tomato</td>
<td>Verticillium alb-a-strum</td>
<td>TIR-NBS-LRR</td>
<td>(Kawchuk et al. 2001)</td>
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</table>

### Table 1. A complete list of isolated plant disease resistance genes (continued).

<table>
<thead>
<tr>
<th>Year</th>
<th>Gene</th>
<th>Plant</th>
<th>Pathogen</th>
<th>Protein</th>
<th>Reference</th>
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<td>R1</td>
<td>Potate</td>
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<td>(Baldova et al. 2002)</td>
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<td>RPP4</td>
<td>Arabidopsis</td>
<td>Peronospora parasitica</td>
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<td>(van der Biezen et al. 2002)</td>
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<td>Dm3</td>
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<td>Hero</td>
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<td>2003</td>
<td>RB</td>
<td>Waxy</td>
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<td>(van der Vossen et al. 2003)</td>
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<td>2003</td>
<td>Erect</td>
<td>Arabidopsis</td>
<td>Plectosphaeriella cucumerina</td>
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<td>(Wang et al. 2005)</td>
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<td>Arabidopsis</td>
<td>Fusarium wilt</td>
<td>CC-NBS-LRR</td>
<td>(Vossen et al. 2005)</td>
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</tbody>
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* eR, enzymatic resistance gene; CC, coiled coil; NBS, nucleotide binding site; LRR, leucine rich repeat; TIR, tol and interleukin receptor; RLP, receptor-like protein; RLK, receptor-like kinase; TM, Transmembrane.
Conservation and Diversity of R Proteins

NBS-LRR (40):
- TIR-NBS-LRR
- CC-NBS-LRR
- Other variants

Other types (22):
- Receptor-like protein (Cf2/4/5/9 RPP27)
- Receptor-like kinase (Xa21/26)
- Protein kinase (Pto)
- Atypical R proteins: RPW8, Xa27
- Enzymatic R (eR) (Hm1, At1/2)

For detail information, see Table 1
Conservation and Diversity of R Proteins

Domains/Motifs of R proteins

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>TIR</td>
<td>Toll / Interleukin 1R</td>
</tr>
<tr>
<td>CC</td>
<td>coiled coil</td>
</tr>
<tr>
<td>NBS</td>
<td>nucleotide binding site</td>
</tr>
<tr>
<td>LRR</td>
<td>leucine-rich repeat</td>
</tr>
<tr>
<td>Kin</td>
<td>kinase domain</td>
</tr>
<tr>
<td>TM</td>
<td>transmembrane domain</td>
</tr>
<tr>
<td>α Helix</td>
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Diagram showing domains and motifs of R proteins with specific examples such as Cf2/4/9, Xa21/26, FLS2, RPW8, and Xa27.
Conservation and Diversity of R Proteins

Some R protein domains are found in animal proteins participating in innate immunity or apoptosis.

TIR
NBS
LRR
Kin

TOLL TLRs
LRR TIR
NOD1/2 CARD
NBS LRR
CED4 Apaf-1
NBS

Innate Immune Response Apoptosis
Over 30 pathogen Avr genes have been isolated and Avr proteins are highly diversified

The time has come to solve the R-Avr recognition enigma

A Key question
Does an R protein physically interacts with the corresponding Avr protein ???

Yes in 3 cases

\[
\begin{align*}
Pto & \text{---AvrPto} \\
Pita & \text{---AvrPita} \\
RRS1-R & \text{---popP2}
\end{align*}
\]

Science, 274:2060-2063; Embo J, 19:4004-4014; PNAS, 100:8024-8029
Step 1  Plant-Pathogen Recognition

Most R and Avr do not directly interact
(Direct R-Avr interaction remains the exception rather than the rule)

“gene-for-gene” model is unable to explain why plants can defend themselves against numerous pathogens with a limited number of \textit{R} genes. (Number of \textit{Avr} \gg \text{number of \textit{R}} genes)

\textit{Pto} (kinase) requires \textit{Prf} (CC-NBS-LRR) for function

\textit{RPS5} (CC-NBS-LRR) requires \textit{PBS1} (kinase) for function

→ “the guard hypothesis”
(Van der Biezen and Jones 1998; Jones and Dangl 2001)
Step 1  Plant-Pathogen Recognition

“The guard hypothesis”

Guard (R)  Guardee (target)  Avr (effector)
Prf, RPS5  Pto, PBS1  avrPto, avrpphB

Defenses
"The guard hypothesis"

Evidence

Guard

RPM1 (CC-NBS-LRR)  

RPS2 (CC-NBS-LRR)  

RPS5 (CC-NBS-LRR)

Guardee

RIN4

PBS1 (kinase)

Pathogen effector

AvrRpm1, AvrB

AvrRpt2

AvrPphB

Avr2

Rcr3 (cysteine protease)

Cf2 (RLP)

Phosphorylation

Degradation

Cleavage

Inhibition
“The guard hypothesis”

Example

RPS2---RIN4---AvrRpt2

Molecular mechanisms of \( R \)-mediated disease resistance

Step 1
- Recognition

Step 2
- Signal initiation

Step 3
- Signal transduction

Step 4
- HR and PR expression
- Activation of defenses
Inter-molecular interaction involving NBS-LRRs

Inactive

Guard (NBS-LRR)

Avr

Other partners

Guardee (host target)

Active

Dangi & Jones  NATURE, 411:826-833 (2001)
Likely R protein partners

RAR1: A component required for function of many NBS-LRRs; Plays a role in R protein accumulation.

SGT1: Interacts with RAR1; Required for function of some NBS-LRR genes; Two separable functions (1) a positive role in R-triggered cell death (2) A negative role in NBS-LRR (RPS5) accumulation.

Hsp90: A chaperone protein required for proper folding of NBS-LRR proteins.

Ready for activation or degradation ??
Intra-molecular interaction of NBS-LRR proteins

Hwang et al, Plant Cell, 12, 1319-1330 (2000)
Intra-molecular interaction of NBS-LRR proteins

Rx protein

Physical Interactions

CC / NBS-LRR
CC-NBS / LRR

Moffett et al, EMBO 21:4511-4519
Intra-molecular interaction of NBS-LRR proteins

Model 1

1. P-loop dependent CC/NBS(?) interaction
2. Recognition of CP
3. Change in nucleotide binding status?
4. Release of active effector molecule(s).

Model 2

1. P-loop independent ARC/LRR interaction
2. Recognition of CP
3. Change in nucleotide binding status?
4. Recruitment of effector molecule(s).

Moffett et al, EMBO J, 21:4511-4519
Molecular mechanisms of $R$-mediated disease resistance

Step 1

Avr → Recognition

Step 2

$R$ → Signal initiation

Step 3

$R^*$ → Signal transduction

Step 4

HR and PR expression → Activation of defenses
Step 3  Signal transduction

 Defense against biotrophs

Two branches at an early step

SA-dependent feedback loop and basal resistance

NPR1-dependent and independent signaling

Basal resistance  HR cell death PR expression  Systemic Acquired Resistance
How NPR1 works

Pathogen infection
SA accumulation
Redox change
Cytoplasm
Nucleus

Signal transduction of plant resistance

Nitric oxide (NO) and Reactive oxygen species (ROS, such as H2O2) are also involved in regulation of HR and disease resistance. But the detail mechanisms are not clear.

Questions to ponder
1. Why can some R gene-mediated resistance be overcome by pathogens in a relatively short period of time?
2. What are the possible advantages of indirect recognition of Avr proteins by plant R proteins?
3. What biological functions may a host target protein (guardian) have? Give 2 examples.
A case study: Broad-spectrum resistance by RPW8

Arabidopsis + Powdery mildew (*Erysiphe*)

Col-0

Ms-0
Compatible interaction between Arabidopsis and Erysiphe
Incompatible interaction between Arabidopsis and Erysiphe
RPW8: A New $R$ Locus in Arabidopsis
The *RPW8* Gene Cluster
RPW8 Encodes A Novel Protein

RPW8 Confers Broad-Spectrum Resistance

Col-0

E. cruciferarum

E. cichoracearum

E. orontii

O. lycopersici

Col-0/RPW8
RPW8 Functions in Tobacco

\[ N. \textit{tabacum} \]
\[ \text{wild type} \]

\[ N. \textit{tabacum} \]
\[ \text{AtRPW8} \]

The RPW8-signaling pathway may be highly conserved
Welcome to Xiao Lab Homepage

The Xiao Lab aims to understand the molecular mechanisms of plant programmed cell death and disease resistance.