Clathrin-mediated endocytosis facilitates internalization of *Magnaporthe oryzae* effectors into rice cells

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M. oryzae effectors

Hypothesis:

*M. oryzae* cytoplasmic effectors are delivered from the BIC and internalized into the rice cell’s cytoplasm by **endocytosis**.

Biotrophic Interfacial Complex (BIC): contains mainly cytoplasmic effectors

The BIC organization on an invasive primary hypha

BICs are formed by multiple vesicles containing mainly cytoplasmic effectors
Dynamics of BIC and cytoplasmic effector delivery

BICs are very dynamic

Hypha undergoing switch
Tagging two mechanisms of plant endocytosis

- **Plant plasma membrane**
- **Plant cytosol**

**clathrin-mediated endocytosis (CME)**
- Clathrin
- Actin

**clathrin-independent endocytosis (CIE)**
- Flotillin
Lti6b (Plant plasma membrane marker protein) localization

Control

Rice cells infected by *M. oryzae*, KV209

- BICs are formed by **plant plasma membrane** (see Lti6b:eGFP localization – green fluorescence)

<table>
<thead>
<tr>
<th>Lti6b:eGFP</th>
<th>Pwl2:mRFP</th>
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<tbody>
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<td><strong>merge</strong></td>
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**Bar chart:**
- BIC on primary hyphae
- BIC on bulbous hyphae
Plant plasma membrane associated effector: Bas83

Bas83 localizes at BICs, EIHM and plasma membrane of vesicles
(see Bas83:mRFP localization – red fluorescence)
Plant Actin localization

- BICs contain large amounts of **plant Actin** (see LifeAct:eGFP localization – green fluorescence)
- BICs contain large amounts of **plant Clathrin**
  (see CLC1:eGFP localization – green fluorescence)
Plant Clathrin dynamics

Rice cells infected by *M. oryzae*, KV209
BICs contain large amounts of plant Clathrin

![Graph showing the percentage of fluorescing BICs for CLC1:eGFP and Flot1:eGFP on primary hyphae and bulbous hyphae.](image)

- **CLC1:eGFP**
  - BICs on Primary Hyphae: 100%
  - BICs on Bulbous Hyphae: 100%

- **Flot1:eGFP**
  - BICs on Primary Hyphae: 40%
  - BICs on Bulbous Hyphae: 40%
Silencing of rice endocytosis machinery

Biotrophic fungal infection requires mainly clathrin-mediated endocytosis.
Silencing of rice endocytosis

Biotrophic fungal infection requires mainly clathrin mediated endocytosis.

Clathrin independent endocytosis
## Chemical plant endocytosis inhibition

### Table 1. Chemical endocytosis inhibitors

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Target</th>
<th>Mode of action</th>
<th>Comments</th>
<th>References</th>
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<tbody>
<tr>
<td>Filipin</td>
<td>CIE*</td>
<td>Binds to sterols in the membrane.</td>
<td>Toxic at higher concentration.</td>
<td>(Rodal et al., 1999)</td>
</tr>
<tr>
<td>Methyl-β-cyclodextrin</td>
<td>CIE</td>
<td>Deplete cellular membranes of sterol by increasing the water solubility of the sterol.</td>
<td>Caveolae-dependent endocytosis.</td>
<td>(Rodal et al., 1999)</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>CME**</td>
<td>Translocates clathrin and AP2 from the cell surface to intracellular endosomes.</td>
<td>Also inhibits CIE in some cells.</td>
<td>(Wang et al., 1993)</td>
</tr>
<tr>
<td>Cantharidin</td>
<td>CME</td>
<td>Affect PP2A (cantharidin)</td>
<td>Affect flg22-mediated FLS2 endocytosis.</td>
<td>(Serrano et al., 2007)</td>
</tr>
<tr>
<td>Fluazinam</td>
<td>CME</td>
<td>Affect PP2A (cantharidin)</td>
<td>Affect flg22-mediated FLS2 endocytosis.</td>
<td>(Serrano et al., 2007)</td>
</tr>
<tr>
<td>Triclosan</td>
<td>CME</td>
<td>Affect PP2A (cantharidin)</td>
<td>Affect flg22-mediated FLS2 endocytosis.</td>
<td>(Serrano et al., 2007)</td>
</tr>
<tr>
<td>Wortmannin</td>
<td>nd***</td>
<td>Inhibits PI3Ks and PI4Ks, block vacuolar trafficking, multivesicular bodies and endocytosis.</td>
<td>Late endosomes lose their clathrin; Potential CME association.</td>
<td>(Bright et al., 2001; Robinson et al., 2008; Sachle et al., 2002)</td>
</tr>
<tr>
<td>Concanamycin A</td>
<td>nd</td>
<td>Targets V ATPase and blocks trafficking at trans-Golgi network, endosome acidification.</td>
<td>Induce acidification, which is crucial for many endocytic pathways.</td>
<td>(Maranda et al., 2001; Robinson et al., 2008)</td>
</tr>
</tbody>
</table>

*CIE: clathrin-independent endocytosis; **CME: clathrin-mediated endocytosis; ***nd: not determined.
Biotrophic fungal infection requires mainly clathrin mediated endocytosis.
Biotrophic fungal infection requires mainly clathrin mediated endocytosis.
Conclusions

• Based on these results we conclude that cytoplasmic effector translocation is mediated by vesicle formation, and may be characteristic of appressoria as well as biotrophic invasive hyphae.

• Clathrin-dependent endocytosis mediates internalization of *M. oryzae* effectors into rice cells

• Our results also suggest a potential role of *M. oryzae* effectors for manipulation of host cell endocytosis.
Thank you for your attention

Acknowledgments:

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Garcia’s Lab

Talbot’s Lab

Nelson’s Lab