Legionella Subvert the Functions of Rab1 and Sec22b to Create a Replicative Organelle

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Abstract

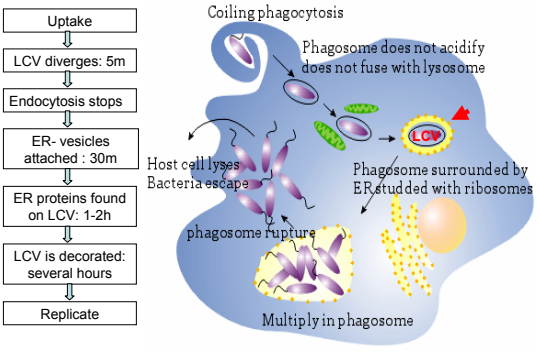
Legionella pneumophila is a bacterial pathogen that infects eukaryotic host cells and replicates inside a specialized organelle that is morphologically similar to the endoplasmic reticulum (ER). To better understand the molecular mechanisms governing transport of the *Legionella*-containing vacuole (LCV), we have identified host proteins that participate in the conversion of the LCV into a replicative organelle. Our data show that Rab1 is recruited to the LCV within minutes of uptake. Rab1 recruitment to the LCV precedes remodeling of this compartment by ER-derived vesicles. Genetic inhibition studies demonstrate that Rab1 is important for the recruitment of ER-derived vesicles to the LCV and that inhibiting Rab1 function abrogates intracellular growth of *Legionella*. Morphological studies indicate that the Sec22b protein is located on ER-derived vesicles recruited to the LCV and that Sec22b is delivered to the LCV membrane. Sec22b function was found to be important for biogenesis of the specialized organelle that supports *Legionella* replication. These studies demonstrate that *Legionella* has the ability to subvert Rab1 and Sec22b function to facilitate the transport and fusion of ER-derived vesicles with the LCV, resulting in the formation of a specialized organelle that can support bacterial replication.

Presented by Jiangsong Jiang

Legionnaire's Disease

- *L. pneumophila* was first found as cause of pneumonia in 1976 at Legionnaires convention in Philadelphia (221 infected, 34 died)
- This disease has highlighted the need to keep air conditioners clean: incidence increased dramatically with central air conditioning in large buildings
- Invades and replicates within a protective phagosome inside alveolar (***LCV: Legionella*-Containing Vacuole**)

Schema for *L. pneumophila* pathway in macrophages



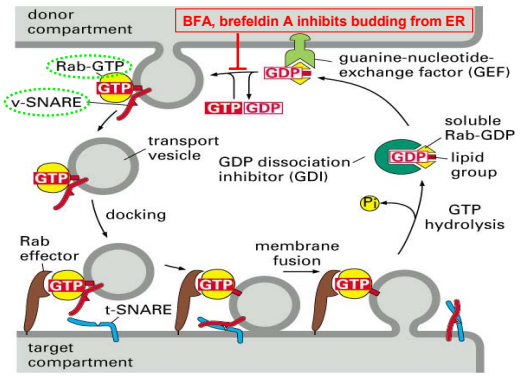
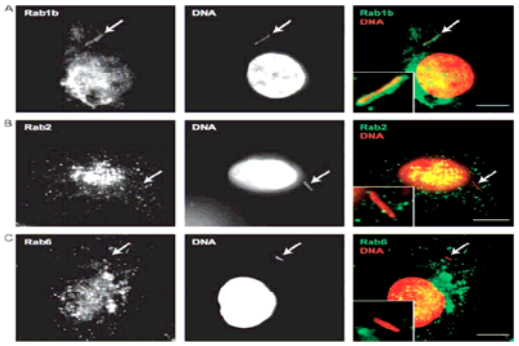
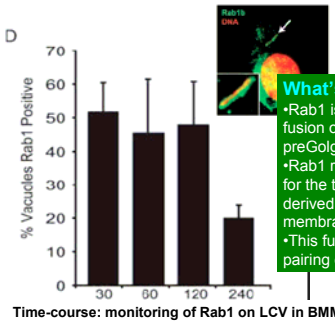


Figure 13–14. Molecular Biology of the Cell, 4th Edition.

Are Rabs involved in *Legionella*-Containing Vacuole?



Specific recruitment of Rab1 to the LCV



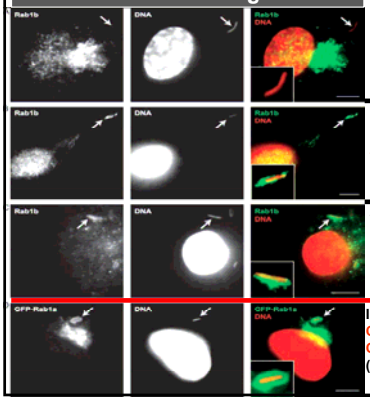
Time-course: monitoring of Rab1 on LCV in BMM cells

What's Rab1?

- Rab1 is a GTPase involved in fusion of ER-derived vesicles with preGolgi and the Golgi apparatus;
- Rab1 recruits factors necessary for the tethering and fusion of ER-derived vesicles with target membranes ;
- This fusion process requires the pairing of v-SNARE and t-SNARE.

Whom does Rab1 hang out with?

Immunofluorescence Microscopy



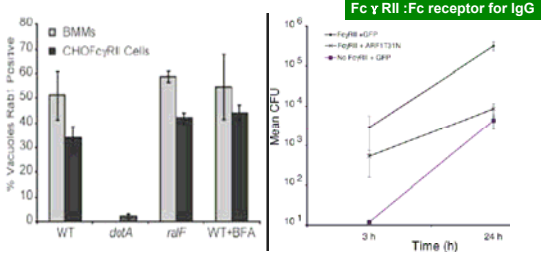
***Legionella dotA* mutant:**
Dot/Icm: Type IV Secretion System
(needed)

***Legionella ralF* mutant:**
RalF: an GTP exchange factor for ARF1 and recruiting ARF1 to LCV
(not required)

With brefeldin A:
BFA: ⊗ (GDP → GTP) Budding from ER
("you can't stop it!")

In CHO-Fc γ RII cells:
CHO cell: nonphagocytic,
CHO-Fc γ RII cell: opsoniztion
(It is the same with BMM)

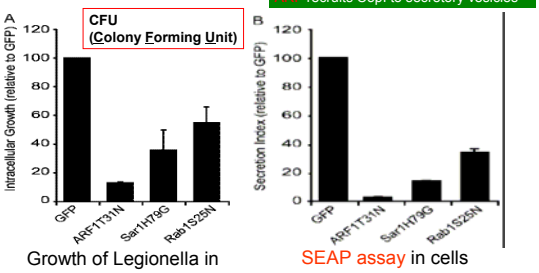
Opsonization
Fc γ RII :Fc receptor for IgG



Requirements for Rab1 to the LCV are similar in BMMs and CHO-Fc γ RII cells ⇒ **Note: "we use CHO-Fc γ RII cells to replace BMMs from now on !"**

Rab1 is important for *Legionella's* intracellular growth

Sar1 recruits CopII to ER membrane
ARF recruits CopI to secretory vesicles



Growth of Legionella in cells with mutant proteins

SEAP assay in cells with mutant proteins
(Secretion of Alkaline Phosphatase)

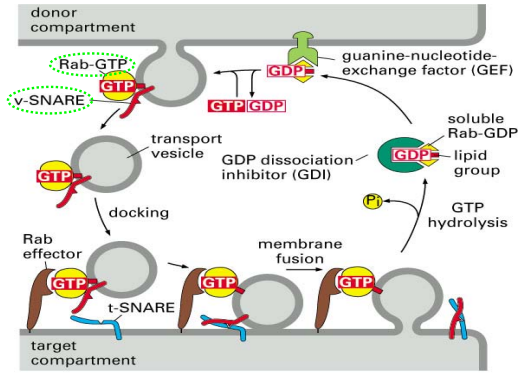
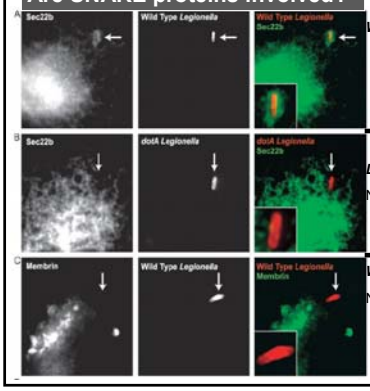


Figure 13–14. Molecular Biology of the Cell, 4th Edition.

Are SNARE proteins involved?

Sec22b: v-SNARE
Membrin: t-SNARE



***WT Legionella*:**
Sec22b is recruited to LCV

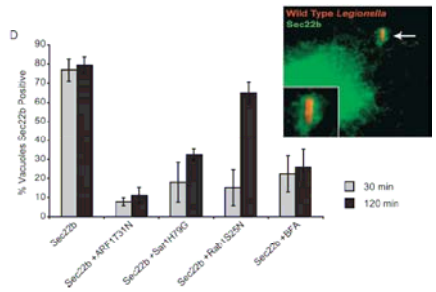
MYC sec22b

***Legionella dotA* mutant:**
No Sec22b is recruited to LCV

***WT Legionella*:**
No Membrin is recruited to LCV

MYC Membrin

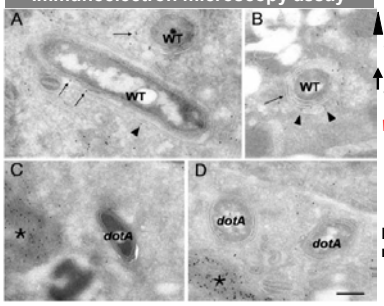
Sec22b staining of vacuoles containing *wt Legionella* in CHO-Fc γ RII cells expressing mutational proteins



Rab1 promotes transport and fusion of ER-derived vesicles containing Sec22b

ER-derived vesicles deliver sec22b to the LCV

Immunoelectron microscopy assay



▲: sec22b on vesicles attached to the LCV

↑: sec22b on the vacuole membrane

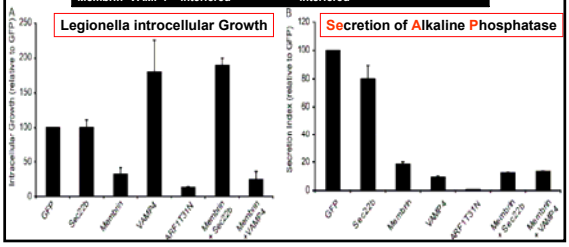
Wild type Legionella

No sec22b on *dotA* mutant *Legionella*

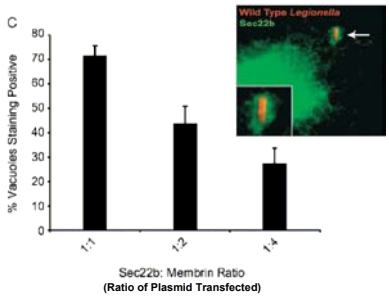
Sec22b, transmembrane protein → ER-derived vesicles fuse with the LCV

Overproduction of SNAREs had different effects

Overproduction	Effect on Legionella introcellular Growth	SEAP	Feature of Protein
Sec22b	did not affect	did not affect	v-SNARE
Membrin	interfered	interfered	t-SNARE
VAMP4	Enhance	Interfered	trans-Golgi SNARE
ARF1T31N	Interfered	Interfered	Dominant negative
Membrin+Sec22b	Enhance	Interfered	-
Membrin+VAMP4	interfered	interfered	-



The ratio of sec22b:membrin affected the delivery of Sec22b to the LCV



Sec22b is important for establishment of the Legionella replicative organelle!

Summary

- Rab1 is specifically recruited by LCV, which is dependent upon Dot/Icm system
- Rab1 mutant Interferes with intracellular replication of *legionella*
- *Rab1 function is associated with the delivery of the sec22b to the LCV*
- Immunoelectron microscopy assay indicated that sec22b is delivered by ER-derived vesicles
- Host ER–Golgi Rab1 and SNARE proteins play an important role in formation of the *Legionella* replicative organelle.

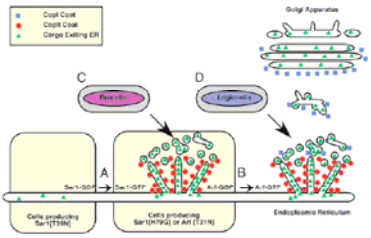
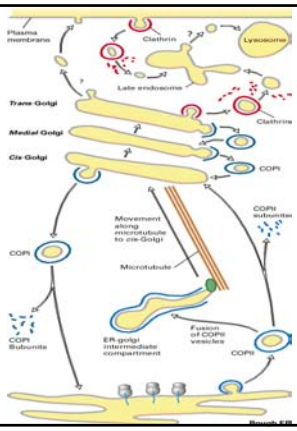
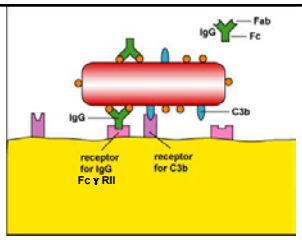


Fig. 1. Vacuoles containing *Brucella* and *Legionella* converge on the secretory pathway at different stages. (*A*) Sar-1 activation leads to the recruitment of the CopII coat complex to ER membranes. CopII recruits cargo that is to be delivered to other organelles and packages the cargo into membrane tubules and vesicles. In cells producing Sar-1[T39N], CopII recruitment to membranes is inhibited, and ER exit sites do not function. In cells producing Sar-1[H79G] protein or Arf[T31N] protein, ER exit sites are functional, but cargo is not transported away from the ER. (*B*) Arf activation leads to the recruitment of the CopI coat complex to early secretory vesicles. The exchange of the CopII coat for the CopI coat on these membranes allows further protein sorting to occur on these vesicles and transport of cargo away from the ER. Cells are able to assemble a functional Golgi apparatus, and the secretory pathway is fully operational. (C) Vacuoles containing *Brucella* require the activities of the Sar-1CopII system for productive interactions with the ER. Cells producing the Sar-1[T39N] protein are nonpermissive for *Brucella* replication. (*D*) Vacuoles containing *Legionella* require the sequential activities of the Sar-1CopII system and the ArfCopI system for productive interactions with the ER. Cells producing either Sar-1[H79G] or Arf[T31N] are nonpermissive for *Legionella* replication.





One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The "tips" of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The "stalk" of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called **opsonization**.

Note: The drug Brefeldin A inhibits activation of the ARF protein by inhibiting nucleotide exchange, and thereby inhibits budding of COPI vesicles

despite in vitro studies demonstrating functional identity between Rab1a and Rab1b, there may be an undetected difference that could change the experimental interpretation

colony forming unit (CFU)
SEAP, secretory alkaline phosphatase;
Fc receptor for IgG (Fc γ RII).

Trafficking: ER to Golgi to Lysosome

2. Types of Coats :

b. CopI: Made of coatamer subunits.
Mediates retrieval of proteins from Golgi to ER (retrograde transport).
COPI vesicles transport ER resident proteins with KKXX or RRXX signals.
Uses GTP binding protein ARF (as does clathrin).
Note: The drug Brefeldin A inhibits activation of the ARF protein by inhibiting nucleotide exchange, and thereby inhibits budding of COPI vesicles.

c. CopII: Mediates forward movement of vesicles from ER to Golgi (anterograde transport).
Regulated by a GTP binding protein Sar1.
Budding of COPII is not inhibited by Brefeldin A (which is specific for Arf).