Molecular dialog between two ingenious opponents-plants and pathogenic bacteria

Magdalena Krzymowska
PAMP TRIGGERED IMMUNITY (PTI)

nonhost, plant is not a host
= pathogen does not colonize a plant)

preformed barriers and inducible response

ancient and most common mechanism

PAMPs (pathogen associated molecular patterns)

LPS, flagellin, EF-Tu methylated bacterial DNA, glucan

Pattern Recognition Receptors PRR
Zigzag model

PTI ETI

Transmission electron micrographs of epidermal cells of tobacco leaves infected with *Pseudomonas syringae* 

Krzymowska et al. Plant J. 2007, 253-64
Structure of Type Three Secretion System of *Pseudomonas syringae* pv. *tomato* DC3000

Structure of Type Three Secretion System
Salmonella subsp. enterica ser. typhimurium

HopQ1 promotes *P. syringae* growth in common bean and tomato

*HopQ1 plays a role in determining the host range of Pseudomonas syringae* avirulence

virulence

HopQ1 promotes *P. syringae* growth in common bean and tomato

*Nicotiana* spp. plants are resistant to *Pseudomonas syringae* expressing *hopQ1*
HopQ1 model (i-tasser)
HopQ1 DXXXDXDD
NH DXDXXXDD

Versées et al., J Mol Biol, 2006
HopQ1 co-purifies with 14-3-3 proteins from \textit{N. benthamiana}

Cultures of \textit{Agrobacterium} carrying:

- Vector
  - Injection into \textit{N. benthamiana}
  - Affinity purification on \textit{Strep}-Tactin resin
  - LC-MS-MS/MS analysis
  - 14-3-3 proteins were not detected

- HopQ1-\textit{Strep}-tag II
  - Injection into \textit{N. benthamiana}
  - Affinity purification on \textit{Strep}-Tactin resin
  - LC-MS-MS/MS analysis
  - Detection of 8 isoforms of 14-3-3 proteins from \textit{N. benthamiana}
Mass spectrometry analysis

<table>
<thead>
<tr>
<th>gi</th>
<th>Description</th>
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<tbody>
<tr>
<td>gi</td>
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<td>221103824</td>
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<tr>
<td>gi</td>
<td>1345675</td>
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The 14-3-3 binding site is conserved in HopQ1, the TTSS effector from *Pseudomonas syringae*, and XopQ, its xenolog from *Xanthomonas* spp.

14-3-3 binding motif

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. syringae</em> pv. <em>phaseolicola</em> 1448A</td>
<td>P V L E R S K S A P A L L T A</td>
</tr>
<tr>
<td><em>P. syringae</em> pv. <em>tomato</em> DC3000</td>
<td>P V L E R S K S A P A L L T A</td>
</tr>
<tr>
<td><em>X. campestris</em> pv. <em>campestris</em> ATCC33913</td>
<td>A V L K R S L S A P A L T A T</td>
</tr>
<tr>
<td><em>X. campestris</em> pv. <em>vesicatoria</em> 85-10</td>
<td>P R H R R A Q S L P A R L T P</td>
</tr>
<tr>
<td><em>X. oryzae</em> pv. <em>oryzae</em> KACC10331</td>
<td>P R H R R T Q S L P A R L T P</td>
</tr>
</tbody>
</table>
Kinase activity capable of phosphorylating HopQ1 is ubiquitously conserved in plants

<table>
<thead>
<tr>
<th>Nicotiana benthamiana</th>
<th>Solanum tuberosum</th>
<th>Zea mays</th>
<th>Arabidopsis thaliana</th>
</tr>
</thead>
<tbody>
<tr>
<td>HopQ1</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CBB</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Recombinant HopQ1 with a C-terminal 6xHis epitope was expressed in E. coli and then incubated with leaf protein extracts from N. benthamiana in buffer containing [γ³²P]ATP. Samples were resolved by SDS-PAGE and analyzed by autoradiography.
The predicted 14-3-3–binding motif of HopQ1 is phosphorylated by plant kinases

hopQ1 expression *N. benthamiana*

recombinant HopQ1 (*E. coli*) incubated with *N. benthamiana* extract
Serine 51 plays a critical role in the phosphorylation of HopQ1
FRET-FLIM analysis showing that HopQ1 S51 is critical for 14-3-3a binding

Lifetime images encoded as pseudo-colors according to the scale shown at the bottom.
HopQ1 binds to 14-3-3a in phosphorylation dependent manner
Interaction with 14-3-3s can affect various aspects of partner proteins

1. Stability, activity, conformation

2. Bridging proteins

3. Subcellular localization

Interaction with 14-3-3s can affect various aspects of partner proteins:

- Stability, activity, conformation
- Bridging proteins
- Subcellular localization

Specifically, the interaction with 14-3-3s can affect phosphorylation, subcellular localization between the nucleus and cytoplasm, and the formation of a dimer.
Subcellular localization of HopQ1 variants

HopQ1

HopQ1-S51A
Co-expression of 14-3-3a and HopQ1 affects nuclear-cytoplasmic partitioning of the binding partners
Interaction with 14-3-3 proteins affects HopQ1 stability in plants.
R18 dramatically reduces stability of *in vitro* assembled complex of HopQ1-Flag and 14-3-3a-Strep incubated with bean crude protein extract.
Assessment of virulence properties of HopQ1 effector mutated to eliminate 14-3-3 binding

\[ P. syringae - \text{HopQ1} = 10^3/\text{ml} \]
\[ P. syringae - \text{control} = 10^3/\text{ml} \]

\[ P. syringae - \text{HopQ1} = 123 \]
\[ P. syringae - \text{control} = 59 \]

HopQ1/HopQ1-S51A

Competitive index

<table>
<thead>
<tr>
<th></th>
<th>2</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>HopQ1/HopQ1-S51A</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

dpi
Small Angle X-ray Scattering (SAXS)

- Low resolution (~20 Å)
- In solution (Tris-buffer pH ~7.5)
- Various conditions (pH, salt, temperature)

X-ray crystallography

- High resolution (~1.20 Å)
- Crystals (non-physiological conditions)
X-Ray scattering

Biological Small Angle X-ray Scattering:

1. Biological SAXS is a solution scattering technique
   a. Change of environmental parameters such as temperature, pH, salt is possible
   b. No protein crystals necessary
2. “Single shot” technique
   a. Only 1-dim data recorded

Low resolution method
No direct recalculation of 3-dim model
p(r) – function describes probability to find a point at distance r from a given point inside particle
Multi Angle Light Scattering

http://www.ap-lab.com/light_scattering.htm
HopQ1-14-3-3a complex

<table>
<thead>
<tr>
<th>Protein</th>
<th>Theoretical mass (kDa)</th>
<th>MALS mass (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-3-3 dimer</td>
<td>60.0</td>
<td>57.1 ± 4%</td>
</tr>
<tr>
<td>HopQ1</td>
<td>49.8</td>
<td>49.4 ± 1.7%</td>
</tr>
<tr>
<td>1:2 HopQ1-14-3-3</td>
<td>109.8</td>
<td>100.0 ± 0.5%</td>
</tr>
</tbody>
</table>
HopQ1-14-3-3a complex

superposition of an envelope model (SAXS) and the predicted HopQ1 - 14-3-3 complex model

M. Taube
M. Kozak
F. Giska

Laboratory of Plant Pathogenesis
HopQ1 forms oligomers

<table>
<thead>
<tr>
<th>Protein</th>
<th>Theoretical mass (kDa)</th>
<th>MALS mass (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HopQ1 monomer</td>
<td>49.8</td>
<td>51.9 ± 2%</td>
</tr>
<tr>
<td>HopQ1 dimer</td>
<td>99.6</td>
<td>93.6 ± 0.7%</td>
</tr>
<tr>
<td>HopQ1 trimer</td>
<td>149.4</td>
<td>138.2 ± 1.2%</td>
</tr>
</tbody>
</table>
Disulphide-linked oligomers of HopQ1

F. Giska, M. Piechocki
Disulphide-linked oligomers of HopQ1
Chelation of calcium ions induces HopQ1 dimer formation in the presence of DTT (gel filtration)

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F. Giska

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Laboratory of Plant Pathogenesis
HopQ1  DXXXDXDD
NH    DXDXXXXDD

Versées et al., J Mol Biol, 2006
Mutation in calcium binding motif leads to dimer formation

101-108

HopQ1  DXXDXXDD

F. Giska
Chelation of calcium ions induces HopQ1 dimer formation in the presence of DTT (SAXS)
Structure of HopQ1 dimer (SAXS analysis)

superposition of envelope model and the predicted HopQ1 model
RihA, NH from *Escherichia coli*, forms tetramers
RihA forms tetramers in the presence of EDTA

<table>
<thead>
<tr>
<th>Protein</th>
<th>Theoretical mass monomer (kDa)</th>
<th>Theoretical mass tetramer (kDa)</th>
<th>MALS mass (kDa)</th>
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</thead>
<tbody>
<tr>
<td>RihA 5 mM DTT</td>
<td>34.9</td>
<td>139.6</td>
<td>132.6 ± 0.6%</td>
</tr>
<tr>
<td>RihA 5 mM DTT</td>
<td>34.9</td>
<td>139.6</td>
<td>132.2 ± 0.7%</td>
</tr>
<tr>
<td><strong>1 mM EDTA</strong></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Conclusions

1. HopQ1 binds host 14-3-3 proteins in phosphorylation-dependent manner.

2. Interaction with 14-3-3 affects steady-state level and subcellular localization of HopQ1.

3. Binding of 14-3-3 by HopQ1 has limited influence on promoting *P. syringae pv. tomato* DC3000D28E growth in *P. vulgaris*.

4. Stoichiometry of HopQ1-14-3-3a complex is 1:2.

5. HopQ1 forms various oligomers.
2nd Annual Conference

ZAKOPANE, 15-17 OCTOBER 2014

The call for abstracts for the 2nd annual conference will open on 4th February 2014.

The second Annual Conference of the SUSTAIN Action is due to take place in Zakopane, Poland from 15th - 17th October 2014. Zakopane is a town in the south of Poland, close to the border with Slovakia.
Jacek Hennig
Fabian Giska
Rafał Hoser
Małgorzata Lichocka
Marcin Piechocki
Wojciech Siwek

Collaboration:
Mirosław Sobczak, WULS
Maciej Kozak, UAM
Gitta Coaker, UC Davis
Justin Lee, IPB Halle

http://plantpath.ibb.waw.pl
Collaborators:
Gitta Coaker (UC Davis)
Elmon Schmelzer (MPIZ)
Maciej Kozak (UAM)
Subcellular localization of HopQ1 variants

![Images of HopQ1 variants](image)

![Bar chart comparing localization of HopQ1 variants](chart)
HopQ1 interaction with 14-3-3s is not critical for its perception by host plants (avirulence)

A

HopQ1-S51A   HopQ1   Control

Agroinfiltration

B

HopQ1-S51A   HopQ1   Control

P. syringae expressing hopQ1

F. Giska
<table>
<thead>
<tr>
<th>gi</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>71725175</td>
<td>type III effector HopQ1 [Pseudomonas syringae pv. phaseolicola 1448A]</td>
</tr>
<tr>
<td>494360</td>
<td>Chain A, The Refined 1.6 Angstroms Resolution Crystal Structure Of The Cc</td>
</tr>
<tr>
<td>3318722</td>
<td>Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX</td>
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<td>vacuolar H+-ATPase B subunit [Nicotiana tabacum]</td>
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<tr>
<td>26986106</td>
<td>vacuolar ATPase subunit B [Mesembryanthemum crystallinum]</td>
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<td>16950587</td>
<td>phosphoenolpyruvate carboxykinase [Lycopersicon esculentum]</td>
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<td>27883932</td>
<td>vacuolar H+-ATPase A1 subunit isoform; V-ATPase A1 subunit isoform [Lycop</td>
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<td>584786</td>
<td>RecName: Full=Abscisic stress-ripening protein 1</td>
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<td>157358208</td>
<td>unnamed protein product [Vitis vinifera]</td>
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<td>putative vacuolar proton ATPase subunit E [Lycopersicon esculentum]</td>
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<td>220938467</td>
<td>phosphoenolpyruvate carboxykinase [Arundinaria sp. PC-2007]</td>
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<tr>
<td>3023189</td>
<td>RecName: Full=14-3-3-like protein C; AltName: Full=14-3-3-like protein B</td>
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<tr>
<td>63053870</td>
<td>phosphoenolpyruvate carboxylase [Alternanthera ficoidea]</td>
</tr>
<tr>
<td>108742705</td>
<td>phosphoenolpyruvate carboxylase [Sesuvium portulacastrum]</td>
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<td>218312</td>
<td>chloroplast elongation factor TuB (EF-TuB) [Nicotiana sylvestris]</td>
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<tr>
<td>15799694</td>
<td>molecular chaperone DnaK [Escherichia coli O157:H7 EDL933]</td>
</tr>
<tr>
<td>44917135</td>
<td>14-3-3 a-1 protein [Nicotiana tabacum]</td>
</tr>
<tr>
<td>221103824</td>
<td>PREDICTED: similar to predicted protein [Hydra magnipapillata]</td>
</tr>
<tr>
<td>1345675</td>
<td>RecName: Full=Catalase isozyme 1</td>
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</table>

F. Giska
Interaction with 14-3-3 proteins increases HopQ1 stability in plants

<table>
<thead>
<tr>
<th></th>
<th>HopQ1</th>
<th>14-3-3a</th>
<th>R18 [mM]</th>
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<tbody>
<tr>
<td>Incubation (1h, 30°C)</td>
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<td></td>
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</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0.3</td>
</tr>
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<td>+</td>
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<td>0</td>
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<tr>
<td>+</td>
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<td>+</td>
<td>0.05</td>
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<td>0.15</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.3</td>
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R18, competitive inhibitor of 14-3-3 binding
Deletion of DXXXDXDXX in HopQ1-1 from *P. syringae* pv. *tomato* DC3000 affects its virulence properties.

Wei Li, Yi-Hsuan Chiang, Gitta Coaker The HopQ1 effector's nucleoside hydrolase domain is required for bacterial virulence in Arabidopsis and tomato, but not host recognition in tobacco. PLoS ONE 2013
FRET-FLIM analysis showing that HopQ1 S51 is critical for 14-3-3a binding

HopQ1-14-3-3a interaction enables energy transfer from CFP to YFP resulting in reduction of CFP fluorescence life-time. Fluorescence life-time of free CFP-14-3-3a 2360 picoseconds.  

E. Schmelzer, M. Lichocka
HopQ1  DXXXDXDD
NH    DXDXXXDD

Ca^{2+}  D107A
D108A

Versées et al., J Mol Biol, 2006
HopQ1 interaction with 14-3-3s is not critical for its perception by host plants (avirulence)

A

HopQ1-S51A    HopQ1    Control

Agroinfiltration

B

HopQ1-S51A    HopQ1    Control

P. syringae pv. syringae expressing hopQ1

F. Giska
**P. syringae – HopQ1** = $10^3$/ml  
**P. syringae – control**  
**P. syringae – HopQ1-S51A** = $10^3$/ml  
**P. syringae – control**

**bean inoculation**

**bacteria isolation, 14 dpi**

**Competitive index assay**

**P. syringae – HopQ1** = 123  
**P. syringae – control** = 59  
**P. syringae – HopQ1-S51A** = 48  
**P. syringae – control** = 43
Transient expression of *hopQ1* in planta

1. **35S Promoter**
   - `hopQ1-Strep-tag`

2. **Agrobacterium tumefaciens**
   - Infiltration into *Nicotiana benthamiana* leaves

3. Incubation, 48h
4. Affinity purification on Strep-Tactin resin
5. Protein identification by LC-MS-MS/MS analysis
Interactions of HopQ1, a type III secretion effector from *Pseudomonas syringae*, with host 14-3-3 proteins

Magdalena Krzymowska