Multilayered strategies for attack, defense and counter-defense in the plant cell wall

- Who is in control?
Men vs. Women

Pathogen

Plant
Pathogen secreted proteins and peptides
- Pathogen wall remodeling
- Plant wall degradation
- Detoxification of antimicrobial compounds
- Proteinaceous Inhibitors

The apoplastic battlefield… attack, defense and counterdefense

Pathogen Associated Molecular Patterns (PAMPs)
- Flagellin (bacteria)
- Necrosis inducing proteins (bacteria, oomycetes, fungi)

Effector proteins
- suppress host defenses

Induced basal defenses

Nucleus

Hypersensitive response
Defining the cell wall proteome: a three-pronged attack

1. *In silico* genome-scale prediction

2. “Traditional” proteome profiling

3. Functional secretion screens

In: Biochemistry and Molecular Biology of Plants (2000) (Eds. Buchanan, Gruissem and Jones)
Phytophthora infestans

Late blight: Important disease worldwide, difficult to control

Irish Potato Famine (1845-1850)

P. infestans and tomato genomes sequenced
**P. infestans** Hemibiotrophy

- Hemibiotrophy: Biotrophy-Necrotrophy
- “Stealth” infection strategy
- Requires suppression of host defense
- Regulation of hemibiotrophy not understood

Coordinated by pathogen secretome?

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Song et al. 2003. PNAS. 100, 9128-33.
Tomato Extracellular Proteins

- PR1, P2 (pathogenesis-related) peroxidases, structural proteins, stellacyanin-like
- Many no predicted function
- Predicted function, surprising location
- No predicted signal sequence

P. infestans Extracellular Proteins

- Elicitins, metallopeptidase, cutinase
- Many unknown (not in EST database)
- Most in planta specific
- Unusual domain structure
Northern analysis: *P. infestans* candidates

- **Constitutive**
  - INF1
  - PiTPE2
  - PiTPE22
  - PiTPE3
  - PiTPE7
  - PiTPE14
  - PiTPE21
  - PiTPE23
  - PiTPE17
- **In planta specific**
  - Mycelium
  - Uninfected leaf
  - 0 24 48 72 96 (hr)

rRNA
PiE12 is expressed during biotrophy.

Npp1 is expressed during necrotrophy.

...and Npp1 is expressed during necrotrophy.
PiE12 Domain Structure

RXLR Motif
(X = QLGAGGG)

Signal Peptide

Nuclear Localization Signals
PiE12 Has a Functional NLS

Light

UV

GFP

PiE12  GFP
Model for PiE12 Trafficking

Xanthomonas campestris

AvrBs3

Effector translocation: bacterial type III secretion system vs. oomycete system

P. infestans

Does PiE12 act as an effector?
Transient expression of *P. infestans* proteins in tomato

- PiE12/SNE1 (Suppressor of Necrosis 1) blocks MAMP-mediated necrosis in tomato and tobacco
and Avr/R-gene mediated programmed cell death

- Avr3a/R3a (oomycetes)
- Pto/AvrPto (bacteria)
- Cf9/Avr9 (fungi)
- Rx2/CP (viruses)

- A new class of effector
- Tip of the iceberg?
Dissecting Hemibiotrophy with RNA-Seq

48 hours 96 hours 144 hours
Tomato

- 93,978 unigenes, most singletons
- 20,220 unigenes ≥ 5 reads, 58% differentially expressed (p=0.05)
- Complex reorganization of host metabolism as early as 48 hai
- 100 biochemical pathways differentially expressed after infection

P. infestans

- 3,495 unigenes ≥ 5 reads, 10% differentially expressed (p=0.05)
- 818 genes (23%) not previously identified in P. infestans genome sequence.
- 10% predicted to be secreted proteins
Putative Effectors

Normalized number of reads

Hours after inoculation

RXLR
CRN
INF
NPP
Candidate HR Suppressor Evaluation: *N. benthamiana* Agroinfiltration Assays

- PiNPP1.1
- Empty vector
- PiNPP1.1
- RLXR18215

Suppression of Cell Death (%)

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<th>96 hai</th>
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Controls
Spatial targeting of defense-associated genes

Epidermis: synthesis of cutin and waxes and, in some cases, flavonoids.

Different patterns of chloroplast/chromoplast morphogenesis are apparent between collenchyma and parenchyma cells.

Differential accumulation of starch granules in the inner pericarp.

Tomato pericarp
Laser Capture Microdissection

Small green fruit

- Transcripts
- Proteins
- Metabolites
454 analysis of tissue specific transcriptomes

- 1.5 million ESTs
- 21,000 unigenes

- 3% tissue specific
- 57% all tissues
‘Defense-associated’ Genes

- Most expressed everywhere
- Surface cell layer and vascular tissues enriched

Parenchyma

Collenchyma

Inner epidermis

Vascular

Outer Epidermis
Evolutionary Time Scales and The Perils of Annotation

Secretion of pathogen wall degrading enzymes

Chitinase and EGase inhibitors?

Pathogen Mycelium

Secretion of plant cell wall degrading enzymes

Polygalacturonase inhibitor protein (PGIP)
Pectate lyase inhibitor protein (PLIP)
Xyloglucanase inhibitor protein (XEGIP)
Xylanase inhibitor protein (XIP/TAXI)
Protease inhibitors

GIP: Glucanase Inhibitor Protein

Polygalacturonase
Pectate lyase
Xyloglucanase
Xylanase
Protease
Co-evolution of GIPs and EGases

Codon evolution analysis indicate that GIPs have 6 amino acids under positive selection.

Who is in control? Neither and both
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