Sorina Popescu
Discovering and Validating Protein Interactions

Boyce Thompson Institute
Mission: To advance and communicate scientific knowledge in plant biology to improve agriculture, protect the environment, and enhance human health
Protein Interactions Underlie Most Cellular Processes

• Within a species
  – Protein complexes and molecular machines
  – Stable complexes and transient/sequential protein interactions
  – Required for normal metabolism and development
  – Parts of dynamic pathways and responses to the environment

• Across species
  – Host-pathogen interactions
  – Defense responses to proteins from disease and insect pathogens (e.g. effectors)
Identifying Protein Interactions

• **Functional Protein Microarrays**
  – Proteins expressed in plants and printed on a glass slide (microarray)
  – Can detect interactions
    • Proteins
    • Small Molecules
  – Can explore enzymatic reactions
    • Autophosphorylation, ubiquitination
    • Find substrate for query enzyme

• **Split Luciferase Complementation**
  – Genes cloned as fusion constructs with Renilla luciferase
    • Bait: C-terminal half
    • Prey: N-terminal half
  – Transfected into protoplasts
  – Luciferase activity (light) if proteins interact
Functional Protein Microarrays

• Full-length cDNA libraries – His/Myc tagged
  – ATEC – 7000 ORFs
  – Expressed in N. benthamiana, purified and printed

• Printed microarrays now available through TAIR
Protein Binding

- Printed proteins – Myc tagged, expressed in *N. benthamiana* and printed onto slide
- Query protein – His tagged, purified from transiently transfected *N. benthamiana*

Experimental: FLS2  
Negative Control: BSA  
Loading Control: Anti-Myc
Other Examples

• **Ca^{2+}**-dependent calmodulin binding

Potential calmodulin targets on microarray are reacted with indicated calmodulin (or control) in the presence of calcium

• **Identification and validation of MKK substrates***

Phosphorylation substrate (MBP) aliquoted in microtiter plates and mixed with MPKs and MKKs.

* Or, which MPKs are activated by which MKKs?
Other Examples

- Salicylic acid (small molecule) binding

Salicylic Acid – a small molecule with key roles in stress resistance

Validation

<table>
<thead>
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<th>SABP2</th>
<th>MES9</th>
<th>cSABP1</th>
<th>cSABP2</th>
<th>cSABP3</th>
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<td>4aSA</td>
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AzSA        | Negative Control | Immunoassay

XL: UV Cross-linking
4aSA: labeled salicylic acid

Magali Moreau

BTI Technology Transfer
Identifying Effector Targets to Enhance Plant Disease Resistance

Effectors: Pathogen proteins that thwart plant basal immune response

R-proteins: Plant resistance proteins that interact with effectors to cause a vigorous immune response

Original figure: http://www.nature.com/nchembio/journal/v5/n5/full/nchembio.164.html
Split Luciferase - Constructs

- Construct library of full-length cDNAs, fused to N-terminal part of *Renilla* luciferase (preys)
  - Focus on kinases, which are likely effector targets
- Create fusion of effector protein gene to C-terminal part of *Renilla* luciferase (bait)
Split Luciferase – Protoplast Transfection

Optimization

Time after transfection

# Protoplasts

[Plasmid DNA]
Validating the Assay

<table>
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<tr>
<th>Effector</th>
<th>AvrPto</th>
<th>AvrPto</th>
<th>AvrPto</th>
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<td>AtFls2</td>
<td>Fen</td>
<td>SIMPK4</td>
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<td>Expected</td>
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Conflicting results in the literature

Positive Reference
Identifying New Effector Targets

Tomato MPK4, MPK6, and BSK7 interact with HopAl1 and HopF2

Functional validation: Silencing *N. benthamiana* BSK7 decreases immune response

**Assay:**
Cell-death protection assay:
1) Infiltrate to induce immunity
   Wait 2 days
2) Infiltrate to test immunity in overlapping regions

**Quantification of the Cell-death Suppression assay**

**Infiltration of Nb leaves**

*P. fluorescens*
Weak pathogen

*P. syringae DC3000*
Strong pathogen

Immune response (No Cell Death)

No Immune response (Heavy Cell Death)
Two approaches for protein interactions

- **Functional Protein Microarrays**
  - *In vitro* interactions
  - Requires a direct/binary interaction
  - Amenable to high throughput
  - Can be used for diverse applications
    - Proteins
    - Small Molecules
    - Enzymatic reactions
  - References

- **Split Luciferase Complementation**
  - *In vivo* system
  - Sensitive – may detect complexes if other subunits are present in protoplasts
  - Medium-throughput
  - Does not require protein production/purification