Legionella pneumophila: an intracellular pathogen of phagocytes

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Legionella pneumophila
- Gram-negative bacterium
- Facultative intracellular pathogen
- Protozoa are natural hosts
- Aerosols can result in human infection
- Replication in macrophages
- Legionnaires' disease

Horwitz and colleagues define the *Legionella* lifecycle
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The Shuman and Isberg laboratories independently identify the dot and icm genes

- Andrea Marra: Complementing an icm defect restores the ability of Legionella 25d to avoid fusion with lysosomes
- Karen Berger: The dotA gene is essential for intracellular replication and avoiding lysosome fusion

Joe Vogel and Gil Segal sequence a total of 24 dot and icm genes

The dot and icm genes define a new type IV secretion system

Host cell plasma membrane
Outer membrane
Inner membrane
A. tumefaciens virB type IVa

Figure by Christie and Vogel

Why is the Dot/Icm system required for intracellular replication?

- Does Dot/Icm control nutrient acquisition into the vacuole?
- Does Dot/Icm poison the cell to make it generally permissive for bacterial replication?
- Does Dot/Icm interfere directly with vesicle transport?
- Are bacterial effector proteins translocated by the Dot/Icm system?
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**Endocytic membrane transport**

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**The Dot/Icm system mediates rapid evasion of endocytic maturation**

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**Understanding Dot/Icm function through coinfection of macrophages**

Joern Coers and Catherine Monahan

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The Dot/Icm system controls transport of the *Legionella*-containing vacuole

<table>
<thead>
<tr>
<th>All bacteria</th>
<th>dotA mutant</th>
<th>Merge-DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>All bacteria + nucleus</td>
<td>dotA mutant</td>
<td>Merge</td>
</tr>
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</table>

*Legionella* subverts vesicular transport between the endoplasmic reticulum and Golgi

Inhibiting Golgi assembly and membrane transport in the late secretory pathway had no effect on *Legionella* replication

Inhibiting membrane transport from the ER interfered with *Legionella* replication

Inhibiting membrane transport in the endocytic pathway enhanced *Legionella* replication

Jonathan Kagan

*Legionella* replication requires vesicular transport out of the endoplasmic reticulum

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ARF function is required for ER remodeling of the Legionella-containing vacuole

ER labeled with YFP-KDEL
No inhibition of ARF

ER labeled with YFP-KDEL
ARF function inhibited with Brefeldin A

ER-derived vesicles associate rapidly with the Legionella-containing vacuole

5 min post infection
15 min post infection

Lew Tilney and Cam Robinson

Legionella utilize host GTPases for biogenesis of a vacuole that supports replication

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The Dot/Icm system modulates Arf

The Dot/Icm system modulates Rab1

Control of Arf and Rab GTPases
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Can we identify Legionella proteins that modulate Arf and Rab1 function?

Identification of raIF (Recruitment of Arf to the Legionella phagosome)

RaIF is present on the cytosolic surface of the Legionella-containing vacuole
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RaIF is delivered into host cells by the Dot/Icm system upon uptake

RaIF defines a bacterial member of the Sec7 family of Arf exchange factors

Activation of Arf by Legionella
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Can Legionella modulate the function of other host GTPases?

Identification of DrrA: a Legionella effector protein that modulates Rab1 activity

DrrA is a novel protein that functions as a guanine nucleotide exchange factor for Rab1
Does **Legionella** have other effectors that target Rab1 specifically?

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DrrA does not require other **Legionella** effectors to localize and activate Rab1

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DrrA is able to dissociate GDI from Rab1

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DrrA has GDI displacement activity

<table>
<thead>
<tr>
<th>Rab-GDI</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
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<tr>
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<td>His-DrrA 451-647</td>
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</table>

DrrA modulates Rab1 function by removing GDI and stimulated GDP for GTP exchange

LepB interacts specifically with Rab1-GTP
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LepB interacts specifically with Rab1-GTP (2)

LepB is a Rab1 GAP

Control of Rab1 membrane cycling by Legionella effector proteins
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**Legionella: the greatest cell biologist on Earth!**

- Phagocytosis
- Trafficking
- Inhibit host defenses
- Egress
- Estimated 150 effectors
- Modulate host transcription/translation
- Cell survival

**Estimated 150 effectors**

**Cell survival**

**Modulate host transcription/translation**

**Inhibit host defenses**

**Legionella**

Phagocytosis

Trafficking

Inhibit host defenses

Egress

Estimated 150 effectors

Modulate host transcription/translation

Cell survival

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